Detection of Coronavirus-Like Immunological Responses Using Enzyme-Linked Immunosorbent Assay (ELISA)

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Please read and understand all aspects of the protocol prior to starting. If you have any questions please contact the approving supervisor.

Approved by:

_____ _____
Laboratory supervisor Date

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1.0 Scope and Application. This protocol was created as a way to detect the patients immunologic response to a coronavirus infection and can be used to detect COVID-19, SARS or MERS as well as others depending on the coating antigen.

2.0 Summary. An Enzyme-Linked Immunosorbent Assay is a standard protocol that has been the “gold standard” for many decades. This protocol has been specifically designed to detect the IgM, IgG, IgG/IgM, IgA or other immunoglobulin responses to a viral infection. The initial response to a viral pathogen can often be detected as an elevation of antigen specific IgM molecules (around day 4) and later by an IgG reaction. As the infection decreases and/or time progresses, the amount of IgM should decrease while IgG remains elevated. The detection of Immunoglobulins may not be an indicator of an active infection but is a surrogate biomarker of an infection that is or has occurred. This protocol is not meant to replace commercially available products but to allow for an alternative, inexpensive and qualitative method for the detection of viral responses that can act as a surrogate biomarker for the purposes of triage and treatment.

3.0 Protocol Distribution and Usage by Personnel.

3.1 Distribution. These Standard Operating Procedures (SOP’s) will be made available to all staff and authorized subcontractors. SOPs are licensed under GPLv3 and Creative Commons-Share alike or equivalent. Feel free to modify but share with others.

3.2 Usage. Relevant SOPs must be followed for all procedures.

3.3 Qualification. Any laboratory personnel trained in or experienced in ELISA techniques.

3.4 Version number. Version number 1.0. This SOP supersedes any previously used methods or techniques.

3.5 Disclaimers. To be in compliance with this procedure, one must be familiar with applicable laboratory processes and all associated SOPs before beginning. Confirm this with a laboratory supervisor.

4.0 Definitions and Abbreviations.

SOP: Standard Operating Procedure
N/A: Not applicable
QA/QC personnel: Quality Assurance/Quality Control personnel. This person may be the study director, QA/QC manager, or other person as chosen by the supervisor.
ELISA: Enzyme-Linked Immunosorbent Assay
dH_2O: Filtered, deionized water

5.0 Health and Safety Warnings. The use of laboratory equipment, supplies, chemicals, and biological products presents a safety and health risk to personnel.

Other applicable SOPs for lab processes detail health and safety risks, and must be read prior to performing this procedure.
Material Safety Data Sheets (MSDS) should be read and understood.
Equipment manufacturer warnings – read and follow manufacturer's guidelines.
Human cells are used, universal precautions must be taken.

6.0 Contraindication for protocol usage.

6.1 Interference. It is possible that an immunosuppressed patient or one that is early on in the invective process may not have sera-converted to recognize the viral response. In these cases, alternative testing such as RT-PCR or ELISA of the viral proteins in biofluids may be necessary for excluding coronavirus infections.

6.2 Enhancement. Depending on the dilution of the biofluid, the non-specific background may increase. Always include appropriate negative controls.

7.0 Equipment and Materials.
Please see appendix for additional information on necessary equipment, supplies and products along with necessary contact information.

7.1 Laboratory equipment.

Vortexer
Micro pipettes
Pipette
ELISA plate

37 degree incubator

7.2 General laboratory supplies.

Phosphate buffered Saline (PBS)
Phosphate buffered saline with 0.05 Tween– 20
Blocking buffer is PBS w Tween -20 and 0.25% Bovine serum albumin
NOTE: 0.05% (w/v) Sodium Azide (NaN3) can be added to PBS. If sodium azide is not used make sure to filter sterilize solutions.
Laboratory grade water

7.3 Consumable products.

Reagent Reservoir (reusable if needed)
Micro pipette tips
96 Well plates (see appendix)
Immulon, Nalgen Nunc or equivalent for protein binding.
Anti-human antibodies
List them here!
Coating Antigen
Viral
Recombinant

8.0 Quality Control.

8.1 Calibration.

Make sure the micro pipettes are calibrated to deliver the appropriate volume
If using a plate reader make sure it is operating correctly and use a calibration plate if needed.

8.2 Quality Control.

8.2.1 Positive control. Positive control will be total human immunoglobulin coated on the plate.

8.2.1.1 Additionally a pool of coronavirus positive samples can be aliquoted, frozen and purified.

8.2.1.2 Alternative for quantification would be to perform a Protein A or Protein A/G sepharose purification of immunoglobulins to act as a direct measurement using total protein concentration of the Ig's.

