Supplementary Materials

Nanozyme-enhanced lateral flow immunochromatographic tests for the sensitive detection of *Francisella tularensis* cells in water samples

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Bacterial strains, cultivation, and inactivation conditions

F. tularensis strains (F. tularensis subsp. holarctica 15 NIIEG, 503, and miura, F. tularensis subsp. tularensis Schu and A-Cole B-399, F. tularensis subsp. mediasiatica 120, and F. tularensis subsp. novicida Utah112) were cultured for 24 h at 37 °C on solid nutrient medium FT-agar with black albumin with the addition of polymyxin B to a concentration of 100 mg/L. The grown cells were suspended in 0.15 M NaCl to a concentration of 5 x 10^9 cells/mL.

Microbial cell concentration was determined by plating microbial suspensions with nutrient media from a series of tenfold dilutions, followed by incubation and counting the resulting colonies. Plating was performed using the surface agar method in triplicate from each dilution. When evaluating the results, plates with bacterial colony counts between 30 and 300 were selected.

The calculation of the cell culture concentration (N) in 1 ml was performed using the formula:

$$N = c / (n_1 + 0.1n_2) \times d,$$

where c is the sum of the counted colonies on all plates; n_1 is the number of plates of the first dilution; n_2 is the number of plates of the second dilution; d is the coefficient of the first dilution; 0.1 is the coefficient taking into account the multiplicity of the first and second dilutions.

For thermal inactivation, the suspension was incubated for 1 h at 70 °C. For chemical inactivation, the suspension was incubated for 24 h at 20–25 °C in the presence of 0.1% thimerosal. Inactivated cells were tested for sterility by plating them on thioglycol medium and FT agar.

Table S1

Conditions during synthesis of AuNPs

AuNPs	Volumes of reagents per 100 mL of the reaction mixture, mL			Concentration in the reaction mixture, mM	
	$HAuCl_4$	Water	Sodium citrate	$HAuCl_4$	Sodium citrate
$AuNPs_{14}$	1.0	97.0	2.0	0.3	0.69
$AuNPs_{28}$	1.0	97.25	1.75	0.3	0.60

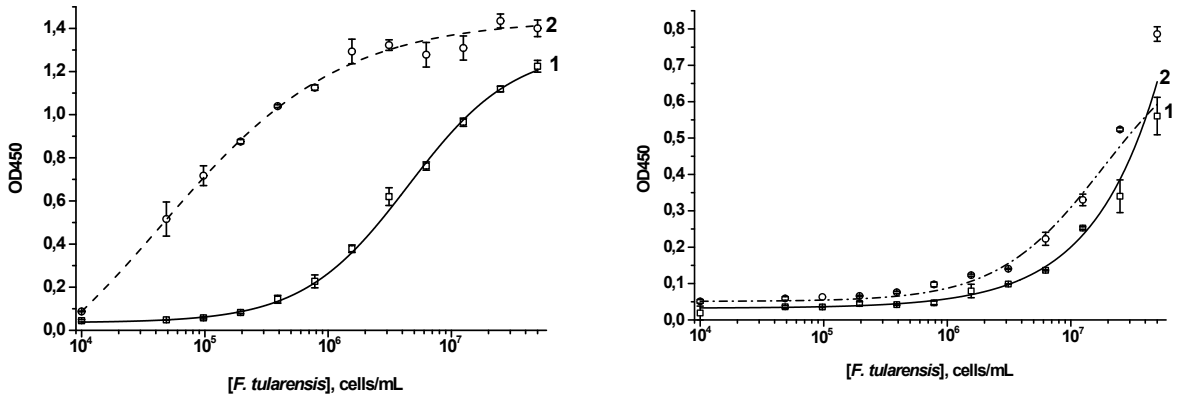

Fig. S1. The dependencies of OD_{450} on cell concentrations obtained as a result of the ELISA using various combinations of immobilized and biotinylated MAb (n = 3). Curves 1 (A, B) – immobilized MAb Fb11, curves 2 (A, B) – immobilized MAb T143, curves 1 and 2 (A) – biotinylated MAb T143, curves 1 and 2 (B) – biotinylated MAb Fb11. Concentrations of both immobilized and biotinylated MAb were 1 μg/mL.

