LABORATORY PROTOCOL

Spa -typing- *Staphylococcus aureus*

PCR AMPLIFICATION and typing of SPA gene

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Version 2

Version 2 reviewed and updated by: EURL-AR

Authors of the document: EURL-AR

HISTORY OF CHANGES				
Version	Sections changed	Description of change	Date	Approval
2	Protocol	Additional methods and tools Primers updated to align with PCR-1 and -2	Dec.. 2022	EURL-AR
1	New document	-	June 2010	EURL-AR

Purpose:

DNA sequencing of the repeat region of the *Staphylococcus* protein A gene (*spa*) can be used for accurate and discriminatory typing of MRSA. The repeats are assigned a numerical code and the *spa*-type is identified from the order of specific repeats. The *spa* fragment resulting from the amplification in this PCR is variable in size due to the repeats and ranges from 180-600 bp depending on the *spa* type present. The *spa* fragment should be amplified from all *S. aureus* strains and lack of 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.

The PCR product of this PCR or the EURL-AR MRSA PCR-1 or PCR-2 can be purified and used directly for sequencing of the *spa* fragment for typing.

PCR Controls:
All *S. aureus* strains should be positive.

PCR
Set up and run the PCR according to the conditions described in the PCR sheet (contains PCR mix and conditions).

Primers

Primer name	Primer # (EURL)	Sequence
spa-1113F	2819	5' –TAAAGACGATCCTTCGGTGAGC– 3'
spa-1514R	2820	5' – CAGCAGTAGTGCCGTTTGCTT – 3'

Run then 5µl of the PCR product on a 2% agarose gel in 1XTBE buffer for 25 min at about 130V with a 100bp Ladder molecular weight marker. Stain the gel in Ethidium bromide about 20-30 min. Destain briefly in milliQ water.

As an alternative to Ethidium bromide, run 5-8µl PCR product on a 2 % agarose gel with SYBR safe stain in 0.5X TBE buffer.

Take a picture in the transilluminator under UV light.
Observe the bands: *spa* should be amplified in all *Staphylococcus aureus* strains but the length of the amplified fragment ranges from 180-600 bp. For *spa* typing the amplified PCR products need to be purified using a common purification kit and sequenced or sent for sequencing.

Sequencing results can be interpreted by using *spa*-typer sequence analysis software such as: Bionumerics *spa* typing plugin; Center for Genomic Epidemiology (CGE) *spa*Typer or Ridom SpaServer to attribute the *spa* type corresponding to the number and sequence of repeats present. Links are listed after references.

References:

Shopsin B, Gomez M, Montgomery SO, Smith DH, Waddington M, Dodge DE, Bost DA, Riehman M, Naidich S, Kreiswirth BN. Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. J Clin Microbiol. 1999 Nov;37(11):3556-63.

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.

Links to software websites:
https://www.bionumerics.com/bionumerics/category/spa-typing
https://cge.food.dtu.dk/services/spaTyper/
http://spaserver.ridom.de/