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Evaluation of DSM/MOLDED cast swabs

Evaluation Report
Final

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Introduction

Test materials are produced in the Netherlands wherever possible, due to the uncertainty of delivery via the regular channels. An initiative between DSM and MOLDED started production of cast nasopharynx swabs. The National Institute for Public Health and the Environment (RIVM) has been involved in evaluating these cast nasopharynx swabs. In this report results are shown of the evaluation of the DSM/MOLDED cast swabs versus a regular used product.

Material & Methods

First we examined whether there are no qRT-PCR inhibiting factors present in the materials of the DSM/MOLDED cast swabs (MOLDED, Rossum, The Netherlands) by comparing them with currently used COPAN 503CS01 FLOQswabs (COPAN ITALIA spa, Brescia, Italy) (n=6). DSM/MOLDED cast swabs were used prior to sterilization (n=6), after Ethylene Oxide (EtO) sterilization (n=6) and after Gamma sterilization (n=6) to see whether the sterilization process has an effect on release of qRT-PCR inhibitors from the material. Of each six swabs were transferred into a transport tube filled with clean 4.5 ml viral transport medium GLY (Mediaproducts B.V., Groningen, The Netherlands), broken at their breaking point and the tubes firmly closed. As a negative control (NC), only a transport tube filled with clean GLY was used. After transfer of swabs to the transport tubes, the tubes are vortexed for 1 minute. After incubating for at least 10 hours at room temperature, tubes were vortexed again and samples for RT-PCR collected The Equine Arteritis Virus (EAV) is used as standard internal control for the qRT-PCR to control for inhibitors. From each sample 200 µl was mixed with 275 µl lysis buffer with EAV included and 450 µl was extracted on a MagNAPure 96 Instrument (Roche) using the MagNA Pure 96 DNA and Viral NA Small Volume Kit (Roche) and eluted in a volume of 50 µl. The E-gene/EAV multiplex PCR was used to test inhibition of EAV amplification.

In order to determine whether the DSM/MOLDED cast swab collects and releases similar amount of human cell material and SARS-CoV-2 as the COPAN FLOQswab, nasopharyngeal specimens have been collected from confirmed COVID-19 patients in different hospitals. Materials for the COPAN FLOQswabs were collected through the left nostril and for the DSM/MOLDED cast swabs througg the right nostril. Each sample is extracted in triplicate and is followed by E-gene/EAV, RdRp-gene and 18S qRT-PCR. For 18S qRT-PCR is to determine how much human DNA is present. E-gene and RdRp-gene primers and probes were as described in the by Corman et al (1). Reaction condition are described in Table 1 and 2.

***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 Mix	6	**RdRp Mix**	3
PCR grade water	4	**PCR grade water**	7
Specimen nucleic acid	5	**Specimen nucleic acid**	5
Total volume	20	**Total volume**	20

***Table 2** . Amplification temperature protocol LC480 mark II.*

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	

For amplification of human cell material 18S human gene target is used as described by André et al(2). Reagents mixture and reaction condition are shown in Table 2 and 3.

Table 3. *For the 18S qPCR the primers and probes where obtained from Eurogentec were premixed at a final concentration of 10 µM primers and 5 µM probes.*

18S gene qRT-PCR	**µl**
4x Taqman Fast Virus MM	5
18S Mix	3
PCR grade water	7
Specimen nucleic acid	5
Total volume	20

The nurses in the different hospitals who collected the swab specimens have been asked what their experience is with the DSM/MOLDED cast swabs compared to the COPAN FLOQswabs. Questions were on ease of inserting, turning and retracting the swabs and ease of transferring the swab to the transport tube with regard to breaking the swabs and closing the tubes.

To be accepted, the cast swabs should fulfil the following criteria compared to COPAN FLOQswabs:

1. No or minimal release of RT-PCR inhibitors
2. Similar or better performance in collecting and releasing human cell material
3. Similar or better performance in collecting and releasing SARS-CoV-2
4. Similar or better ease of handling by those who collect the specimens

Results

Inhibition factors in the DSM/MOLDED cast swabs compared with COPAN FLOQswabs

The DSM/MOLDED cast swabs not-sterilized and sterilized by two different methods, the COPAN FLOQswabs and NC were tested in EAV qRT-PCR. The RNA from these samples were tested in 6-fold in a duplex E-gene and EAV qRT-PCR of which the EAV results were used only (as no SARS-CoV-2 was spiked in the specimens).

