QCMD EQA schemes related to SARS-CoV-2
> Experiences 2020 & 2021 <

Oliver Donoso Mantke
Quality Control for Molecular Diagnostics (QCMD)

EQALM Virtual Symposium
*- Wednesday 13th October 2021-*

Conflict of interest

DISCLOSURE

- I have no potential conflict(s) of interest to report

# Quality-assured diagnostic assays are essential for controlling COVID-19

✓ Extensive **testing is a prerequisite** in containing and mitigating the impact of the pandemic;

✓ **Molecular methods such as RT-PCR are fundamental** to early SARS-CoV-2 detection in suspected cases; **antigen tests can be used to get rapid results** to assess contagiousness of a person on site;

✓ **Many in-house and/or commercial assays** have been developed rapidly and further developed tests are becoming available;

✓ **Validation/monitoring of quality and diagnostic performance** of these tests is an important aspect (e.g., post-market surveillance).

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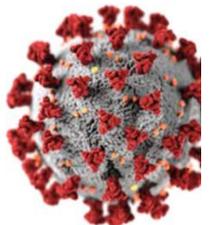
QCMD Respiratory EQA Range: Currently 25 programmes

Adenovirus	Mycobacterium tuberculosis
Atypical mycobacterium	Mycobacterium tuberculosis Drug Resistance
Bordetella pertussis	Mycoplasma pneumoniae
Chlamydia psittaci	Parainfluenza virus
Chlamydophila pneumoniae	Pneumocystis jirovecii pneumonia (PCP)
Coronavirus	Respiratory I
Human metapneumovirus	Respiratory I plus
Influenza A & B virus	Respiratory II
Influenza Typing	Respiratory III
Legionella pneumophila	Respiratory syncytial virus
Measles / Mumps	Rhinovirus
MERS coronavirus	SARS-CoV-2
	SARS-CoV-2 Antigen Testing

for multiplex/cartridge based molecular testing (SARS-CoV-2, Flu A and B, and RSV)

for molecular detection of SARS-CoV-2

NEW in 2021

for SARS-CoV-2 antigen testing (rapid LF tests, automated PoC, or Lab-based immunoassays)

QCMD EQA programmes related to SARS-CoV-2 UKAS accredited to ISO 17043

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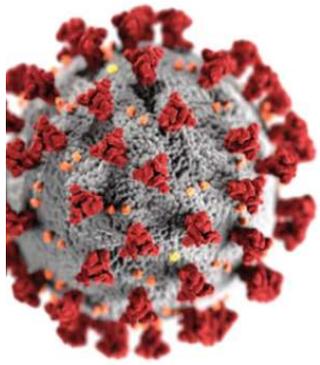
Outlook

- Introduction

- SARS-CoV-2 programme for molecular detection

- SARS-CoV-2 programme for antigen testing

Overview of SARS-CoV-2 QCMD molecular EQA schemes, 2020/2021

Challenge(s) / EQA period:	CVOP20 (S+S2)* Apr-May 2020	SCV2_20C1 Jun-Aug 2020	SCV2_20C2 Oct-Dec 2020	SCV2_20S Oct-Dec 2020	SCV2_21C1A Feb-Mar 2021	SCV2_21C1B May-Jul 2021
No. Registered Laboratories	810	304	300	389	581	845
No. Countries	70	36	35	61	51	60
No. Respondents	688	274	268	335	530	725
No. Datasets Submitted	1011	440	451	497	951	1327
Qual. Results Returned	1011 (100%)	440 (100%)	451 (100%)	497 (100%)	951 (100%)	1327 (100%)
Quant. Results Returned	19 (1.9%)	16 (3.6%)	13 (2.9%)	12 (2.4%)	16 (1.7%)	26 (1.9%)

* Due to high demand for the Coronavirus Outbreak Preparedness (CVOP) pilot we had to split this study into two testing periods where the participants received a panel with the same panel composition and specifications.

- Datasets per applied routine molecular assay workflows (with extraction and amplification method used to test the EQA samples);

- Results/data submission through a dedicated online reporting system (ITEMS).
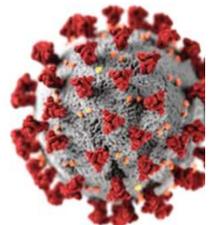
Virus types included

Starting materials for QCMD molecular EQA schemes CVOP and SCV2 in 2020/2021

For SARS-CoV-2 positive panel members (sensitivity samples):

- 10-fold dilutions* of non-infectious SARS-CoV-2 cell culture-derived supernatant (whole virus, inactivated by heat and gamma-irradiation)

SARS-CoV-2 strain: BetaCoV/Munich/ChVir984/2020 (GenBank no. MT270112), Isolate from first generation cases of a cluster identified in Germany/Europe end of January 2020 (Lancet Infect Dis 2020; 20: 920–28).

For inclusion of new variants/strains (consideration of genetic variants) since 2021:

- Inactivated isolates from different regions and outbreak clusters; sequence variants

Pango Lineage B.1.1.298, cluster 5 variant from DK mink associated outbreak (Euro Surveill. 2021; 26(5):2100009);
Pango Lineage B.1.1.25, spike D614G isolate from Bangladesh.

For Specificity control samples:

- Cell culture-derived common human coronaviruses

HCoV-OC43, HCoV-229E, or HCoV-NL63

For Coronavirus negative panel members:

- Transport medium with background human cells

* Calibrated dilution series based on QCMD Internal Reference Materials with values established using an orthogonal droplet digital PCR reference assay [E gene assay modified from Corman VM, et al. Euro Surveill. 2020; 25(3):2000045].

