# Beperkt validatie rapport

# COABS6800 – Sars-CoV-2 op alle respiratoire materialen en faeces

## *Update t.o.v. plan: verificatie materiaal type speeksel*

| | Naam | Functie |
|---|---|---|
| **Opgesteld door:** | [redacted] | [redacted] |
| **Verificatie:** | [redacted] | [redacted] |
| **Verificatie:** | [redacted] [redacted] | [redacted] |
| **Autorisator:** | [redacted] | [redacted] |

## 1. Inleiding

In dec 2019 is het nieuwe virus SARS-CoV-2 ontdekt in China, dat respiratoire infecties veroorzaakte in de bevolking. Als snel bleek dat het virus goed overgedragen kon worden van mens op mens (R0 2-3), en daarnaast ook voor patiënten populatie met co-morbiditeiten (hart afwijkingen, diabetes, mogelijk ook overgewicht) en bij ouderen zware longontstekingen kon veroorzaken, die IC-behoeftig waren en leidde tot een mortaliteit van ~2-4%.
In februari is de eerste patiënt in NL gediagnosticeerd, waarna snel duidelijk werd dat Brabant initieel de haard van de NL epidemie was.

Aangezien Microvida opschalingslab is, is er al een in-house semi-kwantitatieve real time PCR beschikbaar, die gebruik maakt van de MP96 (Roche) (of later gevalideerd: M200SP, Abbott) als extractie systeem. Echter, aangezien er op dit moment schaarste is aan extractie reagentia/disposables voor deze open systemen, is besloten een COABS6800 (Roche) in het lab te installeren, waar inmiddels een CE-IVD gelabelde test voor beschikbaar is om SARS-CoV-2 RNA in nasopharynxswatten en keelwatten in universal transport medium (UTM) te draaien.

Echter, gezien wij verschillende soorten materialen in het lab krijgen, nl naast nasopharynx/keel watten ook diepe luchtweg materialen (dlw) (BAL, sputum) en faeces, willen wel valideren of deze materialen, die voorbewerkt worden volgens standaard protocol voor MP96 extractie, vergelijkbare resultaten geeft in de COBAS6800. Dit zullen we doen door middel van een beperkte validatie, aangezien COBAS6800 & MP96 volledig gevalideerde systemen zijn en vergeleken worden.

Roche COBAS6800 SARS-CoV-2 assay is een volledig geautomatiseerde workflow, waarbij een dual target PCR (RdRp en E-gen) gemultiplexed wordt met een interne controle in een zelfde well. Doorlooptijd is ongeveer 3 uur. Input is minimaal 600ul, waarbij 400ul sample wordt gebruikt in de assay (dus 200ul doodvolume, afhankelijk van gebruikte (secundaire) buis.

### 1.1. Verantwoordelijkheden

Naam 5.1.2e [redacted] beschrijven plan & experimentele opzet, rapport
Naam 5.1.2e [redacted] uitvoeren verificatie
Naam 5.1.2e [redacted] verifiëren plan
Naam 5.1.2e [redacted] utoriseren plan

### 1.2. Tijdsplanning

Periode waarin validatie plaatsvindt : maart/april 2020

### 1.3. Definities en afkortingen

| | |
|---|---|
| UTM | universal transport medium |
| dlw | Diepe lucht weg |
| BAL | Broncho alveolaire lavage |
| VPE | Verpakkings eenheid |
| LBB | Roche MP96 lysis and binding buffer |
| P/N | Part number |
| kt | kamertemperatuur |
| LDA | Lab developed assay / in-house assay |

### 1.4. Onderzoeksmateriaal

Tabel 1. Overzicht benodigdheden COBAS6800 voor SARS-CoV-2 RNA detectie

| Naam reagens/disposables | Storage temperature | P/N | VPE | VPE unit | Totaal aantal testen |
|---|---|---|---|---|---|
| cobas® SARS-CoV-2 -192 | 2–8°C | 09175431190 | 2x96 | tests | 192 |
| cobas® SARS-CoV-2 Control Kit | 2–8°C | 09175440190 | 16 | 1ml | 1536 |
| cobas® Buffer Negative Control Kit | 2–8°C | 07002238190 | 16 | 1ml | 1536 |
| cobas omni Processing Plate | kt | 05534917001 | 32 | PC | 1536 |
| cobas omni Amplification Plate | kt | 05534941001 | 32 | PC | 3072 |
| cobas omni Pipette Tips | kt | 05534925001 | 16 | set | 768 |
| cobas omni Liquid Waste Container | kt | 07094388001 | 3 | containers | 720 |
| cobas omni Lysis Reagent | 2–8°C | 06997538190 | 4 | 875 ml | 1152 |
| cobas omni MGP Reagent | 2–8°C | 06997546190 | 480 | tests | 480 |
| cobas omni Specimen Diluent | 2–8°C | 06997511190 | 4 | 875 ml | 2304 |
| cobas omni Wash Reagent | 15–30°C | 06997503190 | 5 | liter | 288 |
| Solid Waste Bag With Insert | kt | 08030073001 | 20 | stuks | 7680 |
| Lysis/binding buffer (LBB) (Roche) | kt | 03246752001 | 1 bottle | 100ml | |
| Screw cap tube, 7 ml, 82 x 13 mm, round base, PP, with assembled cap, 100 pcs./bag (Sarstedt) | ($1^e$ keus) | 60550115 | 10x100 | 1000 stuks per doos | 1000 |
| serumbuizen Simport 12x82 (VWR) | ($2^e$ keus) | T501-5T | | 1000 stuks per zak | 1000 |
| cobas omni secondary tubes 13x75 (geen srewcap, dus liever niet!) | (laatste keus) | 06438776001 | 50 | | |

Voor overzicht geteste monsters/ panels / positieven controle materialen, zie paragraaf 1.4 van het rapport.

## 2. Prestatiekenmerken

Alle hieronder beschreven experimenten zijn gedaan met samples in Roche lysis and binding buffer (1:1).

### 2.1. Detectie limiet / kwantificatie limiet (aantoonbaarheidsgrens)

De aangegeven LOD van de COBAS SARS-CoV-2 assay wordt aangegeven in TCID50/ml, aangezien op dat moment geen internationale standaard aanwezig was. "*As shown in Table 10, the concentration level with observed hit rates greater than or equal to 95% were 0.009 and 0.003 TCID50/mL for SARS-CoV-2 (Target 1) and pan-Sarbecovirus (Target 2), respectively. As shown in Table 11, the Probit predicted 95% hit rates were 0.007 and 0.004 TCID50/mL for SARS-CoV-2 (Target 1) and pan-Sarbecovirus (Target 2), respectively.*"

Uitvoering:

We kunnen de detectielimiet niet m.b.v. een (internationale) standaard te verifiëren, omdat deze niet beschikbaar is. RIVM heeft een sensitiviteitspanel rondgestuurd waarmee we dit zullen doen.

