# COVID-19 Diagnostic Testing

NAT Technical Screening

| | |
|---|---|
| Name of the device | ~~PRECISION BIOMONITORING INC~~. **TripleLock™ SARS-CoV-2 test strips** (name revised by Elana Cherry, June 2, 2020) |
| Manufacturer | PRECISION BIOMONITORING INC. |
| Application # | **313602** |
| DED Screener | **Catherine Milley** |

| | Guidance | Acceptable | Comment |
|---|---|---|---|
| Device Description | Intended use<br>Testing setting<br>Extraction methods<br>Targeted sequence<br>Probes and primers Sequences | Deficient | Appears to be a rRT-PCR kit, fluorescence based, using "<br>primers and probes targeting the E gene and UTR regions of the SARS-CoV-2-virus"<br><br>Inadequate description – information also references a different test, and that this one is a lyopholised version<br><br>See questions below |
| Limit of Detection | Spiking RNA / inactivated virus into clinical (preferred) or artificial matrix.<br>The matrix should represent the most challenging clinical matrix.<br><br>**Initial study**<br>Dilution series including 3 replicates for each concentration.<br>**Confirmatory study**<br>20 replicates of the final concentration.<br>Acceptance criteria: 19/20 positive | Deficient | See questions below |
| Inclusivity | • Provide results of in sillico analysis including the % identity to published COVID19 sequences.<br>• 100% of the published sequences should be detectable. | Deficient | See questions below |
| Cross-Reactivity | • Provide results of in silico analysis of primers and probes against: common respiratory flora, other viral infections<br>• Wet testing is recommended<br>• Cross-reactivity is defined as greater than 80% homology<br>• Matrix-specific cross-reactivity should be assessed | Deficient | See questions below |
| Precision<br>(This is not an essential requirement) | Conduct internal precision testing (i.e., at the manufacturer's site) in accordance with CLSI, EP5-A2. In 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. | Deficient | See questions below |
| Stability | • Briefly describe stability test plan<br>• 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 | Label states 1 year.<br>See questions below |
| Clinical Evaluation | Known positive samples or contrived clinical samples<br>Minimum of 30 reactive and 30 non-reactive specimens<br>• 20 samples at 1x-2x LoD (95% agreement)<br>• Other concentrations and non-reactive (100% agreement)<br><br>*Seroloical assay* | Deficient | See questions below |

| | | | |
|---|---|---|---|
| | Positive samples should include infection times of 4-10 days and 11-24 days | | |
| Point of Care | Near patient studies performed in clinical setting by intended users. Minimum of 9 operators and questionnaire to assess IFU clarity. | n/a | |
| Labeling | Instructions for use<br>Reagent labels | Y | Text provided for IFU, but not actual document (DSLD deficiency)<br><br>Labels provided. |

Note to DSLD: The device name needs to be corrected in MDS to “TripleLock SARS-COV-2 Test Strips”.

Questions:

Please provide the following information and scientific evidence. As a guide, the expected format for study summaries has been provided below the questions.

1. Provide a complete device description, with details on each component, and rationale for its design, and for your selection of reagents and buffers. Describe the extraction methods and materials, and PCR equipment that have been validated to work with your kit.
2. Provide a 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. Describe the the results expected and acceptance criteria. Ensure you identify the concentration of the positive control relative to the LoD
3. Provide a clear description outlining the specimen types that can be used with the device, and the extraction methods that are to be used for each. 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.
4. Describe the targeted sequences of the SARS-CoV-2 genome. Provide a list of all primers and probe sets and briefly describe what they detect, and include their nucleic acid sequences. Indicate if biotin-streptavidin/avidin chemistry is used in any steps of the test. You may include relevant supporting literature.
5. Provide the intended use, intended users, and the intended testing setting to be used with your device (lab, Point of Care).
6. 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 clinical (preferred) or artificial matrix. The matrix 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. A precise description (with the source and sequence) of the samples used in these studies are needed.
7. Provide the results of your *in silico* analysis of inclusivity, including the % identity to published COVID19 sequences..
8. 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 pyogenes*** |
| | ***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*** |
| | |

9. Provide the study reports for interference testing of endogenous substances (Hb, bilirubin, Proteins, TG, HAMA, RF, Total IgG, Total IgM), and of exogenous substances (common medications).
10. Provide the 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.
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. 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.
12. 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.
13. Provide the Instructions for Use that will accompany the kit.

