INDIAN COUNCIL OF MEDICAL RESEARCH

DEPARTMENT OF HEALTH RESEARCH

Standard Protocol for Validation of Commercial antibody-based ELISA or Rapid diagnostic kit for SARS-CoV-2

Background

- Antibodies to COVID-19 are produced over days to weeks after infection with the virus.
- Studies suggest that most patients develop antibody response around 7-10 days after onset of symptoms (1), indicating that antibody based tests (lateral flow or ELISA) will be of little value in early diagnosis of infection. Only RT-PCR based tests should be used for diagnosis of disease or recent infection.
- Antibody based tests are of value for surveillance purpose in order to evaluate the proportion of infection in the population. It has also been suggested that antibody based test could be used to predict whether an individual is immune to reinfection, although there is no evidence to date to support this.
- It is necessary to validate thesensitivity and specificity of antibody tests, as claimed by the manufacturer, before they are used in the field for surveillance/epidemiological purposes. Test with low sensitivity will miss many infections. Test with low specificity will wrongly show the presence of infection.
- This document provides a guidance for validation of antibody based tests. It applies to the tests for detection of any of the IgM and/or IgG antibodies.

Methods

- **Panel of sera:** A panel of sera from known positive and negative COVID-19 patients is essential for validation.

    - <u>*Known COVID-19 positives:*</u>Since antibody tests are recommended for surveillance purposes, for validation, sera from RT-PCR positive COVID-19 patients should be used as per the following guidelines:

      For IgM /IgG Rapid Tests: *Sera* in the second week of infection (preferable after 10 days of onset of symptoms or the day of RT-PCR diagnosis in asymptomatic individuals) should be used.

      For ELISA kits:

        a. ***For IgM ELISA:*** *Serum samples from RT-PCR positive patients between 7-14 days of onset of illness or day of RT-PCR positivity in asymptomatic individuals.Besides the required number of samples as given in the table below to estimate the sensitivity/specificity, It is desirable to also evaluate these kits against samples from 0-6 days to understand the time when IgM antibodies actually start appearing (atleast 50 such samples should be used).*

        b. ***For IgG ELISA:*** *Serum samples from RT-PCR positive patients between 14-21 days of onset of illness or day of RT-PCR positivity in asymptomatic individuals.*

    - <u>*Known COVID-19 negatives:*</u> Sera collected before COVID-19 pandemic (before December 2019) may be used as known negatives. It is preferable to include sera from patients with

influenza A,influenza B, Chlamydia pneumonia, Mycoplasma pneumoniae, adenovirus, Respiratory Syncytial virus, Hepatitis B surface antibody, *Hepatitis C* antibody, *Treponema pallidum* antibody, HIV, EB virus, *Measles* virus, *cytomegalovirus*, *Mumps, varicella-zoster* virus, other coronaviruses (*SARS CoV, MERS CoV*), whichever are available.

- **Sample size:** The following table provides a minimum number of positive and negative sera that needs to be tested for confidence level of 95% and different levels of precision:

Sensitivity/Specificity	Precision		
	±1%	±3%	±5%
75%	7204	802	290
80%	6148	684	247
85%	4900	546	197
90%	3459	386	140
95%	1826	204	74
98%	754	85	32

A minimum of 100 positive and 100 negative samples should be used for validation. However, it is preferable to have more samples (200 positives and negatives), if available.

- **Test procedure:** Tests should be conducted as per the procedure recommended by the manufacturer. It is preferable that laboratory personnel conducting the tests should be blinded with respect to COVID-19 status of sera.

- **Data analysis**: The data should be analyzed to calculate **sensitivity** (# sera with antibody positive/# sera with PCR positive) and **specificity** (# sera with antibody negative/# sera with PCR negatives) of the test with 95% confidence interval**.**

  Criteria is as follows:

  IgM: Sensitivity- 90% and above, Specificity-99% and above for each batch

  IgG: Sensitivity- 90% and above, Specificity- 95% and above

- **Additional investigations:** It is also suggested that antibody tests be performed on sera from known COVID-19 positives in the first week of infection, to know how early the test comes positive. This may be done subsequent to satisfactory validation of the antibody test kit.

Reference

1. Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. *Nature.* 2020 Apr. DOI: 10.1038/s41586-020-2196-x.

REPORT FORMAT- Report to be sent only to ICMR Hqrs

NAME OF THE VALIDATION CENTRE

PERFORMANCE EVALUATION REPORT FOR ANTIBODY-BASED ELISA OR RAPID DIAGNOSTIC KIT

- Name of the kit
- Name of the manufacturer
- Batch numbers
- Application
- Kit components
- Sample Panel
  - Positive samples
  - Negative samples (provide details)
- Results

		Antibody based ELISA or rapid diagnostic kit		
		Positive	Negative	Total
Name of antibody - based ELISA or rapid diagnostic kit	Positive			
	Negative			
	Total			

	Estimate (%)	95% CI
Sensitivity		
Specificity		

- Conclusions:
  - Sensitivity, specificity
  - Performance: satisfactory or not

(Sensitivity and specificity have been assessed in controlled lab setting using kits provided by the manufacturer from the batch mentioned above)

Disclaimers

1. ICMR's validation process does not approve / disapprove the kit design
2. ICMR's validation process does not certify user friendliness of the kit / assay
3. Validation of a kit by ICMR is not an assurance that the kit specifications would be included in the tendering process.

Note: This report is exclusively for Antibody Kit (Lot No……) manufactured by …… (supplied by …….)

Evaluation Done on ……

Evaluation Done by …….

Signature of Director/ Director-Incharge ……