All samples provided in liquid frozen format, 1 mL
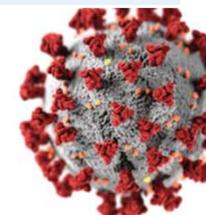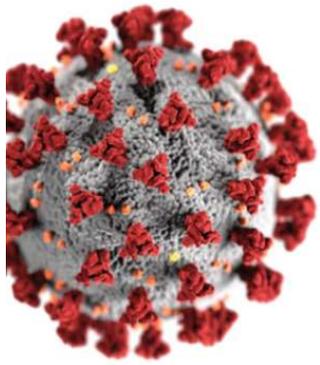
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Outlook

- Introduction

- SARS-CoV-2 programme for molecular detection
  - Variation of Cq values & how to deal with
  - Impact of genetic variations

- SARS-CoV-2 programme for antigen testing

QCMD 2020 EQA schemes (CVOP/SCV2) Qualitative Performance

		Data and Results on the Individual EQA Sample Level											
		CVOP20 (S+S2) Apr-May 2020			SCV2_20C1 Jun-Aug 2020			SCV2_20C2 Oct-Dec 2020			SCV2_20S Oct-Dec 2020		
Sample Content	Viral concentration [dPCR log10 copies/mL]	Assigned as Sample	Percentage Correct [%]	Datasets (n)	Assigned as Sample	Percentage Correct [%]	Datasets (n)	Assigned as Sample	Percentage Correct [%]	Datasets (n)	Assigned as Sample	Percentage Correct [%]	Datasets (n)
SARS-CoV-2	5.30	CVOP20S-07	99.2	1011	-	-	-	-	-	-	-	-	-
SARS-CoV-2	4.30	CVOP20S-01	98.5	1011	-	-	-	-	-	-	SCV2_101S-04	98.8	497
SARS-CoV-2	4.30	CVOP20S-06	98.6	1011	-	-	-	-	-	-	-	-	-
SARS-CoV-2	4.12	-	-	-	SCV2_101C1-02	99.5	440	-	-	-	-	-	-
SARS-CoV-2	3.30	CVOP20S-03	96.6	1011	-	-	-	SCV2_101C2-02	98.7	451	SCV2_101S-05	97.4	497
SARS-CoV-2	3.30	-	-	-	-	-	-	-	-	-	SCV2_101S-07	96.6	497
SARS-CoV-2	3.16	-	-	-	SCV2_101C1-03	98.2	440	-	-	-	SCV2_101S-01	96.0	497
SARS-CoV-2	3.16	-	-	-	-	-	-	-	-	-	SCV2_101S-08	96.2	497
SARS-CoV-2	2.82	-	-	-	SCV2_101C1-04	98.9	440	-	-	-	-	-	-
SARS-CoV-2	2.82	-	-	-	SCV2_101C1-05	96.8	440	-	-	-	-	-	-
SARS-CoV-2	2.48	-	-	-	-	-	-	SCV2_101C2-03	93.1	451	SCV2_101S-03	90.3	497
SARS-CoV-2	2.48	-	-	-	-	-	-	SCV2_101C2-04	93.6	451	SCV2_101S-06	89.3	497
SARS-CoV-2	2.30	CVOP20S-08	85.5	1011	-	-	-	-	-	-	-	-	-
HCoV-NL63	4.64	CVOP20S-02	97.1	1011	-	-	-	-	-	-	-	-	-
HCoV-OC43	4.00	CVOP20S-04	97.5	1011	-	-	-	SCV2_101C2-01	97.8	451	SCV2_101S-10	96.2	497
HCoV-229E	3.93	-	-	-	SCV2_101C1-01	95.9	440	-	-	-	SCV2_101S-02	97.0	497
Transport medium	N/A	CVOP20S-05	98.2	1011	-	-	-	SCV2_101C2-05	99.1	451	SCV2_101S-09	96.8	497
Overall Qualitative Performance													
Percentage of datasets with All panel members correctly identified			82.1	1011 datasets		92.8	440 datasets		88.8	451 datasets		80.5	497 datasets

- Overall qualitative performance: acceptable;
- Commercial assays (84.4% of datasets) and in-house assays (15.6%) performed similarly;
- Analytical sensitivity and specificity remained variable.

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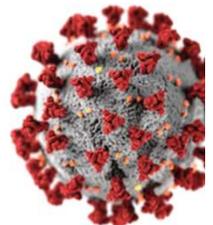
Target genes used for NAT/PCR

SARS-CoV-2 Structure

Spike (S1&S2)
Nucleocapsid (N)
Membrane (M)
Envelope (E)
ssRNA (+sense, ~30kb in length)
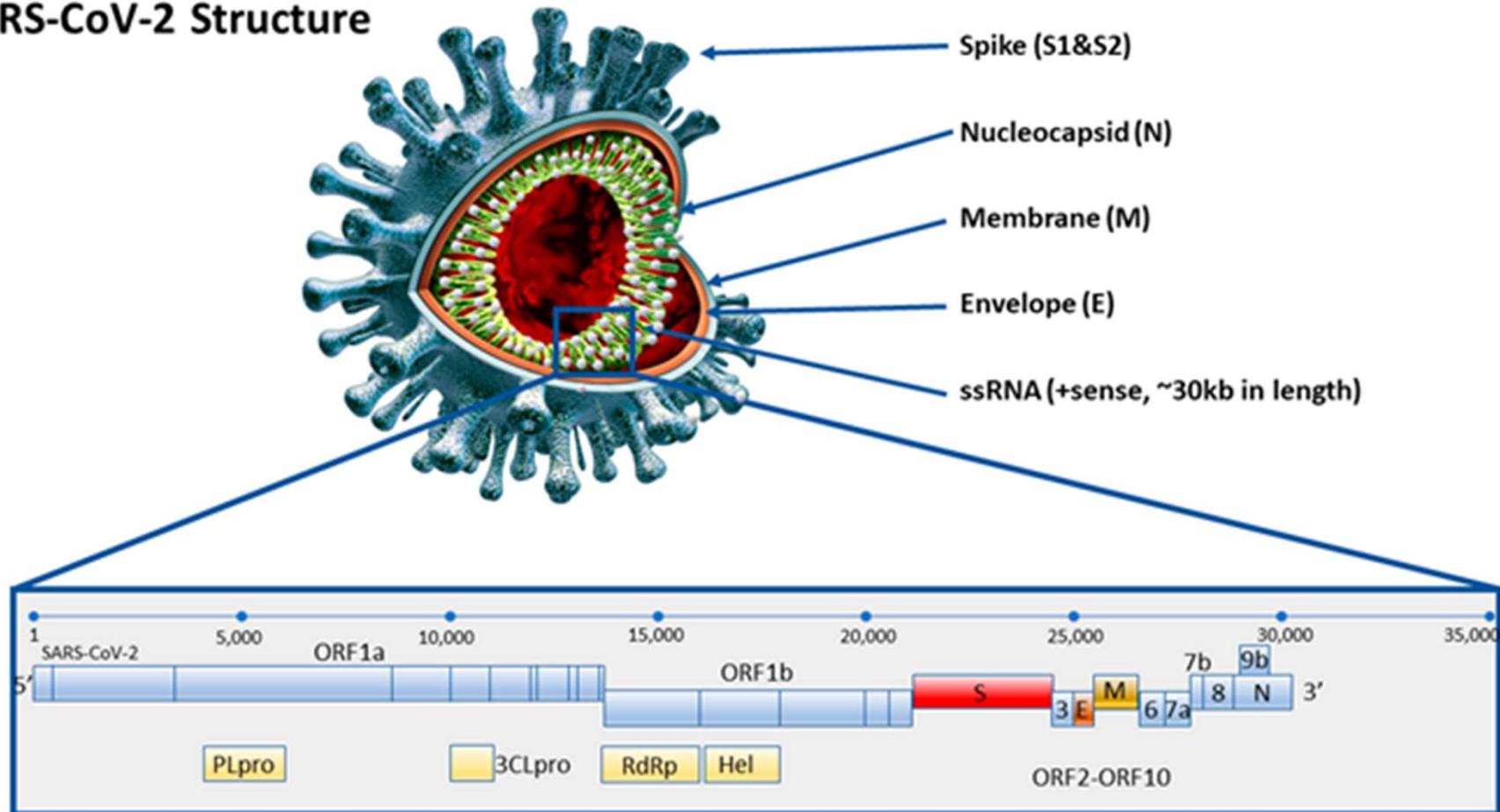
✓ Manufacturers indicate which gene regions their detection systems are targeting (usually two different regions or even more);

