Virtual Proficiency Testing in Food Microbiology

M. Abdelmassih, F. Ferber, E. Pitchugina and A. Gillis

Eurachem, Windsor

27/09/2023

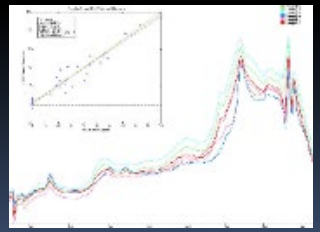
requasud
réseau de laboratoires wallons

Avec le soutien de la
Wallonie

REQUASUD

Belgian network of 13 laboratories

NIR



Nitrates

Mineral-Products

Mineral-Grounds

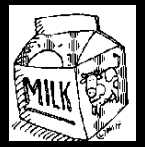
Microbiology

REQUASUD Food Microbiology PT

"Traditional" PT (1989)



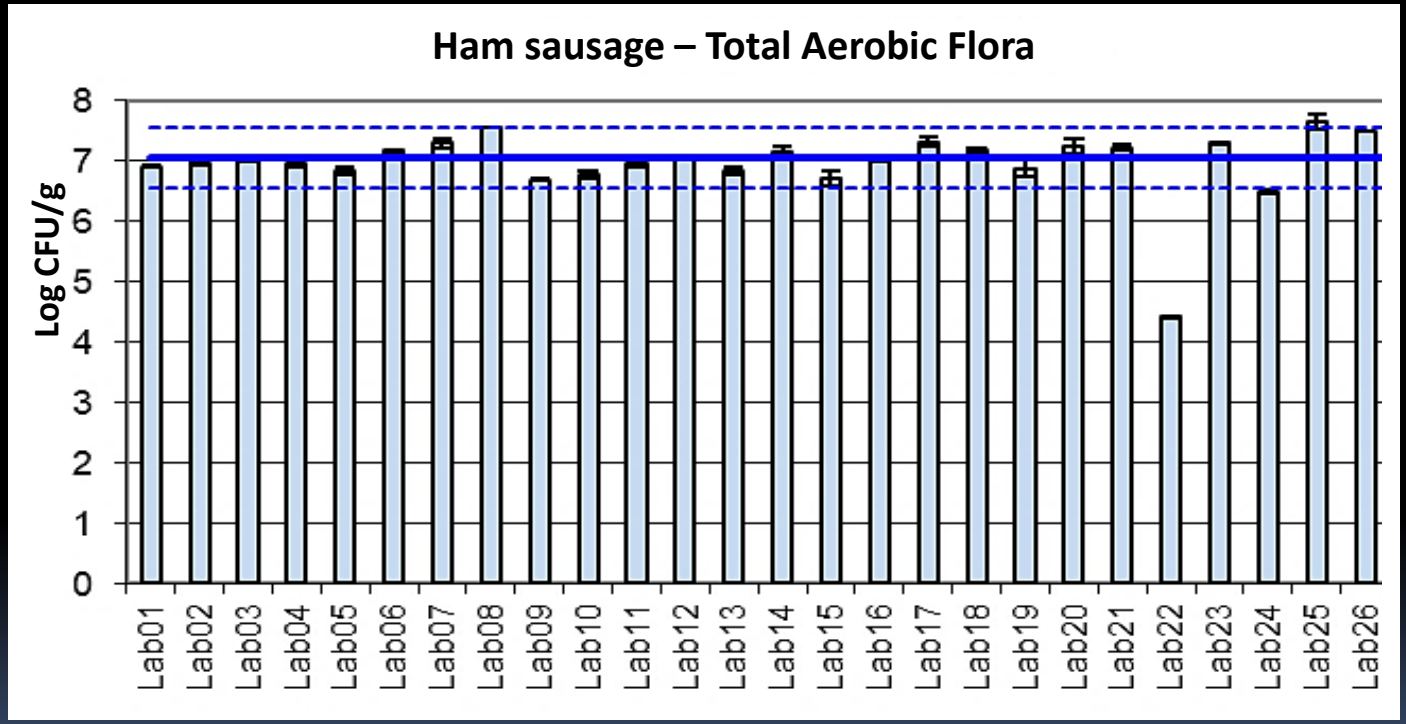




- Total flora
- *Salmonella*
- *S. aureus*
- *L. monocytogenes*
- *B. cereus*
- *Campylobacter*
- *C. perfringens*
- E. *coli* O157:H7
- Enterobacteria
- Coliforms
- Lactic bacteria
- …

