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Microbiologic diagnostic tests when asymptomatic carriers are present

Aspects of the use of conventional throat and nasopharyngeal culture as examples

by

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Abstract

Carriers of potentially pathogenic bacteria simultaneously ill from a viral infection complicate the diagnostic procedure in respiratory tract infections. The present statistical methods available to evaluate common diagnostic tests either ignore the phenomenon of carriers or provide test characteristics that are difficult to apply in clinical decision making. In this dissertation, the influence of carriers on the diagnostic process has been elucidated.

- The etiologic predictive value (EPV) is a new statistical method developed to predict disease caused by the bacteriological findings, taking carriers into consideration. To calculate EPV, it is necessary to have the proportion of positive tests among patients, the proportion of positive tests among a healthy control population and the sensitivity of the test. This enables calculating the positive and negative EPV with a 95% confidence interval.
- A throat culture was found to be a reliable indicator for illness caused by *group A beta-haemolytic streptococci (GABHS)* in adult patients with a sore throat. Positive EPV (PEPV) was 99% (95% confidence interval is 94-100%). A seasonal variation, however, was found in pre-school children (0-6 years of age). A throat culture with growth of *GABHS* was found to be reliable only in the winter season, with a PEPV of 94% (75-100%) as opposed to only 61% (0-91%) in the summer. However, our data did not permit us to conclude that this seasonal variation will be found every year.
- Findings of *Haemophilus influenzae* in a nasopharyngeal culture, taken from patients with a sore throat, may indicate the true etiology of the disease. The prediction in regard to disease caused by *H. influenzae* (PEPV) was 93% (73-99%) for adults ≥16 years of age and 86% (28-99%) for pre-school children 0-6 years of age.
- In adults with a long-standing cough combined with other symptoms of a respiratory tract infection, it was found that growth of *H. influenzae* in a nasopharyngeal culture would indicate the etiology for infection with PEPV 90% (30-99%). Growth of *Moraxella catarrhalis* in a nasopharyngeal sample, taken from a pre-school child with a long-standing cough 0-6 years of age, will indicate the etiology for infection with a PEPV of 90% (66-99%).
- A questionnaire sent to different microbiologic laboratories revealed a substantial variation between different geographical areas' propensity to perform a throat or nasopharyngeal culture. There was also a large variation between the different areas in the outcome of these cultures. It could be shown that the variation in outcome of the cultures makes it difficult to directly apply predictive values calculated from many scientific studies.

Key words: Carriage, respiratory tract infections, predictive value of tests, epidemiology, decision making, streptococcal infections, Streptococcus pyogenes, tonsillitis

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List of publications

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

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II. Gunnarsson RK, Holm SE, Söderström M. The prevalence of potential pathogenic bacteria in nasopharyngeal samples from individuals with a respiratory tract infection and a sore throat – Implications for the diagnosis of pharyngotonsillitis. *Fam Pract* 2001;(Accepted for publication).

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The papers have been reprinted with permission of the journals

Contents

1. Abbreviations and definitions

Abbreviations:

BHS	Beta-haemolytic streptococci
GABHS	Group A beta-haemolytic streptococci
PPV	Positive predictive value
NPV	Negative predictive value
EPV	Etiologic predictive value
PEPV	Positive etiologic predictive value
NEPV	Negative etiologic predictive value

Abbreviations are also introduced in the section 9.1 Appendix: Derivation of the formulae for EPV on page 73.

Definitions

Asymptomatic carriers	Healthy individuals harbouring the agent our test is designed to detect
Symptomatic carriers	Individuals harbouring the agent our test is designed to detect and simultaneously having an illness caused by another agent, usually a virus
Carriers	Carriers with no specification to whether they are asymptomatic or symptomatic

2. Introduction

This dissertation is written from the perspective of the general practitioner. Great numbers of patients in primary health care visit the doctor for respiratory tract infections. In the majority of these cases, the illness is not severe. However, correctly or incorrectly, a large proportion of these patients is treated with antibiotics.

In the early 1990's, I worked as a doctor at a paediatric clinic in the south - western part of Sweden. Respiratory tract infections with sore throat or cough were common complaints. As a young doctor, I tried to assimilate knowledge from more experienced colleagues. However, it was not clear to me when to treat an upper respiratory tract infection with antibiotics. There were several different diagnostic and therapeutic strategies among the doctors for this disorder. Some relied on their clinical judgement, others relied on tests, such as throat or nasopharyngeal cultures.

However, the daily challenge was to decide whether a respiratory tract infection was of viral or bacterial etiology. At the clinic, throat cultures, nasopharyngeal cultures and C-reactive protein (CRP) were tests used in the diagnostic procedure for a large number of patients with upper respiratory tract infections. How useful then was the information obtained by these tests? I found the nasopharyngeal culture to be especially difficult to interpret because potentially pathogenic bacteria were found in tests from most of the patients. Should they then be treated with antibiotics that could eradicate the bacterium found? Most colleagues recommended antibiotic treatment if the condition had not improved spontaneously by the time the results of the nasopharyngeal culture arrived.

The appropriateness of prescribing antibiotic treatment when the nasopharyngeal culture showed growth of potentially pathogenic bacteria was questionable. As one of the senior doctors mentioned, most child patients, as well as healthy children, harbour these bacteria in a nasopharyngeal culture. It was then obvious to me that I, and perhaps many of my colleagues, had not fully understood the consequences of carriers.

How useful are throat and nasopharyngeal cultures in deciding whether the symptomatic infection is of viral or bacterial origin? If one could obtain the answer to this, how should the answer then be presented? At this time, another colleague at the clinic presented different statistical methods of calculating test characteristics. Although I had previously heard of these methods, they became far more relevant to me at this time. Predictive values of throat and nasopharyngeal cultures, taking symptomatic carriers into consideration, would be an aid in understanding the usefulness of these cultures. However, the literature did not provide this information, which lead to this project in the beginning of 1990.

2.1. Respiratory tract infections are common

Respiratory tract infections are very common. Approximately one-third of all visits to doctors in primary health care centres are due to upper respiratory tract infections [1, 2]. This is more common among children with up to 80% of consultations due to respiratory tract infections [2].

In Elfsborg county, Sweden, 426 571 visits were made to doctors in 50 primary health care centres during the year 2000. In total 389 526 diagnoses were set. Seven of the twenty most common diagnoses were respiratory tract infections. All respiratory tract infections may be extracted to compare their relative prevalence (Table I).

Table I– Relationship between different diagnoses of respiratory tract infections in 50 primary health care centres in Elfsborg county in Sweden in the year 2000

Diagnosis[a]	N[b]	P[c]
Upper respiratory tract infection without further definition	16 134	26.5%
Tonsillitis	8 553	14.1%
Acute bronchitis	6 552	10.8%
Acute sinusitis	5 807	9.5%
Unspecified otitis media	4 869	8.0%
Cough	4 324	7.1%
Pharyngitis	3 866	6.4%
Acute otitis media	2 861	4.7%
Pneumonia	2 855	4.7%
Secretory otitis media	2 393	3.9%
Influenza	914	1.5%
Otalgia	550	0.9%
Mononucleosis	422	0.7%
Acute laryngitis	354	0.6%
Scarlatina	209	0.3%
Peritonsillitis	135	0.2%

[a] The first seven diagnoses of respiratory tract infections were present in the twenty most common diagnoses.

[b] Number of this diagnosis

[c] Proportions of all diagnoses of respiratory tract infections (n = 60 818)

Some of these diagnoses overlap. Tonsillitis and pharyngitis may be similar groups of patients. Some patients with cough may have been diagnosed as acute bronchitis or, some with chronic bronchitis or asthma may have been diagnosed as cough.

Common respiratory tract infections constitute a large part of the general practitioners daily workload, thus resulting in high costs for the health care system [3-5]. Opinions vary on the diagnosis and treatment of these infections [6-16]. The increase in antibiotic resistance during the last ten years, has made it obvious that doctors cannot continue to prescribe antibiotics at as before [17-19].

2.2. Infectious diseases and the socio-economic history

Uncomplicated self-limiting respiratory tract infections, usually of viral origin results in many consultations and vast amounts of prescribed antibiotics [5, 13, 14, 20]. However, this is a phenomenon that has only existed during the last few decades. Prior to this, mankind had greater health problems than self-limiting respiratory tract infections. Furthermore, the most important factors for the reduction in mortality related to infectious diseases were previously the improvement of socio-economic conditions rather than antibiotic therapy.

Until the 20th century, epidemic infectious diseases such as plague, malaria and smallpox were a scourge to mankind. During an epidemic a substantial number of the

population died. Most of the survivors gained immunity to the infection. The epidemic ended, but as soon as a new population of uninfected individuals had grown large enough, new epidemics appeared. An example of this is the plague. Historically the plague has intermittently killed large proportions of the population in Europe. From around the 16th century large areas in Europe were gradually cultivated and wooden houses were replaced with stone or half-timbered houses. Thus, rodents could not reside in the ceilings and *Yersinia pestis* could not be as easily transferred to humans in the room below. As a result of these socio-economic changes, Europe did not provide an attractive environment to the rodent population that was the reservoir of *Y. pestis*. The plague disappeared along with the rodent, the black rat, during the 17th and 18th century.

Another example is malaria a common disease for centuries in Europe. As a result of the agricultural changes in the 16th to 19th century, the number of domestic animals increased drastically. The most common malaria mosquito in the middle and northern parts of Europe, *Anopheles atroparvus*, is zoophilic. It means that it prefers cattle and other domestic animals to humans. Since the malaria parasite cannot develop in animals, the basis for its existence disappeared in major parts of Europe. The falling incidence of malaria in Europe began long before the discovery of chloroquine at the end of the 19th century.

Other interesting examples of diseases affected by socio-economic changes in the society are leprosy and tuberculosis. With deteriorating living standards, as in the 13th and 14th century, leprosy was a common endemic disease in Europe. With an improved standard of living, as in the15th century, leprosy disappeared. Tuberculosis seems to be dependent on the social conditions such as the standard of living and the mood in the society [21]. During the 18th century great socio-economic changes increased the average working hours by 50%. At the same time heating of houses and the standard of living deteriorated. In this century and in the beginning of the 19th century tuberculosis became widespread. Thereafter, higher standards of living caused the decline of this disease.

History has shown how socio-economic changes in a society can alter the panorama of infectious diseases. Thus, most infectious diseases may be seen as reflections of the interplay between mankind and the environment [21]. The impact of medical science on major epidemic and endemic infectious diseases, with the exception of smallpox, has been minimal [21, 22]. However, improved hygienic measures introduced by Semmelweis and others in the 19th century, the eradication of smallpox and the introduction of antibiotics after 1945, have further reduced the mortality of infectious diseases [21, 23]. This has subsequently contributed to overpopulation, megacities without proper sanitation, over-exploitation of natural resources, and widespread poverty [21]. This increases the risk for person to person transmission of infectious diseases putting great stress on the existing systems that assure safe water and sanitation [23]. There is reason to believe that the extreme exploitation of the African jungle made it possible for AIDS to reach populated areas [21]. All these changes provides the basis for explosive epidemics of infectious diseases in the developing world [23].

In the developed parts of the world other demographic changes in human and animal populations have occurred that may increase the number of infectious diseases in those populations. Examples of such changes may be, increasing number of children attending child day care, a growing ageing population and increasing number of global travellers. Technical advances made food production more industrialised with intensive animal rearing practices and use of antibiotics.

All these demographic changes enhance the frequency of infectious diseases, the antibiotic usage, and subsequently the development of antibiotic resistance [23]. What might the future consequences be of the present antibiotic usage? What type of problems related to infectious diseases will concern mankind in the future?

2.3. The antibiotic era

Paul Erlich predicted as early as 1906 the possibility of antibacterial compounds (antibiotics) for the treatment of infectious diseases [22]. In 1928 Sir Alexander Flemming discovered penicillin [24]. When sulphonamides were introduced in the 1930s and when Florey and Chain in 1941 learned how to purify benzyl penicillin, the antibiotic era with all its possibilities had definitely begun [25].

In the 1940s, compounds other than benzylpenicillin known to inhibit bacterial growth, were investigated and new antibiotics found [25]. This systematic search expanded during the following decades, but the discovery of new formulae of antibiotics began to decline in the 1960s [26]. A new strategy was needed. The following generation of antibiotics was developed by synthetic modification of known compounds [26]. In the 1980s, there was a greater selection of antibiotics to choose from. However, during the 1980s this phenomenon was repeated and new discoveries of antibiotics declined [25]. Most antibiotics introduced during later years were very similar to their predecessors [25]. New techniques with genetic engineering will hopefully help us to identify new antibiotics [27]. However, antibiotic resistance will probably still remain a threat.

2.4. Resistance to antibiotics

Already in 1909 Paul Erlich predicted that bacteria would develop resistance towards antibiotics. The development of new antibiotics has been followed by the development of antibiotic resistance [23, 28] and antibiotic resistance is a worldwide phenomenon. The three principal mechanisms for bacterial antibiotic resistance are: (i) reduction of the amount of antibiotics within the bacterium, (ii) changes in the bacterium to prevent the drug from binding to the bacterium, and (iii) inactivation of the antibiotics [29]. Antibiotic resistance in *S. pneumoniae* is an example of the second mechanism, i.e. alteration of the different kinds of penicillin binding proteins that resides in the bacterium.

Once a resistant bacterium occurs it will be favoured in relation to sensitive bacteria by the presence of a certain level of antibiotic usage [17]. Several studies reports a link between the development of antibiotic resistance and the use of antibiotics [17, 19]. Theoretical models predict that, antibiotic resistance will not be a problem in a situation with low antibiotic consumption. With increasing antibiotic usage, resistance will increase, at first slowly and then, if antibiotic usage continues to increase, more rapidly to a situation where the resistant strains dominate [17, 18].

In upper respiratory tract infections the resistance to penicillin in *S. pneumoniae* is of vital importance because *S. pneumoniae* may cause lethal pneumonia and meningitis [23]. Antibiotic resistance of *S. pneumoniae* to penicillin was first discovered in Australia in 1967. It has gradually increased worldwide and in many countries 30-50% of all isolates of *S. pneumoniae* are resistant to penicillin. Lower frequencies of pneumococcal resistance have been reported from Germany, the Netherlands, Norway, Finland, Denmark and Sweden.

A few studies report that lowering the antibiotic usage probably decreases the proportion of resistant bacteria [30]. The reason for this could be that developing resistance to antibiotics usually results in lower virulence. In some cases however,

the antibiotic resistant bacterium seems to be almost as virulent as susceptible strains, as is the case with *S. pneumoniae* [31, 32]. In such circumstances, if antibiotic pressure is reduced, it may take longer for antibiotic resistance to disappear than to appear. Furthermore, if the resistant bacteria can accumulate compensatory mutations that restore the virulence, then they may maintain antibiotic resistance even if the usage of antibiotics is reduced [25].

2.5. Respiratory tract infections and antibiotics

As respiratory tract infections represent one of the main reasons for antibiotic therapy [1, 5, 20] the diagnostic procedure for patients with this type of infection is of vital importance if the usage of antibiotics is to be diminished.

The diagnosis and treatment of patients with an upper respiratory tract infection involves two choices. The first is to decide if the etiology of the infection is a virus or a bacterium. The second choice is to decide whether to prescribe antibiotics or not. It is usually not possible to differentiate between viral and bacterial respiratory tract infections on clinical grounds only [1, 33-37]. Microbiological tests such as nasopharyngeal cultures, throat cultures, or rapid tests for detection of *group A beta-haemolytic streptococci (BHS)* may improve our diagnostic accuracy [37-40]. In throat cultures *Beta-haemolytic streptococci (BHS)* are routinely identified by the microbiologic laboratory. In nasopharyngeal cultures *Moraxella catarrhalis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* as well as *BHS* are routinely identified. *Bordetella pertussis* can be detected in nasopharyngeal samples if specifically asked for and by using specific culture techniques.

The use of throat and nasopharyngeal swab *sample*s in patients with respiratory tract infections varies between different countries [41] and between different practices [42, 43]. Recommendations of diagnostic therapeutic procedures in upper respiratory tract infections are sometimes conflicting [7-9].

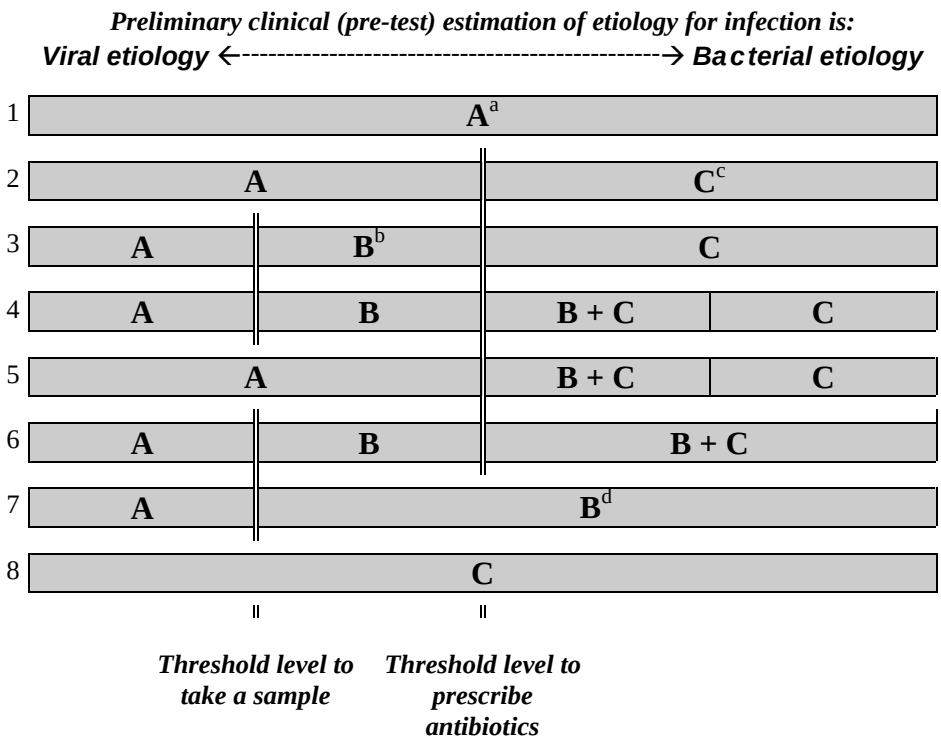
It should be noted that nasopharyngeal cultures are most often used in cases of therapeutic failure when treating acute otitis media and for diagnosis of an infection caused by *B. pertussis*. However, there are some reports or expressed opinions that a nasopharyngeal culture may be used to distinguish between viral or bacterial etiology in pneumonia [44-46], longstanding cough [47-49] or other respiratory tract infections [1, 50, 51].

Although the literature states that it is unreliable to use the clinical picture to distinguish a viral respiratory tract disease from a bacterial one, doctors do make preliminary clinical diagnoses before confirmatory laboratory tests are taken. Based on a preliminary clinical judgement, patients with an upper respiratory tract infection may roughly be divided into three categories: probable viral infection, probable bacterial infection, or an uncharacteristic infection [52, 53]. For some of these patients the doctor may choose to obtain a throat or nasopharyngeal swab sample to confirm the presence of potentially pathogenic bacteria.

2.6. The diagnostic procedure

It is possible to identify eight different diagnostic and therapeutic strategies in the consultation of a patient having a respiratory tract infection. From an individual doctor’s perspective, the chosen strategy is the guide for if and when a sample confirming the presence of potentially pathogenic bacteria should be taken. It also guides in the prescription of antibiotics (Figure 1).

Figure 1 – Doctors will adapt to one of eight possible diagnostic and therapeutic strategies. The doctor usually adheres to the chosen strategy.



[a] No sample to confirm presence of a bacterium and no antibiotic therapy

[b] Swab sample to confirm presence of potentially pathogenic bacteria

[c] Prescribe antibiotics

[d] A sample for epidemiological reasons only

Strategy number one, two and eight (in Figure 1) represents doctors who never use swab samples to confirm the presence of potentially pathogenic bacteria. As seen (in Figure 1) there are two important threshold levels affecting the amount of antibiotics prescribed: the threshold level to obtain a swab sample and the threshold level to prescribe antibiotics. These threshold levels will vary between doctors [41, 42, 54-63]. Findings suggesting that these threshold levels may and could be changed are:

- Doctors in lone practices prescribe more antibiotics than those in health centres with several doctors [56]. This implies that doctors in lone practices may be less influenced by recent medical research concerning antibiotic resistance.
- The need for a return visit by the patient was not higher for low prescribers compared to high prescribers [55, 58]. This implies that low prescribers do prescribe enough antibiotics.
- The influence of diagnostic tests on whether to prescribe antibiotics or not to patients with a respiratory tract infection is usually low [58, 64].
- Doctors who rarely take throat swabs tend to be high prescribers of antibiotics [63].
- A reduction in antibiotic usage for respiratory tract infections can be achieved by an educational programme [59, 65].

A large proportion of patients with uncomplicated respiratory tract infections are treated with antibiotics more often than necessary [5, 55, 63, 66]. The causes for inappropriate antibiotic prescription seems to be:

- It is easy to write a prescription [20] and it is believed to reduce the doctor's workload because patients are believed to be cured [58] and do not need to come back.
- Patients want antibiotics [58] and it is difficult to withstand the social pressure [20].
- Prescribing doctors want to cover all possible etiologic agents [20] to avoid any risk of sequel for the patient [58].
- Antibiotics are perceived to be nontoxic by doctors [20].
- The underlying reasons for a consultation are misinterpreted. Increased anxiety in some parents results in more visits for their children to doctors, and subsequently more prescriptions of antibiotics to the children [58].

None of the most common causes for inappropriate antibiotic prescription involve the use of diagnostic tests to detect presence of potentially pathogenic bacteria. The impact of diagnostic tests in the decision to prescribe or not prescribe antibiotics is often low [58]. How can those few patients with a respiratory tract infection that need antibiotic therapy be identified? A prerequisite for developing and redefining guidelines in this subject is proper information on how to use available tests to confirm or exclude the presence of potentially pathogenic bacteria.

2.7. Evaluation of microbiologic diagnostic tests

A test to diagnose a disease caused by a microbiologic agent usually has a dichotomous outcome: presence or no presence of the etiologic agent. A fundamental prerequisite for its usefulness is that a test designed to detect a bacterium can detect this bacterium better than if the doctor made a guess based on a preliminary clinical observation. In some situations the doctor's guess of viral or bacterial etiology is not much more accurate than setting the diagnosis by flipping a coin. When can it be expected that the test provides more information than a random choice? In order to answer this question the test may be described by means of sensitivity and specificity, or by various indices such as the Youden index [67], the efficiency [68], the index of validity [67] or kappa [69]. The Youden index is dependent on sensitivity and specificity while indices of validity and efficiency are also dependent on the prevalence of disease. Thus they are more informative than the Youden index. The disadvantage of all the indices is that they do not differentiate between the outcome growth of bacteria (T^+) or no growth of bacteria (T^-). In some tests T^- may be highly relevant but T^+ of little value. An example of this is the outcome of throat cultures in children (as will be shown later in this dissertation). However, likelihood ratios or predictive values consider T^+ and T^- separately.

Likelihood ratios depend on sensitivity and specificity alone. Since predictive values also depend on the prevalence of disease they yield more information concerning the evaluation of bacterial cultures than likelihood ratios. The positive likelihood ratios provide information about how much more the odds, for the phenomena the tests is design to detect, increases in case of a positive test. Likelihood ratios cannot be used in clinical practice unless you know the pre-test odds or pre-test probability. The positive predictive value (PPV) provides you with the probability of the phenomenon the test is design to detect.

Although predictive values seem to be the ideal measure of a test it does not take into consideration the presence of symptomatic carriers (individuals harbouring the

agent our test is supposed to detect and at the same time ill by something else, usually a virus). Methods that may consider asymptomatic carriers are relative risk and hypothesis testing.

2.7.1. Sensitivity and specificity

In order to evaluate a test, sensitivity and specificity are most often used [67, 70, 71]. They are calculated by comparing the observed test outcome with the outcome of the gold standard in a sample of n subjects:

	Gold standard is... ...positive	...negative	
Positive test (T^+)	a	b	a+b
Negative test (T^-)	c	d	c+d
	a+c	b+d	

$$\text{Sensitivity} = \frac{a}{a+c} \qquad \text{Specificity} = \frac{d}{b+d}$$

The sensitivity is mathematically independent of the disease prevalence. However, if the test is a microbiologic diagnostic test, in situations with a low disease prevalence, every test will probably be examined less carefully compared to a situation with a higher disease prevalence. Thus, a decrease in the disease prevalence might reduce the sensitivity of the test. A well-known effect on the sensitivity is seen by altering the cut off limit for considering the test as positive, an issue of great interest for manufacturers of rapid tests for detection of *GABHS*. These phenomena can be studied by constructing Receiver Operating Characteristic curves (ROC-curves). As long as the disease prevalence is below 50%, the influence of the disease prevalence on the sensitivity is small [72].

It could be appropriate to say that the sensitivity and the specificity inform you about the health status of your test rather than the health status of your patient [70]. Therefore, there is also a need for another method to evaluate throat and nasopharyngeal culture.

2.7.2. Youden's index

As a measure of a tests efficiency Youden in 1950 suggested an index (J) [67]:

$$J = \text{Sensitivity} + \text{Specificity} - 1$$

This index does not take into account the prevalence of disease and therefore it contains less information than index of validity or efficiency. The Youden index is rarely used.

2.7.3. Index of validity and efficiency

One way of characterising a diagnostic test is to calculate the proportion of correctly classified individuals as an index of validity (I_v).

	Gold standard is... ...positive	...negative	
Positive test (T^+)	a	b	a+b
Negative test (T^-)	c	d	c+d
	a+c	b+d	n=a+b+c+d

$$I_v = \frac{a+d}{n}$$

If the sensitivity and the specificity are equal, then I_v is independent of the disease prevalence [67]. In all other situations, I_v depends on both the sensitivity, the specificity and the prevalence of disease [67]. The efficiency is the same as I_v multiplied by 100 and expressed in per cent [68].

2.7.4. Kappa

The index of validity is the probability of agreement between the test and the gold standard. Kappa is a modification of the index of validity. It compares the found agreement with the agreement that would be expected by chance. To understand the concept, kappa is calculated in an example. In the example a rapid test to detect *GABHS* is evaluated with conventional throat culture as the gold standard [73]:

Outcome of rapid test	Throat culture ...positive	...negative	
Positive test (T^+)	19	2	21
Negative test (T^-)	9	75	84
	28	77	105

$$I_v = \frac{a+d}{n} = \frac{19+75}{105} = 0.895$$

Thus, the index of validity was 0.895, which means that 89.5% of the cases had been correctly classified by the rapid test. Does this indicate that the rapid test is a useful test? By using kappa a better answer may be provided. To calculate kappa the found

index of validity is compared to the index of validity that could be expected if our gold standard and the rapid test worked independently. This means that 26.7% (28/105) of the gold standard tests and 20% (21/105) of the rapid tests will be positive, but that there is no correlation between the outcome of the two tests. Thus, the two tests will only have the same outcome by chance, not because their outcomes are correlated. The probability for both tests to be positive will then be $0.267 \times 0.2 = 0.0534$. The expected number of samples with a positive outcome in both the gold standard and the rapid test is $0.0534 \times 105 = 5.607$. The table may now be completed under independence between the gold standard and the rapid test:

Outcome of rapid test	Throat culture ...positive	...negative	
Positive test (T^+)	5.6	15.4	21
Negative test (T^-)	22.4	61.6	84
	28	77	105

$$I_v = \frac{a+d}{n} = \frac{5.6+61.6}{105} = 0.640$$

How much better is an index of validity of 0.895 compared to an index of 0.640? Kappa is designed to answer this question. Kappa (k) is the ratio between the improvement by using our test (0.895-0.640) and the possible scope for doing better than chance (1-0.640). In our example kappa is

$$k = \frac{0.895 - 0.640}{1 - 0.640} = \frac{0.255}{0.360} = 0.71$$

This could be considered as good agreement between our test and the gold standard [69]. The most common use of kappa is to evaluate inter-rater agreement between different measures of the same event.