Additionally, a known positive serum can be added to the plate.

8.2.2 Negative control. Will be a bovine serum albumin or casein protein diluted in PBS with tween-20 and incubated with antigen coated plate.

9.0 Procedures.

Prior to beginning this procedure fill out the 96 well plate diagram.

NOTE: If this is the first time using the reagents make sure to do a criss-cross serial dilution optimization (Short protocols in Molecular Biology ELISA support protocol 1)

9.1 Coating the Plate with Antigen. All solutions to be made in PBS with sodium azide

- **9.1.1** Make 6 ml of plate of 0.5 µg/ml of recombinant protein
  - **9.1.1.1** Make 1 ml of 0.1 µg/ml of 1:1 mixture of human IgG/IgM for positive control labs (plate manuf. control)
- **9.1.2** Place 50 µl per well of coating antigen or Ig control per well.
- **9.1.3** Tap plate to place coating solution at the bottom of the well
- **9.1.4** Wrap plate(s) in plastic wrap with a moistened paper towel at bottom and incubate overnight at 4 °C (recommended) or 2 hr at 37 °C.
  NOTE: Plates can be stored up to one month at 4 °C with coating antigen.
- **9.1.5** Wash the plate three-times with ddH_2O using an ELISA plate washer or squirt bottle tap technique.
- **9.1.6** Plate is ready for blocking and incubation with patient serum(s)

9.2 Blocking of coated plates to minimize background

- **9.2.1** Verify the wells do not contain wash buffer
- **9.2.2** Add around 100 µL of blocking buffer (PBS w/Tween 20 and BSA). A squirt bottle or multi-channel micro-pipette can be used.
- **9.2.3** Incubate for 30 min at room temp (i.e. 25 deg C)
- **9.2.4** Wash three times with PBS with tween-20
- **9.2.5** Remove excess liquid by gently tapping on paper towels

9.3 .Incubation of the serum with the coated plates

- **9.3.1** Dilute patient serum 1:250 to 1:500 in PBS with tween-20
  - **9.3.1.1** Add either a serum albumin only as a negative control

    -or-
  - **9.3.1.2** May use a standard dilution of known positive patient sample.

9.3.2 Add 100 µL per well, in duplicate (at a minimum)
9.3.3 Wrap in saran wrap or plate cover
9.3.4 Incubate 2 hours at room temperature with gentle shaking if possible
9.3.5 Wash plate three times with PBS with tween-20
9.3.6 Remove excess liquid by gentle tapping on paper towels

9.4 Binding of Horseradish peroxidase (HRP)conjugated secondary antibody for detection antigen|patient immunoglobulin complex

9.4.1 Make 6 ml of anti-Human antibodies conjugated to the appropriate detection enzyme in blocking buffer. Using several goat anti human (H&L chain) polyclonal antibodies 1:8000 per antibody type is a good starting point depending on study options include :
9.4.1.1 Anti-human IgG (recommended for late infection detection)
9.4.1.2 Anti-human IgM (recommended for early infection detection)
9.4.1.3 Anti-human IgA
9.4.1.4 Anti-human IgG/IgM (recommended for screening test 1:1 mix of goat poly clonal antibody diluted around 1:8000)
9.4.2 Incubate for 1 hr with gentle mixing at room temperature
9.4.3 Wash three times as above
9.4.4 Gently tap on paper towels to remove excess liquid

9.5 Color-metric detection antibody|antigen complex

9.5.1 Make 6 ml of colorimetric reagent by diluting the BCIP/NBT Chromogen 1:1
9.5.2 Add 50 µl per well in to each well
9.5.3 Cover plate and incubate in the dark checking every 2 minutes looking for color change.
9.5.4 Add 50 µl stop reagent (2N sulfuric acid) when the +/- control starts to turn but not the negative control Or add stop just as the negative control wells starts to show background when placed on a white sheet of paper (estimated max time @ 15 min)
9.5.5 Once stop has been added take a picture on a white sheet of paper for documentation

9.6 Qualitative reading of the ELISA plate

9.6.1 If no plate reader is available look at the positive and negative controls and set a grading scale of: -, +/-, +, ++,, +++ to indicate strength of the reaction. A

standard of pooled coronavirus positive serum can be used and frozen in aliquots to aid in grading.

