05-03-2000

MRSA-Screen

A slide latex agglutination kit for the rapid detection of PBP2'

CE IVD

Symbols

Batch code
Chargenbezeichnung

Use by
Verwendbar bis

Contains sufficient for <n> tests
Ausreichend für "n" Ansätze

In Vitro Diagnostic Medical Device
In Vitro Diagnostikum

Temperature limitation (Store at)
Zulässiger Temperaturbereich (Aufbewahrung bei)

Catalogue number
Bestellnummer

Contents of kit
Inhalt der Packung

Consult Instructions for Use-
Gebrauchsanweisung beachten

INTENDED USE

MRSA-Screen is a qualitative slide latex agglutination test for the detection of PBP2' (PBP2a) present in isolates of *Staphylococcus aureus* and is thus useful as an aid in identifying methicillin-resistant *Staphylococcus aureus* (MRSA).

SUMMARY AND EXPLANATION

Methicillin-resistant *S. aureus* has become a worldwide concern owing to their increasing frequency in hospitals causing serious staphylococcal infections, including sepsis and endocarditis. Rapid and appropriate antimicrobial therapy, including the administration of vancomycin, is critical for effective treatment. However, conventional methods for identifying MRSA, such as disc susceptibility testing, are not always reliable since phenotypic expression of methicillin resistance is known to be heterogeneous, depending on such factors as incubation time, temperature, NaCl concentration, etc. Difficulties in the differentiation of MRSA from borderline oxacillin-resistance *S. aureus* (BORSA), for example, may also occur. Recent research suggests that in the identification of MRSA, it is more accurate to either directly detect the gene encoding the methicillin resistance determinant (*mecA*) or its product, penicillin-binding protein 2' (2a), or PBP2' (PBP2a), which is found in the cell membrane of MRSA. However, as nucleic acid hybridization and DNA amplification techniques such as PCR for detecting the *mecA* gene are expensive and technically demanding, simple and more inexpensive techniques are required for routine use. MRSA-Screen was developed expressly for this purpose, providing results in 15 minutes with minimal labor and no specialized equipment.

PRINCIPLE OF TEST

MRSA-Screen consists of a latex reagent sensitized with monoclonal antibody against PBP2' together with reagents to rapidly extract PBP2' from the bacterial membranes of MRSA. Extracts are prepared by boiling a suspension of *S. aureus* cells under alkaline conditions, followed by a neutralization and a centrifugation step. The supernatant is then mixed with the latex reagent on a test card and visible clumping or agglutination within three minutes indicates the presumptive presence of PBP2'.

REAGENTS

50-test kit, code 230782

1. **Sensitized Latex** in a dropper bottle (1 x 1.3 mL) with a pink cap. Latex particles are sensitized with a monoclonal antibody prepared against PBP2'. Contains 0.08 w/v% sodium azide as a preservative.
2. **Control Latex** in a dropper vial (1 x 1.3 mL) with a blue cap. Latex particles are sensitized with a monoclonal antibody with no reactivity to PBP2'. Contains 0.08 w/v% sodium azide as a preservative.
3. **Extraction Reagent 1** in a dropper vial (1 x 10 mL) with a green cap. A solution of 0.1 mol/L NaOH.
4. **Extraction Reagent 2** in a dropper vial (1 x 2.5 mL) with a yellow cap. A solution of 0.5 mol/L KH_2PO_4.
5. **Test card**, 30
6. **Mixing stick**, 110
7. **Product insert**

SHELF LIFE AND STORAGE

Store at 2-10 °C. Reagents are stable before and after opening vials when stored under these conditions until the expiry date written on the box.

WARNINGS AND PRECAUTIONS

This product is for in vitro diagnostic use only. As reagents in this kit (Sensitized Latex and Control Latex) are prepared from biological materials, and due to the potential infectious nature of the isolates being tested, proper handling and disposal methods should be established and only trained personnel should be permitted to perform the procedures. Both latex reagents contain 0.08 w/v% sodium azide as a preservative. Disposal by flushing with copious amounts of water is necessary since sodium azide may react with lead or copper piping to form highly explosive sodium azides.

