

Electronic Supplementary Information

Integrated bacterial cell lysis and DNA extraction using paper-based isotachophoresis

Shruti Soni¹ and Bhushan J. Toley^{1,2*},

¹ Department of Chemical Engineering,
Indian Institute of Science, Bangalore
Karnataka, 560012
India

²Center for Biosystems Science and Engineering
Indian Institute of Science
Bengaluru, Karnataka 560012
India

Keywords: Sample preparation, global health, electrokinetics, low resource settings, paper analytical devices, molecular diagnostics

*Correspondence to:

Bhushan J. Toley
Department of Chemical Engineering,
Indian Institute of Science
Malleswaram
Bangalore, Karnataka, 560012
Phone: +91-80-2293 3114
Email: bhushan@iisc.ac.in

Table of Contents

Section S1: Fabrication and detailed design of *p*-ITPrep device

Section S2 Concentration of gDNA in *p*-ITPrep: 10^0 copies/ μ L of *Mtb* genomic DNA

Section S3 Standard 17 as a membrane in *p*-ITPrep

Section S4. Comparison of discontinuous-buffer ITP system vs. single buffer electrophoresis system for the concentration of gDNA in *p*-ITPrep

Section S5. Direct use of paper discs (with extracted gDNA) as a template in PCR

Section S6. Direct use of paper discs (with extracted gDNA) as a template in qPCR

Section S7 Agar plate culture of bacterial samples obtained from ITP (no complex sample)

Section S8 Effect of voltage bias, separation time, and trailing electrolyte on DNA extraction efficiency from saliva samples

Section S9 Proteinase K + Triton X-100 treatment for human serum samples

Section S10 Agar plate culture of bacterial samples obtained from ITP (using complex matrices)

Section S11 Nucleic acid sample preparation from complex samples by filtration

Section S12 Performance of *p*-ITPrep without using a bacterial capture membrane

Section S13 Current vs time plots

Table S1 Comparison of user steps involved in *p*-ITPrep and a Qiagen DNA extraction kit

Table S2 Detailed QIAGEN DNA extraction protocol (QIAamp DNA mini kit)

Table S3 Cost of materials used for fabricating one *p*-ITPrep device

Section S1: Fabrication and detailed design of *p*-ITPrep device

All the designs *p*-ITPrep were made using NanoCAD software. The module parts were cut using a 50 W CO₂ laser cutter (VLS 3.60; Universal Laser Systems, Scottsdale, AZ). The module was fabricated by assembling two parts: 1) two acrylic-based buffer reservoirs for the leading and trailing electrolytes (Fig. 1A (i)) and 2) an 11-layer foldable paper structure (Fig. 1A (ii)). The foldable structure was created using circular discs of Whatman filter paper grade 1 with a diameter of 5 mm and pressure-sensitive adhesive (PSA). **Fig. S1** provides a detailed design of this structure. The TE and LE buffer reservoirs were constructed using three layers of acrylic sheets with varying thicknesses of 2, 3, and 5 mm. These layers were aligned, stacked, and bound together using dichloromethane (DCM). One side of the 3 mm acrylic sheet was engraved with a square (16 X 16 mm²) with a depth of 1 mm to facilitate proper alignment of the foldable paper structure. The 11-layer foldable paper structure was placed between the two reservoirs while properly aligning with the etched part of the reservoir. The horizontal stack of paper discs was aligned with a 3 mm hole present in the center of the 3 mm thick acrylic layer of the reservoir. This arrangement formed a fluidic channel for ions to move between the two reservoirs in the presence of a voltage bias. The paper layers and the two reservoirs were tightly secured to prevent liquid leakage using four adjustable screws positioned at the corners of the acrylic sheets. To prevent electro-migration of bacterial cells towards the anode, a 0.45 μm pore size MCE filter membrane was utilized instead of the first paper layer (Whatman filter grade 1) in contact with the TE reservoir during bacterial experiments. Additionally, this approach facilitated the capture and concentration of live bacterial cells on the filter membrane, which could be utilized for the bacterial culture of dilute samples.

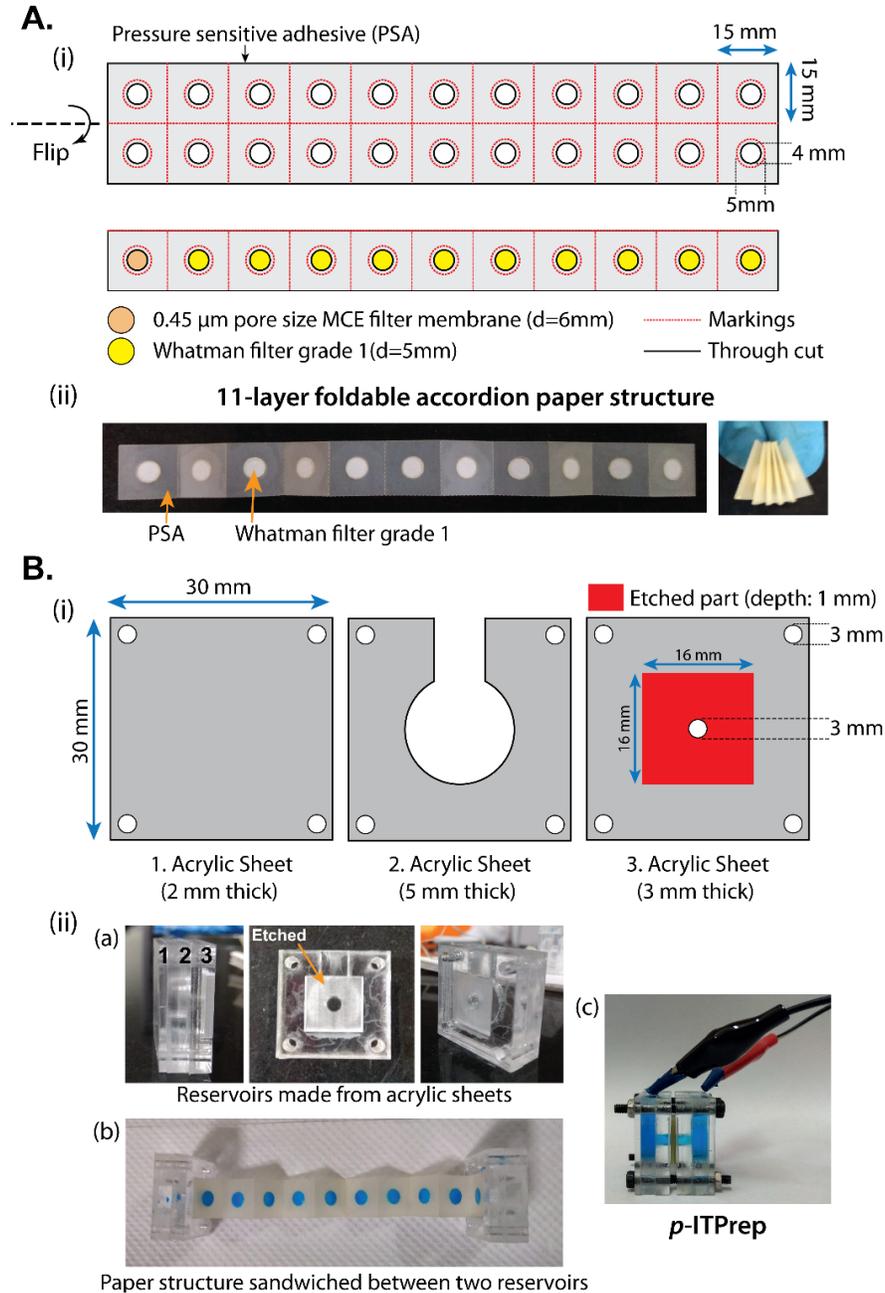
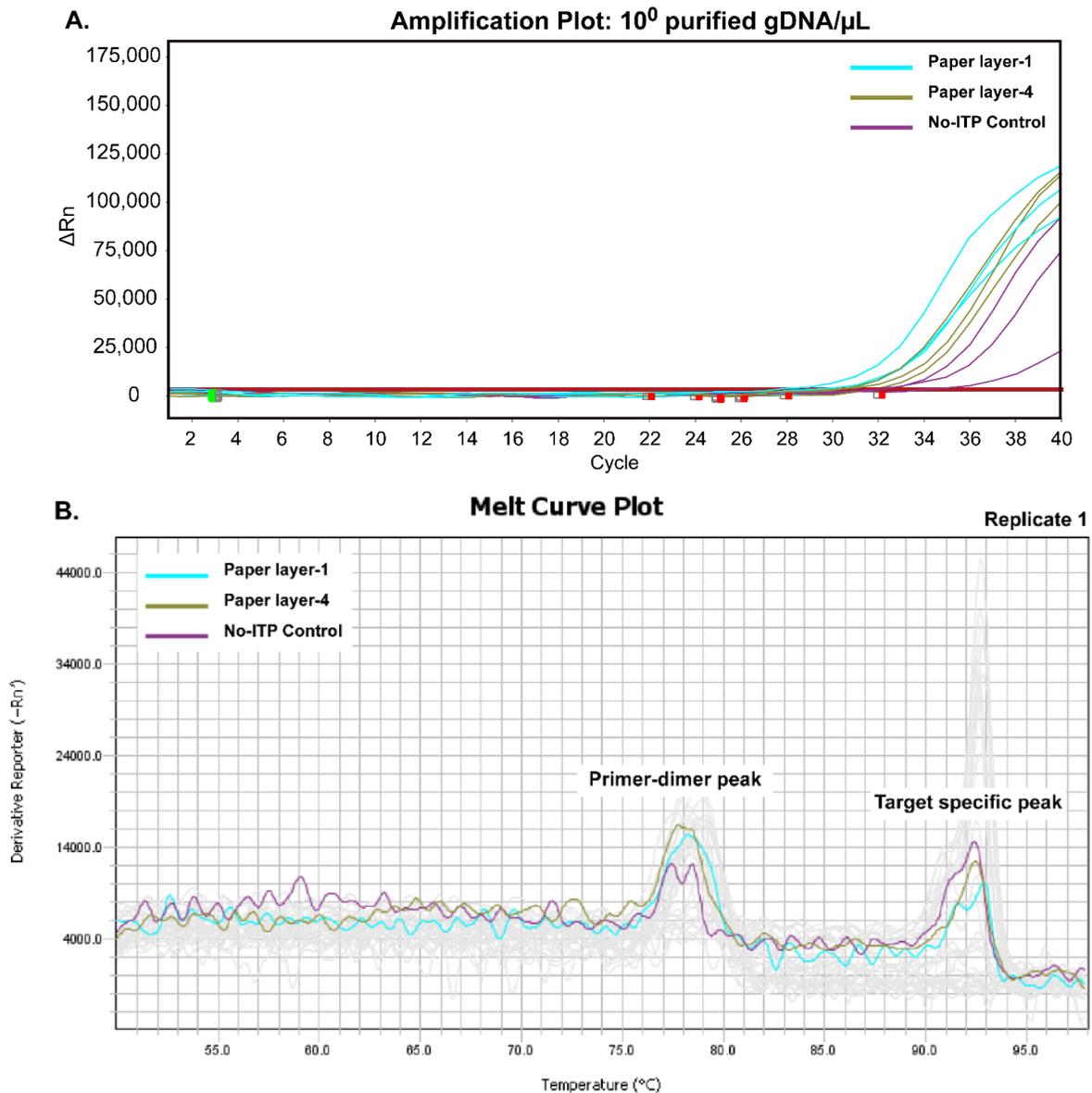