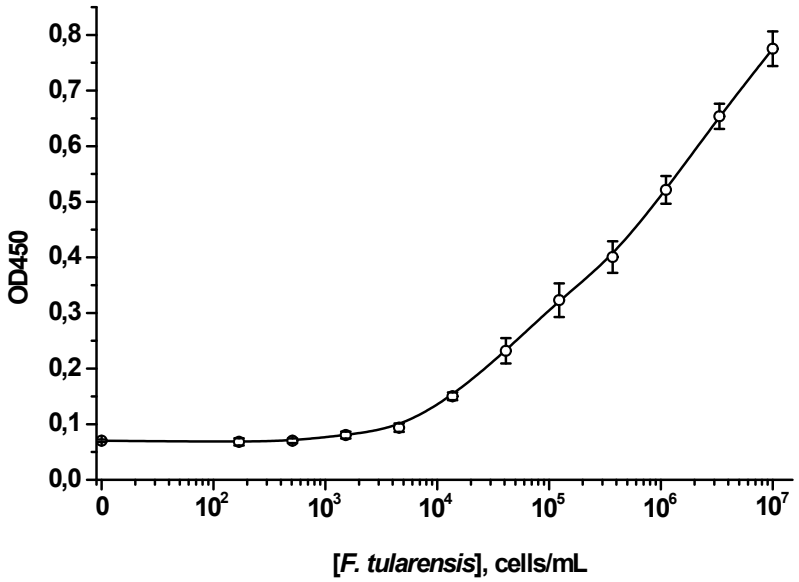

Fig. S2. Calibration curve of *F. tularensis* cells in the ELISA. Concentrations of both immobilized and biotinylated MAb T143 were 1.0 μg/mL (n = 3).

Table S2

Dimensional characterization of AuNPs14, AuNPs28 and Au@Pt NPs by spectrophotometry, TEM, and DLS registration.

NPs	Peak wavelength, nm	Average diameter (by spectrum), nm	Average diameter (by DLS), nm	Pdi (DLS)	Average diameter (by TEM), nm	Ellipticity (TEM)
$AuNP_{14}$	516.5	13.9	13.8	0.2	14.2 ± 1.3	1.2 ± 0.1
$AuNP_{28}$	522.5	28.0	31.4	0.2	28.1 ± 2.3	1.1 ± 0.04
Au@Pt NPs	293	___	54.8	0.6	43.4 ± 10.8	1.1 ± 0.2

Table S3

Surface areas, volumes, weights, and particle concentrations of $AuNPs_{14}$, $AuNPs_{28}$, and Au@Pt NPs.

NPs	Surface area, nm^2	Volume, cm^3	Weight of a single NP, mg	Concentration, NPs/mL
$AuNP_{14}$	531	1.2×10^{-18}	2.2×10^{-14}	2.6×10^{12}
$AuNP_{28}$	2550	12.1×10^{-18}	2.3×10^{-13}	0.3×10^{12}
Au@Pt	5914	42.8×10^{-18}	0.8×10^{-12}	0.1×10^{12}

Table S4

Parameters varied during optimization of *F. tularensis* LFIAs.

Parameter	Varied range	Selected value
AuNPs-based LFIA		
Clone of MAb immobilized in the TZ*	MAb Fb11, MAb T143	MAb T143
Clone of the labeled MAb*	MAb Fb11, MAb T143	MAb T143
Concentration of MAb T143 immobilized in the TZ, mg/mL	0.25–2	1
Concentration of GAMI immobilized in the CZ, mg/mL	0.2–0.6	0.5
OD_{520} of MAb T143–AuNPs	1–6	4
Volume of the test sample, µL	50–100	60
Duration of the incubation of the test strip with the sample	5–15	10
Enhanced Au@Pt NPs-based LFIA		
Concentration of MAb T143 immobilized in the TZ, mg/mL	0.75–2	2
Concentration of DAGI immobilized in the CZ, mg/mL	0.2–0.6	0.5
Dilution of MAb T143–Au@Pt NPs	2–15	12
Type of the peroxidase substrate	TMB, DAB	DAB
Volume of the substrate mixture applied to the test strip, µL	0.5–5	1.2
Duration of the amplification stage, min (for enhanced format)	1–5	2

