- Learn how to isolate and quantify *Legionella* from aqueous sample in less than two hours with the manual immunomagnetic separator (MIMS) and flow cytometric analysis
- Learn how to identify the viable *Legionella* population

## Manual IMS combined with flow cytometry

Manual IMS can be performed with the rqmicro MIMS instrument (manual immunomagnetic separator). It delivers purified and concentrated samples ready for analysis with a standard flow cytometer (Figure 1). Combined with the rqmicro *Legionella* DETECT kits, it provides a complete solution for sample preparation. The target cells are isolated from aqueous samples with different matrices by using antibody-coated magnetic particles. The bacteria are stained with fluorescence-labeled antibodies for subsequent analysis by flow cytometry. Moreover, the use of a viability dye allows discriminating between living and dead cells. Performance tests show a recovery of > 80% and a broad working range. Results are achieved in less than two hours instead of up to 14 days with a 10 - 40 fold higher sensitivity compared to ISO 11731. The lower detection limit is at around 20 - 50 cells per liter depending on complexity of the sample matrix and the flow cytometric instrumentation used.

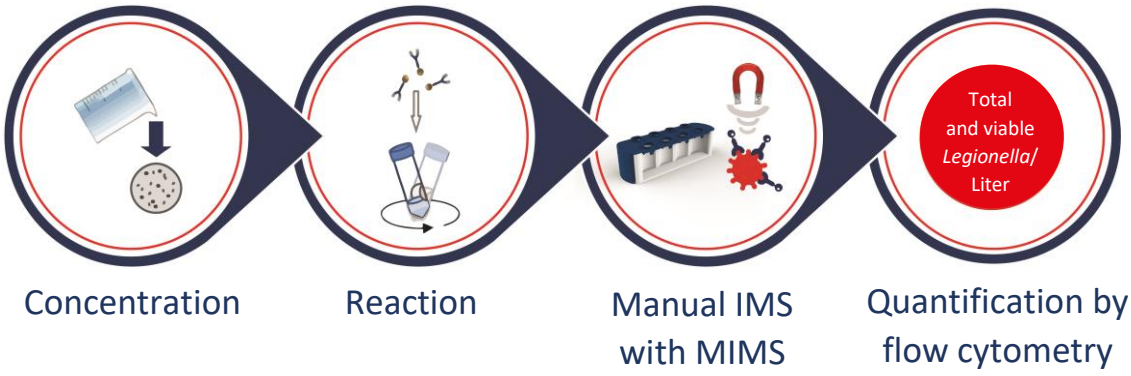



**Figure 1:** Quantitative results in less than 2 hours following a straightforward workflow from filtration to quantification using a standard flow cytometer.

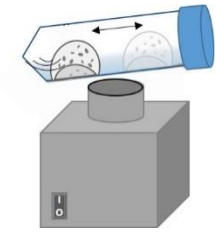
## Protocol

Optional for samples with complex matrices/PLUS Kit: Prefiltration of the total sample volume using a 5 µm filter and collection of the filtrate in a sterile container.

1. Filtrate the desired amount of water with a standard filtration unit, using a 0.2 µm polycarbonate filter provided with the *Legionella* kits.

2. Remove the filter from the filtration unit and place it into a 50 mL tube containing 3 mL of buffer 1 (incubation buffer). The filter should lie flat on the inner wall of the tube.

3. Vortex the 50 mL tube for 60 s in a horizontal position, thereby resuspending the bacterial cells in buffer 1. Remove the filter from the tube.



4. Transfer the 3 mL suspensions in 5 mL tubes.

5. Gently mix the suspension containing the magnetic particles and add 30 µL to each sample. Centrifuge the staining dye for 2 min at maximum speed and add 30 µL to each sample.

6. Incubate the samples for 30 min at RT with gentle shaking or rocking. Protection from light is recommended but not necessary.

7. Transfer the tubes onto the magnet rack. Incubate for 5 min to immobilize the magnetic particles. Then carefully remove the supernatant with a pipette. Take the tubes off the magnet rack and add 3 mL of buffer 2. Mix the samples thoroughly by vortexing and ensure that all magnetic particles are in suspension. Repeat this step.

8. Transfer the tubes onto the magnet rack. Incubate for 5 min to immobilize the magnetic particles. Then carefully remove the supernatant with a pipette. Take the tubes off the magnet rack and add 1 mL of buffer 2 for the final resuspension. Vortex.

