Electronic Supplementary Material (ESI) for Lab on a Chip.

Supporting Information

Lateral flow device for bacteria: From troubleshooting of its microfluidics using bioluminescence to colorimetric-based monitoring of water fecal pollution

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LFS reader operation

The LFS reader analyzes the red color (620 to 750 nm) of the lines present in the LF (i.e., the test line (TL) and the control line (CL), as well as a blank section BK, whose positions are indicated to the software by a barcode. Then, the reader provides a numerical value representing the intensity of the color of each line. These values are divided as (TL-BK)/(CL-BK), providing a ratio that indicates the degree of contamination of the sample analyzed. The higher the TL/CL ratio, the more contaminated the sample.

- Time used to perform the measurements: 5 seconds per snapshot, with three snapshots per sample analyzed to provide an average of all the measurements together.

- System error: If the LF detector fails to read the control line will provide a “Measurement Error” as the control line must always be present. Furthermore, the device calculates de variant coefficient (CV) by taking 10 times the measurement of the same LFS. This CV must be lower than 15% to comply with the reproducibility requirements requested by the CE brand (in the EU).

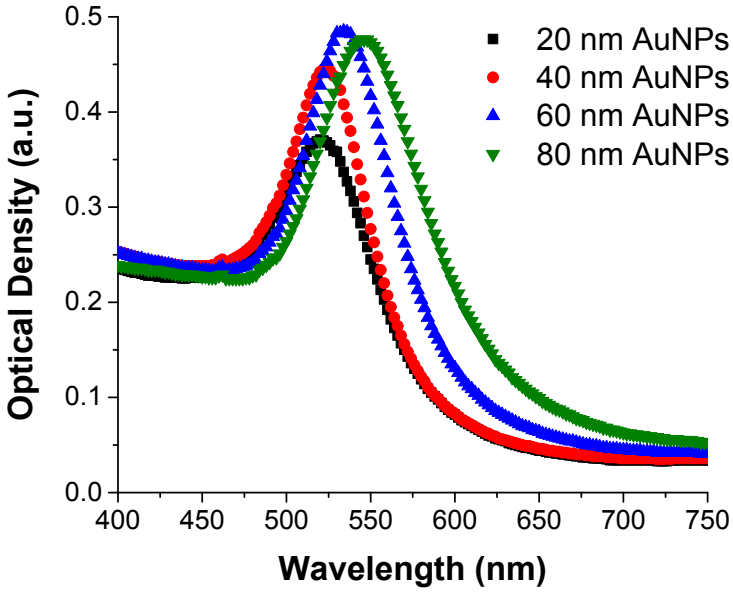


Figure S1. UV-Vis spectra of 20, 40, 60 and 80 nm AuNPs, with maximum absorbance peaks at 520 nm (black squares), 524 nm (red dots), 534 nm (upwards blue triangles), and 546 nm (downwards green triangles), respectively.

