Identification of Bacterial Infection in Patients Presenting with Acute Exacerbation of Chronic Bronchitis, with Particular Reference to Clinical Trials of Antibacterial Drugs.

by

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ABSTRACT

In clinical trials of antibacterial drugs for treatment of acute exacerbation of chronic bronchitis (AECB), a high proportion of patients who have bacterial infection is essential. In 83 randomised clinical trials of antibacterial drugs in AECB or acute exacerbation of chronic obstructive pulmonary disease (AECOPD), published between 1977 and 2007, the percentage of patients who were bacteriologically-positive ranged from 13.9% to 96.0%. There was no evidence that any particular combination of signs and symptoms was associated with a bacterial exacerbation. A study of patients with signs and symptoms of AECB was undertaken to identify entry criteria that would optimise the number of enrolled patients with bacteriologically-proven (culture-positive) exacerbations. This failed to demonstrate that patients with Type I exacerbations (increased sputum purulence, sputum volume, and dyspnoea) had a higher proportion of culture-positive sputum samples than patients with Type II exacerbations (two of these three symptoms). Fisher’s exact test produced a borderline result suggesting that patients with Type II exacerbations possibly had a higher chance of culture-positive sputum samples. From a range of demographic, symptomatic and laboratory investigations, the only factor which emerged as highly significant in distinguishing culture-positive from culture-negative patients was the semi-quantitative Gram stain analysis. Although this technique may offer a useful method of predicting bacterial infection in around 90% of patients, it may not be practical for all clinical trial settings. Nevertheless, identification of bacterial infection in patients with AECB or AECOPD remains an important target for the most cost-effective and scientifically robust clinical trials.
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# Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>α₁-AT</td>
<td>Alpha 1-antitrypsin</td>
</tr>
<tr>
<td>ABECB</td>
<td>Acute bacterial exacerbation of chronic bronchitis</td>
</tr>
<tr>
<td>AECB</td>
<td>Acute exacerbation of chronic bronchitis</td>
</tr>
<tr>
<td>AECOPD</td>
<td>Acute exacerbation of chronic obstructive pulmonary disease</td>
</tr>
<tr>
<td>ATS</td>
<td>American Thoracic Society</td>
</tr>
<tr>
<td>CB</td>
<td>Chronic bronchitis</td>
</tr>
<tr>
<td>CE</td>
<td>Clinically-evaluable (population)</td>
</tr>
<tr>
<td>CF</td>
<td>Cystic fibrosis</td>
</tr>
<tr>
<td>cfu</td>
<td>Colony-forming units</td>
</tr>
<tr>
<td>CL</td>
<td>Containment level (of laboratories)</td>
</tr>
<tr>
<td>COPD</td>
<td>Chronic obstructive pulmonary disease</td>
</tr>
<tr>
<td>CRF</td>
<td>Case report form</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>EOT</td>
<td>End of Therapy (visit)</td>
</tr>
<tr>
<td>FEV₁</td>
<td>Forced expiratory volume in 1 second</td>
</tr>
<tr>
<td>FVC</td>
<td>Forced vital capacity</td>
</tr>
<tr>
<td>GP</td>
<td>General practitioner</td>
</tr>
<tr>
<td>ICH-GCP</td>
<td>International Conference on Harmonisation, Good Clinical Practice</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Interleukin-1β</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin-6</td>
</tr>
<tr>
<td>IL-8</td>
<td>Interleukin-8</td>
</tr>
<tr>
<td>ITT</td>
<td>Intention-to-Treat (population)</td>
</tr>
<tr>
<td>LE</td>
<td>Leucocyte esterase</td>
</tr>
<tr>
<td>LRT</td>
<td>Lower respiratory tract</td>
</tr>
<tr>
<td>mL</td>
<td>Millilitre</td>
</tr>
<tr>
<td>NE</td>
<td>Neutrophil elastase</td>
</tr>
<tr>
<td>NPV</td>
<td>Negative predictive value</td>
</tr>
<tr>
<td>PMN</td>
<td>Polymorphonuclear neutrophils (or leucocytes)</td>
</tr>
<tr>
<td>PP</td>
<td>Per protocol (population)</td>
</tr>
<tr>
<td>PPV</td>
<td>Positive predictive value</td>
</tr>
<tr>
<td>SAA</td>
<td>Serum amyloid A</td>
</tr>
<tr>
<td>SEC</td>
<td>Squamous epithelial cell(s)</td>
</tr>
<tr>
<td>TOC</td>
<td>Test of Cure (visit)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumour necrosis factor-α</td>
</tr>
<tr>
<td>WBC</td>
<td>White blood cell(s)</td>
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2 INTRODUCTION

2.1 History of Chronic Bronchitis

In the British Isles, the diagnosis of bronchitis, and particularly of chronic bronchitis, has been a frequent one ever since the term was introduced into the medical literature in 1808 by Dr Charles Badham [Badham, 1808]. From the early 1900s, doctors had considered that purulent exacerbations of chronic bronchitis might be caused by infection and in 1938, Mulder noted that it was often possible to isolate *Haemophilus influenzae* from the sputum of patients with 'common acute and chronical purulent bronchitis' [Mulder, 1938]. In fact, several bacterial species had been isolated from the sputum of patients with chronic bronchitis [Marshall, 1931; Southwell, 1946], however, because of the lack of effective therapy in the first half of the 20th century, there was a feeling of futility and little motivation for specific research into the disorder [Stuart-Harris *et al.*, 1953; McVay & Sprunt, 1953]. In 1950, a survey was made of the registered disabled persons in four areas of Great Britain; in all four areas chronic respiratory disease was an important cause of disability, and was the leading cause of disability in the north-west area and accounted for more unemployment than any other physical condition [Goodman *et al.*, 1953]. The widespread introduction of antibiotics in the 1950s revived medical interest in chronic bronchitis, stimulated also by an increased incidence of the disease, a growing recognition of the economic impact, and a stable or declining frequency of other infections such as tuberculosis.

In 1953, May reported a significant association between the exacerbation of chronic bronchitis and the presence of bacteria in the sputum, especially of *H. influenzae* and *Streptococcus pneumoniae* [May, 1953]. In the same year Elmes published the results of a study of four antibiotics (penicillin, chloramphenicol, sulphadimidine and aureomycin) with potential to be used as prophylaxis during the winter months [Elmes *et al.*, 1955]. The results showed no long term benefit for any of the four antibiotics used; they displayed too narrow a spectrum of activity for the range of pathogens observed, produced resistance in one or more species, or displayed little effect on the bacterial flora. A few years later a set of two studies over two winters, appeared to
show a benefit of continuous antibiotic therapy during the winter months in the number and severity of exacerbations, however almost one-third of patients reported diarrhoea [Murdoch et al, 1959].

In the early 1950s, the link between cigarette smoking and chronic bronchitis had not been established. However, the observation that the mortality rates due to chronic bronchitis were three to four times higher in men than women of the same age prompted several possible explanations, one of which was the hypothesis that smoking may (at least partly) account for the higher mortality rates in men [Goodman et al, 1953]. Other explanations of this difference between the sexes offered by Goodman were: hormonal or other constitutional differences; the fact that far more men work outdoors in all weathers than women; the financial pressure on men to return to work as soon as possible following acute respiratory illness; the more physically demanding work that men tend to perform, requiring deeper breathing which might induce excessive stretching of the lung tissue that has not recovered after a recent infection. Interestingly, Goodman speculated that this sex difference might narrow over the coming 20 years due to the reduction in men’s physical workload (due to increasing mechanisation), and changes in the smoking habits of the sexes (more women smoking); this phenomenon has indeed been observed over the last few decades [Mannino et al, 2002].

2.2 Terminology

Bronchitis is a respiratory disease in which the mucous membrane in the bronchial passages of the lungs becomes inflamed. The irritated membrane swells due to inflammatory infiltrates and in doing so, it narrows or shuts off the bronchioles in the lungs, resulting in coughing spells and breathlessness because of the obstruction and consequent hypoxia. The cough is normally productive of thick phlegm which may be coloured yellow or green. Bronchitis comes in two forms: acute (lasting less than 6 weeks) and chronic (a long-term disease in which there are underlying physiological changes and sudden worsenings occur regularly).
Acute bronchitis is generally viral in origin, and is often preceded by an upper respiratory tract infection. In otherwise healthy children and adults, acute bronchitis, will normally resolve in 2-3 weeks without the need for an antibiotic. In elderly patients and people with concomitant diseases, a secondary bacterial infection may occur, which requires the addition of an antibiotic.

In chronic bronchitis (CB), there is a constant low level inflammation and swelling of the mucosal membranes that line of the airways. The inflammation stimulates production of mucus causing chronic mucus hypersecretion, which can cause further obstruction of the airways. Periodically, the level of inflammation increases because of viral or bacterial infection, or reaction to airborne pollution or an allergen; this results in a worsening of the condition, termed an acute exacerbation. Sufferers of chronic bronchitis tend to cough up significant quantities of sputum unlike those who do not suffer from chronic bronchitis. Sputum is a complex mixture of respiratory tract mucus, debris, inflammatory cells and exfoliated epithelial cells from upper and lower respiratory tracts.

Chronic bronchitis is defined as a chronic or recurrent productive cough, present on most days for a minimum of three months in the year and for not less than two successive years, in a patient in whom other causes of chronic cough have been excluded [Am Thorac Soc, 1962]. It is a serious long-term disorder that requires regular medical treatment. CB is generally caused by a long-term, irritative assault to the lungs. The most common assault is that from cigarette smoke. In addition to its irritant properties, cigarette smoke causes temporary paralysis of the cilia lining the airways which sweep out debris, irritants and excess mucus. Over time, these cilia are damaged to the extent that they cannot function properly, leaving the lungs clogged with mucus. Obstruction of the airways, especially with mucus, increases the likelihood of bacterial lung infections (acute bacterial exacerbations). Although cigarette smoking is the main cause of chronic bronchitis, long-term exposure to other noxious chemicals or pollutants can also cause chronic bronchitis. Consequently, the vast majority of sufferers are over 35 years of age [Ekberg-Aronsson et al, 2008]. The severity of the disease tends to increase with age, although stopping smoking and/or removing other irritants can reduce
the progress of this disease significantly. However, recurrent exacerbations (bacterial or non-bacterial in nature) will tend to lead to a deterioration in lung function [Donaldson et al, 2002; Chodosh, 2005].

Chronic bronchitis is one form of the complex disease of COPD. It is useful to understand the relationship between chronic bronchitis and COPD as the terms may sometimes appear to be interchangeable. COPD is comprised primarily of two related diseases – chronic bronchitis and emphysema. In both diseases, there is chronic obstruction of the flow of air through the airways and out of the lungs, and the obstruction generally is permanent and progressive over time. Asthma also is a pulmonary disease in which there is obstruction to the flow of air out of the lungs, but unlike chronic bronchitis and emphysema, the obstruction in asthma predominantly is reversible. Between ‘attacks’ of asthma the flow of air through the airways is generally good. In some patients with COPD the obstruction can be partially reversed by medications that enlarge or dilate the airways (bronchodilators) as with asthma. Conversely, some patients with asthma can develop permanent airway obstruction if chronic inflammation of the airways leads to scarring and narrowing of the airways. This process is referred to as lung remodelling. These asthma patients with a fixed component of airway obstruction are also considered to have COPD. There is also frequent overlap among COPD patients: patients with emphysema may have some of the characteristics of chronic bronchitis; similarly, patients with chronic bronchitis also may have some of the characteristics of emphysema.

In its guidelines for the management of COPD, the British Thoracic Society stated that “COPD is a chronic, slowly progressive disorder characterised by airways obstruction (FEV\textsubscript{1} <80\% predicted and FEV\textsubscript{1}/VC ratio <70\%) which does not change markedly over several months” [BTS, 1997]. In this definition FEV\textsubscript{1} is the forced expiratory volume in 1 second, and VC is the vital capacity (also called forced vital capacity or FVC), and is the total volume of air that can be forcibly exhaled after a full inspiration.
2.3 Pathology of Chronic Bronchitis

Ciliated epithelial cells line the upper and lower (trachea to respiratory bronchioles) respiratory tracts. The cilia beat in a two-layered system of fluid, a watery layer lies below the layer of mucus. This mucociliary system forms the first line of defence of the respiratory tract against inhaled particles, including bacteria [Newhouse et al, 1976]. These inhaled particles stick on the mucus layer and are then transported towards the back of the throat by the coordinated movement of the cilia. The mucus that reaches the back of the throat is swept into the oesophagus and is swallowed; alternatively, it may be expectorated. Normally this system works extremely well and the amount and consistency of the mucus does not cause a problem.

Several pathological mechanisms predispose the bronchitic patient to increased mucus production and bacterial infection. Data from animal studies, post-mortem studies and epidemiology surveys suggest that these abnormalities are a result of prolonged exposure to cigarette smoke [Reid, 1988; Tager & Speizer, 1976]. Although tobacco use is the primary risk factor for the development of chronic bronchitis, other risk factors are involved.

In early chronic bronchitis, there is a hypertrophy of the submucosal glands in the walls of the large bronchi. This process results in the mucus hypersecretion that triggers the troublesome 'smoker's cough' of the bronchitic patient. The glycoprotein and lipid composition of this mucus differs from normal bronchial secretions in that it is thick and tenacious, and resists airway clearance. Clinically, the patient often feels well except for a persistent daily cough that is productive of a clear or greyish-white sputum, which if examined is normally non-purulent and sterile. Little or no airways obstruction is present and infection plays a minor role at this stage. As the disease progresses, hypersecretion of mucus starts to involve the smaller airways. Another histological finding at this stage is a proliferation of goblet cells in the respiratory epithelium. Goblet cells are normally found scattered relatively rarely throughout the epithelium of the large bronchi and produce mucin (a glycoprotein), which is the major component of mucus. The result of this proliferation in goblet cells is that mucociliary clearance is further impaired because of the loss of normal ciliated epithelium. In severe chronic
bronchitis, goblet cells may account for the majority of respiratory epithelial cells, and can be found encroaching on the small airways.

Impaction of mucus in the lumen of the small airways along with the structural changes associated with submucosal gland hypertrophy, leads to progressive obstruction of the peripheral airways. Although a low-grade inflammation often exists, the sputum is not markedly purulent with only a moderate number of polymorphonuclear (PMN) leucocytes present. Tobacco smoke has also been shown to inhibit mucociliary function as demonstrated by impaired particle transport \emph{in vitro}, ciliostasis (reduced motility of the bronchial cilia), decreased tracheal mucous velocity in animals, and reduced clearance of inhaled aerosols in humans [Warner, 1977]. In addition to cigarette smoke, viral infection also frequently damages ciliated epithelium, which then impairs mucociliary clearance [Stanley \emph{et al}, 1986; Wilson \emph{et al}, 1987]. In \emph{in vitro} studies subtle defects in the local host immune system have been observed, particularly in patients who continue to smoke; these include impaired neutrophil phagocytosis, impaired bactericidal function, and decreased levels of sputum immunoglobulin A [Green \emph{et al}, 1977, Chodosh, 1987]. Together, these mechanisms impair clearance of inhaled bacteria, and the large bronchi become colonised with the same bacteria that normally inhabit the oropharynx [Irwin \emph{et al}, 1982].

If mucociliary clearance continues to be impaired, these bacteria may persist deep in bronchial epithelium even after antibiotic therapy. There is now the classic state of colonisation whereby organisms are replicating on or in host tissue, but tissue invasion does not take place and the host immune response is minimal. This baseline state of colonisation may periodically progress to active disease. Microscopically, there maybe evidence of bacterial proliferation and acute inflammation with PMN leucocytes. At this point, the patient will complain of worsened cough, sputum production, and dyspnoea.

More recently it has been recognised that patients with alpha 1-antitrypsin (\(\alpha_1\)AT) deficiency have increased inflammation of the larger airways, suggesting that \(\alpha_1\)AT has an important role in protecting these airways from the inflammatory effects of
neutrophil elastase [Hill A et al, 2000]. Furthermore, purified neutrophil elastase has been shown to be a potent secretagogue for goblet cells in vitro, stimulating them to secret mucin [Nadel, 2000]. This suggests a link between the presence of neutrophils in the airways and mucus hypersecretion: the key symptom of chronic bronchitis.

2.4 Acute Exacerbations of Chronic Bronchitis

Acute exacerbations (often just referred to as exacerbations) are worsenings of the chronic bronchitis, which are frequently sudden in onset. They become a regular occurrence for chronic bronchitis patients, and they contrast with the slow progressive worsening of the condition that tends to occur over many years. An acute exacerbation of chronic bronchitis (AECB) may be caused by an acute bacterial or viral infection, environmental pollutants, or an allergic response. Acute infective exacerbations of chronic bronchitis are mucosal infections and in the majority of cases will resolve spontaneously [Wilson, 1992]. Acute exacerbations can cause distress to the patient, result in a reduced quality of life, require prolonged time away from work, and result in additional damage to the lungs. For these reasons most physicians will prescribe an antibiotic to a chronic bronchitis patient who has a significant exacerbation which is productive of purulent sputum, and therefore thought to be caused by a bacterial infection.

The current ‘vicious circle’ hypothesis attempts to explain the pathogenesis of an exacerbation of CB or COPD [Murphy & Sethi, 1992]. It has as the primary initiator, an environmental insult such as pollutants, smoking or a viral infection, which then allows an inflammatory cascade and bacterial numbers to increase. This hypothesis, assumes that the bacteria are opportunistic and that they are contributing to an already established process rather than initiating the process. It also suggests that each significant exacerbation results in further damage to the mucociliary clearance, leaving the airways even more vulnerable to infection. In this way a ‘vicious circle’ is set in place. This hypothesis was originally applied to the progression of bronchiectasis [Cole, 1986]; however, it can also explain the progression of COPD.
Longitudinal studies estimate that each chronic bronchitis patient suffers approximately two to three exacerbations per year [Ball et al, 1995; Miravitlles et al, 2003; Schaberg et al, 2005; Langsetmo et al, 2008]. However many of these exacerbations are unreported; consequently, the reported number of exacerbations per year can be less than one [Langsetmo et al, 2008]. An exacerbation involves the development, or worsening, of one or more of the following signs and symptoms: volume of sputum produced, purulence of the sputum produced, dyspnoea, cough, wheeze, auscultatory findings such as rales (crackling sounds) or rhonchi (whistling/snoring sounds), cyanosis, and fever. Exacerbations are more frequent during the winter months of the year than during the summer months; however, among patients with severe chronic bronchitis and poor lung function, exacerbations can occur all year round.

2.4.1 Treatment of Acute Exacerbations of Chronic Bronchitis

Patients with chronic bronchitis are often prescribed medication on an ongoing basis to help to control their disease. Long-acting inhaled bronchodilators (beta₂-agonists or anticholinergics) should be used to control symptoms and improve exercise capacity in patients who continue to experience problems despite the use of short-acting drugs. Inhaled corticosteroids may be added to long-acting bronchodilators to decrease exacerbation frequency in patients with an FEV₁ less than or equal to 50% predicted who have had two or more exacerbations requiring treatment with antibiotics or oral corticosteroids in a 12-month period [NICE, 2004]. These patients should also be offered annual influenza vaccination and a pneumococcal vaccine. If necessary, theophylline and/or oxygen therapy may be added [NICE, 2004].

During an exacerbation, the following therapy would routinely be prescribed: bronchodilator therapy (with an increase of dose or a change of agent if necessary); inhaled or oral corticosteroids; and oxygen therapy if FEV₁ ≤50% predicted. A mucolytic agent may be considered, and an oral antibiotic would normally be prescribed if the patient had purulent sputum, on the grounds that purulent sputum is associated with bacterial infection [NICE, 2004]. The choice of antibiotic will be discussed in Section 2.5.2. If the patient has severe chronic bronchitis, is frail and/or elderly, or lives
alone without family support, admission to hospital is likely. Social reasons may also influence the decision to admit a patient to hospital.

2.5 The Role of Bacterial Infection

The role of bacteria as pathogens in acute exacerbations of chronic bronchitis (and acute exacerbations of COPD) is controversial and is still not fully understood despite many years of research. The problems that surround the role of bacteria in stable chronic bronchitis and their role causing acute exacerbations are numerous, and include: a) bacteria colonise the lower airways in chronic bronchitis patients, even in the absence of symptoms of an exacerbation; b) the information obtained from cultures of expectorated sputum is limited since the specimens may not reflect the complete picture of the flora of the lower airways, also, it may be contaminated with upper respiratory tract flora; c) patients with chronic bronchitis are a highly heterogeneous population, and as a result bacteria may vary from patient to patient and are likely to play different roles in different individuals; d) evidence indicates that bacteria play a role in only about 50% of infectious episodes; e) the three bacteria most strongly associated with infectious episodes (non-typeable *H. influenzae*, *S. pneumoniae*, and *Moraxella catarrhalis*) are exclusively human pathogens, limiting the use of animal models [Murphy et al, 2000].

Anthonisen and colleagues demonstrated a definite benefit of antibiotic therapy in patients who had at least two of the following three symptoms: increased dyspnoea, increased sputum volume, and increased sputum purulence [Anthonisen et al, 1987]. The benefits were most pronounced among patients who demonstrated all three of these ‘cardinal’ (as termed by Anthonisen) symptoms. In addition, the patients who received antibiotic therapy had a significantly shorter duration of illness than those who received placebo. As expectorated sputum can easily be contaminated with organisms from the upper airways, studies carried out using protected specimen brush technique (which protect against contamination by upper respiratory tract flora), have established the presence of bacteria (>1,000 cfu/mL) in stable COPD disease in 25% of patients, but in 52% of patients during an exacerbation [Monso et al, 1995]. In another study, increased airways inflammation was assessed by measuring levels of interleukin-8 (IL-8), tumour necrosis factor-α (TNF-α), and neutrophil elastase (NE) in patients with AECB who had
non-typeable *H. influenzae*, *Haemophilus parainfluenzae*, or *M. catarrhalis* isolated as the sole pathogen. A control group of patients that were pathogen-negative were also assessed. The results showed that there was increased airways inflammation in patients who had *H. influenzae* or *M. catarrhalis* isolated from their sputum, and therefore support a causative role of these pathogens in AECB [Sethi *et al*, 2000]. A specific study of *H. parainfluenzae* found the organism to be present in 25% of patients with moderate to severe COPD and *in vitro* studies showed that *H. parainfluenzae* could cause extensive damage to epithelial cells and slowed ciliary beat frequency, suggesting that this organism might play a role in the pathogenesis of COPD [Hill *et al*, 2000].

By comparison, Hirschmann considered that any significant role of bacteria in exacerbations of COPD is unproven. He argued that large trials are consistent in showing that antibiotics are not helpful in mild exacerbations; in more severe episodes patients should receive systemic corticosteroids, and that with such therapy antibiotics provide no additional benefit [Hirschmann, 2002].

In a meta-analysis of nine placebo-controlled studies to evaluate the benefit of antibiotics in AECB, there was a small difference in favour of antibiotic therapy [Saint *et al*, 1995]. However, seven of the nine individual studies showed a benefit for antibiotic therapy, giving an advantage on the number of studies in favour of antibiotic therapy. Nevertheless, such a small difference may be viewed as disappointing and it has been suggested that in most cases there is no benefit of antibiotic therapy [Hirschmann, 2000]. Despite the uncertainty regarding the importance of bacteria in exacerbations, antibacterial therapy is widely recommended for exacerbations that are thought to be caused by bacteria (mainly indicated by purulent sputum).

### 2.5.1 The Bacteria of Acute Exacerbations of Chronic Bronchitis

Most studies show *H. influenzae* to be the major organism present in patients with acute exacerbations of chronic bronchitis [Ball & Make, 1998] and in the stable state [Monso *et al*, 1995]. *H. parainfluenzae* may also play an important role [Middleton *et al*, 2003], and Gram-negative opportunistic organisms such as *Escherichia coli* and *Klebsiella*
pneumoniae are increasingly identified in patients with advanced disease [Eller et al, 1998; Miravitlles et al, 1999]. Eller’s study of more than 200 patients demonstrated a correlation between deteriorating lung function and increasing isolation of enterobacteria and Pseudomonas aeruginosa. These pathogens accounted for 63% of all bacteria isolated from patients with an FEV1 of less than 35% predicted. This study also highlights a potential analogy with the colonisation of the lungs and the progression of pathogens that occurs in cystic fibrosis.

It is also recognised that the bacteria which are present during exacerbations are frequently the same as those found in the airways during the stable state [Monso et al, 1995]. Indeed, another study using protected specimen brush technique demonstrated that 25% of stable patients had significant growth of potential pathogens in the lower airways [Pela et al, 1998]; the most commonly isolated organism from patients with exacerbations was S. pneumoniae. In addition, M. catarrhalis is also routinely isolated in patients with exacerbations of AECB [Sethi, 1999, Dever et al, 2002]. However, isolation of a new strain of H. influenzae, M. catarrhalis, or S. pneumoniae has also been associated with acute exacerbations [Sethi et al, 2002].

Atypical pathogens Chlamydia pneumoniae and Mycoplasma pneumoniae are implicated in AECB, but at a fairly low percentage (<5-15%) [Dever et al, 2002]. Many studies in AECB and acute exacerbations of COPD (AECOPD) over the previous decades have missed these atypical pathogens as they are normally confirmed by comparing antibody titres, are difficult to culture, and were not specifically looked for in most clinical trials. A more recent report found that no indication was found for the atypical organisms: Legionella spp, C. pneumoniae, and M. pneumoniae, in stable moderately severe COPD and in exacerbations [Diederen et al, 2007].

2.5.2 Choice of Antibiotic Therapy in Acute Exacerbation of Chronic Bronchitis

An antibiotic (or antibacterial drug) is defined as any compound which either kills or inhibits the growth of bacteria. These two terms are basically interchangeable; the term
antibiotic classically describes a naturally occurring substance that has antibacterial activity; an antibacterial drug is any compound naturally occurring or synthetically derived which has antibacterial activity. In this thesis, the term antibiotic has been used in the broad sense to mean any antibacterial drug.

As previously described, the concept of the beneficial nature of prophylactic antibiotic therapy for the winter months to prevent exacerbations or reduce the number of exacerbations was first explored in the 1950s and remained an intriguing prospect for a number of researchers, and a number of other studies were performed with the aim proving this theory and identifying the best antibacterial agent(s). In a recent review of the well conducted studies, all performed before 1970, the conclusion was that although prophylactic antibiotic therapy can shorten the number of days of illness due to an exacerbation, they have no place in routine treatment because of the risk of antibiotic resistance developing, and the possibility of adverse effects [Staykova et al, 2007].

Except in very specific circumstances, antibiotics are only used in short courses of a maximum of 14 days, but more usually 5-7 days. The prescribing of an antibiotic is normally triggered by the start of an acute exacerbation that is thought to be caused by bacteria (productive of purulent sputum). A number of factors should be considered when selecting an antibiotic for treatment of AECB [revised from Schentag & Tillotson, 1997].

1) Antibiotic spectrum: As the pathogens that cause acute bacterial exacerbations of CB include both Gram-positive and Gram-negative organisms, the selected antibiotic must also have a broad spectrum of activity.

2) Antibiotic resistance: With the increase in antibiotic resistance worldwide, consideration needs to be given to the likelihood of resistance when selecting an antibiotic. Local resistance patterns are most useful; however, the risk factors of each patient should be considered. Factors that put a patient at higher risk of harbouring a resistant pathogen include: recent hospital stay, recent antibiotic treatment, living in community accommodation such as a care home.
3) **Mechanism of action**: Antibiotics with bactericidal activity should be more effective in eradicating pathogens than those that have bacteriostatic activity, as pathogens that are only suppressed may give rise to a relapse, or develop resistance to the antibiotic. There is however no evidence which demonstrates conclusively that bactericidal antibiotics have better outcomes than bacteriostatic ones in the treatment of AECB.

4) **Pharmacokinetics and pharmacodynamics**: Penetration into respiratory secretions is one of the most important pharmacokinetic characteristics of antibiotics used to treat AECB. β-lactam antibiotics generally penetrate poorly into respiratory secretions with concentrations well below those found in serum, yet in clinical trials their efficacy tends to be equivalent to those drugs with better penetration.

5) **Dosing regimens**: The majority of patients are treated in the community and therefore oral antibiotics with once or twice daily dosing are preferable to aid compliance. In addition shorter duration of therapy may help compliance and reduce costs and adverse effects.

6) **Side effects**: The most common side effects of all antibiotics used to treat AECB are gastrointestinal, mainly diarrhoea, nausea, and vomiting. Rash, headache and taste disturbance may also occur, as well as a host of other less common adverse effects. Selecting an antibiotic with a favourable side effect profile is more likely to encourage compliance.

7) **Cost**: Direct comparison of the costs of antibiotic therapies is not straightforward. The cost of each tablet may be misleading if one antibiotic is effective at a dose of one tablet a day for five days, when the other has to taken three times a day for 10 days (5 tablets versus 30 tablets). Other factors that affect cost are the cost of failure (cost of the second-line antibiotics), and the cost of treating side effects associated with antibiotic therapy.
Many groups of antibiotics are used to treat AECB: penicillins (e.g. amoxicillin), cephalosporins (e.g. cefaclor), tetracyclines (e.g. oxytetracycline), macrolides (e.g. clarithromycin), folic acid inhibitors (e.g. trimethoprim), and fluoroquinolones (e.g. ciprofloxacin). There are significant national and local differences in the choice of first line oral antibiotic for patients with AECB of moderate severity, driven predominantly by national and local susceptibility patterns. However, because antibiotics are recommended for patients with purulent exacerbations and antibiotic resistance continues to be a problem, there is an ongoing need for new antibacterial agents to be developed and evaluated in clinical trials in AECB patients.

2.6 Frequency of Chronic Bronchitis and Impact on Healthcare Systems

The changing terminology in this area makes examination of chronic bronchitis difficult. Although some researchers still report data on chronic bronchitis, many groups and organisations (including the World Health Organisation) report on the broader definition of COPD. The data in this section therefore uses data on both definitions; this should not detract from the overall fact that chronic bronchitis is a significant burden to healthcare systems, is a major cause of death, and has considerable cost implications.

The WHO World Health Report of 2002 [WHO, 2002] recorded COPD as the fifth leading cause of death in the world. It has also been identified from a 5 year study, that mortality is significantly related to the frequency of severe exacerbations requiring hospital admission [Soler-Cataluña et al, 2005]. In addition, unlike some other leading causes of death such as cardiovascular disease, which are in decline [Pauwels & Rabe, 2004], further increases in the prevalence of COPD and resultant mortality are anticipated over the next few decades [Murray & Lopez, 1997, Soriano et al, 2000].

In one study of the prevalence of COPD and one study of CB, which were conducted in several countries the prevalences were found to be similar. The prevalence of COPD among adults was found to be between 4% and 10% in countries where it has been rigorously measured [Halbert et al, 2003]. The overall prevalence of chronic bronchitis
was found to be between 1% and 10% of the young adult population (20-44 years) across 16 developed countries [Cerveri et al, 2001]. In studies of specific populations within a country or city more specific and therefore varied prevalences were identified. A study of patients over 45 years of age in Manchester, 26% of patients were found to have chronic airways obstruction [Renwick & Connolly, 1996]. A study in France among people aged 25 years and over, found a prevalence of chronic bronchitis of 4.1%, but a prevalence of chronic cough and/or chronic expectoration of 11.7% [Huchon et al, 2002].

These studies indicate how variable the prevalence of chronic bronchitis can be depending upon the specific population studied. Identifying data that relates the prevalence of chronic bronchitis directly to COPD is difficult; however, von Hertzen and colleagues achieved this in Finland and found that 11% of men and 5.2% of women aged 30 years or above had COPD (FEV₁/FVC <70%), but almost twice as many had chronic bronchitis or emphysema when examined by a physician [von Hertzen et al, 2000]. In addition, COPD (and chronic bronchitis) tend to be under diagnosed; a study in a GP practice in Lincolnshire found that there were 2.69 “true” cases of COPD for every diagnosed case in patients aged 60-74 years [Dickinson et al, 1999].