Also, the following general precautions should be followed:

1. The MRSA-Screen test is intended for *in vitro* diagnostic use.
2. Read instructions completely and carefully before performing the test.
3. Do not freeze the reagents nor use past the expiration date as this may result in poor reagent performance.
4. Bring the kit to room temperature before use each time.
5. Ensure that latex reagents form a homogenous suspension before use by gently inverting and shaking the vials. Avoid extreme or excessive shaking, vortexing, etc. as this may impair reagent performance.
6. Do not interchange reagents between different lot numbers, or caps between different reagent vials, etc.
7. Use aseptic laboratory techniques and never mouth pipette.
8. If reagents come into contact with the skin, mucous membranes or eyes, wash immediately with plenty of water. Extraction Reagents 1 and 2 are slightly basic and acidic, respectively. Seek medical treatment if serious reactions develop.
9. Sterilize all specimens, spills, inoculated products and equipment used in this test by one of the following methods:
    a) soaking in 2 vol% glutaraldehyde solution
    b) soaking in 0.1 w/v% hypochlorite for one hour or more
    c) autoclaving at 121 °C for 20 minutes or more

SPECIMEN HANDLING

In general, standard microbiological procedures should be followed and only isolates with biochemical, growth and morphological characteristics of *S. aureus*, i.e. gram positive cocci that are coagulase positive, or, as identified by a rapid *S. aureus* identification test, should be used. Isolates should be fresh, 18-24 hour cultures grown at 35 °C. Recommended culture media for use with this kit are blood agar plates, such as Tryptic soy agar with 5% sheep blood, Columbia agar with 5% sheep blood and Mueller-Hinton agar. Finally, after extraction of PBP2' and preparation of the test specimen for use in the latex test, perform the test that same day. The test specimen may be stored refrigerated at 2-10 °C for batch testing later in the day or at −80 °C for long term storage.

IMPORTANT PROCEDURAL NOTE

When dispensing latex reagents, hold the vial in a completely vertical position and pause slightly between dispensing of latex reagent drops, such as when performing more than one test at a time. Do not allow reagent vial nozzle tips to come in contact with the specimen on the slide card. After use, ensure that the reagent vial caps are snugly but not too tightly capped.

TEST PROCEDURE

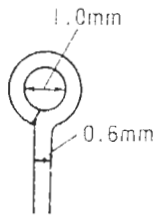
1. Materials and reagents necessary for the test

1) MRSA-Screen

The following materials are required but not provided:

2) Specimen: Isolated colonies of *S. aureus* grown on blood agar plates for less than 24 hours.

3) Equipment: Micropipette and tips(50 µL)
Microcentrifuge tubes(safe lock)
Microbiological loop:
internal volume 1.0 - 1.5 µL(or 5 µL)
(1 mm internal diameter, 0.6 mm thickness)
Boilling water bath or heating block
(95 °C or greater)
Centrifuge/microcentrifuge
Rotary platform(optical)



2. Reagent preparation

All reagents are used as supplied but bring kit to room temperature and ensure latex reagents form homogenous suspensions before use.

3. PBP2' Extraction Procedure

1. Add 4 drops (200 µL) of Extraction Reagent 1 into a microcentrifuge tube or equivalent.
2. Using a 1.5 µL sterile loop (1 mm internal diameter) take sufficient bacterial growth to fill the internal volume and thoroughly suspend in the tube. Repeat a second time. (Gives approx. 1.5 x 10^9 cells/tube)

Note: A 5 µL sterile loop may also be used, taking cell growth to just fill the internal diameter. The total volume of cells in either case should be about 3 – 5 µL. As a guide, this is approximately 25 – 30 small or 4 – 5 large colonies with diameters of 0.5 mm and 2.5 mm, respectively.

3. Cap the tube and place into boiling waterbath or heating block at 95 - 100 °C and heat for 3 minutes. If a heating block is used, ensure that the tube fits snugly into the block well.
4. Remove and allow cooling to room temperature. (For more rapid cooling place on ice.)
5. Add 1 drop (50 µL) of Extraction Reagent 2 into the tube and mix well.
6. Centrifuge at 1500xg for 5 minutes or equivalent, i.e., 3000 rpm with a 15 cm rotor radius; 4500 rpm with a 4.5 cm rotor radius, etc.; use supernatant as the test specimen.

Note: Remove supernatant immediately after centrifugation. When pipetting the specimen for the latex agglutination test below, avoid taking up pelleted cell debris as this may cause non-specific reactions with the latex reagents. The specimen may be stored at 2 - 10 °C for testing later in the day.

