Department of Health & Social Care

Validation Report for Horiba POCKIT

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Assay description

1. What is the principle and method of the assay (Description of the assay according to the manufacturer's Instructions For Use (IFU))

   The POCKIT ™ Central Nucleic Acid Analyzer) is a top sample-in-answer-out molecular detection system. It integrates magnetic bead-based nucleic acid extraction, fluorescence-based insulated isothermal PCR (iiPCR) amplification/detection, and liquid handling technologies to offer a walk-away protocol for nucleic acid detection. Results are displayed on the monitor in less than 1.5 hours; the data is automatically stored in an internal storage space and can be exported via a USB flash drive.

   Intended use: to provide qualitative detection of nucleic acid targets using fluorescence based iiPCR reagents for in vitro diagnostic use. The product is intended for professional use by properly trained laboratory technicians familiar with molecular biology techniques

2. Is the test a stand-alone device/test or to be used in conjunction with other devices/tests?

   The POCKIT™ Central Nucleic Acid Analyzer is intended to be used with the POCKIT ™ Central reagents.

Type of sample to be used in validation

1. Stipulate the sample type (extracted RNA, plasmid, organism) and any sample matrices (saliva, plasma, nasopharyngeal swab etc) in which material is to be spiked. If the material had been extracted stipulate i.e. volume received, volume extracted, volume eluted, volume used in assay. If possible stipulate if any preservatives likely to be present. Shelf life and number of freeze thaw events should also be stated if known. Where dry swabs are to be used, samples will need to be collected prospectively; two swabs per participant, one for new test and one to be tested using reference method; or one swab for new test collected within 24 hours of positive reference method swab.

   Residual VTM samples were used for analysis at Cumbria NHS Trust, Newcastle NHS Trust and Sheffield NHS Trust. Standard materials were used for LOD and performance characteristics analysis; supplied by National Medicines Laboratory (London, UK), based in INSTAND material.

Material ID	**Material Properties**	
G20161	SARS-CoV-2 i $\sim 10^{5}$ RNA copies/mL	
G20162	SARS-CoV-2 iii $\sim 10^{4}$ RNA copies/mL	
G20163	SARS-CoV-2 iii $\sim 10^{3}$ RNA copies/mL	
G20104	CoV-MERS	
G20109	hCoV NL63	
G20111	hCoV 229E	
G20113	hCoV OC43	
G20099	MRC-5	

Table 1. Standard materials

Equipment and reagents

List all equipment required that are supplied by the manufacturer with calibration/service dates where applicable

Product components

- POCKIT™Central reagents(including Extraction Cartridges and Reagent Cartridges)
- POCKIT™CentralNucleic Acid Analyzer×1 unit
- User manual× 1 copy
- Setup Tray ×1 piece
- Warranty card × 1 copy
- Transfer Cartridge×2 pieces(for transportation protection purpose)

Specifications

- Dimensions: 310mm(W) × 480mm(D) × 400mm(H)
- Net weight: 21kg
- Power supply: 100-120/200-240 V AC, 50/60 Hz, 2A
- Fuses: 2A, 250V AC, f 5 × 20 mm fast-acting, low breaking capacity glass tube fuses
- Operation altitude: Under 5,000 meters
- Operating condition: 15-35°C,up to 80% RH
- Transportation condition: -29-50˚C, 55±20% RH
- Storage condition: 10-40˚C, 55±25%RH

Testing Capacity

- Number of sample: 1 -8reactionsper run, per instrument
- Sample input volume:200µl per reaction
- List all reagents required that are not provided by the manufacturer with expiry dates and storage conditions. Include positive and negative control materials.
- 1000-µl micropipette and filter tips
- Disposable gloves
- Uninterrupted power system
- Biological safety cabinet or hood

Performance characteristics

Sensitivity and Linearity

1. Dilution series: Ideally, this should be calculated using a validated standard dilution series. If not possible (as standard material not available), use 5 clinical positive replicates, with a 5 log dilution, plus 5 negatives. If feasible, repeat over several days, different users/machines (feasibility may be limited due to availability of positive material). Where dry swabs are to be used, known amounts of standard material should be added to the swab, and then tested as per IFU.

