FULL-FLEDGED PRODUCT REGISTRATION VALIDATION REQUIREMENTS FOR COVID-19 TESTS (*PROFESSIONAL USE ONLY* TESTS)

Information on product registration submission requirements can be found in the following Guidances, published online at http://www.hsa.gov.sg/medical-devices/guidance-documents.

- "GN-18 Guidance on Preparation of a Product Registration Submission for In Vitro Diagnostic (IVD) Medical Devices using the ASEAN CSDT" under header Product Registration
- "TR-02 Contents of a Product Registration Submission for In-Vitro Diagnostic Medical Devices using the ASEAN CSDT" under header Technical Reference Documents

In addition to the requirements specified in the above Guidances, the following aspects of analytical performance and clinical evidence will also be relevant, for the respective diagnostic tests intended for pre-market submission.

Nucleic Acid Tests

Pre-clinical Validation

- Analytical Sensitivity
  - Limit of Detection (LoD)
    - Probit Analysis to estimate the LoD *or*
    - Confirmation of the LoD concentration with at least 20 replicates
  - Inclusivity
    - *In silico* analysis on SARS-CoV-2 sequences published in the last 6 months on GISAID database
    - Relevant assessments and study reports, which evaluate the impact of new SARS-CoV-2 variants of concern on test kit performance. Otherwise, to provide details of manufacturer's plan to mitigate any risks arising from new SARS-CoV-2 variants of concern, including plans for any on-going or new future studies.

- Analytical Specificity
  - Wet testing cross-reactivity
    - Study on other human coronaviruses (SARS-CoV, MERS-CoV, Coronavirus OC43, Coronavirus NL63, Coronavirus 229E, and Coronavirus HKU1)
    - Study on other respiratory pathogens (e.g. Influenza A virus (H1N1, H3N2), Influenza B virus, Respiratory Syncytial Virus (RSV), Adenovirus)
  - *In silico* cross-reactivity
    - BLAST analysis on the primers/probe
  - Interference
    - Endogenous substances, including but not limited to, mucin and blood for direct swabs
    - Exogenous substances (e.g. drugs), if any

    For interference, testing must be conducted on both positive and negative samples.

- Precision
  - Repeatability/Reproducibility (refer to CLSI EP05-A3)

- Linearity/ Measuring Range
  - For quantitative assays

- Cut off Values (as applicable)

- Sample Matrix Validation
  - Analytical study using contrived specimens on the claimed respiratory specimen type *or*
  - Clinical studies for all other patient sample types (e.g. saliva, oral fluids, anal swabs)

  For respiratory specimen types, studies performed on the most challenging specimen types (e.g. sputum) can be used to cover other matrices.

- Swab Equivalency
  - Where there are different swabs (e.g. different swab suppliers) that are to be supplied with the kit, equivalency study across the different swabs should be provided to validate that the performance of the assay is not affected by the different swabs.

- Viral Transport Media (VTM) Equivalency
  - For all claimed VTMs indicated for use with the assay, equivalency study across the claimed compatible VTMs should be provided.

- Specimen Stability Studies

- Stability Studies
  - Real time or accelerated aging studies on 3 lots of reagent
  - In-use stability studies (e.g. Freeze/Thaw) and onboard stability studies, if applicable, on 1 lot of reagent

  Initial establishment of shelf-life can be based on the data from experience gained with IVD reagents that can reasonably be expected to be comparable with regard to their stability characteristics. (*ref ISO 23640*). However, the claim shall be verified with real time study data.

- Instrument Cross-validation and Extraction Method Validation Studies
  - Limit of Detection study on the different instruments and/or extraction methods

Clinical Validation

- Structured Clinical Study
  - Designed to provide reasonable assurance of clinical performance.
    For detailed requirements, refer to TR-02 Contents of a Product Registration Submission for In Vitro Diagnostic Medical Devices using the ASEAN CSDT.
  - Method of determining clinical status of patients, whose samples will be used to determine sensitivity and specificity, must be consistent
  - Objective analysis of results and overall study conclusion provided
  - Clinical samples ideally should be sourced from a minimum of 2 sites

- Clinical Sensitivity
  - ≥ 95% for respiratory and saliva sample types (applicable after resolution of discrepant results)