9. In order to distinguish between live and dead *Legionella* cells, transfer half of the positive fraction into a fresh 1.5 mL tube and stain with 10 µL/mL viability dye, vortex and incubate for 10 min.

10. Quantify target cells with a standard flow cytometer.

## Configuration of the flow cytometer

Gate cells on green (staining dye) vs. red (viability dye) in order to identify the viable cell population of interest. SCC vs. green can be used in order to obtain additional information for optimal gating.

## Results

### Fixed cells spiked in tap water

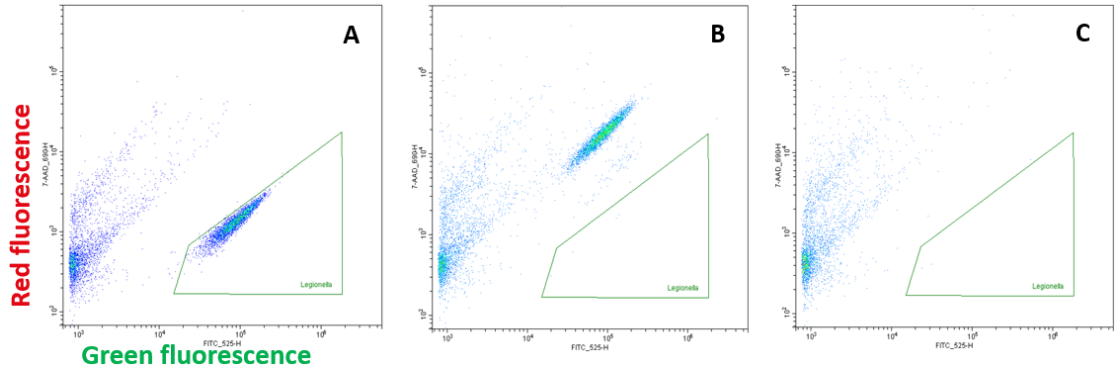



**Figure 2.** Positive control (lyophilized, chemically fixed *Legionella pneumophila* Philadelphia cells) spiked in tap water and processed according to protocol. Total *Legionella* with staining dye (Figure 2A) and viable *Legionella* with staining dye and viability dye (Figure 2B). Non-spiked tap water (negative control) processed according to protocol and stained with staining dye (Figure 2C). Representative plots.

### *L.p.* SG1 cells from culture spiked in EVIAN

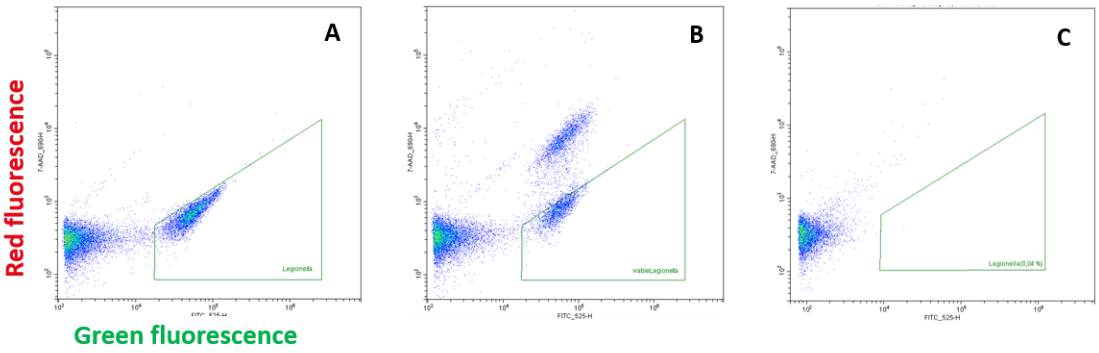



**Figure 3.** *L.p.* SG1 cells spiked in EVIAN and processed according to protocol. Total *Legionella* with staining dye (Figure 3A) and viable *Legionella* with staining dye and viability dye (Figure 3B). Non-spiked EVIAN (negative control) processed according to protocol and stained with staining dye (Figure 3C). Representative plots.

## Reagents

*Legionella pneumophila* SG1 DETECTION Kit.

## Instrument

MIMS

**Contact us:**

**rqmicro Ltd.**
Brandstrasse 24
8952 Schlieren
Switzerland

+41 44 512 51 51

Sales support: sales@rqmicro.com
General information: info@rqmicro.com

www.rqmicro.com