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Cytokines assay to analyze

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Protocol for detection

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We use this protocol and it's working

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Abstract

Abstract

The COVID-19pandemic 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 studyinvestigated the immune and inflammatory profiles of patients with long COVIDsymptoms, 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

PDF

Cytokine Protocol.pd...

589KB

Materials

Materials

- Coupled magnetic beads
- Detection antibody
- Standard
- Quality control
- Detection antibody diluent HB
- Sample diluent HB
- Standard diluent HB
- Streptavidin PE
- Stop Solution
- Wash Buffer Concentrate (20X)
- Adhesive Plate Covers
- Bio-plex magnetic washer
- Bio-Plex® 200 Luminex
- Deionized water

Methods

1 Assay reagents: Prepare the assay diluent, standard diluent HB, detection antibody diluent HB, and sample diluent by gentle mixing until homogeneous. Leave the reagents at room temperature for 30 minutes prior to use.

2 Wash buffer: Dilute the 10X wash buffer with distilled water at a ratio of 1:9.

3 Samples: Dilute serum samples 1:4 with sample diluent before analysis.

4 Standards and controls: Reconstitute the lyophilized standard by adding 250 µL of standard diluent HB. Mix gently by vortexing for 5 seconds and keep the solution on ice for 30 minutes before use.

5 Standard dilutions: Prepare a 4-fold serial dilution of the standard as illustrated. Mix each dilution by vortexing for 5 seconds to ensure homogeneity.

6 **Preparation of coupled beads**
Mix the coupled beads by vortexing for 30 seconds, then dilute to 1× in assay buffer. For a 96-well plate, prepare the working solution by mixing 570 µL of 10× coupled beads with 5130 µL of assay buffer (final volume: 5700 µL). Protect the solution from light.

7 **Bead addition**
Add 50 µL of the prepared 1× coupled beads to each well of a 96-well plate.

8 **Plate washing**
Wash the plate twice with 100 µL of wash buffer per well.

9 **Sample and standard addition**
Add 50 µL of sample, standard, blank, or control to each well.

10 **Incubation**
Seal the plate with sealing tape and incubate at room temperature with shaking at 850 rpm for 30 minutes.

11 **Detection antibody preparation**
Vortex the detection antibody for 5 seconds, then dilute to 1× in detection antibody diluent. For a 96-well plate, prepare the solution by mixing 300 µL of

10× detection antibody with 2700 µL of detection antibody diluent (final volume: 3000 µl).

12 **Plate washing**
Wash the plate twice with 100 µL of wash buffer per well.

13 **Detection antibody incubation**
Seal the plate with sealing tape and incubate at room temperature with shaking at 850 rpm for 30 minutes.

14 **Streptavidin-phycoerythrin (SA-PE) preparation**
Vortex the 100× SA-PE for 5 seconds, then dilute to 1× in assay diluent. For a 96-well plate, prepare the solution by mixing 60 µL of 100× SA-PE with 5940 µL of assay diluent buffer (final volume: 6000 µl). Protect the solution from light.

15 **Plate washing**
Wash the plate three times with 100 µl of wash buffer per well.

16 **SA-PE incubation**
Add 50 µL of the prepared 1× SA-PE solution to each well. Seal the plate and incubate at room temperature with shaking at 850 rpm for 10 minutes.

17 **Final washing**
Wash the plate three times with 100 µL of wash buffer per well.

18 **Bead resuspension**
Resuspend the beads in 125 µL of assay buffer per well. Seal the plate and shake at 850 rpm for 30 seconds at room temperature.

19 **Data acquisition**
Read the plate using the Bio-Plex 3D system with DD Gates to select MagPlex beads.

Protocol references

the Bio-Plex Pro™ Human Cytokine Screening Panel from BIO-RAD, CA, USA