

# Virtual Proficiency Testing in food microbiology

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## INTRODUCTION

REQUASUD has been organizing food microbiology Proficiency Testing (PT) since 1989. One purpose of these PT is to identify the sources of errors in the laboratories, to improve analytical results. However, these “classical” PT, based on the analysis of inoculated food samples, provide little information on the causes of unsatisfactory results, as the analytical process inside the laboratory is a “black box” whose details are not transmitted to the PT provider.



Black box  
Lab result



Errors can occur at the pre-analytical, analytical or post-analytical steps of a microbiological analysis

To identify more precisely the sources of false analytical results, REQUASUD organized **two virtual PT schemes** in 2019 and 2022, each involving 16 laboratories from Belgium and Luxembourg.

→ The aim of these two virtual PTs was to quantify the contribution of the **post-analytical steps** on the total error of analytical results, mainly: **colony counting, tests reading, calculation of final results, encoding and interpretation.**

## MATERIAL AND METHODS

### VIRTUAL PT DESIGN

The “virtual PT samples” sent to the laboratories were **photographs** of Petri dishes or **tables** of enumeration results. Five analytical methods, used routinely by most participants, were proposed in these PT: the enumeration of total aerobic flora, lactic bacteria, *E. coli*, *S. aureus* and *Cl. perfringens*.

#### FIRST VIRTUAL PT (2019)

The samples were **photographs** of Petri dishes and confirmation tests, obtained from real analyses.

Here are some examples:



The analysts (up to 3 operators per laboratory) were asked to return:

- The **number of colonies** enumerated on each dish (dilutions  $10^{-1}$  to  $10^{-5}$ )
- The interpretation of the **confirmation tests** (positive / negative)
- Their **final result**, calculated and expressed in CFU/g
- The **conformity assessment** of the sample, with respect to the legislation

#### SECOND VIRTUAL PT (2022)

To assess exclusively the error linked to **calculation** or **encoding**, the items of this 2<sup>nd</sup> virtual PT were tables of enumeration results, e.g.:

Dilution	Colony count	Operator 1	Operator 2	Operator 3 / LIMS
$10^{-2}$	125	Calculation:	Calculation:	Calculation:
$10^{-3}$	21	Result:	Result:	Result:
$10^{-4}$	3			
$10^{-5}$	0			

Each operator returned a **final result** and a **conformity assessment** (considering the food sample was a “ready to reheat prepared meal”).

The results were log-transformed and statistically analyzed.

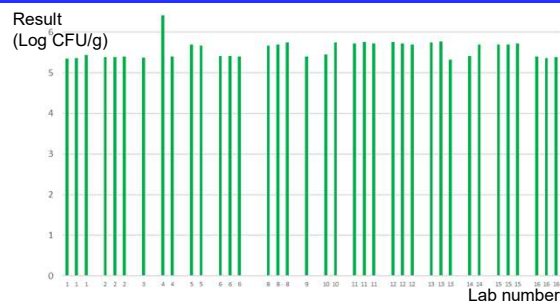
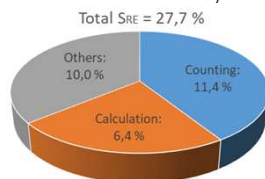
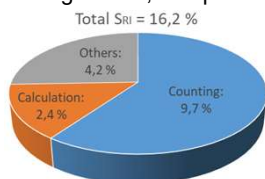
## RESULTS AND DISCUSSION

The **intra- and inter-laboratory variability** ( $S_{RI}$  and  $S_{RE}$ ) were surprisingly high, despite virtual PT schemes were designed to suppress any variability linked to the analytical steps.

All parameters together, the average relative  $S_{RI}$  was **16.2 %**, the largest part of it being attributable to the enumeration of the colonies. The calculation step brought only 2.4 %  $S_{RI}$ , indicating that the labs provided clear and coherent instructions to their operators.

Among laboratories, the average relative  $S_{RE}$  reached **27.7 %**, the main contributions being:

- **Colony-counting** e.g. some operators counted only the large or lenticular colonies.
- **Calculation** e.g. unsuitable formula ; dilution errors which impaired results by a factor 10
- **Others**: Encoding errors ; interpretation of the confirmation tests ; others.



Results of REQUASUD's 2019 virtual PT for the enumeration of total aerobic flora (1-3 operators per lab)

Despite the variability of the numerical results, the **conformity statements** emitted by the different laboratories were quite consistent. The very clear microbiological criteria (“action limits”) provided by the FASFC can be at the origin of this good coherence.

## CONCLUSIONS

Virtual PT schemes represent a privileged observation tool to highlight and quantify the **hidden sources of errors** during food microbiological analyses. This study demonstrated that inappropriate **colony counting, tests interpretation and calculation practices** significantly contribute to the variability of the analytical results. In their attempts to reduce **measurement uncertainty**, food microbiology labs should not only focus on enhancing **analytical precision**: great progress can be achieved by improving the **post-analytical steps**, through providing clear instructions and training to the operators.