Fig. S1 Detailed design of *p-ITPrep*. The *p-ITPrep* features **A.** an 11-layered foldable accordion paper structure consisting of (i) circular discs (diameter 5 mm) of Whatman filter paper grade 1/0.45 μm pore size MCE filter membrane (yellow/orange) and pressure-sensitive adhesive (PSA, grey). The PSA is folded along the red dotted lines after placing the circular paper membrane discs on the circular holes (black outlined circles) in PSA. (ii) The actual picture of the assembled/folded 11-layered structure is shown in (ii). **B.** Two electrolyte reservoirs: (i) fabricated using three layers of the acrylic sheet of varying thicknesses, (ii) (a) which are stacked, aligned, and bound together, with (b) an 11-layered foldable origami paper structure sandwiched between them. The ends of the PSA are peeled off to expose their sticky side that sticks to the etched layer of the reservoir. (c) A photograph of the fully assembled *p-ITPrep* is provided.

Section S2: Concentration of gDNA in p-ITPrep: 10^0 copies/ μL of *Mtb* genomic DNA

To test the limit of detection of our in-house p-ITPrep device, the concentration of gDNA from a sample containing 10^0 gDNA copies/ μL was also examined. An amplified signal in PL-1 and PL-4 with a lower Ct value was observed in the amplification plot (Fig. S2 A.) compared to the No-ITP control. However, it cannot be conclusively concluded that gDNA has been concentrated due to the presence of primer-dimer formation, which contributes to this signal.



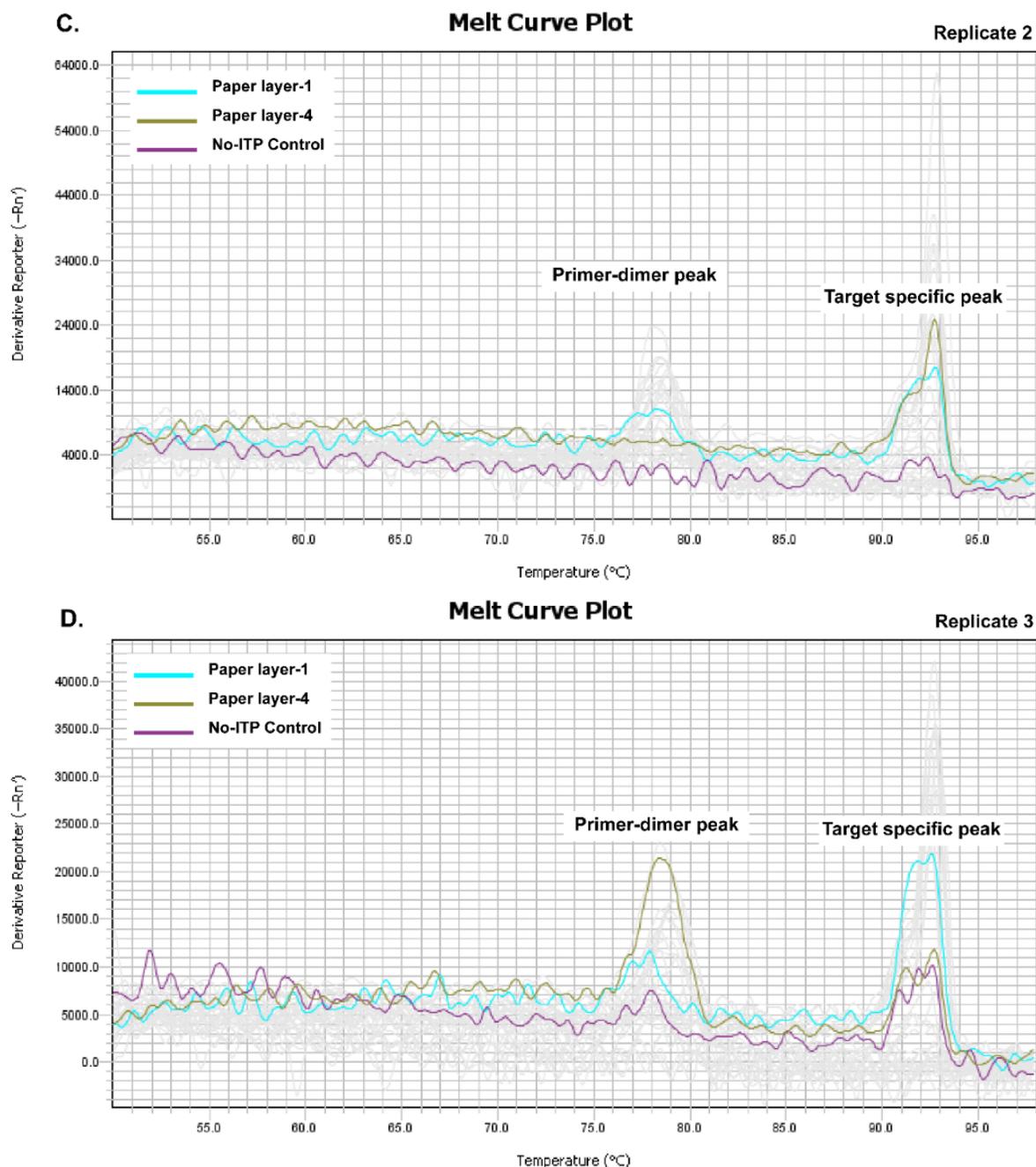


Fig. S2 Concentration of 10^0 copies/ μL purified *Mtb* genomic DNA spiked in TE buffer using *p*-ITPrep. **A.** Amplification plot of No-ITP control (initial DNA concentration of 1 copy/ μL), paper layers 1 & 4. **B-D.** Melt curve plot for three different experimental trials suggesting amplification curve for PL 1, 4, and No-ITP control is a contribution of both target-specific amplicons and primer-dimers. Therefore, we cannot calculate the concentration factors, but amplification plots of PL-1, 4 have a lower Ct value than no amplification plot.

Section S3: Investigating Standard 17 as a paper membrane in *p*-ITPrep

The efficacy of two paper membranes, Standard 17 (glass fiber) and Whatman filter paper grade 1 (cellulose), within the 11-layer foldable structure, was evaluated to achieve maximum DNA concentration factors. However, in the case of Standard 17, no noticeable increase in DNA concentration was observed compared to the No-ITP controls, as shown in Fig. S3. In all paper layers (1-11), the concentration factors (normalized DNA concentration) were found to be <1 , indicating the absence of DNA concentration. Conversely, Whatman filter paper grade 1 resulted in DNA concentration, as illustrated in Fig. 2. Consequently, Whatman filter paper disks were employed within the foldable structure in all subsequent experiments conducted in this study.

