MAST
www.mastgrp.com
Mast Diagnostics, Mast House, Derby Road, Bootle, L20 1EA. Tel: 0151-427 1444 Fax: 0151-944 1332 sales@mastgrp.com

CE

MASTALEX™ - MRSA

RST501. A rapid slide latex test for the detection of penicillin binding protein 2' and the confirmation of Methicillin Resistant *Staphylococcus aureus*.

FOR IN VITRO DIAGNOSTIC USE ONLY

Contents

MASTALEX™ - MRSA contains the following components:

1. Extraction Reagent 1 (Green Coloured Cap). Ready to use. 1 x 10ml of 0.1M sodium hydroxide.
2. Extraction Reagent 2 (Yellow Coloured Cap). 1 x 2.4ml of 0.5M potassium dihydrogen phosphate.
3. Test Latex (Red Coloured Cap). Ready to use. 1 x 1.2ml of latex particles sensitised with anti-PBP2' monoclonal antibodies.
4. Control Latex (White Coloured Cap). Ready to use. 1 x 1.2ml of unsensitised latex particles.
5. One tin containing 100 single use disposable wooden mixing sticks.
6. 1 pack of 24 four-well reaction cards.
7. Instruction leaflet.

The latex reagents contain 0.08% sodium azide as preservative.

Stability and storage

Store unopened at 2-8°C until the expiry date shown on the pack label. Once opened, MASTALEX™ MRSA should be stored at 2-8°C and may be used until the expiry date given on the label. **Do not freeze reagents.**

Warnings and precautions

For *in vitro* diagnostic use only. Observe approved biohazard precautions and aseptic techniques. To be used only by adequately trained and qualified laboratory personnel. Sterilise all biohazard waste before disposal. Sodium azide preservative may be toxic if ingested and may react with lead and copper plumbing to form highly explosive salts. Always dispose of by flushing to drain with plenty of water. Refer to Product Safety Data sheet.

Materials required but not provided

Standard microbiological supplies and equipment such as standard 5µl loops or sterile disposable microbiological loop (internal volume 1.5µl), boiling water bath or heating block, microfuge or bench centrifuge, small tubes and pipettes.

Procedure

A. Sample preparation

Fresh cultures of organisms previously identified by biochemical and morphological tests as *Staphylococcus aureus*, i.e. Gram positive, coagulase positive cocci, should be used in the test. Organisms should be grown on blood agar or other suitable media at 35-37◦C for 18-24 hours.

B. Extraction Procedure

1. Allow the MASTALEX™ MRSA reagents to equilibrate to room temperature before use.
2. Dispense 4 drops (200µl) of Extraction Reagent 1 into a microfuge tube or other suitable plastic tube.
3. Using a standard 5µl sterile loop, take sufficient cells to just fill the internal diameter and thoroughly suspend them in the fluid. The total volume should be 3-5µl or approximately 1.5 x 10^9 cells. Alternatively 25-30 small (0.5mm diameter) or 4-5 large (2.5mm diameter) colonies should be used. If using a 1.5µl disposable sterile loop take a sufficient number of cells to fill the loop and thoroughly suspend them in the fluid. Repeat a second time. Two internal volumes of the loop/tube ≈ 1.5 x 10^9 cells.
4. Cap the tube and place in a boiling water bath or heating block set at 100°C for 3 minutes. If a heating block is used ensure that the tubes fit snugly into the block.
5. Remove the tube from the water bath or heating block and allow to cool to room temperature. Cooling time can be reduced if tubes are placed in a cold water bath or on ice.
6. After cooling add 1 (50µl) drop of Extraction Reagent 2 to the tube and mix well.
7. Centrifuge at 1500 g for five minutes or an equivalent i.e. 3000 rpm in a 15 cm rotor or 4500 rpm in a 4.5 cm rotor Alternatively, a microfuge may be used for 1 - 5 minutes.
8. Use the supernatant as the test specimen in the latex agglutination procedure.
9. **Note:-** Ensure that the precipitated material is not disturbed or used in the agglutination procedure as non-specific agglutination may occur.
10. The test specimen may be stored at 2-8°C for later use that day or stored at -70 - -80°C for longer term storage. For specimens stored at -70 to -80°C avoid repeated freezing and thawing.