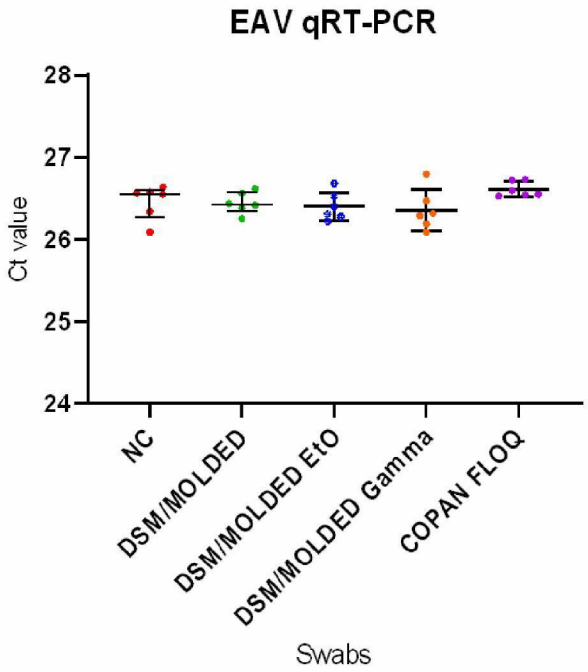


Figure 1. *EAV qRT-PCR results to determine if there are no inhibiting substances release from the DSM/MOLDED cast non-sterilized, EtO and Gamma sterilized swabs compared with the COPAN FLOQ swabs.*

There are no inhibiting factors for the EAV qRT-PCR released from the DSM/MOLDED cast swabs compared with the COPAN FLOQswabs and negative control. Furthermore, the method of sterilization of cast swabs had no effect on potential release of inhibitors. Non-sterilized and sterilized cast swabs showed similar results.

Performance of the DSM/MOLDED cast swabs compared with the COPAN FLOQswabs in the field

Four hospitals where asked to take nasopharyngeal swabs from confirmed COVID-19 patients. From 14 patient samples were collected. The samples were taken with the EtO sterilized DSM/MOLDED cast swabs and the COPAN FLOQswabs. The swabs were transferred on site to viral transport medium GLY and transported to RIVM at ambient temperature and stored at 4°C. These samples were tested for human 18S, SARS-CoV-2 E-gene and RdRp-gene.

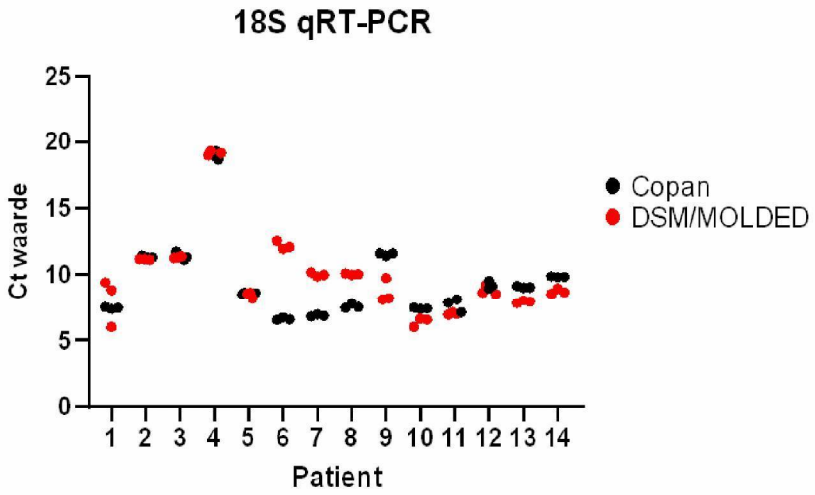
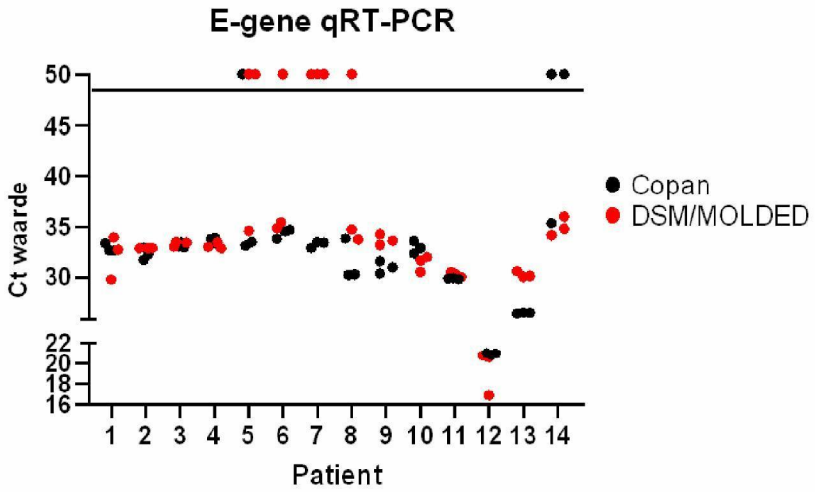