- Clinical Specificity
  - 99% for respiratory and saliva sample types (applicable after resolution of discrepant results)

- Clinical Study Sample Size

  Assays for detection of SARS-CoV-2 infection:
  - For detection of SARS-CoV-2 infection, 300 positive samples should be tested for each claimed sample type, including non-respiratory specimen types such as saliva (at least 20% should be prospectively and/or prospective-retrospectively collected).
  - For assays with genotyping claims, each mutation must have at least 20 positive samples, regardless of sample type. These samples can be a subset of the above stated required positive samples.
  - For assays intended to be used in asymptomatic population, at least 50% of the positive samples should include samples from asymptomatic individuals.
  - 500 confirmed negative patient samples should be tested for each sample type.

  Assays solely for SARS-CoV-2 genotyping:
  - For assays that are solely for genotyping, 200 positive and 100 negative samples should be tested for each claimed sample type, including non-respiratory specimen types such as saliva.
  - Additionally, each mutation must have at least 20 positive samples, regardless of sample type. These samples can be a subset of the above stated required positive samples.

  - For specimen pooling claims, at least 50 positive and 50 negative clinical samples should be tested, individually and in pools.

- Clinical Study Comparator

  Any of the following criteria can be applied:
  - Comparison against actual clinical diagnosis, with clear criteria that defines confirmatory clinical diagnosis for the study patient
  - Method comparison done with an established PCR (e.g. listed on Singapore Medical Device Register). Comparator test(s) used should be clearly identified. Clinical testing laboratory which runs the comparator test should meet at least one of the following criteria:
    - Evidence that clinical lab is a national or country equivalent reference lab (e.g. certified by country's Ministry of Health or Competent Authority to conduct COVID-19 testing)
    - Possess ISO 15189 or CAP accreditation
    - [For SG labs] Listed on MOH's list of approved COVID-19 testing labs (*https://www.moh.gov.sg/licensing-and-regulation/regulations-guidelines-and-circulars/details/list-of-healthcare-institutions-approved-to-provide-sars-cov-2-polymerase-chain-reaction-(pcr)-testing-for-covid-19*)

Antigen Tests

Pre-clinical Validation

- Analytical Sensitivity
  - For qualitative assays
    - Limit of Blank (LoB), if applicable
    - Limit of Detection (LoD)
  - For quantitative assays
    - Limit of Blank (LoB), if applicable
    - Limit of Detection (LoD)
      Limit of Quantification (LoQ)
  - Inclusivity
    - Relevant assessments and study reports, which evaluate the impact of new SARS-CoV-2 variants of concern on test kit performance. Otherwise, to provide details of manufacturer's plan to mitigate any risks arising from new SARS-CoV-2 variants of concern, including plans for any on-going or new future studies.
    - Wet lab testing using recombinant protein for Delta and Omicron Variants of Concern is required. Testing should be conducted close to Limit of Detection (e.g. within 3x LoD, clinical samples with high Ct values).

- Analytical Specificity
  - Cross-reactivity
    - Studies on other human coronaviruses, including SARS-CoV-1, MERS-CoV, Coronavirus OC43, Coronavirus NL63, Coronavirus 229E, and Coronavirus HKU1.
      Studies on other respiratory pathogens (e.g.: Influenza A virus (H1N1, H3N2), Influenza B virus, Respiratory Syncytial Virus (RSV), Adenovirus)
  - Interference
    - Endogenous substances, including but not limited to, mucin and blood for direct swabs
    - Exogenous substances (e.g. drugs), if any

    For interference, testing must be conducted on both positive and negative samples.

- Precision
  - Repeatability/Reproducibility (refer to CLSI EP05-A3)

- Linearity/ Measuring Range
  - For quantitative assays

- High Dose Hook Effect
  - Studies conducted on samples with high antigen titres

- Cut off values (as applicable)

- Sample Matrix Validation
  - Analytical study using contrived specimens on the claimed respiratory specimen type *or*
  - Clinical studies for all other patient sample types (e.g. saliva, oral fluids, anal swabs)

  For respiratory specimen types, studies performed on the most challenging specimen types (e.g. sputum) can be used to cover other matrices.