Update 13-7-2020: RIVM heeft inmiddels ook een speekselpanel, die we zullen gebruiken om dit materiaal type mee te valideren.

Acceptatie criterium:

Er mag niet meer dan 1 verdunning/ 1 log verschil zijn tussen de verschillende real-time apparaten en tussen Roche Cobas6800 SARS-CoV-2 assay en LDA (MP96/Egen)
*Update 15-04-2020: RIVM sensitiviteit panel moet minimaal tot en met verdunning 10-7 (8.26*$^{E}$*1 RdRp dcopies/ml) gedetecteerd worden.*
*Update 13-7-2020: RIVM speekselpanel: er mag niet meer dan 1 log verdunning / 1 log verschil zijn tussen de Cobas6800 SARS-CoV-2 assay en de in-house assay op MP96.*

Resultaat:

Tabel 2. Resultaten sensitiviteitspanel RIVM (swabs in virus transportmedium)

| | | Microvida loc Bravis | | | RIVM | |
|---|---|---|---|---|---|---|
| | | LDA | COBAS | | LDA (reps pos) | |
| **Virus#** | **Load*** | **E-gen** | **RdRp** | **E-gen** | **E-gen** | **RdRp** |
| SARS-CoV-2 10-4 | 8,26E+04 | 21,4 | 25,6 | 26,3 | 23,12 (3) | 25,53 (3) |
| SARS-CoV-2 10-5 | 8,26E+03 | 25,2 | 28,2 | 29,1 | 28,01 (3) | 30,33 (3) |
| SARS-CoV-2 10-6 | 8,26E+02 | 29,6 | 31,9 | 33,4 | 30,72 (3) | 32,76 (3) |
| SARS-CoV-2 10-7 | 8,26E+01 | 33,2 | 33,9 | 35,8 | 33,47 (3) | 35,19 (3) |
| SARS-CoV-2 10-8 | 8,26E+00 | 36,0 | 35,1 | 37,0 | 33,95 (2) | 35,15 (2) |
| SARS-CoV-2 10-9 | 8,26E-01 | 38,3 | neg | neg | neg | neg |
| SARS-CoV-2 10-10 | 8,26E-02 | neg | neg | neg | neg | neg |

*Aantal kopieën RdRP target vastgesteld met dPCR/ml monster

De LDA (MP96/Egen) methode lijkt iets gevoeliger te zijn dan COBAS6800 en RIVM LDA methode. Dit kan verklaard worden, doordat LDA_Bravis gebruikt maakt van een large volume (500ul input) extractie protocol en LDA-RIVM van een small volume extractie protocol. Gevoeligheid van COBAS is gelijkwaardig aan RIVM protocol.

Tabel 3. Resultaten speekselpanel RIVM.

| | LoD speeksel:verdunning | kopieën RdRP/ml |
|---|---|---|
| M2000 CEIVD Bravis | 10-6 | 8.26*10^2 |
| M2000 CEIVD Amphia | 10-6 | 8.26*10^2 |
| MP96 LDA | 10-7 | 8.26*10^1 |
| Cobas6800 | 10-6* | 8.26*10^2 |

* discrepant +/-

Ook hier is de LDA meest gevoelig. Echter, cobas is ook nog eens discrepant (alleen E gen positief) bij verdunning 10-6.

Conclusie:
Acceptatie criterium is gehaald voor materiaal type swabs in virus transportmedium, er is max 1 verdunning (1log) verschil tussen COBAS en LDA (MP96/Egen) methode. Daarnaast is ook voldaan aan het RIVM criterium dat men minimaal verdunning 10-7 moet kunnen aantonen.

Acceptatie criterium is NIET gehaald voor materiaal type speeksel, er is >1 verdunning (1log) verschil tussen COBAS en LDA (MP96/Egen) methode, gezien discrepante uitslag van de cobas6800.

## 2.2. Sensitiviteit, analytisch (incl. interfererende substanties) en diagnostisch

Uitvoering:
In principe niet van toepassing op de kwalitatieve CE-IVD gelabelde assay. Maar aangezien hier bijzonder veel assays van gedraaid zullen worden en we ook andere materialen willen meenemen in deze beperkte validatie, zullen we hier naar kijken.

- Retrospectieve studie arm (pos materialen in -80°C opgeslagen):
  Min 50 klinische materialen (naso-/oropharynx swabs in eswab medium/virus transport medium (bv gly), diepe luchtweg materialen (opgewerkt volgens standaard protocol) en faeces) samples (LDA Ct<35).
- Prospectieve studie arm:
  Idem, maar nu alleen verse klinische materialen (opgeslagen bij +4°C), waarbij min 50 LDA neg materialen worden meegenomen.
- Mogelijke interfererende substanties zullen d.m.v. een interne controle (ICcombi) in de PCR gemonitord worden. We zullen verschillende materialen testen (keel/neusswab, BAL, sputum, feces).

Acceptatie criterium:
Analytische sensitiviteit: 95% van de resultaten moeten overeenkomen met referentie methode (in house MP96/Egen).

Interfererende substanties: er mag niet meer dan 10% remming plaatsvinden in de verschillende sampletypes.

Resultaat:

De resultaten staan uitgebreid beschreven in de publicatie van Stohr et al. (zie bijlage), maar in kort:

- Retrospectieve en prospectieve (relatief veel laag positieven) studie arm, samengevoegde data:
  A total of 377 samples were tested on both the COBAS® 6800 and LDA (retrospectively: n=172; prospectively: n=205). In total, 221 (58.6%) oro-/nasopharyngeal swabs in either E-swab® or GLY-medium, 131 (34.7%) deep respiratory specimens and n=25 (6.6%) swabs of unknown origin were included.

Tabel 4. Kruistabel vergelijking kwal. resultaten LDA (MP96/Egen) vs COBAS6800

| LDA | COBAS® 6800 assay | | |
|---|---|---|---|
| | Positive | Negative | Inconclusive* |
| Positive | 118 | 4 | 22 |
| Negative | 0 | 217 | 16 |

*Single RdRp or E-gene positive

In 57,9% (22/38) van de inconclusieve COBAS6800 resultaten (1 vd 2 target positief), waren positief in de LDA (MP96/Egen), wat de resultaten van de detectielimiet confirmeert (nl LDA is iets gevoeliger), waarvan 72,7% (16/22) een Ct-waarde>35 hadden in de LDA.

Welk interpretatie algoritme is juist bij inconclusieve COBAS6800 resultaten?

