DEPLASP-BAAG: a specific biosensing platform for immuno magnetic capture of milk pathogens

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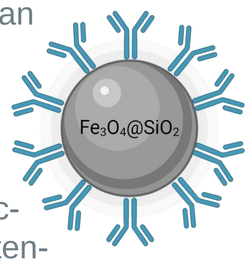
CONTEXT

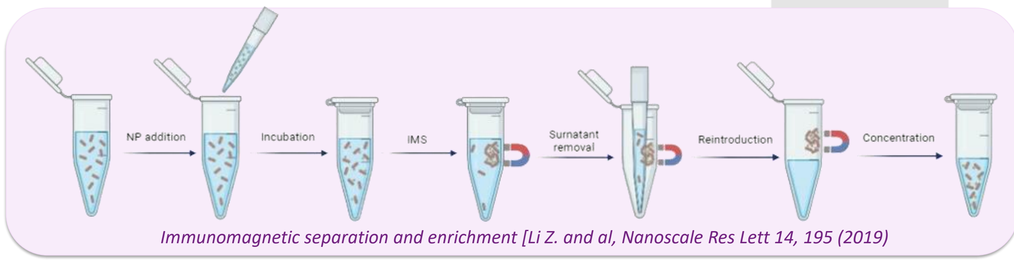
Food industry is increasingly affected by pathogen contamination. In the dairy industry, pathogenic bacteria are present in very low concentrations and can only be detected after 20 to 36 hours best. In addition, European standards stipulate that 25g of milk or cheese must be free from bacteria before it can be marketed. To enable suppliers to act in time to stop the production and marketing of these potentially contaminated products, it is necessary to considerably reduce the analysis time, by a factor of at least 2, and to lower the detection limit.



OBJECTIVE & METHOD

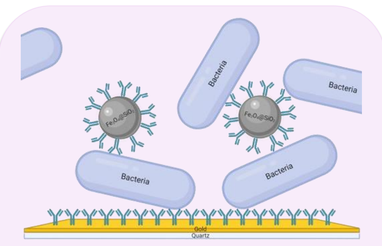
Optimization of the main steps in the analytical process: ***reducing*** the time taken to obtain the first negative result and ***lowering*** the limit of detection of the bacteria. Use of decorated magnetic iron oxide nanoparticles (NP) with antibody will have 2 aims in the project:

Immunomagnetic separation (IMS) : creation of heavy complexes bacteria/nanoparticles (B-NP) by incubation of decorated NP into sample. B-NP complexes are then transferred in a small volume to create an enriched solution of bacteria.



Immunomagnetic separation and enrichment [Li Z. and al, Nanoscale Res Lett 14, 195 (2019)

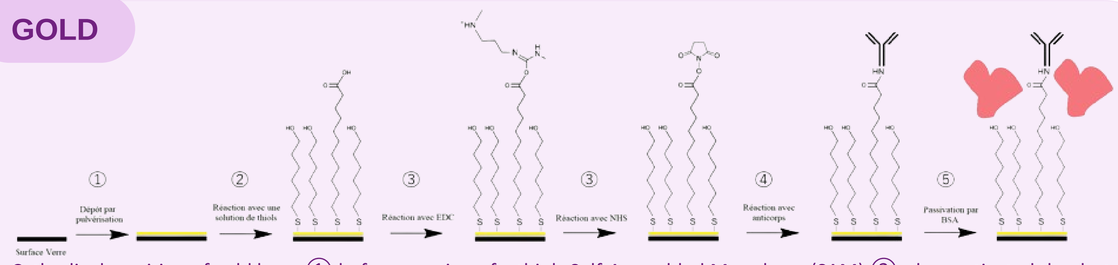
Biodetection via QCM : enriched B-NP solutions analyzed by Quartz Cristal Microblance (QCM). As NP are 40 times heavier than bacteria, the collected signal will be magnified and the detection limit of bacteria will decrease.