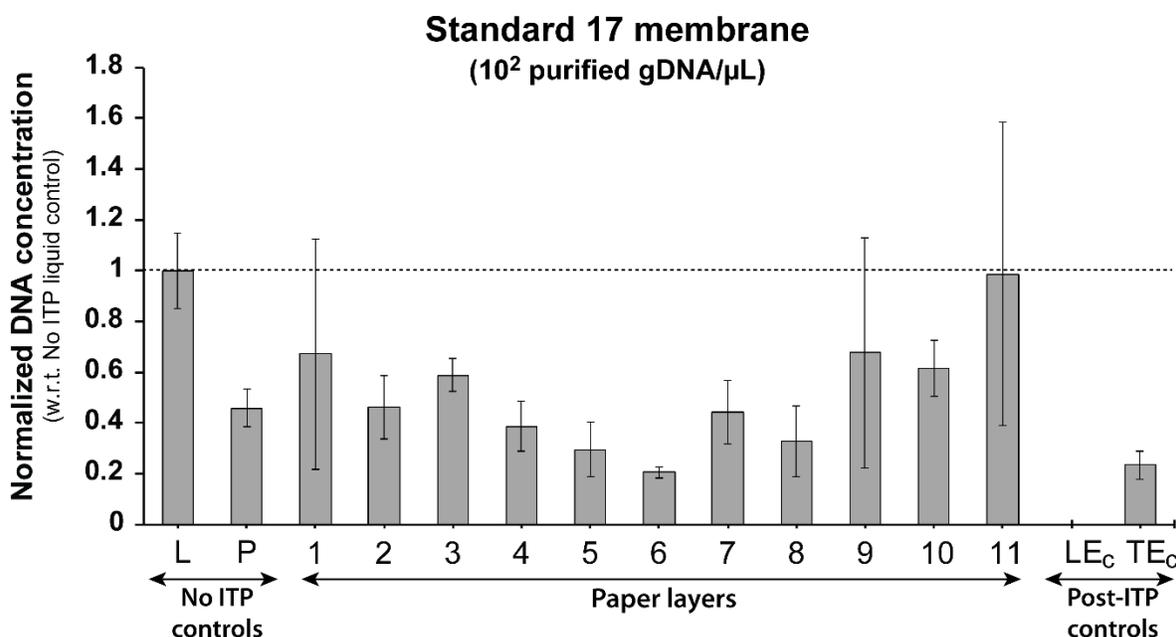
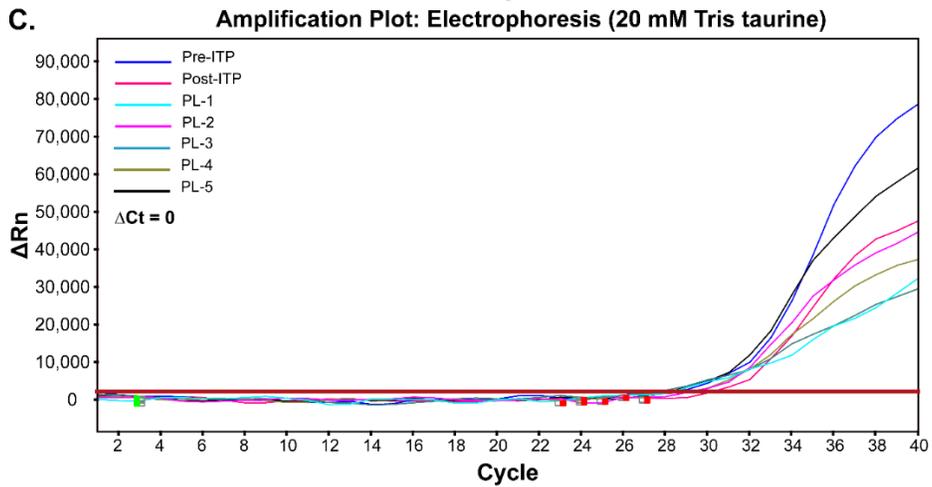
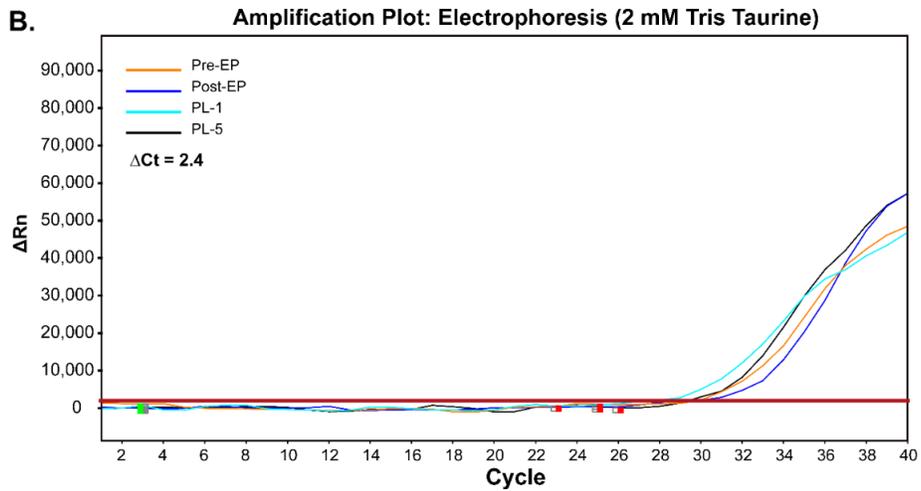
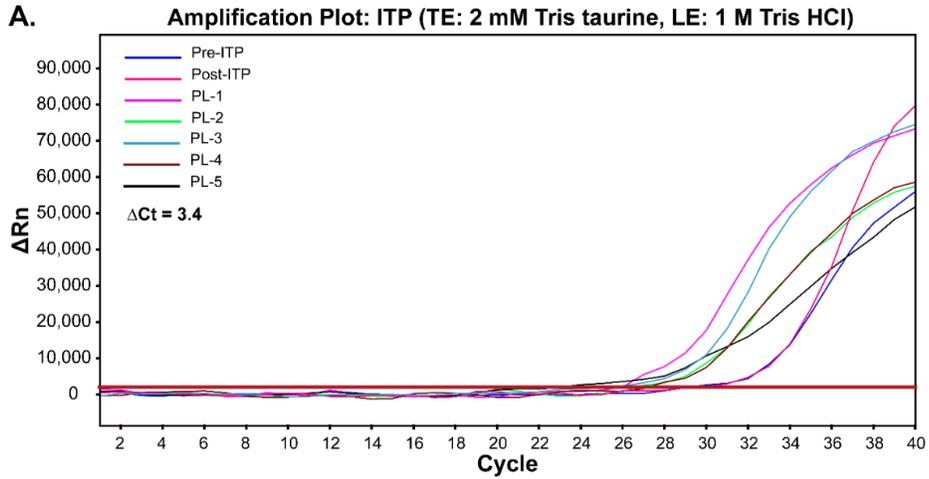


Fig. S3 Quantification of DNA concentrated post-ITP while using Standard 17 as the porous membrane in the 11-layered foldable structure in the *p*-ITPrep. A plot of normalized DNA concentration for No-ITP controls (liquid (L) and paper (P)), paper layers (1 to 11) after ITP and Post-ITP controls (LE_c, TE_c) for the sample with initial genomic *Mtb* DNA of 10² copies/μL suggests no DNA concentration was achieved in any of the paper layers in comparison to the liquid No-ITP control (i.e., initial sample). We see a distribution of DNA in all the paper layers. The device was subjected to 18 V for 15 minutes. All error bars represent standard deviations (n=3).

Section S4: Comparison of discontinuous-buffer ITP system vs. single buffer electrophoresis system for the concentration of gDNA in *p*-ITPrep

A test was conducted to investigate if DNA concentration could be achieved through electrophoresis by filling both reservoirs of the ITP device with the same electrolyte before applying 18V. Various solutions were tested, including 2 mM Tris-Taurine, 20 mM Tris-Taurine, 1X TAE buffer, and 1 M tris HCl, and compared with the discontinuous-buffer ITP system, where 1 M tris-HCl was used as the leading electrolyte (LE) and 2 mM as the trailing electrolyte (TE). Although DNA was detected during electrophoresis in some initial paper layers (except in 1 M tris HCl), the concentration factors were lower than those observed in the ITP process. The assessment was based on the difference in the Ct (threshold cycle) value between the No-ITP control (Pre-ITP control) and the lowest Ct value of any paper layers. [Note: For electrophoresis, both the reservoirs were filled with the same electrolyte, where the cathode-containing reservoir contained 10^2 *Mtb* gDNA copies/ μ L. A voltage bias of 18V, similar to ITP experiments, was applied.



