8. Organisms that possess immunoglobulin binding factors may also agglutinate the test reagent latex.
9. Reaction times longer than the specified 60 seconds may lead to false positive results due to drying.

PERFORMANCE CHARACTERSITICS

		Pulse SA TEST Result	
		Positive	Negative
Reference Method	Positive	15	0
	Negative	1	134

A blind trial with two hundred and fourteen reference strains was tested at a clinical site. These tests involved a number of commonly isolated species together with certain rare species. The Sensitivity (15/15) = 100% and Specificity (134/135) = 99.3% were obtained.

BIBLIOGRAPHY

1. Myrick, B.A., and P.D. Ellner. 1982. Evaluation of the latex slide agglutination test for identification of Staphylococcal aureus. J. Clin. Microbiol. 15:275-277.
2. Kicca, W.C. and P.B. Smith. 1980. Staphylococci, p. 83-87. In E.H. Lennette, A. Balows, W.J. Hausler, Jr. and J.P.Truant (ed.). Manual of Clinical Microbiology 3rd ed. American Society for Microbiology, Washington, D.C.
3. Forsgren, A. 1970. Significance of protein A production by staphylococci. Infect. Immun. 2:672-673.
4. Essers, L. and K. Radebold. 1980. Rapid and reliable identification of *staphylococcal aureus* by a latex agglutination test. J. Clin. Microbiol. 12: 641-643.
5. Phillips, W.E., Jr., and W.E. Kloos. 1981. Identification of coagulase positive Staphylococcus intermedius and Staphylococcus hyicus subsp. hycius isolates from vetinary clinical specimens. J. Clin Microbiol. 14: 671-673

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S. aureus Latex Slide Test

INTENDED USE

The PULSE S. *aureus* LATEX TEST (PULSE SA TEST) is a rapid latex slide agglutination test to detect the presence of both clumping factor and protein A. The presence of these two components are used in the identification of S. *aureus* from other species of Staphylococci. Pure culture suspected of being S. *aureus* should be used.

SUMMARY & PRINCIPLES

The pathogenic nature of *S.aureus* has received wide attention recently especially in cases of suppurative infections, food poisoning and other situations. The identification of *S. aureus* is generally confirmed by the production of coagulase in the microorganism. The tube coagulase test detects free coagulase, an extracellular enzyme produced by *S. aureus* which activates Prothrombin that clots the plasma used in the assay (1). The slide coagulate test detects bound coagulase (clumping factor), a cell associated factor which interacts with fibrinogen and causes the aggregation of *S. aureus* (2).

The demonstration of protein A in over 95% of the human strains of *S. aureus* (3) has provided a basis for another slide procedure using latex particles coated with human plasma (4). The high affinity of protein A to the Fe portion of plasma immunoglobulin G (IgG), and of bound coagulase for fibrinogen in plasma allows rapid agglutination of the coated latex particles in the presence of *S. aureus.* The PULSE SA TEST incorporates the use of specially coated latex particles that will form aggregates in the presence of protein A, or clumping factor or a combination of both; as a basis for identifying *S.aureus* from the other staphylococci.

Some non *S. aureus* species may create non-specific agglutination with the *S. aureus* latex particles. To eliminate these cases, and thereby improve the specificity of the test, a control latex reagent (latex particles not coated with either fibrinogen or human IgG) is provided in the test kit and should be used.

MATERIALS SUPPLIED

***S. aureus* Latex:**	Contains modified latex particles in a stabilized buffer with less than 0.1% sodium azide as preservative.
***S.aureus* Control latex:**	Contains latex particles not coated with fibrinogen or human IgG with less than 0.1% sodium azide as preservative.

Disposable Test Cards.

Additional Items Required:
Primary and Pure Test Culture, Mixing Sticks

STORAGE & STABILITY

When not in use, store reagents at 2 - 8 degree Celsius. DO NOT FREEZE. Prior to use, allow latex reagents to warm up to room temperature. Expiration date is specified on the kit label and on each vial. For optimum stability, only remove one vial of latex reagent for use and return reagent to the refrigerator promptly after use. DO NOT leave reagent at room temperature for prolonged period. Do not use reagent if auto-agglutination is evident or if the expiration date has been exceeded. Bacterial contamination of reagent or specimens might cause false positive agglutination.

