

# Proof of concept of the use of polymer-coated magnetic nanoparticles for concentrating bacteria from broncho-alveolar lavage (BAL)

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# Transparency Declaration

- I have not received research grants or accepted any payment as a company consultant.

# Proof of concept of the use of polymer-coated magnetic nanoparticles for concentrating bacteria from broncho-alveolar lavage

- **Background:**

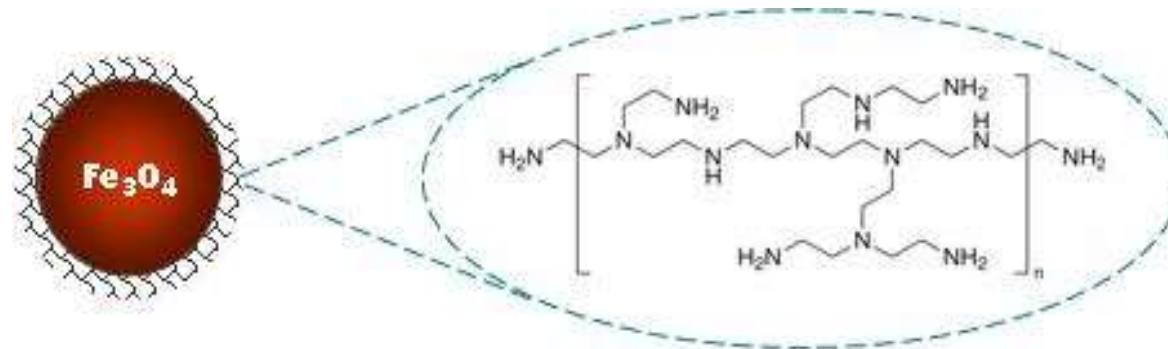
- The **rapid and reliable identification** of microorganisms without expensive and long-term duration techniques is an area of interest in clinical microbiology.
- The application of nanotechnology in biomedical research offers new **methods for the diagnosis.**
- **Unique properties of nanoparticles (NPs)**
  - (1) Magnetic property.
  - (2) 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.

# Proof of concept of the use of polymer-coated magnetic nanoparticles for concentrating bacteria from broncho-alveolar lavage

- **Background:**

- Polymer coated magnetic (PEI) NPs shows adherence to bacterial wall and does not show any bactericidal activity.

Evaluation of Functionalized Magnetic Iron Oxide ( $\text{Fe}_3\text{O}_4$ ) Nanoparticles for Capturing Gram-Positive and Gram-Negative Bacteria.  
Journal of Biomedical Nanotechnology. 2014; 10:, 1–11



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- **Objective:**

- Our objective is to estimate the concordance between results of

- 1) the routine techniques (mainly culture-based) and
- 2) the isothermal amplification test (LAMP)