ISO 17043 (2021)

A traditional quantitative PT result



Outlier

Traditional PT schemes

Black box

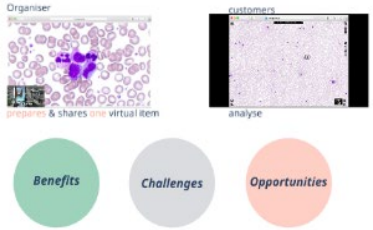
LAB TEST RESULT

Parameter	Method	Result	Units
E. coli	ISO 16649	**9500**	CFU/g
S. aureus	ISO 6888	**140**	CFU/g

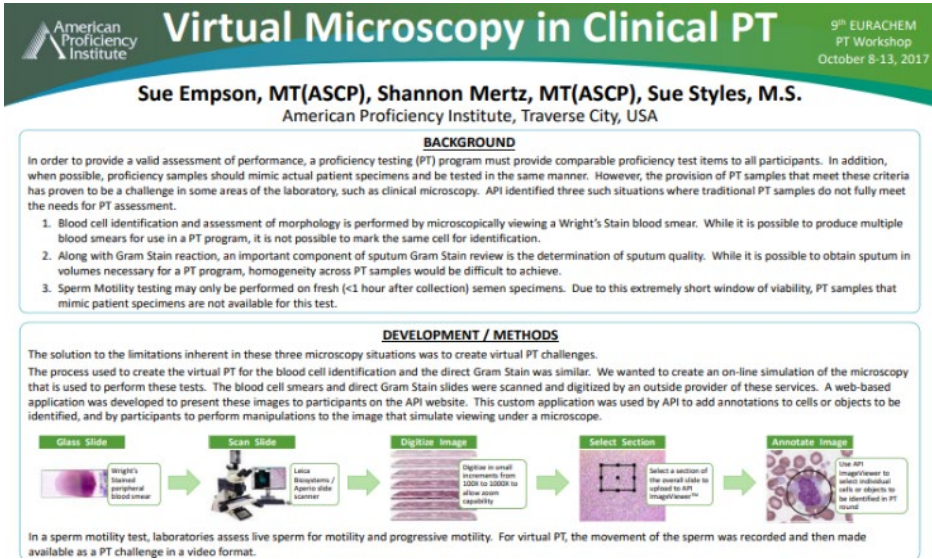
Few information

Eurachem 2017 - 9th International Workshop on PT

Traditional vs virtual PT items



István Juhos
University of Szeged



American Proficiency Institute

Virtual Microscopy in Clinical PT

9th EURACHEM PT Workshop October 8-13, 2017

Sue Empson, MT(ASCP), Shannon Mertz, MT(ASCP), Sue Styles, M.S.
American Proficiency Institute, Traverse City, USA

BACKGROUND

In order to provide a valid assessment of performance, a proficiency testing (PT) program must provide comparable proficiency test items to all participants. In addition, when possible, proficiency samples should mimic actual patient specimens and be tested in the same manner. However, the provision of PT samples that meet these criteria has proven to be a challenge in some areas of the laboratory, such as clinical microscopy. API identified three such situations where traditional PT samples do not fully meet the needs for PT assessment.

1. Blood cell identification and assessment of morphology is performed by microscopically viewing a Wright's Stain blood smear. While it is possible to produce multiple blood smears for use in a PT program, it is not possible to mark the same cell for identification.
2. Along with Gram Stain reaction, an important component of sputum Gram Stain review is the determination of sputum quality. While it is possible to obtain sputum in volumes necessary for a PT program, homogeneity across PT samples would be difficult to achieve.
3. Sperm Motility testing may only be performed on fresh (<1 hour after collection) semen specimens. Due to this extremely short window of viability, PT samples that mimic patient specimens are not available for this test.

DEVELOPMENT / METHODS

The solution to the limitations inherent in these three microscopy situations was to create virtual PT challenges.