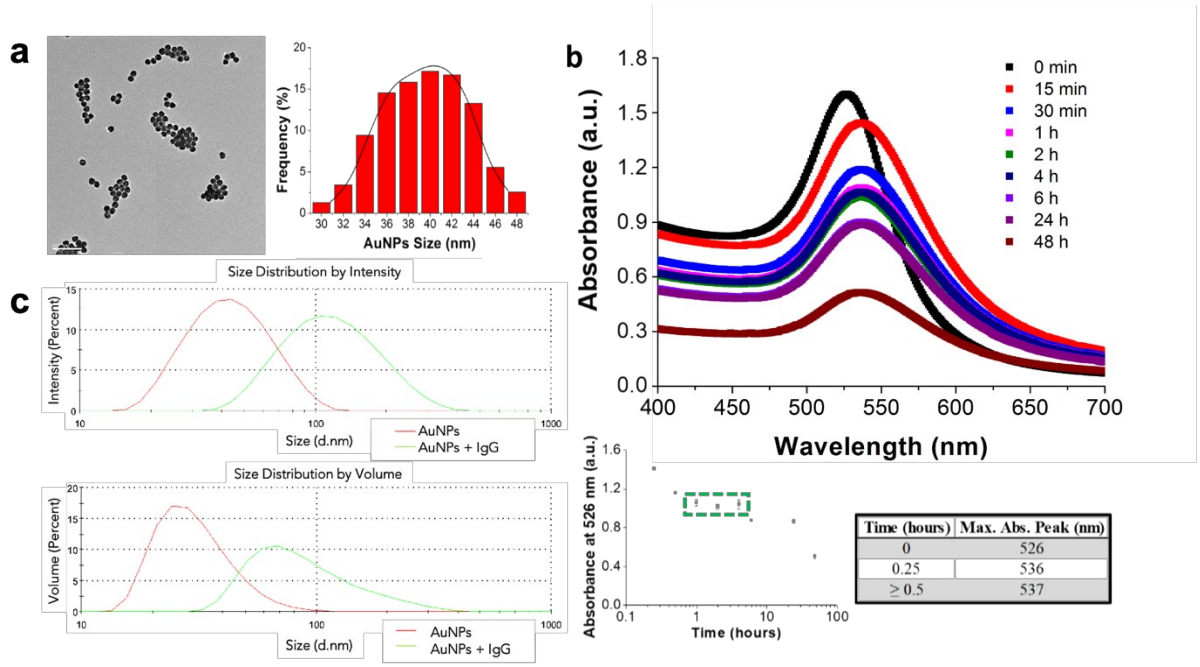


Figure S2. (a) 40 nm AuNPs TEM images and histogram. (b) Spectra of 40 nm AuNPs using different conjugation times with antibodies, from 0 min to 48 hours. The maximum absorbance peak shifts to the right as AuNPs size grows due to the increasing amount of antibodies being conjugated to the AuNPs. On the one hand, too short incubation time does not allow for an optimal antibodies conjugation. On the other hand, too long conjugation time often leads to aggregation and precipitation (i.e. see 48 hours). (c) Average diameters of 40 nm AuNPs unconjugated (red lines) and conjugated to antibodies (green lines) obtained by dynamic light scattering (DLS). Above, size distribution by intensity; below, size distribution by volume.

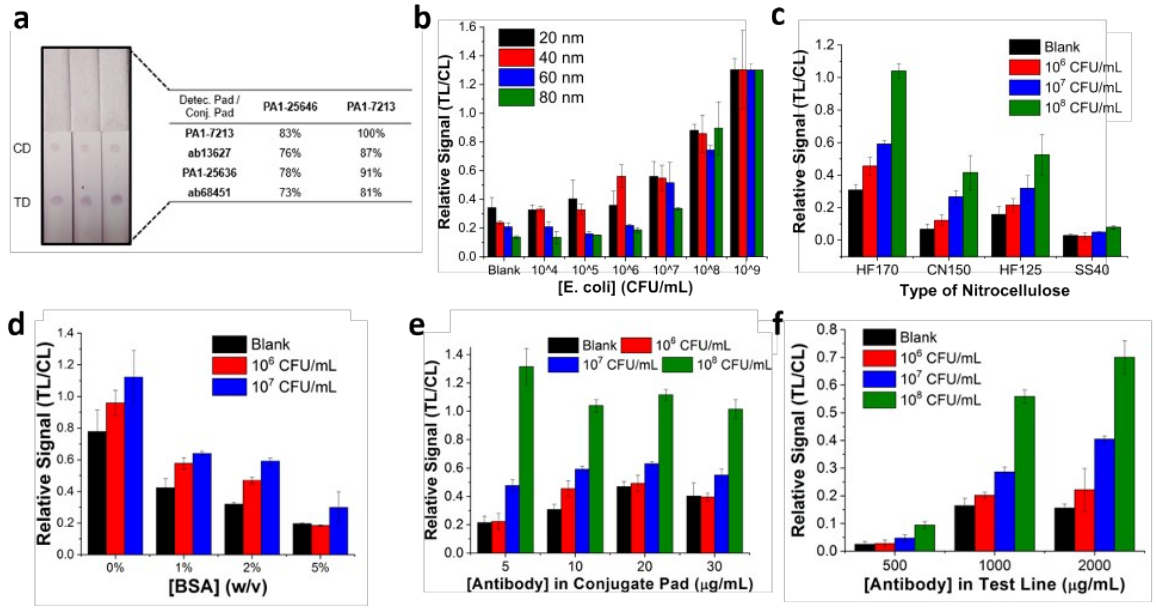
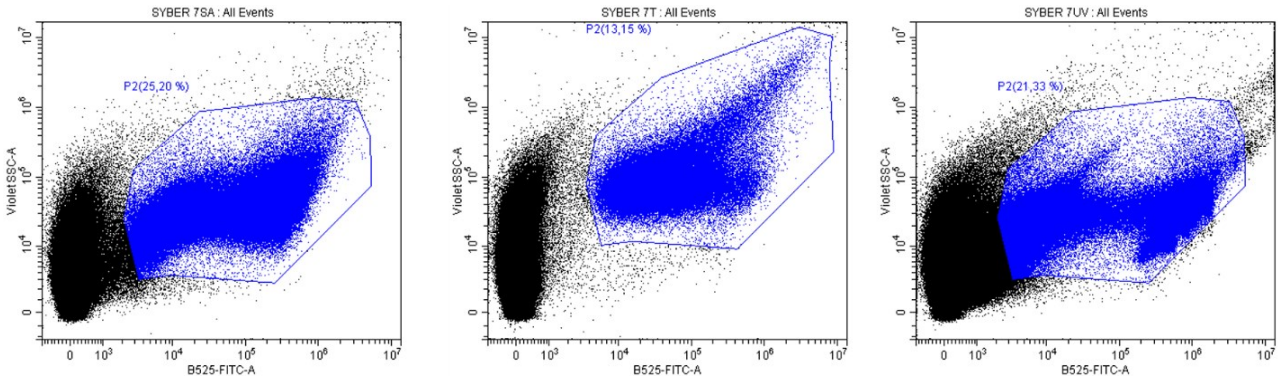