Study format guide

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 method deviation

**June 2, 2020 – Review of Applicant's responses to screening questions, received on May 7, 2020**

| | |
|---|---|
| Name of the device | "TripleLock SARS-COV-2 Test Strips" |
| Manufacturer | PRECISION BIOMONITORING INC. |
| Application # | **313602** |
| DED Screener | **Elana Cherry** |

1. Provide a complete device description, with details on each component, and rationale for its design, and for your selection of reagents and buffers. Describe the extraction methods and materials, and PCR equipment that have been validated to work with your kit.

**Response:** The Precision Biomonitoring TripleLock™ SARS-CoV-2 test is a qualitative RT-qPCR test for detection of novel human coronavirus from clinical-collected nasopharyngeal swabs stored in viral transport media from patients who meet COVID-19 clinical and/or epidemiological criteria for testing. This test is appropriate for laboratory, hospital, clinic or point-of-care use as a diagnostic aid. This test requires the use of an RNA extraction kit (e.g. M1 RNA 2.0 prep kit) and qPCR thermocycler (e.g. Franklin three9 device), sold in Canada by Precision Biomonitoring.
The TripleLock™ SARS-CoV-2 tests is intended for use by registered healthcare professionals who have received specific training on the use of this test from Precision Biomonitoring only.
The Precision Biomonitoring TripleLock™ SARS-CoV-2 test is a multiplex RT-qPCR test designed for use with thermostable reagents that can be shipped, stored and run at room temperature.
To run the test an RNA extraction kit and qPCR thermocycler are also required. The M1 RNA 2.0 Sample Prep Kit (Biomeme Inc. catalogue number 3000536) and the Franklin three9 qPCR thermocycler (Biomeme Inc. catalogue number 1000003) are used for validation. Exact volume pipettes are suggested to simplify the transfer of correct volumes.
The RNA extracted can be run immediately. Each RNA extract is interrogated for three targets simultaneously: E Gene, UTR and RnaseP as a human positive control target.
Primer sequences are provided.

**HC comment:** Note to review: POC is stated, yet the intended user is HCP.

Re subsection "G. Specifications" in the response, the applicant states that "this is being generated at the moment". This is explained later in the document, specifically in Appendix C, whereby the applicant lists the planned validation testing (analytical, clinical, and stability). HC does not normally accept rolling submissions. This is discussed later in this report.
Re "Specimen collection, the applicant states "Specimen collection is not included as part of TripleLock™ SARS-CoV-2. It is intended for use with RNA extracted from viral transport media from a nasopharyngeal swab collection made by a clinician."
The response re provision of device description is acceptable. Details will be evaluated during review.

AI: The device description states that the test is appropriate for "laboratory, hospital, clinic or point-of-care use" … and is "intended for use by registered healthcare professionals". 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. Please provide a near patient study or remove the claim for or point-of-care use. A revised Instructions for Use may be needed to align with your claim.

2. Provide a 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. Describe the the results expected and acceptance criteria. Ensure you identify the concentration of the positive control relative to the LoD

**Response:** A negative control is provided. A positive control is not provided, as the internal control provides this. The internal control is designed to target RNaseP.

**HC comment:** The response is acceptable.

3. Provide a clear description outlining the specimen types that can be used with the device, and the extraction methods that are to be used for each. 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.

**Response:** This device is intended to be used with nasopharyngeal swabs collected into viral transport media. An extraction method suggested for use with the device is the M1 RNA 2.0 sample prep kit.

**HC comment:** Evidence of extraction is required.

AI: Provide evidence of the extraction method used with your device, e.g., the study report documenting it's use and the results. As described in the Further Validation Timetable provided in Appendix C in your response, the results from your wet validation with lyophilized master mix and wet primers and probes, with the M1 RNA prep kit (Biomeme Inc. catalogue number 3000536) for preparation of RNA and run on the Franklin qPCR thermocycler (Biomeme Inc. catalogue number 1000003) are required.

4. Describe the targeted sequences of the SARS-CoV-2 genome. Provide a list of all primers and probe sets and briefly describe what they detect, and include their nucleic acid sequences. Indicate if biotin-streptavidin/avidin chemistry is used in any steps of the test. You may include relevant supporting literature.