A serious disadvantage with indices, like Youden's index, index of validity, efficiency and kappa, is that they do not distinguish between T^+ and T^-. It may often be found that one of the two possible outcomes is informative but not the other. This makes index of validity or efficiency less appropriate as methods for evaluating throat and nasopharyngeal cultures.

2.7.5. Likelihood ratio

How much better is our test than flipping a coin? Likelihood ratios are one method to provide this information. The likelihood ratios give us information about how much the disease probability has changed because of the test results. The formulae for positive likelihood ratio (PLR) and negative likelihood ratio (NLR) are

$$PLR = \frac{\text{Sensitivity}}{1 - \text{Specificity}} \quad \text{and} \quad NLR = \frac{1 - \text{Sensitivity}}{\text{Specificity}}$$

Likelihood ratios of a positive and negative test when flipping a coin are both 1; indicating that the pre-test odds for disease have not been altered by the test. The higher the likelihood ratio of a positive test, the more information will be obtained by a positive test. If the likelihood ratio of a negative test gets close to zero it will yield much more information than flipping a coin. As seen from the formulae above likelihood ratios are solely depending on sensitivity and specificity, and thus they are measures of the health status of your test. Thus, a high PLR does not necessary indicate that a positive test indicates presence of disease [74]. However, it can be shown that in case of a positive test

$$\text{Post - test odds} = \text{Pre - test odds} \times \text{PLR}$$

Thus, likelihood ratios will provide clinically valuable information if you know the pre-test odds for disease. You may then use likelihood ratios to calculate post-test odds which easily can be transformed into post-test probability for disease.

2.7.6. Predictive value of a test

The sensitivity, the specificity, all of the different test indexes mentioned above and the likelihood ratio do not solve the clinical diagnostic problem [70]. These statistical methods provide information of the health status of the test, but not the health status of our patients. In the doctor-patient situation the doctor wants to know the probability of disease in the patient. If the pre-test probability for the bacterial disease is known, then the post-test probability for this disease may be calculated using the likelihood ratio.

An early description of a formula that may be used for direct calculation of post test probability of disease was published in 1763 and is frequently refereed to as the Bayes' theorem [75]. Bayes' theorem can be formulated as

$$P(\mathrm{D}^+ \mid \mathrm{T}^+) = \frac{P(\mathrm{T}^+ \mid \mathrm{D}^+) \times P(\mathrm{D}^+)}{P(\mathrm{T}^+)}$$

$P(\cdot)$ denotes the probability of the condition within parenthesis, i.e. $P(\mathrm{D}^+)$ denotes the probability of disease (= prevalence of disease = pre-test probability of disease) and $P(\mathrm{T}^+)$ the probability of the event of getting a positive test result. $P(\ldots \mid \ldots)$ is the probability of the event indicated before the vertical bar if the conditions stated after the bar is fulfilled. $P(\mathrm{T}^+ \mid \mathrm{D}^+)$ is the probability of a positive test result in patients having the disease, i.e. sensitivity. Bayes' theorem is often transformed to

$$P(\mathrm{D}^+ \mid \mathrm{T}^+) = \frac{\text{Sensitivity} \times P(\mathrm{D}^+)}{\text{Sensitivity} \times P(\mathrm{D}^+) + (1 - \text{Specificity}) \times (1 - P(\mathrm{D}^+))}$$

$P(\mathrm{D}^+ \mid \mathrm{T}^+)$ is often named the positive predictive value (PPV). There is a corresponding negative predictive value (NPV) predicting the absence of disease in case of a negative test result expressed as

$$P(\mathrm{D}^- \mid \mathrm{T}^-) = \frac{\text{Specificity} \times (1 - P(\mathrm{D}^+))}{(1 - \text{Sensitivity}) \times P(\mathrm{D}^+) + \text{Specificity} \times (1 - P(\mathrm{D}^+))}$$

It is easier to understand the predictive values if their calculation is compared with the calculation of the sensitivity and the specificity.

	Gold standard is... ...positive	...negative	
Positive test (T^+)	a	b	a+b
Negative test (T^-)	c	d	c+d
	a+c	b+d	

$$\text{Sensitivity} = \frac{a}{a+c} \qquad \text{Specificity} = \frac{d}{b+d}$$

$$\text{PPV} = \frac{a}{a+b} \qquad \text{NPV} = \frac{d}{c+d}$$

PPV always increases with increasing disease prevalence [76]. PPV is mainly affected by the specificity and the prevalence of the disease [76]. As long as the sensitivity and the specificity are reasonably high, their effect on NPV is negligible. A low prevalence of disease will, if the sensitivity and specificity is reasonably high, result in a high NPV. Increasing the prevalence of disease will only have minimal effect on the NPV until the prevalence of disease reaches a high proportion [76].

For a better understanding, the flipping of a coin may illustrate the relation between sensitivity, specificity and predictive values. A common misconception is to equate flipping a coin with a predictive value of 50% [77]. By flipping a coin, there is a 50% chance that heads will come up (bacterial disease) or tails (viral disease); thus the sensitivity and the specificity are both 50%. Hence the predictive values of flipping a coin depends on the disease prevalence [77]. In the situation with the coin, the PPV will be the same as the disease prevalence and positive + negative predictive value will be 100%. If the disease prevalence is high, then it is possible to achieve a high PPV by flipping a coin and with low disease prevalence flipping a coin will yield a high NPV.

The concept of predictive values has gradually become more common. It is well established that the predictive values in most clinical situations provide more useful information on how to assess the clinical value of a test than sensitivity and specificity alone [52, 70, 74, 76, 78-80].

The event that is being predicted when applying the concept of predictive values to the situation of evaluating throat and nasopharyngeal cultures is the presence of potentially pathogenic bacteria and not if the patient is ill from the potentially pathogenic bacteria isolated! Not all patients with a positive test for presence of potentially pathogenic bacteria have a bacterial infection. Some of these patients may

be just symptomatic carriers of these potentially pathogenic bacteria with a concomitant viral infection. These patients may be misclassified as having an infection caused by the potentially pathogenic bacteria isolated. If the symptomatic carriers suffer from viral infections, antibiotic treatment should usually be avoided. Thus, the clinical value of microbiological testing is related to the prevalence of symptomatic carriers among the patients.

If symptomatic carriers exist and should be treated differently from patients ill from the etiologic agent, then the predictive values of the test is not good enough.

2.7.7. Relative risk

Symptomatic carriers of potentially pathogenic bacteria are common in many patients suffering from a respiratory tract infection. In such cases there is a need of a test evaluation method that involves information about the carriers. The concept of relative risk (RR) could be useful when comparing the outcome of the test in one population with the outcome of the test in another population [81]. When using RR there is no need for a gold standard. RR is thus defined as the increased risk in one study group compared to the risk in another group, for instance patients compared to healthy individuals:

	Study group		
	Patients	Healthy	
Positive test (T^+)	a	b	a+b
Negative test (T^-)	c	d	c+d
	a+c	b+d	

$$RR = \frac{a/(a+c)}{b/(b+d)}$$

Since the subjects of interest are chosen with regard to certain characteristics, such as the presence or absence of a respiratory tract infection, as opposed to the test outcome, then RR is a better choice than odds ratio [81].

2.7.8. Hypothesis test of two independent groups

Another possibility is to utilise information about asymptomatic carriers by comparing the prevalence of bacteria found in patients with healthy individuals. To compare proportions between two independent groups, Chi-square with or without Yates' correction could be useful. Fisher's exact test should be used in case of small numbers. The outcome of the hypothesis testing is a p-value and, $p<0.05$ indicates that the bacterium may be involved as an etiologic agent.

2.8. The choice between different evaluation methods

The only methods available to evaluate the ability of throat and nasopharyngeal cultures to predict viral or bacterial etiology and simultaneously consider the presence of asymptomatic carriers are the relative risk or hypothesis testing (Table II). The disadvantage of these methods is that the results, a relative risk or a p-value, are difficult to apply in clinical decision making. Although the predictive values do not consider carriers, their outcome may be easier to understand in the doctor-patient situation.

Table II – Outline of statistical methods to evaluate common microbiologic diagnostic tests with dichotomous outcome in the presence of asymptomatic carriers

	Separate[a]	Provides information on[b]:			Conclusions on[c]:	
	T^+ and T^-	Tests	Patients	Groups	Agent	Disease
Sensitivity and Specificity	×	×			×	
Youden's index		×			×	
Index of validity and efficiency		×			×	
Kappa		×			×	
Likelihood ratios	×	×			×	
Predictive values	×		×		×	
Hypothesis testing				×		×
Relative risk				×		×

[a] The evaluation method differentiates between growth of bacteria (T^+) and no growth of bacteria (T^-).

[b] An evaluation method provides one of three types of information:
1) The health status of your test, i.e. data about test performance
2) The health status of your patient, i.e. the probability that the patient has….
3) The relationship between groups, i.e. comparison of prevalence between groups

[c] The outcome may lead to different conclusions:
1) All methods using a gold standard predicting the presence of a possible etiologic agent, a bacterium or a virus, only provides information about the probable presence of this agent, not the presence of disease. Methods using a gold standard predicting the disease may provide information about the presence of disease.
2) Methods comparing patients with healthy individuals may provide information with implications about the presence of disease in patients.

A model to evaluate diagnostic tests that might be easy to understand in the doctor-patient situation is calculating both predictive values and likelihood ratios (Table III).

Table III – Interpretation of predictive values in combination with likelihood ratios

Predictive value		Likelihood ratio		
Pos. Pred.	Neg. Pred.	L-pos.	L-neg.	Interpretation
(>60%)		(>1.5)		The test supplies useful information.
(>60%)		(<1.5)		Prior to testing it may be assumed that the patient probably has the disease. The test only increases knowledge marginally.
(<60%)		(>1.5)		The test only provides information of limited clinical value.
(<60%)		(<1.5)		The test is not useful clinically.
	(>90%)		(>0.67)	Prior to testing it may be assumed that the patient probably doesn't have the disease. The test only increases knowledge marginally.
	(>90%)		(<0.67)	The test supplies useful information.
	(<90%)		(>0.67)	The test is not useful clinically.
	(<90%)		(<0.67)	The test only provides information of limited clinical value.

The limits for the likelihood ratios and the predictive values in the table are arbitrarily chosen as examples for easier understanding. Other limits may be more appropriate.

However, when evaluating throat- or nasopharyngeal cultures, the predictive values predict presence of bacterial species, but they do not predict presence of a disease caused by the bacterium found. Predictive values, taking symptomatic carriers into consideration, and predicting a disease caused by the bacterium, would be a superior method of evaluating bacterial cultures used in patients with a respiratory tract infection.

2.9. The gold standard and symptomatic carriers

A gold standard is necessary for calculating sensitivity, specificity, likelihood ratios and predictive values. It is either the accepted reference method or the best known predictor of the truth, hopefully both. In a situation where presence of a marker does not necessarily mean that the individual has a specified disease, there is a difference between predicting the presence of a marker and predicting the presence of a disease [39, 80]. Is the gold standard showing the presence of a marker or the presence of a disease? If the test indicates presence of a marker, for example *GABHS*, that may cause diseases as well as being transitional commensals, then it could be confusing as to what is actually being predicted. Thus, it is obvious that the

question of a proper gold standard ought to be discussed in every evaluation of a test [80, 82].

Predictive value of a direct test to detect *GABHS* has been estimated by using a conventional throat culture as the gold standard [73, 83-86]. A conventional throat culture has also been used as the gold standard to evaluate an office culture [87] or another conventional throat culture [88-90]. These predictive values do not relate to the prediction of streptococcal throat infection caused by *GABHS* but rather to the presence of *GABHS* in the throat [39]. The accepted strategy of not treating symptomatic carriers of *GABHS* sick from other causes, such as a virus, with antibiotics [91-93] creates an obvious need for a distinction between predicting a marker on the one hand and a disease on the other.

This problem has been in focus for years, especially in patients with a sore throat caused by *GABHS*. One attempt to solve the problem was the use of a significant rise in streptococcal antibody titers as the gold standard to predict the presence of a sore throat caused by *GABHS* as opposed to the presence of *GABHS* in the throat. This gold standard has been used to evaluate rapid tests for the detection of *GABHS* [94, 95] and to evaluate conventional throat cultures [95]. The crucial question in every test evaluation is how well the gold standard predicts the truth [70, 71, 80, 96]. Streptococcal antibody titers as the gold standard is questionable since several studies has shown them having great difficulties in predicting true streptococcal disease [91, 97].

In the study by Gerber et al [91] all patients with a sore throat received antibiotics and a throat culture was done. Streptococcal serology for antistreptolysin (ASO) and antideoxyribonuclease B (ADB) was performed in those patients that at the first follow up after 18-24 hours had growth of *GABHS* in the throat culture. A significant rise in antibody titers of two or more dilutions (≥0.2 log rise) between the first blood sample and convalescent sera four weeks later were considered to be a significant rise in streptococcal antibody titers. Thus, all patients belonged to one of three possible groups. Those with a negative throat culture (group one), those with growth of *GABHS* and a rise in streptococcal antibody titers (group two), and finally, those with growth of *GABHS* but no rise in streptococcal antibody titers (group three). The majority (80%) of patients in group one still had a sore throat at the follow up after 18-24 hours and only 32% experienced an overall improvement. In group two and three, only a few had throat pain at the follow up (8% and 9%) and most patients felt an overall improvement in their disease (92% and 91%). Both groups two and three experienced a dramatic improvement with no differences between the groups. This finding contradicts the theory that streptococcal antibody titers can distinguish symptomatic carriers with a viral disease from patients actually ill from *GABHS*. In fact there is no acceptable gold standard predicting throat infection caused by *GABHS* [39, 86].

The situation becomes more difficult if the doctor wants to have predictive values for a nasopharyngeal culture predicting the presence of a disease with bacterial etiology. There are several bacterial species and symptoms to consider compared to the situation with a sore throat caused by *GABHS*. However, some attempts have been made to provide predictive values for nasopharyngeal culture to predict bacterial etiology for otitis media [98]. If the gold standard is the presence of bacteria in a middle ear aspirate [98] and if those are considered to be sterile under normal conditions, then the predictive value may actually predict presence of the disease, acute purulent otitis media, with bacterial etiology. For whooping cough, there might be other ways to find a gold standard predicting the presence of cough caused by *B.*

pertussis [99]. Since asymptomatic carriers of *B. pertussis* are uncommon, one may interpret the predictive values as predicting cough caused by *B. pertussis*. Thus, nasopharyngeal culture is usually used in suspected cases of *B. pertussis* or in the event of therapeutic failure in acute purulent otitis media.

Using nasopharyngeal cultures to predict bacterial etiology of long-standing cough caused by *S. pneumoniae*, *H. influenzae* or M. catarrhalis will result in the same problem as with throat infection caused by *GABHS*. There is no appropriate gold standard predicting the presence of the particular disease. Predictive values will predict presence of bacteria, not presence of disease with bacterial etiology.

In order to predict the presence of the disease "a sore throat caused by *GABHS*" or, "long-standing cough caused by potentially pathogenic bacteria", and not just presence of bacteria, the estimation of the truth has to be made some other way. Finding a gold standard predicting disease caused by the found bacterium is an important challenge for future research [86]. One possible way to solve the problem is the use of a construct validity where one or more logical consequences of the specified disease are selected and defined as the gold standard [82]. In this way the methacoline challenge test was constructed where the response of exposure to methacoline is considered to be a gold standard for asthma [82]. Another way is to find a mathematical model that provides a theoretical gold standard that could be used to calculate the predictive values.

2.10. Aims of the dissertation

2.10.1. General aims

The aim of the present dissertation was to find a method to evaluate microbiological diagnostic tests, such as throat and nasopharyngeal cultures. The new method must consider symptomatic carriers and provide test characteristics with a meaningful interpretation for decision making in the clinical situation. Furthermore the aim was to see if a throat or nasopharyngeal sample could yield etiological information in patients having a respiratory tract infection and suffering from a sore throat or a long-standing cough. The aim was also to compare the outcome of throat and nasopharyngeal cultures between different geographical areas.

2.10.2. Specific aims

- Develop a new statistical method providing predictive values for disease caused by the bacterial specie found in a microbiological diagnostic test.
- Collect throat and nasopharyngeal samples, taken in routine medical care, from patients with a sore throat and provide descriptive statistics for the outcome of these cultures.
- Collect nasopharyngeal samples taken in the routine medical care from patients with long-standing cough and provide descriptive statistics for the outcome of these cultures.
- Collect throat and nasopharyngeal samples from healthy individuals and provide descriptive statistics for the outcome of these cultures.
- Compare the samples from symptomatic patients with the samples from healthy individuals with hypothesis testing, relative risk, and the newly developed statistical method.
- Obtain information on the variation between different geographical areas in the threshold level for taking throat or nasopharyngeal cultures.

- Obtain information on the variation between different geographical areas in the proportion of positive throat and nasopharyngeal cultures.
- Compare the newly found clinical value of throat cultures with descriptions in the literature of how most doctors use these cultures. When necessary, suggest changes concerning the recommendations for the use of throat cultures.

3. Methods

During a winter period (14/1-17/2 1991) and the following summer period (15/7-15/9 1991) throat and nasopharyngeal samples were collected from individuals living in the county of Elfsborg in the southwestern part of Sweden, a mixture of urban, village, and rural populations. The Ethics Committee, Göteborg University, approved the study. A new statistical method was developed to evaluate the data. During 1992 and 2000 a questionnaire was also sent to microbiologic laboratories in Sweden.

3.1. Selection of healthy individuals (I-III)

Throat and nasopharyngeal samples were obtained from healthy pre-school children, school children, and adults.

Samples from pre-school children, ≤6 years of age, visiting child welfare clinics were collected consecutively in four groups, depending on the type of day care. The parents on direct request gave information concerning the form of day care. The four groups were; presence at day care centres ≥30 hours/week (DCC+), presence at day care centres <30 hours/week (DCC-), family day care (FDC), and home care (HC).

Samples from school children, 7-15 years of age, were obtained from children at school. Samples from adults, ≥16 years of age, were obtained consecutively at primary health care centres when visiting as patients with a non-infectious condition.

All individuals lacked signs of respiratory tract infections, had not received antibiotics during the previous four weeks, and did not have known diabetes mellitus or an immunodeficiency disorder. These individuals were considered to represent healthy children and adults.

3.2. Selection of patients (I-III)

During the same periods, the results of cultures were registered from all the consecutive throat and nasopharyngeal samples sent to the microbiological laboratory in Borås with a referral stating that the patient had a sore throat or cough > 9 days. The *sample*s from patients with a respiratory tract infection and the *sample*s from the group of individuals with no sign of respiratory tract infection came from the same geographical area. The doctors, who were encouraged to ask the parents, gave information about type of day care. The different day care groups were the same as presented above (Section 3.1).

During the study periods a special protocol was used. The protocol consisted of written information about the study and was distributed to all primary health care centres and hospitals in the area. Further information to the involved personnel was given via telephone to the chief doctor, or by informing the doctors in person at the clinic. In this written information the doctors were asked to code the referrals stating the main symptom or diagnose. The available codes were:

- Cough > 9 days
- Acute otitis media in a child with a middle ear ventilation tube (grommet)
- Acute otitis media in a child not having a middle ear ventilation tube
- A sore throat

- Sneezing
- Sinusitis
- Other symptoms

Only referrals stating a sore throat or cough > 9 days were included in these studies.

3.3. Processing the throat sample (I)

All throat *sample*s were transported to the same diagnostic laboratory in modified Stuart medium. The swab was gently rolled over 1/3 of the surface of a blood agar plate, followed by streaking with a sterile loop over the rest of the surface. The plate was a double layered selective Columbia blood agar plate with Polymyxin B, Neomycin and Nalidixic acid. The plate was incubated overnight at 37°C in 5% CO_2. The swab was finally inoculated in a tube containing broth with serum for detection of streptolysin S. This tube was incubated overnight at 30°C. If the plate showed growth of *BHS* and the tube showed haemolysis, the sample was considered to contain *BHS*. If both were without signs of beta-haemolytic activity the agar plate was incubated for a further 24 hours. If there was still no sign of beta-haemolytic activity, the culture was declared negative. If the agar plate had beta-haemolytic colonies after the second incubation, another tube was inoculated with some of those colonies and incubated for 4 hours. If only the tube was positive after 24 hours, a new agar plate was inoculated from this broth and left overnight. If in doubt, verification of *BHS* was performed using the Streptex® (Wellcome) latex agglutination method. All growth was estimated semiquantitatively as follows: sparse = 1-10 colonies, moderate = 11-50 colonies, abundant >50 colonies. Each culture showing *BHS* underwent latex agglutination (Streptex®) to determine to which serogroup (Lancefield group) it belonged.

3.4. Processing the nasopharyngeal sample (II, III)

The samples were collected in routine medical care by the ordinary staff, physicians, nurses, or laboratory technician, trained in collecting throat and nasopharyngeal swab samples. The routine method was as follows; insertion of a thin flexible swab through one nasal aperture into the posterior wall of the nasopharynx and then placed in a tube containing modified Stuart medium.

The samples were transported to the same microbiological laboratory in modified Stuart medium. All the samples were inoculated onto blood and haematine agar, incubated in 5% CO_2 atmosphere at 37ºC. If no growth of relevant bacteria was seen after 48 hours the culture was declared negative. Beta-haemolytic streptococci (*BHS*), *Streptococcus pneumoniae, H. influenzae,* and *M. catarrhalis* were identified by standard procedures.

3.5. Questionnaires to microbiologic laboratories (IV)

In March 1992 a questionnaire was sent to all microbiologic laboratories in Sweden (n=30). At the time of the questionnaire, all microbiologic laboratories were publicly financed and responsible for a defined geographical area. All the laboratories routinely performed throat and nasopharyngeal cultures. The laboratories were asked for the size of the population in their area, the total number of throat and nasopharyngeal cultures during the year 1991, and their outcome.

In June 2000 a questionnaire was send to twelve microbiologic laboratories in Sweden. These laboratories were chosen because they all had a computerised system suitable to provide answers to our questions. These laboratories were asked to answer

the same questions regarding the years 1992-1999. A programming instruction accompanied the questionnaire. They were also asked about major changes in the population size during the last 10 years.

3.6. Statistical methods

3.6.1. Comparing results between patients and healthy controls (I-III)

In the hypothesis testing, chi-square with Yates' correction was used. When the numbers were small, Fisher's exact test, two tailed, was used. Relative risk with 95% confidence interval was used to compare groups. The statistical program used was Epi-Info version 6.04c from the Center for Disease Control (CDC) USA and WHO.

3.6.2. Evaluating inquiries to microbiologic laboratories (V)

The number of cultures per 1000 citizens and year was calculated. In a regression model using data from the year 1991 the correlation between the number of cultures per 1000 citizens and the outcome of these cultures in different areas were investigated.

To compare the different areas (laboratories), the difference in the propensity to perform a culture, as measured by number of cultures per 1000 citizens and year, were compared between the different laboratories. In this analysis, data from 1991-1999 was used. To further compare the areas, the differences in the proportion of throat cultures with growth of *GABHS* and the proportion of pertussis cultures with growth of *B. pertussis* between the different laboratories was analysed.

To evaluate changes over time, the same analysis for the comparison of the different areas was made using years as the group variable.

The method used to investigate differences between the groups (laboratories/areas or years) was one way ANOVA. If the variances differed statistically between the groups, tested by the Bartlett´s test for homogeneity of variance, then Kruskal-Wallis one way analysis of variance was used instead.

The statistical programme Epi Info version 6.04c from Center for Disease Control (CDC), USA was used.

3.6.3. Construction of new predictive value (IV)

The new quantity etiologic predictive value (EPV) was first constructed by using mathematical derivation. After the formulae was constructed, it was proven to be correct by replacing each of the included conditional probabilities by the ratio of probabilities that defines them and then simplifying the formulae. EPV was then applied to evaluate throat and nasopharyngeal cultures.

4. Results

A new quantity was defined as the etiologic predictive value (EPV). It predicts the presence of true disease rather than the presence of bacteria. This new method was then used to evaluate the usefulness of throat and nasopharyngeal cultures in patients with a sore throat or long-standing cough.

It was shown that confirming the presence of *GABHS* in adults with a sore throat or in children with a sore throat during the winter season is of high diagnostic value. Finding *H. influenzae* in the nasopharynx may predict the etiology in adult patients suffering from a sore throat. It was also shown that long-standing cough may be caused by *M. catarrhalis* in pre-school children and by *H. influenzae* in adults.

The proportion of positive throat and nasopharyngeal cultures differs between geographical areas. This might be important to consider before applying test evaluations made in another area with another proportion of positive cultures.

4.1. The quantity etiologic predictive value (EPV) (IV)

The etiologic predictive value is a new quantity constructed to provide predictive values predicting presence of a disease rather than presence of a bacterium. Etiologic predictive value also uses information from asymptomatic carriers. Since a specific etiology for disease is being predicted among patients, the term etiologic predictive value (EPV) was chosen. Confidence intervals for EPV were also constructed.

4.1.1. Definitions and expressions for EPV

The doctor usually wants to predict the presence of a specified disease (D), for example throat infection caused by the agent *GABHS*, among patients with similar symptoms (S) caused by various etiologic agents. The symptoms, for example a sore throat and fever, are similar for different etiologic agents. However, the treatment might be very different depending on the etiologic agent. To make a good prediction, a test (T) is often used, for example a throat culture, to detect the presence of a marker (M), for example *GABHS*, associated with the disease (D). Let T^+ indicate a positive test and T^- a negative one. Let M^+ indicate the presence of the marker, for example *GABHS*, and M^- absence. Let D^+ indicate the presence of the disease the doctor wants to predict, for example throat infection caused by *GABHS*, and D^- absence. The population D^- may have symptoms, as long as the symptoms are not caused by *GABHS*. To predict disease caused by *GABHS*, information from a group of healthy individuals (S^-) can be used and the outcome could be compared to that of a group of symptomatic patients (S^+). The healthy control population and the population of patients have to be comparable in respect to confounding factors like age. The patients whose illness is caused by our marker will be described as S^+D^+, and thus, the patients ill from something else than the marker M will be described as S^+D^-. An appropriate term for the population S^+D^- could be symptomatic carriers. In this situation it is appropriate to use $P(\ldots|\ldots)$ for the probability of the event indicated before the vertical bar if the conditions stated after the bar are fulfilled. Positive EPV (PEPV) is defined as $P(D^+|S^+T^+)$ and negative EPV (NEPV) as $P(D^-|S^+T^-)$. For these two predictive values it can be shown that, under reasonable assumptions, the following expressions are valid:

$$P(D^+|S^+T^+) = 1 - \left(\frac{\frac{\text{Sen}}{P(T^+|S^+)} - 1}{\frac{\text{Sen}}{P(T^+|S^+D^-)} - 1} \right)$$

Formula 1 – Positive EPV

$$P(D^-|S^+T^-) = \left(1 + \frac{1 - \text{Sen}}{\text{Sen} - P(T^+|S^+D^-)} \right) \times \left(1 - \frac{1 - \text{Sen}}{1 - P(T^+|S^+)} \right)$$

Formula 2- Negative EPV

where Sen is the sensitivity with which the test T discovers the marker M, i.e.

$$\text{Sen} = P(\text{T}^+ \big| \text{M}^+)$$

The step by step construction of these formulae is shown in the section "9.1 Appendix: Derivation of the formulae for EPV" starting on page 73. A simple proof for the formulae is given in the section "9.2 Appendix: Proving the formulae for EPV" starting on page 83.

4.1.2. Formulae for interval estimate of EPV

When estimating sensitivity and specificity it is appropriate to present an interval estimate [77, 100]. This is rarely done in articles on evaluating diagnostic tests [100]. The precision of predictive values, just as in the case with sensitivity and specificity, is dependent on the size of our sample [100]. It is therefore also appropriate to use some kind of interval estimate for predictive values. Some methods for calculating an interval estimate of predictive values exist but they require a gold standard identifying disease or a previously known prevalence of disease [100, 101]. Both prerequisites may sometimes be difficult to achieve. If previously known prevalence is used the study combines results of independent studies. This might be questionable if the prevalence of the specified disease varies in time and geographical location.