De bijsluiter geeft aan: single RdRp pos, die mag je SARS-CoV2 pos uit doen. Single Egen pos is officieel Sarbeco, dus niet specifiek SARS2 pos, dus moet geconfirmeerd worden. Dit doen we in principe met onze LDA (ook Egen) ook met Ct>35, die confirmeren we ook nog een keer (2xpos = resultaat positief, 1x pos (Ct>35) / 1xneg, resultaat = niet te beoordelen)

Daarom verschillende analyses gedaan met inconclusieve resultaten om te zien wat het effect hiervan is:

- Excludeer deze uit de analyse: McNemar test: no significant difference was observed between the two assay's (p > 0,05). (98,8% overeenkomst LDA/COBAS6800)).
- Interpreteer deze als positief: McNemar test, significant difference, p = 0,01;
- Interpreteer deze als negatief: McNemar test, significant difference, p < 0,01.

➔ Hieruit kan je concluderen dat we de inconclusieve resultaten niet eenduidig als pos of neg moeten interpreteren. Echter, als we deze direct als ntb uitslaan, hebben we (met huidige prevalentie dd14-4-2020) ~1,8% niet te beoordelen als resultaat.

➔ Daarom houden we het huidige test algoritme aan, nl. bij inconclusieve resultaten op de COBAS6800 wordt de (iets gevoeligere LDA) als confirmatie assay in gezet, waarbij dus verwacht 57.9% alsnog positief uitgeslagen kan worden, dus uiteindelijk een ntb% van 0.8% overblijft o.b.v. inconclusieve resultaten. Ook hierbij geldt: COBAS6800 inconclusief + LDA pos = resultaat positief, COBAS6800 inconclusief / LDA neg, resultaat = niet te beoordelen, niet materiaal insturen.

- Voor analyse van semi-kwantitatieve resultaten, zie juistheid/accuraatheid.

- Interfererende substanties.
    - Initieel (retrospectief) geen remmers in uitstrijken (n=83), feces (n=2) en 1 remmer in dlw (1/91=1%), dus ruim onder gestelde 10%.
    - (*update t.o.v. plan*) Na 2 weken in productie, hieronder een tabel met een overzicht van aantal geremde samples op (data van 06-04-2020 t/m 28-04-2020):

Tabel 5. Analyse van geremde samples per sample type op COBAS6800

| Specimen type | N° of samples (%) | |
|---|---|---|
| | valid | invalid |
| Total | 6698 (99.3) | 48 (0.7) |
| Deep respiratory specimen | 379 (99.0) | 4 (1.0) |
| Oro-/nasopharyngeal swab | 5870 (99.4) | 34 (0.6) |
| Swab of unknown origin | 430 (99.3) | 3 (0.7) |
| Feces | 19 (73.1) | 7 (28.9) |

Hierbij is duidelijk dat alle materialen een geremd percentage van <10% hebben, behalve feces, waarbij 28.9% geremd was. Hierna besloten geen feces meer op de COBAS6800 aan te bieden.

Conclusie:
Acceptatie criteria zijn gehaald voor respiratoire uitstrijken, diepe lucht weg monsters (BAL, sputum), maar niet voor feces.

### 2.3. Specificiteit, analytisch en diagnostisch

Bijsluiter van COBAS6800 SARS-CoV-2 CE-IVD assay geeft aan dat deze assay specifiek is voor SARS-CoV-2, dus geen andere coronavirussen detecteert.

Uitvoering:
Het RIVM specificiteitspanel uit -80°C in LBB (2020 EQA panel 2019 nCoV20-01 t/m -10) wordt in lysisbuffer (1:1) geïnactiveerd en aangeboden op de COBAS6800.

Acceptatie criterium:

Er mogen alleen SARS-CoV-2 samples gedetecteerd worden, andere samples met resp virussen moeten negatief zijn .

Resultaat:

Tabel 6. Specificiteitspanel

| | | Microvida loc Bravis | | | RIVM | |
|---|---|---|---|---|---|---|
| | | LDA | COBAS | | LDA (reps pos) | |
| **Virus** | **Ct** | **E-gen** | **RdRp** | **E-gen** | **E-gen** | **RdRp** |
| Influenzavirus A(H3N2) | 21,64 | -1,0 | -1,0 | -1,0 | neg | neg |
| SARS-CoV-2 (d2)# | nvt | 32,6 | 33,5 | 35,5 | 34,76 (3) | 34,99 (3) |
| CoV-OC43 | 27,3 | -1,0 | -1,0 | -1,0 | neg | neg |
| SARS-CoV-2 (d3)#* | nvt | -1,0 | -1,0 | -1,0 | 35,59 (3) | 36,70 (2) |
| Rhinovirus A16 | 25,49 | -1,0 | -1,0 | -1,0 | neg | neg |
| CoV-229E | 32,6 | -1,0 | -1,0 | -1,0 | neg | neg |
| Geen virus | Neg | -1,0 | -1,0 | -1,0 | neg | neg |
| CoV-NL63 | 28,84 | -1,0 | -1,0 | -1,0 | neg | neg |
| Influenzavirus B-Victoria | 28,32 | -1,0 | -1,0 | -1,0 | neg | neg |
| SARS-CoV-2 (d1)# | nvt | 25,0 | 29,2 | 30,1 | 26,88 (3) | 29,41 (3) |

# d1, d2 en d3 geven aan dat d2 een 1:100 doorverdunning is van d1 en d3 een 1:10 doorverdunning van d2.
* d3 is door RIVM aangemerkt als educatief sample.

Conclusie:

Er zijn geen aspecifieke reacties gezien bij andere respiratoire pathogenen.
Acceptatie criterium is gehaald.

## 2.4. Robuustheid

NVT bij kwalitatieve CE-IVD test met kleine aanpassing (verschillende materiaaltypen in LBB).

Uitvoering:
Echter, om te kijken wat de effecten zijn van vries dooi stappen met en zonder lysis (ook i.v.m. studies waarbij dit speelt) worden min 50 positieve materialen (Ct<35 én beide genen positief) die eerder op de COBAS6800 getest zijn, na een vries-dooi stap (van origineel materiaal of materiaal in lysisbuffer) van min 1 nacht -80°C opnieuw in de COBAS6800 getest. Er zal naar kwalitatieve resultaten gekeken worden, en naar semi-kwantitatieve resultaten (vergelijking van Ct-waarden).

Acceptatie criterium:

95% van de resultaten moeten overeenkomen met de eerste COBAS6800 uitslag.

Resultaat:

In totaal zijn 176 cobas pos samples, waarvan 66 samples zonder, en 110 samples met lysis buffer getest, na een o/n vries-dooi stap bij -80°C nogmaals op de cobas getest.
6/176 samples gaf na vries-dooi stap inconclusieve resultaten, wat betekent dat 96.6% van de kwalitatieve resultaten overeen kwam met initiële cobas6800 resultaten. Na vries-dooi cyclus, was 5x RdRp negatief (n=3 zonder lysis, n=2 met lysis) en 1x Egen negatief.

