-- CONFIDENTIAL --

Report on the evaluation of the BIOSYNEX COVID-19 Ag BSS test in mild symptomatic population.

Population/setting	Mild symptomatic population attending GGD/hospital testlanes for COVID-19 testing
Method	Chromatographic immunoassay
Assay	BIOSYNEX COVID-19 Ag BSS
Company	Biosynex, distributor Mediphos
Evaluation type	Clinical prospective field- and technical lab evaluation
Evaluation period	09-11-2020 through 18-12-2020
Test locations	Utrecht, UMCU Groningen, UMCG
Date	07-01-2021
Authors	5.1.2e | UMCU 5.1.2e 5.1.2e | UMCG 5.1.2e 5.1.2e | RIVM 5.1.2e | RIVM 5.1.2e 5.1.2e | RIVM

Introduction

The BIOSYNEX COVID-19 Ag BSS test is CE marked. The assay is a chromatographic immunoassay aimed at qualitative detection of specifically the SARS-CoV-2 antigen in the nasopharynx. The Ministry of Health Welfare and Sport (VWS) requests a validation of SARS-CoV-2 rapid antigen tests before procurement.

Method

The antigen test was clinically evaluated in a population consisting of mainly mild symptomatic cases attending testlocations in Utrecht and Groningen, the Netherlands. Participants were informed of the evaluation on site. Informed consent was requested for a second nasopharyngeal (NP) swab or combined NP and oropharyngeal (NP+OP) swab for the antigen test (as indicated in Tabel 1). The swabs for the antigen test was analyzed on site according to the IFU of the assay. Technicians worked in standard personal protection equipment. The swabs for RT-PCR were analyzed for regular PCR testing.

The antigen test was technically evaluated by diluting SARS-CoV-2 stock provided by Erasmus MC in 10-fold series (10^{-1} to 10^{-8}) viral transport medium (Mediaproducts B.V., Groningen, The Netherlands) with an end volume of 9 ml. The 10-fold series are vortex for 1 minute at room temperature. For each SARS-CoV-2 Rapid-Ag test, 350 µl from each dilution is added to the buffer supplied by the manufacture (n=3). After adding the dilution the procedure is follow as described in the prescription supplied by the manufacture.

Clinical prospective field evaluation

Sensitivity

The sensitivity of the assay with PCR as reference was 88.2% and 61.5% at the Groningen and Utrecht testlocations, respectively (Tabel 1). The tested population at the Utrecht location contained a relative high proportion (31%) of persons with high Ct values. Also used the Utrecht location a triple target PCR (E-, N- and RdRP-gene), whereas most labs only use the E-gene, resulting in very sensitive PCR results. The high proportion of persons with high Ct values are likely explained by 'old' infections. The sensitivity of the assay was correlated to viral load (indicated in this report by Ct values, Tabel 1). The sensitivity of the assay was 88.9% with Ct values below 32, 96.0% with Ct values below 30 and 82.8% when only E-gene PCR at the Utrecht testlocation.

Tabel 1. *Sensitivity and specificity of assay with PCR as reference test in different testlocations*

Location	Studypopulation	Sensitivity with PCR as reference (95%-BI)	Specificity with PCR as reference (95%-BI)	N
UMCG	mild symptomatic (NP+OP)	88.2 % (64.9 – 96.7%)	100 % (98.5 – 100%)	270 (17 PCR+)
UMCU	mild symptomatic (NP)	61.5 % (44.8 – 75.1%) Ct <32: 88.9 % Ct <30: 96.0% E-gen: 82.8 %	100 % (99.3 – 100%)	568 (39 PCR+)

Specificity

The assay had a specificity of 100% (Figure 2).

Technical lab evaluation

Limit of detection

The assay has a lower limit of detection at dilution 10^{-5}, corresponding with TCID50/ml of 3.16E+00 and 4.98E+02 E-gene Copies/ml (Tabel 2). The technical lab evaluation shows 4 levels of sensitivity, based on lower limit of detection and the signal strength of the test result (weak signals are indicated by ^). The sensitivity of the BIOSYNEX COVID-19 Ag BSS test was good (sensitivity level 1) compared to other SARS-CoV-2 Rapid antigen tests.

