Group A Streptococcus

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THE MICRO-ORGANISM AND ITS CLINICAL PRESENTATION

Streptococcus pyogenes (*S.pyogenes*), or Group A Streptococcus (GAS), belongs to the genetically diverse group of Gram-positive cocci that grow with hemolysis, aerotolerant, in chains and are therefore called streptococci. Streptococci are classified in groups and types based on cell wall components. One of the most pathogenic streptococci groups is the beta-hemolytic streptococcus group A. They produce many toxins. GAS is found only in humans and colonizes wounds and mucosal surfaces like the throat.

Other beta-hemolytic streptococci, *S.dysgalactiae subs.equisimilis* and *S.anginosus*, may also show reactivity with group A tests, but do not cause sequelae and do not belong to the *S.pyogenes* [LCI 2023, Murray 2003].

The dynamic interaction between bacterial factors like toxins and host factors leads to a wide range of infections, varying from mild skin infections to more serious infections like necrotising fasciitis [Hand 2020; Brouwer 2023]. In most GAS infections the diagnosis is based on clinical presentation and bacterial cultures but the serological detection of antibodies against toxins (Streptolysine O, Deoxyribonuclease B (anti-DNAse B), Hyaluronidase, Streptokinase and others) can also identify infection recently or in the past.

NON-INVASIVE INFECTIONS

- Impetigo is a common and very contagious superficial skin wound infection in children with distinctive honey-crusted lesions. The most frequently isolated organism is *Staphylococcus aureus*, however non-bullous impetigo can also be caused by GAS. Impetigo generally has a mild disease course, but can be more severe in the case of infected chickenpox lesions.
- Pharyngitis, also known as ‘strep throat’, is an acute infection of the oropharynx which occurs commonly in children. Clinical differentiation from a viral infection can be difficult, but patients with GAS pharyngitis

typically do not have cough, rhinorrhea or hoarseness There is a "Clinical Scoring System to increase the likelihood of GAS pharyngitis" [Zwart 1999].

- Scarlet fever is a mild illness caused by the reaction to exotoxin production of GAS, mostly after a pharyngeal infection. One week after infection a characteristic diffuse erythematous 'sandpaper' like rash, and 'strawberry' red tongue appear.
- Erysipelas is a skin infection involving the dermis layer of the skin and is characterised by a well-demarcated erythema. In patients it can be difficult to distinguish erysipelas from cellulitis, which is also caused by S. aureus.

INVASIVE INFECTIONS

- Necrotising fasciitis is a life-threatening rapidly progressive skin infection caused by GAS and is characterised by necrosis of fascia and subcutaneous tissue. No typical erythema has to be present, but excessive pain in a rapidly deteriorating patient are important clinical clues. Necrotising fasciitis has a high mortality rate, and rapid antibiotic treatment with surgical debridement are essential for a good prognosis.
- Puerperal fever is caused by a post-partum GAS infection of the endometrium. The onset is in the first few days postpartum with clinical symptoms of fever, abdominal pain and sometimes sepsis in normally healthy woman.

Other invasive presentations of GAS infections are septicaemia, meningitis, pneumonia and arthritis.

COMPLICATIONS

Complications of a GAS infection can be due to the direct effect of toxin production by GAS or to the indirect serological response to the GAS infection.

- In rare cases, toxin production by GAS can cause Streptococcal Toxic Shock Syndrome (STSS), which leads to an acute onset of shock and multi-organ failure(mainly after skin infections).
- Infections with select types of GAS, can be complicated by post-streptococcal immunological sequelae, especially in those who are not treated with antibiotics. These disorders develop a couple of weeks after a GAS infection (skin or throat) as result of the host response to the infection:
  - Acute rheumatic fever (ARF; in 0.1-1%, or up till 3% in epidemics, of GAS infections). ARF includes sequelae like carditis, arthritis,

Sydenham chorea, subcutaneous nodules, and erythema marginatum. Recurrent ARF exacerbates existing heart damage with Rheumatic Heart Disease (RHD) as a result. Worldwide there are >15 million cases of RHD, with 282,000 new cases and 233,000 deaths each year [Carrapetis 2005, , Oliver 2021, Thomas 2021].

- Post-streptococcal glomerulonephritis (PSGN) can occur up to 28% after GAS infection and is characterised by a variety of nephrotic disorders [Canon 2021].
- Paediatric autoimmune neuropsychiatric disorders (PANDAS). Children with PANDAS display sudden behavioural or neurological changes after a GAS infection [Hand 2020, Lepri 2019]. It can occur in 1-5% of children and is especially prevalent between ages 5-8 years.

