PERFORMANCE CHARACTERISTICS

Clinical Evaluation

Study I

Total of 61 positive and 105 negative serum or venous whole blood samples were collected at 4 different study sites. These samples were tested with both RT-PCR method for SARS-CoV-2 infection and Ecotest COVID-19 IgG/IgM Rapid Test device for antibodies. The obtained PPA/sensitivity and NPA/specificity results are summarized in following tables.

Table 1. IgG/IgM PPAfor the Ecotest COVID-19 IgG/IgM Rapid Test Device

Site	Days from symptom	# PCR Positive	IgG (Assure Device)			IgM (Assure Device)		
			Antibody Positive	PPA	95%CI	Antibody Positive	PPA	95%CI
(Site 1+3+4) Serum	0-7 days	8	7	87.5%	52.9%-97.8%	8	100%	67.6%-100%
	8-14 days	15	13	86.7%	62.1%-96.3%	13	86.7%	62.1%-96.3%
	≥15 days	25	25	100%	86.7%-100%	21	84%	65.3%-93.6%
(Site 2) Venous Whole Blood	0-7 days	1	1	100%	20.7%-100%	1	100%	20.7%-100%
	8-14 days	3	3	100%	43.9%-100%	3	100%	43.9%-100%
	≥15 days	9	9	100%	70.1%-100%	9	100%	70.1%-100%

Table 2. IgG/IgM NPA for the Ecotest COVID-19 IgG/IgM Rapid Test Device

Site	# PCR Negative	IgG (Assure Device)			IgM (Assure Device)		
		Antibody Negative	NPA	95%CI	Antibody Negative	NPA	95%CI
(Site 1+3+4) Serum	96	96	100%	96.2%-100%	94	97.9%	92.7%-99.4%
(Site 2) Venous Whole Blood	9	9	100%	70.1%-100%	9	100%	70.1%-100%
Combined Sites (Serum + Blood)	105	105	100%	96.5%-100%	103	98.1%	93.3%-99.5%

The NPA/specificity of the Ecotest COVID-19 IgG/IgM Rapid Test Device for IgG/IgM is 99.04%.

Study II: Independent Clinical Agreement Validation

The COVID-19 IgG/IgM Rapid Test Device from Assure Tech. (Hangzhou) Co., Ltd. was tested on 2020-06-15 at the Frederick National Laboratory for Cancer Research (FNLCR) sponsored by the National Cancer Institute (NCI). The test was validated against a panel of previously frozen samples consisting of 30 SARS-CoV-2 antibody-positive serum samples and 80 antibody-negative serum and plasma samples. Each of the 30 antibody-positive samples was confirmed with a nucleic acid amplification test (NAAT) and both IgM and IgG antibodies were confirmed to be present in all 30 samples. The presence of antibodies in the samples was confirmed by several orthogonal methods prior to testing with the Ecotest COVID-19 IgG/IgM Rapid Test Device. The presence of IgM and IgG antibodies specifically was confirmed by one or more comparator methods. Antibody-positive samples were selected at different antibody titers.

All antibody-negative samples were collected prior to 2020 and include: i) Seventy (70) samples selected without regard to clinical status, "Negatives" and ii) Ten (10) samples selected from banked serum from HIV+ patients, "HIV+". Testing was performed by one operator using one lot of the Ecotest COVID-19 IgG/IgM Rapid Test Device. Confidence intervals for sensitivity and specificity were calculated per a score method described in CLSI EP12-A2 (2008).

For evaluation of cross-reactivity with HIV+, it was evaluated whether an increased false positive rate among antibody-negative samples with HIV was statistically higher than the false positive rate among antibody-negative samples without HIV (for this, a confidence interval for the difference in false positive rates was calculated per a score method described by Altman). The results and data analysis are shown in the Tables 3 and 4 below.

