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Staphaurex*

INTENDED USE

Staphaurex* is a rapid slide agglutination procedure for differentiating staphylococci which possess coagulase and/or protein A, particularly *Staphylococcus aureus,* from staphylococci which possess neither of these factors.

SUMMARY AND EXPLANATION OF THE TEST

Plasma coagulase tests are frequently used to assist in the identification of *Staphylococcus aureus.* Two distinct factors are involved independently. The slide coagulase test detects cell-associated clumping factor, sometimes referred to as bound coagulase, which reacts with fibrinogen to cause aggregation of the organisms[11]. The tube coagulase test detects extracellular staphylocoagulase, sometimes called free coagulase, which activates prothrombin thereby initiating clot formation in the plasma. Approximately 97% of human isolates of *S. aureus* possess both factors; strains lacking one of the factors occur in roughly equal proportions[2]. False positive and false negative reactions can be encountered with both tests[2].

Over 95% of human strains of *S. aureus* produce protein A, independently of clumping factor or staphylocoagulase, and this may be cell-associated and/or extracellular[4]. Protein A has a specific affinity for the Fc moiety of immunoglobulin G (IgG).

PRINCIPLE OF THE PROCEDURE

It has been demonstrated that *S. aureus* cultures possessing clumping factor and protein A can be identified using human plasma-coated latex particles, which agglutinate in a rapid slide procedure[1]. The Staphaurex* reagent consists of polystyrene latex particles which have been coated with fibrinogen and IgG. When mixed on a slide with a suspension of *S. aureus* organisms, reaction of clumping factor with the fibrinogen, and/or of protein A with the IgG causes rapid, strong agglutination of the latex particles.

REAGENTS

KIT CONTENTS

Staphaurex*	ZL30/R30859901 120 Tests	ZL31/R30859902 400 Tests
1. Test Latex	3 dropper bottles	10 dropper bottles
2. Disposable Reaction Cards (RT64/R30369001)	1 pack	4 packs
3. Disposable Sampling and Mixing Sticks	2 bundles	5 bundles
4. Instructions for Use	1	1

DESCRIPTION, PREPARATION FOR USE AND RECOMMENDED STORAGE CONDITIONS

See also **Warnings and Precautions.**

The Test Latex is provided ready for use and should be stored in an upright position at 2 to 8°C, where it will retain activity at least until the date shown on the bottle label. Do not freeze. Avoid storage at room temperature (15 to 30°C). Do not stand the reagent in bright light on the bench.

Reaction cards and mixing sticks should be stored at room temperature (15 to 30°C).

TEST LATEX ⚠

Test Latex

3 bottles (ZL30/R30859901) or 10 bottles (ZL31/R30859902) containing a buffered suspension of polystyrene latex, each bottle containing a minimum of 1.7 ml (sufficient for 40 tests). The latex particles are coated with human fibrinogen and IgG. Contains 0.025% Bronidox® preservative.

Materials of human origin have been tested for the presence of hepatitis B surface antigen, anti-HCV and anti-HIV-1/HIV-2 and found to be negative.

WARNINGS AND PRECAUTIONS

IVD

For *in vitro* diagnostic use only.

For professional use only.

Please refer to the manufacture's safety data sheet and the product labelling for information on potentially hazardous components.

HEALTH AND SAFETY INFORMATION

1. CAUTION: This kit contains components of human origin. No test method can offer complete assurance that products derived from human sources will not transmit infection. Therefore, all material of human origin should be considered as potentially infectious. It is recommended that these reagents and test specimens be handled using established good laboratory working practices.

   Test specimens may contain pathogenic organisms and must be handled with appropriate precautions.
2. Non-disposable apparatus should be sterilised by any appropriate procedure after use, although the preferred method is to autoclave for 15 minutes at 121°C. Disposables should be autoclaved or incinerated. Spillage of potentially infectious materials should be removed immediately with absorbent paper tissue and the contaminated areas swabbed with a standard bacterial disinfectant. Materials used to clean spills, including gloves, should be disposed of as biohazardous waste.
3. Do not pipette by mouth. Wear laboratory coat, disposable gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.
4. When used in accordance with the principles of Good Laboratory Practice, good standards of occupational hygiene and the instructions in these Instructions for Use, the reagents supplied are not considered to present a hazard to health.