It has been estimated that at least 12 days are lost from work each year by each AECB patient in Germany, and the overall economic impact of CB amounts to €8.4 billion [Lorenz et al, 2001]. In the USA, 14% of the adult population is affected by COPD, and the annual healthcare costs associated specifically with CB are calculated to be $11.7 billion [Wilson et al, 2000]. In a separate study comparing CB patients aged under 65 and over 65, it was calculated that the total treatment costs for AECB are $1.2 billion for patients aged 65 years and over, and $419 million for patients aged <65 years; the majority of these costs being related to hospitalization [Niederman et al, 1999]. The total economic impact of working days lost has not been estimated in the USA, but would clearly be significant. Whatever the true cost of CB in terms of healthcare costs and the economic impact, this disease has a huge impact to society.
3 RATIONALE, OBJECTIVES, AND SUMMARY OF PROJECT DEVELOPMENT

3.1 Overall Objective

The overall objective of this project was to examine factors that might improve the identification of bacterial infection in patients with a history of chronic bronchitis who present in a GP or hospital setting with signs and symptoms of an acute exacerbation.

A successful outcome would mean that inclusion criteria in clinical trials of antibacterial drugs could be designed to target patients who have a high probability of being included in the microbiological populations (see Section 3.2). These microbiologically-evaluable populations are key in such studies, as these populations are used to evaluate the microbiological efficacy of each antibacterial drug (or combination of drugs) against each species of pathogenic bacteria isolated. The populations may also be used to evaluate the clinical efficacy of the study drugs.

3.2 Clinical Trial Populations for Analysis

Clinical studies in different therapeutic areas have slightly different requirements for their populations for analysis. This section outlines the standard populations in clinical studies of antibacterial drugs, and provides some comparison with clinical studies in other therapeutic areas. A summary of the populations for analysis in antibacterial studies is shown in Table 3-1

In some studies the Intention-to-Treat (ITT) population will be defined as all patients who were enrolled into the study, whether or not they received any study medication. However, in studies of antibacterial drugs, the ITT population is normally all randomised patients who received any study drug. This definition of the ITT population is also the population that is used for all the safety analyses.
In non anti-infective clinical trials the next population is normally called the Modified ITT (MITT, sometimes written as mITT), which will include patients that are confirmed as having the disease under study, and possibly who have received at least one dose of the medication or procedure under study. In studies of antibacterial drugs, this population is still sometimes called the modified ITT and sometimes called the microbiological ITT, which is what it is. The MITT population includes all the ITT patients who had one or more species of bacteria that are recognised pathogens in the infection under study isolated from an appropriate specimen taken at baseline. For example, in a study of AECB isolation of *Streptococcus viridans* from the sputum is unlikely to accepted as a pathogen (as this is normally a commensal of the oropharynx); however isolation of *S. pneumoniae* would be an accepted pathogen.

In antibacterial studies, the Clinically Evaluable (CE) population is the primary population for analysis and the one for which the sample size calculation should be made. The reason for this is because the objective of virtually all antibacterial studies is to demonstrate non-inferiority (or equivalence) and in non-inferiority studies, the CE population is more conservative than the ITT population (whereas the opposite is true when the objective of the study is to demonstrate superiority). The CE population includes all patients in the ITT population who meet pre-defined protocol criteria that will mean that clinical outcome of their infection is likely to be a result of the antibiotic(s) (or placebo) that they have received. An example of such a criterion is that the patient must not have received any concomitant antibacterial therapy, unless this was given for clinical failure.

The Microbiologically Evaluable (ME) population is a combination of the MITT and the CE populations. It will include all the patients who were eligible for the CE population who had one or more species of bacteria that are recognised pathogens in the infection under study isolated from an appropriate specimen taken at baseline.

The ITT and CE populations are used to assess the clinical outcomes; however, some studies will also evaluate the clinical response in the MITT and ME populations as well.
Bacteriological outcomes however can only be assessed in the MITT and ME populations.

Table 3-1: Summary of Populations for Analysis in Antibacterial Studies

<table>
<thead>
<tr>
<th>Population</th>
<th>Abbrev.</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intention-to-Treat</td>
<td>ITT</td>
<td>All randomised patients who receive any amount of study medication.</td>
</tr>
<tr>
<td>Modified or Microbiological Intention-to-Treat</td>
<td>MITT</td>
<td>All ITT population patients with an accepted causative pathogen isolated at baseline.</td>
</tr>
<tr>
<td>Clinically Evaluable or Per Protocol</td>
<td>CE or PP</td>
<td>All patients in the ITT population who meet specific protocol criteria such that the clinical outcome of their infection could be inferred to reflect the effect of the study drug.</td>
</tr>
<tr>
<td>Microbiologically Evaluable</td>
<td>ME</td>
<td>All patients in the CE population who had an accepted causative pathogen isolated at baseline</td>
</tr>
</tbody>
</table>

3.3 Rationale for this Project

Over several years of working on clinical trials of antibacterial drugs in patients with AECB, I had observed the often low proportion (less than half) of patients who had proven bacterial infection i.e. had a bacterial pathogen isolated from their baseline sputum sample. Consequently, a significant proportion of the patients in the CE population, did not have a proven bacterial infection, but were evaluable in terms of clinical response. These patients may or may not have had a bacterial infection, but those who did not have a bacterial infection were adversely affecting the scientific validity of the study; in effect diluting the true clinically evaluable population. I was concerned about the extent to which this could affect the results of a clinical trial, and the conclusions that would be drawn from these studies. It seemed that this could have influenced the move to equivalence (or non-inferiority) studies in this therapeutic area, because proving 10% superiority in a population where perhaps less than 50% of patients have a bacterial exacerbation, would be statistically very difficult [see Figure 8-1]. Therefore, a method that could increase the percentage of microbiologically-evaluable patients in a clinical trial of antibacterial drugs in patients with AECB, would
be a significant advantage to the pharmaceutical industry, and enable more cost-effective studies. In an era where many large pharmaceutical companies are no longer investing in research into new antibacterial drugs [Conly & Johnston, 2006; Tillotson, 2008], a significant improvement in identifying bacterial infection in COPD patients could make this a more attractive investment opportunity.

A patient with AECB that is non-bacterial in origin does not need to be treated with antibacterial drugs. Such drugs will have no effect on the course of the exacerbation, and at a time when attempts are being made to limit the use of antibacterial drugs in order to reduce the spread of antibiotic resistance, treating patients who do not have a bacterial infection is potentially deleterious to the efficacy of the antibiotic used, and exposes the patients to the risk of adverse effects.

A few studies applied techniques that were designed to increase the likelihood of identifying bacterial exacerbations. The simplest method was to screen the sputum for microscopic purulence. This was defined as a sputum sample that contained >25 white blood cells and <10 squamous epithelial cells per low power field (x10 eyepiece and x10 objective lens) [Bartlett, 1974]. The rationale behind this definition was to identify samples that had come from deep in the lungs (few squamous epithelial cells), and had signs of infection by containing significant numbers of white blood cells. This procedure is now recommended by the FDA in its guidance document of 1998 [FDA, 1998]. The more complex method was to screen a Gram stained preparation of the sputum for the presence of bacterial cells. This method could not identify specific species of bacteria, but could identify particular morphological cells types, such as Gram-positive cocci or Gram-negative bacilli. However, much of the variation in clinical trials of antibacterial drugs in AECB patients was the inclusion criteria that different studies used to define an acute exacerbation.

In the 1980s, Anthonisen and co-workers [Anthonisen et al, 1987] defined three types of acute exacerbation based on the presence or absence of three cardinal features as shown in Table 3-2.
Table 3-2: Anthonisen's classification of acute exacerbations of COPD

<table>
<thead>
<tr>
<th>Anthonisen classification</th>
<th>Features</th>
<th>Benefit of an antibiotic from this study*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I exacerbation</td>
<td>increased sputum purulence,</td>
<td>Beneficial</td>
</tr>
<tr>
<td></td>
<td>increased sputum volume,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>increased dyspnoea</td>
<td></td>
</tr>
<tr>
<td>Type II exacerbation</td>
<td>Any 2 of the above 3 features</td>
<td>Some benefit</td>
</tr>
<tr>
<td>Type III exacerbation</td>
<td>Any 1 of the above 3 features</td>
<td>No clear benefit</td>
</tr>
</tbody>
</table>

* Anthonisen et al, 1987

Since the publication of these results, the majority of studies have applied these criteria, or a variation on these criteria. However, some studies included only patients with a Type I exacerbation, whereas others included patients with a Type I or a Type II exacerbation.

My hypothesis was, therefore, that patients with a Type I exacerbation were more likely to have a bacterial infection than those who had a Type II exacerbation; patients with Type III exacerbations would be excluded from the investigation as there was no evidence for the use of antibacterial therapy in these patients. This was the implication of the Anthonisen study, based on the significant benefit of antibiotic therapy over placebo in the Type I patients, which was less clear in Type II patients. However, since no bacteriology was performed in the Anthonisen study, it was impossible for this aspect to be proven.

In addition to this application for the improvement of clinical trials of antibacterial drugs, it has been recognised that antibacterial drugs tend to be over-prescribed for exacerbations of CB/COPD, and that there is a need to identify markers of bacterial infection, which will give a clearer indication for the use of these drugs [Smith et al, 1999].
3.4 Summary of Project Development

This project started with a multicentre investigation into the best indicator of bacterial infection in patients who were diagnosed as having an acute exacerbation of chronic bronchitis. This investigation was designed as part of an MSc degree at the University of Surrey. The investigation started in 1999 and was completed in 2002. At the end of this project, I wanted to take the research further and made the decision not to submit the research for an MSc degree, but to move to a part-time MPhil/PhD course.

On acceptance to the part-time MPhil/PhD course a systematic review was undertaken as background to the project and to test the previously unsupported arguments of the completed investigation that:

i. the proportion of microbiologically-evaluable patients (patients in whom a recognised bacterial pathogen can be isolated from a sputum sample at baseline) in clinical trials of antibiotics in AECB patients is extremely variable, and

ii. in general, this proportion is disappointingly low; frequently less than 50%.

Consequently, clinical efficacy results (clinical cure, clinical failure etc.) may be based on a population in which less than half have a proven bacteriological infection.

The systematic review examined clinical trials of antibacterial drugs in AECB to examine the proportion of patients who were microbiologically-evaluable and what entry criteria were applied to selecting the patients for the study. Other aspects of these clinical studies were also examined (see Section 4).

3.4.1 History of Additional Investigation

An additional investigation was planned to examine the sensitivity and specificity of the presence of leucocyte esterase in the sputum as an indicator of bacterial infection. Leucocyte esterase is most familiar as one of the test patches on a urine dipstick. Leucocyte esterase is an enzyme that identifies the presence of polymorphonuclear neutrophils (PMNs), and is therefore a good suggestive marker of bacterial infection. The benefits of this technique to identify AECB patients who had bacterial infection
were that it would be non-invasive, simple and quick to perform as it would require only a urine dipstick and a fresh sputum sample, and could therefore be performed in a GP surgery. If this procedure offered any advantage over assessing signs and symptoms, as a way of determining whether a patient had a bacterial exacerbation of AECB, it could be a useful tool not only in screening patients for clinical trials of antibacterial drugs for AECB, but also for the decision making process of whether a patient required antibacterial therapy.

A draft protocol was prepared for this additional investigation (Appendix 9.1). However, as further background research was performed, it appeared that there were a number of issues that would make this planned investigation impractical to conduct, and that any benefit that was shown from such an investigation may not be able to be applied in GP surgeries as planned.

The issues that were identified were:

1) **Standardisation of technique.**
The original idea was to place the leucocyte esterase (LE) patch of a urine dipstick in contact with fresh sputum, collected from a patient in a sputum pot. However, the viscosity of sputum can vary significantly and how much a very viscous specimen would 'wet' the LE patch so that it could react with the test agents in the patch was unclear. It seemed that it would be necessary to run a series of tests on sputum and urine dipsticks to validate the methodology that would be used in the investigation. If normal saline had to be added to the specimen, it would then need to be homogenised in order to achieve a uniform sample. This would add to the complexity of the procedure and meant that it would not be practical for most GP surgeries as it was apparent that homogenisers would not be generally available in GP surgeries for this study and for the wider application of this technique.

2) **Safety of handling raw sputum samples.**
Health and safety regulations now mean that sputum samples which could contain a Hazard Group 3 organism (an organism that may cause severe human disease and presents a serious hazard to laboratory workers; it may present a risk of spread to the community but there is usually effective prophylaxis or treatment available), should not
be handled except within a microbiological safety cabinet in a Containment Level 3 (CL3) laboratory, and protective gloves and clothing should be worn [Health Protection Agency, 2008]. Hazard Group 3 organisms include *Mycobacterium tuberculosis*, other *Mycobacterium* species and *Chlamydia psittaci*. The majority of respiratory pathogens including *H. influenzae*, *S. pneumoniae*, and *M. catarrhalis* are in Hazard Group 2 (an organism that may cause human disease and which might be a hazard to laboratory workers but is unlikely to spread to the community). Although these organisms do not require a CL3 laboratory, they do require special precautions, and are not suitable to be handled in most GP surgeries. Although very few patients would be carrying Hazard Group 3 organisms, the risk is present and the general advice is that GPs should not in any way process sputum samples.

The difficulty surrounding being able to conduct this investigation safely in GP surgeries meant that it was impossible to proceed with this study. In addition, as the whole purpose of this research was to identify potential indicators of bacterial infection for application in clinical trials of antibacterial agents, the results of such a study would be irrelevant if they could not be applied directly to a clinical trial situation.

Alternative follow-up investigations were considered. However, the lack of identification within a reasonable time, of a valuable and feasible further investigation, which could be completed within a fixed timeframe, meant that further clinical study work was not possible.

### 3.5 Outcome

It was agreed that I would write up the work that I had already done (the systematic review and the clinical study) and along with further research into the current research into identifying bacterial exacerbations of chronic bronchitis and COPD, submit this as a thesis for an MPhil.
4 SYSTEMATIC REVIEW OF CLINICAL TRIALS OF ANTIBACTERIAL DRUGS IN ACUTE EXACERBATIONS OF CHRONIC BRONCHITIS

4.1 Introduction

From the 1950s to the turn of the millennium more than 200 new antibacterial drugs were developed and marketed. Over this period, the way in which these new drugs were tested in clinical trials improved significantly. In the 1950's clinical trials were often (to modern thinking) not scientifically robust: the doctors and patients frequently knew which drug was being given (not blinded), randomisation was often on the basis of alternate patients receiving study drug or comparator/placebo (a less than ideal system which is open to bias), the numbers of patients studied was small with no statistical sample size calculation and were often based on the number of patients seen in a fixed period of time e.g. 6 months. In addition, the vast majority of these studies were conducted in just one hospital, rather than several centres. This could sometimes result in widely varying conclusions between studies; in addition, the definitions of outcome were often vague.

In the 1980s Phase III and Phase IV clinical trial designs were statistically robust, in that the patient numbers were justified against the success rate anticipated, the power required and the equivalence (or superiority) level to be tested. However, in order to reduce patient numbers and therefore save time and money, the power was often set at only 80% or 85% [personal experience], thus giving a 15% to 20% chance that 'true superiority' or 'true equivalence' would be missed, purely by chance. In addition, equivalence within 15% was often applied in studies; a margin which, at its lower limit, could be argued is hardly equivalent at all.

By the mid 1990s, pivotal clinical trials (the Phase III studies that will be submitted to the Regulatory Agencies to gain a Marketing Authorisation for the product) required large studies that were statistically valid for the outcome that was to be tested. For studies of antibacterial drugs, clinical trials which were designed to prove equivalence between the test drug and the comparator drug were standard [Smith et al, 1998]. Equivalence within
10% of the ‘gold standard’ therapy for that indication was the regulatory standard, and 90% power was expected. Consequently, most two-arm antibacterial studies comprised 500 – 800 patients (depending on the expected success rate). In addition, clinical studies were conducted to high standards as outlined in ICH-GCP (International Conference on Harmonisation, Good Clinical Practice). Throughout this period, clinical trials of antibiotics were conducted in patients with AECB. However, although the scientific robustness as described above had increased significantly, the proportion of patients who had a bacterial pathogen isolated at baseline did not appear to have changed, and remained highly variable. This may have been, in part, caused by the wide range of severity of the underlying chronic bronchitis or COPD e.g. GOLD criteria I – IV [GOLD Report, 2007], which not only could influence the severity of the exacerbation, but also the likelihood of the exacerbation being of bacterial origin.

This systematic review of randomised, controlled, clinical trials of antibacterial agents (antibiotics) in the treatment of patients with an acute exacerbation of chronic bronchitis was, therefore, undertaken in order to attempt to understand the reasons why such variations in the proportions of bacteriologically-proven infections should exist, and examine which (if any) entry criteria improve the proportion of patients from whom a pathogen may be isolated.

4.2 Objective

The objective of this systematic review was to examine the differences in the rates of bacteriologically-positive sputum samples (i.e. sputum samples at study entry from which a recognised pathogen was isolated) in clinical trials of antibacterial drugs in patients with acute exacerbation of chronic bronchitis, and to investigate whether there was any association between the percentage of bacteriologically-positive patients and the following factors:

- definition of chronic bronchitis
- definition of an acute exacerbation
microbiological screening of the sputum sample for white blood cells and squamous epithelial cells, +/- bacterial cells seen on Gram stain performed as an entry criterion

- species of bacteria that were recognised as pathogens
- date of the study

The protocol for this systematic review can be found in Appendix 9.2.

4.3 Selection of Papers

4.3.1 Databases Used

The following databases were used for this study:

a) Ovid Medline

b) Cochrane Library

4.3.2 Search Terms Applied

Medline

The disease that we now call acute exacerbation of chronic bronchitis has had a variety of names and terms over the last 40 years. An examination of the terms used in Medline since 1966 showed that a number of different terms would need to be used in order to identify all papers on AECB since 1966.

The terms used in the Medline search were:

A) Disease definition
   - bronchitis,
   - lung diseases, obstructive,
   - bronchitis, chronic
   - pulmonary disease, chronic obstructive
   - pulmonary emphysema
   - acute exacerbation of chronic bronchitis
B) **Antibiotic therapy definition**
   - anti-infective agents (administration & dosage, therapeutic use)

A) and B)

Limit to: Human, Adult, English language, Randomised controlled clinical trial.

**Cochrane Library**
The search of the Cochrane Library database was simpler than that of Medline as only one term was needed to identify all relevant papers.

**Search term**
   - acute exacerbation chronic bronchitis

**4.3.3 Entry Criteria for Papers**
A number of inclusion and exclusion criteria were required so that only clinical studies with a certain level of scientific robustness would be included in the analysis.

**4.3.3.1 Inclusion criteria**
Papers that were eligible for this systematic review met the following criteria:
   a) Clinical trials of one or more antibacterial agent in the treatment of patients with an acute exacerbation of chronic bronchitis or an acute exacerbation of COPD.
   b) Randomised, controlled, clinical trials.
   c) Published in English.

**4.3.3.2 Exclusion criteria**
The following types of papers were excluded from the analysis:
   a) Letters.
   b) Clinical trials that did not perform or report any bacteriology.
c) Papers that did not give sufficient detail of the study design and/or the bacteriology.
d) Papers reporting interim data.
e) Clinical trials that only examined only one pathogen in AECB.
f) Papers that reported on a subset of a larger study (in such cases efforts were made to identify the publication of the full study).
g) Clinical trials that enrolled fewer than 100 patients.

This last exclusion criterion excluded a significant number of older studies, this aspect was highlighted in the Introduction (Section 4.1). However, with fewer than 100 patients in a two-arm study each patient would account for a minimum of 2%. In a three-arm study that only recruited 60 patients (20 patients per arm), cure rates of 18, 16 and 15 patients, result in the possibly misleading percentage of cure rates of 90%, 80%, and 75% so that a three patient difference in success rate resulted in a 15% difference in cure rates. In the smaller bacteriological populations the differences would be more extreme. It was for this reason that the minimum of 100 patients was applied.

4.4 Information Collected

4.4.1 Overview of Data Collection

Each suitable paper (or potentially suitable paper) was obtained either from my own collection of papers and journals; from online access; from the library at the Royal Society of Medicine; or by ordering the paper from the British Library. Each paper was reviewed and the data from each suitable paper was collected on individual data collection forms (see Data Collection Form on page 39 and Appendix 9.2). At the end of data collection, the data from these forms was transferred to an Excel spreadsheet for analysis.
4.4.2 Data Extracted from the Papers

Each paper identified was given a unique reference number and the following data was collected from each eligible paper:

1. Reference number.
2. 1st Author.
4. Year; volume; and start page of paper.
5. Study drug.
6. Comparator(s).
7. Whether the standard definition of chronic bronchitis was applied in the study (daily production of sputum for 3 months of the year over at least 2 successive years).
8. Minimum and maximum age of patients.
9. The signs and symptoms of an acute exacerbation that were required for entry.
10. Whether there was any screening of the sputum prior to patient enrolment.
11. Whether there were any requirements that had to be met on the quality of the sputum e.g. WBCs; SECs.
12. Whether there was any statistical justification for the sample size.
   If so, whether the a) enrolment target, b) evaluable patient target was reached.
13. The number of patients that were: a) enrolled, b) in the Intention-to-Treat (ITT) population, and c) in the Clinically Evaluable (CE) population.
14. Number of pathogens in the ITT population.
15. Number of bacteriologically-positive patients in the ITT population.
16. Proportion of bacteriologically-positive patients in the ITT population.
17. Number of pathogens in the CE population.
18. Number of bacteriologically-positive patients in the CE population.
19. Proportion of bacteriologically-positive patients in the CE population.
22. The organisms that were recorded as pathogens.
23. Whether organisms that were resistant to the study drugs were excluded.
Data Collection Form for Systematic Review

<table>
<thead>
<tr>
<th>Study Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ref No.: □ □ □</td>
</tr>
<tr>
<td>1st Author: ..................................................</td>
</tr>
<tr>
<td>Journal: ..................................................</td>
</tr>
<tr>
<td>Year/Vol/pg: ..................................................</td>
</tr>
<tr>
<td>Study drug: ..................................................</td>
</tr>
<tr>
<td>Comparator(s): ..................................................</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Population &amp; Entry Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population: Std AECB? Yes □ No □</td>
</tr>
<tr>
<td>if No, specify: ..................................................</td>
</tr>
<tr>
<td>Age range: from □ □ to □ □ OR no upper limit □</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Symptoms req'd for entry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purul sputum: req. □ opt. □; Dyspnoea: req. □ opt. □; ↑ Sputum vol: req. □ opt. □;</td>
</tr>
<tr>
<td>Cough: req. □ opt. □; Wheezing/rales: req. □ opt. □; Fever: req. □ opt. □; FEV1: req. □ opt. □</td>
</tr>
<tr>
<td>Any 1 of optional □ Any 2 of optional □ Any 3 of optional □ Not specified □ Req. only □</td>
</tr>
<tr>
<td>Anything different to above: ............................................................................................................</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sputum criteria for study entry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes □ No □ N/K □</td>
</tr>
<tr>
<td>If Yes, WBCs ≥25: □ SECs ≤10: □ predominant cell types on Gram stain: □</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sputum criteria for culture/to be included Bact. Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes □ No □ N/K □</td>
</tr>
<tr>
<td>If Yes, WBCs ≥25: □ SECs ≤10: □ predominant cell types on Gram stain: □</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Study Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total patients entered: □ □ □</td>
</tr>
<tr>
<td>Stat justif of No.? Yes □ No □ N/K □</td>
</tr>
<tr>
<td>Recruit. target met? Yes □ No □ N/K □</td>
</tr>
<tr>
<td>Clin eval. target met? Yes □ No □ N/K □</td>
</tr>
<tr>
<td>No. in ITT: □ □ □</td>
</tr>
<tr>
<td>No. in PP: □ □ □</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bacteriological population</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITT - No. of paths: □ □ □</td>
</tr>
<tr>
<td>ITT - No. of Bact+ pats: □ □ □ ITT - % of Bact+ pats: □ □ □</td>
</tr>
<tr>
<td>PP - No. of paths: □ □ □</td>
</tr>
<tr>
<td>PP - No. of Bact+ pats: □ □ □ PP - % of Bact+ pats: □ □ □</td>
</tr>
<tr>
<td>ITT - Clinical Success (study drug vs comparator): □ □ □ □% vs □ □ □ □%</td>
</tr>
<tr>
<td>PP - Clinical Success (study drug vs comparator): □ □ □ □% vs □ □ □ □%</td>
</tr>
<tr>
<td>Path: SPN □ HIN □ MCA □ HPA □ SAU □ PAE □ E'bact □ others □ spp N/K □ R-orgs excl. □</td>
</tr>
</tbody>
</table>
4.5 Data Management, Analysis and Reporting

4.5.1 Database

An Excel spreadsheet was created to reflect the data collection form so that the information collected on the data collection forms could be easily transferred to the spreadsheet.

4.5.2 Analysis of Data

Studies varied in whether they reported bacteriology in the ITT and/or CE populations. Therefore, in order to capture the maximum number of good quality studies, studies were accepted for analysis provided that bacteriology was reported in at least one of these populations. The differences in the rates of bacteriologically-positive sputum samples (i.e. sputum samples at study entry from which a recognised pathogen was isolated) in clinical trials of antibacterial drugs in patients with acute exacerbation of chronic bronchitis was examined in both the ITT and CE populations and reported as a median and range.

The association between the percentage of bacteriologically-positive patients and the following factors were analysed:

- definition of chronic bronchitis (ATS definition or alternative)
- definition of an acute exacerbation (Anthonisen Type I and Type II exacerbations or alternative)
- presence or absence of microbiological screening of the sputum sample for white blood cells and squamous epithelial cells, +/- bacterial cells seen on Gram stain performed as an entry criterion
- the species of bacteria that were recognised as pathogens
- date of the study (based on the year that the paper was published).
4.6 Results

4.6.1 Overview of Papers from Search

The search of Medline generated 277 papers that fitted the general criteria, and the Cochrane Library search generated 265 papers. The Medline papers were then screened to check that they were randomised controlled clinical trials of antibiotics in patients with acute exacerbation of chronic bronchitis, and were published in English (inclusion criteria); the Cochrane papers were checked against the Medline list and all duplicates were deleted, the remaining papers were screened as described for the Medline papers. The results of the searches and checks are shown in Table 4-1.

Table 4-1: Results of the database searches

<table>
<thead>
<tr>
<th>Database and search terms</th>
<th>No. of references</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Medline</strong></td>
<td></td>
</tr>
<tr>
<td>A) Terms for disease definition</td>
<td>37,478</td>
</tr>
<tr>
<td>B) Terms for antibiotic therapy</td>
<td>301,063</td>
</tr>
<tr>
<td>A) and B)</td>
<td>2,918</td>
</tr>
<tr>
<td>Limit to: Human, Adult, English language,</td>
<td></td>
</tr>
<tr>
<td>Randomised controlled clinical trial</td>
<td>277</td>
</tr>
<tr>
<td>After screening</td>
<td>132</td>
</tr>
<tr>
<td><strong>Cochrane</strong></td>
<td></td>
</tr>
<tr>
<td>Acute exacerbation chronic bronchitis</td>
<td>265</td>
</tr>
<tr>
<td>After screening</td>
<td>24</td>
</tr>
<tr>
<td><strong>Overall Total from Database Searches</strong></td>
<td>156</td>
</tr>
</tbody>
</table>

The Medline search resulted in 132 papers, and the Cochrane Library search resulted in 24 papers that had not already been listed by Medline. The dates of the papers ranged from 1968 to 2004. In addition, during the identification, and collection of these references, my personal reading in this area provided a further five papers that had not been found on Medline or the Cochrane Library.
4.6.2 Overview of Eligible Papers

Table 4-2 summarises the numbers of eligible and non-eligible papers that were identified in the search of Medline and the Cochrane Library.

Table 4-2: Summary of the database searches

<table>
<thead>
<tr>
<th>Database</th>
<th>Eligible</th>
<th>Non-eligible</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medline</td>
<td>66</td>
<td>66</td>
<td>132</td>
</tr>
<tr>
<td>Cochrane</td>
<td>14</td>
<td>10</td>
<td>24</td>
</tr>
<tr>
<td>Personal</td>
<td>3</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>83</strong></td>
<td><strong>78</strong></td>
<td><strong>161</strong></td>
</tr>
</tbody>
</table>

A total of 161 papers were identified and reviewed. The inclusion criteria had already been applied in the screening of the papers, and this review therefore checked the inclusion criteria, but mainly checked the papers for the exclusion criteria. From this rigorous review 83 papers (52%) were found to be eligible for analysis in this systematic review. The reasons for non-eligibility of the remaining 78 papers are summarised in Table 4-3.

Table 4-3: Summary of the reasons for non-eligibility of papers

<table>
<thead>
<tr>
<th>Reason for non-eligibility*</th>
<th>Medline</th>
<th>Cochrane</th>
<th>Personal</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insufficient or no bacteriology</td>
<td>27</td>
<td>5</td>
<td>1</td>
<td>33</td>
</tr>
<tr>
<td>Insufficient numbers</td>
<td>20</td>
<td>1</td>
<td>1</td>
<td>22</td>
</tr>
<tr>
<td>Insufficient detail</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Mixed LRTIs</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Interim data</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Duplicate paper of study</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Unsuitable design (eg. X-over study, meta-analysis)</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Not a RCT</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Prophylaxis study</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>S. pneumoniae</em> only study</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Not an antibiotic study</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Letter</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

* Papers could have more than one reason for ineligibility. LRTIs - lower respiratory tract infections. RCT - randomised controlled trial.
The eligible papers were published between from 1977 to 2004. All but two of these papers (dated 1977 and 1989), were published after 1990. This suggests that the inclusion and exclusion criteria that were applied regarding the bacteriology, detail of signs and symptoms required and minimum numbers tended to exclude clinical studies from pre-1990.

4.6.2.1 Study population

One of the criteria for this systematic review was that a study had to have enrolled a minimum of 100 patients. This criterion ignored the fact that some studies had 3 arms. The number of patients per arm in the eligible studies ranged from 44 to 1090.

There was considerable inconsistency in the lower age limit that was used in these studies; the number of papers (studies) with each lower age limit is shown in Table 4-4.

Table 4-4: Lower age limit in eligible papers

<table>
<thead>
<tr>
<th>Lower age limit</th>
<th>No. of papers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 18 years*</td>
<td>8</td>
</tr>
<tr>
<td>18 years</td>
<td>47</td>
</tr>
<tr>
<td>35 years</td>
<td>5</td>
</tr>
<tr>
<td>40 years</td>
<td>9</td>
</tr>
<tr>
<td>45 years</td>
<td>1</td>
</tr>
<tr>
<td>‘Adult’</td>
<td>8</td>
</tr>
<tr>
<td>Not specified</td>
<td>5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>83</strong></td>
</tr>
</tbody>
</table>

* Lower age limit: 16 years - 1 paper, 15 years - 1 paper, 13 years - 4 papers, 12 years - 2 papers.

The sample size of the randomised controlled trial was justified in 37 (45%) papers, these papers tended to be the more modern papers, dated from 1993-2004. Thirty-four of these 37 papers were from the period 1997-2004. The sample size was not justified/explained in 46 (55%) papers; these papers were published from 1977-2004, however 26 of these papers were from 1977-1996 inclusive.
4.6.3 Analysis of Systematic Review Objectives

The proportion of bacteriologically-positive patients in each study was identified and the variation between the studies examined.

Each of the factors outlined in Section 4.2 (Objective) was examined against the proportion of bacteriologically-positive patients in each sub-group within that factor.

4.6.3.1 Rates of bacteriologically-positive sputum samples

According to the exclusion criteria b) and c) all studies were required to report adequate detail of the bacteriological data in the ITT and/or CE population. Twenty studies provided bacteriological data on the ITT population only; 34 studies provided bacteriological data on the CE population only; and 29 studies gave this information for both populations. Therefore data on the ITT population was gained from 49 (20 + 29) studies, and on the CE population from 63 (34 + 29) studies.

As the studies varied in the populations in which they reported bacteriology, it was impossible to compare the figures for one population across all of the eligible studies. A few studies required the sputum sample to be bacteriologically-positive to be included in the ITT or CE population. One study required a bacteriologically-positive sample for the patient to be eligible for the study and therefore 100% of patients in the ITT population were bacteriologically-positive. There were also 19 (23%) studies that required a bacteriologically-positive sample for the patient to be eligible for the CE population, and in these studies 100% of patients in the CE population were bacteriologically-positive. These studies which gave 100% bacteriologically-positive patients in the ITT or CE population have been excluded from all the following analyses of the rates of bacteriologically-positive patients (sputum samples), as these were selected populations, and the rates of bacteriologically-positive patients do not reflect the entry criteria that are being examined here. The range of the rates of bacteriologically-positive sputum samples in the ITT and CE populations are shown in Table 4-5. The 25th to 75th percentile in the ITT population was 28.7% - 64.7%, and was 28.2% - 60.4% in the CE population, demonstrating that there was a general wide spread of results and not just a few outliers.
Table 4-5: Rates of bacteriologically-positive sputum samples

<table>
<thead>
<tr>
<th>Population</th>
<th>No. of papers*</th>
<th>Median</th>
<th>Range</th>
<th>25th - 75th Percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITT</td>
<td>48</td>
<td>46.1%</td>
<td>13.9% to 96.0%</td>
<td>28.7% to 64.7%</td>
</tr>
<tr>
<td>CE</td>
<td>44</td>
<td>41.6%</td>
<td>15.6% to 85.6%</td>
<td>28.2% to 60.4%</td>
</tr>
</tbody>
</table>

* excludes papers that had 100% bacteriologically-positive patients, and those where no value was given.