Kwantitatieve analyse laat zien dat het gemiddelde verschil in Ct-value voor en na vries-dooi stap voor het E-gen en RdRP-gen

Tabel 7. Verschil in COBAS6800 Ct-waarde na 1 vries-dooi cyclus.

| lysisbuffer | Egen | RdRp-gen |
|---|---|---|
| Zonder LBB (n=66) | -0.7 (95%CI: -1.2 - -0.2) | -0.3 (95%CI: -0.7- 0.2) |
| Met LBB (n=110) | 0.1 (95%CI: -0.1-0.3) | 0.1 (95%CI: -0.1-0.3) |

Voor Bland-Altman analyses, zie bijlage Stohr et al.

Conclusie:
Er is geen significant verschil gevonden tussen voor/na 1 vries-dooi stap of met/zonder lysisbuffer. Echter, lysisbuffer geeft wel een betere preservatie voor de samples met lage virale load.
Acceptatiecriterium is gehaald.

## 2.5. Juistheid / accuraatheid

NVT bij kwalitatieve CE-IVD test met kleine aanpassing (verschillende materiaaltypen in LBB), en grotendeels ook meegenomen in prestatiekenmerk Detectie limiet / kwantificatie limiet (aantoonbaarheidsgrens), Sensitiviteit, analytisch (incl. interfererende substanties) en diagnostisch en Specificiteit, analytisch en diagnostisch.

*Update t.o.v. plan:*

*Uitvoering:*
*Blant en Altman analyse van de semi-kwantitatieve Ct-waarden van de COBAS6800 en de LDA (MP96/E) gedaan.*

*Acceptatiecriteria:*
*Geen, nl wel/niet accepteren van deze assay zal niet obv van (semi-)kwantitiatieve data gebeuren, gezien het kwalitatieve karakter van de uitgeslagen resultaten. Deze analyse is alleen gedaan om meer inzicht te krijgen in de verschillen in Ctwaarde tussen de twee vergeleken methoden.*

*Resultaten:*
*In deze analyse zijn alleen de samples in de prospectieve studie arm meegenomen, om bias van een evt vries-dooi stap te elimineren. Tabel 4 laat zien dat gemiddeld de Ct-waarden van de COBAS6800 assay hoger zijn dan de LDA (MP96/Egen). Echter, Blant-Altman analyses (zie bijlage, Stohr et al) laten zien dat dit gemiddelde Ct-verschil afneemt naarmate virale load hoger wordt.*

*Tabel 8. Mean difference in Ct-value between the LDA and COBAS® 6800 test per specimen type*

| *Specimen source sample* | *E-gene LDA vs. E-gene COBAS® 6800* | | *E-gene LDA vs. RdRP-gene COBAS® 6800* | |
|---|---|---|---|---|
| | *N of samples positive in both assays* | *Mean difference in Ct-value (95% CI)* | *N of samples positive in both assays* | *Mean difference in Ct-value (95% CI)* |
| *Deep respiratory specimen* | *32* | *3.5 (2.7-4.4)* | *23* | *3.2 (2.3-4.1)* |
| *Oro-/nasopharyngeal swabs* | *43* | *2.3 (2.0-2.7)* | *36* | *1.8 (1.3-2.3)* |
| *Swabs of unknown origin* | *15* | *3.0 (2.4-3.6)* | *13* | *2.2 (1.3- 3.0)* |

*Values above zero indicate a higher Ct value in the COBAS® 6800

### 2.6. Precisie / herhaalbaarheid

NVT bij kwalitatieve CE-IVD test met kleine aanpassing (verschillende materiaaltypen in LBB).

### 2.7. Precisie/ reproduceerbaarheid

NVT bij kwalitatieve CE-IVD test met kleine aanpassing (verschillende materiaaltypen in LBB).

### 2.8. Precisie / meetonzekerheid (5.5.1.4) rondom medische besliswaarde

NVT bij kwalitatieve CE-IVD test met kleine aanpassing (verschillende materiaaltypen in LBB).

### 2.9. Meetinterval

NVT bij kwalitatieve CE-IVD test met kleine aanpassing (verschillende materiaaltypen in LBB).

## 3. Eind conclusie

Gestelde criteria zijn behaald, COBAS6800 kan gebruikt worden voor SARS-CoV-2 moleculaire diagnostiek voor respiratoire uitstrijken en diepe luchtweg materialen, echter is niet geschikt voor faeces i.v.m. te veel remming en speeksel i.v.m. sensitiviteit. Bij inconclusieve resultaten (dus 1 target positief) moet een confirmatie assay worden ingezet op de LDA (MP96/Egen).

Beperkte validatie is afgerond op: 28-04-2020.
Rapport is afgerond op 16-07-2020.

## 4. Implementatie

Implementatie datum is 06-04-2020.

## 5. Bijlagen

Labjournaal: L:\Microvida\Alle afdelingsmedewerkers\PCR\PCR projecten\Validaties\Validatie COBAS6800 SARS-CoV-2\ Labjournaal validatie COBAS6800 SARS-CoV-2.xls
Bijsluiter: L:\Microvida\Alle afdelingsmedewerkers\PCR\PCR projecten\Validaties\Validatie COBAS6800 SARS-CoV-2\ CE-Roche_nCoV-IFU.pdf
Publicatie: L:\Microvida\Alle afdelingsmedewerkers\PCR\PCR projecten\Validaties\Validatie COBAS6800 SARS-CoV-2\publicatie\versie 20200717\2020.08.22 COBAS_SARSCOV_SPSP.doc
RIM rapport sensitiviteit: L:\Microvida\Alle afdelingsmedewerkers\MMM\SARS-CoV-2\RIVM docs\Status laboratorium opschaling SARS-CoV-2_20200701.pdf
Prospectieve risico analyse: L:\Microvida\Alle afdelingsmedewerkers\Kwaliteit\risicoanalyse\PRA definitief\Moleculaire microbiologie\PRA Cobas 6800 mei 2020_v1_RL.xlsx
RIVM validatie speeksel: L:\Microvida\Alle afdelingsmedewerkers\MMM\SARS-CoV-2\RIVM docs\Evaluation of different saliva collection systems_final_version1.pdf.
Implementatie checklist: L:\Microvida\Alle afdelingsmedewerkers\PCR\COBAS6800\Implementatie checklist.doc

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# Clinical performance and sample freeze-thaw stability of the cobas®6800 SARS-CoV-2 assay for the detection of SARS-CoV-2 in oro-/nasopharyngeal swabs and lower respiratory specimens

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Real-time RT-PCR
Viral load

## ABSTRACT

*Introduction:* Studies describing the performance characteristics of the cobas®6800 system for SARS-CoV-2 detection in deep respiratory specimens and freeze-thaw stability are limited. The current study compares the clinical performance of the automated SARS-CoV-2 assay on the cobas®6800 system to a lab-developed assay (LDA) and the cobas impact of freeze-thawing combined with lysis buffer.