It can be shown that, confidence limits for the positive etiologic predictive value are (compare with Formula 1 on page 27)

$$\left(1-\left(\frac{\frac{\text{Sen}}{a}-1}{\frac{\text{Sen}}{d}-1}\right)\right),\left(1-\left(\frac{\frac{\text{Sen}}{b}-1}{\frac{\text{Sen}}{c}-1}\right)\right)$$

Formula 3 - Confidence interval for PEPV

and that confidence limits for the negative etiologic predictive value are (compare with Formula 2 on page 27)

$$\left(\left(1+\frac{1-\text{Sen}}{\text{Sen}-c}\right)\times\left(1-\frac{1-\text{Sen}}{1-b}\right)\right),\left(\left(1+\frac{1-\text{Sen}}{\text{Sen}-d}\right)\times\left(1-\frac{1-\text{Sen}}{1-a}\right)\right)$$

Formula 4 - Confidence interval for NEPV

The step by step construction of these formulae is presented in the section "9.3 Appendix: Derivation of the interval estimate of EPV" starting on page 87.

4.1.3. Estimating EPV from samples

Next step is to explain how to estimate positive and negative etiologic predictive values from samples. The expressions (Formula 1) and (Formula 2) contain the three probabilities

$$\text{Sen},\ P(\text{T}^+ \mid \text{S}^+),\ P(\text{T}^+ \mid \text{S}^+\ \text{D}^-)$$

as well as

$$P(\text{T}^- \mid \text{S}^+),\ \text{and}\ P(\text{T}^- \mid \text{S}^+\ \text{D}^-)$$

Here each of the last two probabilities is the complement of one of the three first, so therefore only the three first require consideration.

Since Sen has nothing to do with the disease D, one may assume Sen to be known from previous experience. The probability $P(T^+ \mid S^+)$ is estimated using test results from symptomatic patients. A small p denotes an estimate of P and N(·) denotes the number of persons with (·):

$$p(T^+ \mid S^+) = \frac{N(T^+S^+)}{N(S^+)}$$

Formula 5 - Probability of a positive test in patients

The remaining probability, $P(T^+ \mid S^+D^-)$, requires careful consideration. It is not possible to differentiate patients with throat pain caused by *GABHS* (S^+D^+) from symptomatic carriers ill by another agent (usually a virus) but carrying *GABHS* ($S^+M^+D^-$). Thus, no scientific study to date has been able to estimate $P(T^+ \mid S^+D^-)$ in our example with throat infection. The relation between the two probabilities may be described as

$$P(T^+ \mid S^+D^-) = P(T^+ \mid S^-) \times q$$

Formula 6 - Probability of a positive test in patients ill from another cause

where the factor q in most situations can be assumed to be 1. Further aspects of q are presented in the discussion (Section 5.1.2 beginning on page 46). The probability $P(T^+ \mid S^-)$, is estimated using test results from healthy individuals:

$$p(T^+ \mid S^-) = \frac{N(T^+S^-)}{N(S^-)}$$

Formula 7 - Probability of a positive test in healthy individuals

By using the expressions (Formula 5 - Formula 7) the probabilities needed to estimate EPV (in Formula 1 and Formula 2) can easily be calculated.

Concerning the estimation of the confidence limits for EPV, the values a, b, c and d are calculated by taking the output from the formulae (Formula 5) and (Formula 7) and inserting it into Formula 8 - Formula 11:

$$a = p(T^+ \mid S^+) - \left(Z_{á/4} \times \sqrt{\frac{p(T^+ \mid S^+) \times \left(1 - p(T^+ \mid S^+)\right)}{\#(S^+)}} \right)$$

Formula 8 - Lower confidence limit for positive tests in patients

$$b = p(T^+ \mid S^+) + \left(Z_{á/4} \times \sqrt{\frac{p(T^+ \mid S^+) \times \left(1 - p(T^+ \mid S^+)\right)}{\#(S^+)}} \right)$$

Formula 9 - Upper confidence limit for positive tests in patients

$$c = p(\mathrm{T}^+ \mid \mathrm{S}^+\mathrm{D}^-) - \left(Z_{\alpha/4} \times q \times \sqrt{\frac{p(\mathrm{T}^+ \mid \mathrm{S}^-) \times \left(1 - p(\mathrm{T}^+ \mid \mathrm{S}^-)\right)}{\#(\mathrm{S}^-)}} \right)$$

Formula 10 - Lower confidence limit for positive test in patients ill from another cause

$$d = p(\mathrm{T}^+ \mid \mathrm{S}^+\mathrm{D}^-) + \left(Z_{\alpha/4} \times q \times \sqrt{\frac{p(\mathrm{T}^+ \mid \mathrm{S}^-) \times \left(1 - p(\mathrm{T}^+ \mid \mathrm{S}^-)\right)}{\#(\mathrm{S}^-)}} \right)$$

Formula 11 - Upper confidence limit for positive test in patients ill from another cause

To obtain a confidence interval for EPV with a confidence level of at least 0.95 let

$$Z_{\alpha/4} = Z_{0.05/4} = Z_{0.0125} = 2.24$$

When *a*, *b*, *c*, and *d* are calculated, confidence limits for EPV may be calculated by using the expressions (Formula 3) and (Formula 4).

4.1.4. EPV applied to evaluate throat culture (I, IV)

Consider the following example: A conventional throat culture has been obtained during a summer period from 36 children 3-15 years of age, showing signs of possible *GABHS*-caused tonsillopharyngitis (I). Among those 36 cultures growth of *GABHS* was found in 11 (31%). If the sensitivity of a throat culture to confirm the presence of *GABHS* in the throat is estimated to be close to 90% [34, 36, 39, 88, 89, 102] and the specificity is estimated to 97% [88, 89] then the prediction, with 95% confidence interval is:

Positive predictive value (PPV):	91%	(74-100%)
Negative predictive value (NPV):	96%	(88-100%)

In this example the high positive predictive value might be interpreted as that a child with a positive throat culture probably has an illness caused by *GABHS*. However, in this case no consideration has been made to the fact that some of the children are ill due to a virus as well as carrying *GABHS*. During the same period of time throat cultures were obtained from 290 healthy children 3-15 years of age living in the same geographical area, and showing no signs indicating possible *GABHS*-caused tonsillopharyngitis (I). Among those 290 cultures, growth of *GABHS* was found in 37 (13%). Assume that the carrier rate of *GABHS* is equal among healthy individuals and among patients with sickness due to a non-*GABHS*-caused tonsillopharyngitis [39, 91, 103, 104], then the prediction with regard to disease truly caused by *GABHS* can be calculated by using the formulae (Formula 1 - Formula 2) and (Formula 5 - Formula 7):

$$p(T^+|S^-) = \frac{N(T^+S^-)}{N(S^-)} = \frac{37}{290} = 0.1276$$

$$p(T^+|S^+) = \frac{N(T^+S^+)}{N(S^+)} = \frac{11}{36} = 0.3056$$

$$p(T^+|S^+D^-) = p(T^+|S^-) \times q = 0.1276 \times 1 = 0.1276$$

$$p(D^+|S^+T^+) = 1 - \left(\frac{\frac{\text{Sen}}{p(T^+|S^+)} - 1}{\frac{\text{Sen}}{p(T^+|S^+D^-)} - 1} \right) = 1 - \left(\frac{\frac{0.9}{0.3056} - 1}{\frac{0.9}{0.1276} - 1} \right) = 0.679$$

$$p(D^-|S^+T^-) = \left(1 + \frac{1 - \text{Sen}}{\text{Sen} - p(T^+|S^+D^-)} \right) \times \left(1 - \frac{1 - \text{Sen}}{1 - p(T^+|S^+)} \right)$$

$$p(D^-|S^+T^-) = \left(1 + \frac{1 - 0.9}{0.9 - 0.1276} \right) \times \left(1 - \frac{1 - 0.9}{1 - 0.3056} \right) = 0.967$$

The calculation of the confidence intervals is not shown. They are obtained by using the formulae (Formula 3 - Formula 4) and (Formula 8 - Formula 11). The formulae will result in a proportion between 0-1. Normally EPVs would be expressed, with 95% confidence interval, in per cent:

Positive EPV (PEPV):	68%	(0-91%)
Negative EPV (NEPV):	97%	(91-100%)

In this example, a positive throat culture does not provide information on the true cause of illness of our children. A negative culture however would provide valuable information and rule out *GABHS* as a possible cause.

Assume another example: A conventional throat culture has been obtained during a winter period from 168 adults ≥16 years of age, having signs indicating a possible *GABHS*-caused tonsillopharyngitis (I). Among those 168 cultures, growth of *GABHS* was found in 32 (19%). During the same period of time and from the same geographical area throat cultures were obtained from 328 healthy adults of matching age having no signs indicating a possible *GABHS*-caused tonsillopharyngitis (I). Among those 328 cultures, growth of *GABHS* was found in 1 (0.3%). The prediction, with 95% confidence interval, with regard to disease truly caused by *GABHS* (EPV) would then be:

Positive EPV (PEPV):	99%	(93-100%)
Negative EPV (NEPV):	97%	(96-99%)

Among these adult patients, both a positive and a negative throat culture would provide valuable information about the true cause of illness.

Our examples show that the negative predictive value of a negative test result predicting absence of bacteria in the throat and the NEPV predicting absence of true disease caused by the same bacterium will be almost the same. However, symptomatic carriers will make a major difference between positive predictive value of a positive test result predicting presence of bacteria in the throat and PEPV predicting true disease caused by the bacterium.

4.1.5. Alterations in the preconditions for EPV

When using the quantity EPV it is important that data from the healthy control group are collected during the same period of time and from approximately the same geographical area as the group of patients. This is necessary because the carrier rate and the prevalence of disease tend to vary by time and place. It can be shown that the effect of symptomatic carriers on NEPV is negligible but the effect is considerable on PEPV. With decreasing rates of symptomatic carriers the EPVs will approach the predictive values as if you were calculating them without taking carriers into consideration. If no symptomatic carriers are present each EPV will be the same as the corresponding usual predictive value, i.e., indicating presence of the marker.

In connection with EPV appears the ratio, theta (q), between the rate of asymptomatic and symptomatic carriers. It should be pointed out that q deals with proportions of individuals and not the quantities (for example the number of bacteria per ml of mucus) of bacteria in each individual. A viral infection may increase the quantity of bacteria in each infected individual but not necessarily increase the proportion of colonised individuals.

If possible, it is best to search the literature for the correct value of q. However, a correct value of q can be established only if a proper gold standard can be defined. If references describing a proper gold standard are available, then it is possible to use this gold standard and directly calculate the predictive value predicting true disease. If this gold standard is complicated, dangerous to the patient or expensive, it could be used in a small pilot study to establish q, the EPV could then be used for the main study. If, however, a proper gold standard cannot be defined, then the only alternative is an estimate based upon reasonable assumptions. The most obvious estimation is to assume that the rate of symptomatic carriers among patients who do not have the specified disease (symptoms caused by another agent) is the same as the rate of asymptomatic carriers among healthy controls. In the case of streptococcal throat infection, many authors make the assumption that q is close to 1 [39, 91, 103, 104]. If q has to be estimated, then it is reasonable to investigate how moderate changes in q will influence the EPV (Table IV). Changes in the sensitivity of the test, in q and in the prevalence of asymptomatic carriers will alter the EPV (Table IV).

Table IV - Alterations in the preconditions for EPV predicting disease caused by group A streptococci.

No alterations in the preconditions for computing EPV[a] in the example[b]	PEPV[c]	99%	(93-99)
	NEPV[d]	97%	(96-99)
Decrease in the sensitivity of the test[e] from 90% to 80%	PEPV	99%	(93-100)
	NEPV	94%	(91-97)
Increase in q [f] from 1.0 to 1.1	PEPV	99%	(92-100)
	NEPV	97%	(96-99)
Increase in the carrier rate [g] from 0.3% to 5%	PEPV	79%	(42-94)
	NEPV	98%	(96-99)
Increase in the carrier rate from 0.3% to 10%	PEPV	53%	(0-81)
	NEPV	99%	(97-100)

[a] Etiologic predictive value, predicting disease caused by group A beta-haemolytic streptococci.

[b] Example described in the text (328 healthy individuals and 168 adult patients with a sore throat. Winter season)

[c] Positive etiologic predictive value

[d] Negative etiologic predictive value

[e] Throat culture to detect group A beta-haemolytic streptococci

[f] Ratio between the proportion of symptomatic versus asymptomatic carriers.

[g] Asymptomatic carriers, i.e. the proportion of positive tests among healthy individuals

Thus, with reasonable changes in the preconditions for computing EPV, it may be shown that the prevalence of disease and the presence of symptomatic carriers are the most important factors in determining the clinical value of a throat culture (Figure 12 on page 57).

4.2. The prevalence of beta-haemolytic streptococci in throat samples from healthy individuals compared to patients with a sore throat (I)

The proportion of positive throat cultures was low before the age of 3 years and in adults ≥16 years of age (Figure 2). In healthy individuals a low prevalence of *GABHS* was seen between 7-9 years of age, 1.6% (95% confidence interval 0-4,7%), (Figure 2).

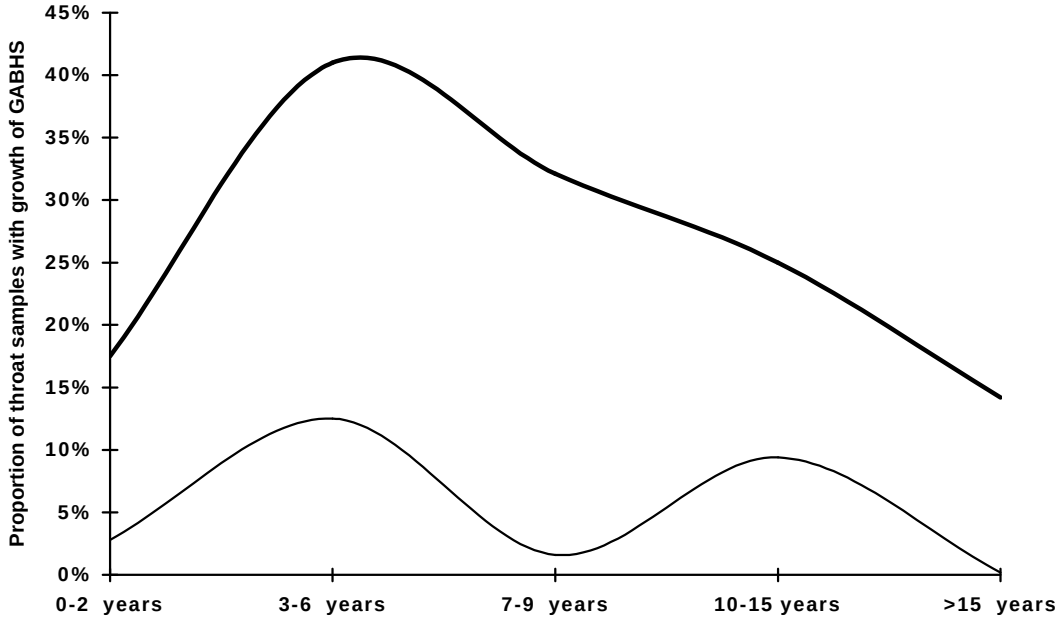


Figure 2 - Influence of age on proportion of throat cultures growth of GABHS.
Thick line = patients with a sore throat and thin line = healthy individuals

A seasonal variation was found that differed for patients with a sore throat compared to healthy individuals. With the exception of the age group 7-9 years, patients with a sore throat had, during the mid-winter season, a higher proportion of cultures with growth of *GABHS* compared to the late summer season (Figure 3).

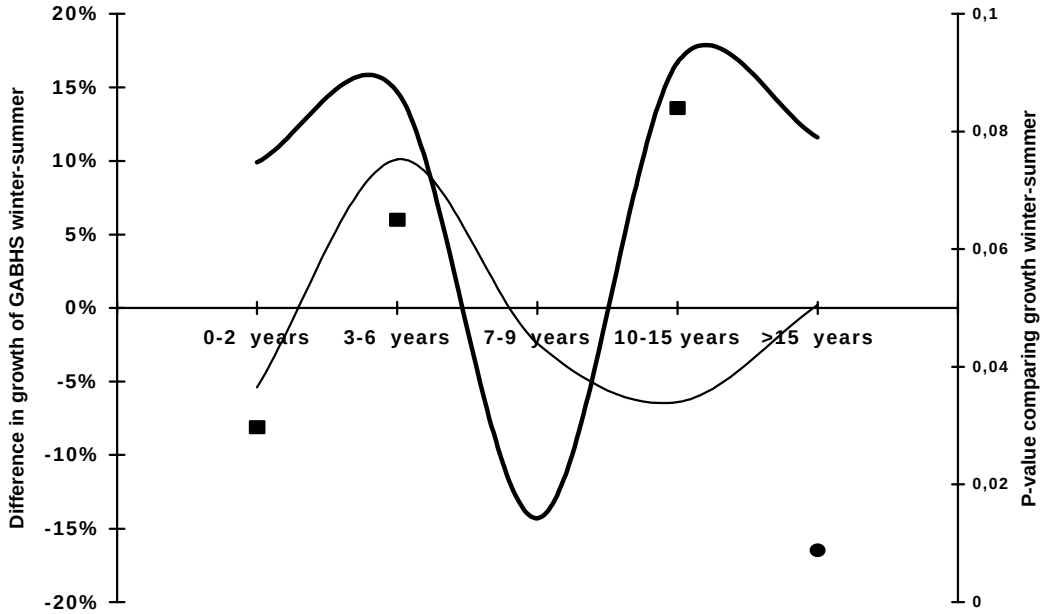


Figure 3 - Difference between winter and summer season in the proportion of positive throat cultures.
Thick line represents patients with a sore throat and thin line healthy individuals.
Squares represent p-values for seasonal comparisons in healthy individuals and a dot represents the same in patients. Missing square or dot indicates p>0.1.

As seen (in Figure 3), the only comparisons with a statistically significant difference of the proportion of positive cultures between seasons was for healthy young children 0-2 years of age and for adult patients.

Comparing difference in the proportion of positive cultures between patients and healthy individuals instead of comparing different seasons will give information of the importance of seasonal variation from a different perspective. The difference in the prevalence of *GABHS* between healthy individuals and patients with a sore throat was smaller during the late summer than during the mid-winter. During mid-winter a statistically significant difference was found between healthy individuals and patients in all age groups. In the late summer a statistically significant difference was only found for children 7-9 years of age and adults ≥16 years of age. Thus, during the summer season there was no statistically significant difference in the proportion of cultures with *GABHS* between healthy children and patients in the age groups 0-2 years, 3-6 years and 10-15 years.

Information concerning type of day care was obtained from 377 of the 400 (94%) non-infectious pre-school children. Specifying only *GABHS* will result in that, during winter season, a positive culture is more likely in healthy pre-school children belonging to DCC+ compared to those in FDC (p=0.04). For the 86 patients in pre-school ages with a sore throat, information was obtained about day care in 57 (66%). However, each day care group was too small to make further analysis among patients meaningful.

Semiquantification of *GABHS* showed that, if *GABHS* was present, abundant growth was found in 85% of the patients with a sore throat and in 41% of the healthy individuals. Sparse growth of *GABHS* was found in 3.7% of patients and in 18% of healthy individuals.

4.3. The prevalence of potentially pathogenic bacteria in nasopharyngeal samples from patients with a sore throat or long-standing cough

Of 108 nasopharyngeal samples with a referral stating that the patient had a sore throat no statistically significant difference was found in the prevalence of any bacterial species, or, in the prevalence of *H. influenzae*, *M. catarrhalis* and *S. pneumoniae*, among patients with a referral stating only sore throat, compared to patients with sore throat in combination with other symptoms. Thus, all referrals including a sore throat were merged into one group of patients. Only two of the 108 samples (2%) came from hospitalised patients.

Of 236 nasopharyngeal samples with a referral stating that the patient had long-standing cough, no statistically significant difference was found in the prevalence of *H. influenzae*, *M. catarrhalis* and *S. pneumoniae* among patients with a referral stating long-standing cough as the only symptom compared to patients with long-standing cough in combination with other symptoms. However, in adults, a difference in the prevalence of any potentially pathogenic bacteria was found (Figure 4).

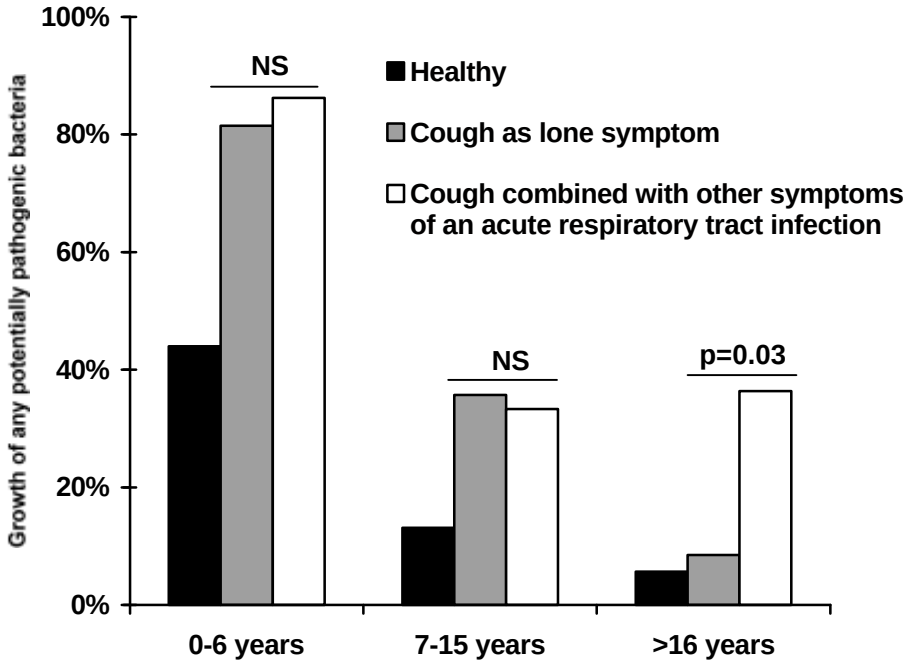


Figure 4 - Proportion of nasopharyngeal samples with growth of any potentially pathogenic bacteria

Thus, for pre-school children and schoolchildren, patients with a referral stating only long-standing cough, and patients with long-standing cough and other symptoms of an acute respiratory tract infection are combined into one group. Very few of the 236 samples (2 = 0.8%) came from hospitalised patients.

The proportion of nasopharyngeal cultures with growth of potentially pathogenic bacteria, such as *S. pneumoniae*, *H. influenzae* and M. catarrhalis decreased with age (Figure 5, Figure 6 and Figure 7)

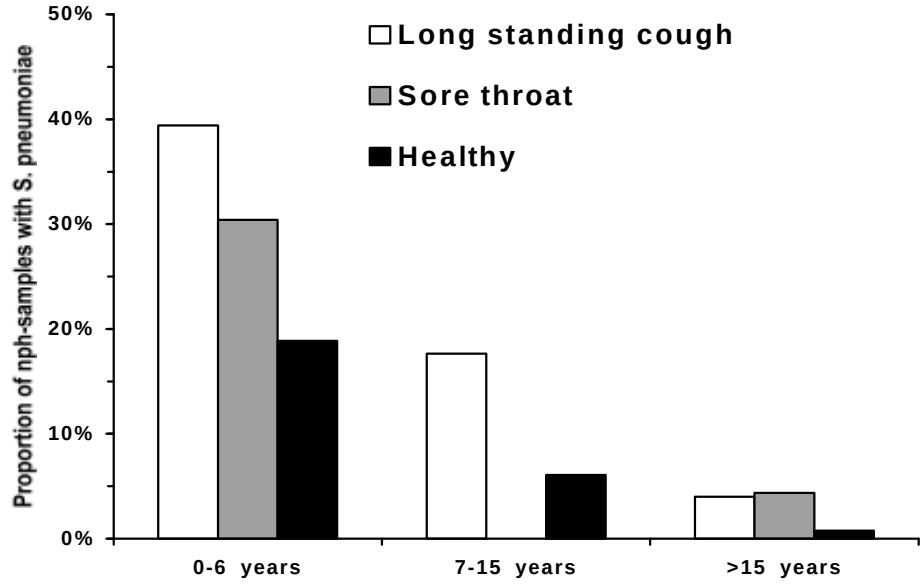


Figure 5 - Influence of age on proportion of nasopharyngeal cultures with growth of S. pneumoniae

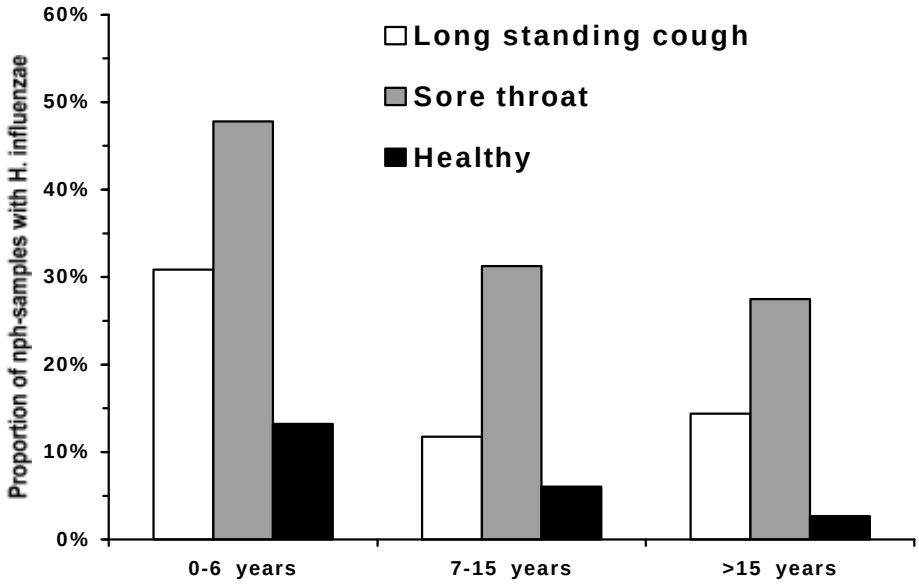


Figure 6 - Influence of age on proportion of nasopharyngeal cultures with growth of H. influenzae

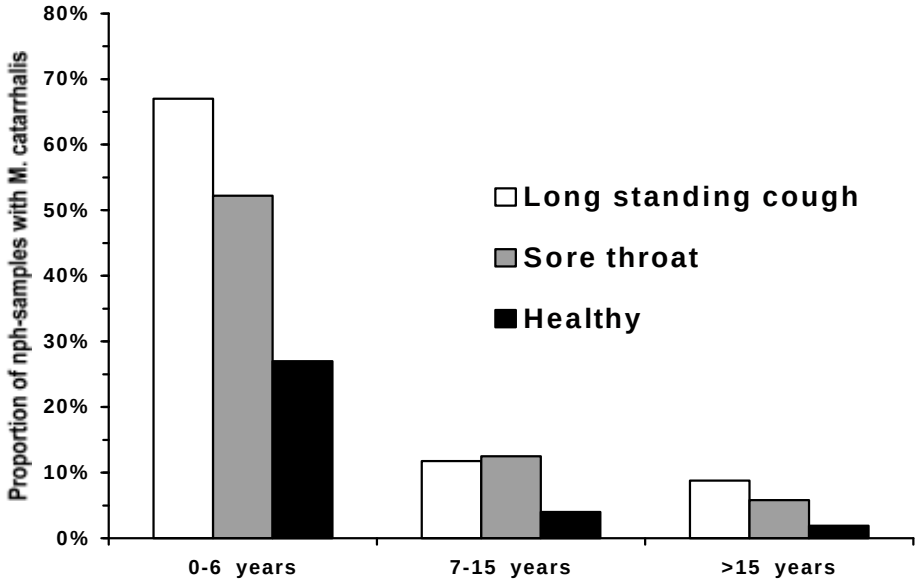


Figure 7 - Influence of age on proportion of nasopharyngeal cultures with growth of M. catarrhalis

If potentially pathogenic bacteria were found in the nasopharynx, from adults and school children with a sore throat, it was usually only one bacterium and the most common finding among them was *H. influenzae*.