Figure 3. *E-gene qRT-PCR results from 14 different patients at 4 different hospitals. The E-gene qRT-PCR is done to determine if there are differences in collected and released SARS-CoV-2 viruses, associated with human cells or trapped in mucus between the DSM/MOLDED cast EtO sterilized swabs and COPAN FLOQswabs. Extraction and qRT-PCR were performed in triplicate per patient.*

Generally, except for patients 6-9, the Ct-value of the 18S qRT-PCR were similar for both type of swabs. Therefore, the amount of human material collected with the DSM/MOLDED cast swabs is similar to that collected with the COPAN FLOQswabs. Patients 6 to 9 had smaller (6-8) or higher (9) amount of human material collected and released using the DSM/MOLDED cast swab compared to COPAN FLOQswab. The specimens of these patients were collected at the same hospital indicating an issue with taking nasopharyngeal specimens through both nostrils consistently;.



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The result of the E-gene qRT-PCR are for the most part similar. Patients 5 to 8 and 14 show inconsistent results for detection of the E-gene. The Ct-values for these samples were close to or passed the limited of detection of the E-gene qRT-PCR. Nevertheless, of the 14 patients, all were positive for at least 1 ou of 3 replicates with COPAN FLOQswab compared with 13 patients using the DSM/MOLDED cast swab.

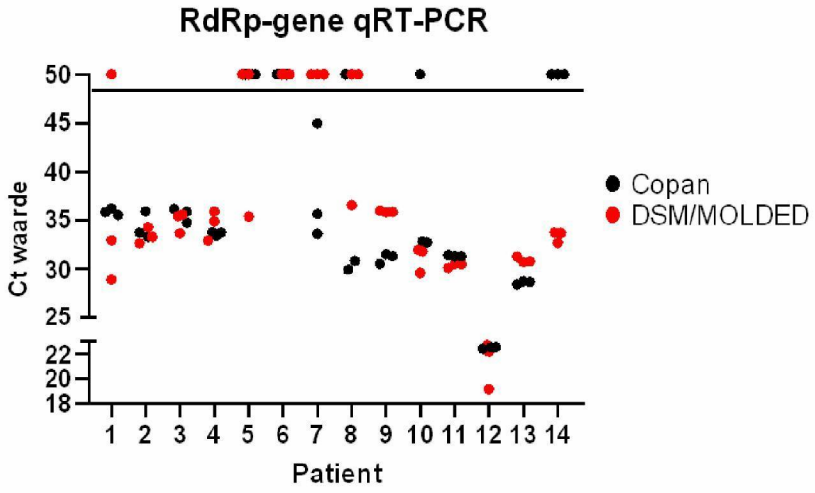

Figure 4. *RdRp-gene qRT-PCR results from 14 different patients of 4 different hospitals. The RdRp-gene qRT-PCR is done to determine if there are differences in collected and released SARS-CoV-2 viruses, associated with human cells or trapped in mucus between the DSM/MOLDED cast EtO sterilized swabs and COPAN FLOQswabs. Extraction and qRT-PCR were performed in triplicate per patient.*

