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# Evaluation of the elution and removal of swabs for SARS-CoV-2 RNA tests from tubes at drive-through sites

## Evaluation Report
## Final

5.1.2e

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## Introduction

In September there was a signal that a drive-through street was removing swabs from the collection tubes with fluid before they were transported to the laboratory. The National Coordination team Diagnostics Chain (LDCK) COVID-19 was wondering if this procedure is validated. After contacting the drive-through street and the involved laboratory, it seemed this procedure was not validated. At laboratories where SARS-CoV-2 diagnostics are performed pipetting robots are used for transferring fluid from the collection tube with the swabs (or swab) to another tube for nucleic acid extraction. Also pipetting steps by hand in the pre-analysis step needs to be performed for this purpose. For example for the fully automated SARS-CoV-2 test at the Panther, the collected medium needs to be transferred to a sample lysis tube because leaving the swab in the lysis tube directly after collection results in very high 'invalid' scores (>8%) because of clotting or dissolvement of swab material. Removing swabs by hand in the laboratory is a labour intensive process due to contamination risks. To limit these pre-analysis steps in the laboratory to be able to increase testing capacity. it would be much preferred to remove the swab with the nose/throat mucus shortly after collection to limit these pre-analysis steps in the laboratory to be able to increase testing capacity. This step has to be performed carefully, since no false positive results should be obtained due to cross-contamination. Also environmental contamination from possible highly positive specimens at the test collection site should be avoided as much as possible. In case the pre-analysis pipetting step in the laboratory can be omitted there are clear advantages: the test turnaround time is shortened and also the chance of mistakes is reduced since there is only one tube that goes directly in the pipetting robot. In addition, for pipetting robots there is a reduced chance of crashes if no swab is present in the tube. National Institute for Public Health and the Environment, Jeroen Bosch Hospital and Public Health Service of Amsterdam started to evaluate the removal of swabs at the specimen collection site (method 2) comparing it to the refence method, leaving the swabs in the tube at the specimen collection site and removing the swab in the laboratory or pipetting the specimen along the swab (method 1).

In order to study how long the duration of elution of such a swab in different buffers should last to give the same analytical sensitivity and specificity when the swab is left in the buffer until arrival in the laboratory, experiments were performed. In addition, the environmental contamination risk at existing test collection facilities was analysed using wipe test of workplace surfaces. Furthermore, a small practice test was performed in a testing street collecting and processing clinical specimens using methods 1 and 2 in parallel. If method 2 provides a similar or better performance than method 1, the new method could be implemented in practice.

## Material & Methods

### *Technical evaluation with SARS-CoV-2*

This evaluation is performed by National Institute for Public Health and the Environment. First inactivated SARS-CoV-2 (hCoV-19/Netherlands/NoordBrabant_10003/2020) in MEM with Hanks' salts; heat inactivated at 60°C for 2 hours; 5.62*10^7 TCID50/ml or 8.26*10^8 digital copies RdRP positive strand RNA/ml was diluted ($10^{-4}$ to $10^{-8}$) in viral transport medium GLY (Mediaproducts B.V., Groningen, The Netherlands). Oropharynx swabs (FLOQswab 53380CS01, COPAN Italia S.p.A., Brescia, Italy) are dipped in each dilution of inactivated SARS-CoV-2 and transferred into a 15 ml tube (Greiner -Bio one, Kremsmünster, Autria) with 1 ml of viral transport medium GLY (n=6) or 1 ml MagNAPure external lysis buffer (LB) (Hoffmann-La Roche, Basel, Switzerland) (n=6). Two different preparation methods are used for comparison. Method 1: swab is left in the 15 ml tube with viral transport medium GLY (n=3) or MagNAPure external lysis buffer (n=3) for 24 hours at room temperature to simulate the normal situation and maximum transporting time. Method 2: swabs are placed into the 15 ml tube and rotated for 10 seconds in the viral transport medium GLY (n=3) or MagNAPure external lysis buffer (n=3) to simulate removal at the specimen collection site. Before removing the swab the tip was squeezed by pressing and twisting the tip of the swab on the side of the tube, after which the swab was carefully removed and transferred to a waste container for potentially contaminated material. The tube with GLY was kept at room temperature for 24 hours to simulate maximum transporting time. After incubation the tubes were vortexed for 1 minute at 2000 rpm. After vortexing from each tube 200 µl was mixed with 275 µl lysis buffer which includes 25 µl Equine Arteritis Virus (EAV). The samples are centrifuged briefly to remove the remaining liquid from the cap. After centrifuging the samples, 450 µl was extracted on a MagNAPure 96 Instrument (Hoffmann-La Roche, Basel, Switzerland) using the MagNAPure 96 DNA and Viral NA Small Volume Kit (Hoffmann-La Roche, Basel, Switzerland) and eluted in a volume of 50 µl. The EAV is used as standard internal control for the qRT-PCR to control for inhibition. The E-gene/EAV multiplex PCR was used to test inhibition of EAV amplification. E-gene primers and probes were as described by Corman et al (1); RdRp-gene