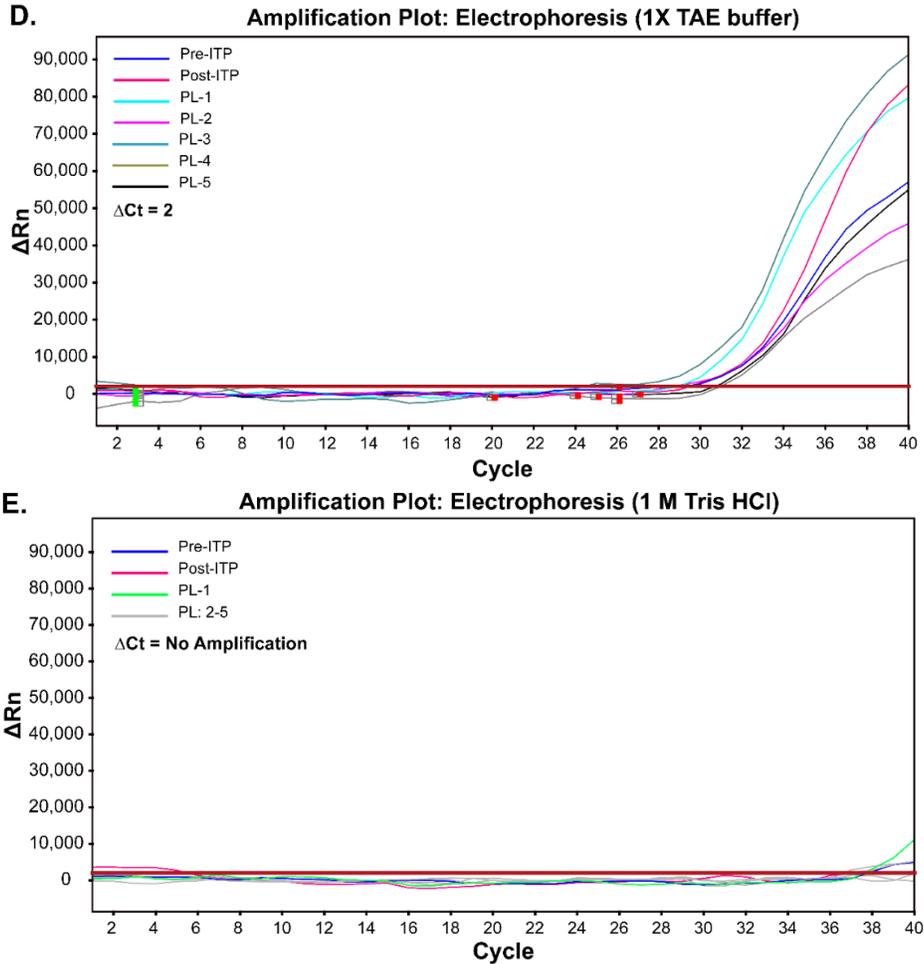


Figure S4. Comparison of isotachopheresis (ITP) and electrophoresis in our paper-based sample preparation module. **A.** For ITP experiments, one reservoir (R1) had 900 μL of gDNA spiked in 2 mM Tris taurine, while the other reservoir (R2) had 900 μL of 1 M Tris HCl. For electrophoresis experiments, both the reservoirs (R1, R2) in the sample preparation module were filled with the same electrolyte; however, in R1, the electrolyte was spiked with gDNA. We tested different electrolytes: **B.** 2 mM Tris taurine, **C.** 20 mM Tris taurine, **D.** 1X TAE buffer (40mM Tris, 20mM Acetate, and 1mM EDTA), and **E.** 1M Tris HCl. The concentration of *Mtb* genomic DNA of 10^2 copies/ μL was kept the same in all the cases.

Section S5: Direct use of paper discs (with extracted gDNA) as a template in PCR

Post ITP, the direct addition of paper discs as a template in the PCR/qPCR reaction mix was also tested. This would eliminate any additional user step of elution. Initially, we found that the paper membrane had inhibitory effects on the PCR/qPCR reaction. To overcome this problem, bovine serum albumin (BSA) and Tween 20 were added to the PCR/qPCR mix to help reduce the non-

specific adsorption of enzymes or reagents onto the paper disk. PCR reactions were followed by analyzing the amplicons in gel electrophoresis.

The DNA concentration achieved by p-ITP SPM against Mtb purified gDNA of two concentrations (10^2 , 10^0 copies/ μL) spiked in TE buffer was first tested by applying 18 V for 15 min. Each paper disc from the foldable structure was removed and used as a template for PCR. The products of PCR amplification were analyzed by gel electrophoresis. In Fig. S4, gel electrophoresis images of the Mtb gDNA concentrated within the paper layers (L1-L11) post-ITP are shown, including some positive (P: 230 bp target; red arrow) and negative controls (N, NP, TH, TT). The qualitative picture of gDNA concentration is depicted in the gel images, where the intensity of gel bands from paper layers (marked with a teal blue rectangle) post-ITP is higher than the No-ITP control (TTD, marked with a green arrow). [Note: The loading of the same paper discs that were included in the PCR mix into the gel wells after the PCR reaction was performed. The liquid amplicons were also loaded and exhibited similar results (not shown here)].

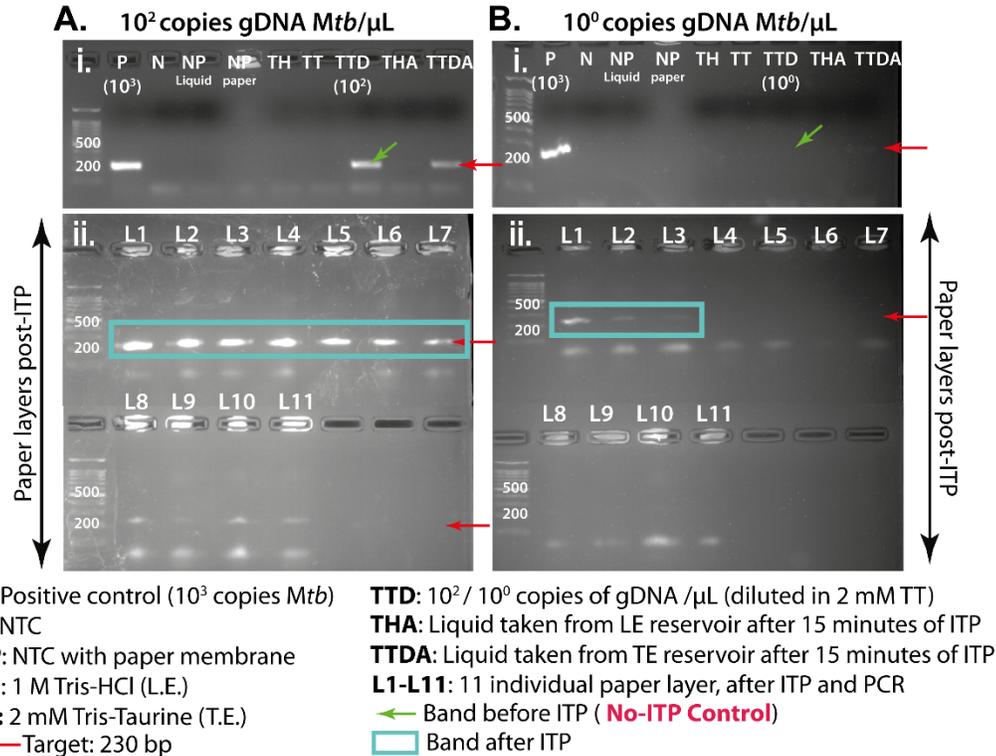


Fig. S5. Qualitative analysis of genomic DNA concentrated using *p*-ITPrep from two different samples. Panel A and B depict samples with an initial concentration of 10^2 copies/ μ L and 10^0 copies/ μ L of *Mtb* gDNA, respectively, diluted in 2 mM tris-taurine buffer (TE). Agarose gel electrophoresis was performed to visualize the amplified products. Panel A(i) and B(i) include various controls, including PCR controls (positive, negative), a No-ITP control, and Post-ITP controls. Panel A(ii) and B(ii) includes the paper layers with concentrated DNA after ITP, which was directly used as a template for PCR. The green arrow indicates the amplified product of the No-ITP Control, while the teal blue rectangle highlights the gel bands of the amplified product of the gDNA concentrated in the initial paper layers of the ITP device post-ITP. The higher intensity of the gel band of the amplicons from the paper layers compared to that of the No-ITP control suggests the concentration of gDNA in the paper layers post-ITP.

Section S6: Direct use of paper discs (with extracted gDNA) as a template in qPCR

Similarly, the paper discs post-ITP were directly used as a template in the qPCR reaction, which provided a quantitative picture of gDNA concentration in the paper layers, as shown in Fig. S5. Comparable results to those obtained by eluting concentrated DNA from the paper discs were obtained.

