COVID-19 Diagnostic Testing

NAT Technical Screening

Name of the device	BKIT VIRUS FINDER COVID-19
Manufacturer	HYRIS LTD
Application #	**315999**
Technology	**PCR**
Test Setting	**POC**
DED Screener	**Catherine Milley**

	Guidance	Acceptable	Comment
Device Description	**Type of Technology** **Instrumentation required** **Sample type/collection methods** **Testing setting:** Laboratory / Point of Care **Extraction methods** **Targeted sequence** **Sequences of Probes and primers** **Controls** (value assignment, supplied with kit) **Detection method**: potential for Biotin interference **Intended use assessed during review**	Deficient	**rRT-PCR, qualitative, POC** **Time to result: approx 2 hours per 8 patient specimens** **nasopharyngeal/ oropharyngeal swabs** **N1 and N2 sequences of the gene N; RP human gene** **The bKIT Virus Finder COVID-19 test is to be used with the bCUBE® 2 miniaturized thermal cycler (produced and sold by Hyris Ltd)** **Hardware (bCube):** **Statement provided regarding compliance with CAN/CSA 61010 electrical standards, and certificate from TUV provided** **Software:** **- bAPP multiplatform web software interface works online, on most operating systems (Windows, Linux, MacOS, iOS, Android). It allows to create and manage custom recipes or launch analyses which are shared among a selected work group (Swarm).** **- bPANEL software interface (for Windows PC / Tablet) allows the user to work totally offline, using recipes created with bAPP.** **Two approaches to extraction described: commercial RNA extraction kits, or direct amplification in bCUBE.** **DNA amplification can be obtained either via isothermal (LAMP) or thermal cycling (Real-Time PCR) protocols. Target DNA detection is obtained via optical measurement of the sample fluorescence, coming by specific fluorophores present in the analysis mixture** **Positive and negative controls are supplied with kit**
Limit of Detection	Spiking RNA / inactivated virus into clinical (preferred) or artificial matrix. The matrix should represent the most challenging clinical matrix. **Initial study** Dilution series including 3 replicates for each concentration. **Confirmatory study** 20 replicates of the final concentration. Acceptance criteria: 19/20 positive	Deficient	LoD study does not follow recommended study format. They tested 6 concentrations, n=2 per concentration Matrix used was water; spiked with extracted RNA
Inclusivity	• Provide results of in silico analysis including the % identity to published COVID19 sequences. • 100% of the published sequences should be detectable.	Deficient	Not provided. Section labelled “inclusivity” does not provide this information
Cross-Reactivity (Exclusivity)	• Provide results of in silico analysis of primers and probes against: common respiratory flora, other viral infections • Wet testing is recommended • Cross-reactivity is defined as greater than 80% homology • Matrix-specific cross-reactivity should be assessed, • Exogenous/Endogenous interferents: these depend on sample type (blood, sputum, stool). The interfering substances studies are not required for the classic/well established PCR (RT-PCR) using respiratory specimens, however for newer molecular type of assays, such as various isothermal methods, testing of potential interferents will be required even for respiratory specimens. Can reference CLSI EP07.	Deficient	Nothing provided
Precision	Conduct internal precision testing (i.e., at the manufacturer’s site) in accordance with CLSI, EP5-A2. In	Deficient	Not provided

	the context of SAP, the 3x5x5 (3 instruments x 5 days x 5 replicates) design is acceptable to provide preliminary estimates of the repeatability (within run) and reproducibility of the assay. Full assessment of repeatability using the 20x2x2 (20 days × 2 run per day × 2 replicates) is expected at time of licensing.		
Stability	**Description of stability test plan** • reagent stability studies do not need to be completed at the time of IO issuance, however the study design should be agreed upon during review and the stability studies started immediately following authorization	Deficient	Not provided IFU states test kit can be stored for 12 months, and 3 freeze-thaw cycles.
Clinical Evaluation	Known positive samples or contrived clinical samples Minimum of 30 reactive and 30 non-reactive specimens • 20 samples at 1x-2x LoD (95% agreement) • Other concentrations and non-reactive (100% agreement)	Deficient	Not provided
Point of Care	**Near patient studies** performed in clinical setting by intended users. Minimum of 9 operators and questionnaire to assess IFU clarity.	Deficient	Not provided
Labeling	**Instructions for use** **Reagent labels** Intended Use Statement will be assessed during review	Deficient	Manual, quick start manual provided for instrument Package insert provided for test kit.
Quality	• **QMS certificate provided?** • **Evidence of lot release programme**	Deficient	Not provided

Preamble:
Ensure that actual study reports are provided when requested. It is generally not sufficient to state performance characteristics without providing supporting scientific evidence. Failure to provide the requested information may result in refusal of your application.

As a guide, the expected format for study summaries has been provided below the questions.

Questions:
You are asked to respond to all the questions in a single, comprehensive package, using a Question and Answer format with references to attachments, as needed. Your response should be submitted in a single e-mail communication; attachments can be included in a compressed zip file format.

