



Faculty of Science, Technology,  
and Health  
Department of Health, Behavior and Society



# Moleculaire diagnostiek COVID-19

(10)(2e)

(10)(2e)

webinar GGD | 11-05-2020



## Sensitiviteit en specificiteit SARS-CoV-2 PCR

- Analytisch
  - Welke minimale hoeveelheid viraal RNA kan een PCR detecteren
  - Hoe effectief is de extractie van RNA uit klinisch materiaal (concentratie factor)
  - Worden geen andere pathogenen dan bedoeld met de PCR gedetecteerd
  - Design PCR voor SARS-CoV-2 detectie
- Klinisch
  - Wordt elke patiënt geïnfecteerd met SARS-CoV-2 infectie gedetecteerd
  - Wordt elke patiënt met COVID-19 gedetecteerd
  - Is een patiënt met een positieve PCR geïnfecteerd met SARS-CoV-2
  - Heeft een patiënt met een positieve PCR COVID-19
  - Type monster
  - Tijdstip monsterafname tov eerste ziektedag
  - Juiste monsterafname



# Monstertypen voor SARS-CoV-2 detectie; PCR

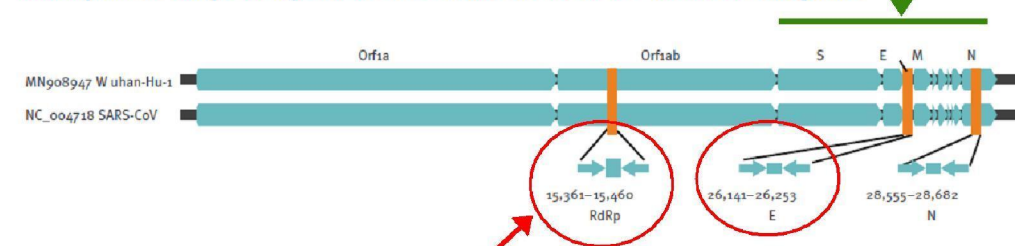
SARS-CoV-2 is primair een respiratoir virus

- Nasofarynxwat/midturbinat
- Orofarynx
- Sputum/BAL
- Speeksel
- Feces
- Bloed
- Urine

Subgenome messengers

FIGURE 1

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



E: envelope protein gene; M: membrane protein gene; N: nucleocapsid protein gene; ORF: open reading frame; RdRp: RNA-dependent RNA polymerase gene; S: spike protein gene.

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

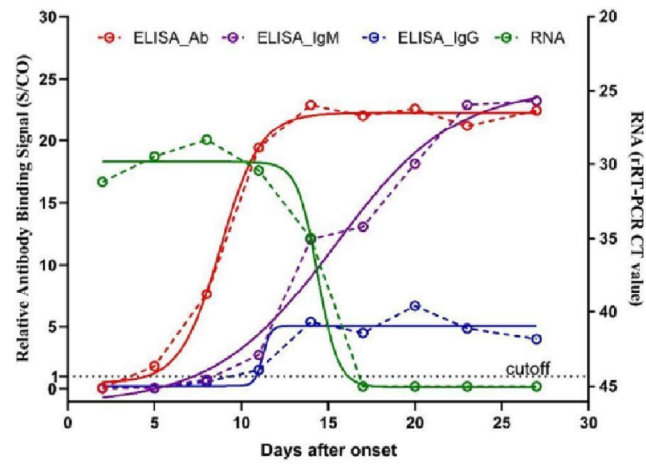
Two probes Sarbeco specific SARS-CoV-2 specific

