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Pharyngeal Colonization with Respiratory Viruses and the Likelihood of Group A Streptococcus (GABHS) Persistence after GABHS Pharyngitis

by

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ABSTRACT

Persistence of group A streptococcus (GABHS) after adequate antibiotic treatment of GABHS pharyngitis is a well-recognized phenomenon for which there is no widely accepted explanation. This prospective cohort study tested the hypothesis that co-infection with a respiratory virus would increase the risk of GABHS persistence after treatment of pharyngitis with penicillin V. Two-hundred and forty-eight of 286 (86.7%) children enrolled with symptomatic pharyngitis were evaluable. One-hundred and four of 248 (41.9%) children with pharyngitis were infected with GABHS; their median age was 6.9 years (range 2.7 to 14.4 years) and there was a male predominance (60/104;57.7 %). Ten of 104 (9.6%) patients with GABHS pharyngitis were co-infected with a respiratory virus. There were no significant differences between the two groups in demographic or clinical characteristics. Persistence occurred in 2/10 (20.0%) of those with viral co-infection and 31/94 (33.0%) of those without it, a difference which was not significant (2-sided FET, P= 0.49). No other clinical or laboratory characteristics measured in this study were associated with persistence. Therefore it was concluded that viral co-infection is not associated with persistence of GABHS after treatment of GABHS pharyngitis.

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DEDICATION

I would like to dedicate this thesis to my daughter, Ileana Camilla, who ensures that I maintain a healthy perspective on my life and work each and every day.

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INTRODUCTION, RATIONALE, AND OBJECTIVES

INTRODUCTION

Group A beta-hemolytic Streptococcus (GABHS) pharyngitis is a common childhood infection. While the discomfort and inconvenience of the illness are the primary concern of the patient and parents, the potential for suppurative and non-suppurative complications are the most important reasons to treat this infection. Suppurative manifestations range from cervical adenitis to peritonsillar or retropharyngeal abscess. Serious invasive infections such as necrotizing fasciitis and streptococcal toxic shock syndrome have recently been noted. Non-suppurative complications include post-streptococcal glomerulonephritis and acute rheumatic fever. Although rheumatic fever can be prevented with adequate therapy, many outbreaks have been noted throughout North America in the last 20 years.

At the same time that there is increasing concern about prevention of complications of GABHS, it has become clear that treatment with antibiotics is not always effective in eradicating pharyngeal GABHS. Not only can bacteriological treatment failure lead to complications in the infected child, but the organism can be transmitted to others as well.

Although many theories have been proposed to explain bacteriological failure (persistence), the pathogenesis remains unexplained despite extensive investigations. One theory, which has not been suggested or investigated to date, is the potential influence of a viral co-infection on the bacteriologic outcome of GABHS pharyngitis.

RATIONALE

If this study is able to demonstrate that respiratory viruses are associated with persistence, it will be a first step towards establishing a causal relationship. Antiviral therapeutic interventions may also result if such an association is demonstrated (e.g. amantidine for influenza A). With continued discovery of antiviral agents, more treatment options for this or other viruses may arise in the future.

STUDY OBJECTIVES

The main objective of this study was to determine whether there is an association between co-infection with a respiratory virus and persistence of GABHS after treatment of GABHS pharyngitis. Subsidiary objectives included a comprehensive description of children presenting with symptoms of pharyngitis, assessment of the relationship between persistence and various other clinical and demographic factors, and evaluation of a new rapid test for GABHS detection.

CHAPTER 2

LITERATURE REVIEW

This chapter provides the background information on the epidemiology, presentation and diagnosis of *S. pyogenes* pharyngitis. The frequency and significance of the phenomenon of persistence is discussed and a definition given. Current and past theories of the pathogenesis of GABHS persistence after treatment of pharyngitis are critically reviewed. Support for the proposed theory that viral co-infection is associated with persistence is detailed along with the laboratory procedures its testing requires.

EPIDEMIOLOGY OF GABHS PHARYNGITIS

Although GABHS is a pathogen found worldwide, it causes different patterns of disease depending on geographic location. Whereas GABHS respiratory infections are uncommon compared to infections of skin in tropical climates, the reverse is true in North America and Europe.[1] Streptococcal pharyngitis has been noted to be one of the most common bacterial infections in the pediatric age group.[2]

Since World War I, in North America GABHS pharyngitis has been primarily an endemic disease with occasional outbreaks.[1] GABHS is estimated to cause 15%[3] of pharyngitis cases, although studies have found figures as low as 5%[4] in adults and as high as 40%[5] or more in children. Among throat swabs cultured at the Alberta Children's Hospital microbiology laboratory in 1995/96, 24.7% were positive for GABHS.

In temperate climates, the incidence of GABHS pharyngitis is at its highest during the coldest months of the year (winter through spring)[6] and has been attributed to crowding.[1] This peak is earlier, more pronounced, and sustained longer the further north

one goes in this climate.[1] Data from the microbiology laboratory at Alberta Children's Hospital confirms a similar GABHS isolation pattern locally, with most isolates occurring from October to June (during 1995/96) with the peak occurring in January/February and the nadir in August of those years (Figure 1).

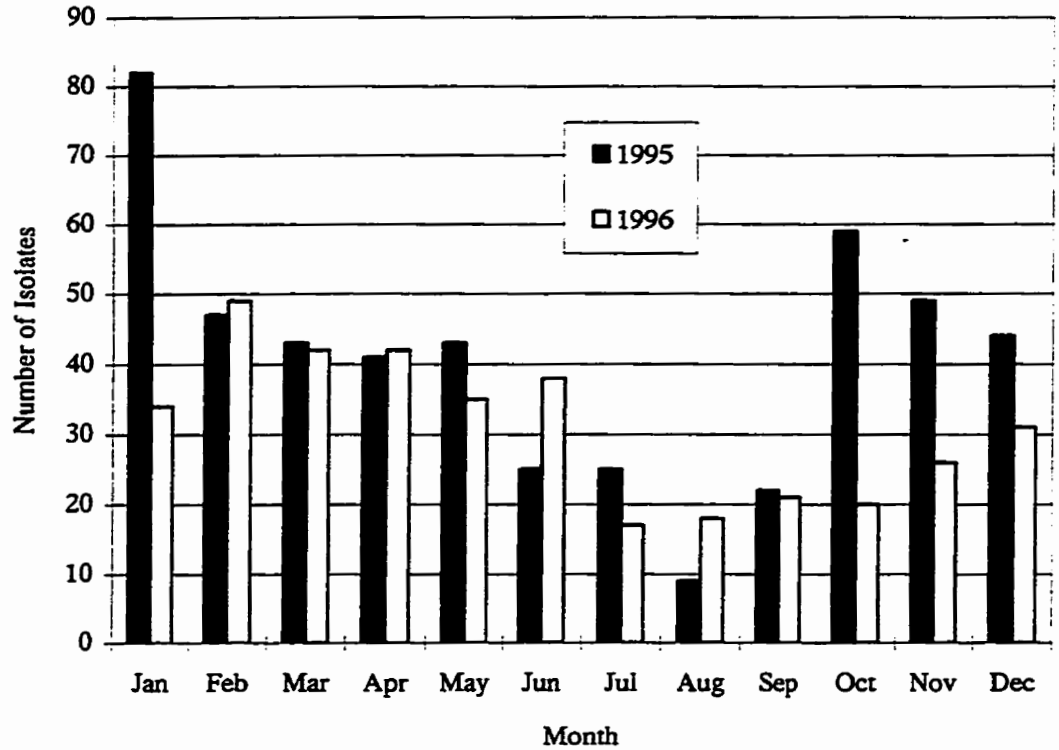
Transmission of GABHS is most often spread by direct person to person contact and aerosolized droplets.[1,2] Crowding of people in army barracks[2,7] or close contact within families[1] have both been shown to facilitate spread of infection. Investigation of outbreaks have also shown that this organism can be transmitted by contaminated food and water.[2]

Streptococcal pharyngitis is primarily a disease of children. The incidence peaks in the young school-aged population (5-9 years old [8-10] although a second peak at 12-13 years of age has also been noted.[8] GABHS is an uncommon cause of pharyngitis in children under two years old[8,9,11] and this age group is usually excluded from enrollment in pediatric pharyngitis studies as a result.

Pharyngeal colonization with GABHS is also very common among children. Up to 20% of school-aged children have been shown to carry this organism,[2] although this figure can vary by season and geographic location. Unlike children with acute pharyngitis, carriers are much less likely to transmit the infection to others.[12]

The epidemiology of this organism has also been studied in terms of the patterns of disease by strain. M and T serotyping is a common means by which group A streptococcal isolates are distinguished. In a review of isolates from the USA submitted to the Centres for Disease Control in Atlanta between 1972 and 1988,[13] respiratory infections (including pharyngitis) made up 47% of the isolates submitted. Overall, M-types 1, 3, 4, 12, and 18 were the only types to contribute less than 1% of the total isolates. Significant changes were noted between 1972-79 and 1989-88 for M1 and M3 (increased) as well as M4 and M12 (decreased). Subsequently, a study examining isolates from 866 children with pharyngitis found five M-types comprised about two-thirds of the total (in decreasing order of frequency): M1, M12, M2, M28 and M5.[14]

Figure 1. GABHS Isolation Frequency at Alberta Children's Hospital: 1995/96



CLINICAL PRESENTATION OF GABHS PHARYNGITIS

Overlap of clinical signs and symptoms in pharyngitis of different etiologies makes prediction of GABHS isolation difficult. Abnormal pharynx, cervical lymphadenopathy, sore throat, headache, halitosis, and temperature greater than 38 °C have been noted to be the most frequent abnormalities in GABHS pharyngitis, whereas cough and rhinorrhea occur in fewer than 20% of cases.[15] Signs and symptoms which have been noted more frequently with GABHS than non-GABHS pharyngitis include sore throat, pain with swallowing, headache, anterior cervical lymphadenopathy, moderate to severe pharyngeal inflammation, and pharyngeal exudate.[8,9] Breese formulated a scoring system to predict GABHS based on clinical data,[16] but recent comparison to culture showed poor positive and negative predictive values (58% and 79% respectively).[17] In spite of this, various combinations of clinical characteristics have been used to select patients into pharyngitis studies in an effort to increase the relative frequency of GABHS isolation.[11,18-21]

BACTERIOLOGICAL DIAGNOSIS OF GABHS INFECTION

Various diagnostic tests have been developed to detect GABHS, including several culture methods and antigen assays. Broth-enrichment and/or dual-plate systems have been used as gold standards in the evaluation of new methods.[22,23] A review of plate cultures agrees that no single medium-atmosphere combination is perfect, but recovery can be maximized by careful collection, swabbing/streaking on plate, inspection at both 24 and 48 hours, and quality control of the medium, incubator, and reagents used.[24] However, the Alberta Children's Hospital microbiology laboratory demonstrated that retention of plates more than 24 hours resulted in only a 1% improvement in yield. (Dr. D. Church, personal communication 1994)

Methods that detect *S. pyogenes* antigen directly from throat swabs have been developed in an attempt to be able to make the diagnosis of GABHS pharyngitis more quickly. Prompt initiation of antibiotics results in faster resolution of symptoms and signs of the infection,[25,26] therefore rapid diagnostic technology is appreciated by patient and physician alike. While most antigen detection systems are very specific, unfortunately sensitivities (even for the same type of test kit) vary from 60 to 90%.[27] A recent study of Directogen 1-2-3 Group A Streptococcal Test demonstrated a positive predictive value of 61% and negative predictive value of 89% (compared to culture) in a population with a GABHS prevalence of 25%.[17] Thus, not only will there be many unnecessary treatments, but also a negative result must be confirmed with traditional culture methods.[17,27]

A new rapid test utilizing a novel technique may be an improvement on previous antigen detection methods. The optical immunoassay technique employs direct visualization of an antigen applied to an antibody monolayer on a film surface.[28] The increased thickness of the film results in a change in its reflective properties, with a positive reaction indicated by a colour change from gold to purple. The initial evaluation included two studies with sensitivities of 97.4% to 98.9% and specificities of 95.6% to 98.9%.[28] Subsequent studies done in office or clinic settings found sensitivities of 77% to 94.8% and specificities of 94.1% to 98.8%[29-31] when the OIA was compared to plate and/or broth culture. Overall the OIA appears to be very specific, and in all but one study had a sensitivity of at least 90%. The latter represents an improvement over earlier types of rapid testing methods for GABHS.

PHARYNGEAL GABHS PERSISTENCE

Epidemiology

GABHS persistence after treatment of pharyngitis, or bacteriological treatment failure, is a common occurrence. Persistence rates after treatment with penicillin benzathine, penicillin V and erythromycin range from 8% to 38%.[8,18,20,32-38] Once

persistence has been documented, the probability of eradication with re-treatment decreases further, with persistent detection of GABHS in 42 to 61% of cases after two or more courses of penicillin.[32,35] Therefore, patients already on antibiotics for as little as one day should not be included in persistence studies, as those who still have GABHS detected may represent a special population. In a study of acute pharyngitis, it was found that the majority was culture negative for GABHS within 24 hours of starting antibiotics.[39] Children on prophylactic antibiotics such as penicillin for prevention of acute rheumatic fever were also excluded. Although recent evidence suggests that cephalosporins may have greater success, failure to eradicate GABHS with this class of antibiotics continues to occur in 6 to 11% of acute GABHS pharyngitis.[20,38] Overall, penicillin is still considered to be the first line antimicrobial in non-allergic patients.[40,41]

Study Populations

Previous GABHS pharyngitis studies have a variety of study population characteristics. Private urban pediatric practices[18,34,42,43] and university/children's hospital outpatient clinics[19,33,35,37,38,44] are most commonly used. Pediatric emergency departments[33] and a rural population[20] appear to be less common. Males usually comprise at least half of the study subjects.[18,19,21,33,34,37] Mean age ranges between 6.5 and 9 years in studies that included patients up to 18 years of age.[19-21,33,37,38,42,44]

Clinical Significance

Persistence of GABHS after treatment presents potential problems both for the individual as well as their contacts. Unlike chronic carriers, patients with persistent GABHS appear to have a higher risk of complications such as acute rheumatic fever,[45,46] and thus represent a distinct group. In view of the recent outbreaks of rheumatic fever,[47] some authorities suggest efforts be made to identify and eradicate persistent GABHS in symptomatic patients.[33] In addition, the recognition of streptococcal toxic shock syndrome has demonstrated that ensuring eradication of this organism in some situations (such as close contacts of cases) may be an important issue.[48] Even if the majority of these patients

do not go on to get complications, the longer organisms persist in the pharynx, the greater the chance of transmission to others. Presumably the transmission risk would be greatest from those who were symptomatic as they more closely resemble acute GABHS pharyngitis than do those who have persistent GABHS but without symptoms.

DEFINITION OF PERSISTENCE

As investigators recognized the need to distinguish persistent infection from re-infection or new infection with GABHS, the definition of persistence became more specific. Early studies defined "persistence" as a positive throat culture as long as three[34] or six weeks[8] after completing treatment. A recent meta-analysis has suggested that GABHS persistence should be defined as the detection of GABHS within two weeks of completing antibiotics.[40] Recent studies have been even more rigorous, repeating cultures within two to seven days of completing treatment to determine whether GABHS persistence occurs.[20,33]

To further distinguish persistence from a new infection, serotyping of initial and post-treatment isolates is advised.[40] Serotyping takes advantage of M and/or T antigens, which are proteins on the cell surface. M typing is done with capillary tube precipitin tests using an extract of the isolate (grown under specific conditions) as the antigen against adsorbed rabbit type-specific immune sera. T typing is performed using a slide agglutination method, which mixes specific T antibodies with, treated whole organisms.[49]

DISTINGUISHING GABHS CARRIAGE FROM PERSISTENCE

Given that treatment is less likely to eradicate GABHS in carriers than those with acute infection,[32,35] it is potentially difficult to distinguish chronic carriers from persisters after therapy. Carriers have been estimated to comprise up to 20% of children presenting

with GABHS pharyngitis.[35] Lack of serologic response has been proposed as one means of detecting chronic carriers,[50] but this definition has been challenged due to the lack of correlation with clinical symptoms.[51,52] In addition, penicillin may blunt anti-streptolysin O responses.[19] Finally, ASOT titres can remain elevated up to 12 months post-infection. The higher the baseline ASOT, the lower the rise in titre.[53] Thus a child who has had a GABHS infection in the preceding few months may not reach a "significant" titre and thus be considered a carrier according to the definition. Serology is therefore not a dependable means of determining carriage.

