Primerdesign™ Ltd

Methicillin-resistant Staphylococcus aureus (MRSA)

mecA (penicillin binding protein 2) & S. aureus FEMB gene (chromosomal gene)

genesig® Standard Kit

150 tests

GENESIG

Kits by Primerdesign

For general laboratory and research use only

Introduction to Methicillin-resistant Staphylococcus aureus (MRSA)

Methicillin-resistant Staphylococcus aureus (MRSA) is a specific strain of the Staphylococcus aureus bacterium that has developed antibiotic resistance to all penicillins, including methicillin and other narrow-spectrum β-lactamase-resistant penicillin antibiotics. The resistant strain, MRSA was first discovered in the UK in 1961 and is now widespread, particularly in the hospital setting where it is commonly termed a superbug.

MRSA may also be known as oxacillin-resistant Staphylococcus aureus (ORSA) and multiple-resistant Staphylococcus aureus, while non-methicillin resistant strains of S. aureus are sometimes called methicillin-susceptible Staphylococcus aureus (MSSA) if an explicit distinction must be made.

Although MRSA has traditionally been seen as a hospital-associated infection, community-acquired MRSA strains have appeared in recent years, notably in the US and Australia. The abbreviations CA-MRSA (community-associated MRSA) and HA-MRSA (hospital-associated MRSA) are now commonly seen in medical literature.

Methicillin resistance arises by acquisition of a staphylococcal cassette chromosome SCCmec, and is conferred by the mecA gene. Expression of this gene yields PBP2a, a penicillin binding protein with reduced affinity for β-lactam rings (the primary active-site of the β-lactam antibiotics).

Some strains of S. aureus over-express β-lactamase and appear to be resistant to oxacillin and, rarely, methicillin despite being mecA-negative. They have slightly raised minimum inhibitory concentrations (MICs) and may thus be described as "minimally resistant". Other strains express modified PBPs (not PBP2) and exhibit varying degrees of β-lactam antibiotic resistance.

Not only are MRSA strains resistant to the usual antibiotics, but a curious interbreeding with community staph has led to an additional worry. Many MRSA isolates found outside of medical facilities, and referred to as CAMRSA (community-acquired MRSA), have acquired the Panton-Valentine leukocidin factor, a gene that produces a series of chemicals that make these MRSA particularly invasive as well as resistant.

Reference
J Clin Microbiol. 2002 May;40(5):1821-3.

Specificity

The Primerdesign genesig Kit for Methicillin-resistant Staphylococcus aureus (MRSA) (MRSA) genomes is designed for the in vitro quantification of MRSA genomes. The kit is designed to have the broadest detection profile possible whilst remaining specific to the MRSA genome.

The primers and probe sequences in this kit have 100% homology with a broad range of MRSA sequences based on a comprehensive bioinformatics analysis.

The mecA gene is a plasmid based gene responsible for antibiotic resistance. FEMB is a chromosomal gene specific to S. aureus. A positive result for both markers has been been validated as a reliable test for MRSA. The primers and probe have 100% homology with all reference sequences in the NCBI data. base including those listed below.

MecA sequence specificity
AM292304, AB266532, AB266531, AB245471, AB245470, AB236888, AY894415, DQ106887, AY786579, AY271717, AB221124, AB221123, AB221122, AB221121, AB221120, AB221119, BA000017, AP006716, CP000046, AM048803, AM048802, CP000255, AJ810121, AJ810120, AB047089, Y14051.1, Y13095.1, Y13096.1, Y00688.1, X52593.1, BA000033, BA000018, AB121219, CP000029, X52592.1, AB063172, AB033763, D86934.2, AB096217, AB063173, AB037671,

FEMB sequence specificity
CP000703.1, BA000017.4, CP000046.1, CP000253.1, CP000255.1, X17688.1, X571857.1, BA000033.2, BA000018.3, BX571856.1

If you require further information, or have a specific question about the detection profile of this kit then please send an e.mail to enquiry@primerdesign.co.uk and our bioinformatics team will answer your question.

