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Clinical agreement study between COVID-19 R-GENE® real-time PCR assay and Allplex SARS-CoV-2 assay on nasopharyngeal swab and saliva paired samples, broncho-alveolar lavages, nasal aspirates, nasal swabs and oropharyngeal swabs

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ABSTRACT

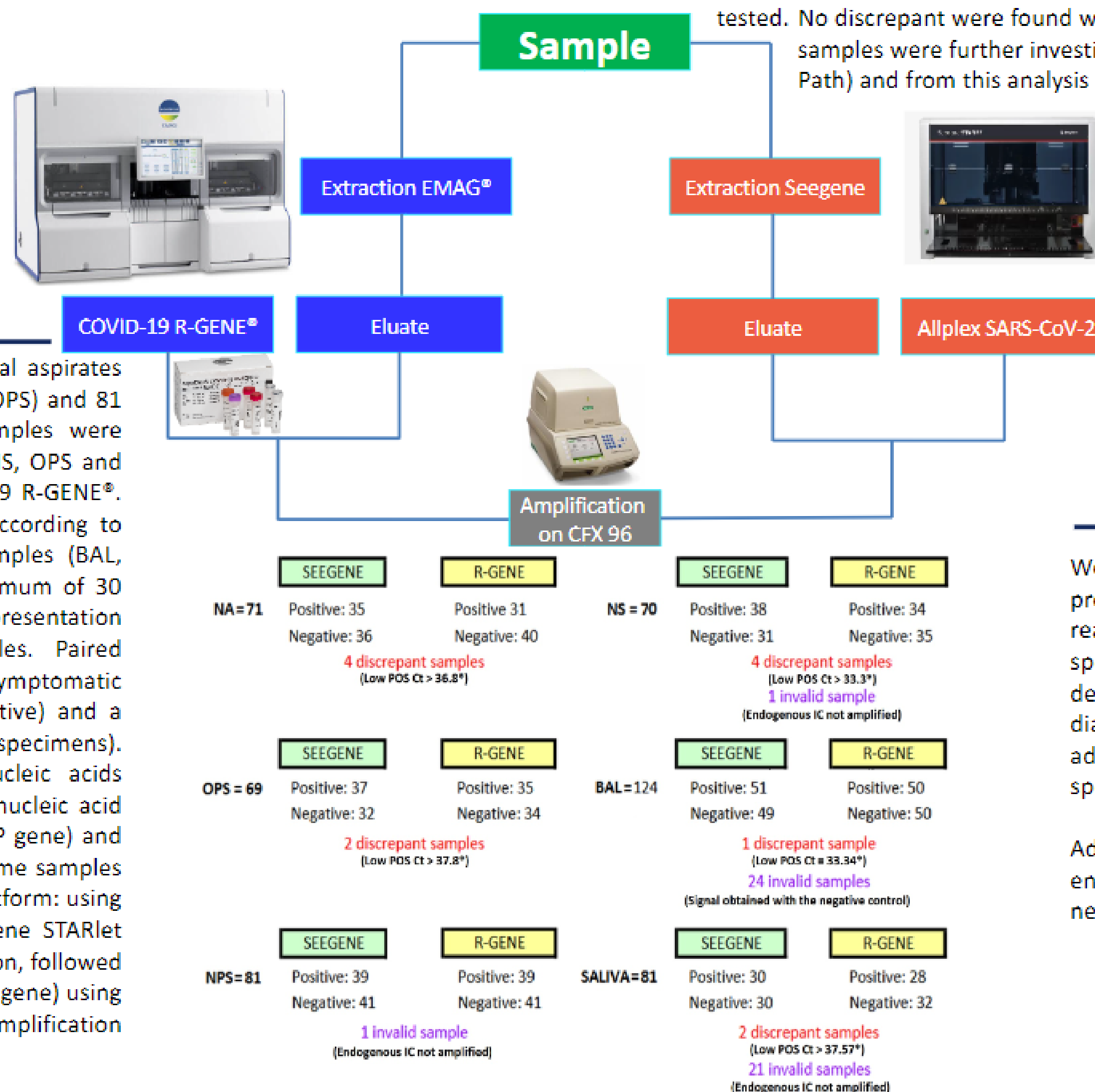
COVID-19 is an ongoing global pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) transmitted between symptomatic and asymptomatic subjects through direct contact, and aerosol droplets. The disease is characterized by fever, dry-cough, dyspnea. Therefore, rapid, sensitive and reproducible diagnostic tests are essential. Validation of diagnostic methods is imperative, requiring procedures with high reliability and accurate repeatability, showing agreement between replicates and a lower incidence of false positive or false negative results.