The most common finding in pre-school children with long-standing cough was *M. catarrhalis* and in adults with long-standing cough it was *H. influenzae*. Growth of more than one bacterium was common in pre-school children with long-standing cough. However, the only combinations of potentially pathogenic bacteria in pre-school children associated with long-standing cough were those including *M. catarrhalis*.

4.4. Evaluation of the usefulness of a culture by using hypothesis testing, relative risk and EPV (I-IV)

Samples were collected from healthy individuals and patients with symptoms of a sore throat or long-standing cough. From patients with a sore throat, 435 throat swab samples and 108 nasopharyngeal swab samples were obtained. From patients with a long-standing cough, 236 nasopharyngeal swab samples were obtained. From healthy individuals 1297 throat swab samples and 618 nasopharyngeal swab samples were obtained. The difference in the proportion of growth between the symptomatic patients and the healthy individuals of the same age was compared by using three statistical methods; hypothesis testing for unmatched groups, relative risk (RR) for a positive culture outcome and the probability for true disease with the etiologic predictive values (EPV).

4.4.1. Comparing outcome of throat and nasopharyngeal cultures from patients with a sore throat and healthy individuals

In throat cultures a seasonal variation was found that was different between healthy individuals and patients with a sore throat. A corresponding seasonal variation in nasopharyngeal cultures was not found. Thus, throat samples were analysed for each season and with the seasons combined. In pre-school children it was found that the clinical value of a positive throat culture is high during the winter season (Table V). However, evidence supporting a clinical value of a positive throat culture among pre-school children during the summer season could not be found (Table V).

Table V – Comparison of the proportion of positive cultures between healthy children and children with a sore throat. Age group 0-6 years

Season and numbers[a]		Throat sample GABHS	Nasopharyngeal sample S. pneumoniae	H. influenzae	M. catarrhalis
Sum	p[b]	NS			
Th: 38/211	RR[c]	2.2 (1.1-4.3)[d]			
N: -----	PEVP[e]	**61 (0-91)**			
	NEPV[e]	98 (94-100)			
Win	p	$<10^{-7}$			
Th: 48/189	RR	11 (4.7-27)			
N: -----	PEVP	**94 (75-100)**			
	NEPV	94 (89-98)			
Sum+Win	p	$<10^{-7}$	NS	0.0003	0.03
Th: 86/400	RR	4.2 (2.6-6.7)	1.6 (0.8-3.2)	3.6 (2.0-6.5)	1.9 (1.2-3.1)
N: 23/159	PEVP	83 (53-94)	50 (0-90)	**86 (28-99)**	72 (0-97)
	NEVP	96 (93-99)	95 (77-100)	81 (43-98)	82 (29-100)

[a] Sum = Summer, Win = Winter, Sum+Win = Summer+Winter. Th: number of throat samples in patients/healthy, N: number of nasopharyngeal samples in patients/healthy

[b] P-value when comparing prevalence of bacteria between patients with sore throat and healthy individuals. NS = Not significant, $p>0.05$.

[c] Relative risk for prevalence of bacteria in patients with a sore throat compared to healthy individuals, 95% confidence interval within parentheses.

[d] It may seem strange that the confidence interval for relative risk does not contain the value 1 when the p-value is above 0.05. There are different ways to calculate p-values and confidence intervals for relative risks. In this case Fishers exact test (two tailed) was used for the p-value and the result was 0.061. Uncorrected chi-square would result in a p-value of 0.03.

[e] Positive (PEPV) and negative (NEPV) etiologic predictive value in %, 95% confidence interval within parentheses.

Furthermore, if *H. influenzae* is found in the nasopharynx in a pre-school child with a sore throat, it suggests that this bacterium is causing the sore throat (Table V).

During the summer season, there were small numbers of school children 7-15 years of age with a sore throat, thus, it was difficult to establish the clinical value of a positive throat culture in the summer season. It seems as if the clinical value of a positive throat culture in children 7-15 years of age is higher in winter compared to summer (Table VI).

Table VI – Comparison of the proportion of positive cultures between healthy children and children with a sore throat. Age group 7-15 years

Season and numbers[a]		Throat sample GABHS	Nasopharyngeal sample S. pneumoniae	H. influenzae	M. catarrhalis
Sum	p[b]	NS			
Th: 15/191	RR[c]	2.6 (1.0-6.5)			
N: -----	PEVP[d]	69 (0-95)			
	NEPV[d]	97 (88-100)			
Win	p	10^{-4}			
Th: 45/190	RR	5.0 (2.4-10)			
N: -----	PEVP	**85 (34-98)**			
	NEPV	96 (91-99)			
Sum+Win	p	10^{-5}	NS	0.004	NS
Th: 60/381	RR	3.5 (2.1-5.6)	-----	5.2 (2.1-13)	3.1 (0.7-13)
N: 16/198	PEVP	78 (30-93)	-----	87 (0-99)	71 (0-98)
	NEVP	97 (93-99)	100 (0-100)	91 (69-100)	98 (90-100)

[a] Sum = Summer, Win = Winter, Sum+Win = Summer+Winter. Th: number of throat samples in patients/healthy, N: number of nasopharyngeal samples in patients/healthy

[b] P-value when comparing prevalence of bacteria between patients with sore throat and healthy individuals. NS = Not significant, p>0.05.

[c] Relative risk for prevalence of bacteria in patients with a sore throat compared to healthy individuals, 95% confidence interval within parentheses.

[d] Positive (PEPV) and negative (NEPV) etiologic predictive value in %, 95% confidence interval within parentheses.

Growth of *GABHS* in a throat culture taken from an adult patient with a sore throat will, with at least 93% probability, indicate the true etiology of the infection. Furthermore, no growth of *GABHS* in a throat culture will, with more than 96% probability, rule out this bacterium as the etiologic agent of the sore throat (Table VII).

Table VII – Comparison of the proportion of positive cultures between healthy adults ³ 16 years and adults with a sore throat.

Season and numbers[a]		Throat sample GABHS	Nasopharyngeal sample S. pneumoniae	H. influenzae	M. catarrhalis
Sum	p[b]	0.0002			
Th: 121/188	RR[c]	-----			
N: -----	PEVP[d]	-----			
	NEPV[d]	99 (98-100)			
Win	p	$<10^{-7}$			
Th: 168/328	RR	62 (8.6-453)			
N: -----	PEVP	**99 (93-100)**			
	NEPV	97 (96-99)			
Sum+Win	p	$<10^{-7}$	NS	$<10^{-7}$	NS
Th: 289/516	RR	85 (13-1679)	5.7 (1.0-33)	10 (4.5-23)	3.0 (0.8-11)
N: 69/261	PEVP	**99 (94-100)**	83 (0-100)	**93 (73-99)**	69 (0-100)
	NEVP	98 (97-99)	99 (97-100)	92 (85-97)	99 (97-100)

[a] Sum = Summer, Win = Winter, Sum+Win = Summer+Winter. Th: number of throat samples in patients/healthy, N: number of nasopharyngeal samples in patients/healthy

[b] P-value when comparing prevalence of bacteria between patients with sore throat and healthy individuals. NS = Not significant, p>0.05.

[c] Relative risk for prevalence of bacteria in patients with a sore throat compared to healthy individuals, 95% confidence interval within parentheses.

[d] Positive (PEPV) and negative (NEPV) etiologic predictive value in %, 95% confidence interval within parentheses.

It was also found that, the correlation for adults having a sore throat and the presence of *H. influenzae* in the nasopharynx was very strong and of the same magnitude as for a sore throat and presence of *GABHS* in throat samples (Table VII).

4.4.2. Comparing outcome of nasopharyngeal cultures from patients with long-standing cough and healthy individuals

On the referral slip, long-standing cough could be either the only symptom, or could be combined with other symptoms. As mentioned, in section 4.3 page 35, adults had a difference in the prevalence of any potentially pathogenic bacteria between patients with cough alone compared to patients with cough combined with other symptoms. Thus, for adults these groups were analysed separately.

Findings of M. catarrhalis in pre-school children suggest that this bacterium is causing the cough (Table VIII). However, since the negative etiologic predictive value was only 57% one may conclude that, absence of M. catarrhalis is not enough to rule out this bacterium as etiologic agent.

Table VIII – Comparison of the proportion of positive cultures between healthy individuals and patients with a long-standing cough > 9 days.

Age, N and symptoms[a]		Nasopharyngeal sample S. pneumoniae	H. influenzae	M. catarrhalis
0-6 years	p[b]	0.0006	0.001	$<10^{-7}$
N: 94/159	RR[c]	2.1 (1.4-3.1)	2.3 (1.4-3.9)	2.5 (1.9-3.3)
S: C+CS	PEVP[d]	68 (12-90)	68 (6-91)	**90 (66-99)**
	NEPV[d]	90 (78-99)	93 (85-100)	**57 (18-80)**
7-15 years	p	NS	NS	NS
N: 17/198	RR	2.9 (0.9-9.3)	1.9 (0.5-8.0)	2.9 (0.7-13)
S: C+CS	PEVP	71 (0-97)	52 (0-95)	69 (0-98)
	NEPV	96 (86-100)	98 (91-100)	98 (90-100)
≥16 years	p	NS	0.004	0.02
N: 81/261	RR	3.2 (0.5-23)	4.1 (1.6-11)	3.9 (1.2-12)
S: C	PEVP	70 (0-100)	78 (0-98)	76 (0-100)
	NEPV	100 (98-100)	98 (95-100)	99 (96-100)
≥16 years	p	0.02	10^{-4}	NS
N: 44/261	RR	8.9 (1.5-52)	7.6 (3.0-19)	2.4 (0.5-12)
S: CS	PEVP	90 (0-100)	**90 (30-99)**	59 (0-99)
	NEPV	98 (96-100)	95 (88-99)	99 (97-100)
≥16 years	p	0.04	10^{-4}	0.003
N: 125/261	RR	5.2 (1.0-27)	5.4 (2.3-13)	34.6 (1.6-13)
S: C+CS	PEVP	82 (0-100)	84 (35-98)	80 (0-100)
	NEVP	99 (98-100)	97 (94-99)	98 (96-100)

[a] Age group in years. N: number of nasopharyngeal samples in patients/healthy. S: C = Only long-standing cough >9 days. S: CS = Long-standing cough combined with other symptoms of an acute respiratory tract infection. S: C+CS = Long-standing cough irrespective of whether combined with other symptoms of an acute respiratory tract infection.

[b] P-value when comparing prevalence of bacteria between patients with sore throat and healthy individuals. NS = Not significant, p>0.05.

[c] Relative risk for prevalence of bacteria in patients with a sore throat compared to healthy individuals, 95% confidence interval within parentheses.

[d] Positive (PEPV) and negative (NEPV) etiologic predictive value in %, 95% confidence interval within parentheses.

Furthermore, if *H. influenzae* is found in a nasopharyngeal swab sample from school children 7-15 years of age, or in adults ≥16 years of age with cough combined with other symptoms of a respiratory tract infection suggests that this bacterium is causing the long-standing cough (Table VIII).

4.5. Throat and nasopharyngeal cultures in different geographical areas (V)

A large variation was found between the different geographical areas in 1991, as represented by the laboratories, in the propensity to perform throat and nasopharyngeal (Table IX) cultures as well as, in the proportion of throat and nasopharyngeal cultures with growth of any potentially pathogenic bacteria (Table X).

Table IX – Variation between different geographical areas in Sweden 1991 in the propensity to send samples for a culture

Culture	Mean[1]	Range[1]	Cultures from a population of [2]
Throat	39.6	13.9-90.9	6 775 195
Nasopharyngeal	45.3	15.3-131	6 775 195
Pertussis	5.9	2.3-10.1	6 053 778

[1] Number of cultures per 1000 citizens reported between different laboratories during 1991

[2] Population covered by the laboratories with sufficient records

Table X – Geographical variations between different geographical areas during 1991 in the proportion of cultures with growth of potentially pathogenic bacteria

	Proportion (%)[1]			
Culture	Mean %	Range %	No of cultures	Cultures from a population of [2]
Throat - BHS[3]	19.1	11.7-39.1	156 221	4 010 318
Throat - GABHS[4]	13.4	3.3-21.6	164 745	4 298 310
Nasopharyngeal[5]	53.3	38.2-78.2	182 878	4 010 318
Bordetella pertussis	24.7	9.2-40.1	29 633	4 772 195

[1] Proportion of cultures with growth of bacteria reported from the laboratories during 1991

[2] Population covered by the laboratories with sufficient records

[3] Beta-haemolytic streptococci of group A, C and G

[4] Group A beta-haemolytic streptococci (syn. Streptococcus pyogenes)

[5] Growth of H. influenzae and/or S. pneumoniae and/or M. catarrhalis

A correlation in the data from 1991, between the propensity to perform a throat, nasopharyngeal or pertussis culture and the proportion of those cultures exhibiting growth of bacteria could not be demonstrated.

If the areas are followed for eight years, the proportion of throat cultures with growth of *GABHS* will vary between 7.2-24% (Figure 8). The proportion of throat cultures with growth of *GABHS* differed between the geographical areas. The statistical parameters was; n=85, p=0.00001, one way ANOVA.

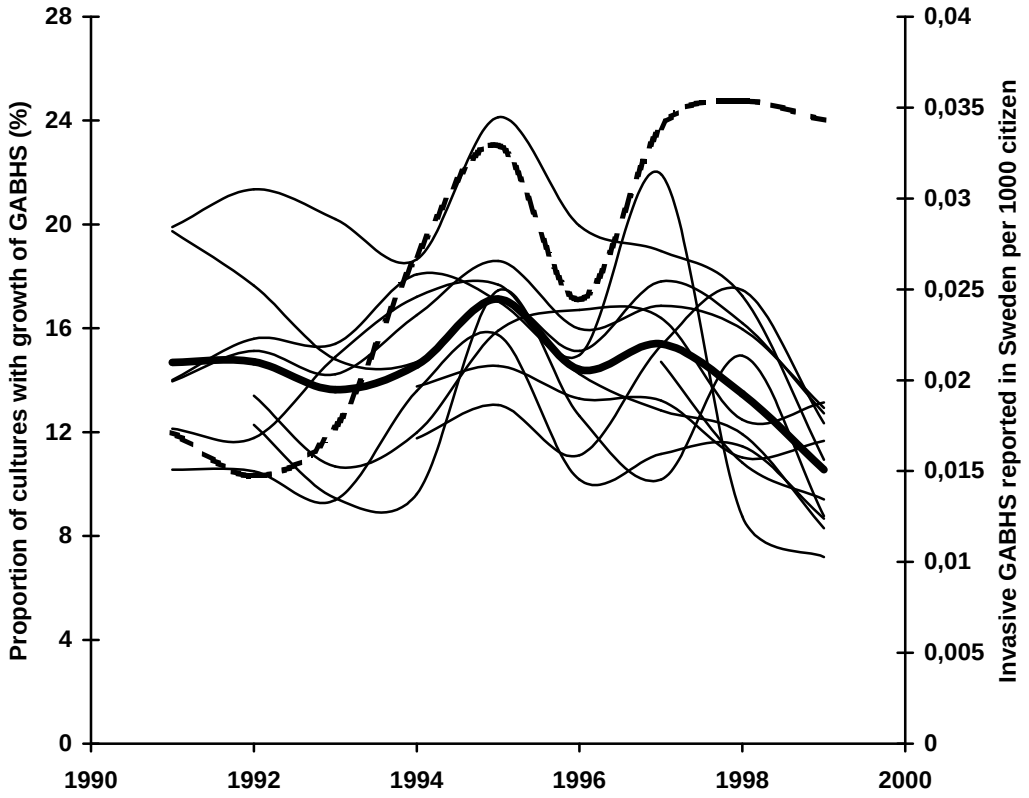


Figure 8 - Variations over time in the proportion of throat cultures with growth of GABHS
Comparison of different geographical areas (thick line is mean for all areas). Dotted line is national statistics of invasive infections with GABHS in Sweden per 1000 citizens.

The proportion of throat cultures with growth of *S. pyogenes* varies over the study period (n=85, p=0.002, one way ANOVA) with a decline during the last year (Figure 8).

5. Discussion

The first important step in the diagnostic procedure of infectious diseases is to decide if antibiotic treatment is a choice to consider in this patient. Is the situation for the patient bad enough to motivate antibiotic treatment if such treatment could improve the condition? If the answer is obviously no, then there is no reason to push the differential diagnosis between viral or bacterial etiology further unless, there is epidemiological reasons for treatment. This is the most important step in the diagnostic procedure. If the answer is yes, then there is a number of questions that needs to be addressed before the prescription eventually can be handed over to the patient (Figure 9).

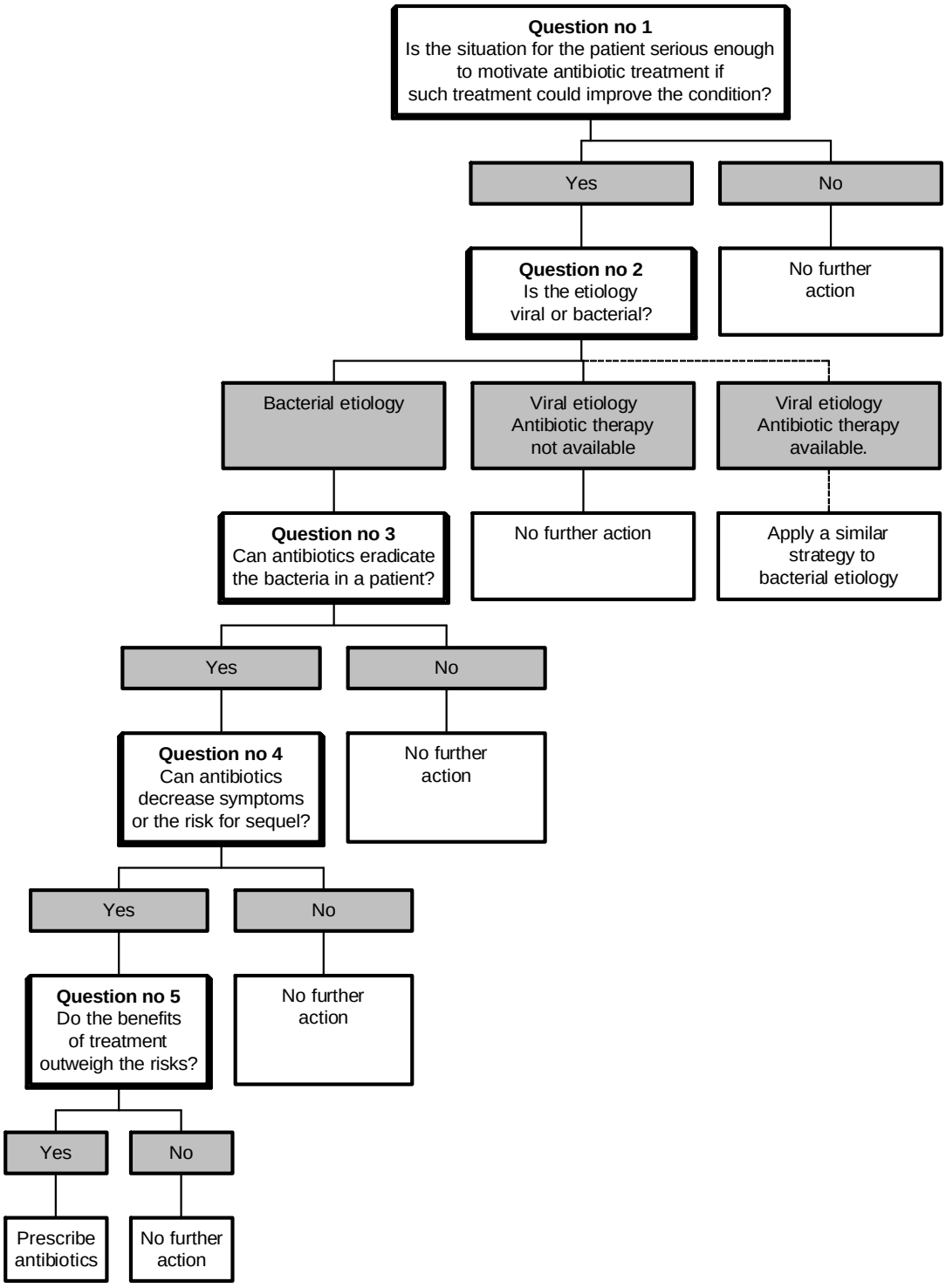


Figure 9 - Five steps before antibiotics may be prescribed.
Epidemiological reasons for treatment is not included in the figure.

If the first question is whether antibiotic treatment might be a choice for this patient or not, then the second question is if the etiology is bacterial or viral. In case of symptomatic carriers, that normally should not be treated, then it is not sufficient to confirm the presence of a potentially pathogenic bacterium in patients. Are there any scientific studies suggesting that the found bacterium is more common among patients than among healthy individuals? If not, then there is very little evidence suggesting that this bacterium is the etiologic agent.

Assume that the answer to question one, three and four is yes and that the second question resulted in bacterial etiology. Before the doctor hands over the prescription, there is a fifth question that needs to be addressed. Are the benefits for the patient large enough to outweigh the ecological disadvantages? The doctor can, by using common sense, answer question number one. Question two, three and four can only be answered by scientific studies. Question number five can not directly be answered by a scientific study. Scientific studies can perhaps provide the information needed, but the fifth question is not a scientific question. Consensus meetings are probably the best way to produce guidelines addressing the fifth question. Antibiotics should not be given unless the answer is yes to all five questions.

A restrictive use of antibiotics is proclaimed both in hospitals and in primary health care. However, antibiotics will remain as an important weapon in the therapeutic arsenal. So long as doctors are faced with therapeutic choices, such as prescribing antibiotics, it will be important to further improve the diagnostic process.

5.1. Methodological aspects

5.1.1. A single estimation and comparison between groups

The proportion of positive samples among patients with a specific symptom, for example a sore throat or long-standing cough, varies in time and geographical area. Thus, a single estimation of the prevalence of bacteria in one group of individuals, usually patients with an infectious disease, is therefore of limited value. However, a comparison of the prevalence of bacteria between two or more groups of individuals, as in this study, is more informative. Such comparisons between groups are only useful if the samples in all groups are obtained from the same geographical area and during the same periods, as in this study.

5.1.2. Estimation of theta (q) (IV)

The relation between the proportion of positive tests in patients sick from another cause (the proportion of symptomatic carriers) and the proportion of positive tests in healthy individuals (the proportion of asymptomatic carriers) is defined as theta (q). Since q is defined by

$$P(\mathrm{T}^+ \left| \mathrm{S}^+\mathrm{D}^- \right.) = P(\mathrm{T}^+ \left| \mathrm{S}^- \right.) \times q$$

and since

$$P(\mathrm{T}^+ \left| \mathrm{S}^+\mathrm{D}^- \right.) < \mathrm{Sen}$$

it can be shown that

$$0 < q < \frac{\mathrm{Sen}}{P(\mathrm{T}^+ \left| \mathrm{S}^- \right.)}$$

There are three principal ways to estimate q:

1. The first one is to define that q =1; i.e. the found agent is of etiologic importance in a population as soon as the proportion of positive tests exceeds that of a healthy control population.

2. The second way is to find a gold standard predicting disease caused by the etiologic agent. This gold standard could be used to estimate *q*. Unfortunately, there is seldom such a gold standard.
3. The third way is to make a reasonable assumption. Assume that there are 500 healthy individuals and that 50 of these are asymptomatic carriers of a bacterium, for example *GABHS*. Assume that 20% of these 500 randomly acquire an acute upper respiratory tract disease caused by a virus. By chance, approximately 10 of these 100 viral infected symptomatic individuals will be symptomatic carriers of *GABHS*. The proportion of symptomatic carriers will initially be the same as the proportion of asymptomatic carriers among healthy individuals. There are several mechanisms that might affect the continuation of events:
   a) Spreading of *GABHS* from symptomatic or asymptomatic carriers might occur to individuals not harbouring *GABHS*. However, since asymptomatic carriers of *GABHS* have a low tendency to spread their bacterium [93] this will be a slow process. Furthermore, if spreading occurs, it will affect both individuals sick due to a virus as well as healthy individuals. It is unlikely that this mechanism alters *q*.
   b) A viral infection might alter the carriage in a symptomatic carrier into disease caused by *GABHS* and thus lower the proportion of symptomatic carriers. A viral infection might alter the properties of mucous membranes in the respiratory tract and as a consequence enhance adhesion or penetration through the mucous membrane. An association between infection with *GABHS* and influenzae A has been shown in vitro [105]. An association has also been seen in vivo so that varicella-zoster seems to increase the risk for invasive infection with *GABHS* [106, 107]. Another study has in vitro showed that a viral infection increases the adhesion of *S. pneumoniae* to epithelial cells [108]. However, the clinical importance of these studies remains to be investigated and it is generally considered that asymptomatic carriers do not have an increased risk for developing sickness caused by *GABHS* [91]. Theoretically this mechanism might result in a slightly lower *q*.
   c) Altering the mucous membrane by a viral infection might alter the number of bacteria per surface unit. If the number of bacteria increase it might enhance the test's ability to discover the bacteria and thus, possibly indirectly increase the proportion of symptomatic carriers. This phenomenon is only likely to occur if the sensitivity of the test is low. Theoretically this mechanism might result in a slightly higher *q*.
   d) Spreading of *GABHS* from patients ill by *GABHS* is more likely to occur than spreading of *GABHS* from carriers [92, 109]. There is a possibility that a viral-infected individual is more susceptible to this spreading of bacteria than healthy individuals. If this were true it would increase *q*. However, there is no clear evidence that this type of spreading is much more likely to occur if the potential recipient has a viral infection compared to healthy individuals. Furthermore, if spreading occurs it will still affect both individuals sick by a virus as well as healthy individuals. Theoretically, this mechanism might result in a slightly higher *q*. However, this remains to be proven.

   As seen, initially $q=1$. It might later deviate slightly from one. The initial phase would probably last a couple of weeks by which time the viral infection will be gone. Thus, it is reasonable to assume that in case of a sore throat caused by *GABHS*, *q* approximates the value of one, an opinion shared by most authorities

[39, 91, 103, 104]. In most other situations where the etiologic predictive value would be of clinical value the assumption that q =1 would apply.

One may find it frustrating that the estimation of q introduces some uncertainty to the etiologic predictive value. However, as shown (Table IV, page 33) this uncertainty is rather small. The possible error caused by estimating q is far less than the error caused by assuming that predicting presence of the etiologic agent is similar to predicting presence of the specified disease.

5.1.3. Selection of patients (I-III, V)

The advantage of defining criteria for selecting patients before the samples are collected is that the study population will be well defined. The disadvantage of this procedure is that the sample may reflect a population that is not the same as the population from whom a throat or nasopharyngeal sample would be taken as part of routine medical care. In this study the focus was on evaluating the outcome of the cultures obtained in routine medical care without an exterior definition of how to select patients appropriate for obtaining a throat or nasopharyngeal sample. Consecutive samples arriving at the microbiologic laboratory stating a specific symptom, like a sore throat or long-standing cough, were used. Thus, the selection of patients in this study could be criticised, as patients were only included when the doctor thought a sample useful to the diagnostic and therapeutic procedure. However, the advantage of this procedure is that the samples might be representative for the population of patients from whom a sample would be taken in routine medical care.

In most scientific studies, where specially trained personnel take the sample from a well defined study population, the proportion of throat cultures with growth of *GABHS* is usually between 20-80% [53, 90, 91, 110-116]. In this dissertation it was shown that the growth of *GABHS* in throat cultures taken in the routine medical care varied between 7.2-24% (Figure 4). Thus, the populations studied in many scientific studies represent other types of populations than that from which throat samples are taken in routine medical care.