ANALYTICAL PRECAUTIONS

1. Do not use the reagents beyond the stated expiry date.
2. Latex reagents should be brought to room temperature (15 to 30°C) before use. Latex reagents which show signs of aggregation or 'lumpiness' before use may have been frozen and should not be used.
3. It is important when using dropper bottles that they are held vertically and that the drop forms at the tip of the nozzle. If the nozzle becomes wet an incorrect volume will form around the end and not at the tip; if this occurs dry the nozzle before proceeding.
4. Do not touch the reaction areas on the cards.
5. Do not interpret agglutination that appears after 20 seconds as a positive result. Prolonged rocking can result in false-positive reactions with some coagulase-negative isolates.
6. Microbiological contamination of reagents must be avoided as this may reduce the life of the product and cause erroneous results.

SPECIMEN COLLECTION AND PREPARATION

For details of specimen collection and treatment a standard text book should be consulted[3]. Cultures may be tested direct from the primary culture plate if there is sufficient growth. Alternatively a subculture should be made on blood or nutrient agar for subsequent testing. The best results are obtained from enriched media such as blood agar or nutrient agar; Columbia CNA agar and Baird-Parker medium also give satisfactory results. THE USE OF FRESH CULTURES GROWN OVERNIGHT IS RECOMMENDED. Growth from DN-ase agar may be tested within 15 minutes of flooding the plate with hydrochloric acid. Organisms growing on high-salt selective media, such as mannitol-salt agar, tend to show 'roughness' or 'stringiness' in the latex, and interpretation of the reactions obtained in the test may be more difficult when these media are used. It is recommended that the culture should be Gram-stained in association with the latex test to confirm the staphylococcal morphology of the organisms.

PROCEDURE

MATERIALS PROVIDED

Sufficient materials are provided for 120 tests (ZL30/R30859901) and 400 tests (ZL31/R30859902), see **Kit Contents.**

TEST PROCEDURE

Please read Analytical Precautions carefully before performing the test.

Step 1	Each bottle of latex reagent contains a minimum of 1.7 ml (sufficient for 40 tests). Shake the latex to obtain an even suspension and dispense a drop into a circle on the reaction card for each culture to be tested.
Step 2	Take a mixing stick and pick up some of the culture by touching it with the flat end of the stick. As a guide, an amount of growth roughly equivalent to six average-sized colonies should be picked. Before sampling colonies from DN-ase agar which has been flooded with hydrochloric acid, tilt the plate so that the growth is not covered by hydrochloric acid.
Step 3	Emulsify the sample of culture in a drop of latex by rubbing with the flat end of the stick. Rub thoroughly, but not too vigorously or the surface of the card may be damaged. Some strains, particularly of species other than *S. aureus* remain difficult to emulsify and this should be noted, since lumps of unemulsified culture can make the latex appear 'rough' or 'stringy' on reading. Spread the latex over approximately half the area of the circle. Discard the mixing stick for safe disposal.
Step 4	Rotate the card gently for up to 20 seconds and examine for agglutination, holding the card at normal reading distance (25 to 35 cm) from the eyes. Do not use a magnifying lens. The patterns obtained are clear cut and can be recognised under any normal lighting conditions.
Step 5	Dispose of the card into disinfectant – do not re-use.

RESULTS

READING OF RESULTS

Positive Result

A positive result is indicated by the development of an agglutinated pattern showing clearly visible clumping of the latex particles with clearing of the milky background (Figure 1). Most positive reactions will be almost instantaneous.

Negative Result

A negative result is indicated when the latex does not agglutinate and the milky appearance remains substantially unchanged throughout the test (Figure 2). It should also be noted that traces of granularity may be seen in negative patterns due to the particulate nature of both reactants.

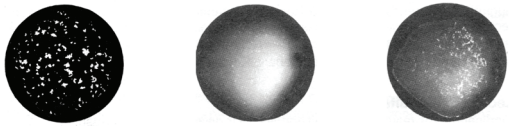
NOTE: Increased granularity may be observed if the Latex Suspensions are rotated for more than 20 seconds.

Rough or stringy reactions appear as white specks or stringy aggregates (Figure 3) and should be interpreted as follows:–

1. When accompanied by a milky background they should be recorded as negative.
2. When accompanied by a clear background they are likely to be positive.

Care should be taken in the interpretation of such results.

