

Controlled immobilization of single-domain antibodies using cellulose-binding modules for AB₅-type toxin detection in paper-based assays

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Comment [GR]: In main text this is mentioned before S1 - should be in the SI in the order listed in main text

Table S1. Detection methods for enterotoxigenic *E. coli* and *V. cholerae*.

Name	Target	Assay type	Sample matrix	Detection	Price	Time	Commercial/ proof of concept	Ref
LFA developed in this research	Cholera toxin (CTX) and enterotoxigenic <i>E. coli</i> heat-labile enterotoxin (LT)	LFA	Human feces and water	12.5 ng/mL CTX and 100 ng/mL LT in human feces 1 ng/mL CTX in water	Low	15 min	Proof of concept	NA
Bioline™ CHOLERA Ag O1/O139	<i>Vibrio cholera</i> O1/O139	LFA	Human feces	LOD not specified Sensitivity: 100% (O1), 100% (O139) (vs. Culture) Specificity: 100% (O1), 100% (O139) (vs. Culture)	Low	15 min	Commercial	(1)
Crystal VC O1/O139	<i>V. cholera</i> O1/O139	Dipstick LFA	Human feces	Not specified	Low	15 min	Commercial	(2)
Aptamer LFA for the detection of cholera toxin	CTX	LFA	Running buffer	2 ng/mL and 5 ng/mL/ 0.6 ng/mL	Low	10-20 min	Proof of concept	(3)
Rapid test for enterotoxigenic <i>E. coli</i> (ETEC)	Heat-labile toxin 1(LT-1)	LFA	Running buffer	62.5 ng/mL of purified LT-1 toxin	Low	15 min	Proof of concept	(4)
Rapid test for ETEC and cross detection of cholera toxin	LT and CTX	LFA	Super-natant	Not specified	Low	15 min	Proof of concept	(5)
ELISA for differentiation of LT and CTX	CTX and LT	ELISA	Culture supernatant or stool extracts	LOD not specified Sensitivity: 87.9% CTX and 100% LT Specificity: 100% CTX and 94.7% LT	Moderate	2-4 h	Proof of concept	(6)
Standard culture + biochemical confirmation	<i>V. cholerae</i> or ETEC	Culture	Human feces/ environmental	Not specified	Low	24-72 h	Commercial	(7)
<i>Vibrio cholerae</i> TaqMan PCR Kit	<i>V. cholerae</i>	qPCR	Human feces	Not specified	Moderate / high	2-4 h	Commercial	(8)
Multiplex PCR direct assay for <i>V. cholerae</i>	<i>V. cholerae</i> genes epsM, ctxA, rfb-O1/O139	Multiplex real-time PCR	Clinical specimens (anal swabs, vomit specimens)	200-1052 copies/mL	Moderate/high	3-5 h	Proof of concept	(9)
ETEC Real-time PCR kits (e.g., VIASURE / RIDA@GENE / YouSeq)	ETEC virulence genes (LT, ST)	qPCR	Human feces	~10 copies/reaction (for some kits)	Moderate / high	2-4 hours	Commercial	(10)
Rapid LAMP-based Diagnostic Test (RLDT) for cholera detection	<i>V. cholerae</i> genes ctxA and O1	Loop-mediated Isothermal Amplification (LAMP)	Human feces and water	~10 ⁴ CFU/gm of human feces 10 bacteria per ml of environmental water	Not specified	< 1 hour	Proof of concept	(11)
RLDT for ETEC	ETEC LT and Shigella (ST) genes	LAMP	Human feces	Sensitivity: 97% for ETEC and 93% for ST	Not specified	< 1 hours	Proof of concept	(12)
Liposome PCR for detection of cholera toxin	CTX	Liposome PCR	Human feces and water	10 pg/mL	Not specified	2-4 hours	Proof of concept	(13)
Dots-based fluorescent aptasensor for detection of cholera toxin	<i>Vibrio cholera</i> O139	Label-free aptamer-based fluorescent biosensor	water	429 CFU/mL	Not specified	Not specified	Proof of concept	(14)

Protocol S1. Covalent conjugation protocol- BioReady 40 nm Carboxyl Gold

Bioready carboxyl-coated gold nanoparticles solution (40 nm, OD 20, nanoComposix, U.S.) was vortexed, and 1 mL of the solution was activated by incubating with 200 µg 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 400 µg N-hydroxysuccinimide (NHS) (Cytiva, Marlborough, USA) for 30 min. The activated gold nanoparticles were washed twice by centrifuging the solution at 3800 RCF for 10 min, discarding the supernatant and resuspending the pellet in reaction buffer (5 mM sodium phosphate, pH 7.4): 1.55 mM sodium dihydrogen phosphate dihydrate (Sigma-Aldrich, Merck, Germany), 3.45 mM sodium phosphate dibasic (Sigma-Aldrich, Merck, Germany), 0.5% 20 K MW PEG (Sigma-Aldrich, Merck, Germany). After washing, 50 µg of anti-CTX/LT V_HH was incubated in the solution for 1 h, and the reaction was quenched for 10 min with 10 µL hydroxylamine (Sigma-Aldrich, Merck, Germany). The washing procedure was repeated three times. After the third centrifugation, the pellet was resuspended in 1 mL conjugate diluent (0.5x phosphate-buffered saline (PBS), pH 7.4), 0.5% IgG and protease-free bovine serum albumin (BSA; Jackson ImmunoResearch, Denmark), 0.5% casein (Carl Roth, Germany), 1% Tween-20 (v/v) (ThermoFisher, Denmark), and 0.05% sodium azide (Ampliqon, Denmark). The gold nanoparticle-coupled V_HH was stored at 4 °C until use.

