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## Moleculaire diagnostiek COVID-19

(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 material (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 patient met een positieve PCR COVID-19
  - Type monster
  - Tijdstip monsterafname tov eerste ziekte dag
  - Juiste monsterafname

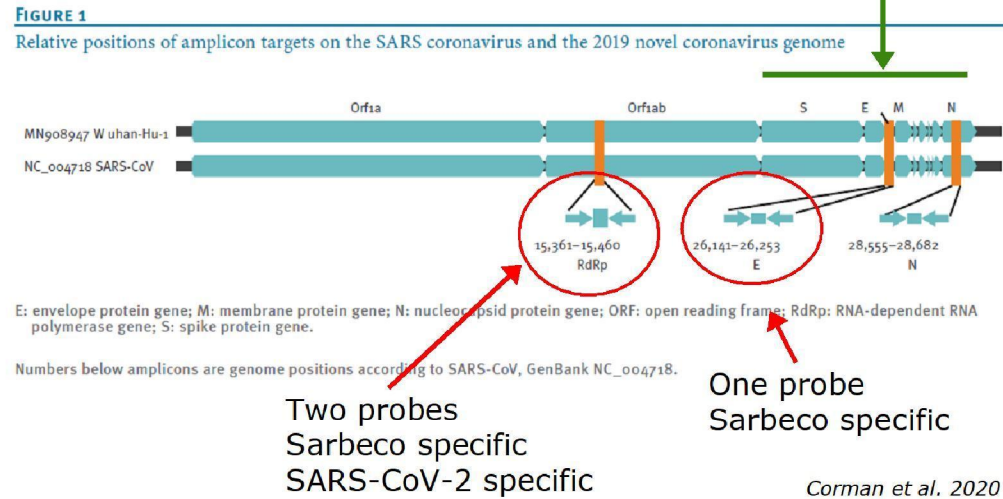


## Monstertypen voor SARS-CoV-2 detectie; PCR

SARS-CoV-2 is primair een respiratoir virus

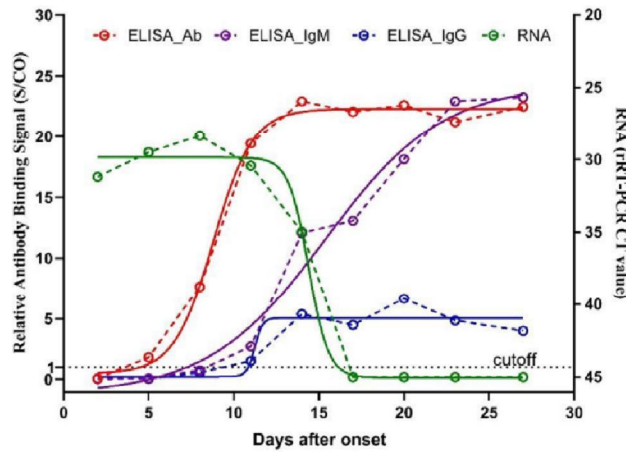
- Nasofarynxwat/midturbinate
- Orofarynx
- Sputum/BAL
- Speeksel
- Feces
- Bloed
- Urine