As the doctors had to characterise the patients' symptoms on the referral slip, it can be postulated that obtaining the samples was preceded by a reflection of its usefulness. Thus, it may be assumed that doctors would not take a throat or nasopharyngeal sample if, after a preliminary clinical evaluation, they found that the patient had a probable viral infection. When the doctors were in doubt about the etiology of the sore throat, the routine procedure to establish the etiology was, during the time period for this study, to test for the presence of *GABHS* by a rapid test or a throat culture. A questionnaire performed 1992 (unpublished data) among 146 doctors in the Elfsborg county revealed that, when they were uncertain about the etiology of a sore throat, 28.3% preferred to use a rapid test to detect *GABHS*, 1.4% never used a test at all and, the remaining 70.3% used a throat culture. Of those 28.3% who preferred a rapid test, 41% would send a throat culture in case of a negative rapid test. A throat or nasopharyngeal culture would most likely not be obtained if the result from a preceding rapid test or throat culture showed the presence of *GABHS*. Thus, some cultures are sent after a preliminary filtering process where some samples with growth of *GABHS* are excluded.

In the study healthy individuals were compared to patients having a sore throat or long-standing cough. Almost all samples were derived from patients that were not hospitalised. Thus, the sample represents patients with a respiratory tract infection having a minor illness.

The number of children in this investigation was small. Furthermore, children do not express symptoms as well as adults. Thus, the findings concerning children should be interpreted carefully. In contrast, the number of adults was large. This made it easier to interpret the findings among them.

5.1.4. Response rate to the study protocol on the referrals of the throat- and nasopharyngeal samples (I-III)

At the time, when the throat cultures were obtained (1991), the tradition was that almost all referral slips were blank. In the study, information was given to all primary health care centres, hospital clinics and emergency wards concerning the study protocol. In all, 520 (18.4%) of 2825 referrals with a throat culture were coded according to the study protocol. Of these 520, 435 (83.7%) stated a sore throat and of the remaining 16.3% the majority used the code for "other symptoms". The cultures with a slip stating "other symptoms" had similar growth of *GABHS* compared to those throat cultures with a referral slip stating a sore throat (Table XI).

Table XI – Growth of GABHS in throat cultures depending on symptom stated on the referral slip

Season	Symptom[a]	0-6 years	7-15 years	≥16 years
Summer	Cough > 9days	0/6[b]	0/2	0/14
	AOM with tube[c]	-----	-----	0/3
	AOM no tube[c]	1/3	0/1	0/3
	Sore throat	17/48	13/45	32/168
	Sneezing	4/9	1/11	2/19
	Sinusitis	-----	0/1	0/7
	Other symptoms	11/25	0/3	5/37
Winter	Cough > 9days	0/1	1/3	0/22
	AOM with tube	-----	-----	0/2
	AOM no tube	-----	-----	0/2
	Sore throat	9/38	4/15	9/121
	Sneezing	0/5	-----	0/12
	Sinusitis	-----	0/2	1/7
	Other symptoms	0/13	2/7	2/21
Summer + winter	Cough > 9days	0/7	1/5	0/36
	AOM with tube	-----	-----	0/5
	AOM no tube	1/3	0/1	0/5
	Sore throat	**26/86 (30%)**	**17/60 (28%)**	**41/289 (14%)**
	Sneezing	4/14	1/11	2/31
	Sinusitis	-----	0/3	1/14
	Other symptoms	**11/38 (29%)**	**2/9 (22%)**	**7/58 (12%)**

[a] Symptom stated on the referral slip. More than one symptom may be stated, thus some cultures may be represented on more than one line.

[b] Number of cultures with growth of GABHS / Total number of cultures

[c] Acute otitis media (AOM) with or without ventilation tube

Consequently, the throat samples with referrals stating “other symptoms” probably represents a population similar to the one with referrals stating a sore throat.

During the year 2000, data from two laboratories could be used to differentiate throat samples taken to trace carriers from other throat samples. The proportion of throat samples taken to trace carriers was only between 2.1-4.9%.

It could be concluded that the overwhelming majority of the throat samples send to the microbiologic laboratory was taken from a population of patients with a sore throat, with or without concomitant symptoms. A small number of throat cultures was taken from adults with a referral stating long-standing cough. None of them had growth of *GABHS*. With the exception of the adults, the growth of any group of *BHS*, or growth of *GABHS* is similar between throat samples with a referral stating a sore throat compared to all referrals (Table XII).

Table XII - Prevalence of BHS in consecutive throat samples arriving at a microbiologic laboratory from the routine medical care

				Growth of beta-haemolytic streptococci							
		Sea-	Total	**All groups**		**Group A**		Group C		Group G	
Age group	Symptom	son[1]	n	n	**%**	n	**%**	n	%	n	%
0-6 years	A sore throat	S	38	9	**24**	9	**24**	0	---	0	---
	All samples[2]	S	345	78	**23**	60	**17**	4	5.1	8	10
7-15 years	A sore throat	S	15	4	**27**	4	**27**	0	---	0	---
	All samples	S	163	40	**25**	24	**15**	3	1.8	6	3.7
≥16 years	A sore throat	S	121	19	**16**	9	**7.4**	6	5.0	4	3.3
	All samples	S	890	123	**14**	78	**8.8**	24	2.7	18	2.0
0-6 years	A sore throat	W	48	20[3]	**42**	17	**35**	0	---	0	---
	All samples	W	229	69	**30**	59	**26**	2	0.9	5	2.2
7-15 years	A sore throat	W	45	15	**33**	13	**29**	1	---	1	---
	All samples	W	149	56	**38**	46	**31**	1	0.7	4	2.7
≥16 years	A sore throat	W	168	36	**21**	32	**19**	0	---	3	1.8
	All samples	W	614	75	**12**	60	**10**	3	0.5	9	1.5

[1] S = Summer (15 July – 15 Sept.). W = Winter (14 Jan. - 17 Feb.)

[2] Includes throat samples with blank referrals as well as referrals with all possible symptoms.

[3] Information about Lancefield’s group is missing in three samples.

Calculating EPV with all consecutive throat samples that arrived to the microbiologic laboratory shows that the difference between these EPVs and the EPVs obtained if only using referrals stating a sore throat is negligible (Table XIII).

Table XIII – EPVs of throat cultures with a referral slip stating that the symptom is a sore throat compared to the EPVs if all throat samples were used

Season[a]	Symptom	EPV[a]	Age group		
			0-6 years	7-15 years	≥16 years
Summer	A sore throat	PEPV	**61 (0-91)**	69 (0-95)	-----
	All samples	PEPV	**45 (0-78)**	38 (0-80)	-----
	A sore throat	NEPV	98 (94-100)	97 (88-100)	99 (98-100)
	All samples	NEPV	99 (97-100)	99 (98-100)	99 (99-99)
Winter	A sore throat	PEPV	**94 (75-100)**	85 (34-98)	**99 (93-100)**
	All samples	PEPV	**92 (77-99)**	87 (65-97)	**98 (90-100)**
	A sore throat	NEPV	94 (89-98)	96 (91-99)	97 (96-99)
	All samples	NEPV	96 (94-98)	96 (93-98)	99 (98-99)
Summer + winter	A sore throat	PEPV	83 (53-94)	78 (30-93)	**99 (94-100)**
	All samples	PEPV	73 (50-87)	72 (45-87)	**98 (93-100)**
	A sore throat	NEPV	96 (93-99)	97 (93-99)	98 (97-99)
	All samples	NEPV	98 (97-99)	98 (96-99)	99 (99-99)

[a] Positive (PEPV) and negative (NEPV) etiologic predictive value in %, 95% confidence interval within parentheses.

The interpretation (of Table XIII) may well be that most blank referrals are taken from patients with a sore throat. Another experience that can be made from the poor response rate is that it is difficult to change the doctor's behaviour. Greater acceptance by doctors to the study protocol requires considerable convincing. However, the more doctors are influenced, the more their behaviour will be altered. The consequence is that their behaviour is no longer representative for routine medical care. It was not the intention to influence the doctors' behaviour in this study. However, their diagnostic strategy may have been altered to some extent. The response rate to our study protocol was poor. However, further analysis, as presented above, indicates that a better response rate in referrals from throat cultures would probably not alter our conclusions.

A similar phenomenon was seen in the referrals belonging to the nasopharyngeal swab samples. Of 3190 nasopharyngeal swab samples, only 685 (21.5%) were coded according to the study protocol. Since nasopharyngeal swab samples are taken for various reasons, a similar analysis of the consequences of the poor response rate like the one made with throat cultures, is difficult to perform. Thus, our conclusions regarding nasopharyngeal cultures must be interpreted with caution.

5.1.5. Sampling techniques (I-III)

The standard procedure in this study for taking throat swab samples was to gently roll a cotton-tipped swab over the surface of the tonsils. To detect presence of *BHS* in patients with a sore throat, oropharynx is superior to nasopharynx [117], oropharynx is superior to anterior nasal swab [117] and oropharynx is superior to saliva cultures [118]. *GABHS* usually adheres to the posterior pharyngeal wall or the surface of the tonsils (or tonsillar fossae), thus a throat sample should be obtained from these locations [119]. This complies with the routine procedure at the time of the study.

The routine method in this study to take a nasopharyngeal sample was to insert a thin flexible cotton wire swab through one nasal aperture into the posterior wall of the nasopharynx. It was recommended to keep the swab in place for 15-30 seconds or

until coughing occurred. Data concerning the compliance to the procedure was not obtained.

Nasopharyngeal culture was used to identify the common potentially pathogenic bacteria *S. pneumoniae*, *H. influenzae*, M. catarrhalis and *GABHS*. They may all be found in the nasopharynx in patients with a respiratory tract infection. However, it has been reported that *H. influenzae* is more easily found if the sample is taken from the oropharynx. In scientific studies, a common procedures reported is to hold the pin in place for at least 30 seconds or until the patient coughs [120]. Protecting the cotton wire swab with a sheath to prevent contamination by nasal flora did not influence the recovery of potentially pathogenic bacteria [121]. The nasopharyngeal samples taken in our study complied fairly well with this procedure. However, the nasopharyngeal swabs in this study might have been held in place for a shorter time than the recommended 30 seconds.

A better sampling technique will increase the proportion of positive cultures [90]. All cultures in this study were collected in routine medical care resulting in an uncontrolled sampling situation. The prevalence of bacteria could be expected to be slightly higher if specially trained personnel had collected the samples. However, since our main purpose was to compare the prevalence of bacteria between healthy individuals and patients, rather than estimate the prevalence in each group, this had only a minimal effect on the outcome. The advantage of using the normal staff was that our study reflected the normal clinical situation.

5.1.6. Culture techniques (I-III)

The culture technique may influence the outcome. For throat cultures most authors consider an anaerobic technique to be superior due to better enhancement of the growth of *BHS* [38, 104, 119, 122-124]. However, there is some belief that the advantages of an anaerobic technique are not obvious [39, 125-127]. There is no support in the literature, to our knowledge, that air with or without 5% CO_2 is always the best choice. In previous scientific studies describing the prevalence of healthy individuals carrying *BHS*, air with or without the addition of 5% CO_2, is most often used [103, 104, 113-115, 117, 128, 129] or, the atmosphere is not described at all [37, 93, 97, 130, 131]. No study mapping the prevalence of *BHS* in healthy individuals had described using an anaerobic atmosphere.

Inhibitors are often used to inhibit the growth of unwanted bacterial species thus, making the culture result easier to interpret. However, inhibitors will to some extent, also inhibit the searched bacteria from growing, subsequently yielding fewer positive cultures. Most authors consider it best to use inhibitors [38, 85, 125, 132]. However, some believe the opposite [133] and others conclude that it is not obvious which alternative is best [119, 123, 124, 127].

Our technique for throat cultures might not have been optimal. Using an anaerobic atmosphere and a non-selective culture media without inhibitors might have yielded higher numbers of *BHS*. However, using a better technique would have only marginally affected the absolute number of positive cultures and not the correlation between the patient groups or the seasons. The semiquantitative method used for the throat cultures might not have fully met scientific stringency, but it was representative of techniques used in routine diagnosis.

While *BHS* is a bacterium that may survive a long transport, *S. pneumoniae*, *H. influenzae* and M. catarrhalis are more sensitive and will not survive a long transport. Most nasopharyngeal samples in our study reached the microbiologic laboratory within 1-8 hours. Only a few samples had a transport period exceeding eight hours.

The transport period in this study slightly exceeds the transport period in many scientific studies [121], however, this may be beneficial since our study reflects the transport period in routine medical care.

5.1.7. Sensitivity of throat and nasopharyngeal culture

Sensitivity is here defined as the probability of the process; sampling, transportation, culturing and reporting, to detect a bacteria that exists in the throat or nasopharynx. Estimating sensitivity is a prerequisite for calculating the EPV, thus it is essential to estimate the sensitivity.

In a microbiologic laboratory with trained personnel, the sensitivity of a conventional throat culture may be estimated at 90% [34, 36, 39, 88, 89, 102]. This estimation of the sensitivity of a conventional throat culture was used in calculating the EPV.

However, no such references could be found for the nasopharyngeal culture. Thus, it was necessary to estimate the sensitivity of the nasopharyngeal culture in some way. One possible option was by using the collected nasopharyngeal swab samples to estimate the sensitivity. Pre-school children during the winter season had the highest proportion of positive cultures and were thus chosen for this estimate.

Of the 711 nasopharyngeal swab samples that arrived during the winter season to the laboratory, taken from patients 0-6 years of age by routine medical care, 82.4% (95% confidence interval 79.6-85.2%) had growth of potentially pathogenic bacteria. The probability of getting a positive culture must therefore be above 79.6%, assuming that no more than 100% of the children actually carry potentially pathogenic bacteria. If the probability of detecting bacteria is >79.6%, the detection involves detecting any of several potentially pathogenic bacterial species. In the collected samples, some had growth of one potentially pathogenic specie, others had growth of several species. The sensitivity of a nasopharyngeal culture to detect a single potentially pathogenic specie can then be calculated to be >81%. If the sensitivity to detect a single bacterium is below 81%, then the finding that more than 79.6% of the children having a positive nasopharyngeal culture cannot be explained unless one assumes that the proportion of children harbouring a potentially pathogenic bacteria is above 100%, (which is impossible). There is no reason to believe that the sensitivity of a nasopharyngeal culture is lower in the other age groups sampled in this study. Thus, the sensitivity 81% was used when calculating the EPVs for nasopharyngeal cultures.

5.2. Factors influencing the clinical value of a culture

5.2.1. The patients age (I-III)

In general, the proportion of positive cultures decreases with age among healthy individuals. This age dependence existed for both throat cultures and nasopharyngeal cultures. Among healthy adults there were practically no asymptomatic carriers. The strong correlation between age and the prevalence of *BHS* has been explained by a mechanism whereby children gradually acquire immunity to the prevalent serologic types of *GABHS* [134]. A similar phenomenon could explain the decrease of potentially pathogenic bacteria in nasopharynx [2]. Thus, throat cultures are more useful in adults than in children when predicting disease caused by *BHS* [135]. A similar reasoning could be applied to the use of nasopharyngeal cultures. However, the indications for using nasopharyngeal cultures are more controversial than throat cultures.

Unexpectedly, among healthy children 7-9 years of age, a lowering of the proportion of cultures positive for *GABHS* was observed (Figure 2 on page 34). The 95% confidence limit for the estimate 1.6% was 0% - 4.7%. Thus, there was a low carriage rate among healthy children 7-9 years of age. This is difficult to explain.

5.2.2. The importance of seasonal variations in throat samples (I)

As seen (in Figure 3 on page 34) patients tend to have a higher proportion of throat cultures positive for *GABHS* in winter compared to summer. Healthy individuals had a less pronounced seasonal variation. However, since only two limited periods were studied during one year, it cannot be concluded that this pattern will be valid every year. Other studies have reported the lack of seasonal variation in the prevalence of *BHS* in healthy individuals [103, 104, 129], but no comparison of the prevalence of *BHS* in age-matched patients was made.

It is well known that streptococcal throat infection, in a temperate climate, is a disease of the winter and early spring [113, 119, 136]. The intention was to compare patients with a sore throat and healthy individuals during periods when maximal and minimal prevalence of *GABHS* among patients could be expected. Therefore the periods mid-winter and late summer were chosen.

For healthy children in age group 3-6 years, the seasonal difference resembled the situation seen in patients, but for the other age groups, the tendency of seasonal variation was opposite to the one seen in patients. The weak links (in Figure 3), due to small numbers, are patients of age group 7-9 and 10-15, especially 7-9. The number of patients in the age group 7-9 was only seven during mid-winter and 21 during late summer. Consequently, there is a wide confidence interval for that estimate and the curve for patients presented (Figure 3) might as well resemble the dotted curve (Figure 10).

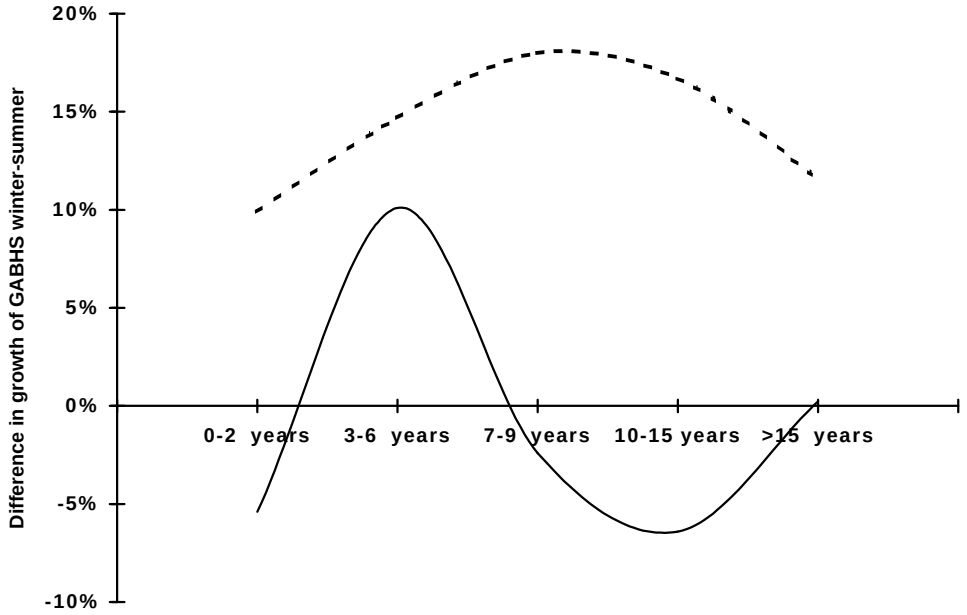


Figure 10 - Presumed difference winter/summer in the proportion of throat cultures with GABHS
Thin line = healthy individuals. Dotted line = presumed for patients if the number of patients aged 7-9 was higher.

The data implies that there is a difference in seasonal variation between patients with a sore throat and healthy individuals. The higher prevalence of *GABHS* in many healthy individuals in the late summer season could reflect a changed streptococcal epidemiology. Thus, a new serotype that had not been circulating for some time, could gain a higher prevalence than the strains that had been present for months. If this was the case, then a similar increase in the prevalence of *GABHS* in patients should been observed, but in fact a decrease in the prevalence of *BHS* from mid-winter to late summer was found in patients. Seasonal variations in the prevalence of *BHS* are normally not correlated to the introduction of a new serotype [131]. In the present study the appearance of a new serotype was not explicitly tested.

Other seasonal factors need to be considered and further evaluated. One factor that may reduce the prevalence of *GABHS* among healthy children is lower attendance at day care centres [137] or schools during longer holidays or vacations. One factor that might increase the prevalence of *GABHS* among healthy children is the effect of crowding on return from holiday. Since the rate of asymptomatic carriers and patients with throat pain showed opposite seasonal fluctuations, one may speculate that *GABHS* are either more virulent during the winter season, or that individuals are more vulnerable to a streptococcal infection during the winter, due perhaps to the higher incidence of viral infections [108].

5.2.3. Day care and asymptomatic carriers of GABHS (I)

Due to the strong correlation between age and type of day care, no conclusions concerning the influence of the type of day care could be drawn in this study. Strömberg et al. [129] found higher rates of asymptomatic carriers in children four years of age not attending day care centres than in those attending day care centres. In Croatia the proportion of asymptomatic carriers of *GABHS* was slightly increased in children attending day care centres compared to children not attending day care centres [104]. However, the difference between the two groups of children was not statistically significant in the investigation by Begovac et al. It is reasonable to assume that day care involving crowding of children increases the carrier rate of *GABHS* in healthy pre-school children, although this has not as yet been shown.

5.2.4. Quantification of growth in throat samples (I)

In patients with an infection caused by *GABHS*, the growth is usually abundant. Therefore, some authors state that quantification is useful in order to distinguish between the symptomatic carrier state and infection caused by *GABHS* [113, 138].

As was expected, it was shown that when *GABHS* was present, abundant growth of *GABHS* was more common in patients with a sore throat when compared to healthy individuals. However, in the present study, asymptomatic carriers often had abundant growth of *GABHS* in throat *sample*s [92, 104, 117]. Thus, presence or absence of abundant growth did not seem to distinguish between symptomatic carriers and those actually ill from *GABHS*.

Some of the patients in our study with a sore throat had sparse growth of *GABHS*. It cannot be stated if they are symptomatic carriers ill from a viral infection, or ill from *GABHS*. In the literature, it is often stated that if growth is sparse it does not exclude symptomatic infection [94, 133]. Furthermore, it was found that most asymptomatic carriers of *GABHS* did not have sparse growth. Thus, presence or not of sparse growth does not seem to distinguish between symptomatic carriers and those actually ill from *GABHS*.

One should also remember that in routine medical care the sampling technique is not controlled and this will further diminish the value of quantification in routine diagnosis. Our conclusion, that quantification alone is unreliable to distinguish the symptomatic carrier state from disease, is in accordance with other studies [104, 117, 123].

5.2.5. The influence of local variations in the proportion of positive throat cultures on predictive values (IV-V)

The proportion of positive throat cultures varies greatly between geographical areas. This suggests that the samples have been taken from different populations. It was shown that predictive values for throat culture, to predict presence of *GABHS* in the patient, calculated from data obtained in one geographical area might not be valid in another area (Figure 11). The variation between areas in the proportion of throat culture positive for *GABHS* varies so that the positive predictive value to predict the presence of *GABHS* in routine medical care might vary between 60-91% (Figure 11).

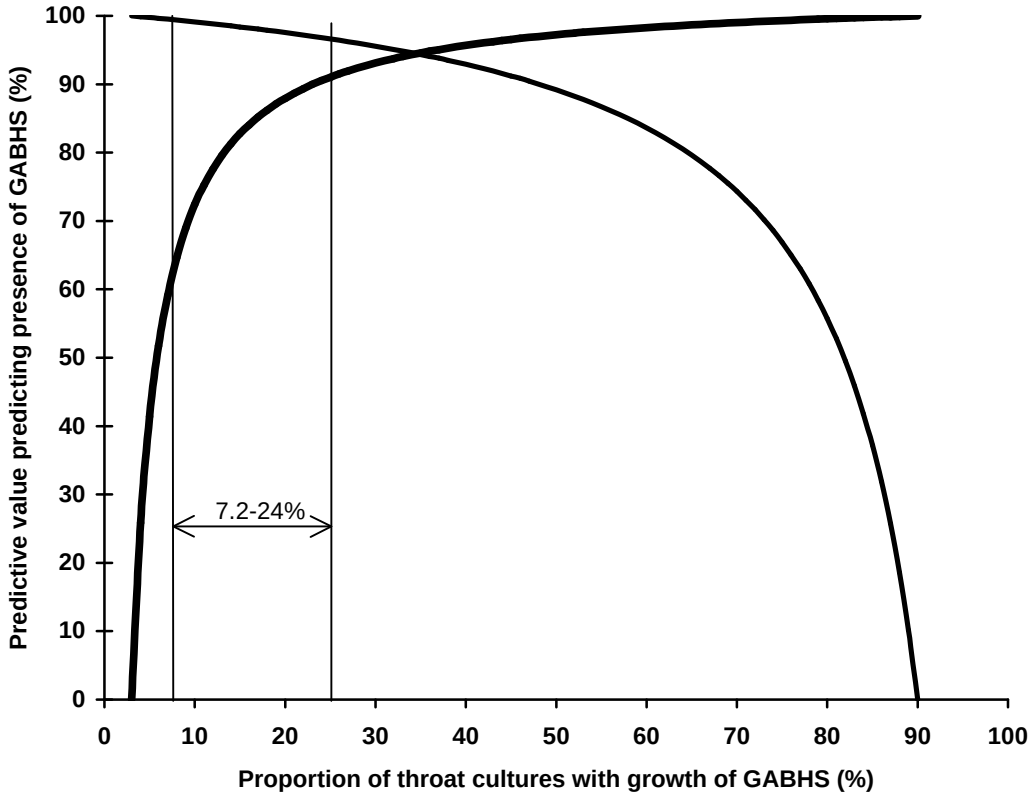


Figure 11 - Predictive values depending on the proportion of positive throat cultures
Proportion of cultures positive for GABHS varies in the routine medical care between 7-24%. Thick line = positive predictive value and thin line = negative predictive value. Sensitivity and specificity are assumed to be 90% and 97%, respectively.

In Figure 11 the presence of *GABHS* is predicted in the throat of the patient. Using EPV instead to predict presence of disease caused by *GABHS* will alter the result.

As seen in Figure 12, PEPV is strongly dependent on the prevalence of symptomatic carriers. If there are no symptomatic carriers, PEPV will be 100%, i.e. a positive culture will always indicate true disease caused by *GABHS*. Adults have very few carriers, therefore PEPV is close to 100% in adults (Table VII on page 41). Asymptomatic carriers of *GABHS* were found to be between 2.4-17.2% in children 3-15 years of age (I) and the proportion of positive cultures among children with a sore throat was 12.5-48.0% (I). If q is considered to be close to one it can be shown that the PEPV might vary from 0% to above 90% (Figure 12). Thus, the interpretation of a positive throat culture in children may vary between geographical areas and in time.

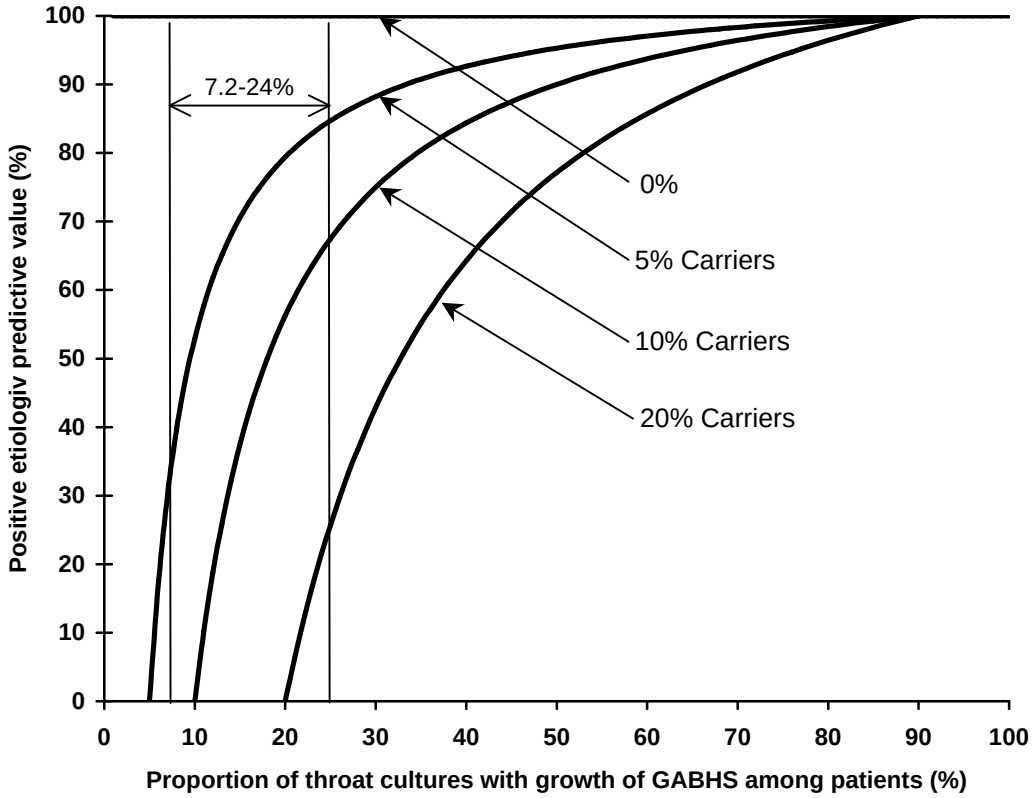


Figure 12 - PEPV depending on carrier rate and proportion of positive throat cultures in patients. Proportion of cultures positive for GABHS varies in the routine medical care between 7.2-24%.One curve for every rate of symptomatic carriers; 0%, 5%, 10% and 20%. Sensitivity estimated to 90%

As observed, symptomatic carriers have a huge impact on the PEPV. However, NEPV is hardly affected by symptomatic carriers (Figure 13).

