Pastorex® *Staph* Plus/Kit
(Identification of *Staphylococcus aureus*)

355-6356
355-6353

DEFINITION

Pastorex® *Staph* Plus Kit is a rapid agglutination test on slides for simultaneous detection of fibrinogen affinity factor ("Clumping factor"), protein A, and capsular polysaccharides of *Staphylococcus aureus*.
The capacity of Pastorex® *Staph* Plus reagent to recognize capsular polysaccharides of *Staphylococcus aureus* enables it to identify, with great sensitivity, strains of both *Staphylococcus aureus* sensitive to meticillin and strains resistant to meticillin, increasingly responsible for serious nosocomial infections.

PRINCIPLE

Pastorex® *Staph* Plus reagent permits simultaneous detection of:

- fibrinogen-affinity factor known as bound coagulase or "Clumping factor".
- protein A, which has an affinity for the "crystallisable fragment" of immunoglobulin G (IgG).
- capsular polysaccharides of *Staphylococcus aureus.*

It has been shown that the presence of protein A and of factor of affinity for fibrinogen enables identification of *Staphylococcus aureus* and detection by means of latex particles sensitized by fibrinogen and IgG.
It has been observed, however, that certain strains of *Staphylococcus aureus* resistant to metacillin were not agglutinated by these latex particles. A recent study of these strains has shown that they all possess capsular polysaccharide. It is therefore probable that the polysaccharide capsule, covering the whole bacterium under certain conditions (fresh isolation, culture conditions, bacterial clone) masks the protein A and the fibrinogen-affinity factor, thus preventing agglutination of latex particles sensitized only by fibrinogen and IgG.
It was on the basis of these observations that the Pastorex® *Staph* Plus reagent was developed (patent pending). This reagent is made up of latex particles sensitized by fibrinogen and IgG on the one hand, and with monoclonal antibodies specific to capsular polysaccharide of *Staphylococcus aureus* on the other. The combination in a single reagent of fibrinogen, IgG and antibodies to capsular anti-polysaccharide means that weakly-capsulated strains of *Staphylococcus aureus* can be recognized just as readily.

In the first case (primary culture, strains resistant to metacillin, strains deficient in protein A and/or fibrinogen-affinity factor) it is above all the capsular anti-polysaccharide antibodies that agglutinate the bacteria. Conversely, in the second case, (strains sensitive to metacillin, or strains that have lost their polysaccharide capsule after sub-culture) bacteria are agglutinated by fibrinogen and IgG. Thus, in the presence of strains of *Staphylococcus aureus* possessing one or more of the following antigens: protein A, factor of affinity for fibrinogen, and capsular polysaccharide, Pastorex® *Staph* Plus suspension agglutinates strongly less than 40 seconds, presenting large clumps readily visible to the naked eye.

PRESENTATION

- **Pack of 50 tests** **code 355-6356**
  Each kit contains:
- 1 instruction sheet
- 1 dropper bottle of ready to use latex reagent (1 ml). Particles of red latex sensitized by fibrinogen, IgG and monoclonal antibodies to capsular anti-polysaccharide of *S.aureus*. Contains 0.02% sodium merthiolate and 0.1% sodium azide.
- 1 dropper bottle of ready to use negative control latex reagent (1 ml). Particles of latex sensitised by a solution of bovine albumin. Contains 0.02% sodium merthiolate and 0.1% sodium azide.
- 15 disposable cards

- **Pack of 5** x **50 tests** **code 355-6353**
  Each kit contains:
- 5 bottles of ready-to-use reagent (1 ml)
- 5 bottles of ready-to-use negative control reagent (1 ml).
- 60 disposable cards
- 1 instruction sheet

STORAGE

- Reagents: + 2°C to 8°C
- The reagents are stable until the expiration date indicated on the label, in the absence of contamination.
- Latex reagents should never be frozen.

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EQUIPMENT REQUIRED (NOT SUPPLIED)
(non-exhaustive)
- Inoculating loop for collecting bacterial colonies
- Disinfecting tank or autoclavable waste bin for throwing away cards and rods after use
- All usual laboratory equipment.

PRECAUTIONS
- Never use out-of-date reagents
- Shake the bottles of latex before collecting specimens.
- Do not touch the reactive surface of cards with the fingers.
- All specimens must be considered potentially contagious.
- It is recommended that disposable gloves be worn.
- Throw away disposable inoculating loops as well as used agglutination cards in an autoclavable waste bin or a disinfection tank.
- Comply with Good Laboratory Practice.

PROTOCOL
Inoculation
- Thoroughly homogenize latex reagents.
- Deposit a drop of latex reagent in circles on the agglutination card.
- Deposit a drop of negative control latex reagent in another circle.
- Collect 1 colony of average dimension using an inoculating loop, and emulsify for 10 seconds with the drop of latex.
- Proceed in the same manner with the negative control reagent.
- Homogenise using gentle circular movements of the card for 30 seconds, holding the card under normal lighting, then take a reading.
- Throw the card in a disinfection container.
- Do not re-use.

READING AND INTERPRETATION
• Positive reaction
This is revealed by the formation of agglutinations only with the latex test, visible with the naked eye under normal lighting, within 40 seconds. The agglutinations of latex particles may be larger or smaller, with a pink base that is more or less milky.

• Negative reaction
In this case, the suspension does not present any agglutinations and will retain its milky appearance with both reagents: latex agglutination test and latex negative control.

• Non-interpretable results
The result is non-interpretable in the case of agglutination of the suspension in both latex reagents: latex agglutination test and latex negative control. In this case, the presence of free coagulase and of thermostabile DNAse should be looked for.

PERFORMANCES / QUALITY CONTROL OF THE TEST
The growth performances of the media are verified with the following strains:

STRAINS	RESULTS OF AGGLUTINATION
Staphylococcus aureus	Presence of agglutination with latex agglutination test No agglutination with latex control reagent
Staphylococcus epidermidis	No agglutination with latex agglutination test No agglutination with latex control reagent

QUALITY CONTROL OF MANUFACTURER
Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the end-product. Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and control of each batch is kept on file.

LIMITATIONS OF THE METHOD
It has been reported that the recently-identified species *Staphylococcus lugdunensis* and *Staphylococcus schleiferi* possess a ffibrinogen-affinity factor and could therefore react with detection tests for clumping factor as a function of strains and isolation medium. *Staphylococcus intermedius* and *Staphylococcus hyicus*, found in animal pathology but very rarely isolated in humans, can present a positive reaction with conventional coagulase tests and can thus also react with detection methods for fibrinogen-affinity factor.

Some *streptococci* have a protein which has an affinity for the crystallizing fragment of immunoglobulins and can react with latex.
Non-specific reactions of latex techniques with several species, including *Escherichia coli* and *Candida albicans* have also been reported. These false-positive reactions are avoided if a Gram staining and catalase are carried out on the test colonies prior to the latex test.

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