Subgenome messengers

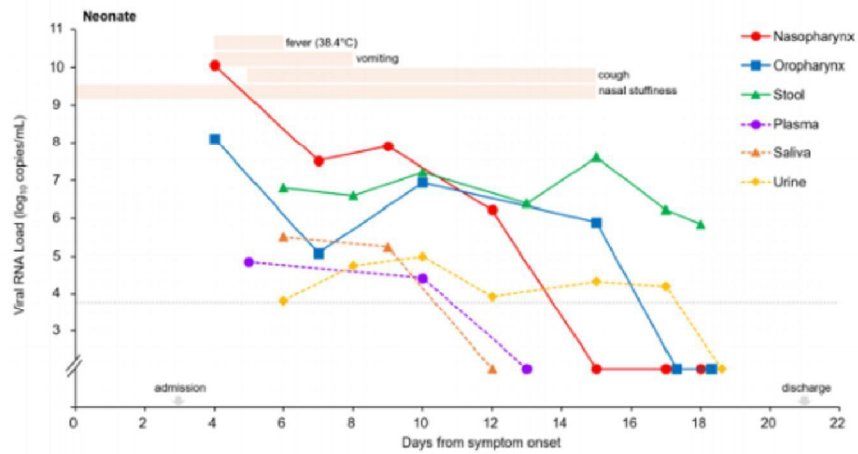




## Kinetiek SARS-CoV-2 detectie in diverse monstertypen



Lou et al. medRxiv

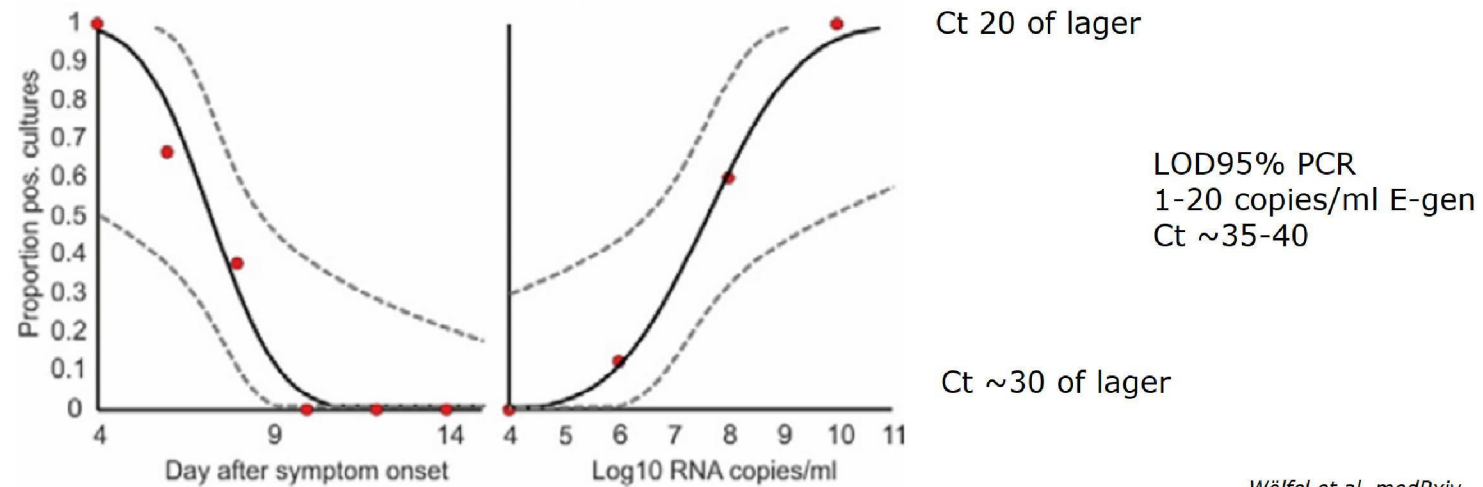


Mi Seon et al. CID



## Relatie PCR positiviteit met besmettelijkheid

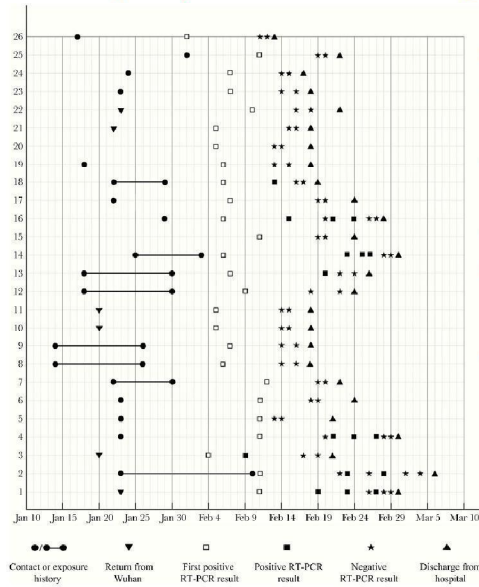
- Aanname – kweekbaarheid virus indicatie voor besmettelijkheid



*Wölfel et al. medRxiv*

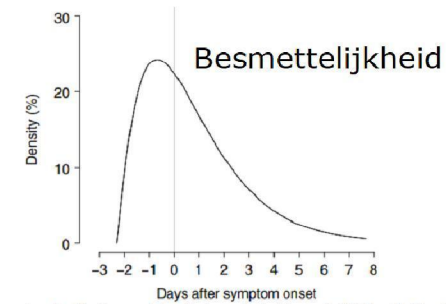
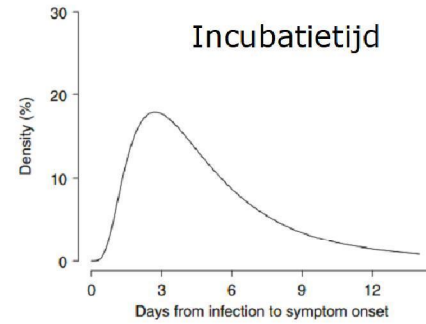


