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Conventional Immunofluorescence assay (MERS-CoV):

Materials:

MERS-CoV immunofluorescence slides with 12 incubation fields (inactivated)
Thin glass cover slips
PBS
Tween20
EUROIMMUN sample buffer (already contains a blocking solution)
Glycerol/Mounting medium for mounting
Human conjugate (Cyanin 2-labeled goat-anti human in glycerol)
Aqua dest.
37°C incubator
Humid chamber
Glass cuvette
Rocking shaker/table
Fluorescence microscope

Information: All slides contain 12 incubation fields with MERS-CoV infected Vero cells mixed 1:1 with non-infected cells. Cells were spotted onto slides and fixed/inactivated with paraformaldehyde (4%) for 30 minutes and ice-cold acetone/methanol (ratio 1:1) for 10 minutes.

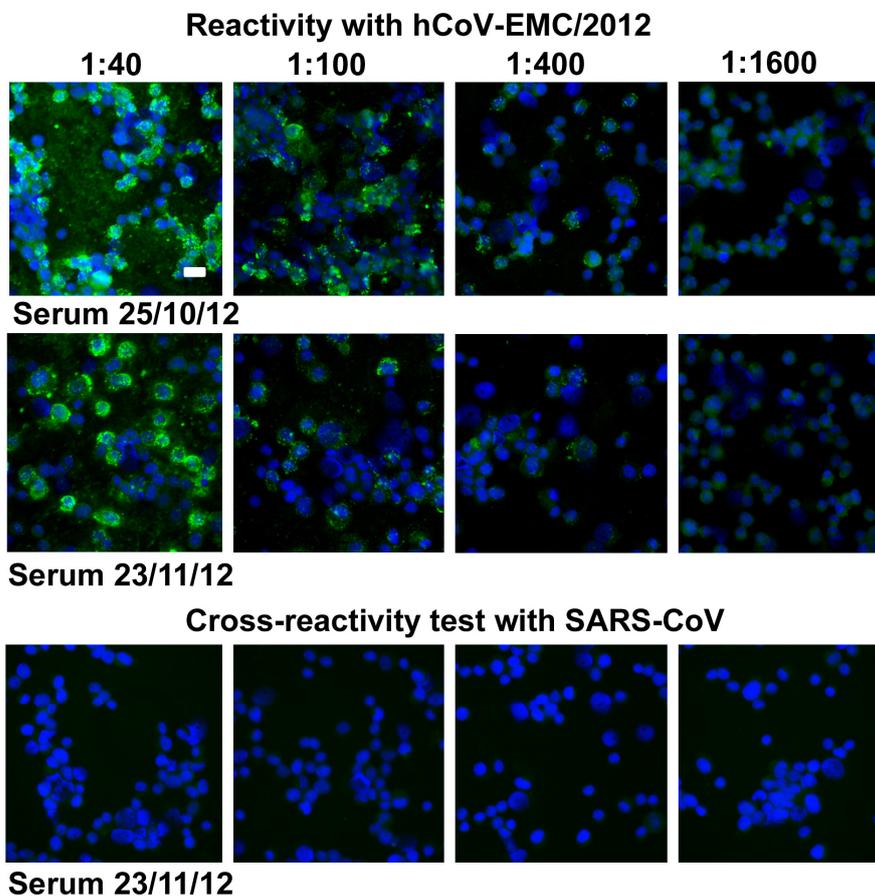
Preparations: Prepare PBS with Tween20 detergent and mix thoroughly before use. (PBS-T can be stored at 4°C for max 1 week). Serum dilutions should be used at the same day.

Procedure:

1. Dilute human sera (25 µl per spot) in dilutions 1:10 and 1:100 in sample buffer (for screening purposes)
2. Apply the 25 µl serum dilutions directly to the spots
3. Incubate at 37°C for 1 h in a humid chamber
4. Rinse the slides with PBS-Tween 0.1% (PBS-T, see preparations) and additionally wash 3 times for 5 minutes with PBS-T in a glass cuvette on a rocking shaker
5. Dry edges of slides with a piece of paper (**Please do not touch the incubation fields**)
6. Prepare the Cyanin 2-labeled human conjugate (green). You will need 25 µl per incubation field. Vortex briefly the stock solution (antibody is in glycerol!) and make a 1:100 dilution in PBS.
7. Add 25 µl human conjugate per field (goat-anti human immunoglobulin labeled with Cyanin 2).
8. Incubate at 37°C for 30 minutes in a humid chamber
9. Rinse with PBS-T and wash 3 times with PBS-T for 5 minutes on a rocking shaker

10. Rinse slides with Aqua dest.
11. Dry edges of slides with a piece of paper (**Please do not touch the incubation fields**) → continued next page
12. Apply one drop of the provided glycerol mounting medium to each spot and add a thin glass cover slip without producing air bubbles (if available, you can also use DAPI stained mounting medium)
13. The glycerol will not solidify. Please store the mounted slides in a horizontal position and keep dark and dry until microscopic analysis.

Example of MERS-CoV (HCoV-EMC/2012) antibody-positive serum samples using spotted slides (DAPI stained nuclei):



Reference:

Corman/Müller et al.; Eurosurveillance, Volume 17, Issue 49, 7 December 2012