**Response:** This assay targets the E Gene and UTR regions of the SARS-CoV-2 genome and RNaseP as a positive internal control. The RNase P gene is a single-copy human gene that encodes the RNA moiety for the RNase P enzyme, used as an endogenous human target in this assay to confirm successful amplification of the sample in the absence of SARS-CoV-2.
The primer sequences are provided.

**HC comment:** The response is acceptable.

5. Provide the intended use, intended users, and the intended testing setting to be used with your device (lab, Point of Care).

**Response:** This device is for use by trained healthcare professional for the in vitro qualitative detection of RNA from the SARS-CoV-2 in nasopharyngeal swabs from patients with or without signs and symptoms of infection who are suspected of COVID-19. The test can be used either in the lab or at the point of care.

**HC comment:** The response is acceptable. The applicant will be notified that POC use must be validated by a Near Patient study (see AI above).

**6.** 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 clinical (preferred) or artificial matrix. The matrix 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. A precise description (with the source and sequence) of the samples used in these studies are needed.

**Response:** Provided in Appendix A

**HC comment:** Appendix A is a preprint of a publication. The LoD study is included. Serial 10-fold dilutions of SARS-CoV-2 RNA were prepared and tested in triplicates. Dilutions of a strong patient sample were also tested. Confirmatory testing, as specified in the question, was not provided.

AI: As previously requested, and as described in the Further Validation Timetable provided in Appendix C in your response, a confirmatory LoD study with 20 replicates of the final concentration is required.

**7.** Provide the results of your *in silico* analysis of inclusivity, including the % identity to published COVID19 sequences.

**Response:** Provided.

**HC comment:** The response is acceptable.

**8.** 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 pyogenes*** |
| | ***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*** |
| | |

**Response:** Provided in Appendix A
**HC comment**: The response is acceptable**.**

**9.** Provide the study reports for interference testing of endogenous substances (Hb, bilirubin, Proteins, TG, HAMA, RF, Total IgG, Total IgM), and of exogenous substances (common medications).

**Response:** N/A

**HC comment**: The response is acceptable as the specimens go through extraction prior to testing**.**

**10.** Provide the 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.

**Response:** Provided in Appendix C

**HC comment:** Appendix C is a "Further Validation Table", listing all the planned validation studies (see boxed information below). With the exception of the preliminary stability study, and perhaps the repeatability study, it will not be possible to evaluate this application in the absence of the data below.

**Further Validation Timetable**

We are requesting time to allow testing of the lyophilised primers/probes.

- Wet validation will be performed with lyophilized master mix and wet primers and probes, with the M1 RNA prep kit (Biomeme Inc. catalogue number 3000536) for preparation of RNA and run on the Franklin qPCR thermocycler (Biomeme Inc. catalogue number 1000003) .
- Wet validation – specificity: May 22, 2020
- Wet validation – clinical sample, including LOD: May 31, 2020
- LOD will be determined with 3 replicates of 3 concentrations, followed by 20 replicates at the LOD.
- Clinical performance testing will be performed with a minimum 30 positive and 30 negative samples.
- The remaining validation will be the fully lyophilized kit, including primers.
- Preliminary stability study: June 15, 2020 Stability of the lyophilized master mix will be determined by running 3 replicates of 1 sample of control plasmid at 1 concentration in the mid rage of the test capability at 3 day intervals over the course of 15 days to begin. This will continue at 1-week intervals up to 3 months minimum.
- Fully lyophilized validation (LOD and clinical testing) with 5-day repeatability : June 15, 2020

- LOD will be determined with 3 replicates of 3 concentrations, followed by 20 replicates at the LOD. Clinical testing will be performed with a minimum 30 positive and 30 negative samples. Repeatability will be tested with 5 replicates of 1 sample at 1 concentration in the mid range of the test capability, on 3 different instruments, repeated over 5 days.
- Complete 20-day repeatability: June 30, 2020. 20-day repeatability will be performed on the end of the 5-day testing above, on the sample sample concentration, but with 2 replicates on 2 instruments, over an additional 15 days.

AI: With regard to the Further Validation Timetable provided in Appendix C in your response, receipt of many of these studies is required for evaluation – and authorization - of your device. As the results from these studies are considered critical for evaluation (particularly the clinical evaluation), Health Canada requests that you submit the information when it is ready, in a single response. Review of your application will be expedited when all the information is received; the process could take longer if information is submitted one report at a time.