Dilution	Pockit results	Pockit results	Pockit results	Pockit results	Cepheid results	Cepheid results	Cepheid results	Copies/ ml
	after light	before light	ratio	result	E ECt	N2 Ct	result	
Neat	1451	953	1.522	Positive	30.2	28.5	detected	$1.00x10^{7}$
1:2	1429	797	1.7916	positive	28.8	27.4	detected	$5.00x10^{6}$
1:4	1577	762	2.0694	positive	30.2	28.5	detected	$2.5x10^{6}$
1:8	1577	767	2.055	positive	30.1	29.6	detected	$1.25x10^{6}$
1:16	1466	739	1.9824	positive	32.9	30.5	detected	$6.25x10^{5}$
1:32	1457	771	1.8884	positive	0	31.7	detected	$3.13x10^{5}$
1:64	1488	800	1.8581	positive	36.2	36.2	detected	$1.56x10^{5}$
1:128	1408	591	2.3821	positive	38.2	33	detected	$7.81x10^{4}$
1:256	1253	688	1.8195	positive	36.3	34.2	detected	$3.91x10^{4}$
1:512	1280	730	1.7539	positive	0	37.4	detected	$1.95x10^{4}$
1:1024	1367	765	1.787	positive	37.8	36.1	detected	$9.77x10^{3}$

Dilution	Pockit results	Pockit results	Pockit results	Pockit results	Cepheid results	Cepheid results	Cepheid results	Copies/ ml
1:2048	1312	752	1.7453	positive	40.2	35.9	detected	$4.88x10^3$
1:4096	1367	766	1.7844	positive	0	41.8	detected	$2.44x10^3$
1:8192	719	794	0.9058	negative	0	39.7	detected	$1.22x10^3$
1:16384	1515	811	1.8671	positive	44.9	39.2	detected	$6.10x10^2$
1:32768	769	811	0.9487	negative	0	40.5	detected	$3.05x10^2$

Table 2. results from dilution series to determine LLOD

Material ID	Day 1	Day 1	Day 1	Day 2	Day 2	Day 2	Day 3	Day 3	Day 3
	run 1 result	run 2 result	run 3 result	run 1 result	run 2 result	run 3 result	run 1 result	run 2 result	run 3 result
SARS-CoV-2 i ~10^5 RNA copies/ mL	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
SARS-CoV-2 iii ~10^4 RNA copies/ mL	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
SARS-CoV-2 iii ~10^3 RNA copies/ mL	Positive	Negative	Negative	Negative	Positive	Negative	Positive	Positive	Negative

Table 3. results from repeated testing of standard materials

2. Linearity and Efficiency. Plot the data from 1. and calculate linearity and efficiency. Compare data with that supplied by the manufacturer, if applicable.

3. Although the assay gives a qualitative result, it also gives a numerical readout. This is not a linear relationship however; therefore, linearity and efficiency cannot be plotted.

4. Lowest Limits of Detection (LLOD). Where a validated standard dilution series was used LLOD should be calculated, using data from 2., in copies/ml (to align with MHRA TPP). Where clinical positive material is used, copies/ml cannot be calculated; median CT value should be given for the lowest dilution detected from the samples used in 1.

5. The company stated LLOD is $6x10^4$ copies/ml, which is outside the current POC TPP criteria of $1x10^4$ copies/ml (LLOD data is not stated within the IFU, but was provided via a powerpoint slide). Results from dilution series demonstrate detection down to 2440copies/ml. Testing of standard material demonstrate consistent detection at $1x10^4$ copies/ml with sporadic detec1tion at $1x10^3$ copies/ml. The LLOD is therefore outside the POC TPP (desirable = 100copies/ml, acceptable = 1000 copies/ml).

Precision and robustness

1. Intra-assay precision: Use the data for 5 replicate values from a single day from Performance Characteristics 1 to calculate Standard Deviation & Coefficient of Variation measurement, with the values for the latter to be <10%.

	Positive Control	**Positive Control**	**Positive Control**	**Positive Control**	**Negative control**	**Negative control**	**Negative control**	**Negative control**
Date tested	result	raw data - after light	raw data - before light	raw data - ratio	result	raw data - after light	raw data - before light	raw data - ratio
24/06/20	positive	1439	756	1.9032	negative	684	743	0.9209
25/06/20	positive	1650	787	2.097	negative	756	771	0.9802
26/06/20	positive	1273	856	1.4864	negative	786	787	0.9987
29/06/20	positive	1440	911	1.5813	negative	729	778	0.9369
30/06/20	positive	1702	904	1.8823	negative	718	747	0.9607
01/07/20	positive	1378	693	1.9885	negative	759	779	0.9745
03/07/20	positive	1546	862	1.7932	negative	725	758	0.9566
06/07/20	positive	1544	888	1.7394	negative	708	710	0.9966
07/07/20	negative	1672	888	1.883	negative	651	652	0.9989
25/06/20	positive	1460	889	1.6417	negative	794	794	1.001
26/06/20	positive	1751	937	1.8675	negative	840	846	0.9924
25/06/20	positive	1798	864	2.081	negativ	986	1067	0.9245

