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# Evaluation of a new method enabling standardization of respiratory RNA virus quantification in nasopharyngeal swabs across molecular systems

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## INTRODUCTION

A network of European laboratories (from EU-RESPONSE project) has been established to accelerate and strengthen the response to new pandemics, especially the emergence of new respiratory viruses. The network's first objective was to develop a unique and robust method for inter-laboratory viral quantification. The EU-RESPONSE project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 101015736.

An initial external quality assessment, based on the Quantification Standard range used in amplification (COVID-19 R-GENE® and QUANTI SARS-CoV-2 R-GENE® IUO), shows that the efficiency of the extraction system influences RNA yield, leading to variations in PCR SARS-CoV-2 Ct values, but not DNA yield (cells). In 2024, efforts focused on a new solution that considers RNA extraction yield variabilities within quantification.

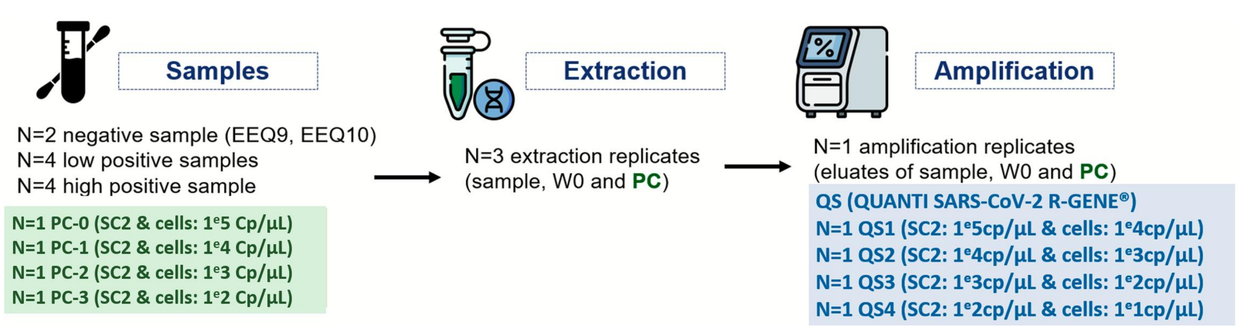
The objective of this study is to evaluate the newly proposed technique for monitoring viral excretion using normalized loads in nasopharyngeal samples (NPS) of respiratory viruses.

## MATERIAL AND METHOD

### Protocol

Using COVID-19 R-GENE® kit, 9 labs quantified ten SARS-CoV-2 (SC2) external samples (prepared by HCL Lyon) using two methods.

- **Method 01**: Amplification Quantitative Standard range (4 QS): QUANTI SARS-CoV-2 R-GENE ® IUO
- **Method 02**: Range of RNA controls for SC2 & DNA for cellular control following the entire process (extraction and amplification steps) called PC-0, PC-1, PC-2, PC-3




### Laboratories and molecular platforms

| Laboratory | Instruments: extraction / amplification |
|---|---|
| Hôpital de la Croix Rousse, CNR Lyon (France) | EMAG® / QS5™ |
| University Hospital Brno, Dpt of Clinical Microbiology and Immunology (Czech) | EZ2 / CFX96™ |
| Evangelismos Hospital Athens Department of Clinical Microbiology (Greece) | QIASymphony / Rotorgene® |
| University of Pécs, Clinical Center / Dpt of Laboratory Medecine (Hungary) | Hamilton / CFX96™ |
| Laboratoire National de Santé (LNS), Microbiology Department (Luxembourg) | easyMAG® / CFX96™ |
| Respiratory Virus Laboratory of the Medical University of Łódź (Poland) | Maxwell® / LC480® |
| INSA – National Reference Lab. for Influenza and Respiratory Viruses (Portugal) | EMAG® / CFX96™ |
| CCL - Cassovia COVID Lab (Solvakia) | RiboSpinvrd™ / CFX96™ |
| LHUB-ULB – Laboratoire Hospitalier Universitaire de Bruxelles (Belgium) | EZ2 / QS6™ |
| Servicio de microbiologia - Hospital Universitario La Paz | MagCore® / CFX96™ |

## RESULTS

The viral loads from each lab were compared to those from CNR – Lyon lab., and expressed as SC2 cp/ 10 000 cells or SC2 cp/ml with the 2 methods.

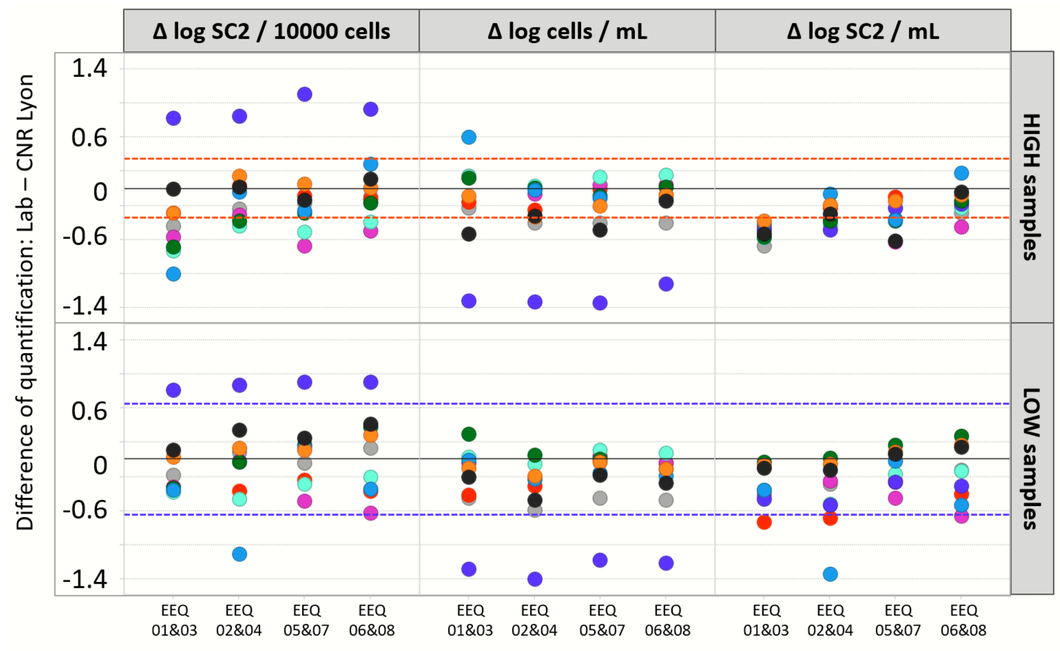
Acceptance criteria (inter-lab consensus): the difference considered as acceptable between labs and CNR are 0.35log10 for sample with Ct < 30 Ct and 0.65log10 for lower viral load > 30 Ct.

