Supplementary information

Rapid detection of total and viable *Legionella pneumophila* in tap water by immunomagnetic separation, double fluorescent staining and flow cytometry

Hans-Anton Keserue[1,2,3], Andreas Baumgartner[2], Richard Felleisen[2], Thomas Egli[1,3]

[1] Swiss Federal Institute for Aquatic Science and Technology (Eawag), Überlandstrasse 133, P.O. Box 611, CH-8600, Dübendorf, Switzerland

[2] Federal Office of Public Health (FOPH), Schwarzenburgstrasse 165, CH-3003, Bern, Switzerland

[3] Institute of Biogeochemistry and Pollutant Dynamics (IBP), ETH Zurich, Universitätsstrasse 16, 8092 Zurich, Switzerland.

Running Title: Rapid detection of viable *Legionella* by FCM

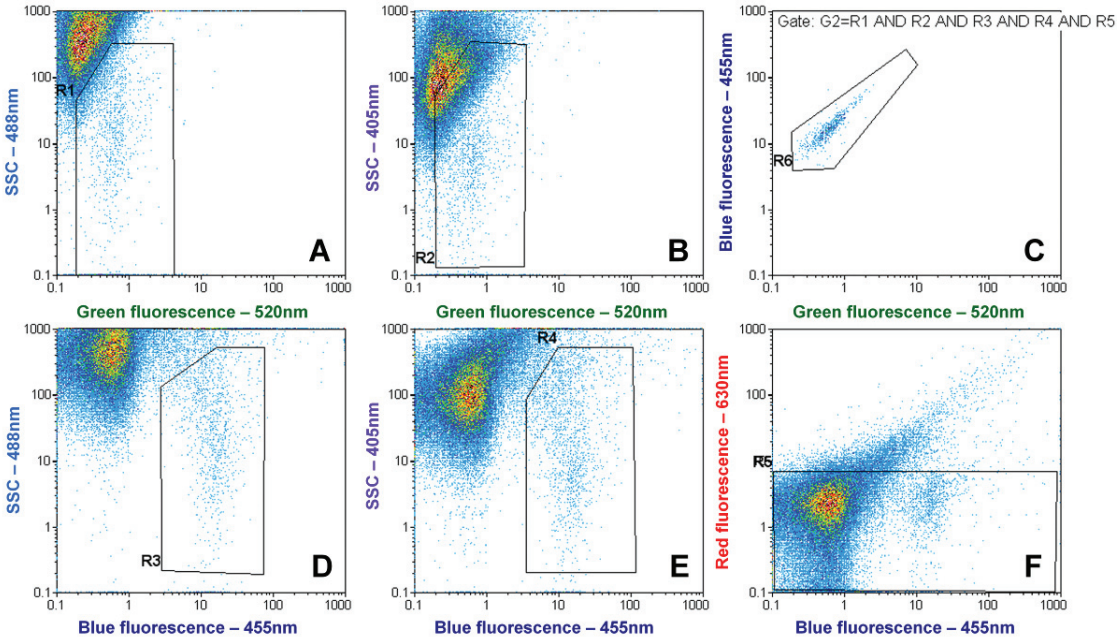


Fig. S 1 Example of FCM dot plots for *Lp* spiked to tap water. Complete view of Fig. 2 Plot A. Gates in plot A, B, D-F are applied to plot C in order to discriminate background signals and membrane-damaged *Lp* cells.

