

SNAPflex: a paper-and-plastic device for instrument-free RNA and DNA extraction from whole blood

Supplementary material

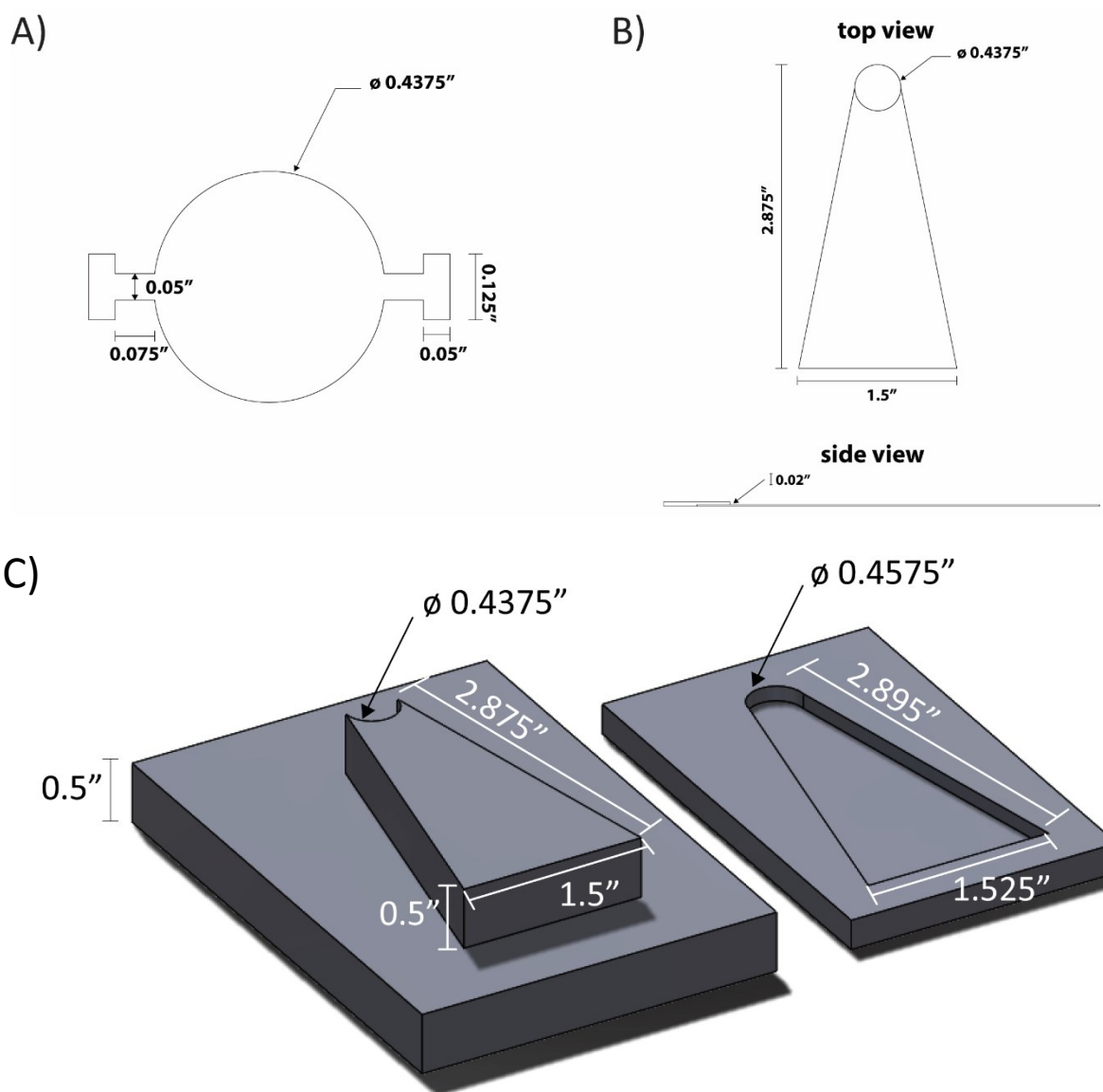
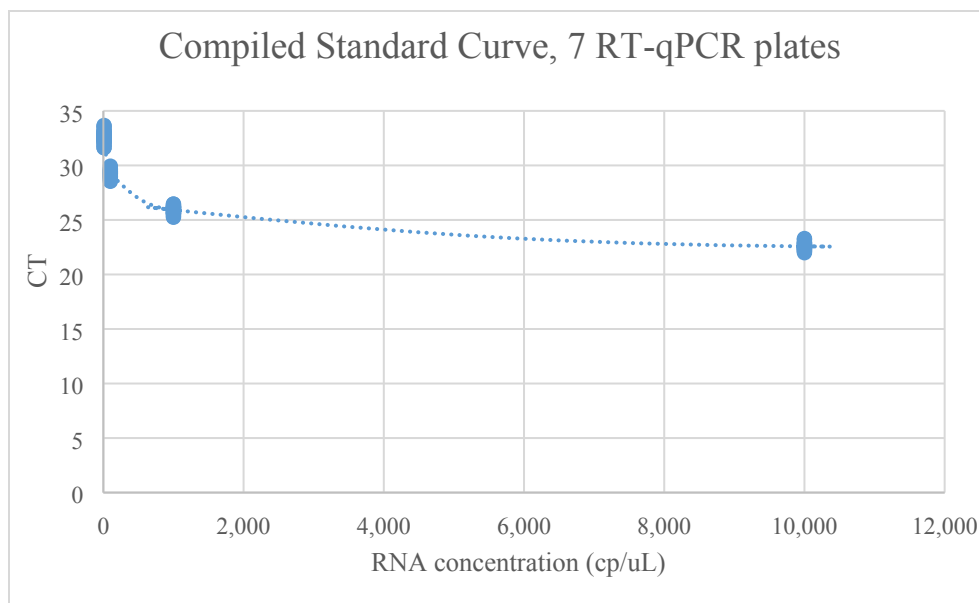


