# LABORATORY PROTOCOL
# MRSA Multiplex PCR-2

## PCR AMPLIFICATION OF MECA, MECC, PVL AND SPA

**April 2024**
**Version 3.1**

Version 3.1 reviewed and updated by: EURL-AR

Authors of the document: EURL-AR

| HISTORY OF CHANGES | | | | |
|---|---|---|---|---|
| Version | Sections changed | Description of change | Date | Approval |
| 3.1 | - | Updated positive control strain | April 2024 | EURL-AR |
| 3 | Title<br><br><br>Purpose<br>Protocol | Defined as MRSA Multiplex PCR-2<br>PCR AMPLIFICATION OF MECA, MECC, PVL AND SPA<br>Purpose updated<br>Alternative methods added for DNA preparations.<br>PCR program included | Nov 2022 | EURL-AR |
| 2 | Background<br>Protocol | Background updated (PVL)<br>Volume of PCR reaction adjusted to 25 µL in total=23 µL+2µL sample DNA in the example of set-up sheet. | Sept. 2012 | Rene Hendriksen |
| 1 | New document | - | August 2012 | Rene Hendriksen |

## 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 changed as a new *mecA* homologue gene (*mecC,* formerly named *mecA*$_{LGA251}$) has been described in *S. aureus*. These findings have raised the need to update the methods for detection of methicillin resistance, which will need to be supplemented with further testing to identify the *mecC.*

## Purpose

The method we recommend and describe below was first described by Stegger *et al,* 2012 and consists of 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 and typing of the *spa* gene and detection of the Panton Valentin Leukocidin (PVL or *LukF* PV) encoding gene.

This PCR method is referred to as the PCR-2 in the “Technical specifications for a baseline survey on the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in pigs” (EFSA, 2022) for detection of *mecC* gene.

## Protocol

### Preparation of DNA templates:

Perform DNA extraction (here described by using Instagene Matrix, Biorad®) or equivalent, or use boiled lysates (cf. PCR-1)

- Suspend a loopful (3-4 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.

The template is ready for use or store DNA samples at -20°C*.

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

**Note**: The PCR results were found to be 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 Positive Control strains:**

A new *mecC* positive control strains of *S. aureus* for this PCR has been distributed in April 2024:

**PCR-2-C3 EURL ST 17.7: *mecC, spa*** (EQAS 2023)

Additional control strains were likewise distributed to be used in the multiplex PCR-1 and PCR-2:

| Control strain ID | Positive control for | Species | MRSA |
|---|---|---|---|
| PCR-1-C1 EURL ST-12.7 | PVL, *scn, spa* | *S. aureus* | No |
| PCR-1-C2 EURL ST-11.3 | *mecA,* CC398, *spa* | *S. aureus* | Yes |

These control strains have previously been distributed to the EURL-AR network laboratories as EQAS strains in 2017 and 2018, in preparation for the MRSA PCR PT in 2024, and can be acquired from the EURL-AR on request.

Previous control strains are still valid:

***spa***: Use *S. aureus* ATCC 29213 or any *S. aureus* strain, (*spa*-control might not be necessary as any other *S. aureus* control strains will also have amplification of *spa*)

***mecA****:* Use *mecA* positive *S. aureus* 50A247

***mecC***: Use *S. aureus* LGA251

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

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

- Take 900 µL $H_2O$
- Add 25 µL *spa*-1113F (100 µM)
- Add 25 µL *mecA*-P4 (100 µM)
- Add 25 µL PVL-F (100 µM)
- Add 25 µL *mecA*$_{LGA251}$ MultiFP (100 µM)
- Vortex s*pa/mecA/mecA*$_{LGA251}$/PVL mix

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

- Take 900 µL $H_2O$
- Add 25 µL *spa*-1514R(100 µM)
- Add 25 µL mecA-P7 (100 µM)
- Add 25 µL PVL-R (100 µM)
- Add 25 µL *mecA*$_{LGA251}$ MultiRP (100 µM)
- 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.

**Template:**

As template for the PCR we recommend to use 2 µl of the above prepared DNA in a 25 µl PCR reaction. For DNA extracted with InstaGene, a 5x dilution can improve the interpretation of the PCR gel.

**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' |

**PCR PROGRAM**

| 1 CYCLE | 30 CYCLES | 1 CYCLE |
|---|---|---|
| 94 ˚C 5 min. | 94 ˚C 30 sec. | 72 $^{O}$C 10 min. |
| | 59 ˚C 60 sec. | Hold ~ 5 ˚C |
| | 72 ˚C 60 sec. | |

**Electrophoresis:**

Run 5-8 µl of the PCR products (no 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. Stain the gel in Ethidium bromide circa 20-30 min. 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 amplification is variable in size and ranges from 180-600 bp depending on the *spa* type 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).
- 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)

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

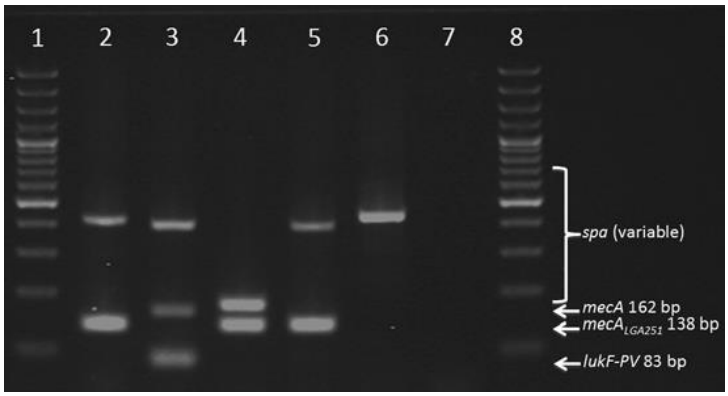


**Figure 1.** Multiplex PCR for detection of *mecA*, *mecC (mecA$_{LGA251}$)*, *lukF-PV (PVL)* and *spa*.

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 ($H_2O$).

**References**:

Stegger M, Andersen PS, Kearns A, Pichon B, Holmes MA, Edwards G, Laurent F, Teale C, Skov R, Larsen AR. (2012) 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.

European Food Safety Authority (EFSA), Marc Aerts, Antonio Battisti, Rene Hendriksen, Jesper Larsen, Oskar Nilsson, José Cortiñas Abrahantes, Beatriz Guerra, Alexandra Papanikolaou, Pierre-Alexandre Beloeil (2022) Technical specifications for a baseline survey on the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in pigs. EFSA Journal Volume 20, Issue10, October 2022. https://doi.org/10.2903/j.efsa.2022.7620

# PCR SAMPLE SHEET (Example for set-up)

**PCR *spa*/PVL/*mecA*/*mecC***

| |
|---|
| 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:<br>*spa* (variable:180-600bp); *mecA* (162 bp); *mecC* (138bp); PVL (~85bp) |
| Remarks: 2 µl of the DNA template. Run: 2% agarose gel run at 130V for 1h<br><br>**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** |

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| 1. | 5 | min at | | 94 | °C |
| 2. | 30 | Cycles | | | |
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