Electronic Supplementary Material (ESI) for Analyst.

Supplemental Data

A dual paper-based nucleic acid extraction method from blood in under ten minutes for point-of-care diagnostics

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Table S1. Properties and applications of filter papers.

Filter Paper	Purpose	Sample type	Composition	Properties	Working mechanism
FTA classic	sample storage and nucleic acid extraction	blood, cultured cells, buccal cells, solid tissue, urine, saliva, plants	chemically coated matrix including surfactants, chelating agents, buffer and free radicals; exact composition not disclosed	nucleic acid preservation for long term room temperature storage, cell lysis, protein denaturation	washing step and purification step required DNA remains tightly bound to the matrix while proteins and inhibitors are washed out
FTA Elute	sample storage and nucleic acid extraction	blood, cultured cells, buccal cells, solid tissue, urine, saliva, plants	chemically coated matrix including chaotropic salts; exact composition not disclosed	nucleic acid preservation for long term room temperature storage and cell lysis	washing step and elution step required; proteins remains tightly bound to the matrix while DNA is eluted
903 Protein Saver	sample storage and nucleic acid extraction	blood, urine	untreated matrix, high purity cotton linters; exact composition not disclosed	Sample preservation	Extraction kit required
Grade 3 Standard Grade	qualitative analytical techniques to determine and identify materials	NA	untreated cellulose filter, particle retention 6 µm, nominal thickness 390 µm	sample carrier, high wet strength, high capacity for precipitate	NA
Fusion 5	lateral flow detection	blood	single layer matrix membrane, particle retention 2.30 µm, thickness 370 µm	blood separator, sample wick, conjugate release, reaction membrane, and absorbent pad	NA

Information from the manufacturers (QIAGEN,Sigma-Aldrich, Cytiva).
https://www.qiagen.com
https://www.sigmaaldrich.com
https://www.cytivalifesciences.com

Table S2. Synthetic DNA sequence: ACTB.

ID	Sequence (5' to 3')
ACTB	TTCCCAGATGAGCTCTTTTTCTGGTGTTTGTCTCTCTGACTAGGTGTCTAAGACAGTGTT
NM_001101.5	GTGGGTGTAGGTACTAACACTGGCTCGTGTGACAAGGCCATGAGGCTGGTGTAAAGCGGC
	CTTGGAGTGTGTATTAAGTAGGTGCACAGTAGGTCTGAACAGACTCCCCATCCCAAGACC
	CCAGCACACTTAGCCGTGTTCTTTGCACTTTCTGCATGTCCCCCGTCTGGCCTGGCTGTC
	CCCAGTGGCTTCCCCAGTGTGACATGGTGTATCTCTGCCTTACAGATCATGTTTGAGACC
	TTCAACACCCCAGCCATGTACGTTGCTATCCAGGCTGTGCTATCCCTGTACGCCTCTGGC
	CGTACCACTGGCATCGTGATGGACTCCGGTGACGGGGTCACCCACACTGTGCCCATCTAC
	GAGGGGTATGCCCTCCCCCATGCCATCCTGCGTCTGGACCTGGCTGGCCGGGACCTGACT
	GACTACCTCATGAAGATCCTCACCGAGCGCGGCTACAGCTTCACCACCACGGCCGAGCGG
	GAAATCGTGCGTGACATTAAGGAGAAGCTGTGCTACGTCGCCCTGGACTTCGAGCAAGAG
	ATGGCCACGGCTGCTTCCAGCTCCTCCCTGGAGAAGAGCTACGAGCTGCCTGACGGCCAG
	GTCATCACCATTGGCAATGAGCGGTTCCGCTGCCCTGAGGCACTCTTCCAGCCTTCCTTC
	CTGGGTGAGTGGAGACTGTCTCCCGGCTCTGCCTGACATGAGGGTTACCCCTCGGGGCTG
	TGCTGTGGAAGCTAAGTCCTGCCCTCATTTCCCTCTCAGGCATGGAGTCCTGTGGCATCC
	ACGAAACTACCTTCAACTCCATCATGAAGTGTGACGTGGACATCCGCAAAGACCTGTACG
	CCAACACAGTGCTGTCTGGCGGCACCACCATGTACCCTGGCATTGCCGACAGGATGCAGA
	AGGAGATCACTGCCCTGGCACCCAGCACAATGAAGATCAAGGTGGGTGTCTTTCCTGCCT
	GAGCTGACCTGGGCAGGTCGGCTGTGGGGTCCTGTGGTGTGTGGGGAGCTGTCACATCCA
	GGGTCCTCACTGCCTGTCCCCTTCCCTCCTCAGATCATTGCTCCTCCTGAGCGCAAGTAC
	TCCGTGTGGATCGGCGGCTCCATCCTGGCCTCGCTGTCCACCTTCCAGCAGATGTGGATC
	AGCAAGCAGGAGTATGACGAGTCCGGCCCCTCCATCGTCCACCGCAAATGCTTCTAGGCG
	GACTATGACTTAGTTGCGTTACACCCTTTCTTGACAAAACCTAACTTGCGCAGAAAACAA
	GATGAGATTGGCATGGCTTTATTTGTTTTTTTTGTTTTG

Table S3. LAMP assay design.