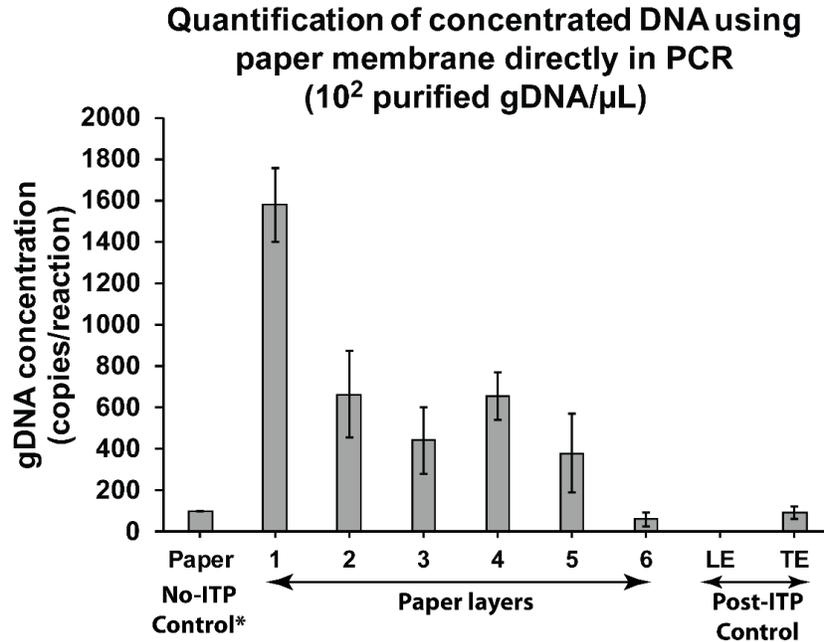


Figure S6. Quantification of concentrated DNA post-ITP in the paper layers using *p*-ITPrep, where post-ITP, we used paper discs directly as a template in the qPCR reaction mix. A plot of normalized DNA concentration for No-ITP control (paper), paper layers (1 to 6) after ITP, and Post-ITP control for sample with initial genomic *Mtb* DNA of 10^2 copies/ μ L. The experiment suggests that post-ITP users can directly use initial paper layers as a template for the downstream amplification, thereby reducing additional user steps (of eluting DNA). The device was subjected to 18 V for 15 minutes. All error bars represent standard deviations ($n=3$).

Section S7: Agar plate culture of bacterial samples obtained from ITP (no complex sample)

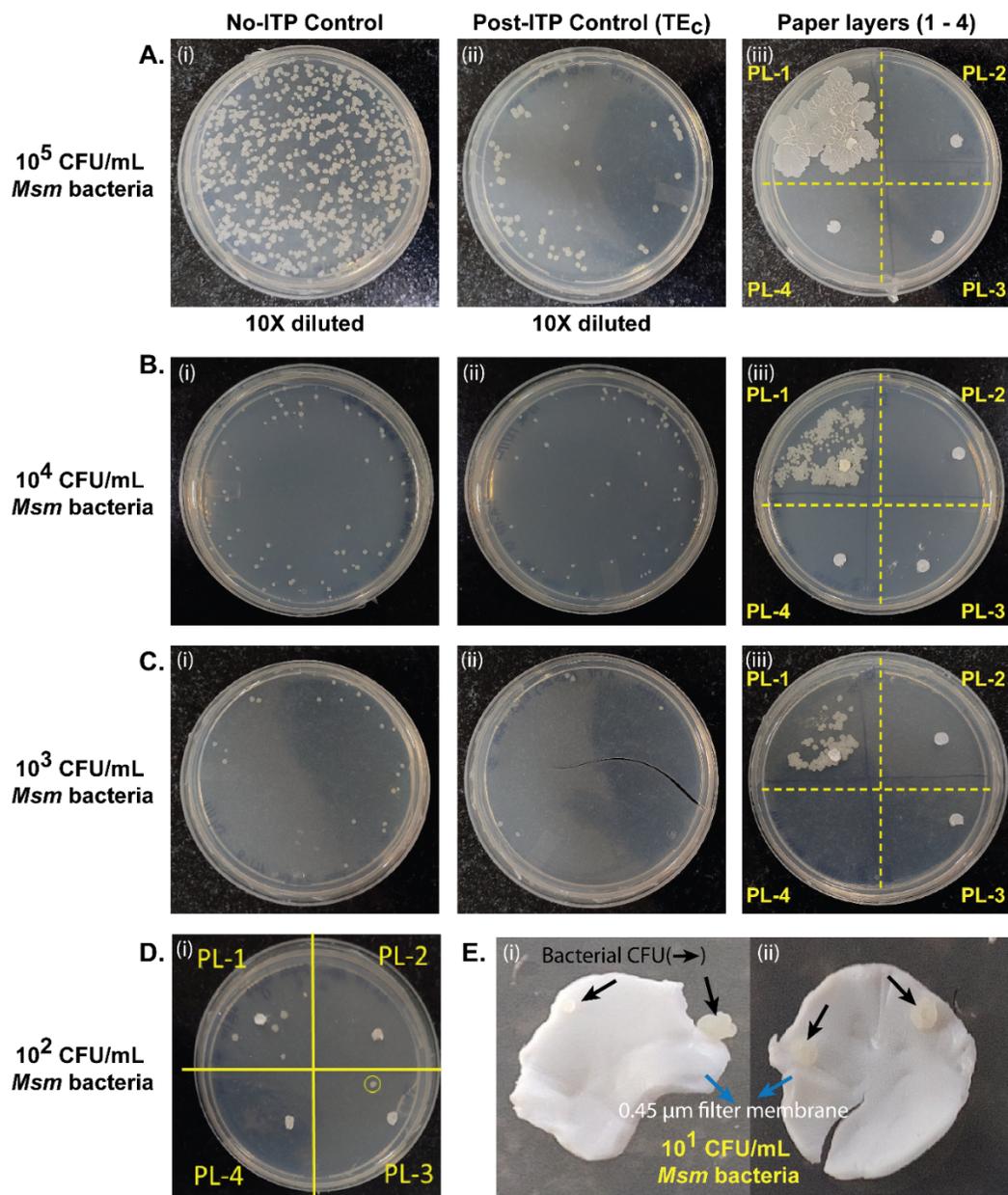
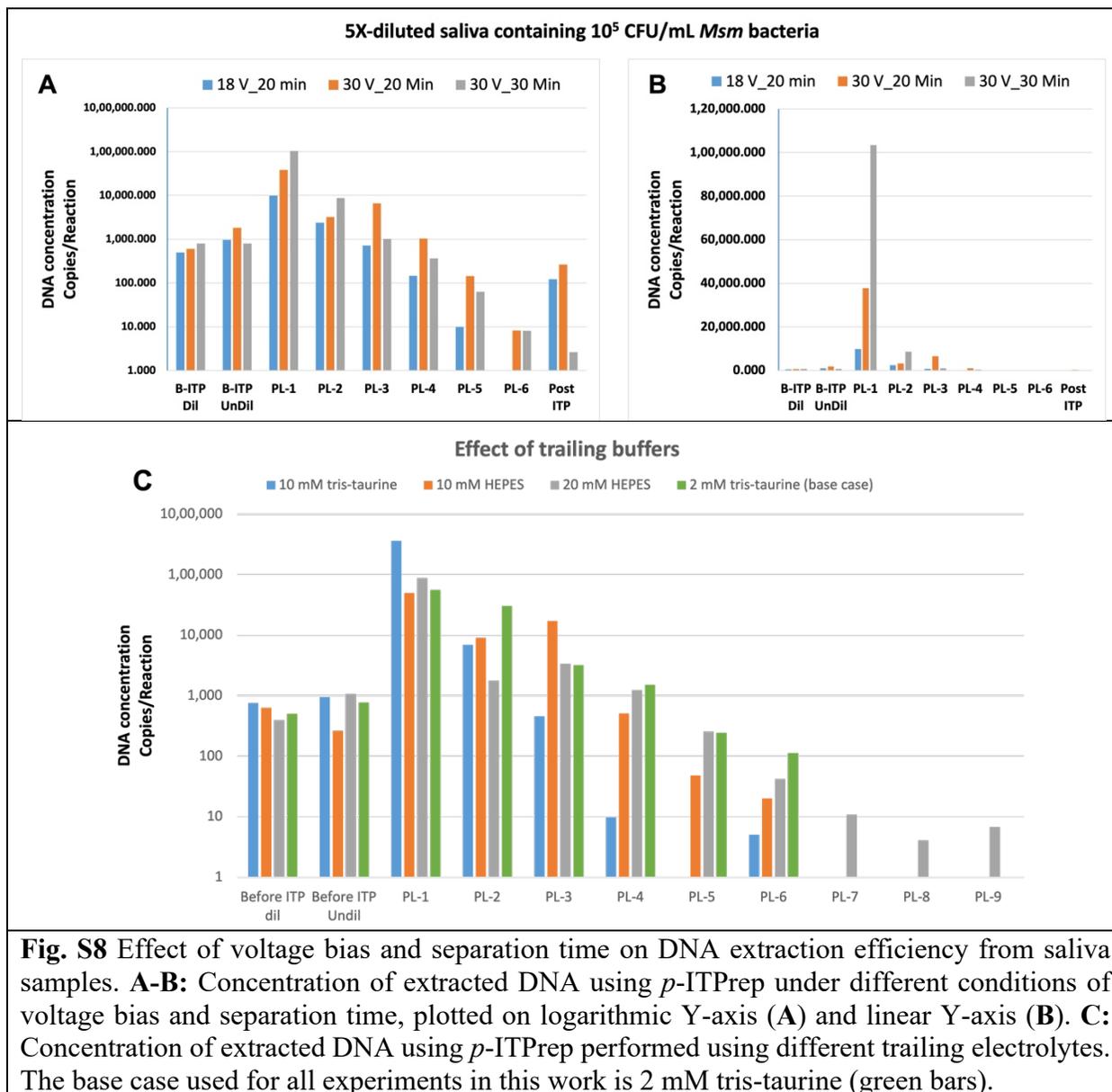


Fig. S7 Cell lysis of bacteria spiked in trailing electrolyte (TE) on applying a voltage bias of 18 V (for 20 min.) in *p*-ITP. Images of agar plates showing *Msm* colony growth in No-ITP Control (bacteria-spiked TE buffer loaded in the TE reservoir), Post-ITP Control (TE_c), and in PL-1 to 4, post-ITP for varying CFU/mL of *Msm* spiked in TE buffer. A. 10^5 CFU/mL, B. 10^4 CFU/mL, C. 10^3 CFU/mL, D. 10^2 CFU/mL, and E. 10^1 CFU/mL. [Note: For 10^1 CFU/mL *Msm* spiked in TE buffer, amplifiable DNA was not detected in the paper layers, although bacterial colonies were observed in the first paper layer for both replicates that were plated.]