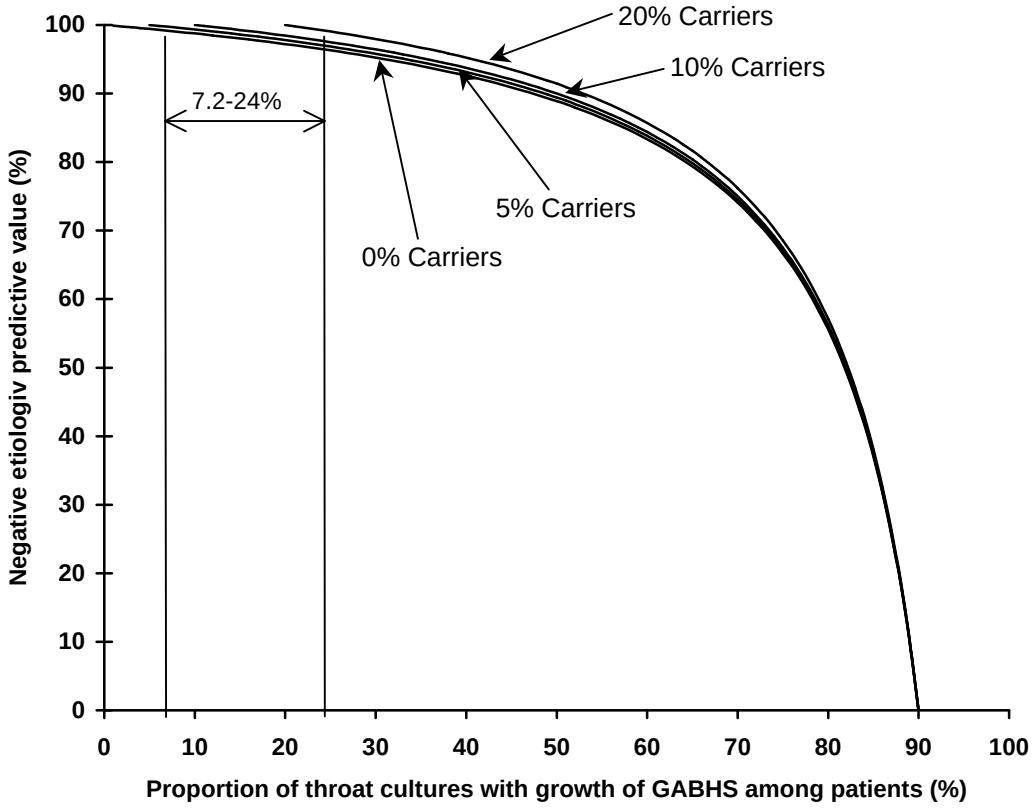


Figure 13 - NEPV depending on carrier rate and proportion of positive throat cultures in patients. Proportion of cultures positive for GABHS varies in the routine medical care between 7.2-24%.One curve for every rate of symptomatic carriers; 0%, 5%, 10% and 20%.. Sensitivity estimated to 90%

It may be concluded that in throat cultures with a sensitivity of approximately 90%, NEPV of a throat or nasopharyngeal culture will be very high and a negative test will in most cases rule out a bacterial etiology. This will be true in all areas within routine medical care. If the sensitivity is lowered to 80%, as would be the case with most rapid tests to detect *GABHS*, the situation will differ slightly. The effect of lowering the sensitivity from 90% to 80% will be minimal for PEPV (Figure 14) but somewhat greater for NEPV (Figure 15).

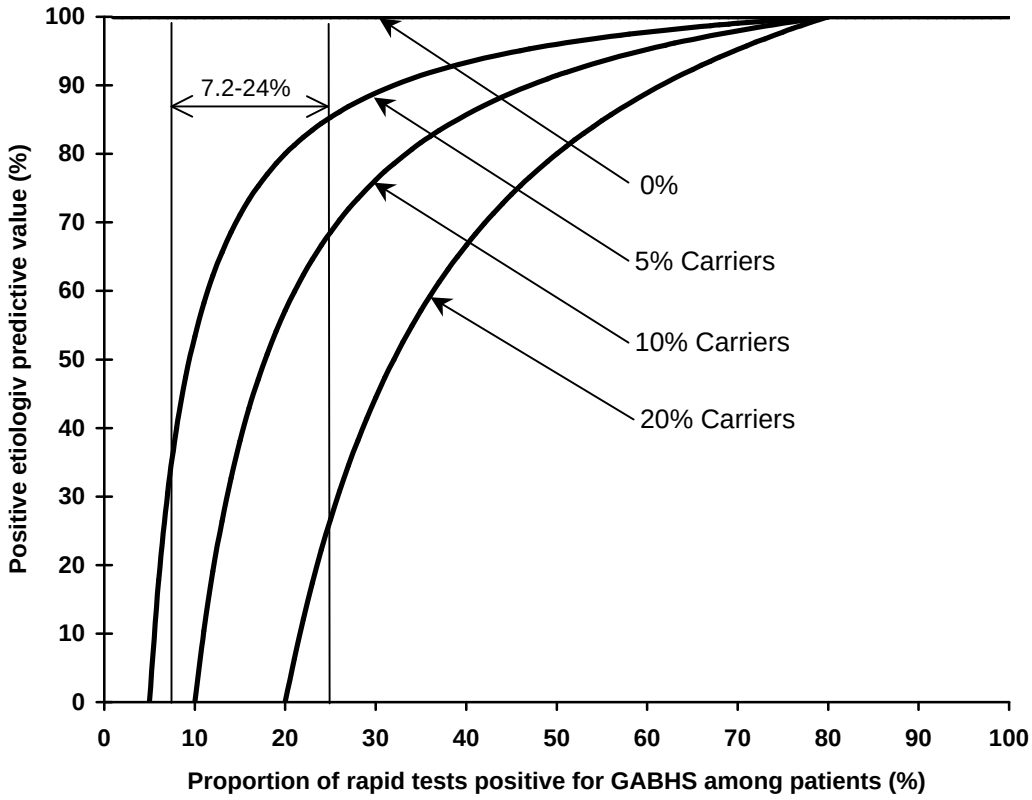


Figure 14 - PEPV depending on carrier rate and proportion of positive rapid tests in patients. Proportion of cultures positive for GABHS varies in the routine medical care between 7.2-24%.One curve for every rate of symptomatic carriers; 0%, 5%, 10% and 20%. Sensitivity estimated to 80%

In rapid tests used to test patients with a sore throat, it can be seen that, a prevalence of *GABHS* of 7.2-24% (as in the routine medical care) will almost always result in a very high NEPV (Figure 15). Thus, a negative rapid test to detect *GABHS* will rule out *GABHS* as the etiologic agent with a very high probability.

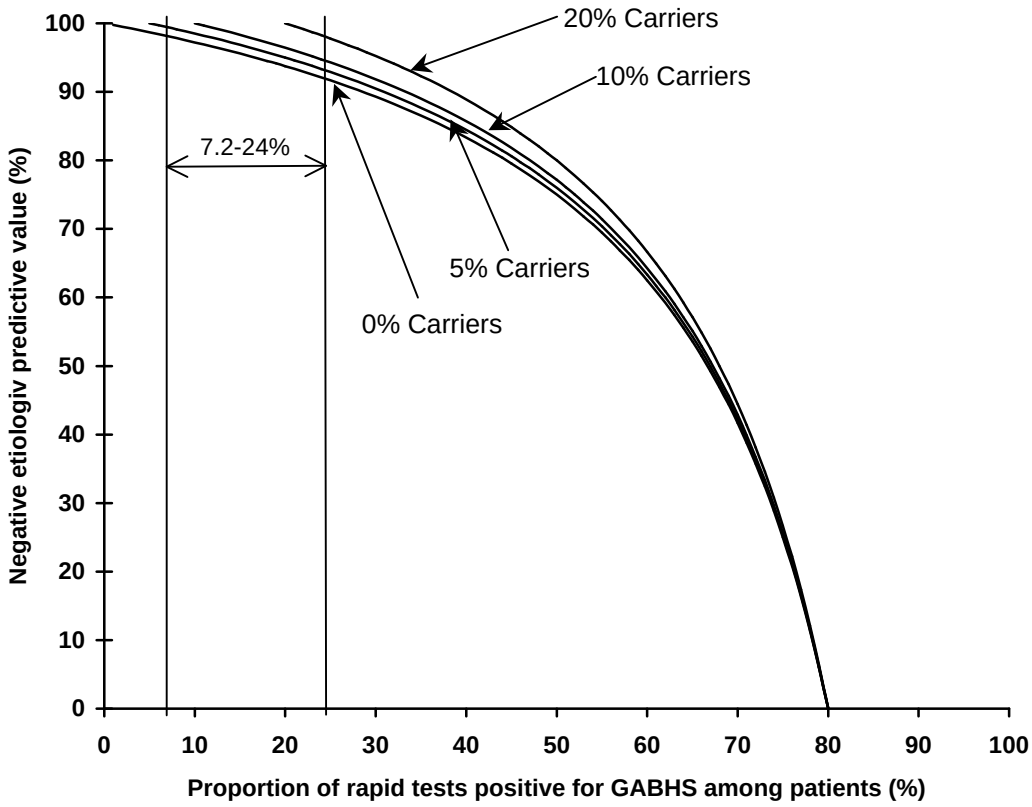


Figure 15 - NEPV depending on carrier rate and proportion of positive rapid tests in patients. Proportion of cultures positive for GABHS varies in the routine medical care between 7.2-24%. One curve for every rate of symptomatic carriers; 0%, 5%, 10% and 20%. Sensitivity estimated to 80%

However, in cases, such as the growth of M. catarrhalis in pre-school children with long-standing cough, there may be a very high proportion of positive nasopharyngeal cultures (67%). During such circumstances, one can see that, the NEPV decreases to a level where one can no longer use a negative test outcome to rule out bacterial etiology.

5.3. Symptomatic carriers – the neglected factor

An individual harbouring a potentially pathogenic bacterium that presently does not cause illness is usually called a carrier of this bacterium. This is a theoretical definition that cannot easily be applied to the clinical situation because it is difficult to know if the bacterium found is involved in the illness. As this question is difficult to answer, it seems as if the problem has been left unattended. If carriers are uncommon, as with adults, then this factor could be ignored. However, for children symptomatic carriers are an important factor.

5.3.1. Carriers and throat samples

Several reasons exist for treatment failure or recurrent infection in immunocompetent individuals after penicillin V treatment of a sore throat.

If a preceding test confirmed the presence of *GABHS* then the reasons for a therapeutic failure or recurrent infection might be: 1; The antibiotic therapy was too short to eradicate the streptococci [139]. 2; The therapy was initiated too soon for the infection to initiate an immune response in the patient This is a possible cause for

treatment failure [140]. 3; A simultaneous viral infection in patients who are just symptomatic carriers of streptococci [116]. 4; Local degradation of penicillin by penicillinase producing bacteria, such as *H. influenzae* and *M. catarrhalis* [141]. 5; Intracellular reservoirs of *GABHS* where penicillin cannot penetrate [142, 143]. 6; Presence of penicillin tolerant *GABHS* [144, 145]. 7; Disturbed ecology, due to previous antibiotic treatment, lacking the protective effect of the normal throat flora especially afforded by the alpha-streptococci [146]. 8; Reacquisition of *GABHS* from other individuals with pharyngotonsillitis or from a carrier [147], although carriers are not very contagious [91, 116].

If the presence of *GABHS* was not confirmed, possible reasons for treatment failure might be: 1; The etiologic agent is a virus, and thus, antibiotic treatment should not have been prescribed. 2; The test failed to detect presence of *GABHS*. Any of the above eight causes might explain the treatment failure. 3; The etiologic agent might be some other bacteria than beta-haemolytic streptococci, that is resistant to penicillin V [148].

Being a symptomatic carrier seems to be a common cause for recurrent sore throat and the presence of *GABHS*. There are several slightly different definitions on the *GABHS* carrier state [91, 149]:

- Presence of *GABHS* for a prolonged period, even when clinically well.
- Presence of *GABHS* in the pharynx without causing symptoms and without evidence of a subsequent immune response.
- Persistence of *GABHS* in the pharynx despite adequate antibiotic therapy.

These definitions have advantages and disadvantages. Common for them is the presence of *GABHS* in healthy individuals or in patients with a sore throat caused by some other etiologic agent than *GABHS*.

The proportion of symptomatic patients with a sore throat who harbour *BHS* exhibit seasonal variability, as well as varying with the epidemiological situation, and age. Thus, the rate may vary from 15% to 30% [110, 150]. However, some of these patients might be symptomatic carriers, with an infection caused by other agents [140].

The rate of asymptomatic carriers of *BHS* has been assessed to 6.7% - 26% [37, 128], and the prevalence of *GABHS* between 0.6% and 11.3% [103, 129]. Our findings are in agreement with these figures. It is assumed that in patients with a sore throat having an infection caused by agents other than *BHS*, the rate of symptomatic carriers of *BHS* is similar to that of healthy individuals [39, 91, 103, 104]. A higher proportion of symptomatic carriers will reduce the clinical value of a positive throat culture (Figure 12 - Figure 15). Based on the results of the present study, it is difficult to use throat cultures during the summer in patients <16 years of age (Table V on page 39 and Table VI on page 40). The clinical value of a negative throat culture is not affected by symptomatic carriers, and a negative throat culture will, with 97% probability, rule out *BHS* as the etiological agent [88, 89].

Equally known is the problem of overtreatment of symptomatic patients with throat infection and no proven growth of *BHS*. However, as this study points out, the problem of overtreating symptomatic carriers of *BHS* must also be considered.

5.3.2. Carriers and nasopharyngeal samples

M. catarrhalis is the most common potential pathogen found in nasopharyngeal samples in healthy children [47, 49, 50, 61, 151]. *H. influenzae* [47, 61, 117, 151,

152] and *S. pneumoniae* [47, 61, 117, 151] are also frequently found in nasopharyngeal samples in healthy children.

The fact that a high proportion of healthy pre-school children harbour these pathogens in the nasopharynx diminishes the value of nasopharyngeal culture for discriminating between bacterial and non bacterial respiratory tract infections [49, 153]. Some authors even considered banning nasopharyngeal cultures [154]. This problem is less pronounced for schoolchildren, and can be ignored completely for adults.

Based on the results in this dissertation it would be reasonable to assume that the finding of M. catarrhalis in nasopharyngeal samples from pre-school children with a respiratory tract infection is of little etiologic value due to the high carrier rate in healthy pre-school children. However, due to the fact that *M. catarrhalis* is very common in children with long-standing cough, growth of this bacterium in a nasopharyngeal sample would suggest that this bacterium is the etiologic agent (Table VIII on page 42).

5.4. The gold standard and etiologic predictive value (IV)

To evaluate a microbiologic diagnostic test a gold standard is needed. The problem of defining a proper gold standard is well known [70, 80, 96, 155]. Methods to calculate predictive values in the absence of a proper gold standard have been previously published [156-158]. These methods calculate a predictive value that predicts the presence of the etiologic agent, HIV or Strongyloides. If the agent is present, then proper treatment is commenced. The methods presented by Joseph et al. [157] and Epstein et al. [156] solve the problem of not having a gold standard predicting the presence of the etiologic agent. If, however, not all individuals carrying the etiologic agent should be treated, as in the case with symptomatic carriers of beta-haemolytic streptococci, then their methods do not solve our problem. In this case a gold standard predicting the presence of a disease rather than the presence of a specific etiologic agent is needed. The method described by Schulzer et al. [158] requires that the gold standard defined to be the same as the test outcome. Their method will then be another way of expressing test-retest variability. Consequently, this solves another problem.

All predictive values are obtained by comparing the outcome of a test with the truth, estimated by using an accepted gold standard. If there are no commonly accepted gold standards, then one has to estimate the truth in another way. In this dissertation, a quantity is introduced using a healthy control group to estimate the truth, and then compute a predictive value. This new quantity is named the Etiologic Predictive Value (EPV).

The purpose of performing a test is to use the outcome to determine the management of the patient. Information about the true etiology of the illness is often difficult to achieve, especially in instances of common bacterial diseases found in primary health care. Predicting the presence of a disease, like EPV, rather than the presence of a marker, is usually more informative.

5.5. The diagnostic value of confirming the presence of potentially pathogenic bacteria

It has been shown in this dissertation that confirming the presence of *GABHS*, in adults with a sore throat or, in children during winter, has a high diagnostic value. It was also shown that long-standing cough may be caused by M. catarrhalis in pre-school children and by *H. influenzae* in adults.

5.5.1. Patients with a sore throat (I-II, IV)

It was found that in patients with a sore throat, growth of *GABHS* in a throat culture is a reliable indicator for true illness caused by *GABHS* in adults ≥16 years of age. In pre-school children 0-6 years of age, a seasonal variation was found. Thus, a throat culture with growth of *GABHS* is reliable in the winter but not in the summer. The data does not permit the conclusion that this seasonal variation is present every year. Although the results in school children 7-15 years of age suggests a seasonal pattern similar to that in pre-school children, the number of school children was too small to be able to draw conclusions regarding the usefulness of a throat culture.

If penicillin does not cure the sore throat then a possible explanation might be that infection is being caused by some other bacteria than beta-haemolytic streptococci that is resistant to treatment with penicillin V [148]. In this study, a high correlation in adults was found between a sore throat and the presence of *H. influenzae*. Previous investigations showed that a high proportion (10-58%) of healthy pre-school children harbour *H. influenzae* in the oropharynx [152] or nasopharynx [117, 151, 152]. Some authors found a throat sample superior to a nasopharyngeal sample for detecting *H. influenzae* [117, 152]. However, in Sweden, the presence of *H. influenzae* is not routinely analysed in throat swabs. *H. influenzae* has been found in removed tonsils [159, 160], in fine-needle aspiration of tonsils among patients with recurrent tonsillitis [161] and in the nasopharynx in patients with pharyngotonsillitis [1, 162]. *H. influenzae* is not clearly implicated as a cause of pharyngotonsillitis [54]. However, many family doctors consider *H. influenzae* as one agent that causes acute pharyngotonsillitis [54], treatment failure [162] or recurrent infection [159, 160, 163]. There are two possible pathogenic mechanisms for *H. influenzae* to be involved; that *H. influenzae* itself is the etiologic agent, or, that it produces betalactamase [160] thus protecting *BHS* from the activity of betalactam antibiotics. If *H. influenzae* is not an etiologic agent in patients with pharyngotonsillitis one could expect the prevalence of *H. influenzae* among healthy individuals and patients with throat infection to be similar. Since a much higher prevalence in patients with sore throat was found compared to healthy individuals one may conclude that, at least in adults, *H. influenzae* may act directly as an etiologic agent. Children do not express a symptom like throat pain as well as adults, and thus, the findings concerning pre-school children are difficult to interpret. The same may be true for children as well, but further testing is needed. The correlation between a sore throat and the presence of *H. influenzae* in the nasopharynx for adults was very strong and of the same magnitude as sore throat and the presence of *GABHS* in throat samples (Table VII on page 41).

It may be concluded that, if *BHS* cannot be detected in patients with a respiratory tract infection and a sore throat, a nasopharyngeal sample could be taken in adults with prolonged symptoms. In adult patients with a proven presence of *BHS* that experience treatment failure or recurrent infection a nasopharyngeal sample could also be considered. It seems advisable to postpone antibiotic treatment until the etiology and the antibiotic sensitivity pattern is established. If *H. influenzae* is found in the nasopharyngeal sample, it is likely to be the etiologic agent. Due to the negative ecological effects of the current antibiotic usage, it could be recommended that pharyngotonsillitis caused by *H. influenzae* only treated in cases with serious or prolonged symptoms.

5.5.2. Patients with a longstanding cough (III, IV)

In this dissertation, long-standing cough in pre-school children correlated with the finding of *M. catarrhalis* in the nasopharynx (Table VIII on page 42) which is in accordance with other studies [47, 48]. Thus, in pre-school children, in whom a bacterial etiology of long-standing cough is suspected, a nasopharyngeal culture might indicate the etiology. However, the chief motive for taking a nasopharyngeal sample is if the result will affect the choice of treatment. Darelid et al found that the presence of *M. catarrhalis* in children with long-standing cough increased the risk of prolonged symptoms and bacterial complications. They further found that proper antibiotic treatment improved the prognosis [48]. However, if it is proven that the etiology is bacterial, and that antibiotic treatment positively alters the prognosis for the patient, one still has to consider whether the benefits of the treatment outweigh the disadvantages [30].

The group of schoolchildren in our investigation was small which might explain why no correlation between potentially pathogenic bacteria and long-standing cough was found. The clinical value of a nasopharyngeal culture for school children with long-standing cough should be further investigated.

The situation is different for adults. Because the carrier rate of common pathogens in the nasopharynx is low in healthy adults [151], the finding of potentially pathogenic bacteria in patients with long-standing cough could provide further information. Such a correlation was found for *S. pneumoniae*, *H. influenzae* and M. catarrhalis. However, it was especially strong for adults with cough combined with other symptoms of respiratory tract infections and growth of *H. influenzae* (Table VIII on page 42). Thus, it could be expected that, in adults with long-standing cough combined with other signs of respiratory tract infection, if the nasopharyngeal culture shows presence of *H. influenzae*, it is likely that this bacterium is involved as etiologic agent.

The finding that adult patients with only cough had more seldom positive nasopharyngeal culture than those with cough combined with other symptoms of a respiratory tract infection (Figure 4 on page 36) implies that the first group contains a higher proportion of patients with asthma or other non-infectious diseases than the other group. This theory is also supported by the fact that *H. influenzae*, the only bacterium associated with cough in adults, was only of importance to those adult patients with cough combined with other symptoms of an upper respiratory tract infection (Table VIII on page 42).

Eradication of M. catarrhalis with erythromycin in adult patients with acute laryngitis has been shown to reduce complaints of cough [164]. However, at inclusion, the erythromycin group had a higher prevalence of M. catarrhalis when compared with the control group, 63% versus 37% ($p=0.02$). Thus, the erythromycin group and the control group might not be comparable. As Schalén et al conclude acute laryngitis in adults is self-limiting, and symptoms are spontaneously reduced after 1 week in most cases. Thus, antibiotic treatment does not seem warranted as a general policy.

It was shown that long-standing cough may be caused by M. catarrhalis in pre-school children and by *H. influenzae* in adults. Nasopharyngeal culture may be used to identify these organisms. In another study it was proven that eradication of M. catarrhalis in children reduces the risk for sequel [48]. The consequences of this

study may be debated. It has not yet been proven that eradication of *H. influenzae* in adults with long-standing cough reduces complaints of cough.

Antibiotic treatment is usually not indicated for children with long-standing cough even if M. catarrhalis is present in the nasopharynx. For adults with long-standing cough, antibiotic treatment is usually not recommended. If *H. influenzae* is found in nasopharynx in adult patients who are professionally dependent on voice function, then antibiotics might be considered.

B. pertussis is a well-known cause for long-standing cough. As is shown (V), the incidence of this disease has decreased substantially during the last four years. Although whooping cough is less common today than it was five years ago, it must still be considered.

5.5.3. Patients with otitis media, pneumoniae and sinusitis

Although it is beyond the purpose of this dissertation to investigate the usefulness of nasopharyngeal samples in patients with otitis media, pneumoniae or sinusitis, a literature review is presented.

The potentially pathogenic bacteria involved in acute otitis media can often be found in nasopharynx [98, 165-167]. Although the positive predictive value predicting presence of bacteria in the middle ear fluid is low for this type of infection [167] a nasopharyngeal culture might have some clinical value especially since it also allows determination of antibiotic resistance patterns of etiologic important bacteria. The negative predictive value of a nasopharyngeal culture predicting absence of bacteria in the middle ear fluid is high in acute otitis media [167].

For patients with pneumonia conflicting results has been presented where some authors found nasopharyngeal culture to be of some value [44, 45] whereas others found it to be of no value [168].

The clinical value of nasopharyngeal culture, in patients with sinusitis, is considered to be small [124].

5.6. When shall the doctor take the test?

It is not advisable to perform a test to detect the presence of potentially pathogenic bacteria in all patients with a respiratory tract disease. How could a physician then refrain from investigating a disease? The answer lies in accepting the uncertainty that is inherent in the practice of all medicine and that the consequences of trying to rule out everything are too great [169].

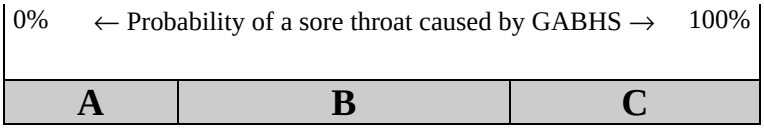
As previously mentioned, there are five important questions in the diagnostic and therapeutic process (Section “5 Discussion” beginning at page 44). If the answer to all of our questions is yes, then the doctor may want to initiate the diagnostic procedure.

Assume the following example with a patient attending for a sore throat. If the doctor is convinced of a viral infection (zone A in Figure 16) then the doctor will probably neither prescribe antibiotics, nor perform a diagnostic test. However, if the patient has many symptoms due to a probable *GABHS* infection, the doctor might prescribe antibiotics without testing (zone C in Figure 16). The literature sometimes mentions a threshold level for performing a test at a pre-test probability for disease caused by *GABHS* that is >10% and a treatment threshold at a >60% probability for the disease [169]. Are these threshold levels appropriate?

Threshold levels in the form of pre-test probability is often difficult to measure. The proportion of patients that are tested or receive a prescription for antibiotics, are quantities that are easier to measure than pre-test probability for disease. In the

literature a throat swab sample would be taken from 22-65% of patients with a sore throat [42, 64, 110, 170] and 52-63% of patients with pharyngitis would, with or without a throat swab sample, immediately receive a prescription for antibiotics [42, 56, 171, 172]. Thus, doctors' behaviour may roughly be described graphically (Figure 16).

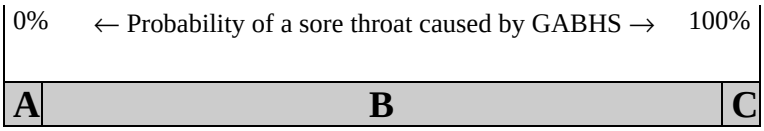
Figure 16 - Threshold levels found in the literature



A = No antibiotics
B = Throat swab sample. Await results
C = Prescribe antibiotics

The clinical usefulness of a throat culture to predict, or exclude, throat infection caused by *GABHS* (Table VII on page 41) suggests that the behaviour in adults should differ from that found in the literature (Figure 17).

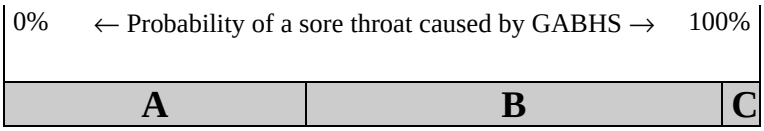
Figure 17 - Threshold levels proposed for adults



A = No antibiotics
B = Throat swab sample. Await results
C = Prescribe antibiotics

Our findings on pre-school children (Table V on page 39) suggest that the clinical value of throat swabs is not as high as in adults. Thus, swabbing in pre-school children having a sore throat and symptoms suggesting a viral infection will result in unnecessary antibiotic treatment of symptomatic carriers having a viral infection. Thus, less swabbing in pre-school children could be recommended (Figure 18).