EPIDEMIOLOGY

GAS infections are prevalent worldwide mainly in children 5-19 years of age. The epidemiology of different types of GAS infection varies between age groups and geographical areas. At any time 5-25% of the population are carriers of GAS. GAS carriage and infection are most common in school-aged children [Hand 2020]. In The Netherlands, 30% of asymptomatic children and 7% of asymptomatic adults are carriers of GAS in the throat. In children aged 5-15 years, 15-30% of all cases of pharyngitis are caused by GAS [Kronman 2014]. When untreated, this is associated with persistence of positive cultures (>50% at 6 weeks) [Wessels 2011]. GAS are transmitted by inhalation of respiratory droplets or by direct and indirect contact with infected tissue, wounds or hands. It is rarely transmitted by food [Zwart 1999].

Non-suppurative, immunological sequalae are mostly seen in areas of crowding and lower socioeconomic status, where the use of antibiotics is limited. ARF and RHD were leading causes of death in children in the 1920s, and decreased and almost disappeared in the 1980s. However, a resurgence of severe GAS infections has been observed in the 2020s, especially in children aged under 5 years, including in high income countries, like The Netherlands [Dunne 2022, Gier 2023].

An important virulence factor of GAS, the M protein, is encoded by the emm gene. The emm types of GAS are differently distributed around the globe[Brouwer 2023]. Since 2022, M1UK, a virulent clone of the emm1 lineage, has emerged in high -income countries and caused a rise of invasive GAS infections [Zhi 2023].

DIAGNOSTIC TESTING

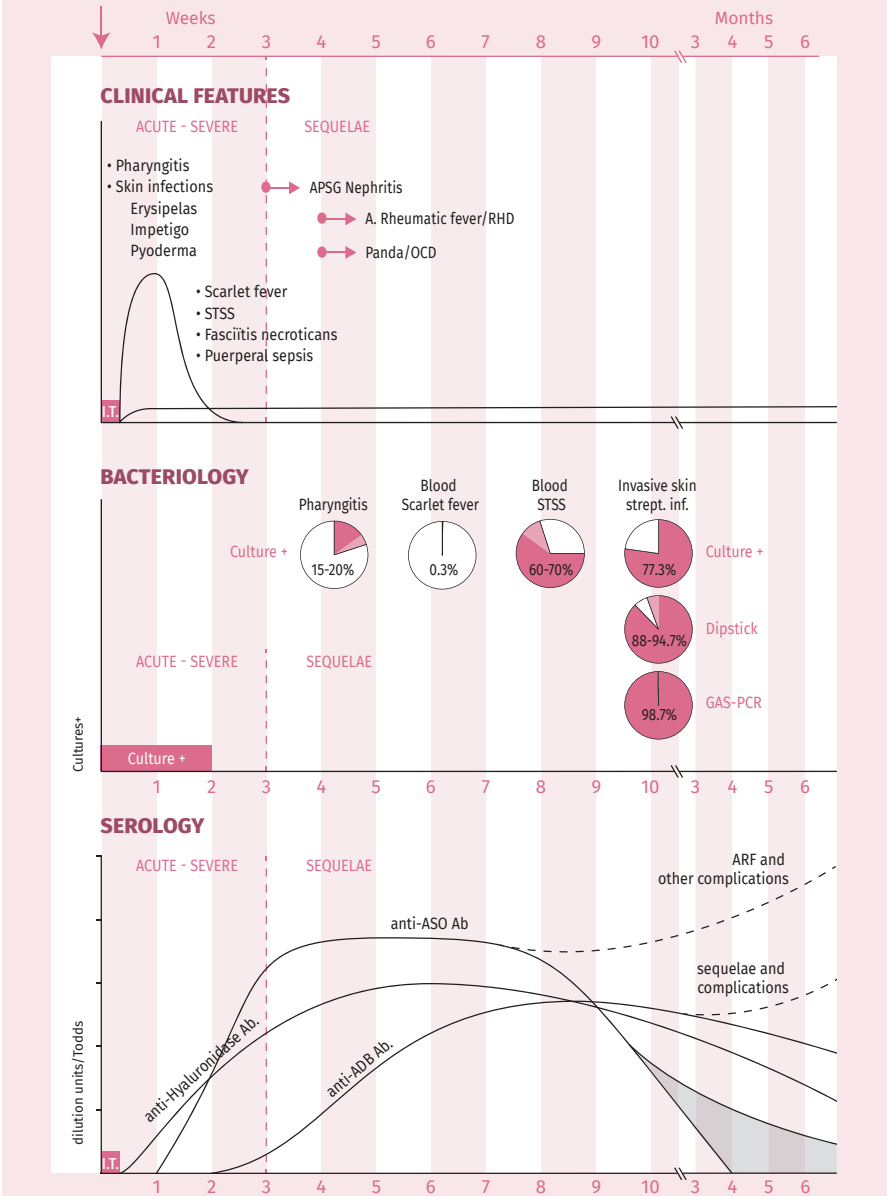
Clinical, bacterial and serological course is depicted in figure 1.