✓ **Using whole, inactivated SARS-CoV-2 in EQA provides the full viral genome ensuring compatibility with all molecular assays.**

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QCMD 2020 EQA scheme SCV2_20S Molecular assay overview

Assays [%], N= 497

- Abbott SARS-CoV-2
- Altona Diagnostics RealStar
- Anatolia Geneworks Bosphore nCoV
- BD SARS-CoV-2
- BGI Real Time PCR
- BioFire FilmArray
- Cepheid Xpert SARS-CoV-2
- Certest Viasure N1 + N2
- Certest Viasure ORF1 & N
- Conventional In-House PCR
- EasyDiagnosis SARS-CoV-2
- Elitech Elite Real Time kit
- Elitech GeneFinder
- Hologic Aptima SARS-CoV-2
- Luminex ARIES
- PerkinElmer SARS-CoV-2 Real Time PCR
- PrimerDesign Genesig
- Real-time In-House PCR
- Roche Cobas SARS-CoV 2
- Seegene Allplex SARS-CoV-2
- Thermofisher TaqPath COVID-19
- TIB MOLBIOL LightMix
- Vitassay SARS-CoV-2
- Other Commercial

Target gene combinations in the performance evaluation [%], N= 497

- E-gene
- E-gene, N-gene
- E-gene, N-gene, ORF1
- E-gene, ORF1
- E-gene, RdRp gene
- E-gene, RdRp gene, N-gene
- E-gene, RdRp gene, N-gene, S-gene
- E-gene, S-gene
- N-gene
- N-gene, ORF1
- N-gene, S-gene, ORF1
- ORF1
- RdRp gene
- RdRp gene, N-gene
- RdRp gene, ORF8
- S-gene
- Other
- Not Known

✓ EQA data provide the landscape of molecular assays currently in use

✓ 86.3% commercial; 13.7% in-house

✓ Major loci targeted:
   N+ORF1, 11.9%
   N, 11.3%
   E+RdRP+N, 10.9%
   E+N, 10.5%
   (…)

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QCMD 2020 EQA scheme SCV2_20S Target Gene Analysis

		Total		Qualitative Results reported and correct, differentiated by Target Gene - Example: SCV2_20S Oct-Dec 2020																	
			Sample Code I Sample Content I (Viral concentration in dPCR log10 copies/mL) I Percentage Correct %																	
Target gene	n	%	SCV2_101S-01 SARS-CoV-2 (3.16) 96.0%		SCV2_101S-02 HCoV-229E (3.93) 97.0%		SCV2_101S-03 SARS-CoV-2 (2.48) 90.3%		SCV2_101S-04 SARS-CoV-2 (4.30) 98.8%		SCV2_101S-05 SARS-CoV-2 (3.30) 97.4%		SCV2_101S-06 SARS-CoV-2 (2.48) 89.3%		SCV2_101S-07 SARS-CoV-2 (3.30) 96.6%		SCV2_101S-08 SARS-CoV-2 (3.16) 96.2%		SCV2_101S-09 CoV negative 96.8%		SCV2_101S-10 HCoV-OC43 (4.00) 96.2%	
			(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)
E-gene	37	7,4	36	97,3	37	100,0	34	91,9	37	100,0	37	100,0	33	89,2	36	97,3	37	100,0	36	97,3	36	97,3
E-gene, N-gene	52	10,5	52	100,0	51	98,1	45	86,5	52	100,0	52	100,0	50	96,2	52	100,0	51	98,1	52	100,0	51	98,1
E-gene, N-gene, ORF1	7	1,4	7	100,0	6	85,7	7	100,0	7	100,0	7	100,0	7	100,0	7	100,0	7	100,0	7	100,0	6	85,7
E-gene, ORF1	23	4,6	21	91,3	23	100,0	18	78,3	23	100,0	22	95,7	20	87,0	21	91,3	21	91,3	23	100,0	23	100,0
E-gene, RdRp gene	24	4,8	24	100,0	24	100,0	23	95,8	24	100,0	23	95,8	22	91,7	24	100,0	23	95,8	24	100,0	24	100,0
E-gene, RdRp gene, N-gene	54	10,9	53	98,1	53	98,1	50	92,6	53	98,1	52	96,3	46	85,2	52	96,3	52	96,3	52	96,3	51	94,4
E-gene, RdRp gene, N-gene, S-gene	19	3,8	19	100,0	18	94,7	17	89,5	19	100,0	19	100,0	16	84,2	19	100,0	19	100,0	19	100,0	17	89,5
E-gene, S-gene	14	2,8	14	100,0	14	100,0	14	100,0	14	100,0	14	100,0	14	100,0	14	100,0	14	100,0	14	100,0	13	92,9
N-gene	56	11,3	49	87,5	52	92,9	49	87,5	53	94,6	50	89,3	46	82,1	51	91,1	52	92,9	50	89,3	51	91,1
N-gene, ORF1	59	11,9	57	96,6	56	94,9	53	89,8	59	100,0	58	98,3	49	83,1	56	94,9	56	94,9	57	96,6	56	94,9
N-gene, S-gene, ORF1	28	5,6	25	89,3	25	89,3	23	82,1	26	92,9	27	96,4	22	78,6	25	89,3	24	85,7	24	85,7	27	96,4
ORF1	27	5,4	25	92,6	27	100,0	25	92,6	27	100,0	26	96,3	26	96,3	27	100,0	25	92,6	27	100,0	27	100,0
RdRp gene	20	4,0	20	100,0	20	100,0	19	95,0	19	95,0	20	100,0	20	100,0	20	100,0	20	100,0	19	95,0	19	95,0
RdRp gene, N-gene	22	4,4	20	90,9	22	100,0	17	77,3	22	100,0	22	100,0	19	86,4	21	95,5	22	100,0	21	95,5	22	100,0
RdRp gene, ORF8	5	1,0	5	100,0	5	100,0	5	100,0	5	100,0	5	100,0	5	100,0	5	100,0	5	100,0	5	100,0	5	100,0
S-gene	9	1,8	9	100,0	9	100,0	9	100,0	9	100,0	9	100,0	7	77,8	9	100,0	9	100,0	9	100,0	9	100,0
Other	33	6,6	32	97,0	31	93,9	32	97,0	33	100,0	32	97,0	32	97,0	32	97,0	32	97,0	33	100,0	32	97,0
Not Known	8	1,6	8	100,0	8	100,0	7	87,5	8	100,0	8	100,0	8	100,0	8	100,0	8	100,0	8	100,0	8	100,0
Total				96.0		97.0		90.3		98.8		97.4		89.3		96.6		96.2		96.8		96.2

