DTU Food
National Food Institute

PROTOCOL FOR PCR AMPLIFICATION OF *MECA*, *MECC* (*MECA*$_{LGA251}$), *SPA* AND *PVL*

RECOMMENDED BY THE EURL-AR

2ST VERSION, SEPTEMBER 2012

Changes from Previous version: Volume of PCR reaction adjusted to 25 µl in total = 23µl+2µl sample DNA in the example of set-up sheet.

Background:

The confirmation of the presence of the *mecA* gene, has until recently been the ”golden standard” for detection of methicillin resistant *S. aureus* (MRSA) worldwide. However, this has been changed as a new *mecA* homologue gene (*mecC,* formerly named *mecA*$_{LGA251}$) has been described in *S. aureus* from humans and cattle by a research group at the University of Cambridge lead by Prof. Mark Holmes. These findings have raised a concern regarding a possible animal origin of the isolates harbouring this gene and also regarding the need to update the methods for detection of methicillin resistance which will need to be supplemented with further testing to identify the *mecC*.

The method we recommend and describe below was first described by Stegger et al, 2012 and consists on a multiplex PCR method which can be used for confirmation of methicillin resistance by amplification of both *mecA* and *mecC,* identification of *S. aureus* by amplification of the *spa* gene (also used for the typing) and detection of the Panton Valentin Leukocidin (PVL or LukF PV) encoding gene.

Protocol

DNA extraction (using Instagene Matrix, Biorad®)

- Suspend a loopful (2-3 colonies of a fresh overnight culture) of *S. aureus* cells in 100 µl lysis buffer (InstaGene Matrix, Biorad®) (use 1.5 ml eppendorf tube), vortex (15 sec) and incubate at 56°C for 1 hour.
- Mix well by vortexing and incubate at 95°C for 1 hour.
- Mix well by vortexing and centrifuge at 13200 rpm for 5min.
- Store DNA samples at -20°C*.

* Vortex and centrifuge the DNA suspension (13200 rpm for 5 min), before use.

Note: The PCR results are more stable and better amplifications were obtained using DNA extracted with this DNA extraction method than when using of boiling lysates. Please note also that there might be differences in the results when setting up the method in different labs, therefore it is advisable to further validate the results obtained and make sure to obtain best sensitivity and specificity of this method.

PCR Controls:

spa: Use *S. aureus* ATCC 29213 or any *S.aureus* strain, (therefore *spa*-control might not be necessary as the other control strains will also have amplification of *spa*)
mecA*:* Use *mecA* positive *S. aureus* 50A247
pvl*:* Use PVL positive *S. aureus*
mecC: Use *S. aureus* LGA251

Preparation of primers *spa*/*mecA*/*mecA*$_{LGA251}$/PVL:

Primermix 1 *spa*/*mecA*/*mecA*$_{LGA251}$/PVL <u>Forward</u> primers:

1. Take 900 µL H_2O

2. Add 25 µL *spa*-1113F (100 µM)

3. Add 25 µL *mecA*-P4 (100 µM)

4. Add 25 µL PVL-F (100 µM)

5. Add 25 µL *mecA*$_{LGA251}$ MultiFP (100 µM)

6. Vortex s*pa/mecA/mecA*$_{LGA251}$/PVL mix

Primermix *spa/mecA/mecA*$_{LGA251}$/PVL <u>Reverse</u> primers:

1. Take 900 µL H_2O

2. Add 25 µL *spa*-1514R(100 µM)

3. Add 25 µL mecA-P7 (100 µM)

4. Add 25 µL PVL-R (100 µM)

5. Add 25 µL *mecA*$_{LGA251}$ MultiRP (100 µM)

6. Vortex *spa*/*mecA*/*mecA*$_{LGA251}$/PVL mix

Sample preparation for PCR

Reaction mix:

At the EURL-AR we have chosen to use a Master mix (DreamTaq™ Green PCR Master Mix) to facilitate the PCR reaction preparation and it has the advantage of also including loading buffer, allowing for direct loading on electrophoresis gel after PCR amplification.

The set up and running conditions are described in the Sample PCR sheet (contains PCR mix and conditions).

Template:

As template for the PCR we recommend to use 2µl of the above prepared DNA in a 25µl PCR reaction.

Primers used in this PCR:

Primer name	Primer # (EURL-AR)	Sequence
spa-1113F	2819	5' – TAAAGACGATCCTTCGGTGAGC – 3'
spa-1514R	2820	5' – CAGCAGTAGTGCCGTTTGCTT – 3'
mecA P4	2821	5' – TCCAGATTACAACTTCACCAGG – 3'
mecA P7	2822	5' – CCACTTCATATCTTGTAACG – 3'
pvl-F	2823	5' – GCTGGACAAAACTTCTTGGAATAT – 3'
pvl-R	2824	5' – GATAGGACACCAATAAATTCTGGATTG – 3'
$mecA_{LGA251}$ MultiFP	2825	5' – GAAAAAAAGGCTTAGAACGCCTC – 3'
$mecA_{LGA251}$ MultiRP	2826	5' – GAAGATCTTTTCCGTTTTCAGC – 3'

Electrophoresis:

Run 5-8µl of the PCR products (you do not need to mix loading buffer for the electrophoresis in case you use the DreamTaq Green Master mix). Run in parallel with a 100bp Ladder molecular weight marker on a 2% agarose gel in TBE 1X. Run for 1h at about 130V.

