



# Protocol for MRSA EQAS 2011

For selective isolation, detection and typing of methicillin resistant *Staphylococcus aureus* (MRSA) from swab samples.

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## 1 Introduction

One of the tasks as the European Union Reference Laboratory for Antimicrobial Resistance (EURL-AR) is to organise and conduct External Quality Assurance Systems (EQAS's). Usually the EQAS's have been focused on susceptibility testing, however, due to the recent concern about FA-MRSA (farm acquired methicillin resistant *Staphylococcus aureus*) detected in European farms and the recently performed baseline screening performed aiming at the detection of FA-MRSA in pig farms, we have now decided to continue the MRSA EQAS to evaluate the proficiency of the laboratories on the selective isolation procedures for detection of MRSA, including their confirmatory testing and *spa* typing.

As referred in the pre-notification, this EQAS is designed specifically for designated NRL-ARs and additional designated laboratories performing the selective isolation and identification of MRSA from pig farms.

**In this MRSA EQAS we have prepared swab samples, which should be processed in a similar way as in the previous EQAS 2010, according to the following description in this protocol.**



## 2 Objectives

The main objective of this EQAS is to support laboratories in assessing and if necessary improve the quality of selective isolation for detection, confirmatory testing and *spa*-typing of MRSA. Furthermore, to assess and improve the comparability of the baseline screening data reported to EFSA by different laboratories within the EU.

## 3 Outline of the MRSA EQAS 2011

### 3.1 Shipping, receipt and storage of strains

In October 2011 all EU appointed National Reference Laboratories (or the alternative laboratories that have processed the MRSA baseline sample analysis) will receive a parcel from the EURL-AR containing eight swab samples which contain a background flora and might contain MRSA (some samples will be negative, or contain other methicillin susceptible or resistant *Staphylococcus* strains).

**NOTE:** The samples should be received and processed immediately upon arrival, or as quickly as possible to assure the stability of the samples.

## 4 Detection and identification of MRSA from swab samples

### 4.1 Selective enrichment and isolation

1. In the laboratory, open carefully the tubes containing the swab samples in transport medium (in a laminar air flow bench and using protective gloves) and inoculate each swab into a tube containing 10 ml of Mueller-Hinton broth supplemented with 6.5 % NaCl. Mix thoroughly.
2. Incubate at 37 °C for 16-20 h.
3. Take one millilitre of this first pre-enrichment culture and then inoculate into 9 ml Tryptone Soya Broth with 3.5 mg/L cefoxitin and 75 mg/L aztreonam. Mix thoroughly.
4. Incubate for 16-20 h at 37 °C.
5. Take one loop-full (10µl loop) of the latter selective enriched culture and streak onto a Brilliance MRSA Chromogenic Agar plate (Oxoid) and a blood agar plate.
6. Incubate 24-48 h at 37 °C.
7. Observe the colony morphology (size and coloration) of the colonies obtained on the Brilliance MRSA Chromogenic Agar plate, choose up to five blue colonies indicative for being MRSA and isolate them **from the MRSA selective plate** onto a new blood agar plate. The additional blood agar plate obtained from inoculation of the selective broth is used for



- parallel observation with the MRSA plate to double-check the colony morphology (to observe typical *S. aureus* morphology and haemolysis), but should not be used for isolation.
8. Incubate the new blood agar plates containing the isolates during 24h at 37°C.
  9. Observe the colony morphology of the isolates on the blood agar plate (colour, appearance, haemolysis). Check for purity and re-isolate if necessary.
  10. Presumptive isolates of methicillin resistant *Staphylococcus aureus* should at this stage either be stored under appropriate conditions (–80°C) for later identification and characterisation or processed immediately.

