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# Clinical validation of a freeze-dried multiplex One-Step RT-qPCR assay for UK VUI 202012/01 VARIANT of SARS-CoV-2 discrimination in clinical samples.

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## **INTRODUCTION**

Rapid increase in COVID-19 cases is due to new phylogenetic cluster including UK-variant VUI202012/01 defined by multiple spike protein mutations (deletion 69-70, deletion 144, N501Y, A570D, D614G, P681H, T6171, S982A, D1118H). Often  $\Delta$ 69-70 has not been detected by RT-PCR causing false negative. Avoiding it is possible thanks to molecular assay developed by Bioside: qualyfast®SARS-CoV-2 UK-Variant qPCR (quantitative Polymerase-Chain-Reaction) assay which allows to specifically identify the UK-variant.

#### **AIM**

Validate the performance of new **Bioside qualyfast®SARS-CoV-2 UK-Variant qPCR assay** for the concurrent discrimination of SARS-CoV-2 UK-Variant in clinical samples

- S-gene target;
- screening phase facilitation: reduction of sequencing work and detection times for promptly infection identification;
- reduction of false negative.

# **METHOD**

qualyfast®SARS-CoV-2 UK-Variant freeze-dried innovative assay developed by:

- proprietary Bioside technology;
- **lyophilized form**: solid cake stabilized at room temperature;
- simple to use;
- detection at the same time both SARS-CoV-2 UK-Variant ( $\Delta$ 69-70 in S-gene) and Sarbecovirus (E-gene) from clinical samples.

54 nasopharyngeal swabs were analyzed; the genomic extraction was performed using both silica-based columns and magnetic beads and the RNA was analyzed with qualyfast®SARS-CoV-2 UK-Variant obtaining results in two hours.

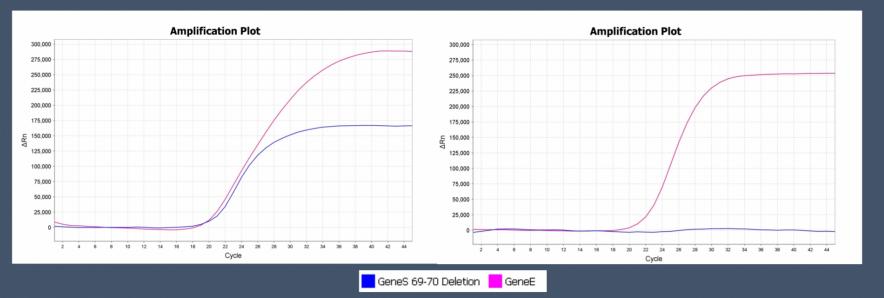




#### **RESULTS**

qualyfast®SARS-CoV-2 UK-Variant allows simultaneous discrimination of SARS-CoV-2 UK-Variant from not-UK-Variant in positive samples (see Figure 1).

The assay shows **100% specificity and 96% sensitivity** for SARS-CoV-2 UK-Variant (see Table 1) No cross-reaction with other SARS-CoV-2 Variant. This proves that primers and probes were optimized, analytical specificity and sensitivity maximized.



positive

27

27

Specificity

100%

negative total negative Sensitivity

Table1: Assay sensitivity and specificity on 223 samples.

27

96%

26

Fiigure1: qPCR discrimination between SARS-CoV-2 UK-Variant and Sarbecovirus in positive samples.

## **CONCLUSIONS**

Bioside has developed qualyfast® SARS-CoV-2 UK-Variant, a One Step RT qPCR assay by a proprietary freeze-drying technology.

- ✓ Lyophilized and multiplexing Bioside technology accurately detected positive samples for SARS-CoV-2 discriminating simultaneously UK-Variant with higher specificity to improve patient prognosis.
- ✓ Assay in lyophilized form stable at room temperature for 12 months is really easy to use, permitting virological analysis within 2 hours for rapid monitoring of infection in order to guarante a large-scale population molecular tracing.
- ✓ Bioside multiplexing assay also reduces analytical costs and time of analysis, enables Point-of-Care tests and labor saving.

  Our mission is to detect and isolate quickly new Sars-CoV-2 UK-Variant by integrating standard laboratory molecular tests in order to guarantee a massive screening of the population. Our work continued and focused on identifying different variants of SARS-CoV-2 targeting the relevant mutations of each. We then validated qualyfast® SARS-CoV-2 Variant Multiplex assays which allows to discriminate:

UK Δ69-70, Δ144, N501Y

South Africa Δ241-243, N501Y, E484K

Brazil/Japan P.1 H655Y, N501Y, E484K

Indian L452R, P681R

Indian B.1.617.2-B.1.617.3 Δ157-158, L452R, P681R

Niger Δ144,E484K

Brazil P.2 E484K

Philippines and Columbia E484K,N501Y

Bioside lyophilized assays can detect:  $\Delta 69-70$ ,  $\Delta 144$ , N501Y, E484K, L452R, P681R,  $\Delta 157-158$ ,  $\Delta 241-243$ , H655Y to immediately select the most popular SARS-CoV-2 Variants.

# CONTACT INFORMATION

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