MASTALEX™ MRSA RST501

Performance data

General description of the product and intended use

Product name	MASTALEX™ MRSA
Catalogue number:	RST501
Manufacturer:	Mast Group Ltd., Mast House, Derby Road, Bootle, Liverpool, Merseyside. L20 1EA. UK.
Product description:	The MASTALEX™ MRSA kit 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). Ready to use. 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. 100 single use disposable mixing sticks. 6. 1 pack of 24 four-well reaction cards. 7. Instruction leaflet.
Intended purpose:	The MASTALEX™ MRSA kit A rapid slide latex test for the detection of penicillin binding protein 2' and the confirmation of Methicillin Resistant *Staphylococcus aureus*.
Functionality:	MASTALEX™ MRSA is intended for presumptive identification and confirmation of Methicillin Resistant *Staphylococcus aureus* from clinical samples.
Type of test:	Qualitative. There is no minimum level of detection assigned as there is no Methicillin Resistant *Staphylococcus aureus* from clinical samples.
Type of specimen:	Swab specimens normally taken from skin or sites in the body and cultured in the laboratory on a suitable agar or broth medium isolate and identify a *Staphylococcus aureus* and then grown on a suitable medium to obtain pure colonies of the organism.
Intended user:	Trained laboratory technical staff.

Description and explanation of product basis and use

Staphylococcus aureus has been well known as a major pathogen for many years causing septicaemia, endocarditis, osteomyelitis, abscesses, pneumonia, wound infections, impetigo, boils, and a variety of toxin mediated diseases[1]. Before the antibiotic era *S. aureus* bacteraemia resulted in a mortality rate of 80%[2]. The introduction of penicillins in the 1940s dramatically changed this situation. However, it was not long before *S. aureus* strains resistant to penicillins appeared and so semi-synthetic penicillins such as methicillin were introduced from 1959. Only 2 years after their introduction the first methicillin-resistant *Staphylococcus aureus* strain (MRSA)[3] was described and the first nosocomial outbreak was reported soon afterwards[4]. During the 1970s little notice was taken of MRSA as most strains were non-multiply resistant to other classes of antibiotics and outbreaks of infection seemed manageable. Interest was rekindled in the early 1980s with the recognition that single strains were causing nosocomial outbreaks in a number of hospitals in the south-east of England and in other countries notably Australia and USA[5]. A survey in 1988 identified 14 strains of MRSA as causing nosocomial outbreaks affecting more than one hospital in England and Wales[6]. These strains were termed epidemic MRSA (EMRSA) strains; EMRSA-1 was by far the most common affecting 50 hospitals. Since 1990 two new epidemic strains have emerged EMRSA-15 and EMRSA-16[7]. Of particular concern is EMRSA-16 which is resistant to many anti-staphylococcal antibiotics including erythromycin, gentamicin, neomycin, ciprofloxacin and trimethoprim. MRSAs and multiple resistant *S. aureus* have now been reported throughout the world with varying degrees of prevalence.

Bacteria have surface membrane localised proteins with binding affinity for β-lactam antibiotics called penicillin binding proteins (PBPs) which are expressed constitutively. Penicillin -susceptible staphylococci have 5 PBPs labelled PBP1, PBP2, PBP3, PBP3' and PBP4 and these act as peptidoglycan transpeptidases[8]. In classical methicillin resistance an additional PBP is present and is referred to as PBP2a or PBP2'[9,10]. PBP2' has a low binding affinity for β-lactam antibiotics and for this reason unsaturated PBP2' may take over as the main transpeptidase for continued peptidoglycan synthesis at methicillin concentrations which have resulted in saturation and inhibition of the normal PBPs[8]. The chromosomal located gene *mecA* is the structural gene responsible for encoding PBP2' and its expression is regulated by a number of plasmid and chromosomal-borne genes[11]. It is noteworthy that methicillin resistance may also be associated with mechanisms independent of *mecA* resulting in borderline resistance. These include β-lactamase hyper-production, production of methicillinases, acquisition of structurally modified normal PBPs, or the appearance of small colony variants of *S. aureus*[8].