4.6.3.2 Definition of chronic bronchitis

The definition of chronic bronchitis of “the daily production of sputum for at least three consecutive months over two consecutive years” has generally been accepted as the “gold standard” definition of chronic bronchitis for around four decades. Despite this, not all clinical trials of chronic bronchitis patients adopted this definition. Of the 83 papers, 59 (71%) used this standard definition of chronic bronchitis; 23 (28%) papers did not define their definition of chronic bronchitis; and just 1 (1%) paper used a different definition (history of recurrent productive cough).

Since many of the papers in which the definition of chronic bronchitis was not stated may have applied this standard definition, and only one paper specifically used a different definition, it is not possible to compare the rates of bacteriologically-positive sputum samples among studies that applied the standard definition with those that used a different definition. Among the papers that used the standard definition of chronic bronchitis, the rates of bacteriologically-positive sputum samples in the ITT populations ranged from 16.7% to 87.1% (33 studies; median 45.6%), and in the CE population ranged from 15.6% to 85.6% (33 studies; median 42.9%). There was therefore no evidence that the standard definition of chronic bronchitis significantly affected the rate of bacteriologically-positive patients. In the one paper that used the definition of recurrent productive cough, the bacteriologically-positive rate in the ITT population was 34.0%, the bacteriology of the CE population was not analysed.
4.6.3.3 Definition of an acute exacerbation

The definition of an acute exacerbation was assessed according to the Anthonisen classification [Anthonisen et al, 1987]. In 10 (12%) papers, patients were required to have a Type I exacerbation to be eligible for the study, and in 14 (17%) papers, patients were required to have a either a Type I or II exacerbation to be eligible for the study. All the other studies had different criteria, although some only included minor variations.

The rates of bacteriologically-positive sputum samples in the 10 studies that specified only Type I exacerbations, ranged from 13.9% to 66.0% in the ITT population (5 studies; median 45.6%), and from 26.2% to 67.2% in the CE population (7 studies; median 42.7%). In the 14 studies that allowed Type I or Type II exacerbations, the rates of bacteriologically-positive sputum samples in the ITT population ranged from 16.7% to 44.2% (12 studies; median 27.9%), and in the CE population, 15.6% to 44.2% (9 studies; median 27.5%).

Among the other 59 studies, the rates of bacteriologically-positive sputum samples in the ITT population ranged from 20.0% to 96.0% (31 studies; median 57.6%), and in the CE population ranged from 16.0% to 85.6% (28 studies; median 56.5%), as shown in Table 4-6. Therefore, although there seemed to be a suggestion that Type I exacerbation tended to have higher rates of bacteriologically-positive sputum samples than Type II exacerbations, the studies that used other varied criteria appeared to have at least as good, if not better, rates of bacteriologically-positive sputum samples than Type I exacerbations. It is possible that although the Anthonisen Type I symptoms are a useful guide to a bacterial exacerbation; other medical history, disease criteria, and signs and symptoms are more significant in predicting a bacterial exacerbation.
Table 4-6: Rates of bacteriologically-positive patients according to the definition of an acute exacerbation

<table>
<thead>
<tr>
<th></th>
<th>ITT population</th>
<th>CE population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total papers</td>
<td>Median % Bact-pos range (n)</td>
</tr>
<tr>
<td>Type I exacerbation</td>
<td>10</td>
<td>45.6% 13.9 - 66.0% (5)</td>
</tr>
<tr>
<td>Type I or II exacerbation</td>
<td>14</td>
<td>27.9% 16.7 - 44.2% (12)</td>
</tr>
<tr>
<td>Other studies</td>
<td>59</td>
<td>57.6% 20.0 - 96.0% (31)</td>
</tr>
</tbody>
</table>

If macroscopically purulent sputum is taken as the major indicator of a bacterial exacerbation, 31 (37%) of the 83 studies applied this as a requirement for enrolment to the study, the remaining studies did not specify this as an entry requirement, although many of the patients would have had purulent sputum, so this is not a precise comparison. In the studies that required purulent sputum for entry, the percentage of patients in the ITT populations who were bacteriologically-positive (20 of the 31 studies gave details on the ITT population) ranged from 13.9% to 81.1%. The studies that did not require purulent sputum for entry, showed a similarly wide range of bacteriologically-positive ITT patients (19.7% to 87.1%) in 27 of the 52 studies that gave details on the ITT population.

4.6.3.4 Microbiological screening of the sputum sample

A total of 20 (24%) studies had applied criteria for microscopic evaluation of the sputum samples (generally >25 WBCs and <10 SECs) that had to be met in order for the patient to be eligible for the study. The rates of bacteriologically-positive sputum samples in the ITT population in these studies ranged from 54.6% to 96.0% (7 studies; median 67.3%), and in the CE population ranged from 24.5% to 85.1% (13 studies; median 57.8%). The rates of bacteriologically-positive sputum samples in the remaining 63 studies that did not apply microscopic screening of the sputum samples ranged from 13.9% to 87.1% in the ITT population (41 studies; median 42.5%), and in the CE population ranged from 15.6% to 85.6% (31 studies; median 40.4%). These results are summarised in Table 4-7.
Table 4-7: Rates of bacteriologically-positive patients according to microbiological screening of sputum purulence.

<table>
<thead>
<tr>
<th></th>
<th>No. of papers</th>
<th>WBC* screen</th>
<th>SEC* screen</th>
<th>Bact cells on Gram-stain</th>
<th>% Bact-pos ITT (median)</th>
<th>% Bact-pos CE (median)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microbiological</td>
<td>20</td>
<td>17</td>
<td>16</td>
<td>8</td>
<td>54.6 - 96.0%</td>
<td>24.5 - 85.1%</td>
</tr>
<tr>
<td>screening of sputum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(67.3%)</td>
<td>(57.8%)</td>
</tr>
<tr>
<td>purulence</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No microbiological</td>
<td>63</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>13.9 - 87.1%</td>
<td>15.6 - 85.6%</td>
</tr>
<tr>
<td>screening of sputum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(42.5%)</td>
<td>(40.4%)</td>
</tr>
<tr>
<td>purulence</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

WBC: white blood cells; SEC: squamous epithelial cells; * No. of papers using this criterion in the screening.

In the ITT populations, microbiological screening of the sputum appears to increase the lowest value of the range of bacteriologically-positive patients. However, this difference is only marginal in the CE populations. The large difference in the lowest value of the range between the ITT and CE populations in the studies that applied microscopic screening makes this finding uncertain.

In addition, it appeared that in 28 studies (34%) some degree of microscopic screening of the sputum was performed to qualify the sputum for culture, this was generally >25 WBCs and <10 SECs, but sometimes only one of these two criteria was applied.

4.6.3.5 Species of bacteria that were recognised as pathogens

Across all the studies there was a large variation in the species of bacteria that were classed and reported as pathogens. Three papers did not specify any of the organisms isolated, leaving 80 papers from which the following results were obtained. Only *H. influenzae* was isolated and reported as a pathogen in every study. *S. pneumoniae* was isolated and reported in 77 (96%) studies and *M. catarrhalis* was isolated and reported in 75 (90%) studies. A few studies seemed to isolate several *S. pneumoniae* but no *M. catarrhalis*, and a few studies appeared to find the reverse. As both of these pathogens are frequently isolated from patients with AECB, this appears to be an unusual finding.

Other pathogens were more difficult to interpret as it was only possible to know if an organism had been accepted as a pathogen if it was reported. It was therefore generally not possible to know whether a particular organism had not been isolated, or had been
isolated and was not classified as a pathogen. *Staphylococcus aureus* was reported in 61 (76%) studies; *H. parainfluenzae* was reported in 49 (61%) studies; Enterobacteriaceae were reported in 45 (56%) studies; *P. aeruginosa* was reported in 36 (45%) studies; and "other", often unspecified, organisms were reported in 50 studies. Resistant isolates were excluded from the results or from the microbiologically-evaluable (ME) population (CE population that had a pathogen isolated at baseline) in 12 (15%) studies.

Enterobacteriaceae was a well-reported group of organisms, either by individual species (e.g. *E. coli, K. pneumoniae* etc.) or collectively termed coliforms or enterobacteria. Enterobacteriaceae have been recorded as occurring in the lungs of AECB patients, particularly those with advanced disease. There is a body of opinion that believes that these are almost always colonisers [Hirschmann, 2000], and another that believes that they can commonly be the cause of exacerbations [Eller et al, 1998; Miravitlles et al, 1999]. The rates of bacteriologically-positive sputum samples in the studies that included Enterobacteriaceae as pathogens, in the ITT population ranged from 16.7% to 96.0% (26 studies; median 43.4%), and in the CE population, ranged from 15.6% to 85.1% (21 studies; median 31.5%). Rates in studies that did not include Enterobacteriaceae as pathogens, in the ITT population ranged from 13.9% to 87.1% (21 studies; median 49.4%), and in the CE population ranged from 16.0% to 85.6% (21 studies; median 44.8%).

### 4.6.3.6 Date of the study

From 1997, studies increased in size quite dramatically, possibly as a result of the International Conference on Harmonisation Good Clinical Practice (ICH-GCP). ICH-GCP is an international ethical and scientific quality standard for designing, conducting, recording and reporting trials that involve human participants. The guidelines were developed in order to provide clinical trials with a unified standard across the European Union, Japan and the United States and were labelled ICH-GCP at the International Conference on Harmonisation, 1996. Almost all studies from 1997 onwards involved populations of at least 350 patients. Only 16 of the 54 (30%) studies published in 1997 or later had less than 350 patients, and 24 (44%) studies had more than 500 patients. Prior to 1997, only 12 of the 29 (41%) studies involved populations larger than 200
patients; and only 7/29 (24%) had 350 or more patients. In addition, 20 of the 22 papers that were excluded on the grounds of involving of less than 100 patients were published pre-1997.

The rates of bacteriologically-positive sputum samples in the 29 pre-1997 studies, ranged from 13.9% to 96.0% in the ITT population (14 studies; median 60.2%), and from 16.0% to 85.1% in the CE population (8 studies; median 38.2%). In this time period, 13 of the 29 (45%) studies required a pathogen to be isolated for a patient to be clinically evaluable, and therefore these studies gave 100% bacteriologically-positive in the CE population. In some of these studies, therefore, there was a large difference in size between the ITT population and the CE population; the largest difference observed was in a study with 802 ITT patients, of which only 182 (23%) patients were clinically evaluable. Although not all of these patients would have been clinically non-evaluable for the reason of “non-isolation of a pathogen”, this was always the major reason that patients were clinically non-evaluable.

The rates of bacteriologically-positive sputum samples in the 54 studies from 1997 onwards, ranged from 16.7% to 87.1% in the ITT population (34 studies; median 45.4%), and from 15.6% to 85.6% in the CE population (36 studies; median 41.6%). In this time period, only 6 of the 54 (11%) studies required a pathogen to be isolated for a patient to be clinically evaluable, and therefore these studies gave 100% bacteriologically-positive in the CE population.

4.6.3.7 Analysis of the studies with >60% bacteriologically-positive patients

Table 4-8 summarises the 21 studies, dating from 1992 to 2000, which had rates of bacteriologically-positive patients of greater than 60%, either from the ITT or CE populations. The level of 60% was chosen because it is significantly above the median value of 46.1% ITT and 41.6% CE, and yet is low enough to include at least 20 studies.

These 21 studies were reporting on the activity of a range of antibacterial agents that were being developed in the 1990s, many of which were quinolones. Eleven of these studies applied the standard definition of chronic bronchitis, while the other 10 studies
did not give a definition of chronic bronchitis. Most studies used a lower age limit of 18 years, however younger and older lower age limits were also used. In only 2 studies were patients required to have a Type I exacerbation according to the Anthonisen criteria. There was microscopic screening of sputum purulence prior to entry to the study (i.e. only patients with microscopically purulent sputum were allowed to enter the study) in 10 of the 21 (48%) studies. This is a higher proportion than for the overall database, in which 24% studies specified microscopic screening of the sputum prior to entry. Most studies reported a wide range of pathogens, however one study reported only 3 species (*H. influenzae*, *S. pneumoniae*, and *M. catarrhalis*) [O’Doherty et al, 1998], and one study reported only 2 species (*H. influenzae*, and *M. catarrhalis*) [Perez-Gonzalvo et al, 1996].
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Table 4-8: Summary of the data from the 21 studies with rates of bacteriologically-positive sputum samples >60%.
4.7 Final Review of the Literature

A review was performed of the literature from March 2004 to July 2008, so that more recent randomised, controlled trials of antibacterial agents in AECB could be examined and compared with the finding of the original systematic review.

This further review identified 12 papers dating from December 2004 to February 2007. No eligible study published more recently than February 2007 was identified. Of these 12 papers [see Section 10.3], one was found to have been included in the original systematic review, one was found to be not eligible on the basis of insufficient bacteriological data, and one paper was a follow-up paper from a previous publication which had been included in the original systematic review. The remaining nine papers were reviewed using the same data form as previously.

4.7.1 Results

There were no major differences in these nine papers that would affect the results and conclusions of the systematic review.

A couple of trends in the more recent papers were however noted:

- The lower age limit in six of these nine papers (67%) was 30 years or above (1 x 30yr; 2 x 35yr; 2 x 40yr; and 1 x 45yr). This older age limit in two-thirds of studies compares with only 18% of the studies (15/83) in the original review.

- All nine papers (100%) specified Anthonisen criteria for the exacerbation; this compares with only 24 studies (29%) in the original review. Four of these nine studies (44%) specified a Type I or Type II exacerbation, compared with 14 studies (17%) in the original review. Five of the nine studies (56%) specified Type I exacerbation only, compared with 10 studies (12%) in the original review.

- All nine papers (100%) specified that microscopic purulence of each sputum sample (defined as >25 leucocytes (or WBCs or PMNs) and <10 squamous epithelial cells) was examined. Most specified that only sputum samples that
met this criterion would be cultured, other were less clear about this point. In the original review, descriptions of microscopic screening were often unclear, however it appeared that 28 studies (34%) performed some sort of microscopic screening of the sputum before culture.

With regard to the main objective of the proportion of bacteriologically-positive sputum samples, there was an almost equally wide range of values in these nine studies as there was in the original 83 studies. The nine recent studies showed a range of bacteriologically-positive sputum samples from 18% to 63% (median 31.2%): this compares with a range from 14% to 96% (median 46.1%) in the original review. The implication of these findings is that we are not getting any better at identifying patients with bacterial exacerbations of chronic bronchitis.

4.8 Summary and Conclusions

This systematic review was set up to document in a consistent manner, the variation in the rates of bacteriologically-positive patients in randomised, controlled clinical trials of antibacterial agents in patients with AECB. The results of these studies from 1966 to present, demonstrate some interesting points.

The vast majority of clinical trials published between 1966 and 1989 inclusive, were not eligible for this systematic review. The major reasons for this were study populations of less than 100 patients, and either no bacteriology, or insufficient detail of the bacteriology performed. Consequently 81 of the 83 eligible papers were published between 1990 and 2004.

This systematic review has confirmed that in studies of antibacterial agents in randomised controlled clinical trials in AECB patients, there is a large variation in the proportion of patients that have a bacterial pathogen isolated from their sputum at study entry. The signs and symptoms of an acute exacerbation that are used as entry criteria vary considerably between studies; however, there is no evidence that any particular combination of signs and symptoms are linked with a bacterial exacerbation, this is likely to be patient specific. The only factor that appeared to
affect the proportion of patients that had a bacterial pathogen isolated from their sputum at study entry (bacteriologically-positive) was the microscopic screening of a sputum sample of potential patients, prior to entry into the study. Patients were then only allowed to enter the study if their sputum sample “passed” this screening. This screening takes time to perform and the patient is not able to enter the study and start medication until the results of this screening are available. This adds an extra complication to a clinical trial, particularly one conducted in general practice, and it is therefore, not surprising that many clinical trials do not use microbiological screening of sputum purulence. However, several studies (particularly more recent studies) apply this criterion to the evaluability of the sputum sample for culture in accordance with FDA guidance [FDA, 1998] In addition, even in studies where microbiological screening of sputum purulence has been applied, it does not appear to guarantee a high rate of bacteriologically-positive patients. The review of nine more recent papers dating from December 2004 to February 2007 suggests that despite a greater adoption of Anthonisen Type I (and Type II) exacerbations as entry criteria, and microscopic screening of all sputum samples for eligibility for culture, there has been no improvement in the proportion of patients enrolled in these studies who have respiratory pathogens isolated from their sputum at baseline, and who can therefore be classed as having a bacteriologically-proven exacerbation.

This review has confirmed how difficult it is to distinguish between exacerbations caused by bacteria and those caused by other agents, and that there has been no improvement over the last 40 years, in our ability to identify patients with bacterial exacerbations.
5 A MULTICENTRE INVESTIGATION INTO THE BEST INDICATOR OF BACTERIAL INFECTION IN PATIENTS WITH ACUTE EXACERBATION OF CHRONIC BRONCHITIS

5.1 Introduction

The generally accepted description of chronic bronchitis was defined by the American Thoracic Society in 1962 [Am Thorac Soc, 1962], although it had been attempted a few years earlier by a group in London who had defined it as 'the production of phlegm on most days for at least three months in each year' [Fletcher et al, 1959]. Almost 50 years later, the description of sputum production for at least three months of the years and for at least two successive years remains the most frequently used definition of this complex disease. As previously described, exacerbations of CB may occur due to a number of factors that include exposure to airborne irritants, or as a result of infection by bacteria, or viruses. It has been proposed that at least 50% of exacerbations are caused by bacteria [Fagon et al, 1990; Miravitlles et al, 1999; Sethi, 2000,], however many clinical trials of antibacterial drugs in patients with AECB, fail to achieve this percentage of patients who have respiratory pathogens cultured from their sputum.

Such studies aim to select patients who have an acute bacterial exacerbation, although there appears to be no simple and reliable method of distinguishing between exacerbations caused by bacteria and those caused by other agents. This lack of clarity as to the nature of exacerbations, may contribute to the fact that the criteria used for selecting AECB patients for entry into clinical trials of antibacterial drugs are not consistent, and the proportion of bacteriologically-positive patients in such clinical trials shows considerable variation. The proportion of patients in studies of AECB over the last 10 years, who have a bacterial pathogen isolated from a baseline sputum sample can vary between 17% [Wilson et al, 2002] and 87% [DeAbate et al, 1999]. Although it has been suggested that a sputum sample which contains few epithelial cells, abundant neutrophils and a predominant morphological type of bacteria, can indicate a bacterial exacerbation [Murray & Washington, 1975; Chodosh, 1991], and that applying this criterion for entry into a clinical trial may increase the likelihood of
enrolling patients with bacterial exacerbations, only a minority of trials have used this procedure as part of the entry criteria.

Anthonisen and co-workers [Anthonisen et al, 1987] demonstrated a 20% difference in success rates between antibiotic and placebo-treated patients when the patients had increased dyspnoea, sputum volume and sputum purulence. Exacerbations with fewer symptoms showed less difference between antibiotic and placebo, possibly indicating that these were not bacterial exacerbations. It is unfortunate that sputum microbiology was not performed in this study as this could have added weight to the argument for the use of an antibiotic in these categories of patients. It was concluded by Staley and co-workers [Staley et al, 1993] that in view of these results future studies with an active control group should be designed to include only patients in whom exacerbations are likely to have a bacterial cause. It was claimed that the characteristics of increased cough and/or dyspnoea and increased sputum volume and purulence are associated with bacterial infection [Periti et al, 1990; Rademaker et al, 1990], however, many recent studies have produced a disappointing percentage of bacteriologically-proven infections, 19% [Zervos et al, 2003], 26% [Wilson et al, 2004] and 27% [Starakis et al, 2004] even when these criteria were applied.

Alternatively, in studies where only culture-positive patients are eligible for the clinically evaluable (CE) population, then entry criteria attempt to optimise the proportion of patients that will have a pathogen isolated from the sputum; nevertheless, because patients are almost always enrolled on the basis of clinical signs and symptoms and not based on any bacteriological factors (e.g. microscopically-purulent sputum, bacterial cells seen on a Gram-stained sputum preparation) around 50% of the enrolled patients may fail to be eligible for the CE analysis [Shah et al, 1999; Chodosh et al, 2000]. This makes for very inefficient clinical studies, in which many (non-evaluable) patients do not contribute substantially to the efficacy conclusions, or in which the conclusions may have been based on a population where only a small proportion of patients have proven bacterial exacerbations, and where the effects of any one antibacterial agent are diluted by the substantial proportion of patients who did not have a bacterial exacerbation and for whom antibacterial therapy would have no beneficial effect.
This clinical study aimed to examine several aspects of the chronic bronchitis patients who develop an exacerbation and would be entered into clinical trials of antibacterial drugs, to see if patients who have a Type I exacerbation (worsening of sputum volume, sputum purulence and dyspnoea), were more likely to have a bacterial pathogen than those who had a Type II exacerbation (any two of these signs and symptoms). Additional factors were also to be examined to see if a better understanding of patients with a bacterial exacerbation could be formed. These factors were: microscopic purulence (>25 neutrophils/<10 epithelial cells per low power field) and Gram staining of the sputum to identify the presence of bacterial cells by their shape and Gram-staining properties (Gram-positive or Gram-negative). The study also examined the implications of a 24 hour delay in getting a sputum sample to the laboratory, as occurs when a central laboratory is used for culture and testing of the samples. Patients often report that they cough up the most purulent sputum soon after rising in the morning, therefore the study also looked at the isolation rates of potential pathogens in sputum samples produced soon after rising, with those produced later in the day. In addition, there had been recent research which reported a progression of bacterial species from *S. pneumoniae* and *H. influenzae* through coliforms (or other Enterobacteriaceae), to *P. aeruginosa*, as the severity of the COPD progressed [Eller et al, 1998; Miravitlles et al, 1999], and the time since the previous exacerbation for which an antibiotic was prescribed, compared with the bacterial species that were isolated was investigated.

5.2 Objectives

5.2.1 Primary Objective

This prospective study aimed to examine how effective various criteria are in indicating bacterial infection in patients with AECB. The criteria to be examined were:

a. Anthonisen Type I clinical signs and symptoms (increased sputum purulence; increased sputum volume; increased dyspnoea), or Anthonisen Type II clinical signs and symptoms (any 2 of the above signs and symptoms).

b. The combination of >25 neutrophils and <10 epithelial cells per x100 field.
c. The presence of a morphological bacterial cell type in a Gram stained preparation of sputum, of greater than 1 cell per oil immersion field.

5.2.2 Secondary Objectives

The study also examined:

1. The implications of a 24 hour delay in getting a sputum sample to the laboratory.
2. The isolation rates of potential pathogens in sputum samples produced soon after rising, with those produced later in the day.
3. Any association between isolation of a bacterial species and the time since the previous exacerbation will be examined.

5.3 Study Design

This was a multicentre, epidemiological study of chronic bronchitis patients presenting with signs and symptoms of an acute exacerbation to six GP sites and one hospital site in Scotland, and was conducted according to the study protocol dated 5 February 1999 (Appendix 9.3). The signs and symptoms of infection, the neutrophils and epithelial cells in the sputum; and the Gram stain results were compared with the results of the sputum culture in order to identify the best indicator of bacterial infection.

The original study was set up with four GPs in Edinburgh area, however, as interest in the study among Edinburgh GPs was disappointing it was decided to involve a large medical centre in Coatbridge and to set up the microbiology laboratory at Monklands Hospital in nearby Airdrie as the second central laboratory. In addition, Monklands Hospital itself became an investigative site and patients who were being admitted for AECB were screened for the study. Over a year later, when recruitment had stopped altogether in Edinburgh and Coatbridge, it was decided to involve a few Glasgow GP practices that had a proven track record in recruiting AECB patients into clinical
studies. To service these GP practices, the Southern General Hospital was set up as the third central laboratory. These changes were documented in protocol amendment 1, dated 4 August 2001 (Appendix 9.3).

5.3.1 Entry Criteria

As this was an epidemiological study, no exclusion criteria based on safety factors were applicable although investigators were advised that patients with significant bronchiectasis should not be enrolled. Patients were not allowed to enter the study more than once.

Inclusion criteria

1. Adult patients with evidence of chronic bronchitis as defined by: chronic cough and sputum production on most days over a period of 3 months for at least 2 consecutive years.

2. Patients with at least two of the following symptoms: increased sputum purulence; increased sputum volume; increased dyspnoea.

3. Patients who were able to produce a sputum sample at the visit (alternatively, the patient could take a sputum pot away and return a sample the following morning, provided that no antibiotic treatment had been started).

4. Patients who had not yet received an antibiotic for this exacerbation.

5. Patients who had consented to the study.

5.3.2 Study Sites, Laboratories and Methodology

The study was performed at six general practice surgeries and at one hospital in Scotland (Glasgow, Edinburgh and Coatbridge/Airdrie). Sputum samples were taken from the GP surgeries to one of three accredited hospital laboratories in Scotland either by a regular van service or by taxi. Each investigator completed a short case
report form for each patient (Appendix 9.4), recording: date of the visit, basic details of demography, smoking history, date of the last chest infection for which an antibiotic was given, date and time of production of the sputum sample, and extent of pre-infection and current dyspnoea, sputum volume and sputum purulence.

The laboratories also completed a short report form (Appendix 9.4) for each sputum sample, reporting: macroscopic appearance of the specimen, microscopic purulence (> or ≤25WBCs and < or ≥10 squamous epithelial cells per x100 field) and the presence and quantity of morphological bacterial cell types from a Gram-stained preparation; interpreted as: +++ = >25 cells; ++ = 10-25 cells; + = <10 cells. Part of the specimen was then cultured immediately and any organism grown at ≥10³ colony forming units/mL was identified. The species classed as pathogens when found in these numbers were: H. influenzae, H. parainfluenzae, S. pneumoniae, M. catarrhalis, S. aureus, P. aeruginosa, Enterobacteriaceae and any other species that the laboratory would normally report from a sputum sample. The remaining part of the specimen was left at room temperature for 24 hours and then cultured, so that the results could be compared with those of the immediate culture. This procedure of delayed culture was designed to simulate transport of a sputum sample to a central laboratory with delivery within 24 hours.

Antibacterial therapy was not part of this study and therefore participating investigators decided separately whether or not each patient required antibacterial treatment, and what follow up was required. This data was not collected as part of the study. Investigators were informed of the results of the sputum culture and any susceptibility testing that the laboratory routinely performed in order to assist with the patient management.

5.3.3 Ethical Aspects

Local ethics committee approval was obtained for all participating sites and the submissions and dates of approval are shown in Table 5-1
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<td>Greater Glasgow LREC (additional sites)</td>
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* submission, approval with investigator

#### 5.3.3.1 Patient information and informed consent

All patients were given a patient information sheet that explained the study. Patients were encouraged to discuss any concerns with the doctor. If the patient had any doubts about taking part in the study, they were not entered.

All patients who agreed to take part in the study were asked to sign the consent form. No patient was allowed to enter the study unless they had signed and dated the consent form. A copy of the patient information and the consent form are included in the appendices to the study protocol (Appendix 9.3).

#### 5.3.3.2 Confidentiality

All data recorded on the CRF and entered onto the study database was anonymous. Patients were assigned a unique patient number when they consented to the study, and this was the only identification of the patients' data on the CRF or in the database. If any clarification was required between two or more patients, then the dates of birth were used.

#### 5.3.3.3 Approval of the study protocol and amendments

The study protocol was approved by the following people:

- Carol Burley  project leader
- Dr R.G. Masterton  project supervisor
It was agreed that any amendments to the protocol which did not involve patient numbers or the statistical plan could be approved by the project leader and the project supervisor only. Further statistical advice could be sought from any qualified statistician working in pharmaceutical research.

5.3.4 Monitoring

A selection of CRFs was monitored by Carol Burley (CB) as planned, to assess the accuracy of the data. Monitoring took place at the majority of sites and covered the clinical and microbiological aspects of the data. All monitoring was performed in a confidential manner and no record of the patients' names was made. Any errors in the data identified at the monitoring visits were corrected in the CRF and (if applicable) in the database.

5.3.5 Amendments to the Study Design

One protocol amendment was required during this study; the justifications for this amendment are given below and the changes to the protocol are detailed here and in the Study Procedures (Section 5.4).

Primary Justification
The original study was planned to only take place in Edinburgh with Lothian general practitioners. Because of the lack of response in several centres and the need to get the 120 samples completed as quickly as possible, it was decided to extend the study to a wider area of Scotland.

Secondary Justifications
It became clear that it would only be possible to monitor a selection of the patient notes, rather than all of them. It was then necessary to make this clearer in the protocol and in the patient information and consent form.
The funding for this project, in the form of an educational grant, was clarified as being received from Hoechst Marion Roussel (Aventis), as CB was no longer working for that company.

The changes of address and telephone number for CB and Dr Masterton were detailed.

**Confirmation of Scientific and Ethical Content**

None of the changes in the protocol amendment were thought to affect the scientific content of the study as the laboratories at Monklands Hospital and Southern General Hospital, Glasgow agreed to process the sputum samples as laid out in the protocol. The changes did not compromise patient treatment or confidentiality in any way.

### 5.4 Study Procedures

#### 5.4.1 Overview of Data Collection

Data for this study were collected on individual case report forms (CRFs) provided to each investigator and to the laboratory. A CRF was completed for each eligible patient who consented to take part in the study. Any patient who failed to produce a sputum sample before any antibiotic treatment was started was not eligible for the project.

Data for this study therefore came from two sources: the investigator completed the first section of the CRF (clinical data) at the time of the patient visit, and the laboratory completed section two of the CRF (laboratory results).

A copy of the laboratory culture results with routine antibiotic susceptibility results, where an organism was isolated, was sent to the investigator by post. The laboratory request form to be completed by the investigator for study sputum samples was identified by means of an adhesive label. This label was added in order to alert the laboratory that the sample should be processed according to the protocol and the results recorded on a study CRF.
5.4.1.1 Clinical data

The following data was collected by the investigator in the CRF provided for each patient:

- date of visit
- entry criteria
- confirmation of patient consent
- assignment of unique patient number
- basic patient demography
- date of last exacerbation (for which an antibiotic was prescribed)
- time and date of sputum sample
- rating of symptoms of dyspnoea, sputum volume and sputum purulence:
  - prior to this infection (pre-infection)
  - currently

5.4.1.2 Laboratory data

All sputum samples were transferred to the local hospital laboratory and processing commenced within 6 hours. The instructions for processing, to be applied to all three central laboratories are outlined below:

On arrival at the laboratory, samples were logged and any queries about the patient number resolved. The sample was split into two aliquots of equal purulence, using sterile forceps.

Aliquot 1 had the following tests/examinations performed:

i. Assessment of purulence of the sample by appearance:

<table>
<thead>
<tr>
<th>Description</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear, non-viscous</td>
<td>non-sputum sample</td>
</tr>
<tr>
<td>Grey/white, viscous</td>
<td>mucoid</td>
</tr>
<tr>
<td>Up to 50% yellow-green</td>
<td>mucopurulent</td>
</tr>
<tr>
<td>&gt;50% yellow-green</td>
<td>purulent</td>
</tr>
</tbody>
</table>

ii. Low power microscopy of cells (total magnification x100):

- epithelial cells: <10 per field / >10 per field
- neutrophils: >25 per field / ≤25 per field
iii. Gram stain and quantitative assessment of bacterial cell types:

<table>
<thead>
<tr>
<th>No. Bacterial Cells</th>
<th>Recorded as</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-9 per oil immersion field</td>
<td>+</td>
</tr>
<tr>
<td>10-25 per oil immersion field</td>
<td>++</td>
</tr>
<tr>
<td>&gt;25 per oil immersion field</td>
<td>+++</td>
</tr>
</tbody>
</table>

iv. Culture of purulent section of sputum for identification of pathogen with a semi-quantitative interpretation using a dilution technique (see Appendix V of protocol, in Appendix 9.3)

<table>
<thead>
<tr>
<th>CFU/mL</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^3 - 10^4$</td>
<td>Light growth</td>
</tr>
<tr>
<td>$10^5 - 10^6$</td>
<td>Moderate growth</td>
</tr>
<tr>
<td>$\geq 10^6$</td>
<td>Heavy growth</td>
</tr>
</tbody>
</table>

Aliquot 2 was left at room temperature for 24 hours to simulate transport of a sputum sample to a central laboratory by overnight courier. After 24 hours a purulent section of the sputum was cultured as point iv. above.

