

National Institute for Public Health  
and the Environment  
*Ministry of Health, Welfare and Sport*

## SARS-CoV-2 preparedness using Outbreak Assistance Laboratory (OAL) network.



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NVMM | 1 April 2020

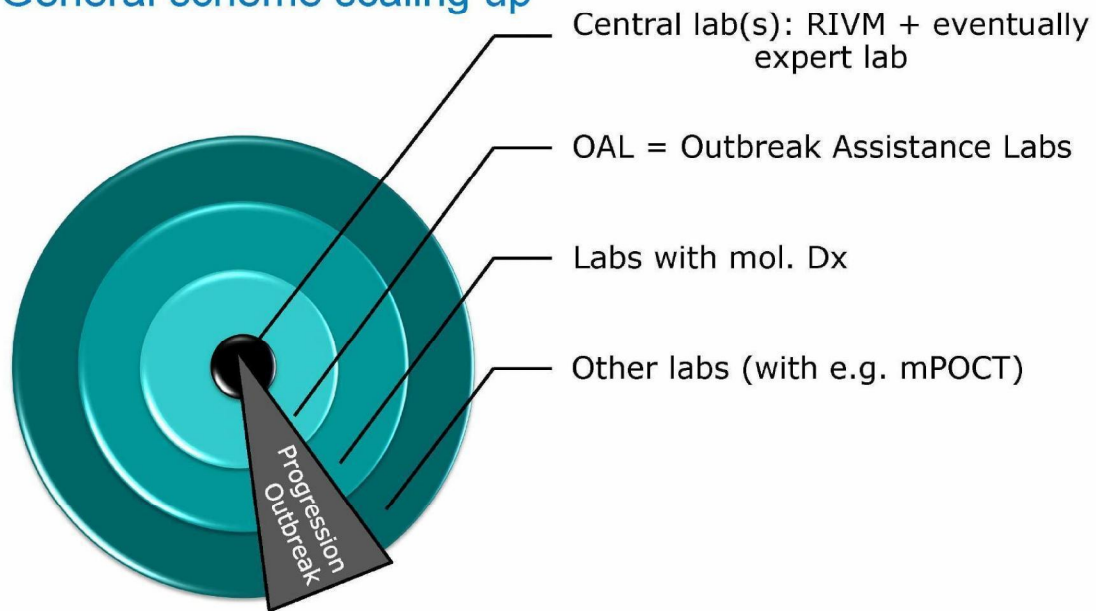


## Emerging virus, what is needed for appropriate MDx?

- Sequences (or pre-selected primers and probe)
- Positive control material
- Validation
  - Positive control material
  - Clinical materials from repository / first few 100 patients
- Roll-out to other labs
  - Implement on own molecular platform
  - Validate with provided positive control material and clinical materials from own repository
  - Participate in EQA exercise
- When available, repeat validation with positive clinical materials

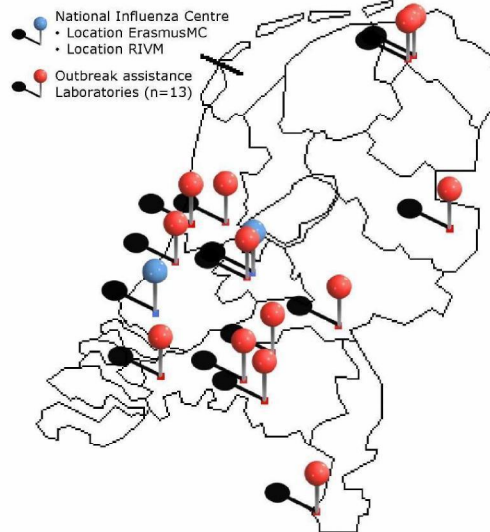


## General scheme scaling up





## Outbreak Assistance Laboratories



### Minimal requirements

- 24 hours, 7 days a week availability
- Minimum of 100 samples a day, during 2-3 months
- Able to unpack samples possibly containing BSL-3 organisms
- Routine molecular diagnostics
- Internal control used for routine diagnostics
- 8 hours turnaround time (*24 hrs during 2009 pandemic*)
- Willing to participate in quality control programs and to share results

### PREPAREDNESS PLAN

- Case load estimation by syndromic surveillance
- Provision of validated protocols
- Stockpiling reagents, kits
- Many discussions on possible scenario's

van Asten L *et al.* J Clin Virol. 2009 Jul;45(3):185-90.



