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Comparative Evaluation of Mispa i2 and Ichroma Systems with Manual Latex Agglutination for Anti-Streptolysin O Measurement in Clinical Laboratories

Haniyeh Ahmed Mohamed Argiah *
Laboratory Department, College of Medical Technology -Ajdabiya, Libya

التقييم المقارن لنظامي *Mispa i2* و*Ichroma* مع اختبار تراص اللاتكس اليدوي لقياس مضاد الستربتوليزين *O* في المختبرات السريرية

هنية أحمد محمد أرجيعه *
قسم المختبرات، كلية التقنية الطبية - أجدابيا، ليبيا

*Corresponding author: hanaahmad436@gmail.com

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Abstract:

Streptococcus pyogenes (Group A Streptococcus) is a common bacterium responsible for pharyngitis, with the potential for serious post-infectious complications such as rheumatic fever and glomerulonephritis. Accurate laboratory detection of anti-streptolysin O (ASO) antibodies is critical for clinical diagnosis and management. This study aimed to evaluate the performance of the Mispa i2 and Ichroma automated analyzers against the conventional manual latex agglutination method in measuring ASO titers. A cross-sectional study was conducted on 20 serum samples from patients presenting with symptoms suggestive of streptococcal infection. Results indicated that the Mispa i2 analyzer showed the highest diagnostic yield, identifying 16 positive cases (80%), followed by the Ichroma system with 13 cases (65%), while the rapid latex test identified 12 cases (60%). The findings suggest that the Mispa i2 analyzer provides superior diagnostic accuracy, simplicity, and reproducibility, making it a suitable choice for routine ASO titration in clinical microbiology laboratories.

Keywords: *Streptococcus pyogenes*, Anti-Streptolysin O (ASO), Mispa i2, Ichroma, Latex Agglutination, Clinical Diagnosis.

الملخص

تعد بكتيريا العقدية المقيحة (*Streptococcus pyogenes*) المسببة للمجموعة (A) من المكورات العقدية عاملاً شائعاً لالتهاب الحلق، مع احتمالية حدوث مضاعفات خطيرة بعد العدوى مثل الحمى الروماتيزمية والتهاب كبيبات الكلى. يُعد الكشف المخبري الدقيق عن أضداد الستربتوليزين O (ASO) أمراً حاسماً للتشخيص والإدارة السريرية. هدفت هذه الدراسة إلى تقييم أداء جهازي التحليل الآلي Mispa i2 وIchroma مقارنة بطريقة تراص اللاتكس اليدوية التقليدية في قياس عيارات ASO. أجريت دراسة مقطعية على 20 عينة مصل من مرضى يعانون من أعراض تشير إلى عدوى عقدية. أشارت النتائج إلى

أن جهاز Mispa i2 أظهر أعلى كفاءة تشخيصية، حيث حدد 16 حالة إيجابية (80%)، يليه نظام Ichroma بـ 13 حالة (65%)، بينما حدد اختبار اللاتكس السريع 12 حالة (60%). تشير النتائج إلى أن جهاز Mispa i2 يوفر دقة تشخيصية وبساطة وقابلية تكرار فائقة، مما يجعله خياراً مناسباً لتقدير عيارات ASO بشكل روتيني في مختبرات علم الأحياء الدقيقة السريرية.

الكلمات المفتاحية: العقدية المقيحة، مضاد الستربتوليزين O (ASO)، جهاز Mispa i2، جهاز Ichroma، تراص اللاتكس، التشخيص السريري.

Introduction

The ASO test, an acronym for anti-streptolysin O, detects antibodies formed in the body when it is infected by group A streptococci (Ahmed, 2022). It detects whether a person is infected with these bacteria. This test is performed by taking and examining a blood sample. A normal ASO level in the blood is less than 200 IU/mL. Infection with Group A streptococci increases the ASO titer level. Diseases that cause an increase in the level of ASO, including rheumatic fever, include levels that may reach approximately 800 IU/mL, where the analysis of ASO is one of the most common tests for detecting rheumatic fever (Ahmed, 2022). Scarlet fever is another disease that causes an increase in the level of ASO. In addition to cases of bacterial endocarditis caused by Staphylococcus aureus (Nelson et al., 2016). These bacteria are responsible for many throat infections and usually respond well to antibiotic treatment. However, if a Strep A infection is not diagnosed or poorly treated, the toxin produced by the bacteria (streptolysin O) can lead to complications such as severe rheumatism or glomerulonephritis (inflammation of the kidneys). Anti-ASO antibodies develop 1 week to 1 month after infection with Streptococcus A, peak around 4 to 6 weeks after disease onset and then decline, but may remain detectable for several months after recovery (Ahmed, 2022). A negative or very low result, especially if it recurs after 10 to 14 days, indicates that the person may not have had a recent bacterial infection. However, there are rare exceptions. If the concentration is high or increasing, a recent bacterial infection is likely. The ASO blood test cannot be used to predict whether complications will occur or to predict the type and severity of disease. If symptoms of rheumatic fever or glomerulonephritis are present, a higher ASO titer can help confirm the diagnosis. The ASO titer (Anti-Streptolysin O) test is a blood test that checks for bacterial infections. When you come into contact with harmful bacteria, your body produces antibodies to defend itself against these; these antibodies are specific to the bacteria it fights off (Nelson et al., 2016). Usually, when you have a bacterial infection such as strep throat, you receive antibiotics that kill Streptococcus bacteria. However, some people don't develop any symptoms during a streptococcal infection and may not know they need treatment. When this happens, an untreated infection can lead to complications in the future. These sequelae are known as poststreptococcal complications. The letter "O" indicates that this is an alpha oxygen. SLO has direct toxic effects on the heart tissue. During streptococcal infection, SLO stimulates the production of specific anti-streptolysin (ASO) antibodies, which neutralize the hemolytic properties of the antigen (Ahmed, 2022; Nelson et al., 2016). Anti-Streptolysin O (ASO or ASLO) is the antibody that develops in response to streptolysin O, which is an immunogenic and oxygen-sensitive hemolytic toxin produced by most group A streptococci, as well as certain strains of groups C and G. The ASO titer test is a blood assay designed to detect a streptococcal infection. Streptococcus pyogenes is a species of Gram-positive, aerotolerant bacteria in the genus Streptococcus. These bacteria are extracellular and are made up of non-motile, non-sporing cocci (round cells) that tend to link in chains. They are clinically important for humans, as they are an infrequent but usually pathogenic part of the skin microbiota that can cause Group A streptococcal infections. S. pyogenes is the predominant species harboring the Lancefield group A antigen and is often called group A

Streptococcus (GAS). However, both Streptococcus dysgalactiae and the Streptococcus anginosus group can also possess the group A antigen. When grown on blood agar, group A streptococci typically produce small (2–3 mm) zones of beta-hemolysis, indicating complete destruction of red blood cells. The name "group A (beta-hemolytic) Streptococcus" (GABHS) is also used (Government of Canada, 2001).

Aim of the Study

The aim of this study is to evaluate the ability of the tests (Mispa i2 and Ichroma) to correctly classify patients as positive or negative for Strep A infection and to compare the results with the traditional manual latex detection method. This is intended to help prevent the occurrence of pathogens and serious health complications from the infection and to enable doctors to provide appropriate treatment based on the test results.