Table S2. Overview of donor details for fecal samples

Sample	Age	Gender	Race	Weight (kg)	Height	BMI	Health Conditions	Current Medications	Bristol Stool Chart
1	70	Female	Caucasian	No information	No information	None	No information	No information	Type 4 Normal
2	66	Male	Caucasian	99.8	6'2	28,2 overweight	None	None	Type 5 Lacking fibre
3	50	Male	Caucasian	83.9	5'9	27,3 overweight	None	None	Type 2 Mild constipation
4	51	Female	Caucasian	72.6	5'8	24,3 healthy	None	None	Type 2 Mild constipation
5	19	Female	Caucasian	54.4	5'4	20,6 healthy	None	None	Type 3 Normal
6	53	Female	Asian	68	5'8	22,8 healthy	Anxiety	Lexapro	Type 5 Lacking fibre
7	198	Female	Asian	69.4	5'3	27,1 overweight	None	None	Type 2 Mild constipation
8	67	Female	Caucasian	81.6	5'4	30,9 obese	Hypothyroidism, Migraines, Osteoarthritis	Cytomel, PRN Maxalt, PRN Acetaminophen, PRN Ibuprofen, Multivitamin, Vitamin D	Type 4 Normal
9	24	Male	Asian	72.6	5'8	24,3 healthy	Bilateral Conductive Deafness	Pantoprazole, Sertraline	Type 1 Severe constipation
10	25	Female	Caucasian	70.3	5'2	28,3 overweight	None	Birth Control	Type 3 Normal
11	28	Male	Caucasian	108.9	6'7	27 overweight	Depression	Zoloft	Type 6 Mild diarrhea

1 **Table S3.** Overview of the experiments performed with the developed lateral flow assays. The variable being optimized is underlined.

Experiment	Replicates (n=)	Matrix	Test and control lines	AuNP volume	Sample to RB ratio	% sample	Stock concentration of target	Absolute concentrations
Selection of CBM	2	Running buffer (PBS with 0.3% Tween-20 (v/v))	T: 1.5 mg/mL anti-CTX/LT <u>V_HH-CBM1</u> or anti-CTX/LT <u>V_HH-CBM2</u> No C	2 μ L	20:80	20	CTX-B 1000 ng/mL 100 ng/mL 0 ng/mL	250 ng/mL 25 ng/mL 0 ng/mL
Determination of test line concentration	2	Running buffer (PBS with 0.3% Tween-20 (v/v))	T: <u>1.5 or 2 mg/mL</u> anti-CTX/LT <u>V_HH-CBM1</u> No C	2 μ L	20:80	20	CTX-B 10,000 ng/mL 1000 ng/mL 100 ng/mL 0 ng/mL	2,500 ng/mL 250 ng/mL 25 ng/mL 0 ng/mL
Comparison of V _H H versus V _H H-CBM test line	2	Running buffer (PBS with 0.3% Tween-20 (v/v))	T: 2 mg/mL <u>anti-CTX/LT V_HH</u> or anti-CTX/LT <u>V_HH-CBM1</u> No C	2 μ L	20:80	20	CTX-B 1000 ng/mL 100 ng/mL 10 ng/mL 1 ng/mL 0 ng/mL	250 ng/mL 25 ng/mL 2.5 ng/mL 0.25 ng/mL 0 ng/mL
Selection of running buffer	2	Running buffer (PBS with <u>0%, 0.1%, 0.2% or 0.3% Tween-20</u> (v/v))	T: 1.5 mg/mL anti-CTX/LT <u>V_HH-CBM1</u> No C	2 μ L	20:80	20	CTX-B 10,000 ng/mL 0 ng/mL	2,500 ng/mL 0 ng/mL
Optimization of gold conjugate (AuNP) volume	2	Running buffer (PBS with 0.3% Tween-20 (v/v))	T: 1.5 mg/mL anti-CTX/LT <u>V_HH-CBM1</u> No C	<u>2, 3 or 4 μL</u>	20:80	20	CTX-B 10,000 ng/mL 0 ng/mL	2,500 ng/mL 0 ng/mL
Selection of conjugate pad buffer	2	Running buffer (PBS with 0.3% Tween-20 (v/v))	T: 2 mg/mL anti-CTX/LT <u>V_HH-CBM1</u> No C	4 μ L in 4 μ L <u>5 mM borate buffer with 5% sucrose, PBS with 5% sucrose or conjugate diluent</u>	20:80	20	CTX-B 10,000 ng/mL 0 ng/mL	2,500 ng/mL 0 ng/mL
Selecting sample to running buffer ratio	2	Fecal sample #1 (1:1 in water)	T: 2 mg/mL anti-CTX/LT <u>V_HH-CBM1</u> No C	4 μ L in 4 μ L 5 mM borate buffer with 5% sucrose	<u>1:99</u> <u>5:95</u> <u>10:90</u> <u>20:80</u> Running buffer (PBS with 0.3% Tween-20 (v/v))	1 5 10 20	CTX-B 10 ng/mL 0 ng/mL	0.1, 0.5, 1 or 2.5 ng/mL 0 ng/mL
Determination of limit of detection (LOD) in running buffer	3	Running buffer (PBS with 0.3% Tween-20 (v/v))	T: 2 mg/mL anti-CTX/LT <u>V_HH-CBM1</u> C: 0.25 mg/mL anti-mouse F(ab') ² specific	4 μ L in 4 μ L 5 mM borate buffer with 5% sucrose	5:95	5	CTX-B and LT-B 100,000 ng/mL 10,000 ng/mL 1000 ng/mL 100 ng/mL 10 ng/mL 1 ng/mL 0 ng/mL	5000 ng/mL 500 ng/mL 50 ng/mL 5 ng/mL 0.5 ng/mL 0.05 ng/mL 0 ng/mL
Determination of	3	<u>Fecal sample #1</u> (1:1 in water)	T: 2 mg/mL anti-CTX/LT <u>V_HH-CBM1</u>	4 μ L in 4 μ L 5 mM borate	5:95 Running buffer	5	CTX-B and LT-B 1000 ng/mL	