Techniques

- CULTURE: in most GAS infections the diagnosis is based on clinical presentation and cultures of the throat, fascia, pus, blood or cerebrospinal fluids that can be obtained during acute infection. Growth on a blood-agar plate exhibits complete hemolysis, called beta-hemolysis. Streptococci can be identified in culture using MALDI-TOF-MS. However, the definitive species identification is still based on the Lancefield grouping (A, B, C, F and G), which is determined using agglutination testing [Cherkaoui 2011].
- RAPID ANTIGEN TESTING (dipstick): after extracting antigens from throat swabs, components of the cell wall can be identified using latex agglutination or enzyme-immunoassay (EIA) for diagnosing pharyngitis in settings of otherwise inappropriate antibiotic use [Luo 2019] or rapid results in emergency cases. Unfortunately, due to the high GAS carrier rate in the age groups which presents with pharyngitis, the clinical applicability is limited.
- MOLECULAR TESTING: for GAS is available, consisting of rapid and standard polymerase chain reaction (PCR) tests. However, the use of molecular testing is limited since it cannot differentiate between GAS infection and carriage in the throat [Tanz 2019]. Molecular testing can be used in acute infections in which cultures are negative due to the use of antibiotics, but an invasive GAS infection is suspected based on clinical presentation and/or Gram stain.
- SEROLOGY: can be used for measuring the humoral response against extracellular antigens of GAS. Serology is not useful for diagnosis of an acute infection, since serum antibody levels require at least 10-14 days to rise, or even longer in case of dermal infections and is therefore used mainly in case of complications or sequelae. There are no accurate diagnostic cut-offs and studies show different figures for decrease of titres in time [Johnson 2010], >60% titres remain high one year after an infection. All available kits read the response from all antibodies, IgG, IgM and IgA. The two clinically important antibodies are anti-streptolysin O (ASO) and anti-DNAse B (ADB)(see subchapter “interpretation”). Titers vary according to age (also between individuals of the same age), area and climate [Steer 2015]!
  - Most important for serology is the Streptolysine O (Oxygen-labile toxin that binds to the cholesterol in membranes making these permeable, this in contrast to Streptolysine S=oxygen stable which is not antigenic) [Sierig 2003].

FIGURE 1.

CLINICAL, BACTERIAL AND SEROLOGICAL COURSE AFTER *STREPTOCOCCUS A* INFECTION



RHD	Rheumatic Heart Disease
ARF	Acute Rheumatic Fever
OCD	Obsessive Compulsive Disorder
PANDAS	Paediatric Autoimmune Neuropsychiatric Disorder
APSG/PSGN	Acute Post Streptococcal Glomerulonephritis

*Dipstick is a rapid antigen test

[Martin 2006, Gazzano 2016, Stevens 2000, Steer 2015, Steer 2009, Johnson 2010]

Streptolysin O is a haemolysin and is toxic to a variety of cells. It is produced by almost all strains of GAS, as well as many group C and group G streptococci. ASO is detectable one week after infection, peaks between 3 and 5 weeks, and declines after 8 weeks. ASO is mainly detected after pharyngeal infections and can remain negative after skin infections. ASO is produced in 80% of GAS-pharyngitis cases, but in only 25% of GAS skin infections. False positives will be found from group C and G streptococcal infections. ASO titers are disturbed by cholesterol/liver disturbances.

ADB is a GAS specific response, directed towards an enzyme which participates in the degradation of deoxyribonucleic acid (DNA). ADB peaks at 6 to 8 weeks and begins to decline at 12 weeks [Steer 2009, Steer 2015]. Antibodies directed towards DNAse B remain detectable for longer than antibodies directed against Streptolysin O.