Review of Literature

Classification of *Streptococcus pyogenes*

Taxonomic Rank	Taxon
Domain	Bacteria
Phylum	Bacillota
Class	Bacilli
Order	Lactobacillales
Family	Streptococcaceae
Genus	*Streptococcus*
Species	*S. pyogenes*

Streptococci are Gram-positive, non-motile, non-spore-forming, catalase-negative cocci that occur in pairs or chains. Older cultures may lose their Gram-positive character. Most streptococci are facultative anaerobes, whereas others are obligate (strict) anaerobes, and the majority require enriched media such as blood agar. Group A streptococci frequently colonize the throats of asymptomatic individuals and may also colonize the skin, rectum, and vagina (Public Health Agency of Canada, 2001; World Health Organization [WHO], 2005).

Streptococcal infections are ordinarily spread through direct person-to-person contact. In cases of pharyngitis and respiratory infections, droplet nuclei from saliva or nasal secretions serve as the primary mode of transmission. Crowding—such as that occurring in schools or military barracks—favors the interpersonal spread of the organism during community outbreaks, and fomites can also act as sources of streptococcal transmission (WHO, 2005). A variety of clinical presentations may occur, including pharyngitis, otitis media, peritonsillar abscess (quinsy), skin and soft tissue infections (pyoderma, impetigo, erysipelas, and scarlet fever), pneumonia, and puerperal fever (WHO, 2005; Centers for Disease Control and Prevention [CDC], 2021).

Post-infectious complications of group A Streptococcus (GAS) infections include acute rheumatic fever—which can result in secondary aortic and mitral valve injury—and glomerulonephritis. Pharyngeal strains of GAS can lead to either syndrome, whereas skin infections are exclusively associated with acute glomerulonephritis. Outbreaks of pharyngitis

and impetigo among school-age children in group settings are particularly common (CDC, 2021).

Globally, severe *S. pyogenes* infections account for at least 517,000 deaths each year, with rheumatic heart disease alone responsible for 233,000 of these fatalities. In the United States, approximately 1,800 invasive *S. pyogenes* disease-related deaths are reported annually; necrotizing fasciitis carries a mortality rate of about 30%, and streptococcal toxic shock syndrome presents a mortality rate ranging from 30% to 70% (Carapetis et al., 2005). Different clinical manifestations of this bacterium vary in frequency across different regions of the world. *Streptococcus* species commonly colonize the throat, genital mucous membranes, rectum, and skin. Among healthy individuals, 1% to 5% experience colonization in the throat, vagina, or rectum, whereas colonization rates among healthy children range from 2% to 17% (Carapetis et al., 2005).

Transmission of the bacterium occurs via four primary routes: inhalation of respiratory droplets, direct skin contact, contact with contaminated objects, surfaces, or dust, and less frequently through food. These bacteria are responsible for 15% to 30% of all pharyngitis cases in children, whereas 5% to 20% of pharyngitis cases in adults are streptococcal in origin (Carapetis et al., 2005).

Diagnosis

The anti-streptolysin O latex agglutination test is utilized for both qualitative and quantitative determination of anti-streptolysin O (ASO or ASLO) antibodies in human serum. *Streptococcus pyogenes*, or group A beta-hemolytic streptococci (GAS), produces various toxins that function as antigens. Streptolysin O, a notable exotoxin, was first discovered by Todd in 1932 (Todd, 1932).

Individuals infected with group A hemolytic streptococci produce specific antibodies against these exotoxins, including anti-streptolysin O. The concentration of this antibody in a patient's serum reflects the degree of the immune response associated with hemolytic streptococcal infection. The conventional procedure for determining the anti-streptolysin titer relies on the inhibitory effect of the patient's serum on the hemolytic capacity of pre-prepared, reduced streptolysin O. Because the antibody-antigen neutralization occurs independently of the direct hemolytic activity of streptolysin O, this property enables the development of reliable qualitative and quantitative assays to measure anti-streptolysin O levels via latex particle agglutination on a slide (Haber & Feingold, 1998).

Principle of the Test

The ASO latex reagent is a stabilized, buffered suspension of polystyrene latex particles coated with streptolysin-O. When the latex reagent is mixed with serum containing antibodies to streptolysin-O, agglutination occurs. The latex reagent has been adjusted so that agglutination occurs only when the level of antibodies to streptolysin-O is greater than 200 IU/mL, a threshold determined by epidemiological and clinical studies as indicative of disease (Haber & Feingold, 1998).

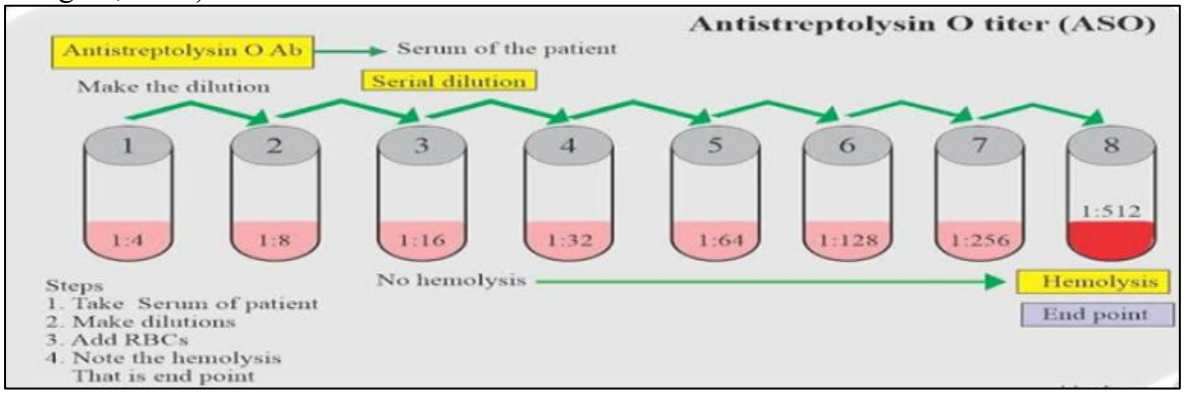

Figure 1: ASO latex test

Clinical Significance of the ASO Test

An anti-streptolysin titer greater than 200 IU/mL is considered a positive test in adults, whereas a titer less than 200 IU/mL indicates a negative test. Measurement of the ASO antibody titer is important in investigating post-streptococcal diseases, particularly acute post-streptococcal glomerulonephritis and rheumatic fever. Over 80% of patients with acute rheumatic fever and 95% of patients with acute glomerulonephritis exhibit elevated ASO titers. The antibody level starts to rise one to three weeks after a streptococcal infection, peaks in three to five weeks, and then returns to an insignificant level over six to twelve months. A positive test can indicate a current or recent group A, C, or G streptococcal infection (Haber & Feingold, 1998).

Test Components

Reagents

1. **ASO Latex Reagent:** A suspension of polystyrene particles coated with streptococcal exoenzymes. Mix well before use.
2. **ASO Positive Control:** A stabilized human serum containing at least 200 IU/mL of ASO, which is reactive with the test reagent.
3. **ASO Negative Control:** A stabilized human serum containing less than 200 IU/mL of ASO, which is non-reactive with the test reagent.

Additional Materials

- Agglutination slide
- Disposable stirring sticks

Figure 2: ASO test kit

Materials Required

1. Timer
2. Test tubes and rack
3. Serological pipettes

Qualitative Evaluation

1. Allow all reagents and samples to reach room temperature before testing. Shake all reagents well.
2. Place one drop (approximately 40 µL) of the positive control (P) in field 1 of the agglutination slide.
3. Place one drop (approximately 40 µL) of the negative control (N) in field 2 of the agglutination slide.
4. Place 40 µL of each undiluted patient sample on the remaining fields of the agglutination slide, using a new serological pipette for each sample.
5. Gently resuspend the latex reagent (A) and add one drop (40 µL) to each test field.
6. Mix well using separate stirring sticks.
7. Gently rock the slide for 3 minutes by hand or use a rocking shaker at 80–100 rpm.