All results were recorded in the laboratory CRF.

5.5 Data Handling and Statistical Procedures

5.5.1 Sample Size Justification and Statistical Analysis

All patients entered into this study had either a Type I exacerbation (a worsening of sputum volume, sputum purulence and dyspnoea) or a Type II exacerbation (a worsening of any two of these symptoms) of chronic bronchitis. The primary outcome was to examine whether Type I exacerbations (all 3 symptoms) were associated with a higher proportion of bacteriologically-positive (culture-positive) patients, than Type II exacerbations (2 symptoms). The assumptions made in order to calculate the study sample size were:
• 50% of patients would have a Type I exacerbation and 50% would have a Type II exacerbation.

• 35% of patients with a Type II exacerbation would be culture-positive

It was calculated that a total of 112 patients would provide 85% power of detecting a 25% increase in patients with a Type I exacerbation (i.e. 60% culture-positive) using a Chi-squared test at the 5% level. To allow for a small number of unevaluable patients (e.g. sputum samples lost or broken, report forms not completed, etc.) 120 patients were recruited.

Data on all patients with a Type I or Type II exacerbation and for whom a clinical and laboratory CRF were completed adequately were entered into the analysis. This was the only population for analysis. The positive culture rate between the Type I exacerbation patient group and the Type II exacerbation patient group was planned to be compared using a Chi-squared test at the 5% level. The sensitivity and specificity, and positive and negative predictive values were calculated and tabulated for comparison.

5.5.2 Changes to the Planned Statistical Tests

On review of the numbers of patients in the result categories of the primary outcome, it was decided that the Chi-squared test was not the most appropriate test for these data because of the value of seven (patients) in the Type II exacerbation, culture-negative group. Initially, a Chi-squared with Yates’ correction test was performed, however it was a later agreed that the Fisher’s exact test would be the most suitable test. A Fisher’s exact test was therefore performed on all the primary outcome factors. Further discussion on the choice of statistical test and the differences in the results between these tests of significance can be found in Section 6, Statistical Methods.

During submission for publication in the Journal of Infection, the reviewers requested that likelihood ratios were also presented. The results of this statistical test are also presented here.
5.6 Results

Ethics Committee approval for the first set of sites in the Edinburgh area was obtained on 1 April 1999. The first patient was consented into the study on 16 November 1999 and the last patient consented into the study was on 6 February 2003.

5.6.1 Study Population

Overall, 120 patients were enrolled into this study. In 12 patients the investigator CRF was not received and in 7 patients the laboratory CRF was not completed, leaving 101 patients in which both sets of information were received. Of these 101 patients, four were enrolled with only one Anthonisen symptom and were not eligible for the analysis. A total of 97 patients were therefore included in the analyses. The demographics, relevant history, and sputum appearance of the 97 eligible patients are presented in Table 5-2.

There were more females than males recruited into the study (57.7% vs 42.3%), which seems contrary to the incidence of chronic bronchitis in the general population. However, the age range of males and females were very similar, and although any adult patient (aged 18 years or above) could enter this study, the youngest patient entered was 39 years; the oldest patient enrolled was 90 years old. The older character of this population can be seen by the fact that the mean age was close to 65 years. Overall, there were 48 patients under the age of 65 years, and 49 patients aged 65 years or over.

As expected, the vast majority of patients were either current smokers or ex-smokers (not smoked for >6 months), with only 11.3% of patients reported as non-smokers (never smoked). Information on passive smoking at home or at work was not collected. The date of the most recent acute infective exacerbation that the patient had suffered was also recorded (i.e. the last acute exacerbation for which an antibiotic was prescribed). Over half of the patients had suffered an acute exacerbation within the 3 months prior to the current exacerbation; however, almost 20% of patients had not had an exacerbation in the 10 months prior to the current exacerbation. Over two-
thirds of patients were reported to have a worsening of all three cardinal symptoms (sputum purulence, sputum volume, and dyspnoea).

Sputum samples were examined macroscopically by the local laboratory, and recorded as purulent, mucopurulent, mucoid, or non-sputum sample. Approximately 70% of sputum samples were reported to be of purulent or mucopurulent appearance. Assessment of microscopic purulence (>25 white blood cells and <10 squamous epithelial cells) was also performed by the laboratory. Sputum samples were evenly split between purulent (49.5%) and non-purulent (50.5%). The local laboratory was also asked to record the morphological cell types and seen on the Gram stain. Over 90% of sputum samples had bacteria seen on the Gram-stained preparation, and in only 7 specimens were no bacteria observed.
Table 5-2: Patient demographics, history and sputum details (N=97)

<table>
<thead>
<tr>
<th>Patient status</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>41</td>
<td>42.3</td>
</tr>
<tr>
<td>Female</td>
<td>56</td>
<td>57.7</td>
</tr>
</tbody>
</table>

**Smoking History**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current smoker</td>
<td>45</td>
<td>46.4</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>41</td>
<td>42.3</td>
</tr>
<tr>
<td>Non-smoker</td>
<td>11</td>
<td>11.3</td>
</tr>
</tbody>
</table>

**Last LRTI**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 mth ago</td>
<td>24</td>
<td>27.9</td>
</tr>
<tr>
<td>1-3 mth ago</td>
<td>26</td>
<td>30.2</td>
</tr>
<tr>
<td>4-6 mth ago</td>
<td>17</td>
<td>19.8</td>
</tr>
<tr>
<td>7-9 mth ago</td>
<td>3</td>
<td>3.4</td>
</tr>
<tr>
<td>10-12 mth ago</td>
<td>9</td>
<td>10.5</td>
</tr>
<tr>
<td>&gt;12 mth ago</td>
<td>7</td>
<td>8.1</td>
</tr>
<tr>
<td>Missing</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

**Anthonisen Cardinal Symptoms**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>All 3 symptoms</td>
<td>68</td>
<td>70.1</td>
</tr>
<tr>
<td>Any 2 symptoms</td>
<td>29</td>
<td>29.9</td>
</tr>
</tbody>
</table>

**Sputum: macroscopic appearance**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purulent</td>
<td>31</td>
<td>32.3</td>
</tr>
<tr>
<td>Mucopurulent</td>
<td>36</td>
<td>37.5</td>
</tr>
<tr>
<td>Mucoid</td>
<td>26</td>
<td>27.1</td>
</tr>
<tr>
<td>Non-sputum sample</td>
<td>3</td>
<td>3.1</td>
</tr>
<tr>
<td>Missing</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

**Sputum: microscopic purulence**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purulent</td>
<td>48</td>
<td>49.5</td>
</tr>
<tr>
<td>Non-purulent</td>
<td>49</td>
<td>50.5</td>
</tr>
</tbody>
</table>

**Gram stain morphology**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organisms seen</td>
<td>90</td>
<td>93.1</td>
</tr>
<tr>
<td>None seen</td>
<td>7</td>
<td>6.9</td>
</tr>
</tbody>
</table>

**Age**

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>65.8</td>
<td>39-90</td>
</tr>
<tr>
<td>Females</td>
<td>62.9</td>
<td>42-86</td>
</tr>
<tr>
<td>All</td>
<td>64.1</td>
<td>39-90</td>
</tr>
</tbody>
</table>

1. Assessed by the laboratory.
2. Purulent: >25 white blood cells and <10 squamous epithelial cells; non-purulent: ≤25 white blood cells or ≥10 squamous epithelial cells.
5.6.2 Pathogens Isolated from Study Patients

In total, 58/97 (60%) sputum samples grew a pathogenic organism in adequate quantity (≥10^3 cfu/mL) when cultured at the local laboratory; the remaining 39 (40%) sputum samples grew either insufficient organisms or only normal upper respiratory tract flora. The species of organisms grown in these 58 patients is shown in Table 5-3. A total of 68 organisms were grown from the 58 patients, 48 patients had infection with only one organism, and 10 patients were infected with two organisms. No patient was found to have more than two pathogenic organisms. *H. influenzae* was the predominant pathogen; present in 30 of the 58 sputum samples and accounting for 45% of all pathogens isolated. *H. influenzae* was the major organism among single pathogen infections and in the infections where two pathogens were isolated.

*S. pneumoniae*, *M. catarrhalis* and coliforms were found in similar numbers overall; however, *M. catarrhalis* and coliforms were mainly found as single pathogens, whereas the majority of *S. pneumoniae* were found in combination with other pathogens.

<table>
<thead>
<tr>
<th>Organism combinations isolated</th>
<th>Number of occurrences (organisms)</th>
<th>% of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. influenzae</em></td>
<td>23 (23)</td>
<td>39.7</td>
</tr>
<tr>
<td><em>M. catarrhalis</em></td>
<td>7 (7)</td>
<td>12.1</td>
</tr>
<tr>
<td><em>S. pneumoniae</em></td>
<td>4 (4)</td>
<td>6.9</td>
</tr>
<tr>
<td>Coliforms</td>
<td>8 (8)</td>
<td>13.8</td>
</tr>
<tr>
<td><em>H. parainfluenzae</em></td>
<td>1 (1)</td>
<td>1.7</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>4 (4)</td>
<td>6.9</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>1 (1)</td>
<td>1.7</td>
</tr>
<tr>
<td><em>H. influenzae + S. pneumoniae</em></td>
<td>5 (10)</td>
<td>8.6</td>
</tr>
<tr>
<td><em>H. influenzae + M. catarrhalis</em></td>
<td>1 (2)</td>
<td>1.7</td>
</tr>
<tr>
<td><em>H. influenzae + S. aureus</em></td>
<td>1 (2)</td>
<td>1.7</td>
</tr>
<tr>
<td><em>M. catarrhalis + S. pneumoniae</em></td>
<td>1 (2)</td>
<td>1.7</td>
</tr>
<tr>
<td><em>S. aureus + coliform</em></td>
<td>1 (2)</td>
<td>1.7</td>
</tr>
<tr>
<td><em>S. aureus + E. faecalis</em></td>
<td>1 (2)</td>
<td>1.7</td>
</tr>
<tr>
<td>Number of patients with 2 pathogens</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Number of patients (organisms)</td>
<td>58 (68)</td>
<td>100</td>
</tr>
</tbody>
</table>

* Enterococcus faecalis
5.6.3 Primary Outcome: Analysis of the Indicators of Bacterial Infection

The primary objective of this study was to examine how effective three factors were in indicating bacterial infection in patients with AECB. These were:

- The presence of increased sputum purulence, increased sputum volume, and increased dyspnoea (Anthonisen Type I exacerbation), compared with the presence of any two of these symptoms (Anthonisen Type II exacerbation).
- The combination of >25 neutrophils and <10 epithelial cells per x100 field (microscopic evaluation of purulence).
- The presence of a morphological bacterial cell type in a Gram stained preparation of sputum, of greater than 1 cell per oil immersion field.

Macroscopic purulence was also evaluated as a primary outcome measure although this was not specified in the protocol. The reason for this deviation was the publication of the paper of Stockey et al [2000] on sputum colour as an indicator of bacterial infection, which received significance acceptance.

5.6.3.1 Positive sputum culture according to Anthonisen Type I or Type II exacerbations

Table 5-4 shows that of the 68 patients that had all three symptoms present (a Type I exacerbation), 36 (53%) had a pathogen isolated from their sputum (culture-positive), and 32 (47%) patients were culture negative. Among the patients that had two of the three symptoms (Type II exacerbation), 22 (76%) patients were culture-positive and 7 (24%) patients were culture-negative. The figures would appear to suggest that patients are more likely to be culture-positive with only two of these three signs/symptoms present than with all three signs/symptoms present, and a Fisher’s exact test showed this result to be just significant at the 5% level (p=0.043). Although in many respects the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) are irrelevant the values are 62%, 18%, 57%, and 24% respectively.
Table 5-4: Culture results of the major indicators of bacterial infection (N=97)

<table>
<thead>
<tr>
<th>Patient status</th>
<th>n</th>
<th>Culture-positive n (%)</th>
<th>Culture-negative n (%)</th>
<th>Fisher’s exact test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptoms</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-symptoms (Type I)</td>
<td>68</td>
<td>36 (52.9%)</td>
<td>32 (47.1%)</td>
<td>p = 0.043</td>
</tr>
<tr>
<td>2-symptoms (Type II)</td>
<td>29</td>
<td>22 (75.9%)</td>
<td>7 (24.1%)</td>
<td></td>
</tr>
<tr>
<td>Macroscopic purulence*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purulent</td>
<td>67</td>
<td>45 (67.2%)</td>
<td>22 (32.8%)</td>
<td>p = 0.045</td>
</tr>
<tr>
<td>Non-purulent</td>
<td>29</td>
<td>13 (44.8%)</td>
<td>16 (55.2%)</td>
<td></td>
</tr>
<tr>
<td>Microscopic purulence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purulent</td>
<td>48</td>
<td>35 (72.9%)</td>
<td>13 (27.1%)</td>
<td>p = 0.013</td>
</tr>
<tr>
<td>Non-purulent</td>
<td>49</td>
<td>23 (46.9%)</td>
<td>26 (53.1%)</td>
<td></td>
</tr>
<tr>
<td>Gram stain: bacterial cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seen</td>
<td>90</td>
<td>56 (62.2%)</td>
<td>34 (37.8%)</td>
<td>p = 0.113</td>
</tr>
<tr>
<td>Not seen</td>
<td>7</td>
<td>2 (28.6%)</td>
<td>5 (71.4%)</td>
<td></td>
</tr>
</tbody>
</table>

* 1 sputum sample was not assessed for macroscopic purulence and was excluded from these macroscopic purulence results. The sample was culture-negative

5.6.3.2 Positive sputum culture according to microscopic and macroscopic sputum purulence

The macroscopic purulence of the sputum samples was assessed visually by the laboratory before any other tests were performed. A total of 67 sputum samples were reported as macroscopically purulent, of which 45 (67%) were culture-positive and 22 (33%) were culture-negative. Twenty-nine samples were not macroscopically purulent, and of these 13 (45%) were culture-positive and 16 (55%) were culture-negative. One sputum sample was not assessed for macroscopic purulence, and this specimen was culture-negative. The Fisher’s exact test showed this result to be marginally significant (p = 0.045). The sensitivity of this simple screening was reasonably good (78%), but the specificity was poor (45%). The PPV was 67%, and the NPV was 55%. These values indicate that this would not be a particularly robust test.

Forty-eight sputum samples were found to be microscopically purulent; of these, 35 (73%) samples were culture-positive and 13 (27%) samples were culture-negative (Table 5-4). Of the 49 sputum samples that were non-purulent, 23 (47%) samples...
were culture-positive and 26 (53%) samples were culture-negative. These results suggest that microscopically purulent sputum samples are more likely to be culture-positive; however among the non-purulent samples, almost half were culture positive. The Fisher's exact test showed this result to be significant (p = 0.013); however the sensitivity and specificity, and PPV and NPV were insufficient for this test to be particularly useful in a clinical trial setting (sensitivity: 60%; specificity: 67%; PPV 73%; NPV 53%).

An interesting comparison was that between the microscopic and the macroscopic purulence. As can be seen in Table 5-5, microscopic and macroscopic purulence were often not in agreement.

<table>
<thead>
<tr>
<th></th>
<th>Culture +ve n (%)</th>
<th>Culture -ve n (%)</th>
<th>Total n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macro-purulent + Micro-purulent</td>
<td>32 (71%)</td>
<td>13 (29%)</td>
<td>45</td>
</tr>
<tr>
<td>Macro-purulent not Micro-purulent</td>
<td>13 (59%)</td>
<td>9 (41%)</td>
<td>22</td>
</tr>
<tr>
<td>Micro-purulent not Macro-purulent</td>
<td>3 (100%)</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Neither</td>
<td>10* (37%)</td>
<td>17 (63%)</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>58</td>
<td>39</td>
<td>97</td>
</tr>
</tbody>
</table>

* These 10 patients’ samples grew 5 *H.influenzae*; 2 *S.pneumoniae*; 3 coliforms; and 1 *S.aureus*

Very few sputum samples were microscopically purulent but not macroscopically purulent, and all of the three samples that were, grew a pathogen (culture-positive). Excluding this very small group, the most definitive results were macroscopically and microscopically purulent, and culture-positive; and not macroscopically or microscopically purulent, and culture-negative.

The results of this study, found 45 of 67 (67%) visibly purulent sputum samples grew a recognised bacterial pathogen. However, 13 of 29 (45%) visibly non-purulent samples grew a bacterial pathogen (Table 5-6). The main three bacteria of AECB (*H. influenzae*, *S. pneumoniae* and *M. catarrhalis*), were predominantly isolated from visibly purulent sputum samples, nevertheless a significant proportion (mean: 22%) of
the total were isolated from visibly non-purulent samples. Of the bacteria isolated in this study, *M. catarrhalis* was isolated only from visibly purulent sputum, whereas, *S. aureus* was isolated from a greater proportion of visibly non-purulent than purulent sputum samples. On the results of this study, visibly (macroscopically) purulent sputum does not appear to be a particularly robust feature to distinguish bacterial from non-bacterial exacerbations.

Table 5-6: Examination of the pathogens isolated from macroscopically purulent and non-purulent sputum samples (N=96)

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Purulent sputum (N=67)</th>
<th>Non-purulent sputum (N=29)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td><em>H. influenzae</em></td>
<td>23</td>
<td>34.3</td>
<td>7</td>
</tr>
<tr>
<td><em>S. pneumoniae</em></td>
<td>8</td>
<td>11.9</td>
<td>2</td>
</tr>
<tr>
<td><em>M. catarrhalis</em></td>
<td>9</td>
<td>13.4</td>
<td>0</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>4</td>
<td>6.0</td>
<td>3</td>
</tr>
<tr>
<td>coliforms</td>
<td>6</td>
<td>9.0</td>
<td>3</td>
</tr>
<tr>
<td>others</td>
<td>3</td>
<td>4.5</td>
<td>0</td>
</tr>
<tr>
<td>Total pathogens</td>
<td>53</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Total patients with a pathogen</td>
<td>45</td>
<td>67.2</td>
<td>13</td>
</tr>
<tr>
<td>Total patients with no pathogen</td>
<td>22</td>
<td>32.8</td>
<td>16</td>
</tr>
</tbody>
</table>

There were 67 macroscopically (visibly) purulent sputum samples, 29 non-purulent sputum samples and 1 sputum sample that was not assessed; this sample did not grow a pathogen.

5.6.3.3 Positive sputum culture according to bacterial cells seen on Gram-stain

Bacterial cells were seen on the Gram stain in the vast majority of sputum samples (90/97 samples, 93%). However, pathogens were isolated from only 56 (62%) of these 90 samples; the remaining 34 (38%) samples were culture-negative i.e. no pathogen isolated (Table 5-4). Among the 7 sputum samples in which no bacterial cells were seen, 2 (29%) samples were culture-positive and 5 (71%) were culture-negative. A Fisher’s exact test shows this result to be not significant (p = 0.113). The two patients who had no bacterial cells types seen on the sputum using the non semi-quantitative Gram stain, but were culture-positive (Table 5-4), were found to have an organism (*H. influenzae* and *S. aureus*) that was only present as light growth. This finding might
suggest that these were part of the oropharyngeal flora, or were colonisers in the lower respiratory tract rather than the cause of the acute exacerbation.

5.6.4 Analysis of the Effect of a 24-hour Delay in Culturing in the Organisms Isolated

In some clinical studies, sputum specimens are transported to central laboratories for analysis. This often means a delay of around 24 hours before culturing, and the specimens are often not refrigerated during transportation. The effect of this delay on the accuracy of the culture results was investigated. Table 5-7 shows the results of the 24 hour culture, compared with the results of the immediate culture. Of the 39 specimens that were negative on immediate culture, 38 were also negative at 24 hours, and the remaining 1 specimen grew a *Pseudomonas* (species unknown) at 24 hours. Of the 58 specimens that were culture positive on immediate culture, 54 grew exactly the same organism(s) at 24 hours, and in 4 specimens the 24 hour culture was not performed.

5.6.5 Analysis of the Difference between Early Morning Sputum Samples and those Produced Later in the Day

Patients with CB often report that they produce the most purulent sputum soon after rising in the morning. It is therefore often believed that this is the best specimen from which to obtain bacteria. This study compared the culture results of specimens obtained at or before 9am with those produced later in the day. Table 5-7 presents the results of this comparison; the time of day of the specimen was available in 92/97 patients. Thirty-four (37%) of the 92 specimens in which time of day was known were produced at 9am or earlier; of these 22 (65%) were culture-positive, and 12 (35%) were culture negative. Fifty-eight (63%) specimens were produced after 09:00 hours, of which 35 (60%) were culture-positive and 23 (40%) were culture-negative. If the time cut-off is moved to 10am, the proportions of culture-positive and culture-negative specimens are almost identical. Only 4 sputum samples were produced at 8am or earlier, so at this time point it is not feasible to assess with the data.
Table 5-7: The effect of re-culturing after 24 hours at room temperature, and the influence of time of day of sputum sample on culture results.

<table>
<thead>
<tr>
<th></th>
<th>Culture-positive n (%)</th>
<th>Culture-negative n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Immediate vs 24 hr culture results</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immediate culture results</td>
<td>58</td>
<td>39</td>
</tr>
<tr>
<td>Re-culture results @ 24hr(^1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Culture-positive at immediate culture(^2)</td>
<td>54 (93%)</td>
<td>0</td>
</tr>
<tr>
<td>Culture-negative at immediate culture(^2)</td>
<td>1 (3%)(^3)</td>
<td>38 (97%)</td>
</tr>
<tr>
<td><strong>Time of day of sputum sample</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 9:00 am</td>
<td>22 (65%)</td>
<td>12 (35%)</td>
</tr>
<tr>
<td>&gt; 9:00 am</td>
<td>35 (60%)</td>
<td>23 (40%)</td>
</tr>
<tr>
<td>Time of day not known</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>

1. 4 specimens did not have a 24 hour culture performed.
2. Percentage of the immediate culture result
3. Pseudomonas spp.

5.6.6 Analysis of the Relationship between Frequency of Exacerbations and Incidence of Bacterial Infection and the Species Isolated

Forty-two (72\%) of the 58 culture-positive specimens were from patients who had their last exacerbation within the previous 6 months; only 11 (19\%) culture-positive patients had their last exacerbation more than 6 months previously, and in 5 culture-positive patients the date of the previous exacerbation was not known. Table 5-8 presents the pathogens that were grown from the sputum samples according to the time of the previous exacerbation. Because so few pathogens were grown from patients who had their last exacerbation more than 6 months ago, it is not possible to discern any trends from these data. *H. influenzae* was the most frequently isolated pathogen in every time period (time since the previous exacerbation); infection with more than one pathogen was the second most common infection, and of these *H. influenzae* was involved in the infection in 5/10 patients. In the patients that harboured Enterobacteriaceae, all had their last exacerbation within the previous 6 months, however with small numbers of patients that had their last exacerbation longer than 6 months previously, it is not possible to put any great value on this finding.
Table 5-8: Pathogens isolated from patients according to the time since their last exacerbation.

<table>
<thead>
<tr>
<th>No. patients</th>
<th>Culture-positive (%)</th>
<th>HIN</th>
<th>MCA</th>
<th>SPN</th>
<th>Ent</th>
<th>Others²</th>
<th>Mixed culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1 mth</td>
<td>24</td>
<td>13</td>
<td>6</td>
<td>-</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>1-3 mth</td>
<td>26</td>
<td>17</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>4-6 mth</td>
<td>17</td>
<td>12</td>
<td>6</td>
<td>3</td>
<td>-</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>7-9 mth</td>
<td>3</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10-12 mth</td>
<td>9</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>&gt;12 mth</td>
<td>7</td>
<td>5</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Not known</td>
<td>11</td>
<td>5</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Totals</td>
<td>97</td>
<td>58</td>
<td>23</td>
<td>7</td>
<td>4</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>


1. Percentage of the number of patients in that time period.
2. 1-3 mth: *S. aureus* – 3; *H. parainfluenzae* – 1; *P. aeruginosa* – 1. 10-12 mth: *S. aureus* – 1.

### 5.6.7 Examination of a Semi-Quantitative Approach to the Gram Stain

Since none of the planned analyses showed a sufficient difference which might be useful in predicting bacterial infection in AECB patients, a closer examination of the Gram stain results was undertaken. As presented earlier, 90 of the 97 (93%) patients had bacterial cells seen on the Gram stain, which did not prove to be very helpful. However, a more quantitative analysis of these results proved to be far more useful.

The results were assessed according to the laboratory Gram stain reports of <10 cells (+), 10-25 cells (++) or >25 cells (+++) of each morphological bacterial cell type seen per x1000 field (e.g. Gram-positive cocci). The results of this examination took into account the number of morphological bacterial cell types seen, and the highest classification (numbers) of cells seen of any of the morphological cell types. The categories for this examination were:

a. 1 morphological cell type seen at >25 cells per field gave 8/8 (100%) culture-positive specimens
b. 1 morphological cell type seen at 10-25 cells per field gave 14/16 (88%) culture-positive specimens
c. >1 morphological cell type seen, at least one type present at >25 cells per field gave 17/19 (89%) culture-positive specimens
d. >1 morphological cell type seen, at least one type present at a maximum of 10-25 cells per field gave 4/9 (44%) culture-positive specimens.

e. 1 (or more) morphological cell type present, but at <10 cells per field gave 13/38 (34%) culture-positive specimens.

f. No bacterial cells seen.

The first three categories (a. b. and c.) outline culture-positive rates that were considerably higher than the overall culture-positive rate of 60%, and of those seen in any of the other sub-groups. The last three categories (d. e. and f.) outline culture-positive rates that were well below the overall culture-positive rate seen in this study. Figure 5-1 presents the results of this semi-quantitative analysis in graphical format.

Figure 5-1: The proportion of patients that were bacteriologically-positive against the quantity and number of different bacterial cell types seen on the Gram stain.

![Graph showing percentage of subjects bacteriologically positive for various cell counts.]

Key: +++ = >25 cells/field; ++ = 10-25 cells/field; + = <10 cells/field.

There appeared to be a clear difference between the Gram stains that produce high proportions of bacteriologically-positive patients (black bars) and those that do not white bars). When these types of patients were grouped together the distinction could
be seen more clearly (Table 5-9). In this table ‘semi-quantitative Gram stain: bacterial cells – significant No. seen’, equals the total of the three black bars on the graph (categories a.-c.) and the ‘non-significant No. seen’ equals the total of the three white bars (categories d.-f.). The major factor here is that very few patients who had a significant number of bacterial cells seen on the Gram-stain were culture-negative.

Table 5-9: Culture results of the semi-quantitative approach to the Gram stain

<table>
<thead>
<tr>
<th>Patient status</th>
<th>Culture-positive n (%)</th>
<th>Culture-negative n (%)</th>
<th>Fisher’s exact test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Semi-quantitative Gram stain: bacterial cells</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significant No. seen</td>
<td>39 (90.7%)</td>
<td>4 (9.3%)</td>
<td>p = &lt;0.0001</td>
</tr>
<tr>
<td>Non-significant. No. seen</td>
<td>19 (35.2%)</td>
<td>35 (64.8%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>58</td>
<td>39</td>
<td></td>
</tr>
</tbody>
</table>

Of the 19 sputum samples that produced a Gram stain that was classed as non-significant under this semi-quantitative approach and which were culture-positive (see Table 5-9), five (26%) samples had only light growth of any organism grown; the remaining 14 (74%) sputum samples grew at least one organism that had moderate or heavy growth. In comparison, the 39 sputum samples where the Gram stain was classed as significant under this semi-quantitative approach and which were culture-positive (see Table 5-9), just two (5%) samples had light growth, these organisms were *S. pneumoniae* and *Proteus spp*.

Applying this method of a quantitative Gram stain whereby a single cell type present at ≥10 cells per (x1000) field, or more than one cell type present, but with at least one cell type present at >25 cells per field is predictive of a bacterial infection. A Fisher’s exact test showed this result to be highly significant (p = <0.0001), with a sensitivity of 67%, and a specificity of 90% (positive predictive value: 91%, negative predictive value 65%).
5.7 Summary and Conclusions

This study was set up to examine a number of factors relating to acute exacerbations of chronic bronchitis in the context of patient entry criteria for antibacterial clinical trials in AECB. The main aim of the study was to seek to identify study entry criteria that would optimise the number of enrolled patients with bacteriologically-proven exacerbations, and thus the microbiologically-evaluable study populations. The achievement of this endpoint was set to enable the most cost-effective management of AECB clinical trials. Fifty-eight (60%) of the 97 evaluable patients in this study had a culture-positive sputum sample. This percentage of culture-positive samples was generally good, and was higher than the median value of 46% found in the systematic review.

The primary objective of this study was to investigate the hypothesis that patients with Type I exacerbations had a higher percentage of culture-positive sputum samples than patients with Type II exacerbations. The study failed to confirm this hypothesis and a Fisher's exact test produced a borderline result which suggested that patients Type II exacerbations possibly had a higher chance of having a culture-positive sputum sample. This result is discussed fully in the Discussion (Section 8).

From a range of demographic, symptomatic and laboratory investigations, the only factor which emerged as highly significant in distinguishing culture-positive from culture-negative patients was the semi-quantitative Gram stain analysis. This technique may offer a useful method of accurately predicting bacterial infection, in around 90% of patients. However, the technique involves considerable laboratory expertise and co-operation in achieving a rapid turnaround of results prior to patient randomisation and hence may not be suitable for all clinical trial settings.
6 STATISTICAL METHODS

6.1 Statistical Tests Applied in the Clinical Study

Data produced from a scientific study requires the application of statistical analysis in order that the conclusions of the study may be viewed with confidence. The specific tests that are selected must be appropriate to the particular type of data that has been collected, and the hypothesis that is to be tested.

In this study, the primary and secondary outcomes were categorical data as all the factors being examined were assessed as to whether the patient had a pathogen at baseline, or not (culture +ve, or culture -ve). The results of each of the primary endpoints (see Table 5-4) produced a 2 x 2 frequency table as shown in the example below:

**Table 6-1: Format of primary endpoint for analysis**

<table>
<thead>
<tr>
<th></th>
<th>Culture +ve</th>
<th>Culture -ve</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I exacerbation</td>
<td>36</td>
<td>32</td>
<td>68</td>
</tr>
<tr>
<td>Type II exacerbation</td>
<td>22</td>
<td>7</td>
<td>29</td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
<td>39</td>
<td>97</td>
</tr>
</tbody>
</table>

When planning this study, it appeared that the most appropriate statistical test for this type of data would be a Chi-squared test at the 5% level and this was stated in the statistical section of the study protocol [Appendix 9.3].

6.1.1 The Chi-squared Test

The Chi-squared analysis is used to compare two or more frequencies, to investigate the probability that their values depart from what would be expected by chance alone on the null hypothesis that their frequencies are the same. Interpretation of the Chi-squared value is based upon comparing the results seen in the experiment against results that could occur due to chance alone and using this to establish the statistical significance of the results. For frequency comparisons like those in this study,
statistical significance shows the probability that the differences between observed and expected frequencies are due to chance factors assuming no difference i.e. the null hypothesis is true. To determine a critical value that might be termed statistically significant, an alpha level must be set. The alpha level is traditionally set at .05 (although occasionally it may be set at .01).

There are a number of key assumptions in a Chi-squared test and these are:

- Random sample data.
- A sufficiently large sample size: applying a Chi-squared test to small samples risks an unacceptable rate of Type II errors. There is no accepted minimum number however a number greater than 50 would generally be seen as acceptable.
- Adequate cell sizes: when this assumption is not met for integer data, Yates' correction is applied (see Section 6.1.2 on Yates’ correction).
- Independence: observations must be independent. The same observation can only appear in one cell.
- Non-directional hypotheses: the Chi-squared test tests the hypothesis that two variables are related only by chance.
- Finite values: observations must be grouped in categories.

The major assumption made in the design of this study was that 50% of patients entered into the study would have a Type I exacerbation and 50% would have a Type II exacerbation. This assumption was not met and the proportions were approximately 70% to 30% respectively. This resulted in a small number of patients in the Type II exacerbation, culture-negative group. For this reason, a Chi-squared test with Yates’ correction was initially performed on the data.