## Support and requirements OAL and other labs

### Support

1. OAL – implementing PCR distributed by expert labs in the absence of commercial available tests and absence or sporadic presence of emerging pathogen; proficiency and sensitivity testing
2. 2nd and 3rd level labs – implementing approved in-house PCR or commercial PCR; primers and probes if needed, proficiency and sensitivity testing

### For accepting testing results as definitive:

1. Proficiency panel fully correct
2. Run control dilution series satisfactory or better
3. Confirmation 5 +ve and 10 -ve clinical specimens at one of the expert labs (RIVM or Erasmus MC) with fully correct results



## Timeline SARS-CoV-2 preparedness activities

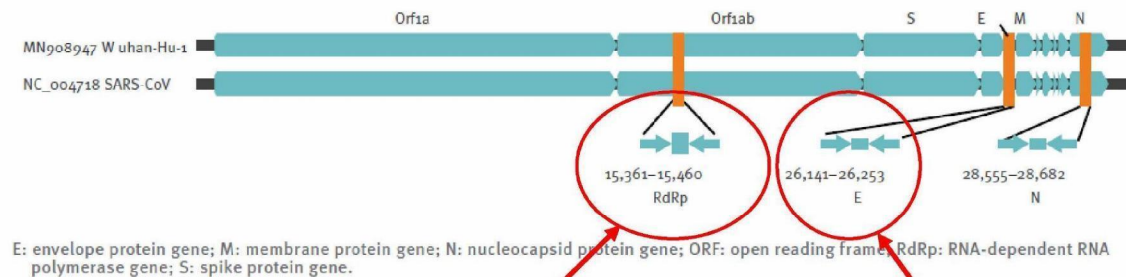
- |              |   |
|--------------|---|
|              | <ul style="list-style-type: none"> <li>● 1 Dec 2019 - – patients in Wuhan with unexplained pneumonia</li> <li>● 8 Jan 2020</li> <li>● 9 Jan – first report discovery new coronavirus</li> <li>● 10 Jan – full genome sequence available</li> <li>● 12 Jan 2020 – PCR protocol available developed by Corman et al.</li> <li>● 12 Jan 2020 – Primers and probes ordered; request Corman et al. to help with validation</li> <li>● 17 Jan 2020 – Validation at Erasmus MC and RIVM completed; diagnostic testing started</li> <li>● 23 Jan 2020 – OAL approached</li> <li>● 23 Jan 2020 – Preparation primers/probes kits, EQA panel, positive control started</li> <li>● 31 Jan 2020 – QC pass failed for some EQA specimens; QC positive control SARS-CoV passed</li> </ul> |
| 7 days       |   |
|              | <ul style="list-style-type: none"> <li>● 3 Feb 2020 – primers/probes received; QC failed – contaminated with E-gene synthetic material</li> <li>● 6-10 Feb 2020 – new manufacturer found; QC test and final batches passed</li> <li>● Week of 10/2 – distribution primers/probes kits, EQA panel and positive control</li> <li>● 24 Feb 2020 – EQA panel results returned</li> </ul>  |
| 7 days delay |   |



## 2019-nCoV RT-PCR

**FIGURE 1**

Relative positions of amplicon targets on the SARS coronavirus and the 2019 novel coronavirus genome



Numbers below amplicons are genome positions according to SARS-CoV, GenBank NC\_004718.