Table 2. *Results of the diluted SARS-CoV-2 stock read out. The dilution is done in triplicate in each SARS-CoV-2 Rapid-Ag test. Colored boxes show the categorization of the SARS-CoV-2 Rapid-Ag test in sensitivity levels.*

Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	
TCID50/ml	3.16E+04	3.16E+03	3.16E+02	3.16E+01	3.16E+00	3.16E-01	3.16E-02	3.16E-03	
E-gene Copies/ml	4.98E+06	4.98E+05	4.98E+04	4.98E+03	4.98E+02	4.98E+01	4.98E+00	4.98E-01	
Ct-value E-gene qRT-PCR	10.86	14.43	17.77	20.97	24.02	27.34	30.18	34.29	Sensitivity level:
Test A	(3/3)	(3/3)	(3/3)	(3/3)	(3/3) ^	(0/3)	(0/3)	(0/3)	
Test B	(3/3)	(3/3)	(3/3)	(3/3)	(3/3) ^	(0/3)	(0/3)	(0/3)	
Test C	(3/3)	(3/3)	(3/3)	(3/3)	(3/3) ^	(0/3)	(0/3)	(0/3)	1
Test D	(3/3)	(3/3)	(3/3)	(3/3)	(3/3) ^	(0/3)	(0/3)	(0/3)	
BIOSYNEX COVID-19 Ag BSS	(3/3)	(3/3)	(3/3)	(3/3)	(3/3) ^	(0/3)	(0/3)	(0/3)	
Test F	(3/3)	(3/3)	(3/3)	(3/3)	(0/3)	(0/3)	(0/3)	(0/3)	
Test G	(3/3)	(3/3)	(3/3)	(3/3)	(0/3)	(0/3)	(0/3)	(0/3)	2
Test H	(3/3)	(3/3)	(3/3)	(1/3)	(0/3)	(0/3)	(0/3)	(0/3)	
Test I	(3/3)	(3/3)	(3/3)	(3/3) ^	(0/3)	(0/3)	(0/3)	(0/3)	3
Test J	(3/3)	(3/3)	(3/3)	(0/3)	(0/3)	(0/3)	(0/3)	(0/3)	
Test K	(3/3)	(3/3)	(3/3)	(0/3)	(0/3)	(0/3)	(0/3)	(0/3)	4

^ These SARS-CoV-2 Rapid-Ag tests have a weak signal.

Ease of use

The assay has, compared to other SARS-CoV-2 rapid antigen test, some disadvantages concerning ease of use. The buffer is not pre-filled per tube. The tubes are relatively small in comparison to the swab, causing poor fit and need to remove the swab (with biosafety and contamination risks). The quality of the extraction tube and nozzle is not very good. The testkit does not contain a positive control (swab). The test is less suitable to perform high volumes of tests, as is the case for large testlanes.

Conclusion

Based on the data presented the assay is in agreement with the criteria proposed by WHO (*1*); sensitivity ≥80% and specificity ≥97% for detection of SARS-CoV-2 infected cases with RT-PCR as a reference at the Groningen testlocation. The overall sensitivity at the Utrecht testlocation was below 80%, however this is due to the relative large proportion of high Ct-value samples. The results of the technical lab validation shows good sensitivity (sensitivity level 1) of the assay. Since the

studypopulation and proportion high Ct-value samples seems to differ between validation studies, the technical lab evaluation is leading for the decision of procurement. The BIOSYNEX COVID-19 Ag BSS test can be procured by the Ministry of Health Welfare and Sport (VWS).

Reference

1. https://www.who.int/publications/i/item/advice-on-the-use-of-point-of-care-immunodiagnostic-tests-for-covid-19-scientific-brief