*was selected in the sandwich ELISA for immobilization in the wells of the microplate and transferred to LFIA conditions

**was selected in the sandwich ELISA for labeling by biotin and transferred to LFIA conditions

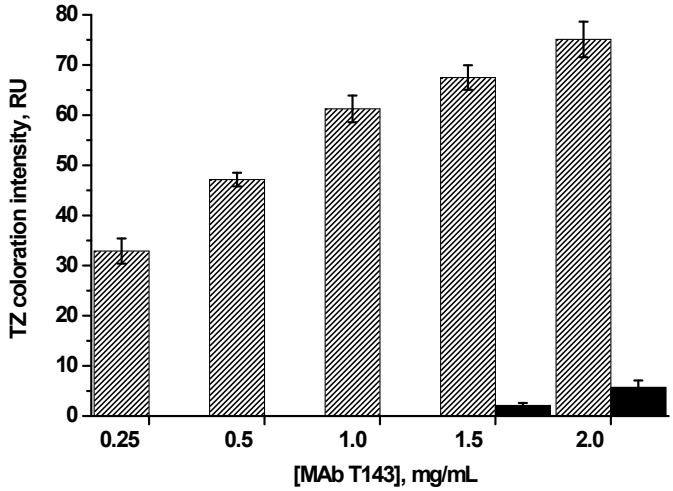

Fig. S3. Dependency of the TZ coloration intensity on the immobilized MAb T143 concentration (n = 3). The concentration of *F. tularensis* cells was 3×10^7 (shaded bars) and 0 (black bars) cells/mL.

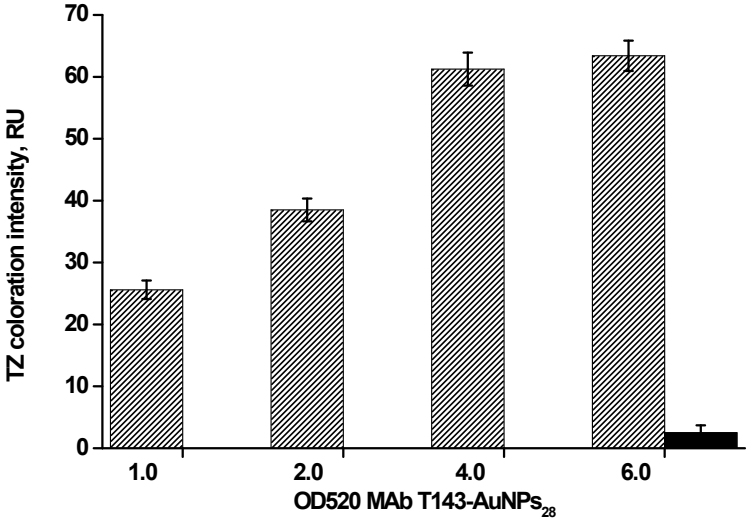

Fig. S4. Dependency of the TZ coloration intensity on the OD_{520} of the MAb T143–$AuNPs_{28}$ conjugate (n = 3). The concentration of *F. tularensis* cells was 3×10^7 (shaded bars) and 0 (black bars) cells/mL.