Generally high success rates for the qualitative detection of the SARS-CoV-2 positive dilutions and negative samples, represent correct reported results regardless of the underlying target genes (success rates were high for all EQA schemes in 2020 and so far in 2021).

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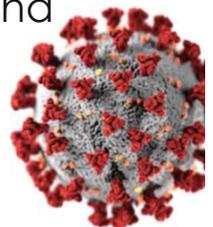
QCMD 2020 EQA scheme SCV2_20S Quantification Cycle Value Analysis

Reported quantification cycle (Cq) values, differentiated by Target Gene - Example: SCV2_20S Oct-Dec 2020																												
Target gene	Sample Code I Sample Content I (Viral concentration in dPCR log10 copies/mL) I Percentage Correct %																												
	SCV2_101S-04 SARS-CoV-2 (4.30) 98.8%				SCV2_101S-05 SARS-CoV-2 (3.30) 97.4%				SCV2_101S-07 SARS-CoV-2 (3.30) 96.6%				SCV2_101S-01 SARS-CoV-2 (3.16) 96.0%				SCV2_101S-08 SARS-CoV-2 (3.16) 96.2%				SCV2_101S-03 SARS-CoV-2 (2.48) 90.3%				SCV2_101S-06 SARS-CoV-2 (2.48) 89.3%			
	(n)	Median	Low	High	(n)	Median	Low	High	(n)	Median	Low	High	(n)	Median	Low	High	(n)	Median	Low	High	(n)	Median	Low	High	(n)	Median	Low	High
E-gene	127	29.1	16.8	45.3	126	32.0	28.0	45.0	127	32.1	28.0	45.0	116	33.1	28.8	39.2	126	33.1	15.0	45.0	104	35.5	19.4	45.0	113	35.4	26.5	45.0
N-gene	164	29.5	17.9	38.0	169	32.8	21.9	39.7	166	32.7	19.4	40.0	152	33.4	20.0	39.8	167	33.5	24.4	40.4	142	35.4	25.0	42.6	161	35.4	24.8	41.4
S-gene	30	28.1	24.8	33.6	30	31.0	19.2	39.3	29	30.1	21.3	36.2	29	31.6	21.1	39.7	25	31.4	24.2	38.8	25	34.6	30.8	39.0	24	34.5	31.0	39.8
ORF1	76	29.8	17.1	50.0	76	33.0	20.9	50.0	76	32.9	21.1	50.0	72	33.7	22.0	50.0	75	33.9	23.2	50.0	58	35.7	24.7	50.0	59	35.7	24.2	50.0
ORF8	7	29.5	23.2	37.0	7	33.2	27.0	38.0	6	33.4	27.3	35.3	5	32.3	28.5	36.3	6	33.9	28.9	34.9	6	34.7	32.6	36.5	6	35.2	32.3	37.3
RdRp gene	64	30.0	22.6	39.0	61	33.0	24.7	38.2	63	33.0	26.2	37.8	59	34.0	25.6	38.3	62	34.0	26.4	38.6	53	36.3	29.3	45.0	50	36.6	29.3	45.0
Total		29.3				32.5				32.4				33.0				33.3				35.4				35.5		

Considerable spread in reported Cq values for all SARS-CoV-2 positive samples with respect to the underlying target genes reflects differences in the applied methods and current lack of standardization (observation was made for all EQA schemes in 2020 and is also true for 2021).

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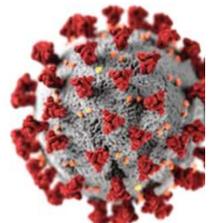
QCMD 2020 EQA scheme SCV2_20S Variation of Cq values by different assays

SCV2_101S-04 (4.30) N-gene, n=164, Median 29.5, 17.9-38.0

- All reporting Cq values for N-gene (n=164)
- BD SARS-CoV-2 (n=5)
- Cepheid Xpert SARS-CoV-2 (n=20)
- Certest Viasure N1+N2 (n=5)
- Certest Viasure ORF1 + N (n=5)
- EasyDiagnosis SARS-CoV-2 (n=5)
- Elitech GeneFinder (n=4)
- Luminex ARIES (n=1)
- PerkinElmer SARS-CoV-2 Real Time PCR (n=2)
- Real time In-House PCR (n=17)
- Seegene Allplex SARS-CoV-2 (n=30)
- Thermofisher TaqPath COVID-19 (n=12)
- Vitassay SARS-CoV-2 (n=4)
- Other (n=54)

SCV2_101S-04 (4.30) RdRP-gene, n=64, Median 30.0, 22.6-39.0

- All reporting Cq values for RdRP-gene (n=64)
- Elitech Elite Real Time kit (n=5)
- Elitech GeneFinder (n=4)
- PrimerDesign Genesig (n=4)
- Real-time In-House PCR (n=8)
- Seegene Allplex SARS-CoV-2 (n=17)
- TIB MOLBIOL LightMix (n=5)
- Other (n=21)

