Key Code TSMX6555C
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Penicillin-Binding Protein (PBP2′) Latex Agglutination Test

REF DR0900A..... Σ 50 **EN**

1. INTENDED USE

This test is a rapid latex agglutination assay, detecting PBP2′ (also called PBP2a)[7], in isolates of *Staphylococcus*, as an aid in identifying methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant coagulase-negative staphylococci.

2. PRINCIPLES OF THE TEST

Staphylococci are a leading cause of nosocomial and community-acquired infections worldwide[2]. In many institutions, approximately 25% to 50% of *S. aureus* strains and 75% of coagulase-negative staphylococci (CoNS) are resistant to methicillin[1,2]. MRSA are of particular concern because of the ease with which certain epidemic strains spread and colonise debilitated patients. Treatment of sensitive strains with penicillinase-resistant penicillins (PRP), is preferred as beta-lactam drugs are more easily absorbed into body fluids and tissues, cause fewer complications from treatment, and do not select for vancomycin-resistant organisms. Reliable identification of methicillin-resistance is therefore important.

Strains of *S. aureus* with reduced susceptibility to PRP are categorised as follows:

iii(i) methicillin-resistant *S. aureus* (MRSA), which produce the low-affinity penicillin-binding protein PBP2′, encoded by the mecA gene[3,6]

ii(ii) borderline methicillin-resistant *S. aureus* (BORSA), generally considered to be due to hyperproduction of type A - beta-lactamase[10]

(iii) strains with modified PBPs due to altered penicillin-binding capacity or hyper-production of PBPs (MODSA)[1,2.] MODSA have only rarely been isolated and their clinical response to beta-lactam therapy has not been well studied. Thus for clinical purposes, with rare exceptions, the presence of PBP2′ is responsible for methicillin-resistance in the treatment of infections with *S. aureus* and CoNS[2,6.]

The methicillin-resistant phenotype can be highly heterogeneous, making it difficult to detect by conventional antimicrobial susceptibility test methods, such as Minimum Inhibitory Concentration (MIC), disc and agar screen. The accuracy of these methods is affected by inoculum size, incubation time and temperature, medium, pH, salt concentration and other factors[8,9]. In addition, these culture methods require 24 hours incubation for accurate results. CoNS often produce lower amounts of PBP2′ and require induction by exposure to one of the PRPs to produce sufficient product to be detected[2,3,12].

Detection of the mecA gene has been considered the gold standard in the determination of methicillin-resistance because of its accuracy, but this method is labour-intensive and expensive to perform[1,6]. The Oxoid PBP2′ Latex Test has the advantage of direct detection of the PBP2′ protein performed in a rapid timeframe with minimal labour. It has the potential for being even more accurate than the detection of the mecA gene, as false-positive results will not occur with strains that possess mecA but are unable to produce the protein product of the gene. In addition, the assay does not detect strains that are hyperproducers of either beta-lactamase or PBPs.

The Oxoid PBP2′ test has previously been evaluated worldwide, demonstrating its high sensitivity and specificity[4,5,11].

Latex particles sensitised with a monoclonal antibody against PBP2′ will specifically react with methicillin-resistant staphylococci to cause agglutination visible to the unaided eye.

3. COMPONENTS OF THE KIT

TEST LATEX	DR0901 Test synthetic latex particles sensitised with a monoclonal antibody against PBP2′
CONTROL LATEX	DR0902 Control synthetic latex particles coated with rabbit antibodysensitised with a monoclonal antibody of the same IgG subclass showing no reactivity with proteins of *S. aureus*
EXTRACTION REAGENT 1	DR0903 Extraction Reagent 1
EXTRACTION REAGENT 2	DR0904 Extraction Reagent 2
	Test Cards
	Mixing Sticks
	Instruction Leaflet

4. MATERIALS REQUIRED BUT NOT PROVIDED

- Micropipette and tips (50µl)
- Microbiological loops (5µl/1µl)|
- Boiling water bath or heating block
- Centrifuge (1500 x g)
- Microcentrifuge tubes (safe lock)
- Suitable laboratory disinfectant

5. PRECAUTIONS

IVD This product is for in vitro diagnostic use only.

The heating time should be three minutes. Heating for more than five minutes may lead to a decrease in sensitivity. Heating for only one minute or less may lead to non-specific agglutination. When removing the supernatant for use in the test following centrifugation, withdraw the pipette carefully to avoid solid material at the bottom of the tube. Carry over of solid material may cause non-specific agglutination. Shake the Latex Reagents well to form a homogeneous suspension before use.

Reagents contain 0.095% sodium azide as a preservative. Sodium azide is toxic and may react with lead or copper plumbing to produce metal azides which are explosive by contact detonation. To prevent azide accumulation in plumbing, flush with copious amounts of water immediately after waste disposal.

As specimen materials may contain pathogenic organisms, handle with appropriate precautions. The extraction procedure may not kill bacteria; therefore the extract must be handled with the same precautions.

Extraction Reagents 1 and 2 contain a mild irritant and a weak acid. Avoid direct contact by wearing suitable protective equipment. If the material comes into contact with the skin, mucous membranes or eyes immediately wash the area by rinsing with plenty of water.