1. Provide a complete device description, with details and rationale for its design, and for your selection of all reagents. Include a detailed description of all components, including their composition and source.
2. Describe all instruments required to perform the test, from sample collection to result. Provide details on the reaction settings required (temperature, time). As you have identified that both isothermal and thermal cycling can be used, details are requested for both approaches.
3. Provide a detailed description of all controls used with the kit (e.g. negative control, positive control, internal control), including a rationale for their selection, and their source. Identify the specific sequences of targets, primers and probes, where relevant. Describe the recommended frequency of use, thet results expected and the acceptance criteria. Ensure you identify the concentration of the positive control relative to the LoD
4. Provide a clear description outlining the specimen types that can be used with the device, the extraction methods that are to be used for each, and the specimen volume required. Note that the evidence you provide in support of your device must include all labelled sample types, or you must provide evidence that these sample types are equivalent.
5. Provide a study report, or a detailed summary of methods and results, to support the claimed Limit of Detection (LoD)/analytical sensitivity. LoD can be determined by spiking RNA or inactivated virus into a clinical (preferred) or an artificial matrix. The matrix selected should represent the most challenging clinical matrix. The initial study requires a dilution series including 3 replicates for each concentration. The confirmatory study with 20 replicates of the final concentration is needed. Include in your responses a detailed description of the samples (live or inactivated virus, viral RNA) used in these studies, including their source. As you have identified that both isothermal and thermal cycling can be used, details are requested for both approaches.
6. Provide a description of your *in silico* analysis of inclusivity, including the database search parameters, the number of SARS-CoV-2 sequences analyzed, the date the analysis was performed, etc.. Provide a summary of the results , including the % identity to current published COVID19 sequences, a description of any mismatches and a discussion of their effect on the results of your assay..
7. Provide results of matrix-specific cross reactivity studies demonstrating that the following pathogens are not cross-reacting with the assay. *In silico* analysis and all currently available results of wet testing should be submitted. Note: For wet testing, concentrations of 10^6 CFU/ml or higher for bacteria and 10^5 pfu/ml or higher for viruses is

recommended.
Note: If *in silico* analysis reveals ≥ 80% homology between the cross-reactivity microorganisms and your test primers/ probe(s), we recommend that you perform a microbial interference study with SARS-CoV-2 and the microorganisms that your test primers/ probe(s) have homology to, or provide an appropriate scientific rationale which supports the clinical utility of your test given your results.

High priority pathogens from the same genetic family	High priority organisms likely in the circulating area
Human coronavirus 229E	**Adenovirus (e.g. C1 Ad. 71)**
Human coronavirus OC43	**Human Metapneumovirus (hMPV)**
Human coronavirus HKU1	**Parainfluenza virus 1-4**
Human coronavirus NL63	**Influenza A & B**
SARS-coronavirus	**Enterovirus (e.g. EV68)**
MERS-coronavirus	**Respiratory syncytial virus**
	Rhinovirus
	Chlamydia pneumoniae
	Haemophilus influenzae
	Legionella pneumophila
	Mycobacterium tuberculosis
	Streptococcus pneumoniae
	Streptococcus pyrogenes
	Bordetella pertussis
	Mycoplasma pneumoniae
	***Pneumocystis jirovecii* (PJP)**
	Pooled human nasal wash - *to represent diverse microbial flora in the human respiratory tract*
	Candida albicans
	Pseudomonas aeruginosa
	Staphylococcus epidermis
	Staphylococcus salivarius

8. Provide the study reports for interference testing of endogenous substances (whole human blood, human DNA) and of exogenous substances (common medications), or a scientific rationale for why these studies are not required. As you have identified that both isothermal and thermal cycling can be used, details are requested for both approaches.
9. Provide study reports for precision testing. Conduct internal precision testing (i.e., at the manufacturer's site) in accordance with CLSI EP5-A2. In the context of the Interim Order, the 3x5x5 (3 instruments x 5 days x 5 replicates) design is acceptable to provide preliminary estimates of the repeatability (within run) and reproducibility of the assay. Full assessment of repeatability using the 20x2x2 (20 days × 2 run per day × 2 replicates) is expected at time of authorization. As you have identified that both isothermal and thermal cycling can be used, details are requested for both approaches.
10. Stability (Shelf life and Shipping/transport stability)
Provide all evidence currently available supporting the stability of test kit, including sample stability. Alternatively, submit a plan for stability studies. Note that reagent stability studies do not need to be completed at the time of IO issuance, however the study design will be assessed during review of your submission, and we will require that the stability studies be started no later than immediately following authorization. Provide the claim you are making for stability of your device and how you arrived to this claim.
11. Provide the reports for any Clinical Performance Studies using known positive samples or contrived clinical samples. A minimum of **30 reactive and 30 non-reactive specimens** is needed. Validation of the reactive and non-reactive samples using a reference standard is needed, and details on the reference standard used must be provided (name and manufacturer). For reactive samples, 20 samples at 1x-2x LoD demonstrating 95% agreement is needed. Other concentrations and non-reactive samples should demonstrate 100% agreement. A statistical rationale for the sample size of the study should also be provided. As you have identified that both isothermal and thermal cycling can be used, details are requested for both approaches.
12. For tests intended to be use for point of care testing, a near patient study, performed in the intended use setting by intended users, is required. The study should be performed by a minimum of 9 operators, under the intended conditions of use. It should include a questionnaire to assess clarity of the instructions for use, the ability of the users

to understand and interpret the result and to operate the device, as well as the robustness of the device. As you have identified that both isothermal and thermal cycling can be used, details are requested for both approaches.

13. Provide labels for all kit components, including all reagents etc.
14. Provide evidence of current ISO 13485 or MDSAP certification, or equivalent for the bKit Virusfinder test kit.

Guide to study reports/summaries format

a) Study Title
b) Objectives
   - Provide a short description of the objective
c) Methodology
   - Sample type: description of the matrix
   - Number of samples tested (pos & neg)
   - Sample characterization: Name of assay or method used to characterize the samples
   - Testing algorithm: time-point, replicates, run, days, site, etc
d) Results
   - Tabular format whenever possible
   - Statistical analysis
   - Discrepant results (explanation and resolution)
   - Results for each setting and/or sample type
e) Conclusion
   - Clear conclusion supporting the performance claim
   - Rationale for any method deviations