One probe Sarbeco specific

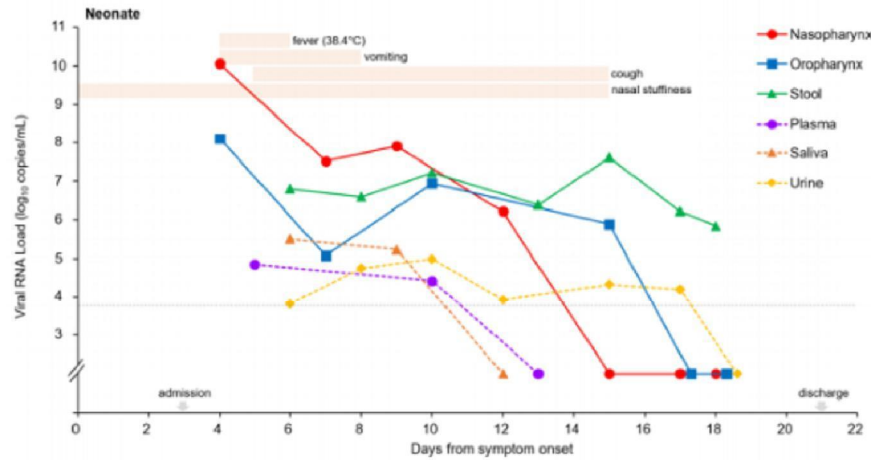
Corman et al. 2020



## Kinetiek SARS-CoV-2 detectie in diverse monstertypen



Lou et al. medRxiv

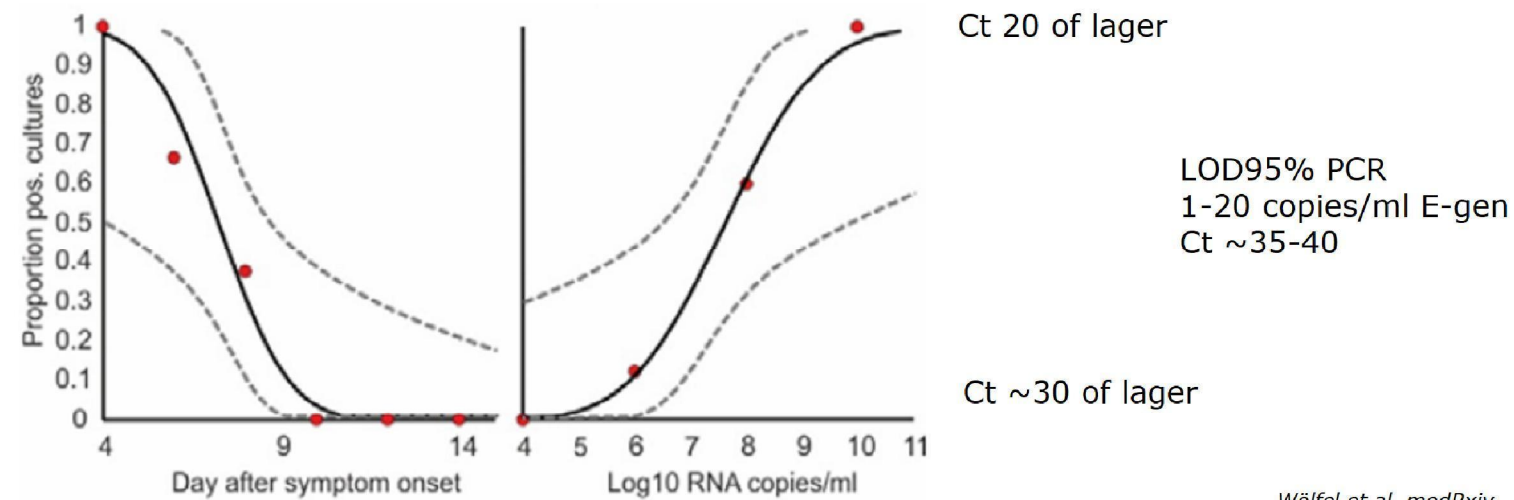


Mi Seon et al. CID



## Relatie PCR positiviteit met besmettelijkheid

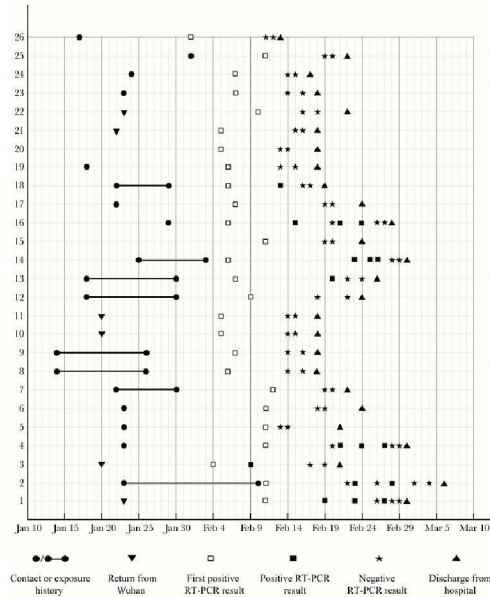
- Aanname – kweekbaarheid virus indicatie voor besmettelijkheid



Wölfel et al. medRxiv

