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# Direct detection of MRSA in surveillance samples by Xpert MRSA

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BILULU

## ABSTRACT

**Objectives:** Evaluation in a multi-centre setting of the performance of a rapid commercial molecular technique (Cepheid's Xpert MRSA assay) for the detection of MRSA in surveillance samples of high-risk patients in comparison to standard culture technique.

**Methods:** High-risk patients (n=236) were sampled from April till June 2007 in 5 Belgian hospitals. Separate nasal, throat and perineal swabs were collected using the Cepheid collection device (Double Copan Swab). In addition, swabs were taken according to each specific hospital procedure.

One part of the double Copan nose, throat and perineum swabs was pooled and vortexed in the Elution Reagent and then transferred to the Xpert MRSA cartridge. Molecular testing was performed on the GeneXpert according to the manufacturer's instructions. The system combines extraction, real-time PCR and detection in one hour.

The other part of the double swabs was pooled, frozen and sent to 1 laboratory that performed the reference culture, using TSB with 5% NaCl and subculture after 24 hours on a chromogenic agar plate.

The own procedure swabs (nose, throat and perineum) were cultured in-house, also using selective enrichment and chromogenic agar according to local procedures.

**Results:** 27 Samples (11,4%) showed inhibition on the GeneXpert and were not included in the analysis.

The Xpert MRSA assay identified 97.4% of the specimens positive for MRSA and 86.5% of the specimens negative for MRSA. For the samples tested, the Positive Predictive Value was 62.3% and the Negative Predictive Value was 99.3%.

The PPV of the GeneXpert results versus the Reference culture and versus the In-house culture method differed significantly (62.3% versus 80.3%). Freezing and thawing might have been injurious to the viability of MRSA. For almost all hospitals, the PPV decreased significantly when performed with the Reference Culture method.

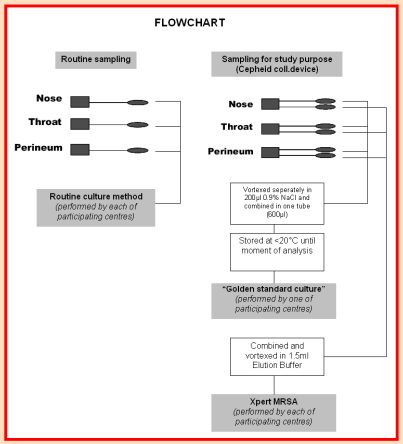
**Conclusion:** The Cepheid's Xpert MRSA assay proves to be an efficient test to rule out MRSA (NPV=99.3%). The test provides rapid results (about one hour) which might be helpful in prevention and control of MRSA in hospitals. However, because of a high rate of 'invalids' and a low PPV, a culture method is still needed to confirm a positive result.

## INTRODUCTION

In Belgium, MRSA is responsible for approximately 25% of nosocomial infections. Controlling MRSA is a primary focus of most hospital infection control programs. Currently, the standard surveillance method for detecting MRSA is culture, which is very laborious and time intensive. A rapid and more sensitive method for surveillance of MRSA could represent a definite advantage for infection control programs. We evaluated the performance of the Cepheid Xpert MRSA assay, which is a commercial molecular assay based on real-time PCR and which has the ability to provide results in about one hour.

## METHODS (1)

***Specimen collection and processing***




A total of 236 samples was collected from April 2007 till June 2007 in 5 different hospitals in Belgium:

- Imelda Ziekenhuis, Bonheiden (IMB)
- Onze-Lieve Vrouw Ziekenhuis, Aalst (OLV)
- Sint-Lucas Ziekenhuis, Gent (SLG)
- Ziekenhuis Oost-Limburg, Genk (ZOL)
- Virga Jesseziekenhuis, Hasselt (VJZ)

Separate nasal, throat and perineal swabs were collected using the Cepheid collection device (Double Copan Swab). In addition, swabs were taken according to each specific hospital procedure.

## METHODS (2)

***Culture and MRSA identification***

All samples were cultured in-house using chromogenic agar with or without enrichment according to local practices. Confirmation and identification of positive samples was performed according to the Belgian national guidelines (2003).

A frozen aliquot of each sample was also sent to one center (VZJ, Hasselt) for reference culture:
100 µL of the NaCl solution was inoculated into a staphylococcal enrichment broth (Tryptic Soy Broth containing 5% NaCl). After 24h incubation at 35°C in ambient air, the enrichment broth was subcultured on a selective MRSA-screening agar.
The plates were incubated for 24 hours at 35°C and inspected for suspicious colonies. MRSA-negative cultures were incubated for another 24 hours and inspected again.

The identification of the isolated MRSA strains was confirmed by biochemical analysis, tube coagulase test and slide coagulase test. Methicillin resistance was confirmed by disk diffusion with Oxacillin and Cefoxitin disks on MH II agar.

***Molecular testing using the GeneXpert***

Molecular testing was performed on the GeneXpert system (Cepheid) according to the manufacturer's instructions. The system combines sample preparation with real time PCR amplification and detection in one hour.
Every participating hospital performed the analysis on their own instrument.
Each test included a Sample Processing Control (spores of Bacillus globigii) that verifies if lysis of MRSA has occurred if present and verifies that specimen processing was adequate. This control also detects specimen-associated inhibition of the real-time PCR assay.