With the continuously high prevalence of MRSA world-wide and the increasing problem of epidemic nosocomial multi-drug resistant strains, appropriate management and treatment of infection is important. Antimicrobial therapy is critical in such infections but conventional methods for identifying

MRSA, such as 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[12]. Recent research suggests that it is more appropriate to either directly detect the *mecA* gene coding for methicillin resistance or its product PBP2', when identifying MRSA. Methods for detecting the *mecA* gene e.g. PCR, can take time and are too expensive for routine testing[13]. MASTALEX™-MRSA is a sensitive and rapid slide agglutination assay which detects PBP2' present in MRSA, using latex sensitised with a monoclonal antibody directed against PBP2'.

MASTALEX™ MRSA kit is a rapid latex test for the detection of penicillin binding protein 2' and the confirmation of Methicillin Resistant *Staphylococcus aureus*. The method of use is described in Mast product IFU154. The procedure involves alkaline extraction followed by heat treatment of bacterial samples followed by testing the supernatant obtained following extraction with latex particles sensitised with anti-PBP2' monoclonal antibodies.

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°C to 37°C for 18 to 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 to 5µl or approximately 1.5×10^9 cells. Alternatively 25 to 30 small (0.5mm diam.) or 4 to 5 large (2.5mm diam.) 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 $\approx 1.5 \times 10^9$ 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 15cm rotor or 4500 rpm in a 4.5 cm rotor Alternatively, a microfuge may be used for 1 to 5 minutes.
8. Remove the supernatant immediately after centrifugation and use as the test specimen in the latex agglutination procedure.

Note:- Ensure that the precipitated material is not disturbed or used in the agglutination procedure as non-specific agglutination may occur.

9. The test specimen may be stored at 2 to 8°C for later use that day or stored at minus 70°C to minus 80°C for longer term storage. For specimens stored at minus 70°C to minus 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 50µl 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 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

- 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 aureu*s (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'. It is recommended that alternative antimicrobial susceptibility procedures should be conducted following standard methods e.g. Clinical and Laboratory Standards Inst. (CLSI®).
- 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

- 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.
- Other mechanisms of methicillin resistance exist which are not detected by this kit, including the hyperproduction of β-lactamase (BORSA – borderline oxacillin resistant Staphylococcus aureus) and other altered PBPs (MODSA – modified Staphylococcus aureus).
- The test should not be performed on a direct specimen such as blood culture.

References

1. Arbuthnot JP. Staphylococcus: skin and wound infections; abscess; osteomyelitis; food poisoning. Ch. 15 pp 203-210. In: Medical Microbiology. 14th ed. Greenwood D, Slack RCB and Peutherer JF, editors. 1992: Churchill Livingstone.
2. Smith IM, Wickers AB. Natural history of 338 treated and untreated patients with staphylococcal septicaemia (1936-1955). *Lancet.* 1960; **1**: 1318-1322.
3. Jevons PM. Celbenin resistant staphylococci. *Letter BMJ.* 1961; **1**: 40-46.
4. Stuart GT, Holt RJ. Evolution of natural resistance to the newer penicillin. *BMJ.* 1963; **1**: 1309-1311.
5. Kerr S, Kerr GE, Mackintosh GA, Marples RR. A survey of methicillin resistant *Staphylococcus aureus* affecting patients in England and Wales. *J Hosp Infect.* 1990; **16**: 35-48.

6. Epidemic methicillin resistant *Staphylococcus aureus*. *Communicable Disease Report.* 1995; **5**: 165.
7. Brakstad OG, Maeland JA. Mechanisms of methicillin resistance in staphylococci. Review article. *APMIS.* 1997; **105**: 264-276.
8. Pavillard R, Harvey K *et al*. Epidemic of hospital acquired infection due to methicillin-resistant *Staphylococcus aureus* in major Victoria hospitals. *Med J Aust.* 1982; **1**: 451-454.
9. Georgopapadakou NH, Smith SA, Banner DP. Penicillin-binding proteins in a *Staphylococcus aureus* strain resistant to specific β-lactam antibiotics. *Antimicob Agents Chemother*. 1982; **22**: 172-175.
10. Hartman BJ, Tomasz A. Low affinity penicillin-binding protein with β-lactam resistant *Staphylococcus aureus*. *J Bact.* 1984; **158**: 513-516.
11. Song MD, Wachi M, Doi M, Ihno F, Matsuashi M. Evolution of an inducible penicillin-target binding protein in methicillin resistant *Staphylococcus aureus* by gene fusion. *FEBS Lett*. 1987; **221**: 167-171.
12. Cookson B, Phillips I. Methicillin resistant staphylococci. *J Appl Bact Symposium Suppl.* 1990; 55S-70S.
13. Hiramatsu K, Kihara H, Yokota T. Analysis of borderline strains of methicillin resistant *Staphylococcus aureus* using polymerase chain reaction. *Microbiol Immunol.* 1992; **36**: 445-453.
14. Andrews JM, Boswell FJ, Wise R. Establishing MIC breakpoints for coagulase-negative staphylococci to oxacillin. *J Antimicrob Chemother.* 2000; **45**: 259-261.
15. Graham JC, Phillips JA, Murphy OM *et al*. Use of Mastalex to detect methicillin resistance in coagulase-negative staphylococci. *J Antimicrob Chemother.* 2000; **46**: 850.
16. Brown DFJ, Walpole Evaluation of the Mastalex latex agglutination test for methicillin resistance in *Staphylococcus aureus* grown on different screening media. *J Antimicrob Chemother*. 2001; **47**: 187-189

