1. PRINCIPLE OF THE TEST

This test is used to detect inactive bacteria. In this case, the sample of interest is coated and fixed on to a glass slide. The presence of the antigen is detected using specific antibodies. After incubation and washing, a second antibody coupled to FITC is added to the slide. The secondary antibody is specific for the species that the primary antibody is raised in. The result is visualized using a microscope which has an ultra-violet light. The sensibility of this test is generally in the region of 10^2 to 10^3 bacteria / ml.

2. PROTOCOL

ANTIGENS

- Clean the glasses with alcohol before use.
- Extract the sample to be tested by grinding 0.5g of tissue in 2ml of **Phosphate buffered saline (PBS) 1X.** Homogenize. In some case, the recommended amount of buffer may have to be increased if the extract is too viscous for pipetting.
- Place a discrete 20 µl drop of bacterial test suspension on a clean glass slide. Repeat for controls.
- Fix the samples by heating at 60°C for 20 minutes using a thermo-stated heating plate.

Probe-Ab — color code: blue

- Dilute the probe antibody in the **PBS Buffer 1X** as recommended on the bottle label (we recommend to test the dilution under your own lab conditions. The slide, incubation chambers and opticals of the microscope used may influence the working dilution and are different from lab to lab. Titers may vary from 250 to 5000 times)
- Add 20µl to each test spot (appropriate the volume according to the dilution).
- Incubate during 30 minutes at room temperature in a tightly closed humid box.
- Wash slide gently two times for 7 minutes with **PBS Buffer 1X**. Rince with distilled water and carefully remove excess moisture.

FITC-Conjugate-Ab — color code: Brown

- Dilute the FITC-Anti-Species antibody in the **PBS Buffer 1X** as recommended on the bottle label.
- Add 20µL to each test spot.
- Incubate during 30 minutes at room temperature in a tightly closed humid box.
- Wash slide gently two times for 7 minutes with **PBS Buffer 1X**. Rince with distilled water and carefully remove excess moisture.

REVELATION

- Spread the buffered glycerol (or alternative mounting medium) in droplets over the glasses and cover this with a fitting standard object glass.
- Examine using a UV microscope.

3. RECOMMENDATIONS

- **Storage**

  Store all the reagents and buffers at recommended temperature

- **Safety**

  Avoid the direct contact with eyes or skin or ingestion of the various compounds

4. BUFFER FORMULATIONS

- **PBS 1X**

Dissolve in 1000 ml distilled water:

NaCl	8 g
Na_2HPO_4-$12H_2O$	2.9 g
KH_2PO_4	0.2 g
KCl	0.2 g
NaN_3	0.2 g

The pH of this buffer is 7.4

- **Buffered Glycerol**

Dissolve in 100 ml distilled water:

Na_2HPO_4-$12H_2O$	3.2 g
Na_2HPO_4-$2H_2O$	0.15 g
Glycerol	50 ml

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