Performance of a cylindrical microarray-based system for rapid molecular pathogen detection

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Background: Molecular-based diagnostics are essential for rapid pathogen identification, and increases sensitivity compared to culture for fastidious/non-cultivable microbes and after antimicrobial administration.

We use broad-range 16S rRNA gene PCR with Sanger sequencing (16S) and/or selected in-house specific PCRs routinely based on a clinical microbiologist's assessment of samples. Specific PCRs offer high sensitivity but are limited to predefined targets, while 16S can detect a wider pathogen range but relies on sequencing, prolonging turnaround times and requiring specialized expertise for interpretation.

Cube Dx (St.Valentin (Austria)) has developed a cylindrical microarray-based assay identifying >100 targets from 16S rRNA PCR-amplicons. The system (Hyborg-platform) provides semi-quantitative results within approximately 15 minutes. This study compares Hyborg results with standard diagnostics (SD).

Methods: Retrospective analysis of eluates (Roche MagNA Pure 96 or ELITech ELITe InGenius, pretreatment = mechanical lysis) from all samples submitted to 16S and/or selected in-house specific PCRs at Haukeland university hospital (Bergen (Norway)) during May 2025. Two µl eluate was analysed with a RUO-version of Cube Dx's Bacteria xC PCR-kit on a QuantStudio 5 platform (Thermo Fisher), followed by analysis of the PCR-product on the Hyborg.

Results: 74 samples were included (pleural effusion (41), synovial fluid (9), CSF (1), BAL (1), abscesses (11), bone (4), heart valve (1) and tissues (6)), from 46 men and 28 women (median age 62 years (IQR 28; range 0–87)).

The median turnaround time from sample arrival to end-of SD-analysation was 2.56 days (IQR 2.16; range 0.53–6.17 days).

Culture results were available for 57 (78%) samples; 8 (15%) were growth positive. Both molecular SDs and Hyborg detected ≥1microorganism(s) in 28 (38%) samples. Hyborg identified eight additional pathogens across five samples, whereas seven were missed (Figures 1+2). Culture identified six additional microbes; however, all were considered of uncertain significance. Possible 16S contaminants were identified by Hyborg in 46 samples (Figure 3).

Pathogen detection by selected in-house specific PCRs vs. Cube Dx`s Bacteria xC PCR/Hyborg microarray

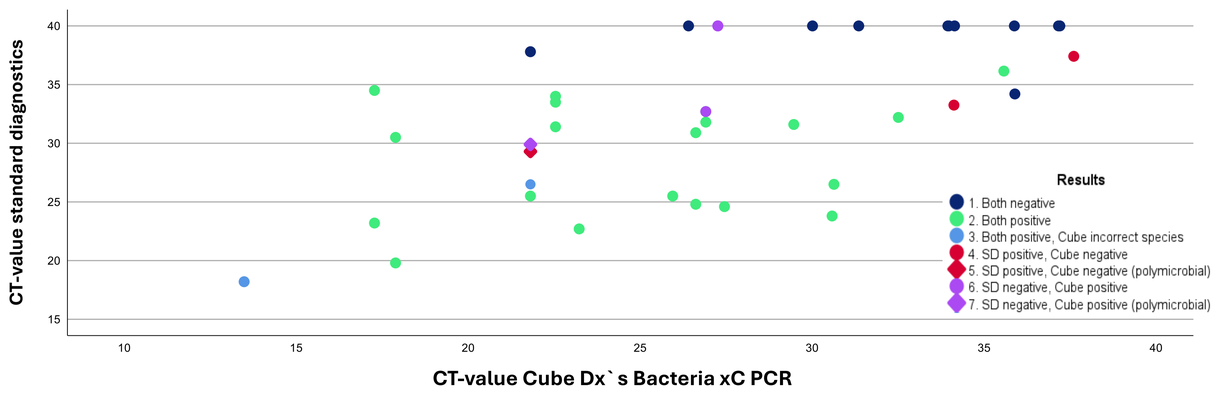

Figure 1 Comparison of standard diagnostic (SD) results obtained using selected in-house specific PCRs with retrospective analysis performed using Cube Dx's Bacteria xC PCR and microarray detection on the Hyborg system for samples received at Haukeland University Hospital in May 2025. The following microbes (n =) were detected in Results category:

1. None
2. *Parvimonas micra* (2), *Fusobacterium nucleatum* (6), *Staphylococcus aureus* (2), *Streptococcus anginosus group* (6), *Streptococcus pneumoniae* (5)
3. *Klebsiella oxytoca* (SD) vs. *Klebsiella michiganensis* (Cube) and *Staphylococcus aureus* vs. *Staphylococcus spp.*
4. *Borreliella burgdorferi* (2)
5. *Parvimonas micra*
6. *Granulicatella adiacens, Streptococcus mitis*
7. *Prevotella intermedia*

Pathogen detection by 16S rRNA-PCR/Sanger vs Cube Dx`s Bacteria xC PCR/Hyborg microarray

