## Rapid and screening methods

$$\text{Sensitivity} = \text{percent positives identified} = \frac{\text{True positives}}{\text{True positives} + \text{false negatives}} \times 100\%$$

$$\text{Specificity} = \text{percent negatives identified} = \frac{\text{True negatives}}{\text{True negatives} + \text{false positives}} \times 100\%$$

$$\text{Efficiency} = \text{percent correct results} = \frac{\text{True positives} + \text{True negatives}}{\text{Total number of samples}} \times 100\%$$

1. A rapid test based on a biosensor for the determination of celiac disease is under development. The comparison of patients under biopsy (which is the gold standard test) n=100 celiac patients and 100 control patients without suffering the disease provided 4 false positives and 3 false negatives. Determine Specificity, Sensitivity and Efficiency for the test.

2. A WHO has assessed and today listed the ReEBOV Antigen Rapid Test Kit (Corgenix, USA) as eligible for procurement to Ebola affected countries. The test was evaluated under WHO's Emergency Assessment and Use, a procedure established to provide minimum quality, safety and performance assurance for diagnostic products in the context of the Ebola emergency.Ebola is currently being tested in laboratories largely through the detection of the virus's nucleic acid (genetic material), using commercial or in-house tests. Nucleic acid tests (NATs) are more accurate but are complex to use and require well-established laboratories and fully trained personnel. In addition, turn-around time can vary between 12 and 24 hours. The ReEBOV Antigen Rapid Test, which can provide results within 15 minutes, is based on detection of the Ebola protein rather than nucleic acid. When compared with the results of a NAT previously listed by WHO and currently being used in the field (RealStar® Filovirus Screen RT-PCR Kit 1.0, altona Diagnostics GmbH), ReEBOV provided the following results:

| SAmple | RT-PCR (RNA copies mL-1) | LFA | Validation result |
| --- | --- | --- | --- |
| 1 | $1.8 \times 10^7$ | positive | |
| 2 | $3.5 \times 10^9$ | positive | |
| 3 | $1.5 \times 10^4$ | negative | |
| 4 | 0 | negative | |
| 5 | 0 | poitive | |
| 6 | $4.0 \times 10^8$ | positive | |
| 7 | $3.3 \times 10^9$ | positive | |

| 8 | $1.6 \times 10^6$ | positive | |
|---|---|---|---|
| 9 | $2.0\ 10^5$ | positive | |
| 10 | $9.0\ 10^6$ | positive | |
| 11 | 0 | negative | |
| 12 | 0 | negative | |
| 13 | 0 | negative | |
| 14 | 0 | negative | |
| 15 | 0 | negative | |

Provide the values for the stadistics parameters. How many samples are correctly identify of Ebola infected patients? Which is the vakue for those not infected with the virus.

3. **Validation of a Qualitative Antibiotic Susceptibility Test**. Medical laboratories are frequently asked to determine which antibiotic compounds are effective against clinically isolated microorganisms.

An accepted method for determining antibiotic susceptibilities involves culturing the microorganism on an agar plate in the absence or presence of antibiotic on a small filter-paper disk place on the agar surface. Growth of the culture (reproduction) is then allowed to occur during overnight incubation at 37 ª C. A ring with no growth around a filter-paper disk indicates that the microorganism is susceptible to that antibiotic. This method requires at least 8 h and may require several days, depending on the growth rate of the organism.
In the proposed new method, microorganisms suspended in a liquid culture are incubated at 37 º C for 20 min in the absence or presence of an antibiotic, using the same drug concentration as is present in the accepted agar plate method. Respiratory activity (breathing) is then measured by a new electrochemical method. If respiratory activity is <90% of the control measurement, made in the absence of antibiotic, the microorganism is susceptible to the antibiotic. The new method requires only 25 min, and can therefore provide results (and effective treatment regimes) much more rapidly than the accepted method.
Validation was performed using a common laboratory strain of E. coli with 13 antibiotics possessing different mechanisms of action, using both the accepted and the new method. Comparison of results for each antibiotic allowed classification of decreased respiratory activity by the new method:
* A true positive shows no growth by the agar plate method and decreased respiratory activity by the new method.
* A true negative involves growth on agar, and no charge or an increase in respiration.
* A false positive involves growth on the agar plate but decreased respiration in the new method.
* A false negative result occurs if there is no growth around the filter paper disk by the agar plate method, while the new method shows no change or an increase in respiratory activity.
The following table summarizes the results obtained in this validation experiment.

| Antibiotic | Agar plate result | Respiration result | Validation result |
|---|---|---|---|
| Penicillin G | No growth | Decrease | |
| D-Cycloserine | No growth | No change | |
| Vancomycin | No growth | Decrease | |
| Bacitracin | Growth | Increase | |
| Cephalosporin C | No growth | Decrease | |
| Tetracycline | No growth | Decrease | |

| Erythromycin | Growth | No change | |
|---|---|---|---|
| Chloramphenicol | No growth | Decrease | |
| Streptomycin | Growth | No change | |
| Nalidixic acid | No growth | Increase | |
| Rifampicin | No growth | Decrease | |
| Trimethoprim | No growth | Decrease | |
| Nystatin | Growth | Decrease | |

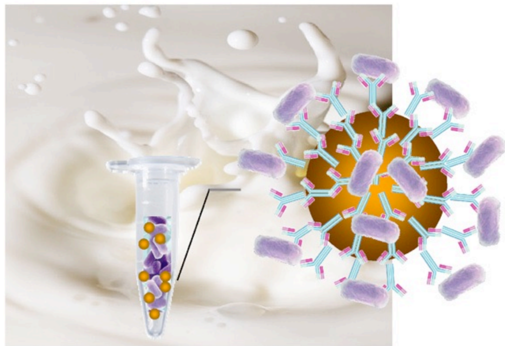
4. **Magneto Immunosensor for *Salmonella*.** *Salmonella* has been one of the most frequently occurring foodborne pathogens affecting the microbial safety of foods, including milk.