The process used to create the virtual PT for the blood cell identification and the direct Gram Stain was similar. We wanted to create an on-line simulation of the microscopy that is used to perform these tests. The blood cell smears and direct Gram Stain slides were scanned and digitized by an outside provider of these services. A web-based application was developed to present these images to participants on the API website. This custom application was used by API to add annotations to cells or objects to be identified, and by participants to perform manipulations to the image that simulate viewing under a microscope.

In a sperm motility test, laboratories assess live sperm for motility and progressive motility. For virtual PT, the movement of the sperm was recorded and then made available as a PT challenge in a video format.

Traditional PT schemes

Black box

LAB TEST RESULT

Parameter	Method	Result	Units
E. coli	ISO 16649	9500	CFU/g
S. aureus	ISO 6888	140	CFU/g

Few information

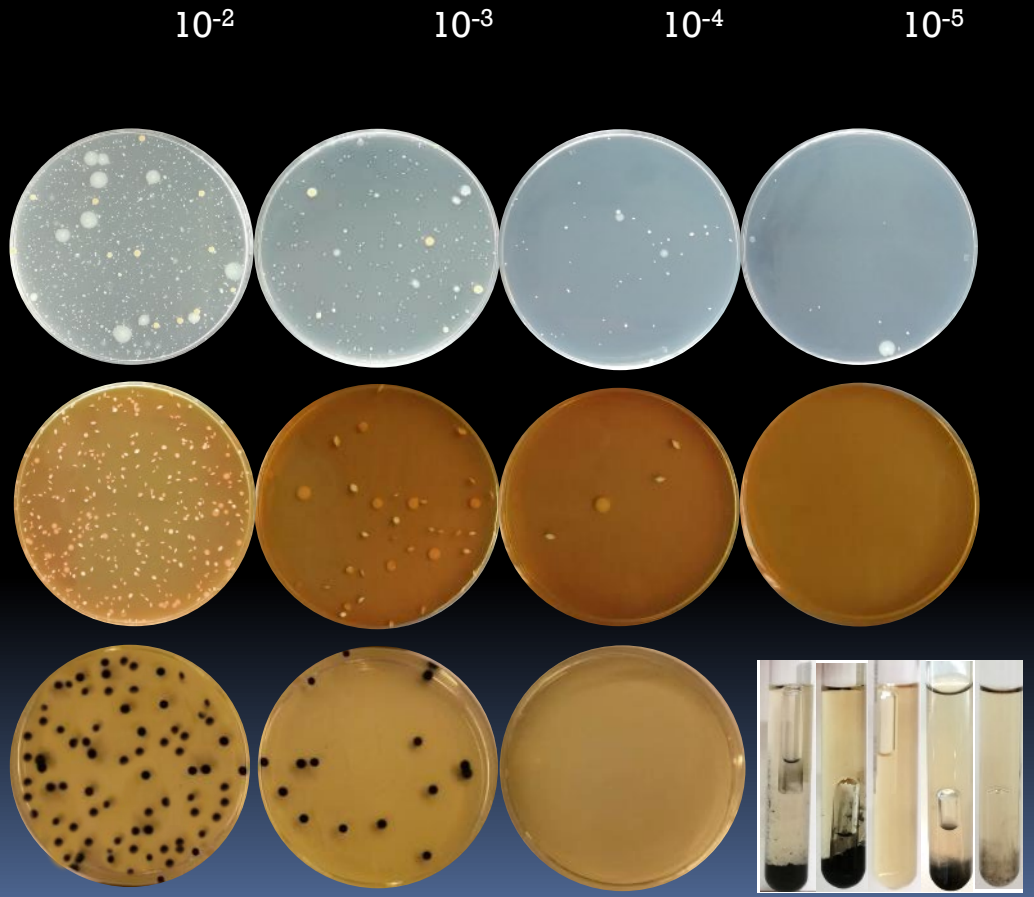
Virtual PTs in 2019 and 2022

- 16 laboratories
- Purpose: To quantify the contribution of analytical and post-analytical steps to the total error of analytical results.