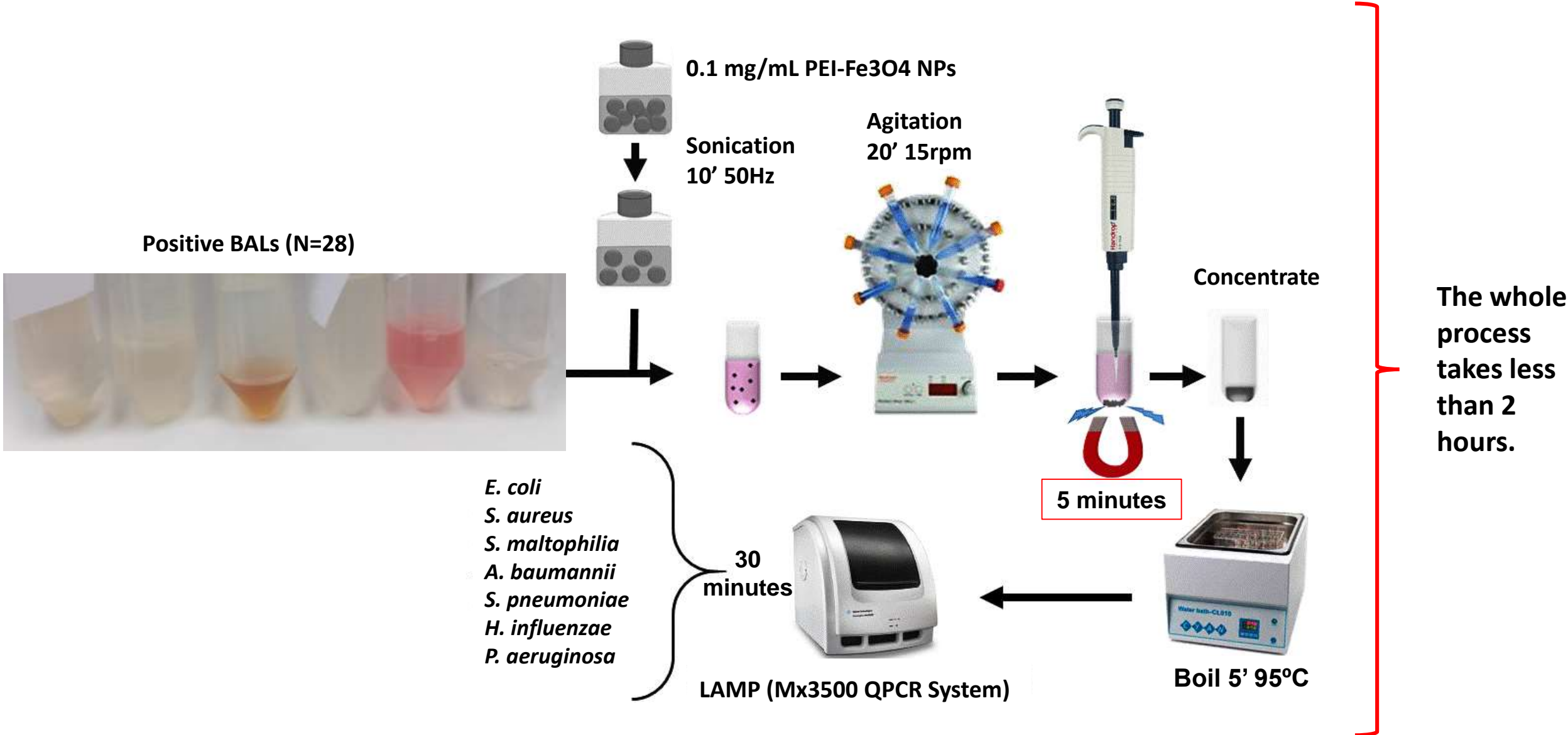
- without polymer coated magnetic (PEI) NPs
- using polymer coated magnetic (PEI) NPs as bacterial concentrators in broncho-alveolar lavage (BAL) samples

# Proof of concept of the use of polymer-coated magnetic nanoparticles for concentrating bacteria from broncho-alveolar lavage

