Comparison of the GeneXpert PA assay with semi-quantitative and quantitative cultures and O-antigen acetylase gene-based qPCR for the detection of *Pseudomonas aeruginosa* in endotracheal aspirate samples

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Introduction

Ventilator-associated pneumonia (VAP) is the most common nosocomial infection in intensive care units (ICUs), with *Pseudomonas aeruginosa (P. aeruginosa)* as one of the most important etiologies. While culture remains the gold standard, effective and rapid laboratory diagnosis is critical for timely treatment of VAP. Culture-based methods detect viable pathogens, but also endure a relatively extended time period (1). On the other hand, polymerase chain reaction (PCR)-based detection offers benefits, such as lower limits of detection (LOD) and shorter time to detection (2). **To investigate this, we compared detection of *P. aeruginosa* in endotracheal aspirates (ETA) by three assays: GeneXpert assay (Cepheid, ref: RPA-10), in-house quantitative real-time PCR (qPCR) and quantitative bacterial culture.**

Methods

80 Fresh ETA samples from University Hospital Antwerp (Routinely collected from patients between May 2017 – September 2018)

Homogenisation and liquefaction with N-acetylcysteine

GeneXpert (Cepheid)

GeneXpert assay was performed according to manufacturer's instructions within 48h of collection of the ETA.

GeneXpert Cartridge (Cepheid)

Quantitative culture

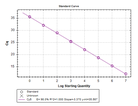
plated on:
(1) chromogenic ChromID *P. aeruginosa* (bioMerieux)
(2) blood agar

with and without brain heart infusion (BHI) enrichment.

Eddyjet (IUL Instruments)

In-house q-PCR

(1) DNA extraction: NucliSENS® easyMAG®, bioMérieux
(2) Taqman qPCR, targeting the O-antigen acetylase gene
(3) CFX96 TouchTM, Bio-Rad



Standard curves were set up using Avogadro's constant.

Results

Of 41 samples positive for *S. aureus* by culture, 39 samples were positive by GeneXpert, and 37 by in-house qPCR. Of culture-negative samples (n=39); 38 samples were also negative by GeneXpert, while 27 were negative by qPCR (Table 1).

		GeneXpert		UA qPCR	
		Pos (n=40)	Neg (n=40)	Pos (n=49)	Neg (n=31)
qCulture*	Pos (n=40)	39	1	37	3
	Neg (n=40)	1	39	12	28

*Culture is based on the presence of *S. aureus* on blood agar, PAID plate or after enrichment

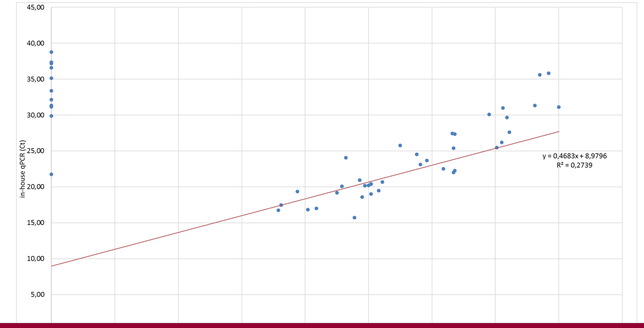
False negative results with GeneXpert (1/41) and *in-house* qPCR (3/41, C_T= 0.00) may be attributed to presence of substances interfering with PCR.

		Extended gold standard*		Sensitivity (%)	Specificity (%)
		+	-	(95% CI)	(95% CI)
GeneXpert	+	40	0	97.56 (87.14 - 99.94)	100
	-	1	39		
Q-culture	+	40	0	97.56 (87.14 - 99.94)	100
	-	1	39		
Enrichment Q-culture	+	40	0	97.56 (87.14 - 99.94)	100
	-	1	39		
qPCR	+	38	11	92.68 (80.08 - 98.46)	71.79 (55.13 - 85.00)
	-	3	28		
SQ-culture	+	40	0	97.56 (87.14 - 99.94)	100
	-	1	39		

* *P. aeruginosa* detected by SQ-culture plus one sample that showed *P. aeruginosa* presence by the other four methods but not by SQ-culture

A total of 13 samples had discrepant results between *P. aeruginoasa* detection by GeneXpert PCR and the in-house qPCR. There was two *P, aeruginosa* positive by GeneXpert that was negative by culture.

Differences between TaqMan qPCR and GeneXpert might be due to different sample preparation (untreated versus liquefied), and the washing step, which removes extracellular DNA prior to the GeneXpert. Of note the GeneXpert performs a PCR in a single-use cartridge and provides results in less than 1 hour after a sample is received



Conclusion

Compared to culture as a reference standard, GeneXpert assay showed 100% specificity and 97,6% sensitivity, while in-house PCR showed 71,8% specificity and 92,7% sensitivity. This first evaluation of the GeneXpert PA assay with ETA samples found it to be highly sensitive, specific, user-friendly (hands-on time ~5 min.), and rapid (~66 min. assay time).

References

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(2) **Yang S**, **Rothman RE**. 2004. Lancet Infect. Dis. **4**:337–348.

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