PASTOREX™ STAPH-PLUS

Σ 5 x 50  REF 56353
Σ 1 x 50  REF 56356

Latex agglutination test for the identification of Staphylococcus aureus

IVD

CE
1- **INTENDED USE**

PASTOREX™ STAPH-PLUS is a rapid slide agglutination test for the simultaneous detection of the fibrinogen affinity (clumping factor), protein A, and capsular polysaccharides of *Staphylococcus aureus*.

2- **SUMMARY AND EXPLANATION**

*Staphylococcus aureus* is one of the most frequently encountered pathogens in clinical specimens. The rapid distinction between this species and other, less virulent, staphylococci is of great importance for an appropriate patient management. The test for the detection of free coagulase production permits the identification of *Staphylococcus aureus* (11, 12).

However, this test takes 4 to 24 hours, and plasma may have lot-to-lot variation that can affect the reaction (16).

Agglutination reagents have been developed, giving a more rapid and reliable detection of *Staphylococcus aureus* (3). These agglutination tests use latex sensitised with fibrinogen and IgG, in order to detect the clumping factor and protein A, which are biochemical characteristics of *S. aureus*.

However, it has been observed that certain strains of *Staphylococcus aureus* (essentially the methicillin-resistant strains) are not agglutinated by these agglutination tests (15). A study of these strains has shown that they all possess capsular polysaccharide (4). It is therefore probable that the polysaccharide capsule which envelopes all the bacteria under certain conditions (direct specimens, culture isolation, and bacterial clone), masks protein A and clumping factor, thereby preventing agglutination of the latex particles only sensitised by fibrinogen and IgG.

3- **PRINCIPLE**

The PASTOREX™ STAPH-PLUS was designed to allow simultaneous detection of the following three components:

1) the fibrinogen affinity factor, also referred to as bound coagulase or ”clumping factor“;

2) protein A, which possesses an affinity for the crystallisable fragment (Fc) of gamma immunoglobulins (IgG);

3) capsular polysaccharides of *Staphylococcus aureus*.

The reagent is made of latex particles sensitised by fibrinogen and IgG as well as specific monoclonal antibodies (Institut Pasteur patent) raised against capsular polysaccharides of *Staphylococcus aureus* (1, 5, 6, 8, 10). The combination of fibrinogen, IgG and anti-capsular monoclonal antibodies in the same reagent allows the recognition of highly encapsulated strains of *Staphylococcus aureus* as well as poorly encapsulated strains. For highly encapsulated strains, anti-capsular polysaccharide antibodies agglutinate the bacteria. For strains that have lost their polysaccharide capsule, the bacteria are agglutinated by fibrinogen and IgG.
Culture isolates of *Staphylococcus aureus* are mixed with the latex reagent on a slide. Following complete mixing, the slide is evaluated visually for the presence of agglutination, which indicates the presence of *Staphylococcus aureus* organisms.

### 4- PRESENTATION

1. **PASTOREX™ STAPH-PLUS**, 50-test kit, code 56356
   - **Latex test**: 1 dropper bottle of 1 ml of red latex sensitised by bovine albumin solution, fibrinogen, IgG, and monoclonal antibodies directed against capsular polysaccharides of *Staphylococcus aureus*.  
     Preservative : <1.5 % ProClin™ 300
   - **Negative control**: 1 dropper bottle of 1 ml of negative control reagent of red latex sensitised by bovine albumin solution.  
     Preservative : <1.5 % ProClin™ 300.
   - 16 disposable agglutination cards
   - 150 rods

2. **PASTOREX™ STAPH-PLUS**, 5 x 50 test kit, code 56353
   - **Latex test**: test reagent, 5 dropper bottles (1 ml).
   - **Negative control**: negative control reagent, 5 dropper bottles (1 ml).
   - Disposable agglutination cards, 4 x 16
   - Rods, 3 x 100

### 5- STORAGE

Once open, all reagents are stable until the expiry dates indicated on the label, if stored at +2-8°C and in absence of microbial contamination. Store the latex reagent bottles upright.