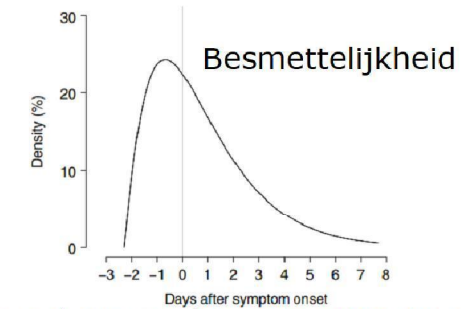
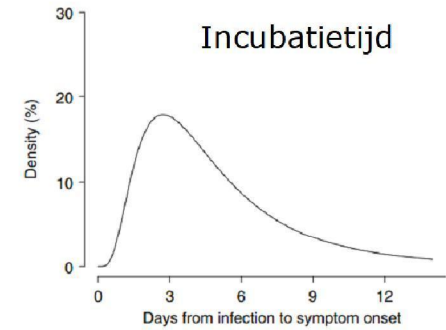


## Asymptomatische gevallen



- 26 asymptomatische gevallen
- Gevonden bij contactonderzoek
- Persistent asymptomatisch tot PCR negatief en ontslag
- 10 wel typische CT
- 2 betrokken bij transmissie

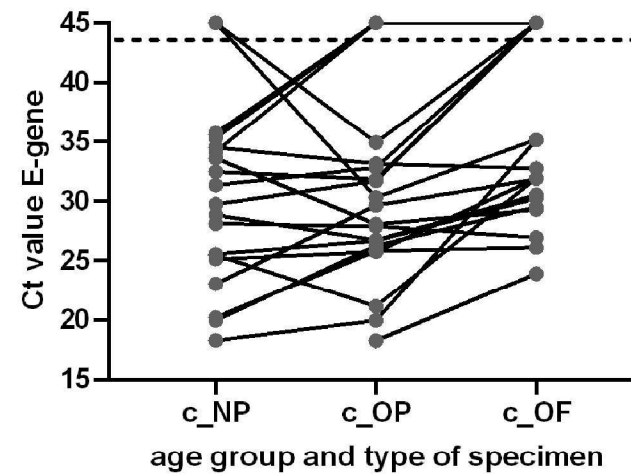
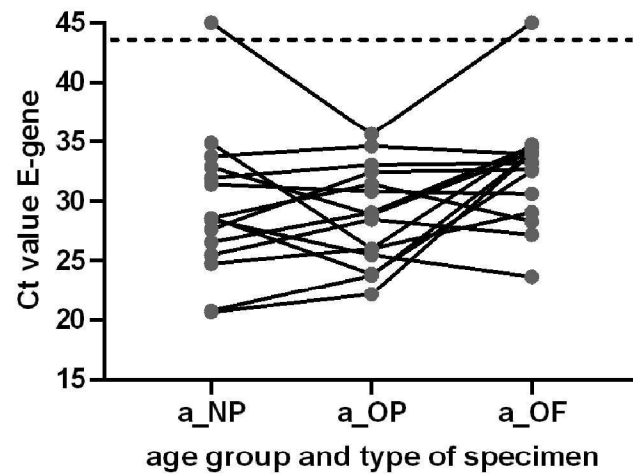
*Pan et al. J Infect Dis, jiaa205*