primers and probes have been modified form the original Corman primers and probe conferring the primers SARS-CoV-2 specific and the analytical sensitivity comparable to that of the E-gen RT-PCR. Modified primers and probe: RdRp_SARS-F2 GTGAAATGGTCATGTGTGGCGG; RdRp_SARS-R2 CAAATGTTAAAAACACTATTAGCATAAGCA; RdRp_SARS-P2.2 CCAGGTGGAACCTCATCAGGAGATGC; EAV as described by Scheltinga et al (2). Reaction condition of Corman et al are described in Table 1 and 2 . All tests are run on the Light Cycler 480 I (LC480) (Hoffmann-La Roche, Basel, Switzerland) and performed according to the manufacturer's instruction.

*Technical evaluation with human albumin*

A small experiment was performed at the Jeroen Bosch Hospital, which involved 12 samples collected from three healthy individuals. Using nasopharynx swabs (Disposable Sampling Swab, Type:A-04, SJK Global, LLC, Olathe, KS 66061) eight swabs were taken from the cheek mucosa (two individuals, 4 swabs each) and four swabs from the nasopharynx (1 individual). The swabs were transferred into a tube with 0,71 ml lysis buffer to which 0,5 ml Tris-EDTA solution had been added (lysis-TE) and removed after 2, 5 and 20 seconds of moving the swab up and down in the lysis-TE, while the fourth swab was removed after 2 hours.
In a small second experiment four nasopharynx and four oral swabs were collected from one healthy individual. Sets of one nasopharynx swab and one oral swab were washed together in lysis-TE by putting the swabs in the liquid and twisting the two sticks between the thumb and forefinger for a given time interval. Before removing the swabs the tips of the swabs were squeezed out by pressing and twisting the tip of the swab on the side of the tube above the liquid. Two sets were eluted for 5 seconds and one set for 20 seconds. The fourth set of two swabs was eluted for 60 seconds and removed after 1,5 hours. To get an estimate of the quantities of human DNA present in de solutions an MagNAPure96 nucleic acid isolation was carried out followed by a real time PCR targeting the human albumin gene as described by Pongers-Willemse et al. (3).

*Clinical field evaluation*

This evaluation was performed by the Public Health Service of Amsterdam. First, a combined throat/ nasopharynx swab was collected in viral transport medium GLY for routine testing using SARS-CoV-2 TMA test (Hologic, Marlborough, United States of America). Routine testing is performed by first transportation of the viral transport medium GLY tube to the laboratory. At the pre-analysis lab 0.5 ml viral transport medium GLY is transferred to a SLT tube containing 0.71 ml of lysis buffer (Hologic, Marlborough, United States of America). Those persons who agreed to participate by signing an informed consent also allowed the sampling using a second swab with again probing the throat and nose. This second swab was eluted for 5 seconds in an SLT tube containing 0.71 ml lysis buffer (Hologic) which was supplemented with 0.5 ml viral transport medium GLY. Before removing the swab the tip of the swab was squeezed out by pressing and twisting the tip of the swab on the side of the tube. The tubes with samples are transported to the laboratory and were tested directly in the Panther Hologic machine. In total 25 persons with COVID-19 symptoms agreed and participated.