Section S8: Effect of voltage bias, separation time, and trailing electrolyte on DNA extraction efficiency from saliva samples



Pooled human saliva was diluted 5X in the TE buffer, and the diluted sample contained 10^5 CFU/mL *Msm* bacteria. Three different combinations of voltage bias and separation times were used in this experiment: i) 18V, 20 min (base case); ii) 30V, 20 min; and iii) 30V 30 min. Increasing voltage as well as separation time increased the concentration of extracted DNA. Compared to the base case, condition iii) provided an approximately 10X higher concentration of extracted DNA.

Extracted DNA concentrations are plotted on a logarithmic y axis (Fig. S8 A) and on a linear y axis (Fig. S8 B). In a separate experiment, the device was operated with 4 different trailing electrolytes: i) 2 mM tris taurine (base case), ii) 10 mM tris taurine, iii) 10 mM HEPES, and iv) 20 mM HEPES. Tris taurine at 10 mM appeared to produce the maximum concentration of DNA in PL1.

Section S9: Proteinase K + Triton X-100 treatment for human serum samples

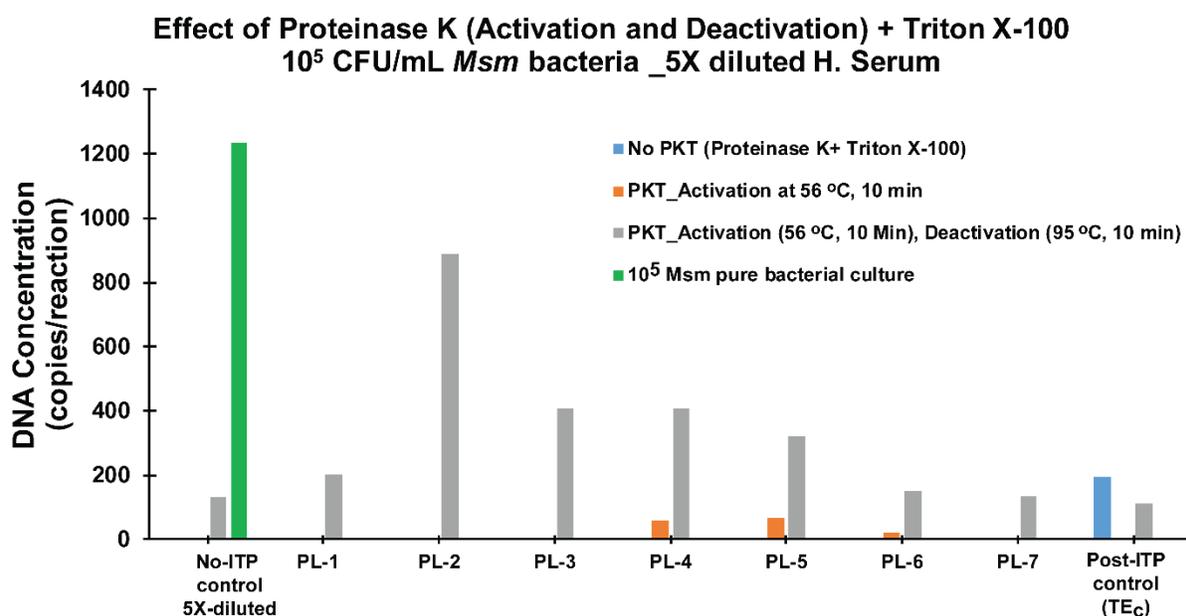


Fig. S9 Effect of proteinase K (PK) and Triton X-100 treatment on DNA isolation from complex human blood serum samples spiked with 10⁵ CFU/mL of *Msm*. Before subjecting the samples to ITP in our device, we applied three different conditions: (i) no proteinase K –Triton X-100 (*blue*), (ii) addition of proteinase K and Triton X-100 (referred as PKT) to the *Msm* spiked serum sample followed by activation of PK at 56 °C for 10 min prior to ITP (*orange*), and (iii) addition of PKT to *Msm* spiked serum sample followed by activation of PK 56 °C for 10 min and deactivation of PK 95 °C for 10 min (*grey*). *Green* column represents the DNA concentration of pure bacteria culture diluted in autoclaved DI water to a final concentration of 10⁵ CFU/mL.

No amplifiable DNA was detected in any of the paper layers in the absence of PKT treatment due to the presence of PCR inhibitory molecules in the serum that possibly co-focused with gDNA in

the PLs. Upon introducing an activation step of PK prior to ITP, we detected amplifiable DNA in PLs-4,5,6, but not in the initial layers, presumably due to the presence of activated PK, a known PCR inhibitory molecule. However, by introducing an additional step of deactivating PK, we were able to detect DNA in the initial PLs, and the DNA concentration was higher than in the previous condition.

Section S10: Agar plate culture of bacterial samples obtained from ITP (using complex matrices)

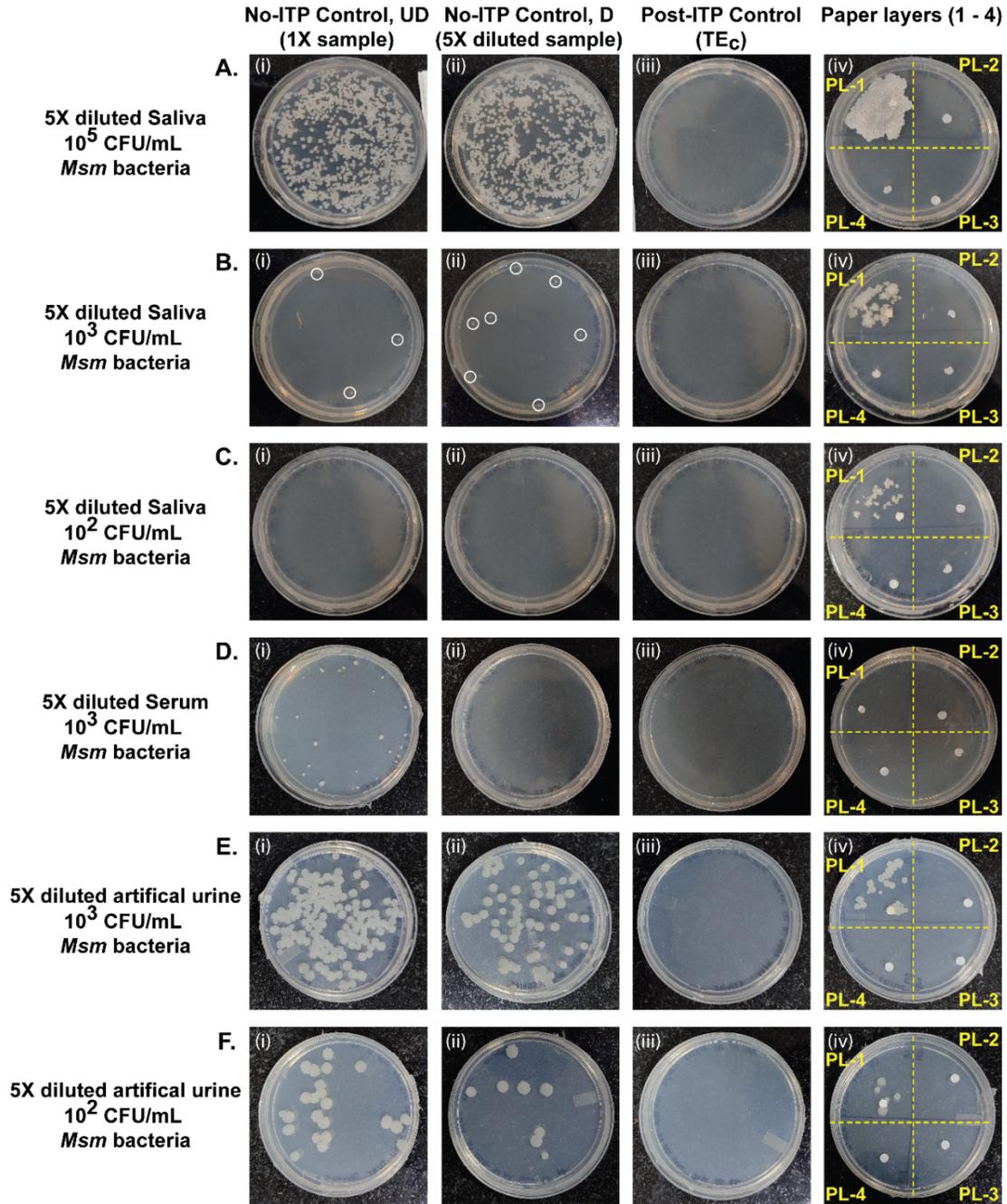


Fig. S10 Cell lysis of bacteria spiked in different crude biological samples on applying a voltage bias of 18 V (for 20 min.) in *p*-ITPrep. Images of agar plates showing *Msm* colony growth before-ITP, after-ITP, and in PL-1 to 4, post-ITP for varying CFU/mL of *Msm* spiked in different biological samples. *Pooled human saliva*: A. 10⁵ CFU/mL, B. 10³ CFU/mL, C. 10² CFU/mL; *Human blood serum*: D. 10³ CFU/mL, and *Artificial urine*: E. 10³ CFU/mL, F. 10² CFU/mL. [Note: We used new agar powder for the artificial urine experiments, which may have contributed to the larger size of *Msm* colonies observed. All the agar plates were imaged approximately 72 hours after plating using a mobile phone camera: Redmi Note 10 pro max.]