The test reports

Reports relating to the use of the MRSA latex agglutination test kit for the detection of penicillin binding protein 2' and the confirmation of Methicillin Resistant *Staphylococcus aureus* and potential use with coagulase-negative staphylococci are as follows:

1. Nakatomi Y, Sugiyama J. A rapid latex agglutination assay for the detection of Penicillin-Binding Protein 2'. M*icrobiol Immunol*. 1998; **42**: 739-743.
2. Brown DFJ, Walpole E. Evaluation of the Mastalex latex agglutination test for methicillin resistance in *Staphylococcus aureus* grown on different screening media. *J Antimicrob Chemother*. 2001; **47**: 187-189.
3. Internal evaluation reports for Mastalex MRSA at Edinburgh University Medical School; Clatterbridge Hospital, Wirral; PHL Cambridge. All held on file at Mast Group Ltd.
4. van Griethuysen A, Pouw M, van Leeuwen N, Heck M, Willemse P, Buiting A, Kluytmans J. Rapid slide latex agglutination test for detection of methicillin resistance in *Staphylococcus aureus*. *J Clin Microbiol.* 1999; **37**: 2789-2792.
5. Cavassini M, Wenger A, Jaton K, Blanc DS, Bille J. Evaluation of MRSA-Screen, a simple Anti-PBP 2a slide latex agglutination kit, for rapid detection of methicillin resistance in *Staphylococcus aureus*. *J Clin Microbiol.* 1999; **37**: 1591-1594.
6. van Leeuwen B, van Pelt C, Luijendijk A, Verbrugh HA, Goessens WHF. Rapid detection of methicillin resistance in *Staphylococcus aureus* isolates by the MRSA-Screen latex agglutination test. *J Clin Microbiol.* 1999; **37**: 3029-3030.
7. Marriott DJE, Karaagiannis T, Harkness JL, Kearney P. Further evaluation of the MRSA-Screen kit for rapid detection of methicillin resistance. *J Clin Microbiol.* 1999; **37**: 3783-3784.
8. Andrews JM, Boswell FJ, Wise R. Establishing MIC breakpoints for coagulase-negative staphylococci to oxacillin. *J Antimicrob Chemother.* 2000; **45**: 259-261.
9. Bowers KM, Wren MWD, Shetty NP. Screening for methicillin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci: an evaluation of three selective media and Mastalex-MRSA latex agglutination. . Br *J Biomed Sci*. 2003; **60**: 71-74.
10. Graham JC, Phillips JA, Murphy OM, Kearns AM, Gould FK, Freeman R. Use of Mastalex to detect methicillin resistance in coagulase-negative Staphylococci. *J Antimicrob Chemother*. 2000; **46**: 850.
11. Cavassini M, Wenger A, Jaton K, Bille J, Blanc DS. Further evaluation of the MRSA-Screen kit for rapid detection of methicillin resistance. (Authors' reply) *J Clin Microbiol.* 1999; **37**: 3783-3784.

Performance evaluation data

Study 1

An original evaluation of the MRSA-Screen rapid latex agglutination PBP 2' assay by Nakatomi and Sugiyama[1] tested 319 clinical isolates of *Staphylococcus aureus* that were previously classified as either methicillin-resistance *Staphylococcus aureus* (MRSA) or methicillin-sensitive *Staphylococcus aureus* (MSSA) by antibiotic susceptibility testing procedures using Oxacillin agar screening medium

Staphylococcus aureus classification according to susceptibility test results	Number PBP2' positive by latex test	Number PBP2' negative by latex test	Total
MRSA	231	1*	232
MSSA	0	87	87
Total	231	88	319

*This strain was identified as MRSA by susceptibility testing, PBP2' negative by latex agglutination but was confirmed as *mecA* gene negative by PCR.