6.1.2 Yates’ Correction

Yates' correction (named after the English statistician Frank Yates who published the correction in 1934), is a conservative adjustment to the Chi-squared test when applied to tables with one or more cells with frequencies less than five. It is only applied to 2 x 2 frequency tables (1 degree of freedom). A certain amount of controversy
surrounds the use of Yates' Correction and some statisticians apply it to all 2 x 2 tables since the correction gives a better approximation to the binomial distribution. Yates' correction is conservative in the sense that it makes it more difficult to establish significance and can tend to overcorrect. This can therefore lead to an overly conservative result that wrongly fails to reject the null hypothesis. However, in situations with large sample sizes, using the correction will have little effect on the value of the test statistic, and hence the p-value obtained.

6.1.3 Comparing the Results of Different Statistical Tests

Later discussions on the most appropriate test for the data resulted in a decision in favour of the Fisher's exact test being performed on these data and this is the test that is presented in the study results in Section 5 of this thesis.

A comparison of these three different tests on the primary endpoint is displayed in Table 6-2. This shows that the Chi-squared test indicates that the difference in the culture-positive rates between patients with a Type I exacerbation and Type II exacerbation is statistically significant, whereas when the Yates' correction is added this difference becomes non-significant. The Fisher's exact test again indicates a statistically significant difference (p=0.043), however the result can still be viewed as borderline significance.

Table 6-2: Comparison of Chi-squared test, Chi-squared test with Yates' correction, and Fisher's exact test on the primary endpoint

<table>
<thead>
<tr>
<th>Culture +ve</th>
<th>Culture -ve</th>
<th>Chi-squared ($\chi^2$) test</th>
<th>$\chi^2$ test with Yates' correction</th>
<th>Fisher's exact test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I exacerbation</td>
<td>36</td>
<td>32</td>
<td>4.44</td>
<td>3.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type II exacerbation</td>
<td>22</td>
<td>7</td>
<td>p=0.043</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

As previously mentioned the level of significance (alpha) is traditionally set at 0.05, however, this is only a generally accepted standard that allows 5% error. Any result must be assessed against the possibility that this could be a chance occurrence. If a more rigorous level of significance of 0.01 was imposed for all the primary endpoints
of this study (see Table 5-4), none of the results would be significant. However the semi-quantitative Gram stain would remain highly significant.

6.2 Diagnostic Statistical Analyses

When confronted with a patient who complains of a particular set of symptoms, a doctor needs to make a diagnosis. The doctor will rely on the combination of signs and symptoms, and quite possibly one or more laboratory tests. In some cases, a diagnosis will depend almost solely on the result of a laboratory test. The results of a laboratory test may seem very straightforward e.g. pregnant or not-pregnant. However, the majority of these tests do not have clear cut results – even in a pregnancy test false negatives are not unknown. For most laboratory tests the results provide only a probability of the patient having or not having the disease being tested for. The consequence of this is that the doctor may have to decide how definitive this result is in his whole picture of the patient’s medical condition and whether further tests or procedures are necessary in order to make a firm diagnosis.

This study employed microbiology tests in addition to signs and symptoms in an attempt to predict bacterial infection in patients with chronic bronchitis who presented with an acute exacerbation. As previously explained, acute exacerbations of chronic bronchitis may be caused by infection with bacteria or viruses, or a reaction to an allergen such as pollution, smoke, or dust. The ability to distinguish reliably between these aetiologies would be extremely useful as it would mean that antibiotics could be targeted at the patients who have a bacterial aetiology and will benefit from antibiotic therapy. In this study, the aim was to be able to identify patients who had a high chance of having a bacterial cause of their AECB. In this way a clinical trial of antibacterial drugs would have a high proportion of patients who had pathogenic bacteria grown from their sputum, and would therefore be eligible for the microbiological analysis populations.
6.2.1 Application of Diagnostic Statistical Tests

Laboratory tests are aimed at identifying the truth about whether a patient has a particular disease or not. In a broad sense this could be likened to a jury trying to establish the truth about whether an accused person is innocent or guilty. A jury may correctly find the accused guilty, or correctly find him innocent; alternatively, they may find him guilty when he is innocent, or find him innocent when he is guilty. In a laboratory test, these jury verdicts relate to correctly identifying disease or non-disease; or diagnosing disease when the patient does not have the disease, or diagnosing that the patient is clear of the disease when they actually have it. Diagnostic statistical tests can apply numbers to the laboratory results in order to provide the likelihood of this result being the correct result. There are a number of diagnostic statistical tests that can be applied and each one gives a specific piece of information about the laboratory result. Usually several diagnostic statistical tests will be performed to give a full picture of the relevance of a single laboratory test.

6.2.2 Sensitivity and Specificity

Sensitivity and specificity give the proportions of patients who are correctly identified as having, or not having the disease in question. Sensitivity is the measure of the percentage of patients with the condition in question who have a positive test result. Specificity is the measure of the percentage of patients without the disease who have a negative test result. That is:

- Sensitivity = how good the test is at detecting disease
- Specificity = how good the test is at identifying normal

Sensitivity and specificity are the most widely used statistics used to describe a diagnostic test. However, because it is not generally known whether or not the patient has the disease (which is why the test is being done), sensitivity and specificity do not provide the complete information that is required to interpret the test results.
6.2.3 Positive and Negative Predictive Values

Positive and negative predictive values take the sensitivity and specificity and apply them in another way to in order to suggest the value of a positive or negative test result. The positive predictive value (PPV) of a test is the probability that the patient has the disease when restricted to those patients who test positive. The negative predictive value (NPV) of a test is the probability that the patient will not have the disease when restricted to all patients who test negative.

- PPV = how often a patient with a positive test has the disease
- NPV = how often a patient with a negative test does not have the disease.

The positive predictive value and negative predictive value should not be applied to a sample where the prevalence of the disease was artificially controlled. For example, the PPV or NPV is meaningless in a study where healthy and diseased patients were specifically enrolled in a one to one ratio.

6.3 The Effect of Prevalence

Prevalence describes how common a disease is in the general population at any point in time e.g. 3% of UK adults have diabetes mellitus. The prevalence of a disease or abnormality is therefore an important factor in interpreting the results of a diagnostic test. Sensitivity and specificity remain unaffected by the prevalence, but PPV and NPV are significantly affected. When the true prevalence of the disease or abnormality being tested for is unknown, it makes the calculation of PPV and NPV unreliable.

If a disease has a prevalence of 10% in a given population, and a particular laboratory test for the disease has a sensitivity and a specificity each of 95%, the positive predictive value of the test can be calculated using Bayes’ theorem.

The two mutually exclusive possibilities are that the person has disease or is normal. The ‘prior probability’ of having disease is 10% (0.1) and of being normal is 90% (0.9). If a person has the disease, the probability of having a positive test result is
0.95. If a person is normal, the probability of having a positive test result is 0.05. These probabilities are obtained from the sensitivity and specificity values given above. If the sensitivity is 95%, then the false negative rate is 5%; and if the specificity is 95%, then the false positive rate is 5%. The joint probabilities are the products of multiplying the prior probability and the probability of a positive test result. The posterior (or final) probability for each of the two mutually exclusive states is obtained by dividing the joint probability value by the sum of the two joint probability values.

<table>
<thead>
<tr>
<th></th>
<th>Disease</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior probability</td>
<td>0.1</td>
<td>0.9</td>
</tr>
<tr>
<td>Probability of a positive test result</td>
<td>0.95</td>
<td>0.05</td>
</tr>
<tr>
<td>Joint probability</td>
<td>0.1 x 0.95 = a</td>
<td>0.9 x 0.05 = b</td>
</tr>
<tr>
<td>Posterior probability</td>
<td>( \frac{a}{a+b} = 0.68 )</td>
<td>( \frac{b}{a+b} = 0.32 )</td>
</tr>
</tbody>
</table>

In this case, with a disease prevalence of 10%, a person with a positive test result has a 68% probability of having disease, and a 32% probability of being normal. However, in a population in which the disease prevalence is 0.1% (0.001), the calculations are as follows:

<table>
<thead>
<tr>
<th></th>
<th>Disease</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior probability</td>
<td>0.001</td>
<td>0.999</td>
</tr>
<tr>
<td>Probability of a positive test result</td>
<td>0.95</td>
<td>0.05</td>
</tr>
<tr>
<td>Joint probability</td>
<td>0.001 x 0.95 = a</td>
<td>0.999 x 0.05 = b</td>
</tr>
<tr>
<td>Posterior probability</td>
<td>( \frac{a}{a+b} = 0.019 )</td>
<td>( \frac{b}{a+b} = 0.981 )</td>
</tr>
</tbody>
</table>

\[
PPV = \frac{\text{sensitivity} \times \text{prevalence}}{\text{sensitivity} \times \text{prevalence} + (1 - \text{specificity}) \times (1 - \text{prevalence})}
\]

\[
NPV = \frac{\text{specificity} \times (1 - \text{prevalence})}{(1 - \text{sensitivity}) \times \text{prevalence} + \text{specificity} \times (1 - \text{prevalence})}
\]
In this situation, the interpretation has changed appreciably; a person with a positive test result has only a 1.9% probability of having disease and a 98.1% probability of being normal.

The prevalence of a disease in the population, therefore, has an important influence on the positive predictive value of a laboratory test. With increasing disease prevalence, the more likely it becomes that a person with a positive test result has the disease, and the less likely it becomes that a positive result is a false positive.

6.4 Application of Diagnostic Testing in this Study

6.4.1 Patients with AECB

The overall prevalence of chronic bronchitis is difficult to assess as different studies have found different rates depending on the country, the age of the population studied and other factors [Cerveri et al, 2001; Huchon et al, 2002; von Hertzen et al, 2000]. However this study was not aimed at identifying chronic bronchitis, it was about identifying bacterial exacerbations in patients with chronic bronchitis. This adds two more factors into the equation: chronic bronchitis patients who have an exacerbation, and an exacerbation that is caused by a bacterial infection.

Patients who have chronic bronchitis can be identified by their history cough and sputum production (daily production of sputum for 3 consecutive months over 2 successive years). Also, an acute exacerbation is relatively easy to identify and is normally defined as a worsening of at least two of the following three symptoms: worsening cough, worsening sputum volume, and worsening sputum purulence. The problem arises in identifying the cause of the acute exacerbation, as this can be due to viral infection, bacterial infection, or pollution/noxious chemicals. Bacterial infection has been estimated to account for approximately 50% of acute exacerbations [Sethi, 2000]. This prevalence of bacterial infection in acute exacerbations of chronic bronchitis makes the high sensitivity of the semi-quantitative analysis of a Gram stained sputum specimen more relevant. A patient with a positive result on the semi-quantitative analysis of the Gram-stained sputum specimen (sensitivity = 0.67,
specificity = 0.9 in this study) would have an 87% chance (PPV) of having an acute bacterial exacerbation of chronic bronchitis, based on a prevalence of acute bacterial exacerbations of 50%, applying the formula given earlier in this section. The negative predictive value would be 73%.

6.5 Summary and Conclusions

Selection of the most appropriate statistical test to be applied in a research project is important. In this clinical study, the statistical test that was first proposed for the primary outcome was based on the assumption that there would be no number lower than 17 in the 2 x 2 frequency table. There were far fewer patients with Type II exacerbations than expected and the lowest number in the actual 2 x 2 table was seven. Although opinions vary, a Yates' corrected Chi-squared appeared to be suitable, and this suggested that there was no difference between Type I and Type II exacerbations with regard to the proportion of patients who would produce a culture-positive sputum sample. Further discussion indicated that the Fisher's exact test would be the optimum test for this data. The Fisher's exact test suggested that the difference in culture-positive rates between Type I and Type II exacerbations was significant, but remained borderline. It is recognised that Yates' correction tends to overcorrect (and in this case resulted in a significant result) and therefore it is not surprising that the Fisher's exact test changed the borderline non-significant result into significance. Overall, the main conclusion has to be that the results are borderline. The results could reflect a real difference, but then again, they might not. Furthermore, it is possible that the difference being observed (Type I or Type II exacerbation) is not the difference that is causing the difference in culture-positive rates; this could be something totally different or a combination of factors that happen to have come together in a higher proportion of patients with a Type II exacerbation than those with a Type I exacerbation.

The patients who were eligible for the study were not a random sample of the population, they were patients with a documented history of chronic bronchitis, and they had presented to the doctor with signs and symptoms of an acute exacerbation. The only question regarding these patients was whether this acute exacerbation was
caused by a bacterial infection or not. However, this is exactly the population that should be tested and not a random sample of the population. Therefore, the proportion of acute exacerbations of chronic bronchitis that have a bacterial aetiology, reported as being around 50% [Sethi, 2000] is a realistic prevalence to apply to the results obtained in this clinical trial.

Calculation of the positive and negative predictive values to these results applying the rate of prevalence of 0.5 (50%) gave a positive predictive value of 0.87 (87%), and a negative predictive value of 0.73 (73%). These vary from the PPV and NPV given in Section 5.6.7 because of the rate of prevalence applied. In the clinical study, the PPV and NPV were calculated on the basis of the results obtained in the study. However in the study, the prevalence i.e. the rate of culture-positive patients, was 60% (Section 5.6.2). Using the study prevalence of 60%, the PPV was 0.91 and the NPV was 0.65; whereas, using the documented prevalence of 50%, the PPV was lower at 0.87 but the NPV was higher at 0.73. Whichever prevalence is applied, this technique of semi-quantitative analysis of a Gram stained sputum samples for bacterial cell types appears to have a good PPV and an acceptable NPV.
This study has not given a definitive answer to the initial question posed and further research is warranted in this important area. Distinguishing bacterial infection from viral infection or from non-infectious causes in exacerbations of chronic bronchitis has not been a key area of research in the last 10 years. Although the term chronic bronchitis has appeared in the literature relatively rarely since the turn of the century, research remains active in COPD. During the early part of the clinical study in this thesis, Professor Stockley's team in Birmingham were examining the association of certain characteristics of purulent sputum and bacterial exacerbations. This is a very simple test that can match the colour of the expectorated sputum to the chances of the exacerbation being bacterial in nature [Stockley et al, 2000]. The majority of the current research in COPD is centred on the inflammatory process in COPD and the inflammatory markers, through which the progression of the disease may be monitored; however, the role of some of this work could prove useful in identifying bacterial infection if it is found that the presence (or abundance) of certain inflammatory markers are associated with bacterial infection. White et al [2003], identified that resolution of bronchial inflammation following AECB is related to bacterial eradication, and patients in whom bacteria continue to be cultured in their sputum have partial resolution of inflammation which may reflect continued stimulation by the remaining bacteria. This link between inflammation and bacteria should provide a marker for bacterial exacerbations, but to date no clear and reliable biomarker has come to light. Nevertheless, there are some promising candidates for predicting bacterial infection, and all of these require further research.

7.1 Sputum colour

As explained in the Introduction, purulent sputum has long been associated with bacterial infection. Stockley et al suggested that it should be possible to separate the presence of bacteria as commensals in the airway from those causing an infection [Stockley et al, 2000], they proposed that an active infection would be accompanied
by activation of secondary host defenses, which include increased neutrophil recruitment to the airways. This neutrophil influx should result in a change in secretions from mucoid to purulent (because the enzyme myeloperoxidase in the neutrophils is greenish in colour). This process would then reverse once the bacterial load had been reduced or eliminated following antibiotic therapy. The results showed a positive association between green or yellow-green sputum and bacterial infection. This is a quick and simple indicator of bacterial infection, as it can be applied using a colour chart along with a sputum sample. Nevertheless, this approach is not perfect; the sensitivity was good at 94.4%, but the specificity was only 77%. This level of specificity means that almost a quarter of patients who do not have green sputum will have significant numbers of bacteria in their sputum.

In another study [Allegra et al, 2005], not only was purulent sputum colour associated with bacterial infection, but additionally deeper colours of sputum were associated with increased yield of Gram-negatives including *P. aeruginosa* and Enterobacteriaceae. The results of the bacterial species cultured from mucoid and purulent sputum, and from patients with an FEV₁ of 35-50% and those with an FEV₁ of <35% of predicted is interesting, although hardly conclusive. Increasing sputum purulence colour from whiteish, through yellowish, greenish, and brownish, showed a percentage of no significant bacterial growth (<10⁶ cfu/mL) of 22%, 2%, 8%, and 24%, suggesting that patients with the least purulent and the most purulent sputum samples had the lowest frequency of bacterial infection. In the study of Allegra et al the sensitivity and specificity were not given, however it could be assumed that the specificity of purulent-coloured sputum samples would not be high, because of the relatively high level of no significant bacterial growth (24%) in patients with the highest level of sputum purulence (brownish).

The results of my clinical study (Section 5) indicated that although more respiratory pathogens were isolated from visibly purulent sputum than non-purulent sputum (53 pathogens from 67 specimens versus 15 pathogens from 29 specimens). The sensitivity was adequate at 78%, however the specificity (45%) was not particularly good as 13 of the 29 visibly non-purulent sputum samples grew a respiratory pathogen.
The complication of identifying bacterial infection is that in patients with moderate to severe chronic bronchitis there is often colonisation of the lungs, so that there are bacteria permanently present in the lungs. Consequently, an effective indicator of bacterial infection has to be able to distinguish between colonisation and active bacterial infection. In addition, the effect of a viral infection on sputum colour remains unclear. Therefore whether this is an effective indicator of bacterial infection or just infection, has not been proven conclusively. This concept is therefore deserving of further research and validation.

The level of C-reactive protein (CRP) was also examined in the study of Stockley et al, 2000. It was found that CRP was higher overall in patients with purulent sputum than those with non-purulent sputum and therefore appeared to be linked with bacterial exacerbations. CRP is often used in hospital as an indicator of infection, particularly in pneumonia where there is a more profound systemic response. However, other authors have found CRP to be an unreliable measure of bacterial infection in AECB [Dev et al, 1998].

7.2 Changes in FEV₁/FVC

The values of FEV₁ and FVC as a measure of lung function are regularly assessed in chronic bronchitis patients. A sudden deterioration in FEV₁ and/or FVC is a sign of an exacerbation; however not necessarily a bacterial or even infectious exacerbation. Therefore, such a sudden deterioration in FEV₁ and FVC cannot, in isolation, indicate a bacterial exacerbation. However, in combination with other changes in a patient's condition such as a change to the production of purulent sputum, it can help to support a diagnosis of a bacterial exacerbation. Van der Valk et al concluded from their study that patients presenting with an exacerbation who have a negative result of a sputum Gram stain, do not have a clinically relevant decrease in lung function, and who have experienced less than 2 exacerbations of COPD in the previous year do not require antibiotic treatment [van der Valk et al, 2004].
7.3 Leucocyte Esterase in Sputum

Leucocyte esterase (LE) is most familiar as one of the tests on a urine dipstick and will indicate urinary infection. This test shows the presence of polymorphonuclear neutrophils (PMNs) in urine through the detection of LE enzyme activity. Perhaps because of its simplicity and speed of result, the LE test has been evaluated in ascitic fluid [Butani et al, 2004] and effusion fluid in otitis media [Lebovics et al, 1993]. In relation to infections of the lung, the test has been assessed in the diagnosis of infectious pleural effusions [Azoulay et al, 2000], in spontaneous bacterial empyema [Castellote et al, 2005], and in bronchoalveolar lavage fluid as a potential screening for ventilator-associated pneumonia [Jacobs et al, 2000]. These studies have reported variable levels of reliability of the LE test.

A study published in 2004 reported results of a study to test the usefulness of the reagent strip for examining sputum samples, not to identify infection, but to determine sputum quality; to differentiate between specimens from the lower respiratory tract (LRT) and those from the upper respiratory tract [Gal-Oz et al, 2004]. The results of this study found that the specific gravity test was a sensitive test for the evaluation of sputum quality. Surprisingly, the LE test was found to be ineffective for the purpose of evaluating sputum. The authors suggested that enzymes such as amylase, glycoproteins, mucins, immunoglobulins, lysozymes, and other proteins that are found in saliva may cross-react with the LE reagent.

7.4 Procalcitonin

Procalcitonin is a small (13 kilodaltons) protein that is normally undetectable in plasma. Procalcitonin increases markedly in bacterial infections, especially those associated with sepsis; however, it does not appear to be increased by inflammation that is autoimmune or due solely to viral infection [Martinez, 2007].

In 2003, Polzin and colleagues examined the value of procalcitonin as an indicator of bacterial infection in respiratory tract infections including hospital-acquired pneumonia, community-acquired pneumonia and acute exacerbation of chronic
bronchitis. Significantly elevated procalcitonin levels were found in patients with LRT infections compared with the control group, but these levels were below the recommended cut-off level of 0.5 ng/mL. Therefore, in regard to the currently recommended cut-off level, procalcitonin was not found to be a useful parameter in the diagnosis of LRT infections [Polzin et al, 2003].

In a recent publication, Stolz et al report their study of procalcitonin-guided antibiotic therapy in exacerbations of COPD [Stolz et al, 2007]. The results showed that using procalcitonin levels to guide the requirement for antibiotics (cut-off >0.25 ng/mL), reduced the exposure of patients to antibiotics. They also found that over the following 6 months there was no increased antimicrobial usage. However, they found that there was no correlation between procalcitonin levels and sputum bacteriology, but as no molecular genotyping was performed, they could not determine whether a high procalcitonin was associated with new bacterial strains. Similarly, it was found that procalcitonin was not effective in identifying bacterial from non-bacterial infection in acute otitis media [Echols et al, 2008].

7.5 Serum Amyloid A

The most recent biomarker to be investigated in acute exacerbations of COPD is serum amyloid A (SAA). SAA is an acute phase protein secreted from the liver, which is induced by inflammatory mediators including interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumour-necrosis factor-α (TNF-α). An investigation by Bozinovski et al set out to assess SAA as a biomarker for acute exacerbations of COPD [Bozinovski et al, 2008]. They found that SAA was associated with infection, but it could not effectively distinguish between bacterial and viral infections. The group also examined the other inflammatory markers C-reactive protein (CRP), IL-6, and procalcitonin, but found IL-6 and procalcitonin to be uninformative. The study concluded that SAA was more sensitive than CRP alone or in combination with dyspnoea.
7.6 A Proposal for Future Research

All of the above biomarkers of infection attempt to identify the patient's immune response to the bacterial challenge. However, an individual's response to infection is not always predictable. A lowered immune response can be caused by other medical conditions, drugs such as steroids, and advanced age; this results in markers such as these not responding to indicate infection. It is well recognised for example that elderly patients who develop pneumonia sometimes do not have a fever and do not display a rise in white cell count or CRP.

As identified previously, in AECB and AECOPD the situation is complicated by the fact that many patients have bacteria colonising their lungs. Because of this, it is often difficult to know the significance of bacteria that are found in a sputum sample; these could be the bacteria of an active bacterial infection, or they could be a sample of the colonising bacteria and the exacerbation is actually viral in origin. It has also been noted that a viral infection that causes an exacerbation might lead to a secondary bacterial infection. Overall, the picture is extremely complicated and often very difficult to interpret in individual cases.

The clinical study detailed in Section 5, concludes that a semi-quantitative Gram stain is the best indicator of bacterial infection. This method relies on higher numbers of bacteria present on the Gram stain indicating infection. Higher numbers of bacteria present, particularly of one species, is certainly suggestive of a bacterial infection and this seems to be the best indicator of a bacterial exacerbation that we currently have.

A major hurdle seems to be how to distinguish between colonising bacteria and those that are causing an infection. It may be that the bacteria themselves could give away their activity and that this is where the breakthrough might come in identifying bacterial exacerbations. Colonising bacteria are relatively sessile, their numbers are kept steady, the rate of reproduction is low, and overall the bacterial cell metabolism is comparatively slow. In comparison, bacteria causing an infection are in active growth phase, their numbers will be increasing, the rate of reproduction is high, and they will have a rapid metabolism. If a test can be found that will accurately identify rapidly growing bacteria, this would be the best indicator of active infection. One
possible chemical that could be used to assess bacterial metabolism is bacterial ATP. There are existing tests to detect bacterial ATP; however, a methodology would have to be determined which could separate colonising (sessile) bacteria from those causing an infection. There are likely to be other bacterial products of metabolism which could also be used to identify rapidly growing pathogenic bacteria.

7.7 Summary and Conclusions

The link between sputum colour, FEV$_1$, and bacterial infection is a complex one. Purulent sputum, as indicated by sputum colour, appears to be a simple and reasonably effective indicator of bacterial infection, and more work in this area is warranted in order to quantify more accurately, the sensitivity and specificity of this indicator in large numbers of patients in a multicentre study. It is however, noteworthy that in both the Stockley et al and the Allegra et al studies [Stockley et al, 2000; Allegra et al, 2005], a proportion of patients with mucoid sputum had significant growth of such pathogens as *H. influenzae* and *M. catarrhalis*, and therefore it appears that caution should be used in applying this technique too strictly. In the clinical study described in this thesis, the association between sputum colour (visibly purulent or not) and bacteriologically-positive sputum samples was present, but was relatively weak, and other indicators were more strongly associated with bacteriologically-positive sputum.

Further research in this area should examine sputum colour in a large, multicentre population of chronic bronchitis or COPD patients, with a full statistical calculation of the number of patients required to statistically prove the value to be measured. This research should start with stable chronic bronchitis patients and then follow them through an exacerbation and back to the stable condition. Sputum samples would be collected in the initial stable state, a few times during an exacerbation, and then again on at least two occasions when the patient has recovered from the exacerbation and is in the stable state again. Research into measures of lung function such as FEV$_1$ and FVC may be useful when combined with other biomarkers, but there is currently little benefit in further research into this area alone in respect of identifying indicators of bacterial exacerbations of chronic bronchitis.
The study of Gal-Oz et al. [2004] into LE is interesting [Gal-Oz et al., 2004], as this was the marker that I was originally planning to examine, following the original research into signs and symptoms and Gram stain results. Section 3.3.1 details the reasons why this line of research was abandoned. My reasons for abandoning this planned research were based on practical grounds of GPs handling sputum samples in an uncontrolled environment, another problem was seen to be the identification of the best way to test thick sputum and whether it might need to be homogenised with some normal saline before testing. The paper of Gal-Oz suggests that had I continued with this research into the evaluation of LE test on sputum samples, it would not have provided helpful results. Further research of LE activity in raw sputum using reagent strips would appear to be fruitless.

Procalcitonin appears to have potential as a biomarker for bacterial infection, but there is still no conclusive evidence that this has good sensitivity and specificity. In addition, it is not certain what level of procalcitonin provides a high chance (>95%) that the exacerbation has a bacterial cause. This may be because of a wide variation between patients in the response of procalcitonin to bacterial infection. Research into SAA is still at an early stage. SAA appears to be associated with infection, but this may be viral only, bacterial only, or viral and bacterial. Bozinovski et al. [2008] comment that it is currently not possible to detect SAA in sputum reliably, although the group will be attempting to develop methods to achieve this. An interesting result from this study was that procalcitonin was not found to be informative, possibly because procalcitonin levels might better reflect pneumonia than acute exacerbations of COPD. Another possibility is that procalcitonin levels soar in sepsis, pneumonia often involves sepsis but acute exacerbations of COPD do not. Although SAA may prove to be useful in predicting the severity of an exacerbation and suggesting whether there is an infectious cause or not. In isolation, it currently does not appear to hold potential for indicating bacterial infection; however, there may be potential for SAA measured in sputum in combination with one or more other markers.

The results of my clinical project suggest that further research into a semi-quantitative Gram-stain approach, possibly in combination with macroscopic and/or microscopic
purulence could be warranted. A population of patients who have an acute exacerbation of chronic bronchitis, even if they satisfy the Anthonisen Type I criteria are a varied group with regard to, in their non-exacerbation state: FEV₁ and FVC, volume of sputum produced, colour of sputum produced, degree of colonisation, organisms with which they are colonised, concomitant diseases etc. It is quite possible that each of these factors may affect how the semi-quantitative Gram-stain, and macroscopic and microscopic purulence should be interpreted. The ideal would be to know each CB patient's baseline levels so that significant changes in status, particularly in the bacterial load or in the bacterial species (or strains) present, could be identified and then treated.

In conclusion, sputum colour as an indicator of bacterial infection has gathered much support since the paper of Stockley and colleagues [Stockley et al, 2000]. Sputum colour is certainly a quick, simple and non-invasive method of assessing the likelihood of bacterial infection and could be applied easily to clinical trials of antibacterial drugs. From the work of Stockley et al [2000], the sensitivity and specificity of sputum colour suggest that a positive sputum colour (purulent sputum) will indicate bacterial involvement; however, a negative sputum colour (mucoid sputum), does not totally rule out bacterial involvement. My own results on the macroscopic appearance of sputum showed similar results. Unless a better understanding of individuals' baseline status is possible, it seems likely that no single biomarker in isolation will be able to identify the aetiology of an exacerbation of chronic bronchitis.

The future may lie in developing a simple combination of tests that will confirm that a patient is starting to have an acute exacerbation and indicate the causative agent. The method of testing may also be innovative involving testing saliva or exhaled breath. Alternatively, it may be possible to track each patient's disease status in detail so that the changes in a specific patient, which indicate a bacterial infection in the lungs, but which would not suggest a bacterial infection in another patient, can be identified. Furthermore, if markers of bacterial metabolism can be quantified to separate colonising bacteria from pathogenic bacteria, and if this marker (or markers) could be incorporated into a simple, non-invasive test, this could be of great importance not
only in the area of AECB and AECOPD, but also in several other diseases where the presence of bacteria alone is not enough to be able to diagnose infection. Diseases such as cystic fibrosis and bronchiectasis would certainly come into this category, but so too might certain skin and soft tissue infections.
8 DISCUSSION AND OVERALL CONCLUSIONS

8.1 Acute Exacerbations of Chronic Bronchitis

As previously detailed, chronic bronchitis is a disease of mucus hypersecretion caused by an inflammatory response to an irritant or allergen. The predominant trigger in chronic bronchitis is cigarette smoke, however other industrial chemicals and pollutants can initiate and maintain this inflammatory response. The airways inflammation is thought mainly to be due to neutrophil recruitment and release of elastase in response to the trigger factors such as cigarette smoke [Riise et al, 1995]. Chronic bronchitis itself is a slowly progressive disease, however, the acute exacerbations that occur at intervals in chronic bronchitic patients tend to accelerate the progression of this disease [Donaldson et al, 2002; Spencer & Jones, 2003; Hurst et al, 2005; Martinez et al, 2006]. Studies of protected specimen brush technique in patients with stable chronic bronchitis (patients not undergoing an acute exacerbation) showed that a quarter of patients had bacteria (predominantly *H. influenzae*) colonising their lungs even when they were not suffering an exacerbation [Monzo et al, 1995]. Murphy and Sethi proposed the hypothesis of a 'vicious cycle' of bacterial colonisation leading to weakened host defences in the respiratory tract, which predisposes to bacterial infection which further weaken the host defences [Murphy & Sethi, 1992]. This theory had already been put forward for progression of bronchiectasis [Cole, 1989; Cole & Wilson, 1989] and it seemed that it could be relevant also in COPD (and chronic bronchitis).

Another comparison that might be made is with patients who have cystic fibrosis (CF). In this condition, the sufferers have bacteria colonising their lungs almost from birth, with the species of bacteria changing as the patient gets older. The change over time of bacterial species in CF bears many similarities to COPD, from Gram-positive species such as *S. pneumoniae* and *S. aureus* to the Gram-negative organisms *H. influenzae* and *coliforms*, eventually to *P. aeruginosa*, *Stenotrophomonas maltophilia* and *Burkholdaria cepacia*. In CF patients, adverse changes in sputum purulence and dyspnoea are assumed to be a bacterial infection, and the patient is treated with a course of antibiotics. In the diseases of bronchiectasis, COPD, and CF advances...
made in one of this trio of lung diseases should be investigated in the other two diseases.

The issue of bacterial colonisation of the LRT in patients with moderate to severe CB/COPD leads us to the major problem surrounding identification of an acute exacerbation caused by bacterial infection – if a significant proportion of patients with chronic bronchitis permanently have bacteria in their respiratory tract, does isolation of bacteria in the sputum have any significance during an acute exacerbation? The answer to this question may lie in the number of colony forming units (cfu) per millilitre (mL) that are grown, or more simply a minimum number of bacterial cells of the same morphological type that can be seen in a Gram-stained sputum specimen, as examined in the clinical study in this report.