LAMP assay design
A LAMP assay targeting the human house-keeping gene beta-actin (ACTB) was designed using the software Primer Explorer version 5.0 (`http://primerexplorer.jp/lampv5e/index.html`), based on the sequences retrieved from NCBI GenBank database (`https://www.ncbi.nlm.nih.gov/genbank/`) with accession number: $NC\_000007.14 : c5530601 - 5527148$, and $NM\_001101.5$. Sequences were aligned using the MUSCLE algorithm (Edgar et al., 2004) in Geneious 10.0.5 software, and primers were designed to detect both mRNA and DNA. All primers used in this study were purchased from IDT and resuspended in 1X TE buffer at 200 µM. Primer sequences are provided in Fig. 2C.
Analytical sensitivity of the LAMP-ACTB assay
Analytical sensitivity was evaluated using 10-fold dilutions of synthetic DNA in nuclease-free water ranging from 10 to 1×10^7 copies per reaction. Each condition was run in triplicates using the LC96 real-time instrument.

Table S4. Properties and applications of detergents used in this study.

Detergent	Type	CAS Number	CMC (mM) 25 °C	MW (Da) Monomer	Strength	Protein Interaction	Applications
Tween 20	non-ionic	9005-64-5	0.06	1228	mild	Non-denaturing	mammalian cells lysis,and pre-extraction of membranes to remove peripheral proteins
Triton X-100	non-ionic	9002-93-1	0.20-0.90	625	mild	Non-denaturing	solubilization and isolation of proteins
Igepal CA-630	non-ionic	9002-93-1	0.08	680	mild	Non-denaturing	solubilization, isolation and purification of membrane protein complexes
CHAPS hydrate	zwitterioinic	331717-45-4	6	615	med	Non-denaturing	solubilization of membrane proteins and receptors, breaking protein-protein interactions
Sodium dodecyl sulfate	ionic (anionic)	151-21-3	7-10	289	harsh	Denaturing	cell lysis, electrophoresis, hybridization

Information from the manufacturer, Sigma-Aldrich (MERCK).

Table S5. Pathogen-specific LAMP assays used in this study.

ID	Sequence	Reference
Ecoli_malB_F3	GCCATCTCCTGATGACGC	Hill 2008
Ecoli_malB_B3	ATTTACCGCAGCCAGACG	
Ecoli_malB_BIP	CTGGGGCGAGGTCGTGGTATTCCGACAAACACCACGAATT	
Ecoli_malB_FIP	CATTTTGCAGCTGTACGCTCGCAGCCCATCATGAATGTTGCT	
Ecoli_malB_LF	CTTTGTAACAACCTGTCATCGACA	
Ecoli_malB_LB	ATCAATCTCGATATCCATGAAGGTG	
LAMP-PfK13_F3	GGAGCAGCTTTTAATTACCTT	Malpartida-Cardenas 2019
LAMP-PfK13_B3	ATGACATGAATTTAGAACTTCGCC	
LAMP-PfK13_LF	AATATGTTATGTTCATTATCAA	
LAMP-PfK13_LB	GAGAAAAAAATGAATTTTGGAGCT	
LAMP-PfK13_FIP	TGGTTGATATTGTTCAACGGAATCT-ATCAAATATATGTTGTTGGAGGT	
LAMP-PfK13_BIP	TGGCAATTTCTA AATGGTGTACCA-ATAAGAATCTGACAATGTGGC	
LAMPcov_F3	ACC AAT AGC AGT CCA GAT GA	Rodriguez-Manzano 2021
LAMPcov_B3	CAC GAT TGC AGC ATT GTT AGC	
LAMPcov_LF	GGA CTG AGA TCT TTC ATT TTA CCG T	
LAMPcov_LB	ACT GAG GGA GCC TTG AAT ACA	
LAMPcov_FIP	TCTGGCCCAGTTCCTAGGTAGTCCAGACAAATTCGTGGTGG	
LAMPcov_BIP	GGACTTCCCTATGGTGCTAACAAACGGGTGCCAATGTGATCT	