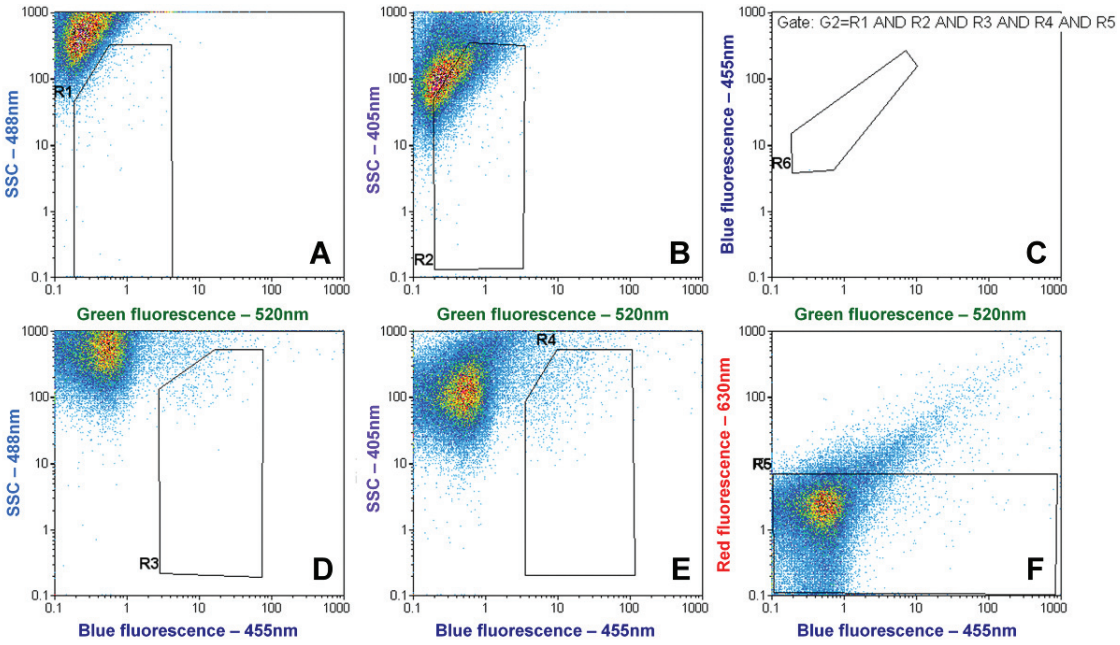


Fig. S 2 Example of FCM dot plots for *Lp* spiked to tap water. Complete view of Fig. 2 Plot B. Gates in plot A, B, D-F are applied to plot C in order to discriminate background signals and membrane-damaged *Lp* cells.

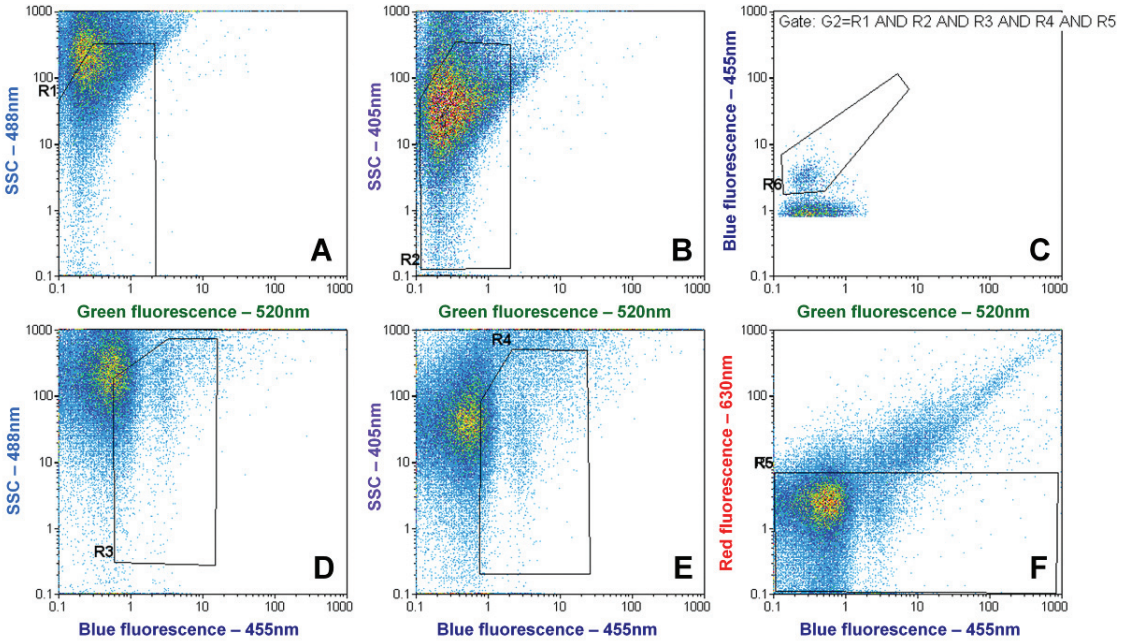


Fig. S 3 Example of FCM dot plots for *Lp* spiked to tap water. Complete view of Fig. 2 Plot D. Gates in plot A, B, D-F are applied to plot C in order to discriminate background signals and membrane-damaged *Lp* cells.