In addition at the Jeroen Bosch Hospital, Ct-values for human albumin gene were determined in 20 clinical specimen obtained from the drive-through test location: 10 clinical specimen obtained with method 1 (in 3 ml viral transport medium GLY) and 10 clinical specimen obtained with method 2 (1,21 ml Lysis-TE) with 20 seconds elution.

*Workplace surface contamination*

This evaluation was performed by National Institute for Public Health and the Environment. For this evaluation two large testing streets agreed to participate; one drive-through street using the reference method 1 and the other drive-through street removing the swabs similar to method 2 described above. To check for contamination oropharynx swabs (FLOQswab 53380CS01, COPAN Italia S.p.A., Brescia, Italy) have been used to swab different surfaces such as the side of the table were the collecting tubes with specimen are located (dirty side), the side of the table were all the clean and sterile materials are located (clean side) and other locations such as walls and floors (other). The 2 locations that agreed to participate were Location 1 (n=40 swabs collected) and location 2 (n=19 swabs collected). After the swabs were taken they were transferred into a 15 ml tube with 1 ml viral transport medium GLY. After incubating for at least 12 hours the tubes were vortexed for 5 minutes at 2000 rpm. After vortexing, from each tube 200 µl was mixed with 275 µl (of which 25 µl is EAV) lysis buffer. The samples were centrifuged briefly to remove the remaining liquid from the cap. Extraction

of total nucleic acid and qRT-PCR for E-gene/EAV and RdRp-gene were performed at National Institute for Public Health and the Environment as described above.

***Table 1.*** *For the SARS-CoV-2 the primers and probes obtained from Biolegio were premixed at a final concentration of 10 µM primers and 5 µM probes.*

| E-gene/EAV qRT PCR | µl | RdRp-gene qRT-PCR | µl |
|---|---|---|---|
| 4x Taqman Fast Virus MM | 5 | 4x Taqman Fast Virus MM | 5 |
| E+EAV PP Mix | 3 | RdRp PP Mix | 3 |
| PCR grade water | 7 | PCR grade water | 7 |
| Specimen nucleic acid | 5 | Specimen nucleic acid | 5 |
| Total volume | 20 | Total volume | 20 |

***Table 2.*** *Amplification temperature protocol for SARS-CoV-2 E-gene and RdRp-gene target with LC480 mark I.*

| PCR Program | Segment number | Temp Target (°C) | Hold Time (sec.) | Slope (°C/sec.) | Acquisition mode | |
|---|---|---|---|---|---|---|
| **Reverse Transcription** | **1** | **50** | **900** | **EXTERNAL*** | | |
| **Denaturation/Inactivation** | **1** | **95** | **120** | **EXTERNAL*** | | |
| **Denaturation** | 1 | 95 | 60 | 4.4 | None | LC 480 |
| **Amplification** | 1 | 95 | 10 | 4.4 | None | |
| **(cycles:50)** | 2 | **60** | 30 | 2.2 | Single | |
| **Cooling** | 1 | 40 | 30 | 4.4 | None | |

**External means in heating blocks and not in the thermal cycler.*

## Results

### Technical evaluation with SARS-CoV-2

To demonstrate the performance of method 2 versus the reference method 1, tubes with the diluted SARS-CoV-2 in viral transport medium GLY or MagNAPure external lysis buffer are collected with method 1 and 2, respectively, were tested for SARS-CoV-2 using qRT-PCR (E-gene and RdRp-gene). Each dilution is done in triplicate. These results are translated to percentage (%) positive.