Figure 18 - Threshold levels proposed for pre-school children



A = No antibiotics
B = Throat swab sample. Await results
C = Prescribe antibiotics

Since NEPV in throat cultures are very high, it might be more appropriate to exclude the C-zone completely from Figure 17 and Figure 18.

Consequentially, a throat swab sample should be taken from a larger proportion of adults with a sore throat than from pre-school children. Unfortunately doctors tend to do the opposite. McIsaac and Goel found that a throat swab sample was usually taken from 41% of children with a sore throat but from only 22% of adults [64].

Children have far more episodes of sore throat than adults do. Thus, the ecological impact of improved diagnosis and treatment is greater if applied to children rather than adults. In future guidelines for the management of sore throats, doctors should

not prescribe antibiotics unless presence of *GABHS* is established. Furthermore, they should refrain from both testing and prescribing antibiotics if symptoms in the children suggest viral etiology.

5.7. Will the test result help?

This dissertation originated with routine medical work as a doctor. Several questions concerning the diagnostic procedure of patients with respiratory tract infections were raised on a day to day basis. To obtain an answer, it was necessary to engage in theoretical discussions in the area of statistics and collected samples from healthy individuals and patients had to be evaluated. Let us now return to the routine medical work and see if these thoughts can be applied. Assume the following:

You have had a three-week vacation and Monday is your first day back at the primary health care centre. The other doctors are on vacation and you are the only doctor on duty. Thus, your schedule is already full when the nurse presents you with an unscheduled patient “that will not take long”. It is a father with his six year old son. It is his fourth visit in the last 8 months. They have met different doctors every time but this is his first visit to you. His father informs you that on the first visit eight month ago, the boy complained of a sore throat. The consulting doctor they visited said; “I can see that this is a classical strep throat”. No tests were made, penicillin was prescribed and the boy’s condition improved. Two months later he suffered again from a sore throat. A rapid test to detect *GABHS* was positive and penicillin was prescribed once again. After another two months the sore throat was back again. At this third visit, a rapid test to detect *GABHS* was again positive. The boy was prescribed cephalosporin (an antibiotic with a wider spectrum than penicillin) for ten days and the symptoms disappeared. Three days ago, the boy again started to complain about a sore throat. The same day the father took his soon to your primary health care centre. A throat swab sample was taken and sent to the nearby microbiological laboratory. He did not prescribe antibiotics, but told them to call the primary health care centre on Monday. The results arrived early this morning showing abundant growth of *GABHS*. The doctor on duty last Friday is now on vacation so you take the case. The father already knows about the test results and informs you that he only wants a prescription of antibiotics for his son.

The boy seems unaffected by his illness. When you look into the boy’s throat there is only slight redness. He has neither enlargement of lymph nodes nor fever. What is the etiology to the boy’s illness? Is it the same etiology in all four episodes, or is it different illnesses? When you ask about other symptoms, the father says that in the previous episodes his son always experienced some hoarseness and coughing a couple of days after the onset of the sore throat. Antibiotic therapy has always led to improvement within 4-8 days of treatment. At two of the episodes he had mild fever. At the third episode he had none and has no fever today.

The nurse senses your hesitation. She asks the laboratory technician about the clinical value of the throat culture. The laboratory technician says that this test is very reliable. She knows that the sensitivity of the culture to find presence of *GABHS* in the throat can be estimated to be close to 90% [34, 36, 39, 88, 89, 102] and the specificity can be estimated to 97% [88, 89].

Furthermore, the laboratory technician has read that the positive predictive value is 100% [36]. The high specificity and the high positive predictive value make it likely that there are *GABHS* in the throat of this boy. It is also well known that *GABHS* may cause pharyngotonsillitis, but is the boy’s illness caused by *GABHS* today? The

symptoms resemble that of a viral infection. Could he be a symptomatic carrier of *GABHS* suffering from a viral infection?

The outcome of the test is only useful for the doctor if it has been evaluated by any of the previously mentioned statistics (Section 2.7 page 13). Before the doctor interpreting the test outcome in the light of the test-statistics presented in a previous study, there are some questions that need to be addressed. Which evaluation method was used? Does this method take carriers into consideration? Are the test statistics applicable in this case?

The sensitivity and the specificity informs you of the health status of the test used, but provides little information about the health status of the patient (Table II on page 20). Since the issue of carriers is highly interesting in this case, an evaluation method considering carriers is needed. In a study a comparison of the proportion of individuals with *GABHS* in the throat was made between healthy pre-school children and children of the same age having a sore throat (this dissertation). During summer, the p-value was 0.06, RR was 2.2 (1.1-6.7) and PEPV was 61% (0-91%) (Table V on page 39). If seasonal variation is not considered then the p-value will be very low ($<10^{-7}$) and the RR was 4.2 (2.6-6.7). The estimate of PEPV is slightly higher with 83% (53-94%).

It seems that the p-value is very sensitive and goes below the magic limit of 0.05 long before the PEPV reaches high values. Thus, it may be inappropriate to evaluate a microbiologic test and make a comparison with a healthy control population using only hypothesis testing. Hypothesis testing will most likely overestimate the clinical importance of finding a bacterium in a patient. RR does not quickly increase to extreme values. In this sense RR resembles EPV. However, EPV is more easily applied to the clinical situation than RR. It is easy to understand that, in this boy, the probability for disease caused by *GABHS* may be estimated to 61%. However, there is a considerable uncertainty and the 95% confidence limits varies between 0% and 91%. If one ignores the seasonal variation the probability for illness caused by *GABHS* increases to 83%, although some uncertainty remains, because the lower 95% confidence limit is only 53%.

In this situation, you may think it true that the conventional throat culture is reliable in detecting *GABHS* in the throat, but it does not necessarily indicate that the boy is ill from *GABHS*. The symptom of hoarseness, cough and low fewer as well as the fact that in previous episodes it took 4-8 days of antibiotics before improvement, are all factors strongly indicating a viral disease in each episode. His present sore throat is probably also a viral disease. Your decision is not to prescribe antibiotics in this case. After an explanation and discussion with the boys father you agree to await spontaneous recovery and re-evaluate only if symptoms get worse.

In other cases with long-standing cough or a sore throat caused by *H. influenzae*, a similar reasoning may be applied. However, there is one important distinction. While more doctors agree to treat sore throats caused by *GABHS*, fewer would agree to treat a sore throat caused by *H. influenzae*, or long-standing cough caused by *H. influenzae* or *M. catarrhalis*. There is some evidence that treatment of *M. catarrhalis* in children with long-standing cough may benefit from antibiotic treatment. It has not been proven that a patient having a sore throat caused by *H. influenzae* has an increased risk for a severe sequel. Neither is there any scientific randomised placebo controlled trials evaluating the effect of antibiotic treatment on sore throats caused by *H. influenzae*. Thus, before the question of performing a test is addressed, the doctor must decide if the grounds for antibiotic treatment are adequate to initiate a further diagnostic procedure.

6. Summary and conclusions

An important factor influencing the diagnostic procedure in uncomplicated respiratory tract infections is symptomatic carriers with illness caused by viral infection. In this dissertation, the influence of symptomatic carriers on the diagnostic process has been clarified and included in the calculation of the predictive value of throat and nasopharyngeal cultures.

A new statistical method was developed, the etiologic predictive value (EPV), that made it possible to predict disease caused by the bacteriological findings while taking symptomatic carriers into account. To calculate the EPV, it is necessary to obtain data concerning the proportion of positive tests among patients, the proportion of positive tests among a healthy control population and the sensitivity of the test. If this information is acquired, the positive and NEPV, with 95% confidence interval, can be calculated.

In this study the proportion of cultures with growth of potentially pathogenic bacteria in patients with respiratory tract symptoms has been compared with a control group of healthy individuals from the same area. The comparison has been made with hypothesis testing, relative risk and the EPV.

Adult patients ≥16 years of age with symptoms of a sore throat and growth of *GABHS* in a throat culture was found to be a reliable indicator for true illness caused by *GABHS*, with a PEPV of 99% (95% confidence interval is 94-100%). In pre-school children (0-6 years of age) there was a seasonal variation in the proportion of children harbouring *GABHS* both in symptomatic patients and healthy individuals. During winter, a throat culture with growth of *GABHS* is reliable, with a PEPV of 94% (75-100%), but not in summer where PEPV is only 61% (0-91%). Our data does not permit us to conclude that this seasonal variation is present every year.

Another observation was that findings of *H. influenzae* in a nasopharyngeal culture, taken from patients with a sore throat, may indicate the true etiology for disease. The prediction in regard to true disease caused by *H. influenzae* (PEPV) is 93% (73-99%) for adults ≥16 years of age and 86% (28-99%) for pre-school children 0-6 years of age. However, the number of school children was too small to be able to make any conclusions regarding the value of nasopharyngeal cultures.

In adults with a long-standing cough combined with other symptoms of a respiratory tract infection, it was found that growth of *H. influenzae* in a nasopharyngeal culture would probably indicate the true etiology for infection. The prediction to true disease caused by *H. influenzae* is 90% (30-99%). Growth of *M. catarrhalis* in a nasopharyngeal sample, taken from pre-school children with a long-standing cough 0-6 years of age, will indicate the true etiology for infection with a probability of 90% (66-99%). However, absence of *M. catarrhalis* results in a NEPV of only 57% (18-80%). Thus, absence of *M. catarrhalis* in these children does not seem to rule out *M. catarrhalis* as a possible etiologic agent to the long-standing cough.

The results of a questionnaire sent to different microbiologic laboratories revealed that there was a substantial variation between different geographical areas in the propensity to perform throat or nasopharyngeal cultures. There was also a large variation between different areas in the outcome of these cultures. The variation in outcome of the cultures makes it difficult to directly apply predictive values calculated from scientific studies.

7. Future perspectives

The need for further research, concerning the diagnostic process of respiratory tract infections, will partly be determined by the future development of bacterial resistance to antibiotics. If antibacterial resistance increases, doctors might have to refrain from treating uncomplicated respiratory tract infections. If so, then the diagnostic procedure in these conditions will not be an important field for research. If however, antibacterial resistance does not increase, and if doctors continue to treat a few selected uncomplicated respiratory tract infections with antibiotics, then further research is necessary. Long-term multicenter studies where patients with a specified condition or symptom are compared to matched control groups are needed. These studies could answer questions such as:

- How will the EPV vary between seasons if variations in the prevalence of potentially pathogenic bacteria are observed for several years?
- How does the EPV vary between different geographical areas?
- Will likelihood ratios add further clinical information to the EPV?

The EPV could be a valuable evaluation method beyond bacterial cultures in patients with a respiratory tract infection. In the diagnostic process of urinary tract infections there is, as with respiratory tract infections, carriers that the diagnostic process should consider. Thus, another issue suitable for future research is if the EPV is also useful in the diagnostic process of urinary tract infections? There are many clinical situations where the method EPV might add diagnostic information (Table XIV).

Table XIV – Example of clinical situations where the EPV might add diagnostic information

Symptom	Disease	Test to be evaluated	Etiology	Sensitivity[a]
A sore throat	A sore throat caused by GABHS	Throat culture	GABHS	GABHS in the throat
A sore throat	A sore throat caused by GABHS	A rapid test to detect presence of GABHS	GABHS	GABHS in the throat
A sore throat	A sore throat caused by H. influenzae	Nasopharyngeal culture	H. influenzae	H. influenzae in the nasopharynx
Long-standing cough	Long-standing cough caused by H. influenzae	Nasopharyngeal culture	H. influenzae	H. influenzae in the nasopharynx
Long-standing cough	Long-standing cough caused by M. catarrhalis	Nasopharyngeal culture	M. catarrhalis	M. catarrhalis in the nasopharynx
Frequent urge to urinate	Frequent urge to urinate caused by E. Coli	Urine culture	E. Coli	E. Coli in the urine
Low back pain	Low back pain caused by a herniated disc in the lumbar region	Specific pattern on a non invasive diagnostic method, for example ultrasound	Herniated disc in the lumbar region	Herniated disc in the lumbar region
Low back pain	Low back pain caused by a herniated disc in the lumbar region	Pain reaction when performing a specific orthopaedic diagnostic manoeuvre	Herniated disc in the lumbar region	Herniated disc in the lumbar region
Chest pain	Chest pain caused by coronary heart disease	A specific EKG-pattern	Coronary heart disease	Coronary heart disease

[a] Sensitivity of the test to be evaluated established with a gold standard predicting presence of…
To calculate EPV this sensitivity is used together with information about the proportion of positive tests among patients and healthy controls.

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9. Appendix

9.1. Appendix: Derivation of the formulae for EPV

(This section explains how the etiologic predictive values were constructed. It can be omitted without loss of understanding.)

Let us begin by making some definitions:

S^+	=	Symptoms, for example a sore throat, is present
S^-	=	Absence of symptoms, i.e. the individual is healthy
D^+	=	Presence of a specified disease, for example a sore throat caused by *GABHS*
D^-	=	Absence of the same disease
M^+	=	Presence of etiologic agent, for example *GABHS*
M^-	=	Absence of the same etiologic agent
T^+	=	Positive test, for example growth of *GABHS* in a throat culture
T^-	=	Negative test, for example no growth of *GABHS* in a throat culture
T^+S^+	=	Positive tests in symptomatic patients
T^-S^+	=	Negative test in symptomatic patients
T^+S^-	=	Positive tests in healthy individuals = Asymptomatic carriers
T^-S^-	=	Negative tests in healthy individuals
Sen	=	Sensitivity of the test

Assume an example of a patient having a sore throat. Let us perform a throat culture. Four situations can then theoretically be identified (Table XV).

Table XV - True positive and true negative test outcome

	S^+D^+ Illness caused by GABHS	S^+D^- Illness caused by another agent
Positive test among patients (S^+T^+)	**True positive**	Carrier
Negative test among patients (S^+T^-)	False negative	**True negative**

However, this is not a true description of all the possible alternatives. The sensitivity and the specificity of the test must also be considered. Thus, there are eight possible situations (Table XVI).

Table XVI - Expanded description of true positive and true negative test outcome

	S^+D^+ Illness caused by GABHS		S^+D^- Illness caused by another agent	
	$S^+D^+M^+$ Marker Present	$S^+D^+M^-$ No marker	$S^+D^-M^+$ Marker Present	$S^+D^-M^-$ No marker
Positive test among patients (S^+T^+)	**True Positive**	False Positive	Real Positive	False Positive
Negative test among patients (S^+T^-)	False Negative	Real Negative	False Negative	**True Negative**

In order to simplify further derivations of formulae Table XVI will be redrawn (Table XVII).

Table XVII - Expanded description with mathematical denotations

	S^+D^+ Illness caused by GABHS		S^+D^- Illness caused by another agent	
	$S^+D^+M^+$ Marker Present	$S^+D^+M^-$ No marker	$S^+D^-M^+$ Marker Present	$S^+D^-M^-$ No marker
Positive test among patients (S^+T^+)	X_1	X_2	X_3	X_4
Negative test among patients (S^+T^-)	Y_1	Y_2	Y_3	Y_4

The X- and Y-terms are introduced in order to simplify the following formulae. When the marker is the only possible etiologic agent for our specified disease, for example *GABHS* for the disease streptococcal throat infection caused by *GABHS*, then X_2 and Y_2 will by definition be zero. In this case Table XVII may be simplified (Table XVIII):

Table XVIII - Simplification of the expanded description

	S^+D^+ Illness caused by GABHS	S^+D^- Illness caused by another agent
Positive test among patients (S^+T^+)	X_1	$X_3 + X_4$
Negative test among patients (S^+T^-)	Y_1	$Y_3 + Y_4$

The relation between X_1 and Y_1 may be provided by the sensitivity (Sen). To estimate the relation between X_3+X_4 and Y_3+Y_4 a healthy control-group will be used as a comparison (Table XIX).

Table XIX - Some patients compared to healthy individuals

	S^+D^- Illness caused by another agent		S^-D^- Healthy control group	
	$S^+D^-M^+$ Marker Present	$S^+D^-M^-$ No marker	$S^-D^-M^+$ Marker Present	$S^-D^-M^-$ No marker
Positive test (T^+)	X_3	X_4	X_5	X_6
Negative test (T^-)	Y_3	Y_4	Y_5	Y_6

The main interested is in the test outcome among patients with the specified disease not present (S^+D^-) (symptomatic carriers) and in the healthy control group (S^-D^-) (asymptomatic carriers). There is no point in dividing them into false negative-true negative respectively false positive-true positive. Since sensitivity and specificity are almost equal in all columns (Table XIX) one may merge:

$$X_3 + X_4 = X_7$$
$$X_5 + X_6 = X_8$$
$$Y_3 + Y_4 = Y_7$$
$$Y_5 + Y_6 = Y_8$$

Table XIX may then be simplified (Table XX):

Table XX - Simplified comparison between patients and healthy individuals

	S^+D^- Illness caused by another agent	S^-D^- Healthy control group
Positive test (T^+)	X_7	X_8
Negative test (T^-)	Y_7	Y_8

The probability of a positive test among healthy is:

$$P(T^+ \mid S^-) = \frac{X_8}{X_8 + Y_8}$$

Let us now define the factor q as the ratio between the probability for a positive test among those patients who do not have the specified disease and among healthy controls. The probability of a positive test among patients who do not have specified etiology for disease is will then be:

$$P(T^+ \mid S^+D^-) = P(T^+ \mid S^-) \times q$$

Table XVIII may now be redrawn (Table XXI).

Table XXI - Mathematical denotations for symptomatic patients

	S^+D^+ Illness caused by GABHS	S^+D^- Illness caused by another agent
Positive test among patients (S^+T^+)	X_1	X_7
Negative test among patients (S^+T^-)	Y_1	Y_7

The probability of a positive test among healthy individuals, $P(T^+ \mid S^+)$, and q will show us the relationship between X_7 and Y_7. Sensitivity of our test (Sen) will provide the relation between X_1 and Y_1. With these known relationships all values may be calculated (Table XXI). Let us begin by estimating Y_7:

$$P(T^+ \mid S^+D^-) = \frac{X_7}{X_7 + Y_7} \qquad X_7 = \left(X_7 \times P(T^+ \mid S^+D^-)\right) + \left(Y_7 \times P(T^+ \mid S^+D^-)\right)$$

$$Y_7 \times P(T^+ \mid S^+D^-) = X_7 - \left(X_7 \times P(T^+ \mid S^+D^-)\right)$$

$$Y_7 = \frac{X_7 - \left(X_7 \times P(T^+ \mid S^+D^-)\right)}{P(T^+ \mid S^+D^-)}$$

Formula 12

Let us now continue to find a formula for X_7. As seen in Table XXI:

$$X_1 = S^+T^+ - X_7$$

Formula 13

In the same way Y_7 can be described:

$$Y_7 = S^+T^- - Y_1$$

Formula 14

As mentioned previously, the sensitivity of our test will describe the relation between X_1 and Y_1 (Table XXI).

$$\text{Sen} = \frac{X_1}{X_1 + Y_1} \qquad X_1 = (\text{Sen} \times X_1) + (\text{Sen} \times Y_1) \qquad X_1 - (\text{Sen} \times X_1) = \text{Sen} \times Y_1$$

$$(X_1 \times (1 - \text{Sen})) - (\text{Sen} \times Y_1) = 0$$

Formula 15

In Formula 15 replace X_1 according to Formula 13. This results in a new expression (Formula 16).

$$\left(\left(S^+T^+ - X_7\right)\times\left(1-\text{Sen}\right)\right)-\left(\text{Sen}\times Y_1\right)=0$$

Formula 16

In Formula 12 replace Y_7 according to Formula 14. This also results in a new expression (Formula 17).

$$S^+T^- - Y_1 = \frac{X_7 - \left(X_7 \times P(T^+ \left| S^+D^- \right.)\right)}{P(T^+ \left| S^+D^- \right.)}$$

$$Y_1 = S^+T^- - \left(\frac{X_7 - \left(X_7 \times P(T^+ \left| S^+D^- \right.)\right)}{P(T^+ \left| S^+D^- \right.)}\right)$$

Formula 17

In Formula 16 replace Y_1 according to Formula 17. After several simplifications there is an expression for X_7 (Formula 18).

$$\left(\left(T^+S^+ - X_7\right)\times\left(1-\text{Sen}\right)\right)-\left(\text{Sen}\times\left(T^-S^+ - \left(\frac{X_7 - \left(X_7 \times P(T^+ \left| S^+D^- \right.)\right)}{P(T^+ \left| S^+D^- \right.)}\right)\right)\right)=0$$

$$T^+S^+ - \left(\text{Sen}\times T^+S^+\right) - X_7 + \left(\text{Sen}\times X_7\right) - \left(\text{Sen}\times T^-S^+\right) + \left(\frac{\left(\text{Sen}\times X_7\right) - \left(\text{Sen}\times X_7 \times P(T^+ \left| S^+D^- \right.)\right)}{P(T^+ \left| S^+D^- \right.)}\right)=0$$

$$T^+S^+ - \left(\text{Sen}\times N_2^+\right) - \left(\text{Sen}\times T^-S^+\right) = X_7 - \left(\text{Sen}\times X_7\right) - \left(\frac{\left(\text{Sen}\times X_7\right) - \left(\text{Sen}\times X_7 \times P(T^+ \left| S^+D^- \right.)\right)}{P(T^+ \left| S^+D^- \right.)}\right)$$

$$T^+S^+ - \left(\text{Sen}\times T^+S^+\right) - \left(\text{Sen}\times T^-S^+\right) = \left(1-\text{Sen}-\left(\frac{\text{Sen} - \left(\text{Sen}\times P(T^+ \left| S^+D^- \right.)\right)}{P(T^+ \left| S^+D^- \right.)}\right)\right)\times X_7$$

$$X_7 = \frac{T^+S^+ - \left(\text{Sen}\times T^+S^+\right) - \left(\text{Sen}\times T^-S^+\right)}{1-\text{Sen}-\left(\frac{\text{Sen} - \left(\text{Sen}\times P(T^+ \left| S^+D^- \right.)\right)}{P(T^+ \left| S^+D^- \right.)}\right)}$$

Formula 18

Since all necessary parts are defined, let us now construct the positive etiologic predictive value. The formula for positive etiologic predictive value is (see Table XXI):

$$P(\mathrm{D}^+|\mathrm{S}^+\mathrm{T}^+) = \frac{\mathrm{X}_1}{\mathrm{S}^+\mathrm{T}^+}$$

$$P(\mathrm{D}^+|\mathrm{S}^+\mathrm{T}^+) = \frac{\mathrm{S}^+\mathrm{T}^+ - \mathrm{X}_7}{\mathrm{S}^+\mathrm{T}^+}$$

Formula 19

In Formula 19 replace X_7 according to Formula 18 and then one will, after several steps of simplification, end up with a preliminary expression for the positive etiologic predictive value (Formula 20).

$$P(\mathrm{D}^+|\mathrm{S}^+\mathrm{T}^+) = \frac{\mathrm{S}^+\mathrm{T}^+ - \left(\dfrac{\mathrm{S}^+\mathrm{T}^+ - (\mathrm{Sen}\times\mathrm{S}^+\mathrm{T}^+) - (\mathrm{Sen}\times\mathrm{S}^+\mathrm{T}^-)}{1-\mathrm{Sen}-\left(\dfrac{\mathrm{Sen}-(\mathrm{Sen}\times P(\mathrm{T}^+|\mathrm{S}^+\mathrm{D}^-))}{P(\mathrm{T}^+|\mathrm{S}^+\mathrm{D}^-)} \right)} \right)}{\mathrm{S}^+\mathrm{T}^+}$$

Divide both the numerator and the denominator with S^+T^+.

$$P(\mathrm{D}^+|\mathrm{S}^+\mathrm{T}^+) = \left(\frac{\mathrm{S}^+\mathrm{T}^+}{\mathrm{S}^+\mathrm{T}^+} \right) - \left(\frac{\mathrm{S}^+\mathrm{T}^+ - (\mathrm{Sen}\times(\mathrm{S}^+\mathrm{T}^+ + \mathrm{S}^+\mathrm{T}^-))}{\mathrm{S}^+\mathrm{T}^+ \times \left(1-\mathrm{Sen}-\left(\mathrm{Sen}\times\left(\dfrac{1-P(\mathrm{T}^+|\mathrm{S}^+\mathrm{D}^-)}{P(\mathrm{T}^+|\mathrm{S}^+\mathrm{D}^-)} \right) \right) \right)} \right)$$

Since $S^+T^+ + S^+T^-$ is S^+ one get

$$P(\mathrm{D}^+|\mathrm{S}^+\mathrm{T}^+) = 1 - \left(\frac{\mathrm{S}^+\mathrm{T}^+ - (\mathrm{Sen}\times\mathrm{S}^+)}{\mathrm{S}^+\mathrm{T}^+ \times \left(1-\left(\mathrm{Sen}\times\left(1+\left(\dfrac{1-P(\mathrm{T}^+|\mathrm{S}^+\mathrm{D}^-)}{P(\mathrm{T}^+|\mathrm{S}^+\mathrm{D}^-)} \right) \right) \right) \right)} \right)$$

$$P(D^+ \mid S^+T^+) = 1 - \left(\frac{1 - \left(\frac{Sen \times S^+}{S^+T^+} \right)}{1 - \left(Sen \times \left(1 + \left(\frac{1 - P(T^+ \mid S^+D^-)}{P(T^+ \mid S^+D^-)} \right) \right) \right)} \right)$$

Formula 20

The probability of a positive test among patients can be transformed to another expression (Formula 21).

$$P(T^+ \mid S^+) = \frac{S^+T^+}{S^+}$$

$$\frac{S^+}{S^+T^+} = \frac{1}{P(T^+ \mid S^+)}$$

Formula 21

In Formula 20 replace S^+/S^+T^+ according to Formula 21. After further simplification one will end up in the correct formula for the positive etiologic predictive value.

$$P(D^+ \mid S^+T^+) = 1 - \left(\frac{1 - \left(\frac{Sen}{P(T^+ \mid S^+)} \right)}{1 - \left(Sen \times \left(1 + \frac{1}{P(T^+ \mid S^+D^-)} - \frac{P(T^+ \mid S^+D^-)}{P(T^+ \mid S^+D^-)} \right) \right)} \right)$$

$$P(D^+ \mid S^+T^+) = 1 - \left(\frac{1 - \left(\frac{Sen}{P(T^+ \mid S^+)} \right)}{1 - \left(\frac{Sen}{P(T^+ \mid S^+D^-)} \right)} \right)$$

The formula for the positive etiologic predictive value will appear nicer if one multiply the numerator and the denominator with –1.

$$P(D^+ \mid S^+T^+) = 1 - \left(\frac{\frac{Sen}{P(T^+ \mid S^+)} - 1}{\frac{Sen}{P(T^+ \mid S^+D^-)} - 1} \right)$$

This is the expression for positive etiologic predictive value presented earlier (Formula 1 on page 27).