An alternative means of predicting carriage versus acute infection on the initial throat swab is the quantitative measurement of a positive GABHS culture. Breese *et al*[15] developed a "positivity ratio" which is calculated as follows:

$$\frac{\text{sum of 3+ and 4+ cultures}}{\text{sum of rare, 1+, 2+, 3+, 4+ cultures}} \times 100$$

The higher the calculated ratio, the fewer the low-colony count positives are included in the population, and therefore the lower the risk of inclusion of carriers. This ratio can be used to assess the potential presence of carriers by comparing it to the ratios calculated by the same authors for different populations in whom GABHS is detected.[15]

PATHOGENESIS THEORIES OF GABHS PERSISTENCE

GABHS Cellular Components

The major cellular components of this organism which contribute to its pathogenicity include several substances in its cell wall: lipoteichoic acid (LTA), capsular polysaccharide, and M protein. LTA mediates adherence to epithelial cells, while M protein and the hyaluronic acid capsule resist phagocytosis and killing by

polymorphonuclear leukocytes.[49] Additional cell surface virulence factors may include such proteins as C5a peptidase,[49,54] protein Sir[55] and protein Arp.[56]

M protein is generally considered to be the major determinant of virulence for *S. pyogenes*.[49] Work in a rat model has demonstrated that although M protein is not necessary for initial colonization, it may play a role in persistence of colonization.[57] Whether this is true in human GABHS infections has not been determined.

Many different M types exist, and most have been associated with uncomplicated pharyngitis.[14,58] Predominant serotypes vary over time and by geographic location,[13,14,59] but certain M-types have been associated with invasive disease[14,59-62] and others with non-suppurative complications.[59,60] The potential relationship of certain M type(s) to persistence of GABHS after treatment of pharyngitis has not been investigated to date.

Antimicrobial Tolerance and Resistance

Tolerance and resistance to antimicrobials have also been postulated to influence bacteriologic outcome. Tolerance refers to the phenomenon of inhibition but not killing of a bacteria by an antibiotic (usually a beta-lactam), and usually is defined in the laboratory as an MBC/MIC ratio of greater than 32.[63] In some studies, penicillin tolerance has been demonstrated to be common and may be associated with GABHS treatment failure[64-66] but has been refuted by other studies,[38,67,68] so its importance is unclear.

To date, penicillin-resistant GABHS has not been identified from clinical specimens. A review of pharyngeal isolates collected between 1989 and 1992 in the United States found that 90% of isolates had MICs for penicillin ≤ 0.012 μg/mL and none had an MIC greater than 0.024 μg/mL.[58] A review of 1000 *S. pyogenes* isolates from the Calgary Regional Health Authority in April - May 1996 did not identify either any penicillin-resistant strains or strains exhibiting an increase in MICs. (D. Church, personal communication 1997) GABHS isolates with elevated MIC values for penicillin have been produced in the laboratory,[69] so it is possible that resistant strains will eventually be isolated from humans. At this time, however, penicillin resistance does not explain persistence.[70]

Resistance to erythromycin has been reported to varying degrees worldwide. Resistance has increased as high as about 20% from pharyngeal isolates[71] in Finland, for example, and was associated with an increased use of this drug. Recently, Coonan and Kaplan found a resistance rate of only 3.5% in the United States, with similar MICs for other macrolides.[58] Newer macrolides such as azithromycin may be less efficacious than penicillin for reasons other than resistance.[72] Macrolides are an alternate treatment GABHS pharyngitis[73] and given the predominant use of beta-lactams, probably do not contribute significantly to persistence either in clinical practice or in most studies.

Timing of Antibiotic Initiation

Early initiation of antibiotics for *S. pyogenes* pharyngitis has been shown in two studies to lead to an increased risk of treatment failure.[19,42] The authors postulated that early treatment leads to a diminished host immune response and therefore impairs their ability to eradicate GABHS. These studies suffered from several methodologic weaknesses including unclear definition and measurement of compliance, re-culturing patients not only at the end of therapy but also several months later, counting the same subject more than once (e.g. in some cases patients had both a "relapse" and a later "recurrence"), and a lack of serotyping.[74] Their conclusions were refuted by a subsequent study which clearly defined compliance, counted only one "recurrence" per subject, and did serotyping to distinguish acquisition of new strains from persistence of the initial isolate.[18] However, Gerber *et al* did not distinguish those in whom GABHS was detected four to six days after completing antibiotics (first follow-up visit) from cases with GABHS up to two months post-treatment.[18] As the latter group may not be "persisters" in the strict definition of the term, the results are not conclusive.

Lack of Compliance

Non-adherence to physicians' instructions can include failure to fill a prescription, omission of medication doses, errors in dosing, as well as early discontinuation of medication.[75] In one study of GABHS pharyngitis, almost two-thirds of patients

discontinued penicillin by the sixth day of the course of treatment.[7] Subsequently it was shown that detection of GABHS three weeks after starting therapy was significantly more likely in non-compliant patients than compliant ones.[43]

Measurement of compliance in pharyngitis studies has varied. In several cases, it has not been assessed at all.[8,19,32,38] Several methods that have been used include patient report, medication measurement, and laboratory testing appropriate to the antibiotic used.[75] Urine testing for GABHS pharyngitis treated with penicillin consists of observation of inhibition of growth of *Micrococcus lutea* by patient urine specimens collected on one or more occasions during treatment.[18,43,44] Measurement of residual medication at the follow-up visit is another option[35] and has frequently been combined with urine testing,[7,42] patient record of doses taken,[21,37] or both[20,34,76] in previous studies. Comparison of patient report to the other methods suggests that it is usually not as dependable, but medication measurement appears to be comparable to urine testing results[7] and does not require the expense and inconvenience of the latter. Another shortcoming of urine testing is that it does not allow determination of the degree of compliance. In contrast, the other two methods can be used to measure the number of doses taken, which may provide a more refined measurement of compliance.

Specific definitions of compliance based on number of doses taken have been used in some studies published since 1986. In two pharyngitis studies, compliance was defined as the patient taking 90% or more of doses,[20,34] while one study of treatment of carriers[6] and another of acute pharyngitis[76] judged patients to be compliant if they took 80% of the prescribed doses of antibiotic. Justification or explanation of either definition is lacking.

Host Immune Response

The host immune response has been suggested to play a role in GABHS persistence and is thought to consist of several components: M type-specific immunity, mucosal immunity, immunity to extracellular enzyme products and heterotypic immunity.[77] Although there has been speculation about their contribution to persistence, in vivo evidence is non-existent.

Vaccine studies suggest that M type-specific immunity have protective effects. Local immunization with single type-specific vaccines has successfully prevented colonization in mice[74] and colonization as well as clinical pharyngitis in humans[78] with the same serotype. Using a conserved region from M5 covalently linked to the B subunit of cholera toxin, researchers were able to evoke protective immunity and prevent colonization and death from M type 24 strains, suggesting that broadly cross-protective vaccine may be possible.[79] The greatest benefit to such vaccines and therefore also natural immunity is likely in the prevention of acquisition rather than reducing the occurrence of persistence after a clinical infection.

The role of local immunity to other proteins has also been investigated. Total salivary IgA levels have been observed to rise during upper respiratory tract infections in children, peaking two to six days after onset of symptoms.[80] This response appears to increase with age. Non strain-specific cell surface proteins including protein Arp[55] and protein Sir[56] have been shown to bind IgA, and secretory IgA against a similar antigen, C5a peptidase, has been detected in over 90% of salivary specimens after streptococcal pharyngitis.[54] Although it is interesting to speculate about the role of such local immune responses, its importance in clinical infection including prevention of persistence is unknown at this time.

Beta-Lactamase-Producing Flora (BLPF)

The antagonistic effect of beta-lactamase producing organisms on penicillin has been extensively studied. Beta-lactamases produced by *S. aureus, H. influenzae, H. parainfluenzae, M. catarrhalis, Fusobacterium sp., Prevotella sp., Porphyromonas sp.,* and *Bacteroides spp.* may inactivate penicillin, thus preventing the eradication of GABHS. [37] Beta-lactamase activity has been detected more frequently in patients with treatment failure than asymptomatic GABHS carriers.[36] In addition, beta-lactamase producing organisms were detected significantly more often in children who did not clear GABHS after penicillin V treatment than in those who were cured.[37] However, treatment

failures were included if they occurred from one to 42 days post-treatment, which is not an ideal definition for persistence.

Investigators have compared the treatment failure rates of oral penicillin versus various beta-lactamase resistant antibiotics. Bacteriological cures have been shown to be more frequent in children treated with amoxicillin-clavulinate compared to penicillin.[33] Similar results have been demonstrated for cephalosporins such as cefaclor or cefixime compared to penicillin V.[20,34] However, in one of these studies, the follow-up period was greater than two weeks post-therapy.[34] Repeating the analysis including only documented persisters within two weeks of therapy yielded a non-significant result. Pichichero and Margolis[81] attempted to address some of the limitations of this study and previous ones using meta-analysis. Although they were able to show that cephalosporins resulted in significantly more cures than penicillin, the difference was only 8%. Furthermore, the study inclusion criteria were suboptimal, defining neither the desired follow-up interval nor compliance, and requiring only that GABHS be confirmed on culture at onset and end of therapy to be considered a treatment failure, but not specifying the isolates be concordant serotypes.

The beta-lactamase producing flora theory has been challenged by authors of a study who found *no difference* between penicillin versus amoxicillin-clavulinate in eradication of GABHS, in spite of their differences in activity against BLPF.[76] This study was well-designed and conducted, avoiding most of the methodologic problems evident in earlier publications. It is therefore still controversial whether BLPF account for GABHS persistence and unclear whether cephalosporins are preferred over penicillin.

Eradication of Protective Pharyngeal Flora

The relationship between other normal pharyngeal microbes and GABHS has garnered increasing attention. Normal oropharyngeal flora is known to be inhibitory to the growth of GABHS.[82]. In one study, a group of children were followed for 20 weeks, measuring the inhibitory activity of oral flora (alpha and non-hemolytic streptococci, *Neisseria sp.*) against GABHS. GABHS-inhibitory activity was found to be reduced in

those who become colonized with GABHS compared to those who did not, suggesting a protective effect of normal flora.[83] If this flora is reduced for any reason, including killing by an antibiotic such as penicillin, there may also be a decreased ability to clear GABHS once someone has become infected.

Two studies have investigated the role of GABHS-inhibiting pharyngeal alpha-streptococci in penicillin treatment failures.[21,36] Roos et al.[36] compared alpha-streptococcal inhibition of GABHS strains of patients with persistent infection after treatment to that of asymptomatic GABHS carriers in the same household. Inhibitory activity was significantly less in the persisters than the carriers. While this suggests that carriage and persistence are different conditions, the useful comparison would have been inhibitory activity *at the time of diagnosis* in patients who went on to be cured versus those who were bacteriological treatment failures after penicillin. Brook and Gober[21] did exactly that and found inhibitory alpha-streptococci significantly less often among those with bacteriologic treatment failures than those who cleared GABHS. Unfortunately, the definition of "treatment failure" was very broad, and included detection of GABHS up to 42 days after completion of therapy. Serotyping was not done, so re-infection with the same strain or infection with a different one could not be ruled out.

One group of investigators[84] went on to examine a prophylactic role of alpha streptococci. A suspension of alpha streptococci was sprayed into the throat twice a day for ten days to prevent recurrent episodes of GABHS pharyngitis. Unfortunately, their treatment group had a past history of multiple recurrences and their control group had none. A marked difference was observed in spite of this. The result is interesting but routine cultures were not done at any point, particularly in the immediate post-treatment period, so it is difficult to extrapolate from clinical recurrence to bacterial persistence. Overall, evidence supporting the role of GABHS-interfering flora in persistence is promising but far from conclusive.

Beta-lactamase Producing Flora and Microbial Interference

A combination of the effects of BLPF and microbial interference has also been proposed. In a mouse model, subcutaneous abscesses containing different combinations of GABHS, *S. salivarius*, and *S.aureus* were created and treated with either penicillin or cefprozil.[85] Unlike penicillin, cefprozil eradicated both GABHS and the potentially GABHS-protective *S. aureus* (suggesting a role for BLPF) but not the (desirable) GABHS-inhibitory *S. salivarius*. Human studies that examined both theories were limited as discussed above.[21,36]

VIRAL CO-PATHOGENICITY

A co-pathogenicity theory is an attractive one given the many microorganisms which inhabit the upper respiratory tract. In addition to bacteria, viruses also commonly inhabit the nasopharynx but have not been considered as a potential cause of persistence; therefore information about this potential risk factor does not exist. There is in vitro evidence to suggest that acute viral infections have local and systemic effects that may make secondary bacterial infections of the respiratory tract more difficult to combat. This appears to be the case in acute otitis media (AOM), and may be similar for pharyngitis. Viruses are certainly a common cause of upper respiratory tract infections, and have been shown to co-exist with GABHS pharyngeal infections. This evidence, albeit circumstantial, suggests viral co-infection may be an important influence on bacteriological outcome in pharyngitis.

Epidemiology of Respiratory Virus Infections: Temporal Relationship with GABHS Pharyngitis

In order for viral infections to play an important role in GABHS pharyngitis, there must be an epidemiological overlap between the two types of infection. Similar to the pattern found in GABHS infections, the late fall through to spring are also the seasons in

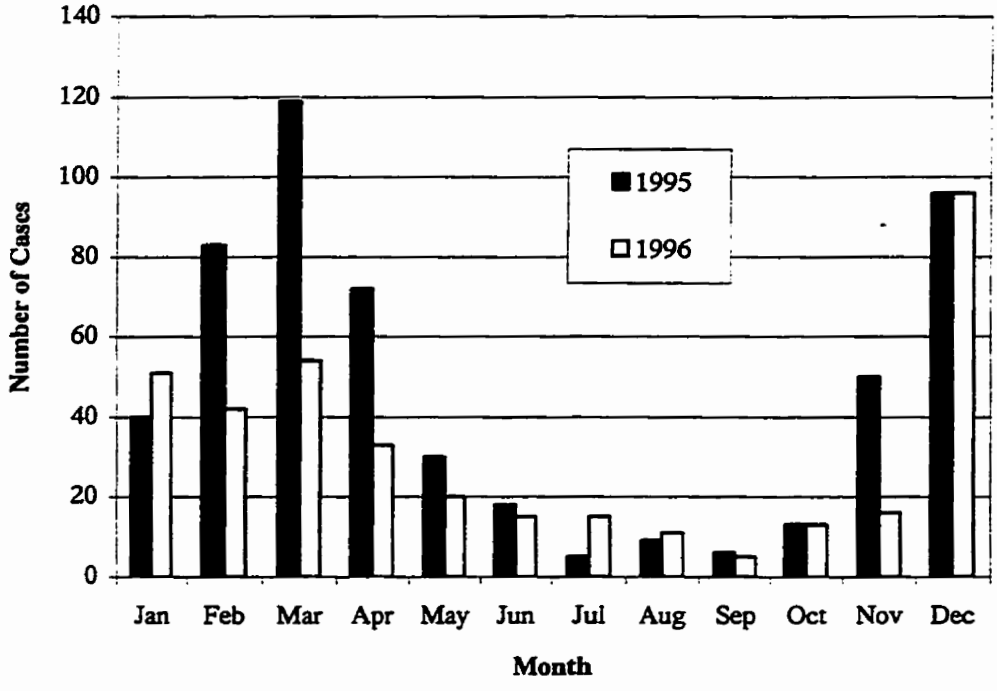
which most viral respiratory infections are at their peak.[86] Locally, this also appears to be the case using Southern Alberta Provincial Laboratory data for respiratory virus isolation and serology for 1995 and 1996 (Figure 2). Given the seasonal similarity between these infections, dual infections may not be uncommon.

Effects of Viral Infections on the Respiratory Tract

Infection with a viral respiratory pathogen has been shown to have direct effects on respiratory epithelial cells. Sequential epithelial samples during and after upper respiratory tract infections demonstrated marked focal cellular abnormalities, including cilia with aberrant microtubular patterns. Changes were most marked in the first week of illness, with gradual return to normal thereafter.[87] These ciliary defects may be associated with impaired mucociliary function, resulting in decreased bacterial clearance. Streptococcal species have also been shown to adhere better to virus-infected than uninfected cell cultures[88] and a similar advantage may exist for GABHS. Eradication may be more difficult as a result.

Viral infections may also induce alterations in the host immune response. In particular, viruses can depress polymorphonuclear (PMN) leukocyte function, including adherence, chemotaxis, phagocytosis, and bactericidal activities.[89] Most investigators have focused on influenza virus, although similar PMN abnormalities have been shown with parainfluenza virus, herpes simplex virus, and respiratory syncytial virus. In addition, a synergistic inflammatory reaction may result from dual infection. Viral infections trigger release of cytokines, which may be augmented by bacteria and their breakdown products. This may increase inflammation and with it clinical severity as shown in cattle with a herpesvirus infection.[90]

Figure 2. Number of Cases of Respiratory Virus Infections by Month: 1995/96*



*Includes adenovirus, influenza A and B, parainfluenza, respiratory syncytial virus, and rhinovirus.

