Penicillin-Binding Protein (PBP2/ Latex Agglutination Test

**INTENDED USE**

This test is a rapid latex agglutination assay, detecting PBP2 (also called PBP2a), a gene in staphylococci, as an aid in identifying methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-susceptible S. aureus (MSSA).

**PRINCIPLES OF THE TEST**

Staphylococci are a leading cause of nosocomial and community-acquired infections worldwide. In many institutions, approximately 25% to 50% of S. aureus bloodstream cultures are methicillin-resistant, and require induction by exposure to one of the PRPs to produce sufficient product to be detected. The use of fresh (18-24 hours) cultures is recommended. However, in some laboratories, particularly for strains other than S. aureus epidermidis, results can be verified by retesting with a fresh culture.

**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 cause false-negative results.
- Reagents containing >0.15% sodium azide may be used for one year or less in sensitivity to agglutination, when reusing the supernatant for the test following centrifugation, and will not significantly affect the test results.
- Store the reagents at the specified temperature.
- Any agglutination should be confirmed by retesting with a fresh extract. If, upon retesting, the result is again indeterminate, the methicillin resistance can 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.

**11. LIMITATIONS**

- Intermittent results should be re-tested with a fresh extract. If, upon retesting, the result is again indeterminate, the methicillin resistance can 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.

**MATERIALS REQUIRED BUT NOT PROVIDED**

- Two 20-μl or 100-μl loops
- Microbiological loops (1μl/1μl)
- Boiling water bath or heating block
- Centrifuge (10,000 rpm)
- Microcentrifuge tubes (safe lock)
- Suitable laboratory disinfectant

**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 PROCEDURE**

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

1. **Positive Control**

   - Test a strain that possesses mecA and do not have the gene for any other known PBP.
   - Add 5 μl of broth culture and 1 μl of Control Latex to each 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, Catalogue No. 85010032). Place for 5 minutes one loop of culture on the primary isolation plate. For each new lot of the kit and weekly thereafter, the following control procedures must be performed.

3. **Indeterminate Control**

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

4. **5. PRECAUTIONS**

   - Do not use kits outside their expiry date.

   **8. IMPORTANT PROCEDURE NOTE**

   Do not allow the reagents to become contaminated by allowing the dropper tip to touch the surface of the control reaction. Ensure that the caps are not removed or placed to re-use after preparing and drying out the reagents. After use return the kit to the refrigerator ensuring that the test time is kept in an upright position.

5. **PREPARATION OF CULTURE**

   Colonies may be tested from any of the following culture media: Tryptone Soy Agar (Tryptic Soy Agar) with 5% sheep blood (TSA blood), Columbia Agar with 5% sheep blood, Mueller-Hinton Agar. The use of fresh cultures is recommended. However, cultures 24-48 hours old may be tested, if necessary to obtain sufficient growth, but the supernatant can only be easily absorbed into body fluids and tissues, cause fewer complications from treatment, and do not select for vancomycin-resistant organisms. Reliable identification of methicillin-resistant strains is important.

6. **STRAINS OF S. aUREUS WITH REDUCED SUSCEPTIBILITY TO PBP2/ ARE CATEGORISED AS follows:**

   - **mecA-negative S. aureus (MRSA), which produce the low-affinity penicillin-binding protein PBP2/ encoded by the meca gene**

   - **mecA-positive S. aureus (MSSA), generally considered to be due to hyperproduction of type β-penicillinase**

   - **mecA variants, which produce penicillin-binding proteins for which there is no inducible resistance to PBP2/ and require induction by exposure to one of the PRPs to produce sufficient product to be detected**


7. **F. THE USE OF FRESH (18-24 HOURS) CULTURES IS RECOMMENDED. HOWEVER, IN SOME LABORATORIES, PARTICULARLY FOR STRAINS OTHER THAN S. AUREUS EPIDERMIDIS, THE RESULTS CAN BE VERIFIED BY RETESTING WITH A FRESH CULTURE.**

8. **H. SOME ORGANISMS MAY HAVE A LOW LEVEL METHICILLIN-RESISTANCE OR, IN RARE CASES, PRODUCE PBP2/ IN LOW AMOUNTS, AND GIVE A FALSE-NEGATIVE RESULT.**


10. **J. THE PENICILLIN-BINDING PROTEIN (PBP2/) LATEX AGGLUTINATION TEST HAS BEEN EVALUATED IN FOUR GEOGRAPHICALLY DISTINCT REGIONS WITH FOUR CHLORAMPHENICOL-RESISTANT S. AUREUS, 201 REAGENTS WERE TESTED WITH NCCLS METHODS AND WITH THE OXOID PBP2/ TEST FROM EACH OF THREE MEDIA. ONE WEAK FALSE-NEGATIVE OXIDPair reaction (negative on repeat) was found from TSA with 5% sheep blood, Columbia Blood Agar Base. Despite a high degree of background clearing should be used to interpret the result. An opaque background indicates a negative result and a clear background is interpreted as a positive result.**

11. **K. LIMITATIONS**

   - Intermittent results should be re-tested with a fresh extract. If, upon retesting, the result is again indeterminate, the methicillin resistance can 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 (MDSA), and borderline resistant strains of S. aureus (BORA) do not possess PBP2/ and are not expected to react with the Oxoid PBP2/ test from each of three media. One weak false-positive OXIDPair reaction (negative on repeat) was found from TSA with 5% sheep blood, Columbia Blood Agar Base. Despite a high degree of background clearing should be used to interpret the result. An opaque background indicates a negative result and a clear background is interpreted as a positive result.
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

15. SYMBOL LEGEND

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