| | | | | | |
|---|---|---|---|---|---|
| Belgium | | Hungary | | Portugal | |
| Greece | | Luxembourg | | Czech Rep. | |
| Spain | | Poland | | Slovakia | |

Dotted line:
+/-0.35 log qty
+/-0.65 log qty

### Method 01: Using QUANTIFICATION Standard SARS-CoV-2 R-GENE ® IUO

**Delta log SC2/10000 cells – cells/mL - SC2/mL per sample (Lab X – HCL EMAG CNR results)**



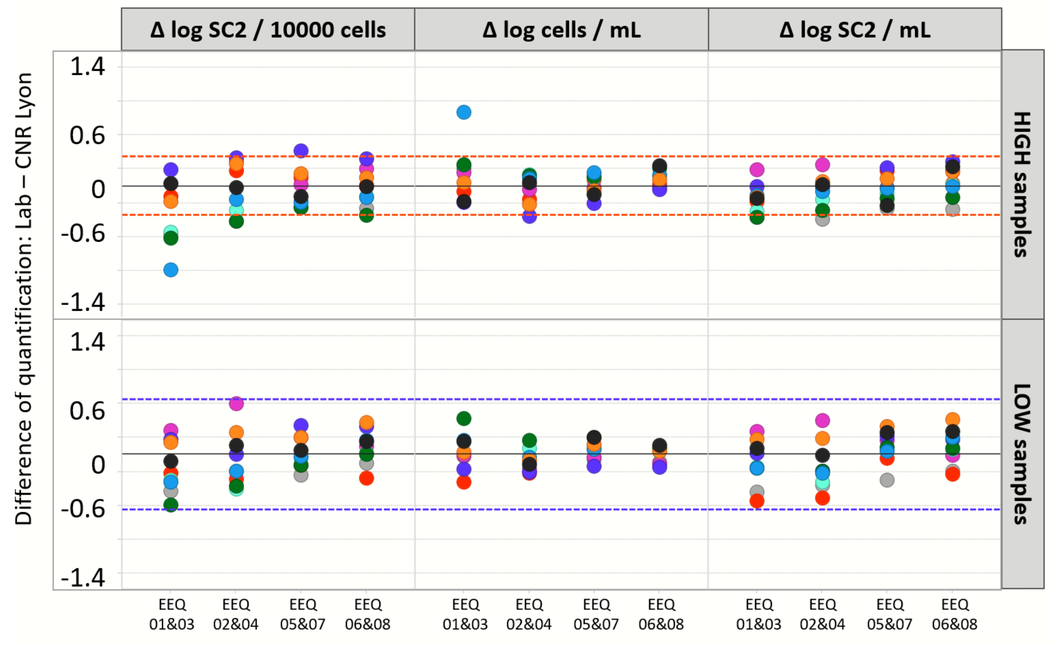

**Log SC2/10000 cells quantification:**
- Mean quantification difference ≈ -0. 07log
- % sample within acceptance criteria = **72,2%**

**Log SC2/ mL quantification:**
- Mean quantification difference ≈ -0. 29 log
- % sample within acceptance criteria = **63,8%**

Approximately 68% of samples have comparable SC2/mL and SC2/10000 cells quantification. The method taking into account cell quantification improve a little bit interlaboratory homogeneity. We confirm that extraction efficiency influences the yield of RNA and DNA differently inducing quantification variabilities

### Method 02: Using RNA & DNA standard following the entire process

**Delta log SC2/10000 cells – cells/mL – SC2/mL per sample (Lab X – HCL EMAG CNR results)**




**Log SC2/10000cells quantification :**
- Mean quantification difference ≈ -0. 06 log
- % sample within acceptance criteria = **91,8%**

**Log SC2 / mL quantification:**
- Mean quantification difference ≈ -0. 03 log
- % sample within acceptance criteria = **95.8%**

More than 90% of the samples have comparable SC2/mL and SC2/10000 cells quantification with negligible mean bias. Despite a few cases, this method allows a robust and homogeneous inter-laboratory quantification using various extraction and amplification platforms.

Using quantification standards following the full process, the method 02 significantly improves the quantification homogeneity between labs compared to the method 01 using QS in direct amplification. The quantification in SC2/ 10000 cells versus the SC2/mL are similar. These results demonstrated the importance of considering RNA & DNA extraction efficiencies between systems.

## CONCLUSION

**Thanks to the VIRvOLT lab network, we implemented a robust method to monitor the viral load in nasopharyngeal swabs, to provide comparable results between labs using various molecular platforms. This allows a decentralization of testing of new drug efficacy, more rapid in case of epidemic/pandemic.**