LOD in a fecal sample			C: 0.25 mg/mL anti-mouse F(ab') ² specific Configuration 1	buffer with 5% sucrose	(PBS with 0.3% Tween-20 (v/v))		100 ng/mL 10 ng/mL 1 ng/mL 0 ng/mL	50 ng/mL 5 ng/mL 0.5 ng/mL 0.05 ng/mL 0 ng/mL
Optimization of running buffer for testing fecal samples		Fecal sample #1 (1:1 in water)	T: 2 mg/mL anti-CTX/LT V _H H-CBM1 C: 0.25 mg/mL anti-mouse F(ab') ² specific Configuration 1	4 µL in 4 µL 5 mM borate buffer with 5% sucrose	5:95 Running buffer (PBS with 0.3% Tween-20 (v/v) and 0 or 0.5% BSA)	5	CTX-B 100 ng/mL 0 ng/mL	5 ng/mL 0 ng/mL
Determination of optimal conjugate pad positioning	2	Fecal sample #1 (1:1 in water)	T: 2 mg/mL anti-CTX/LT V _H H-CBM1 C: 0.25 mg/mL anti-mouse F(ab') ² specific <u>Configuration 1: mouse IgG-anti-CTX/LT VHH (mouse IgG-AuNP closest to the nitrocellulose membrane)</u> <u>Configuration 2: anti-CTX/LT VHH-mouse IgG (anti-CTX/LT VHH closest to the nitrocellulose membrane)</u>	4 µL in 4 µL 5 mM borate buffer with 5% sucrose	5:95 Running buffer (PBS with 0.3% Tween-20 (v/v) and 0 or 0.5% BSA)	5	CTX-B 100 ng/mL 0 ng/mL	5 ng/mL 0 ng/mL
Optimization of sucrose concentration in conjugate pad buffer	2	Fecal sample #1 (1:1 in water)	T: 2 mg/mL anti-CTX/LT V _H H-CBM1 C: 0.25 mg/mL anti-mouse F(ab') ² specific Configuration 2	4 µL in 4 µL 5 mM borate buffer with 5% or 10% sucrose	5:95 Running buffer (PBS with 0.3% Tween-20 (v/v) and 0.5% BSA)	5	CTX-B 100 ng/mL 0 ng/mL	5 ng/mL 0 ng/mL
Determination of LOD for optimized conditions	3	Fecal sample #1 (1:1 in water)	T: 2 mg/mL anti-CTX/LT V _H H-CBM1 C: 0.25 mg/mL anti-mouse F(ab') ² specific Configuration 2	4 µL in 4 µL 5 mM borate buffer with 10% sucrose	5:95 Running buffer (PBS with 0.3% Tween-20 (v/v) and 0.5% BSA)	5	CTX-B, LT-B, CTX, LT and STX-B 1000 ng/mL 100 ng/mL 10 ng/mL 1 ng/mL 0 ng/mL	50 ng/mL 5 ng/mL 0.5 ng/mL 0.05 ng/mL 0 ng/mL
Determination of LOD for optimized conditions	3	Fecal sample #1 (1:1 in water)	T: 2 mg/mL anti-CTX/LT V _H H-CBM1 C: 0.25 mg/mL anti-mouse F(ab') ² specific Configuration 2	4 µL in 4 µL 5 mM borate buffer with 10% sucrose	5:95 Running buffer (PBS with 0.3% Tween-20 (v/v) and 0.5% BSA)	5	CTX 100 ng/mL 50 ng/mL 25 ng/mL 12.5 ng/mL 0 ng/mL	5 ng/mL 2.5 ng/mL 1.25 ng/mL 0.625 ng/mL 0 ng/mL
Testing 10 additional fecal samples	2	Fecal sample #1-#11 (1:1 in water)	T: 2 mg/mL anti-CTX/LT V _H H-CBM1 C: 0.25 mg/mL anti-mouse F(ab') ² specific Configuration 2	4 µL in 4 µL 5 mM borate buffer with 10% sucrose	5:95 Running buffer (PBS with 0.3% Tween-20 (v/v) and 0.5% BSA)	5	CTX 1000 ng/mL 500 ng/mL 250 ng/mL 100 ng/mL 0 ng/mL	50 ng/mL 25 ng/mL 12.5 ng/mL 5 ng/mL 0 ng/mL
Determination of sample to running buffer ratio for	2	Tap water	T: 2 mg/mL anti-CTX/LT V _H H-CBM1 C: 0.25 mg/mL anti-mouse F(ab') ² specific Configuration 2	4 µL in 4 µL 5 mM borate buffer with 10% sucrose	<u>100:0, 50:50 and 20:80</u> Running buffer (PBS with 0.3% Tween-20 (v/v) and	100 50 20	CTX-B 1 ng/mL	1, 0.5 or 0.25 ng/mL

testing tap water					0.5% BSA)			
Determination of LOD in tap water	2	Tap water	T: 2 mg/mL anti-CTX/LT V _H H-CBM1 C: 0.25 mg/mL anti-mouse F(ab') ₂ specific Configuration 2	4 μL in 4 μL 5 mM borate buffer with 10% sucrose	50:50 Running buffer (PBS with 0.3% Tween-20 (v/v) and 0.5% BSA)	50	CTX-B 100 ng/mL 10 ng/mL 1 ng/mL 0.1 ng/mL 0 ng/mL	500 ng/mL 50 ng/mL 5 ng/mL 0.5 ng/mL 0 ng/mL