Since all GAS strains produce DNAses and ADB is not produced by other streptococci, ADB has better sensitivity for detecting sequelae after pharyngeal and skin infections [Kaplan 1998]. Nephelometry is the preferred method to detect the serological response, since enzyme-linked immunosorbent assays (ELISAs) give varying results, depending on which test is used. Serological results generated by an ELISA must be carefully interpreted [Feijen 2001]. Aged related cut-offs are not given here since they differ by geographical location and have to be established by labs locally (see also in subchapter “interpretation”).

- OTHER TESTS WHICH ARE NO LONGER USED IN THE NETHERLANDS:
  - Testing of hyaluronidase is used in the UK but no longer performed in the Netherlands since a. only 50% of GAS strains produce hyaluronidase and b. other haemolytic streptococci and other bacteria may produce hyaluronidase [Watanabe 1976] and c. it is produced mainly in skin infections.
  - Streptozyme tests at least 5 different exoenzymes. It is a rapid but also an unreliable test. The exoenzyme concentrations produced by GAS vary and other bacteria may produce these exoenzymes as well [Klein 1971].
  - As a second test if no other is available these tests could be used knowing its limitations!

PRACTICAL USE OF SEROLOGY

Serological testing of GAS infections and sequelae is challenged by the presence of GAS-carriage in all age groups, slow decrease of mean population titers after childhood, and fluctuating (high or very low)

titers in many individual persons. The only proper testing in all patients consists in testing with 2 methods (ASO plus ADB) on a serum pair [Blyth 2006].

Screening

Since antibody levels of ASO and ADB reach their peaks at multiple weeks after infection and can remain high for some time, ASO and ADB testing is not useful for diagnosing acute pharyngitis. However, it can support the use of antibiotic treatment in recurrent pharyngitis, to prevent non-suppurative sequelae of a GAS infection, especially in high-incidence countries for ARF [Hand 2020]. If screening for rheumatic fever is performed, it also needs a serum-pair (at least 2-4 weeks apart) and at least 2 different tests (ASO, and ADB or Anti-Hyaluronidase) to make firm conclusions for preventative treatment.

Suspected infection in immunocompetent child or adult

Non-suppurative sequelae of a GAS infection occur usually 1 to 3 weeks after GAS pharyngitis, or 3 to 6 weeks after a skin infection. Patients cannot always remember the episode of pharyngitis and throat cultures are negative in 75% of patients when the symptoms manifest. Even when cultures are negative for GAS, antibiotic treatment for infection or eradication of GAS carriage is recommended to reduce symptoms, prevent recurrences of PANDAS and ARF, and to reduce the risk of developing rheumatic heart disease (RHD) [Hand 2020, Lepri 2019]. Most accurate is a demonstration of a rising titer regardless of the value found (Johnson 2010, Blyth 2005) against age-stratified ULN (Upper Limit of Normal) values. If a second convalescent sample is not possible an initial ASO/ASB titer above the ULN for age is sufficient for diagnosis (Steer 2015).

Suspected chronic infection in immunocompromised patients

Serological testing is always challenging in immunocompromised hosts. However, non-suppurative sequelae are the result of the immunological response after a GAS infection. Therefore, the serologically tested population will mostly consist of immunocompetent children [Cunningham 2019]. Immunocompromised hosts will be mostly at risk for invasive GAS infection, where culturing body fluids is the preferred diagnostic technique. A rising titer demonstrates an acute infection regardless of the initial values. A single initial titer above the ULN for age proves an active infection if clinical features are found [Steer 2015].

INTERPRETATION OF SEROLOGY

To diagnose a GAS infection with serology, a 2-fold rise in convalescent ASO and ADB titers has to be observed. It is not recommended to compare the titers of a single time-point against a reference upper limit of normal (ULN) [Johnson 2010]. The ULN varies between age groups and between individuals of the same age, season and geographical location [Shet 2002; Kaplan 1998]. These epidemiological data are not always known and ULN could be higher than expected [Danchin 2005]. In practice even though the titers of patients with ARF are mostly far above the ULN, a rise in titer regardless of the initial value is still preferred, because:

- The rise of antibodies after GAS acquisition will not always peak above the ULN.
- Antibodies can remain high after GAS infection or during GAS carriage and do not always represent the current infectious episode.
- The measured antibody levels can differ between different tests used [Fijen 2001].
- Use of a single serum with high ULN cut-offs undercounts definite and probable ARF diagnoses and other sequelae or complications [Steer 2015].

The timing of the determination of the titers is important since ASO and ADB peak and wane at different moments. Therefore, ASO and ADB should be determined at the start of symptoms and after 4 to 8 weeks.