Kit contents

- **mecA (penicillin binding protein 2) primer/probe mix (150 reactions BROWN)**
  FAM labelled
- **FEMB gene (chromosomal gene) primer/probe mix (150 reactions BROWN)**
  FAM labelled
- **mecA (penicillin binding protein 2) positive control template (for Standard curve RED)**
- **FEMB gene (chromosomal gene) positive control template (for Standard curve RED)**
- **RNase/DNase free water (WHITE)**
  for resuspension of primer/probe mixes
- **Template preparation buffer (YELLOW)**
  for resuspension of positive control templates and standard curve preparation

Reagents and equipment to be supplied by the user

Real-time PCR Instrument

DNA extraction kit
This kit is recommended for use with genesig Easy DNA/RNA extraction kit. However, it is designed to work well with all processes that yield high quality DNA with minimal PCR inhibitors.

oasig™ lyophilised or Precision®PLUS 2X qPCR Master Mix
This kit is intended for use with oasig or PrecisionPLUS 2X qPCR Master Mix.

Pipettors and Tips

Vortex and centrifuge

Thin walled 1.5 ml PCR reaction tubes

Kit storage and stability

This kit is stable at room temperature but should be stored at -20ºC on arrival. Once the lyophilised components have been resuspended they should not be exposed to temperatures above -20°C for longer than 30 minutes at a time and unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances.

If a standard curve dilution series is prepared this can be stored frozen for an extended period. If you see any degradation in this serial dilution a fresh standard curve can be prepared from the positive control.

Primerdesign does not recommend using the kit after the expiry date stated on the pack.

Suitable sample material

All kinds of sample material suited for PCR amplification can be used. Please ensure the samples are suitable in terms of purity, concentration, and DNA integrity (An internal PCR control is supplied to test for non specific PCR inhibitors). Always run at least one negative control with the samples. To prepare a negative-control, replace the template DNA sample with RNase/DNase free water.

Dynamic range of test

Under optimal PCR conditions genesig MRSA detection kits have very high priming efficiencies of >95% and can detect less than 100 copies of target template.

Notices and disclaimers

This product is developed, designed and sold for research purposes only. It is not intended for human diagnostic or drug purposes or to be administered to humans unless clearly expressed for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. During the warranty period Primerdesign genesig detection kits allow precise and reproducible data recovery combined with excellent sensitivity. For data obtained by violation to the general GLP guidelines and the manufacturer's recommendations the right to claim under guarantee is expired. PCR is a proprietary technology covered by several US and foreign patents. These patents are owned by Roche Molecular Systems Inc. and have been sub-licensed by PE Corporation in certain fields. Depending on your specific application you may need a license from Roche or PE to practice PCR. Additional information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing at Roche Molecular Systems, 1145 Atlantic Avenue, Alameda, CA 94501 or Applied Biosystems business group of the Applera Corporation, 850 Lincoln Centre Drive, Foster City, CA 94404. In addition, the 5' nuclease assay and other homogeneous amplification methods used in connection with the PCR process may be covered by U.S. Patents 5,210,015 and 5,487,972, owned by Roche Molecular Systems, Inc, and by U.S. Patent 5,538,848, owned by The Perkin-Elmer Corporation.

Trademarks

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The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG. BI, ABI PRISM® GeneAmp® and MicroAmp® are registered trademarks of the Applera Genomics (Applied Biosystems Corporation). BIOMEK® is a registered trademark of Beckman Instruments, Inc.; iCycler™ is a registered trademark of Bio-Rad Laboratories, Rotor-Gene is a trademark of Corbett Research. LightCycler™ is a registered trademark of the Idaho Technology Inc. GeneAmp®, TaqMan® and AmpliTaqGold® are registered trademarks of Roche Molecular Systems, Inc., The purchase of the Primerdesign reagents cannot be construed as an authorization or implicit license to practice PCR under any patents held by Hoffmann-LaRoche Inc.

