SARS-CoV2 antigeen Diasorin

Validatie Microvida

19-01-2021

Indeling

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2. In house klinische validatie: Microvida locatie ETZ.
3. Teststraat klinische validatie: GGD teststraat Tilburg.

Technische validatie: methode

- Diluting a cell cultured SARS-CoV-2 strain:
  - (SARS hCoV-19/Netherlands/NoordBrabant_10003/2020 SARS-CoV-2)
  - median tissue cultured infectious dose (TCID50) of 5.62 x 10^7 per mL in 10-fold series (10^{-1} – 10^{-8})
  - In triplicate testing of both SARS-CoV2 Antigen assay using the following volumes of virus stock.
    - Diasorin antigen assay and Roche antigen assay performed on soaked nasopharynx swabs
    - Diasorin antigen assay performed on 1000 uL of the dilution series added to 1000 uL lysis buffer.
    - Diasorin antigen assay performed on 350 uL of the dilution series added to 1000 uL lysis buffer.
    - Roche antigen assay performed on 350 uL of the dilution series added to 320 uL of RRA extraction buffer.
  - Duplicate qRT-PCR (Allinity, abbott) on 500 µl of the dilution series, qRT-PCR was performed in duplex, the Ct-value is the mean

Technische validatie: resultaten

- SARS-CoV-2 Ag assays performed in triplicate on nasopharynx **swabs** soaked in each dilution.

Dilution step	TCID50/ml	Ct-value qRT-PCR*	Diasorin antigen (RLU signal range)	Roche antigen
10^-1	5,62E+06	10.23	3/3 (>100000)	3/3
10^-2	5,62E+05	13.17	3/3 (14436-16112)	3/3
10^-3	5,62E+04	16.75	3/3 (2247-2437)	3/3
10^-4	5,62E+03	20.20	3/3 (328-272)	3/3
10^-5	5,62E+02	24.33	0/3 (86-89)	0/3
10^-6	5,62E+01	28.02	0/3 (53-62)	0/3
10^-7	5,62E+00	31.21	0/3 (52-60)	0/3
10^-8	5,62E-01	35.32	0/3 (56-65)	0/3
blanc	0	>50	0/3	0/3

Technische validatie: resultaten

- SARS-CoV-2 Ag assays performed in triplicate on each dilution using two different volumes.

Dilution step	TCID50/ml	Ct-value qRT-PCR*	Diasorin (RLU signal range) 1000 uL	Diasorin (RLU signal range) 350 uL	Roche
10^-1	5,62E+06	10.23	3/3 (>100000)	3/3 (>100000)	3/3
10^-2	5,62E+05	13.17	3/3 (20926-22301)	3/3 (>100000)	3/3
10^-3	5,62E+04	16.75	3/3 (10753-12422)	3/3 (10753-12422)	3/3
10^-4	5,62E+03	20.20	3/3 (2319-2614)	3/3 (1524-1700)	3/3
10^-5	5,62E+02	24.33	3/3 (297-352)	1/3 (173-232)	3/3
10^-6	5,62E+01	28.02	0/3 (64-82)	0/3 (54-67)	0/3
10^-7	5,62E+00	31.21	0/3 (31-40)	0/3 (47-58)	0/3
10^-8	5,62E-01	35.32	0/3 (34-40)	0/3 (39-45)	0/3
blanc	0	>50	0/3	0/3	0/3

In house klinische validatie: Methode

- Persons presenting at the Municipal Health Service (GGD) test service received a combined oro-/nasopharyngeal swabs.
  - Suspended in 3mL GLY-medium
    - qRT-PCR performed on 500uL uGLY-medium (allinity, abbott)
    - Diasoring antigen assay performed on 1000uL GLY-medium suspended in 1000uL lysis buffer
      - Total suspension volume of original swab is 3mL GLY + 1000uL lysis buffer -> **1:4.**
      - **Inactivation period of 2-hours.**
- Cut-off positive samples of Diasorin antigen Assay: **200.**
- Comparing sensitivitiy specificity of Diasorin antigen assay with PCR:
  - Stratified for Ct-value:
    - Ct-value < 30
    - Ct-value < 25

In house klinische validatie: Resultaten

- A total of n=248 samples were included:
  - N=74 (29,9%) positive qRT-PCR for SARS-CoV-2 RNA.
  - N=174 (70,1%) negative qRT-PCR for SARS-CoV-2 RNA.

		qRT-PCR result			
		positive			negative
DAA	positive	54			0
		Ct > 30	Ct: 25-30	Ct <25	
		0	1	53	
	negative	20			174
		Ct > 30	Ct: 25-30	Ct <25	
		11	5	4	

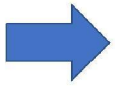
Specificity:
- 100%

Sensitivity:
- Overall: 72,9%
- Ct-value < 30: 85,7%
- Ct-value < 25: 92,9%.

