Unilabs	**Document index:** MF12A **Author:** P J Ford **Authorised by:** N Barrett

Title: Validation and Verification Form (SOP MP12)

Validation and Verification Number	Change Control Form Number

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Department:	Clinical Pathology	**Implementation Date:**	30/04/2020
Prospective / Concurrent / Retrospective Validation / Verification?		Verification	

1. Validation / Verification Title:
Implementation of the OnSite COVID-19 IgG/IgM Rapid Test (CTK Biotech) at Unilabs UK

2. Detailed Description of Validation / Verification:
SARS-CoV-2 belongs to the family of coronaviruses which are capable of causing illnesses ranging from the common cold to more severe respiratory disease. SARS-CoV-2 infections results in the so-called COVID-19 disease. The infected patients have a wide range of clinical symptoms ranging from fever, lethargy, dry cough to severe respiratory distress and death. Most patients recover without special treatment. Around 1 out of 6 patients who get COVID-19 become seriously ill and develop difficulty in breathing. Older people and those with underlying medical problems, like high blood pressure, heart problems or diabetes, are more likely to develop serious illness. Human to human transmission of the virus has been confirmed and occurs primarily via respiratory droplets from coughs and sneezes within a range of 6 feet. Viral RNA has also been found in stool samples from patients. It's possible that the virus can be infectious even during the incubation period but this remains to be shown. Currently, the laboratory method for detecting SARS-CoV-2 infection is reverse transcription-polymerase chain reaction (RT-PCR). However, this method requires sophisticated equipment and highly trained laboratory technicians. Moreover, viral load decreases rapidly 9 or 10 days after the onset of symptoms. During the acute phase of infection, the titre of IgM to SARS-CoV-2 rises rapidly and peaks around 2-3 weeks after the infection. SARS-CoV-2 specific IgG antibodies appear shortly after IgM and persist for months. It is unknown if SARS-CoV-2 infection and the presence of specific antibodies

leads to immunity or could potentially result in re-infection. Even so, SARS-CoV-2 specific antibodies may be useful markers for epidemiological surveys.

The OnSite COVID-19 IgG/IgM Rapid Test detects anti-SARS-CoV-2 IgG and IgM antibodies in human serum, plasma or whole blood. The Test is CE marked for use in Laboratory settings only and the Instructions For Use must be followed to retain result integrity.

Unilabs UK requires a serological assay to offer to its customers.

Unilabs UK will procure, verify and implement the OnSite COVID-19 IgM/IgG Rapid Test in its laboratories at Stephenson Way and 100a New Cavendish Street.

The OnSite COVID-19 IgM/IgG Rapid Test is a lateral flow chromatographic immunoassay. The test strip in the cassette consists of: 1) a coloured conjugate pad containing SARS-CoV-2 recombinant antigens with colloidal gold (SARS-CoV-2 conjugates) and a control antibody conjugated with colloidal gold, 2) a nitrocellulose membrane strip containing two test lines (G and M lines) and a control line (C line). The G line is pre-coated with antibodies for the detection of anti-SARS-CoV-2 IgG, the M line is pre-coated with antibodies for the detection of anti-SARS-CoV-2 IgM, and the C line is pre-coated with a control line antibody.

When adequate volume of specimen is dispensed into the sample well of the test cassette, the specimen migrates by capillary action along the cassette strip. Anti-SARS-CoV-2 IgG, if present in the specimen, will bind to the SARS-CoV-2 conjugates. The immunocomplex is then captured by the pre-coated antihuman IgG, forming a coloured G line, indicating an anti-SARS-CoV-2 IgG positive test result, suggesting a recent or past infection. Anti-SARS-CoV-2 IgM, if present in the specimen, will bind to the SARS-Cov-2 conjugates. The immunocomplex is then captured by the pre-coated anti-human IgM, forming a coloured M line, indicating an anti-SARS-CoV-2 IgM positive test result and suggesting an acute SARS-CoV-2 infection. An IgM and IgG double positive result suggests a late acute infection.

Absence of any test lines (G or M) suggests a negative result. Each test contains an internal control (C line). If the C line does not develop, the test result is invalid, and the specimen must be retested with another device.