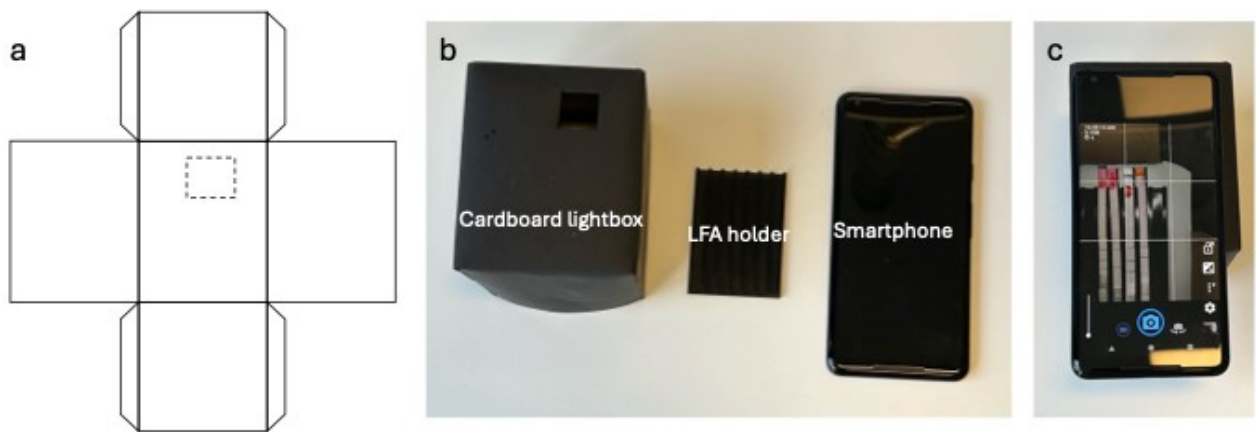


Figure S1. Cardboard lightbox for reading LFA under controlled lighting conditions. **(a)** Cardboard box net, **(b)** photo of the disassembled parts of the platform, and **(c)** photo of the assembled platform with the smartphone recording developed LFAs. The lightbox was made from cardboard, as the cube of the net could be incorporated as part of the test packaging at a later stage.

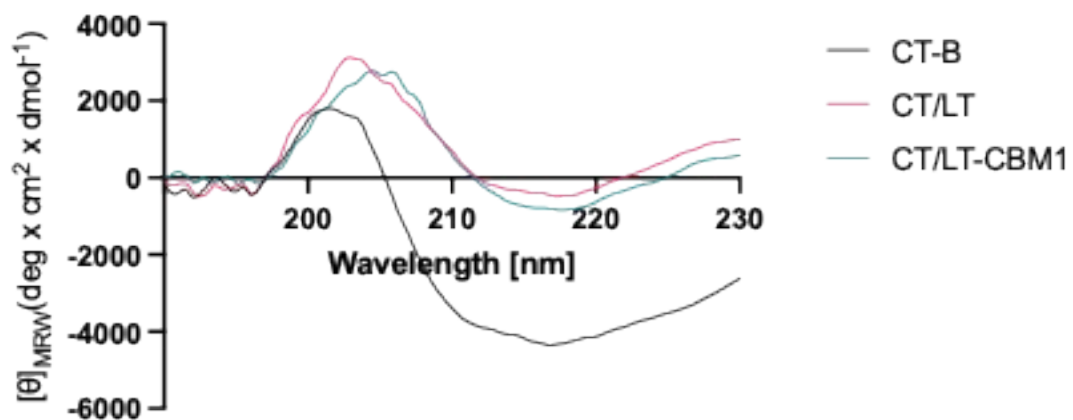


Figure S2. Graphical representation of circular dichroism (CD) measurement of recombinantly expressed cholera toxin B-subunit (black), anti-CTX/LT detection single-domain antibody ($V_{\text{H}}\text{H}$) (pink) and anti-CTX/LT-CBM1 capture $V_{\text{H}}\text{H}$ (green). The x-axis shows wavelength in nanometers, and the y-axis shows the mean residue molar ellipticity.

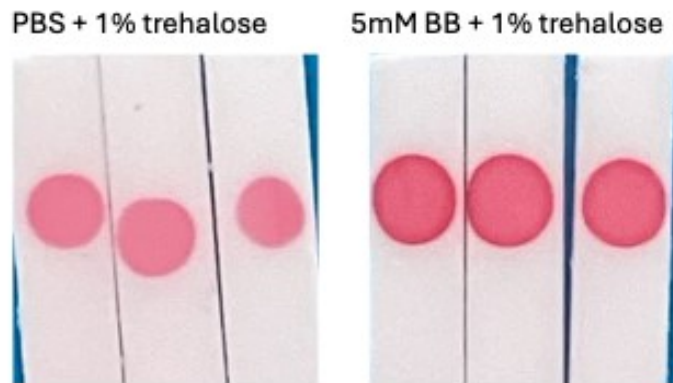


Figure S3. Establishing the optimal immobilization buffer using Ponceau S stain. Comparison between PBS+1% trehalose and 5 mM borate buffer+1% trehalose.