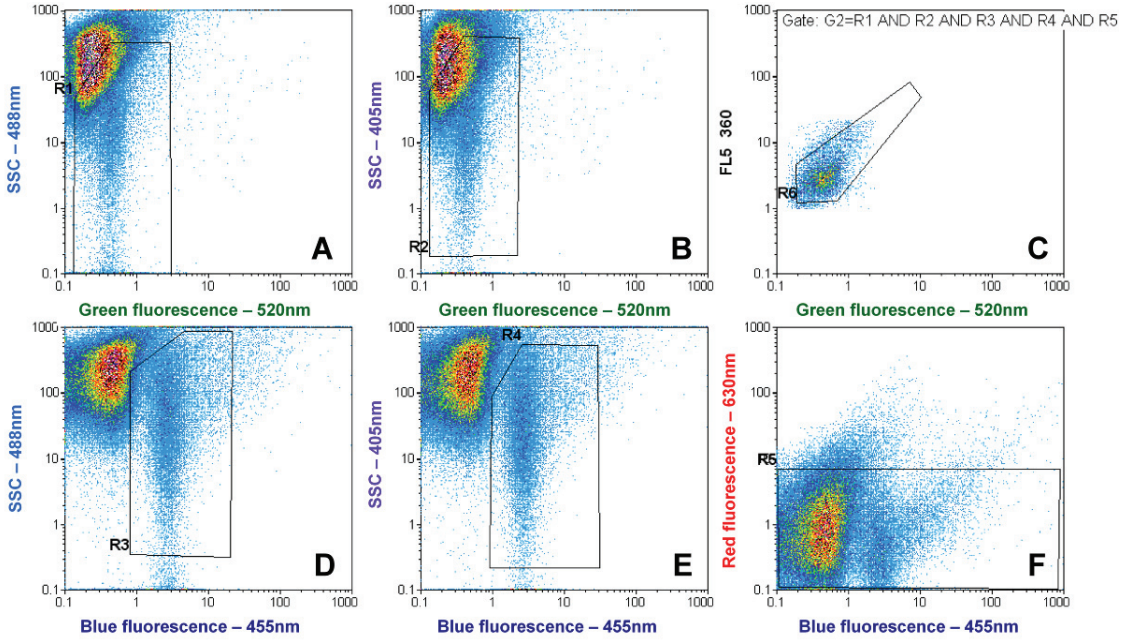


Fig. S 4 Example of FCM dot plots for *Lp* spiked to tap water. Complete view of Fig. 2 Plot E. Gates in plot A, B, D-F are applied to plot C in order to discriminate background signals and membrane-damaged *Lp* cells.