*Methods:* Both retrospective and prospectively selected deep respiratory samples and oro- and nasopharyngeal samples in either E-swab® or GLY- were tested using the SARS-CoV-2 assay on the cobas®6800 System and compared to a lab developed assay. Additonally, SARS-CoV-2 RNA stability was assessed after one freeze-thaw cycle with or without lysis buffer.

*Results:* In total, 221 (58.3 %) oro- and nasopharyngeal swabs, 131 (34.6 %) deep respiratory specimens, and n = 25 (6.6 %) swabs of unknown origin were included to study clinical performance. Only 4 samples gave discrepant results, all being positive in the LDA and not the cobas®6800 system. For stability testing, 66 samples without and 110 with lysis buffer were included. No clinically significant difference was found in test results after one freeze-thaw cycle and addition of lysis buffer.

*Conclusion:* Based on our findings, the cobas®6800 SARS-CoV-2 RNA assay yielded similar results as the LDA in oro-/nasopharyngeal swabs and deep respiratory specimens. Moreover, the cobas®6800 SARS-CoV-2 RNA assay yielded similar results before and after a freeze-thaw cycle, with better preservation of low viral loads in lysis buffer.

## 1. Introduction

In December 2019, severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) was first identified as a causal agent for cases of viral pneumonia in Wuhan, China [1,2]. Since then, SARS-CoV-2 has spread across the globe causing millions of cases of coronavirus disease 2019 (COVID-19). The detection of SARS-CoV-2 RNA with (real-time) reverse transcriptase polymerase chain reaction (RT-PCR) assays is the cornerstone of COVID-19 diagnostics, and a variety of different PCR assays have been made publicly available since the onset of the pandemic [3,4]. Widespread laboratory testing of potentially infected patients has a central role in attempts to mitigate the spread of SARS-CoV-2 [5], and requires rapid upscaling of the test capacity by automation [6–8]. Several automated systems, like the cobas®6800 assay, have been validated for the detection of SARS-CoV-2 in oro-/-nasopharyngeal swabs in virus transport medium [9–11], but performance data for other clinical specimen types are limited. Several studies involving SARS-CoV-2 detection using PCR assays use samples which

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have undergone a freeze-thaw cycle [9,12,13]. Currently, no data is available on the effect of a freeze-thaw cycle, on the performance of the cobas®6800 assay. Although SARS-CoV-2 should be handled as a biosafety level 2 agent for nucleic acid amplification tests according to the World Health Organization, most automated systems do not operate on BSL2 level and should, therefore, include an external lysis step prior to loading [14]. The objective of this study is to compare the clinical performance of the automated SARS-CoV-2 assay on the cobas®6800 assay (Roche Diagnostics, Switzerland) to an ISO15189:2012 lab-developed assay (LDA) for the detection of SARS-CoV-2 RNA in externally lysed (oro-/nasopharyngeal) swabs (E-swab® (Copan, Italy) or swabs in GLY-medium) and lower respiratory tract specimen. The time from r of sample receipt by the laboratory until result was compared between the LDA and automated cobas®6800 assay. Furthermore, the Ct-values of both cobas®6800 assay targets in oro-/-nasopharyngeal and lower respiratory samples were compared before and after a freeze thaw cycle, with and without additional lysis buffer.

## 2. Methods

### 2.1. Setting and study population

All oro-/nasopharyngeal and lower respiratory samples collected from patients submitted to Microvida laboratory for microbiology as part of routine clinical diagnostics were deemed eligible for inclusion in the study. Microvida laboratory for microbiology receives samples from two general, two teaching hospitals, long-term care facilities and general practitioners in the south of the Netherlands.

### 2.2. SARS-CoV-2 semi-quantitative real-time RT-PCR using cobas®6800 assay and lab-developed assay

Before testing on the cobas®6800 SARS-CoV-2 PCR assay, samples were pre-treated in a BSL3/BSL2 laminar flow cabinet. Oro-/nasopharyngeal swabs (E-swab® in Amies (Copan, Italy) or GLY-medium) were vortexed. Subsequently, 600 μL of E-swab®- or GLY-medium was added to a collection tube containing 600 μL of MagnaPure LC lysis- and binding buffer (LBB) (Roche diagnostics, The Netherlands) and mixed by pipetting. External lysis was allowed for at least 15 min at room temperature. Lower respiratory specimens (sputa, bronchial fluid, and bronchoalveolar lavage fluid) were pre-treated by placing a flake of material in a tube containing 600 μL of LBB and Sentosa SX lysis beads (Vela Diagnostics, Germany) and shaking the suspension in the MagNA lyser (Roche, Switzerland) for 70 s at 5000 rpm. Thereafter, the tubes were centrifuged for 1 min at 6000 *g*, and external lysis was completed at room temperature for at least 15 min. Following pre-treatment, all samples were analysed on the cobas®6800 with the cobas® SARS-CoV-2 assay according to the manufacturer's instructions. In short, after loading pre-lysed samples, nucleic acid extraction was automatically performed, followed by an internally controlled semi-quantitative real-time reverse transcriptase polymerase chain reaction (sqRT-PCR) on the envelope (E) gene and the RNA dependent RNA polymerase (RdRp) gene and automated analysis of the sqRT-PCR results. The ISO15189:2012 accredited, internally controlled, single-target (Sarbeco-specific E-gene), sqRT-PCR lab-developed assay (LDA) was performed as described previously by Kluytmans-van den Bergh et al. [15]. The cobas®6800 assay was considered positive when both the E-gene and RdRp-gene targets were detected; inconclusive when either E-gene or RdRp-gene target was detected; negative when both targets were undetectable. The cobas®6800 results were considered invalid when either the E-gene or RdRp-gene target did not produce a result, due to internal/external invalid controls.

### 2.3. Assay comparison

Clinical performance was evaluated both retrospectively and prospectively. For the retrospective evaluation, stored (−80 °C or +4 °C (for a maximum of 7 days)) LDA confirmed SARS-CoV-2 positive ($Ct<35$) and LDA SARS-CoV-2 negative oro-/nasopharyngeal, and lower respiratory samples were randomly selected from samples sent to the laboratory for routine testing from the March 1–31, 2020, ensuring the complete dynamic range of Ct-values and variety of sample types. The cobas®6800 assay results were compared to historical data generated using the LDA. For prospective evaluation, samples from patients submitted to Microvida laboratory for microbiology between the April 4–11, 2020, were stored at +4 °C until testing using both the cobas®6800 SARS-CoV-2 PCR assay and LDA. Samples with an inconclusive result in the cobas®6800 assay but not included in the initial comparison ($n = 36$), were additionally tested in the LDA and included to evaluate samples with viral loads near the lower limit of detection in more detail.