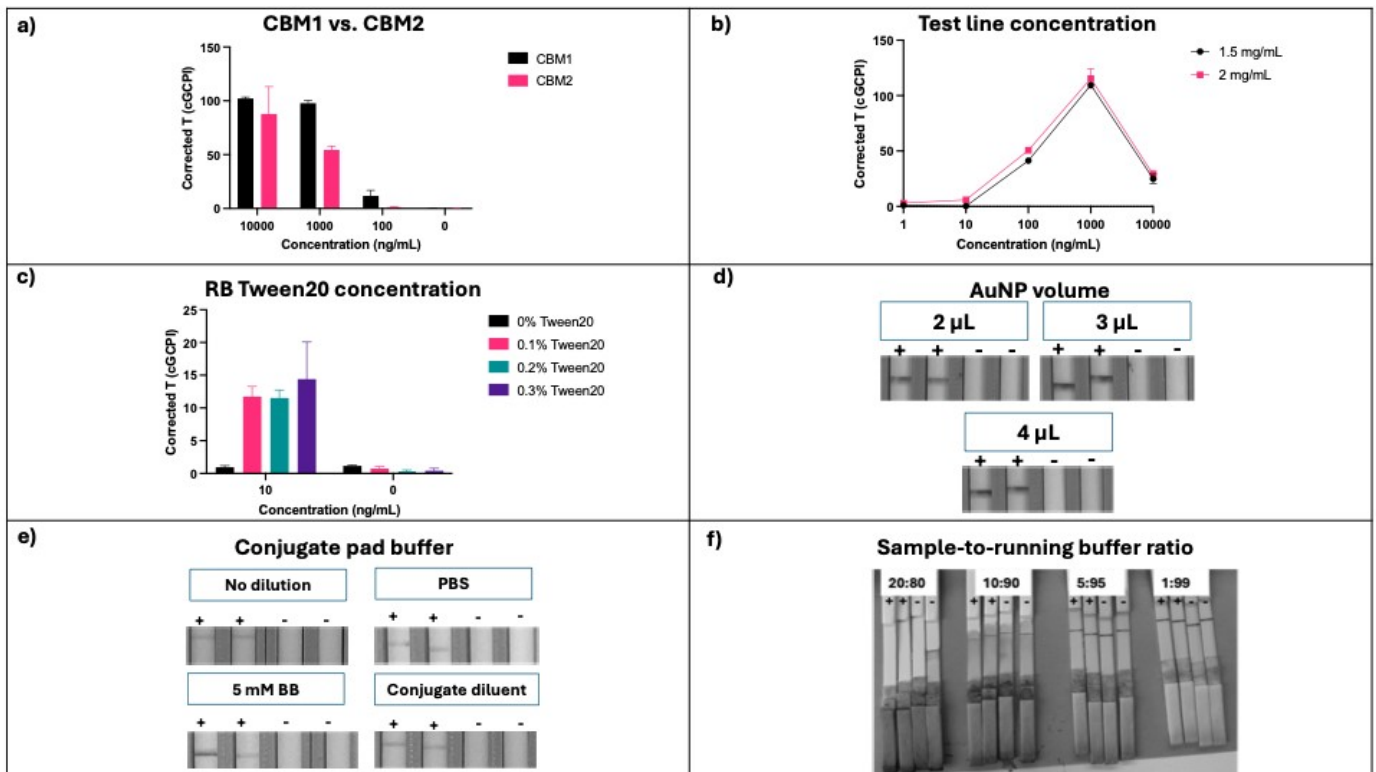


Figure S4. Smartphone photographs, calibration curves, or bar graphs establishing the optimal LFA conditions. Establishing (a) if CBM1 or CBM2 is better for immobilization of the single-domain antibody, (b) the optimal test line concentration (1.5 or 2 mg/mL), (c) the Tween-20 concentration in the running buffer (0, 0.1, 0.2, 0.3%), (d) the volume of AuNP conjugate used per test (2, 3, or 4 µL), (e) the optimal conjugate pad buffer (i.e., PBS, 5 mM borate buffer, conjugate diluent or no conjugate pad buffer) and (f) the sample-to-running buffer ratio (20:80 (µL), 10:90 (µL), 5:95 (µL), or 1:99 (µL)). Error bars display the standard deviation between duplicates.

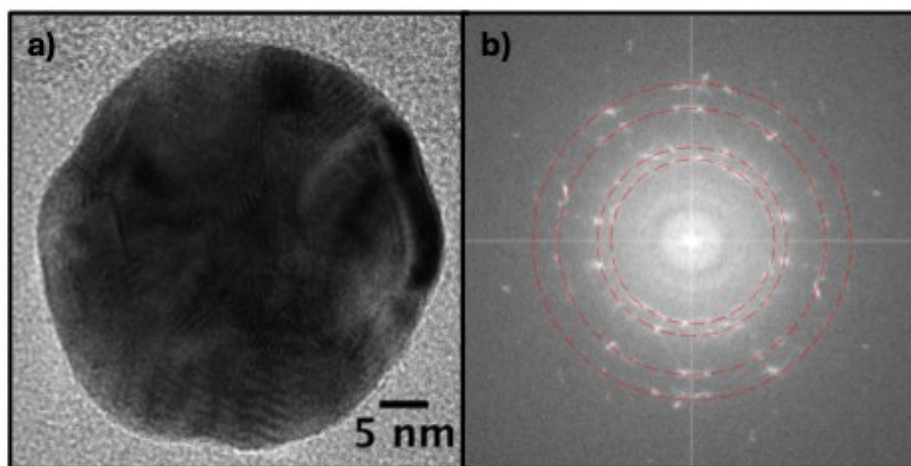


Figure S5. (a) High-resolution bright-field transmission electron microscopy (HR-BF-TEM) image of a gold nanoparticle (AuNP). (b) Fast-Fourier Transform (FFT) of (a) showing the equivalent of a typical electron diffraction of an Fm-3m Au poly-crystal, with the dashed circles overlaying the {111}, {200}, {220} and {311} planes, from the inside out, respectively.