- **Cq values are dependent on the applied molecular assay and the targeted genes;**

- **Without standardization it is difficult to set universal quantification cycles, and therefore Cq values are not a good measure for determining viral loads in SARS-CoV-2 positive samples which is important e.g., for estimating infectiousness/contagiousness;**

- **Laboratories will need suitable quantitative reference materials to anchor their Cq values.**

All datasets with Cq values for all main targets, n=468: Median 29.3, 16.8 - 50.0
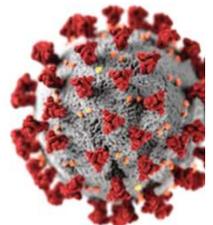
Digital PCR (dPCR) value assigned concentrations for comparing sensitivity

Overview of digital PCR

DNA — Partition → PCR →
- Absolute quantification
- Endpoint detection (0 or 1)
- Sensitive or precise
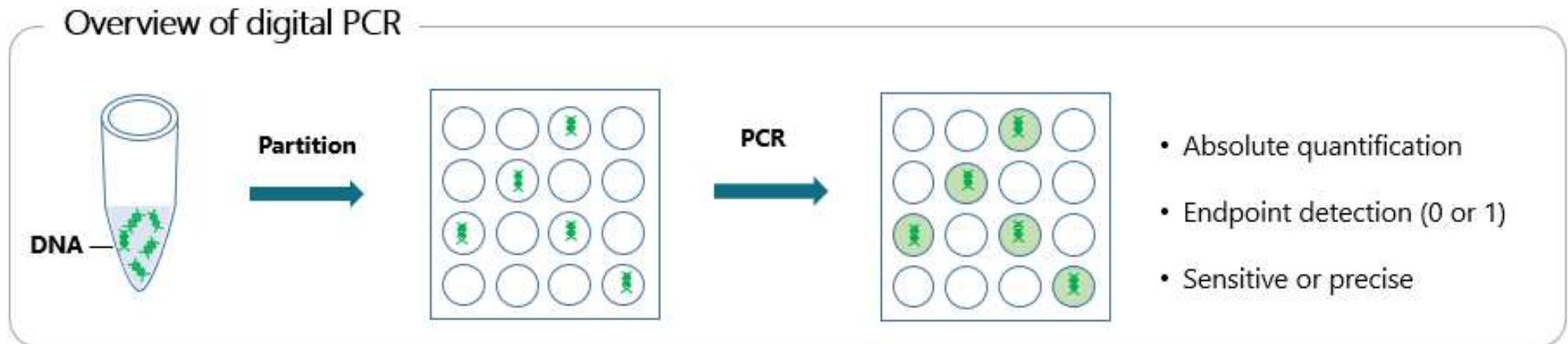
✓ In the absence of an International Standard or Certified Reference Material (CRM), QCMD use Internal Reference Materials (IRMs). For SARS-CoV-2, with values established using ddPCR reference assays. This supports the consistency and traceability of the EQA materials, and helps also to aid the comparison of results across laboratories;

✓ **ISO 17511:2020** (*In vitro diagnostic medical devices - Requirements for establishing metrological traceability of values assigned to calibrators, trueness control materials and human samples*): dPCR is considered as reference measurement procedure for assigning DNA copy number concentration which can be used in combination with WHO standards or on their own;

✓ IRMs can be calibrated back when an International Standard (IS) is available.

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ddPCR calibrated reference materials – characterization

Fully traceable to the 1st WHO IS for SARS-CoV-2 RNA 20/146 & at clinically appropriate levels

Product Code	Expected value Log$_{10}$ dC/ml*	Expected value Log$_{10}$ IU/ml*
SCV2AQP01-S01	6.0	6.88
SCV2AQP01-S02	5.0	5.88
SCV2AQP01-S03	4.0	4.88
SCV2AQP01-S04	3.7	4.57
SCV2AQP01-S05	3.0	3.88
SCV2AQP01-S06	2.7	3.57
SCV2AQP01-S07	2.0	2.88
SCV2AQP01-S08	1.7	2.57
SCV2AQP01-S09	Below LoD	Below LoD

Values in digital copies and international units.
* Molecular workflow specific: here in-house N-gene assay

Distribution of clinical positives observed in a clinical study plotted against the QCMD analytical/linearity panel

Distribution of positive clinical samples

n= 262
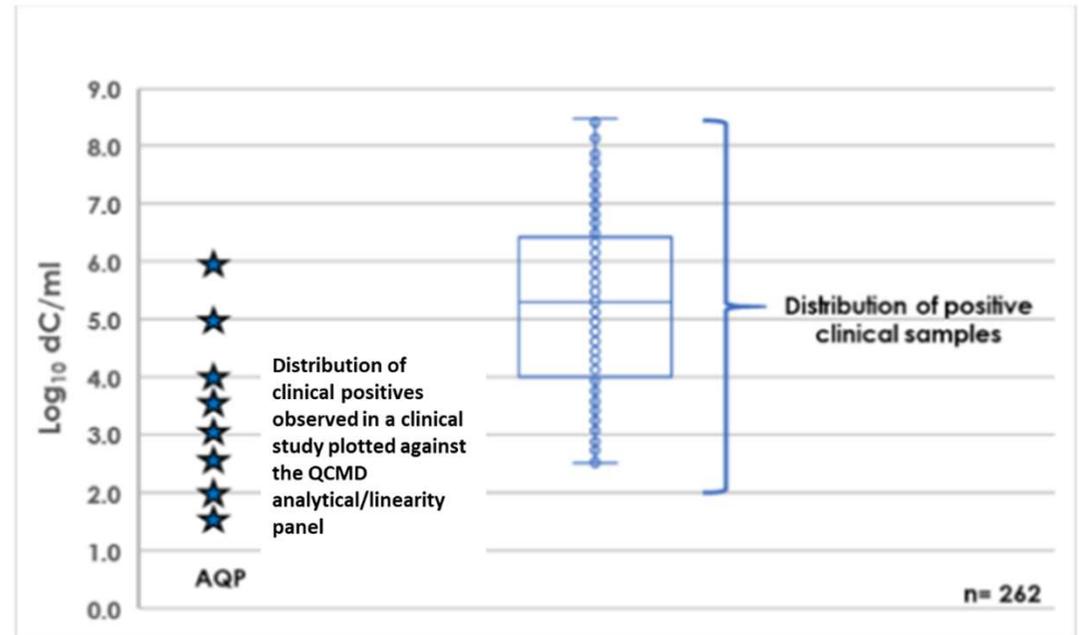
Performance evaluation for clinical range: Typical cohort of asymptomatic and symptomatic population tested (n= 1174). Figure shows the distribution of 262 positive patient samples identified over 60 consecutive days of testing.