	Positive Control	**Positive Control**	**Positive Control**	**Positive Control**	**Negative control**	**Negative control**	**Negative control**	**Negative control**
					e			
26/06/20	positive	1459	917	1.5905	negative	992	1057	0.9392

Table 4. Results from positive and negative controls performed over successive days

Positive control for one day (26/06/20): mean 1.65; SD 0.20; CV 11.95%; UoM 23.43%
Negative control for one day (26/06/20): mean0.98; SD 0.03; CV 3.35%; UoM6.56%

2. Inter-assay precision: Use the data for 5 replicate values data from multiple days from Performance Characteristics 1 for Standard Deviation & Coefficient of Variation with the values for the latter to be <15%

3. Positive control on successive days: mean 1.81; SD 0.17; CV 9.26%; UoM 18.15%

4. Negative control on successive days: mean 0.97; SD 0.03; CV 2.90%; UoM 5.68%

5. Repeatability: Spike 30 negative samples with known amount of agent/positive material (suggested 3x the LLOD), all should be positive.

6. Data for this section is not available

Analytical specificity (Interferences and cross-reactions)

Cross-reactivity to non-target samples/organisms. A range of samples either direct clinical samples or spiked samples that are known positives for other diseases, both closely related (i.e., other coronaviruses), syndromic diseases (i.e., other respiratory viruses and bacteria) and common diseases (i.e. HIV, HBV, HCV, VZV, EBV, CMV) should be tested.

	Day 1	**Day 1**	**Day 1**
Material ID	run 1 result	run 2 result	run 3 result
CoV-MERS	Negative	Negative	Negative

Table 5. cross-reactivity check

No cross reactivity was seen to the organisms tested above

Diagnostic sensitivity and specificity (Clinical validation with confirmed positives and negatives)

1. Diagnostic sensitivity. Confirmed clinical samples from patients (positive PCR result) should be used. Preferably, depending on the availability of samples, ~150 samples should be included to align with MHRA TPP. Clinical sensitivity (95% CI) and positive predictive value (PPV) should be calculated in comparison with a CE reference method that itself has good sensitivity and specificity.

		Comparator RT-PCR	**Comparator RT-PCR**	**Comparator RT-PCR**
		Positive	Negative	Total
Pockit	Positive	156	7	163
Pockit	Negative	7	305	312
Pockit	Total	163	312	475

Table 6. Comparison with reference method (note prevalence 34.3%)

Sensitivity = 95.7% (95% CI 91.0-98.1%), this meets the acceptable criteria for sensitivity of the POC TPP (desirable>97% = acceptable = >80%). The CT values of the seven false negative samples were 21, 35, 29, 27.9, 36.6, 36.2 and unavailable for n = 1.

CT value range	**Positive on Pockit/positive on comparator**	**Sensitivity (%)**
≤25 (low)	45/46	97.8
25-<30 (medium)	53/55	96.4
≥30 (high)	16/20	80

CT range for 121/163 positive samples (CT values not available for 42 positive samples)

2. Diagnostic specificity. Confirmed clinical samples from patients (negative PCR result) should be used. Preferably, depending on the availability of samples, ~250 samples should be included to align with MHRA TPP. Clinical specificity (95% CI) and negative predictive value (PPV) should be calculated in comparison with a CE reference method that itself has good sensitivity and specificity.

3. Specificity = 97.7% (95% CI 95.2-99.0%), this meets the acceptable criteria for specificity of the POC TPP (desirable>99% = acceptable = >95%). Two samples that gave a false positive result at one centre were removed from the analysis as they repeated positive on a second comparator PCR, but with very late CT values that are potentially beyond the LLOD of the assay.

Summary

TVG uses a wide range of sites in order to validate new technologies/tests. These independent sites use a range of RT-qPCR assays against different genomic regions and it is recognised that for some assay comparisons the sensitivity of RT-qPCR assay(s) may subtly differ from the true sensitivity of the test if compared to the same genomic region. The POCKIT detects the ORF1ab genomic region, while several of the comparator assays used here detected sub genomic regions, such as E and N; this may slightly decrease the performance of the assay in question to that seen here.

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