

Session: OS202 MALDI-TOF - diagnostics for the micro lab in the 21st century

Category: 4b. Diagnostic bacteriology – non-culture based, including molecular and MALDI-TOF

25 April 2017, 13:42 - 13:52
OS1027

Proof of concept using PEI-nanoparticles for concentrating bacteria from broncho-alveolar lavage

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Background: The application of nanotechnology in biomedical research offers new methods for the diagnosis of lower respiratory tract infections. Nanoparticles (NPs) are small particles that comprise 1-100nm in diameter and have unique properties and present affinity for bacterial walls. Furthermore, NPs present a large surface-to-volume ratio that allows biomolecule binding (functionalization) and can be modified for certain uses such as biosensors and drug-carriers. One example is polymer coated magnetic (PEI) NPs which do not show any bactericidal activity.

Our objective was estimate the concordance between the results of the routine techniques with an isothermal amplification test using PEI NPs as bacterial concentrators in broncho-alveolar lavage (BAL) samples.

Material/methods: We tested a method for detecting bacteria with Real Time Loop-mediated isothermal Amplification (LAMP) determinations using PEI NPs as bacterial concentrators in 28 BAL samples collected in our hospital. The study protocol was approved by the Ethics Committee of the Hospital Clínic of Barcelona. The following bacteria were analysed: *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Stenotrophomonas maltophilia*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Haemophilus influenzae*. Briefly, PEI NPs were previously sonicated and added to each sample. The mixture was then homogenated in a rotator shaker for 20 minutes, and the PEI NPs bacteria complex was separated from the sample using a magnet. Finally, we prepared LAMP to detect bacteria in vortexed and boiled pellets. Microorganism detection and identification took less than 2 hours (Graph 1). The Cohen's kappa coefficient was calculated to analyse the results and to establish concordance between the gold standard technique (routine) and the probe test.

Results: Table 1 shows the routine techniques compared with LAMP test with and without PEI NP. On comparing the results of the routine diagnosis with LAMP determinations without NPs the Cohen's kappa coefficient showed a moderate correlation of 0.59 according to the Kappa interpretation table. Comparison of routine techniques with the results of the LAMP procedure with PEI NPs showed a Cohen's kappa coefficient of 0.65, indicating good correlation between the techniques.

Table 1. Comparison between routine results and LAMP test with and without PEI NPs.

LAMP test	Routine results			Cohen's kappa coefficient
	Concordant results		Non-concordant results	
	Positive	Negative		
Without NPs	11	171	14	0.59
With NPs	18	158	20	0.65

Conclusions: We found a good concordance between the gold standard, mainly culture based analysis, and LAMP determinations with concentrated BAL samples with PEI NPs, suggesting that concentration of bacteria increase sensitivity of the LAMP assay.

Graph 1. Schematic representation of sample processing.

