# Summarizing report Eurofins 5.1.2a TC 17 September 2020

## Present

**Eurofins NMDL-LCPL, Netherlands**

5.1.2e

5.1.2e

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**Eurofins** 5.1.2a

5.1.2e

5.1.2e

5.1.2e

**RIVM**

5.1.2e

**LCDK**

Apologies from 5.1.2e

Notified for not attending 5.1.2e

## Issue

There was a report from a municipal health service on percentage SARS-CoV-2 positive specimens coming from Eurofins 5.1.2a that were considerable higher than expected based on previous testing of specimens submitted to a laboratory in the Netherlands; increase from 1-2% at the Dutch lab to up to 9% at 5.1.2a An investigation was started whether the PCR workflow used at Eurofins 5.1.2a ould be a cause of this difference.

## Investigation and results

Previously to this investigation Eurofins 5.1.2a passed the quality check by testing the RIVM specificity and sensitivity panels using the workflow intended to be used for specimens coming from the Netherlands. The workflow includes Molgen Pureprep Pathogen extraction reagent on Kingfisher Flex followed by real-time RT-PCR using the ViroBoar RT-PCR kit (CE-IVD) on Roche LC 480 II thermal cycler. Primers and probe used are for N1-gene CDC and E-gene WHO/Corman et al.

The specificity panel includes three specimens containing various concentrations of SARS-CoV-2 of which one at educational level (around the limit of detection (LOD) of several workflows being used in The Netherlands) and seven specimens containing other respiratory viruses among which seasonal human coronaviruses (n=6) or no virus (n=1). The sensitivity panel includes seven specimens from a 10-fold dilution series of SARS-CoV-2. Three of the sensitivity panel specimens from this dilution series are included in the specificity panel. Of the seven sensitivity panel specimens five are expected to become positive in RT-PCR of which one is educational.

Using this workflow the Eurofins lab correctly identified the SARS-CoV-2 containing specimens in the specificity panel and none of the other respiratory virus containing specimens or the no-virus specimen generated a signal. Important to mention is that the core specimens had Ct values below 39 for both the N1 and E gene targets whereas the educational specimen had a Ct value of 41.4 in the N1 gene RT-PCR and no Ct value in the E-gene RT-PCR. In the sensitivity panel the Eurofins lab detected SARS-CoV-2 in the core and educational SARS-CoV-2 containing specimens that should become positive in RT-PCR. Also here the core specimen had Ct values for both gene targets below 39 whereas the educational specimen had Ct 37,19 for the E-gene and 40,93 for the N1-gene RT-PCR. Together these results show that the performance of the workflow used at the Eurofins lab

meets the performance of the 60-70% top performing laboratories in The Netherlands that are capable of detecting the educational specimens in one or both panels.

Using this validated workflow the Eurofins lab analyzed 25,778 specimens (dd. 16 Sept 2020). The Eurofins laboratory reported to CoronIT, according to the kit IFU, applying a Ct cutoff value of 39. All Ct values below Ct 39 were reported as positive. All Ct values between 39 and 42 as positive with a remark stating: "*Bepaling is correct uitgevoerd, maar op basis van het resultaat kan niet worden geconcludeerd of de uitslag van de bepaling positief of negatief is. Indien klinisch relevant aub opnieuw materiaal insturen.* All Ct values above were reported as negative.

Upon questions from local municipal health services (GGDs) it became clear that the remark field for ambiguous positive samples is only visible in CoronIT and not in HPzone, the contact tracing tool. After discussions on the higher percentage positives detected by the Eurofins laboratory it was decided to classify all ambiguous results (negative specimens positive for a single gene, usually the N1-gene RT-PCR and with a Ct value of >39), as ambiguous with a remark (dd. 10-9-2020). However as HPzone does not get this remark transferred the municipal health service did not know what to do with an ambiguous result. Therefore these specimens were subsequently reported as SARS-CoV-2 negative as of 11-09-2020. However, although it was suggested that single gene positive specimens could be the cause of the increased percentage positives actually only 181/25,778 specimens showed such a result, which is only a contribution of 0.7% to the percentage positive. This does not explain the increase from 1-2% to 7-10% after switching from laboratory. To exclude false positive as a possible cause an increased number of specimens were sent for confirmation by the RIVM laboratory.

Three sets of specimens from the municipal health service that were analyzed at the Eurofins German laboratory were submitted:

- 20 specimens that were positive in the N1 and E gene RT-PCR, all with Ct values below 39.
- 20 specimens that were negative in the N1 and E gene RT-PCR
- 17 SARS-CoV-2 positive specimens from one day (dd 9-9-2020) containing specimens that were positive in the N1 and E gene RT-PCR (n = 13; Ct range 14.79 - 36.63) or N1 gene only (n = 4; Ct range 36.55 - 38.78 ).

All specimens were analyzed at RIVM using the routinely used RT-PCR assays targeting the E-gene (Corman et al.) and the RdRP-gene (modified from Corman et al. to have near equal sensitivity as the E-gene RT-PCR. All 20 N1 and E gene RT-PCR positive specimens were confirmed by being positive in the RIVM E-gene and RdRP-gene RT-PCR assays. Of the 20 N1 and E gene negative specimens 18 were confirmed negative. Two were positive in the RIVM E-gene RT-PCR only with Ct values 34.3 or 34.5, Ct values at the LOD of the used RT-PCR assays. Of the third group 12 of the 13 double gene positive specimens were confirmed twelve of which being positive in the RIVM E-gene and RdRP-gene RT-PCR assays and one in the E-gene RT-PCR assay only. The later specimen had Ct values in the Eurofins E-gene and the N1-gene RT-PCR of 32.31 and 36.34 respectively, indicating low viral load. Of the four Eurofins N1-gene positive specimens only, two were confirmed by being positive in both the RIVM E-gene and RdRP-gene RT-PCR assays, one was confirmed by RIVM E-gene RT-PCR assay only and one could not be confirmed.

Overall, 4 (7%) of the 57 specimens could not be confirmed. All 4 were low to very low viral load specimens with Ct values around the LOD of either assay from Eurofins lab or RIVM. Other specimens with low or very low viral load positive by the Eurofins lab could be confirmed by RIVM. Taken the results of the panels and the confirmations together it is clear that similar to the RT-PCR

assays at RIVM, the RT-PCR assays at the Eurofins lab generate single gene positive results with high Ct values at low to very low viral load. This the result of statistical chance, slight differences in LOD between RT-PCR assays and differential production of subgenomic messengers of the E and N1 genes.

In conclusion, there is no evidence that the RT-PCR assays utilized at the Eurofins laboratory generates false positive signals, even if Ct is higher than 39. Therefore, the increased percentage positive is a valid observation, assuming the original laboratory has similar performance. Therefore, cross validation of the original laboratory with similar specimens used for the current confirmations is recommended to find out whether the observed increase in percentage positives is true or a result of underreporting by the original laboratory.

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