8. Read immediately under direct light.

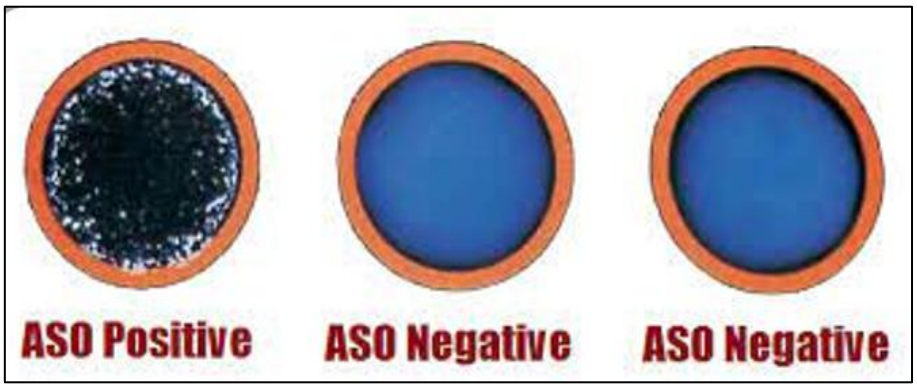

Figure 3: ASO latex test

Semi-Quantitative Evaluation

1. Allow all reagents and samples to reach room temperature prior to testing. Shake all reagents well before use.
2. Prepare at least five serial dilutions per patient sample (for example, 1:2, 1:4, 1:8, 1:16, 1:32) using buffered saline.
3. Place one drop (approximately 40 µL) of the positive control (P) on field no. 1 of the agglutination slide.
4. Place one drop (approximately 40 µL) of the negative control (N) on field no. 2 of the agglutination slide.
5. Place 40 µL of each sample dilution onto the remaining fields of the agglutination slide, using a different serological pipette for each dilution.
6. Gently resuspend the latex reagent (A) and add one drop (40 µL) to each test field.
7. Mix well using separate stirring sticks for each field.
8. Gently rock the slide for 3 minutes by hand or use a rocking shaker set at 80–100 rpm.
9. Read the results immediately under direct light.

Semi-Quantitative Test Evaluation

A positive reaction is indicated by any observable agglutination in the reaction mixture. Record the last dilution that shows a positive reaction. The concentration of ASO can be determined by multiplying the reciprocal of the last positive dilution factor of the sample by the concentration of the positive control (200 IU/mL).

The titer of the serum is the reciprocal of the highest dilution that exhibits a positive reaction.

$$\text{IU/mL of sample} = \text{concentration of positive control (200 IU/mL)} \times \text{titer}$$

Example:

Table 1: Latex Test Sample Dilution

Dilution	ASO IU/mL
1:2	200
1:4	400
1:8	800

Dilution	ASO IU/mL
1:16	1600
1:32	6400

Expected Values

Although normal values can vary with age, season, and geographical area, the upper limit of normal anti-streptolysin O titers for preschool children is less than 100 IU/mL, and for school-age children or young adults, it is usually between 166 and 250 IU/mL (Todd, 1932). In general, the average can be established at less than 200 IU/mL. Because of this variation, titers above the upper limits may indicate a streptococcal infection, but only a two-dilution rise in titer between acute- and convalescent-stage specimens should be considered clinically significant (Haber & Feingold, 1998). Following an acute streptococcal infection, the anti-streptolysin O titer usually rises after one week, increases to a maximum level within 3 to 5 weeks, and typically returns to pre-infection levels in approximately 6 to 12 months (Todd, 1932).

Interpretation of Results – Qualitative

Agglutination indicates an ASO concentration greater than or equal to 200 IU/mL in the serum sample. Sera that yield a positive result should be retested and titrated using the semi-quantitative assay protocol.

Interpretation of Results – Semi-Quantitative

The highest dilution at which visible agglutination occurs is considered the endpoint titer. The corresponding ASO concentration (in IU/mL) is calculated as the product of the endpoint dilution factor and the assay cut-off value, as shown in the following table. For example, if the endpoint dilution is 1:8, the corresponding ASO serum concentration would be 8 × 200, or 1600 IU/mL.

Treatment

Pharyngitis caused by group A beta-hemolytic streptococci (GABHS) is one of the most common infections diagnosed in children. Orally administered penicillin is the standard treatment recommended for GABHS pharyngitis, but its use is limited by the need for multiple daily doses and a relatively long (10-day) treatment regimen. Erythromycin, clindamycin, and many cephalosporins are known to be safe and effective alternatives to penicillin for treatment (Bisno et al., 2002). There is a multivalent inactivated vaccine against several types of streptococci, including *Streptococcus pyogenes*, called "Vacuna antipiogena polivalente BIOL," and it is recommended that it be administered in a series of five doses over five weeks (Rubinstein et al., 1988).

Previous Studies

Group A *Streptococcus* (GAS) is the cause of a wide range of acute suppurative diseases and, after a latent period, non-pyogenic diseases such as rheumatic fever and post-streptococcal glomerulonephritis. Diagnosis of the latter group requires evidence of prior GAS infection. Bacteria produce a range of extracellular antigens, including streptolysin O, which stimulate an antibody response in the host. An elevation in anti-streptolysin O titer (ASOT) indicates prior invasive infection. In clinical practice, often only one ASOT measurement is available, and its timing in relation to a possible GAS injury is unknown (Shulman et al., 2004).

A cross-sectional study was conducted in Behera Governorate in 2020 (El-Gazzar et al., 2020). The aim of the study was to determine the upper limit of the normal range for anti-streptolysin O in healthy school children aged between 6 and 15 years in Behera Governorate. Samples were collected and tested in the Clinical Pathology Laboratory of Al-Azhar University Hospital, Damietta. A total of 3,000 serum samples (1,609 males and 1,391 females) were collected from children aged 6–15 years and tested for anti-streptolysin O (ASO) titer by

measuring turbidity. The results showed that the normal value of the ASO standard increased significantly during early childhood and then gradually decreased with age. The estimated 80th percentile titer of the upper normal value at age 10 years was 287 IU/mL for ASO. This study provides the upper limit of the normal value of the ASO titer for schoolchildren, noting values of more than 400 IU/mL in specific groups (El-Gazzar et al., 2020).

In another study conducted in Ethiopia in 2019 (Biresaw et al., 2019), the normal and reference values of anti-streptolysin O titer were compared in rheumatic heart disease (RHD) patients and healthy children. Cross-sectional study methods were used to collect blood samples from RHD patients undergoing secondary prophylaxis and apparently healthy children at the Debre Berhan Children's Clinic. Blood samples were collected to determine the ASO titer. Data were analyzed using SPSS version 21, and a p-value less than or equal to 0.05 was considered statistically significant. A total of 123 children receiving secondary prophylaxis for rheumatic heart disease in the age range of 5 to 15 years were included. Of these, 65 (52.8%) were male and 58 (47.2%) were female. The ASO upper limit of normal (ULN) for all RHD subjects was 800 IU/mL. The ASO ULN for both male and female children in all age groups with RHD was also 800 IU/mL. Additionally, 127 healthy children aged 5–15 years were selected and screened for ASO ULN. The ASO ULN for all healthy subjects was 360 IU/mL. The ASO ULN for both male and female healthy children was 320 IU/mL, while the highest ASO ULN level observed in the 9–12-year age group was 400 IU/mL. This study revealed that most children with RHD had a recent streptococcal infection, as indicated by an elevated ASO titer, and showed that the ASO ULN titer of healthy children is approximately similar to those reported in different countries (Biresaw et al., 2019).

