Validation Report			
Title: Validation of Revital Viral Transportation Medium			
	NAME	**SIGNATURE**	**DATE**
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APPROVED BY	Isabella Ochola		25th August 2020

Laboratory section: VEC LAB	Validation report no.: 1/2020

Purpose: (Tick any of the following appropriately)

☐√**Initial validation** ☐Re-Validation ☐Other: _____

DESCRPTION OF THE EQUIPMENT/METHOD TO BE VALIDATED: (Describe the name of the method, test(s) to be validated/name and manufacturer of equipment/instrument)
Name of Equipment/Method: Revital Viral Transportation Medium
Equipment Appliance no.: N/A
Date of validation: 30th June – 18th August 2020

INTRODUCTION

This validation was carried out to verify the capability of the Revital Viral Transport Medium (VTM) to enhance and eventually lead to detection of the SARS-CoV-2 virus by molecular method using real time polymerase chain reaction (PCR) at KWTRP, Kilifi.

The Revital VTM are manufactured by Revital HealthCare (EPZ) LTD in compliance to ISO 13485: 2016, ISO 9001: 2015 and WHO-GMP.

The aim of this validation was to evaluate the performance of Revital viral transport medium (VTM) compared to that of KEMRI-VTM. This was done to help in informing whether Revital VTM could be used as a substitute for the KEMRI-VTM.

VALIDATION SCOPE

Assess the degree of concordance between the Revital VTM and that of the validated comparator, KEMRI VTM

RESPONSIBILITIES

Competent staff assigned to perform SARS-CoV-2 assay

VALIDATION PROTOCOL/METHODOLOGY

Validation Requirements

Procedure of sample analysis involved the use of SOPs below;

1. Extraction of RNA using LVEC 055
2. PCR assay using LVEC 057

Test Samples

- Nasopharyngeal and oral pharyngeal swabs (NP/OP) obtained from patients and people seeking COVID 19 test (n=19). These were collected in both Revital and KEMRI-VTM
- Revital VTMs spiked with 100ul of material containing known positive SARS-CoV-2 and known negative material (n=20) compared to data obtained from samples collected in KEMRI VTM.

Testing conditions to be used

Methodology:

1) Throat and nasal swabs were collected from 19 individuals seeking SARS-CoV-2 test as per sample collection SOP using VTM under validation and the validated KEMRI-VTM randomly.

2) Twenty Revital VTMs were spiked with 100 µl of SARS-CoV-2 positive sample with varying Ct value in the range of 22-36.79

5) RNA was extracted from all 36 samples using viral RNA extraction kit routinely used for detection of SARS-CoV-2 samples in the laboratory

6) SARS CoV-2 target genes were tested by real time PCR incorporating both known positive and negative controls.

Data to be collected

There were two sets of data that were collected for this validation, PCR results obtained from

1. Lab based: These were Revital VTMs spiked with known positive and known negatives. See appendix 1, date done 30th June 2020
2. Field based: These were obtained from patients/people from the specimen collection sites directly. See appendix 1, date done 3rd July to 17th August 2020

Results:

1. Detection of SARS-CoV-2 in spiked samples(n=20)

2. Detection of SARS-CoV-2 in directly collected samples (n=19)

Results Analysis: 2 × 2 contingency table

	Positive Comparative Method	Negative Comparative Method	Total
Positive Test Method	A	b	a+b
Negative Test Method	C	d	c+d
Total	a+c	b+d	N

Percent Agreement = 100% (a+d)/N

Agreement of test method with comparative method (Positive)=100% (a / a + c)

Agreement of test method with comparative method (Negative)=100% (d / b + d)

N = Total number of samples used for validation

Results 1: Spiked and Direct Samples (combined)

	KEMRI VTM Positive Comparative Method	KEMRI VTM Negative Comparative Method	Total
REVITAL VTM Positive Test Method	a (8)	b (1)	a+b (9)
REVITAL VTM Negative Test Method	c (4)	d (26)	c+d (30)
Total	a+c (12)	b+d (27)	N (39)

Agreement of test method (REVITAL VTM) with comparative method (Positive) = 100% (a / a + c)

= (8/12) *100
= 67%

Agreement of test method with comparative method (Negative)=100% (d / b + d)

= (26/27) *100
= 96.3%

See Appendices 1 for results

Acceptance Criteria

- Performance of method under Validation must show sensitivity and specificity of at least 65% and 90% agreement with the comparative method respectively.