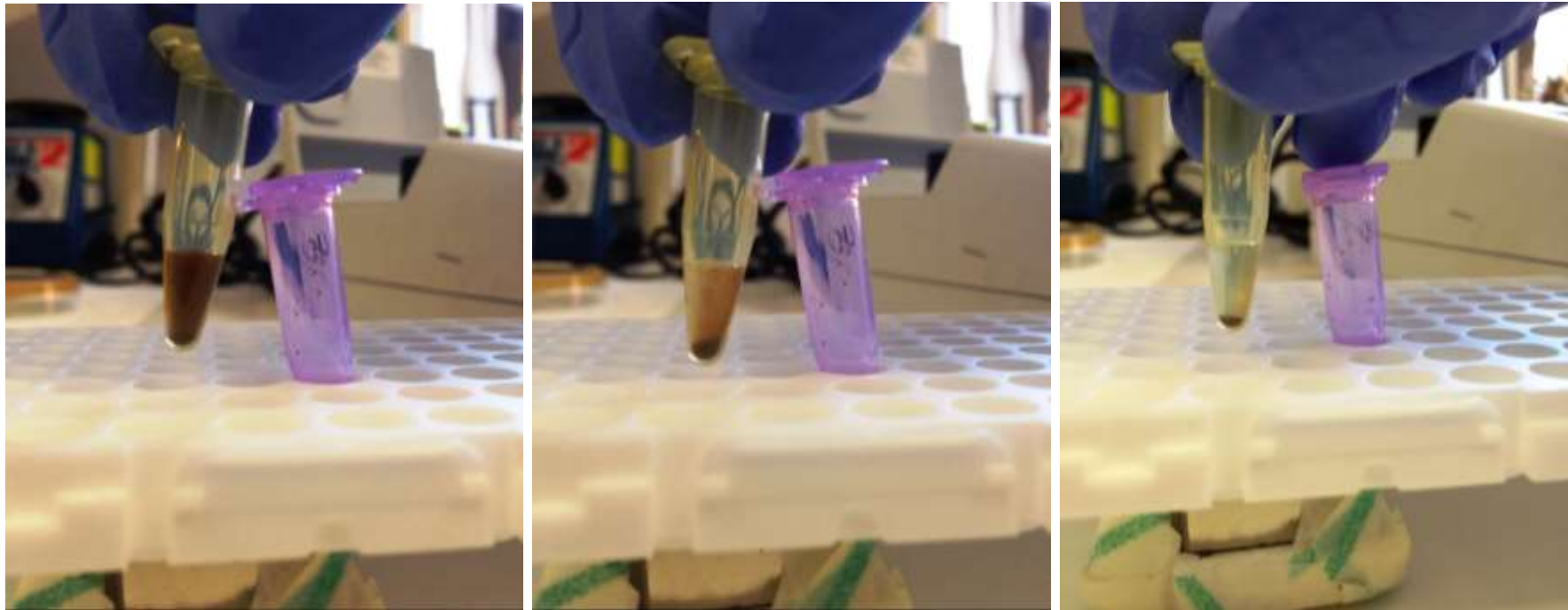
- **Materials/Methods:**

- We tested a method for detecting bacteria with Real Time Loop-mediated isothermal Amplification (LAMP) using PEI NPs as bacterial concentrators in 28 known positives BAL samples.
- 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*
  - *Haemophilus influenzae*