In another similar study in Britain in 1990 (Gerber et al., 1990), serum samples were collected in the acute and convalescent phases from 50 patients with group A streptococcal pharyngitis. Anti-streptolysin O (ASO) titers were determined for each serum sample using both a standard neutralization test and a latex agglutination test (Rheumagen ASO; Biokit Inc., New Britain, Conn.). When the ASO titers derived by the two methods were compared, the correlation coefficient was 0.93. When the ability of the latex agglutination (LA) test to show a significant elevation in ASO titer (greater than or equal to a two-dilution rise) was compared with the standard neutralization test, the LA test demonstrated a sensitivity of 91%, a specificity of 86%, a positive predictive value of 83%, and a negative predictive value of 92%. Triplicate LA test determinations were performed on a subset of 31 serum samples; for 29 of them (94%), repeated ASO titers were all within 1 dilution of each other. The width of the 95% confidence interval for the triplicate measurements for each serum sample was ± 32.8 IU. The study found that Rheumagen ASO is a simple and rapid procedure for the measurement of ASO titers that produces results that are highly reproducible, show minimal variance, and are comparable to ASO titers obtained using the standard neutralization test (Gerber et al., 1990).

In a study by Qotbi et al. (2012) conducted in Egypt regarding the over-diagnosis of acute rheumatic fever based on anti-streptolysin O titer (ASOT) in endemic areas, 660 children (9.2 ± 1.7 years of age) were consecutively recruited and categorized as follows: G1 (control group, $n = 200$ healthy children), G2 ($n = 20$ with a first acute rheumatic fever [ARF] attack), G3 ($n = 40$ with recurring ARF), G4 ($n = 100$ with rheumatic heart disease [RHD] on long-acting penicillin), G5 ($n = 100$ with acute follicular tonsillitis), and G6 ($n = 200$ healthy children with a history of recurrent follicular tonsillitis three times a year). Serum ASOT was measured by latex agglutination. The upper limit of normal ASOT (80th percentile) was 400 IU in G1, 200 IU in G4, and 1600 IU in G6. Significantly high levels were seen in the first ARF attack when compared to groups 1 and 5 ($p < 0.001$ and $p < 0.05$, respectively). ASOT was significantly elevated in children over the age of 10, during the winter season, and in those with acute rheumatic carditis. ASOT showed a significant direct correlation with the number of tonsillitis attacks ($p < 0.05$). Egyptian children exhibit an upper limit of normal ASOT as

high as 400 IU, which must be taken into account when interpreting values in suspected ARF. Elevation of ASOT is less pronounced in recurrent ARF than in a first attack, acute tonsillitis, and recurrent tonsillitis. Basal levels of ASOT increase with age, but the pattern of increase during infection incidence is not age-dependent (Qotbi et al., 2012).

A community-based cross-sectional study in Ujjain in 2017 (Mahore et al., 2017) was conducted on normal healthy children aged 5–15 years, divided into Group 1 (5–10 years) and Group 2 (11–15 years), after informed consent was obtained from their parents. Blood samples were collected after thorough sterilization of the area. A serum sample ASO assay was performed using TURBILYTE Anti-streptolysin 'O' Diagnostic Reagent for the in vitro quantitative determination of serum ASO on photometric systems. Of the total 200 children included in the study, 100 were in Group 1 and 100 in Group 2; 118 (59%) were male and 82 (41%) were female. The mean ASO titer for Group 1 was 105.69 IU, and for Group 2, it was 144.73 IU, with standard deviations of 3.675 and 5.823, respectively ($p < 0.05$). Out of the total 200 students, 160 were living in crowded conditions. The mean ASO for children with crowded living conditions was 131.41 IU (standard deviation = 53.472), and the mean ASO for children with uncrowded living conditions was 100.42 IU (standard deviation = 39.30) ($p < 0.05$). The study concluded that the upper limit of normal was greater in Group 1 children ($p < 0.05$, 200 vs 135). No statistically significant difference in ASO titer according to sex was found. The mean ASO titer was statistically higher (131.41 IU) in children living in crowded conditions compared to those living in non-crowded conditions (100.41 IU) (Mahore et al., 2017).

The past two decades have brought alarming increases in severe streptococcal diseases globally (Lamagni et al., 2008). To investigate and compare the epidemiological patterns of these diseases within Europe, data were collected through the European Union-funded program FP-5 (Strep-EURO). Potential population surveillance for invasive *Streptococcus pyogenes* infection was performed using a standardized case definition. A total of 5,522 cases were identified in 11 countries during this period. Reported infection rates varied, reaching 3 per 100,000 population in northern European countries. The seasonal patterns of infection showed remarkable congruence between countries. The risk of infection was higher among the elderly, and rates were higher in males than in females in most countries. Skin lesions and wounds were the most common predisposing factors, recorded in 25% of cases, while 21% had no predisposing factors reported. The skin and soft tissues were the most common foci of infection, with 32% of patients developing cellulitis and 8% developing necrotizing fasciitis. The overall 7-day case fatality rate was 19%, and among patients who developed streptococcal toxic shock syndrome, the rate was 44%. The findings from Strep-EURO confirm the high incidence of severe *S. pyogenes* disease in Europe and establish targets for public health intervention while raising awareness across the region (Lamagni et al., 2008).

A national survey was initiated in France to assess the burden of GAS infections, describe their clinical characteristics, and evaluate the molecular characteristics of the GAS strains responsible for these infections (Plainvert et al., 2012). The survey was carried out across 194 hospitals, accounting for 51% of hospital admissions in France. Group A invasive streptococcal infection causes significant morbidity and mortality. Clinical, predisposing, and demographic data were obtained, and all GAS isolates were typed by *emm* sequence. A total of 664 cases of invasive GAS infection were identified, with an annual incidence of 3.1 per 100,000 population. Overall mortality was 14%, increasing to 43% in cases involving streptococcal toxic shock syndrome. Bacteremia without a specific focus (22%) and skin/soft tissue infections (30%) were the most common clinical presentations. Necrotizing fasciitis was common in adults (18%) and uncommon in children (3%). The three dominant *emm* types—*emm1*, *emm89*, and *emm28*—accounted for 33%, 16%, and 10% of GAS isolates, respectively. The *emm1* subtype was associated with fatal outcomes and was more common in children than

in adults. Invasive GAS infection represents one of the most severe bacterial diseases in France, especially in individuals under 50 years of age or when associated with toxic shock syndrome (Plainvert et al., 2012).

Materials and Methods

The cross-sectional study was conducted at the Al-Madinah Medical Laboratory in Ajdabiya from October 10 to October 12, 2022. The participants included 20 individuals aged between 5 and 55 years who exhibited symptoms of a Strep A infection. These symptoms included sore throat, difficulty swallowing, redness and swelling of the tonsils, swelling of the lymph nodes in the neck, fever, headache, and fatigue.

The anti-streptolysin O (ASO) titer is a blood test that measures antibodies against streptolysin O, a substance produced by group A *Streptococcus* bacteria. Antibodies are proteins produced by our bodies when they detect harmful substances, such as bacteria. Alternative names for the test include ASO titer and ASLO.