### 2.4. Time-to-result and invalid results

Additional samples were included to evaluate the time-to-result for the LDA and the cobas®6800 assay and to evaluate the number of invalid results in the cobas®6800 assay. The time-to-result, i.e. the time between sample receipt by the laboratory until generation of the SARS-CoV-2 RNA result, was extracted from the laboratory information system from samples received from March 1–31, 2020, for the LDA and from samples received from April 4–28, 2020 for the cobas®6800 assay. The number of invalid results was calculated for all samples tested on the cobas®6800 assay from April 4–28, 2020.

### 2.5. Freeze-thawing samples with and without lysis buffer

From April 28- July 1, 2020 lower respiratory and oro-/nasopharyngeal samples, submitted to Microvida laboratory for microbiology for routine clinical diagnostics and that tested positive for SARS-CoV-2 RNA using the cobas®6800 assay, were stored overnight at −80 °C with or without external lysis buffer. Following overnight freezing, the selected samples were thawed at room temperature and re-tested using the cobas®6800 assay. The cobas®6800 SARS-CoV-2 Ct-values were compared before and after this one freeze-thaw cycle for each sample.

### 2.6. Statistical analysis

A McNemar test for paired samples was performed using Researchpy v0.1.9 and Pandas v1.0.3. Bland-Altman plots comparing the cobas®6800 and LDA SARS-CoV-2 Ct-values of the prospectively collected samples and comparing the cobas®6800 SARS-CoV-2 Ct-values before and after a freeze-thaw cycle, were drawn using Matplotlib v3.2.1. The mean of the differences (and 95 % confidence interval) between the cobas®6800 assay and LDA Ct-values and between the cobas®6800 SARS-CoV-2 Ct-values before and after a freeze-thaw cycle was calculated using NumPy v1.18.4 and SciPy v1.4.1. In the Bland-Altman analysis, a Ct-value of 40 was imputed for a negative result in samples with discrepant results. Samples with imputed values were excluded from calculating the mean difference in Ct-value. A Mann-Whitney *U* test was performed between the median Ct-values of the LDA E-gene, cobas®6800 E-gene and cobas®6800 RdRp of the prospectively collected deep respiratory specimens and oro-/nasopharyngeal swabs using SciPy v1.4.1. The adjusted Wald confidence interval of the proportion of invalid test results per specimen type was calculated using NumPy v1.18.4 and SciPy v1.4.1.

## 3. Results

### 3.1. Assay comparison

A total of 377 samples were tested on both the cobas®6800 assay and LDA (retrospectively: $n = 172$; prospectively: $n = 205$). In total, 221

(58.6 %) oro-/nasopharyngeal swabs in either E-swab® or GLY-medium, 131 (34.7 %) lower respiratory specimens and n = 25 (6.6 %) swabs of unknown origin were included. In total 118 (31.3 %) samples were positive in both the cobas®6800 assay and LDA, 217 (57.6 %) samples were negative in both assays, 38 (10.1 %) samples had an inconclusive result in the cobas®6800 assay (single RdRp or E gene detectable) and cobas®6800 assay-LDA discrepant results were found in only 4 (1.1 %) samples (all being positive in the LDA and negative cobas®6800 assay) (Table 1). Two of these latter 4 samples were of lower respiratory origin and 2 samples were oro-/nasopharyngeal swabs. The samples with inconclusive results in the cobas®6800 assay were either single E-gene (n = 36) or RdRP-gene target (n = 2) positive. Twenty-two (57.9 %) of these samples with inconclusive results cobas®6800 assay, still had SARS-CoV-2 RNA detectable in the LDA (Table 1). However, the LDA E-gene Ct-values were higher than 35.0 in 16 (72.7 %) of these samples, confirming low viral loads. If all samples with inconclusive results in the cobas®6800 assay were excluded from the analysis, no significant difference was observed between the two assays ($p > 0.05$) (Table 1). Nonetheless, if all inconclusive results in the cobas®6800 assay were considered either positive or negative, the number of discrepant results increased to 20 and 26 respectively resulting in significant difference (all positive: $p = 0.01$; all negative: $p < 0.01$) (Table 1).

The mean difference in Ct-value between the LDA E-gene and cobas®6800 assay E-gene results of the prospectively collected samples was 3.8 (95 % confidence interval (CI): 3.4–4.2). Ct-values being lower in the LDA as compared to the cobas®6800 assay (including all sample types without inconclusive results) (Fig. 1a). The mean difference in Ct-values between the cobas®6800 assay RdRP-gene target and the E-gene LDA of the prospectively collected samples was 3.2 (95 %CI: 2.7–3.7). However, the difference in Ct-value between the LDA E-gene and the cobas®6800 E-gene or RdRp results decreased with increasing mean Ct-value (Fig. 1a and b respectively). The mean difference in E-gene or RdRp Ct-value between the LDA and the cobas®6800 assay was higher in the lower respiratory samples when compared to oro-/nasopharyngeal swabs (Table 2). No statistically significant difference was detected between the median Ct-values of the LDA E-gene, cobas®6800 E-gene and cobas®6800 RdRp gene of the prospectively collected deep respiratory specimens and oro-/nasopharyngeal swabs (Supplementary table S1).

### 3.2. *Time –to-result and invalid results*

Additional samples were included to evaluate the time-to-result for the LDA and the cobas®6800 assay and to evaluate the number of invalid results in the cobas®6800 assay. A total of 6609 samples were tested for SARS-CoV-2 RNA using the LDA from the 1st of 31st of March. The median number of hours between the sample arriving in the laboratory until result (time-to-result) during this period, was 20.6 h. From the April 6, 2020, until April 28, 2020, 6746 samples were tested for SARS-CoV-2 RNA presence on the cobas®6800 assay. During this period, the median time to result was 7.2 h. In 48 (0.7 %; 95 %CI: 0.5–0.9%) of these 6746 samples the cobas®6800 SARS-CoV-2 RNA tests yielded invalid results (Table 3) compared to 36 out 6609 samples (0.5 %; 95 %CI: 0.4–0.8%) for the LDA. The percentage of samples with invalid results in the cobas®6800 assay was similar between the lower respiratory samples and the oro-/nasopharyngeal swabs being 1.0 % (95 %CI: 0.3–2.8 %) and 0.6 % (95 %CI: 0.4–0.8%), respectively (Table 3). However, the percentage of invalid results was higher in the fecal samples tested being 28.9 % (95 %CI: 13.5–46.3 %) as compared to both the oro-/nasopharyngeal swabs and lower respiratory samples (Table 3).