9.6.2 Using the pre-filled 96 well plate template record the graded

9.7 (OPTIONAL) Quantitative reading of the ELISA Plate
NOTE: Please use manufacturers recommendation and internal SOP for reading the plate

9.7.1 Read the plate at 450 nm and a background correction of 540 or 570 nm.

10.0 Data Analysis and Calculation.

10.1 Basic

10.1.1 Given the grading scale report the patient reaction with a +/- being a possible positive result depending on background. Ones that are border line can be diluted less or put as questionable and run again with new serum collection 12-24 hours later.

10.2 Quantitative

10.2.1 Read the plate at both 450nm and 570nm total $OD_{adjusted} = OD_{450} - OD_{540}$

10.2.2 If an exact amount of anti-viral human immunoglobulin (i.e. Protein A or similar purified total Ig) use the dilution in columns 1 and 2 to act as the standard curve

10.2.3 Calculate the estimated amount of protein based upon the curve fit to the standard curve.

10.2.4 Report as positive any sample that fits to a level greater than a pre-set minimum dilution or any sample that has a minimum adjusted OD greater than the maximum negative control reading.

Note: This should be regionally standardized and operate according to known statistical protocols. If you have any questions please contact a statistician.

11.0 Waste Management.

All sharps must be disposed of following legally approved methods.
All pipettes are to be rinsed with 10% bleach, autoclaved, or both before disposal.
Liquid waste must be diluted so that final concentration of bleach

is 10% and/or autoclaved.
Waste disposal must be in compliance with all applicable laws and regulations.

12.0 References.

12.1 Short Protocols in Molecular Biology

12.2 Short Protocols in Immunology

12.3 Reference preprint.

12.4 WHO website

12.5 FDA 21 CFR section 58 website

13.0 Appendix.

13.1 96 well plate diagram

Plate ID: Date: Technician:

	1	2	3	4	5	6	7	8	9	10	11	12
A	Positive Ctr	Positive Ctr										
B	1:50 + serum	1:50 + serum										
C	1:100 +	1:100 +										
D	1:200 +	1:200 +										
E	1:400 +	1:400 +										
F	1:800 +	1:800 +										
G	1:1600 +	1:1600 +										
H	Negative Ctr	Negative Ctr										

13.2 List of product vendors.

Thermo-Fisher
VWR
R&D
Southern Biotech
RayBiotech
Nalgene
Corning
Promega
et al. (need to get list and part numbers)

14.0 Revision History.

Reviewed

Initials				
Date				

Version 1.0 – Initial Release

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Serological test is an efficient supplement of RNA detection for confirmation of SARS-CoV-2 infection

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Abstract
Until now, viral RNA detection is almost the only way to confirm the infection of SARS-CoV-2 in practice. But varied reasons lead to the low sensitivity by RNA detection, which proposes serious challenge to disease control. We tested the performance of detecting total antibody(Ab) and IgM antibody in serum by the methods of chemiluminescence, Enzyme Linked Immunosorbent Assay(ELISA), and colloidal gold. Data showed that the sensitivity and specificity for total Ab and IgM detection were high by all the three methods, and compared with IgM, the sensitivity was higher for total Ab detection. Evidence from studies showed viral RNA testing combining with serological testing could increase the sensitivity of diagnosis, while remaining the high specificity. Specific serolody test for SARS-CoV-2 has great value for clinical practice and public health.

Keywords: SARS-CoV-2; diagnosis; antibody; serology; screening

On March 7, 2020, The World Health Organization (WHO) declared that beyond 90 countries/territories/areas reported more than 100,000 cases (including 80,000 cases from China) and more than 3,400 deaths of COVID-19.[1] Given the rapid spread of COVID-19, WHO raised the risk level of COVID-19 from "high" to "very high" on February 28, 2020, and call on all countries to give the highest priority to the prevention and control of COVID-19 epidemics.[2] Currently, viral RNA detection by quantitative real-time polymerase chain reaction (PCR) and/or sequencing is almost the only way to confirm the diagnosis of SARS-CoV-2 infection in practice. However, the reported positive rate of PCR in COVID-19 patients was not high, and patients often need to be sampled several times and at multiple sites before final diagnosis. Some highly suspected patients who were epidemiologically linked to SARS-CoV-2 exposure strongly and with typical lung radiological findings remained RNA negative in their upper respiratory tract samples. Besides, RNA detection requires high-quality swab specimen. Consequently, these troubles propose serious challenge to disease control and preventive quarantine.

In addition, patients with mild symptom, asymptomatic infection, and those who are