Co-Infection in Acute Otitis Media

Co-infection with respiratory viruses appears to be related to persistent bacterial infection in acute otitis media (AOM). Arola *et al*[2] found respiratory viruses in middle ear fluid or pharynx significantly more often in children with symptoms persisting more than 48 hours after starting therapy than the new cases of AOM. In two prospective studies, investigators compared the clinical course of children co-infected with a virus and bacteria to those with only a bacterial pathogen in middle ear fluid.[91,92] Both demonstrated that eradication of bacteria was significantly less common in those with viral co-infection. In a subsequent study identifying the viral isolates, rhinovirus was found to be associated with a higher rate of bacteriologic failure than other respiratory viruses.[93] If viruses have similar effects in the pharynx, they may also result in impaired clearance of bacterial infection.

GABHS and Viral Co-Infection

Although *S. pyogenes* and viruses cause most of the cases of pharyngitis,[28] few studies have attempted to identify co-infection with two or more pathogens. Viral co-infection is uncommon in adults, probably because GABHS occurs infrequently.[94] On the other hand, viral and GABHS co-infection can occur in 8% of children with pharyngitis.[5] Influenza A was the most common followed by poliovirus 1, respiratory syncytial virus (RSV), herpes simplex virus (HSV), parainfluenza 3, and adenovirus. Rhinovirus was not detected in this particular study.

VIRAL HANDLING AND ISOLATION TECHNIQUES

A study involving viral detection requires careful attention to how samples are taken, handled, as well as cultured. For some respiratory viruses such as rhinovirus and RSV, nasopharyngeal aspirates provide a higher yield than nasopharyngeal (NP) swabs. However, the swab is the preferred specimen for adenovirus. A nasopharyngeal swab is

technically easier to perform, particularly on an uncooperative child, and may be the best option especially when one of several possible viruses may be present.[95] The NP swab is inserted into the nose towards the posterior nasopharynx and rubbed against it. Calcium alginate swabs should not be used as (unlike rayon, cotton, Dacron and polyester) this material is toxic to herpes simplex virus.[96] The swab can then placed in one of several different liquid media for transport.

Specimens for virus isolation should be kept at refrigerator temperature or on wet ice, then transported as soon as possible. In general, viral detection is better the faster this can be done, although some viruses are more stable than others.[96]

If samples are to be stored for more than 24 hours, infectivity is best maintained if frozen at 70°C with optimal concentrations of sucrose.[96,97] Repeated freezing and thawing should be avoided as each cycle reduces the viral yield.[95]

Specimens are inoculated onto different cell lines depending on which viruses are sought. Specimens are allowed to adsorb to the cells, then a maintenance medium is added, and cultures are incubated at 37 and/or 33°C. Virtually all cell lines of mammalian origin will support growth of herpes simplex virus,[94] while several (including HEp-2 cells) permit growth of adenoviruses.[98] Parainfluenza viruses will grow in primary human embryonic cells and, along with influenza virus types A and B, can be isolated in rhesus monkey kidney cell cultures.[99,100] Rhinoviruses can be isolated in human embryonic kidney and lung cells,[101] while respiratory syncytial virus (RSV) grows in HEp-2 and monkey kidney cells.[102] Cultures are examined for cytopathic effect at seven and 14 days. If CPE is not sufficient to identify a virus, or if it does not always occur, results are confirmed using techniques like immunofluorescence, hemagglutination, or electron microscopy for adenovirus,[98] fluorescent antibody testing for RSV,[102] and hemadsorption inhibition or immunofluorescence for influenza A and B[100] and parainfluenza.[99] Fluorescent antibody testing of clinical specimens is available with commercial kits for adenovirus, influenza viruses, parainfluenza virus, and respiratory syncytial virus.

CHAPTER 3

METHODS

The methods section includes a discussion of the study design, study population, and the inclusion and exclusion criteria. The study protocol, including subject enrollment and follow-up as well as laboratory procedures, is provided in detail. Specific definitions of study variables and a description of their measurement are given. Ethical approval of this study is noted and the process of obtaining informed consent is discussed. The determination of sample size is explained followed by a description of the data management and analysis.

STUDY DESIGN

This study was conducted using a prospective community cohort design. Children presenting with symptoms of pharyngitis to participating community pediatricians and two emergency departments in Calgary were enrolled, from whom a population of children with GABHS pharyngitis were identified, specimens taken to determine their viral co-infection status, then followed prospectively to determine their outcome.

SUBJECTS

Study Population

The target population was all children with acute GABHS pharyngitis in Calgary who were treated with penicillin. The study sample consisted of children between two and

18 years of age who presented to one of eleven participating pediatricians or two emergency departments in Calgary during the fall through spring months of 1994 - 96 with acute pharyngitis by study definition, and consented to participation in the study. This group was a non-random convenience sample.

Inclusion Criteria

Children were recruited if they met the following inclusion criteria: were two to 18 years of age and had clinical pharyngitis consisting of one symptom (sore throat, sore neck, refusal to eat, or fever) and at least one clinical sign on examination (pharyngeal injection, pharyngeal exudate, temperature > 38.4 °C, or tender cervical lymphadenopathy).

Exclusion Criteria

Children were excluded if they had a known allergy to penicillin, used any antibiotic within 72 hours prior to presentation, a past history of acute rheumatic fever, or if they refused to participate in the study.

Identification of Potential Study Subjects

In offices and emergency departments where patient flow patterns allowed, potential study participants were identified in advance of seeing the physician if they complained of one of the symptoms in the inclusion criteria. The patient and / or guardian were given a study information sheet to read while waiting to see the study physician (Appendix 1).

PROTOCOL

Physician Recruitment

We recruited physicians for this study by sending out letters inviting participation in the study to pediatricians practicing in the city of Calgary as well as the director of the emergency department of the Alberta Children's Hospital. Subsequently, the director of another community hospital in Calgary, the Rockyview General Hospital (RGH) requested to be involved as well. There is no general pediatric care provided from the ACH site other than the emergency department.

The Community Group A Streptococcal Study Group membership consisted of eleven pediatricians with community practices and 24 physicians from the two emergency departments. The pediatricians' offices and the patients they serve are found in all quadrants of the city. A survey of the pediatricians revealed that most practices were comprised of more than 1500 patients, the majority of whom were ten years of age or younger and Caucasian. Primary care made up the majority of the practice in two-thirds, followed by consultation pediatrics. The two emergency departments are located in the southwest area of the city, and the participating physicians included eleven pediatricians and eight family or emergency physicians from ACH, and five family/emergency physicians from RGH.

Members of the Study Group were provided with the background information for the study and the protocol and given a complete information package. All physicians were instructed on the techniques to be used for throat and nasopharyngeal swabs. Dr. Kuhn and the research assistant visited each office and emergency department. The details of the study discussed with nursing and reception staff and adjustments made to ensure the study could fit into the individual office flow. All enrollment materials were supplied.

Patient Enrollment

Patient enrollment followed the procedure shown in Appendix 2. When a patient and guardian agreed to enter the study a consent was signed using a triplicate NCR form

(Appendix 3), with one copy given to the patient and their family, one placed in the patient's chart, and one sent to the study office with the specimens. Each physician was identified with a letter-code and each patient assigned a unique number-code (Appendix 4). A pre-coded enrollment form was completed by the physician documenting the history and physical findings (Appendix 5). The form was faxed to the study office and the original included in the patient's chart. A logbook of enrollments was maintained in each office. Two swabs were taken from each patient including a rayon oropharyngeal swab for rapid detection and culture of GABHS, and a rayon nasopharyngeal swab for viral detection and culture. Swabs were placed in the refrigerator while awaiting pick-up by the delivery service. All patients were given a prescription for penicillin (approximately 50 mg/kg/d divided three times a day for ten days) using pre-written prescription forms (in 5 kg weight increments) as shown in Appendix 6. The physician could choose to direct the patient to fill the prescription immediately *or* do so only if instructed to do so by office or study staff. All patients positive for GABHS were contacted by telephone. Patients were provided with a diary in which to record the doses of antibiotics taken. This was then placed in a prominent location at home such as the refrigerator (Appendix 7). All participating children received a copy of *Kids Doc,* an information sheet from Alberta Children's Hospital, as well as a thank you letter from the investigators (Appendix 8).

Enrollments occurred Monday through Friday in physician offices and throughout the week in the emergency departments. The majority of patients enrolled in physician offices were seen in the afternoons, therefore an automatic pick-up service was arranged for Tuesday through Friday mornings, and late Friday afternoons. A Monday morning pick-up was made from RGH to retrieve Sunday specimens. Patients were not enrolled from this site on Saturdays. Specimens and a copy of the consent form were picked up and delivered to the Alberta Children's Hospital microbiology lab within 1½ hours. Specimens from patients enrolled in the ACH emergency department were sent directly to the lab, and consents and enrollment forms collected by the research assistant. Participating physicians/emergency departments were sent the result of rapid GABHS detection and culture results by fax.

Patient Follow-up

Patients found to be GABHS infected were asked to return for a follow-up appointment with the pediatrician or an investigator at the Alberta Children's Hospital two to five days after completion of the antibiotics, returning their medication diary and container. The offices arranged for these appointments when families were phoned with positive throat swab results, and faxed this information to the study office. Follow-up of patients enrolled through the emergency departments was done at the ACH outpatient department by one of the investigators. Reminder calls were made to all patients a few days prior to the follow-up appointment.

At the follow-up appointment, a visit #2 checklist was completed and faxed to the study office (Appendix 9), repeat swabs of throat and nasopharynx were done and sent to the microbiology lab, and the medication diary and container collected. All children were given a certificate of appreciation at visit #2 (Appendix 10), and $5 given to cover parking expenses for those who came to Alberta Children's Hospital. Lab results were faxed to the offices and further treatment decisions left to the physician's discretion. Patients were instructed to return for a third visit to their pediatrician or to contact an investigator if symptoms of pharyngitis recurred within one month after this second visit (Appendix 11).

Diaries and containers were retrieved from the enrollment sites at appropriate intervals and the number of doses of medication taken was noted.

Physician Follow-up and Communication

Ongoing communication with the many participating physicians was maintained through regular contact with Dr. Kuhn and the research assistant, as well as group meetings. A bi-weekly bulletin was sent to participating physicians throughout the study containing information about enrollments and providing feedback about study procedures (Appendix 12). Offices were visited regularly to pick-up patient medication bottles and diaries, and to replenish supplies. The Community Group A Streptococcus Study Group met at the end of the first season of enrollment to review the study progress and at the end

of the study to discuss the results. Separate meetings with the emergency department physicians were arranged as necessary.

The research assistant was available during regular business hours for questions or concerns from participants or physicians. An investigator could be contacted at any time through the ACH switchboard or messages could be left on voice mail. Instructions for contacting an investigator were clearly indicated on all patient and physician study material.

Laboratory Testing

Throat swab

Oropharyngeal cultures were placed into transport medium. Specimens were refrigerated until the time of the pick-up. In the laboratory, the throat swab was inoculated onto a blood agar plate and incubated anaerobically at 37°C. Plates were inspected at 24 hours for beta-hemolytic colonies. The foam plug at the base of the swab transport tube was incubated in Todd-Hewitt (TH) broth aerobically, subcultured to BAP at 24 hours and inspected after 24 hours of anaerobic incubation. Gram positive cocci that were catalase negative, and bacitracin sensitive were typed using the PathDX® kit until March/95 and OXOID® Diagnostic Reagents thereafter. All GABHS isolates were sent for M and T typing at the National Center for Streptococcus in Edmonton, Alberta.

Rapid detection of GABHS using Optical Immunoassay (OIA, Biostar Inc., Boulder, CO.) was done using the swab after it had been planted for culture. This procedure is described in the original study by Harbeck et al.[41] Three drops of 0.3 M acetic acid were added to the swab in an extraction tube, incubated for two minutes, then neutralized with three drops of 1.5 M 3-(*N*-morpholino)-2-hydroxypropanesulfonic acid buffer with 0.2% Tween 20. A horseradish peroxidase-labeled rabbit anti-*S.pyogenes* antibody was added and immune complexes allowed to form. A sample was placed on the slide test surface and incubated for two minutes. Unbound sample was removed by washing with two ml of water. The substrate (tetramethylbenzidine with H_2O_2) was applied for four minutes to react with the bound enzyme-labeled antibody. After washing

with water, a negative reaction was indicated by a gold colour (i.e. unchanged from an unused test surface) and a positive reaction appeared as a purple spot. A control was included with each test.

Nasopharyngeal swab

All nasopharyngeal (NP) swabs were placed in viral transport medium and sent to the Northern Alberta Provincial Lab for viral testing. Requisitions accompanied specimens from patients with a positive OIA test. Swabs were frozen at -70°C from those patients in whom GABHS was not detected, thawed and tested when found to be GABHS positive on culture or at the end of the study if no GABHS was detected. Virology testing was not done initially for patients who were GABHS negative because of the cost of the testing.

In addition to viral culture, direct fluorescent antibody (DFA) testing of the sample for parainfluenza virus, influenza, and RSV were done when each virus was in season during the first year of the study. As this method did not yield any additional results and sensitivity was low, DFA testing was discontinued in the second year of the study. Specimens were inoculated onto four cell lines including african green monkey kidney and HEp-2 cells at 37°C, and rhesus monkey kidney and human embryonic lung cells at 33°C. Cultures were examined for cytopathic effect at seven and 14 days. Positive results were confirmed using electron microscopy for adenovirus and rhinovirus, DFA for RSV, and hemadsorption followed by DFA for influenza A and B and parainfluenza.

Laboratory flow

All swabs were delivered to the Alberta Children's Hospital microbiology lab by 10:30 a.m. Monday through Friday and again at 6 p.m. on Friday. NP swabs were couriered to the Northern Alberta Provincial Laboratory in Edmonton the same day for specimens received during the week and on Saturday morning if received on Friday night (Appendix 13).

MEASUREMENT OF VARIABLES

Predictor Variable

Respiratory virus co-infection status was determined by detection (or lack thereof) of a virus (adenovirus, influenza A or B virus, rhinovirus, respiratory syncytial virus, parainfluenza virus) using the methods described above.

Outcome Variable

GABHS persistence was defined as the detection of GABHS on the throat swabs taken on initial presentation with pharyngitis and at follow-up two to five days after completion of antibiotic in a given patient. A positive GABHS result included a positive by any laboratory method described above (rapid test, plate or broth culture with any number of colony forming units). GABHS persistence was further distinguished as either *definite* (the two isolates were shown to have the same M and T type) or *possible* (missing one isolate so that M and T types cannot be compared). A patient who was GABHS positive but with a *different* M and T type at any follow-up visit was considered to have GABHS re-infection.

Other Variables

Patient demographics (age, sex, year of enrollment) and symptoms (sore throat, sore lymph glands, fever) were taken by patient history. Clinical signs (temperature, pharyngeal infection, tonsillo-pharyngeal exudate, enlarged or tender lymph glands) and timing of antibiotic initiation (immediately or after a positive throat swab result) were documented by the study physician on the enrollment form. A patient was defined as GABHS infected if any GABHS tests were positive (optical immunoassay, plate culture, broth culture). Colony counts were also provided by the microbiology lab, with rare to 2+ considered a "low" colony count and 3+ or 4+ considered a "high" colony count. M and T type of GABHS isolates were noted. Viral cultures were considered positive or not, and further described by viral type. Compliance was assessed by measuring the number of

penicillin doses taken according to the patient diary and measurement of residual medication by a principle investigator or research assistant. At least one of the two measures had to be available to determine compliance status. Compliance was defined as documented consumption of at least 80% (24 doses) of the prescribed penicillin within ten days of starting therapy. Where there was a difference in the number of doses taken by the two methods, the lower number was used to determine compliance status.

ETHICS

The study protocol was approved by the Ethics Committee at the Alberta Children's Hospital and the University of Calgary. Informed consent was obtained from each participant and / or their guardian and consisted of an explanation by the study physician, reading the consent form (which included the justification and description of the study in layman's terms), and asking questions. If the physician felt that they understood the contents of the consent, the participating child/parent signed the form along with the physician and a witness. They were invited to contact an investigator at any time (phone numbers were provided on all study material) if they had any further questions.