Conclusion

(Tick any of the following appropriately)

- The results showed 67% and 96.3% concordance for sensitivity and specificity respectively when Revital VTM was compared with the KEMRI VTM which were within the expected acceptance criteria

☐√ Validation results acceptable ☐ validation results not acceptable

Comments:

Limitation

For spiked samples; viral replicability / recoveries from samples with late CT of >35 is not assured

References

1. **Priya Ranganathan, C. S. Pramesh, Rakesh Aggarwal:** Common pitfalls in statistical analysis: Measures of agreement. Perspective in Clinical Research. 2017 Oct-Dec; 8(4): 187–191. doi: 10.4103/picr.PICR_123_17

2. Jessica Watson GP, Penny F Whiting, John E Brush: Interpreting a covid-19 test result. BMJ 2020;369:m1808 doi: 10.1136/bmj.m1808 (Published 12 May 2020)

Appendix 1: Validation Results

Validation Results done30th June - 17th August 2020

	KEMRI-VTM			REVITAL -VTM		
Date Done	ID A	CT VALUE	RESULTS	ID B	CT VALUE	RESULTS
30-Jun-20	P-37	31.98	POS	P-37	35.39	POS
30-Jun-20	P-119	35.07	POS	P-119	Undetermined	NEG
30-Jun-20	P-41	Undetermined	NEG	P-41	Undetermined	NEG
30-Jun-20	P-163	35.66	POS	P-163	Undetermined	NEG
30-Jun-20	P-155	Undetermined	NEG	P-155	Undetermined	NEG
30-Jun-20	P-185	Undetermined	NEG	P-185	Undetermined	NEG
30-Jun-20	P-232	27.99	POS	P-232	Undetermined	NEG
30-Jun-20	P-305	33.38	POS	P-305	34.2	POS
30-Jun-20	P-367	33.8	POS	P-367	35.58	POS
30-Jun-20	P-248	Undetermined	NEG	P-248	Undetermined	NEG
30-Jun-20	P-279	Undetermined	NEG	P-279	Undetermined	NEG
30-Jun-20	P-351	Undetermined	NEG	P-351	Undetermined	NEG
30-Jun-20	P-418	36.79	NEG	P-418	Undetermined	NEG
30-Jun-20	P-336	Undetermined	NEG	P-336	Undetermined	NEG
30-Jun-20	P-372	Undetermined	NEG	P-372	Undetermined	NEG
30-Jun-20	P-487	22.77	POS	P-487	27.32	POS
30-Jun-20	P-427	Undetermined	NEG	P-427	Undetermined	NEG

30-Jun-20	P-515	32.88	POS	P-515	Undetermined	NEG
30-Jun-20	P-572	23.21	POS	P-572	26.97	POS
30-Jun-20	P-444	Undetermined	NEG	P-444	Undetermined	NEG
02-Jul-20	P26502_A	Undetermined	NEG	P26502_B	Undetermined	NEG
02-Jul-20	P26506_A	Undetermined	NEG	P26506_B	Undetermined	NEG
03-Jul-20	P27227	Undetermined	NEG	P27227B	Undetermined	NEG
29-Jul-20	37914A	Undetermined	NEG	37914B	Undetermined	NEG
29-Jul-20	37911A	Undetermined	NEG	37911B	Undetermined	NEG
29-Jul-20	37913A	Undetermined	NEG	37913B	Undetermined	NEG
29-Jul-20	37912A	Undetermined	NEG	37912B	Undetermined	NEG
29-Jul-20	37907A	Undetermined	NEG	37907B	Undetermined	NEG
29-Jul-20	37909A	Undetermined	NEG	37909B	Undetermined	NEG
29-Jul-20	37906A	Undetermined	NEG	37906B	Undetermined	NEG
29-Jul-20	37908A	Undetermined	NEG	37908B	Undetermined	NEG
29-Jul-20	37910A	Undetermined	NEG	37910B	Undetermined	NEG
30-Jul-20	P38786A	Undetermined	NEG	P38786B	Undetermined	NEG
30-Jul-20	P38787A	Undetermined	NEG	P38787B	Undetermined	NEG
30-Jul-20	P38788A	Undetermined	NEG	P38788B	Undetermined	NEG
08-Aug-20	41803 A	26.604	POS	41803 B	28.256	POS
08-Aug-20	41804 A	Undetermined	NEG	41804 B	34.981	POS
17-Aug-20	44776A	32.68	POS	44776C	33.94	POS
17-Aug-20	44777A	35.73	POS	44777C	32.86	POS

Appendix 2: Internal Quality Control per run

Run 103 30/Jun/20

CONTROLS	CT VALUE	VALIDITY
NC	Undetermined	Valid
NTC	Undetermined	Valid
PC1	26.95890236	Valid
PC 2	28.41980171	Valid

RUN 133 31/Jul/2020

CONTROLS	CT VALUE	VALIDITY
NC	Undetermined	Valid
NTC	Undetermined	Valid
PC1	25.57100296	Valid
PC 2	28.51928329	Valid

RUN 141 08/Aug/2020

CONTROLS	CT VALUE	VALIDITY
NC	Undetermined	Valid
NTC	Undetermined	Valid
PC1	24.98480797	Valid
PC 2	31.06588364	Valid

RUN 150 17/Aug/2020

CONTROLS	CT VALUE	VALIDITY
NC	Undetermined	Valid
NTC	Undetermined	Valid
PC1	22.18182373	Valid
PC 2	23.72153854	Valid

Disclaimers:

1. KWTRP's validation process does not approve / disapprove the kit design
2. KWTRP's validation process does not certify user friendliness of the kit / assay
3. Validation of a kit by KWTRP is not an assurance that the kit specifications would be included in the tendering process
4. This report is based on sampling done with the stated time period and takes into consideration limitations in the assays and has not control over how samples are collected which can impact the test result