## 4.2 Identification of MRSA

Presumptive MRSA isolates should be confirmed as *Staphylococcus aureus* isolates carrying the *mecA* gene by PCR. There is no need to perform other screening methods (such as screening with either oxacillin or ceftioxin) if the isolates have been obtained from selective isolation, thus, the presence of the *mecA* gene can be directly confirmed by PCR amplification. The species identification is simultaneously confirmed by using a multiplex PCR protocol including the amplification of 16S rDNA (internal control of the PCR reaction), nuclease (specific for *Staphylococcus aureus* species) and *mecA*-gene, which should be performed according to the EURL-AR recommended protocol ([http://www.eurl-ar.eu/data/images/meca-pcr\\_protocol%2006.02.08.pdf](http://www.eurl-ar.eu/data/images/meca-pcr_protocol%2006.02.08.pdf)).

## 4.3 Spa typing

*Spa* typing of the MRSA isolates may be performed additionally if the laboratory has the capacity to perform and analyse the *spa*- typing data. In case you decide to include *spa* types in the data submitted, these will be evaluated on the accuracy of the *spa* typing.

## 5 Reporting of results and evaluation

Fill in your results in the enclosed test form. Please enter your results into the interactive web database. Please read the detailed description below before entering the web database. When you enter the results via the web, you will be guided through all steps on the screen and you will immediately be able to view and print a submission report of your results. Please submit results by latest December 16<sup>th</sup> 2011. After the deadline, the database will be closed and you will be able to view and print an automatically generated report evaluating your results.

If you experience difficulties entering the data, please return results by e-mail, fax or mail to the EURL-AR.



All results will be summarized in a report which will be made available to all participants. The data in the report will be presented with laboratory codes. A laboratory code is known to the individual laboratory, whereas the entire list of laboratories and their codes is confidential and known only to the EURL-AR and the EU Commission. All conclusions are public.

If you have any questions, please do not hesitate to contact the MRSA EQAS Coordinator:

Lina Cavaco

The National Food Institute

Technical University of Denmark

Kemitorvet Building 204 Ground floor,

DK-2800 Kongens Lyngby

Denmark

Tel: +45 3588 6269

Fax: +45 3588 6341

E-mail: [licav@food.dtu.dk](mailto:licav@food.dtu.dk)

## 6 How to enter results in the interactive database

Please read this passage before entering the web page. Before you go ahead, you need your test form.

You are able to browse back and forth by using the forward and back keys or click on the EURL logo.

You enter the EURL-AR EQAS 2011 start web page (<http://thor.dfvf.dk/crl>) then write your username and password in low cases and press enter. Your username and password is the same as in the previous EQAS's arranged by The National Food Institute. If you have problems with the login please contact us.

Click on "[MRSA tests](#)" to start entering your data regarding the MRSA EQAS.

Please read carefully the instructions on the webpage and start by answering to the questionnaire on the work performed in your laboratory relative to MRSA by clicking on "[General MRSA questionnaire](#)".

Please choose the options that more correctly describe your work on MRSA and before you leave this page click on "[Save page](#)" which will take you back to the previous menu.



Then, we will ask you to fill in the methods used in a second page which you will reach by clicking on “[Methods for MRSA test samples.](#)”

In the next page you navigate to fields with the Tab-key and mouse.

Fill in what kind of method you have used for the selective isolation and confirmation of MRSA in this EQAS.

Click on "[Save and go to the next page](#)”

In the data entry pages for each sample “[EURL-MRSA 3.1, ...](#)”, you enter the obtained results for each of the MRSA EQAS samples.

If you wish so, you will have the option to include the *spa*-typing results.

If you did not perform *spa* typing leave the field blank, in case you have obtained an isolate but it is not a methicillin resistant *Staphylococcus aureus*, choose “not applicable (N/A)”, otherwise if you have performed the *spa* typing, choose the *spa* type from the list. Click on "[save and go to next page](#)" to navigate to the next sample results, until you finish to upload all your data.

From the last result sheet you get into the general menu, from where you can review the input pages, approve your input and finally see and print the evaluated results:

Browse through the pages and make corrections if necessary. Remember to save a page if you make any corrections. If you save a page without changes, you will see an error screen, and you just have to click on "[back](#)" to get back to the page and "[go to next page](#)" to continue.

At the end, approve your input. Be sure that you have filled in all the results before approval, as **YOU CAN ONLY APPROVE ONCE!** The approval blocks your data entry in the interactive database, but allows you to see the submitted results.