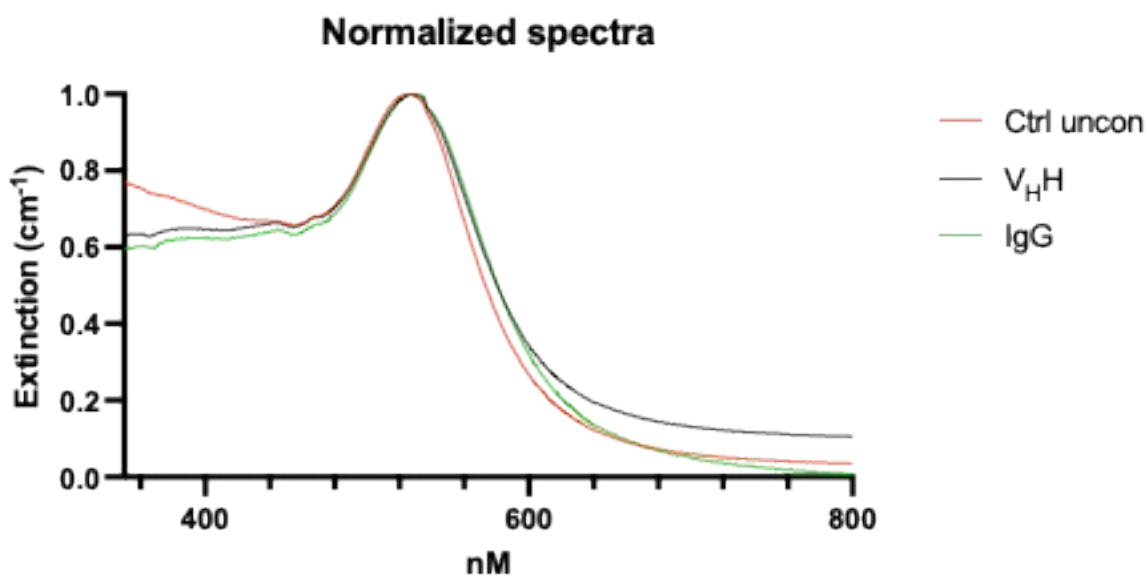


Figure S6. Graphical representation of normalized spectra from ultraviolet-visible spectroscopy. In red is the unconjugated gold (control), in black is the gold coupled anti-CTX/LT single-domain antibody, and in green is the gold coupled mouse immunoglobulin G. On the x-axis is the wavelength in nanometers, and on the y-axis is the extinction in cm⁻¹.

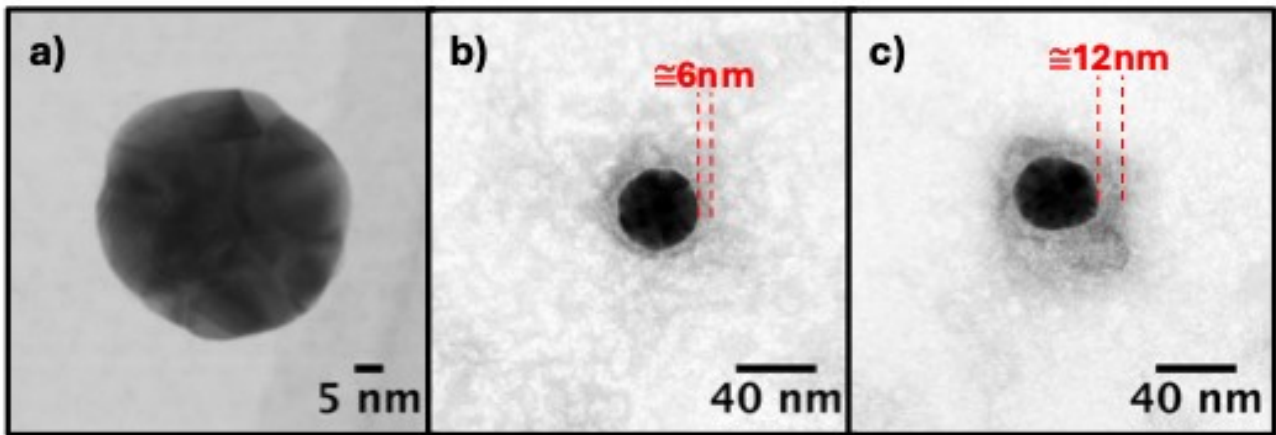


Figure S7. Bright-field scanning transmission electron microscopy (BF-STEM) of the (a) bare gold nanoparticle (AuNP), (b) anti-CTX/LT single-domain antibody-AuNP ($V_{\text{H}}\text{H}$), and (c) anti-CTX/LT $V_{\text{H}}\text{H}$ -AuNP in the presence of antigen (cholera toxin B subunit). The darkest regions correspond to denser AuNPs, while the slightly darker “halo” around the AuNPs is due to the functionalization with the anti-CTX/LT $V_{\text{H}}\text{H}$ (6 nm wide:b) and $V_{\text{H}}\text{H}$ plus antigen (12 nm wide:c).