All work undertaken will be performed at the CL2 laboratories and within MSC (microbiology safety cabinet) at the laboratories at Stephenson Way and 100a New

Cavendish Street. The current guidance for laboratories (link below) will be followed for all actions:

https://www.gov.uk/government/publications/wuhan-novel-coronavirus-guidance-for-clinical-diagnostic-laboratories/wuhan-novel-coronavirus-handling-and-processing-of-laboratory-specimens

The test shall be offered to our clients as a test to help confirm if a patient has previously had COVID-19, not as a diagnostic test for active infection.

3. User Requirements:

To verify the CTK Biotech performance claims for the OnSite COVID-19 IgM/IgG Rapid Test.

The Information for Users (IFU) claims:

- Liquid velocity: not less than 10 mm/min
- Sensitivity: 100% with standard control specimens
- Accuracy: positive and negative 100% with 3 replicates of QC
- Intra-lot Precision: 100% for 10 replicates of 2 positive controls. No significant difference in signal intensity; 100% for 10 replicates of negative control. No significant difference in signal intensity.
- Inter-lot Precision: 100% for 10 replicates from 3 continuous lots of 2 positive controls. No significant difference in signal intensity; 100% for 10 replicates from 3 continuous lots of negative QC. No significant difference of signal intensity.
- Interference: none for bilirubin (up to 15 mg/dL), triglycerides (up to 400 mg/dL), haemoglobin (up to 20 g/dL), rheumatoid factor (up to 3250 IU/mL).
- Cross-reactivity: no false positives for 5 patients who were COVID-19 negative but presented with similar clinical symptoms. 5 specimens each with the following disease states were tested and showed no significant cross-reactivity: BHV, HCV, HIV, pneumonia, mycoplasma, tuberculosis, syphilis, dengue and pneumonia chlamydia.
- Clinical performance: 126 specimens in total compared with a PCR kit (85 positive + 41 negative).
    - IgG sensitivity 98.82% (95% CI: 93.63%-99.79%)
    - IgG specificity 100% (95% CI: 1.43%-100%)
    - IgM sensitivity 88.24% (95% CI: 79.68%-93.48%)
    - IgM specificity 100% (95% CI: 86.01%-95.63%)

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Prepared by (name / signature / date):	**Jonathan Upton 29/04/2020**

4. Impact / Risk Assessment:

1. Impact assessment of change:

   The new Onsite COVID-19 IgG/IgM rapid Test will be implemented using existing processes and procedures but with some extra steps. Following verification of processes, the new test should be able to function as per manufacturer's recommendations.

Consequence score: High impact on the laboratory's function -	5
Likelihood score: This will probably never happen/recur -	2
Impact / risk score: Consequence score x Likelihood score (5x2) -	10 'Medium'

2. Requirements as a result of change:

   a) Formal acceptance by Unilabs management that the new reporting process are fit for purpose (e.g. safe, correct design, materials are deemed suitable for Unilabs' business purposes).
   b) Verification of all laboratory examination procedures, equipment and instruments that are transferred to the laboratory for use according to the latest version of the standard operating procedure (SOP): MP012 'Validation and Verification'. Details of this should be maintained with the examination procedures, equipment and instruments in question and cross referenced within this document.
   c) Health and Safety authorisation according to relevant Unilabs policies and procedures.

Compliance requirements in accordance with ISO15189:2012 standards.

Prepared by (name / signature / date):	**Jonathan Upton 29/04/2020**

5. Verification Master Plan (VMP):

The purpose of this verification is to determine if the Onsite COVID-19 IgM/IgG Rapid Test is suitable for use at Unilabs UK.

- To determine the intra-assay precision, one known SARS-Cov-2 PCR positive sample was run 8 times in one day.