THE LATEX REAGENTS SHOULD NOT BE FROZEN.

### 6- NECESSARY MATERIAL NOT PROVIDED

- Loop for collection of bacterial colonies
- Disinfectant tank or autoclave bag for disposal of used cards

### 7- PRECAUTIONS

The Pastorex™ Staph-Plus kit is intended for culture confirmation testing only, and should not be used on direct clinical samples. The quality of results depends on complying strictly with Good Laboratory Practice.

- All the reagents and the sample should be used at a temperature of between 18 and 30°C.
- Do not touch the reaction surface of the agglutination cards.
- Shake the bottles of latex before use.
• Wipe the tip of the reagent dropper bottle in order to obtain well-calibrated drops.
• Hold the reagent bottle vertical to deposit drops.
• Use the plastic stir rods supplied in the kit for mixing the latex reagent and the bacterial colonies. Do not use wooden sticks.
• Change of mixing stick for each reaction.
• Discard all disposable material used in an autoclavable waste bin or disinfectant bath.

HYGIENE AND SAFETY INSTRUCTIONS
Always observe the current techniques and precautions concerning protection against microbiological hazards.

Caution: These reagents contain ProClin™ 300 < 1.5%. For risks and safety recommendations refer to the table at the end of the package insert

8- PROCEDURE

1) SPECIMEN PREPARATION
Specimens used with this kit should be pure and fresh. Recommended isolation medias, or equivalent medias, are as follows:

Agar Media
• Trypto-casein-soy agar
• Columbia agar + sheep blood
• Columbia agar
• Blood agar
• Mannitol salt agar
• Baird-Parker media with additives
• MRSASelect™

Perform Gram stain and catalase testing on the cultured organisms. Colonies that are tested with the PASTOREX™ STAPH-PLUS reagent should be Gram positive cocci that are catalase positive.

2) AGGLUTINATION REACTION
1. Thoroughly homogenize the latex reagents by shaking. Vortex reagent if necessary.
2. Deposit a drop of latex test reagent into one of the circles of the agglutination card.
3. Deposit a drop of negative control latex reagent in another circle.
4. Take 1 to 3 Gram positive catalase positive colonies with a loop or plastic stir rod and emulsify them in a drop of latex for 10 seconds.
5. Repeat step 4 for the negative control latex.
6. Homogenise by gently rotating the card. Results must be read within 30 seconds of beginning the card rotation.
7. Evaluate results according to the following criteria and then discard the card into a disinfectant container. Do not re-use the card.

9- INTERPRETATION OF RESULTS

Positive reaction
A positive reaction is evidenced by the formation of aggregates with the reagent test only, visible to the naked eye under normal lighting within 30 seconds of beginning the card rotation. The aggregates of latex particles may be of varying sizes with a more or less cloudy, pink background.
A slow and weak agglutination could signify a non-specific agglutination.

Negative reaction
In a negative reaction, the suspension does not produce any aggregates and retains its milky appearance.

Non-interpretable results
An non-interpretable result corresponds to agglutination of the suspension by the negative control latex. In this case, identify using another method such as testing for the presence of free coagulase and heat-stable DNAse.

10- QUALITY CONTROL OF THE TEST

Evaluate the latex reagent at each use by verifying the absence of any agglutination when depositing the latex on the card. The latex should be periodically tested with previously identified strains of *Staphylococcus aureus* and *Staphylococcus epidermidis*. The latex test reagent must show presence of agglutination with *Staphylococcus aureus* and absence of agglutination with *Staphylococcus epidermidis*. The negative control latex must show an absence of agglutination with both organisms.

