Broad-Range 16S/18S PCR and Sequencing Diagnosis of Infectious Endocarditis Using Automated Pathogen Enrichment and DNA Extraction

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Background

Microbiological culture specimen in infectious endocarditis (IE) patients is often negative due to fastidious growth requirements or inhibition of strains from antibiotic treated patients. Direct molecular testing does not depend on growth of pathogens, but can generate false positive results because of potential microbial DNA contamination during handling. Also, routine molecular diagnosis spends long hands-on time on sample processing, in particular on DNA extraction. Therefore, automated solutions are required.

Materials / Methods

Specimens included blood (1ml), excised heart valves, pacemaker electrodes, haematomas, pericardial abscesses and thrombi. Samples were processed according to instructions of the Micro-Dx™ kit and run on the SelectNA™*plus* robot (Molzym, Bremen, Germany). Eluates were analysed by 16S and 18S Real-Time PCR assays provided with Micro-Dx™. Amplicons from positive samples were sequenced and Blast-analysed (www.sepsitest-blast.net).

Fig. 1: The SelectNA™*plus* desk top robot for the automated extraction of microbial DNA from liquid and tissue samples (see table, right). The instrument is operated using the Mico-Dx™ kit. The robotic process includes the MolYsis™ removal of human DNA by DNase digestion, vacuum filtration of microorganisms on a membrane, *in situ* cell lysis and isolation of microbial DNA (see scetch below). Tissue samples are digested by proteinase K (10min) before extraction.

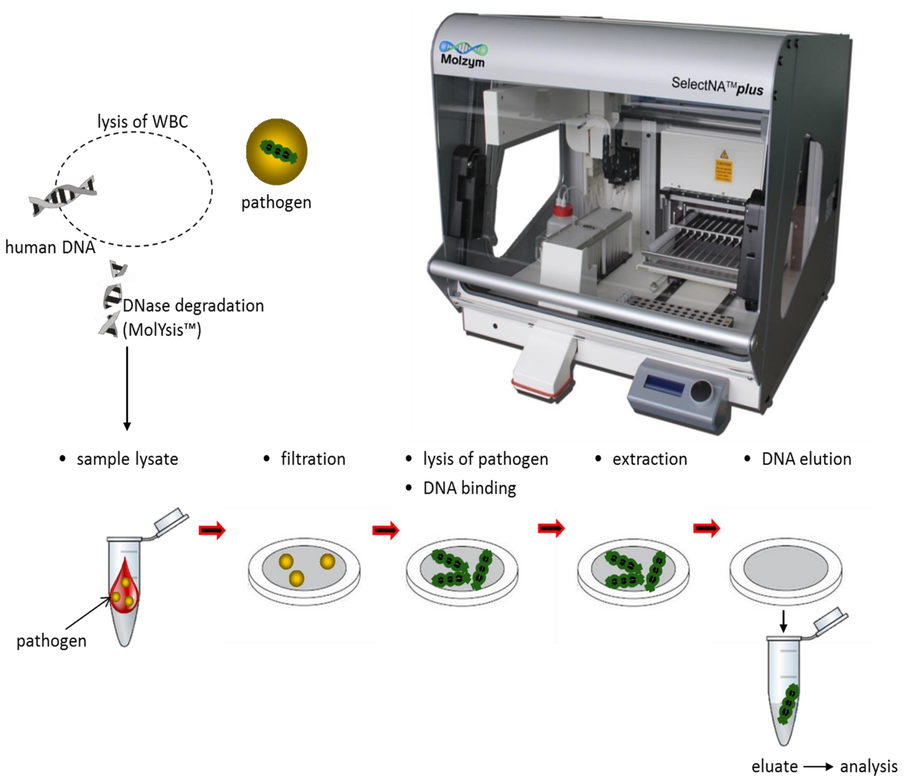

Table 1: IE patients (Duke criteria) and specimens used.

Patients	55
Samples	103
Patients with culture results	52
Samples	97
Specimens	blood, valves (aortic, mitral, tricuspidal), pacemaker, pericard biopsies, haematomas, pericardial abscesses, thrombi

Results

Aim:

To compare the clinical performance of the new completely automated, walk-away microbial DNA extraction system, SelectNA™*plus*/Micro-Dx™ (PCR) with culture diagnosis.