- To determine the inter-assay precision, one known SARS-CoV-2 PCR positive sample was run 3 times over 5 days.
- To determine accuracy qualitatively, a method comparison study was undertaken comparing the ONSITE test with the ISIA test using SARS-CoV-2 positive patients.
- To determine if line intensity and clarity is dependent on antibody concentration, 2 serial dilations will be performed with a known positive sample and 2 different diluents separately – a known negative sample, and 0 calibrator for immunoglobulins with a know concentration of 0 g/L for IgG and IgM.
- To determine the analytical specificity of the assay, 134 negative samples from Barking, Havering and Redbridge Hospital University NHS Trust were obtained. These samples were collected prior to 2019 for Downs screening and have been made available for research purposes. In addition, 20 PCR SARS-CoV-2 negative but other corona virus or common influenza virus PCR positive samples were obtained from Unilabs SA Laboratoire Central de Suisse Romande for testing for potential cross-reactivity.
- To determine the clinical sensitivity of the kit, 25 SARS-CoV-2 PCR positive serum samples were obtained from patients within a time frame of <14 days from the PCR test. These have been designated as acute samples. Also, 19 SARS-CoV-2 PCR positive serum samples were obtained from patients after a time frame of >14 from the initial PCR test. These have been designated as convalescent samples.

All samples used in the study were collected in plain blood collection tubes or gel barrier collection tubes and were centrifuged to obtain the serum. All samples were either stored at 2-8°C and then frozen at -20°C long term.

Clinical sensitivity was calculated as (TP/TP+FN)x100 with a two-sided Wilson 95% confidence interval (CI 95%). Sensitivity will be calculated for the IgG, IgM and IgG/IgM (both, either, or).
Clinical specificity was calculated as (TN/TN+FPx100) with a two sided Willson 95% confidence interval (CI 95%). Specificity will be calculated for the IgG, IgM and IgG/IgM (booth, either, or).
All calculations will be made at https://www.medcalc.org/calc/diagnostic_test.php

Prepared by (name / signature / date):	**Jonathan Upton 29/04/2020**

6. Report

Precision
Intra-assay precision

The results showed concordant responses from 8 replicates run on the same day. No apparent variation in line intensity was observed (Table 1).

Inter-assay precision

The results from the inter-assay precision study are ongoing. 3 samples, negative, faint IgG, IgG reactive and faint IgM will be analysed over 5 days.

Table 1. Intra-assay precision. 8 replicates of a positive result analysed 8 time in one day.

				Results		
PCR date	Sample date	Date Tested	Lab No.	Control Line	IgM	IgG
22-Mar	01-Apr	08/04/2020	1	Reactive	Faint	Reactive
22-Mar	01-Apr	08/04/2020	2	Reactive	Faint	Reactive
22-Mar	01-Apr	08/04/2020	3	Reactive	Faint	Reactive
22-Mar	01-Apr	08/04/2020	4	Reactive	Faint	Reactive
22-Mar	01-Apr	08/04/2020	5	Reactive	Faint	Reactive
22-Mar	01-Apr	08/04/2020	6	Reactive	Faint	Reactive
22-Mar	01-Apr	08/04/2020	7	Reactive	Faint	Reactive
22-Mar	01-Apr	08/04/2020	8	Reactive	Faint	Reactive

Accuracy

At the time of verification, no suitable standard or external quality control (EQA) material was available to assess bias. Method comparison studies (ONSITE CTK compared to ISIA) in patients with COVID-19 disease (23 pairs were available) gave 87% and 78% concordance for IgG and IgM, respectively in a head-to comparison (Table 2).

Table 2. Method comparison study. ISIA versus ONSITE.

ISIA IgG	**ONSITE IgG**	**ISIA IgM**	**ONSITE IgM**
(+)	POS	POS+	FAINT
NEG	NEG	NEG	NEG
NEG	NEG	NEG	NEG
NEG	NEG	NEG	NEG
(+)	POS	(+)	POS
NEG	NEG	NEG	NEG
NEG	NEG	NEG	NEG
(+)	FAINT	(+)	FAINT
NEG	FAINT	(+)	FAINT
NEG	NEG	(+)	FAINT

(+)	POS	NEG	FAINT
POS+++	FAINT	(+)	FAINT
POS++	POS	POS++	POS
POS++	POS	(+)	POS
POS+	POS	(+)	FAINT
NEG	FAINT	(+)	FAINT
(+)	FAINT	NEG	FAINT
(+)	POS	NEG	FAINT
POS+	POS	NEG	FAINT
(+)	NEG	(+)	FAINT
POS+++	POS	POS+	FAINT
POS++	POS	(+)	FAINT
(+)	FAINT	NEG	FAINT

(+) denotes faint positivity with the ISIA device.

