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Clinical performance of broad-range 16S and 18S rRNA gene PCR/Real-Time PCR and sequencing compared to culture diagnosis: a systematic review and meta-analysis

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BACKGROUND

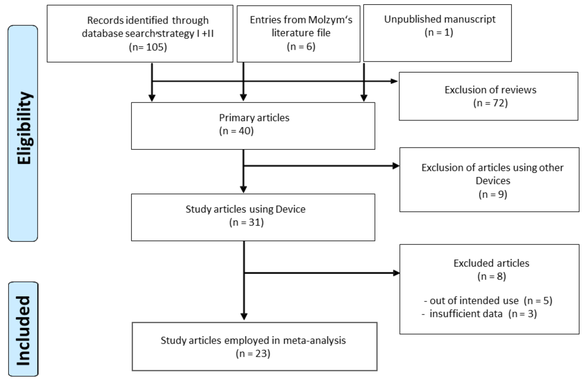
The identification of infectious agents remains a multifactorial challenge. Routine culture methods are often negative due to the administration of antibiotics or specific growth requirements of fastidious microorganisms. On the other hand, culture-independent approaches are often described with lack in sensitivity, specificity and identification performance. Molzym has developed culture-independent molecular testing solutions (MMDx), SepsiTest™-UMD, UMD-SelectNA™ and Micro-Dx™ including the automated system SelectNA™*plus* for the *in vitro* diagnosis of pathogens, comprising the most common, but also rare, fastidious and non-growing bacteria, as well as fungi directly from body fluids, tissues or swabs in 7 hours. MMDx are based on a single protocol of unique microbial DNA enrichment and extraction (MolYsis™), followed by broad-range 16S & 18S rRNA gene PCR or Real-Time PCR and sequencing analysis. The aim of this systemic review and meta-analysis was to determine the clinical performance of MMDx in comparison to culture diagnosis as standard.

MATERIAL & METHODS

A systematic review of peer-reviewed articles on MMDx published in international journals and a meta-analysis of the data was performed according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). Two reviewers independently appraised the quality of studies and extracted data. The risk of bias for diagnostic test accuracy was conducted by using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2). Statistical analysis was carried out using MetaXL (EpiGear International). Pooled and subgroup analysis of diagnostic sensitivities, specificities, and added-value of MMDx (expressed as true pathogen identification in culture-negative samples) were reported in addition to positivity, false-positive and false-negative rates.

RESULTS

Figure 1: Results of literature search and selection of articles: PRISMA Workflow



RESULTS

Table 1: Positivity, false-positive and false-negative rates of MMDx compared to conventional culture (pooled results)

Parameter	Device	Reference standard	Diff. significant?
POSITIVITY	**35%** (95% CI: 34%, 37%)	**21%** (95% CI: 19%, 22%)	**Yes** (p=0.001)
FALSE POSITIVE	**6%** (95% CI: 5%, 7%)	**9%** (95% CI: 6%, 11%)	**No** (p=0.306)
FALSE NEGATIVE	**4%** (95% CI: 3%, 5%)	**15%** (95% CI: 11%, 18%)	**Yes** (p=0.000)

Table 2: Diagnostic sensitivity and specificity of MMDx compared to conventional culture and added value of MMDx to all samples (subgroup analysis)

Disease	Sensitivity	Specificity	Added value per all samples
SEPSIS / BLOODSTREAM INFECTIONS	83% (95% CI: 73%, 95%)	87% (95% CI: 84%, 90%)	8% (95% CI: 6%, 12%)
BONE & JOINT INFECTIONS	86% (95% CI: 67%, 100%)	98% (95% CI: 94%, 100%)	13% (95% CI: 0%, 35%)
INFECTIVE ENDOCARDITIS	86% (95% CI: 81%, 92%)	67% (95% CI: 45%, 99%)	13% (95% CI: 6%, 26%)
MENINGITIS	94% (95% CI: 87%, 100%)	74% (95% CI: 61%, 90%)	47% (95% CI: 18%, 100%)
VARIOUS ROUTINE INFECTIONS	85% (95% CI: 80%, 90%)	84% (95% CI: 67%, 100%)	18% (95% CI: 9%, 36%)
TOTAL Including all 23 studies	**87%** (95% CI: 83%, 91%)	**87%** (95% CI: 79%, 95%)	**18%** (95% CI: 9%, 34%)

Table 3: Diagnostic added value of MMDx by identification of additional pathogens in comparison to culture (subgroup analysis)

Disease	Culture-positive rates	Added-Value over culture (expressed as the % of true infections identified in culture-negative samples) Added-value% – sensitivity% – specificity%	% of absolute added-value in addition to culture
SEPSIS / BLOODSTREAM INFECTIONS	13%	**8%** - 83% - 87%	+62%
BONE & JOINT INFECTIONS	13%	**13%** - 86% - 98%	+100%
INFECTIVE ENDOCARDITIS	31%	**13%** - 86 % - 67%	+42%
MENINGITIS	28%	**47%** - 94% - 74%	+168%
VARIOUS ROUTINE INFECTIONS	21%	**18%** - 85% - 84%	+86%
TOTAL Including all 23 studies	20%	**18% - 87% - 87%**	**+ 90%**

RESULTS

The state-of-the-art search resulted in 105 literature citations. This selection was supplemented by 6 articles from the Molzym database and one unpublished manuscript, so that 112 references were available. After excluding 72 reviews, 40 primary articles with study results remained. After applying specific selection criteria, a total of 23 studies analyzing 4.419 samples from 2.378 patients/episodes were included for data extraction (Figure 1). Among the studies, diseases such as sepsis (8/23) [1,2,3,4,5,6,7,8], infective endocarditis (5/23) [9,10,11,12,13], bacterial meningitis (2/23) [15,16], joint infections (1/23) [14] and various diseases from routine diagnostics (7/23) [17,18,19,20,21,22,23] were investigated with MMDx.

The following results have been determined:

- The median positivity rate with MMDx (35%) was significantly higher in comparison to conventional culture (21%) (Table 1).
- The false positive rate for culture was 9%, for MMDx 6% (Table 1).
- MMDx failed to find true pathogens grown in culture in 4% of cases (all studies), while up to 15% of the reference culture was false negative (Table 1).
- The pooled diagnostic sensitivity of species identification among diverse clinical materials of various diseases was 87%. The pooled specificity was 87% (Table 2).
- Added value: 18% additional pathogens were found by MMDx in culture-negative samples, in addition to the 20% identified by culture (Table 3).
- MMDx results had a direct impact on patient management with the adjustment of the antibiotic treatment protocol in 16% to 25% of positive cases [10, 23].

CONCLUSION

Many infection-causing microorganisms remain unidentified by culture. The **performance of MMDx showed**: detection of extra pathogens in addition to culture; earlier detection of pathogens than culture; broad spectrum of pathogens ID (with difficult-to-cultivate, rare or inhibited microorganisms); **Clinical added-value**: MMDx proved to ID infectious pathogens which are not detected by culture; contributed in adapting antimicrobial therapy (narrow therapy selection or de-escalation of antibiotic regimen). **MMDx is** thus an **effective** tool for **diagnosing** pathogens causing life-threatening diseases such as **sepsis**, **bone and joint infections**, **infective endocarditis and meningitis**, with the advantage of being culture-independent.

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