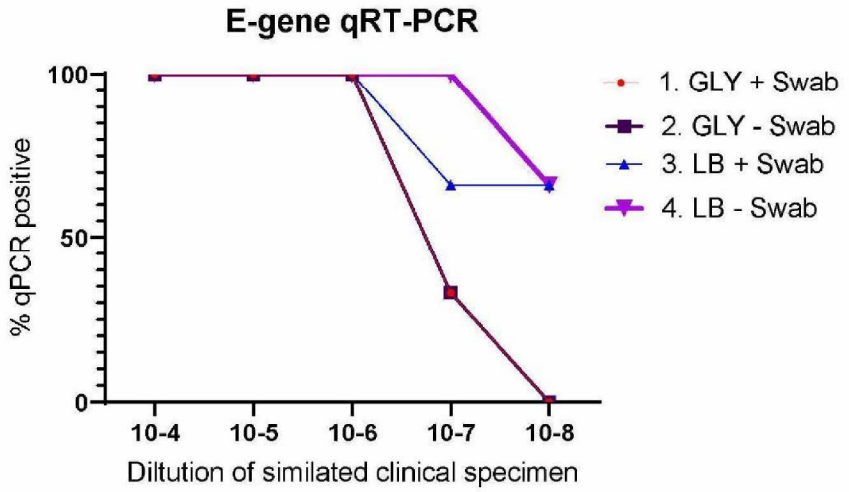


***Figure 1.*** *E-gene qRT-PCR results of the technical evaluation with SARS-CoV-2. 1) Using method 1 (swab remaining in tube) with viral transport medium GLY. 2) Using method 2 (swab removed) with viral transport medium GLY. 3) Using method 1 with MagNAPure external lysis buffer. 4) Using method 2 with MagNAPure external lysis buffer.*

The results of E-gene qRT-PCR (figure 1) show that MagNaPure external lysis buffer method 2 (4 in figure 1) is better compared with method 1 (3 in figure 1). Method 1 is 100% positive in dilution $10^{-6}$ and method 2 in dilution $10^{-7}$. The viral transport medium GLY Method 2 (2 in figure 1) results are similar to method 1 (1 in figure 1). Method 1 and 2 are 100% positive in the $10^{-5}$ dilution. It seems there is more release of viral RNA from the swab in de MagNAPure external lysis buffer compared with release of virus in viral transport medium GLY.

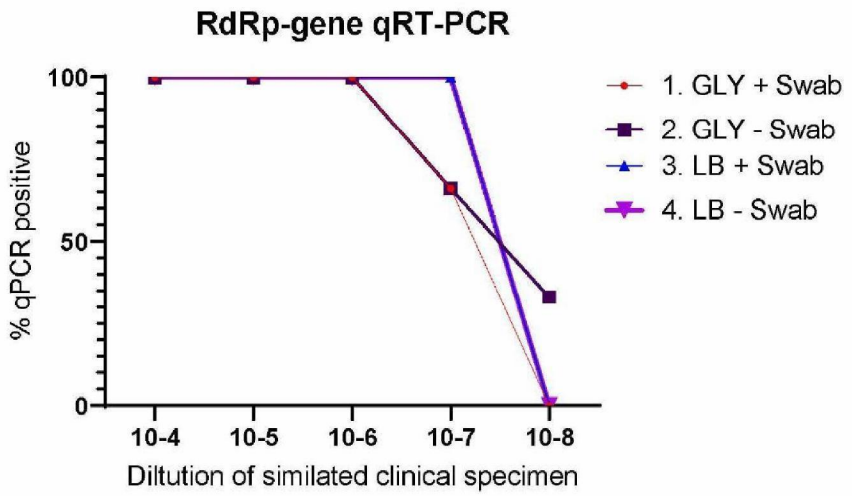


***Figure 2.*** *RdRp-gene qRT-PCR results of the technical evaluation with SARS-CoV-2. 1) Using method 1 (swabs remaining in tube) with viral transport medium GLY. 2) Using method 2 (swab removed) with viral transport medium GLY. 3) Using method 1 with MagNAPure external lysis buffer. 4) Using method 2 with MagNAPure external lysis buffer.*

The results of RdRp-gene qRT-PCR (figure 2) show that MagNaPure external lysis buffer method 2 (4 in figure 2) has similar results with method 1 (3 in figure 2). Method 1 and 2 are 100% positive in dilution $10^{-7}$. The viral transport medium GLY Method 2 (2 in figure 2) results are similar to method 1 (1 in figure 2). Method 1 and 2 are 100% positive in the $10^{-6}$ dilution. Similar to the E-gene qRT-PCR results there is more release from the swab in de MagNAPure external lysis buffer compared with de viral transport medium GLY.

