Is there a need for 'a grey zone' for Xpert MRSA rapid testing?

K.Van Vaerenbergh[1], A.Boel[1], R.Cartuyvels[4]*, G.Coppens[5]*, J.Frans[2]*, A.M.Van den Abeele[3]*, H.De Beenhouwer[1]* on behalf of the BILULU * study group

[1] Onze-Lieve-Vrouw Ziekenhuis, Aalst, [2] Imelda Ziekenhuis, Bonheiden, [3] AZ Sint-Lucas, Gent, [4] Virga Jesseziekenhuis, Hasselt, [5] Ziekenhuis Oost-Limburg, Genk, Belgium.

Corresponding author: Moorselbaan 64, 9300 Aalst, BELGIUM   Phone: +32 53 72 42 74   Fax: +32 53 72 45 88   Email: kristien.van.vaerenbergh@olvz-aalst.be

BILULU

www.bilulu.be

ABSTRACT

Objectives: Xpert MRSA (GX) testing was introduced in routine screening for MRSA because of the high negative predictive value (NPV). In this setting the potential benefit of the use of an equivocal interpretative category ('**grey zone**') was evaluated in order to ameliorate the reliability of a fast screening pathway.

Methods: Among five microbiology laboratories the MRSA screening procedure was standardized for pooled samples. Indications for GX screening were well defined. The follow-up of patients in decolonisation was done by culture on chromogenic media after non-selective enrichment. GX gives a positive/negative result based on a cut-off Ct value of 36 cycles. Ct values of all negative GX tests were reanalyzed and any sample with a Ct > 36 was cultured.

Results: During a first extensive phase of evaluation, all samples were cultured and GX tested. This evaluation showed a high NPV (99,3 %) and an acceptable sensitivity (>95%) of GX in our patient populations, permitting the use of GX to give fast and reliable exclusion of MRSA positivity. Because of the rather low positive predictive value (80,3 %), positive results of GX needed to be confirmed by culture. GX was introduced in routine since January 2009. During 2009, 4745 samples were analysed with GX. For 4082 samples, GX was negative. Culture was positive for 513 of the 663 positive GX samples, giving a PPV of 77.3 %. 137 samples had a negative GX result with a Ct above 36. Of these 29 (21.2%) were positive with culture. Thus considering an equivocal interpretative category **5% more positive samples** were recovered.

Conclusion: A 'fast-screening' pathway based on Xpert MRSA gives rapid and reliable exclusion of MRSA. However in our patient population 5% of the positive samples were GX negative but with a Ct above 36. In order to increase the sensitivity of the fast screening pathway, introduction of an equivocal interpretative category -to be confirmed by culture- seems mandatory.

INTRODUCTION

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. We introduced Xpert MRSA testing in routine screening for its high negative predictive value, resulting in fast exclusion of MRSA. To ameliorate the reliability of a fast screening pathway, we evaluated the need for a 'grey zone', an equivocal interpretative category.

METHODS (1)

Specimen collection and processing
Among 5 microbiology laboratories the MRSA screening procedure was standardised with well defined criteria for Xpert MRSA screening :

1. Previously being MRSA positive
2. Recent hospitalisation
3. Resident in a chronic care facility
4. Room mate of a MRSA positive patient

A total of 4745 samples was collected for Xpert MRSA analysis.
Separate nasal, throat and perineal swabs were collected using E-swab. After pooling the liquid medium, Xpert MRSA was performed on 150 µl of pooled medium.

Molecular testing using the Xpert MRSA

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 its own instrument.

The manufacturer's interpretation of Xpert MRSA is based on a Ct cut-off value, with samples being recorded as **MRSA positive** if the **Ct ≤ 36** and **MRSA negative** if the **Ct > 36** regardless of the evidence of amplification.

METHODS (2)

Molecular testing using the Xpert MRSA
Because of the performance characteristics of GeneXpert (high NPV~ 99% and sensitivity~96%, lower PPV ~ 80%), a positive GX result was confirmed by culture, a negative result was reported without any further confirmation.

For this evaluation, analysis of amplification data was performed for all samples with negative Xpert MRSA result. All samples with Ct value above 36, regardless of the endpoint values, were cultured