"Sandwich" type device for trapping pathogens on QCM quartz

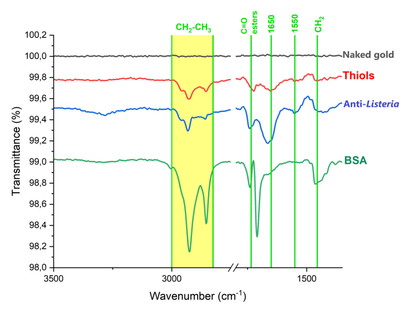
SURFACE FUNCTIONALIZATION

GOLD



Cathodic deposition of gold layer ① before creation of a thiols Self-Assembled Monolayer (SAM) ②, then activated thanks to carboxylic function ③ before antibody grafting ④ and Bovine Serum Albumin (BSA) passivation ⑤.

IR

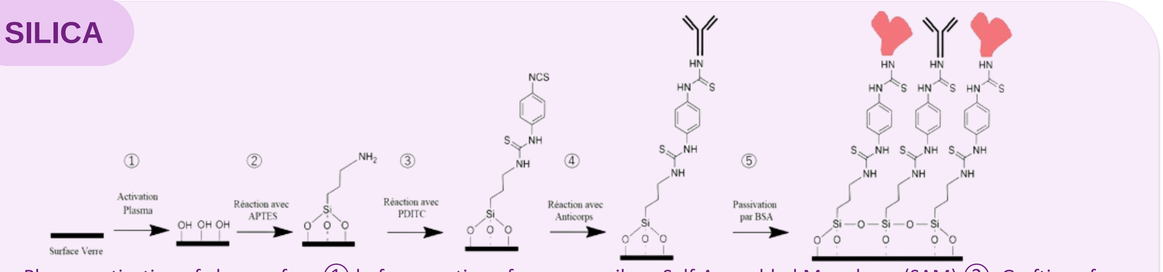


IR spectra after each gold functionalization step

→ Caracteristic bands around 3000 cm^{-1} show thiol carbon chains presence.

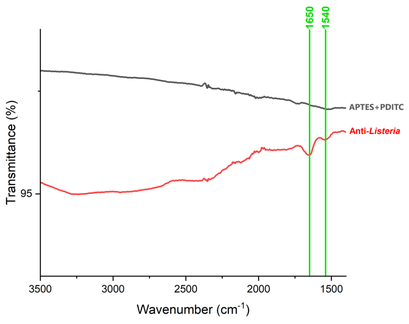
→ Caracteristic bands between 1650 and 1550 cm^{-1} show amide I and amide II bonds presence, from antibodies and BSA.

SILICA



Plasma activation of glass surface ① before creation of an organosilane Self-Assembled Monolayer (SAM) ②. Grafting of a thiocyanate spacer ③ before antibody grafting ④ and Bovine Serum Albumin (BSA) passivation ⑤.

IR



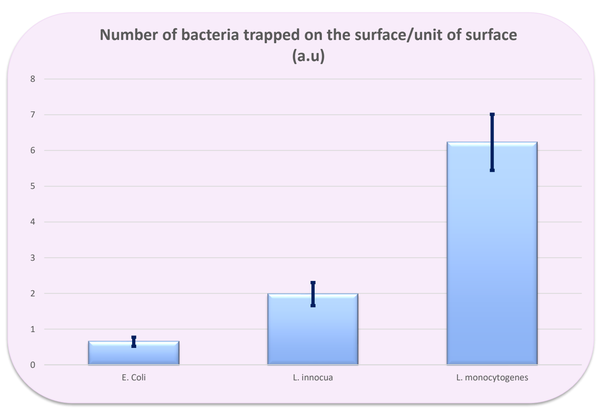
IR spectra after each glass functionalization step

→ **Validation of protocols for immobilizing antibodies on surfaces**

ANTIBODY SPECIFICITY

Bacteria capture on gold surfaces functionalized with *anti-Listeria monocytogenes* antibodies in static conditions.

E. Coli | *L. innocua* | *L. monocytogenes*



→ 3 times more *L. monocytogenes* than *L. innocua* captured by antibody, 10 times more than *E. coli* (negative control).

→ **Validation of the specificity of the antibody in static conditions**

CONCLUSION & PROSPECTS

Specificity of anti-*Listeria monocytogenes* in simple fluids validated. → Specificity in complex samples (milk for example) to investigate.

Specificity of anti-*Listeria monocytogenes* in static condition validated. → Investigation for dynamic conditions.

QCM measurements with bacteria alone and B-NP complexes : thanks to nanoparticles of known size and mass, we can determine the mass of bacteria captured on the quartz, and estimate the number of bacteria.

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