**Table 1**
Qualitative SARS-CoV-2 RNA results of all (retrospective and prospective) samples tested in the LDA and cobas®6800 assay.

| Result in LDA | Result in COBAS® 6800 system | | |
|---|---|---|---|
| | Positive | Negative | Inconclusive* |
| Positive | 118 | 4 | 22 |
| Negative | 0 | 217 | 16 |

* Single RdRp or E-gene positive.

### 3.3. *Effect on ct-value of one freeze-thaw cycle for samples with and without lysis buffer*

A total of 176 samples were collected to be frozen (−80 °C) and thawed once, of which 66 samples without and 110 samples with lysis buffer. One sample frozen without lysis buffer was positive (Ct-value: 34.7) in the E-gene target before freezing and negative following freezing. Five samples (n = 3 without lysis buffer, n = 2 with lysis buffer) were positive in the RdRP-gene target before freezing and negative following freezing (mean Ct-value: 31.4 (range 29.4–33.5). The mean difference in Ct-value before and after a freeze-thaw cycle of the E-gene and RdRP-gene target respectively were: −0.7 (95 %CI: −1.2 to −0.2) and -0.3 (95 %CI: -0.7–0.2) in the samples frozen without lysis buffer; and 0.1 (95 %CI: -0.1–0.3) and 0.1 (95 %CI: -0.1–0.3) in the samples frozen with lysis buffer. In the samples frozen without lysis buffer and with a relatively medium-low load of SARS-CoV-2 RNA (Ct-value cobas®6800 E-gene > 30.0), the difference in Ct-value before and after a freeze-thaw cycle increased for both the E-gene and the RdRP-gene target (Figs. 2**a**; 3 a). The difference in Ct-values did not increase in the samples containing lysis buffer with a relatively medium-low SARS-CoV-2 RNA load (Ct-value cobas®6800 E-gene > 30.0) (Figs. 2**b**; 3 b).

## 4. Discussion

Based on our findings, the cobas®6800 SARS-CoV-2 RNA assay yielded similar qualitative results compared to the LDA in externally lysed (oro-/nasopharyngeal) swabs (E-swab® or swabs in GLY-medium) and pre-treated lower respiratory specimens. However, when cobas®6800 assay inconclusive (single RdRp or E gen positive) results were incorporated in the analysis, the results of both test assays significantly differed. This may be caused by the relatively high number of inconclusive results in the samples included in the study (38/377 (10 %)) with the aim to study clinical sensitivity, as compared to previous studies and our routine clinical samples (92/6698 (0.3 %)) [9,11]. As in previous studies, these inconclusive samples predominantly contained relatively low SARS-CoV-2 RNA loads (Ct-value cobas®6800 E-gene > 35.0) [11]. Most of these samples were only positive in the cobas®6800 E-gene target, which may be explained by the presence of more E gene target RNA due to generation of subgenomic RNA, while this phenomenon is not described for the RdRp gene. A higher sensitivity of the E-gene target as compared to the RdRP target for the detection of SARS-CoV-2 using PCR has already been described in other studies [16–18]. On the other hand, two samples were single positive in the RdRP target. This could be related to a previously described C26340 T mutation in the SARS-CoV-2 E-gene, resulting in a mismatch with primers/probes and thus escape from detection in samples with lower viral loads [19]. This supports the dual-target detection strategy for fast-evolving viruses to limit this risk for false-negative results. Inconclusive results due to low viral loads can be expected during the very beginning or at the end of the course of infection and should therefore be reported as such. A new sample should be requested, which either may confirm rising viral load or undetectable viral RNA.

Analysis of semi-quantitative results showed the mean Ct-value of both cobas®6800 targets were 3.2 and 3.8 higher than the Ct-value of the E gene target in the LDA assay, for RdRp and E gene respectively. Though tempting, this cannot be interpreted as an 8.6–13.0 times less sensitivity based on this Ct-difference alone (calculated with 100 % PCR reaction efficiency), since in samples with a relatively low SARS-CoV-2 RNA load, the difference in Ct-value between the cobas®6800 assay targets and LDA tended to be much lower. This effect confirmed

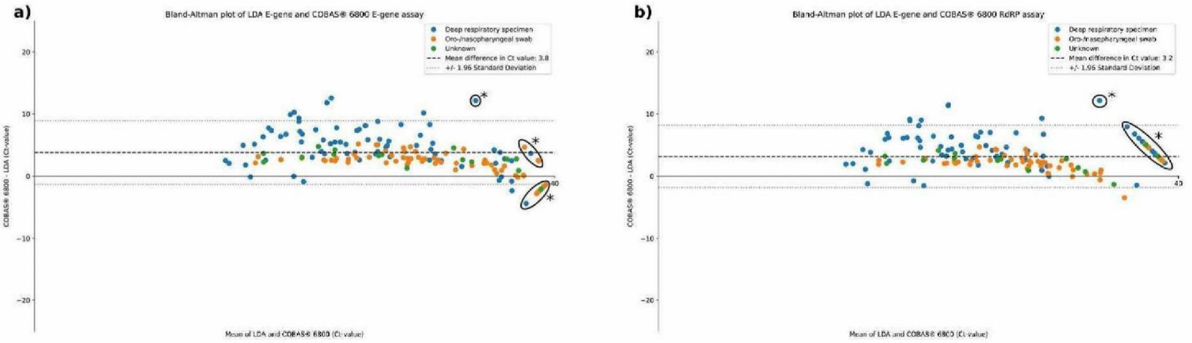

**Fig. 1.** Bland-Altman plot of the LDA and the cobas®6800 assay semi-quantitative results per specimen type, Ct-value of: **a)** cobas®6800 assay E-gene and LDA E-gene targets **b)**. cobas®6800 assay RdRP-gene and LDA E-gene targets. *Samples with imputed results for either the LDA or cobas®6800 assay E-gene assay. Total number of samples: 160 (**a**), 144 (**b**).

**Table 2**
Mean difference in Ct-value between the LDA and cobas®6800 system per specimen type of the prospectively collected samples.

| Specimen source sample | E-gene COBAS® 6800 - E-gene LDA | | RdRP COBAS® 6800 - E-gene LDA | |
|---|---|---|---|---|
| | N of samples positive in both assays | Mean difference in Ct-value* (95% CI) | N of samples positive in both assays | Mean difference in Ct-value* (95% CI) |
| Deep respiratory specimen | 32 | 3.5 (2.7–4.4) | 23 | 3.2 (2.3–4.1) |
| Oro-/nasopharyngeal swabs | 43 | 2.3 (2.0–2.7) | 36 | 1.8 (1.3–2.3) |
| Swabs of unknown origin | 15 | 3.0 (2.4–3.6) | 13 | 2.2 (1.3–3.0) |

[*] Values above zero indicate a higher Ct value in the COBAS® 6800.

