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Recombinant immunofluorescence assay (rIFA) for the detection of MERS-CoV (recombinant spike)

Recovery of spike-plasmid (can be provided upon request)

- Cut out marked spot from filter paper
- Soak in 50 µl of sterile PCR-grade water for 5 min
- Transform 5 µl in Top10 *E. coli* (Invitrogen Cat# C4040-03) as described in the manual
- Plate bacteria on a LB Agar plate containing 50 µg/ml ampicillin and incubate O/N at 37°C
- Pick single colony and grow in LB medium containing 50 µg/ml ampicillin for mini- or midi-DNA preparation
- Sequence plasmid for confirmation of correct sequence

Expression of recombinant spike proteins in Vero cells

- Add 2.5 µg of plasmid DNA to 500 µl Opti-Pro (serum-free medium)
- Briefly vortex and centrifuge
- Add 7.5 µl FuGene HD (Promega Cat# E2311) without touching the wall of the tube with the tip
- Incubate 15' at room temperature
- Harvest Vero cells and resuspend in DMEM 10% FCS without antibiotics
- Count cells and seed 1×10^6 cells/well in a 6-well plate in 2.5 ml DMEM 10% FCS without antibiotics
- Drop the transfection mix on the cells
- Gently shake the plate
- Incubate for 24 h at 37°C in a cell culture incubator

Preparation of rIFA slides

- Prepare multitest cover slides (e.g. 12 spots, diameter 5 mm, Cat. No. 40-412-05, Dunn Labortechnik, Asbach, Germany) by rigorous washing and autoclaving
- Harvest transfected Vero cells by trypsin-treatment and resuspend in 5 ml DMEM
- Count cells and centrifuge at 300 x g for 5 min
- Resuspend in calculated amount of DMEM 10% FCS without antibiotics to achieve a cell density of 2.5×10^5 cells/ml.
- Place multitest cover slides in a humid chamber and apply 50 μ l of cells to each spot
- Incubate at 37°C in a cell culture incubator for 6 h to allow cells to attach to the glass surface
- Gently wash slides 2x with PBS
- Fix cells with ice-cold acetone/methanol (ratio 1:1) for 10 min
- Dry slides O/N at room temperature
- Slides can be stored at 4°C under dry conditions for approx. 1 month

rIFA to detect human immunoglobulin IgG

1. For IgG screening purposes: Dilute human sera (30 μ l per incubation field) in e.g. 1:40 and 1:100 and 1:400 in EUROIMMUN sample buffer (includes blocking solution)
2. Apply the 30 μ l serum dilutions to the fields on the cover slides
3. Incubate at room temperature for 30 min to 1 h
4. Rinse the slides with PBS-Tween 0.1% (PBS-T) and additionally wash 2 times for 5 minutes with PBS-T in a cuvette on a rocking shaker.
5. Carefully dry the edges of each incubation field with a piece of cotton
6. Add 25 μ l secondary antibody to each field. Use IgG conjugate (goat anti-human immunoglobulin labeled with FITC, Cy2, Alexa488)
7. Incubate at room temperature for 30 minutes
8. Rinse and wash 2 times with PBS-T for 5 minutes on a rocking shaker
9. Rinse slides with water
10. Dry edges of slides with a piece of cotton/paper
11. Apply one drop of DAPI ProLong mounting medium or glycerin buffer directly to each incubation field and add a glass cover slip.
12. Keep dark and dry for storage until microscopic analysis

Remark: For differential rIFA other humanpathogenic CoV spikes (HCoV-NL63, -229E, -OC43, -HKU-1, SARS-CoV) should be included.