Colony-counting, Test reading, Calculation, Interpretation

Virtual PT 2019 – Pictures from real analyses

1. Total aerobic flora
2. Lactic acid bacteria
3. *Clostridium perfringens*



Technical hurdles:

- Quality of photographs
- Enumerate on screen / no rotation

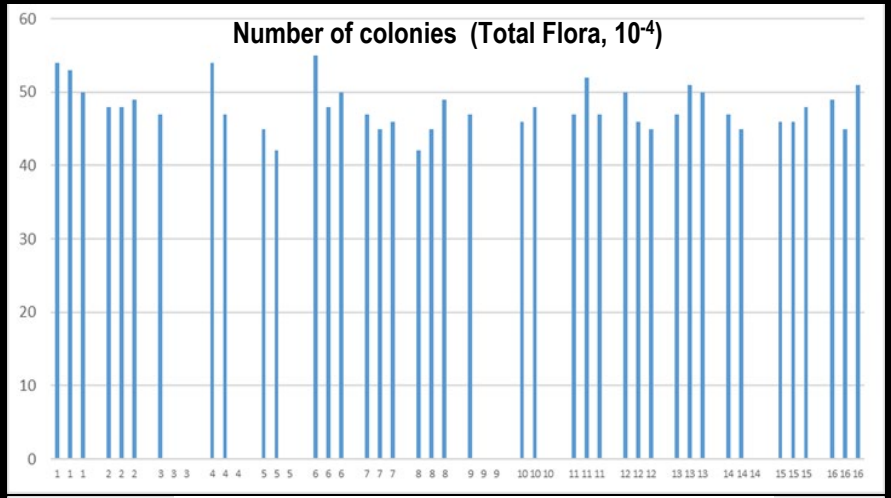
Lab reporting:

- Colony counts
- Interpretation of confirmation tests (+/-)
- Calculation of final result
- Sample conformity

Sources of variability (based on PT 2019 results):

