

RESULTS OF THE PROFICIENCY TESTING OF DETECTION, IDENTIFICATION AND QUANTIFICATION OF PARASITIC HELMINTH EGGS IN THE YEAR 2010

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Proficiency Testing (PT) participation allows to the laboratories the check of the test results obtained by using routine analytical methods and have objective evidence of their technical performance. This activity allows comparing the results of participating laboratories to assess their reliability, improve the test procedures and develop new methods in line with continuous improvement.

Technical training of the participating laboratories in the first PT of parasitic helminth eggs is higher in the detection and identification in the quantification of these, so we conclude that the quantification technique requires improvements to increase the recovery rate of helminth eggs.

1. INTRODUCTION

Wastewater is a valuable resource, however without a properly developed framework policy, safe and efficient management of this resource can not be achieved. Wastewater reuse increases the risk of intestinal nematode infection.

In this communication we show the results of technical competence of laboratories participating in the Proficiency Testing of detection, identification and quantification of helminth eggs by Hydrolab Microbiologica organized in 2010.

2. METHODS

PT of Parasitic Helminth Eggs consists of two rounds. Two parameters are analyzed in each round: quantification of parasitic helminth eggs in a reference sample and detection and identification of parasitic helminth eggs in 15 photographs of natural samples evaluated by PhD. Humbert Salvadó (Professor of Parasitology, University of Barcelona).

PT begins with sending a newsletter detailing the conditions of confidentiality, reference samples, statistical analysis, results report and the schedule of exercise.

Thirty technicians of twenty six different laboratories have taken part in the PT this year. Each laboratory is assigned a code that identifies it throughout the process, ensuring its confidentiality.

2.1. Quantification of parasitic helminth eggs:

Samples of the first round of PT were infected with 20 parasitic helminth eggs, with homogeneity RSD of 7.4% and stability of 2 months (Norma Oficial Mexicana NMX-AA-113-SCFI-1999). The samples of the second round were infected with 37 eggs, with homogeneity RSD of 4% and stability of 6 months (4% of RSD).

The quantitative samples were analyzed according to the following protocols:

- Water samples: Baillenger method modified (Ayres *et al.*, 1996).
- Sand samples: NTJ 13R (1998).

Results above the titled value and negative results have been previously eliminated and were not considered for statistical analyses. Accepted results were subjected to logarithmic conversion to transform a normal distribution.

We have considered three assigned values taking into account the following considerations:

- Titled value: Number of eggs inoculated in the samples.
- Value of 50% recovery: The analysis technique has certain limitations which limits the recovery efficiency of 30 to 75%, with an average recovery of 50% (Malicki *et al.*, 2001).
- Consensus value: Average scores of accepted results.

The value assigned to 50% recovery is considered the most appropriate for the analysis of helminth eggs. We provide the other two assigned values to increase the information obtained from the assigned value of 50% recovery.

2.2. Detection and identification of parasitic helminth eggs:

We have considered two assigned values taking into account the following considerations:

- Titled value: Results provided by a technical expert (PhD. Humbert Salvadó, professor of Parasitology, University of Barcelona).
- Consensus value: Average of accepted results.

The titled assigned value is considered the most appropriate for this exercise. We provide the other assigned value to increase the information obtained from the titled assigned value.

The results have been compared with the assigned values and assigned to four categories: presumptive positives confirmed positives, presumptive negatives confirmed negatives, presumptive positives confirmed negatives and presumptive negatives confirmed positives.

From these categories, we have estimated the following parameters in response to ISO/TR 13843:2000: sensitivity, specificity, false positive rate, false negative rate, efficiency and selectivity.

The selectivity is a discriminative parameter: <-2 are invalid results, >-2 and <-1 are questionable results, and >-1 are satisfactory results.

2.3. Z-score:

We used the Z-score to evaluate the effectiveness of participants for each exercise:

$$Z = \frac{X - X^*}{\sigma^*}$$

X: Laboratory results

X*: Assigned value

σ^* : Assigned standard deviation

The Z-score is a statistic that measures the deviation of the laboratory result to the assigned value against the reference standard deviation.

| | |
|---------------|------------------------|
| $ Z \leq 2$ | Satisfactory results |
| $2 < Z < 3$ | Questionable results |
| $ Z \geq 3$ | Unsatisfactory results |

3. RESULTS AND CONCLUSIONS

3.1. Quantification of parasitic helminth eggs:

Table 1 shows the average results of the first and second round of the exercise to quantification of parasitic helminth eggs. The recovery rate in the two rounds of the exercise has been low, and in any cases the results of the technicians (Fig 1).