## Asymptomatische gevallen



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

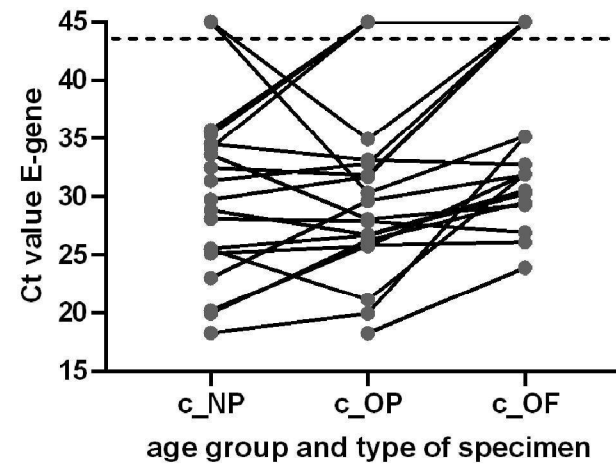
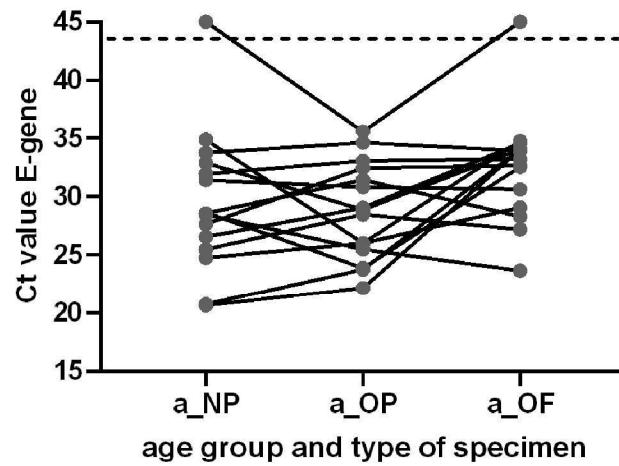
*Pan et al. J Infect Dis, jiaa205*