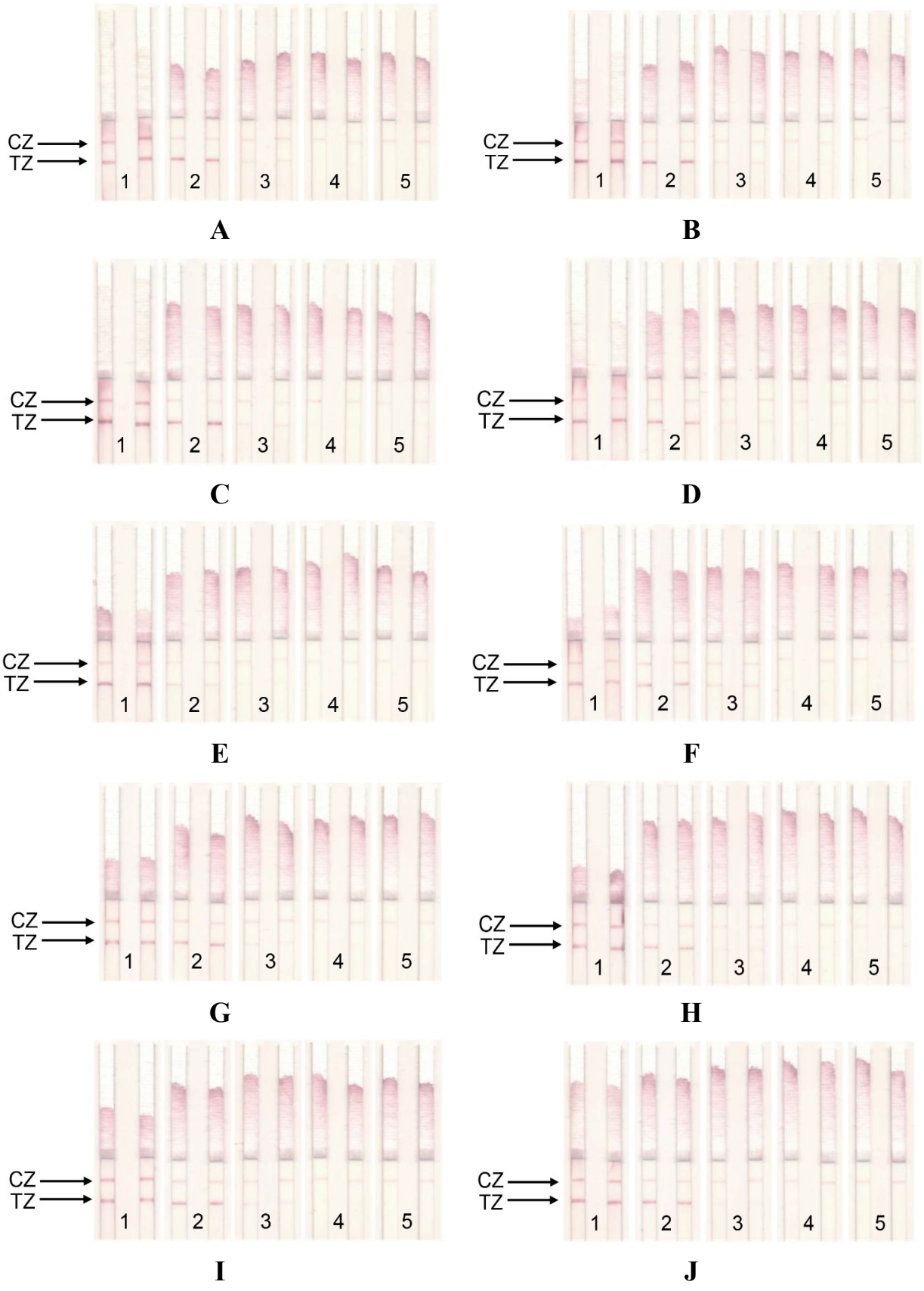

Fig. S5. Test strips after the LFIA of *F. tularensis* holarctica P-464 (A), holarctica XM1 (B), holarctica A-1045 (C), holarctica C-12 (D), holarctica 55 (E), mediasiatica 117 (F), mediasiatica A-178 (G), mediasiatica K-334 (H), mediasiatica A-823 (I), nearctica 8859 (J). Cell concentrations

are indicated on the bottom of the test strips: 1.0×10^7 (1), 1.0×10^6 (2), 1.0×10^5 (3), 1.0×10^4 (4), 1.0×10^3 (5).

Fig. S6. Images of test strips after the enhanced LFIA with TMB (A) or DAB (B) as a peroxidase substrate. The concentration of cells was 1.0×10^6 cells/mL.