Figure S3. (a) Optimization of the antibodies chosen for the detection of several strains of *E. coli*. Four different antibodies were tested on the detection pad (left row) and two different antibodies were tested on the conjugate pad (upper line) by using a concentration of 10^7 CFU/mL of a pool of *E. coli* strains. The table shows the normalized results of the ratio TD/CD, being TD = test dot, and CD = control dot. The combination of the polyclonal antibody PA1-7213 both in the conjugate and in the detection pad yields the best results (b) Bar chart representing the sensitivity of LFS anti-E. coli using 20 nm (black), 40 nm AuNPs (red), 60 nm (blue), and 80 nm (green) AuNPs. The mathematical detection limit (LOD) was calculated as follows: the calibration curve for the detection of *E. coli* with our LFS yields the following formula: y = a·ln(x) + b, where "a" and "b" are values provided by the logarithmic fitting, and "y" is the value of "the blank plus three times the standard deviation of the blank". Therefore, "x", the detection limit is calculated as: $x = e^{\frac{(Blank + 3 \cdot SD_{Blank} - b)}{a}}$. (c) Bar chart of LFS anti-*E. coli* sensitivity using four different types of nitrocellulose as detection pad. (d) Bar chart of LFS anti-*E. coli* sensitivity showing the different blocking conditions tested on the nitrocellulose pad. (e) Bar chart of LFS anti-*E. coli* sensitivity showing different antibody concentration tested on the conjugate pad. (f) Bar chart of LFS anti-*E. coli* sensitivity showing different antibody concentration tested on the test line (TL).



Sample 1		Sample 2		Sample 3	
LFS	Flow Cytometry	LFS	Flow Cytometry	LFS	Flow Cytometry
10^7 CFU/mL	$1.6 \cdot 10^7$ CFU/mL	10^7 CFU/mL	$5.1 \cdot 10^6$ CFU/mL	10^7 CFU/mL	$1.2 \cdot 10^7$ CFU/mL

Figure S4. Flow cytometry results that confirm the accurate concentration estimated by our LFS in river water (10^7 CFU/mL). We tested 3 different river samples spiked with *E. coli* in our LFS, estimating a concentration of 10^7 CFU/mL for all of them. These results match very well with those obtained by flow cytometry.

Table S1. Calibration curves and detection limits related to the Figures 2b, 2c and 2d.

ID.	Sample	Calibration Curve	Detection Limit (LOD)
Figure 2b	Sensitivity before filtration	$y = 0.14 \cdot \ln(x) - 1.53$	10^6 CFU/mL
Figure 2b	Sensitivity after filtration	$y = 0.08 \cdot \ln(x) - 0.39$	10^4 CFU/mL
Figure 2c	*E. coli* ATCC11303	$y = 0.09 \cdot \ln(x) - 0.91$	10^6 CFU/mL
Figure 2c	*E. coli* ATCC11775	$y = 0.06 \cdot \ln(x) - 0.52$	10^6 CFU/mL
Figure 2c	*E. coli* ATCC25922	$y = 0.07 \cdot \ln(x) - 0.66$	10^6 CFU/mL
Figure 2d	*E. coli*	$y = 0.09 \cdot \ln(x) - 1.09$	10^6 CFU/mL
Figure 2d	*Salmonella*	$y = 3.05 \cdot x - 0.44 \cdot x^2 + 0.02 \cdot x^3 - 6.91$	-
Figure 2d	*A. fischeri*	$y = 7.09 \cdot x - 0.96 \cdot x^2 + 0.04 \cdot x^3 - 16.94$	-
Figure 2d	*E. coli* + *Salmonella*	$y = 0.09 \cdot \ln(x) - 0.96$	10^6 CFU/mL
Figure 2d	*E. coli* + *A. fischeri*	$y = 0.09 \cdot \ln(x + 194671.22) - 0.92$	10^6 CFU/mL

Table S2. Reproducibility intra-assay (within the same batch) and inter-assay (using different batches) of three batches of LFS tested with triplicates of three different bacterial concentrations (10^6, 10^7 and 10^8 CFU/mL).

[*E. coli*] (CFU/mL)	Intra-assay RSD (%)	Inter-assay RSD (%)
10^6	8.0	14.3
10^7	7.7	14.9
10^8	7.9	10.1

Table S3. Sensitivity, detection limit and % recovery of different water samples spiked with *E. coli* and tested with specific anti-*E. coli* LFS.

Water Sample	Calibration Curve	Mathematical LOD (CFU/mL)	% Recovery for 109 CFU/mL
Tap water	$y = 0.10 \cdot \ln(x) - 1.23$	$1.22 \cdot 106$	100%
River water	$y = 0.08 \cdot \ln(x) - 1.01$	$1.93 \cdot 106$	90%
Inlet Sewage water	$y = 0.07 \cdot \ln(x) - 0.84$	$2.01 \cdot 106$	84%
Middle Sewage water	$y = 0.08 \cdot \ln(x) - 0.97$	$8.34 \cdot 106$	80%
Outlet Sewage water	$y = 0.07 \cdot \ln(x) - 0.72$	$1.65 \cdot 106$	88%