Extraction-free Molecular Identification of Bacteria/Fungi
Directly from Broncho Alveolar Lavage (BAL) Samples

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AIM AND SCOPE

The lungs are the primary target for airborne pathogens causing pneumonia. The diagnosis of pneumonia is often based on Bronchoalveolar Lavage (BAL) followed by culturing or molecular (DNA-based) methods.

This application note describes Cube Dx's extraction free molecular multiplex test and first clinical results on 79 patient samples (BAL). The simplified workflow offers results with a minimum of hands-on in less than 2 hours for a broad panel of pathogens. The aim was to demonstrate the clinical usability of LINA – a modulation buffer and protocol for direct use of BAL samples – in combination with Cube Dx's compact sequencing of bacteria, resistance genes, and fungi in a pilot study. The results of three methods were compared against each other: Cube Dx' LINA + compact sequencing, the Unyvero system (molecular), and the gold standard culture followed by MALDI-TOF identification.

METHODS

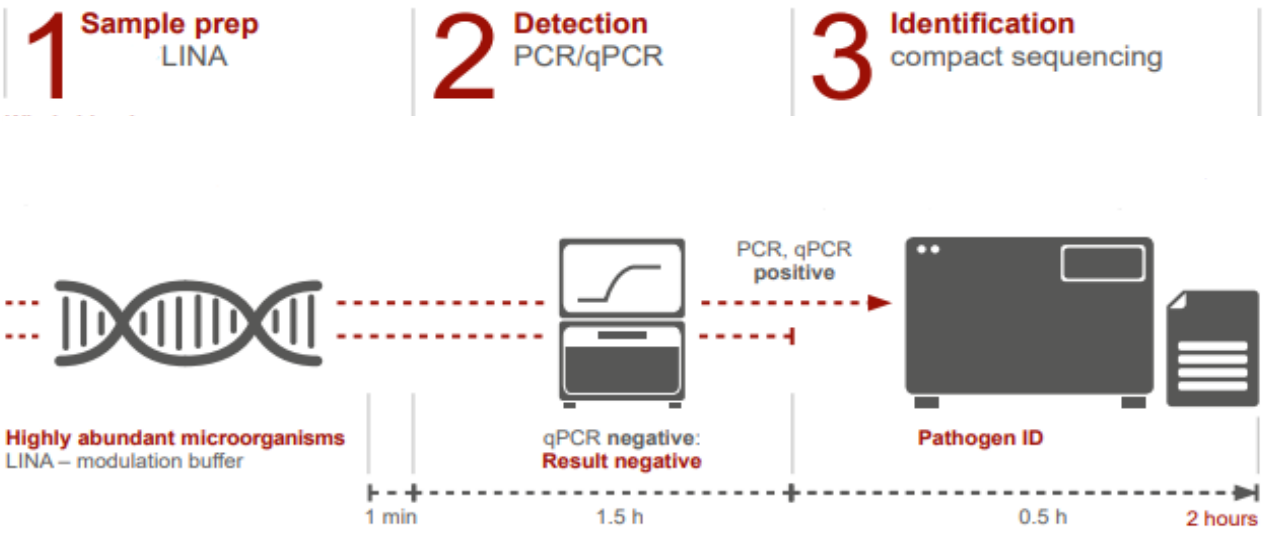
BAL samples from 79 patients (Institute for Medical Microbiology, University Hospital Essen / Germany) were analyzed by the traditional blood culture methods, the Unyvero system, and Cube Dx's compact sequencing technology.

Compact sequencing combines sensitive PCR testing and highly specific array diagnostics. The workflow for Cube Dx divides into three simple steps:

First, aliquots (20µl) of the BAL samples were directly transferred to the ready-to-use LINA modulation buffers (8mL each). The mixtures were briefly vortexed.

Second, 20µl of the BAL-LINA mixture was pipetted into the 'Ready-to-use' PCR-Master mixes of Bacteria (16S) and Fungi (28S) and thereafter loaded and processed in the PCR machine.

Third, the PCR products (fluorescent-labeled single-stranded DNA) were transferred into the test cartridge (hybcell) and analyzed by the hyborg fully automated. The resulting report shows genera and species of bacteria and fungi present in the samples.



Cube Dx' Pneumonia Test Panel

Bacteria: Acinetobacter baumannii complex, Acinetobacter calcoaceticus complex, Actinobacillus pleuropneumoniae, Citrobacter freundii complex, Citrobacter koseri, Enterobacter cloacae, Enterobacter cloacae complex, Escherichia coli, Haemophilus haemolyticus, Haemophilus influenzae, Klebsiella (Enterobacter) aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae (variicola), Legionella pneumophila, Moraxella catarrhalis, Morganella morganii, Proteus spp., Proteus mirabilis, Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus aureus, Stenotrophomonas maltophilia, Streptococcus pneumoniae, Streptococcus pyogenes
Fungi: Candida spp., Candida albicans, Candida dubliniensis, Candida parapsilosis, Candida tropicalis, Candida glabrata, Candida auris

RESULTS

The 'true' result was assumed as the result that at least two of the three methods provided. For one sample no agreement between two of the three methods could be found, so the number of samples for the analysis was reduced to 78. For 31 samples the result was correctly classified as positive, for 32 samples the result was correctly classified as negative. From 9 false-positive results, 5 showed Haemophilus influenzae. Of 6 false-negative results, 3 did not indicate Staphylococcus aureus.

		Concession-Results		Overall Correctness	Sensitivity	Specificity
		Positive	Negative			
Cube Dx	Positive	31	9	81%	84%	78%
	Negative	6	32			
Total		78				

CONCLUSION

The LINA modulation buffer in combination with compact sequencing promises to be a simple and fast alternative to other commercially available molecular-based testing technologies. The technology can easily be handled in PCR laboratories and the hands-on is reduced to a minimum. The turnaround time is around 2 hours and the throughput is higher than comparable multiplex technologies. Minor improvements will further reduce false positive and negative results before entering into the clinical routine.

ADVANTAGES

1. Extraction free testing of highly abundant microorganisms in BAL
2. From sample to result in 2 hours
3. Sensitive, parallel identification of bacteria, fungi, and potentially resistance genes
4. Identification of multiple pathogens/mixed infections
5. Simple handling and optimized workflow with 24/7 availability and immediate results

REFERENCES

Georges O, Risso K, Lemiale V, Schlemmer F. Place du lavage broncho-alvéolaire dans l'exploration d'une pneumopathie de l'immunodéprimé [The place of bronchoalveolar lavage in the diagnosis of pneumonia in the immunocompromised patient]. Rev Mal Respir. 2020 Oct;37(8):652-661. French.