Table S6. Duality of DBSFP. Optimisation of washing step and gel electrophoresis

Optimisation of washing step
Optimisation of the washing step in eluted disk and in-situ disk methods. W1 (no wash) W2 (1 wash, 1500 μL); W3 (2 washes, 600 μL); W4 (3 washes, 400 μL); W5 (1 wash, 1500 μL); W6 (2 washes, 600 μL); W7 (1 wash, 1500 μL); W8 (2 washes, 600 μL); W2 to W4 using a vortex, W5 and W6 by shaking, and W7 and W8 passive.
Gel electrophoresis
Restriction analysis and gel electrophoresis of amplified products with the RE MseI showing bands at 152 bp and 51 bp. Lane M shows Quick-Load 1 kb Plus DNA Ladder, lane 1 and 9 show NTC, lane 2-4 show digested amplified products from eluted disk method at RT, 63 ^{o}C and 95 ^{o}C respectively; lane 5-9 show digested amplified products in-situ disk method at RT, RT, 63 ^{o}C and 95 ^{o}C respectively. Low part of the gel shows amplified products, not digested. Lane M shows Quick-Load 1 kb Plus DNA Ladder, lane 1 and 5 show NTC, lane 2-4 show amplified products from eluted disk method at RT, 63 ^{o}C and 95 ^{o}C respectively; lane 6-8 show amplified products from in-situ disk method at RT, 63 ^{o}C and 95 ^{o}C respectively.

A

		K2EDTA			LiHep		
		Mean (min)	**STD (min)**	**Reps**	**Mean (min)**	**STD (min)**	**Reps**
With RT	RT	8.54	0.55	4/4	8.93	0.75	4/4
	40	9.23	0.57	4/4	9.11	0.57	3/4
	63	No Ct	No Ct	0/4	10.08	1.63	4/4
	80	11.02	No Ct	1/4	9.34	0.91	3/4
	95	9.77	0.95	2/4	8.06	0.15	4/4
Wihout RT	RT	11.97	3.70	2/4	9.14	0.30	2/4
	40	17.40	0.29	2/4	10.66	2.04	4/4
	63	#DIV/0!	#DIV/0!	0/4	11.50	2.58	3/4
	80	9.62	#DIV/0!	1/4	9.64	0.50	4/4
	95	9.46	1.05	2/4	8.53	0.28	4/4

Reactions stopped at 20 minutes

B

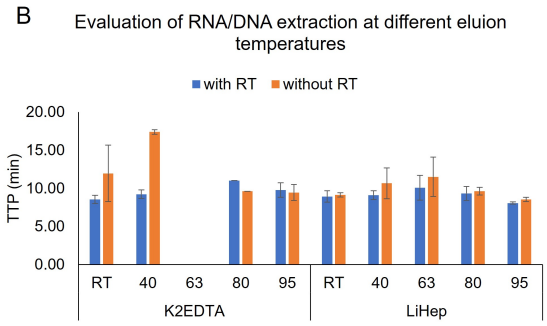
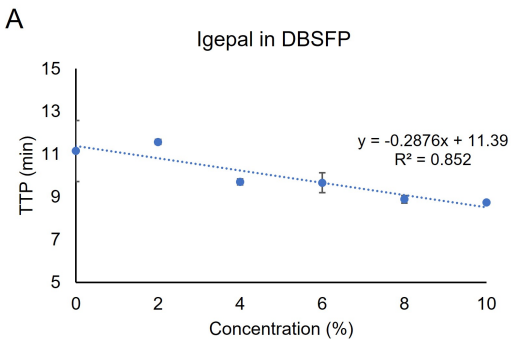


Figure S1. NAE recovery using DBSFP at various elution temperatures and whole blood stored in different anticoagulants. (A) Matrix table detailing the elution temperatures (RT, 40, 63, 80 and 95 ^{o}C), the addition of reverse transcriptase (with RT), the anticoagulants (K_2EDTA or LiHep), the mean of the TTP values and the standard deviation (STD), and the number of replicates that amplified (reps). (B) Plot of the values shown in (A) for a visual representation.