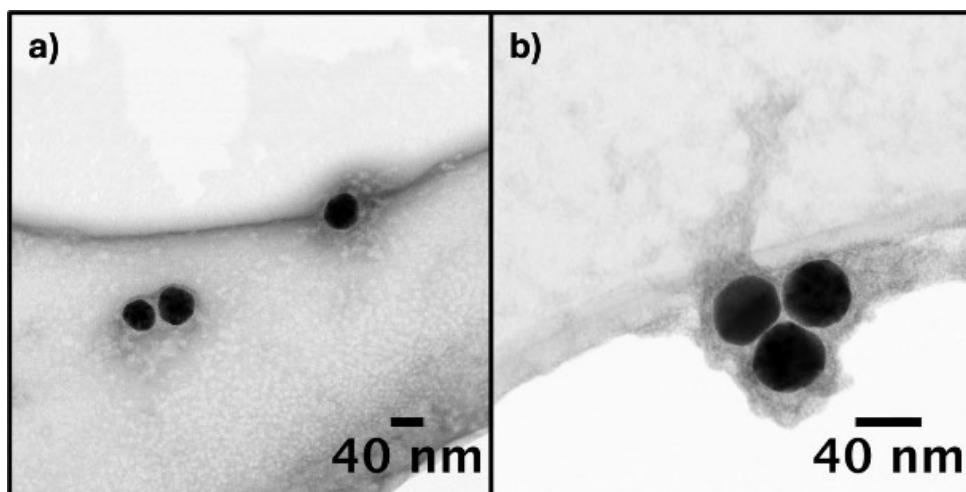


Figure S8. Bright-field scanning transmission electron microscopy (BF-STEM) images of (a) anti-CTX/LT $V_{\text{H}}\text{H}$ -AuNP and (b) anti-CTX/LT $V_{\text{H}}\text{H}$ -AuNP in the presence of antigen (cholera toxin B subunit), supporting the presence of the antigen around the particles by means of an increased agglomeration.

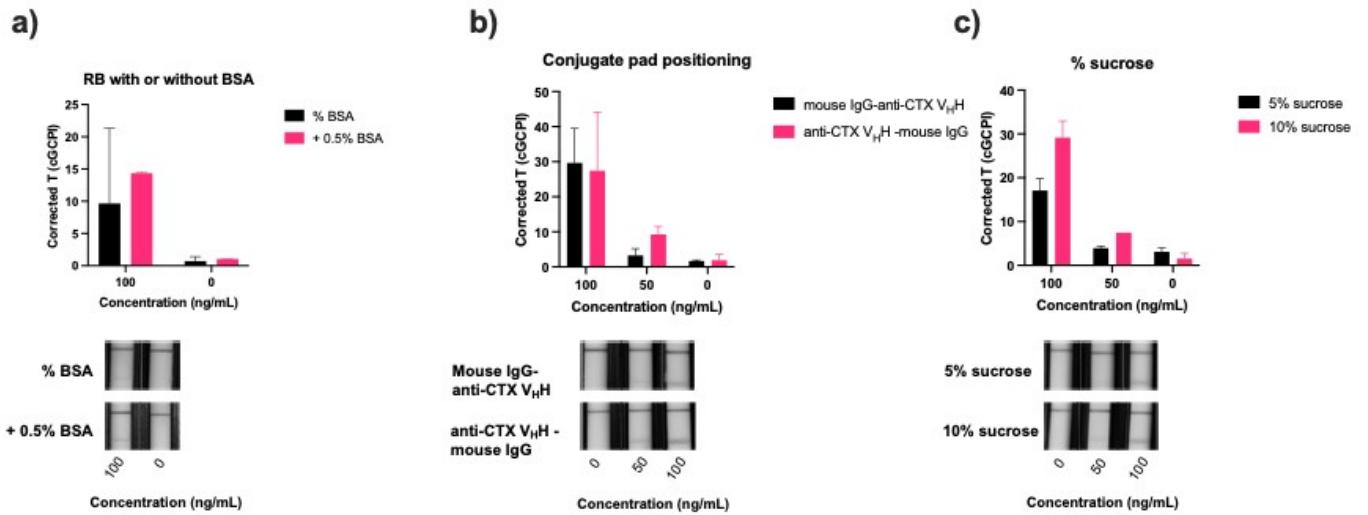


Figure S9. Bar graphs and smartphone photographs of the assay optimization conditions tested in duplicates in a decreasing concentration range (100-0 ng/mL) of cholera toxin B-subunit (CTX-B) in spiked fecal sample. Bar graphs are plotted as the corrected green channel pixel intensity (cGCPI) of the test line against CTX-B concentrations. **(a)** Comparing running buffer (RB) with or without 0.5% Tween-20, **(b)** establishing the optimal positioning of the two conjugate pads, i.e., mouse IgG-anti-CTX/LT V_HH (mouse IgG-AuNP closest to the nitrocellulose membrane) or anti-CTX/LT V_HH-mouse IgG(anti-CTX/LT V_HH closest to the nitrocellulose membrane), and **(c)** testing 5% versus 10% sucrose in the conjugate buffer. Error bars display the standard deviation (n = 2) between replicates.

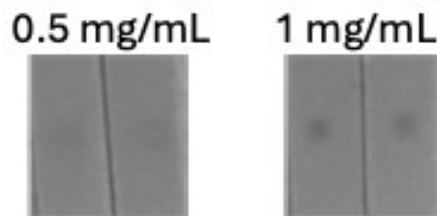


Figure S10. Testing anti-alpaca IgG (0.5 mg/mL or 1 mg/mL) as the control line antibody.