Wedzicha’s group in London [Seemungal et al, 2001; Wedzicha, 2004] studied the prevalence of respiratory viruses in acute exacerbations of COPD and in the stable state, and found that patients with moderate to severe COPD can have viruses present in their lungs during the stable state. However, this was not compared with the presence of bacteria in the stable state in these patients; nevertheless, it is likely that at least some of these patients were also colonised with bacteria. It has been reported that, when properly defined, 80% of AECB are likely to be infectious in origin, approximately 50% bacterial, and 30% viral (with or without a superimposed bacterial infection) [Sethi, 2000]. However other studies have shown that almost two-thirds of exacerbations of COPD follow on from a symptomatic cold and that respiratory viruses may therefore be responsible for in excess of 50% of exacerbations [Seemungal et al, 2001]. These overlapping percentages could suggest more than a simple bacterial or viral infection in a significant number of patients with AECB or AECOPD.

Many exacerbations of CB/COPD (particularly in patients with underlying moderate to severe disease), could therefore be of a more complex aetiology than has been previously suggested. It seems likely that in some patients a severe exacerbation may be a combination of viral and bacterial infection. From the evidence that patients with moderate to severe COPD can have viruses present in their lungs during the stable
state, it is probable that these patients are colonised with one or more species of virus [Seemungal et al, 2001], and it is likely that many of these patients are also colonised with bacteria. Although most studies have focussed on either bacterial infections or viral infections in exacerbations of CB or COPD, a recent study which tested for bacteria and viruses in sputum samples found that a quarter of all patients with an acute exacerbation appeared to be co-infected [Papi et al, 2006]. The implications of these findings may be wide-reaching and could require a review of the management of CB and COPD patients. The role of atypical pathogens, such as *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Legionella pneumophila* in exacerbations of CB or COPD however, is unclear [MacNee, 2008].

It is therefore possible that any disruption to the equilibrium in the LRT could allow both a viral and bacterial infection either in parallel, possibly in different areas of the LRT, or sequentially. Such exacerbations would be severe, would invoke a profound inflammatory response, and could be difficult to treat. This type of co-existing bacterial and viral infection could account for some of the clinical failures of antibiotic therapy, when the isolated species of bacteria shows susceptibility to the antibiotic. The full picture in such patients might be that the bacterial infection was successfully treated but the viral infection was still present and still causing clinical signs and symptoms of infection.

### 8.2 Review of the Objectives of this Research

This research project was inspired by my personal dismay in the late 1990s, that many clinical trials of antibiotics in patients with acute exacerbation of chronic bronchitis have so few bacteriologically-proven infections (patients who have a pathogen isolated at baseline). It seemed to me that this was unacceptable on several levels:

- As the primary efficacy outcome is almost always clinical response at the test of cure (TOC) visit, if less than half of the patients had a proven bacterial exacerbation, then it is possible that in more than half of the patients who were clinical cures at TOC, the cure is unrelated to the antibiotic therapy.
• If the above occurs, then any benefit that one antibiotic has over another will
be impossible to demonstrate by clinical response (but might be possible by
examining bacterial eradication rates); in every clinical population for analysis
the patients with non-bacterial exacerbations would be diluting the difference
in effect between the two (or more) treatment arms. It is therefore possible to
see how even a comparison of an antibiotic against a placebo could show very
little difference between the clinical response rates in the two treatment
groups.

• In terms of the clinical trial and in the wider general prescribing, many
patients with an acute exacerbation of CB will receive antibiotics who will not
benefit from them and might possibly be harmed by receiving them. These
patients could suffer side effects from the antibiotic or could later discover that
their treatment had lead to the development of an antibiotic-resistant strain of
bacteria in their lungs, or they could develop *Clostridium difficile*-associated
diarrhoea, particularly if they had been treated in hospital.

• An antibiotic has the power to kill bacteria and that is what it should be tested
for. This is relatively easy in a petri dish, but somewhat more difficult in the
human body. As previously discussed, chronic bronchitis is a complex
inflammatory disease and the periodic acute exacerbations are similarly
complex. Therefore, the only fair test of an antibiotic is in a patient who has a
bacterial infection. An antibiotic is not an anti-inflammatory, anti-viral,
bronchodilator, decongestant, mucolytic, or antitussive and so it cannot be
assessed in patients who have a non-bacterial cause of their AECB.

In order to demonstrate the effect on clinical success rates, of only 30% of clinical
trial patients having a bacterial exacerbation of CB (or COPD), we can consider the
following example:

In a 500 patient study of two antibiotics, one antibiotic (Drug A) has a success rate of
95% in acute bacterial exacerbations of chronic bronchitis (ABECB), and the other
(Drug B) has a success rate of 85%. If all the patients have ABECB, then the
difference between the two antibiotic treatments should be approximately 10%.
However, the effect on the clinical success rates if only 30% of patients in the study have a bacterial exacerbation is dramatic and is shown in Figure 8-1.

**Figure 8-1: Diagram of the effect of only 30% patients having a bacterial exacerbation on a 10% difference between antibiotics**

<table>
<thead>
<tr>
<th>Drug A</th>
<th>Drug B</th>
</tr>
</thead>
<tbody>
<tr>
<td>250 patients</td>
<td>250 patients</td>
</tr>
<tr>
<td>75 Bact +ve</td>
<td>75 Bact +ve</td>
</tr>
<tr>
<td>175 Bact -ve</td>
<td>175 Bact -ve</td>
</tr>
<tr>
<td>95% success</td>
<td>85% success</td>
</tr>
<tr>
<td>small proportion of failures</td>
<td>small proportion of failures</td>
</tr>
</tbody>
</table>

Overall Clin Success: 244 (97.6%)  
Overall Clin Failure: 6 (2.4%)  
Overall Clin Success: 237 (94.8%)  
Overall Clin Failure: 13 (5.2%)

In this scenario, a theoretical 10% difference in success rate was reduced to 2.8%. Consequently, any study designed to demonstrate 10% superiority, or even 5% superiority, would be doomed to fail. The reason for this is that the 70% of patients who do not have a bacterial infection have exactly the same chance of clinical success regardless of the drug to which they are randomised.

In this way, it is not surprising that in comparative studies of two antibacterial therapies in AECB or AECOPD, little difference in clinical success rates between drugs is observed, and equivalence of the two drugs is all that can be achieved. In addition, the standard time to perform the assessment that is used for the primary results (the Test of Cure [TOC] visit) is approximately 2 weeks after the end of therapy, or 3-4 weeks after the onset of the exacerbation; this happens to coincide with the time that an exacerbation caused by viruses or environmental triggers might be expected to resolve spontaneously. It is for this reason that the majority of patients with non-bacterial exacerbations will have an outcome of cure at the TOC visit. This factor almost certainly complicates the results of clinical trials of antibiotics in patients with AECB.
This project was therefore designed to explore aspects of AECB that could be used to increase the proportion of patients in clinical trials of antibiotics in AECB that have a bacterial pathogen isolated at baseline from a sputum sample. The study of Anthonisen and co-workers [Anthonisen et al, 1987] is recognised as landmark study in trials of AECB/COPD patients [Miravitlles & Torres, 2004]. However, the Anthonisen study did not include microbiology and therefore the suggestion that Type I (and to a lesser extent Type II) exacerbations are bacterial in aetiology was not fully evidence-based. I could not identify any publications that had subsequently compared the proportion of patients with a Type I exacerbation that had culture-positive sputum samples with those who had a Type II exacerbation in order to investigate whether there was a difference. I therefore wondered whether this simple test of patient signs and symptoms could help to improve the proportion in clinical trials of antibiotics that had a bacterial pathogen isolated from their baseline sputum sample.

A multicentre, placebo-controlled study in acute exacerbations of COPD, which was conducted in Italy, found that a five-day course of antibiotic gave a significantly lower percentage of clinical failures (13.6% versus 49.7%) than five days of placebo [Allegra et al, 1991]. Like Anthonisen’s study, this clinical trial did not report microbiology, which could have supported the findings and therefore strengthened the robustness of these results. In addition, patients did not have to have purulent sputum to register as an exacerbation for which an antibiotic might be given. Nevertheless, this study provides additional evidence of the benefit that antibiotic therapy can provide.

More recent clinical trials in AECB/AECOPD normally screen the baseline sputum sample for microscopic purulence (>25 PMNs and <10 SECs per low power field) as recommended by the FDA [FDA, 1998]. The results of this screen are used either to exclude patients whose sputum samples did not meet these criteria, or just to exclude the sputum samples from bacterial culture on the basis of poor quality, ensuring that these patients will not be microbiologically-evaluable. A few studies screened a Gram-stained preparation of sputum for the presence of bacterial cells. I decided also to look at these microscopic screening techniques to investigate whether one or both
could significantly improve the proportion of patients who had a baseline pathogen, and would therefore be microbiologically-evaluable, allowing a true assessment and comparison of two or more antibiotics' activities.

8.3 Clinical Trials of Antibacterial Drugs over the Last 50 Years

In the past 50 years, antibiotics have been critical in the fight against many diseases and infections. Their discovery was one of the leading causes for the marked rise in average life expectancy in the 20th century and their significance to public health is immense. The introduction of penicillin into general clinical practice in 1944 meant that previously deadly illnesses such as scarlet fever, and Strep throat became curable. Today, we depend heavily on antibiotics, although with the rise of antibiotic resistance we are having to reassess our perhaps over zealous use of these drugs. In England in 2007, in excess of 39 million prescriptions were written for antibiotics, at a value of over £173 million [Dept of Health website, 2008]. At an average cost per prescription of £4.43 they are among the lowest cost drugs in use and considerably less than the current prescription charge of £7.10. However, the most frequent users of antibiotics are children and the elderly, who are exempt from prescription charges.

8.3.1 The Systematic Review of Clinical Trials of Antibiotics in AECB Since 1966

In the systematic review, the majority of studies used 18 years as the lower age limit for the clinical trial, despite the fact that true chronic bronchitis is relatively rare in people under 35 years of age; as evidenced by a retrospective study of over 100 patients admitted to hospital for AECOPD where the lowest patient age was 38 years [Smith et al, 1999]. Therefore a lower age of 35 years would be more in keeping with the disease. The use of 18 years as a lower age limit is likely to reflect the indication that the sponsor company has for a licensed antibiotic, or wishes to achieve. The risk of applying a lower age limit of 18 years in these clinical trials is that some doctors who are less familiar with the condition or who wish to enter as many patients into the clinical trial as possible, may in error enrol young adults who have a combination of asthma and allergic rhinitis rather than a true acute exacerbation of chronic bronchitis.
Overall the rates of bacteriologically-positive patients varied between 13.9% and 96.0%: an enormous variation. The 25th to 75th percentile in the ITT populations was 28.7% - 64.7%, which is still a wide range, thus demonstrating that the spread of bacteriologically-positive rates is not accounted for by a few outliers at each end of the range.

It was surprising that despite a recognised definition of chronic bronchitis since 1962, 28% of the papers did not specify that this definition had been applied in the entry criteria and one paper specifically used a variation on the standard definition. When it comes to the definition of an acute exacerbation, arguably the most recognised definition and classification is that described by Anthonisen et al [1987] and around which this project was based. According to Anthonisen’s data, Type I and II (but predominantly Type I) exacerbations are generally considered to benefit from antibiotic therapy; whereas, Type III exacerbations are accepted as not benefiting from antibiotic therapy. This review found that in the clinical trials that used Anthonisen’s definition of Type I and Type II exacerbations, the rates of bacteriologically-positive patients were higher in patients with a Type I exacerbation than those with a Type II exacerbation. This difference in the proportion of bacteriologically-positive patients between Type I and Type II exacerbations supported the objectives of the clinical study that formed the main part of this project. However, those studies that used various other combinations of signs and symptoms at baseline, had rates of bacteriologically-positive patients that were at least as high if not higher those in Type I exacerbations. Overall, this suggests that signs and symptoms are a complex issue and are not a good guide to bacterial infection.

Twenty studies screened the sputum samples of potential patients for WBCs, SECs (and a bacterial cell type) on a Gram stained specimen, and only those patients whose samples met the entry criteria were allowed to enter the study. This criterion appeared to produce a higher rate of bacteriologically-positive patients, compared with the studies where this was not done. However, the data were not conclusive.

The 80 studies that reported individual pathogens, varied considerably in the species of bacteria that were isolated and reported as pathogens. *H. influenzae, S. pneumoniae*
and *M. catarrhalis* were reported by virtually all the studies; however species such as *H. parainfluenzae, S. aureus, P. aeruginosa* and the Enterobacteriaceae were reported in only a proportion of studies. In most cases, when a species was absent from the reporting, it was difficult to determine whether the reason was that the species had not been isolated from any patient (as they are found less commonly than *H. influenzae, S. pneumoniae* and *M. catarrhalis*), or whether the species had been isolated, but was not accepted as a pathogen. What was evident from the results was that it was not a simple case that the more species that were included as pathogens, the higher the rate of bacteriologically-positive patients; the main reason for this is that these species are isolated in only a few patients.

A specific examination of the studies that had rates of bacteriologically-positive patients of greater than 60% in the ITT or CE population, showed that the only factor that appeared to have an impact on the bacteriologically-positive rate was microbiological screening of sputum purulence (possibly with the presence of bacterial cells), as there were twice as many studies (as a percentage) that had applied this screening as an entry criteria, in this group of studies than in the whole dataset.

### 8.4 Review of the Clinical Study

#### 8.4.1 Objectives of the Clinical Study and the Difficulties, Solutions and Limitations

The clinical study was designed to investigate three potential indicators (primary objective) of a bacterial exacerbation in patients presenting to their GP or at hospital admission with AECB.

- The primary indicator to be evaluated (and the one upon which the sample size was calculated) was whether patients with a Type I exacerbation of CB (all three cardinal signs/symptoms) had statistically higher probability of having a bacterial pathogen isolated from their sputum than patients with a Type II exacerbation (two of the three signs/symptoms present).

The other two primary objective indicators investigated were:
• Microscopic purulence of the sputum at baseline i.e. whether the sputum had >25 PMNs and <10 SECs (microscopically purulent), or not
• The presence of bacterial cells on a Gram-stained preparation of sputum at baseline.

In addition, other factors that might affect the presence of baseline pathogens in the sputum were examined including: the numbers of months since the previous exacerbation for which an antibiotic was given and the relationship between this and the species or bacteria grown; the time of day that the sputum sample was produced; and delaying culture of part of the sputum sample for 24 hours at room temperature.

Unlike more recent studies of acute exacerbations of COPD, this study did not measure lung function of the patients. The measurement of FEV₁ and FVC has most value when a series of measurements can be taken from each patient, ideally a) stable state, b) during an acute exacerbation, c) just after the acute exacerbation, d) a few weeks later in the stable state again. In this way the baseline value (the first stable state) can be recorded for each patient and it is against this that all later measurements will be evaluated. The sequence of deterioration of lung function during the acute exacerbation, followed by improvement of the lung function again after the exacerbation can be assessed. One of the major factors is whether lung function returns to the baseline value, or whether each time the patient has an acute exacerbation a further small proportion of lung function is lost. The measurement of FEV₁ and FVC was considered in the design stage of the study, but as all the data to be recorded by the investigators was to be collected at one visit (when the patient first presented with signs and symptoms of an acute exacerbation), it was thought that a single set of measurements from each patient would not have a great deal of value. It was therefore decided not to collect lung function data.

The study started with four GP surgeries in Edinburgh, however because of a lack of interest in this study in the initial period of recruitment of investigators (it was originally planned to have at least six investigative sites in Edinburgh), the study was extended to Coatbridge to a large Medical Centre. Monklands Hospital, which was originally involved as the laboratory for the Coatbridge GP practice then also became
an investigative site, recruiting patients from its admissions of patients with AECB. Over a year later, because of poor recruitment figures in Edinburgh and Coatbridge, a further four general practices in Glasgow that had a good record of recruiting AECB patients for clinical trials joined the study. By extending the study to Glasgow, it was eventually possible to meet the recruitment target of 120 patients. However, these 120 patients only yielded 97 evaluable patients instead of the intended 112 patients. These reduced numbers of evaluable patients will have affected the power of the study, but only in a minor way. Nevertheless, as the power of the study was set at 85% any reduction could be of concern. Extending the study outside Edinburgh meant that other laboratories were required to conduct the sputum culture from sites in Coatbridge and Glasgow. The outcome was that the study had three central laboratories instead of the original plan for a single central laboratory. Although the laboratories all agreed to adhere to the methodology for sputum culture given in the protocol, it was later found out that the methodology of Airdrie and Glasgow hospitals was almost identical, but this was different to that of the Edinburgh laboratory and the methodology given in the protocol. The laboratories at Airdrie and Glasgow performed the cultures according to their standard procedures and not to the protocol. There were two key differences in technique/interpretation, these were: a) sputum samples were not homogenised before culture, but a piece of the most purulent section of sputum was removed and cultured; and b) the numbers of organisms used to rank the Gram-stain preparation varied from that used in Edinburgh. As there were only four eligible patients enrolled in Edinburgh, and only three of them had cells on the Gram stain, the interpretation used in the analysis of the Gram stain results from these three patients was that of Airdrie and Glasgow hospital laboratories. The differences between the two laboratories in the numbers of organisms required in each growth group (+, ++, and ++++) are shown in Table 8-1.

### Table 8-1: Differences in the interpretation of Gram stain quantitative assessment between the 3 central laboratories

<table>
<thead>
<tr>
<th>Gram stain quantitative assessment</th>
<th>No. of cells per oil immersion field</th>
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<td>Edinburgh lab</td>
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<tr>
<td>+</td>
<td>1</td>
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<tr>
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<td>+++</td>
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The use of three central laboratories lead to a lack of standardisation in this study and that was not ideal. Nevertheless, the results were made as consistent as possible in the circumstances, and it is believed that the overall results are robust.

8.4.2 Discussion of the Clinical Study Results

The primary outcome of this study suggested that patients with Type II exacerbations were more likely to have bacterial involvement than those in patients with Type I exacerbations. However, this difference was borderline and given the reduced number of patients in the evaluable population (97 instead of 112) and the fact that the distribution of Type I and Type II exacerbations was very unequal 7:3 instead of an expected 1:1 distribution, this result may have occurred purely by chance. This difference between Type I and Type II exacerbations is counterintuitive and contrary to previous studies where there has been a suggestion that patients with Type I exacerbations are more likely to have a bacterial cause than patients with a combination of Type I and Type II exacerbations. As stated, although the statistical design of the study was based on there being a 1:1 ratio of patients with Anthonisen Type I and Type II exacerbations, the actual ratio was approximately 7:3. This imbalance may have reflected investigator and/or patient bias in confirming that all three symptoms had worsened, leading to a more varied collection of patients in the Type I group than the Type II group and consequently, the more accurate reporting of just two symptoms. However, the severity of dyspnoea, sputum volume and sputum purulence in the pre-exacerbation state in these patients did not indicate that patients with a Type II exacerbation had more severe chronic bronchitis in the stable state than those who were reported to have a Type I exacerbation, which could have explained this difference in bacterial exacerbations. In hindsight, stratifying for Type I and Type II exacerbations would have improved the design of the study. This would have guaranteed an even distribution of Type I and Type II exacerbations which would have allowed a better statistical analysis. If this type of study is repeated, stratification is recommended.

The secondary outcome of microscopic purulence (>25 WBCs and <10 squamous epithelial cells), showed significance, though the sensitivity and specificity meant that
the test is probably not useful in a clinical or research study setting. Geckler and colleagues found a correlation of 94% between potential pathogens grown from sputum samples containing <10 squamous epithelial cells and >25 white blood cells per low power (x100) field and paired transtracheal aspirates [Geckler et al, 1977]. However, the study presented in this thesis worked within conventional clinical practice where the position is less clear cut. Although the test of >25 WBCs and <10 epithelial cells has been used in many studies to distinguish between bacterially and non-bacterially infected sputum specimens, the findings in this study challenge the rigid application of this test. The usefulness of this investigation was questioned in another clinical trial in AECB, and based on the culture results it was decided retrospectively not to apply it [Wilson et al, 1999].

There was virtually no difference between the results of the immediate sputum culture and the culture of the same specimen following 24 hours at room temperature. This lack of any significant difference in the culture results was surprising. The question of whether a delay in culturing sputum samples leads to a difference in the culture results was investigated by Gould et al [1996]. Gould et al, stored the sputum samples for 48 hours at 4°C and found a discrepancy of 5–25% between early and late testing; however, the three laboratories taking part in this study accounted for much of this difference. It was concluded by the authors that methodology rather than storage time was responsible for most of the variation. There is therefore no great discrepancy between the results of Gould et al and the results of the study presented in this thesis.

There was similarly no difference between the proportion of culture-positive sputum samples that were produced early in the day (<09:00 hours) with those produced after this time; however, the major factor here may not be the precise time of the day, but that it is the first sputum sample produced after waking up in the morning, and this information was not known. Many of the sputum samples that were produced at or before 09:00 hours may not have been the first sputum sample of the day, and this could have affected the results.
In this study, the date of the most recent exacerbation for which an antibiotic was prescribed was collected, rather than the number of exacerbations that the patients had suffered in the previous 12 months; a parameter which is more usually collected in clinical trials. Collecting the date of the most recent exacerbation may have been a less reliable measure of the severity of their disease, but it was a simple and objective value for the investigators to record. It is recognised that CB patients may not visit their GP for every exacerbation. GPs will have a record of each exacerbation for which a surgery (or home) visit was made or for which there was a direct hospital admission but some exacerbations may go unrecorded. However, asking the patient how many exacerbations they have had in the previous 12 months may be unsubstantiated and possibly under or over-estimated. A total of 26 patients had the previous exacerbation less than one month before the current presentation, and it is therefore questionable whether the current exacerbation was indeed a new episode, or whether it was a relapse of the previous exacerbation; it was in this group of patients that one of the lowest culture-positive rates was seen. Overall, there were no particularly significant trends seen in the species of pathogen isolated in the different time periods since the previous exacerbation; however, the numbers of patients in several of the time periods were small and this makes any assessment unreliable. Nevertheless, the finding that Enterobacteriaceae were only isolated from patients who had had an exacerbation within the previous 6 months is consistent with the theory that Enterobacteriaceae are mainly found in the lungs of patients with poor lung function, and therefore patients who have more frequent exacerbations [Eller et al, 1998; Sayiner et al, 1999].

Medici and colleagues [Medici et al, 1988] found that in a comparison of Gram stain and culture, microscopic examination of the Gram stain was found to be superior to culture, with around 50% of organisms microscopically identified as pneumococci failing to grow. This is an interesting observation, but offers little information about the pathogenicity of these organisms. It is not surprising to find bacteria in the LRT of these patients. The question is therefore, are they causing the exacerbation? The results of Medici and colleagues do not provide such information.
It was when the pattern of the Gram stain results was examined that a distinct relationship between the number of bacterial cells seen on the Gram stain and the subsequent culture results was observed. This observation was then formalised into a full analysis. Accepting all the reservations of a post-hoc analysis, the semi-quantitative analysis of the Gram stain results provided the best indicator of bacterial infection. A single bacterial cell type present at ≥10 cells per field (x 1000), or more than one bacterial cell type present with at least one cell type at >25 cells per field was seen in over 90% of the culture-positive specimens. The benefit of quantitative Gram stain analysis has been described previously [Chodosh et al, 1991]. Chodosh suggested that chronic bronchitis patients in stable state (between exacerbations) have few bacteria on a Gram stain. However, during a bacterial exacerbation, Chodosh demonstrated that thresholds can be applied which, when exceeded in a sputum sample, indicate that the exacerbation is of bacterial origin. The criteria given were >12 Haemophilus-like organisms, >8 pneumococcus-like organisms, or >18 Moraxella-like organisms per oil-immersion field. The technique applied in this study however, is simpler than that of Chodosh because the same numbers are used for all relevant cell types. Furthermore, the technique of Chodosh requires extensive microbiology experience and is very user specific. The classification of the numbers of cell types seen in this study was based on standard laboratory practice and for the purpose of identifying acute bacterial exacerbations of CB may not be optimal. Nevertheless, in this study the semi-quantitative investigation appeared to provide the possibility of a potential classification method for predicting bacterial infection in AECB patients. The results of this clinical study were published first in summary as a poster presentation [Burley, 2003], and then as an original research paper [Burley et al, 2007 and Appendix 9.5].

It has been established that frequent exacerbations in patients with chronic bronchitis or COPD increases the decline in lung function and also quality of life [Donaldson et al, 2002; Spencer& Jones, 2003; Hurst et al, 2005; Martinez et al, 2006]. In addition, the frequency of exacerbations is related to the underlying severity of COPD [Hurst & Wedzicha, 2007]. Consequently, any medical intervention, antibiotic therapy or other intervention, which can decrease the frequency of exacerbations, will be of significant long-term benefit to these patients. One of the first steps along this path will be to be
able to identify correctly those patients who have an acute exacerbation caused by bacteria. It is hoped that this research may contribute in a small way to this goal.

8.4.3 Comparison with Other Research

The ATS definition of chronic bronchitis has stood the test of time; nevertheless, the condition goes beyond this definition in terms of the inflammation and in more severe cases, colonization of the airways. The 'vicious circle' hypothesis proposed by Murphy and Sethi in 1992 gave a revised view of chronic lung disease and how the presence of bacteria results in an alteration in the host defences in the respiratory tract, which in turn predispose to further infection [Murphy & Sethi, 1992]. This hypothesis lends support to the argument that antibiotics are useful in the treatment of exacerbation thought to be bacterial in nature as their use will return the bacterial load to a low level, and therefore minimise the damage to host respiratory defences.

Stockley and co-workers established that the colour of the sputum can help to predict whether or not an exacerbation is caused predominantly by bacteria [Stockley et al, 2000]. This is simple technique, which is detailed in Section 7.1, could easily be used in the community, without the need for an immediate laboratory examination of the sputum sample. Assessing purulent sputum by colour against the culture-positive results gave a positive predictive value of 83.9%, which is good, but lower than the PPV of 90.7% that was achieved using the semi-quantitative Gram stain analysis recommended in the clinical study presented in this thesis.

Van der Valk et al investigated predictors of bacterial involvement in exacerbations of COPD. They examined patients' lung function and found that a combination of a positive Gram stain result associated with a decrease in the FEV1 (>12%, and of ≥200mL), and 2 or more exacerbations in the preceding year best predicted a bacterial exacerbation (positive predictive value 67%; negative predictive value 100%) [van der Valk et al, 2004]. These positive and negative predictive values are almost the reverse of those from the study presented in this thesis (which had a high PPV and a lower NPV), and which used only a semi-quantitative Gram stain result. The purpose of being able to predict bacterial involvement was different in these two studies. In van der Valk's study the aim was to assist the appropriate use of antibiotics, and in
In this regard a high negative predictive value was essential. In the current study the intention was to be able to distinguish as accurately as possible those patients that were highly likely to have a bacterial exacerbation so that within a clinical trial population there would be a high proportion of bacteriologically-proven infections and a high proportion of patients in the microbiologically-evaluable population.

The objective in clinical trials of antibiotics in patients with AECB is to have a high proportion of culture-positive patients to enable a more relevant comparison of two (or more) antibacterial drugs. In terms of PPV and NPV, a high PPV is most important as this indicates that few of the patients enrolled into the study on the basis of the test result will be culture-negative (few false positives). A number of patients may be excluded from the study on the basis of the test who later turn out to be culture-positive (false negatives), but this is of less importance to the final robustness of the clinical trial. In clinical practice however the situation is reversed, a high rate of false-negatives will create a problem as these are patients who need an antibiotic, but on the basis of the test may be refused this treatment. Instead, treating a small proportion of patients unnecessarily with antibiotics (the false positives) is likely to be the more acceptable option.

Bacterial colonisation of the LRT brings into question all assumptions that bacteria grown from expectorated sputum are pathogenic. However, it is difficult to argue that none of these culture-positive patients is suffering a bacterial exacerbation. So what is the truth? It has been reported that a common cold precedes the majority of exacerbations of COPD [Wedzicha, 2004], but are all of these exacerbations exclusively viral in nature? There is now good evidence that many exacerbations are triggered by respiratory viruses, and the following viruses have been identified with acute exacerbations: cold viruses, influenza and parainfluenza viruses, and respiratory syncytial virus (RSV) [Sethi, 2000]. In a proportion of these patients it also seems possible that the viral infection allows the colonising bacteria, or a newly arrived strain of existing bacteria to flourish and develop into a secondary infection. It is possible that a high yield (>10^7 cfu/mL) of a bacterial species from a sputum sample is currently our best indicator of active infection, until a more precise marker of bacterial infection is found. In this way a semi-quantitative Gram stain makes sense
in being able to suggest possible active bacterial infection, before the culture of the sputum sample is performed.

8.5 Overall Conclusions

1. Chronic bronchitis is a complex disease and the terminology for the condition has changed over the last 40-50 years. This is reflected in the number of different terms that had to be used in the systematic review in order to identify the disease in the years since 1966.

2. Over the last 50 years, the design and statistical rigour of clinical trials of antibiotics in AECB has improved significantly.

3. The proportion of patients with AECB who have a respiratory pathogen isolated from their sputum in significant numbers shows wide variation (14% to 96% in the systematic review). However, there was no clear association between the proportion of bacteriologically-proven AECB and signs/symptoms, or sputum sample quality.

4. The Anthonisen et al [1987] classification of exacerbations may be useful in guiding antibiotic therapy; however this study provided no evidence that there was a higher proportion of bacteriologically-positive patients among those who had Type I exacerbations than patients who had Type II exacerbations. The results were counter-intuitive and the Fisher’s exact test gave a borderline result. This outcome was possibly the result of the very uneven distribution of Type I and Type II patients in this study (approx. 7:3 instead of 1:1).

5. Applying a semi-quantitative screen of a Gram-stained preparation of sputum gave the best indication of bacterial infection. In total, 91% of samples that had at least 10 bacterial cells per oil immersion field (x 400 magnification) as the only bacterial morphological cell type present, or at least 25 cells per field with multiple bacterial morphological cells types present, grew a recognised
pathogen. The test had a sensitivity of 67% and a specificity of 90%; a positive predictive value of 91%, and a negative predictive value of 65%.

6. In large multinational clinical trials the shipment of sputum samples to a local accredited laboratory and the rapid processing of the sample and reporting back to the general practitioner may not be possible. Consequently, this procedure may have some limitations for community-based studies.

7. Current research in the area of AECB is identifying several potential biomarkers of inflammation and infection. Currently none of these have conclusively demonstrated efficacy in identifying acute bacterial exacerbations of CB from non-bacterial exacerbations.

8. Research to understand more clearly the whole disease area is still required, and patients in whom antibiotics will be of benefit in exacerbations requires further clarification. Nevertheless, new antibiotics will continue to be developed and these must be evaluated rigorously in patients with acute exacerbations of CB/COPD. Therefore, any simple technique that can be applied in clinical trials of these antibiotics in acute exacerbations of CB/COPD, which can reliably and consistently provide a high proportion of patients who have culture-positive sputum samples at baseline may have clinical benefits (in terms of more appropriate prescribing of antibiotics), as well as making clinical trials easier and more scientifically robust.
9 APPENDICES

9.1 Proposed Further Research Project: Draft Protocol

9.2 Systematic Review: Protocol (including Data Collection Form)

9.3 Clinical Study: Protocol and Amendment

9.4 Clinical Study: Data Collection Forms

9.5 Clinical Study: Publication in Journal of Infection, Sep. 2007
APPENDIX 9.1

Proposed Further Research Project: Draft Protocol
STUDY PROTOCOL

PhD/AECB-II/03

A prospective, multicentre comparison of sputum colour and leucocyte esterase against standard symptoms, as an indicator of bacterial infection in patients with acute exacerbation of chronic bronchitis.

Draft 1: 17 Jun 2003

PhD Project of: Carol Burley
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Supervisor:

Tel:
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1. INTRODUCTION AND STUDY RATIONALE

Chronic bronchitis was defined by the American Thoracic Society in 1962 as 'the daily production of sputum for at least three consecutive months over two consecutive years'\(^{(1)}\) and over 40 years later, this description remains the best definition that we have of this disease. Clinical trials of antibacterial drugs in patients with acute exacerbations of chronic bronchitis (AECB), aim to select patients who have an acute bacterial exacerbation.

Exacerbations of chronic bronchitis may occur due to a number of factors that include exposure to airborne irritants, or as a result of infection by bacteria, or viruses. There is no reliable method of distinguishing between exacerbations caused by bacteria and those caused by other agents. The criteria that are used for selecting AECB patients for entry into clinical trials of antibacterial drugs are not consistent, and the proportion of bacteriologically positive patients shows considerable variation. The proportion of patients in studies of AECB, who have a bacterial pathogen isolated from a sputum sample can vary between 25%\(^{(2)}\) and 73%\(^{(3)}\). Although a Gram stain of the sputum may be helpful if it shows abundant neutrophils and a predominant morphological type of organism, few clinical trials use this procedure as part of the entry criteria, possibly because it presents logistical problems and a delay in being able to enrol and treat the patients.