Study 2

van Griethuysen et al[4] evaluated the MRSA-Screen rapid latex agglutination PBP 2' assay in comparison with other standard tests used including oxacillin agar screen test and PCR detection of the *mecA* gene. They tested a total of 563 *Staphylococcus aureus* isolates of which 296 had previously been classified as methicillin-susceptible isolates. The results of their test are shown in the following table:

PCR detection results	Total no. of isolates	MRSA Screen test		Oxacillin agar screen test	
		Positive	Negative	Positive	Negative
mecA positive	267	263	4	250	17
mecA negative	296	0	296	0	296
Total	563	263	300	250	313

Analytical sensitivity of MRSA Screen = 98.5%

Analytical sensitivity of Oxacillin agar screen test = 93.6%

Analytical specificity of both tests = 100%

Measurand – Presence or absence of PBP2' proteins extracted from bacterial isolates

Cut-off – Agglutination of latex particles by slide agglutination test within 3 minutes = positive

No agglutination of latex particles = negative

Conclusion: The MRSA Screen test is a rapid, easy-to-perform and highly reliable, sensitive and specific test for the detection of methicillin-resistance in *Staphylococcus aureus*. Results are available in approximately 20 minutes whereas PCR detection of the *mecA* genes and susceptibility method take several hours

Study 3

Cavassini et al[5] evaluated the MRSA-Screen rapid latex agglutination PBP 2' assay in comparison with other standard tests used including oxacillin disc susceptibility testing, oxacillin-salt agar screen test and PCR detection of the *mecA* gene. They tested a total of 200 *Staphylococcus aureus* isolates of which 120 had previously been classified as methicillin-susceptible isolates (MSSA) and 80 as methicillin-resistant isolates (MRSA). The results of their test are shown in the following table:

Test method	MRSA (mecA gene positive) N=80			MSSA (mecA gene negative) N=120		Sensitivity (%)	Specificity (%)
	True Positive	False negative	Indeterminate	True negative	False positive		
MRSA Screen	80	0	0	119	1*	100	99.2
Oxacillin disc diffusion	49	20	11	116	4	61.3	96.7
Oxacillin salt agar screen	66	14	0	118	2	82.5	98.3

* = This isolate when retested gave no agglutination.

Analytical sensitivity of MRSA Screen = 100%
Analytical specificity of both tests = 99.2%
Measurand – Presence or absence of PBP2' proteins extracted from bacterial isolates
Cut-off – Agglutination of latex particles by slide agglutination test within 3 minutes = positive
No agglutination of latex particles = negative

Conclusion: The MRSA Screen test is a rapid, easy-to-perform and shows higher sensitivity and specificity compared to the standard disc diffusion test and oxacillin salt agar screening test for the detection of methicillin-resistance in *Staphylococcus aureus*.

Study 4

van Leeuwen et al[6] evaluated the MRSA-Screen rapid latex agglutination PBP 2' assay in comparison with *mecA* PCR test as the "gold standard" standard test. They tested a total of 246 bacterial isolates including 90 MRSA, 106 MSSA, 10 methicillin-resistant *Staphylococcus epidermidis* (MRSE), 10 *Micrococcus* species, 10 members of the genus *Enterobacteriaceae*, 10 Strepto*coccus pneumoniae* and 10 *Enterococcus* species. The results of their test are shown in the following table:

Organism	Total no. of isolates	MRSA Screen test		Oxacillin agar screen test	
		Positive	Negative	Positive	Negative
MRSA	90	90	0	87	3
MSSA	106	0	106	0	106
MRSE	10	10	0	10	0
Others	40	0	40	0	40

Analytical sensitivity of MRSA Screen = 97%

Analytical specificity of both tests = 100%

Measurand – Presence or absence of PBP2' proteins extracted from bacterial isolates

Cut-off – Agglutination of latex particles by slide agglutination test within 3 minutes = positive

No agglutination of latex particles = negative

Conclusion: The MRSA Screen test is a rapid, and highly sensitive and specific test for the detection of methicillin-resistance in *Staphylococcus aureus*. A positive result is a definite indication of presence of an MRSA however for a negative result they recommend that the assay should be repeated following exposure of the strain to methicillin in order to induce the methicillin resistance.