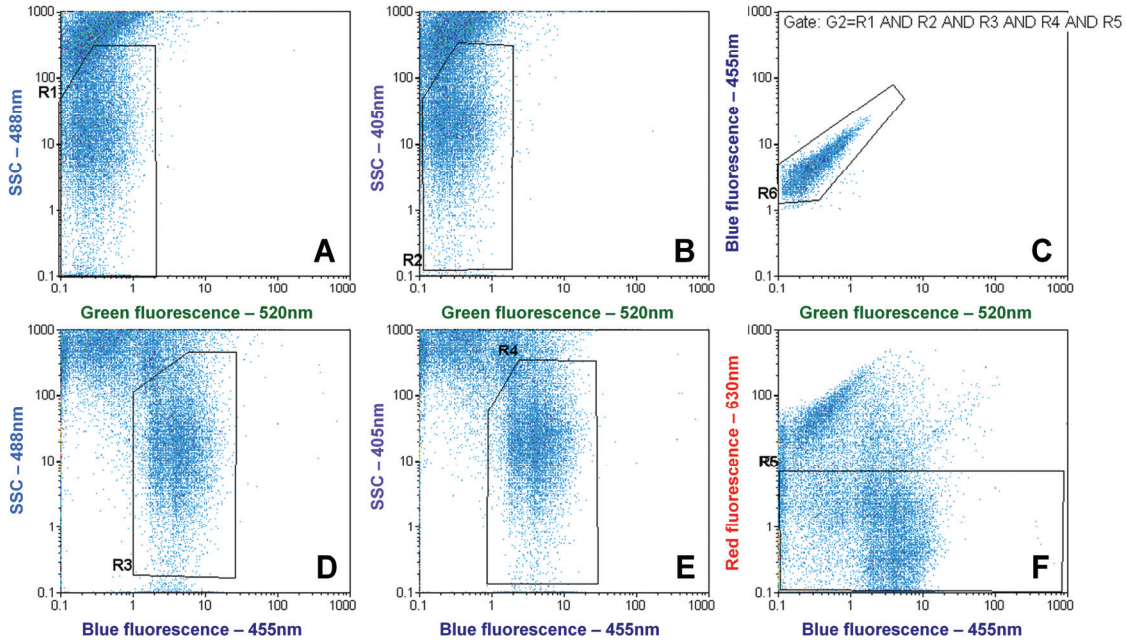


Fig. S 5 Example of FCM dot plots for *Lp* spiked to tap water. Complete view of Fig. 2 Plot F. Gates in plot A, B, D-F are applied to plot C in order to discriminate background signals and membrane-damaged *Lp* cells.

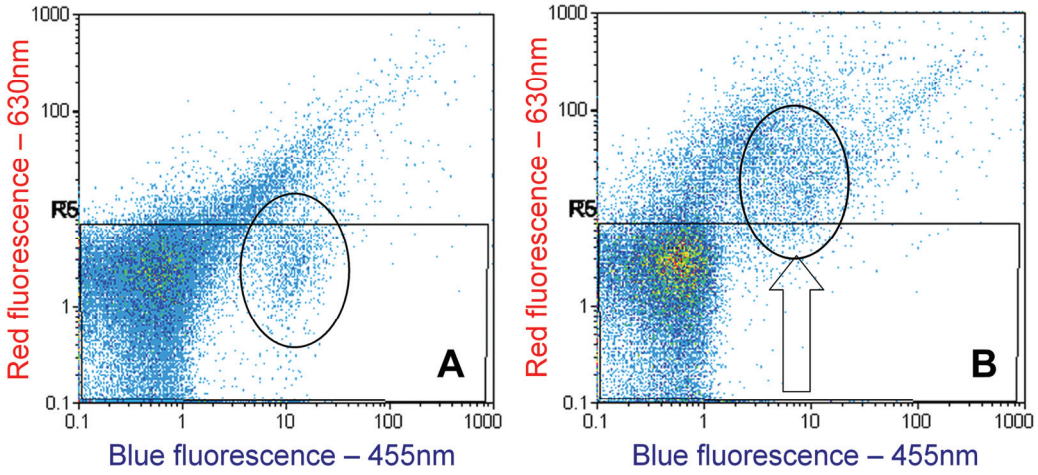


Fig. S 6 Example of blue versus red fluorescence dot plot demonstrating the increase of red fluorescence in membrane damaged *Lp* cells. Plot A shows the cells before heat treatment and plot B is after heat treatment of 90° C for one minute. Before heat treatment the *Lp* cells lie inside the gate (A) and after (B) they pass outside the gate and are thus discriminated as membrane-deficient.