6. STORAGE

Store the kit at 2-8°C. Under these conditions the reagents will retain their reactivity until the expiry date shown on the box.

7. CONTROL PROCEDURES

For each new lot of the kit and weekly thereafter, the following control procedures must be performed.

1. Positive Control
Use a known MRSA strain such as ATCC® 43300 (Thermo Scientific Culti-Loops™ R4609022). Follow the method given in the test procedure. Ensure that agglutination occurs within 3 minutes.

2. Negative Control
Use a known Methicillin-Sensitive *Staphylococcus aureus* (MSSA) strain such as ATCC® 25923 or ATCC® 29213, (Thermo Scientific Culti-Loops® R4607010 or R4607011). Follow the method given in the test procedure. Ensure that no agglutination occurs within 3 minutes. Do not use the test if reactions with the control organisms are incorrect.

3. Do not use kits beyond their expiry date.

8. IMPORTANT PROCEDURE NOTE

Do not allow the reagents to become contaminated by allowing the dropper tip to touch the specimen on the reaction card. Ensure that the caps are securely fitted after use to prevent contamination and drying out of the reagents. After use return the kit to the refrigerator ensuring that the bottles are stored in an upright position.

9. PREPARATION OF CULTURE

Colonies may be tested from any of the following culture media:

Tryptone Soya Agar (Tryptic Soy Agar) with 5% sheep blood (TSA blood), Columbia Agar with 5% sheep blood, Mueller-Hinton Agar. The use of fresh (18-24 hours) cultures is recommended. However, cultures 24-48 hours old may be tested, if necessary to obtain sufficient growth. The performance data quoted in this leaflet were generated using these media as part of the submission to the North American Food and Drugs Administration. Data on file show that the test is also effective with colonies of *S. aureus* from Tryptone Soya Agar and Columbia Agar with 5% horse blood, Iso-Sensitest Agar and DSTA. These media are commonly used in Europe but not in North America, and so they were not used in the trials used to generate the data shown in the Performance Characteristics section.

Special requirements:

The PBP2′ test should be performed only on *Staphylococcus* species (Gram-positive cocci). A coagulase or equivalent test must be performed in order to determine if the isolate is *S. aureus* or another species of *Staphylococcus*.

Inoculum preparation:

For *S. aureus*, the test can be performed from well-isolated colonies on the primary isolation plate, if there is sufficient growth, or from a subculture of the isolate. Other microbiota that are present on the plate have not been shown to interfere with the assay. For coagulase-negative staphylococci, an induction is needed for the production of sufficient PBP2′.

1. Prepare a broth suspension of the organism equivalent to a 1 McFarland standard and streak a lawn on TSA with blood, Columbia Blood Agar, or Mueller-Hinton Agar. Alternatively, inoculate the agar plate with several colonies and streak in four quadrants.
2. Place an oxacillin disc (1µg/ml) onto the lawn of organisms or in the main inoculum quadrant.
3. Incubate at 37°C for at least 24 hours but not more than 48 hours.

Caution: To avoid false negative results,do not perform the test unless sufficient inoculum is available. Always harvest organisms from close to the disc.

10. TEST METHOD

PBP2′ Extraction Procedure

1. Add four drops of Extraction Reagent 1 into a microcentrifuge tube.
2. Approximately 1.5 x 10^9 (3-5µl) cells should be tested. This may be achieved by using a sterile 5µl loop to remove sufficient growth to fill the internal diameter of the loop. Alternatively, a sterile 1µl loop may be used to remove several colonies and scoop up a heaped loopful 1mm high covering the external diameter of the loop. Suspend the test culture in the microcentrifuge tube. Vortex, if clumps are present. A very turbid suspension should be visible.
3. Place the tube into boiling water or heating block (OVER 95°C) and heat for three minutes.
4. Remove the microcentrifuge tube and allow it to cool to room temperature.
5. Add one drop of Extraction Reagent 2 into the tube and mix well.
6. Centrifuge at 1500 x g for five minutes, (i.e., 3000rpm at 15cm rotation radius or 4500rpm at 4.5cm rotation radius). Use the supernatant for the test.

Latex Agglutination Procedure

1. For each supernatant to be tested, label one circle of the test card for testing with Test Latex and another for testing with Control Latex.
2. Mix the Latex reagents well by vigorous shaking, to smooth out the latex prior to each use, and add one drop of Test Latex or Control Latex to each labelled circle.
3. Place 50µl of supernatant on the Test circle and the Control circle, being careful to avoid the pellet. Mix the latex and supernatant in each circle thoroughly with a mixing stick.
4. Pick up and rock the card for up to three minutes and look for agglutination under normal lighting conditions. Record the results of the test and control reactions.
5. Dispose of reaction card safely into disinfectant or infectious waste.

11. READING AND INTERPRETATION OF RESULTS

Agglutination is seen with Test but not Control Latex within 3 minutes	PBP2′ Positive (MRSA)
No agglutination in either Latex Reagent within 3 minutes	PBP2′ Negative (MSSA)
Agglutination is seen with the Control Latex within 3 minutes	Indeterminate

Strength of agglutination reaction

Negative (–) = a homogeneous suspension of particles with no visible clumping

Weak positive (+) = small but definite clumps against a clouded background

Strong positive (+) = large and small clumps against a slightly clouded background or large clumps against a very clear background.