SAMPLE SIZE JUSTIFICATION

The primary outcome measure was the frequency of persistent GABHS infection after completion of antibiotic therapy, comparing children who were co-infected with a virus to those who were not co-infected. If approximately 10% of all GABHS-infected children were co-infected with a virus, then a virus positive: virus negative ratio of 1:9 would be expected. Assuming that the rate of persistence would be 20% and that a four-fold increase in rate of persistence in the presence of viral co-infection was clinically

significant, a minimum sample of 60 (6 virus-infected, 54 virus-negative) would provide a power of 0.80 and a two-sided alpha of 0.05 (calculated using Epi Info version 6.0). Allowing for participant losses of 25 % due to refusal to participate, refusal of repeat swabs, medication side effects or allergy resulting in discontinuation of treatment, at least 80 children with GABHS pharyngitis were required. This number was expected to come from a cohort of 320 children presented with signs and symptoms of pharyngitis by our definition, assuming a 25% incidence of GABHS among those with pharyngitis in this population.

ANALYSIS

Data Management

All the data were entered into the database program Microsoft Access® version 2.0. Following data cleaning, analyses were performed using Stata®.

Statistics

Descriptive statistical analyses were performed on individual variables including frequencies for categorical variables and mean, median, range and standard error for continuous variables with graphical illustration as appropriate. Bivariate analyses were done when potential differences were noted in factors of interest. These were compared using two-sided Fisher's exact test for categorical and Student's t-test for continuous variables. Chi-square for trend was used where appropriate.

The strength of association between viral co-infection and GABHS persistence was presented in the form of a relative risk (RR) with a two-tailed 95% confidence interval and tested using a two-sided Fisher's exact test. The small sample size in some categories precluded further stratified analyses on potential confounding and interacting variables. A retrospective power calculation was done using Epi Info version 6.0.

CHAPTER 4

RESULTS

This chapter includes a description of the enrollment timeline and itemizes the number and reasons for subjects to be excluded from the analysis. Demographic information for those considered eligible is described along with the clinical presentation and etiology of pharyngitis. In the main analysis, the association of viral co-infection and GABHS persistence is measured and tested. An exploration of the relationship between outcome and other potential risk factors including age, gender, presenting symptoms and signs, timing of antibiotic initiation, compliance, and M-type is shown. Finally, the performance of the new rapid test, the OIA, is assessed.

ENROLLMENTS

Study Timeline

A total of 286 children were enrolled in the study between November 1994 and March 1996. The target sample size was not reached by June of 1995, therefore the study was continued for an additional year. Enrollments were suspended between June and August 1995 given the low incidence of streptococcal pharyngitis in the summer but high ongoing costs of conducting the study. Thus, participants were enrolled over 16 months during two fall/winter seasons. Two hundred and twenty-three children (78.0%) were enrolled in the first year of the study. Although efforts were made to document the number of patients identified but not enrolled on the basis of penicillin allergy or refusal for any reason, insufficient data was submitted by participating physicians for analysis.

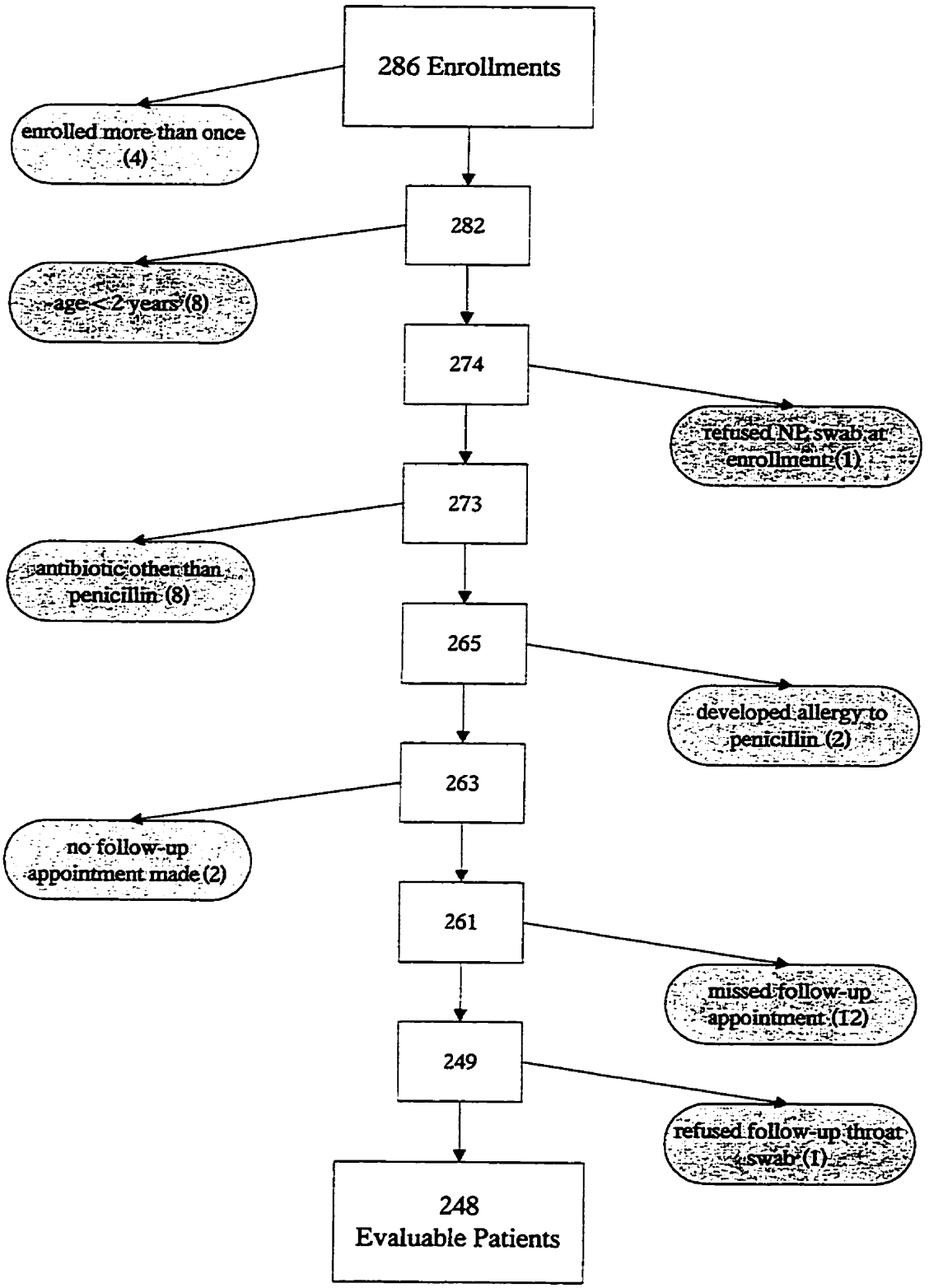
Exclusions

Thirty-eight children were excluded from the analysis leaving 248 (86.7%) evaluable. The sequence of and reasons for these exclusions are shown in Figure 3. Twenty patients were excluded from the 157 enrollments in offices (12.7%) and 18/129 (14.0%) from the emergency department enrollments. Exclusion from the analysis for 22/38 (58%) patients were due to errors made by participating physicians, 14/38 (37%) were due to patient errors, and two cases of previously unknown type 1 penicillin allergy (5%) were unavoidable. Physicians either contravened the inclusion criteria (including underage patients) or did not adhere to other steps in the study protocol (enrolled same patient more than once, used non-penicillin treatment, did not make a follow-up appointment for a GABHS infected patient). Patients did not follow directions, either refusing swabs or missing their follow-up appointment despite reminder calls. All but one of the patients who missed their follow-up appointment were subjects who had been enrolled through the emergency department.

Site and Timing of Eligible Enrollments

More than half of all eligible enrollments occurred in the offices of pediatricians (137/248, 55.2%), the remainder originating from the emergency department. The majority (195/248, 78.6%) were enrolled in the first year of the study, of whom almost two-thirds (119/195, 61.0%) had been enrolled in a pediatric office.

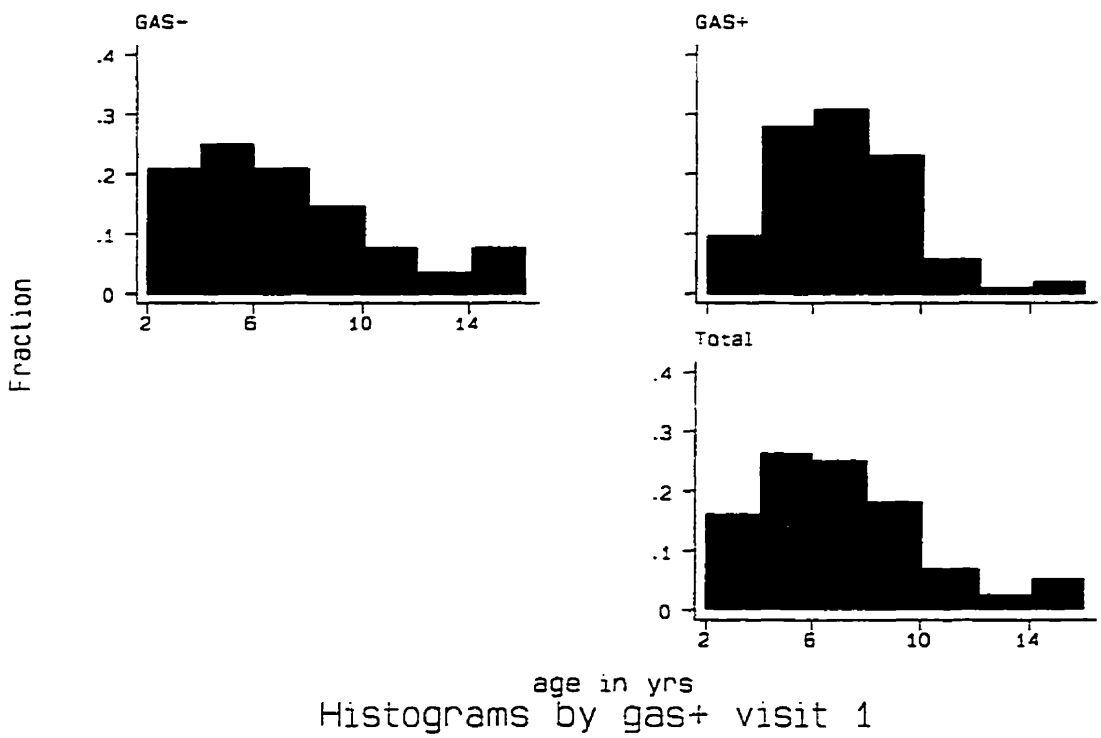
Figure 3. Flow Chart of Patient Exclusions



DEMOGRAPHICS

The 248 evaluable children had a median age of 6.6 years (mean 7.1 years, range 2.2 - 15.9 years). GABHS negative children had a median age of 6.4 years (mean 7.1 years, range 2.2 - 15.9 years) compared to 6.9 years (mean 7.1 years, range 3.4 - 14.4 years) in the GABHS infected (Figure 3). Overall there were almost equal numbers of each gender enrolled (126 male; 50.8%), but there were more males among enrollees with group A streptococcal pharyngitis (60/104, 57.7%) than those without GABHS (66/144, 45.8%; P=0.07). Comparison of demographics between subjects enrolled through the emergency department and the pediatric offices is shown in Table 1.

Figure 4. Histograms For Age In GABHS Infected, Uninfected, and All Patients.*



* Mean age in years (SD) was 7.1 (2.5) for GABHS positive and 7.1 (3.6) for GABHS negative patients.

Table 1. Demographics of 248 Subjects Enrolled Through the Emergency Department Compared to Those Enrolled in Pediatric Offices

Demographic Feature	Pediatric Offices (137) *n (%)*	Emergency Departments (111) *n (%)*
Total enrollments	137 (55.2)	111 (44.8)
Median age in years (range)†	7.0 (2.2 - 15.9)	6.1 (2.2 - 15.9)
Male gender	63 (46.0)	63 (56.8)
GABHS positive	48 (35.0)	56 (50.5)

† Mean age in years (SD) = 7.3 (3.1) for enrollments via offices and 6.8 (3.2) through emergency departments

PRESENTATION AND CLINICAL COURSE OF PHARYNGITIS

Etiology of Pharyngitis

One or more organisms were identified in the throat/NP swabs of 120/248 (48.4%) enrollments (Table 2). Group A beta-hemolytic streptococcus was cultured from 104/120 (86.7%) children and one or more other beta-hemolytic streptococci were isolated in 6/120 (5.0%). The seven isolates of non group A streptococcus in six patients included one group B, four group C, and two group G beta-hemolytic streptococci. A respiratory virus was cultured in 20/120 (16.7%) patients, ten of whom (8.3% of total) had no other presumptive etiology for their clinical pharyngitis.

Table 2. Identification of Potential Pathogens from Throat and Nasopharyngeal Swabs in 248 Patients

Etiology	*n (%)*
No pathogen identified	128 (49.4)
Total pathogens*	131 (50.6)
Group A β-hemolytic streptococci	104 (40.2)
Non group A β-hemolytic streptococci	7 (2.7)
Respiratory virus	20 (7.7)

* Number of total pathogens exceeds 120 as more than one organism identified in some patients

Respiratory virus isolation was slightly more common among those with GABHS pharyngitis (10/104, 9.6%) than those without (10/144, 6.9%). Viral pathogens found in both groups included adenovirus (8/20, 40.0%), parainfluenza (4/20, 20.0%), influenza A (5/20, 25.0%), and influenza B (3/20, 15.0%). Rhinovirus, respiratory syncytial virus, and herpes simplex virus were not isolated in any nasopharyngeal specimens (Table 3).

Table 3. Number and Type of Respiratory Viruses Isolated in GABHS Infected and Uninfected Children

Respiratory Virus	Group A β-Hemolytic Streptococcus			
	Positive (10)		Negative (10)	
	Year 1 *n*	Year 2 *n*	Year 1 *n*	Yyear 2 *n*
Adenovirus	3	0	1	4
Influenza A	2	2	1	0
Influenza B	2	0	1	0
Parainfluenza	0	1	3	0

Clinical Presentation and Treatment

The presentation and treatment of enrollments are shown in Table 4. The most common symptom reported at presentation was sore throat (232/248, 93.5%) and most frequent sign was an inflamed pharynx (233/248, 94.0%). Compared to children with non-GABHS pharyngitis, those who were GABHS positive were more likely to complain of sore lymph nodes (45.1% vs. 68.3%; P<0.001) and fever (60.4% vs. 77.9%; P<0.01), and to have a pharyngeal exudate (30.6% vs. 44.2%; P=0.03) and enlarged/tender lymph nodes (51.4% vs. 71.2%; P<0.01). Physicians prescribed antibiotics immediately at enrollment (before the results of the throat swab were known) in 104/248 (42%) patients, but this was significantly more common among children who were subsequently confirmed to have GABHS infection (59.6% vs. 29.2%; P<0.001).

Table 4. Clinical Presentation and Treatment of All Patients, GABHS-infected and Uninfected Patients

	All Patients (248)	GABHS Positive (104)	GABHS Negative (144)
	n (%)	*n (%)*	*n (%)*
Symptom			
Sore throat	232 (93.5)	101 (97.1)	131 (91.0)
Sore lymph nodes[*]	136 (54.8)	71 (68.3)	65 (45.1)
Fever[†]	168 (67.7)	81 (77.9)	87 (60.4)
Clinical findings			
Inflamed pharynx	233 (94.0)	98 (94.2)	135 (93.8)
Exudate[‡]	90 (36.3)	46 (44.2)	44 (30.6)
Tender lymph nodes[†]	148 (59.7)	74 (71.2)	74 (51.4)
Median temperature in°C (range)	37.7 (35.5-40)	37.9 (36-40)	37.6 (35.5-39.7)
Antibiotics given immediately[*]	104 (41.9)	62 (59.6)	42 (29.2)

Difference between GABHS positive and negative patients: [*]P < 0.001; [†]P < 0.01; [‡] P = 0.03 by 2-sided Fisher exact test

Presenting characteristics did not appear to differ according the site of patient enrollment except for history of fever and physician documentation of pharyngeal exudate (Table 5). In both cases these features were significantly more common among patients enrolled in the emergency departments than those enrolled in pediatric offices.

Table 5. Clinical Presentation and Treatment by Site of Enrollment

	Pediatric Offices (137)	Emergency Departments (111)
	n (%)	*n (%)*
Symptom		
Sore throat	129 (94.2)	103 (92.8)
Sore lymph nodes	77 (56.2)	59 (53.2)
*Fever**	85 (62.0)	83 (74.8)
Clinical findings		
Inflamed pharynx	129 (94.2)	104 (93.7)
*Exudate**	42 (30.7)	48 (43.2)
Tender lymph nodes	83 (60.6)	65 (58.7)
Median temperature in°C (range) †	37.8 (35.9-40)	37.6 (35.5-40)
Antibiotics given immediately	58 (42.3)	46 (41.1)

* Difference between enrollment sites by two-sided Fisher exact test, P<0.05. † Mean temperature (SD) for enrollments = 37.8 (1.0) via pediatric offices and 37.6 (1.2) through emergency departments.