SENSITIVITY AND SPECIFICITY

Sensitivity and specificity are optimised when measuring both ASO and ADB, and the diagnosis is based on a two-fold increase in titer [Johnson 2010]. See Table 1 for the sensitivity and specificity for a single versus a double serological test [Blyth 2005]. Sensitivity is influenced by the timing of testing. Recognition of a recent infection does not depend on a starting titer nor the maximum titer, but on a rising titer. The best

TABLE 1.

SENSITIVITY AND SPECIFICITY OF SEROLOGICAL TESTING		
Serological test*	**Sensitivity**	**Specificity**
Single test	70.5-72.7	86.4-93.2
Combination ASO & ADB	95.5 %	88.6 %

*A single anti-streptococcal test versus combinations of anti-streptococcal tests !
[Blyth 2005]

results are with the ASO and ADB combination. Other tests do not increase sensitivity nor specificity. In practice an Upper limit of normal (ULN) is used with a 80% safety score which signifies a high number of false negatives with clinical consequences [Blyth 2006].

PITFALLS

- Timing of testing: the rise in ASO and ADB titers could be missed when the serological time-points are not adequately timed after the GAS infection and the start of symptoms.
- False positive reactions are caused by Streptococcus group C and G.
- False negatives are numerous if only a single test is used for a diagnosis of GAS infection.

REFERENCES

Brouwer S, Rivera-Hernandez T, Curren BF et al. Pathogenesis, epidemiology and control of Group A Streptococcus infection. Nat Rev Microbiol. 2023 Jul;21(7):431-447. doi: 10.1038/s41579-023-00865-7.

Blyth CC, Robertson PW. Anti-streptococcal antibodies in the diagnosis of acute and post-streptococcAL DISEASE: streptokinase versus streptolysine O and deoxyribonuclease B.Pathology 2006;38(2): 152-156.

Canon JW, Zhang J, Bennett J et al. The economic and health burdens of diseases caused by group A Streptococcus in New Zealand. Int J Inf Dis 2021;103:176-181.

Carapetis JR The global burden of GAS. Lancet Infect Dis 2015;5:685-94.

Cherkaoui A, Emonmet S, Fernandez J et al. Evaluation of matrix-assisted laser desortion ionization-time of flight mass spectrometry for rapid identification of beta-hemolytic streptococci. J.Clin Microb 2011;49(8):3004-5.

Cunningham MW. Molecular Mimicry, Autoimmunity, and Infection: The Cross-Reactive Antigens of Group A Streptococci and their Sequelae. Microbiol Spectr. 2019 Jul;7(4):10.1128/microbiolspec.GPP3-0045-2018. doi: 10.1128/microbiolspec.GPP3-0045-2018.

Danchin MH, Carlin JB, Devenish W et al. New normal ranges of antistreptolysin O and antideoxyribonuclease B titers for Australian children J Paediatr Child Health 2005 Nov;41(11):583-6. doi: 10.1111/j.1440-1754.2005.00726.

Dunne EM, Hutton S, Peterson E et al Increasing incidence of invasive Group A Streptococcal Disease, Idaho, USA 2008-2019. Emerging inf. dis. 2022;28(9): 1785-1795.

Fijen CAP, Molenaar M, Hollingsworth-Vendel I et al. De test bepaalt de uitslag. Vergelijking van twee commerciële ELISA's voor de bepaling van anti-DNase-B. Nederlands Tijdschrift voor Medische Microbiologie. 2001 May; 9(2):42-44.

Gazzano V, Berger A, Benito Y et al. Reassessment of the role of rapid antigen tests in diagnosis of invasive group A streptococcal infections. J.Clin Micr 2016;54(4): 994-999.

Gier de B, Marchal N, Beer-Schrman de I et al Increase in invasive group A streptococcal (Streptococ. pyrogenes) infections (iGAS) in young children in the Netherlands, 2022. Eurosurveillance 2023: 28(1):pii:2200941

Hand RM, Snelling TL, Carapetis JR. Group A Streptococcus. Hunter's Tropical Medicine and Emerging Infectious Diseases. 2020 : 429–438. Published online 2019 May 28. doi: 10.1016/B978-0-323-55512-8.00040-5.

Jack S, Morehead NJ, Meager J et al. Streptococcal serology in acute rheumatic fever patients: Findings from 2 high-income, high burden settings. The Pediatric Inf Dis J 2019;38(1):e1-e6.