Test Performed

Patients may require this test if they exhibit symptoms of a previous infection caused by group A *Streptococcus*. Some illnesses caused by this bacterium include bacterial endocarditis (an infection of the inner lining of the heart), a kidney problem called glomerulonephritis, rheumatic fever (which can affect the heart, joints, or bones), scarlet fever, and strep throat. The ASO antibody may be found in the blood weeks or months after the strep infection has resolved.

Normal Results

A negative test result indicates the absence of a bacterial infection. The healthcare provider may perform the test again in 2 to 4 weeks, as a previously negative test may turn positive upon repetition (meaning it detects ASO antibodies). An abnormal or positive test result indicates a recent bacterial infection, even in the absence of symptoms.

- **Sample required:** A blood sample drawn from a vein.
- **Test preparation required:** Patients may be asked to fast (refrain from eating) for 6 hours before the test.

This test measures the amount of ASO in the blood.

Ichroma ASO Test

Figure 4: Ichroma ASO test

The Ichroma ASO test is a fluorescence immunoassay (FIA) for the quantitative determination of anti-streptolysin O (ASO) in humans (Boditech Med, 2020). The sample type for the

Ichroma ASO test is human serum or plasma. It is recommended to analyze the sample within 24 hours of collection. Serum or plasma must be separated from the clot by centrifugation within 3 hours of blood collection. Samples stored frozen at −20°C for up to 3 months show no performance differences. Once frozen, the sample should be used only once for testing, as repeated freezing and thawing can alter test values.

Principle

The test uses a sandwich immunodetection method. Detector antibodies in the buffer bind to antigens in the sample, forming antigen-antibody complexes that migrate onto a nitrocellulose matrix to be captured by immobilized antibodies on the test strip. A higher concentration of antigens in the sample forms more antigen-antibody complexes, leading to a stronger fluorescence signal from the detector antibodies. This signal is processed by the Ichroma instrument to determine the ASO concentration in the sample.

Components

- **Cartridges:** The cartridge contains the membrane (test strip), which features SLO protein at the test line and chicken IgY at the control line. All cartridges are individually sealed in an aluminum foil pouch containing a desiccant inside a box.
- **Detection Buffer:** Contains SLO protein-fluorescence conjugate, anti-chicken IgY-fluorescence conjugate, mouse IgG as a blocker, sucrose, bovine serum albumin (BSA) as a stabilizer, and sodium azide in phosphate-buffered saline (PBS) as a preservative. All detection tubes are packed in a pouch.
- **Diluent:** Contains detergent, bovine serum albumin (BSA) as a stabilizer, and sodium azide in phosphate-buffered saline (PBS) as a preservative. The diluent is dispensed in a vial and packed in a box.

Test Procedure

Multi Mode

1. Open the diluent container and transfer 500 µL of diluent to the detection buffer using a pipette.
2. Transfer 5 µL of the sample (human serum, plasma, or control) to the detection buffer using a pipette.
3. Close the lid of the detection buffer and mix the sample thoroughly by shaking 10 to 15 times. Ensure the sample mixture is used immediately.
4. Pipette 75 µL of the sample mixture and load it into the sample well of the cartridge.
5. Leave the sample-loaded cartridge at room temperature for 12 minutes. Scan the cartridge immediately after the incubation time elapses to avoid inaccurate results.
6. Insert the cartridge into the cartridge holder of the Ichroma instrument, ensuring proper orientation as indicated by the marked arrow before pushing it fully in.
7. Press "Select" or tap the "START" button on the instrument to initiate the scanning process.
8. The instrument will immediately scan the cartridge.
9. Read the test result on the instrument's display screen.

Single Mode

1. Follow the test procedure steps 1 through 4 of the multi mode.
2. Insert the cartridge into the holder of the ichroma™ test instrument, ensuring proper orientation using the marked arrow before pushing it fully in.
3. Tap the "START" button on the ichroma instrument.
4. The cartridge enters the instrument, which automatically starts scanning after 12 minutes.
5. Read the test result on the instrument's display screen.

Interpretation of Test Results

The ichroma instrument automatically calculates and displays the ASO concentration of the test sample in IU/mL. The cut-off reference range is 160 IU/mL.

Table 2: ichroma™ ASO Test Limit

Age	Concentration of ASO
Adult	< 166 IU/mL
Preschool age	< 100 IU/mL
School age	< 250 IU/mL

Limitations of the Test System

1. The test may yield false-positive results due to cross-reactivity and/or non-specific binding to sample capture antibodies or reagent components.
2. The test may yield false-negative results if antigens fail to bind to antibodies, most commonly because antigens are masked by unknown components, preventing detection or capture.
3. Instability or degradation of the antigen over time and/or temperature may also cause false-negative results by rendering the antigen unrecognizable to the antibody.
4. Technical or procedural errors can interfere with the test and cause inaccurate results.
5. Deterioration of test components/reagents or the presence of interfering substances in test samples can cause erroneous results. Any clinical diagnosis based on test results must be carefully considered and supported by a comprehensive evaluation by a specialist, including clinical symptoms and other relevant diagnostic findings.

Mispa i2 Test

Figure 5: Mispa i2 test device

The Mispa-i2 is a semi-automated specific protein analyzer that offers high accuracy and supports the clinical management of various protein assays and disease states. It utilizes unique Unique Dual Scale (UCS) technology, combining both nephelometry and turbidimetry, allowing the instrument to automatically switch between modes to provide optimal sensitivity and linearity.

Intended Use

This reagent is intended for the in-vitro quantitative determination of anti-streptolysin O (ASO).

Measurement Methodology

- Ready-to-use prefilled cartridges.
- Linear up to 1000 IU/mL.
- No calibration required.
- Detection limit is less than 50 IU/mL.
- **Sample:** Fresh serum (do not use hemolyzed or lipemic samples).

Principle

When an antigen-antibody reaction occurs between ASO in the sample and streptolysin-O sensitized latex particles, lattice formation (stacking) occurs, which is proportional to the ASO concentration in the sample. The actual concentration is determined by interpolation from a prepared calibration curve using titrators of known concentration.

Reagent Composition: ASO Cartridge

Table 3: Mispa i2 Test Reagent Composition

Reagent	Well	Description
ASO R1	Well No. 3	Glycine buffer solution
ASO R2	Well No. 4	ASO latex suspension particles coated with streptolysin-O

Reagent Preparation

The reagents are ready to use and supplied in pre-filled cartridges. Sampling, dispensing, and mixing are performed automatically by the machine.

Reagent Storage and Stability

Sealed cartridges are stable until the expiration date stated on the kit label. Store at 2°C to 8°C and protect from light. Do not freeze.

Detector Deterioration

Turbidity or sedimentation in any cartridge component indicates deterioration, and the cartridge must be discarded. If the values fall outside the recommended acceptable range or indicate reagent instability (such as for Agape protein controls), the associated results are not valid, and the sample should be retested with a new cartridge.

Procedure

Test details and calibration data are provided on the smart card. Insert the smart card into the card reader slot. Select the test function from the main screen and allow the holder to exit the equipment. Take a pre-filled ASO cartridge and gently press it before adding the sample to remove any air bubbles.

Add 80 µL of the sample to the designated well of the cartridge (Well No. 6). Place the cartridge in the holder and select the "OK" button on the device. The cartridge slides into the device and is detected for testing. If the cartridge is not properly detected, the equipment will make two more attempts. Upon successful detection of the cartridge, the screen displays the corresponding test name along with calibration details. Select the "Save" button to save the test calibration, and select "OK" to match the calibration data to the patient entry.