In conclusion of this part: there are no clear differences in sensitivity for both the E-gene and RdRp gene target detection between method 1 (swab not removed) versus method 2 (swab removed).

## Technical evaluation with human albumin

After incubating the swabs for 2, 5, 20 seconds and 2 hours in lysis-TE, the samples were tested for the relative quantities of the human albumin gene in a real-time PCR. Human albumin gene content was considered indicative of the amount of human sample eluted in the collection medium.

***Table 3.*** *Albumin real-time PCR results of 3 types of specimen incubated for 2, 5, 20 seconds and 2 hours.*

| Person / specimen | 2 sec | 5 sec | 20 sec | 2 h |
|---|---|---|---|---|
| **1 (Cheek mucosa)** | 26.29 | 24.72 | 23.77 | 23.24 |
| **2 (Cheek mucosa)** | 23.73 | 24.75 | 23.09 | 25.45 |
| **3 (Nasopharynx)** | 25.33 | 23.66 | 22.74 | 21.03 |

Persons 1 and 3 showed that the longer the swab is eluted in the tube with lysis-TE, the higher the quantity of human albumin gene is. Person 2 demonstrated too high a variability between the different time points, so no time effect was seen.

***Table 4.*** *Combined nasopharynx and oropharynx swabs eluted together in a single tube, albumin real-time PCR and Panther SARS-CoV-2 TMA test results from 1 healthy individual, incubated for 2, 5, 20 seconds and 1,5 hours.*

| System | Detection Target | 5 sec | 5 sec | 20 sec | 1,5 h | NC |
|---|---|---|---|---|---|---|
| MagNaPure 96 | SARS-CoV-2 | UVL | UVL | UVL | UVL | UVL |
| | Phocine distemper virus (PDV) | 28,12 | 29,25 | 29,24 | 30,96 | 30,79 |
| | Albumin | 21,51 | 22,20 | 21,06 | 31,55 | Undet |
| Panther TMA test | SARS-CoV-1 (RLU) | 263 | 273 | 276 | 261 | ND |

*Undet = Undetectable*
*RLU = Relative Light Units*
*ND = Not Done*

As expected the elution of nasopharynx and oropharynx swab combined in a single tube leads to a higher quantity of albumin.
The relatively high albumin and PDV Ct-values after 1,5 hour incubation with 60 seconds of washing the swabs was probably due to overloading of the beads used for DNA-isolation and/or inhibition of the PCR due to the abundance of human DNA.

## Clinical field evaluation

After the swabs were incubated in the SLT-GLY medium and removed on site or transported in the tube to the lab, the samples were tested on the Panther TMA SARS-CoV-2 test. This to show whether the two different methods yield the same result or not.

***Table 5.*** *Panther TMA SARS-CoV-2 test results of clinical evaluation. Swabs left in the tube compared with swabs removed from tube.*

| Method | Total of samples tested | Total positive | Total negative |
|---|---|---|---|
| Swab left in GLY tube | 25 | 4 | 16 |
| Swab removed from SLT+GLY | 25 | 4 | 16 |

The Results of the clinical field evaluation show that the different pre-analyses methods gave the same patients positive in the Panther TMA SARS-CoV-2 test.

Albumin Ct-values of 10 samples from a drive-through location obtained with method 1 were 25,3 ± 0,9 (250 µl of Gly-medium, + 250 µl MP96 lysis buffer, elution 100 µl), while Ct-values of 10 samples, also from a drive through location obtained with method 2 were 23,3 ± 0,7 (500 µl of Lysis-TE directly in MP96, elution in 100 µl). The 2 Ct difference between these two methods can be explained by the volume of collection medium (3 ml viral transport medium GLY versus 1,21 ml Lysis TE) and the input in the MP96 (250 µl versus 500 µl) for method 1 and method 2, respectively.