The result of the RdRp-gene qRT-PCR are for the most part equivalent to those for the E-gene qRT-PCR, although more heterogenous due to lower sensitivity and several specimens containing viral loads close to or beyond the LOD. For patient 5 to 8 most of the 3 replicates were negative in the qRT-PCR. Whereas the E-gene qRT-PCR is more sensitive than the RdRP-gene qRT-PCR, it is predictable that where the Ct value of most clinical specimens was around the detection limit, more negative results were expected for the RdRP-gene qRT-PCR compared to the E-gene qRT-PCR. Of the 14 patients 10 were positive with COPAN FLOQswab and the DSM/MOLDED cast swab. Of the other 4, 1 patient was negative with both swab types, 2 only positive with DSM/MOLDED cast swab and 1 only positive with COPAN FLOQswab.

Feedback from the field

After the nurses at the hospitals had taken the samples from the confirmed COVID-19 patients, the nurses were asked for feedback on working with the DSM/MOLDED cast swabs. The questions that have been asked were; 1) What the experienced comfort was when working with it; 2) How the patient experienced it; 3) Whether the breaking point of the swab breaks properly; and 4) How the flexibility was of the swab when trying to reach the nasopharynx.

Table 4. *Nurses' reactions from 4 hospitals about the ease of use of the DSM/MOLDED cast swabs.*

	Positive	Neutral	Negative	No reaction
Working comfort	1/4	2/4	0/4	1/4
Comfort for patient	0/4	1/4	0/4	3/4
Breaking point	3/4	0/4	0/4	1/4
Flexibility	2/4	0/4	1/4	1/4

One of the hospitals did not reply on the asked feedback. Most of the reactions about the DSM/MOLDED cast swab were positive or neutral. The nurses were very positive about the breaking point. The DSM/MOLDED cast swab breaks easier at the breaking point compared to the COPAN FLOQswabs, There was one negative reaction about the flexibility of the swab.

Conclusion

For the qRT-PCR's there are no inhibiting factors released from the material that has been used for the DSM/MOLDED cast swabs. Also no differences before or after EtO or Gamma sterilization. The sterilization method going to be used is EtO. The EtO sterilization method is chosen, because it is more efficient and more swabs can be sterilized in one batch. The amount of human material collected with the DSM/MOLDED cast swabs is acceptable, as overall the the results for the 18S qRT-PCR are similar for both swab types. Comparison of the collection and release of SARS-CoV-2 was a bit complicated by the presence of a very low viral load in some of the specimens, resulting in negative qRT-PCR assays using both type of swabs. Nevertheless, if specimens are collected in the right way, as has been demonstrated by nurses of most laboratories, SARS-CoV-2 detection is equally sensitive using the DSM/MOLDED cast swabs or COPAN FLOQswbs. The ease of use of DSM/MOLDED cast swabs is comparable with that of the COPAN FLOQswabs. The nurses were very impressed by how good the break point breaks. Taken all the results together we conclude that the DSM/MOLDED swabs are comparable with the COPAN FLOQswabs and can be used in clinical practice.

References

1. Corman VM, Landt O, Kaiser M, Molenkamp R, 5.1.2e, Chu DK, Bleicker T, Brünink S, Schneider J, Schmidt ML, Mulders DG, Haagmans BL, van der Veer B, van den Brink S, Wijsman L, Goderski G, Romette JL, Ellis J, Zambon M, Peiris M, 5.1.2e, Reusken C, Koopmans MP, Drosten C. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020 Jan;25(3):2000045.
2. Murielle André, Sylviane Reghin, Estelle Boussard, Laurent Lempereur, Stéphane Maisonneuve. Universal real-time PCR assay for quantitation and size evaluation of residual cell DNA in human viral vaccines. J.biologicals.2016.03.002.