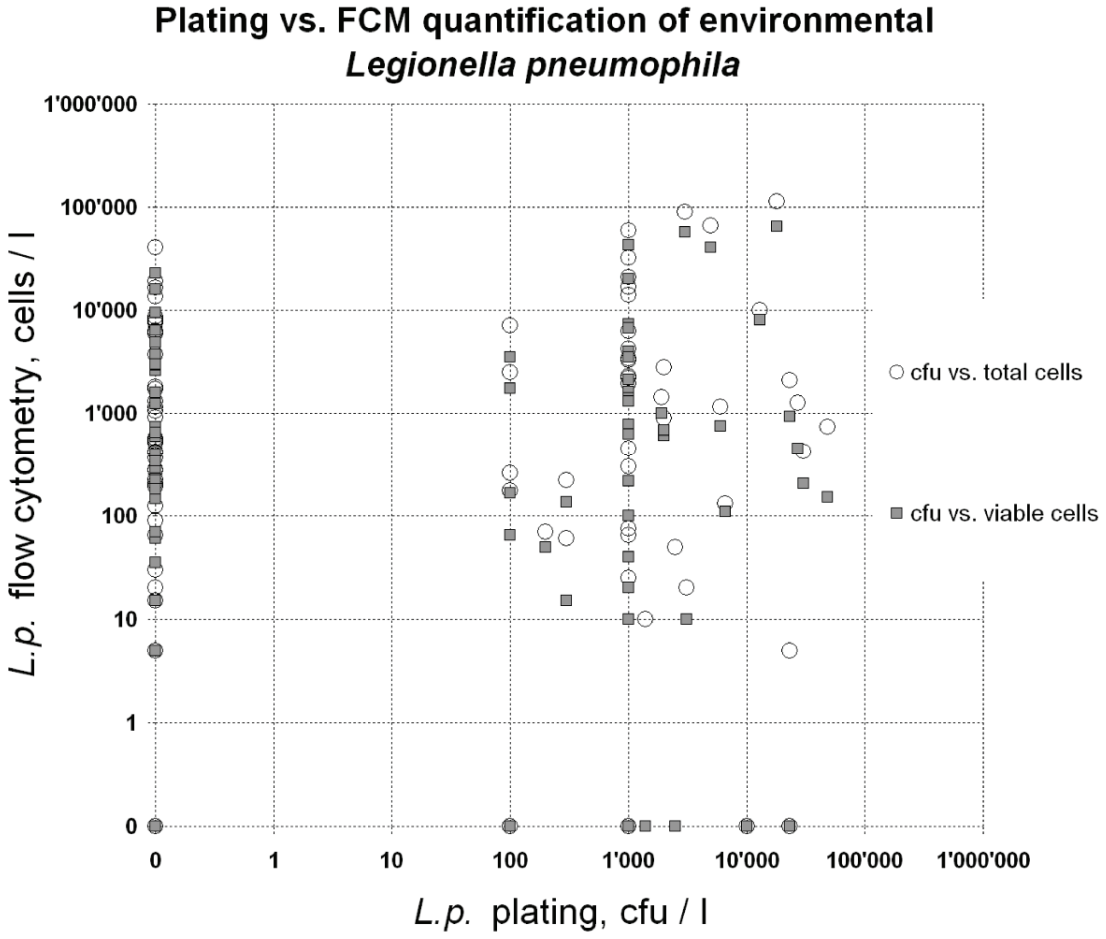


Fig. S 7 Correlation of *Lp* plating versus Flow cytometry for total and viable *Lp.*