Demographic Details

Enter the patient's demographic details and select the "Start" button to run the examination. Status updates and a progress bar will be displayed during the test.

- After the sample has been analyzed, the test results will be printed and displayed on the screen.
- The machine will eject the cartridge holder to allow removal of the used cartridge.
- Remove the cartridge, press "Exit," select "OK" to proceed, or select "Home" from the main menu.

Figure 6: Steps for Mispa i2 device

Reference Group

The following values can be used as a guideline:

- **Serum (adults):** Up to 200 IU/mL.
- **Children under 5 years:** Up to 100 IU/mL.

The results obtained for patient samples should be evaluated in conjunction with clinical findings for proper interpretation and diagnosis.

Performance Characteristics

Linearity

The reagent is linear up to 1000 IU/mL. If the concentration exceeds the upper limit of linearity, dilute the sample with normal saline, repeat the assay, and multiply the final result by the dilution factor.

Comparison

A comparative study has been performed between the Mispa i2 ASO and other internationally available reagents, yielding a correlation coefficient of $r = 0.9976$ and a regression equation of $y = 1.0052x$.

Smart Card Calibration System Features

1. Innovative smart card technology.
2. Easy automated calibration.
3. Multiple calibration points.
4. Enables calibration of the specified parameter.
5. Inline calibration data.
6. No reagent waste.
7. No calibration failures.

Parameters at a Glance

- **ASO:** 50–1000 IU/mL, 60 days stability

Static Analysis of Data

Data were analyzed using SPSS version 10. Data were entered into the data display window, checked, and reviewed, and frequency tables were produced for all variables and observations. Descriptive statistics were used to analyze the data and reveal associations between relevant variables.

Results

Table 4: Age Group

Age Average	Number	Percent
5–25	12	60%
26–45	5	25%
46–55	3	15%

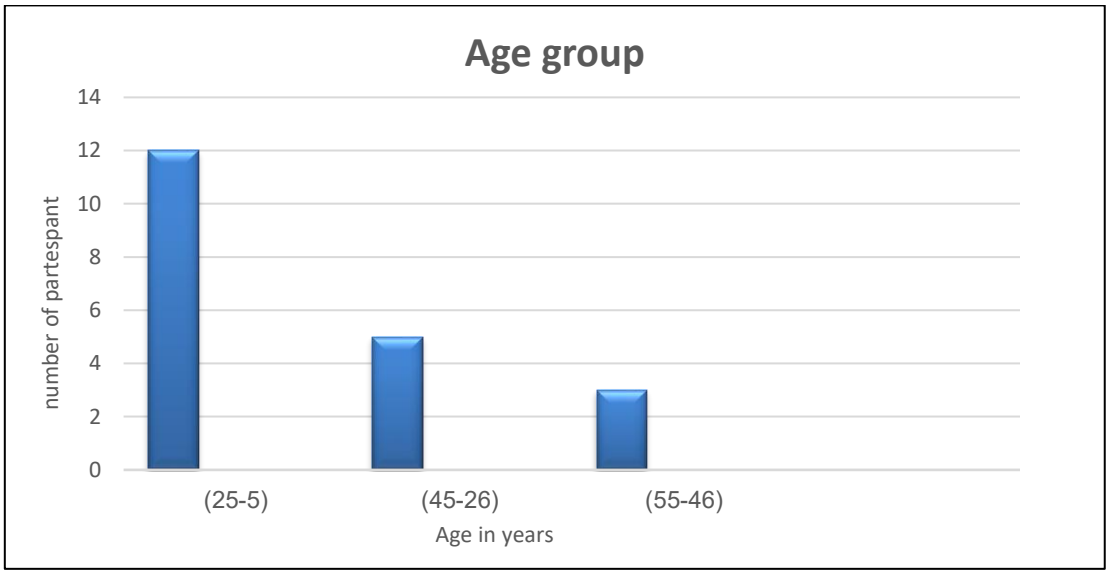

Figure 7: Age group in bar chart.

In Table 4, the age groups participating in the study were included. Most of the participants were in the age group between 5 and 25 years old, totaling 12 people and representing 60% of the participants, while the smallest number of participants was over the age of 45 years, numbering 3 people and representing 15% of the participants.

Table 5: Gender Group

Gender	Number	Percent
Male	9	45%
Female	11	55%

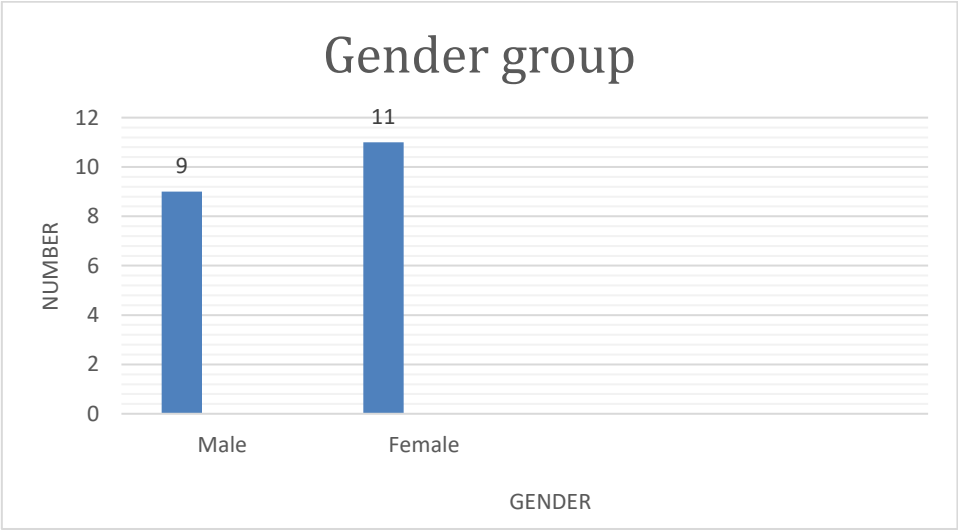

Figure 8: Gender group in bar chart.

In Table 5, the gender of the study participants was included. Most of the participants were female, numbering 11 and representing 55% of the participants, while the least number of participants were male, with 9 people, representing 45% of the participants.

Table 6: Rapid Latex Test

LT Test	Result (Positive: ≥ 200, Negative: < 200)
Number of Participants	20
Positive	12 (60%)
Negative	8 (40%)

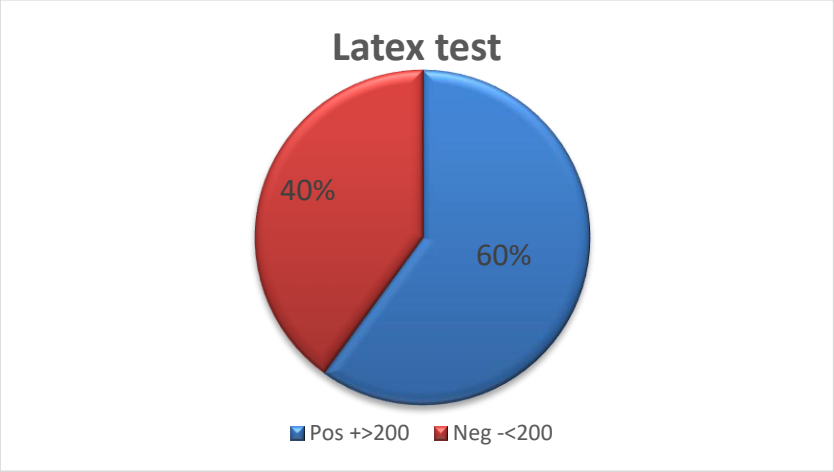

Figure 9: Rapid latex test in pie chart.