Outcome:

Samples (97) from 52 patients under suspect of having IE according to Duke criteria (Table 1) were diagnosed in parallel by 16S/18S PCR/sequencing and culturing.

Table 2: PCR and culture results.

	PCR pos	PCR neg	Sum
Culture pos	19	3	22
Culture neg	11	19	30
Sum	30	22	**52**

PCR result (Tables 3, 4):

True: identical by species or genus to culture in the same or other sample of a patient

Possible: culture negative/discrepant; organism found by PCR in another sample of a patient or grown earlier or identified in other material or judged relevant by clinical consideration of treating physician

False: PCR negative, culture positive

Table 3: Single infections.

Organism	PCR	Culture	Both	Relevance PCR [a]
Streptococci, total	**8**	**3**	**3**	
S. mitis/oralis	2	1	1	possible
S. gallolyticus	2	1	1	possible
S. gordonii	2	1	1	possible
S. salivarius	1	0	0	possible
S. pneumoniae	1	0	0	possible
Staphylococci, total	**7**	**4**	**4**	
S. aureus	2	1	1	possible
S. epidermidis	1	1	1	n.a.
S. haemolyticus	1	1	1	n.a.
S. hominis	1	0	0	possible
S. lugdunensis	2	1	1	possible
Enterococcus faecalis	5	3	3	2x possible
Abiotrophia defectiva	1	1	1	n.a.
Corynebacterium diphtheriae	1	0	0	possible
Propionibacterium acnes	0	2	0	2x false
Pseudallescheria boydii	1	1	1	n.a.
Sum	**23**	**14**	**12**	

[a] At culture-negative results; n.a., not applicable

Table 4: Mixed infections and discrepant results.

Organism	PCR	Culture	Relevance PCR
Mixed infections	*S. mitis/oralis*	*S. mitis/oralis,* *S. epidermidis*	true
	S. anginosus	*S. anginosus* *S. hominis*	true
	Enterococcus spp., *S. hyicus*	*E. faecium*	true (*Enterococcus* spp.)
	S. hominis	*S. hominis,* *M. luteus*	true
	Enterobacter cloacae	*E. cloacae,* *E. faecium*	true
	negative	*C. parapsilosis,* *S. hominis,* *S. epidermidis*	false
Discrepant	*S. dysgalactiae*	aerobic spore former	possible
	Bartonella quintana	*S. pneumoniae*	possible
Sum positives	**7**	**8**	

Table 2:

- Positivity of culture vs. PCR: 22/52 (42%) and 30/52 (58%).
- Sensitivity of PCR vs. culture: 19/22 (86%).
- PCR found 11 culture-negative patients being positive with pathogens.

Table 3:

- PCR missed 2 cases of valves infected by *Propionibacterium acnes* and 1 case of mixed infection of valve prosthesis by *Candida parapsilosis*, *Staphylococcus hominis* and *S. epidermidis* (Table 4).
- On the other hand, PCR detected 11 cases of possible infection of valves and blood in culture-negative patients: *Streptococcus pneumoniae, S. gallolyticus, S. gordonii, S. oralis/mitis, S. salivarius, Staphylococcus aureus, S. hominis, S. lugdunensis, Enterococcus faecalis, Corynebacterium diphtheriae*.

Table 4:

- More mixed infections were detected by culture (5/52, 10%) than PCR (1/52, 2%).
- PCR identified at least one of the mixed species in culture, except 1 case (*C. parapsilosis, S. hominis, S. epidermidis*).
- In 2 cases, PCR detected species (*S. dysgalactiae, B. quintana*) that were discrepant to culture and considered possibly true.

Summary / Conclusions

SelectNA™*plus*/Micro-Dx™ (PCR) was run with a variety of specimens from IE patients, using an automated uniform protocol and compared to culture results. A high concordance of positive PCR results was observed with culture (86%). Organisms included IE-typical streptococcal, staphylococcal and enterococcal pathogens as well as rare bacteria (*A. defectiva, C. diphtheriae, B. quintana, E. cloacae*) and a fungus (*P. boydii*). PCR identified >3-times more pathogens alone (11/52; 21%) than culture (3/52, 6%).

The good clinical performance and the great reduction of handling limited to loading of the instrument with consumables and samples make SelectNA™*plus*/Micro-Dx™ a promising, versatile tool for culture-independent molecular analysis of IE infections.

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