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Human ACE2 ELISA

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Protocol for detection

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We use this protocol and it's working

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Abstract

The COVID-19
pandemic has led to a significant number of individuals experiencing persistent post-acute sequelae, commonly referred to as long COVID, following SARS-CoV-2 infection. Similar symptoms, though less frequent, have also been observed after COVID-19 vaccination.
This study investigated the immune and inflammatory profiles of patients with long COVID symptoms, focusing on differences between post-infection and post-vaccination cases. A total of 110 patients (61 post-infection and 49 post-vaccination) were assessed at three and six months after symptom onset, alongside 40 controls. Serological testing was performed for SARS-CoV-2-specific antibodies, including neutralizing, non-neutralizing, and anti-ACE2 antibodies, and cytokine profiling was conducted using a 19-marker panel.
Post-infection patients exhibited elevated and increasing levels of anti-ACE2 antibodies and persistently high levels of pro-inflammatory cytokines such as IL-1β, IFN-γ, and MCP-1, indicating ongoing immune dysregulation and possible autoimmunity. In contrast, post-vaccination patients demonstrated stable antibody levels and cytokine profiles comparable to controls, suggesting a more regulated and transient immune response. These findings highlight the immunological heterogeneity of long COVID and support the need for targeted diagnostic and therapeutic approaches tailored to the underlying immune mechanisms in post-infection and post-vaccination subgroups.

Attachments

ACE-2 Protocol.pdf

78KB

Guidelines

Sample Preparation Guidelines:

- If samples will not be tested immediately, freeze them after collection.
- Avoid multiple freeze–thaw cycles of frozen samples.
- Thaw samples completely and mix gently (do not vortex) prior to analysis.
- Do not use hemolyzed or lipemic sera.
- Pre-dilute samples using 1X Assay Diluent for serum, plasma, and cell culture supernatant samples.
- Dilute serum 8-fold.

Materials

- Human ACE2 Antibody Coated wells, 96-well plate
- Human ACE2 Biotin Conjugate
- Human ACE2 Standard, recombinant human ACE2
- Assay Diluent (5X)
- Streptavidin-HRP (150X)
- TMB Substrate
- Stop Solution
- Wash Buffer Concentrate (20X)
- Adhesive Plate Covers
- Plate washer-automated or manual (manifold dispenser)
- Microtiter plater reader with software capable of measuring at 450 nm
- Deionized water

1 For the standard curve, add 100 µL of each standard to the appropriate wells for samples, add 100 µL
of diluted samples to the designated wells.

2 Cover the plate and incubate for 2.5 hours at room temperature or overnight at 4 °C with gentle shaking.

3 Discard the solution and wash the plate four times with 1× wash Buffer. Fill each well with 300 µL of wash Buffer using a multi-channel pipette or an auto washer. Complete removal of liquid at each wash is critical for optimal performance. After the final wash, remove any residual buffer by aspiration or decanting. Invert the plate and gently blot against clean paper towels.

4 Add 100 µL of prepared biotin conjugate to each well.

5 Incubate for 1 hour at room temperature with gentle shaking.

6 Discard the solution and repeat the wash

7 Add 100 µL of prepared Streptavidin-HRP solution to each well.

8 Incubate for 45 minutes at room temperature with gentle shaking.

9 Discard the solution and repeat the wash.

10 Add 100 µL of TMB Substrate to each well. The solution will start to turn blue.

11 Incubate for 30 minutes at room temperature in the dark with gentle shaking.

12 Add 50 µL of Stop Solution to each well and gently tap the plate to mix. The color will change from blue to yellow.

13 Read the absorbance at 450 nm. Read the plate within 30 minutes after adding the Stop Solution.

Protocol references

Human ACE2 ELISA Kit
Catalog Number EH489RB (96 tests)