Linearity

The result from the dilution studies (Table 3) showed that the development of the line in the reader window is independent of sample concentration. Using either negative sample or 0 calibrator as diluents gave similar intensity and clarity of line throughout the different relative concentration ranges.

Table 3. Dilution recovery studies.

Sample no.		1	2	3	4	5
	Positive Sample Vol. µL	100	75	50	25	0
	Negative Sample Vol. µL	0	25	50	75	100
Results	Control	Reactive	Reactive	Reactive	Reactive	Reactive
	IgG	Reactive	Reactive	Reactive	Reactive	Negative
	IgM	Faint	Faint	Faint	Faint	Negative
Sample no.		1	2	3	4	5
	Positive Sample Vol. µL	100	75	50	25	0
	0 Calibrator Ig Vol. µL	0	25	50	75	100
Results	Control	Reactive	Reactive	Reactive	Reactive	Reactive
	IgG	Reactive	Reactive	Reactive	Reactive	Negative

	IgM	Faint	Faint	Faint	Faint	Negative

Analytical Specificity/ Cross-reactivity

Of the 20 potentially cross-reactive samples, all gave negative results for COVID-19 IgG and IgM (Table 4). Of the 134 negative Downs screening samples, all gave negative results for IgG and IgM.

Table 4. Cross-reactivity with closely related viruses.

Organism	**n**
INFLUENZA A	6
INFLUENZA AH3	1
CORONAVIRUS NL63	3
CORONAVIRUS HKU1	8
CORONAVIRUS 229E	1
CORONAVIRUS OC43	1

Clinical Specificity and Sensitivity

Acute Presentation

Of the 25 acute samples tested, 10 were IgG positive, 13 IgM positive and 13 IgG or IgM positive. The results are concordant with ELISA assays (EuroImmun and Epitope kits). The clinical performance in this group is shown in Table 5. The overall specificity is 100% with a sensitivity of 52%.

Table 5. Clinical performance of the ONSITE kit during acute presentation.

	Clinical Performance IgG		Clinical Performance IgM		Clinical Performance overall (IgG and IgM combinations)	
Statistic	Value	95% CI	Value	95% CI	Value	95% CI
Sensitivity	40.00%	21.13% to 61.33%	52.00%	31.31% to 72.20%	52.00%	31.31% to 72.20%
Specificity	100.00%	97.63% to 100.00%	100.00%	97.63% to 100.00%	100.00%	97.63% to 100.00%
Positive Likelihood Ratio						

Negative Likelihood Ratio	0.6	0.44 to 0.83	0.48	0.32 to 0.72	0.48	0.32 to 0.72
Disease prevalence	13.97%	9.25% to 19.92%	13.97%	9.25% to 19.92%	13.97%	9.25% to 19.92%
Positive Predictive Value	100.00%		100.00%		100.00%	
Negative Predictive Value	91.12%	88.17% to 93.39%	92.77%	89.51% to 95.07%	92.77%	89.51% to 95.07%
Accuracy	91.62%	86.56% to 95.23%	93.30%	88.58% to 96.49%	93.30%	88.58% to 96.49%

Convalescent period

Of the 19 convalescent samples tested, 18 were IgG positive, 19 IgM positive and 19 IgG or IgM positive. The results are concordant with an ELISA assay (EuroImmun) except one IgG result which was found to be negative. The clinical performance in this group is shown in Table 6. The overall specificity is 100% with a sensitivity of 100%.

Table 6. Clinical performance of the ONSITE kit during convalescence.

