MBPCR019 Staphylococcus Cassette Chromosome (SCC) *mec* typing MRSA Detection Kit (Multiplex)

Description:

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are prevalent pathogens in both healthcare facilities and in the community. Methicillin resistance among staphylococci is mediated by a low-affinity, penicillin-binding protein (PBP2a) encoded by the *mec*A gene. *mec*A is carried on a mobile genetic element called SCC*mec* or staphylococcus cassette chromosome *mec* element, which integrates in a site-specific manner into the staphylococcus genome. This element harbors the two main genetic complexes: the *mec* gene complex, which mediates methicillin resistance and the *ccr* gene complex, which codes for the site-specific cassette chromosome recombinases (*ccr*) responsible for its mobility. Till date, eleven SCC*mec* (I – XI) types have been characterized based on different combinations of the *mec* and *ccr* gene complexes and according to variations in the "joining" regions (J-region). The widely circulating types are SCC*mec* types I – VI.

Intended Use:

The Staphylococcus Cassette Chromosome (SCC) mec typing MRSA Detection Kit (Multiplex) is a qualitative *in vitro* test designed for amplification of targeted genes with specifically designed primers. This multiplex PCR contains 7 sets of primers targeting SCC*mec* types I – VI. The kit also targets *mec*A – the methicillin resistant determinant as an internal control.

NOTE: The Staphylococcus Cassette Chromosome (SCC) mec typing MRSA Detection Kit (Multiplex) is for *in vitro* use only.

Principle:

HiMedia's Staphylococcus Cassette Chromosome (SCC) mec typing MRSA Detection Kit (Multiplex) is a qualitative conventional PCR kit which focuses on simultaneous amplification of 7 targets specific for types I – VI and *mec*A – the methicillin resistant determinant as an **internal control** using specific primers. The amplified target is confirmed by using agarose gel electrophoresis. This kit also contains **positive control.**

Positive control: This is a control reaction using a known template (target pathogen). A positive control is usually used to check that the primers have been designed properly and the PCR conditions have been set up correctly.

Polymerase Chain Reaction (PCR) is a very sensitive and specific method for amplification based detection of target genes. The three steps of a successful PCR reaction include Denaturation, Annealing and Extension. The double-stranded DNA melts and forms single stranded DNA at high temperature (Denaturation). Sequence-specific primers bind to the target sequence on single-stranded DNA at lower temperature (Annealing). Taq DNA Polymerase adds dNTPs onto the single stranded DNA at intermediate temperature (Extension). These 3 steps of PCR are usually repeated upto 30 to 40 times in each PCR assay.

Features:

- Fast and simple

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- Sensitive and specific results
- Guaranteed reproducible results
- Rapid detection of all relevant clinical pathogens

Unit Definition:
1U is defined as amount of enzyme that is required to catalyze the incorporation of 10 nmoles of dNTP into acid-insoluble material in 30 minutes at 74°C.

Storage and Shelf-life:
The provided kit has a shelf-life of 6 months when stored at -20°C. Repeated thawing and freezing of PCR reagents should be avoided, as this may reduce the sensitivity. If reagents are to be used multiple times, we recommend storing reagents as aliquots to avoid repeated freeze and thaw. Degradation of sample DNA specimens can also reduce sensitivity of the assay. The kit provided is stable for 6 months when stored at mentioned conditions. HiMedia does not recommend using the kit after the expiry date stated on pack.

Kit Contents:

Components	Reagents provided for 10R (reactions)	Reagents provided for 25R (reactions)	Reagents provided for 50R (reactions)
2X PCR Master Mix **(MBT061)**	260µL	650µL	1.5 ml
SCC*mec* Primer Mix	180µL	420µL	820 µL
Nuclease free water **(ML065)**	1 ml	2 ml	4 ml
6X Loading Dye **(ML015)**	30µL	75µL	150 µL
100 bp DNA Ladder **(MBT049)**	60µL	150µL	300 µL
Positive control DNA	55 µL	130 µL	260µL

Sample Material Preparation:
Various clinical isolates either hospital or community based can be examined. Extract bacterial DNA using HiMedia HiPurA Bacterial DNA Extraction kit (MB505) or equivalent extraction kit according to manufacturer's instructions.

Enrichment of pathogens (if required):
- In order to ensure sensitive detection of pathogens, the pathogens need to be enriched in broth.

General Preparation Instructions:
- Before use, all PCR components should be completely thawed on ice (4°C).
- Perform the amplification reactions in a clean area.
- Use of aerosol barrier pipette tips is recommended to reduce contamination risks from extraneous DNA templates.
- Extract and store positive control material (if used) separately from all other reagents to avoid contamination and add it to the reaction mix in a separate area.
- Centrifuge the components briefly once thawed.

Protocol:

Preparation of PCR Reaction Mixture

Add 25 µl of 2X PCR Master Mix **(MBT061)** in a PCR tube

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In the same tube, add 16 µl of SCC*mec* Primer Mix (10 pmoles concentration provided)

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Add 3-5 µl of **template DNA** (upto 50 ng of extracted DNA)

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Add nuclease free water **(ML065)** to make the final volume to 50 µl

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Centrifuge the tube briefly at 6000 rpm for about 10 seconds.