OBJECTIVES

Evaluate the performances of COVID-19 R-GENE® real time PCR assay comparing with Allplex™ SARS-CoV-2 assay (routinely used in our laboratory) on residual samples collected for diagnostics of asymptomatic and symptomatic patients of Romagna area (Italy).

METHODS

A total of 124 bronchoalveolar lavages (BAL), 71 nasal aspirates (NA), 70 nasal swabs (NS), 69 oropharyngeal swabs (OPS) and 81 paired nasopharyngeal swabs (NPS) and saliva samples were collected between April and September 2022. BAL, NS, OPS and NA are not claimed in the intended use of COVID-19 R-GENE®. The two methods were performed simultaneously according to the manufacturers' instructions. The respiratory samples (BAL, OPS, NS, NA) were selected in order to have a minimum of 30 negative and 30 positive samples with equal representation between high, medium and low positive samples. Paired specimens (NPS and saliva) were collected from an asymptomatic population (a minimum of 10 positive and 10 negative) and a symptomatic population (20 positive and 20 negative specimens). The BioMérieux EMAG® system was used for nucleic acids extraction. The COVID-19 R-GENE® kit was used for nucleic acid amplification to detect: SARS-CoV-2 (N gene and RdRP gene) and an endogenous internal control (HPRT1 gene). The same samples were simultaneously retested with the comparison platform: using Seegene's STARMag 96x4 Universal kit on the Seegene STARlet system with *One Step* protocol for nucleic acid extraction, followed by nucleic acid amplification (N gene, RdRP gene and E gene) using the Seegene Allplex Sars-CoV-2 kit. The nucleic acid amplification of both assays was performed on Bio-Rad CFX96.



RESULTS

The present study shows an optimal agreement of positive and negative results between COVID-19 R-GENE® and Allplex™SARS-CoV-2. **Discrepant results have been found in very low percentages ranging from 1% to 4%. The discrepant specimens where all found as very low positive in Allplex assay** (very high threshold cycle (> 34 Ct) values) but negative with R-GENE® assay. In details, we found 2 discrepants among 69 OPS samples; 4 discrepant among 71 NA samples; 4 discrepant among 70 NS samples; 1 discrepant among 124 BAL samples and 2 discrepant among 81 saliva samples between the two methods tested. No discrepant were found with the NPS between both methods. All discrepant samples were further investigated with a third reference method (Thermofisher Taq Path) and from this analysis we obtained low positive results except for one nasal sample negative with Taq Path. We obtained 23 **invalid BAL** samples because a positive signal (37 Ct) was found with the negative control W0 (possible contamination). We collected 22 saliva, 1 NPS, 1 BAL and 1 NS that gave invalid result (endogenous internal control non amplified or ≥35 Ct). One saliva was confirmed negative after investigation, (endogenous internal control: 33,3 Ct).

CONCLUSIONS

We have demonstrated that the COVID-19 R-GENE® assay provides comparator-like efficiency without risk of cross-reacting effects or false negative results. The sensitivity and specificity parameters were fully met. This preliminary study demonstrated that COVID-19 R-GENE® is suitable for the diagnosis of COVID-19 on NPS and saliva, and also on four additional samples, not claimed, i.e. BAL, NA, NS and OPS specimens.

Additionally, the COVID-19 R-GENE® provides an endogenous internal control that allows to validate the negative results as true, by validating the sample quality.