Johnson DR, Kurlan R, Leckman J, Kaplan EL. The human immune response to streptococcal extracellular antigens: clinical, diagnostic, and potential pathogenic implications. Clin.Infect Di 2010 ; 50(4):481-90

Kaplan EL, Rothermel CD, Johnson DR. Antistreptolysin O and anti-deoxyribonuclease B titers: normal values for children ages 2 to 12 in the United States. Pediatrics. 1998 Jan;101(1 Pt 1):86-8. doi: 10.1542/peds.101.1.86.

Klein GC, Jones WL Comparison of the streptozyme test with the antistreptolysin O, antideoxyribonuclease B, and antihyaluronidase tests. Applied Microb 1971; vol 21(2): 257-259.

Kronman MP, Zhou C, Mangione-Smith R et al. Bacterial prevalence and antimicrobial prescribing trends for acute respiratory tract infections. Pediatrics. 2014;134(4):e956.

Murray PR, Baron LJ, Jorgensen JH. Clinical Microbiology 8th edition 2003, chapter 29: Streptococcus. Ruoff KL, Whiley RA, Beighton D RA.

LCI. Groep A-streptococcen infectie (RIVM) 2023.

Lepri G, Rigante D, Bellando Randone S et al. Clinical-Serological Characterization and Treatment Outcome of a Large Cohort of Italian Children with Pediatric Autoimmune Neuropsychiatric Disorder Associated with Streptococcal Infection and Pediatric Acute Neuropsychiatric Syndrome J Child Adolesc Psychopharmacol 2019 Oct;29(8):608-614. doi: 10.1089/cap.2018.0151.

Luo R, Sickler J, Vahidnia F. Diagnosis and Management of Group a Streptococcal Pharyngitis in the United States, 2011-2015. BMC Infect Dis 2019 Feb 26;19(1):193. doi: 10.1186/s12879-019-3835-4.

Oliver J, Bennett J, Thomas S et al Preceding group A streptococcus skin and throat infections are individually associated with acute rheumatic fever: evidence from New Zealand. BMJ Global Health 2021;6:e007038

Shet A, Kaplan EL. Clinical use and interpretation of group A streptococcal antibody tests: a practical approach for the pediatrician or primary care physician. Pediatr Infect Dis J. 2002 May;21(5):420-6; quiz 427-30. doi: 10.1097/00006454-200205000-00014.

Sierig G, Cywes C, Wessels MR et al. Cytotoxic effects of streptolysine o and streptolysine s enhance the virulence of poorly encapsulated group a streptococci. Infection and Immunity 2003;71(1):446-55.

Steer AC, Vidmar S, Ritika R et al.Normal ranges of streptococcal antibody titers are similar wether stereptococci are endemic to the setting or not. Clin and Vacc Immunol 2009,172-175.

Steer AC, Smeesters PR, Curtis N. Streptococcus serology, secrets for the specialist. The ped.inf dis journal 2015;34(11):1250-1252.

Stevens DL, Kaplan EL Streptococcal infectionsO.U.Press 2000. Streptococcal pharyngitis, Rheumatic fever, Streptococcal pyoderma, acute post-streptococcal glomerulonephritis, Life-threatening streptococcal infections: scarlet fever, necrotizing fasciitis, bacteremia and streptococcal toxic shock syndrome (Stevens)

Tanz RR, Ranniger EJ, Rippe JL et al. Highly Sensitive Molecular Assay for Group A Streptococci Over-identifies Carriers and May Impact Outpatient Antimicrobial Stewardship. Pediatr Infect Dis J. 2019 Aug;38(8):769-774. doi: 10.1097/INF.0000000000002293.

Thomas S, Bennett J, Jack S et al. Descriptive analysis of group A Streptococcus in skin swabs and acute rheumatic fever, Auckland, New Zealand, 2010-2016. The Lancet Regional Health-Western Pacific 2021;8:100101.

Watanabe N Antihyaluronidase level in children with rheumatic fever and other streptococcal infections. Japanese Heart J 1976;17(5):580-91.

Zhi X, Li HK, Li H et al. Emerging Invasive Group A Streptococcus M1UK Lineage Detected by Allele-Specific PCR, England, 20201. Emerg Infect Dis. 2023 May;29(5):1007-1010. doi: 10.3201/eid2905.221887.

Zwart S. Sore throat, streptococci and penicillin. Thesis 1999.Chapter 1: general overview and research questions.

KEYWORDS

Streptococcal infection , Strep throat, GAS infection.