In house klinische validatie: Resultaten

- Distribution of RLU results of the DAA of qRT-PCR positive and negative samples.

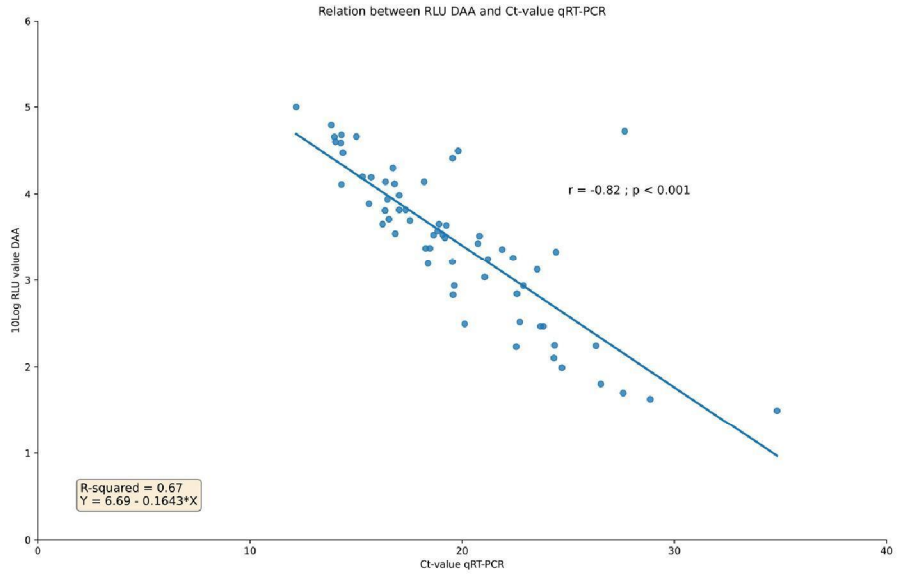
Current cut-off



RLU signal in DAA	qRT-PCR result	
	positive	negative
<22	11	156
22-40	1	17
40-60	2	1
60-80	1	0
80-100	1	0
100-120	0	0
120-140	1	0
140-160	0	0
160-180	3	0
180-200	0	0

In house klinische validatie: Resultaten

- Relation between the 10log transformed RLU values of the DAA and Ct-values of the qRT-PCR.



Teststraat klinische validatie: methode

- On location testing for COVID-19 using Diasorin antigen assay performed on the LIAISON XL.
- Two alterations in the procedure:
  - Combined oro-/nasopharyngeal swabs direct in 500uL GLY-medium + 500 uL lysis buffer. (according to manufacturers instruction)
    - Dilution swab **in 1mL instead of 4mL.**
  - Inactivation period of **30 minutes instead of 2 hours**.

Teststraat klinische validatie: methode

On site clinical validation: comparing PCR to Diasorin antigen test:

- After 30 minutes inactivation.
- After 2 hours inactivation. (subset)

Diluting samples 1:1 in GLY medium to asses the effect of sample dilution.

Teststraat klinische validatie: resultaten

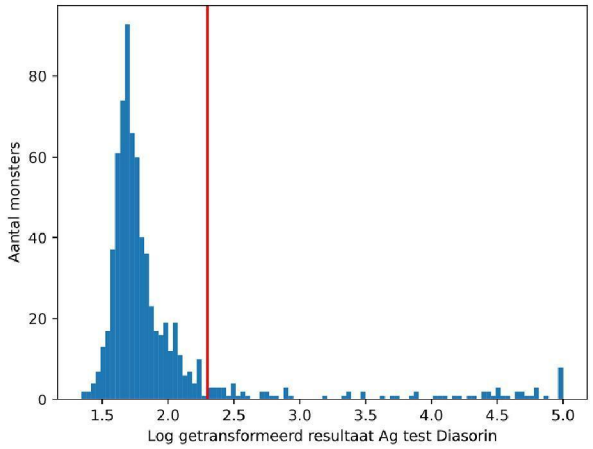
- A total of n=823 samples were included:
  - N= 90 (10,9%) positive qRT-PCR for SARS-CoV-2 RNA.
  - N= 733 (89,1%) negative qRT-PCR for SARS-CoV-2 RNA.
- After 30 minutes inactivation and using cut-off 200.

		qRT-PCR result			
		positive			negative
DAA	positive	58			23
		Ct > 30	Ct: 25-30	Ct <25	
		0	4	54	
	negative	32			710
		Ct > 30	Ct: 25-30	Ct <25	
		19	10	3	

Specificity:
- 96,7%

Sensitivity:
- Overall: 64,4%
- Ct-value < 30: 81,7%
- Ct-value < 25: 94,7%.

