Update on protocols

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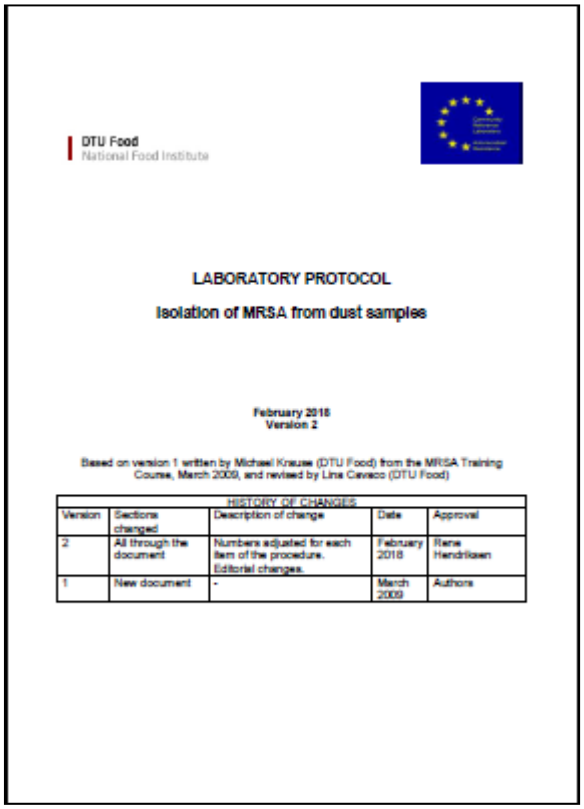
REVISION:
Isolation of MRSA



DTU Food
National Food Institute

LABORATORY PROTOCOL

Isolation of MRSA from dust samples

February 2018
Version 2

Based on version 1 written by Michael Krause (DTU Food) from the MRSA Training Course, March 2009, and revised by Lina Cavaco (DTU Food)

HISTORY OF CHANGES

Version	Sections changed	Description of change	Date	Approval
2	All through the document	Numbers adjusted for each item of the procedure. Editorial changes.	February 2018	Rene Hendriksen
1	New document	-	March 2009	Authors

Old protocol

Enrichment

Five dust samples in 300mL 6.5 % NaCl Müller Hinton Broth (37°C, 16-20 h.)

Selective enrichment

1 ml 6,5 % NaCl Müller Hinton Broth in 9 ml TSB with 3.5 mg/L Cefoxitin and 75 mg/L Aztreonam (37°C, 16-20 h.)

Isolation

Oxoid *Brilliance*^{TM} MRSA Agar – streak with a 10 µL inoculation loop (37°C, 24 –48 h.)

Cultivation

Suspect colonies to nutrient blood agar (37°C, 24 h.)

Identification

Multiplex PCR for the detection of the *mec*A or *mec*C gene

Revised protocol

RAPID COMMUNICATIONS

Evaluation of a widely used culture-based method for detection of livestock-associated meticillin-resistant *Staphylococcus aureus* (MRSA), Denmark and N… 2014 to 2016

Samples from countries with high… of MRSA in pig populations (nasal s… abs, environmental samp…

Country	1-S			Total
		4	57	221
C…	…ve	10	1,863	1,873
	Total	174	1,920	2,094

1-S method has higher sensitivity (lower ratio of false negative results)

No gold standard thus difficult to assess sensitivity, specificity, PPV, NPV – BUT the methods yielded significantly different results

Revised protocol

	Action
1	Collect samples for the MRSA monitoring, according to the guidelines listed in table 1 and Table 2 of the EFSA document on Technical specifications on the harmonised monitoring and reporting of antimicrobial resistance in methicillin-resistant *Staphylococcus aureus* in food-producing animals and food. EFSA J. 2012; 10(10):2897. Available from: https://www.efsa.europa.eu/en/efsajournal/pub/2897

Revised protocol

2	Cover the samples in Mueller-Hinton broth containing 6.5% sodium chloride (NaCl) and incubate at 35-37°C for 16-24 h.	The pre-enrichment step selects for staphylococci and other salt-tolerant bacteria.
3	Spread a 10-µl loopful of the broth on *Brilliance* MRSA 2 agar (Oxoid) and incubate at 35-37°C for 16-24 h.	Denim blue colonies are presumptive MRSA.

Revised protocol

4	Subculture presumptive MRSA colonies on blood agar and incubate at 35-37°C for 22-24 h.	MRSA colonies on blood agar are greyish or yellowish and usually surrounded by a zone of haemolysis. The catalase test can be used to distinguish staphylococci from enterococci, which sometimes produce a similar colony morphology on *Brilliance* MRSA 2 agar.

Revised protocol

5	Confirm presumptive MRSA colonies by PCR, according to the protocol described in the document entitled "Protocol for PCR Amplification of *mecA*, *mecC*, *spa* and *pvl*" on the EURL-AR website.	MRSA isolates are positive for *spa* and either *mecA* or *mecC*, whereas the presence of *pvl* is more variable. MRSA-confirmed isolates can be further *spa*-typed in order to determine the corresponding Clonal Complex (CC). Isolates for which no CC can be inferred from the *spa*-type should be further typed by MLST-typing. These methods are described in the documents entitled "Protocol for *spa*-typing" and "MLST typing" on the EURL-AR website.

THANK YOU FOR YOUR ATTENTION!