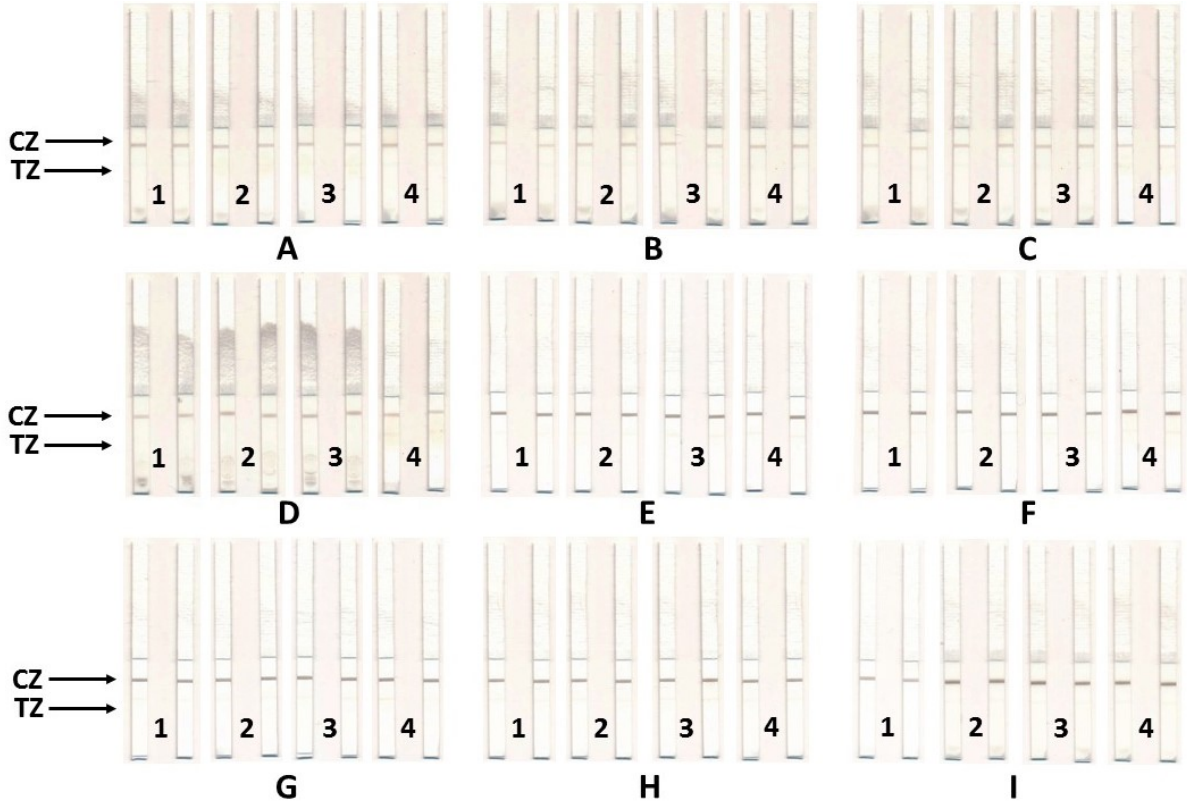

Fig. S7. Test strips after the LFIA of different bacterial pathogens: *Brucella abortus* (A), *Escherichia coli* 0157:H7 ATCC51658 (B), *Listeria monocytogenes* ATCC52658 (C), *Pseudomonas aeruginosa* ATCC27853 (D), *Salmonella* Enteritidis 3-2 (E), *Salmonella* Paratyphi A56 (F), *Salmonella* Typhimurium (G), *Yersinia enterocolitica* H-26-04 (H), and *Yersinia pseudotuberculosis* 4320 (I). Cell concentrations are indicated on the bottom of the test strips: 1.0×10^7 (1), 1.0×10^6 (2), 1.0×10^5 (3), 0 (4).