Let us know continue with constructing the negative etiologic predictive value. In Table XXI on page 76 one can see that

$$P(D^- \mid S^+T^-) = \frac{Y_7}{S^+T^-}$$

Formula 22

In Formula 22 replace Y_7 according to Formula 12 on page 76:

$$P(D^- \mid S^+T^-) = \frac{\left(\frac{X_7 - \left(X_7 \times P(T^+ \mid S^+D^-)\right)}{P(T^+ \mid S^+D^-)} \right)}{S^+T^-}$$

$$P(D^- \mid S^+T^-) = \frac{X_7 - \left(X_7 \times P(T^+ \mid S^+D^-)\right)}{P(T^+ \mid S^+D^-) \times S^+T^-}$$

Formula 23

In Formula 23 replace X_7 according to Formula 18 on page 77:

$$P(D^- \mid S^+T^-) = \frac{\left(\frac{S^+T^+ - \left(Sen \times S^+T^+\right) - \left(Sen \times S^+T^-\right)}{1 - Sen - \left(\frac{Sen - \left(Sen \times P(T^+ \mid S^+D^-)\right)}{P(T^+ \mid S^+D^-)} \right)} \right) - \left(\left(\frac{S^+T^+ - \left(Sen \times S^+T^+\right) - \left(Sen \times S^+T^-\right)}{1 - Sen - \left(\frac{Sen - \left(Sen \times P(T^+ \mid S^+D^-)\right)}{P(T^+ \mid S^+D^-)} \right)} \right) \times P(T^+ \mid S^+D^-) \right)}{P(T^+ \mid S^+D^-) \times N_2^-}$$

$$P(D^- \mid S^+T^-) = \frac{\left(\frac{S^+T^+ - \left(Sen \times S^+T^+\right) - \left(Sen \times S^+T^-\right)}{1 - Sen - \left(\frac{Sen - \left(Sen \times P(T^+ \mid S^+D^-)\right)}{P(T^+ \mid S^+D^-)} \right)} \right) \times \left(1 - P(T^+ \mid S^+D^-)\right)}{P(T^+ \mid S^+D^-) \times S^+T^-}$$

$$P(D^-|S^+T^-) = \frac{\left(\frac{S^+T^+ - (Sen \times S^+)}{1 - Sen - \left(Sen \times \left(\frac{1 - P(T^+|S^+D^-)}{P(T^+|S^+D^-)}\right)\right)}\right) \times (1 - P(T^+|S^+D^-))}{P(T^+|S^+D^-) \times S^+T^-}$$

$$P(D^-|S^+T^-) = \frac{(S^+T^+ - (Sen \times S^+)) \times (1 - P(T^+|S^+D^-))}{\left(1 - Sen - \left(Sen \times \left(\frac{1 - P(T^+|S^+D^-)}{P(T^+|S^+D^-)}\right)\right)\right) \times P(T^+|S^+D^-) \times S^+T^-}$$

$$P(D^-|S^+T^-) = \frac{(S^+T^+ - (Sen \times S^+)) \times (1 - P(T^+|S^+D^-))}{\left(1 - Sen - \left(Sen \times \left(\frac{1}{P(T^+|S^+D^-)} - \frac{P(T^+|S^+D^-)}{P(T^+|S^+D^-)}\right)\right)\right) \times P(T^+|S^+D^-) \times S^+T^-}$$

$$P(D^-|S^+T^-) = \frac{(S^+T^+ - (S_e \times S^+)) \times (1 - P(T^+|S^+D^-))}{\left(1 - Sen - \left(\frac{Sen}{P(T^+|S^+D^-)} - Sen\right)\right) \times P(T^+|S^+D^-) \times S^+T^-}$$

$$P(D^-|S^+T^-) = \frac{(S^+T^+ - (Sen \times S^+)) \times (1 - P(T^+|S^+D^-))}{\left(1 - \frac{Sen}{P(T^+|S^+D^-)}\right) \times P(T^+|S^+D^-) \times S^+T^-}$$

$$P(D^-|S^+T^-) = \frac{(S^+T^+ - (Sen \times S^+)) \times (1 - P(T^+|S^+D^-))}{(P(T^+|S^+D^-) - Sen) \times S^+T^-}$$

$$P(D^-|S^+T^-) = \left(\frac{1 - P(T^+|S^+D^-)}{P(T^+|S^+D^-) - Sen}\right) \times \left(\frac{S^+T^+ - (Sen \times S^+)}{S^+T^-}\right)$$

$$P(D^-|S^+T^-) = \left(\frac{1 - P(T^+|S^+D^-)}{P(T^+|S^+D^-) - Sen}\right) \times \left(\frac{S^+T^+ - (Sen \times S^+)}{S^+ - S^+T^+}\right)$$

$$P(\mathrm{D}^-|\mathrm{S}^+\mathrm{T}^-) = \left(\frac{1-P(\mathrm{T}^+|\mathrm{S}^+\mathrm{D}^-)}{P(\mathrm{T}^+|\mathrm{S}^+\mathrm{D}^-)-\mathrm{Sen}}\right)\times\left(\frac{\frac{\mathrm{S}^+\mathrm{T}^+}{\mathrm{S}^+}-\mathrm{Sen}}{1-\frac{\mathrm{S}^+\mathrm{T}^+}{\mathrm{S}^+}}\right)$$

$$P(\mathrm{D}^-|\mathrm{S}^+\mathrm{T}^-) = \left(\frac{1-P(\mathrm{T}^+|\mathrm{S}^+\mathrm{D}^-)}{P(\mathrm{T}^+|\mathrm{S}^+\mathrm{D}^-)-\mathrm{S_e}}\right)\times\left(\frac{P(\mathrm{T}^+|\mathrm{S}^+)-\mathrm{Sen}}{1-P(\mathrm{T}^+|\mathrm{S}^+)}\right)$$

$$P(\mathrm{D}^-|\mathrm{S}^+\mathrm{T}^-) = \left(\frac{1-P(\mathrm{T}^+|\mathrm{S}^+\mathrm{D}^-)}{P(\mathrm{T}^+|\mathrm{S}^+\mathrm{D}^-)-\mathrm{Sen}}\right)\times\left(\frac{1-1+P(\mathrm{T}^+|\mathrm{S}^+)-\mathrm{Sen}}{1-P(\mathrm{T}^+|\mathrm{S}^+)}\right)$$

$$P(\mathrm{D}^-|\mathrm{S}^+\mathrm{T}^-) = \left(\frac{1-P(\mathrm{T}^+|\mathrm{S}^+\mathrm{D}^-)}{P(\mathrm{T}^+|\mathrm{S}^+\mathrm{D}^-)-\mathrm{Sen}}\right)\times\left(\left(\frac{1-\mathrm{Sen}}{1-P(\mathrm{T}^+|\mathrm{S}^+)}\right)+\left(\frac{(-1)\times\left(1-P(\mathrm{T}^+|\mathrm{S}^+)\right)}{1-P(\mathrm{T}^+|\mathrm{S}^+)}\right)\right)$$

$$P(\mathrm{D}^-|\mathrm{S}^+\mathrm{T}^-) = \left(\frac{1-P(\mathrm{T}^+|\mathrm{S}^+\mathrm{D}^-)}{P(\mathrm{T}^+|\mathrm{S}^+\mathrm{D}^-)-\mathrm{Sen}}\right)\times\left(\left(\frac{1-\mathrm{Sen}}{1-P(\mathrm{T}^+|\mathrm{S}^+)}\right)-1\right)$$

Add +Sen and –Sen to the left parenthesis.

$$P(\mathrm{D}^-|\mathrm{S}^+\mathrm{T}^-) = \left(\frac{1-P(\mathrm{T}^+|\mathrm{S}^+\mathrm{D}^-)+\mathrm{Sen}-\mathrm{Sen}}{P(\mathrm{T}^+|\mathrm{S}^+\mathrm{D}^-)-\mathrm{Sen}}\right)\times\left(\left(\frac{1-\mathrm{Sen}}{1-P(\mathrm{T}^+|\mathrm{S}^+)}\right)-1\right)$$

$$P(\mathrm{D}^-|\mathrm{S}^+\mathrm{T}^-) = \left(\frac{\mathrm{Sen}-P(\mathrm{T}^+|\mathrm{S}^+\mathrm{D}^-)}{P(\mathrm{T}^+|\mathrm{S}^+\mathrm{D}^-)-\mathrm{Sen}}+\frac{1-\mathrm{Sen}}{P(\mathrm{T}^+|\mathrm{S}^+\mathrm{D}^-)-\mathrm{Sen}}\right)\times\left(\frac{1-\mathrm{Sen}}{1-P(\mathrm{T}^+|\mathrm{S}^+)}-1\right)$$

Multiply both parentheses with –1:

$$P(\mathrm{D}^-|\mathrm{S}^+\mathrm{T}^-) = \left(\frac{P(\mathrm{T}^+|\mathrm{S}^+\mathrm{D}^-)-\mathrm{Sen}}{P(\mathrm{T}^+|\mathrm{S}^+\mathrm{D}^-)-\mathrm{Sen}}+\frac{\mathrm{Sen}\text{-}1}{P(\mathrm{T}^+|\mathrm{S}^+\mathrm{D}^-)-\mathrm{Sen}}\right)\times\left(1-\frac{1-\mathrm{Sen}}{1-P(\mathrm{T}^+|\mathrm{S}^+)}\right)$$

$$P(\mathrm{D}^-|\mathrm{S}^+\mathrm{T}^-) = \left(1+\frac{\mathrm{Sen}\text{-}1}{P(\mathrm{T}^+|\mathrm{S}^+\mathrm{D}^-)-\mathrm{Sen}}\right)\times\left(1-\frac{1-\mathrm{Sen}}{1-P(\mathrm{T}^+|\mathrm{S}^+)}\right)$$

Multiply the numerator and the denominator in the left fraction with –1

$$P(\mathrm{D}^{-}\,|\,\mathrm{S}^{+}\mathrm{T}^{-}) = \left(1+\frac{1-\mathrm{Sen}}{\mathrm{Sen}-P(\mathrm{T}^{+}\,|\,\mathrm{S}^{+}\mathrm{D}^{-})}\right)\times\left(1-\frac{1-\mathrm{Sen}}{1-P(\mathrm{T}^{+}\,|\,\mathrm{S}^{+})}\right)$$

This is the expression for negative etiologic predictive value (Formula 2 on page 27).

9.2. Appendix: Proving the formulae for EPV

(This section shows how one can prove that the formulae for etiologic predictive values are correct. It can be omitted without loss of understanding.)

The simplest way to convince oneself about the truth of Formula 1 on page 27 is to rearrange the formulae and then replace each of the three conditional probabilities by that ratio of probabilities which defines it, and then solve Formula 1 for Sen.

$$P(\mathrm{D}^{+}\,|\,\mathrm{S}^{+}\mathrm{T}^{+}) = 1-\left(\frac{\frac{\mathrm{Sen}}{P(\mathrm{T}^{+}\,|\,\mathrm{S}^{+})}-1}{\frac{\mathrm{Sen}}{P(\mathrm{T}^{+}\,|\,\mathrm{S}^{+}\mathrm{D}^{-})}-1}\right)$$

Multiply both sides with the denominator

$$\left(P(\mathrm{D}^{+}\,|\,\mathrm{S}^{+}\mathrm{T}^{+})\right)\times\left(\frac{\mathrm{Sen}}{P(\mathrm{T}^{+}\,|\,\mathrm{S}^{+}\mathrm{D}^{-})}-1\right) = \frac{\mathrm{Sen}}{P(\mathrm{T}^{+}\,|\,\mathrm{S}^{+}\mathrm{D}^{-})}-1-\left(\frac{\mathrm{Sen}}{P(\mathrm{T}^{+}\,|\,\mathrm{S}^{+})}-1\right)$$

$$\frac{\mathrm{Sen}\times P(\mathrm{D}^{+}\,|\,\mathrm{S}^{+}\mathrm{T}^{+})}{P(\mathrm{T}^{+}\,|\,\mathrm{S}^{+}\mathrm{D}^{-})} - P(\mathrm{D}^{+}\,|\,\mathrm{S}^{+}\mathrm{T}^{+}) = \frac{\mathrm{Sen}}{P(\mathrm{T}^{+}\,|\,\mathrm{S}^{+}\mathrm{D}^{-})} - \frac{\mathrm{Sen}}{P(\mathrm{T}^{+}\,|\,\mathrm{S}^{+})}$$

$$\frac{\mathrm{Sen}\times P(\mathrm{D}^{+}\,|\,\mathrm{S}^{+}\mathrm{T}^{+})}{P(\mathrm{T}^{+}\,|\,\mathrm{S}^{+}\mathrm{D}^{-})} - \frac{\mathrm{Sen}}{P(\mathrm{T}^{+}\,|\,\mathrm{S}^{+}\mathrm{D}^{-})} + \frac{\mathrm{Sen}}{P(\mathrm{T}^{+}\,|\,\mathrm{S}^{+})} = P(\mathrm{D}^{+}\,|\,\mathrm{S}^{+}\mathrm{T}^{+})$$

$$\mathrm{Sen}\times\left(\frac{P(\mathrm{D}^{+}\,|\,\mathrm{S}^{+}\mathrm{T}^{+})}{P(\mathrm{T}^{+}\,|\,\mathrm{S}^{+}\mathrm{D}^{-})} - \frac{1}{P(\mathrm{T}^{+}\,|\,\mathrm{S}^{+}\mathrm{D}^{-})} + \frac{1}{P(\mathrm{T}^{+}\,|\,\mathrm{S}^{+})}\right) = P(\mathrm{D}^{+}\,|\,\mathrm{S}^{+}\mathrm{T}^{+})$$

$$\mathrm{Sen}\times\left(\frac{1}{P(\mathrm{T}^{+}\,|\,\mathrm{S}^{+})} + \frac{P(\mathrm{D}^{+}\,|\,\mathrm{S}^{+}\mathrm{T}^{+})-1}{P(\mathrm{T}^{+}\,|\,\mathrm{S}^{+}\mathrm{D}^{-})}\right) = P(\mathrm{D}^{+}\,|\,\mathrm{S}^{+}\mathrm{T}^{+})$$

$$\mathrm{Sen}\times\left(\frac{1}{P(\mathrm{T}^{+}\,|\,\mathrm{S}^{+})} - \frac{1-P(\mathrm{D}^{+}\,|\,\mathrm{S}^{+}\mathrm{T}^{+})}{P(\mathrm{T}^{+}\,|\,\mathrm{S}^{+}\mathrm{D}^{-})}\right) = P(\mathrm{D}^{+}\,|\,\mathrm{S}^{+}\mathrm{T}^{+})$$

$1-P(\mathrm{D}^{+}\left|\mathrm{S}^{+}\mathrm{T}^{+}\right.)$ can be replaced with $P(\mathrm{D}^{-}\left|\mathrm{S}^{+}\mathrm{T}^{+}\right.)$

$$\boxed{\mathrm{Sen}\times\left(\frac{1}{P(\mathrm{T}^{+}\left|\mathrm{S}^{+}\right.)}-\frac{P(\mathrm{D}^{-}\left|\mathrm{S}^{+}\mathrm{T}^{+}\right.)}{P(\mathrm{T}^{+}\left|\mathrm{S}^{+}\mathrm{D}^{-}\right.)}\right)=P(\mathrm{D}^{+}\left|\mathrm{S}^{+}\mathrm{T}^{+}\right.)}$$

Replacing each of the conditional probabilities by that ratio of probabilities which defines it yields

$$\boxed{\mathrm{Sen}\times\left(\frac{1}{\frac{P(\mathrm{S}^{+}\mathrm{T}^{+})}{P(\mathrm{S}^{+})}}-\frac{\frac{P(\mathrm{S}^{+}\mathrm{T}^{+}\mathrm{D}^{-})}{P(\mathrm{S}^{+}\mathrm{T}^{+})}}{\frac{P(\mathrm{S}^{+}\mathrm{T}^{+}\mathrm{D}^{-})}{P(\mathrm{S}^{+}\mathrm{D}^{-})}}\right)=\frac{P(\mathrm{S}^{+}\mathrm{T}^{+}\mathrm{D}^{+})}{P(\mathrm{S}^{+}\mathrm{T}^{+})}}$$

$$\boxed{\mathrm{Sen}\times\left(\frac{P(\mathrm{S}^{+})}{P(\mathrm{S}^{+}\mathrm{T}^{+})}-\frac{P(\mathrm{S}^{+}\mathrm{D}^{-})\times P(\mathrm{S}^{+}\mathrm{T}^{+}\mathrm{D}^{-})}{P(\mathrm{S}^{+}\mathrm{T}^{+})\times P(\mathrm{S}^{+}\mathrm{T}^{+}\mathrm{D}^{-})}\right)=\frac{P(\mathrm{S}^{+}\mathrm{T}^{+}\mathrm{D}^{+})}{P(\mathrm{S}^{+}\mathrm{T}^{+})}}$$

$$\boxed{\mathrm{Sen}\times\left(\frac{P(\mathrm{S}^{+})}{P(\mathrm{S}^{+}\mathrm{T}^{+})}-\frac{P(\mathrm{S}^{+}\mathrm{D}^{-})}{P(\mathrm{S}^{+}\mathrm{T}^{+})}\right)=\frac{P(\mathrm{S}^{+}\mathrm{T}^{+}\mathrm{D}^{+})}{P(\mathrm{S}^{+}\mathrm{T}^{+})}}$$

Multiply both sides with $P(\mathrm{S}^{+}\mathrm{T}^{+})$

$$\boxed{\mathrm{Sen}\times\left(P(\mathrm{S}^{+})-P(\mathrm{S}^{+}\mathrm{D}^{-})\right)=P(\mathrm{S}^{+}\mathrm{T}^{+}\mathrm{D}^{+})}$$

$P(\mathrm{S}^{+})-P(\mathrm{S}^{+}\mathrm{D}^{-})$ can be replaced with $P(\mathrm{S}^{+}\mathrm{D}^{+})$

$$\boxed{\mathrm{Sen}\times P(\mathrm{S}^{+}\mathrm{D}^{+})=P(\mathrm{S}^{+}\mathrm{T}^{+}\mathrm{D}^{+})}$$

This shows that what one have to prove is that

$$\mathrm{Sen}=\frac{P(\mathrm{S}^{+}\mathrm{T}^{+}\mathrm{D}^{+})}{P(\mathrm{S}^{+}\mathrm{D}^{+})}$$

Formula 24

and here the right hand side equals $P(\mathrm{T}^{+}\left|\mathrm{S}^{+}\mathrm{D}^{+}\right.)$.

By definition

$$\mathrm{Sen}=P(\mathrm{T}^{+}\left|\mathrm{M}^{+}\right.)$$

and since the test (T) pays attention neither to symptoms (S) nor to the cause of symptoms, if any, one can enter an arbitrary combination of S^{-}, S^{+} and D^{-}, D^{+} after the vertical bar.

Right now we prefer the combination S^+D^+ which gives

$$\text{Sen} = P(T^+ \mid S^+M^+D^+);$$

but M^+D^+ equals D^+, which means that we have

$$\text{Sen} = P(T^+ \mid S^+D^+),$$

which is what we set out to prove.

The result for the NEPV (Formula 2 on page 27) can be proved in the same way: straight-forward computation shows that (Formula 2) is also equivalent to (Formula 24):

$$P(D^- \mid S^+T^-) = \left(1 + \frac{1-\text{Sen}}{\text{Sen} - P(T^+ \mid S^+D^-)}\right) \times \left(1 - \frac{1-\text{Sen}}{1 - P(T^+ \mid S^+)}\right)$$

$$P(D^- \mid S^+T^-) = \left(\frac{\text{Sen} - P(T^+ \mid S^+D^-)}{\text{Sen} - P(T^+ \mid S^+D^-)} + \frac{1-\text{Sen}}{\text{Sen} - P(T^+ \mid S^+D^-)}\right) \times \left(\frac{1 - P(T^+ \mid S^+)}{1 - P(T^+ \mid S^+)} - \frac{1-\text{Sen}}{1 - P(T^+ \mid S^+)}\right)$$

$$\Downarrow$$

$$P(D^- \mid S^+T^-) = \frac{\text{Sen} - P(T^+ \mid S^+D^-) + (1-\text{Sen})}{\text{Sen} - P(T^+ \mid S^+D^-)} \times \frac{1 - P(T^+ \mid S^+) - (1-\text{Sen})}{1 - P(T^+ \mid S^+)}$$

$$P(D^- \mid S^+T^-) = \frac{1 - P(T^+ \mid S^+D^-)}{\text{Sen} - P(T^+ \mid S^+D^-)} \times \frac{\text{Sen} - P(T^+ \mid S^+)}{1 - P(T^+ \mid S^+)}$$

$1 - P(T^+ \mid S^+D^-)$ with $P(T^- \mid S^+D^-)$ and $1 - P(T^+ \mid S^+)$ can be replaced with $P(T^- \mid S^+)$

$$P(D^- \mid S^+T^-) = \frac{P(T^- \mid S^+D^-)}{\text{Sen} - P(T^+ \mid S^+D^-)} \times \frac{\text{Sen} - P(T^+ \mid S^+)}{P(T^- \mid S^+)}$$

Replacing each of the conditional probabilities by that ratio of probabilities which defines it yields

$$\frac{P(S^+T^-D^-)}{P(S^+T^-)} = \frac{\frac{P(S^+T^-D^-)}{P(S^+D^-)}}{\text{Sen} - \frac{P(S^+T^+D^-)}{P(S^+D^-)}} \times \frac{\text{Sen} - \frac{P(S^+T^+)}{P(S^+)}}{\frac{P(S^+T^-)}{P(S^+)}}$$

$$\frac{P(S^+T^-D^-)}{P(S^+T^-)}\times\left(\text{Sen}-\frac{P(S^+T^+D^-)}{P(S^+D^-)}\right)=\frac{P(S^+T^-D^-)}{P(S^+D^-)}\times\left(\frac{\text{Sen}-\frac{P(S^+T^+)}{P(S^+)}}{\frac{P(S^+T^-)}{P(S^+)}}\right)$$

Divide both sides with $P(S^+T^-D^-)$

$$\frac{1}{P(S^+T^-)}\times\left(\text{Sen}-\frac{P(S^+T^+D^-)}{P(S^+D^-)}\right)=\frac{1}{P(S^+D^-)}\times\left(\frac{\text{Sen}-\frac{P(S^+T^+)}{P(S^+)}}{\frac{P(S^+T^-)}{P(S^+)}}\right)$$

$$\frac{1}{P(S^+T^-)}\times\left(\text{Sen}-\frac{P(S^+T^+D^-)}{P(S^+D^-)}\right)=\frac{1}{P(S^+D^-)}\times\left(\frac{P(S^+)\times\left(\text{Sen}-\frac{P(S^+T^+)}{P(S^+)}\right)}{P(S^+T^-)}\right)$$

Multiply both sides with $P(S^+T^-)$

$$\text{Sen}-\frac{P(S^+T^+D^-)}{P(S^+D^-)}=\frac{1}{P(S^+D^-)}\times\left(P(S^+)\times\left(\text{Sen}-\frac{P(S^+T^+)}{P(S^+)}\right)\right)$$

Multiply both sides with $P(S^+D^-)$

$$\text{Sen}\times P(S^+D^-)-P(S^+T^+D^-)=P(S^+)\times\left(\text{Sen}-\frac{P(S^+T^+)}{P(S^+)}\right)$$

$$\text{Sen}\times P(S^+D^-)-P(S^+T^+D^-)=P(S^+)\times\text{Sen}-P(S^+T^+)$$

$$\text{Sen}\times P(S^+D^-)-P(S^+)\times\text{Sen}=P(S^+T^+D^-)-P(S^+T^+)$$

Multiply both sides with -1

$$\text{Sen}\times P(S^+)-\text{Sen}\times P(S^+D^-)=P(S^+T^+)-P(S^+T^+D^-)$$

$$\text{Sen}\times P(S^+)-\text{Sen}\times P(S^+D^-)=P(S^+T^+D^+)$$

Replace $P(S^+)-P(S^+D^-)$ with $P(S^+D^+)$

$$\text{Sen}\times P(S^+D^+)=P(S^+T^+D^+)$$

and this can be transformed to Formula 24 on page 84 where the rest of the proof is given.

9.3. Appendix: Derivation of the interval estimate of EPV

(This section explains how the confidence intervals for etiologic predictive values were constructed. It can be omitted without loss of understanding.)

The formulae for EPV (Formula 1) and (Formula 2) express PEPV and NEPV by means of $P(T^+ \mid S^+)$, $P(T^+ \mid S^+D^-)$ and Sen. Here Sen is assumed to be known, while the first two probabilities have to be estimated. Now we shall show how confidence intervals for $P(T^+ \mid S^+)$ and $P(T^+ \mid S^+D^-)$ can be translated into confidence intervals for PEPV and NEPV.

Let us start by pointing out that for any reasonable test T we have

$$P(T^+ \mid S^+D^-) < P(T^+ \mid S^+D^+)$$

which is the same as

$$P(T^+ \mid S^+D^-) < P(T^+ \mid M^+)$$

and we can write this as:

$$P(T^+ \mid S^+D^-) < Se$$

Formula 25

Furthermore $P(T^+ \mid S^+)$ is a weighted average of $P(T^+ \mid S^+D^-)$ and Sen:

$$P(T^+ \mid S^+) = P(D^- \mid S^+) \times P(T^+ \mid S^+D^-) + P(D^+ \mid S^+) \times P(T^+ \mid S^+D^+)$$

and since

$$P(T^+ \mid S^+D^+) = P(T^+ \mid M^+) = Sen$$

we obtain

$$P(T^+ \mid S^+) = P(D^- \mid S^+) \times P(T^+ \mid S^+D^-) + P(D^+ \mid S^+) \times Sen$$

Hence $P(T^+ \mid S^+)$ is between $P(T^+ \mid S^+D^-)$ and Sen:

$$P(T^+ \mid S^+D^-) < P(T^+ \mid S^+) < Sen$$

Formula 26

In the formula for PEPV (Formula 1 on page 27) we can see that the PEPV, $P(D^+ \mid S^+T^+)$, is an increasing function of $P(T^+ \mid S^+)$ and a decreasing function of $P(T^+ \mid S^+D^-)$ while for NEPV both relations are reversed.

Thus, a lower limit for $P(T^+ \mid S^+D^-)$ and an upper for $P(T^+ \mid S^+)$ will give us an upper limit for the positive etiologic predictive value and a lower one for the negative etiologic predictive value; limits in the other direction are obtained in an analogous fashion. Suppose we have the following confidence intervals:

$$a < P(T^+ \mid S^+) < b$$

Formula 27

$$c < P(\mathrm{T}^+ \,|\, \mathrm{S}^+\mathrm{D}^-) < d$$

Formula 28

Confidence intervals will then be given by (Formula 3 - Formula 4 beginning on page 28 and Formula 8 - Formula 11 beginning on page 29). As concluded above (in Formula 26) we always have

$$P(\mathrm{T}^+ \,|\, \mathrm{S}^+) > P(\mathrm{T}^+ \,|\, \mathrm{S}^+\mathrm{D}^-) \text{ and } \mathrm{Se} > P(\mathrm{T}^+ \,|\, \mathrm{S}^+)\,.$$

If however $d > a$, Formula 3 on page 28 will return a lower limit below zero. The same will occur in Formula 4 on page 28 if $b > \mathrm{Se}$. If this occurs we take the lower limit to be zero. If $b > \mathrm{Se}$, Formula 3 will return an upper limit above 1. The same will occur in Formula 4 if $d > a$. If so we take the upper limit to be 1.

If each of the two confidence intervals (*a*, *b*) and (*c*, *d*) has confidence level 1-â, we can, according to Bonferroni, state with at least 1-2â confidence that both intervals are correct simultaneously. Whenever that happens, both intervals (Formula 3) and (Formula 4) will be correct. Hence we choose â=á/2 where 1-á is the desired confidence level for the intervals for PEPV and NEPV. As an extra bonus, the confidence intervals for PEPV and NEPV will have the simultaneous confidence level 1-á.

Thus, confidence limits for the etiologic predictive values are as in Formula 3 and Formula 4 (in Section 4.1.2 on page 28).

10. References

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13. Original publications

I. Gunnarsson RK, Holm SE, Söderström M. The prevalence of beta-haemolytic streptococci in throat specimens from healthy children and adults. Implications for the clinical value of throat cultures. *Scand J Prim Health Care* 1997;**15**(3):149-55.

II. Gunnarsson RK, Holm SE, Söderström M. The prevalence of potential pathogenic bacteria in nasopharyngeal samples from individuals with a respiratory tract infection and a sore throat – Implications for the diagnosis of pharyngotonsillitis. *Fam Pract* 2001;(Accepted for publication).

III. Gunnarsson RK, Holm SE, Söderström M. The prevalence of potentially pathogenic bacteria in nasopharyngeal samples from individuals with a long-standing cough - clinical value of a nasopharyngeal sample. *Fam Pract 2000*;**17**(2):150-5.

IV. Gunnarsson RK, Lanke J. The predictive value of microbiologic diagnostic tests if asymptomatic carriers are present. *Submitted manuscript*.

V. Gunnarsson RK, Holm SE, Kahlmeter G, Söderström M. Geographical variations in the propensity to perform upper respiratory tract cultures in Sweden do not correlate to findings of pathogens in the cultures. *Manuscript*.

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