In Table 6, for the rapid latex test, the number of study participants examined with RLA was 20. Their examination results showed 12 positive samples (60%) and 8 negative samples (40%).

Table 7: Ichroma Test

Ichroma Test	Result (Positive: ≥ 166, Negative: < 166)
Number of Participants	20
Positive	13 (65%)
Negative	7 (35%)

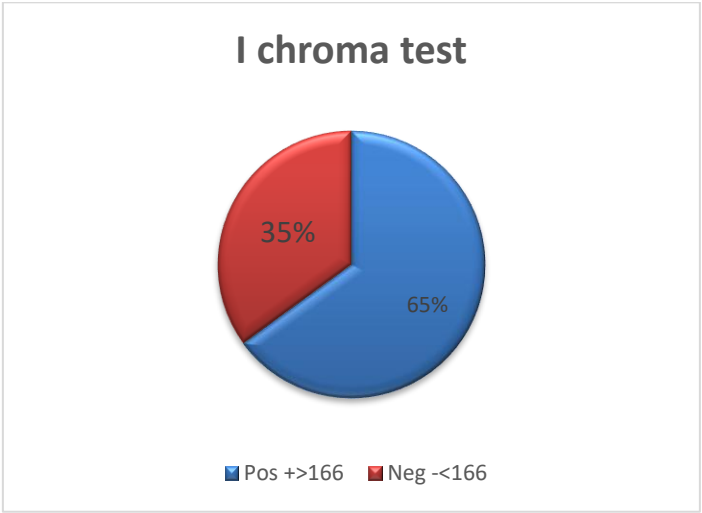

Figure 10: Ichroma test in pie chart.

In Table 7, the examination was performed with the Ichroma device for the same 20 people participating in the study. Their examination results showed 13 positive samples (65%) and 7 negative samples (35%).

Table 8: Mispa i2 Test

Mispa i2 Test	Result (Positive: ≥ 200, Negative: < 200)
Number of Participants	20
Positive	16 (80%)
Negative	4 (20%)

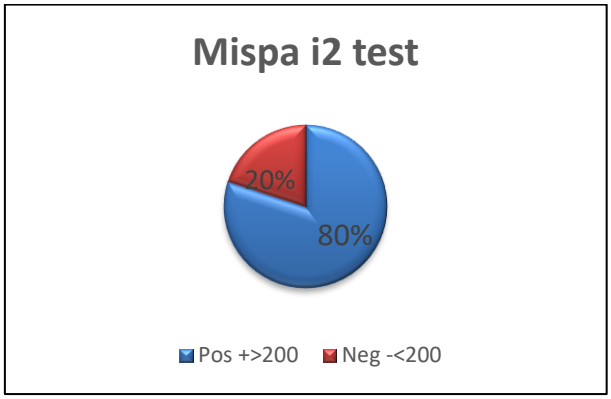

Figure 11: Mispa i2 test in pie chart.

In Table 8, the Mispa i2 device was examined on the same 20 subjects participating in the study. The examination results showed 16 positive samples, representing 80% of the total, and 4 negative samples, representing the remaining 20%.

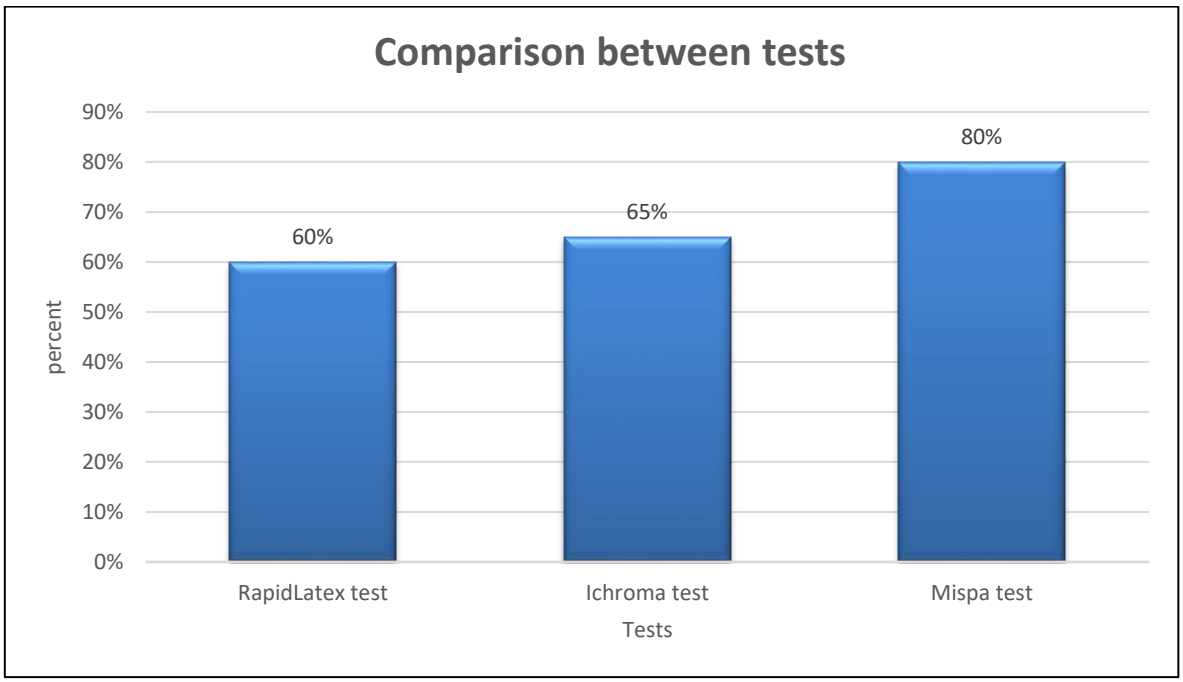

Figure 12: Comparison of the results of the three tests.

In Figure 12, the three tests used in the research were compared across the same 20 participants. The examination results showed 16 positive samples (80%) for the Mispa i2 device, 13 positive samples (65%) for the Ichroma device, and 12 positive samples (60%) for the rapid latex test.

Discussion

Invasive group A streptococcal infection is uncommon, but serious, and is associated with high mortality rates. Periodic relapses in invasive group streptococcal infection have been reported in industrialized countries from the 1980s onwards, with current estimates of the incidence in these countries being around 3–4 per 100,000 population. Infants, pregnant women, and the elderly are at increased risk of invasive group A streptococcal infection (Steer et al., 2005). Group A Streptococcus possesses an array of virulence factors that support its invasive capacity. In approximately 10% of cases, the superantigen toxins produced by the bacteria stimulate a large proportion of T cells, leading to streptococcal toxic shock syndrome (Stevens et al., 2005). Given the rapid clinical progression, effective management of invasive group A streptococcal infection hinges on early recognition of the disease and prompt initiation of supportive care, often intensive care, along with antibacterial therapy. In cases of toxic shock syndrome, distinguishing between streptococcal and staphylococcal infections is often difficult before cultures are available; thus, the choice of antibacterial should include coverage of both organisms. In addition, clindamycin is an important adjuvant antibacterial because of its antitoxin effects and excellent tissue penetration. Early initiation of treatment with intravenous immunoglobulin should be considered in cases of toxic shock syndrome and severe invasive infection, such as necrotizing fasciitis. Early surgical debridement of necrotic tissue is also an important part of the management of necrotizing fasciitis (Stevens et al., 2005).

