Project SOP D-JRP17-WP5.SOP2 – Visualization of intracellular bacteria after infection (IPMA)

Workpackage 5

Responsible Partner: WBVR

Contributing partners: ANSES, APHA, BfR, FLI, IZSAM

GENERAL INFORMATION

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VISUALIZATION OF INTRACELLULAR BACTERIA AFTER INFECTION (IPMA)

1. IPMA (Immunoperoxidase Monolayer Assay)

Prepare wells for IPMA

- Pipet medium off wells
- Wash cells twice with 1mL PBS
- Carefully pipet last PBS out of well
- Dry wells in hood
- Put plates in sealbag
- Store at -20C until IPMA

IPMA

Materials:

- Suitable primary antibody (eg positive serum or specific mAb)
- Secondary HRP conjugate
- PBS + blocking buffer (eg 5% horse serum)
- Xx% Triton-X-100
- Wash buffer A: PBS
- Wash buffer B: PBS + 0.05% TW80
- 1ml AEC solution (stock 4 mg/ml in DMSO) + 19ml substraatbuffer +10µl H_2O_2 30%

Procedure

- Fix cells in 4% PFA and take out of BSL3
- Wash 5x with PBS
- Add 500 ul triton-X 1%, incubate 5 minutes at RT
- Wash 5x in PBS
- Add PBS + blocking buffer (eg 5% horseserum)
- Incubate 1 hr at RT
- Empty wells
- Add primary antibody in blocking buffer, incubate 1 h at RT
- Wash 5x in PBS
- Add secondary antibody in blocking buffer
- 1 h at RT
- Wash 5x in PBS
- Add AEC buffer
- Incubate 15 min at RT
- Wash 5x
- Fill wells with PBS
- Check under microscope