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Place the tubes in the PCR machine and set the recommended PCR program (mentioned below)

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Interpret the data using Agarose gel electrophoresis

NOTE: (Optional) – The user can also set up an additional PCR reaction containing 5µl Positive control DNA (provided) in a separate tube.

A. SCC*mec* typing PCR program:

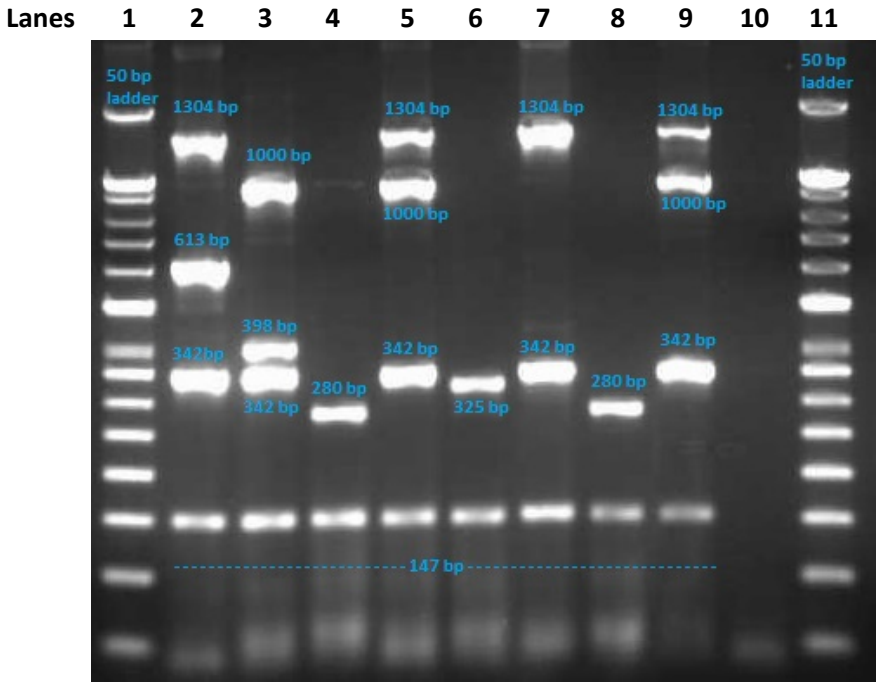
Step	Temperature (°C)	Time (minute:second)	No. of cycles
Initial Denaturation	94	15:00	1
Denaturation	94	00:30	30
Annealing	56	01:30	30
Extension	72	01:30	30
Final Extension	72	10:00	1
Post run	4	Hold	-

B. After amplification, store the products at 4°C overnight or -20°C for long term storage.

C. Agarose Gel Electrophoresis

1. 10µL of the amplicons along with 1µL of 6X DNA loading dye **(ML015)** are separated on a 2% of Ultra resolution agarose gel **(MB073)** prepared in 1X TAE buffer **(ML010)** under an electric current of 20V/cm for 90 mins.
2. Load 5µL of 50bp DNA ladder **(MBT084)** in separate well.

Gel image representing amplification of SCC*mec* types using clinical samples.



Lanes	1 & 11	2	3	4	5	6	7	8	9	10
Identification	50 bp DNA Ladder	SCC*mec* type								Negative control
		I	II	III	IVa	V	VI	III	IV	

Specifications:

Sensitivity: Detectable upto 10^3 cfu/ml.

Quality Control:

Each lot of HiMedia's Staphylococcus Cassette Chromosome (SCC) mec typing MRSA Detection Kit (Multiplex) is assayed for contaminating endonuclease, exonuclease, and non-specific DNase activities. Functionally tested in DNA amplification.

Troubleshooting Guide:

Sr.No.	Problem	Cause	Solution
1.	No amplification	Degraded samples	1. Check the integrity of DNA using agarose gel electrophoresis.
			2. Use freshly prepared DNA to ensure the availability of intact template sequence for efficient amplification.
		Error in protocol setup	Verify that the correct reagent volumes, dilutions and storage conditions have been used.

2.	Variability between replicates	Error in reaction set-up	Prepare large volume reaction mix, vortex thoroughly and aliquot appropriately into reaction tubes.
		Air bubbles in reaction mix	Briefly centrifuge reaction samples/plate prior to running on a PCR machine.
		Pipetting error	Replicates can show increased variation due to poor laboratory techniques or imprecise pipettes.
3.	Amplification in negative control	Reagents contaminated	1. Replace all critical solutions 2. Repeat the analysis of all tests with fresh aliquots of critical reagents.

Safety Information

The Staphylococcus Cassette Chromosome (SCC) mec typing MRSA Detection Kit (Multiplex) is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

Product Use Limitation & Warranty

HiMedia guarantees the performance of product in the manner described in the product literature. Staphylococcus Cassette Chromosome (SCC) mec typing MRSA Detection Kit (Multiplex) is designed and sold for research and *in vitro* purposes only. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of HiMedia products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Technical Assistance:

At HiMedia we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance mail at mb@himedialabs.com

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