## Workplace surface contamination

To get an impression of the workplace contamination in different drive-through streets, surfaces were sampled with swabs. The swabs were placed in 1 ml viral transport medium GLY and tested for E-gene and RdRp-gene with qRT-PCR. Drive-through street location 1 uses the reference method 1 (leaving the swabs in the tubes). Location 2 removes the swab after 2-5 seconds (method 2).

***Table 6.*** *Results of swabs taken of different surfaces such as dirty side and clean side of the table and other (Ct values between brackets).*

| Location | Total of samples tested | Dirty side* positive | Clean side** positive | Other positive | Total Negative |
|---|---|---|---|---|---|
| 1 | 40 | 1[1] (39.77) | 0 | 1[1] (36.02) | 38 |
| 2 | 19 | 5[2] (34.32-35.78) | 0 | 1[3] (32.05) | 13 |

** Dirty side = the side of the table were the collecting tubes with specimen are*

*** Clean side = the side of the table were all the clean and sterile materials are*

[1] *Samples were only qRT-PCR positive for the E-gene, not for the RdRp-gene.*

[2] *3 samples were positive for both targets (E-gene and RdRp-gene), 2 samples are only positive in the RdRp-gene qRT-PCR.*

[3] *Sample is positive for both targets (E-gene and RdRp-gene).*

The results of the workplace surface contamination show that the reference method 1 causes less surface contamination (5%) compared with method 2, where the swab is removed from the tube in the drive-through street (31%). At both locations no contamination was detected at the clean side of the table.

## Conclusion

### *Technical evaluation with SARS-CoV-2*

Removing the swab after 10 seconds have similar sensitivity results compared with leaving the swabs in the tube. This means removing the swab has no negative effect on the results.

### *Technical evaluation with human albumin*

Washing the two (throat and nasopharyngeal) swabs in medium for 5 to 20 seconds (method 1) provides sufficient elution of human DNA, comparable with removal of swabs in the laboratory (method 2).

### *Clinical field evaluation*

The concordance of the results of the Panther TMA SARS-CoV-2 test with the two different methods was 100%. This was however a very small evaluation, with only 4 positive samples. More SARS-CoV-2 positive samples need to be tested, ideally with a wide range of viral loads.

### *Workplace surface contamination*

Removing the swabs from the tubes at the drive-through test location led to more workplace surface contamination. Whether the viruses are still able to infect people is unknown.

There are a number of things that need to be done to prevent contamination such as:

1. Rotating the swabs in the medium and not pulling the swab up and down.
2. Removing the swab so close as possible above the waste bin and not 0.5 to 1 meter away from the waste bin.
3. Regular monitoring to be sure that no contamination occurs and everyone sticks to the protocol.

To monitor workplace surface contamination and cross contamination of samples, follow these steps:

1. Make sure there is an open viral transport medium or lysis buffer tube on the table where the clinical samples are placed (dirty side of the table). At the end of the day test these tubes with viral transport medium or lysis buffer for SARS-CoV-2.
2. At the end of the day take a swab of the workplace surface (dirty and clean side of the table) and placed the swab into a tube with viral transport medium or lysis buffer. Test these swabs for SARS-CoV-2.

Protocol for removal of swabs for SARS-CoV-2 RNA tests from tubes at drive-through sites:

1. Take a nasopharynx and/or oropharynx swab(s) from the patient.
2. Open the tube with viral transport medium or lysis buffer.
3. Transfer the nasopharynx and/or oropharynx swab(s) into the tube with viral transport medium or lysis buffer.

4. Gentle rotated the swab(s) in the tube for 5-10 seconds.
5. Before removing the swab the tip is squeezed by pressing and twisting the tip of the swab on the side of the tube.
6. Remove the swab(s) carefully from the tube so close as possible above the waste bin.
7. Close the tube with viral transport medium or lysis buffer.

## References

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