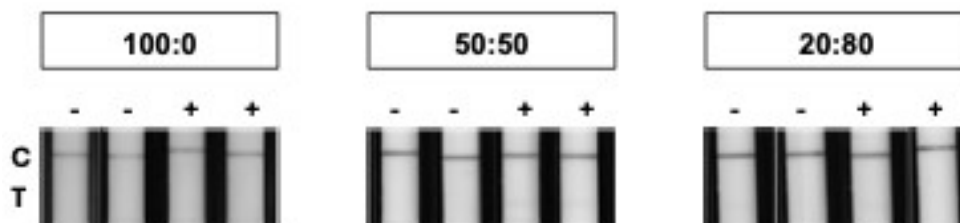


Figure S11. Smartphone photographs of sample-to-running buffer ratios tested in tap water spiked with 1 ng/mL or 0 ng/mL CTX-B.

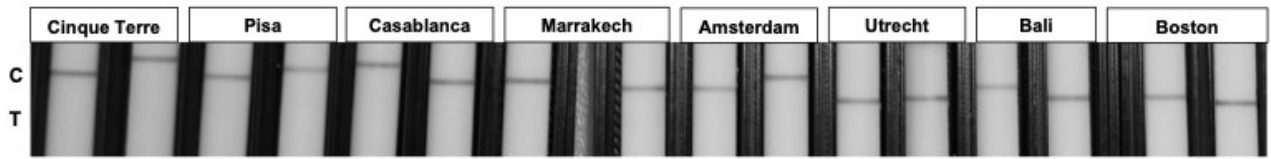


Figure S12. Smartphone photographs of blank tap water samples from eight different cities.

References

1. Abbott. Bioline™ CHOLERA Ag O1/O139. V. CHOLERA O1/O139 ANTIGEN TEST.
2. Global Task Force on Cholera Control S laboratory working group/ wash working group. Rapid Diagnostic Test (RDT) for cholera detection Quick Reference Guide For more detailed instructions please refer to the manufacturer's package insert. Report.
3. Frohnmeier E, Tuschel N, Sitz T, Hermann C, Dahl GT, Schulz F, et al. Aptamer lateral flow assays for rapid and sensitive detection of cholera toxin. *Analyst*. 2019;144(5):1840–9. doi:10.1039/C8AN01616J
4. Henrique C, Caetano BA, Mitsunari T, dos Santos LF, Piazza RMF, Rocha LB. Large-scale evaluation of a rapid diagnostic test for diarrhea caused by enterotoxigenic *Escherichia coli* targeting the heat-labile toxin. *J Microbiol Methods*. 2018 Jan;144:125–7. doi:10.1016/j.mimet.2017.11.020
5. Arimitsu H, Sasaki K, Tsuji T. Immunochromatographic detection of the heat-labile enterotoxin of enterotoxigenic *Escherichia coli* with cross-detection of cholera toxin. *J Microbiol Methods*. 2017 Jan;132:148–52. doi:10.1016/j.mimet.2016.12.007
6. Koike N, Okada K, Yabushita Y, Zhang DY, Yamamoto K, Miwatani T, et al. Rapid and differential detection of two analogous enterotoxins of *Vibrio cholerae* and enterotoxigenic *Escherichia coli* by a modified enzyme-linked immunosorbent assay. *FEMS Immunol Med Microbiol*. 1997 Jan;17(1):21–5. doi:10.1111/j.1574-695X.1997.tb00992.x
7. Chowdhury F, Ross AG, Islam MT, McMillan NAJ, Qadri F. Diagnosis, Management, and Future Control of Cholera. *Clin Microbiol Rev*. 2022 Sep 21;35(3). doi:10.1128/cmr.00211-21
8. Lasec Group. VIBRIO CHOLERAEE TAQMAN PCR DETECTION KIT DX, 24 REACTIONS.
9. Yan Y, Zhan L, Zhu G, Zhang J, Li P, Chen L, et al. Direct and Rapid Identification of *Vibrio Cholerae* Serogroup and Toxicogenicity by a Novel Multiplex Real-Time Assay. *Pathogens*. 2022 Jul 30;11(8):865. doi:10.3390/pathogens11080865
10. certest. VIASURE Real Time PCR Detection Kits. *E. coli* ETEC + EIEC.
11. Chakraborty S, Velagic M, Connor S. Development of a simple, rapid, and sensitive molecular diagnostic assay for cholera. *PLoS Negl Trop Dis*. 2023 Feb 6;17(2):e0011113. doi:10.1371/journal.pntd.0011113
12. Chowdhury G, Ghosh D, Zhou Y, Deb AK, Mukhopadhyay AK, Dutta S, et al. Field evaluation of a simple and rapid diagnostic test, RLDT to detect *Shigella* and enterotoxigenic *E. coli* in Indian children. *Sci Rep*. 2024 Apr 16;14(1):8816. doi:10.1038/s41598-024-59181-6
13. Frohnmeier E, Frisch F, Falke S, Betzel C, Fischer M. Highly affine and selective aptamers against cholera toxin as capture elements in magnetic bead-based sandwich ELAA. *J Biotechnol*. 2018 Mar;269:35–42. doi:10.1016/j.jbiotec.2018.01.012
14. Karthikeyan M, Venkatasubbu GD, Rathinasabapathi P. A label-free carbon dots-based fluorescent aptasensor for the detection of *V. cholerae* O139. *Diam Relat Mater*. 2023 Aug;137:110173. doi:10.1016/j.diamond.2023.110173