Culture and MRSA identification
Samples with positive GX or with negative GX and Ct≠0 were cultured using chromogenic media after enrichment.
Identification of suspicious colonies was assessed with at least coagulase tube test with rabbit plasma or slide agglutination in combination with the detection of DNAse. Screening tests for MecA mediated oxacillin resistance were used according to CLSI guidelines.
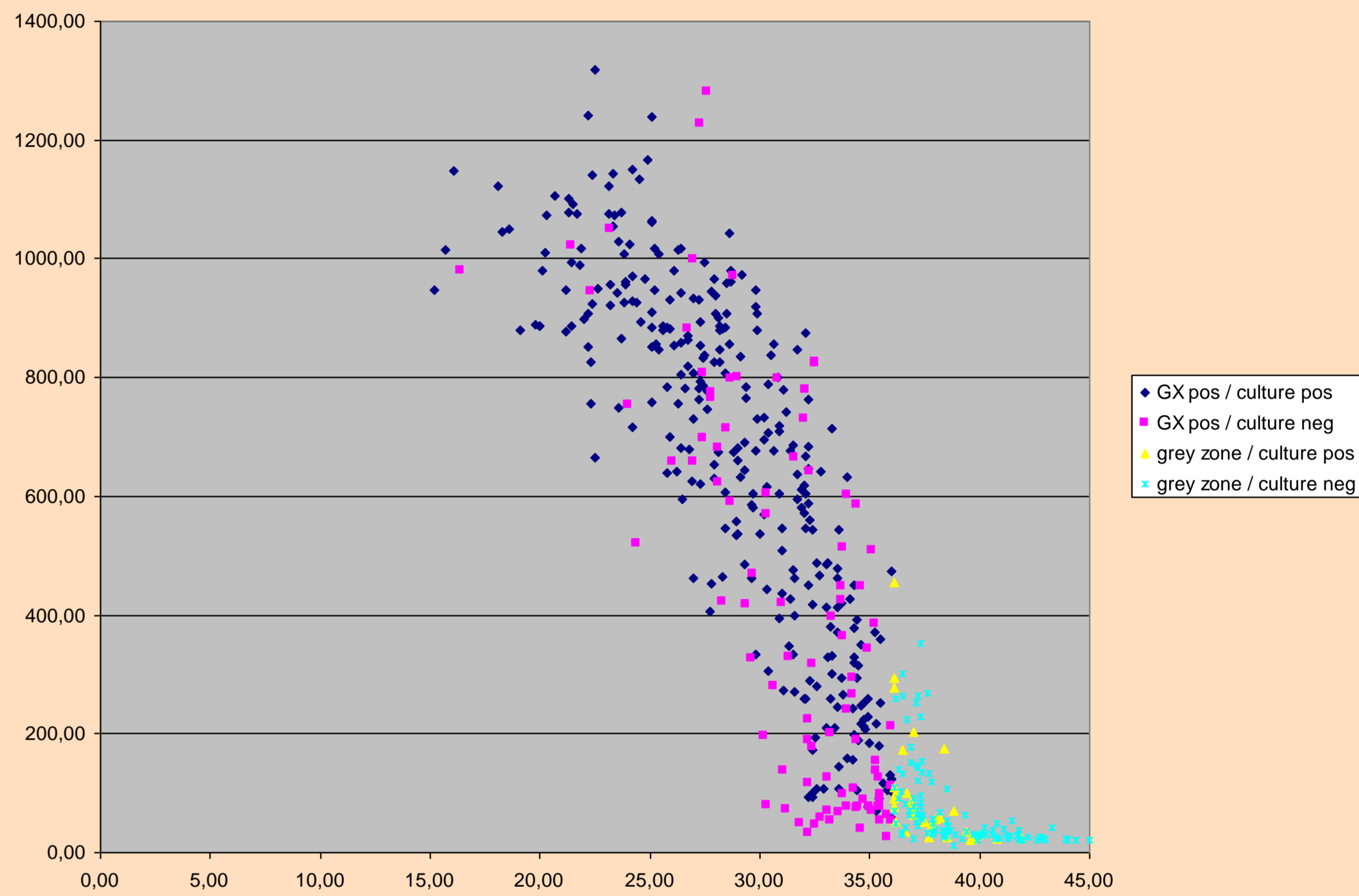
RESULTS (1)

• 4754 samples analysed with Xpert MRSA during 2009 were included in this evaluation.

• 663 samples tested positive on GX (14%). For 513 of them, culture was positive (11%) resulting in a positive predictive value of 77%.

• 4082 (86%) samples tested negative on GX. For 3945 samples (83%) with a Ct sample = 0, no culture was performed.

•The remaining 137 samples with negative GX had a Ct > 36. In the different labs, 3% (range 1.6 to 5.3%) of the negative GX samples had a result in this 'grey zone'. Culturing this 'grey zone' showed that 29 (21,2 %) were positive for MRSA.

• Culture of samples with a 'grey zone' result, increases the sensitivity of GX with 5.3% (range 4 to 9%) in the different labs.

• In the strategy we use, implementation of a 'grey zone' increases the number of samples that need to be cultured with 20%. (from 14 to 17% of all samples)

• Endpoints for samples with a 'grey zone' result are similar for samples with positive and negative culture.