A



B

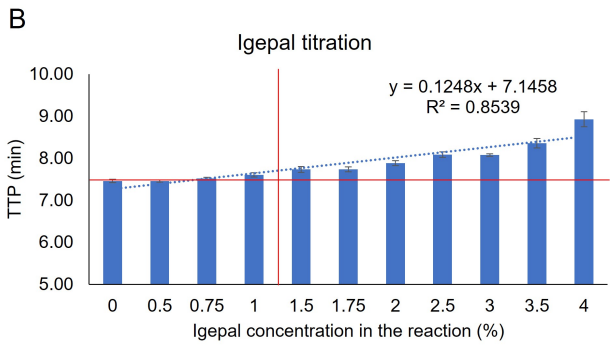


Figure S2. Effect of the addition of igepal on filter paper for NAE from DBSFP at RT conditions. (A) TTP values obtained using the LAMP-ACTB assay for detection of the ACTB gene. Standard curve showing a linear decreasing trend in the TPP values as the concentration of igepal was increased. (B) Titration of igepal directly in the reaction at concentrations ranging from 0% to 4%.

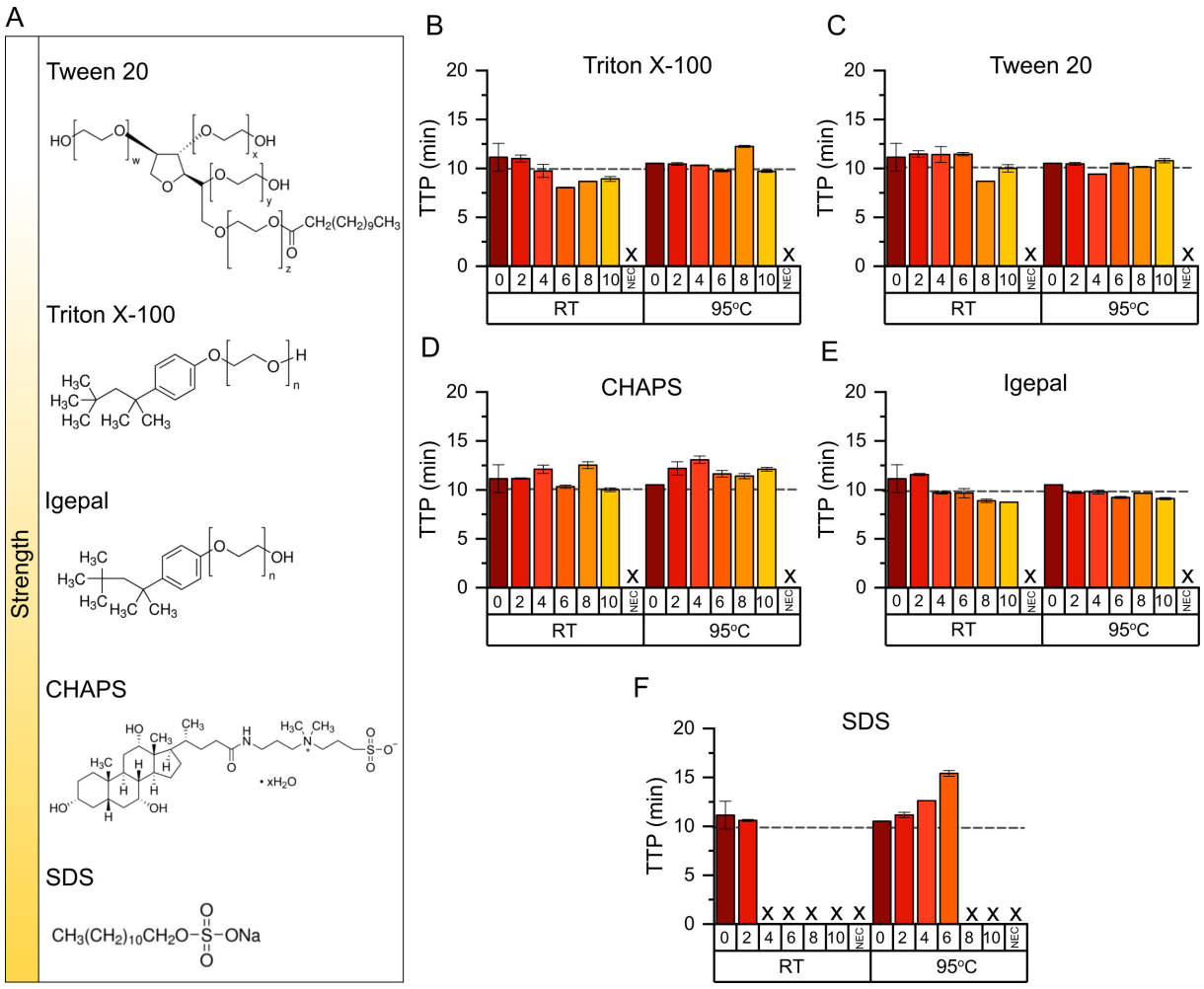


Figure S3. Evaluation of detergents for DBSFP lysis with the *eluted disk* method. TTP values using the LAMP-ACTB assay plotted against the different detergent concentrations and incubation temperatures. (A) Chemical structure of the detergents used in this study. (B) Triton X-100, (C) Tween 20, (D) CHAPS, (E) Igepal and (F) SDS. The "0" denotes no detergent addition and NEC denotes negative extraction control which consisted of disks without dried blood.