Anthonisen and co-workers\(^{(4)}\) demonstrated a 20% difference in success rates between antibiotic and placebo-treated patients when they had the three 'cardinal symptoms' of increased dyspnoea, sputum volume and sputum purulence (Type I exacerbation). Exacerbations with fewer symptoms showed less difference between antibiotic and placebo, possibly indicating that many of these were not bacterial exacerbations. It is unfortunate that sputum microbiology was not performed in this study as this could have added weight to the argument for the use of an antibiotic. It was concluded by Staley \(et\) \(al\)\(^{(5)}\) however, that in view of these results future studies with an active control group should be designed to include only patients in whom exacerbations are likely to have a bacterial cause. The characteristics of increased cough and/or dyspnoea and increased sputum volume and purulence have been demonstrated to be associated with bacterial infection\(^{(6,7)}\), but still the majority of studies produce a disappointing percentage of bacteriologically proven infections e.g. 44%\(^{(6)}\), 39%\(^{(6)}\), 27%\(^{(10)}\), 34%\(^{(11)}\).

The correlation of potential pathogens grown from sputum samples and paired transtracheal aspirates has been shown to be 94% in sputum samples with <10 squamous epithelial cells and >25 white blood cells per low power (x100) field\(^{(12)}\). Gram staining has been reported to confirm the presence of bacteria characterized by their morphology. Using an oil immersion field (x1000) ≥8 pneumococcus-like, ≥12 hemophilus-like, or ≥18 moraxella-like organisms are likely to indicate acute bacterial exacerbation; stable patients have few bacteria per high power field\(^{(13)}\). In a previous comparison of the usefulness of the categories of: Anthonisen type II exacerbation (2/3 symptoms), Anthonisen type I exacerbation (all 3 symptoms), >25 neutrophils/<10 epithelial cells per low power field and a Gram stain of the sputum as indicators of bacterial infection, it was found that screening the sputum samples for purulence and bacterial cell types, significantly increased the proportion of patients in which a pathogen was grown\(^{(14)}\). This study therefore demonstrated the benefit of applying a screening procedure to sputum samples prior to enrolling patients into a clinical study however, in large, multinational, Phase III clinical trials this screening is logistically difficult, adds a significant expense to the study, delays the entry of patients into the study and consequently delays the start of antibacterial therapy, which may create ethical concerns. Ideally what is needed is a simple method of identifying bacterial infection that could be applied by the general practitioners, and would give a high degree of sensitivity and specificity, similar to that achieved by Gram stain screening the sputum.
This study aims to examine two simple methods of testing sputum samples from patients with AECB for bacterial infection, and compare them with the number of bacteriologically-positive patients using just the standard inclusion criterion of two or all three of Anthonisen's cardinal symptoms. The two methods to be tested are:

i. sputum colour, matched against a colour chart based on the work of Stockley et al.\(^{(15)}\)

ii. presence of leucocyte esterase in the sputum, using reagent strips; this technique has been used successfully in other infection types\(^{(16,17)}\) and may be useful in AECB.

These two methods applied separately or in combination may offer a convenient, simple, inexpensive and reliable method for GPs to assess the likelihood of bacterial infection in patients presenting with AECB that could be applied to the inclusion criteria of clinical studies of antibacterial agents, potentially offering a simple and effective means of ensuring a significantly higher proportion of bacteriologically-evaluable patients.

2. OBJECTIVES

2.1 Primary objective

- To compare the sensitivity and specificity of a) sputum colour and b) leucocyte esterase (tested on expectorated sputum with a urine dipstick) as assessed by the GP, with Anthonisen (type I and II) symptoms as indicators of bacterial infection in patients with acute exacerbation of chronic bronchitis.

2.2 Secondary objectives

a) To compare the accuracy and specificity of sputum colour assessed by the GP with the presence of predominant organism on a Gram-stained sputum preparation, as indicators of bacterial infection in patients with an acute exacerbation of chronic bronchitis.

b) To compare the accuracy and specificity of leucocyte esterase (tested on expectorated sputum with a urine dipstick) assessed by the GP with the presence of predominant organism on a Gram-stained sputum preparation as indicators of bacterial infection in patients with an acute exacerbation of chronic bronchitis.

c) To examine the effect of combining sputum colour and presence of leucocyte esterase as an indicator of bacterial infection in these patients, and how this compares with Anthonisen (type I and II) symptoms and with the presence of predominant organism on a Gram-stained sputum preparation.

d) To compare the assessment of sputum colour and leucocyte esterase performed by the laboratory with that of the GP to assess the reproducibility of these methods.

e) To examine the effect of using each of these four methods (symptoms, sputum colour, leucocyte esterase and predominant organism on Gram stain) as the main inclusion criterion in clinical trials of antibacterial agents in the patients with an acute exacerbation of chronic bronchitis, in terms of the proportion of bacteriologically-evaluable subjects and the additional costs that would be incurred.
3. STUDY DESIGN

This study is a prospective, multicentre, non-interventional study of documented chronic bronchitic patients presenting to a general practitioner with signs and symptoms of an acute exacerbation.

4. STUDY DURATION AND DATES

The study is planned to commence in <MONTH YEAR> and finish <MONTH YEAR>. In order to recruit the required number of subjects, a second winter season may be required, in which case the study will finish during the winter season 2004/05 when the <100 subjects> (rough calculation) have been enrolled.

5. SELECTION OF SUBJECTS

5.1 Number of subjects

It has been calculated that XXX subjects will be required to identify a 20% improvement in the number of subjects with a proven bacterial infection between either sputum colour and Anthonison type I and II symptoms or a positive leucocyte esterase test and Anthonison type I and II symptoms (see section 7.1 for details of the sample size justification).

No centre will recruit more than <32 subjects>.

5.2 Entry criteria

Inclusion criteria

➢ Adult patients aged 35 years and above with evidence of chronic bronchitis as defined as: chronic cough and sputum production on most days over a period of 3 successive months for at least 2 consecutive years.

➢ Patients with at least two of the following symptoms: increased sputum purulence; increased sputum volume; increased dyspnoea.

➢ Patients who are able to produce a sputum sample at the visit (alternatively, the patient may take a sputum pot away and return a sample the following morning, provided that no antibiotic treatment has been started).

➢ Patients who have consented to the study.

Exclusion criteria

➢ Patients who have significant bronchiectasis (>100mL sputum daily).

➢ Patients who have cystic fibrosis.

➢ Patients who have already received antibiotic treatment for this episode.

➢ Patients who have received an antibiotic for any reason in the previous 7 days.

➢ Patients who have previously entered this study.
6. STUDY PROCEDURES

6.1 Overview of data collection

Data for this study will be collected on individual case report forms (CRFs) provided to each investigator and to the laboratory. A CRF will be completed for each eligible patient who consents to take part in the study. Any patient who fails to produce a sputum sample before antibiotic treatment is started will not be eligible for the project, and will be replaced.

Data for this study will come from two sources. The investigator will complete the first section of the CRF (clinical data) at the time of the patient visit. The laboratory will complete section two of the CRF (laboratory results). A copy of the laboratory results with routine antibiotic susceptibility results, where an organism is isolated, will be sent to the investigators by post. The laboratory request forms will be identified as sputum samples for this research project by means of an adhesive label.

6.2 Clinical data

The following data will be collected by the investigator in the CRF provided for each patient:
- date of visit
- entry criteria
- confirmation of patient consent
- assignment of unique patient number
- basic patient demography
- relevant medical history checklist
- number of exacerbations in the previous 12 months for which an antibiotic was prescribed
- time and date of sputum sample
- rating of symptoms of dyspnoea, sputum volume and sputum purulence:
  a) prior to this infection (pre-infection)
  b) currently
- ranking of the sputum colour according to the supplied colour chart
- test for the presence of leucocyte esterase in the sputum sample using the dipsticks provided

6.3 Laboratory data

All sputum samples will be transferred to the agreed local microbiology laboratory by the routine collection service or in special circumstances by a taxi service which will be setup as necessary. All samples will be identified by a study specific adhesive label, which will identify the study subject number.

On arrival at the microbiology laboratory, samples will be logged and any queries about the subject number resolved.

The sputum sample will have the following tests/examinations performed:

1. Assessment of purulence of the sample by appearance:

<table>
<thead>
<tr>
<th>Clear, non-viscous</th>
<th>non-sputum sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grey/white, viscous</td>
<td>mucoid</td>
</tr>
<tr>
<td>Up to 50% yellow-green</td>
<td>mucopurulent</td>
</tr>
<tr>
<td>&gt;50% yellow-green</td>
<td>purulent</td>
</tr>
</tbody>
</table>
2. Ranking of the sputum colour according to the supplied colour chart

3. Test for the presence of leucocyte esterase in the sputum sample

4. Low power microscopy of cells (total magnification x100):
   - epithelial cells: <10 per field / ≥10 per field
   - neutrophils: >25 per field / ≤25 per field

5. Gram stain and quantitative assessment of bacterial cell types:

<table>
<thead>
<tr>
<th>CFU/mL</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10 per oil immersion field</td>
<td>+</td>
</tr>
<tr>
<td>10–25 per oil immersion field</td>
<td>++</td>
</tr>
<tr>
<td>&gt;25 per oil immersion field</td>
<td>+++</td>
</tr>
</tbody>
</table>

6. Culture of purulent section of sputum for identification of pathogen with a semi-quantitative interpretation using a dilution technique (see Appendix V)

<table>
<thead>
<tr>
<th>CFU/mL</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>10^3 – 10^4</td>
<td>Light growth</td>
</tr>
<tr>
<td>10^5 – 10^6</td>
<td>Moderate growth</td>
</tr>
<tr>
<td>≥10^6</td>
<td>Heavy growth</td>
</tr>
</tbody>
</table>

All results will be recorded in the laboratory CRF.

7. DATA HANDLING AND STATISTICAL PROCEDURES

7.1 Sample size justification

Data from a previous study has shown that a maximum of 60% of subjects with two or three symptoms of an acute exacerbation (Anthonisen type I & II exacerbations) will have a proven bacterial infection (?pathogen grown at ≥10^5 CFU/mL) however, many clinical trials produce a much lower proportion of subjects with proven bacterial infection. In order to have a 90% chance (power) of demonstrating an increase of 20% or greater in the proportion of subjects with a proven bacterial infection in subjects with a positive sputum colour (from the colour chart) or with a positive leucocyte esterase test (using the dipsticks provided), a total of X subjects will be required.

Assuming that 10% of subjects will be non-evaluable (non-eligibility or missing test results) ZZZ subjects will be recruited and a one-sided (5%) chi-squared test will be applied to each of the two primary analyses.

7.2 Data management

All data collected in the CRFs will be entered into a computer database for analysis. All data entered onto this database will be anonymous. Subject data will be identified only by the subject number, with the date of birth as a qualifier.

7.3 Study populations

Data from all subjects who consented and were given a subject number will be entered into the database. All subject data that has all the major fields completed will be entered into the analysis population. This will be the only population for analysis.
Major protocol violations

Investigator CRF
- non-fulfilment of any of the entry criteria
- missing or incomplete ratings of symptoms of dyspnoea, sputum volume and sputum purulence pre-infection and currently
- missing ranking of the sputum colour according to the supplied colour chart
- missing result of the test for the presence of leucocyte esterase in the sputum sample

Laboratory CRF
- missing or incomplete microscopy of cells (WBCs and epithelial cells)
- missing or incomplete Gram stain and quantitative assessment of bacterial cell types
- missing or incomplete culture of sputum sample

7.4 Statistical methods

The positive culture rate between the 2-symptom group and the 3-symptom group will be compared with a one-sided chi-squared test at the 5% level.

The sensitivity and specificity will be calculated under all prediction schemes and tabulated for comparison.

7.5 Interim analyses

No interim analysis is planned for this study.

8. ETHICAL ASPECTS

8.1 Subject information and informed consent

All patients will be given a patient information sheet (see Appendix III) that explains the study. Patients will be encouraged to discuss any concerns with the doctor. If the patient has any doubts about taking part in the study, they will not entered.

All patients who agree to take part in the study will be asked to sign the consent form (see Appendix IV). No patient may enter the study unless they have freely signed and dated the consent form.

8.2 Confidentiality

All data recorded on the CRF and entered onto the study database will be anonymous. Patients will be assigned a unique patient number when they consent to the study, and this will be the only identification of the patients' data on the CRF or in the database.
8.3 Approval of the study protocol and amendments

The study protocol and any amendments will be reviewed and approved by the following people:

Carol Burley PhD student
<To be assigned> project supervisor

9. MONITORING

A selection of the investigator and laboratory CRFs will be monitored by Carol Burley (CB) to ensure the completeness and accuracy of the data, and access to the patients' GP medical notes will be required. All monitoring will be performed in a confidential manner and no record of the patients' names will be made.
10. REFERENCES


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Protocol Approval Form

PhD/AECB-II/03

A prospective, multicentre comparison of sputum colour and leucocyte esterase against standard symptoms, as an indicator of bacterial infection in patients with acute exacerbation of chronic bronchitis.

This study protocol was subject to critical review and has been approved by the signatories below.

The information it contains is consistent with the moral, ethical and scientific principles governing clinical research as set out in the Declaration of Helsinki and Good Clinical Practice guidelines.

PhD student

_________________________  ________________________
Carol Burley                Date

Project Supervisor

_________________________  ________________________
Dr                          Date
Investigator Protocol Agreement

A prospective, multicentre comparison of sputum colour and leucocyte esterase against standard symptoms, as an indicator of bacterial infection in patients with acute exacerbation of chronic bronchitis.

Declaration of Investigator

I have been adequately informed about the study PhD/AECB-II/03. I have read this study protocol and agree that it contains all the information required to conduct the study. I agree to conduct the study as set out in this protocol.

I agree to provide a signed and dated copy of my curriculum vitae before the study starts.

Investigator

________________________________________
Print Name

_________________________  __________________________
Signed                     Date

Co-investigator (if applicable)

________________________________________
Print Name

_________________________  Position
Signed                     Date
A study to compare simple indicators of bacterial infection in patients with acute exacerbation of chronic bronchitis.

Your doctor is taking part in a small research study to test two simple ways of telling if patients, like yourself, have a chest infection caused by bacteria (germs) and compare these against the symptoms that you are suffering. We are asking whether you would agree to help with this research.

Approximately XXX patients with symptoms of a chest infection will take part in this study. Worsenings (exacerbations) of chronic bronchitis are sometimes caused by bacteria, in which case an antibiotic might be helpful, or they might be caused for other reasons (e.g. pollution, viruses), in which case an antibiotic would not help at all. The problem is that it is difficult to tell what is causing each worsening. This study will examine and compare two simple methods 1) comparing the colour of your sputum against a colour chart, 2) testing your sputum with a test stick that is often used to test urine samples for bacterial infection. This study involves no experimental (trial) medicine.

If you agree to take part, a few notes will be made about the nature of your current and previous chest infections and about some other medical problems you may have (such as heart problems) and you will be asked to produce a good sputum sample for analysis - that is all. You will not have to make any other visits to your GP or have any other samples taken as part of this study.

Your doctor will give you regular medication for your chest infection if it is necessary. The results of the laboratory tests on your sputum sample will be sent to your GP within 5 days. You will be able to find out the results of these tests by contacting your surgery after this time.

All records kept for this project will be anonymous. You will be identified by a number and only your doctor will know who this number refers to. If the results of this project are published, your identity will remain confidential.

The information written down in the study report form may be checked against your medical notes, to confirm the completeness and accuracy of the data. This is standard practice in such research work and all monitoring will be performed in a confidential manner by the post-graduate researcher.

You are under no obligation to take part in this study. You may choose not to take part without having to give a reason. Your treatment and the attitude of your doctor towards you will not be affected if you decide not to take part. Please feel free to ask your doctor any questions you may have about this study.

Thank you for reading this sheet.
A study to compare simple indicators of bacterial infection in patients with acute exacerbation of chronic bronchitis.

1. I agree to take part in this study comparing indicators of bacterial infection in patients with acute exacerbation of chronic bronchitis.

2. I have been given a patient information sheet and have received an explanation of the nature and purpose of the study and what I am expected to do.

3. I confirm that I have informed the doctor of any antibiotics that I have received for this chest infection, from other doctors.

4. I understand that I am under no obligation to take part in this project and that my treatment and the attitude of the doctor towards me will not be affected if I decide not to take part.

5. I understand that I will not be referred to by name in any report or publication concerning this project.

6. I understand that the project leader of this study will wish to inspect my medical records to verify the information collected. By signing this document I give my permission for this review of my records.

NAME OF PATIENT: ............................................................
SIGNATURE OF PATIENT: ............................................................
DATE OF SIGNATURE: ............................................................

I confirm that I have explained the nature and purpose of the project to the above named patient, and that he/she freely consented to participate.

NAME OF INVESTIGATOR: ............................................................
SIGNATURE OF INVESTIGATOR: ............................................................
DATE OF SIGNATURE: ............................................................
APPENDIX 9.2

Systematic Review: Protocol (including Data Collection Form)
PROTOCOL

SR/0401

A Systematic Review of Clinical Trials of Antibiotics in Patients with Acute Exacerbations of Chronic Bronchitis, to Examine the Variation in the Proportion of Bacteriologically-positive Subjects and any Association with the Entry Criteria.

PhD Project of:
Postgraduate Medical School,
University of Surrey
Stirling House
Surrey Research Park
Guildford
Surrey. GU2 7DJ

Tel: 
E-mail: MATERIAL REDACTED AT REQUEST OF UNIVERSITY

Supervisor: 
Postgraduate Medical School,

Tel: 
E-mail: 

Date: 03 March 2005
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1. INTRODUCTION

Virtually all clinical trials of antibacterials drugs in patients with acute exacerbation of chronic bronchitis (AECB) use the definition of chronic bronchitis that was published by the American Thoracic Society in 1962 as 'the daily production of sputum for at least three consecutive months over two consecutive years' [1]. Exacerbations of chronic bronchitis may occur due to exposure to airborne irritants, or as a result of infection by bacteria or viruses. Unfortunately, there is no reliable method of distinguishing between exacerbations caused by bacteria and those caused by other agents. The criteria that are used for selecting AECB patients for entry into clinical trials of antibacterial drugs are not consistent, and the proportion of bacteriologically-positive patients shows considerable variation.

Anthonisen and co-workers [2] demonstrated a 20% difference in success rates between antibiotic and placebo-treated patients when they had increased dyspnoea, sputum volume and sputum purulence (Type I exacerbation). Exacerbations with fewer symptoms showed less difference between antibiotic and placebo, possibly indicating that these were not bacterial exacerbations. The characteristics of increased cough and/or dyspnoea and increased sputum volume and purulence have been demonstrated to be associated with bacterial infection [3,4]; nevertheless, the majority of studies produce a disappointing percentage of bacteriologically-proven infections, sometimes below 20% [5, 6, 7]. Given the significant variation in the percentage of patients from whom a bacterial pathogen is isolated in clinical trials, and yet the standard definitions of chronic bronchitis, and to a lesser extent the definition of an acute exacerbation, it seems strange that such a wide variation should exist. A systematic review of randomised, controlled, clinical trials of antibacterial agents (antibiotics) in the treatment of patients with an acute exacerbation of chronic bronchitis will be undertaken in order to attempt to understand the reasons why such variations in the proportions of bacteriologically-proven infections should exist, and potentially to propose some entry criteria to improve the proportion of subjects from whom a pathogen may be isolated.

2. OBJECTIVE

To examine the differences in the rates of bacteriologically-positive sputum samples (i.e. sputum samples at study entry from which a recognised pathogen was isolated) in clinical trials of antibacterial drugs in patients with acute exacerbation of chronic bronchitis, and to investigate whether there is any association between the percentage of bacteriologically-positive subjects and the following factors:

- definition of chronic bronchitis
- definition of an acute exacerbation
- microbiological screening of the sputum sample for white blood cells and squamous epithelial cells, +/- bacterial cells seen on Gram stain (this can either be done as an entry criterion, or as a criterion for culture of the sputum or for the patient/pathogen to be eligible for the microbiologically-evaluable population)
- species of bacteria that were recognised as pathogens
- date of the study

Following analysis of the data, other associations or factors may be investigated and presented. These will be presented as non-planned analyses.
3. SELECTION OF PAPERS

3.1 Databases to be Used

The databases that will be used for this study will be:
   a) Ovid Medline
   b) Cochrane Library

3.2 Search Terms Used

Medline

A) Disease definition
   o bronchitis,
   o lung diseases, obstructive,
   o bronchitis, chronic
   o pulmonary disease, chronic obstructive
   o pulmonary emphysema
   o acute exacerbation of chronic bronchitis

B) Antibiotic therapy definition
   o anti-infective agents (administration & dosage, therapeutic use)

A) and B)

Limit to: Human, Adult, English language, Randomised controlled clinical trial.

Cochrane Library

   o Acute exacerbation chronic bronchitis

3.3 Entry Criteria for Papers

Inclusion criteria

Papers that will be eligible for this systematic review will meet the following criteria:
   • Clinical trials of one or more antibacterial agent in the treatment of patients with an acute exacerbation of chronic bronchitis.
   • Randomised, controlled, clinical trials.
   • Published in English.

Exclusion criteria

The following types of papers will be excluded from the analysis:
   • Letters.
   • Clinical trials that did not perform or report any bacteriology.
   • Papers that did not give sufficient detail of the study design and or the bacteriology.
• Papers reporting interim data.
• Clinical trials that only examined one pathogen in AECB.
• Papers that report on a subset of a larger study (in such cases efforts will be made to identify the publication of the full study).
• Clinical trials that enrolled less than 100 patients.

4. INFORMATION TO BE COLLECTED

4.1 Overview of Data Collection

Each suitable paper (or potentially suitable paper) will be obtained either from my own collection of papers and journals; from online access; from the library at the Royal Society of Medicine; or by ordering the paper from the British Library. Each paper will be read and the data from each suitable paper will be collected on individual data collection forms (see Appendix I). At the end of data collection, the data from these forms will be transferred to an Excel spreadsheet for analysis.

4.2 Data to be Extracted from the Papers

Each paper identified will be given a unique reference number and the following data will be collected from each eligible paper:

> Reference number.
> 1st Author.
> Journal.
> Year; volume; and start page of paper.
> Study drug.
> Comparator(s).
> Whether the standard definition of chronic bronchitis was applied in the study (the daily production of sputum production for a minimum of 3 consecutive months of the year over at least 2 successive years).
> Minimum and maximum age of subjects.
> The signs and symptoms of an acute exacerbation that are required for entry.
> Whether there is any screening of the sputum prior to subject enrolment.
> Whether there are any requirements that have to be met on the quality of the sputum e.g. WBCs; SECs.
> Whether there was any statistical justification for the sample size. If so, whether the a) enrolment target, b) evaluable subject target was reached.
> The number of subjects that were enrolled
  Number of subjects in the ITT population
  Number of subjects in the clinically evaluable (CE) population.
> Number of pathogens in the ITT population
  Number of bacteriologically-positive subjects in the ITT population
  Proportion of bacteriologically-positive subjects in the ITT population.
Number of pathogens in the CE population
Number of bacteriologically-positive subjects in the CE population
Proportion of bacteriologically-positive subjects in the CE population.

Clinical success rates in the ITT population
Clinical success rates in the CE population.

The organisms that are recorded as pathogens
Whether organisms that are resistant to the study drugs are excluded.

There will also be the opportunity to comment on any unusual aspects of the design in each study.

5. DATA MANAGEMENT, ANALYSIS AND REPORTING

5.1 Database

An Excel spreadsheet will be created to reflect the data collection form so that the information collected on the data collection forms can easily be transferred to the Excel spreadsheet.

5.2 Analysis of Data

The differences in the rates of bacteriologically-positive sputum samples (i.e. sputum samples at study entry from which a recognised pathogen was isolated) in clinical trials of antibacterial drugs in patients with acute exacerbation of chronic bronchitis will be examined in both the ITT and CE populations and reported as a median and range.

Any association between the percentage of bacteriologically-positive subjects and the following factors will be analysed and reported:
- definition of chronic bronchitis (ATS definition or alternative)
- definition of an acute exacerbation (Anthonisen Type I and Type II exacerbations or alternative)
- presence or absence of microbiological screening of the sputum sample for white blood cells and squamous epithelial cells, +/- bacterial cells seen on Gram stain done as an entry criterion, or as a criterion for culture of the sputum or for the patient/pathogen to be eligible for the microbiologically-evaluable population.
- the species of bacteria that were recognised as pathogens
- date of the study (based on the year that the paper was published).

Following completion of the above analyses, other associations or factors may be investigated and presented. These will be presented as non-planned analyses.

5.3 Systematic Review Report

Once the analysis has been completed, a systematic review report will be prepared, which will form part of the final thesis. It is anticipated that the results from this review will determine the direction of the subsequent stages of this research project.
6. REFERENCES


APPENDIX I

**Study Details**

| Ref No.: | - | 1st Author: |
| Journal: | | Year/Vol/pg: |
| Study drug: | | Comparator(s): |

**Population & Entry Criteria**

| Population: Std AECB? | Yes | No |
| if No, specify: | | |

**Age range:** from to OR no upper limit

**Symptoms req'd for entry**

- Purulent sputum: req. option
- Dyspnœa: req. option
- Sputum vol: req. option
- Cough: req. option
- Wheezing/rales: req. option
- Fever: req. option
- FEV1: req. option

Any 1 of optional Any 2 of optional Any 3 of optional Not specified Req. only

Anything different to above:

**Sputum criteria for study entry**

- Yes | No | N/K

If Yes, WBCs >25: SECs ≤10: predominant cell types on Gram stain:

**Sputum criteria for culture/to be included Bact. Population**

- Yes | No | N/K

If Yes, WBCs >25: SECs ≤10: predominant cell types on Gram stain:

**Study Numbers**

| Total patients entered: | Stat justif of No.? | Yes | No | N/K |
| Recruit. target met? | Yes | No | N/K |
| No. in ITT: | No. in PP: |

**Bacteriological population**

| ITT - No. of paths: | ITT - No. of Bact+ pats: ITT - % of Bact+ pats: |
| PP - No. of paths: | PP - No. of Bact+ pats: PP - % of Bact+ pats: |
| ITT - Clinical Success (study drug vs comparator): | vs |
| PP - Clinical Success (study drug vs comparator): | vs |

Path: SPN | HIN | MCA | HPA | SAU | PAE | E'bact | others | spp N/K | R-orgs excl.
APPENDIX 9.3

Clinical Study: Protocol and Amendment
A local multicentre investigation into the best indicator of bacterial infection in patients with acute exacerbation of chronic bronchitis.

Investigator: Multicentre GP Study, Edinburgh

Principal Investigator: Dr I. McKay
Rose Garden Medical Centre
4 Mill Lane, Leith
Edinburgh. EH6 6TL

Laboratory: Department of Microbiology
Western General Hospital
Edinburgh
EH4 2XU

MSc Project of: Carol Burley (Project Leader)
c/o HPRU, University of Surrey
Milford Hospital
Godalming
Surrey. GU7 1UT

Tel: 01895 837622

Supervisor: Dr R.G. Masterton
Department of Microbiology
Western General Hospital
Edinburgh EH4 2XU

Tel: 0131 537 1925

Date: 05 February 1999
LIST OF INVESTIGATORS

Only Lothian practices will be taking part in this study and the specific practices are listed below:

Dr I. McKay (Principal Investigator)
Rose Garden Medical Centre
4 Mill Lane,
Leith
Edinburgh. EH6 6TL

Dr D.G. Maxwell
Leith Walk Surgery
60 Leith Walk
Edinburgh. EH6 5HB

Dr N. Hewitt
The Long House Surgery
73, East Trinity Road
Edinburgh. EH5 3EL

Dr J. Shaw
The Surgery
8a Bridge Street
Musselburgh
Midlothian. EH21 6BL
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5. SELECTION OF SUBJECTS
   - Number of subjects
   - Entry criteria

6. STUDY PROCEDURES
   - Overview of data collection
   - Clinical data
   - Laboratory data

7. DATA HANDLING AND STATISTICAL PROCEDURES
   - Sample size justification
   - Data management
   - Study populations
   - Statistical methods
   - Interim analyses

8. ETHICAL ASPECTS
   - Subject information and informed consent
   - Confidentiality
   - Approval of the study protocol and amendments

9. MONITORING

10. REFERENCES

11. APPENDICES

   I  Protocol Approval Form
   II Investigator Protocol Agreement
   III Patient Information Sheet
   IV Patient Consent Form
   V Laboratory Techniques
1. INTRODUCTION AND STUDY RATIONALE

Chronic bronchitis was defined by the American Thoracic Society in 1962 as ‘the daily production of sputum for at least three consecutive months over two consecutive years’\(^{(1)}\) and almost 40 years later, this description remains the best definition that we have of this disease. Clinical trials of antibacterial drugs in patients with acute exacerbations of chronic bronchitis (AECB), aim to select patients who have an acute bacterial exacerbation. Exacerbations of chronic bronchitis may occur due to a number of factors that include exposure to airborne irritants, or as a result of infection by bacteria, or viruses. There is no reliable method of distinguishing between exacerbations caused by bacteria and those caused by other agents. The criteria that are used for selecting AECB patients for entry into clinical trials of antibacterial drugs are not consistent, and the proportion of bacteriologically positive patients shows considerable variation. The proportion of patients in studies of AECB, who have a bacterial pathogen isolated from a sputum sample can vary between 27%\(^{(2)}\) and 73%\(^{(3)}\). Although a Gram stain of the sputum may be helpful if it shows abundant neutrophils and a predominant morphological type of organism, few clinical trials use this procedure as part of the entry criteria.

Anthonisen and co-workers\(^{(4)}\) demonstrated a 20% difference in success rates between antibiotic and placebo-treated patients when they had increased dyspnoea, sputum volume and sputum purulence (Type I exacerbation). Exacerbations with fewer symptoms showed less difference between antibiotic and placebo, possibly indicating that these were not bacterial exacerbations. It is unfortunate that sputum microbiology was not performed in this study as this could have added weight to the argument for the use of an antibiotic. It was concluded by Staley et al\(^{(5)}\); however, that in view of these results future studies with an active control group should be designed to include only patients in whom exacerbations are likely to have a bacterial cause. The characteristics of increased cough and/or dyspnoea and increased sputum volume and purulence have been demonstrated to be associated with bacterial infection\(^{(6,7)}\), but still the majority of studies produce a disappointing percentage of bacteriologically proven infections, approx. 42%\(^{(8)}\), 39%\(^{(9)}\) and 48%\(^{(10)}\).

The correlation of potential pathogens grown from sputum samples and paired transtracheal aspirates has been shown to be 94% in sputum samples with <10 squamous epithelial cells and >25 white blood cells per low power (x100) field\(^{(11)}\). Gram staining has been reported to confirm the presence of bacteria characterized by their morphology. Using an oil immersion field (x1000) ≥8 pneumococcus-like, ≥12 haemophilus-like, or ≥18 moraxella-like organisms are likely to indicate acute bacterial exacerbation; stable patients have few bacteria per high power field\(^{(12)}\). Medici et al\(^{(13)}\) found that in a comparison of Gram stain and culture, microscopic examination of the Gram stain was found to be superior to culture, with around 50% of organisms microscopically identified as pneumococci failing to grow.

The issue of delay in processing sputum samples has been investigated by Gould et al\(^{(14)}\), although they stored sputum samples for 48 hours at 4°C. They found only a 5–25% discrepancy between early and late testing. However, the three different laboratories taking part accounted for much of this difference. It would appear from these results that methodology rather than storage time was responsible for the variation in viability of the organisms.

This study aims to examine the usefulness of the categories of: Anthonisen type II symptoms, Anthonisen type I symptoms, >25 neutrophils/<10 epithelial cells per low power field and a Gram stain of the sputum as indicators of bacterial infection. The study will also examine the implications of a 24 hour delay in getting a sputum sample
to the laboratory, as occurs when a central laboratory is used for culture and testing of the samples. Patients often report that they cough up the most purulent sputum soon after rising in the morning. The study will also look at the isolation rates of potential pathogens in sputum samples produced soon after rising, with those produced later in the day.