Figure 1 Figure 2 Figure 3



QUALITY CONTROL

Quality control testing should be run with each shipment and new kit lot number received. Each laboratory should follow their state and local requirements.

Under normal circumstances it will become apparent in day-to-day testing if the reagent fails to operate properly. **The latex suspension should always be inspected for granularity as it is dropped onto the test card.** Some granularity can be removed by shaking vigorously but if there is evidence of auto-agglutination, the suspension should not be used. In addition, known stock cultures of *S. aureus* and *S. epidermidis* should be used periodically as controls.

INTERPRETATION OF RESULTS

A positive reaction indicates the presence of either coagulase or protein A, or both, in the culture under test and a negative result indicates their absence.

LIMITATIONS OF THE PROCEDURE

1. Specimens grown on high-salt-supplemented media such as mannitol-salt agar tend not to emulsify well giving 'rough' or 'stringy' reactions (see Reading of Results) and may be relatively weak in their protein A and coagulase content.
2. Some species of Staphylococcus in addition to *S. aureus* notably *S. hyicus* and *S. intermedius* may give positive results in conventional coagulase tests[9], and may also react in the latex procedure. If necessary these species may be identified by biochemical test procedures, but they are not considered to be of major clinical significance in man.
3. Some other coagulase negative staphylococcal species, such as *S. capitis* possess plasma protein binding factors[8], but these do not react in the Staphaurex* Test. However, a few strains identified biochemically as *S. saprophyticus* have given weak positive reactions and further identification of urinary isolates may be required.
4. Some streptococci and possibly other organisms possess immunoglobulin or other plasma protein binding factors which can react in the latex test[5,6,7,10] and there are several species such as *Escherichia coli* and *Candida albicans* which are able to non-specifically agglutinate latex particles. To eliminate potential interference from these organisms a Gram stain should be performed so that only organisms with staphylococcal morphology are tested.

EXPECTED RESULTS

Strong agglutination with *S. aureus* cultures, no agglutination with staphylococci which possess neither clumping factor or protein A.

SPECIFIC PERFORMANCE CHARACTERISTICS

Staphaurex* was evaluated in five centres on a total of 940 routine (presumed staphylococcal) clinical isolates. The cultures were also tested by two or more of the following established procedures: slide coagulase, tube coagulase, DN-ase, biochemical tests (Table 1).

a) **Sensitivity**

525 of the clinical cultures tested gave a positive reaction in at least one of the established tests for *S. aureus* identification and 516 of these samples were positive in two or more tests **(Table 1).**

Staphaurex* correctly identified 522 of the 525 presumed *S. aureus* cultures. Two of the three cultures which gave a negative result did not possess clumping factor, protein A or produce free coagulase and were subsequently identified as staphylococcal species other than *S. aureus*. The third sample was not available for further identification. The sensitivity of Staphaurex* was calculated to be 99.8% (522/523).

b) **Specificity**

Staphaurex* gave a negative result with 413 of the 415 cultures which did not react in any of the established tests for *S. aureus* identification (specificity 99.5%, Table 1). The two cultures which gave a positive result with Staphaurex* were both identified as *S. saprophyticus.*

c) **Predictive Values**

The predictive values of positive and negative Staphaurex* tests were 99.6% (522/524) and 99.8% (415/416) respectively.

Table 1

Identification of S. aureus: correlation between established laboratory tests[a] and Staphaurex* on 940 routine clinical cultures

	Staphaurex* +	–	Totals
S. aureus – positive result in two or more established tests[a]	515	1[b]	516
S. aureus – positive result in one established test[a]	7	2[c]	9
Not *S. aureus*	2[d]	413	415
Totals	524	416	940

[a] Slide coagulase, tube coagulase, DN-ase, biochemical tests.

[b] Culture was not available for further investigation.

[c] These cultures did not possess clumping factor, protein A or produce free coagulase and were found to be Staphylococci other than *S. aureus* by an independent Reference Laboratory.

[d] Both cultures identified as *S. saprophyticus.*

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PACKAGING

REF ZL30/R30859901..... 120 tests
ZL31/R30859902..... 400 tests

Symbol legend

REF	Catalog Number
IVD	*In vitro* diagnostic medical device
	Consult instruction for use (IFU)
	Temperature limitation (Storage Temp.)
LOT	Batch code (Lot Number)
	Use by (Expiration Date)
	Caution, consult accompanying documents
	Manufacturer

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