Teststraat klinische validatie: resultaten



VS

RLU signal in DAA	qRT-PCR result	
	positive	negative
<22	11	156
22-40	1	17
40-60	2	1
60-80	1	0
80-100	1	0
100-120	0	0
120-140	1	0
140-160	0	0
160-180	3	0
180-200	0	0

Teststraat klinische validatie: resultaten

- Increasing the Cut-off: 200 -> 300 -> 400.

200

Specificity:
- 96,7%

Sensitivity:
- Overall: 64,4%
- Ct-value < 30: 81,7%
- Ct-value < 25: 94,7%.

300

Specificity:
- 98,2%

Sensitivity:
- Overall: 63,3%
- Ct-value < 30: 80,3%
- Ct-value < 25: 93,0%.

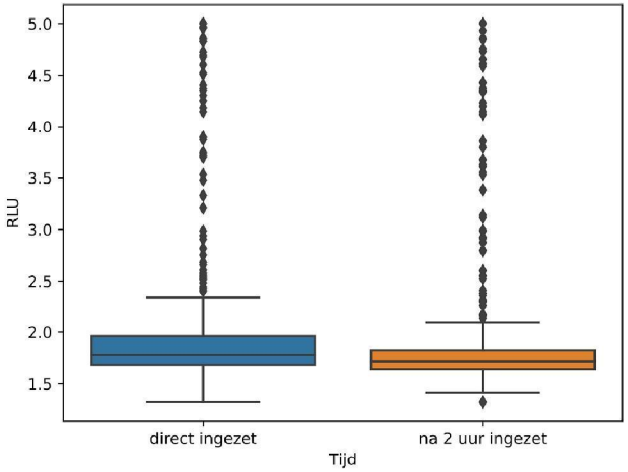
400

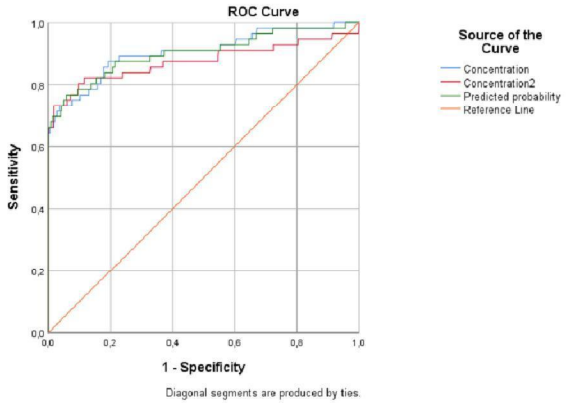
Specificity:
- 99,2%

Sensitivity:
- Overall: 62,2%
- Ct-value < 30: 78,9%
- Ct-value < 25: 93,0%.

Resultaten: verschil locatie ETZ en Teststraat

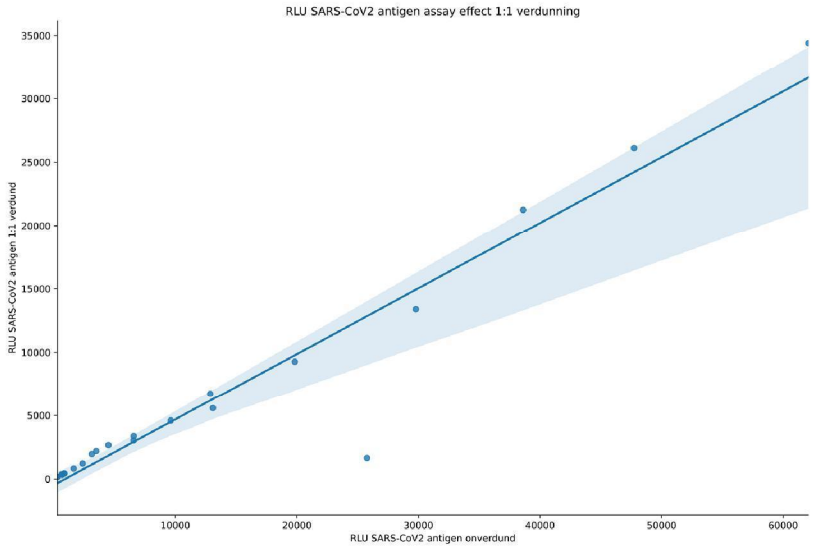
- Effect inactivation duration on measured RLU:
- A total of n=558 samples were included:
  - N= 56 (10,0%) positive qRT-PCR for SARS-CoV-2 RNA.
  - N= 502 (90,0%) negative qRT-PCR for SARS-CoV-2 RNA.





Resultaten: verschil locatie ETZ en Teststraat

- Effect sample dilution on measured RLU.



Discussie: heden in gebruik

- Currently
  - we are using a cut-off of 400.
    - To optimize specificity
  - Samples are inactivated for 30 minutes
    - Results are faster available
    - No clear benefit of increasing activation time given the current cut-off