**Table 3**
Number of samples with a valid or invalid test result per specimen type in the cobas®6800 assay.

| Specimen type | N° of samples (%) | |
|---|---|---|
| | valid | invalid |
| Total | 6698 (99.3) | 48 (0.7) |
| Deep respiratory specimen | 379 (99.0) | 4 (1.0) |
| Oro-/nasopharyngeal swab | 5870 (99.3) | 34 (0.7) |
| Swab of unknown origin | 430 (99.3) | 3 (0.7) |
| Feces | 19 (73.1) | 7 (28.9) |

observations in a previous study [10]. The difference in Ct-values between the cobas®6800 and LDA targets was higher in lower respiratory specimens, with no significant effects on qualitative results in this sample type.

The proportion of invalid results in the cobas®6800 assay, measured in nearly 6700 clinical samples, was similar between the pre-treated lysed lower respiratory samples and lysed swabs, but significantly higher in fecal samples. Therefore, we conclude that stool is not an appropriate sample type for analysis on the cobas®6800 SARS-CoV-2 assay in our hands. The median time-to-result was far lower using cobas®6800 assay, which can partly be due to the cobas' efficient workflow. With the most efficient LDA workflow, time to result can be as fast as 4.5–6 hours compared to 2.5–3 hours for the cobas®6800. However, due to a limited and delayed supply of extraction reagents and disposables for the MagnaPure96 system at the time, samples were tested in full run batches to optimize test capacity and efficacy, prolonging the LDA time-to-result. Since there were no difficulties regarding the supply of reagents for the cobas®6800 assay during this

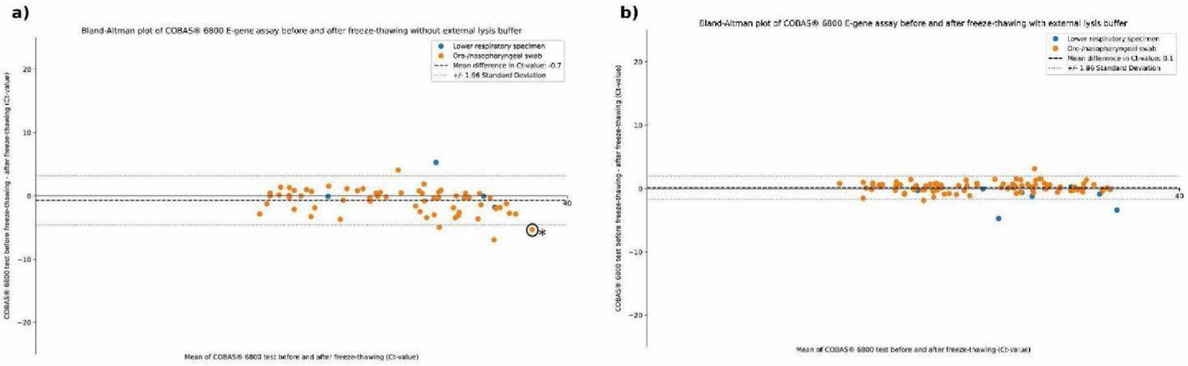

**Fig. 2.** Bland-Altman plot of the cobas®6800 assay E-gene target semi-quantitative results before and after freeze-thaw cycle per specimen type **a)** without lysis buffer; **b)** with lysis buffer. * Samples with imputed results for either the cobas®6800 assay E-gene or RdRP-gene assay before or after freeze thawing. Total number of samples is 66.

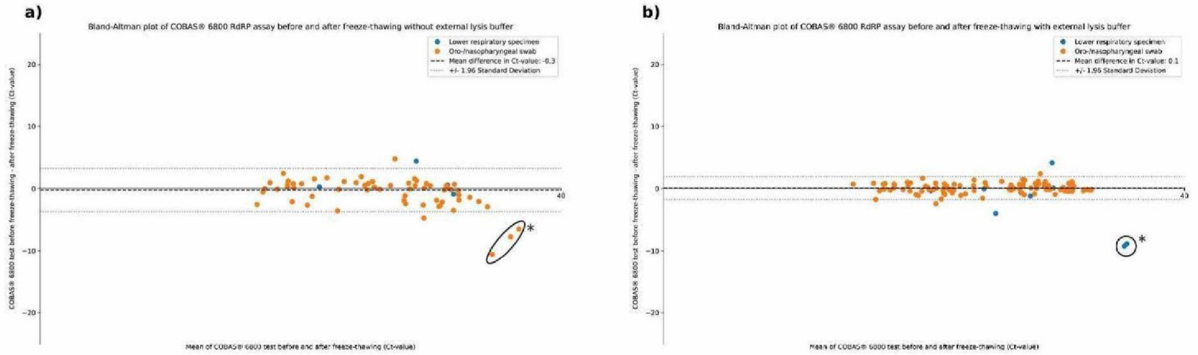

**Fig. 3.** Bland-Altman plot of the cobas®6800 assay semi-quantitative results before and after freeze-thawing with lysis buffer per specimen type: **a)** E-gene target; **b)** RdRP-gene target. * Samples with imputed results for either the cobas®6800 assay E-gene or RdRP-gene assay before or after freeze thawing. Total number of samples is 110.

study period, samples were tested as soon as they entered the laboratory, which reduced the time-to-result.

Analysis of the semi-quantitative results of the Ct-values generated by the cobas®6800 assay revealed similar results in samples before and after freeze-thawing, with and without external lysis buffer. Despite this, 6 samples converted from positive to inconclusive result after a single freeze-thaw cycle. Moreover, in the absence of external lysis buffer the difference in Ct-values of both targets before and after a freeze-thaw cycle increased in samples with a relatively high SARS-CoV-2 load. The current study only evaluated the performance of the cobas®6800 assay before and after freeze thawing. Future studies should evaluate the effect of freeze-thawing on the performance of other assays for SARS-CoV-2 detection.

Concluding, based on our findings, the cobas®6800 SARS-CoV-2 RNA assay yielded similar results as the LDA in lysed (oro-/nasopharyngeal) swabs and lower respiratory specimens. Moreover, the cobas®6800 SARS-CoV-2 RNA assay yielded similar results before and after a freeze-thaw cycle, with better preservation of low viral loads in lysis buffer.

## 5. Credit author statement

JS analysed and interpreted the data, and drafted the manuscript. MW performed the experiments. ME performed the experiments. BD analysed and interpreted the data, and revised the manuscript. MK analysed and interpreted the data, and revised the manuscript. AB analysed and interpreted the data, and revised the manuscript. JK analysed and interpreted the data, and revised the manuscript. SP performed the experiments, analysed and interpreted the data, and revised the manuscript. All authors read and approved the final manuscript.

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None to report.

## Declaration of Competing Interest

The authors report no declarations of interest.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jcv.2020.104686.

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