- Swab Equivalency
  - Where there are different swabs (e.g. different swab suppliers) that are to be supplied with the kit, equivalency study across the different swabs should be provided to validate that the performance of the assay is not affected by the different swabs.

- Specimen Stability Studies

- Stability Studies
  - Real time or accelerated aging studies on 3 lots of reagent
  - In-use stability studies (e.g. open pouch), if applicable, on 1 lot of reagent

  Initial establishment of shelf-life can be based on the data from experience gained with IVD reagents that can reasonably be expected to be comparable with regard to their stability characteristics. (*ref ISO 23640*). However, the claim shall be verified with real time study data.

- Reading Time and Sample Volume Validation Studies
  - For rapid antigen tests

- Usability Studies
  - For Point-of-Care tests (including specimen collection procedure, if the collection kit is specified as a closed system with main test)

Clinical Validation

- Structured Clinical Study
  - Designed to provide reasonable assurance of clinical performance.
    For detailed requirements, refer to TR-02 Contents of a Product Registration Submission for In Vitro Diagnostic Medical Devices using the ASEAN CSDT.
  - Method of determining clinical status of patients, whose samples will be used to determine sensitivity and specificity, must be consistent
  - Objective analysis of results and overall study conclusion provided
  - Clinical samples ideally should be sourced from a minimum of 2 sites

- Clinical Sensitivity
  - > 90% within 7 days of symptom onset
    Clinical performance data, stratified by days post-symptom onset and Ct values, should be provided

- Clinical Specificity
  - 99%

- Clinical samples
  - 300 positive samples should be tested for each claimed sample type (at least 20% should be prospectively and/or prospective-retrospectively collected).
  - For assays intended to be used in asymptomatic population, at least 50% of the positive samples should include samples from asymptomatic individuals. Asymptomatic positive samples should cover both high Ct (> 30) and low Ct (< 30) values.
  - 1000 confirmed negative patient samples should be tested, including within ~100 symptomatic PCR negative patients with no exposure to SARS-CoV-2.
    Alternatively, a comprehensive cross-reactivity study against other respiratory viruses may be considered in place of symptomatic PCR negative patient samples.
  - For specimen pooling claims, at least 50 positive and 50 negative clinical samples should be tested, individually and in pools.

- Clinical Study Comparator
  Any of the following criteria can be applied:
  - Comparison against actual clinical diagnosis, with clear criteria that defines confirmatory clinical diagnosis for the study patient
  - Method comparison done with an established PCR or antigen test (e.g. listed on Singapore Medical Device Register). Comparator test(s) used should be clearly identified. Clinical testing laboratory which runs the comparator test should meet at least one of the following criteria.
    - Evidence that clinical lab is a national or country equivalent reference lab (e.g. certified by country's Ministry of Health or Competent Authority to conduct COVID-19 testing)
    - Possess ISO 15189 or CAP accreditation
    - [For SG labs] Listed on MOH's list of approved COVID-19 testing labs (https://www.moh.gov.sg/licensing-and-regulation/regulations-guidelines-and-circulars/details/list-of-healthcare-institutions-approved-to-provide-sars-cov-2-polymerase-chain-reaction-(pcr)-testing-for-covid-19)

Serology Tests

Pre-clinical Validation

- Analytical Sensitivity
  - For quantitative assays
    - Limit of Blank (LoB), Limit of Detection (LoD), Limit of Quantification (LoQ)
  - For qualitative assays
    - Limit of Detection (LoD)
  - Inclusivity
    - Justification for why tests will not be affected by SARS-CoV-2 variants of concern

- Analytical Specificity
  - Cross-reactivity
    - Studies on antibodies to other human coronaviruses, including Coronavirus OC43, Coronavirus NL63, Coronavirus 229E, and Coronavirus HKU1.
    - Studies on antibodies of other respiratory pathogens (e.g. Influenza A virus (H1N1, H3N2), Influenza B virus, Respiratory Syncytial Virus (RSV), Adenovirus)
    - Studies on antibodies against the organism in expression system used to produce the recombinant antigens (e.g. anti-E.coli, anti-yeast)
  - Interference
    - Endogenous substances, including but not limited to hemoglobin, bilirubin, protein, triglyceride
    - Exogenous substances (e.g. drugs), if any

    For interference, testing must be conducted on both positive and negative samples.