Two probes  
 Sarbeco specific  
 SARS-CoV-2 specific

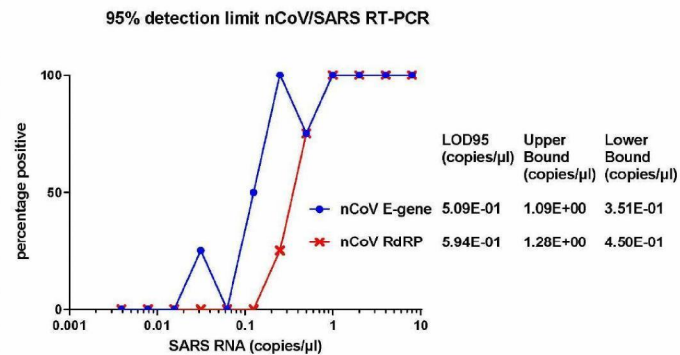
One probe  
 Sarbeco specific

Corman et al. 2020



## SARS-CoV-2 RT-PCR RIVM validation

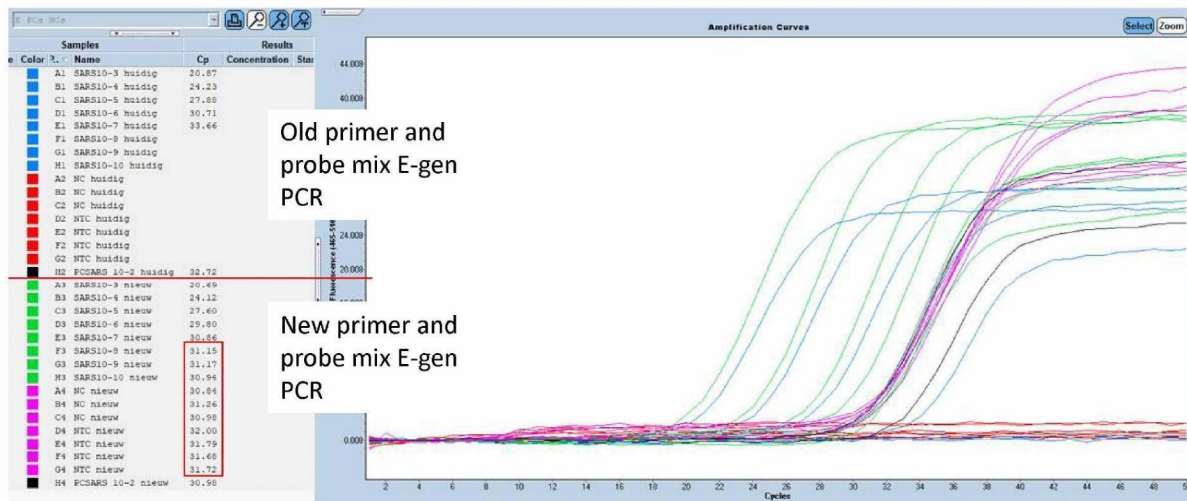
Clinical specimens with known viruses/bacteria	Number of samples tested in both assays	Ct range
CoV-HKU1	1	NA
CoV-OC43	5	22.7 – 29.9
CoV-229E	3	25.1 – 28.5
Influenza virus A(H1N1)pdm09	10	18.9 – 28.1
Influenza virus A(H3N2)	10	17.4 – 27.7
Influenza virus B/Victoria	7	19.8 – 29.9
Influenza virus B/Yamagata	10	23.5 – 29.1
Rhinovirus	13	13.4 – 28.0
Enterovirus (for types see supplementary excel file)	18	19.6 – 28.9
Respiratory syncytial virus A	10	18.0 – 29.8
Respiratory syncytial virus B	11	21.0 – 29.4
Parainfluenza virus 3	1	26
Human metapneumovirus	3	26.7 – 27.6
Adenovirus	2	21.0 – 27.3
Human Bocavirus	1	35 (borderline)
Mycoplasma spp.	1	26.8



*C. Pneumoniae*  
*C. Psittaci*  
 QCMD MERS-CoV panel



## Contamination of primer and probes





## Preparing OALs

Primers and probes thoroughly Qced for possible contamination  
RIVM and EMC protocols to guide implementation on own platform

Runcontrol of SARS-CoV-1 RNA (HKU39849) [since 26/2 also SARS-CoV-2  
BetaCoV/Germany/BavPat1/2020]

Labs asked to perform implemented PCR on 10-fold dilution series; analytical sensitivity