11. QUALITY CONTROL OF THE MANUFACTURER

All manufactured reagents are prepared according to our quality system starting from reception of raw material to the final commercialization of the product.
Each lot is submitted to quality control assessments and is only released to the market, after conforming to the pre-defined acceptance criteria. The records relating to production and control of each single lot are kept within Bio-Rad.
The performance of PASTOREX™ STAPH-PLUS has been evaluated in several different laboratories (2, 13, 14). A total of 440 strains of *S. aureus* and 138 staphylococcal strains other than *S. aureus* were tested with the kit. Culture identification was carried out using gram stain, catalase activity, and the coagulase tube test. Discrepant samples were subsequently tested by biochemical analysis and by a commercialized alternative rapid test. *S. aureus* strains were also checked for their susceptibility to methicillin. The results for sensitivity testing on methicillin-resistance *S. aureus* (MRSA) and methicillin-sensitive *S. aureus* (MSSA), as well as the total results for all *Staphylococcus aureus*, are reported in Table 1. The results for specificity testing on staphylococcal strains other than *S. aureus* are reported in Table 2.

**Table 1**

**Performance of PASTOREX™ STAPH-PLUS on MRSA and MSSA isolates**

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Positive</th>
<th>Negative</th>
<th>Relative Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA</td>
<td>217*</td>
<td>217</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>MSSA</td>
<td>222</td>
<td>222</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>Total <em>S. aureus</em></td>
<td>439*</td>
<td>439</td>
<td>0</td>
<td>100%</td>
</tr>
</tbody>
</table>

*one non-interpretable result was excluded*

**Methicillin resistant *S. aureus***

As reported in Table 1, 217 of 217 well-defined MRSA isolates were correctly identified by the PASTOREX™ STAPH-PLUS kit. The sensitivity of PASTOREX™ STAPH-PLUS for MRSA was estimated to be 100%, excluding the non-interpretable result which represents 0.4% of the strains analyzed.

**Methicillin sensitive *S. aureus***

All of the 222 MSSA that were cultured gave a positive result with the PASTOREX™ STAPH-PLUS, as shown in Table 1. The sensitivity obtained with this population of MSSA was 100%.
Other Staphylococci

Table 2

Performance of PASTOREX™ STAPH-PLUS on other staphylococcal strains

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Positive</th>
<th>Negative</th>
<th>Relative Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other staphylococcal strains</td>
<td>138</td>
<td>1</td>
<td>137</td>
<td>99.3%</td>
</tr>
</tbody>
</table>

138 staphylococcal isolates other than S. aureus, including S. epidermidis, S. haemolyticus, S. hominis, S. saprophyticus, S. schleiferi, S. lugdunensis, and other species, were also analyzed with the PASTOREX™ STAPH-PLUS. A negative result was obtained for 137 of the 138 isolates, as shown in Table 2. The discrepant result was identified as S. lugdunensis and it was also positive with an alternative rapid test.

13- LIMITATIONS OF THE PROCEDURE

Staphylococcus lugdunensis and Staphylococcus schleiferi have been reported to possess a fibrinogen affinity factor (7,9) and may react with the detection test for clumping factor, depending on the strains and the isolation medium. As some strains of Staphylococcus, are known to cause non-specific aggregation of latex particles, particularly staphylococcus saprophyticus, it is recommended that the control latex provided in the kit be used with each organism that is tested. Staphylococcus intermedius and staphylococcus hyicus, which are found in animal pathology but are very rarely isolated in man, may present a positive reaction with the classical coagulase tests and may therefore theoretically also react with fibrinogen affinity factor detection tests.

The possibility of cross-reactions should not be forgotten. Certain streptococci possess a protein with an affinity for the Fc fragments of immunoglobulins and may therefore react with the latex. Non-specific reactions of latex techniques have also been reported for several species including Escherichia coli and Candida albicans (17). It is recommended to perform a Gram stain and catalase test on the colonies to be tested prior to the latex test.

False negative reactions can occur if the Staphylococcus aureus that is isolated does not produce the fibrinogen affinity factor (clumping factor), protein A, or capsular polysaccharides against which the specific monoclonal antibodies were raised. False negative results can occur with an insufficient inoculum.


