Indirect immunofluorescence assay (IIFA) of IgM Abs

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- 1 Prepare: The preparation of the reagents and of the serum and plasma samples is described on page 4 of this test instruction.
- 2 Pipette: Apply 30 μ l of diluted sample to each reaction field of the reagent tray, avoiding air bubbles. Transfer all samples to be tested before starting the incubation (up to 200 droplets). Use a polystyrene pipetting template.
- 3 Incubate: Start reactions by fitting the BIOCHIP slides into the corresponding recesses of the reagent tray. Ensure that each sample makes contact with its BIOCHIP and that the individual samples do not come into contact with each other. Incubate for

- 4 min at room temperature (+18°C to +25°C).
- 5 Wash: Rinse the BIOCHIP slides with a flush of PBS-Tween using a beaker and immerse them immediately afterwards in a cuvette containing PBS-Tween for at least 5 min. Shake with a rotary shaker if available. Wash max. 16 slides then replace PBS-Tween with new buffer.
- 6 Pipette: Apply 25 µl of fluorescein labelled anti-human globulin to each reaction field of a clean reagent tray. Add all droplets before continuing incubation. Use a stepper pipette. The labelled anti-human serum should be mixed thoroughly before use. To save time, conjugate can be pipetted onto separate reagent trays during the incubation with the diluted sample.
- 7 Incubate: Remove one BIOCHIP slide from cuvette. Within five seconds blot only the back and the long sides with a paper towel and immediately put the BIOCHIP slide into the recesses of the reagent tray. Do not dry the areas between the reaction fields. Check for correct contact between the BIOCHIPS and liquids. Then continue with the next BIOCHIP slide. From now on, protect the slides from direct sunlight. Incubate for 30 min at room temperature (+18°C to +25°C).
- 8 Wash: Fill cuvette with new PBS-Tween. Rinse the BIOCHIP slides with a flush of PBS-Tween using a beaker and put them into the cuvette filled with the new PBS-Tween for at least 5 min. Shake with a rotary shaker if available. Wash max. 16 slides then replace PBS-Tween, with new buffer.
- 9 Embed: Place embedding medium onto a cover glass - drops of max. 10 µl per reaction field. Use a polystyrene embedding template. Remove one BIOCHIP slide from PBS Tween and dry the back, all four sides, as well as the surface around, but not between the reaction fields with a paper towel. Put the BIOCHIP slide, with the BIOCHIPS facing downwards, onto the prepared cover glass. Check immediately that the cover glass is properly fitted into the recesses of the slide. Correct the position if necessary.
- 10 Evaluate: Read the fluorescence with the microscope. General recommendation: objective 20x (tissue sections, infected and transfected cells), 40x (cell substrates). Excitation filter: 450-490 nm, colour separator: 510 nm, blocking filter: 515 nm. Light source: mercury vapour lamp, 100 W, EUROIMMUN LED, EUROStar Bluelight
- 11 Evaluate: fluorescence microscopy