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ddPCR calibrated reference materials help laboratories to anchor their Cq values for each assay/target gene and to identify LOD

QCMD analytical/linearity panel	dC/mL	Log10 dC/mL	PCR (E gene)		Ingenius			Cepheid		Qiastat			FilmArray				M2000 (RdRp/Ngene)		Alinity M (RdRp/Ngene)	
			Result	Ct value	RdRP-gene	N- gene	E-gene	E- gene	N-gene	Result	E- gene	IC	Result	1	2	IC	Result	Ct value	Result	Ct value
SCVA2AQP01-S01	1000000	6	Positive	21.87	24.22	24.17	22.27	21.5	23.5	Positive	29.9	33.8	Positive	8.43	9.83	15.16	Positive	13,43	Positive	26,09
SCVA2AQP01-S02	100000	5	Positive	24.66	27.09	27.11	25.46	24.9	26.7	Positive	30.5	31	Positive	11.2	12.66	14.36	Positive	16,79	Positive	30,22
SCVA2AQP01-S03	10000	4	Positive	27.87	30.07	29.74	28.77	28.6	30.6	Positive	32.5	31.5	Positive	14.5	15.93	14.5	Positive	20,14	Positive	33,22
SCVA2AQP01-S04	5000	3.7	Positive	28.52	31.11	30.62	29.73	29.3	31.3	Positive	35.5	31.5	Positive	14.8	16.8	14.9	Positive	21,37	Positive	33,82
SCVA2AQP01-S05	1000	3	Positive	30.57	34.39	32.8	32.88	31.2	34.1	Positive	36.2	31.3	Positive	18.86	20.76	15.4	Positive	23,63	Positive	36,29
SCVA2AQP01-S06	500	2.7	Positive	31.89	43.97	34.08	35.45	32.2	34.4	Positive	33.5	32	Positive	19.7	21.16	15.1	Positive	24,67	Positive	37,42
SCVA2AQP01-S07	100	2	Positive	34.26	Not detected	36.4	Not detected	36.6	38.9	Negative	Not detected	33.9	Positive	20.43	21.93	13.63	Positive	26,27	Positive	38,36
SCVA2AQP01-S08	50	1.7	Positive	35.01	Not detected	40.14	Not detected	36.6	38.6	Negative	Not detected	33.2	Positive	27.03	24.1	15.16	Positive	26,45	Positive	37,56
SCVA2AQP01-S09	Negative	-	Negative	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Negative	Not detected	34	Negative	Negative	Negative	14.43	Negative	Not detected	Negative	Negative

Example for one laboratory and their different tests used

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SARS-CoV-2
QCMD molecular EQA schemes, 2020/2021

Conclusions

- ✓ The **EQA data provide an overview of molecular assays** for SARS-CoV-2 detection currently in use;

- ✓ The **overall qualitative performance** of the participating laboratories was at an **acceptable** level;

- ✓ The schemes revealed that **analytical sensitivity and specificity remained variable**, and affected laboratories should review their procedures (to prevent false-positive/indeterminate results) and/or strive to improve sensitivity;

- ✓ **For future quantification, it will be necessary to make results among laboratories comparable** (therefore, guidelines, the 1st WHO IS for SARS-CoV-2 RNA 20/146 developed by NIBSC*, and reference materials towards calibrated concentrations are welcome!).

*Bentley et al., Collaborative Study for the Establishment of a WHO International Standard for SARS-CoV-2 RNA. 2020, WHO Expert Committee on Biological Standardization. WHO/BS/2020.2402
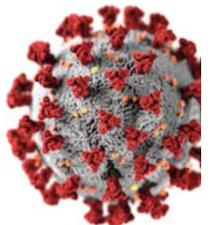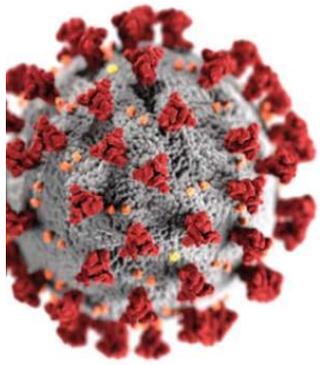
Outlook

- Introduction

- SARS-CoV-2 programme for molecular detection
  - Variation of Cq values & how to deal with
  - Impact of genetic variations

- SARS-CoV-2 programme for antigen testing

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QCMD 2021 EQA scheme SCV2_21C1B Qualitative Results

Intended Results / Panel Composition

Sample Code	Sample Content	Matrix	Sample Relationships [1]	Detection Frequency [2]	Sample Status [3]	Percentage Correct (All) [4]	
						(%)	(n)
SCV2_21C1B-01	SARS-CoV-2 Lineage B.1	Transport Medium	4.13 dPCR Log10 Copies/ml (DS1_1)	Frequently Detected	CORE	99.1	1327
SCV2_21C1B-02	SARS-CoV-2 Lineage B.1.1.298	Transport Medium	2.51 dPCR Log10 Copies/ml	Frequently Detected	CORE	96.4	1327
SCV2_21C1B-03	SARS-CoV-2 Lineage B.1	Transport Medium	2.00 dPCR Log10 Copies/ml (DS1_3)	Detected	CORE	87.3	1327
SCV2_21C1B-04	SARS-CoV-2 Lineage B.1.1.25	Transport Medium	2.94 dPCR Log10 Copies/ml	Frequently Detected	CORE	97.2	1327
SCV2_21C1B-05	SARS-CoV-2 Lineage B.1	Transport Medium	3.15 dPCR Log10 Copies/ml (DS1_2)	Frequently Detected	CORE	97.7	1327