Stain the gel in Ethidium bromide circa 20-30min.

De-stain briefly in milliQ water.

Take a picture in the transilluminator under UV light. Observe the bands and interpret the results according to the description below and the figure (Figure 1):

- *Spa* - the *spa* fragment resulting from the amplification is variable in size and ranges from 180-600bp depending on the *spa* type present and this fragment should be amplified from all *S. aureus* strains (no amplification of the *spa* fragment indicates the isolate is not a *S. aureus* and further identification procedures might be necessary to determine the species, in case this is necessary).
- Methicillin resistance: any amplification of the *mecA* or the *mecC* gene confirms methicillin resistance
    - *mecA* - the *mecA* fragment to be amplified has an expected size of 162bp.
    - *mecC* - the amplified fragment is expected to be 138 bp.
- PVL - an amplified fragment of 85bp indicates the presence of the gene encoding the Panton Valentine Leukocidin (PVL) which might be present in some isolates.

Note: The PCR product of the multiplex can be purified and used for sequencing the *spa* fragment for *spa* typing, directly.

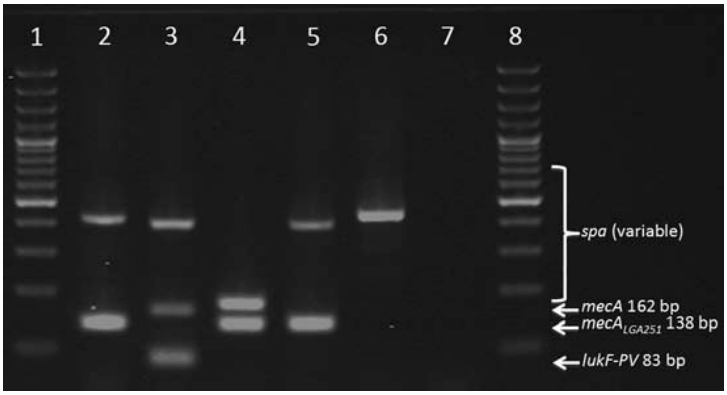

Figure 1. Multiplex PCR for detection of *mecA*, *mecC* (*mecA*$_{LGA251}$), *lukF-PV (PVL)* and *spa*.

Lanes 1 and 8: 100-bp ladder.

Lane 2: *mecC* positive MRSA (*spa* and *mecC* amplification).

Lane 3: *pvl* positive MRSA (*lukF-PV*, *spa* and *mecA* amplification).

Lane 4: MRSA (*spa* t528=one *spa* repeat and *mecC* amplification)

Lane 5: MRSA (*spa* t843 and *mecC* amplification)

Lane 6: MSSA. (*spa* amplification only)

Lane 7: negative control (H_2O).

Reference:

Stegger M, Andersen PS, Kearns A, Pichon B, Holmes MA, Edwards G, Laurent F, Teale C, Skov R, Larsen AR. Rapid detection, differentiation and typing of methicillin-resistant *Staphylococcus aureus* harbouring either *mecA* or the new *mecA* homologue *mecA(LGA251)*. Clin Microbiol Infect. 2012 Apr;18(4):395-400.

DTU Food
National Food Institute

PCR SAMPLE SHEET (Example for set-up)

PCR *spaIpvIImecAImecC*

Primer 1: Primer mix containing: 2819-2821-2823-2825
Primer 2: Primer mix containing: 2820-2822-2824-2826
DNA polymerase: DreamTaq™ Green PCR Master Mix
PCR products: spa(variable:200-600bp); *mecA* (162 bp); *mecC* (138bp); pvl (~85bp)
Remarks: 2 µl of the DNA template. Run: 2% agarose gel run at 130V for 1h **Reference:** Stegger M, Andersen PS, Kearns A, Pichon B, Holmes MA, Edwards G, Laurent F, Teale C, Skov R, Larsen AR. Rapid detection, differentiation and typing of methicillin-resistant *Staphylococcus aureus* harbouring either *mecA* or the new *mecA* homologue *mecA(LGA251)*. Clin Microbiol Infect. 2012 Apr;18(4):395-400.

No. of reactions	1	**14**
PCR H_2O	6,5	**91**
2xGreen PCR Master Mix	12,5	**175**
dNTP	0	**0**
25 mM $MgCl_2$	0	**0**
Primer 1 (0,5 µl of each)	2	**28**
Primer 2 (0,5 µl of each)	2	**28**
Taq polymerase	0	**0**
Total volume	23	**322**

1.	5	min at	94	°C
2.	30	Cycles		
	30	sec at	94	°C
	1	min at	59	°C
	1	min at	72	°C
3.	10	min at	72	°C
4.		hold at	4	°C

M:	
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	

M:	
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	

M:	
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	