- Precision
  - Repeatability/Reproducibility (refer to CLSI EP05-A3)

- Linearity/ Measuring Range
  - For quantitative assays

- High Dose Hook Effect
  - Studies conducted on samples with high antibody titres

- Cut off values (as applicable)

- Sample Matrix Validation
  - For venous whole blood, plasma and serum, 25 positive and 25 negative samples are ideally required to demonstrate sample matrix equivalency
  - For capillary serum or plasma, 50 positive and 25 negative samples are required
  - If specific anticoagulants are recommended as part of claimed sample type, validation study conducted must include these anticoagulants. 25 positive and 25 negative samples are required for each anticoagulant
  - For rapid test with capillary whole blood claim, clinical studies using patient samples are required

- Specimen Stability Studies

- Stability Studies
  - Real time or accelerated aging studies on 3 lots of reagent
  - In-use stability studies (e.g. open pouch), if applicable, on 1 lot of reagent

  Initial establishment of shelf-life can be based on the data from experience gained with IVD reagents that can reasonably be expected to be comparable with regard to their stability characteristics. (*ref ISO 23640*) However, the claim shall be verified with real time study data. Additional condition will be imposed on approved devices, to submit completed real time stability study to HSA for assessment.

- Reading Time and Sample Volume Validation Studies
  - For rapid serology tests

- Usability Studies
  - For Point-of-Care tests (including specimen collection procedure, if the collection kit is specified as a closed system with main test)

Clinical Validation

- Structured Clinical Study Evidence

- Designed to provide reasonable assurance of clinical performance.
  For detailed requirements, refer to TR-02 Contents of a Product Registration Submission for In Vitro Diagnostic Medical Devices using the ASEAN CSDT.
- Method of determining clinical status of patients, whose samples will be used to determine sensitivity and specificity, must be consistent
- Objective analysis of results and overall study conclusion provided
- Clinical samples ideally should be sourced from a minimum of 2 sites

- Clinical Sensitivity
  - Clinical performance data, either stratified by days post-symptom onset, or days post-PCR positive, should be provided
  - IgM > 90% at 7 days (8-14 days) post-symptom onset or post-PCR positive
  - IgG > 90% at 14 days post-symptom onset or post-PCR positive

- Clinical Specificity
  - 99% for IgM and IgG (for lab-based serology tests)

- Clinical samples
  - 300 positive samples should be tested for each claimed sample type (at least 20% should be prospectively and/or prospective-retrospectively collected)
  - 1000 pre-pandemic or confirmed serology negative patient samples should be tested, including within ~100 symptomatic PCR negative patients with no known prior exposure to SARS-CoV-2. Alternatively, a comprehensive cross-reactivity study against other respiratory viruses may be considered in place of symptomatic PCR negative patient samples.

- Clinical Study Comparator
  - Either comparison against actual clinical diagnosis, with clear criteria that defines confirmatory clinical diagnosis for the study patient,
  - Or method comparison done with an established PCR or serology test (e.g. listed on Singapore Medical Device Register). Comparator test(s) used should be clearly identified. Clinical testing laboratory which runs the comparator test should meet at least one of the following criteria.
    - Evidence that clinical lab is a national or country equivalent reference lab (e.g. certified by country's Ministry of Health or Competent Authority to conduct COVID-19 testing)
    - Possess ISO 15189 or CAP accreditation
    - [For SG labs] Listed on MOH's list of approved COVID-19 testing labs (https://www.moh.gov.sg/licensing-and-regulation/regulations-guidelines-and-circulars/details/list-of-healthcare-institutions-approved-to-provide-sars-cov-2-polymerase-chain-reaction-(pcr)-testing-for-covid-19)
  - For assays with claims to detect neutralizing antibodies, method comparison against neutralization assays (e.g. Plaque Reduction Neutralisation Test (PRNT), Microneutralization assay (MNA)) is required.

- Additional Clinical Requirements
  - Data to demonstrate performance has been evaluated in vaccinated individuals. Otherwise, details concerning manufacturer's recommendation to users in this regard.