**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. 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.

**Response:** Provided in Appendix A

**HC comment**: The clinical study in Appendix A does not meet the requirements of the IO.

The applicant "analyzed 4192 clinical specimens across two hospital sites and instruments (CFX96 and Rotor Gene). E gene positivity within the triplex assay was used as an internal reference. There was a total of 88 E gene positives and 4104 E gene negatives. There was also 88 UTR positives and 4104 UTR negatives, demonstrating 100% concordance between UTR and E gene readouts. The UTR assay therefore displays 100% clinical sensitivity and specificity, and comparable performance across different instruments."

The results between the 2 theromocyclers were compared without appropriate calculation of sensitivity and specificity. A suitable reference standard was not used.

| | POS | NEG | Total |
|---|---|---|---|
| Rotor Gene | 49 | 2644 | 2693 |
| CFX96 | 39 | 1460 | 1499 |
| Total | 88 | 4104 | 4192 |

The response is not acceptable.

AI: The clinical study provided in Appendix A does not meet the requirements of the Interim Order. As per your Further Validation Timetable in Appendix C, Health Canada notes that a new clinical performance study is planned with a minimum 30 positive and 30 negative samples. Validation of the reactive and non-reactive samples using a reference standard is needed. The reference should be a comparator RT-PCR assay that is authorized under the Interim Order. In the absence of Health Canada authorization, Emergency Use Authorization from the United States Food and Drug Administration, or declaration of eligibility for procurement

by the World Health Organization under the Emergency Use Listing Procedure, will be accepted. Verify the associated links to determine if the comparator RT-PCR assay is eligible for use as a comparison for the clinical study:
Health Canada COVID-19 Authorization
Emergency Use Authorization from the United States Food and Drug Administration
World Health Organization under the Emergency Use Listing Procedure

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.

In the clinical study provided in Appendix A, results from Rotor Gene and CFX96 were compared to each other. Note that this does not assess the clinical sensitivity and specificity of your test, which is required for evaluation.

**12.** 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.

**Response:** Provided in Appendix C:

Preliminary stability study: June 15, 2020 Stability of the lyophilized master mix will be determined by running 3 replicates of 1 sample of control plasmid at 1 concentration in the mid rage of the test capability at 3 day intervals over the course of 15 days to begin. This will continue at 1-week intervals up to 3 months minimum.

**HC comment:** Additional stability studies are required.
AI: In your stability plan, please include the protocol for studies that will assess the shelf-life, in-use stability, shipping, and freeze-thaw stability. The preliminary stability validation plan submitted will not yield sufficient information required for authorization. You may want to refer to the studies as per ISO 23640:2011 and CLSI EP25-A:2009.

**13.** Provide the Instructions for Use that will accompany the kit.
**Response:** Provided in Appendix B

**HC comment:** An IFU is provided. However, it will require revision at review to include the required elements.

**ADDITIONAL QUESTIONS:**

**1.** The device description states that the test is appropriate for “laboratory, hospital, clinic or point-of-care use” … and is “intended for use by registered healthcare professionals”. 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. Please provide a near patient study or remove the claim for or point-of-care use. A revised Instructions for Use may be needed to align with your claim.

2. Provide evidence of the extraction method used with your device, e.g., the study report documenting it's use and the results. As described in the Further Validation Timetable provided in Appendix C in your response, the results from the planned wet validation with lyophilized master mix and wet primers and probes, with the M1 RNA prep kit (Biomeme Inc. catalogue number 3000536) for preparation of RNA and run on the Franklin qPCR thermocycler (Biomeme Inc. catalogue number 1000003) are required.

3. As previously requested, and as described in the Further Validation Timetable provided in Appendix C in your response, a confirmatory LoD study with 20 replicates of the final concentration is required.

4. With regard to the Further Validation Timetable provided in Appendix C in your response, receipt of many of these studies is required for evaluation – and authorization - of your device. As the results from these studies are considered critical for evaluation (particularly the clinical evaluation), Health Canada requests that you submit the information when it is ready, in a single response. Review of your application will be expedited when all the information is received; the process could take longer if information is submitted one report at a time.