Principles of the test

The kit contains two primer and probe sets. The FEMB primer and probe set are designed to detect all Staphylococcus aureus sequences regardless of the antibiotic resistance profile. The MecA primer and probe set is specific to the mobile genetic element that contains the MecA antibiotic research gene. Samples that test positive for MecA and FEMB are confirmed to contain the MRSA stain. Samples that test positive for MecA but are negative for FEMB indicate that the MecA element has been detected in an alternative bacterial strain.

Real-time PCR

mecA and FEMB specific primer and probe mixes are provided and these can be detected through the FAM channel.

The primer and probe mixes provided exploit the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridize to the MRSA DNA. Fluorogenic probes are included in the reaction mixtures which consists of a DNA probe labeled with a 5`-dye and a 3`-quencher. During PCR amplification, the probe is cleaved and the reporter dye and quencher are separated. The resulting increase in fluorescence can be detected on a range of qPCR platforms.

Positive control

For copy number determination and as a positive control for the PCR set up, the kit contains a positive control template. This can be used to generate a standard curve of mecA and FEMB copy number / Cq value. Alternatively the positive control can be used at a single dilution where full quantitative analysis of the samples is not required. Each time the kit is used, at least one positive control reaction must be included in the run. A positive result indicates that the primers and probes for detecting the target MRSA gene worked properly in that particular experimental scenario. If a negative result is obtained the test results are invalid and must be repeated. Care should be taken to ensure that the positive control does not contaminate any other kit component which would lead to false-positive results. This can be achieved by handling this component in a Post PCR environment. Care should also be taken to avoid cross-contamination of other samples when adding the positive control to the run. This can be avoided by sealing all other samples and negative controls before pipetting the positive control into the positive control well.

Negative control

To validate any positive findings a negative control reaction should be included every time the kit is used. For this reaction the RNase/DNase free water should be used instead of template. A negative result indicates that the reagents have not become contaminated while setting up the run.

Resuspension protocol

To minimize the risk of contamination with foreign DNA, we recommend that all pipetting be performed in a PCR clean environment. Ideally this would be a designated PCR lab or PCR cabinet. Filter tips are recommended for all pipetting steps.

1. **Pulse-spin each tube in a centrifuge before opening.**
   This will ensure lyophilised primer and probe mix is in the base of the tube and is not spilt upon opening the tube.

2. **Resuspend the kit components in the RNase/DNase free water supplied, according to the table below.**
   To ensure complete resuspension, vortex each tube thoroughly.

Component - resuspend in water	Volume
Pre-PCR pack	
mecA primer/probe mix (BROWN)	165 µl
FEMB primer/probe mix (BROWN)	165 µl

3. Resuspend the positive control templates in the template preparation buffer supplied, according to the table below:
   To ensure complete resuspension, vortex each tube thoroughly.

Component - resuspend in template preparation buffer	Volume
Post-PCR heat-sealed foil	
mecA Positive Control Template (RED) *	500 µl
FEMB Positive Control Template (RED) *	500 µl

* This component contains high copy number template and is a VERY significant contamination risk. It must be opened and handled in a separate laboratory environment, away from the other components.

qPCR detection protocol

1. **For each DNA sample prepare a reaction mix according to the table below:**
   Include sufficient reactions for positive and negative controls.

Component	Volume
oasig or PrecisionPLUS 2X qPCR Master Mix	10 µl
mecA or FEMB primer/probe mix (BROWN)	1 µl
RNase/DNase free water (WHITE)	4 µl
Final Volume	**15 µl**

2. **Pipette 15µl of this mix into each well according to your qPCR experimental plate set up.**

3. **Prepare DNA templates for each of your samples.**

4. **Pipette 5µl of DNA template into each well, according to your experimental plate set up.**
   For negative control wells use 5µl of RNase/DNase free water. The final volume in each well is 20µl.