NB Occasionally, negative reactions may have a finely granular appearance or reactions can be stringy. In such cases, the degree of background clearing should be used to interpret the result. An opaque background indicates a negative result and a clear background should be interpreted as a positive result.

12. LIMITATIONS

Indeterminate results should be re-tested with a fresh extract. If, upon retesting, the result is again indeterminate, the methicillin resistance must be determined by other methods.

False-negative results can occur if insufficient culture is used for testing. In such cases, the test should be repeated with sufficient culture.

True positive results generally have strong reactions. False-positive reactions have been known to occur rarely, but are generally limited to weak reactions. Such results can be verified by retesting with a fresh culture.

Modified *S. aureus* (MODSA), and borderline resistant strains of *S. aureus* (BORSA) do not possess PBP2′ and are not expected to react in this assay.

Some organism strains may have a low level methicillin-resistance or, in rare cases, produce PBP2′ in low amounts, and give a false negative result.

Because of limitations in sensitivity and specificity of the CLSI susceptibility test methods for coagulase-negative staphylococci[9], particularly for strains other than *Staphylococcus* epidermidis, the results with the PBP2′ assay may not agree with the results of standard susceptibility testing. Strains with MICs of ≥0.5µg/ml should be considered methicillin-resistant, regardless of the PBP2′ assay results.

13. PERFORMANCE CHARACTERISTICS

1. The Oxoid Penicillin-Binding Protein Latex Agglutination Test has been evaluated in four geographically diverse laboratories with fresh clinical isolates of *Staphylococcus aureus*.
201 isolates were tested with NCCLS methods and with the Oxoid PBP2′ test from each of three media. One weak false-positive Oxoid Latex reaction (negative on repeat) was found from TSA with blood. All positive reactions were strong, except 3 weak (but positive) reactions from Mueller-Hinton Agar. The sensitivity of the Latex test in detecting MRSA on each medium was 100%; the specificity was 99% for TSA with blood and 100% for tests from all other media.

Results of testing of 201 fresh clinical isolates of S. aureus from 4 laboratories:

	MRSA	BORSA[A]	MSSA
No. tested	68	3	130
Growth on Oxacillin Salt Agar (NCCLS)	68	0	0
MIC ≥4µg/ml (NCCLS)	68	0	0
Positive Latex from TSA Blood	68	0	1 (1)[B]
Positive Latex from Columbia Blood	68	0	0
Positive Latex from Mueller-Hinton	68 (3)[B]	0	0

[A] MICs were 2µg/ml and mecA was negative.

[B] Number of weak reactions shown in parentheses.

2. Testing of challenge strains of S. aureus in three geographically distinct areas:
Three laboratories each examined a set of previously collected challenge strains of *S. aureus* and compared the PBP2′ results to the NCCLS methods of determination of MRSA. A total of 724 strains were tested from TSA with blood. The sensitivity of the Oxoid PBP2′ test was 98.5% and the specificity was 100%. Sensitivities of the agar screen and MIC methods were 98.7% and 99.2% and specificities were 90.0% and 88.7%. Two strains of MODSA were also tested separately; both strains gave a negative result with the Latex test.

3. Testing Coagulase-negative staphylococci
Two laboratories tested coagulase-negative staphylococci for PBP2′ after induction with oxacillin discs. One laboratory tested

115 methicillin-resistant strains and 45 methicillin-susceptible strains, including 58 fresh clinical isolates. The sensitivity was 96.5% for testing from TSA blood and 95.6% for Mueller-Hinton Agar. The specificity was 100% from TSA blood and 98% from Mueller-Hinton Agar. Obtaining a good inoculum was more difficult with Mueller-Hinton Agar. The second laboratory tested 212 methicillin-resistant strains and 203 methicillin-susceptible strains with a sensitivity of 99.5% and specificity of 99.5%, using growth on Columbia Agar for the inoculum.

4. Reproducibility

Ten different well-characterised *S. aureus* strains (3 MRSA, 3 MSSA, 3 BORSA and 1 MODSA) were sent to three geographically diverse laboratories with each strain submitted 5 times in a coded and blinded fashion. All 150 test results agreed with the expected results for 100% reproducibility. The three mecA positive strains were positive each time they were tested (45/45 tests). The three MSSA and the three BORSA strains gave negative results each time the test was performed (90/90 tests). The MODSA strains had an MIC of 16µg/ml to oxacillin and gave a negative result in the Oxoid Latex Test, as expected, each time the test was performed (15/15 tests).

14. REFERENCES

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15. SYMBOL LEGEND

REF	Catalogue Number
IVD	*In Vitro* Diagnostic Medical Device
	Consult Instructions for Use (IFU)
	Temperature Limitations (Storage temp.)
	Contains sufficient for <N> tests
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)
	Manufactured by

CE

IFU X6555C, Revised October 2015 Printed in the UK

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