PRECAUTIONS

This product is for In Vitro Diagnostic Use Only and should be used by properly trained staff. Appropriate precautions should be taken against microbial hazards. The preservative sodium azide may react with metal plumbing to form explosive metal oxides. In disposal, flush with a large volume of water to prevent metal azide build up. All materials should be autoclaved (or sterilized) after use. Good Laboratory Practice should be employed to use this product.

SPECIMEN COLLECTION

Consult a standard microbiological text book for methods relating to specimen collection and the preparation of primary cultures on agar plates. Cultures should be fresh 24 hour growths and may be tested directly from the plate. If there is insufficient growth, sub- culture to blood agar base or nutrient agar and incubate overnight at 37ºC. Organisms grown on high salt media, such as mannitol salt agar, may show signs of stringiness when mixed with the reagents. Any discrepancies can be eliminated by parallel use of the control latex with the test latex. Alternatively, the user can sub-culture to blood agar base or nutrient agar to avoid the problem.

PROCEDURE

1. Resuspend *S.aureus* latex reagent by gentle inversion of the bottle for a few seconds.
2. Dispense one drop of *S. aureus* latex reagent onto a test circle on the test card. If there is any sign of auto-agglutination, discard bottle and use a new bottle.
3. Collect 2 to 4 colonies of pure culture suspected of being staphylococci with the mixing stick and transfer onto the same test circle containing reagent. Mix and emulsify the culture with the reagent.
4. Rotate the slide by hand for a maximum of 60 seconds observing for visible agglutination. The agglutination patterns are clear cut and recognized under normal lighting conditions.
5. Positive reactions usually take 5-20 seconds.
6. In the case of rough or stringy samples, carry out the above procedure using the **control latex** and using the same sample culture. Positive results with the *S. aureus* latex reagent should also be tested with the **control latex** to eliminate the possibility of non-specific agglutination.
7. Report result and discard used materials into a biohazard receptacle.

RESULTS

Positive Result: Visible clumping with substantial clearing of *S.aureus* latex reagent indicates a positive reaction. In most cases the reaction is instantaneous. Rough or smooth filamentous reactions with clear background also indicate a positive reaction.

Negative Result: No agglutination occurs or when there is little change in the milky appearance of the reagent. Filamentous agglutinates as well as fine agglutinates with milky background also indicate negative reaction.

NOTE: Agglutination of the Pulse *S.aureus* latex reagent without agglutination of the *S. aureus* control reagent indicates the presence of either clumping factor or protein A; or both.
If the control reagent also shows agglutination then other biochemical tests will be necessary.

QUALITY CONTROL PROCEDURE

A control latex is provided and should be used to verify that organism under test does not agglutinate latex particles non-specifically.
Periodically check the following:
1. The test reagent agglutinates with a known *S.aureus* strain.
2. The test and control reagents do not auto agglutinate in normal saline solution.

As a Positive Control, the ATCC ® 25923 S. *aureus* can be used and the *S. aureus* latex reagent should show strong agglutination when tested. Similarly, the ATCC 12228 ® S. *epidermidis* can be used as a Negative Control.

LIMITATIONS OF THE PROCEDURE

1. The results of this test SHOULD NOT be used as a single diagnostic tool to make a clinical diagnosis. Instead, the test results must be evaluated together with other clinical findings and observed symptoms to aid in the final diagnosis.
2. This test is designed to be performed by hand rotation. The use of a mechanical rotator could yield false positive/negative results.
3. Media containing high salt concentrations (e.g. Mannitol-salt agar) inhibit protein A production. Colonies from these media should not be used as they would give false negative or weak reactions.
4. Some species of staphylococcus other than *S.aureus* (notably *S. intermedius* and *S.hyicus*) may give positive results in conventional coagulase tests (5) and may also agglutinate latex reagents. Further biochemical tests will be required in these cases. However, *S. intermedius* and *S.hyicus* are not commonly found in human specimens. Some strains of *S. saprophyticus* may yield false positive results and further testing of urinary staphylococcal isolates may be required.
5. Rare species such as *S.lugdunensis* and *S.schleiferi* have been reported as clumping factor positive. Novobiocin resistant strains may also give false positive results using latex based tests.
6. Several species such as *E.coli* and *C.albicans* are capable of non-specifically agglutinating latex particles.
7. Gram stain should be performed to insure that only organisms with staphylococcal morphology are used to eliminate potential interference by other organisms.