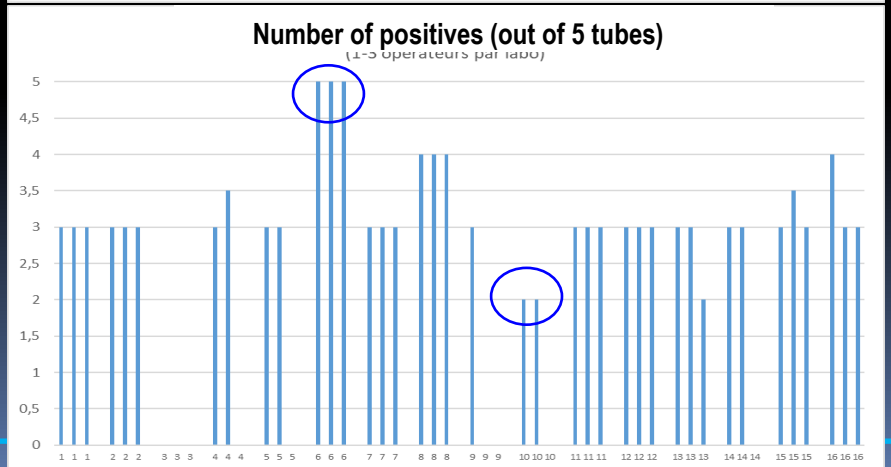
PT-2019

1. Colony counting



Mean: 48
sR_{intra}: 5,31 %
sR_{inter}: 6,32 %

2. Confirmation



PT-2019

3. Calculation

$$N = \frac{\sum C}{1{,}1 . V . d}$$

Dilution	Colonies
10^{-1}	205 >150
10^{-2}	16
10^{-3}	0

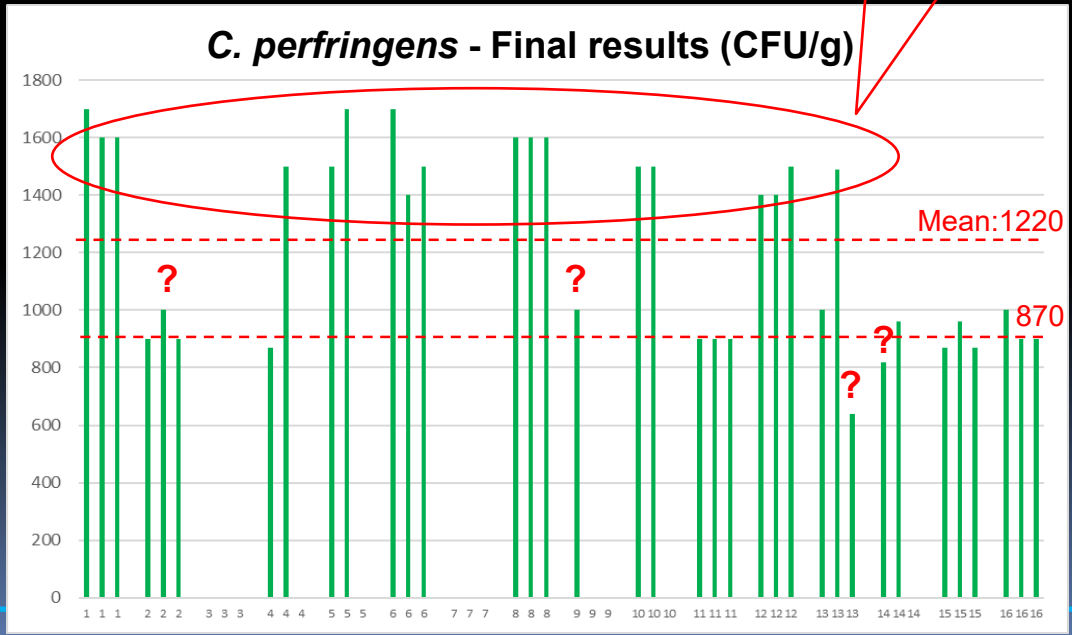
$$N = \frac{\sum C}{1{,}1 . V . d} * \frac{Nb\ positives}{5}$$

= (16+0 / $1{,}1.10^{-2}$) * 3/5

= 870 CFU/g

Expected result
(**ISO 7218** – General requirements for food microbiological analyses)

No confirmation ratio and/or excluded 0



➔ *Virtual PT 2022*

To assess **calculation practices**

16 laboratories

1-3 operators / lab

+ Confirmation: 3/5 positives

	Total flora	***E. coli***	***S. aureus***	***C. perfringens***
10^{-1}	>300	7	196	14
10^{-2}	>300	2	13	1
10^{-3}	>300	0	0	0
10^{-4}	289	0	0	0
Calculs				
Results				

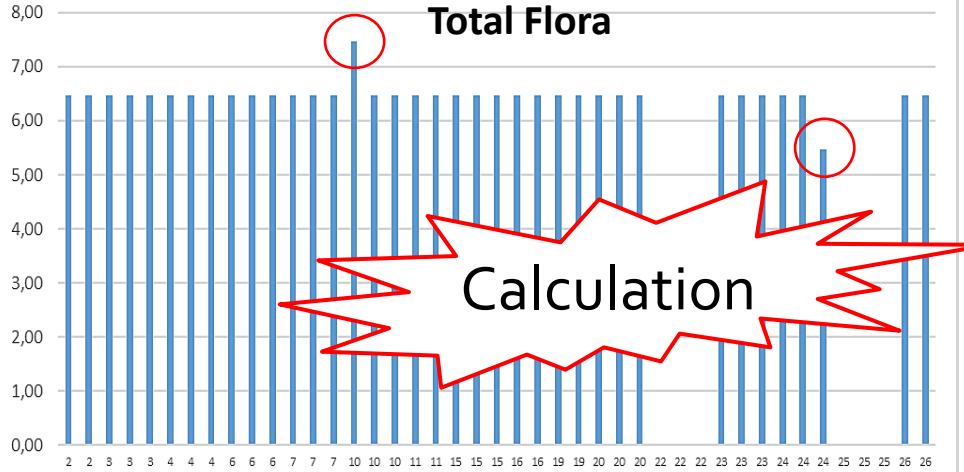
Expected results
(**ISO 7218** – General requirements for food microbiological analyses)