	Clinical Performance IgG		Clinical Performance IgM		Clinical Performance overall (IgG and IgM combinations)	
Statistic	Value	95% CI	Value	95% CI	Value	95% CI
Sensitivity	94.74%	73.97% to 99.87%	100.00%	82.35% to 100.00%	100.00%	82.35% to 100.00%
Specificity	100.00%	97.63% to 100.00%	100.00%	97.63% to 100.00%	100.00%	97.63% to 100.00%
Positive Likelihood Ratio						
Negative Likelihood Ratio	0.05	0.01 to 0.35	0		0	
Disease prevalence	10.98%	6.74% to 16.62%	10.98%	6.74% to 16.62%	10.98%	6.74% to 16.62%

Positive Predictive Value	100.00%		100.00%		100.00%	
Negative Predictive Value	99.35%	95.81% to 99.90%	100.00%		100.00%	
Accuracy	99.42%	96.82% to 99.99%	100.00%	97.89% to 100.00%	100.00%	97.89% to 100.00%

Discussion

Precision and accuracy are both adequate for the ONSITE device. There is no apparent cross-reactivity with closely related viruses. The assay compares well with another assay (ISIA). Detailed method comparison studies with other devices and assays have been undertaken and the data is available on file pending further analyses.

The manufacturer claims a sensitivity of 98.8% (84/85) for IgG and 88.2% (75/85) for IgM. This is comparable with the results of this verification study for IgG (94.74%), IgM (100%) and IgG+IgM (100%) in convalescent patients where an immune response is expected. The result from the acute cohort of patients compared less well and is likely to be due to the early timing of sampling. It is important to note that the timing is based on the date on which the PCR test for SAR-CoV-2 was undertaken rather than the onset of symptoms.

The specificity was found to be 100% for IgG, IgM and IgG+IgM, which is comparable to the manufacture's claims of 100%.

It is interesting to note that IgM had a higher sensitivity (100%) than IgG (94.74%). This is likely attributable to the time of sampling and the clinical presentation of the patients, which remains unknown. Future studies should aim to acquire samples after a longer period of convalescence (>24 days) from multiple cohorts of patients with varying severity of symptoms (asymptomatic, mild and severe).

The laboratory has subscribed to the UKNEQAS COVID-19 antibody scheme to monitor performance and inter-laboratory agreement. At the present time, no third-party internal quality control (IQC) material is available for routine use in the UK. Unilabs will continue with post-marketing surveillance and procure a suitable IQC material when available. In the interim, pooled anonymised patient sera will be used.

Conclusion

Title: Validation and Verification Form (SOP MP12)

The Onsite COVID-19 IgG/IgM Rapid Test from CTK Biotech shows suitable performance for routine clinical use within the laboratory for the detection of immunoglobulins for SARS-CoV-2.

Clinical Reports

The possible combinations of reporting nomenclature are:

SARS-CoV-2 IgG Ab - Negative
SARS-CoV-2 IgM Ab - Negative

SARS-CoV-2 IgG Ab - Negative
SARS-CoV-2 IgM Ab - Positive

SARS-CoV-2 IgG Ab -Positive
SARS-CoV-2 IgM Ab - Negative

SARS-CoV-2 IgG Ab - Negative
SARS-CoV-2 IgM Ab - Negative

SARS-CoV-2 IgG Ab - Negative
SARS-CoV-2 IgM Ab - Indeterminate

SARS-CoV-2 IgG Ab - Indeterminate
SARS-CoV-2 IgM Ab – Negative

SARS-CoV-2 IgG Ab - Positive
SARS-CoV-2 IgM Ab - Indeterminate

SARS-CoV-2 IgG Ab - Indeterminate
SARS-CoV-2 IgM Ab - Positive

SARS-CoV-2 IgG Ab - Positive
SARS-CoV-2 IgM Ab - Positive

All clinical reports will have the following comments appended:

Negative: Antibodies not detectable. The absence of antibodies does not imply susceptibility to infection. Correlate results with the clinical presentation.

Indeterminate: the result was either borderline or unequivocal and therefore not possible to determine with certainty. Please repeat test.

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Positive: Antibodies detected. The presence of antibodies does not always imply immunity. Correlate results with the clinical presentation

The testing was performed using CTK Biotech Onsite COVID-19 IgG/IgM rapid Test. The testing is not currently UKAS accredited.

Signatures (name / signature / date):	
Prepared by:	**Jonathan Upton & Dr R.Sodi, 05/05/2020**
Authorised by QMT:	
Authorised by Laboratory Operations Director:	
Authorised by Medical Director / Consultant:	**Dr R.Sodi,05/05/2020**