4. Latex Agglutination Procedure

1. For each specimen, allot and label two circles on the test card, one as test and the other as control.
2. Place 50 µL of the specimen onto each of the test and the control circles.
3. To the test circle, add 1 drop (25 µL) of Sensitized Latex and to the control circle, 1 drop (25 µL) of Control Latex.
4. With separate mixing sticks, thoroughly mix each reagent with specimen over the area of the circle.
5. Rotate the test card by hand or mechanical rotary platform for 3 minutes. Place the test card on the bench and read the agglutination patterns by eye. Record the results.

INTERPRETATION

1. Judgement is made as follows:

	Test Latex	Control Latex	Interpretation	Report
Strong agglutination (3+)	+	−	+ (PBP2' positive)	MRSA
Agglutination against slightly turbid background (2+)	+	−	+ (PBP2' positive)	MRSA
Slight agglutination against turbid background (1+)	+	−	+ (PBP2' positive)	MRSA
	+	+	Indeterminate	Do not report
No agglutination	−	−	−	*mecA* negative

Note: The degree of positivity shoud not be interpreted to indicate levels of antibiotic resistance.

2. Precautions for interpretation

1. A positive reaction is indicated by the clear development of an agglutination pattern within 3 minutes. Although the size and appearance of the aggregates of latex particles may vary, agglutination is visible to the unaided eye. A negative reaction is indicated by a homogenous suspension or milky appearance of both latex reagents, although traces of granularity may be seen due to the particulate nature of the latex reagents. Care should be taken in not overreading such granularity as true positive reactions. (Also, see Limitations of the Procedure below)
2. Specimens showing indeterminant results should be retested, taking care to follow the protocol exactly as written, particularly with respect to the length of boiling, centrifugation and agglutination steps. Boiling for less than 3 minutes or insufficient centrifuging may result in non-specific reactions and boiling for over 5 minutes may decrease sensitivity.
3. Specimens repeatedly showing indeterminate results should be tested by another method.

QUALITY CONTROL

Proper reactivity of the Sensitized Latex and Control Latex should be confirmed with previously identified MRSA and MSSA upon receipt of this kit and periodically in accordance with the laboratory's standard quality control practice to serve as both a reagent and procedural control. Recommended strains for this purpose are *S. aureus* ATCC 43300 (MRSA) and *S. aureus* ATCC 25923 (MSSA), respectively. The former should show clear agglutination with the Sensitized Latex reagent with a strength of about 2+ within 3 minutes, while the latter should show a negative reaction. The Control Latex must not show agglutination with either organism.

Control strain	Test Latex	Control Latex	Interpretaion
S. aureus ATCC 43300	+	−	+
S. aureus ATCC 25923	−	−	−

LIMITATIONS OF THE PROCEDURE

1. Negative results obtained with this kit should be considered 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.
2. In very rare cases, false negatives will occur if the strain produces low amounts of PBP2'. Accordingly, other antibiotic susceptibility testing methods as recommended by the current National Committee for Clinical Laboratory Standards (NCCLS) should be performed.
3. It is strongly recommended that laboratory personnel using this kit thoroughly familiarize themselves with the procedure and reading of agglutination patterns before employing the test routinely in a clinical setting.
4. Other mechanisms of methicillin resistance exist which are not detected by this kit, including the hyperproduction of ß-lactamase (BORSA) and other altered PBPs (MODSA).
5. The test should not be used to detect *mecA* in coagulase-negative staphylococci.
6. The test should not be performed on a direct specimen such as a blood culture, etc.

PERFORMANCE CHARACTERISTICS

MRSA-Screen has been evaluated at a number of geographically diverse laboratories using clonally distinct isolates of *S. aureus* (1, 5, 6, 7, 8, 9 and others on file). The test was also evaluated and compared to the NCCLS reference methods for microbroth dilution and oxacillin agar screen tests with 6 µg/mL oxacillin on 726 clinical isolates of coagulase-positive *S. aureus* collected at 3 North American sites to challenge the test for a wide range of phenotypically distinct strains of *S. aureus* as well as on 201 fresh isolates of *S. aureus* at 4 North American sites. Below summarizes agreement between MRSA-Screen and oxacillin agar screen:

Ox MIC	Oxacillin Screen Agar Sensitive (n=433)			Oxacillin Screen Agar Resistant (n=494)		
	MRSA-Screen		Indeterminate	MRSA-Screen		Indeterminate
	Positive	Negative		Positive	Negative	
≤0.5	0	350	0	2	0	0
1	2	41	0	1	0	0
2	1	28	0	1	3	0
4	1	8	0	3	12	0
8	1	1	0	3	20	0
≥16	0	0	0	446	3	0
TOTAL	5	428	0	456	38	0

Agreement	**98.8%**	**92.3%**
Confidence interval	**98.1 - 99.9%**	**89.6 - 94.4%**
n	433	494
p	98.8%(428/433)	92.3%(456/494)
confidence level	95%	95%

		MIC		
		Positive	Negative	
MRSA-Screen	Positive	454	7	461
	Negative	44	422	466
		498	429	927

Agreement 94.5% (876/927)

		Oxacillin Agar		
		Positive	Negative	
MRSA-Screen	Positive	456	5	461
	Negative	38	428	466
		494	433	927

Agreement 95.4% (884/927)

Note 1 : Overall agreement between the MRSA-Screen and the oxacillin agar screen was 95.4%. *MecA* analysis by PCR for forty two of the discrepant strains showed 85.7%(36/42) agreement with MRSA-Screen and 14.3% (6/42) with oxacillin agar screen.

Note 2 : For the 201 fresh isolates of *S. aureus* tested, the agreement between the MRSA-Screen test and both the oxacillin agar screen and microbroth dilution were 100% (201/201) for the detection MRSA on Columbia blood agar and Mueller-Hinton agar, while for TSA blood agar the agreement was 99.5% (200/201)

REPRODUCIBILITY

Ten different well-characterized *S. aureus* strains (3 MRSA, 3 MSSA, 3 BORSA and 1 MODSA) were sent to three laboratories with each strain submitted five times in 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 tested (45/45); the three MSSA and the three BORSA, negative each time tested (90/90); and the MODSA, which had an MIC of 16 µg/mL to oxacillin, similarly, was negative in all tests (15/15) as expected.

PACKAGE CONTENTS

MRSA-Screen

50 test kit, Code Number 230782

REFERENCES

1) Cavassini M., et. al, Evaluation of MRSA-Screen, a simple anti-PBP2a slide latex agglutination kit, for rapid detection of methicillin resistance in *Staphylococcus aureus*. J. Clin. Microbiol., 1999, **37**, p. 1591-1594.
2) Chambers, H. F., Methicillin resistance in Staphylococci: Molecular and biochemical Basis and Clinical Implications. Clin. Microbiol. Rev., 1997, **10**, p. 781-791.
3) Hartman, B. J., and Tomasz A., Low affinity penicillin-binding protein associated with beta lactam resistance in *Staphylococcus aureus*. J. Bacteriol., 1984, **158**, p. 513-516.
4) O'Hara, D. M., and Reynolds P. E., Antibody used to identify penicillin binding protein 2' in methicillin-resistant strains of *Staphylococcus aureus*: FEBS Left. 1987. Vol212, p237-241.
5) Nakatomi, Y. and Sugiyama, J., A rapid latex agglutination assay for the detection of penicillin binding protein 2'. Microbiol. Immunol., 1998, **42**, p. 739-743.
6) van Griethuysen, et. al, Rapid slide latex agglutination test for rapid detection of methicillin resistance in *Staphylococcus aureus*. J. Clin. Microbiol., 1999, **37**, p. 2789-2792.
7) van Leeuwen, et. al, Rapid detection of methicillin resistance in *Staphylococcus aureus* isolates by the MRSA-Screen latex agglutination test. J. Clin. Microbiol., 1999, **37**, p. 3029-3030.
8) Louie, L., et. al., Evaluation of three rapid methods for detection of methicillin resistance in *Staphylococcus aureus*. J. Clin. Microbiol., 2000, **38**, p. 2170-2173.
9) Yamazumi, T., et. al, Comparison of the Vitek-gram positive susceptibility 106 card and the MRSA-Screen latex agglutination test for determining oxacillin resistance in clinical bloodstream isolates of *Staphylococcus aureus*. J. Clin. Microbiol., 2001, **39**, p. 53-56.