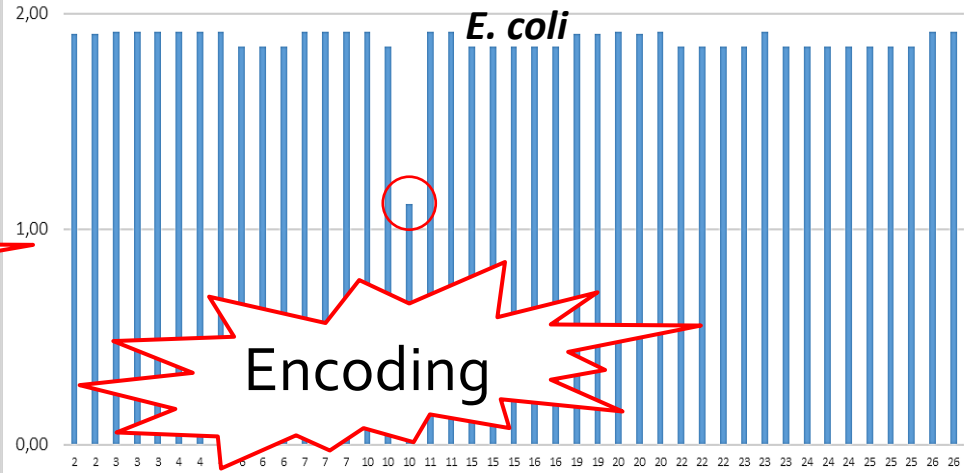
$$N = \frac{\sum C}{1{,}1 . V . d}$$

	Total flora	*E. coli*	*S. aureus*	*C. perfringens*
10^{-1}	>300	7	196	14
10^{-2}	>300	2	13	1
10^{-3}	>300	0	0	0
10^{-4}	289	0	0	0
Calculs	$\frac{289}{1 . 10^{-4}}$	$\frac{7 + 2}{1{,}1 . 1 . 10^{-1}}$	$\frac{13 + 0}{1{,}1 . 1 . 10^{-2}}$	$\frac{14 + 1}{1{,}1 . 1 . 10^{-1}} . \frac{3}{5}$
Results	$2{,}9 . 10^{6}$	$8{,}2 . 10^{1}$	$1{,}2 . 10^{3}$	$8{,}2 . 10^{1}$

Final results [Log CFU/g]