Figure S1: A) Dimensions of glass fiber paper membrane; B) Dimensions of top view and side view of chromatography paper waste pad, C) Dimensions of press used to generate the protrusion in the chromatography paper waste pad. Ahlstrom chromatography paper cut into the appropriate dimensions was placed into the bottom portion of the mold, the top portion of the mold is sealed on top and pressurized to incorporate the protrusion.



Slope	-3.34	standard error, 0.03
Intercept	35.9	standard error, 0.1
R ²	0.99	
Calculated Efficiency	99.21%	
Equation of the line:	$C_T = -3.34066 \cdot \log(\text{quantity}) + 35.94307$	

Figure S2: Compiled standard curve used to analyze all stability samples. RNA stocks went through an unexpected freeze-thaw during storage at -80°C. To avoid variability between quantification due to this error, all samples were quantified by RT-qPCR and the C_T values were compared against this master standard curve from 9 separate known successful experiments rather than an internal standard curve. The raw C_T values and the corresponding calculated quantities are included in Table S1.

Table S1: C_T values and calculated quantities using “master” standard curve for RNA stability.

Experiment Replicate	Day	Temperature	CT	Ct Mean	Ct SD	Calculated Quantity (copies/ μ L)	Calculated Quantity (copies/mL)	Mean Quantity (copies/mL)	Quantity Standard Dev (copies/mL)	Recovery
1	0	Room Temp	29.90	29.81	0.12	64.32	64322.89	68876.66	5816.98	68.88%
1	0	Room Temp	29.85	29.81	0.12	66.88	66877.48			
1	0	Room Temp	29.67	29.81	0.12	75.43	75429.60			
1	0	Room Temp	29.92	30.08	0.21	63.62	63615.88	57440.56	7836.59	57.44%
1	0	Room Temp	30.31	30.08	0.21	48.62	48624.79			
1	0	Room Temp	30.00	30.08	0.21	60.08	60081.01			
1	1	Room Temp	29.70	29.54	0.16	74.32	74319.28	83027.16	9026.64	83.03%
1	1	Room Temp	29.38	29.54	0.16	92.34	92341.93			
1	1	Room Temp	29.54	29.54	0.16	82.42	82420.28			
1	1	Room Temp	30.02	30.02	0.05	59.36	59364.26	59447.53	1973.74	59.45%
1	1	Room Temp	29.97	30.02	0.05	61.46	61461.60			
1	1	Room Temp	30.06	30.02	0.05	57.52	57516.74			
1	7	Room Temp	29.74	29.79	0.07	71.80	71800.64	69595.71	3423.03	69.60%
1	7	Room Temp	29.75	29.79	0.07	71.33	71334.16			
1	7	Room Temp	29.87	29.79	0.07	65.65	65652.32			
1	7	Room Temp	29.71	29.65	0.09	73.60	73602.77	76407.36	5008.68	76.41%
1	7	Room Temp	29.55	29.65	0.09	82.19	82190.02			
1	7	Room Temp	29.71	29.65	0.09	73.43	73429.29			
1	14	Room Temp	29.43	29.63	0.19	89.30	89300.17	78241.95	10491.79	78.24%
1	14	Room Temp	29.64	29.63	0.19	77.00	76998.20			
1	14	Room Temp	29.81	29.63	0.19	68.43	68427.46			
1	14	Room Temp	29.87	30.02	0.17	65.72	65716.22	59523.63	6738.74	59.52%
1	14	Room Temp	29.99	30.02	0.17	60.51	60507.70			
1	14	Room Temp	30.20	30.02	0.17	52.35	52346.96			
1	1	37C	29.76	29.76	0.06	71.18	71184.46	70860.22	3024.16	70.86%
1	1	37C	29.83	29.76	0.06	67.69	67687.00			
1	1	37C	29.70	29.76	0.06	73.71	73709.19			
1	7	37C	29.74	29.69	0.07	72.12	72124.86	74435.60	3663.21	74.44%
1	7	37C	29.73	29.69	0.07	72.52	72522.68			
1	7	37C	29.61	29.69	0.07	78.66	78659.28			
1	14	37C	29.96	29.91	0.06	61.65	61645.86	64120.02	2498.01	64.12%
1	14	37C	29.91	29.91	0.06	64.07	64072.99			
1	14	37C	29.85	29.91	0.06	66.64	66641.22			
1	14	37C	29.88	29.97	0.23	65.14	65144.89	61735.44	9182.39	61.74%
1	14	37C	30.23	29.97	0.23	51.34	51335.99			
1	14	37C	29.81	29.97	0.23	68.73	68725.42			