5. **If a standard curve is included for quantitative analysis, prepare a reaction mix according to the table below:**

Component	Volume
oasig or PrecisionPLUS 2X qPCR Master Mix	10 µl
mecA and FEMB primer/probe mix (BROWN)	1 µl
RNase/DNase free water (WHITE)	4 µl
Final Volume	**15 µl**

6. Preparation of a standard curve dilution series.

1) Pipette 90µl of template preparation buffer into 5 tubes and label 2-6
2) Pipette 10µl of Positive Control Template (RED) into tube 2
3) Vortex thoroughly
4) Change pipette tip and pipette 10µl from tube 2 into tube 3
5) Vortex thoroughly

Repeat steps 4 and 5 to complete the dilution series

Standard Curve	Copy Number
Tube 1 Positive control (RED)	2 x 10^5 per µl
Tube 2	2 x 10^4 per µl
Tube 3	2 x 10^3 per µl
Tube 4	2 x 10^2 per µl
Tube 5	20 per µl
Tube 6	2 per µl

7. Pipette 5µl of standard template into each well for the standard curve according to your experimental plate set up.
The final volume in each well is 20µl.

Amplification protocol

Amplification conditions using oasig or PrecisionPLUS 2X qPCR Master Mix.

	Step	Time	Temp
	Enzyme activation	2 min	95 °C
Cycling x50	Denaturation	10 s	95 °C
	DATA COLLECTION *	60 s	60 °C

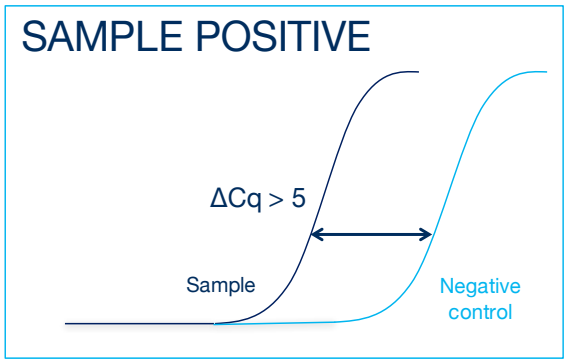
* Fluorogenic data should be collected during this step through the FAM channel

Interpretation of results

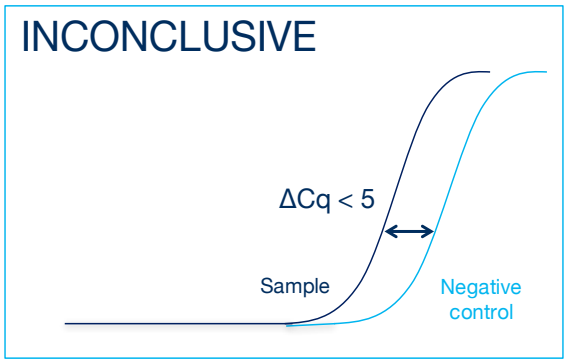
Target	Positive control	Negative control	Interpretation
+	+	-	**POSITIVE QUANTITATIVE RESULT** calculate copy number
-	+	-	**NEGATIVE RESULT**
+ / -	+	≤ 35	**EXPERIMENT FAILED** due to test contamination
+ / -	+	> 35	*
+ / -	-	+ / -	**EXPERIMENT FAILED**

Positive control template (**RED**) is expected to amplify between Cq 16 and 23. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised

*Where the test sample is positive and the negative control is positive with a Cq > 35, the sample must be reinterpreted based on the relative signal strength of the two results:



If the sample amplifies > 5 Cq earlier than the negative control then the sample should be reinterpreted (via the table above) with the negative control verified as negative.



If the sample amplifies < 5 Cq earlier than the negative control then the positive sample result is invalidated and the result should be determined inconclusive due to test contamination. The test for this sample should be repeated.