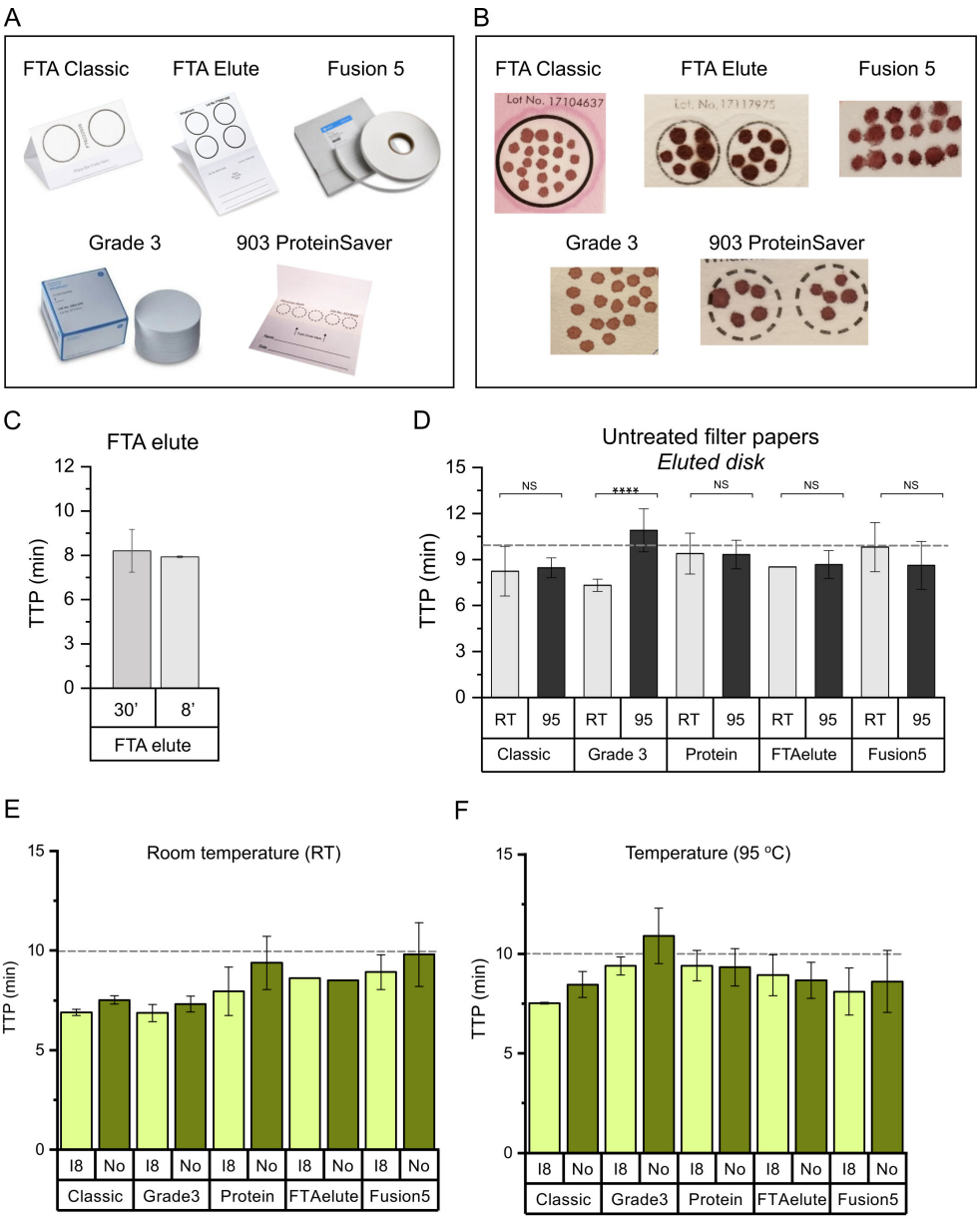


Figure S4. Evaluation of untreated filter papers for NAE from DBSFP. (A) Photographs of the filter papers used in this study. (B) Photographs of DBSFP after the pre-treatment with igepal 8% v/v and spotted blood: Classic, FTAelute, Fusion 5, Grade3 and Protein cards. (C) Comparison between the recommended protocol for FTA elute cards (30 min incubation at 95 ^{o}C) and the *eluted disk* method (8 min incubation at 95 ^{o}C) to demonstrate incubation time reduction did not have a negative effect. (D) TTP values using the LAMP-ACTB assay with the *eluted disk* method and incubation at RT or 95 °C with the different filter papers. (E) TTP values using the LAMP-ACTB assay with the *eluted disk* method and incubation at RT using pre-treated (I8) or untreated (No) filter papers. (F) TTP values using the LAMP-ACTB assay with the *eluted disk* method and incubation at 95 °C using pre-treated (I8) or untreated (No) filter papers.