MAIN ANALYSIS: COMPARISON BY VIRUS CO-INFECTION STATUS

Comparison by Risk Factor

Of the 104 children with GABHS pharyngitis, 10 (9.6%) were found to be co-infected with a respiratory virus. Comparison of children with and without viral co-infection revealed a statistically significant difference in the measured temperature (P<0.01) (Table 6). No other statistically significant differences were found.

Table 6. Comparison of Characteristics by Risk Factor

Characteristic	Virus Co-infected (10) *n (%)*	Virus Uninfected (94) *n (%)*
Male sex	5 (50.0)	55 (58.5)
Median age in yrs (range)*	6.8 (2.7-14.4)	6.9 (2.3-15.9)
Symptoms		
Sore throat	9 (90.0)	92 (97.9)
Sore lymph nodes	5 (50.0)	66 (70.2)
Fever	9 (90.0)	72 (76.6)
Clinical signs		
Inflamed pharynx	9 (90.0)	89 (94.7)
Exudate	4 (40.0)	42 (44.7)
Tender lymph nodes	6 (60.0)	68 (72.3)
Median temperature in ° C (range) †	38.6 (37.9 - 40)	37.6 (36 - 40)
Antibiotics given immediately	5 (50.0)	57 (60.6)

* Mean age (SD) = 7.4 (3.1) for virus co-infected and 7.0 (2.5) for virus uninfected patients. † Mean temperatures in °C (SD) were 38.7 (0.7) in viral co-infected and 37.6 (1.0) in non virus co-infected, difference (t-test with unequal variances) P<0.01; measurements available for 9/10 viral co-infected, 73/94 non virus co-infected.

Relationship between Viral Co-infection and GABHS Persistence

There were a total of 33/104 (31.7%) children in whom GABHS was detected after treatment with penicillin V ("persisters"). Of these, two (6.1%) had viral co-infection compared to 8/71 (11.3%) non-persisters. The relative risk of GABHS

persistence with respiratory virus co-infection was 0.57 (95% CI 0.16; 2.03, Taylor series). Fisher's exact test (two-sided) yielded a P-value of 0.50 (Table 7).

Table 7. Risk of Viral Co-infection and GABHS Persistence

Viral Co-infection	GABHS Persistence*		
	Yes (33)	No (71)	Total (104)
	N	*n*	*n*
Yes	2	8	10
No	31	63	94

*2-sided Fisher exact test, P = 0.50; RR=0.57 (95% CI = 0.17 - 2.17)

Inclusion of Missing Data

Fifteen of the 38 (39.5%) patients excluded from the main analysis were lost to follow-up or refused follow-up cultures. These children comprised 11.6% of all GABHS infected subjects. Nasopharyngeal swabs of two of these 15 children (14.3%) were positive for a respiratory virus (one each of influenza A and B). As the outcome was unknown in these children, the analysis was repeated assuming the most extreme cases of bias to examine for their potential effects on the results of the study. First, patients who were lost to follow-up and were virus co-infected were all assumed to be persisters while those who were not virus co-infected were assumed to be non-persisters (Table 8). Then, the reverse outcomes were assumed for each risk factor group (Table 9). Neither assumption lead to a significant association between viral co-infection status and GABHS persistence.

Table 8. Analysis Including Missing Data: Virus Co-infected Assumed to be Persisters and Non Virus Co-infected Assumed to be Non-persisters

Viral Co-infection	GABHS Persistence*		
	Yes (35) *n*	No (84) *n*	Total (119) *n*
Yes	4	8	12
No	31	76	107

*2-sided Fishers exact test, P=0.75; RR=1.15 (95% CI = 0.49 - 2.70)

Table 9. Analysis Including Missing Data: Virus Co-infected Assumed to be Non-persisters and Non Virus Co-infected Assumed to be Persisters

Viral Co-infection	GABHS Persistence*		
	Yes (33) *n*	No (86) *n*	Total (119) *n*
Yes	2	10	12
No	44	63	107

*2-sided Fishers exact test, P =0.12; RR=0.41 (95% CI = 0.11 - 1.47)

Exclusion of Uncertain Data

Four of the persisters identified met the definition for *possible* rather than *definite* persisters. When these four cases were excluded and the analysis repeated the difference in outcome frequency between the two groups were not significant (Table 10).

Table 10. Analysis of Results Using a Strict Definition of Persistence

Viral Co-infection	GABHS Persistence*		
	Yes	No	Total
	(29)	(71)	(100)
	N	*n*	*n*
Yes	2	8	10
No	27	63	90

*2-sided Fishers exact test, P = 0.72; RR=0.67 (95% CI = 0.19 - 2.40)

Symptomatic versus Asymptomatic Persisters

Eleven of 33 (33.3%) persisters were symptomatic at the time of their follow-up visit (Table 11) with one to five symptoms or signs each (mean = 2.6). Mean colony counts were similar in asymptomatic (3.3) and symptomatic (3.5) persisters. Apparent differences in M type frequency were not significant (M1, P=0.14; M6, P=0.10) (Table 12).

Table 11. Clinical Presentation in Eleven Symptomatic Persisters

Clinical Presentation	*n (%)*
Sore throat	8 (72.7)
Sore glands	4 (36.3)
Fever	4 (36.3)
Inflamed pharynx	9 (81.8)
Exudate	2 (18.2)
Enlarged/tender lymph nodes	7 (63.6)

Table 12. M-Types in Symptomatic and Asymptomatic Persisters

M Type	Symptomatic (11) n (%)	Asymptomatic (22) n (%)
1	0	5 (22.7)
3	2 (18.2)	4 (18.2)
4	2 (18.2)	2 (9.1)
6	2 (18.2)	0
12	3 (27.3)	5 (22.7)
28	1 (9.1)	2 (9.1)
77	1 (9.1)	2 (9.1)
Non-typeable	0	2 (9.1)

RELATIONSHIP OF OTHER FACTORS TO PERSISTENCE OF GABHS

Demographic and Clinical Characteristics

Recorded data on patient demographics (year of enrollment, sex, age), clinical presentation (symptoms and signs), treatment (immediate versus delayed initiation of antibiotics), and behavior (compliance with medication) were compared for persisters and non-persisters (Table 13).

Compliance was examined in greater detail without reference to a predetermined definition. Diary information was available for 30/33 (91%) persisters and 67/71 (94%) non-persisters and residual medication recorded for 25/33 (76%) persisters and 56/71 (79%) non-persisters. There were no differences between persisters and non- persisters in the mean number of penicillin doses measured by either diary (28 in both groups) or medication measurement (29.6 for persisters versus 29.0 for non-persisters, difference P=0.94).

Table 13. Comparison of Characteristics Between Persisters and Non-persisters

Characteristic	GABHS Persistence	
	Yes (33)	No (71)
	n (%)	*n (%)*
Demographics		
Enrollment in 1st year of study	26 (78.7)	49 (69.0)
Male gender	21 (63.6)	39 (54.9)
Median age in yrs (range)*	6.3 (2.3-14.4)	6.3 (2.7-15.9)
Clinical presentation		
Sore throat	32 (97.0)	69 (97.2)
Sore lymph nodes	23 (69.7)	48 (67.6)
Fever†	22 (66.7)	59 (83.1)
Inflamed throat	30 (90.9)	68 (95.8)
Exudate	16 (48.5)	30 (42.3)
Tender lymph nodes	25 (75.8)	49 (69.0)
Median temperature in°C (range) ‡	37.6 (36-40)	38.0 (36.1-40)
Antibiotics given immediately	18 (54.6)	44 (62.0)
Compliant with therapy	30 (90.9)	66 (93.0)

*Mean age in years (SD) = 6.5 (2.4) for persisters and 7.3 (2.6) for non-persisters, P = 0.07 (2-sided FET). † P = 0.13 (2-sided FET). ‡ Mean temperatures (SD) = 37.6 (1.0) for persisters and 37.9 (1.0) for non-persisters; data recorded for 27 persisters and 55 non-persisters

Laboratory Data

Colony count

The proportion of patients with low colony counts on the enrollment throat swab was compared between persisters and non-persisters. Plate cultures were positive in 98/104 cases (94.2%), including all 33 persisters and 65/71 (91.5%) non-persisters. The frequency of a low colony count (rare to 2+ colonies) on the initial culture was similar for persisters (6/33, 18.2%) and non-persisters (11/65, 16.9%). A $\chi 2$ for trend confirmed that there was no association between decreasing colony count and GABHS persistence (P=0.46; Table 14).

Table 14. Frequency of High/Low Colony Counts for Two Outcome Groups.

	Persistence of GABHS	
Colony Count	Yes (33)	No (65)*
	n (%)	*n (%)*
Rare	1 (3.0)	3 (4.6)
1 +	0	3 (4.6)
2 +	5 (15.2)	5 (7.7)
3 +	5 (15.2)	8 (12.3)
4 +	22 (66.7)	46 (70.8)

* Six swabs were broth culture positive but plate culture negative, therefore total available specimens with colony counts (98) is less than the total number of GABHS positive patients

GABHS serotype

M and T serotyping was performed on 100/104 (96.2%) initial isolates. Of the four with no results, three were OIA positive but culture negative and in one case the isolate was not saved. The relative frequencies of M-types were similar between persisters and non-persisters except for M12 (more common among persisters), although the difference was not quite statistically significant (Table 15).

CHILDREN WITH RECURRENT SYMPTOMS AFTER FOLLOW-UP VISIT

Nine children returned to a study physician with a recurrence of symptoms within one month of the follow-up visit. Five were found to be GABHS-infected, of whom one was a continuing persister (never cleared M12 strain), one was a relapse (cleared at first follow-up but the original strain, M77, was detected on this return visit), one was a re-infection (M4 at enrollment and M12 with recurrent symptoms), and two were unknown because of lack of one or more of the isolates to compare serotypes (M4 and M28 at enrollment but OIA positive only with recurrent episode).

Table 15. Outcome by GABHS M-Type of Isolate at the Time of Enrollment

M-Type	GABHS Persistence		
	Yes (33)	No (67)*	Total (100)
	n (%)	*n (%)*	*n (%)*
1	5 (15)	9 (13)	14 (14)
2	0	2 (3)	2 (2)
3	6 (18)	15 (22)	21 (21)
4	4 (12)	6 (9)	10 (10)
6	2 (6)	4 (6)	6 (6)
12†	8 (24)	7 (10)	15 (15)
28	3 (9)	10 (15)	13 (13)
62	0	3 (4)	3 (3)
77	3 (9)	5 (7)	8 (8)
Non-typeable	2 (6)	6 (9)	8 (8)

*Three specimens were OIA positive only at enrollment and one isolate was lost.
† Difference between groups, P=0.08 (2-sided Fishers exact test)

LABORATORY TESTING FOR GABHS

Comparison of Optical Immunoassay to Culture Methods

The performance of the Optical Immunoassay rapid detection method for GABHS was compared to both broth and plate culture for all eligible patients. Plate culture was the most likely to be positive (98/248, 40%) followed by broth culture (94/248, 38%) and OIA (93/248, 38%). When OIA results were compared to the *combination* of culture methods, there were no false positive OIA results but 11/155 (7.1%) false negative tests (Table 16). Comparisons to the individual culture methods are shown in Tables 17 and 18 with the associated test performance results shown in Table 19. All percentages shown denote column totals.

Table 16. OIA Results Compared to Broth or Plate Culture

Optical Immunoassay	Broth or Plate Culture		
	Positive (104)	Negative (144)	Total (248)
	n (%)	*n (%)*	*n (%)*
Positive	93 (89.4)	0 (0.0)	93 (37.5)
Negative	11 (10.6)	144 (100.0)	155 (62.5)

Table 17. OIA Results Compared to Broth Culture

	Broth Culture		
Optical Immunoassay	Positive (94)	Negative (154)	Total (248)
	n (%)	*n (%)*	*n (%)*
Positive	86 (91.5)	7 (4.5)	93 (37.5)
Negative	8 (8.5)	147 (95.5)	155 (62.5)

Table 18. OIA Results Compared to Plate Culture

	Plate Culture		
Optical Immunoassay	Positive (98)	Negative (150)	Total (248)
	n (%)	*n (%)*	*n (%)*
Positive	89 (90.8)	4 (2.7)	93 (37.5)
Negative	9 (9.2)	146 (97.3)	155 (62.5)

Table 19. OIA Performance Compared to Culture Methods

OIA Performance Test	Comparative Culture		
	Broth (%)*	Plate (%)	Broth or Plate (%)
Sensitivity	91	91	89
Specificity	95	97	100
Positive predictive value	92	96	100
Negative predictive value	95	94	93

*Denotes performance of OIA compared to the culture method(s) in each column

OIA Performance by Inoculum of GABHS

OIA performance was also assessed in terms of the inoculum of GABHS; with rare to 2+ colonies classified as "low" and 3+ or 4+ as "high" colony counts (Table 20). A positive OIA rate of 99% in throat swabs with a high inoculum contrasted with a 53% of those with a low colony count ($P<0.001$ by 2-sided FET).

Table 20. OIA Performance by Colony Count on Plate Culture.

Optical Immunoassay	Colony Count		
	High (81)	Low (17)	Total (98)*
	n (%)†	*n (%)*	*n (%)*
Positive	80 (98.8)	9 (52.9)	89 (90.8)
Negative	1 (1.2)	8 (47.1)	9 (9.1)

*Total number of isolates that had positive plate cultures; † Denotes percentage of column totals

CHAPTER 5

DISCUSSION

This chapter includes an assessment of the strengths and weaknesses of the study. Results from the preceding chapter are interpreted and discussed, including the relationship between GABHS persistence and viral co-infection as well as other selected variables. Conclusions about the performance of the optical immunoassay are drawn and it's potential utility placed in context. Finally, implications of this study with respect to current management as well as future research into the phenomenon of GABHS persistence are discussed.

STUDY STRENGTHS AND WEAKNESSES

Design Issues

The nature of the association postulated in this study and the type of variables that required measurement necessitated a prospective cohort design. Given that nasopharyngeal swabs for viral culture are not a routine test when children present with pharyngitis, this information was not available to conduct a retrospective cohort or case control study using existing records.

The benefits of this design were an opportunity to keep participating physicians and patients blinded to patient risk factors (thus reducing one form of bias) and the ability to calculate a relative risk ratio for the association of interest. The main drawback of this prospective cohort design was the cost associated with data collection over a two-year period. However, this had the positive effect of reducing potential "year effects". In other words, a predominant outbreak of a single virus in one year could result in a false negative result if it was not one of the viruses that is associated with GABHS persistence. By extending the enrollment time period, the potential skewing effects of a single unusual year should have been reduced.

Recruitment

Sample size

A retrospective calculation demonstrated that the study had a power of 97% to detect a four-fold (alpha=0.05, two-sided) difference in frequency of persistence between the viral co-infected and virus uninfected individuals based on the acquired sample size and frequency of viral co-infection. There were sufficient enrollments to detect up to a 3.5-fold difference with 80% power. A smaller difference is not likely to have any clinical importance.

Generalizability of results

This study sample was intended to reflect a general pediatric population between the ages of two and 18 in Calgary who have GABHS pharyngitis. There were roughly equal numbers of males and females enrolled, with an age distribution similar to other GABHS pharyngitis studies. [19-21,33,37,38,42,44] The lack of individuals over 16 is likely a reflection of both the site of enrollments (in pediatric health facilities) and the epidemiology of the disease, but absence of older children is neither unusual compared to

other pharyngitis studies [19,21,38,43,44] nor would it be expected to change the results of the study.

The variety of enrollment sites in this study ensured a mix of subjects, which likely improved on the generalizability of results compared to similar studies reported in the literature. Enrollments were almost equal between emergency departments and pediatrician offices. Participating pediatricians covered all quadrants of the city in an effort to enroll patients from a variety of social strata. One might be concerned that pediatricians would attract patients from families of high socioeconomic status and potentially threaten external validity as a result, however participating pediatricians reported a range of patient population characteristics as shown in the methods. It would have been ideal to have collected more detailed information directly from subjects, but this was not done. Although an attempt to collect information on patients who were excluded or refused enrollment was made, it was not done consistently by participating physicians and did not include demographic details, therefore a comparison of the actual study sample to the intended sample could not be made. Given the significantly higher frequency of a history of fever and pharyngeal exudates in the emergency department enrollments, these subjects may have been slightly sicker (as one might expect) than the office enrollments, but they did not appear to differ in any clinically important demographic variables. Thus, the differences in training between some emergency physicians (family practitioners/emergency medicine specialists) and the office pediatricians did not appear to have a big impact on the study population and therefore presumably not the results.

Overall the study sample appeared to be an improvement on that described in previous studies as well as being reasonably representative of the target population in spite of the use of convenience sampling.