5. The clinical study provided in Appendix A does not meet the requirements of the Interim Order. As per your Further Validation Timetable in Appendix C, Health Canada notes that a new clinical performance study is planned with a minimum 30 positive and 30 negative samples. Validation of the reactive and non-reactive samples using a reference standard is needed. The reference should be a comparator RT-PCR assay that is authorized under the Interim Order. In the absence of Health Canada authorization, Emergency Use Authorization from the United States Food and Drug Administration, or declaration of eligibility for procurement by the World Health Organization under the Emergency Use Listing Procedure, will be accepted. Verify the associated links to determine if the comparator RT-PCR assay is eligible for use as a comparison for the clinical study:

   Health Canada COVID-19 Authorization

   Emergency Use Authorization from the United States Food and Drug Administration

   World Health Organization under the Emergency Use Listing Procedure

   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.

   In the clinical study provided in Appendix A, results from Rotor Gene and CFX96 were compared to each other. Note that this does not assess the clinical sensitivity and specificity of your test, which is required for evaluation.

6. In your stability plan, please include the protocol for studies that will assess the shelf-life, in-use stability, shipping, and freeze-thaw stability. The preliminary stability validation plan submitted will not yield sufficient information required for authorization. You may want to refer to the studies as per ISO 23640:2011 and CLSI EP25-A:2009.

**June 15, 2020 – Review of Applicant's responses to screening questions, received on June 15, 2020**

| | |
|---|---|
| Name of the device | "TripleLock SARS-COV-2 Test Strips" |
| Manufacturer | PRECISION BIOMONITORING INC. |
| Application # | **313602** |
| DED Screener | **Elana Cherry** |

1. The device description states that the test is appropriate for "laboratory, hospital, clinic or point-of-care use" … and is "intended for use by registered healthcare professionals". 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. Please provide a near patient study or remove the claim for or point-of-care use. A revised Instructions for Use may be needed to align with your claim.

**Response:** Reference of point of care use has been removed from the description and instructions for use. (See Appendix A)

**HC comment**: Appendix A is a revived IFU. The response is acceptable.

2. Provide evidence of the extraction method used with your device, e.g., the study report documenting it's use and the results. As described in the Further Validation Timetable provided in Appendix C in your response, the results from the planned wet validation with lyophilized master mix and wet primers and probes, with the M1 RNA prep kit (Biomeme Inc. catalogue number 3000536) for preparation of RNA and run on the Franklin qPCR thermocycler (Biomeme Inc. catalogue number 1000003) are required.

**Response:** The M1 RNA prep kit was used for LOD testing (Appendix C), wet specificity testing (Appendix C), and clinical testing (Appendix D), demonstrating its efficacy.

**HC comment**: The response is acceptable.

3. As previously requested, and as described in the Further Validation Timetable provided in Appendix C in your response, a confirmatory LoD study with 20 replicates of the final concentration is required.

**Response:** The results presented in Appendix C indicate that the LOD for TripleLock SARS-COV-2 Test Strips run using wet reagents on the Franklin PCR equipment is 50 copies of SARS-CoV-2 in a sample input of 50 uL into the M1 sample prep. This translates to ~200 copies in the final RT-qPCR reaction. Appendix C contains complete detail.

**HC comment**: The response is acceptable.

4. With regard to the Further Validation Timetable provided in Appendix C in your response, receipt of many of these studies is required for evaluation – and authorization - of your device. As the results from these studies are considered critical for evaluation (particularly the clinical evaluation), Health Canada requests that you submit the information when it is ready, in a single response. Review of your application will be

expedited when all the information is received; the process could take longer if information is submitted one report at a time.

**Response:** The adjusted timeline for completion of these tests is listed in Appendix B. Supply delays have delayed progress to a degree, however validation with wet primers and probes has been completed (Appendix E). We are continuing to validation with the fully lyophilized test.
Wet validation will be performed with lyophilized master mix and wet primers and probes, with the M1 RNA prep kit (Biomeme Inc. catalogue number 3000536) for preparation of RNA and run on the Franklin qPCR thermocycler (Biomeme Inc. catalogue number 1000003).

- Wet validation – specificity: completed and included in response document.
- Wet validation – LOD completed; clinical testing completed for 68 previously tested patient samples (see Appendix C)
- LOD will be determined with 3 replicates of 3 concentrations, followed by 20 replicates at the LOD.
- Clinical performance testing will be performed with a minimum 30 positive and 30 negative samples.
- The remaining validation will be with the fully lyophilized kit, including primers.
- The stability study has been updated and included in the response document.