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



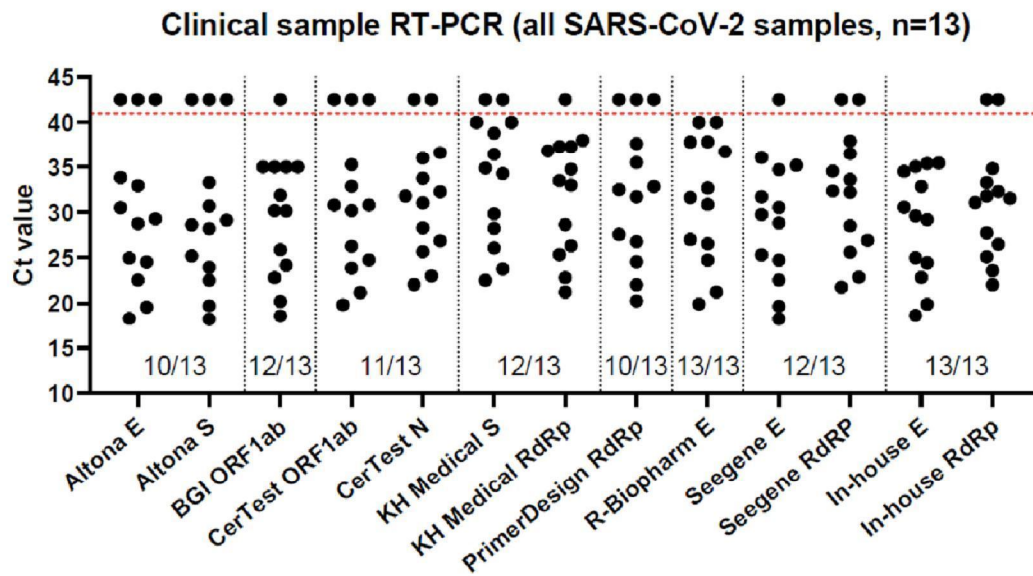
## Speeksel alternatief? FFX studie



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



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



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

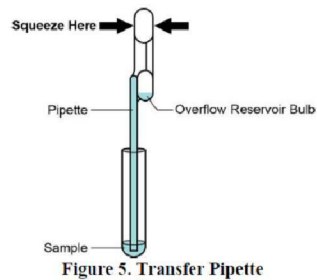


Figure 5. Transfer Pipette



- 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

| Patient ID                     | Views                        | Test Result  | Analyte Result | Detail | Errors         | History     | Support |
|--------------------------------|------------------------------|--------------|----------------|--------|----------------|-------------|---------|
| S07                            | Result View<br>Primary Curve | Analyte Name | Ct             | EndPt  | Analyte Result | Probe Check | Result  |
| Sample ID                      |                              | E            | 27.1           | 445    | POS            | PASS        |         |
| Sensilutetspane07              |                              | N2           | 28.6           | 265    | POS            | PASS        |         |
|                                |                              | SPC          | 27.7           | 414    | NA             | PASS        |         |
| Assay: Xpert Xpress SARS-CoV-2 |                              |              |                |        |                |             |         |
| Version: 1                     |                              |              |                |        |                |             |         |
| Test Type: Specimen            |                              |              |                |        |                |             |         |
| Sample Type: Other             |                              |              |                |        |                |             |         |
| Other Sample Type:             |                              |              |                |        |                |             |         |
| Notes:                         |                              |              |                |        |                |             |         |
| Module Name: A3                |                              |              |                |        |                |             |         |
| Reagent Lot #: 09032           |                              |              |                |        |                |             |         |
| Start Time: 04/15/20 09:43:22  |                              |              |                |        |                |             |         |
| End Time: 04/15/20 10:32:16    |                              |              |                |        |                |             |         |
| Status: Done                   |                              |              |                |        |                |             |         |
| User: <None>                   |                              |              |                |        |                |             |         |

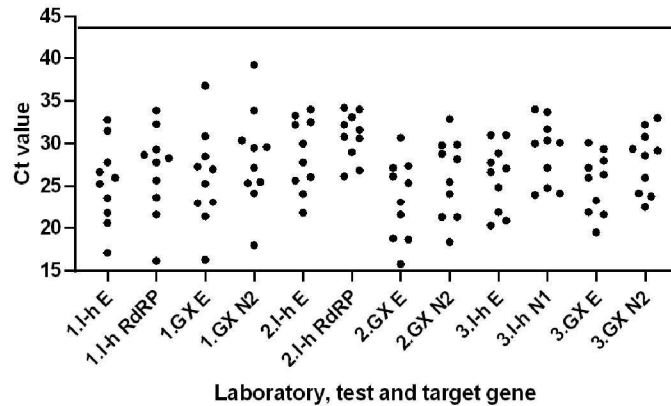
  

| Views                        | Legend   |
|------------------------------|--|
| Result View<br>Primary Curve | <input checked="" type="checkbox"/> E: Primary   |
|                              | <input checked="" type="checkbox"/> N2: Primary  |
|                              | <input checked="" type="checkbox"/> SPC: Primary |