Table 3. Summary Results

Ecotest COVID-19 IgG/IgM Rapid Test Device		Comparator Method			Total
		Positive (IgM/IgG) +	Negative (IgM/IgG) -	Negative, HIV+	
Positive	IgM+/IgG+	27	0	0	27
	IgM+, IgG-	3	1	0	4
	IgM-, IgG+	0	0	0	0
Negative	IgM-/IgG-	0	69	10	79
Total (n=110)		30	70	10	110

Table 4. Summary Statistics

Measure	Estimate	Confidence Interval
IgM+ Sensitivity (PPA)	(30/30) 100%	(88.7%; 100%)
IgM- Specificity (NPA)	(79/80) 98.8%	(93.3%; 98.8%)
IgG+ Sensitivity (PPA)	(27/30) 90.0%	(74.4%; 96.5%)
IgG- Specificity (NPA)	(80/80)100%	(95.4%; 100%)
Combined Sensitivity	(30/30) 100%	(88.7%; 100%)
Combined Specificity	(79/80) 98.8%	(93.3%; 98.8%)
Combined PPV for prevalence = 5%	80.8%	(40.9%; 96%)
Combined NPV for prevalence = 5%	100%	(99.4%; 100%)
Cross-reactivity with HIV+	(0/10) 0% not detected	----------

Study III

Total of 42 positive and 113 negative fingerstick whole blood samples were collected and tested at 3 different POC sites. These samples were tested with both RT-PCR method for SARS-CoV-2 infection and Ecotest COVID-19 IgG/IgM Rapid Test device for antibodies. The PPA/sensitivity and NPA/specificity results are summarized in following tables.

Table 5. IgG/IgM PPA for the Ecotest COVID-19 IgG/IgM Rapid Test Device

Site	Days from symptom	# PCR Positive	IgG (Assure Device)			IgM (Assure Device)		
			Antibody Positive	PPA	95%CI	Antibody Positive	PPA	95%CI
(Site 1+2+3)	0-7 days	2	0	0%	0%-57.5%	2	100%	42.5%-100%
	8-14 days	12	10	83.3%	55.2%-95.3%	10	83.3%	55.2%-95.3%
	≥15 days	28	28	100%	91.2%-100%	25	89.3%	72.8%-96.3%

Site 1		IgG			IgM			IgG/IgM		
Days from symptom	# PCR positive	Antibody positive	PPA	95% CI	Antibody positive	PPA	95% CI	Antibody positive	PPA	95% CI
0-7 days	0	0	NA	NA	0	NA	NA	0	NA	NA
8-14 days	0	0	NA	NA	0	NA	NA	0	NA	NA
≥15 days	11	11	100%	80.3%-100%	10	90.9%	62.3%-98.4%	11	100%	80.3%-100%
Site 2		IgG			IgM			IgG/IgM		
Days from symptom	# PCR positive	Antibody positive	PPA	95% CI	Antibody positive	PPA	95% CI	Antibody positive	PPA	95% CI
0-7 days	2	0	0%	0%-57.5%	2	100%	42.5%-100%	2	100%	42.5%-100%
8-14 days	7	6	85.7%	48.7%-97.4%	7	100%	72.1%-100%	7	100%	72.1%-100%
≥15 days	9	9	100%	76.9%-100%	9	100%	76.9%-100%	9	100%	76.9%-100%
Site 3		IgG			IgM			IgG/IgM		
Days from symptom	# PCR positive	Antibody positive	PPA	95% CI	Antibody positive	PPA	95% CI	Antibody positive	PPA	95% CI
0-7 days	0	0	NA	NA	0	NA	NA	0	NA	NA
8-14 days	5	4	80%	37.6%-96.4%	3	60%	23.1%-88.2%	5	100%	64.9%-100%
≥15 days	8	8	100%	74.7%-100%	6	75%	40.9%-92.9%	8	100%	74.7%-100%

Table 6. IgG/IgM NPA for the Ecotest COVID-19 IgG/IgM Rapid Test Device

(Site 1+2+3)	# PCR Negative	IgG (Assure Device)			IgM (Assure Device)		
		Antibody Negative	NPA	95%CI	Antibody Negative	NPA	95%CI
Combined Sites	113	113	100%	97.7% -100%	113	100%	97.7%-100%