The genus Streptococcus includes coccus-shaped, Gram-positive organisms organized into chains. They act as commensal, pathogenic, and opportunistic pathogens of humans and animals. Most medically important Streptococcus species have a specific ecological niche. Several families of antibiotics are used to treat streptococcal infections; first-line treatments include penicillin, and patterns of resistance in Streptococcus isolates have been reported worldwide (Bisno et al., 2002). A study similar to ours was conducted in January 1989 (Gerber et al., 1990) and compared the latex agglutination test with the hemolysin inhibition method for the detection of anti-streptolysin O (ASO) antibodies in 428 serum samples. After a slight modification of the latex method to produce maximum stacking, good agreement was observed between the results obtained by the two methods. The sensitivity of the latex test was 83.6%, the specificity was 93.3%, the positive predictive value was 86.5%, and the negative predictive value was 91.6%. It was convenient, less labor-intensive than the hemolysin test, and allowed for economical testing of small numbers of sera (Gerber et al., 1990).

In our study, anti-streptolysin antibodies were estimated in 20 samples from children, young adults, and the elderly by means of latex testing and examination using two devices: Ichroma and Mispa i2. The latex test and the Mispa i2 device detect a titer of 200 IU/mL and above, while the Ichroma device detects a titer of 166 IU/mL or more for diagnosis. Antibodies appear in children over 5 years old due to infection with Streptococcus pyogenes. In a similar study of a rural population in Ceylon in 1990 (Fernando et al., 1990), measurements of anti-streptolysin O were determined in the serum of children to determine the upper limit of normal ASO titer in non-rheumatic children and to compare ASO with the results of surveys conducted in other countries. Swabs were taken from the throats and sores of children specially screened and cultured for group A hemolytic streptococcus. Of 257 children, 29.5% had an ASO titer greater than 166 Todd units. The largest number of children showed ASO values between 100 and 166 units. The proportion of children who showed values over 166 units increased with age until a maximum of 54% was reached between 9 and 10 years. Group A hemolytic streptococci were isolated from five throat swabs, but no isolates were found from ulcers. The values obtained from this research were compared with those of other developing countries (Fernando et al., 1990).

Furthermore, investigating alternative antibacterial agents and phytochemical profiles against relevant pathogens provides broader insights into managing bacterial resistance (Salem &

Alhadad, 2026; Alshawish et al., 2025; Khalil et al., 2025). Our study showed that children are the most vulnerable age group, and the incidence increases in equal proportions between females and males across all countries. Multiple studies similar to ours (Carapetis et al., 2005; Ayoub & Wannamaker, 1962), conducted in different countries with varying economic situations and environments, showed that antibodies increased gradually with age, from 11.8% in the 4–8-year age group to 15.8% in the 9–12-year age group. All of these participants were from lower socioeconomic groups. ASO was positive in 16.7% of youth from a lower socioeconomic status, while it was only positive in 9.2% of those from a higher socioeconomic status. Additional evaluations involving botanical and natural extracts further emphasize the continuous global research effort into alternative antimicrobial mechanisms (Salem et al., 2025; Salem, 2024). Another study showed a total of 522 patients with rheumatic carditis: only 23.4% had no antibodies or levels less than 200 IU/mL, while 77% were positive (26.9% had levels greater than 400 IU/mL and 49.7% had levels of 200 IU/mL) (Carapetis et al., 2005). In another study, throat swabs and anti-ASO antibodies were administered simultaneously to 76 patients clinically diagnosed with acute bacterial pharyngitis. Group A hemolytic streptococcus was isolated in 64% of the patients, and anti-streptolysin antibodies were detected in 62% of the patients. School health records of more than 50,000 pupils were surveyed, and the prevalence of rheumatic heart disease was estimated to be 0.17% among higher socioeconomic groups (Carapetis et al., 2005). Related bioactive essential oils and specific pathogen isolation studies also underline the diagnostic and therapeutic relevance in local and regional laboratories (Soof et al., 2025; Ben Hsin et al., 2025; Salem & Lakwani, 2024).

Conclusion

While the incidence of many diseases has decreased in developed countries, regions of the world with low incomes and poor infrastructure still suffer a high burden of Streptococcus pyogenes (group A streptococcus) diseases, resulting in millions of deaths annually (Carapetis et al., 2005). The majority of these deaths are due to the development of rheumatic heart disease (RHD), which remains a concern in both developed and developing countries. In wealthier countries, the prevalence of rheumatic heart disease is much lower. The majority of deaths associated with S. pyogenes are attributed to clinical manifestations associated with invasive disease. Our general understanding of the epidemiology of group A streptococcus and associated diseases remains relatively poor compared with other infectious diseases. Many countries with well-established infectious disease surveillance programs conduct relatively little surveillance for diseases caused by Streptococcus pyogenes. However, this has improved over the years, with many countries establishing invasive group A streptococcal infection as a legally notifiable disease. To fully understand the epidemiology of these diseases—specifically how they spread, host and strain characteristics that are important for forward transmission, disease severity, and competition between and within species for ecological niches—researchers will need to conduct comprehensive investigations that follow a large group of individuals for an extended period of time. Understanding these factors will also enable the development of effective prevention strategies.

The magnitude and severity of the disease burden of S. pyogenes highlight the importance of epidemiological surveillance for detecting changes across the disease distribution in different populations. Since the early 1980s, there have been some notable changes in the global epidemiology of group A streptococcal infections, particularly in the reporting of invasive cases. Outbreaks of both suppurative and non-suppurative Streptococcus pyogenes infections were frequently reported in the 1980s and 1990s (Efstratiou, 2000). The increased incidence of invasive S. pyogenes infection is often associated with a specific clone. The incidence of invasive S. pyogenes infection varies with time and geographic area, which presumably reflects population susceptibility to particular strains as well as natural variations in dominant species.

Variations in species distribution may also result in fluctuations in infection severity and in overall mortality rates. S. pyogenes infection can occur in people of any age; however, the prevalence of infection is higher in children, presumably due to a combination of multiple exposures (in schools or nurseries, for example) and host immunity. The prevalence of nasopharyngeal infection is highest among children over three years of age and is described as a risk in school-age children. The disease in neonates is uncommon, possibly reflecting protective immunity acquired through the placenta. In this paper, we have focused on the epidemiology of S. pyogenes infection, with an emphasis on novel detection methods being applied in global medical laboratories, as well as the prevention, control, and management of this devastating disease (Carapetis et al., 2005; Efstratiou, 2000).

Recommendations

Group A Streptococcus bacteria (group A strep) can cause many different types of infections. The best way to protect yourself from group A infections is to practice good hygiene, e.g.:

1. Wash your hands often. Washing your hands is the right way to prevent all kinds of infections.
2. Cover your mouth. Teach your children to cover their mouths with their elbow or a tissue when they cough or sneeze.
3. Avoid sharing personal items. Do not share drinking glasses or eating utensils.
4. A laboratory examination is necessary if you have any similar symptoms such as fever, cough, inflammation in the lymph nodes and chest, or skin infections. Interpretation of the results in this context is prone to misdiagnosis. To improve the diagnosis of a previous GAS infection, two consecutive ASOT measurements are recommended.
5. It is preferable to use examinations performed with developed, fast, and reliable devices, such as the Mispa i2 device.
6. Use oral penicillin for 10 days, or intramuscularly (IM), as the drug of choice to treat infected patients. This treatment is cost-effective and has a narrow spectrum of activity. Severe invasive Streptococcus pyogenes infections may also be treated with vancomycin or clindamycin.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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