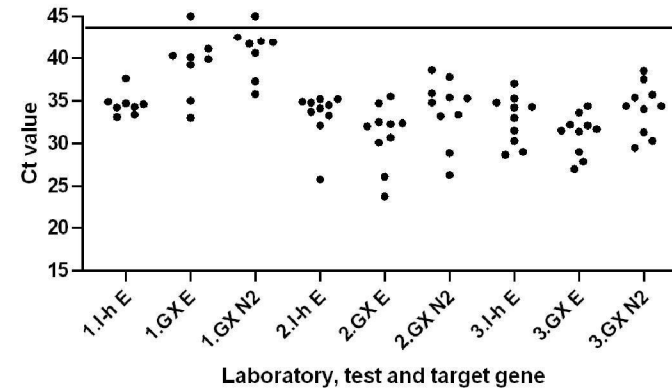


## Cepheid, Xpert® Xpress SARS-CoV-2 – clinical specimens

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

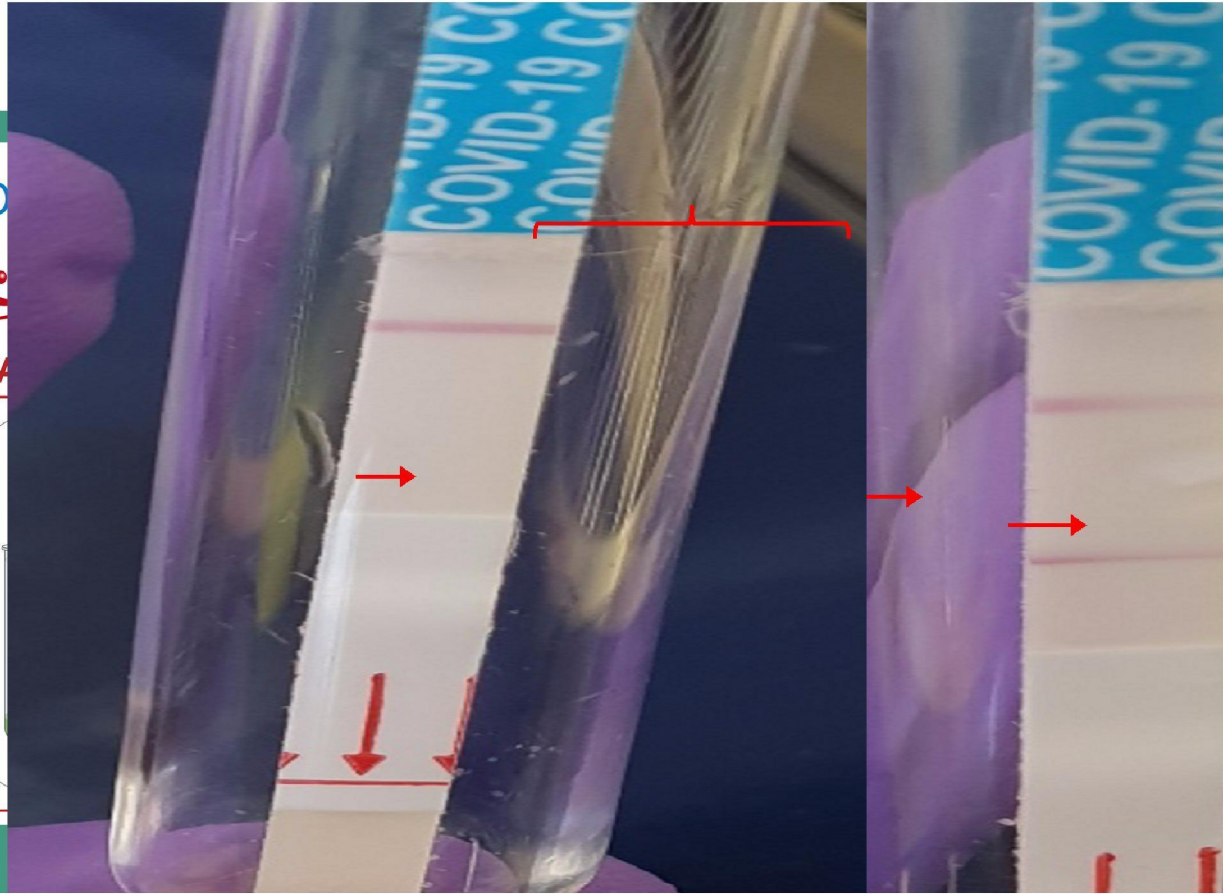
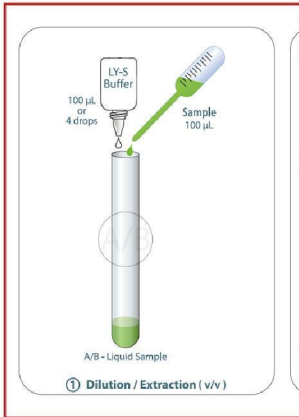


Low viral load (n=28)  
Positive for E-gene only



# CORIS Ag PO

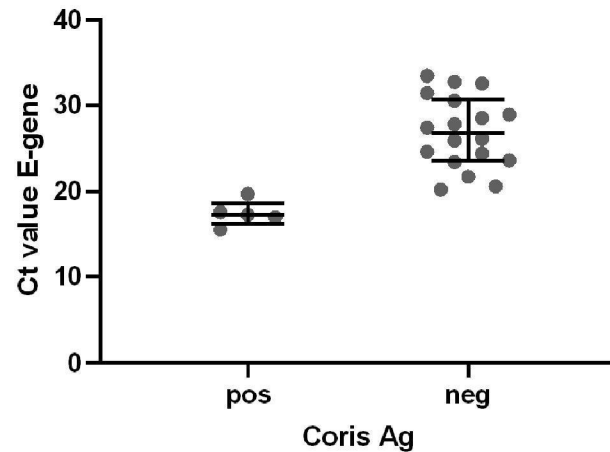
## COVID-19 A





## 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%.