Section S11: Nucleic acid sample preparation from complex samples by filtration

To test whether separation of bacteria from the other components of the complex sample achieved by non electrolytic methods may yield detectable DNA, we tried concentration of bacteria suspended in complex matrices via filtration through a 0.45 μm MCE filter. *Msm* bacteria were spiked in three complex matrices – saliva, urine, and serum, at 10^5 , 10^4 , 10^3 , and 10^2 CFU/mL. A makeshift filtration tube was created by removing silica columns from Qiagen purification tubes and replacing them with 2 layers of 0.45 μm MCE filter. All samples solutions (200 μL) were pushed through these filters by centrifugation. The entangled bacteria were then released from the MCE filter by the addition of 5 μL of TE buffer into them followed by centrifugation in the other direction. The resulting liquid was used as template for PCR for the determination of DNA concentration. Results thus obtained are shown in Fig. S11. Saliva, urine, and serum samples containing 10^5 (Fig. S11A), 10^4 (Fig. S11B), 10^3 (Fig. S11C), and 10^2 (Fig. S11D) CFU/mL *Msm* bacteria underwent filtration. Blue and grey bars represent DNA concentrations obtained by qPCR in the sample before filtration and after filtration, respectively.

Saliva

For saliva samples, there was an increase in the concentration of detectable DNA for all starting copy numbers (Fig. S11 A-D). For concentrations of 10^3 and 10^2 CFU/mL, DNA was not detectable directly from the original sample, but DNA could be detected post filtration, which shows that the filtration method may be an effective method for sample preparation for saliva.

Urine

Results for urine were inconsistent – no consistent increase in the DNA concentration was observed after filtration. This may be possible because of adsorption of urinary components (perhaps salts) on the filter, which ends up in the PCR and affects its efficiency.

Serum

For serum, at high concentrations of 10^5 and 10^4 CFU/mL, some improvement in the detectable DNA concentration was observed. For lower concentrations of 10^3 and 10^2 CFU/mL, DNA was not detectable before or after filtration.

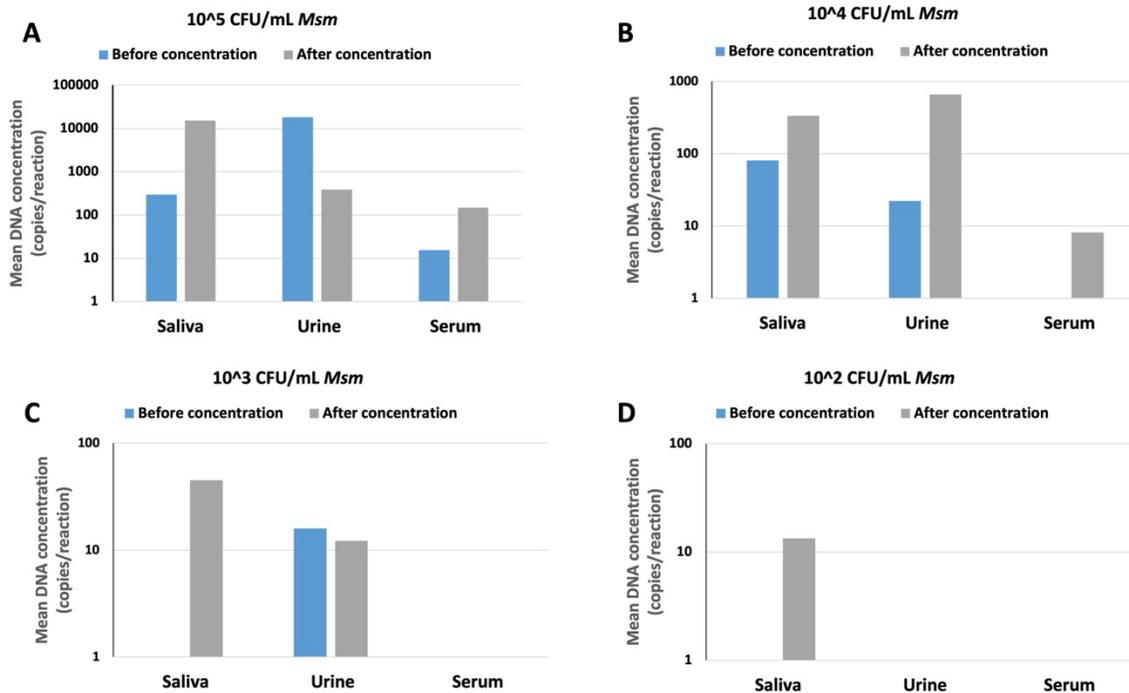


Fig. S11. Concentration of bacteria by filtration. **A-D:** Saliva, urine, and serum samples containing 10^5 (A), 10^4 (B), 10^3 (C), and 10^2 (D) CFU/mL *Msm* bacteria underwent filtration through a $0.45 \mu\text{m}$ MCE filter. The captured bacteria were eluted, and qPCR was conducted by spiking the concentrated bacteria as the template. Blue bars represent DNA concentration obtained by qPCR in the original sample and grey bars represent DNA concentrations obtained from concentrated samples.

Overall, while not the most efficient method, for some samples, especially for saliva, this method may be used as an effective sample preparation method for nucleic acid amplification tests. However, compared to the ITP-based method described in this manuscript, the filtration method is more cumbersome and requires more user steps.

Section S12: Performance of p-ITPrep without using a bacterial capture membrane

Since live bacteria entrapped by the MCE filter membrane at layer 1 were successfully detected by PCR, we investigated whether the inclusion of the MCE filter membrane at layer 1 is essential. Even if live bacteria migrate to subsequent layers, they might still be detectable via PCR. To address this, human saliva containing 10^5 CFU/mL *Msm* was introduced into two p-ITPrep devices: one incorporating the MCE filter membrane at layer 1 (base case) and the other substituting the MCE membrane with a Whatman Grade 1 filter paper, identical to the subsequent layers. A direct comparison of DNA concentrations obtained from all paper layers in the two devices is presented in Fig. S12. Blue bars represent the base case, while orange bars represent the configuration excluding the MCE bacterial capture membrane. The results indicate no significant difference in detectable DNA concentrations between the two devices. These findings suggest that p-ITPrep devices may be operable without incorporating a bacterial capture membrane. However, further experiments are required to comprehensively assess and validate the performance of these two configurations.

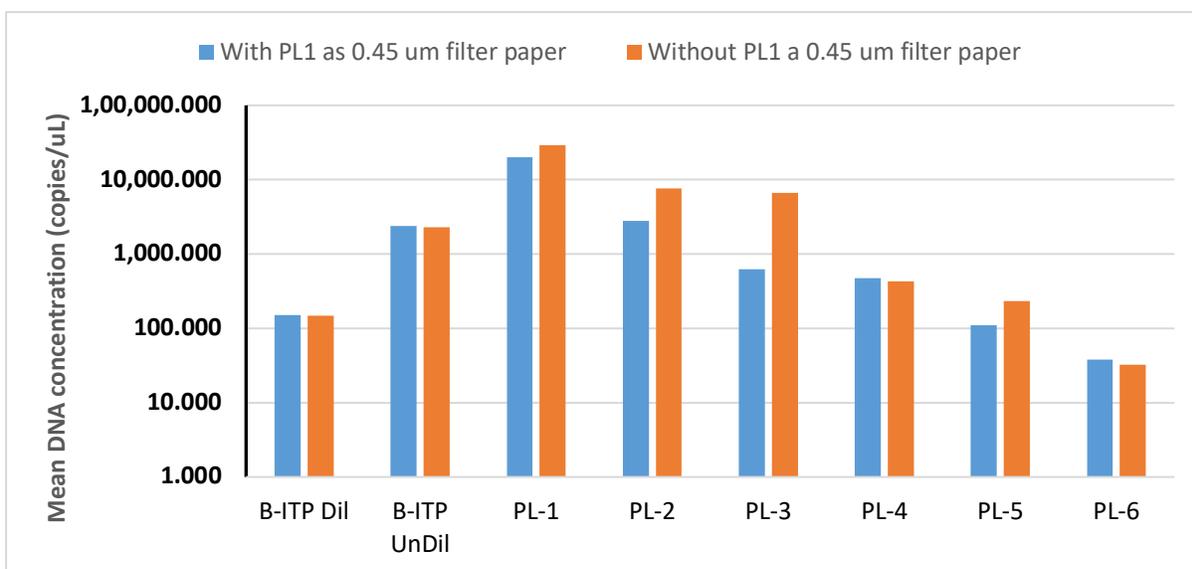


Fig. S12. Comparison of p-ITPrep with and without a bacterial capture membrane at layer 1.