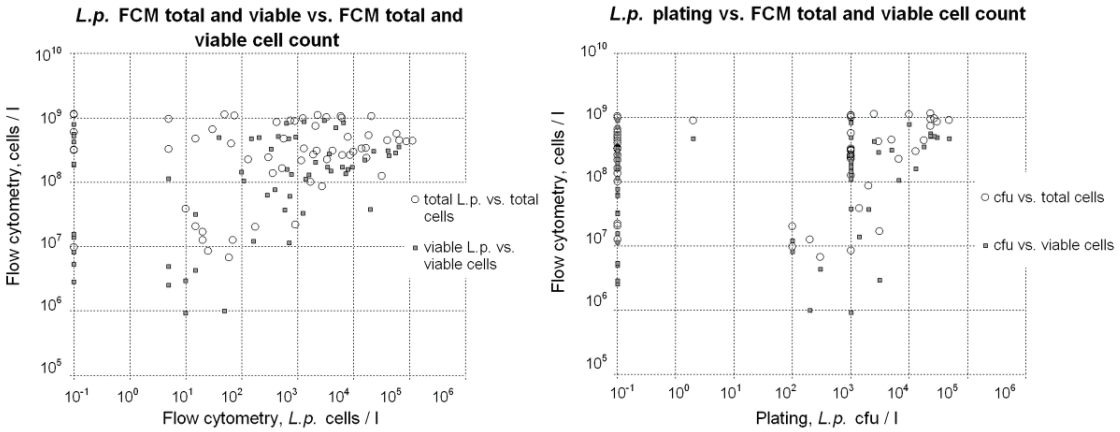
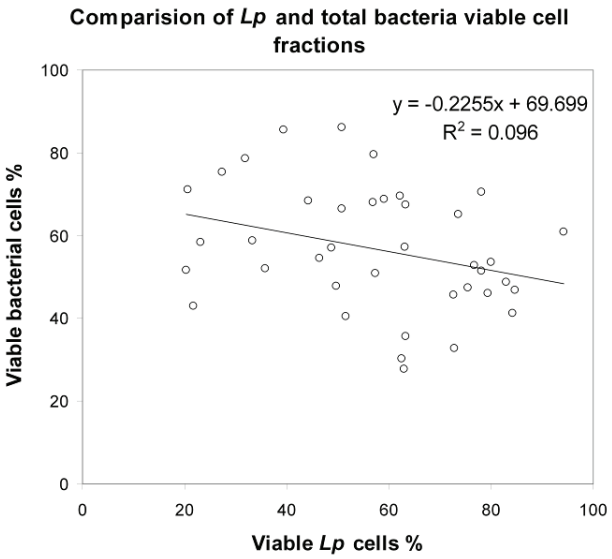


Fig. S 8 Correlations of *Lp* detection by FCM and plating versus total and viable cell count (TCC, VCC). Left plot shows FCM total and viable *Lp* versus TCC and VCC. Right plot shows *Lp* plating versus TCC and VCC.



96

97 **Fig. S 9 Linear correlation of the viable fraction of *Lp* and total cells**

98 **measured by FCM.**

99

100

101

102

103

104

105

Table S 1 Value table of the spiking experiments calibration series (Fig. 3). Recovery is displayed in percent ± the root mean square deviation (RMSD), taking into account the error propagation from seeding to recovery.

Seeding Levels					Recovered Cells*				Recovery			
	L.p. total		*L.p.* viable		*L.p.* total		*L.p.* viable		*L.p.* total		*L.p.* viable	
n	Value ± SD	SD %	Value ± SD	SD %	Value ± SD	SD %	Value ± SD	SD %	%	RMSD %	%	RMSD %
15	0	0	0	0	26.0 ± 8.0	30.6	8.0 ± 2.5	30.9	-	-	-	-
3	41.7 ± 7.6	18.3	36.7 ± 7.6	20.8	32.3 ± 15.6	26.7	37.0 ± 5.4	11.9	**77.6**	**53.7**	**100.9**	**25.2**
3	421.7 ± 29.3	6.9	403.3 ± 28.4	7.0	377.3 ± 31.6	7.8	365.3 ± 18.0	4.8	**89.5**	**11.4**	**90.6**	**9.2**
3	1'920 ± 53	2.8	1'853 ± 76	4.1	1'792 ± 63	3.5	1'712 ± 13	0.7	**93.4**	**4.6**	**92.4**	**4.5**
3	4'147 ± 68	1.6	4'013 ± 45	1.1	3'792 ± 135	3.7	3'534 ± 74	2.1	**91.5**	**4.0**	**88.0**	**2.5**
3	50'512 ± 952	1.9	48'962 ± 579	1.2	38'062 ± 312	0.8	34'585 ± 1'084	3.1	**75.4**	**2.6**	**70.6**	**3.6**
3	450'167 ± 24'939	4.9	430'482 ± 16'017	3.7	382'199 ± 15'492	4.1	345'345 ± 10'081	2.9	**84.9**	**7.0**	**80.2**	**5.5**
							Averages:		**85.4**	**13.9**	**87.1**	**8.4**

*after subtracting the average background signals