It might be argued that the high compliance rate found in this study may have influenced the results and made them less generalizable. In order to be sure that it was primarily the effects of the predictor that were being observed it was important to control as many factors as possible. Alternatively a longer study conducted at great expense

would be necessary to acquire a large enough sample size to permit analyses stratified on compliance. As poor compliance would be equally likely in both groups and may lead to persistence, its presence could move the results towards the null hypothesis. Thus while the actual frequency of persistence may not be generalizable, it is its relationship to viral co-infection that is of primary interest in a study aiming to understand potential causality of a single factor.

Loss to Follow-up

Loss to follow-up occurred in only 15 GABHS-infected patients (15/129; 11.6%). Two (13.3%) were positive for the risk factor, a prevalence similar to those who completed the study (10/104; 9.6%). As re-analysis demonstrated, assuming extreme bias in outcome by risk factor status did not result in significant differences between the two groups. Thus loss to follow-up did not bias the results of the study.

Measurement

The strengths and weaknesses of the tools of measurement used in this study were discussed in detail in the literature review. Measurement of the predictor variable (viral co-infection) was the best possible under the circumstances considering the need to use a simple sampling technique, gather samples from a large number of sites, and ensure prompt delivery to a lab capable of producing dependable results. Detection of GABHS to establish infection status and outcome may have been limited somewhat by the use of a single swab, although false negative results would have been more likely for the OIA than the other techniques because of the order of testing. Given that a positive result was defined as GABHS detected by any one of the three methods (which included the gold standard of broth culture), it was virtually impossible to miss a positive result. Measurement of clinical variables was limited only by the variation in clinician assessments, but was not expected to be a major concern. The data forms were consistently completed for all patients with the exception of temperature as noted in the results.

INTERPRETATION OF RESULTS: VIRAL CO-INFECTION AS A RISK FACTOR FOR BACTERIAL PERSISTENCE

Viral Co-Infection is Not a Predictor of GABHS Persistence

The results of this study suggest that respiratory virus co-infection is *not* associated with bacterial persistence following treatment of GABHS pharyngitis with penicillin V. The sample size of this study was not large enough to assess for the presence of effect modification through stratified analysis or modeling, therefore it is possible that there is an association between viral co-infection and persistence for a certain subgroup. However, the clinical relevance of an association for a subgroup would be questionable.

Viral Co-infection and Bacterial Persistence in AOM versus Pharyngitis

At first glance, it is puzzling that viral co-infection appears to play a role in bacterial persistence in acute otitis media but not GABHS pharyngitis. Although the two upper respiratory tract infections would presumably share many of the effects of a viral infection that are postulated to lead to bacterial persistence, the anatomic differences may be key. The increased inflammatory response in the middle ear, unlike the pharynx, may increase obstructive edema, block the eustachian tubes and result in delayed clearance of middle ear fluid and bacteria.[93] This difference may explain why viral co-infection appears to be important in one but not the other.

Additional Interpretations of the Negative Association

Viral co-infection influences the course but not the outcome of GABHS pharyngitis

While a viral infection may not affect the bacteriologic outcome of GABHS pharyngitis, it may influence the acquisition and early course of GABHS pharyngitis. Effects of a viral infection on nasal epithelial cells[87,88] and the immune response[89,90] may inhibit initial efforts to clear the organism and thus slow clinical and/or bacteriological response. However, their influence should settle as the viral infection resolves. This might

account for the resolution of GABHS infection in our viral co-infected patients by the time of their reassessment at the end of therapy.

Reverse association: Viral co-infection reduces the risk of persistence

As two-sided alpha limits were used in the sample size calculation, the results may also be interpreted as suggesting a possible *reverse* association but of a lesser magnitude than postulated for the sample size calculation. Repeating the study using a larger sample size might provide sufficient power to confirm the association of viral infection and clearance of GABHS. On the other hand, a statistically significant but smaller association would be of questionable clinical significance. It is also difficult to postulate the pathophysiological steps by which a viral co-infection may actually be beneficial by increasing eradication of GABHS.

Potential Influence of Misclassification Bias on Study Results

Predictor variable

Misclassification of viral co-infected children as virus negative is a concern in this study. Viability may have been compromised by excess transportation time or by freezing of some specimens. Tracking of specimens confirmed that they reached the virology laboratory within 24 - 32 hours of collection and upon arrival were set up for culture immediately, so transportation time was minimized. Viral infectivity may have been reduced by the freezing and thawing process, even under ideal conditions,[96] but this could have occurred for only 13 specimens that were OIA negative but culture positive 24-48 hours later.

The absence of certain viruses among viral co-infected patients may also suggest some false negative results. Herpes simplex virus, respiratory syncytial virus, and rhinovirus were not detected although a previous pharyngitis study isolated all three in GABHS co-infected patients.[5] However, broader inclusion criteria in the latter study may have selected for a viral-caused illness with GABHS carriage rather than GABHS infection. Thus a different spectrum of viruses in the two studies is not surprising. This

difference may also be explained by variation in predominant viruses in different geographic locations and in different years. This study would likely have been more protected from the effects of the latter given that it was conducted over a two year period.

Assuming there were some specimens falsely identified as virus-negative, one would expect a non-differential distribution of false negatives between GABHS persisters and non-persisters given that their outcomes were unknown when specimens were handled. This would result in a shift towards the null hypothesis. A high differential false negative rate would be necessary to change what was really a positive association. Judging by the 8% co-infection rates found previously[5] compared to the 9.4% found here, it is unlikely that the false negative viral culture rate was excessively high. Predictor variable misclassification is therefore unlikely to have significantly influenced the results.

Outcome variable

A high rate of persistence (31.7%) was noted in this study. While rates up to 38% have been reported,[33] studies utilizing similar methods (follow-up cultures within 2 weeks of completing treatment, and serotyping of isolates) were found to have a mean persistence rate of 11.3% (95% CI 9.2 - 13.9) by meta-analysis.[40] Thus, the persistence rate in this study appears to be high.

No other factors were found to be associated with persistence (as discussed in the next section in greater detail), including compliance,[51] early antibiotic use,[19,42] or young age.[6] Although persisters did tend to be younger than non-persisters, the age distribution and mean age of the study population was not skewed towards younger patients compared to other pharyngitis studies, including those with lower persistence rates.[38,76] Potential influences such as alpha streptococci and beta-lactamase producing flora were not measured in this study. Their association with persistence is uncertain, but if either does play a role, it is difficult to speculate why this study population would be so different from those in other pharyngitis studies.

False-positive GABHS results on follow-up throat swabs are another potential explanation for the high persistence rate. However, there were only three patients who

were OIA positive but culture negative on follow-up throat swab. Theoretically, the OIA in these cases may have detected non-viable organisms.[28] However, assuming these three were misclassified as persisters when they were actually non-persisters, the results of the analysis do not change (association between viral co-infection and persistence, P=0.72).

If chronic GABHS carriers were included inadvertently in this study, given that eradication is reduced compared to those with acute infection,[32,35] some carriers may have been misclassified as persisters. Although the chance of including carriers in acute pharyngitis studies is thought to be as high as 20% by some authors,[35] two pieces of evidence go against their presence in our GABHS positive population. The distribution of viral co-infection in persisters compared to non-persisters and the frequency of different colony counts in the GABHS positive subjects both suggest that inclusion of carriers is unlikely.

If some of the subjects classified as persisters in this study were actually chronic carriers of GABHS whose pharyngitis was caused by another pathogen, such as a virus, one would expect there to be more viral-infected children among these patients than the GABHS negative group. In fact, there were *fewer* persisters (6.1%) than non-persisters (11.3%) infected with a virus. This goes against the inclusion of carriers in the former.

Bacterial growth can also help distinguish colonization from infection. Among 33 persisters, only one had cultures with 1+ colonies or less, and a $\chi 2$ for trend suggested that a decreasing colony count was not associated with persistence. The colony count profile of the GABHS positive subjects closely resembled that of an "infected" group rather than a "colonized" group as shown in Table 21.

Table 21. Comparison of Colony Count to GABHS Infection and Carriage

Colony Count	Breese *et al.*[15]		Present Study
	% of Carriers	% of Infected	% of GABHS Positive
1+ and less	29.7	2.2	7.1
2+	17.8	11.0	10.2
3+	20.5	14.3	13.3
4+	32.0	72.5	69.4

The "degree of positivity ratio" as described in chapter 3[15] 'captures' this data in a single number. The higher the ratios, the higher the chance of a positive culture representing a true infection for a given group of patients. The positivity ratio in this study (87.7%) was almost identical to that found in the "infected" group in the study of Breese et al (84.4%) suggesting a high chance of true GABHS infection among those who were GABHS positive in this study.

Although serology was not performed in this study, its omission does not weaken the results. The majority (59.6%) of the patients in this study were advised to begin taking penicillin immediately after enrollment, which has been shown to blunt anti-streptolysin O responses.[19] Children with *S. pyogenes* infections in the months preceding the study may also have had a non-diagnostic rise in titre as a result of a higher baseline ASOT titre.[53] For these reasons as well as inconsistent correlation with clinical symptoms,[51,52] this is not considered a dependable means of distinguishing carriers from those with acute infection.[103]

If misclassification due to GABHS carriage did occur in spite of the evidence to the contrary, could this have produced the results obtained? A differential bias in favor of the null hypothesis would only result if carriers were also more likely to be virus *negative* than the true persisters. As viral infections are thought to cause symptoms in most cases of carriers with pharyngitis, this should not be the case. While a non-differential bias (if carriers were no more or less likely to have had viral co-infection than true persisters) could also shift the results towards the null hypothesis, it could not push it to the other side of the null as occurred here.

INTERPRETATION OF RESULTS: RELATIONSHIP OF OTHER FACTORS TO GABHS PERSISTENCE

There were no other factors found to have a definite association with GABHS persistence in this study, although three variables demonstrated a trend towards an association with persistence: age, fever, and M-type. Other factors such as early initiation of antibiotics and lack of compliance clearly did not have a relationship to this outcome.

Age

There appeared to be a trend towards younger age among persisters compared to non-persisters in this study. Although age has not been a factor of investigation in acute pharyngitis outcomes, a study of carriers treated with rifampin or cefixime also noted persistence to be significantly more common in children under 15 years of age compared to older patients.[6] If an association exists, why adults may be more likely to eradicate GABHS is unknown. It may be the result of a differential immune response by age. In one study measuring immunoglobulins to streptococcal C5a peptidase (SCP) with and without infection, the serum IgG response to this antigen increased with increasing age.[54] The same was not true for the secretory IgA response, however. Although the age difference between persisters and non-persisters is almost significant in our study, it is not very

large. At this point it is still unclear whether there is a clinically important difference in GABHS clearance rates with age and, if it exists, the reasons for it. It should taken into account in future studies, but is unlikely to be a major predictor of persistence.

Fever

Patient report of fever also bordered on statistical significance in this study, and may be a marker for severity of illness that in turn may influence outcome. However, objective measures such as temperature measured in the physician's office was neither clinically or statistically different by outcome. Thus, this variable is unlikely to be either statistically or clinically useful.

Serotype

Of all the serotypes identified in this group of patients, only M12 appeared to have a potential relationship to persistence. M12 is known to be a common cause of uncomplicated streptococcal pharyngitis[14] and has also been found to be common in post streptococcal glomerulonephritis.[60] Although there has been a report of it being a frequent cause of invasive infections in Ontario,[61] more often M1[59,61,62] and sometimes M3[59] are implicated. A common finding in these isolates is the gene(s) encoding streptococcal pyrogenic exotoxin such as *spe*A. However, there are no studies with information examining M type with respect to risk of bacteriologic persistence with *S. pyogenes.* There is also a lack of information regarding the specific properties of M12 strains that may explain their increased propensity for this outcome. More investigation is required before conclusions can be drawn regarding the role of M-type in GABHS persistence.

Early Antibiotic Use

The results of this study confirmed that of prior investigators that immediate initiation of antibiotics compared to a 24-48 hour delay did not increase the risk of persistence.[18] Instead, this approach has the added advantage of reducing the infectious period and therefore transmission to others as well as complications in the index case.[104]

Furthermore, this approach has been shown to result in a more rapid subjective and objective clinical improvement.[25,26] Not only is this factor unlikely to be important in the pathogenesis of persistence, but delaying treatment is not a logical or defensible approach in acute pharyngitis, particularly if rapid testing is incorporated into therapeutic decision-making.

Compliance

While early studies showed that compliance with oral antibiotic therapy was essential for GABHS eradication,[43] the association was not demonstrated by this study. None of the persisters took fewer than 24 (80%) doses of medication by either patient report or measurement of residual medication. The fewest number of doses recorded among non-persisters was 21 according to patient diary. This information confirms earlier suggestions that oral penicillin with optimal counseling (explaining the necessity for completing the course of antibiotics as well as providing written material to that effect) could result in eradication rates as good as intramuscular penicillin and better than that of oral medication without counseling.[44] The environment of this study could be argued to have provided comparable "counseling" of patients. As a result, there may not have been enough non-compliant patients (8%) to determine the role of compliance. Unfortunately, most studies either don't provide a definition of compliance or use a single urine measurement to determine it, so it is difficult to compare results. The important conclusion, however, is that persistence still occurs despite good patient compliance.

Unknown Variables

There were several pieces of information not collected in this study for which association to outcome is unknown. These include certain symptoms and signs that classically are not associated with GABHS (e.g. rhinorrhea, cough, congestion) but if present in a patient later found to have GABHS after treatment may suggest a chronic GABHS carrier with non-GABHS pharyngitis (such as a virus) rather than a true GABHS persister. Secondly, if funding had permitted, it would have been desirable to do throat

swabs on subjects to isolate both beta-lactamase-producing organisms as well as GABHS-inhibitory flora given that both may play a partial role in the development of persistence.

UTILITY OF OPTICAL IMMUNOASSAY IN DIAGNOSIS OF GABHS PHARYNGITIS

Comparison to Broth and Plate Cultures

Broth culture is usually considered to be more sensitive than plate cultures, and therefore is the gold standard for assessing a new test for GABHS in throat swabs. Only plate cultures provide colony count results, which were also required for this study. To minimize cost and discomfort to the patients, only one throat swab was taken at each visit. There may have been some false negative broth culture tests as a result of using the foam plug from the throat swab transport container, whereas plate culture was done from the throat swab directly. Although it was interesting to compare the OIA to each culture method, a combination of results from the two methods was considered the best comparison to assess the performance of the rapid test in this situation. Previous authors have cautioned that the reference method must be accurate in order to consider comparison with a new test reliable.[24]

Specificity

The OIA was very specific test (100%) for GABHS in the pharynx compared to a combination of the two culture methods. One could be very confident that a patient with a negative test did not have GABHS present in the pharynx. Previous generations of rapid GABHS tests have also been increasingly specific,[17,23] although the OIA was particularly high.

Sensitivity

The weakness of the optical immunoassay was an unacceptably low sensitivity (89%). This lack of sensitivity appeared to be related to bacterial load, as false negative tests were more frequent among specimens with low colony counts than high colony counts. However, this may be an underestimate of the actual test sensitivity given the methodology used in this study. The OIA was done using the throat swab after it had been plated for culture, which may have decreased the number of organisms below the level of detection and increased the number of false negatives. An ideal testing scenario would include a throat swab for each testing method used if taken simultaneously.

Previous studies provide disparate estimates of OIA sensitivity. The majority of estimates are higher than the 89% found in our study[28,30,31] although all of these studies received some support from the company that markets this test kit. Another study conducted at a primary care centre at a teaching hospital found a sensitivity of only 77%,[29] in spite of the fact that a separate throat swab was used for the OIA test. Rigorous blinding by the investigators may have minimized bias that may have been possible in other studies as well as our own.

Predictive Values

In this population with a GABHS prevalence of 42%, the positive predictive value was excellent (100%) and the negative predictive value was 93%. In everyday practice the prevalence of GABHS may be lower because of less selective use, and therefore the PPV would drop. In other words, one could not be sure that a specimen with a positive OIA test is truly GABHS positive and inappropriate treatment may result.

Practical Application of the OIA

The performance of the OIA suggests it may be useful if used thoughtfully. A positive test can be relied upon, obviating the need for culture. In view of a lower NPV and the importance of diagnosing GABHS infections to prevent sequelae, practitioners should submit a swab for culture to be used if the OIA is negative.

FUTURE DIRECTIONS IN INVESTIGATION OF GABHS PERSISTENCE AFTER TREATMENT OF PHARYNGITIS

This study was unable to demonstrate a role for viral co-infection in GABHS persistence after pharyngitis. Other theories have either been discounted or remain uncertain. One area of investigation, which remains understudied, is the relationship of immune response to outcome. The role of mucosal immunity in particular is unknown at present.