Table 1. Results of quantification analysis of helminth eggs (eggs/10 L).

| | First Round (20 eggs/sample) | | | Second Round (37 eggs/sample) | | |
|------------------------------|------------------------------|--------------|---------------|-------------------------------|--------------|---------------|
| | Result | Recovery (%) | $\log_{10} X$ | Result | Recovery (%) | $\log_{10} X$ |
| Average | 6,7 | 34% | 0,72 | 18,0 | 49% | 1,14 |
| σ^* | 4,16 | 21% | 0,36 | 11,21 | 30% | 0,41 |

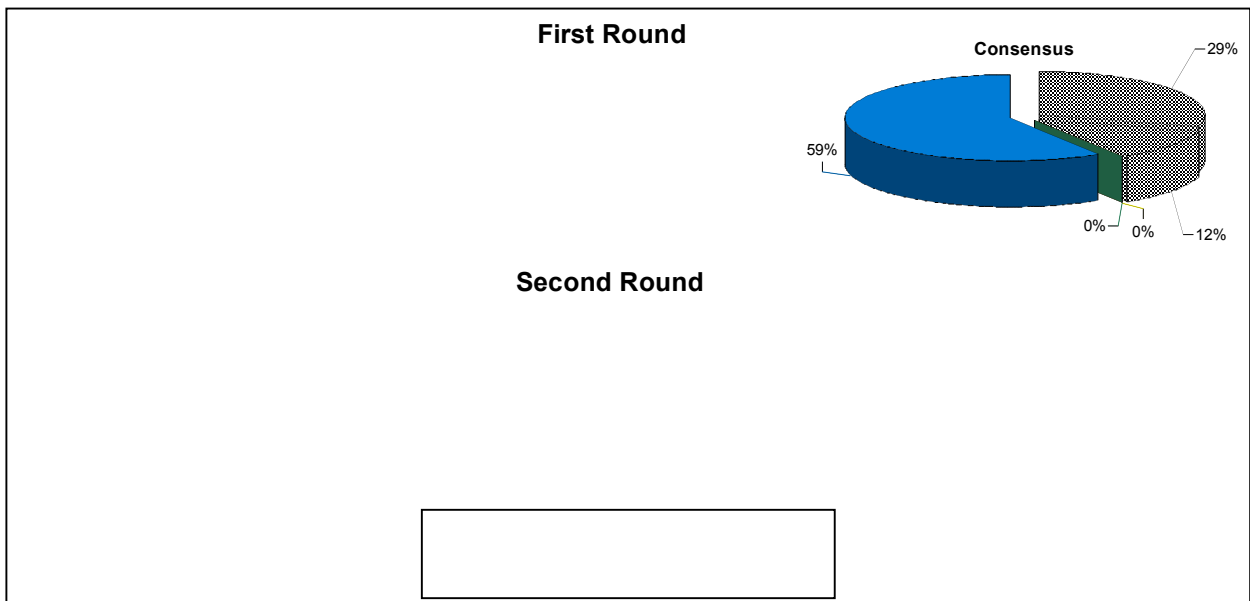


Fig. 1. Distribution of acceptance/rejection of quantitative test results of parasitic helminth eggs from the Z-score values.

3.2. Detection and identification of parasitic helminth eggs:

Technical competence of participants in the detection and identification of parasitic helminth eggs is high: satisfactory results in all cases exceed 50% comparing to the titled value. The Z-score value is more restrictive than the selectivity value (Fig. 2).



Fig 2. Distribution of acceptance/rejection of the results for detection and identification of parasitic helminth eggs attending the Z-score values and the selectivity values.

4. REFERENCES

- Ayres *et al.* 1996. Analysis of Wastewater for Use in Agriculture – A laboratory Manual of Parasitological and Bacteriological Techniques (WHO, 1996, 36 p.).
- ISO/TR 13843:2000. Water Quality – Guidance on Validation of Microbiological Methods. Geneva: International Organisation for Standardization.
- Malicki, J., A. Montusiewicz, and A. Bieganski. 2001. Improvement of counting helminth eggs with internal standard. *Water Res.* 35:2333–2335.
- Norma Mexicana NMX-AA-113-SCFI/1999, Análisis de Agua.- Determinación de Huevos de Helminto. Método de Prueba. (Diario Oficial de la Federación 5 de agosto de 1999).
- Norma NTJ 13R. 1998. Higiene de los areneros en áreas de juegos infantiles. Serie Normas tecnológicas de jardinería y paisajismo. Colegio Oficial de Ingenieros de Cataluña.