Study 5

Brown et al[2] evaluated the performance of Mastalex MRSA rapid latex agglutination PBP 2' assay in comparison with 52 MRSA and 27 MSSA, strains of *Staphylococcus aureus* grown on various culture media including blood agar ± 2mg/l oxacillin, Mast and Oxoid Mannitol Salt Agar (MSA) ± 2mg/l oxacillin and Baird Parker Medium + 8mg/l ciprofloxacin. The results of their test are shown in the following table:

Medium	*MecA* gene status	No. isolates tested	No. strains growing	No. Mastalex MRSA positive	Mean time to agglutination
Blood agar/no oxacillin	+	52	52	52	46
	-	27	27	0	-
Blood agar + 2mg/l oxacillin	+	52	52	52	22
	-	27	5	0	-
Mast MSA/no oxacillin	+	52	52	48	91
	-	27	27	0	-
Mast MSA + 2mg/l oxacillin	+	52	50	49	76
	-	27	9	0	-
Oxoid MSA/no oxacillin	+	52	52	36	95
	-	27	27	0	-
Oxoid MSA + 2mg/l oxacillin	+	52	51	46	82
	-	27	9	0	-
Baird Parker Medium + ciprofloxacin 8mg/l	+	52	26	25	46
	-	27	5	0	-

Analytical sensitivity of Mastalex MRSA on blood agar with or without oxacillin = 100%

Analytical specificity of both tests = 100%

Measurand – Presence or absence of PBP2' proteins extracted from bacterial isolates

Cut-off – Agglutination of latex particles by slide agglutination test within 3 minutes = positive

No agglutination of latex particles = negative

Conclusion: The Mastalex MRSA test is a rapid, and highly sensitive and specific test for the detection of methicillin-resistance in *Staphylococcus aureus* if colonies are grown on blood agar with or without oxacillin. Caution is needed when the test is used with colonies grown on Mannitol Salt Agar, or Baird Parker Agar with ciprofloxacin.

Study 6

Bowers et al[9] evaluated the performance of Mastalex MRSA rapid latex agglutination PBP 2' assay in comparison with 125 strains of *Staphylococcus aureus* and 40 strains of coagulase-negative staphylococci compared to the Stokes' disc diffusion method. The results of their test are shown in the following table:

A. Results for *Staphylococcus aureus* strains

Test	Test result	*MecA* PCR		Total
		Positive	Negative	
Mastalex MRSA	Positive	87	1	88
	Negative	3	34	37
Total		90	35	125
Stokes' disc diffusion	Resistant	87	1	88
	Sensitive	3	34	37
Total		90	35	125

Sensitivity 96.67%; Specificity 97.14%

Positive predictive value 96.86%; Negative predictive value 91.89%

B. Results for coagulase negative staphylococci strains

Test	Test result	*MecA* PCR		Total
		Positive	Negative	
Mastalex MRSA	Positive	17	0	17
	Negative	9	14	23
Total		26	14	40
Stokes' disc diffusion	Resistant	20	0	20
	Sensitive	6	14	20
Total		26	14	40

Mastalex MRSA

Sensitivity 65.3%; Specificity 100%

Positive predictive value 100%; Negative predictive value 60.87%

Stokes' disc diffusion

Sensitivity 76.92%; Specificity 100%

Positive predictive value 100%; Negative predictive value 70.0%

Conclusion: The Mastalex MRSA test provides a reliable and rapid detection of methicillin-resistance in *Staphylococcus aureus* when grown in pure culture on enriched media. However it is not recommended for the detection of methicillin-resistance in coagulase negative staphylococci.

Additional reports on Coagulase negative staphylococci testing

As methicillin-resistant coagulase negative staphylococci possess PBP2' it would be expected that the Mastalex MRSA rapid latex agglutination could be used for testing for methicillin resistance. Andrews et al[8] used the Mastalex MRSA rapid latex agglutination PBP 2' assay to test 200 strains of coagulase negative staphylococci and found only 2 discrepant results compared to PCR results with one strain of *Staphylococcus warneri* and one strain of *Staphylococcus hominis* were latex positive and PCR negative. Other reports from Marriott *et al*[7], Cavisinni *et al*[11] and Graham *et al*[10] noted that sensitivity may vary considerably from 50 to 88% mainly due to false, weakly false positive agglutination reactions. They advised therefore that the Mastalex MRSA rapid latex agglutination should not be reliably used for organisms other than *Staphylococcus aureus*.