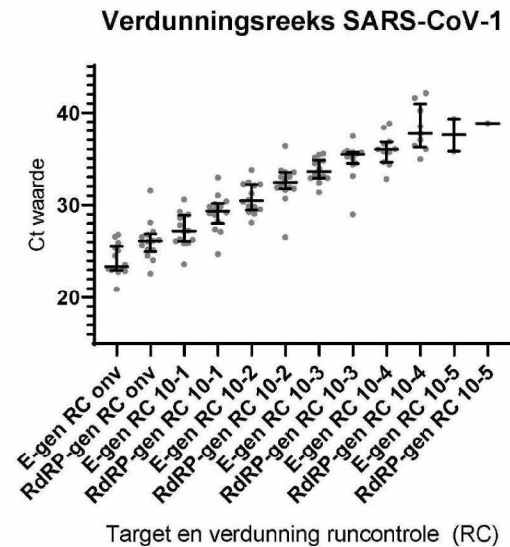
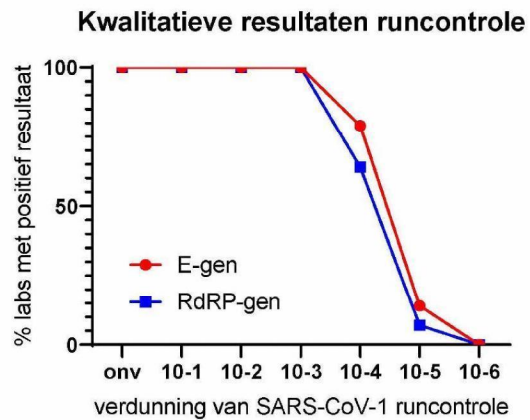
Proficiency panel for 'clinical' sensitivity and specificity

Extraction using one platform and testing with implemented PCR

Panel coding	Virus	Target specifieke Ct	E-gen	RdRP-gen
EQA_CoV20-01	CoV-NL63	25.59	Neg	Neg
EQA_CoV20-02	SARS-CoV-1 (d1)	nvt	24.22	24.73
EQA_CoV20-03	Inf A(H3N2)	19.77	Neg	Neg
EQA_CoV20-04	Geen virus	nvt	Neg	Neg
EQA_CoV20-05	SARS-CoV-1 (d3)	nvt	31.98	32.03
EQA_CoV20-06	CoV-229E	27.78	Neg	Neg
EQA_CoV20-07	Inf B/Victoria	22.27	Neg	Neg
EQA_CoV20-08	CoV-OC43	28.98	Neg	Neg
EQA_CoV20-09	SARS-CoV-1 (d2)	nvt	28.21	28.8
EQA_CoV20-10	Rhinovirus A16	25.3	Neg	Neg