Pango Lineage B.1.1.298, cluster 5 variant from DK mink associated outbreak
Pango Lineage B.1.1.25, spike D614G isolate from Bangladesh
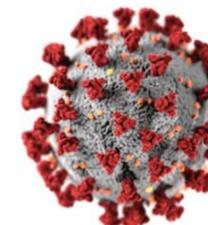
QCMD 2021 EQA scheme SCV2_21C1B Impact on Performance

|	Qualitative Results reported and correct differentiated by Target Gene - SCV2_21C1B May-Jun 2021										
	Sample Code I Sample Content I (Viral concentration in dPCR log10 copies/mL) I Percentage Correct %										
Target gene	Total		SCV2_21C1B-01 Lineage B.1 (4.13) 99.1%		SCV2_21C1B-02 Lineage B.1.1.298 (2.51) 96.4%		SCV2_21C1B-03 Lineage B.1 (2.00) 87.3%		SCV2_21C1B-04 Lineage B.1.1.25 (2.94) 97.2%		SCV2_21C1B-05 Lineage B.1 (3.15) 97.7%
	n	%	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)
E-gene	76	5,73	75	98,68	76	100,00	61	80,26	74	97,37	76	100,00
E-gene, N-gene	198	14,92	197	99,49	196	98,99	192	96,97	197	99,49	196	98,99
E-gene, N-gene, ORF1	24	1,81	23	95,83	21	87,50	14	58,33	22	91,67	22	91,67
E-gene, ORF1	55	4,14	55	100,00	53	96,36	49	89,09	54	98,18	55	100,00
E-gene, RdRp gene	20	1,51	20	100,00	19	95,00	18	90,00	20	100,00	20	100,00
E-gene, RdRP gene, N-gene	86	6,48	85	98,84	84	97,67	75	87,21	83	96,51	84	97,67
E-gene, RdRP gene, N-gene, S-ge	66	4,97	66	100,00	61	92,42	53	80,30	62	93,94	63	95,45
E-gene, RdRp gene, S-gene	10	0,75	10	100,00	9	90,00	9	90,00	9	90,00	9	90,00
E-gene, S-gene	15	1,13	15	100,00	15	100,00	15	100,00	15	100,00	15	100,00
N-gene	115	8,67	114	99,13	108	93,91	101	87,83	111	96,52	110	95,65
N-gene, Nsp2 Gene	12	0,90	11	91,67	11	91,67	12	100,00	11	91,67	11	91,67
N-gene, ORF1	183	13,79	181	98,91	175	95,63	153	83,61	175	95,63	180	98,36
N-gene, S-gene	7	0,53	7	100,00	7	100,00	7	100,00	7	100,00	7	100,00
N-gene, S-gene, ORF1	90	6,78	90	100,00	87	96,67	83	92,22	87	96,67	89	98,89
Not Known	46	3,47	44	95,65	44	95,65	42	91,30	44	95,65	44	95,65
ORF1	87	6,56	87	100,00	86	98,85	79	90,80	87	100,00	87	100,00
Other	23	1,73	22	95,65	21	91,30	18	78,26	21	91,30	23	100
RdRp gene	51	3,84	51	100,00	50	98,04	35	68,63	51	100,00	46	90,20
RdRP gene, N-gene	73	5,50	73	100,00	69	94,52	60	82,19	72	98,63	72	98,63
RdRp gene, N-gene, S-gene	17	1,28	17	100,00	17	100,00	15	88,24	17	100,00	17	100,00
RdRP gene, ORF1	7	0,53	7	100,00	7	100,00	7	100,00	7	100,00	7	100,00
RdRp gene, ORF8	15	1,13	15	100,00	15	100,00	15	100,00	15	100,00	15	100,00
RdRp gene, S-gene	5	0,38	5	100,00	5	100,00	4	80,00	5	100,00	5	100,00
S-gene	7	0,53	6	85,71	5	71,43	5	71,43	6	85,71	6	85,71
S-gene, M-gene	17	1,28	17	100,00	16	94,12	17	100,00	16	94,12	16	94,12
S-gene, ORF1	22	1,66	22	100,00	22	100,00	19	86,36	22	100,00	22	100,00
Total				99.1		96.4		87.3		97.2		97.7

BE236

"Concerning QCMD 2021 SARS-CoV-2 EQA programme: Thank you for the individual reports. I submitted results for different assays that we use in our laboratories. ==For the ThermoFisher TaqPath Covid-19 assay I submitted for the sample codes SCV2_21C1B-01 -> 05 three results because we detect 3 target genes: ORF1ab gene, N-gene and S-gene. For the sample SCV2_21C1B-02 I submitted the CT values 27.4 (ORF1ab gene), 28.01 (N-gene) and not detected / negative for the S-gene.== Because of that last result we received a detection score 3 in the last report (*only S gene as target*). ==We investigated this sample SCV2_21C1B-02 on 5 different platforms of ThermoFisher in our different laboratories and the S-gene was consistently not detected. Was this sample SCV2_21C1B-02 an Uk (alpha) variant? Because of the del 69/70 of this variant we observe a S-dropout on our ThermoFisher system.== "

Virus	Alternative virus name	Pango Lineage	Clade	GISAID accession ID	AA substitutions
hCoV-19/Denmark/DCGC-3024/2020	SARS-CoV-2/hu/DK/CL-5/1	B.1.1.298	GR	EPI_ISL_616802	**Spike D614G**, Spike H69del, **Spike I692V, Spike M1229I**, Spike V70del, **Spike Y453F**, N G204R, N R203K, N S194L, **NS3 H182Y**, NSP1 M85del, NSP3 N1263del, NSP12 P323L, **NSP12 T739I**, NSP15 T112I
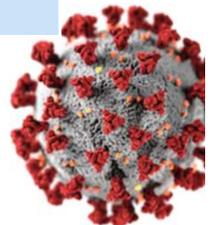
Future considerations

- Apart from sensitivity aspect, inclusion of new strains/variants
  - VOC: alpha, beta, gamma, delta
  - VOI: eta
  - Others: mink cluster 5, diff. other strains

  (MDCG 2021-21, Aug 2021: consideration of isolates from different regions and outbreak clusters; sequence variants)
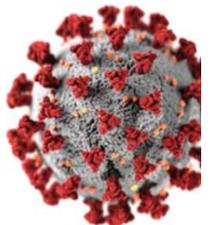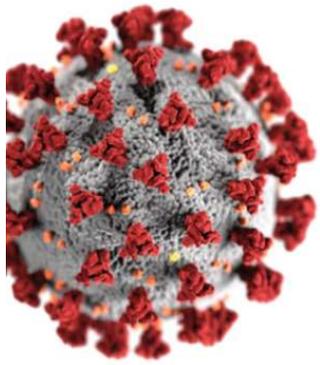
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- SARS-CoV-2 programme for antigen testing

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QCMD 2021 EQA pilot SCV2Ag21C1A Qualitative Results