*He et al. Nature Medicine 26;May 2020: 672-675*



## Speeksel alternatief? FFX studie

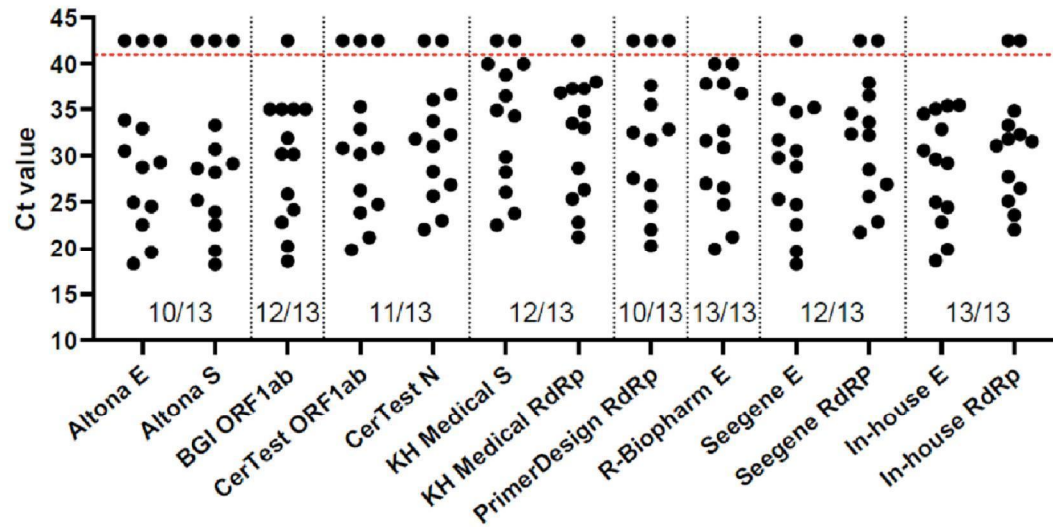


a=volwassen; c=kind; NP=nasofarynxwat; OP=orofarynxwat; OF=speeksel



## Real-time RT-PCR kits – clinical specimens

Clinical sample RT-PCR (all SARS-CoV-2 samples, n=13)



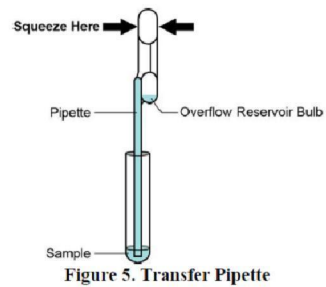
1 target only and Ct > 35  
= weak positive?

At RIVM approx. 3.6% of  
specimens tested for  
primary diagnosis have Ct  
>34.5, the point where  
kits are starting to fall off