# Materials/Methods: workflow



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Concentration at: 0 seconds

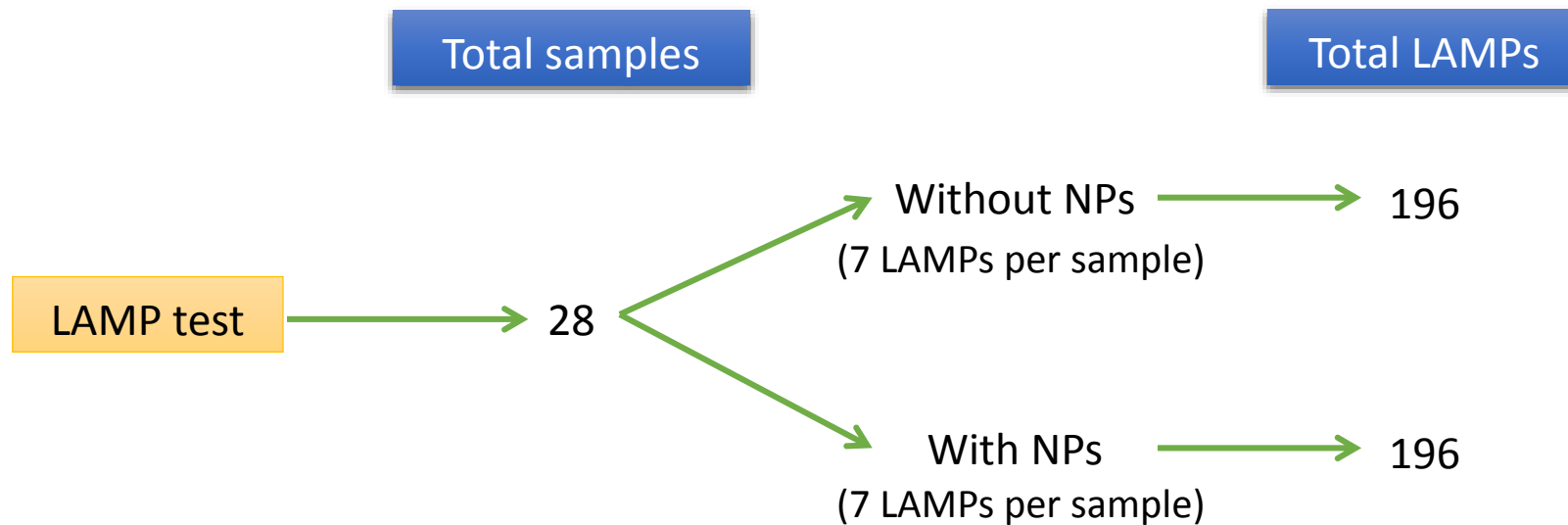
15 seconds

and 30 seconds



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- Sample tested:



# Results:

Sample	LAMP Experimental Results		Routine diagnosis
	Without NP	With PEI-NPs	Causative agent
1			<i>A. fumigatus</i>
2	PAER	PAER	PAER
3	HINF	HINF, ABAU	HINF
4	ABAU	ABAU	ABAU
5		SMAL	PAER
6		SPNE	SPNE, <i>S. salivarius</i> , <i>P. jirovecii</i>
7		SMAL	PAER
8		SAUR, HINF	SAUR, HINF, <i>S. pyogenes</i>
9	SAUR	SAUR	SAUR
10		PAER, HINF	PAER
11	PAER	SMAL	PAER
12	SAUR	SAUR	SAUR
13		HINF	HINF
14			<i>E. ludwigii</i>
15	HINF	HINF, PAER	HINF, SAUR
16			<i>P. mirabilis</i>
17		SMAL, SAUR, PAER	SMAL
18			ABAU, <i>H. parainfluenzae</i>
19		SMAL	SMAL
20			<i>C. parapsilosis</i>
21		SMAL	SMAL, <i>A. fumigatus</i>
22		SMAL, SPNE	PAER
23			<i>C. parapsilosis</i>
24	SAUR	SAUR	SAUR
25	SAUR	SAUR	SAUR
26	PAER	PAER	PAER
27	PAER	PAER	PAER
28		HINF	<i>C. albicans</i>

Escherichia coli	ECOL
Staphylococcus aureus	SAUR
Streptococcus pneumoniae	SPNE
Stenotrophomonas maltophilia	SMAL
Acinetobacter baumannii	ABAU
Pseudomonas aeruginosa	PAER
Haemophilus influenzae	HINF

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- Results:**

LAMP test	Routine (culture-based) results				
	Concordant results			Non-concordant results	Total
	Positive	Negative	Total concordant		
Without NPs	11	171	182	14	196
With NPs	18	158	176	20	196

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- **Results:**

- Non-concordant results:

- Sampling.
    - Quantity of pathogens presents in the BALs.
    - Antibiotic therapy.
    - Contamination of sample.
    - Procedure error.
    - Cross reaction.
    - Improved sensitivity by LAMP with NPs method.

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- **Results:**

Cohen's kappa coefficient		
LAMP test	Without NPs	0.59
	With NPs	0.65

Value of Kappa	Strength of agreement
<0.20	Poor
0.21 – 0.40	Fair
0.41 – 0.60	Moderate
0.61 – 0.80	Good
0.81 – 1.00	Very good

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- **Conclusion:**

- We found a good concordance between the culture-based routine techniques results and LAMP determinations with concentrated BAL samples with PEI NPs, suggesting that concentration of bacteria increases sensitivity of the LAMP assay.
- Bacterial concentration from BAL with NPs and their detection using LAMP assay can be performed in less than 2 hours.

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- Acknowledgments:



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