Site 1	IgG			IgM			IgG/IgM		
# PCR negative	Antibody negative	NPA	95% CI	Antibody negative	NPA	95% CI	Antibody negative	NPA	95% CI
20	20	100%	88.1%-100%	20	100%	88.1%-100%	20	100%	88.1%-100%
Site 2	IgG			IgM			IgG/IgM		
# PCR negative	Antibody negative	NPA	95% CI	Antibody negative	NPA	95% CI	Antibody negative	NPA	95% CI
53	53	100%	95.1%-100%	53	100%	95.1%-100%	53	100%	95.1%-100%
Site 3	IgG			IgM			IgG/IgM		
# PCR negative	Antibody negative	NPA	95% CI	Antibody negative	NPA	95% CI	Antibody negative	NPA	95% CI

40	40	100%	93.7%-100%	40	100%	93.7%-100%	40	100%	93.7%-100%

The NPA/specificity of the Ecotest COVID-19 IgG/IgM Rapid Test Device for IgG/IgM in fingerstick whole blood samples is 100%.

Cross Reactivity

There was no cross-reactivity with plasma specimens meeting the disease state shown below. No IgM or IgG false positive results were observed with the following potential cross-reactants:

Table 7. Cross-reactivity Study Data of Ecotest COVID-19 IgG/IgM Rapid Test Device

Conditions	Number of samples	Conditions	Number of samples
Anti-HAV IgM +	5	Lyme disease+	5
Anti-HEV IgG +	2	P. falciparum +	5
HBsAg +	5	P. vivax +	5
Anti-HCV +	5	Toxoplasma IgM +	5
Anti-HIV +	5	HAMA +	1
Anti-Rubella IgM +	5	RF +	5
Anti-CMV IgM +	5	ANA+	5
Anti-HSV-I IgM +	5	Anti-Influenza A IgM +	3
Anti-HSV-II IgM +	5	Anti-Influenza B IgM +	1
EBV IgM +	4	Anti-RSV IgM +	3
Anti-Dengue IgM +	5	Legionella pneumophila IgM+	2
Anti-Yellow fever +	5	Anti-Adenovirus IgM +	1
Anti-Zika IgG +	5	Anti-Mycoplasma pneumonia IgM +	3
Chagas Ab+	5	Anti-Chlamydia pneumonia IgM +	3
Anti-Syphilis IgG +	4	Anti-Chlamydia pneumonia IgG +	2
Anti-Tuberculosis +	5	Measles IgG +	1
Typhoid IgM +	5	Mumps IgG +	1

Interfering Substances

The assay performance of COVID-19 IgG/IgM Rapid Test Device is not affected by substances at concentrations listed below.

Table 8. Interference Study Data of Ecotest COVID-19 IgG/IgM Rapid Test Device

Interfering substances	Concentration of analyate
Blood analytes	
Albumin	5 g/dL
Anticoagulants	
EDTA (sodium salt)	3.4 µmol/L
Heparin	3000 U/L
Sodium citrate	5 mg/mL
Potassium oxalate	2 mg/mL
Abnormal blood sample	
Visual hemolysis (Hemoglobin)	20 g/dL
Icteric (Bilirubin)	5 mg/dL
Lipemic (Triglycerides)	500 mg/dL
Common medicines	
Acetylsalicylic acid	3.62 mmol/L
Ascorbic acid (Vitamin C)	342 µmol/L
Amoxicillin	206 µmol/L
Fluconazole	245 µmol/L
Ibuprofen	2425 µmol/L
Loratadine	0.78 µmol/L
Nadolol	3.88 µmol/L
Naproxen	2170 µmol/L
Paroxetine	3.04 µmol/L
Anti-malarial medicines	
Quinine	148 µmol/L
Anti-tuberculosis medicines	

Rifampicin	78.1 μmol/L
Isoniazid	292 μmol/L
Ethambutol	58.7 μmol/L
Common consumables	
Coffee (caffeine)	308 μmol/L
Alcohol (ethanol)	86.8 mmol/L