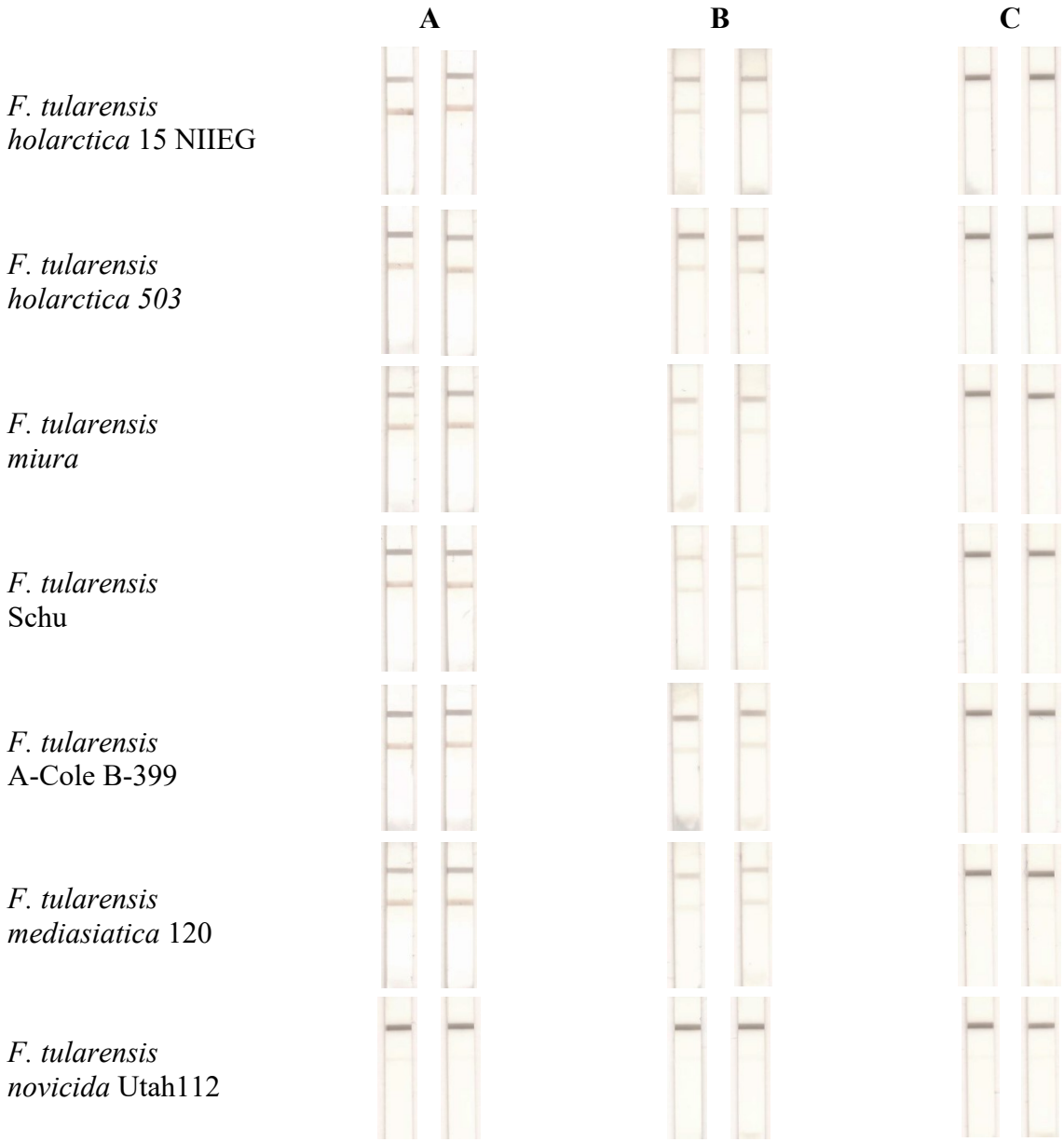

Fig. S8. Images of test strips after the LFIA of *F. tularensis* cells in blood serum. For sample preparation, serum was 10-fold diluted by PBST. Cell concentrations in serum were 1.0×10^7 (A), 1.0×10^5 (B), and 0 cells/mL (C).