Official agencies for food safety, such as US Food and Drug Administration (FDA), US Department of Agriculture (USDA), Association of Official Analytical Chemist International (AOACI), International Organization of Standardization (ISO), recommend classical culture methods for recovering *Salmonella* spp. from food. However, the development of new methodologies with the advantages of rapid response and sensitivity is a challenge for food hygiene inspection for screening-out negative samples.

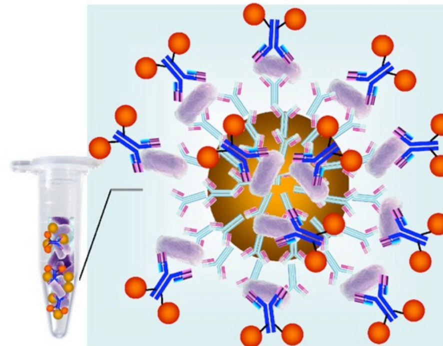
A novel method for the sensitive detection of Salmonella was performed. In this approach, the bacteria are captured from food samples and preconcentrated by immunomagnetic separation. During the immunomagnetic separation, the enzymatic labeling of the bacteria is also performed using a polyclonal anti-Salmonella-HRP antibody. Then, the modified magnetic beads are easily captured by a magneto electrode which is also used as the transducer for the electrochemical detection.

The details of the procedure is shown in the following scheme:

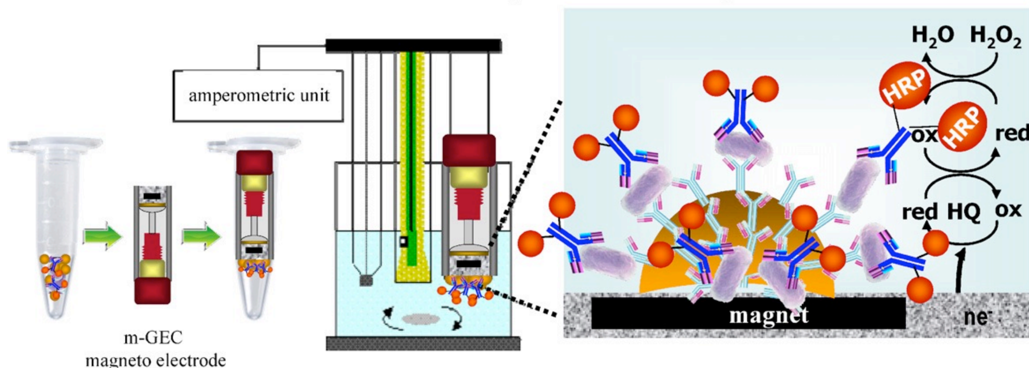
A.- Immunomagnetic separation of *Salmonella* from milk samples



B.- Immunological reaction with the anti Salmonella-HRP antibody



C.- Electrochemical magneto immunosensing



In order to validate the new procedure for the screening of milk contaminated samples in farms, 35 negatives samples of milk were analysed with the standard the gold standard microbiological culture method as well as by the novel approach, giving the following signals

0,025; 0,020: 0,040; 0,040; 0,045; 0,045; 0,040; 0,040; 0,035; 0,035; 0,040; 0,030; 0,030; 0,030; 0,030; 0,050; 0,050; 0,050; 0,055; 0,055; 0,040; 0,045; 0,040; 0,045; 0,045; 0,045; 0,045; 0,045; 0,035; 0,035; 0,035; 0,035; 0,035; 0,040; 0,040;

After that, 30 samples were also analysed, obtaining the following results:

| Mostra Nª | Resultat Cultiu (CFU/25 ml) | Resultat Immunoassaig | Mostra Nº | Resultat Cultiu (CFU/25 ml) | Resultat Immunoassaig |
|---|---|---|---|---|---|
| **1** | 3 | 0,060 | **16** | 45 | 4,990 |
| **2** | 0 | 0,050 | **17** | 0 | 0,025 |
| **3** | 0 | 0,045 | **18** | 0 | 0,03 |
| **4** | 15 | 1,650 | **19** | 63 | 6,965 |
| **5** | 255 | 28,05 | **20** | 29 | 3,230 |
| **6** | 0 | 0,060 | **21** | 0 | 0,055 |
| **7** | 0 | 0,020 | **22** | 0 | 0,060 |
| **8** | 126 | 13,850 | **23** | 76 | 8,450 |
| **9** | 2 | 0,070 | **24** | 115 | 12,695 |
| **10** | 0 | 0,020 | **25** | 178 | 19,795 |
| **11** | 1 | 0,060 | **26** | 0 | 0,060 |
| **12** | 56 | 6,210 | **27** | 217 | Out of range |
| **13** | 79 | 8,725 | **28** | 2 | 0,065 |
| **14** | 0 | 0,035 | **29** | 0 | 0,030 |
| **15** | 22 | 2,455 | **30** | 302 | Out of range |

Taking into account that the Real Decreto 1679/1995, BOE 24-09-94 requires absence of *Salmonella* in 25 g of samples,

a) Calculate the cut-off value

b) Codify the signal in a binary form

c) Calculate specificity, sensitivity and efficiency of the new method

d) Is the method able to be use for the screening of milk samples in a farm?