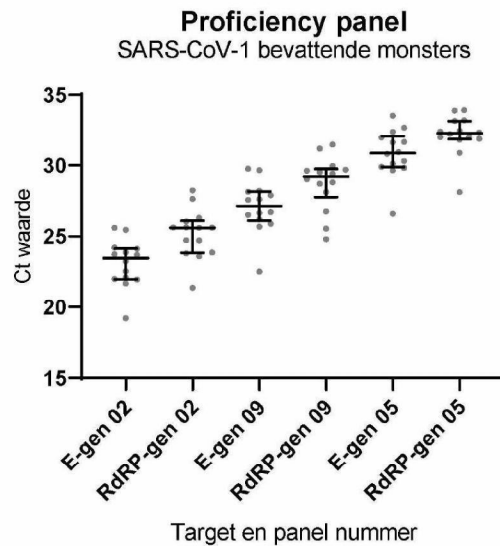


## Runcontrol dilution series OAL 24 February 2020





## Proficiency panel SARS-CoV-1 containing specimens

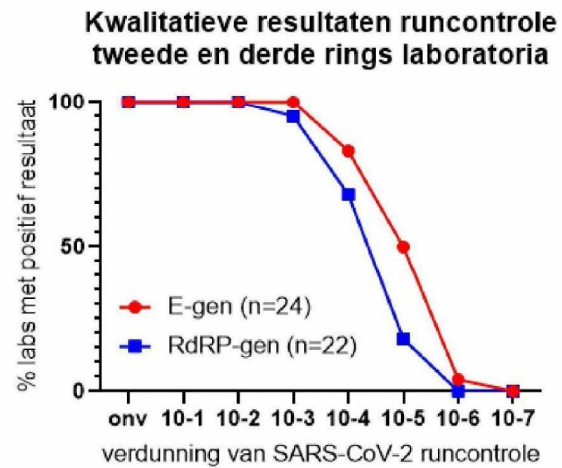
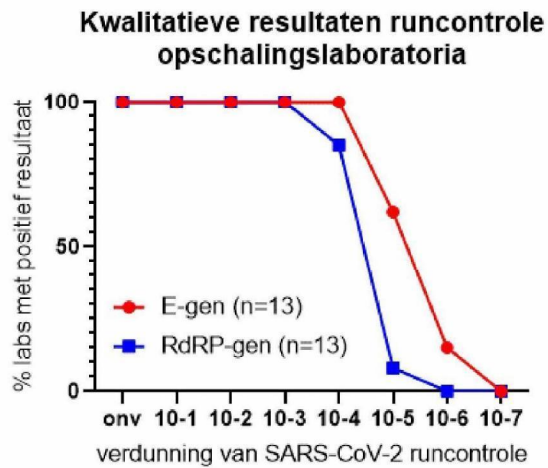


CoV-NL63, CoV-229E, CoV-OC43,  
A(H3N2), B/Vic, Rhinovirus A16,  
all negative with E-gene and  
RdRP-gene PCR

**Conclusion**  
OAL well prepared for SARS-  
CoV-2 testing

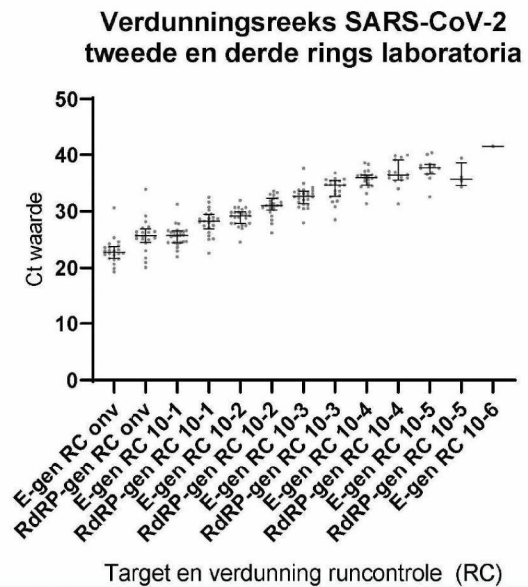
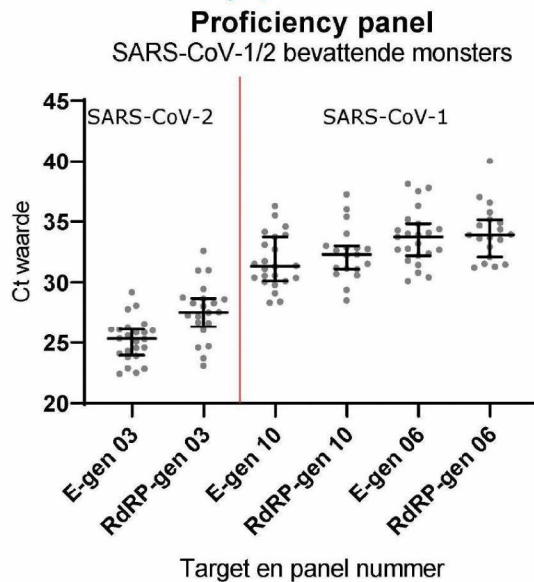


## 2<sup>nd</sup> and 3<sup>rd</sup> ring laboratories





## Proficiency panel with SARS-CoV-1 and -2





## Acknowledgements

- RIVM/IDS laboratory
  - Gabriel Goderski
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- Erasmus Medical Centre 
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  - Annemiek van der Eijk
  - Bart Haagmans
  - Marion Koopmans
  - En veel meer collega's....
- Nivel 
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- All other laboratories implementing diagnostics
- Johan Reimerink
- Gert-Jan Godeke
- Harry Vennema
- Erwin Duizer
- Rogier Bodewes
- En veel meer college's...