2. OBJECTIVES

2.1 Primary objective

• To identify the best indicator of bacterial infection, in patients with acute exacerbation of chronic bronchitis. The indicators that will be studied will be:
  1) Anthonisen type II clinical signs and symptoms (at least 2 out of the following signs/symptoms: increased sputum purulence; increased sputum volume; increased dyspnoea)
  2) Anthonisen type I clinical signs and symptoms (all 3 of the above signs/symptoms)
  3) The combination of >25 neutrophils and <10 epithelial cells per x100 field
  4) The presence of a morphological type of organism in a Gram stain of greater than 1 cell per oil immersion field.

2.2 Secondary objectives

f) To compare the pathogens isolated from the sputum samples within 6 hours (same day) and 24 hours (next day), and assess the implications of this to clinical trials using a central laboratory.

g) To compare the proportion of bacteriologically positive sputum samples collected at 9:00am or earlier, with those collected later in the day.

h) To examine the link between isolation of a bacterial species and the time since the previous exacerbation.

3. STUDY DESIGN

This is a local, multicentre, non-drug study of chronic bronchitis patients presenting with signs and symptoms of an acute exacerbation to 3–6 GPs in the Edinburgh, Lothian area. The signs/symptoms of infection, the neutrophils/epithelial cells in the sputum and the Gram stain results will be compared with the results of the sputum culture in order to identify the best indicator of bacterial infection.
4. STUDY DURATION AND DATES

The study is planned to commence in February 1999 and finish by December 1999.

The start and finish dates are dependent upon local research ethics committee approval and the rate of patient recruitment, and may therefore vary from those given above. The study will finish when the 120 patients have been recruited.

5. SELECTION OF SUBJECTS

5.1 Number of subjects

It is estimated that 120 patients will be required to identify the best indicator of bacterial infection in patients with acute exacerbation of chronic bronchitis (see section 7.1 for details of the sample size justification). A minimum of 12 and a maximum of 48 patients will be recruited by each of the 3 to 6 investigators taking part in the project.

5.2 Entry criteria

As this is a study that involves no study treatment, no exclusion criteria based on safety factors are applicable. Patients may not enter the study more than once.

Inclusion criteria

➢ Adult patients with evidence of chronic bronchitis as defined by: chronic cough and sputum production on most days over a period of 3 months for at least 2 consecutive years.

➢ Patients with at least two of the following symptoms: increased sputum purulence; increased sputum volume; increased dyspnoea.

➢ Patients who are able to produce a sputum sample at the visit (alternatively, the patient may take a sputum pot away and return a sample the following morning, provided that no antibiotic treatment has been started).

➢ Patients who have not yet received an antibiotic for this exacerbation.

➢ Patients who have consented to the study.
6. STUDY PROCEDURES

6.1 Overview of data collection

Data for this study will be collected on individual case report forms (CRFs) provided to each investigator and to the laboratory. A CRF will be completed for each eligible patient who consents to take part in the study. Any patient who fails to produce a sputum sample before antibiotic treatment is started will not be eligible for the project, and will be replaced.

Data for this study will come from two sources. The investigator will complete the first section of the CRF (clinical data) at the time of the patient visit. The laboratory will complete section two of the CRF (laboratory results). A copy of the laboratory results with routine antibiotic susceptibility results, where an organism is isolated, will be sent to the investigators by post. The laboratory request forms will be identified as sputum samples for this research project by means of an adhesive label.

6.2 Clinical data

The following data will be collected by the investigator in the CRF provided for each patient:

- date of visit
- entry criteria
- confirmation of patient consent
- assignment of unique patient number
- basic patient demography
- date of last exacerbation
- time and date of sputum sample
- rating of symptoms of dyspnoea, sputum volume and sputum purulence:
  a) prior to this infection (pre-infection)
  b) currently

6.3 Laboratory data

All sputum samples will be transferred to the Department of Clinical Microbiology at the Western General Hospital, Edinburgh and processing commenced within 6 hours. This will be achieved by the routine collection service.

On arrival at the Department of Clinical Microbiology, samples will be logged and any queries about the patient number resolved. The sample will be split into two aliquots of equal purulence, using sterile forceps.
Aliquot 1 will have the following tests/examinations performed:

7. Assessment of purulence of the sample by appearance:

<table>
<thead>
<tr>
<th>Condition</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear, non-viscous</td>
<td>non-sputum sample</td>
</tr>
<tr>
<td>Grey/white, viscous</td>
<td>mucoid</td>
</tr>
<tr>
<td>Up to 50% yellow-green</td>
<td>mucopurulent</td>
</tr>
<tr>
<td>&gt;50% yellow-green</td>
<td>purulent</td>
</tr>
</tbody>
</table>

8. Low power microscopy of cells (total magnification x100):
   - epithelial cells: <10 per field / >10 per field
   - neutrophils: >25 per field / <25 per field

9. Gram stain and quantitative assessment of bacterial cell types:

<table>
<thead>
<tr>
<th>Count</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 per oil immersion field</td>
<td>+</td>
</tr>
<tr>
<td>2-10 per oil immersion field</td>
<td>++</td>
</tr>
<tr>
<td>&gt;10 per oil immersion field</td>
<td>+++</td>
</tr>
</tbody>
</table>

10. Culture of purulent section of sputum for identification of pathogen with a semi-quantitative interpretation using a dilution technique (see Appendix V)

<table>
<thead>
<tr>
<th>CFU/mL</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^3 - 10^4$</td>
<td>Light growth</td>
</tr>
<tr>
<td>$10^5 - 10^6$</td>
<td>Moderate growth</td>
</tr>
<tr>
<td>$\geq 10^6$</td>
<td>Heavy growth</td>
</tr>
</tbody>
</table>

Aliquot 2 will be left at room temperature for 24 hours to simulate transport of a sputum sample to a central laboratory by overnight courier. After 24 hours a purulent section of the sputum will be cultured as point 4. above.

All results will be noted in the laboratory CRF.

**7. DATA HANDLING AND STATISTICAL PROCEDURES**

**7.1 Sample size justification**

All patients are required to have two out of the three major symptoms of acute exacerbation of chronic bronchitis to enter the study, however, some patients will only have two symptoms, whereas the remainder of patients will have all three symptoms. Assuming 50% of patients will have two symptoms only, with the remaining 50% having three symptoms, and a 2-symptom positive culture rate of 35%, a total of 112 patients will provide 85% power of detecting a 25% increase in the 3-symptom group (60% positive culture rate) using a one-sided (5%) chi-squared test. To allow for a small number of unevaluable samples, 120 patients will be recruited.
7.2 Data management

All data collected in the CRFs will be entered onto a computer database.

7.3 Study populations

Data on all patients where a sputum sample was received and cultured by the laboratory will be entered into the analysis. This will be the only population for analysis. Any patient who successfully produced a sputum sample, but for reasons of breakage or non-receipt by the laboratory, no sputum sample results are available, will not be entered into the analysis, but will be listed separately in any report or publication. Patients who are unable to produce a sputum sample are not eligible to enter the study.

7.4 Statistical methods

The positive culture rate between the 2-symptom group and the 3-symptom group will be compared with a one-sided chi-squared test at the 5% level.

The sensitivity and specificity will be calculated under all prediction schemes and tabulated for comparison.

7.5 Interim analyses

No interim analysis is planned for this study.

8. ETHICAL ASPECTS

8.1 Subject information and informed consent

All patients will be given a patient information sheet (see Appendix III) that explains the study. Patients will be encouraged to discuss any concerns with the doctor. If the patient has any doubts about taking part in the study, they will not entered.

All patients who agree to take part in the study will be asked to sign the consent form (see Appendix IV). No patient may enter the study unless they have signed and dated the consent form.
8.2 Confidentiality

All data recorded on the CRF and entered onto the study database will be anonymous. Patients will be assigned a unique patient number when they consent to the study, and this will be the only identification of the patients' data on the CRF or in the database.

8.3 Approval of the study protocol and amendments

The study protocol and any amendments will be approved by the following people:

Carol Burley          project leader
Dr R.G. Masterton    project supervisor
David Shaw           statistician

9. MONITORING

All CRFs will be monitored by Carol Burley (CB) to ensure the completeness and accuracy of the data and access to the patients' medical notes will be required. All monitoring will be performed in a confidential manner and no record of the patients' names will be made. All monitoring visits will be made from Buckinghamshire where CB is based. Travel costs will be paid for by CB's employers, who are covering the costs of the MSc course, and will cover the laboratory costs.
10. REFERENCES


11. APPENDICES

I  Protocol Approval Form
II Investigator Protocol Agreement
III Patient Information Sheet
IV Patient Consent Form
V Laboratory Techniques
A local multicentre investigation into the best indicator of bacterial infection in patients with acute exacerbation of chronic bronchitis.

This study protocol was subject to critical review and has been approved by the signatories below.
The information it contains is consistent with the moral, ethical and scientific principles governing clinical research as set out in the Declaration of Helsinki and Good Clinical Practice guidelines.

Project Leader

Carol Burley Date

Project Supervisor

Dr R.G. Masterton Date

Biometrician

David Shaw Date
Investigator Protocol Agreement

A local multicentre investigation into the best indicator of bacterial infection in patients with acute exacerbation of chronic bronchitis.

Declaration of Investigator

I have been adequately informed about the study MSc/CB/98/01. I have read this study protocol and agree that it contains all the information required to conduct the study. I agree to conduct the study as set out in this protocol.

I agree to provide a signed and dated copy of my curriculum vitae before the study starts.

Investigator

__________________________________________
Print Name

__________________________________________  ________________
Signed                                      Date

Co-investigator (if applicable)

__________________________________________  ________________
Print Name                                      Position

__________________________________________
Signed                                      Date
Patient Information Sheet

A local multicentre investigation into the best indicator of bacterial infection in patients with acute exacerbation of chronic bronchitis.

Your doctor is taking part in a small research study to examine the best way to tell if patients, like yourself, have a chest infection caused by bacteria (germs). We are asking whether you would agree to help with this research. Approximately 120 patients in the Edinburgh area will take part in this study.

This study involves no experimental (trial) medicine.

If you agree to take part, a few notes will be made about the nature of your chest infection and you will be asked to produce a good sputum sample for analysis - that is all. You will not have to make any other visits to your GP or have any other samples taken as part of this study.

Your doctor will give you regular medication for your chest infection if it is necessary. The results of the laboratory tests on your sputum sample will be sent to your GP within 5 days. You will be able to find out the results of these tests by contacting your surgery after this time.

All records kept for this project will be anonymous. You will be identified by a number and only your doctor will know who this number refers to. If the results of this project are published, your identity will remain confidential.

Some of the information written down in the study report form will be checked against your medical notes, to confirm the completeness and accuracy of the data. This is standard practice in such research work and all monitoring will be performed in a confidential manner by the organiser of the study.

You are under no obligation to take part in this study. You may choose not to take part without having to give a reason. Your treatment and the attitude of your doctor towards you will not be affected if you decide not to take part.

Please feel free to ask your doctor any questions you may have about this study.

Thank you for reading this sheet.
Patient Consent Form

A local multicentre investigation into the best indicator of bacterial infection in patients with acute exacerbation of chronic bronchitis.

1. I agree to take part in this study into the best indicator of bacterial infection in patients with acute exacerbation of chronic bronchitis.

2. I have been given a patient information sheet and have received an explanation of the nature and purpose of the project, and what I am expected to do.

3. I confirm that I have informed the doctor of any antibiotics that I have received for this chest infection, from other doctors.

4. I understand that I am under no obligation to take part in this project and that my treatment and the attitude of the doctor towards me will not be affected if I decide not to take part.

5. I understand that I will not be referred to by name in any report or publication concerning this project.

6. I understand that the project leader of this study will wish to inspect my medical records to verify the information collected. By signing this document I give my permission for this review of my records.

NAME OF PATIENT: ..............................................................

SIGNATURE OF PATIENT: ......................................................

DATE OF SIGNATURE: ............................................................

I confirm that I have explained the nature and purpose of the project to the above named patient, and that he/she freely consented to participate.

NAME OF INVESTIGATOR: ......................................................

SIGNATURE OF INVESTIGATOR: .............................................

DATE OF SIGNATURE: .............................................................
Laboratory Techniques

SPUTUM CULTURE METHOD

The above respiratory secretion specimens must be processed in the Category 3 laboratory using the safety cabinet and wearing protective gloves and clothing.

METHOD

1. Record the appearance of the specimen.

2. Make a heat-fixed smear and stain with Gram's stain and examine for the presence of pus cells, epithelial cells and organisms. Record the results according to the protocol definitions.

3. Using a sterile pastette add an equal volume of sputolysin to the aliquot of the sputum sample selected for processing.

4. Vortex the sample until it is liquefied.

5. Add 0.1ml of the liquefied sputum (actually now 1:2) to 9.9ml of saline giving a 1:200 dilution.

6. Then add 0.1ml of this preparation to another 9.9ml of saline giving a 1:2000 dilution.

7. Inoculate each plate as follows with 0.1ml of the appropriate sputum dilution (mark plates accordingly) and spread over the entire surface of the plate using a spreader.

<table>
<thead>
<tr>
<th></th>
<th>BA</th>
<th>BA + Optochin disc</th>
<th>Chocolate/Bacitraci</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>neat liquefied sputum</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(actually 1:2 dilution)</td>
</tr>
<tr>
<td>1:200 dilution</td>
<td>1:200 dilution</td>
<td>1:200 dilution</td>
<td></td>
</tr>
</tbody>
</table>

8. Incubate the media as follows:-

<table>
<thead>
<tr>
<th>Media</th>
<th>Atmosph</th>
<th>Temp</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>BA</td>
<td>CO₂</td>
<td>37℃</td>
<td>24hrs</td>
</tr>
<tr>
<td>BA + Optochin</td>
<td>AnO₂</td>
<td>37℃</td>
<td>24hrs</td>
</tr>
<tr>
<td>Chocolate/Bacitra</td>
<td>CO₂</td>
<td>37℃</td>
<td>24hrs</td>
</tr>
</tbody>
</table>

If there is no growth after 24hrs, re-incubate for a further 24hrs.

9. Count the number of colonies of pathogenic organisms and calculate the number of colony forming units/ml of sputum.

10. Identify suspected pathogens according to the standard laboratory methods.
PROTOCOL AMENDMENT NO.1

A local multicentre investigation into the best indicator of bacterial infection in patients with acute exacerbation of chronic bronchitis

MSc/CB/98/01

Date of Amendment: 04 August 2001

Date of original protocol: 05 February 1999

Dates of previous amendments: N/A
1. JUSTIFICATION FOR AMENDMENT

Primary Justification

The original study was planned to only take place in Edinburgh with Lothian general practitioners. Because of the lack of response in several centres, and the need to get the 120 samples completed by Spring 2002, it has been agreed to extend the study. Centres will now include Monklands Hospital, Airdrie, one GP practice in Coatbridge, and two GP practices in the area of Glasgow Royal Infirmary.

This means that a number of references to the study taking place only in the Edinburgh area will need to be changed.

Secondary Justifications

As a student it will only be possible to monitor a small selection of the patient notes and this has been made clearer in the protocol and in the patient information and consent form.

The funding for this project has been clarified as being received from Hoechst Marion Roussel as the student is no longer employed by this company, however the educational grant remains and will be accounted for as part of the dissertation.

The change of address and telephone number of the student and supervisor have been detailed.

Confirmation of Scientific and Ethical Content

All the changes to the Final Protocol dated 5th February 1999 are detailed in this protocol amendment.

None of these changes will affect the scientific content of the study as the laboratories at Monklands Hospital and Glasgow Royal Infirmary have agreed to process the sputum samples as laid out in the protocol. The changes will not compromise patient treatment or confidentiality in any way.
2. INDIVIDUAL CHANGES

Unless otherwise specified, the page numbers and section numbers given refer to the original protocol.

Page 1. Title

The title:
A local multicentre investigation into the best indicator of bacterial infection in patients with acute exacerbation of chronic bronchitis

Will be amended to read:
A multicentre investigation into the best indicator of bacterial infection in patients with acute exacerbation of chronic bronchitis

Page 1. Principal Investigator and Laboratory

The text:

Investigator: Multicentre GP Study, Edinburgh

Will be changed to read:
Investigator: Multicentre Study

And the designations:

Principal Investigator: Dr I. McKay
Rose Garden Medical Centre
4 Mill Lane, Leith
Edinburgh. EH6 6TL

Laboratory: Department of Microbiology
Western General Hospital
Edinburgh
EH4 2XU

will be deleted and detailed on page 2 under List of Investigators and Laboratories:
MSc Project of: Carol Burley (Project Leader)  
c/o HPRU, University of Surrey  
Milford Hospital  
Godalming  
Surrey. GU7 1UT  
Tel: 01895 837622  
Supervisor: Dr R.G. Masterton  
Department of Microbiology  
Western General Hospital  
Edinburgh EH4 2XU  
Tel: 0131 537 1925

Will be corrected to read:  
MSc Project of:  

MATERIAL REDACTED AT REQUEST OF UNIVERSITY  

Tel:  
Supervisor: Dr R.G. Masterton  
Deputy Medical Director & Director of Clinical Developments  
The Royal Infirmary of Edinburgh  
Lauriston Place  
Edinburgh EH3 9YW  
Tel: 0131 536 3014

Page 2. List of Investigators

The sentence:  
Only Lothian practices will be taking part in this study and the specific practices are listed below:

Dr I. McKay (Principal Investigator)  
Rose Garden Medical Centre  
4 Mill Lane, Leith  
Edinburgh. EH6 6TL  

Dr D.G. Maxwell  
Leith Walk Surgery
LIST OF INVESTIGATORS AND LABORATORIES

Practices in Edinburgh, Glasgow and Coatbridge/Airdrie will be taking part in this study. The specific practices and hospital laboratories are listed below:

**Edinburgh**

**Investigators:** Dr I. McKay  
Rose Garden Medical Centre  
4 Mill Lane  
Leith  
Edinburgh. EH6 6TL

Dr N. Hewitt  
The Long House Surgery  
73, East Trinity Road  
Edinburgh. EH5 3EL

**Laboratory:** Dept of Microbiology  
Western General Hospital  
Edinburgh. EH4 2XU

**Glasgow**

**Investigators:** Dr G. McKaig  
Shettleston Health Centre  
420 Old Shettleston Road  
Glasgow. G32 7JZ

and at:  
Baillieston Health Centre  
20 Muirside Road  
Glasgow. G69 7AD

**Laboratory:** Dept of Bacteriology  
Glasgow Royal Infirmary  
Glasgow. G4 0SF
This is a local, multicentre, non-drug study of chronic bronchitis patients presenting with signs and symptoms of an acute exacerbation to 3-6 GPs in the Edinburgh, Lothian area.

Will be changed to read:

This is a multicentre, non-drug study of chronic bronchitis patients presenting with signs and symptoms of an acute exacerbation to six GP and Hospital centres in Scotland.

The study is planned to commence in February 1999 and finish by December 1999.

Will be changed to read:

The study started in October 1999 and is expected to finish by March 2002.

All sputum samples will be transferred to the Department of Clinical Microbiology at the Western General Hospital, Edinburgh and processing commenced within 6 hours.
All sputum samples will be transferred to the local hospital laboratory and processing commenced within 6 hours.

The following sentence in paragraph 2:

On arrival at the Department of Clinical Microbiology, samples will be logged and any queries about the patient number resolved.

Will be changed to read:

On arrival at the laboratory, samples will be logged and any queries about the patient number resolved.

Page 11. Section 8.3 Approval of the study protocol and amendments

Section 8.3:

The study protocol and any amendments will be approved by the following people:

Carol Burley  project leader
Dr R.G. Masterton  project supervisor
David Shaw  statistician

Will be changed to read:

The study protocol will be approved by the following people:

Carol Burley  project leader
Dr R.G. Masterton  project supervisor
David Shaw  statistician

Any amendments to the protocol which do not involve the patient numbers or the statistical plan will be approved by the project leader and the project supervisor only. Further statistical advice may be sought from any qualified statistician working in pharmaceutical research.

Page 11. Section 9 Monitoring

The paragraph:

All CRFs will be monitored by Carol Burley (CB) to ensure the completeness and accuracy of the data and access to the patients' medical notes will be required. All monitoring will be performed in a confidential manner and no record of the patients' names will be made.
All monitoring visits will be made from Buckinghamshire where CB is based. Travel costs will be paid for by CB's employers, who are covering the costs of the MSc course, and will cover the laboratory costs.
Will be changed to read:

A selection of the CRFs will be monitored by Carol Burley to check the completeness and accuracy of the data. Access to the medical notes of the selected patients will be required. All monitoring will be performed in a confidential manner and no record of the patients' names will be made. Monitoring visits will be made from Buckinghamshire where CB is based. A small fee will be paid to investigators for their time and to the laboratory to cover their costs. These monies will be paid from an educational grant for this project from Hoechst Marion Roussel.
**Patient Information Sheet**

_A local multicentre investigation into the best indicator of bacterial infection in patients with acute exacerbation of chronic bronchitis._

Your doctor is taking part in a small research study to examine the best way to tell if patients, like yourself, have a chest infection caused by bacteria (germs). We are asking whether you would agree to help with this research. Approximately 120 patients in the Edinburgh area will take part in this study.

This study involves no experimental (trial) medicine.

If you agree to take part, a few notes will be made about the nature of your chest infection and you will be asked to produce a good sputum sample for analysis - that is all. You will not have to make any other visits to your GP or have any other samples taken as part of this study.

Your doctor will give you regular medication for your chest infection if it is necessary. The results of the laboratory tests on your sputum sample will be sent to your GP within 5 days. You will be able to find out the results of these tests by contacting your surgery after this time.

All records kept for this project will be anonymous. You will be identified by a number and only your doctor will know who this number refers to. If the results of this project are published, your identity will remain confidential.

Some of the information written down in the study report form will be checked against your medical notes, to confirm the completeness and accuracy of the data. This is standard practice in such research work and all monitoring will be performed in a confidential manner by the organiser of the study.

You are under no obligation to take part in this study. You may choose not to take part without having to give a reason. Your treatment and the attitude of your doctor towards you will not be affected if you decide not to take part.

Please feel free to ask your doctor any questions you may have about this study.

Thank you for reading this sheet.
A local multicentre investigation into the best indicator of bacterial infection in patients with acute exacerbation of chronic bronchitis.

1. I agree to take part in this study into the best indicator of bacterial infection in patients with acute exacerbation of chronic bronchitis.

2. I have been given a patient information sheet and have received an explanation of the nature and purpose of the project, and what I am expected to do.

3. I confirm that I have informed the doctor of any antibiotics that I have received for this chest infection, from other doctors.

4. I understand that I am under no obligation to take part in this project and that my treatment and the attitude of the doctor towards me will not be affected if I decide not to take part.

5. I understand that I will not be referred to by name in any report or publication concerning this project.

6. I understand that the project leader of this study will wish to inspect my medical records to verify the information collected. By signing this document I give my permission for this review of my records.

NAME OF PATIENT: ............................................................

SIGNATURE OF PATIENT: ............................................................

DATE OF SIGNATURE: ............................................................

I confirm that I have explained the nature and purpose of the project to the above named patient, and that he/she freely consented to participate.

NAME OF INVESTIGATOR: ............................................................

SIGNATURE OF INVESTIGATOR: ............................................................

DATE OF SIGNATURE: ............................................................
Patient Information Sheet

A multicentre investigation into the best indicator of bacterial infection in patients with acute exacerbation of chronic bronchitis.

Your doctor is taking part in a small research study to examine the best way to tell if patients, like yourself, have a chest infection caused by bacteria (germs). The aim of this project is to help to improve the design of future antibiotic clinical trials in chest infections. We are asking whether you would agree to help with this research. Approximately 120 patients in Glasgow, North Lanarkshire and Edinburgh area will take part in this study.

This study involves no experimental (trial) medicine.

If you agree to take part, a few notes will be made about the nature of your chest infection and you will be asked to produce a good sputum sample for analysis - that is all. You will not have to make any other visits or have any other samples taken as part of this study.

Your doctor will give you regular medication for your chest infection if it is necessary. If you are attending your GP surgery, the results of the laboratory tests on your sputum sample will be sent to your GP within 5 days. You will be able to find out the results of these tests by contacting your surgery after this time.

All records kept for this project will be anonymous. You will be identified by a number and only your doctor will know who this number refers to. Some of the information written down in the study report form may be checked against your medical notes, to confirm the completeness and accuracy of the data. This is standard practice in research work and all such data checking will be performed in a confidential manner.

This research is being organised and monitored by a post-graduate student and will form part of a MSc degree. If the results of this project are published, your identity will remain confidential.

You are under no obligation to take part in this study. You may choose not to take part without having to give a reason. Your treatment and the attitude of your doctor towards you will not be affected if you decide not to take part.

Please feel free to ask your doctor any questions you may have about this study.

Thank you for reading this sheet.
Patient Consent Form

A multicentre investigation into the best indicator of bacterial infection in patients with acute exacerbation of chronic bronchitis.

1. I agree to take part in this study into the best indicator of bacterial infection in patients with acute exacerbation of chronic bronchitis.

2. I have been given a patient information sheet and have received an explanation of the nature and purpose of the project, and what I am expected to do. I have also had the opportunity to ask questions.

3. I confirm that I have informed the doctor of any antibiotics that I have received for this chest infection, from other doctors.

4. I understand that I am under no obligation to take part in this project and that my treatment and the attitude of the doctor towards me will not be affected if I decide not to take part.

5. I understand that I will not be referred to by name in any report or publication concerning this project.

6. I understand that the organiser of this study may wish to inspect my medical records to verify the information collected. By signing this document I give my permission for this review of my records.

NAME OF PATIENT: ..............................................................

SIGNATURE OF PATIENT: ..............................................................

DATE OF SIGNATURE: ..............................................................

I confirm that I have explained the nature and purpose of the project to the above named patient, and that he/she freely consented to participate.

NAME OF INVESTIGATOR: ..............................................................

SIGNATURE OF INVESTIGATOR: ..............................................................

DATE OF SIGNATURE: ..............................................................
3. SIGNATURES

I agree to the changes listed above:

Project Leader
Date: ________  Signature: ___________________________
Carol Burley

Project Supervisor
Date: ________  Signature: ___________________________
Dr R.G. Masterton

Investigator
Date: ________  Signature: ___________________________
Name (block letters): ___________________________
APPENDIX 9.4

Clinical Study: Data Collection Forms
Investigator Case Report Form

MSc/CB/98/01

A local multicentre investigation into the best indicator of bacterial infection in patients with acute exacerbation of chronic bronchitis.

MSc Project of: MATERIAL REDACTED AT REQUEST OF UNIVERSITY

Supervisor: Dr R.G. Masterton Medical Director Ayrshire & Arran Acute Hospitals Trust Crosshouse Hospital Kilmarnock Ayrshire KA2 0BE

Tel: 01563 577004

Local Laboratory: Dept of Bacteriology Southern General Hospital Glasgow

University Address: Dept of Pharmaceutical Medicine HPRU, University of Surrey Egerton Road Guildford Surrey GU2 5XP

Please fax completed forms (excluding this page) to Carol Burley at: or post to above home address keeping a copy for your file.

Please remember to complete patient number on each page – thank you.
ENTRY CRITERIA

Date of Visit: [ ] [ ] [ ]

All Entry Criteria must be answered ‘Yes’ for patient to be eligible

1. Patient has history of chronic bronchitis [ ] No [ ] Yes

2. Patient has at least 2 symptoms of an exacerbation from:
   - increased sputum purulence
   - increased sputum volume
   - increased dyspnoea
   [ ] No [ ] Yes

3. Patient is able to produce a sputum sample [ ] No [ ] Yes

4. Patient has NOT taken an antibiotic for this episode [ ] No [ ] Yes

5. Patient has consented to the study [ ] No [ ] Yes

Patient No: [ ]

I confirm that this patient has consented to take part in this study and has signed and dated the consent form provided.

_________________________  __________________________
Signed                     Date
<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Date of Birth:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender:</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Patient is a:</td>
<td>Smoker</td>
<td>Ex-smoker (&gt; 6 mth)</td>
</tr>
<tr>
<td>Date of last chest infection for which an antibiotic was given:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time and date patient produced the sputum sample:</td>
<td>Hrs</td>
<td>Mins</td>
</tr>
<tr>
<td></td>
<td></td>
<td>/</td>
</tr>
</tbody>
</table>
### PATIENT SIGNS / SYMPTOMS

#### 7. Dyspnoea:

- **a) Pre-infection**
  - 1. none
  - 2. only on unusual exertion
  - 3. during normal activity
  - 4. at rest

- **b) Currently**
  - 1. no change
  - 2. worse

#### 8. Sputum Volume in 24 hrs:

- 1. no sputum
- 2. up to 1 tablesp. (<15ml)
- 3. 1-4 tablesp. (15-60ml)
- 4. >4 tablesp. (>60ml)

#### 9. Sputum Purulence:

- 1. no sputum
- 2. grey/white (mucoid)
- 3. up to 50% yellow/green (mucopurulent)
- 4. >50% yellow/green (purulent)

### DECLARATION OF THE INVESTIGATOR

I confirm that, to the best of my knowledge, the information in this case report form is complete and accurate.

Signature of Investigator ____________________________ Date ____________________________

---

Page 3
# Laboratory Case Report Form

**MSc/CB/98/01**

**A local multicentre investigation into the best indicator of bacterial infection in patients with acute exacerbation of chronic bronchitis.**

<table>
<thead>
<tr>
<th>Patient Initials:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patient Date of Birth:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day Month Year</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patient No.:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time &amp; Date of Receipt of Sample:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day Month Year Hrs Mins</td>
</tr>
</tbody>
</table>

24 hr clock

**MSc Project of:**
Carol Burley  
4, Franklin Court  
Furlong Close  
Bourne End  
Bucks SL8 5AT

**Tel:** 01628 851351  
**Fax:** 01628 819187

**University Address:**
Dept of Pharmaceutical Medicine  
HPRU, University of Surrey  
Egerton Road  
Guildford  
Surrey, GU2 5XP

**Supervisor:** Dr R.G. Masterton  
Ayrshire & Arran Acute Hospitals Trust

**Tel:** 01563 577004

Please fax completed forms to Carol Burley at: 01628 819187  
or post to above home address keeping a copy for your file.  
Please remember to complete patient number on each page – thank you.
# Sputum Sample Results

1. **Appearance of Sputum Sample:**
   - 0: non-sputum sample
   - 1: mucoid
   - 2: mucopurulent
   - 3: purulent

2. **Cells:**
   - Epithelial Cells: 1: < 10, 2: ≥ 10
   - Neutrophils: 1: > 25, 2: ≤ 25

3. **Gram Stain – Cell Types Seen:**
   0: none, 1: Gram-negative bacilli, 2: Gram-negative cocci, 3: Gram-positive cocci, 4: Gram-positive bacilli

4. **Culture – Immediate Growth:**
   - Light, Moderate, Heavy
   - Organism(s) isolated:
     0: none, 1: H. influenzae, 2: H. parainfluenzae, 3: M. catarrhalis, 4: S. pneumoniae, 5: S. aureus, 6: coliform(s), 7: other

   Please specify: __________________________

Page 2
## SPUTUM CULTURE RESULTS @ 24 hr.

5. Culture – @ 24 hr.

<table>
<thead>
<tr>
<th>Organism(s) isolated</th>
<th>Light</th>
<th>Growth</th>
<th>Heavy</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H. influenzae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H. parainfluenzae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. catarrhalis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. pneumoniae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>coliform(s)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

please specify: _______________________

6. other

please specify: _______________________

## DECLARATION OF THE LABORATORY

I confirm that, to the best of my knowledge, the information in this case report form is complete and accurate.

Name *(Please print)* ____________________________

Position ____________________________

Signature ____________________________ Date ____________________________
APPENDIX 9.5

Clinical Study: Publication in Journal of Infection, Sep. 2007
10 REFERENCES

10.1 References in Thesis


80. NICE COPD guideline February 2004 (www.nice.org.uk/CG012NICEguideline).


96. Schentag JJ, Tillotson GS. Antibiotic selection and dosing for the treatment of acute exacerbations of COPD. *Chest* 1997; 112: 314S-19S.


100. Sethi S. Infectious etiology of acute exacerbations of chronic bronchitis. *Chest* 2000; 117(Suppl.): 380S-5S.


10.2 References Reviewed for Systematic Review


S30. Castaldo RS, Celi BR, Gomez F, LaVallee N, Souhrada J, Hanrahan JP. A comparison of 5-day courses of dirithromycin and azithromycin in the treatment of


10.3 References Reviewed in the Late Review (Mar-04 to Jul-08)


