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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 1x10^6 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 2.5x10^5 cells/ml.
- ➔ Place multitest cover slides in a humid chamber and apply 50 µl 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 µl 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 µl 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 µl 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.