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SARS-CoV-2 – 2024

ROUND I - FEBRUARY

INSTRUCTIONS

ISO 17043
Nº 2 / PPI007

A) SAMPLES PREPARATION

1. INTRODUCTION

In the rounds of this proficiency testing scheme a homogeneous and stable material will be supplied in liquid format. It is a material that contains SARS-CoV-2 from a natural origin.

The supplied material includes:

- One vial (A) containing around 1 mL of sample.
- One vial (B) containing around 1.8 mL of sample.
- One plastic bottle containing 500 mL of matrix water.

After reception, the vials should be stored in refrigeration at (5±3°C) until their analysis.

The analysis of the sample should be done **as soon as possible**. Recommended deadline to do it: **04th March 2024.**

2. EQUIPMENTS AND MATERIALS

In this round, the following reagents and equipment are needed to prepare the sample which be analysed from the supplied materials:

- Refrigerator thermostatically controlled at around (5±3°C)
- Vortex
- Sterile graduated pipettes.
- Automatic positive displacement micropipettes or micropipettes that incorporate tips with a filter barrier.

3. PROCEDURE

Vial A

1. Visually check that the received vials are well sealed.
2. Mix samples thoroughly by using a pipette or vortex.
3. Take three aliquots from the sample for direct extraction and purification of RNA following the standard methods used in the laboratory **(do not carry out any previous process of dilution or concentration of the sample)**. The volume processed will agree with the established protocol for extraction and purification of RNA normally used in the laboratory.
4. Once each aliquot is prepared, perform the **RT-qPCR** analysis, always following the usual procedure of the laboratory.
5. Submit the result obtained for each aliquot and for any analysed gene, and additionally a global **Final Result** for sample A.

Vial B

1. Visually check that the received vials are well sealed.
2. Mix samples thoroughly by using a pipette.
3. For the study of the **DIRECT SAMPLE**:

   3.1. Take **200 µL** of the received suspension and carry out the extraction and direct purification of the RNA following the usual laboratory procedure (**Do not carry out any previous concentration process of the sample**).

   3.2. Proceed to carry out the RT-qPCR reaction in triplicate, following the procedure usually used in the laboratory.

   3.3. Submit the result obtained in each assay and for any amplified gene, and additionally a global **Final Result** for direct sample B.

4. For the study of the **PREPARED SAMPLE**:

   4.1. Add the remaining content of the vial (1600 µL approx.) to the 500mL matrix supplied and thoroughly homogenize the mixture.

   4.2. Concentrate the sample following the usual laboratory procedure for concentration of virus in water.

   4.3. Take three aliquots of the concentrate and perform RNA purification and extraction. The volume processed and the work protocol must be in accordance with the RNA extraction and purification protocol usually used in the laboratory.

   4.4. Once each aliquot is prepared, proceed to carry out the RT-qPCR reaction, following the usual procedure used in the laboratory.

   4.5 Submit the result obtained for each aliquot and for any amplified gene, and additionally a global **Final Result** for prepared sample B.

The statistical evaluation of the results will be made based on the reported Final Result for each option.

B) PARAMETERS TO PERFORM

VIAL A: SARS-CoV-2

- Qualitative determination: **Detected / Not Detected**
- Quantitative determination: **GC/mL**

VIAL B SARS-CoV-2

- Qualitative determination: **Detected / Not Detected**
- Quantitative determination:
  - Direct sample: **GC/200µL**
  - Prepared sample: **GC/500mL**

C) RESULTS SUBMISSION INSTRUCTIONS

In order to make sure that your results are correctly handled, it is important to take into account the following instructions when you fill in the "Results Data Sheet" through our website:

- Express the results **in qualitative form**, for all the analysed genes, by indicating "Detected" or "Not Detected" in each result cell for the 3 replicates and also submit a global final result for each sample in the Final result cell.
- Express the results **in quantitative form**, by indicating the concentration of each identified gene for the 3 replicates and also submit a global final result for each sample in the Final result cell.
- Express the results **in numerical form**, in the units that are indicated and y without any symbol to separate the thousand places. Do not express the results in exponential format or using abbreviations.
- If an analysis was not performed, leave the corresponding cell empty (blank).
- Due to the characteristics of the RT-qPCR technique and in order to be able to make a better evaluation of the submitted results, **it is important to complete all the requested information on the technical data**.
- The information included in "Comments'' field, will be considered as additional information. Please submit all queries that require an answer from ielab by telephone (+34 966 10 55 01) or by email (comercial@ielab.es).

Note: *Results that do not follow these instructions cannot be considered in the statistical analysis. (Results will not appear in the round report).*

You will be able to modify your results through our website until the results submission deadline. Once the round is closed, all the results saved in our database will be studied. Anytime you enter or modify your results, you will receive a confirmation email to the contact address registered in your personal data.

RESULTS REPORT DEADLINE: Friday 15th March 2024