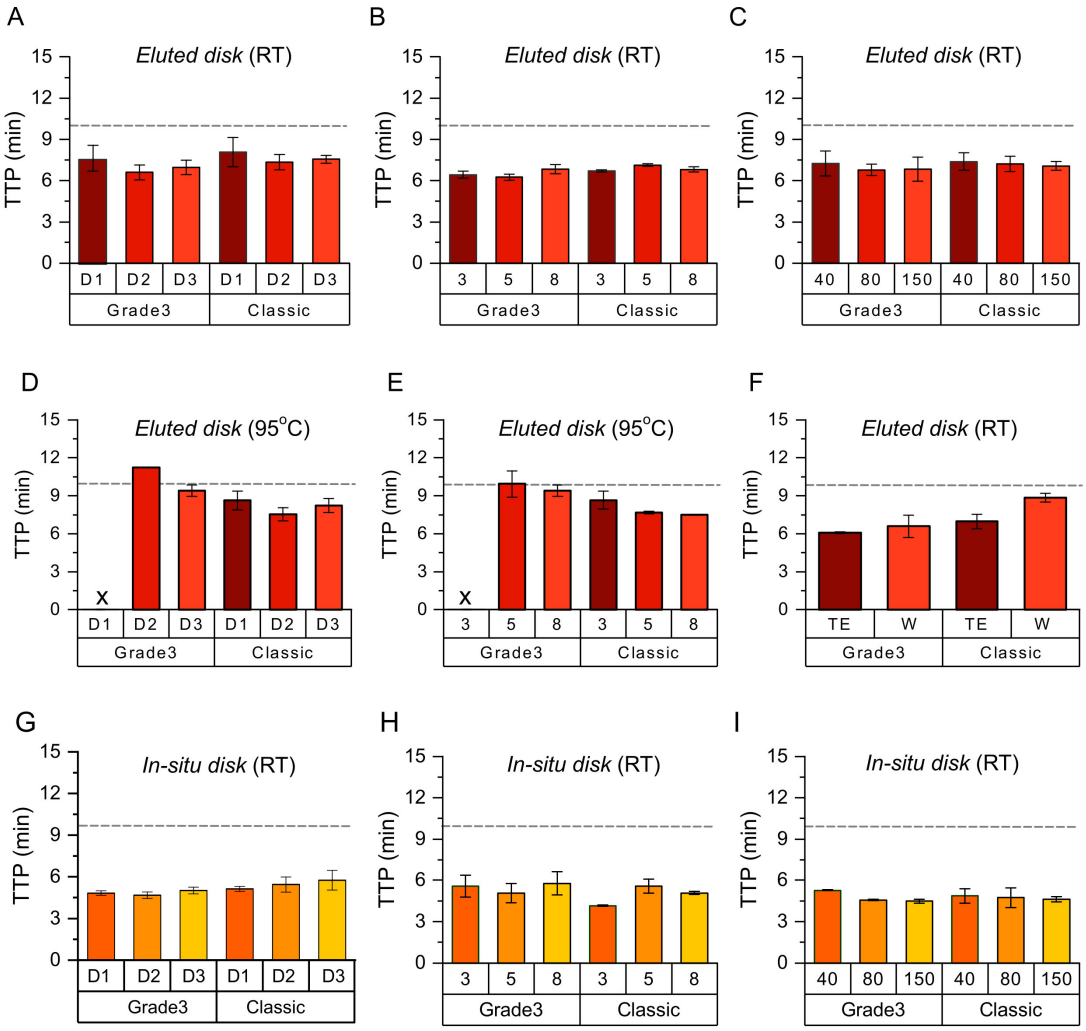


Figure S5. Optimisation of NAE from DBSFP using the *eluted disk* method. (A) Elutions from incubation at RT using one disk (D1), two disks (D2) or three disks (D3) from grade 3 filter paper or FTA classic cards. (B) Elutions from incubation at RT using three disks during different times: 3 min (3), 5 min (5) or 8 min (8) from grade 3 filter paper or FTA classic cards. (C) Elutions from 5 min incubation at RT using three disks in nuclease-free water at different volumes 40 μL, 80 μL, 150 μL, from grade 3 filter paper or FTA classic cards. (D) Elutions from incubation at 95 °C using one disk (D1), two disks (D2) or three disks (D3) from grade 3 filter paper or FTA classic cards. (E) Elutions from incubation at 95 °C using three disks during different times: 3 min (3), 5 min (5) or 8 min (8) from grade 3 filter paper or FTA classic cards. (F) Elutions from 5 min incubation at RT using three disks in nuclease-free water (W) or Tris-EDTA 1× buffer (TE) from grade 3 filter paper or FTA classic cards. (G) Disks from incubation at RT using one disk (D1), two disks (D2) or three disks (D3) from grade 3 filter paper or FTA classic cards. (H) Disks from incubation at RT using three disks during different times: 3 min (3), 5 min (5) or 8 min (8) from grade 3 filter paper or FTA classic cards. (I) Disks from 5 min incubation at RT using three disks in nuclease-free