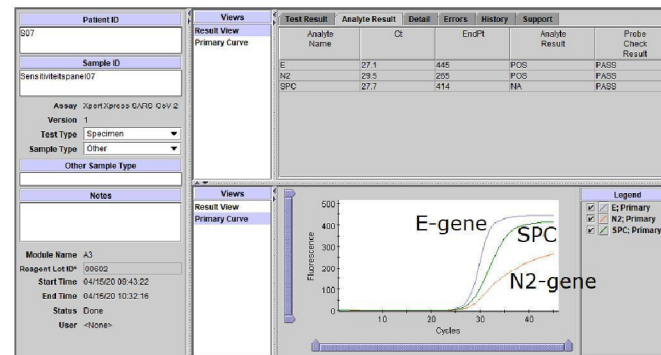




## Cepheid, Xpert® Xpress SARS-CoV-2



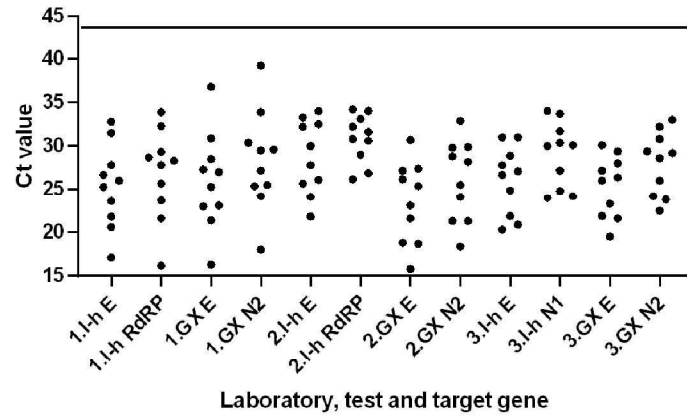
- 300 µl VTM in cartridge
- 600 µl aspirate in 3 ml VTM; 300 µl in cartridge
- Insert cartridge and start machine
- ~45 min later read results