Section S13: Current vs time plots

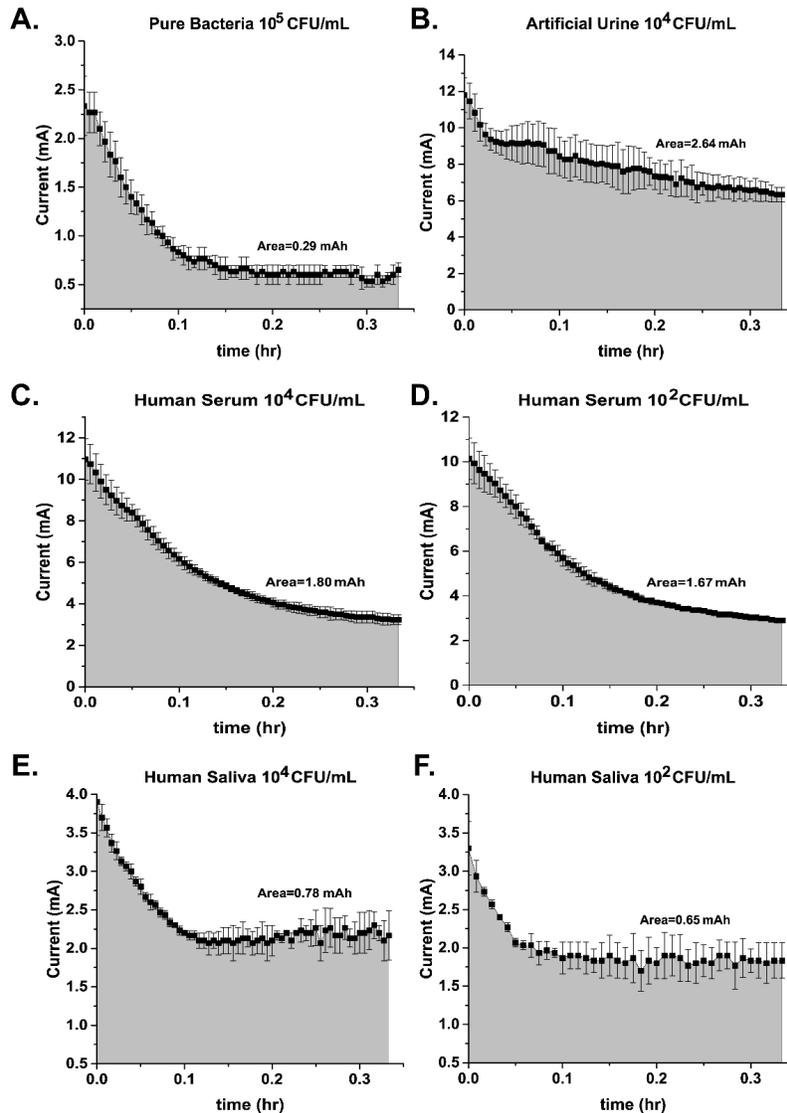


Fig. S13 Current vs time plot of ITP experiments in *p*-ITPrep.

Experiments were run at a constant voltage of 18 V for 20 min (0.33 hr) using DC power supply. Here we plot the average current vs time ($N=3$ *p*-ITPrep devices) for different biological matrices: pure *Msm* bacteria 10^5 CFU/mL (A), artificial urine spiked with 10^4 *Msm* CFU/mL, human serum spiked with 10^4 *Msm* CFU/mL (C), 10^2 *Msm* CFU/mL (D), human saliva spiked with 10^4 *Msm* CFU/mL (E), and 10^2 *Msm* CFU/mL (F). We calculated the energy required for each *p*-ITPrep run by finding the area under the curve of the current vs time plot. Energy requirement for the ITP runs varied in between 0.29 to 2.64 mAh for different biological matrices.

Table S1. Comparison of user steps involved in *p*-ITPrep and a Qiagen DNA extraction kit

QIAGEN DNA mini kit			<i>p</i> -ITPrep		
Sr. No.	Steps	Time (min)	Sr. No.	Steps	Time (min)
1.	Spin the bacterial sample, and decant the supernatant.	5	1.	Add buffer and bacterial sample into the reservoir	2*
2.	Add ATL buffer and vortex	2	2.	ITP process (18 V)	15-20
3.	Add Proteinase K & vortex	2	3.	Elute concentrated DNA from paper layers	5
4.	Incubate at 56 °C	10			
5.	Spin the tubes	0.5			
6.	Add RNase A and incubate at Room temperature (RT).	2			
7.	Add lysis buffer (AL) & vortex	2			
8.	Incubate at 70 °C	10			
9.	Spin the tubes	0.5			
10.	Add ethanol and vortex	1			
11.	Transfer the sample to the silica spin column				
12.	Spin at 8000 RPM	1			
13.	Add wash buffer (AW1) and spin at 8000 RPM	1			
14.	Add wash buffer (AW2) and spin at 14000 RPM	3			
15.	Add elution buffer (AE) and incubate at RT	5			
16.	Spin at 8000 RPM	1			
	No. of user steps	16		No. of user steps	3
	Total time (min)	46 -60		Total time (min)	~30 min

*for the blood serum sample, an additional step of Proteinase K addition, PK-activation (56 °C, 10 min), and PK-deactivation (95 °C, 10 min) step is involved in a tube before loading the same in the reservoir.

Table S2. Detailed QIAGEN DNA extraction protocol (QIAamp DNA mini kit)

1.	<i>Mycobacterium smegmatis</i> (<i>Msm</i>) spiked (5×10^4 CFU/mL) in pooled human saliva/human blood serum/artificial urine was transferred to 1.5 mL microcentrifuge tubes and spun at 7500 RPM for 5 min. The supernatant was discarded, and 200 μ L of ATL buffer was added to the tube and vortexed.
2.	20 μ L of Proteinase K (51304) was added and vortexed for 30 sec.
3.	This was followed by incubation at 56 °C for 10 min.
4.	Tubes were centrifuged at 6000 RPM in a micro-centrifuge for 30 sec
5.	4 μ L of RNase A (100 mg/mL) was added and incubated at RT for 2 minutes.
6.	200 μ L of AL buffer (lysis buffer) was added and vortex vigorously.
7.	Tubes were incubated at 70 °C for 10 min.
8.	Tubes were centrifuged at 6000 RPM for 30 secs, and 200 μ L of 100% Ethanol was added, followed by vortexing for 1 min.
9.	Lysate was transferred to the silica column provided by QIAGEN.
10.	Tubes were centrifuged at 8000 rpm for 1 min, and the flow through was discarded.
11.	The spin column was transferred to a 2 mL collection tube, and 500 μ L AW1 (wash buffer) was added and spun at 8000 rpm for 1 min.
12.	The collection tubes were discarded, and the spin column was transferred into another 2 mL collection tube. 500 μ L AW2 (wash buffer) was added and spun at 12000 RPM for 4.5 minutes.
13.	The flow-through was discarded, and the spin column was transferred into another 2 mL collection tube. 200 μ L AE (elution buffer) was added and incubated at room temperature for 5 min.
14.	The column was centrifuged at 8000 RPM for 1 min, and PCR/qPCR was performed on the extracted DNA.

Table S3. Cost of parts used for fabricating one *p*-ITPrep device

Note that these costs are based on small batch purchases of laboratory supplies alone, and are not representative of manufacturing or sales cost, which would be determined by scale and market conditions.

	Material	Total dimension of the material (mm)	Total area (mm²)	Total cost of the material (INR)	Material dimension in the device (mm)	No. of piece/device	Total area in the device (mm²)	Cost INR (\$)
1.	5 mm transparent acrylic sheet, A3	297 x 420	1,24,740	880	30 x 30	2	1800	12.7 (0.16)
2.	3 mm transparent acrylic sheet, A3	297 x 420	1,24,740	520	30 x 30	2	1800	7.5 (0.092)
3.	2 mm transparent acrylic sheet, A3	297 x 420	1,24,740	440	30 x 30	2	1800	6.3 (0.078)
4.	Whatman filter paper grade 1	100 circles (dia. 150 mm)	17,66,250	2000	5 mm circular disk	11	215.88	0.24 (0.003)
5.	3M Pressure sensitive adhesive (PSA)	2100 x 1220	25,62,000	38,300	30 x 165	1	4950	74 (0.91)
Total material cost for one <i>p</i>-ITPrep device INR (\$)								100.74 (1.23)
6.	Platinum wire (dia. 0.5 mm)	120 mm length (dia. 0.5 mm)		3,363	30	2		1682 (20.6)
7.	9 V battery			160		2		320 (3.9)
Cost of reusable/ancillary component INR (\$)								2002 (24.5)