R43:

(GB) May cause sensitisation by skin contact.
(FR) Peut entraîner une sensibilisation par contact avec la peau.
(ES) Posibilidad de sensibilización en contacto con la piel.
(IT) Può provocare sensibilizzazione per contatto con la pelle.
(DE) Sensibilisierung durch Hautkontakt möglich.
(PT) Pode causar sensibilização em contacto com a pele.
(SE) Kan ge allergi vid hudkontakt.
(DK) Kan give overfølsomhed ved kontakt med huden.
(PL) Może powodować uczulenie w kontakcie ze skórą.
(LT) Gali sukelti alergiją suspilietus su oda.
(HU) Bőrrel érintkezve túlérzékenységet okozhat (szennibilizáló hatású lehet).
(EE) Kokkupuuvel nahaga võib põhjustada ülitundlikkust.
(SK) Môže spôsobiť senzitizáciu pri kontakte s pokožkou.
(CZ) Může vyvolat senzibilizaci při styku s kůží.
(RO) Poate provoca o sensibilizare în contact cu piele.
(BG) Възможна е сензитизация при контакт с кожата.
(LV) Saskaroties ar ādu, var izraisīt paaugstinātu jutīgumu.
(MT) Jista' jikkaguna sensitizzazzjoni meta jniss il-gildà.
(NL) Kan overgevoeligheid veroorzaken bij contact met de huid.
(SI) Stik s kožo lahko povzroči preobčutljivost.
(FI) Ihokosketus voi aiheuttaa herkistymistä.

S24-37-60:

(GB) Avoid contact with skin. Wear suitable gloves. This material and its container must be disposed of as hazardous waste.
(FR) Éviter le contact avec la peau. Porter des gants appropriés. Éliminer le produit et son récipient comme un déchet dangereux.
(ES) Evítete el contacto con la piel. Úsense guantes adecuados. Eliminense el producto y su recipiente como residuos peligrosos.
(IT) Evitare il contatto con la pelle. Usare guanti adatti. Questo materiale e il suo contenitore devono essere smaltiti come rifiuti pericolosi.
(PT) Evitar o contacto com a pele. Usar luvas adequadas. Este produto e o seu recipiente devem ser eliminados como resíduos perigosos.
(GR) Να φροντίσετε κατάλληλα γάντια. Το υλικό και ο περιπατής του να θεωρηθούν έτσι όπως καταίσθησης αποτρέπονται.
(LT) Vengti patekimą ant odos. Mūšėti tinkamas pirštinės. Šios medžiagos atliekos ir jos pakuotė turi būti šalinamos kaip pavojingos atliekos.
(SK) Zabraňte kontaktu s pokožkou. Noste vhodné rukavice. Tento materiál a príslušná nádoba musia byť zlikvidované ako nebezpečný odpad. Tento materiál a príslušná nádoba musia byť zlikvidované ako nebezpečný odpad.
(CZ) Zamezte styku s kůží. Používejte vhodné ochranné rukavice. Tento materiál a jeho obal musí být zneškodněný jako nebezpečný odpad.
(RO) A se evita contactul cu pielea. A se purta mănuși corespunzătoare. Acest produs și ambalajul său se vor depozita ca un deșeu periculos.
(BG) Да се избягва контакт с кожата. Да се носят подходящи ръкавици. Този материал и неговата опаковка да се третират като опасен отпадък.

• Evita l-kuntatt mal-ġilda. Ilbes ingwanti adatt. Dan il-materjal u l-kontenitur tiegħu għandhom jintremew ma’skart perikoluż.

• Aanraking met de huid vermijden. Draag geschikte handschoenen. Deze stof en de verpakking als gevaarlijk atval afvoeren.

• Preprečiti stik s kožo. Nositi primerne zaščitne rokavice. Snov/pripravek in embalažo odstraniti kot nevarni odpadek.

• Varottava kemikaalin joutumista iholle. Käytettävä sopivia suojakäsineitä. Tämä aine ja sen pakkaus on käsiteltävä ongelmajätteenä.