Intended Results / Panel Composition

Sample Code	Sample Content	Matrix	Reference Value[1]	Detection Frequency[2]	Sample Status[3]	Percentage Correct (All)[4]	
						(%)	(n)
SCV2Ag21C1A-01	SARS-CoV-2	P9 RNAssist	6.4 log10 dc/ml	Detected	EDUCATIONAL	66.9	143
SCV2Ag21C1A-02	SARS-CoV-2	P9 RNAssist	7.4 log10 dc/ml	Detected	CORE	89.7	143
SCV2Ag21C1A-03	True Negative	P9 RNAssist		Negative	CORE	90.3	143
SCV2Ag21C1A-04	SARS-CoV-2	Transport Medium	6.4 log10 dc/ml	Frequently Detected	CORE	96.6	143
SCV2Ag21C1A-05	SARS-CoV-2	Transport Medium	5.4 log10 dc/ml	Infrequently Detected	EDUCATIONAL	33.8	143

All samples provided in liquid format under ambient conditions, 0.55 mL

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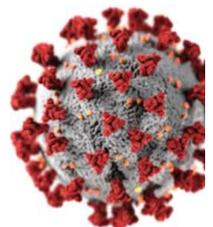
QCMD 2021 EQA pilot SCV2Ag21C1A Test Methods Performance

Qualitative Results reported and correct differentiated by Test Method - SCV2AgC1A Jun-Jul 2021							
Test Method	**Total**		Sample Code I Sample Content I (Viral concentration in dPCR log10 copies/mL) I Percentage Correct %				
	n	**%**	SCV2Ag21C1A-01 SARS-CoV-2 (6.4) 66.9%	SCV2Ag21C1A-02 SARS-CoV-2 (7.4) 89.7%	SCV2Ag21C1A-03 True negative 90.3%	SCV2Ag21C1A-04 SARS-CoV-2 (6.4) 96.6%	SCV2Ag21C1A-05 SARS-CoV-2 (5.4) 33.8%
			(%)	(%)	(%)	(%)	(%)
Lateral Flow Rapid Antigen Test	97	67,8	55,7	87,6	87,6	95,9	28,9
Automated PoC Test Platform	42	29,4	92,8	97,6	95,2	100	40,5
Laboratory ELISA/Immunoassay Test	4	2,8	50,0	50,0	100	100	100
Total			66.9	89.7	90.3	96.6	33.8
				in virusPHIX-P9™		in Transport medium	

- Generally, reduced performance in P9 stabilization buffer
  (potential reason: matrix effect, samples seem to be more viscous)
- Performance in TM: Lab-based immunoassays > automated PoC > LF tests
- Positivity starts to tail off at 6.4 log10 dC/ml (definitely cut-off for LF tests)

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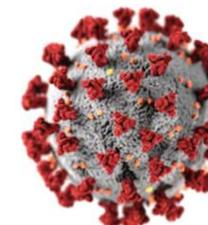
Future considerations

- Future matrix should be TM

- Inclusion of new strains/variants
    - VOC: alpha, beta, gamma, delta
    - VOI: eta
    - Others: mink cluster 5, alpha N variant, diff. other strains
    (MDCG 2021-21, Aug 2021: consideration of genetic variants)

Acknowledgements

QCMD Experts & Partners Network SARS-CoV-2
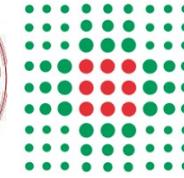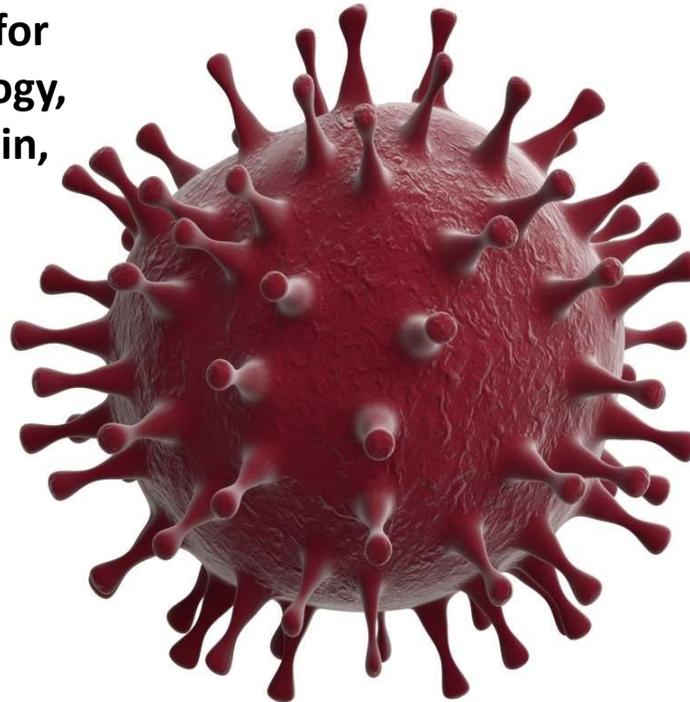
For more details

RAPID COMMUNICATION

International external quality assessment for SARS-CoV-2 molecular detection and survey on clinical laboratory preparedness during the COVID-19 pandemic, April/May 2020

Veerle Matheeussen[1,6] , Victor M Corman[2,6] , Oliver Donoso Mantke[3,6] , Elaine McCulloch[3] , Christine Lammens[1] , Herman Goossens[1] , Daniela Niemeyer[2] , Paul S Wallace[3] , Paul Klapper[4] , Hubert GM Niesters[5] , Christian Drosten[2] , Margareta Ieven[1] , on behalf of the RECOVER project and collaborating networks[7]

1. Department of Medical Microbiology, Vaccine and Infectious Disease Institute (VAXINFECTIO), University of Antwerp, Wilrijk, Belgium
2. National Consultant Laboratory for Coronaviruses, Institute of Virology, Charité-Universitätsmedizin Berlin, Berlin, Germany
3. Quality Control for Molecular Diagnostics (QCMD), Glasgow, United Kingdom
4. School of Biological Sciences, Division of Infection, Immunity and Respiratory Medicine, The University of Manchester, Manchester, United Kingdom
5. Division of Clinical Virology, Department of Medical Microbiology, University Medical Center Groningen, Groningen, the Netherlands
6. These authors contributed equally to this work and share first authorship
7. Details on these projects are noted in the Acknowledgements

Correspondence: Oliver Donoso Mantke (oliverdonoso@qcmd.org)

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