*Figure 1: Xpert MRSA cartridge, reagents and the 4-site GeneXpert system*

## DISCUSSION (1)

Results of all 236 samples were analyzed together to determine clinical sensitivity and specificity. A total of 27 samples showed inhibition on the GeneXpert system and were therefore not included in the analysis.

**Table 1: results of the GeneXpert versus the Reference "golden standard" culture method.**

| Table 1 | Ref. Culture + | Ref. Culture - | Total (N=236) |
|---|---|---|---|
| GeneXpert + | 38 | 23 | 61 |
| GeneXpert - | 1 | 147 | 148 |
| Total | 39 | 170 | 209 |
| Invalids | 1 | 26 | 27 |

- Sensitivity = 97.4%
- Specificity = 86.5%
- Positive Predictive Value (PPV) = 62.3%
- Negative Predictive Value (NPV) = 99.3%
- GeneXpert invalid samples (inhibition) = 11.4%. All invalid samples (except 1) were negative for culture

**Table 2: results of the GeneXpert versus the in-house culture method.**

| Table 2 | In-house Culture + | In-house Culture - | Total (N=236) |
|---|---|---|---|
| GeneXpert + | 49 | 12 | 61 |
| GeneXpert - | 3 | 145 | 148 |
| Total | 52 | 157 | 209 |
| Invalids | 1 | 26 | 27 |

- Sensitivity = 94.2%
- Specificity = 92.4%
- Positive Predictive Value (PPV) = 80.3%
- Negative Predictive Value (NPV) = 98.0%
- GeneXpert invalid samples (inhibition) = 11.4%. All invalid samples (except 1) were negative for culture

## DISCUSSION (2)

We also compared our results with the clinical performance tests carried out by Cepheid (REF GXMRSA-100N-10). The Xpert MRSA assay identified 86.3% of the specimens positive for MRSA and 94.9% of the specimens negative for MRSA. For the subjects tested (1077 patients), the positive predictive value was 80.5% and the negative predictive value was 96.6%.

| Table 3 | Culture + | Culture - | Total (N=1077) |
|---|---|---|---|
| GeneXpert + | 182 | 44 | 226 |
| GeneXpert - | 29 | 819 | 848 |
| Total | 211 | 863 | 1074 |
| Invalids | | | 3 |

*Table 3: Overview of the results performed on the GeneXpert (Cepheid) and Culture method by Cepheid*

Cepheid included only nasal specimens in their study, while we pooled nasal, throat and perineum specimens. This could possibly explain our higher rate of invalid samples. Our results show a higher sensitivity. (In our results, nasal swabs sometimes gave negative culture results while for the same patient, the culture for the throat or perineum was MRSA positive.)

The Positive Predictive Value (PPV) of the GeneXpert versus our own in-house culture and versus the Culture method by Cepheid is equal (80.3% and 80.5%), but there is a significant difference with the PPV from our Reference culture method (62.3%). The reason for this could be that all samples for reference culture were frozen (-20°C) prior to culture. Freezing and thawing might have been injurious to the viability of MRSA cells. For almost all institutes, the PPV decreased significantly when performed with the Reference Culture method.

| Table 4 | PPV in-house culture | PPV reference culture |
|---|---|---|
| Hospital 1 | 87.5% | 62.5% |
| Hospital 2 | 76.5% | 41.2% |
| Hospital 3 | 88.9% | 66.6% |
| Hospital 4 | 76.1% | 71.4% |
| Hospital 5 | 83.3% | 83.3% |
| Mean | 80.3% | 62.3% |

*Table 4: Positive Predictive Values (PPV) of the GeneXpert versus the in-house culture and the reference culture for every hospital separately.*

The Negative Predictive Value (NPV) of the GeneXpert versus all three culture methods (Reference, in-house and Cepheid culture) is very high and comparable which indicates that the Xpert MRSA assay is a reliable test to determine if a patient is MRSA negative.

When we plot the GeneXpert Ct-values versus the MRSA reference culture results (positive/negative), most discrepancies are situated at higher Ct-values (Ct >=30), indicating a low DNA concentration. However, some samples with relatively low Ct-values (Ct = 25-29) still have a negative culture result. When we compare the Ct-values versus the MRSA in-house culture method, the 5 negative culture samples with the lowest Ct-values were found positive with the in-house culture method.
We can conclude that discrepancies between Culture and GeneXpert results are mostly found in the higher Ct range (indicating that DNA might be present in the samples from non-viable MRSA).

## CONCLUSIONS

The Cepheid's Xpert MRSA assay proves to be an efficient test to determine if a patient is negative for MRSA (NPV=99.3%). The test provides rapid results in one hour and might help prevent and control the spread of MRSA in hospitals. The GeneXpert system can be used 24 hours a day because it's easy to use.
However, if the result is positive, because of the low PPV, a culture method is still needed to confirm or verify the positive result.