Amplification curve for sample in 'grey zone'
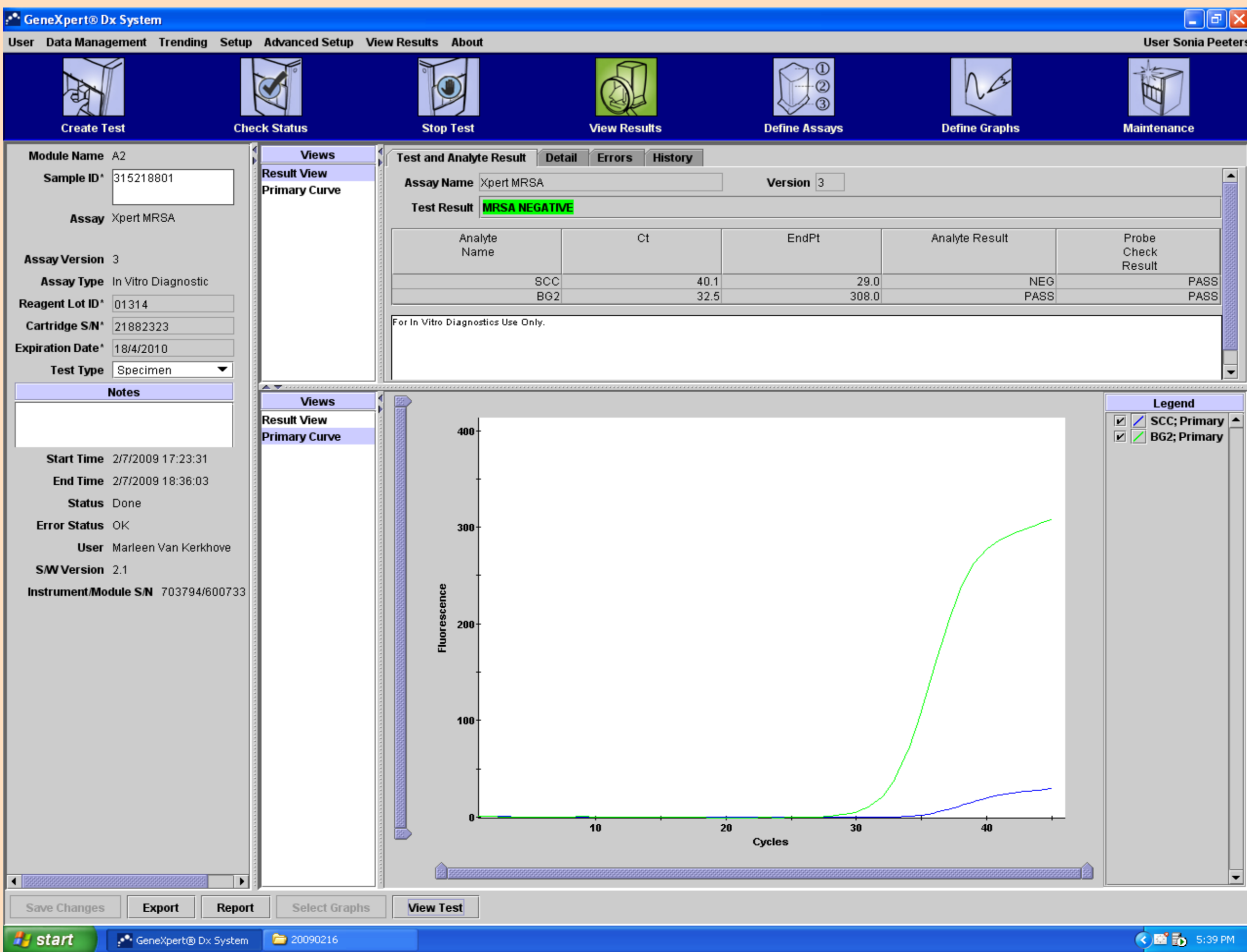
RESULTS (2)

Distribution of samples with a Ct ≠ 0, considering CT and endpoint

Results of MRSA Xpert versus culture

LAB	TOTAL	NEG GX	POS GX	POS GX POS CUL	EQUIV. GX	EQUIV GX POS CUL	% POS SAMPLES
1	2078	1748	274	218	56	13	11
2	532	393	117	98	22	5	19.3
3	406	353	38	30	15	3	8
4	1175	966	173	115	36	6	10
5	554	485	61	52	8	2	10
TOT	4745	3945 (83%)	663 (14%)	513	137 (3%)	29	11.5 %

CONCLUSIONS

With a high negative predictive value the Cepheid's Xpert MRSA assay proves to be an efficient test to exclude MRSA. In our patient population the sensitivity can be increased with 5% by introducing a third interpretative category ('grey zone') for negative samples with a Ct above 36. It seems mandatory to exclude for these samples the presence of MRSA with culture in order to ameliorate the reliability of this fast screening pathway.