Mucosal Immunity and Persistence

Mucosal immunity is the first line of defense in the body and therefore may be important in acquisition as well as resolution of GABHS pharyngitis. Secretory IgA inhibits attachment to epithelial cells, but neither promotes phagocytosis nor activates complement. It has been suggested that the latter may reduce opsonophagocytic-enhancing activity of IgG and impede the systemic immune response, leading to continued infection or colonization.[77] The reverse association is also plausible. If the anti-attachment role is the more important of the two, an inadequate IgA response may increase the risk of bacterial persistence. This hypothesis has not been suggested or tested to date.

Streptococcus pyogenes has several IgA-binding regions, including C5a peptidase, to which children produce a specific secretory IgA response.[54] However, not all children (only 87% of those under eleven years of age) in this study had a measurable response. If this or another specific IgA play a role in fighting this infection and not all hosts are able to mount this response,[54,80] then those with an impaired IgA response may be more likely to have persistent GABHS isolation. Given the possible variation in immune response with age,[54] the latter must be taken into account a potential confounder.

In order to test this hypothesis, sputum samples and throat swabs could be taken from children with GABHS pharyngitis at presentation and at follow-up to see if a

specific IgA response occurs or not, then the frequency of persistence compared for the two groups.

Other Important Factors

Although important contributors to resolution of GABHS pharyngitis such as local immune response are yet to be identified, it is unlikely that a single factor accounts for all cases of persistence. Previous studies have not considered the possibility of a multifactorial model. Among the various theories, the protective effect of alpha-streptococci with high GABHS-suppressive activity appears to be the most promising and should be included. Although the evidence is conflicting for other theories, future studies should collect data on patient demographics, clinical characteristics, treatment, compliance, and co-existing BLPF as well as GABHS-inhibitory flora. If an association is found between a factor of interest and persistence, then logistic regression could be applied including some of these other factors as appropriate. It may then be possible to derive a model, which includes several factors, and have predictive utility. Depending on the factors included, the model may have applications in clinical practice, either for identifying high-risk patients (which may influence treatment choice) or to provide additional interventions.

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APPENDIX 1

INFORMATION SHEET

GROUP A STREPTOCOCCUS PHARYNGITIS AND VIRUSES STUDY
Community Streptococcus Study Group

You or your child are coming to see the pediatrician because of a sore throat, sore neck, fever, or refusal to eat. If the doctor thinks that the "strep" bug is the cause, you may be asked if you would like to participate in this study. Here is some background information about our study and 'strep throat'.

Group A Streptococcus (strep) is the germ that causes 'strep throat' in children and adults. Strep throat is most common in North America during the winter time, and is usually accompanied by the symptoms of fever, sore throat, and swollen neck glands. It is usually treated with a 10 day course of an antibiotic such as penicillin. Although the symptoms improve in most patients who receive this treatment, the germ remains in the throat of between 8% and 38% of all children who are treated. Many studies have shown that the symptoms of strep throat are most likely to return in persons in whom the germ is not fully cleared by antibiotics.

At the present time, we do not know why the germ, strep, is not cleared with antibiotics in some children. We know that it is not because strep is resistant to penicillin. Many researchers have also tried to look for other bacterial germs in the throat that may prevent the penicillin from working properly, but so far, no such germ has been shown to be responsible. However, no one has looked at the possibility that the presence of viral germs in children with strep throat may make it harder to clear strep from the throat.

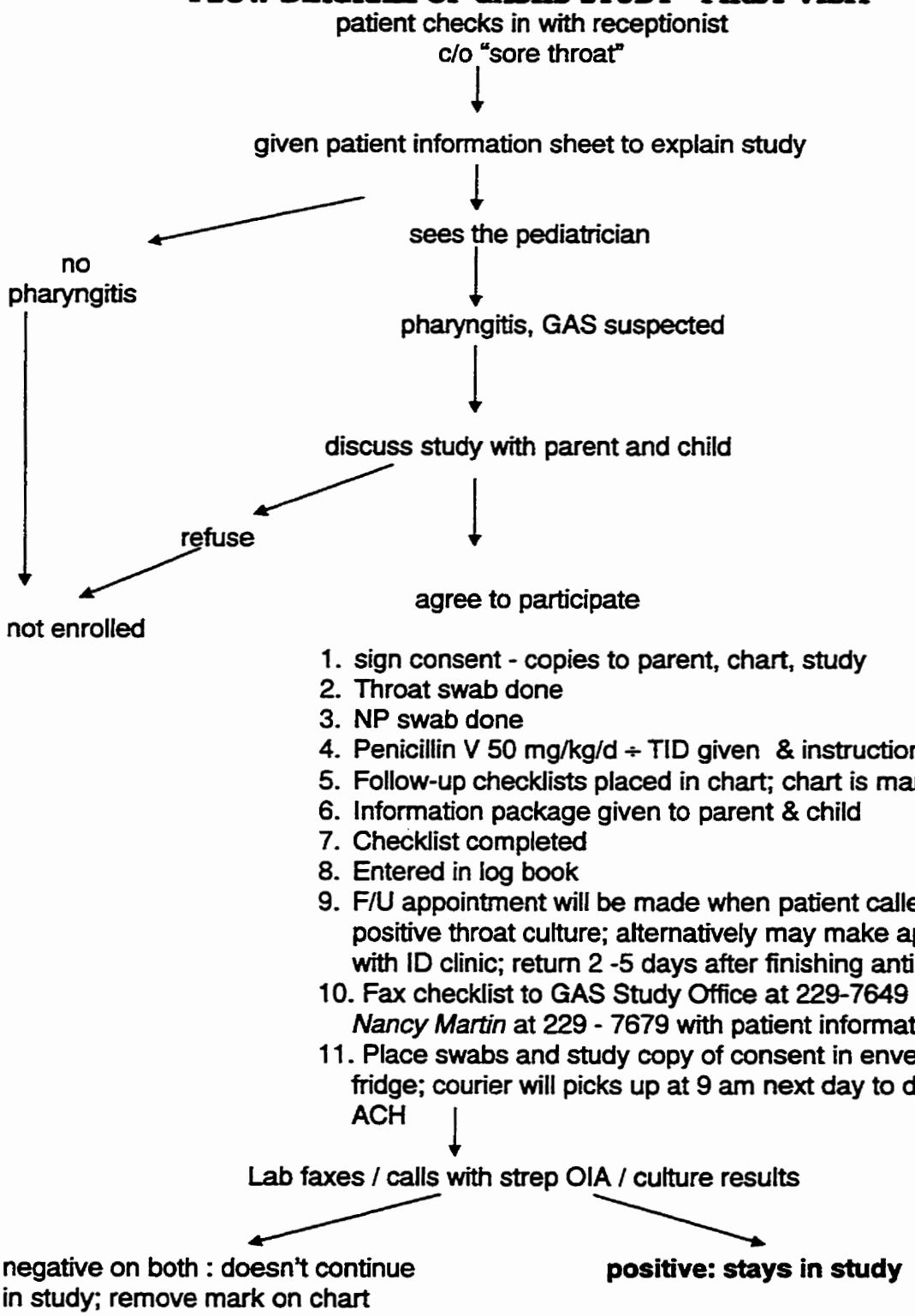
We would like to study the relationship between the presence of viral germs in the nose and throat and ability to clear strep in children with strep throat. We are suspicious of a possible link because strep throat is most common in the winter, at the same time during which colds (caused by viral germs) are most common. In fact, most (up to 75%) cases of sore throat are due to viral germs, and will not be affected by penicillin. Thus, we want to see if the presence of these viruses in children with strep throat makes it harder for the children to clear the strep after treament with penicillin. If this is true, it may open other avenues for treament in the future. Participation in this study would not include any changes in the treatment of you/your child's infection. However, in addition to the throat swab that is usually done to see if the cause of the throat infection is strep, we will need to take a second swab to look for viruses. This swab has to be taken from the back of the nose, and is more irritating than the throat swab for strep. Both sets of swabs would be repeated following completion of the 10 days of antibiotics, and once more if the same symptoms come back within 1 month of completing treatment.

We will offer you the choice of returning to your doctor, or coming to the Alberta Children's Hospital Clinic for the swabs to be done following treatment. We will provide $5 to pay for your parking if you choose to have these follow up swabs done at the Children's hospital. Any information obtained from you or your child will be strictly confidential and no information will be released or published that may indicate the identity of you or your child.

Thank you for considering participating in this study.

APPENDIX 2

FLOW DIAGRAM OF GABHS STUDY - FIRST VISIT



APPENDIX 3

CONSENT FORM

This consent form, a copy of which has been given to you, is only part of the process of informed consent. It should give you a basic idea of what the research is about and what your participation will involve. If you would like more detail about something mentioned here, or information not included here, you should feel free to ask. Please take the time to read this carefully and understand any accompanying information.

Group A Streptococcus (strep) is the germ that causes 'strep throat' in children and adults. Strep throat is most common in North America during the winter time, and is usually accompanied by the symptoms of fever, sore throat, and swollen neck glands. It is usually treated with a 10-day course of an antibiotic such as penicillin. Although the symptoms improve in most patients who receive this treatment, the germ remains in the throat of between 8% and 38% of all children who are treated. Many studies have shown that the symptoms of strep throat are most likely to return in person in whom the germ is not fully cleared by antibiotics.

At the present time, we do not know why the germ. Strep, is not cleared with antibiotics in some children. We know that it is not because strep is resistant to penicillin. Many researchers have also tried to look for other bacterial germs in the throat that may prevent the penicillin from working properly, but so far, no such germ has been shown to be responsible. However, no one has looked at the possibility that the presence of viral germs in children with strep throat may make it harder to clear strep from the throat.

We would like to study the relationship between the presence of viral germs in the nose and throat and ability to clear strep in children with strep throat. We are suspicious of a possible link because strep throat is most common in the winter, at the same time during which colds (caused by viral germs) are moist common. In fact, most (up to 75%) cases of sore throat are due to viral germs, and will not be affected by penicillin. Thus, we want to see if the presence of these viruses in children with strep throat makes it harder for the children to clear the strep after treatment with penicillin. If this is true, it may open other avenues for treatment in the future. Participation in this study would not include any changes in the treatment of you/your child's infection. However, in addition to the throat swab that is usually done, to see if the cause of the throat infection is strep, we will need to take a second swab to look for viruses. This swab has to be taken from the back of the nose, and is more irritation that the throat swab for strep. Both sets of swabs would be repeated following completion of the 10 days of antibiotics, and one more if the same symptoms come back within 1 month of completing treatment, We will offer you the choice of returning to your doctor, or coming to the Alberta Children's Hospital Clinic, for the swabs to be done following treatment. We will provide $5 to pay for your parking if you choose to have these follow-up swabs done at the Children's hospital. Any information obtained from you or your child will be strictly

confidential and no information will be released or published that may indicate the identity of you or your child. Thank you for considering participation in this study.

Your signature on this form indicates that you have understood to your satisfaction the information regarding your participation in the research project and agree (for your child) to participate as a subject. In no way does this waive your legal rights nor release the investigators, sponsors, or involved institutions from their legal and professional responsibilities. You are free to withdraw (your child) from the study at any time without jeopardizing his/her health care. Your continued participation should be as informed as your initial consent, so you should feel free to ask for clarification or new information throughout your participation. If you have further questions, please contact:

	Phone number
Dr. Susan Kuhn or	229-7211, pager 1323
Dr. H. Dele Davies	229-7813
Dr. Taj Jadavji	229-7813
Dr. Deirdre Church	229-7381

If you have any questions concerning your rights as a possible participant in this research, please contact the office of Medical Bioethics, Faculty of Medicine, The Univer4sity of Calgary, at 220-7990.

_____
Name of child

_____ _____
Name and signature Date

_____ _____
Investigator Date

_____ _____
Witness (Name and signature) Date

A copy of this consent form will be given to you. Please keep it for your records and future reference. The investigator will, as appropriate, explain to your child the research and his or her involvement, and will seek his or her ongoing cooperation throughout the project.

APPENDIX 4

CODING SYSTEM

X 0000 1

doctor code-letter | patient # | visit #

example: your office is assigned patient #'s 1 to 100
Johnie is the first patient you enter.
This is his initial visit (study entry) and your letter code is "Z".
Therefore Johnie's swabs are labelled as:

code: Z 1000-**1**

Johnie comes for a followup in 12 - 15 days (2/5 after he finishes abx)
These swabs are now labelled as:

code: Z 1001-**2**

DOCTOR CODES:

A Starr Cardwell (A 1000 - 1099)
B Janice Heard (B 1100 - 1199)
C Peter Nieman (C 1200 - 1299)
D John Wu (D 1300 - 1399)
E **ER** (E 1400 - 1599) C. Njissen-Jordan, M. Kalny, R. Galbraith, M. O'Byrne, N. Cooper, D. Smith, P. Stone, J. Grant, P. Downey
F Heidi Schroter (F 1600 -1699)
G Theo Govender (G 1700 - 1799)
H David Truscott (H 1800 - 1899)
J Ted Prince (J 1900 - 1999)
K Monique Wright (K 3000 - 3099)
L Elizabeth Shyleyko (L 3100 - 3199)
M Margaret Clarke (M 3200 - 3299)

For those who exceed their alloted one hundred, no problem! Then we assign from 2000 to 3000 in a similar fashion.

APPENDIX 5

OFFICE CHECK-LIST: *INITIAL VISIT*

PATIENT NAME_____ **CODE#**_____
Date of Birth (yr/mo/d)_____ Male ☐ Female ☐
Today's Date (yr/mo/d)_____
Phone # Home:_____
Work:_____ Name of Contact:_____

PHYSICIANS: PLEASE CHECK THE FOLLOWING AS APPROPRIATE:

			yes	*no*
SYMPTOMS	sore throat		☐	☐
	sore glands		☐	☐
	fever		☐	☐
SIGNS	inflamed pharynx		☐	☐
	pharyngeal exudate		☐	☐
	tender, enlarged lymph nodes		☐	☐
	temperature:_____°	oral ☐ tympanic ☐ rectal ☐ axillary ☐		

CONSENT SIGNED - a copy to family, a copy in chart, a copy with swabs ☐

			yes	*no*
SWABS DONE	time _____	THROAT	☐	☐
		NASOPHARYNGEAL	☐	☐

if no, give reason: _____

PENICILLIN 50 mg/kg/d divided TID (max 500 mg TID)	Given now	☐	
	Will give if GAS +ve		☐

	yes	*no*
Would the patient consider answering further study questions by telephone?:	☐	☐

OFFICE STAFF - PLEASE CHECK AS APPROPRIATE

FOLLOW-UP (if strep positive) WILL BE AT:	This office	☐
	ACH ID clinic	☐

	yes	*no*
PATIENT INFORMATION PACKAGE - given to family	☐	☐
ORANGE DOT - placed on chart to mark it	☐	☐
FOLLOW-UP CHECKLISTS - left in patient chart	☐	☐
VISIT #1 ENTERED IN LOGBOOK	☐	☐
SWABS AND STUDY COPY OF CONSENT - placed in fridge in delivery envelope	☐	☐

YOUR INITIALS _____

*****PLEASE FAX THIS FORM TO THE STUDY OFFICE AT *229-7649* THEN KEEP THIS COPY IN PATIENT'S CHART*****

APPENDIX 6

PRESCRIPTION FOR GROUP A STREP STUDY

The nurse or physician has taken a swab of your child's throat to determine if he/she has an infection caused by a germ or bacterium called ' Streptococcus' which is treatable with antibiotics. office will call you in 2 to 3 days to tell you if the test is positive for Streptococcus. If you do not receive a phone call telling you the test is positive, please destroy the prescription. Sore throats or fevers are frequently caused by viruses which do not respond to antibiotics and we advise against the unnecessary use of antibiotics which may have unwanted side effects.

*If the test is **positive** you should have the attached prescription filled at your pharmacy. The office will call to set up a follow-up appointment for 2 - 5 days after the antibiotics are finished.*

It is very important to continue the treatment for a "Strep Throat' for the entire 10 days. This is to prevent very serious complications of the infection such as rheumatic fever which may result in heart disease. The patient will feel better very quickly once the medicine is started and it is quite easy to forget to give the medicine.

TO THE PHARMACIST: DISPENSE WITH SAFETY LABEL AND SAFETY CLOSURE. Must include the generic name of the drug, the proprietary name, if necessary, the strength and quantity dispensed. **SAFETY CLOSURE:** Required for tablets and capsules, unless these are available in prepackaged format which provides a comparable safeguard.