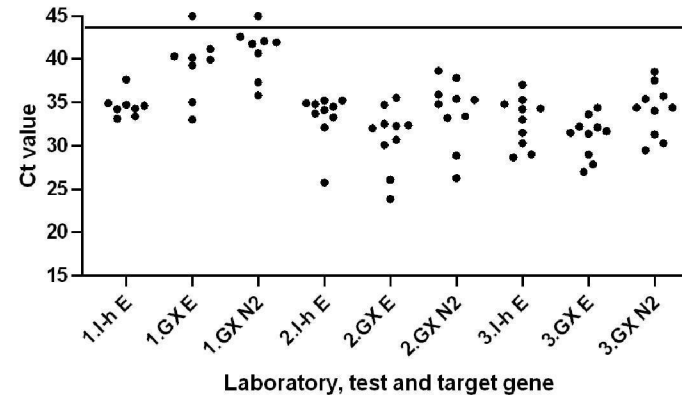


## Cepheid, Xpert® Xpress SARS-CoV-2 – clinical specimens

Moderate to high viral load (n=30)  
Positive for 2 targets

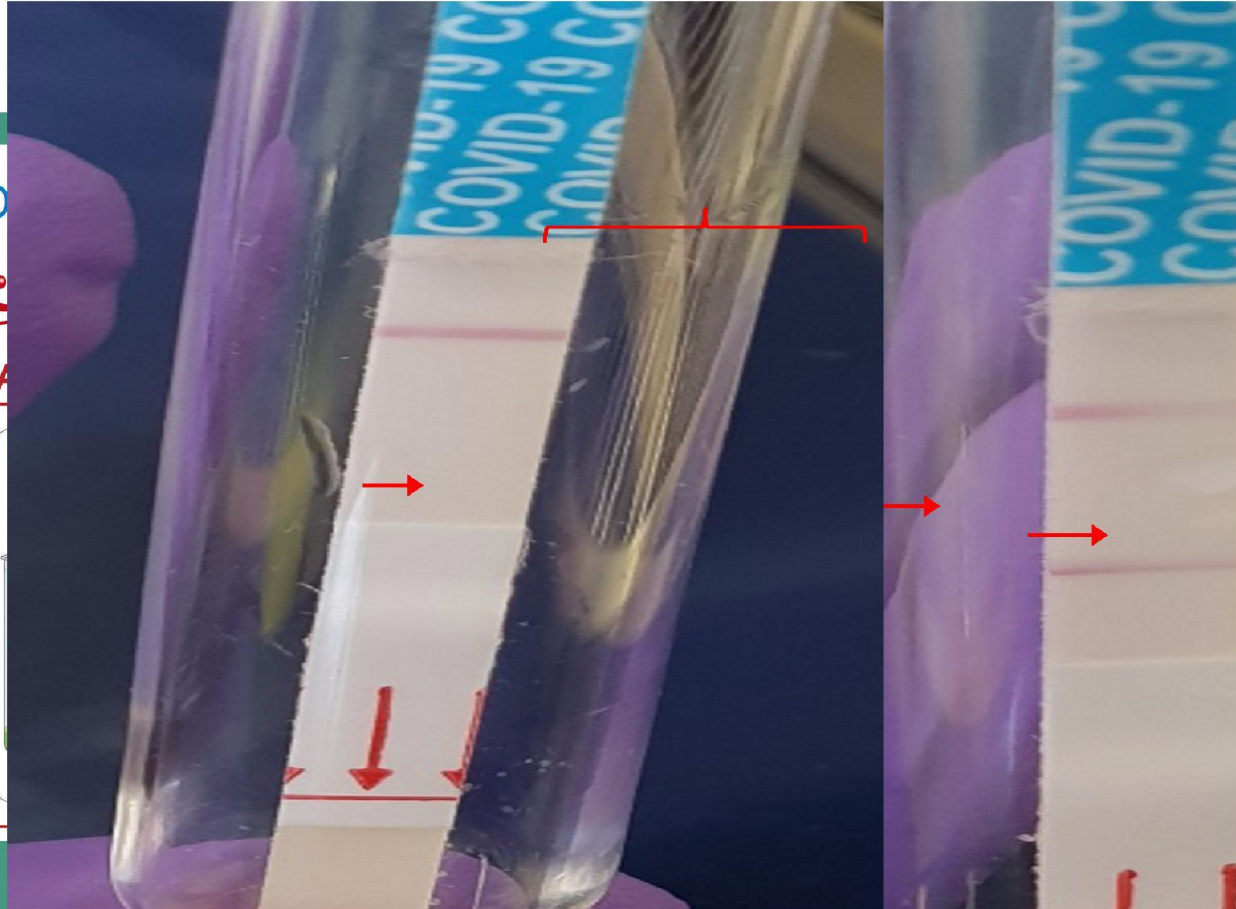
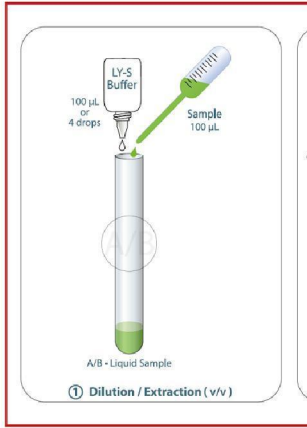


Low viral load (n=28)  
Positive for E-gene only



# CORIS Ag PO

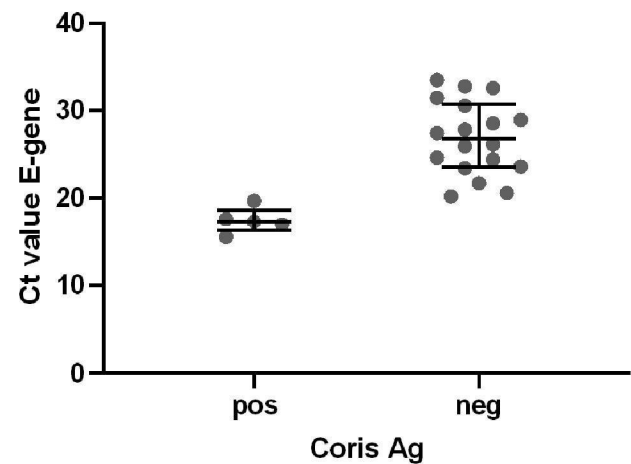
## COVID-19 A





## CORIS Ag POCT

Clinical specimens nose/throat combined



- LOD with SARS-CoV-2  $\sim 2 \cdot 10^4$  TCID<sub>50</sub> and a Ct  $\sim 23$  (E-gene PCR).
- Sensitivity not influenced by GLY or other VTM.
- Did not detect other common respiratory viruses.
- Clinical specimens dropped off at about Ct 20 (E-gene PCR).
- Applying this test during routine testing in the SARS-CoV-2 outbreak in the Netherlands would result in false negative incidence rate of 57-75%.