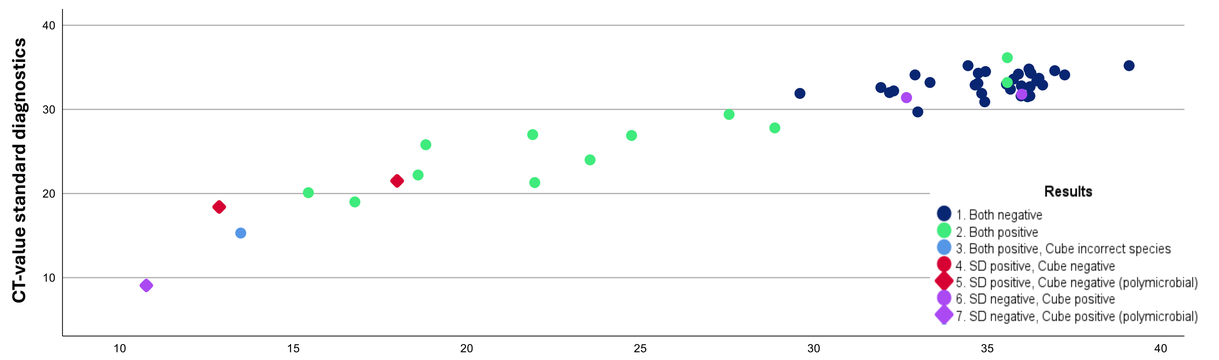

Figure 2 Comparison of standard diagnostic (SD) results obtained using in-house 16S rRNA PCR followed by Sanger sequencing with retrospective analysis performed using Cube Dx's Bacteria xC PCR and microarray detection on the Hyborg system for samples received at Haukeland University Hospital in May 2025.
The following microbes were detected in Results category:

1. None
2. *Clostridium perfringens* (2), *Escherichia coli* (3), *Finegoldia magna* (1), *Fusobacterium nucleatum* (2), *Parvimonas micra* (3), *Staphylococcus aureus* (1), *Staphylococcus capitis* (1), *Staphylococcus warneri* (1), *Streptococcus anginosus group* (1), *Streptococcus pneumoniae* (1)
3. *Klebsiella oxytoca* (SD) vs. *Klebsiella michiganensis* (Cube) (Both belong to the *Klebsiella oxytoca* species complex)
4. None
5. *Aggregatibacter aphrophilus, Campylobacter gracilis, Streptococcus mitis, Abiotrophia defectiva*
6. *Clostridium perfringens, Enterococcus faecium, Fusobacterium, Parvimonas*
7. *Campylobacter*

Conclusion: Both molecular standard diagnostics and Hyborg outperformed culture. The Hyborg system shows promising potential for rapid molecular pathogen detection. Interpretation of possible 16S contaminants may be challenging in some cases, but this limitation is inherent to 16S-based diagnostic methods in general.

A **Cube Dx result report**

Sample [#]
Date
Remarks
Liquids 2: CS-Buffer (2816010004)/S: System Liquid (2802010004)

Test hybcell Bacteria DNA xC (4)
Profile Sepsis (17,07,2025)
hybcell 2718A250548

Control|s		
Controls	PASSED	

Parameters	Result	Representation
Internal Control (IC)	Detected	
BACTERIA	Detected	

Off-profile parameters	Result	Representation
Bacteria Pan	Detected	100 — 99999
Gram neg	Detected	100 — 99999
Gram pos	Detected	100 — 99999
Cutibacterium acnes	Detected	100 — 99999
Staphylococcus	Detected	100 — 99999

Figure 3 A) Example of sample with Cube Dx results interpreted as 16S contaminants

B **Cube Dx detections considered possible contaminants**

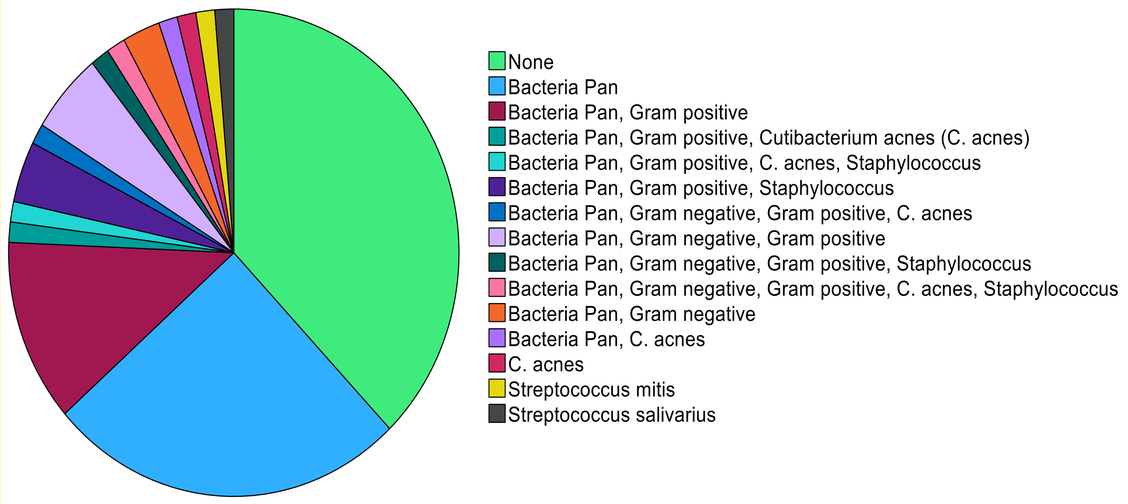

Figure 3 B) Overview of all Cube Dx results interpreted as 16S contaminants

References: Kommedal Ø et al. Dual priming oligonucleotides for broad-range amplification of the bacterial 16S rRNA gene directly from human clinical specimens. J Clin Microbiol. 2012 Apr;50(4):1289-94. doi: 10.1128/JCM.06269-11.
Kommedal Ø et al. Microbiological diagnosis of pleural infections: a comparative evaluation of a novel syndromic real-time PCR panel. Microbiol Spectr. 2024 Jun 4;12(6):e0351023. doi: 10.1128/spectrum.03510-23.

Ethics/Funding: Cube Dx provided assay materials but was not involved in data analysis/interpretation. The regional ethics committee waived the requirement for informed patient consent

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