water at different volumes 40 μL, 80 μL, 150 μL, from grade 3 filter paper or FTA classic cards. (A)-(I) TTP values from the amplification of the human ACTB gene using the LAMP-ACTB assay. Dash line at y-axis sets a threshold at 10 min; for each condition, n = 4.

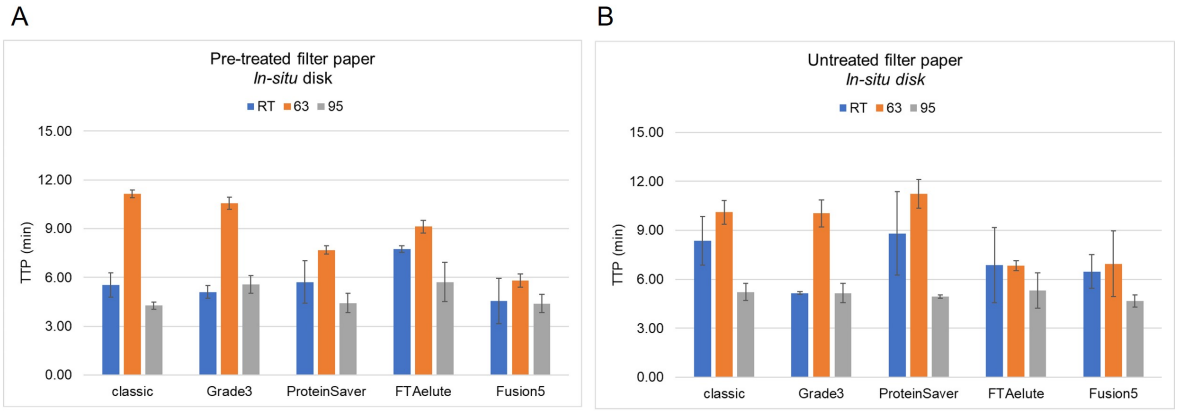


Figure S6. Evaluation of filter papers for NAE from DBSFP using the *in-situ disk* method. (A) TTP values using the LAMP-ACTB assay with pre-treated filter papers with igepal 8% v/v and incubation at RT, 63 °C or 95 °C. (B) TTP values using the LAMP-ACTB assay with untreated filter papers with igepal 8% v/v and incubation at RT, 63 °C or 95 °C.

```
clc
clear all

testfiledir = 'C:\Users\kenny\OneDrive - Imperial College London\...';
matfiles = dir(fullfile(testfiledir, '*.jpg'));
nfiles = length(matfiles);

fid=cell(nfiles,2);
for i = 1 : nfiles
    fid{i,1}= matfiles(i).name;
    fid{i,2} = imread( fullfile(testfiledir, matfiles(i).name) );
    fid{i,3}=fid{i,2}(:,:,1);%R
    fid{i,4}=fid{i,2}(:,:,2);%G
    fid{i,5}=fid{i,2}(:,:,3);%B
    fid{i,6}=fid{i,4}-fid{i,5};%G-B
    fid{i,7}=im2bw(fid{i,6},0.1);
end

%%
x=1;y=1;
figure;
subplot(2,1,1);
imshow(fid{x,2});
title(['Raw at ', fid{x,1}]);
subplot(2,1,2);
imshow(fid{x,7})
title(['G-B channel at ', fid{x,1}]);
```

Figure S7. Script implemented in MATLAB for colorimetric detection of nucleic acid amplification using a ratiometric approach.