C. Latex Agglutination Procedure

Note:- Ensure that the latex reagent bottles are brought to room temperature and that the Test and Control Latex reagents are sufficiently shaken before use to give a uniform suspension directly before use.

Note:- when multiply dispensing hold the bottle in a completely vertical position and pause slightly between dispensing drops. Do not allow reagents to come into direct contact with the specimen on the reaction card while dispensing. After use ensure that all reagent bottles are securely capped.

1. For each specimen to be tested place 50 µl of the supernatant into two circles of a pre-labelled Test Card. To one circle add one drop (25µl) of Test Latex and to the other circle add one drop (25µl) of Control Latex.
2. Mix the supernatant and latex together on each well using separate mixing sticks as provided, and spread the mixture over the area of the black circle on the reaction card. Rotate the slide by hand or using a mechanical mixing table for three minutes and observe for agglutination by eye.
3. After three minutes, place reaction cards on the bench and observe the circles for signs of agglutination and record the results.

Interpretation of results

- A positive agglutination reaction with the Test Latex only and not the Control Latex indicates that the organism contains PBP2' and should be reported as a presumptive methicillin-resistant *Staphylococcus aureus* (MRSA). Degrees of positivity may be scored as follows:

Strong agglutination against a clear background.	3+
Agglutination against a slightly turbid background.	2+
Slight agglutination against a turbid background.	1+
Homogeneous white suspension with no visible agglutination.	-

- A negative reaction with both Test and Control Latex indicates that the organism contains no PBP2' and should be reported as a presumptive methicillin-sensitive *Staphylococcus aureus* (MSSA).
- If a positive agglutination reaction is seen with the Control Latex the test should be classified as indeterminate and repeated.
- Non-specific reactions may result if the amount of cells used is too great.
- Indeterminate results should be retested. When doing so ensure that the heating and centrifugation steps are followed as given in the procedure. Heating for more than five minutes may lead to a decrease in sensitivity and heating for one minute or less may lead to non-specific agglutination. If on retesting the sample still gives an indeterminate result, an alternative method should be used e.g. antimicrobial susceptibility or Polymerase Chain Reaction (PCR) testing.
- In very rare cases false negatives may result if the *S. aureus* isolate produces low levels of PBP2', although prolonged incubation for 10 minutes should result in a true positive result. On the other hand, some MSSA strains may show false positive reactions due to the prolonged reaction time. Accordingly, any strain showing agglutination after 3 minutes should be retested using an inducer medium containing oxacillin or ceftizoxime. In addition antimicrobial susceptibility testing, PCR testing etc. is recommended. If MIC values are required standard methods should be followed e.g. National Clinical Laboratory Standards (NCCLS).
- Negative results obtained with this kit should be considered along with other clinically relevant data when diagnosing an MRSA infection. In particular, retesting should be performed if during the course of a *S. aureus* infection prognosis indicates treatment failure etc.

Limitations of use

- The test is designed for presumptive identification of MRSA organisms. Other organisms producing the PBP2' gene product may also give a positive result.
- Some organism strains may have a low level of methicillin resistance or in rare cases produce PBP2' in low amounts. Appropriate antimicrobial susceptibility testing is recommended for such cases.
- Methicillin-resistant coagulase negative staphylococci (CNS) produce PBP2' and this kit will detect its presence however a full validation of its diagnostic use for CNS has not been made. It is thus not recommended for use with CNS.

Quality control

Check for signs of deterioration. Quality control must be performed with at least one organism to demonstrate a positive reaction and at least one organism to demonstrate a negative reaction. Do not use the product if the reactions with the control organisms are incorrect. The list below illustrates a range of performance control strains which the end user can easily obtain.

Test Organisms	Result	
	Test latex	Control latex
Staphylococcus aureus ATCC® 25923	No agglutination	No agglutination
Staphylococcus aureus ATCC® 33591 (MRSA)	Agglutination	No agglutination

References

Bibliography available on request.

IFU154 DNH 02/06 V6