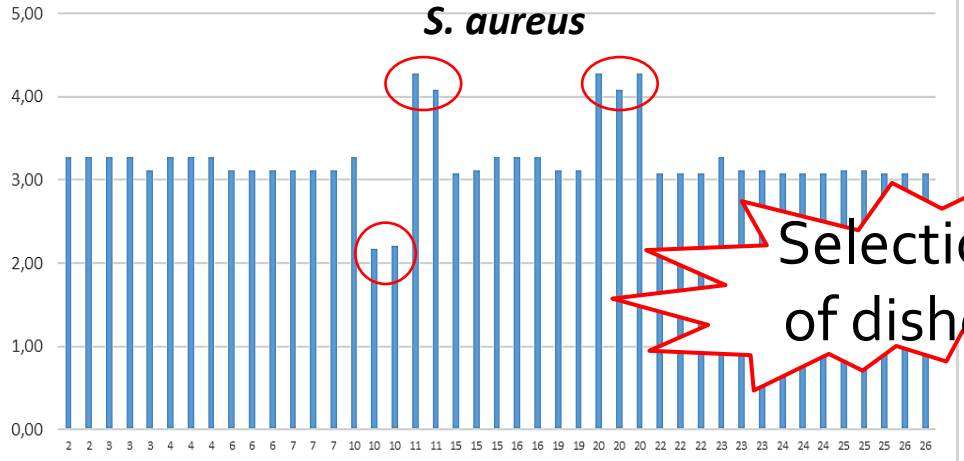
PT-2022

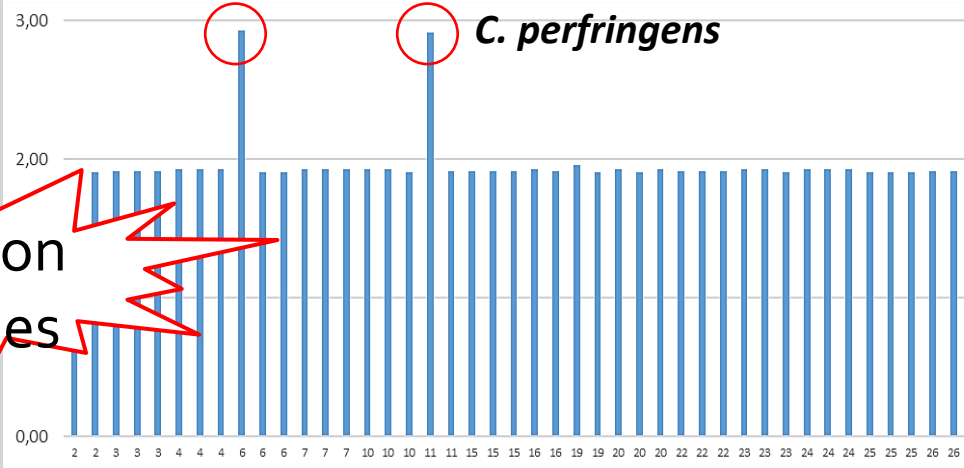




Calculation

Encoding





Selection of dishes

PT 2019 and 2022 – General observations

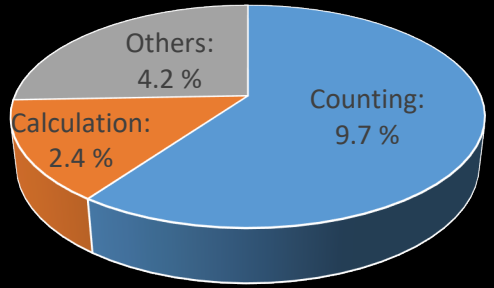
Intra-lab:

- Results quite coherent
- Sporadic errors

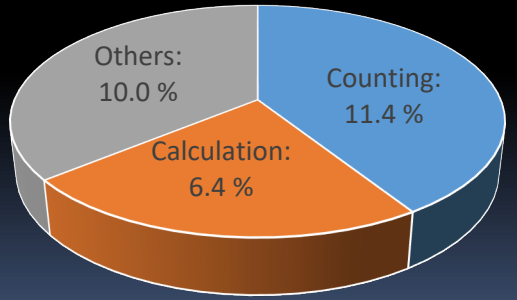
Inter-lab:

- Different practices
- Counting, calcul, interpretation

Total S_{RI} = 16,2 %

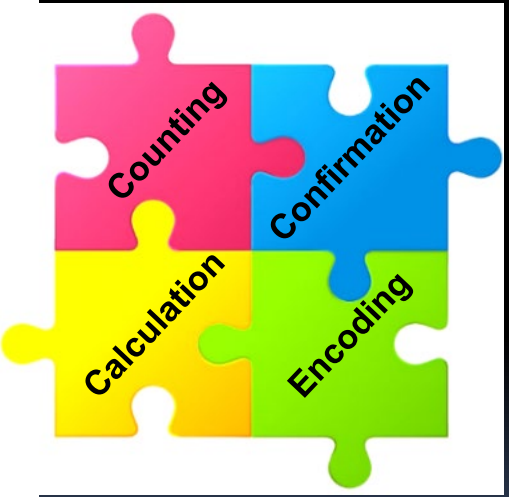


Total S_{RE} = 27,7 %



Conclusions

Virtual PT ➔ Identify hidden sources of errors



➔ Enumeration, confirmation, calculation and encoding errors contribute to analytical uncertainty in microbiology.

➔ Improvement areas

Perspectives

- ✓ To reduce MU in food microbiology : clear instructions and training
  - ➔ *Training (18/11/22) to harmonize calculation practices among labs*
  - ➔ *Revision of ISO 7218 (2024)*
- ✓ Further virtual PT to highlight (many) other hidden sources of errors…

Thank you !

Florence Ferber
REQUASUD Coordinator

Viviane Planchon
REQUASUD President

Elena Pitchugina
REQUASUD Statistician

Alain Dubois
Director of photography

Thibaut Cugnon
REQUASUD Min/Org quality

requasud
réseau de laboratoires wallons

UCLouvain