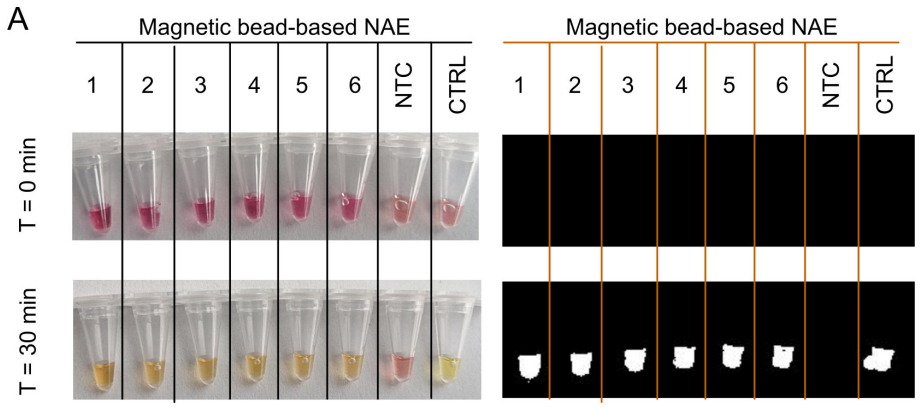


Figure S8. Colorimetric detection from sample-to-result with nucleic acids extracted using a commercial magnetic bead-based NAE method. Samples used included whole blood in K2EDTA (1-3) and whole blood in LiHep (4-6); non-template control (NTC); and positive control (CTRL) which consisted of purified human genomic DNA (Promega).

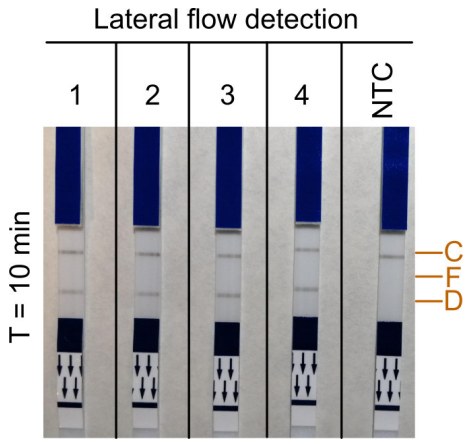


Figure S9. Lateral flow detection of *P. falciparum* DNA from extracted nucleic acids from the *eluted disk* method. C-Control; F-FAM; D-DIG. LAMP amplification using only *P.falciparum* specific assay.

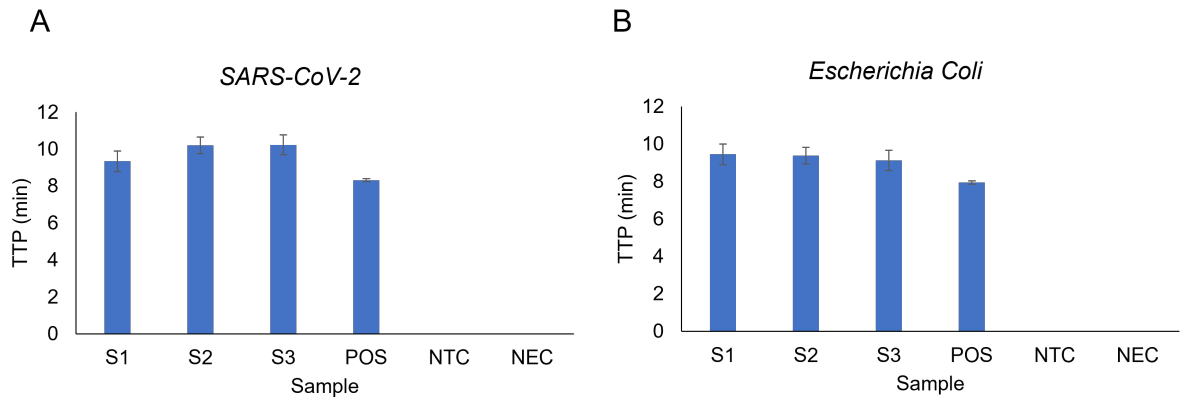


Figure S10. TTP values obtained with LAMP from the extraction of viral particles and bacterial nucleic acids using the *eluted disk* method. (A) SARS-CoV-2 viral particles at 4×10^3, 2×10^3 and 4×10^2 copies per μL were spotted in the DBSFP and eluted. Concentration per reaction per sample as follows: S1 (1.2×10^3), S2 (6×10^2) and S3 (1.2×10^2). NTC (non-template control) and NEC (negative extraction control). (B) One colony of *Escherichia Coli* was diluted into 200 μL of water, spotted in the DBSFP and eluted. Three biological replicates were performed (S1 to S3). NTC (non-template control) and NEC (negative extraction control).