PATIENT NAME: _____

ADDRESS : _____

AGE: _____

_____
patient label or stamp

10 - 14 kg	☐	PENICILLIN V LIQUID: 300 mg/5ml 90 cc; SIG: 3 ml TID for 10 days
15 - 19 kg	☐	PENCILLIN V LIQUID : 300 mg/5ml 150 cc: SIG: 5 ml TID for 10 days
20 - 24 kg	☐	PENICILLIN V LIQUID: 300 mg/5ml 180 cc: SIG: 6 ml TID for 10 days
25 - 29 kg	☐	PENICILLIN V LIQUID: 300 mg/5ml 225 cc; SIG: 7.5 ml TID for 10 days
OR	☐	PENICILLIN V 300 mg Tablets #45 SIG: 1 and 1/2 tablets (450mg) TID for 10 days
≥ 30 kg	☐	PENICILLIN V 250 mg Tablets #60 SIG: 2 tablets (500mg) TID for 10 days

_____
Date

_____
Physician Signature

_____
Name in block letters

DO NOT REPEAT
Prescription not good after 7 days

APPENDIX 7

STREP INFORMATION SHEET FOR FAMILIES
Please post on your fridge

NAME :_____ CODE #:_____

- If you/your child are prescribed Penicillin for strep throat, please **MARK OFF THE DOSES IN THE LITTLE DIARY** as you/your child takes it. The diary can be placed on your fridge or some other easy-to-see place.
- If you have any problems with the antibiotic CALL YOUR DOCTOR
- Please **KEEP** the antibiotic container and this diary when you have finished the prescription. **If** the strep bug is found on the throat swab test, the office will call to tell you this. An appointment will be made at this time to return **2 to 5 days after finishing the Penicillin.** You can write it in below for a handy reminder:
- *Return Appointment is on (date):* _____
  *at (time):*_____

- Please bring this DIARY and the ANTIBIOTIC CONTAINER with you for this visit
- We will call you to confirm your follow-up appointment. Those returning to the Infectious Disease Clinic, held on Friday mornings in the DAT area on the main floor of the Alberta Children's Hospital, will be given $5 for parking costs.
- **If the same symptoms of sore throat return within a month after this second visit, please return to your doctor OR the Infectious Disease Clinic.**

ANTIBIOTIC DIARY:

Date started: _____ *Date finished:*_____

	DAYS PENICILLIN TAKEN									
	1	2	3	4	5	6	7	8	9	10
DOSE										
morning										
afternoon										
evening										

If you have any questions or concerns about the study call our 24-hour line *229 - 7679* or contact Dr. Kuhn 229-7211 or Dr. Davies 229 - 7813.

APPENDIX 8

THANK YOU LETTER

Dear participant:

We would like to thank you very much for agreeing to participate in this study of "strep throat" and viral infections. Research into pediatric health problems such as this one would not be possible without the involvement of people such as you and your child.

As you know, not all children with sore throats will have the "strep" bug in their throats. Very often these are due to viruses, such as those causing "colds".

Therefore, you will only be called for a follow-up appointment if the "strep" but is found on your child's throat swab. Even if the throat swab test is negative, however, you have brought valuable information to our study about pharyngitis in children.

We would therefore like to express our appreciation of your involvement in the study to this point. Enclosed is a copy of the Alberta Children's Hospital Pediatric Residents' "Kids Doc". We hope you find it informative and helpful!

The Community Group A Streptococcus Study Group
Alberta Children's Hospital

APPENDIX 9

OFFICE CHECK-LIST: VISIT #2

Mandatory Follow-up for Strep Positive Patients

PATIENT NAME: _____ **CODE # :** _____

Today's Date (yr/mo/d): _____

PHYSICIANS -PLEASE CHECK THE FOLLOWING AS APPROPRIATE:

		yes	no
COLLECTED:	ANTIBIOTIC DIARY	☐	☐
	ANTIBIOTIC CONTAINER	☐	☐
	if no, give reason: _____		
REPEATED:	THROAT SWAB ☐ ☐		
	NASOPHARYNGEAL SWAB	☐	☐
	time _____		
	if no, give reason: _____		

		yes	no
IS PATIENT SYMPTOMATIC ?		☐	☐
If yes then complete the following:			
SYMPTOMS	sore throat	☐	☐
	sore glands	☐	☐
	fever	☐	☐
SIGNS	inflamed pharynx ☐ ☐		
	pharyngeal exudate	☐	☐
	tender, enlarged lymph nodes	☐	☐
	temperature:_____° oral ☐ tympanic ☐ rectal ☐ axillary ☐		
WERE ANTIBIOTICS PRESCRIBED:		☐	☐
if yes, which one? _____			
INSTRUCTED PATIENT TO RETURN IF SYMPTOMS RECURR		☐	☐

OFFICE STAFF - PLEASE CHECK AS APPROPRIATE:

	yes	no
VISIT #2 ENTERED IN LOGBOOK	☐	☐
CERTIFICATE OF APPRECIATION - given to patient	☐	☐

YOUR INITIALS _____

*****PLEASE FAX THIS FORM TO THE STUDY OFFICE AT *229-7649* THEN KEEP THIS COPY IN PATIENT'S CHART*****

APPENDIX 10

Certificate of Appreciation

awarded to

_____

For enthusiastic participation in the study of strep throat infections in children

presented by

The Community Group A Streptococcus Study Group
Alberta Children's Hospital

_____ _____

(Attending Doctor's Name) ***Date***

APPENDIX 11

OFFICE CHECK-LIST: VISIT # 3

Follow-up if Symptoms Recurr Within a Month of Visit #2

PATIENT NAME: _____ **CODE # :** _____

Today's date (yr/mo/d): _____

PHYSICIANS: PLEASE CHECK THE FOLLOWING AS APPROPRIATE:

		yes	no
SYMPTOMS:	sore throat	☐	☐
	sore glands	☐	☐
	fever	☐	☐
SIGNS:	inflamed pharynx	☐	☐
	pharyngeal exudate	☐	☐
	tender, enlarged lymph nodes	☐	☐
	temperature:_____° oral ☐ tympanic ☐ rectal ☐ axillary ☐		
		yes	*no*
REPEATED:	THROAT SWAB	☐	☐
	NASOPHARYNGEAL SWAB	☐	☐
	*Time :*_____		
	if no, give reason: _____		
		yes	*no*
WERE ANTIBIOTICS PRESCRIBED:		☐	☐
	*if yes, which one?*_____		

OFFICE STAFF - PLEASE COMPLETE:

	yes	*no*
VISIT #3 ENTERED IN LOGBOOK	☐	☐

YOUR INITIALS _____

*****PLEASE FAX THIS FORM TO THE STUDY OFFICE AT *229-7649* THEN KEEP THIS COPY IN PATIENT'S CHART*****

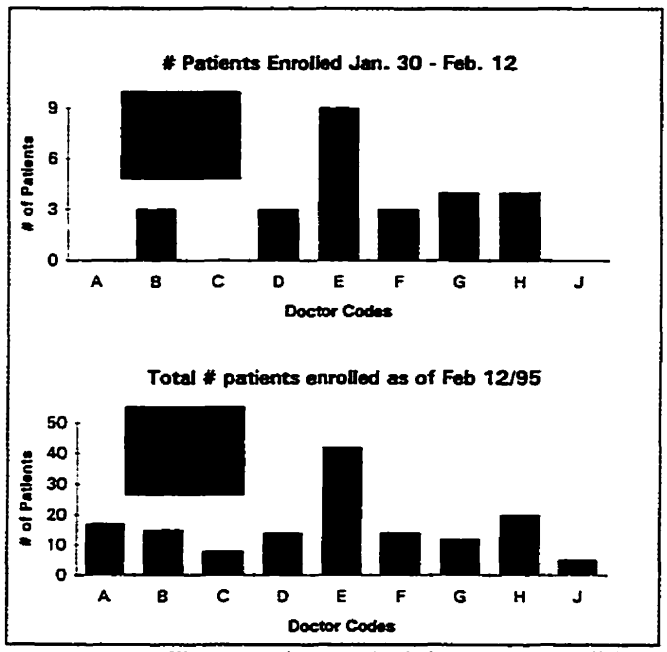
APPENDIX 12

COMMUNITY G.A.S. STUDY

NEWSBULLETIN

Feb. 14/95 Issue No. 10

As of February 12, 147 patients have been entered into the study. The past two weeks saw a slight increase in the number of patients enrolled in our study as compared to the previous two weeks. Seven more cases of Group A Strep were reported, bringing the total to 56 positives (22% of desired total number of cases). Two new GAS persisters have been identified, bringing our total to 15 persisters.



The Virology Lab in Edmonton reports that they are seeing an increasing number of viral isolates, particularly Influenza A & B and RSV. During this past two week period, another two patients enrolled in the study were reported to have Influenza B.

There have been an increasing number of antibiotic containers sent to the Microbiology lab with the specimens and consent forms. Space for the Group A Strep study is very limited in the lab and so we would ask the offices to please keep these containers at their offices in the white box provided for the G.A.S. study. If the box is getting full, please contact the study office and someone will come by and pick up the antibiotic containers and diaries.

Also, we would like to add a quick reminder about follow-up visits. Please remember to call or fax the study office and let us know when a patient will be coming back for their second visit. This is so that the study coordinator can call and remind the patients to bring their antibiotic diaries and containers with them to their follow-up appointment.

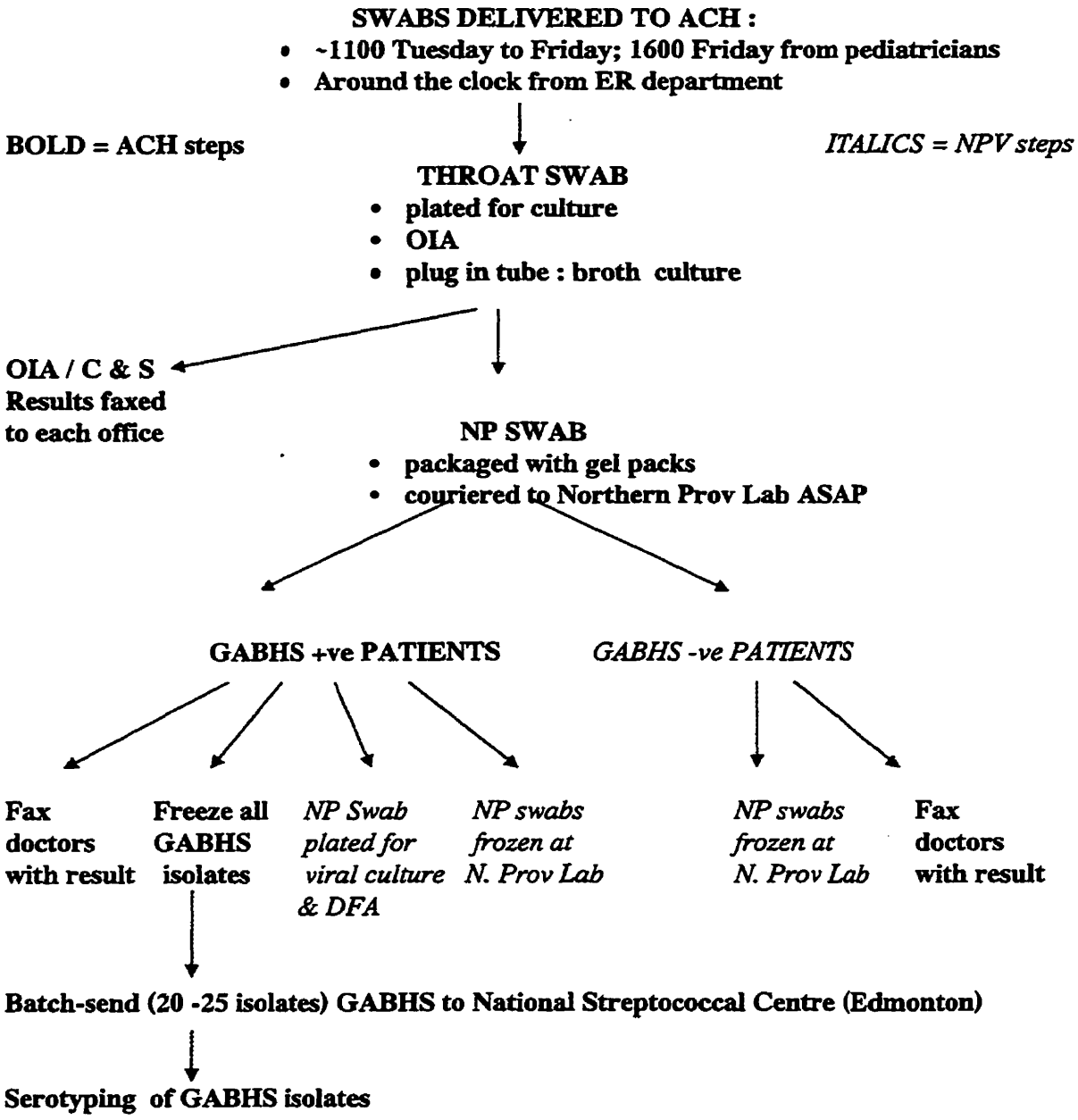
As offices are closed on Monday, February 20, courier pick-ups will resume on Wednesday, February 22, for the remainder of that week.

Happy Valentine's Day!

The Community Group A Strep Study Office

APPENDIX 13

LABORATORY FLOW SHEET



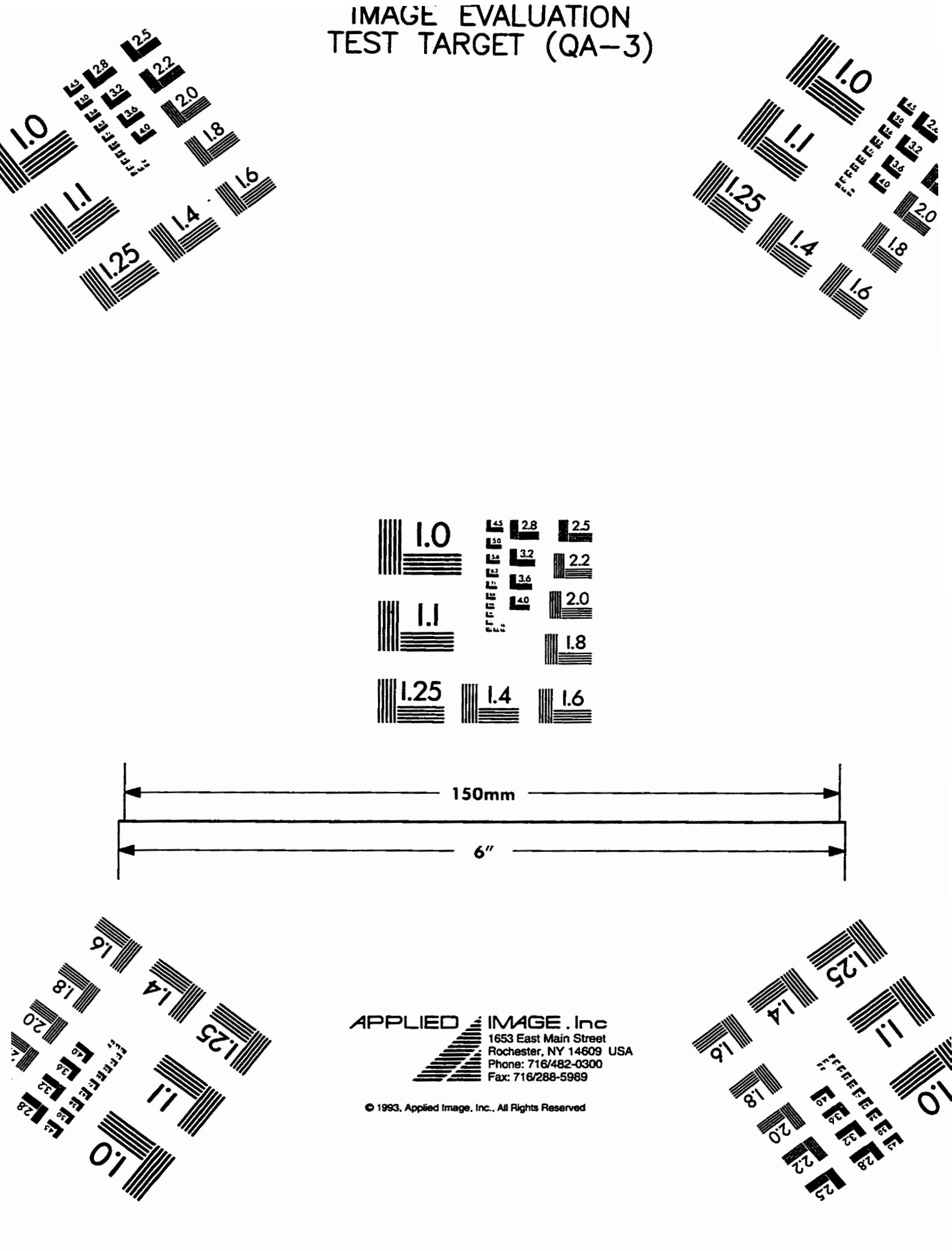


IMAGE EVALUATION
TEST TARGET (QA-3)
1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
150mm
6"
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