Fully lyophilized validation (LOD and clinical testing) with 5-day repeatability: June 30, 2020

- LOD will be determined with 3 replicates of 3 concentrations, followed by 20 replicates at the LOD. Clinical testing will be performed with a minimum 30 positive and 30 negative samples and performance will be compared to the CDC assay approved for FDA emergency use (from IDT; 2019-nCov CDC EUA Kit, product number 10006770)
- Repeatability will be tested with 5 replicates of 1 sample at 1 concentration in the mid-range of the test capability, on 3 different instruments, repeated over 5 days.
- Complete 20-day repeatability: July 15, 2020
  20-day repeatability will be performed on the end of the 5-day testing above, on the same sample concentration, but with 2 replicates on 2 instruments, over an additional 15 days.

**HC comment**: The response is acceptable

**5.** The clinical study provided in Appendix A does not meet the requirements of the Interim Order. As per your Further Validation Timetable in Appendix C, Health Canada notes that a new clinical performance study is planned with a minimum 30 positive and 30 negative samples. Validation of the reactive and non-reactive samples using a reference standard is needed. The reference should be a comparator RT-PCR assay that is authorized under the Interim Order. In the absence of Health Canada authorization, Emergency Use Authorization from the United States Food and Drug Administration, or declaration of eligibility for procurement by the World Health Organization under the Emergency Use Listing Procedure, will be accepted. Verify the associated links to determine if the comparator RT-PCR assay is eligible for use as a comparison for the clinical study:

Health Canada COVID-19 Authorization

Emergency Use Authorization from the United States Food and Drug Administration

World Health Organization under the Emergency Use Listing Procedure

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.

In the clinical study provided in Appendix A, results from Rotor Gene and CFX96 were compared to each other. Note that this does not assess the clinical sensitivity and specificity of your test, which is required for evaluation.

**Response:** We have added comparison to a reference test to our clinical evaluation plans (see Appendix B for modified plan, and Appendix D for clinical validation results compared to a reference test).

**HC comment**: Appendix D contains information regarding specificity, not clinical evaluation.

Clinical Evaluation is found in Appendix E.

Clinical samples were obtained and tested at St. Joseph's Hospital in Hamilton, ON. Each clinical sample was performed with two tests: the CDC 2019-nCoV Kit and the Precision Biomonitoring TripleLock SARS-COV-2 assay. The report's conclusion is as follows: "A total of 34 positive and 34 negative clinical patient samples were tested with the Precision Biomonitoring TripleLock SARS-COV-2 assay and with a reference assay (CDC EUA Test Kit). The concordance between the Precision Biomonitoring TripleLock SARS-COV-2 assay and the reference assay was 94% (64/68)."

The response is acceptable for screening purposes.

6. In your stability plan, please include the protocol for studies that will assess the shelf-life, in-use stability, shipping, and freeze-thaw stability. The preliminary stability validation plan submitted will not yield sufficient information required for authorization. You may want to refer to the studies as per ISO 23640:2011 and CLSI EP25-A:2009.

**Response:** Since this product is stored, shipped and used at ambient temperature, the shipping and shelf-life studies have been combined, and a freeze-thaw stability study is not required. Appendix F includes the updated plan.

**HC comment**: The response is acceptable for screening purposes.

## File Disposition:

1. **Background/*Antécédents***
   The applicant has requested authorisation for the above named device under the ***Interim order respecting the importation and sale of medical devices for use in relation to COVID-19.***

   In their original application, the applicant did not provided adequate evidence to allow for a full assessment the safety, effectiveness and quality of the subject device. As a result, additional information was sought, as documented above.

2. **Evaluation/*Évaluation***

   All responses to requests for additional information are considered acceptable to be screened into review.

3. **Conclusion**
   The applicant has provided the required level of scientific evidence to allow for an assessment of device safety, effectiveness and quality, as required under the IO, and as outlined in the ***Guidance on Requirements for serological antibody tests submitted under the COVID-19 Interim Order***.

4. **Recommendation**

   **Recommend for Review……. X.  June 15 2020**
   **Recommend for Rejection…….**