2	0	Room Temp	29.19	29.15	0.04	105.2891765	105289.1765	108420.6576	2720.810228	108.42%
2	0	Room Temp	29.13	29.15	0.04	109.7669037	109766.9037			
2	0	Room Temp	29.12	29.15	0.04	110.2058927	110205.8927			
2	0	Room Temp	29.58	29.64	0.06	80.5028448	80502.8448	77274.89201	3234.910573	77.27%
2	0	Room Temp	29.70	29.64	0.06	74.03306826	74033.06826			
2	0	Room Temp	29.64	29.64	0.06	77.28876297	77288.76297			
2	1	Room Temp	29.45	29.40	0.12	88.0532572	88053.2572	91287.32589	7743.354429	91.29%
2	1	Room Temp	29.26	29.40	0.12	100.1234417	100123.4417			
2	1	Room Temp	29.49	29.40	0.12	85.68527875	85685.27875			
2	1	Room Temp	29.16	29.19	0.06	107.0832574	107083.2574	105459.5297	4129.022574	105.46%
2	1	Room Temp	29.25	29.19	0.06	100.7654682	100765.4682			
2	1	Room Temp	29.14	29.19	0.06	108.5298635	108529.8635			
2	7	Room Temp	29.33	29.48	0.13	95.13582733	95135.82733	86359.01247	7604.565864	86.36%
2	7	Room Temp	29.55	29.48	0.13	82.2052594	82205.2594			
2	7	Room Temp	29.55	29.48	0.13	81.73595069	81735.95069			
2	7	Room Temp	29.38	29.47	0.09	92.01086714	92010.86714	86707.25003	5093.612183	86.71%
2	7	Room Temp	29.55	29.47	0.09	81.85348343	81853.48343			
2	7	Room Temp	29.48	29.47	0.09	86.25739953	86257.39953			
2	14	Room Temp	30.28	30.33	0.05	49.43201598	49432.01598	47992.24683	1618.441562	47.99%
2	14	Room Temp	30.38	30.33	0.05	46.24054265	46240.54265			
2	14	Room Temp	30.32	30.33	0.05	48.30418185	48304.18185			
2	14	Room Temp	29.78	29.82	0.14	70.14179037	70141.79037	68150.35386	6362.414547	68.15%
2	14	Room Temp	29.98	29.82	0.14	61.0304251	61030.4251			
2	14	Room Temp	29.71	29.82	0.14	73.27884611	73278.84611			
2	1	37C	29.66	29.57	0.11	75.833681	75833.681	81022.40057	6213.987377	81.02%
2	1	37C	29.45	29.57	0.11	87.90879146	87908.79146			
2	1	37C	29.60	29.57	0.11	79.32472924	79324.72924			
2	1	37C	29.61	29.55	0.11	78.54922269	78549.22269	82304.68607	6226.176965	82.30%
2	1	37C	29.42	29.55	0.11	89.4916221	89491.6221			
2	1	37C	29.61	29.55	0.11	78.87321342	78873.21342			
2	7	37C	29.58	29.63	0.08	80.51903901	80519.03901	77680.22897	4248.532962	77.68%
2	7	37C	29.59	29.63	0.08	79.72577712	79725.77712			
2	7	37C	29.72	29.63	0.08	72.79587078	72795.87078			
2	7	37C	29.65	29.71	0.05	76.55268257	76552.68257	73602.71211	2564.760576	73.60%
2	7	37C	29.73	29.71	0.05	72.35411712	72354.11712			
2	7	37C	29.74	29.71	0.05	71.90133665	71901.33665			
2	14	37C	29.56	29.48	0.10	81.53890123	81538.90123	85978.60409	5819.688813	85.98%
2	14	37C	29.37	29.48	0.10	92.56715747	92567.15747			
2	14	37C	29.52	29.48	0.10	83.82975357	83829.75357			
2	14	37C	29.93	29.97	0.12	63.01134624	63011.34624	61488.83167	4800.839438	61.49%
2	14	37C	30.10	29.97	0.12	56.11135067	56111.35067			

2	14	37C	29.88	29.97	0.12	65.34379809	65343.79809			
3	0	Room Temp	29.15	29.37	0.21	108.0861824	108086.1824	93259.56812	13685.7533	93.26%
3	0	Room Temp	29.57	29.37	0.21	81.11038983	81110.38983			
3	0	Room Temp	29.41	29.37	0.21	90.58213216	90582.13216			
3	1	Room Temp	29.73	29.68	0.15	72.26732368	72267.32368	75387.90025	8065.65584	75.39%
3	1	Room Temp	29.51	29.68	0.15	84.54761651	84547.61651			
3	1	Room Temp	29.79	29.68	0.15	69.34876057	69348.76057			
3	1	Room Temp	30.39	30.32	0.16	45.91512602	45915.12602	48537.32191	5437.3277	48.54%
3	1	Room Temp	30.42	30.32	0.16	44.90801511	44908.01511			
3	1	Room Temp	30.14	30.32	0.16	54.7888246	54788.8246			
3	7	Room Temp	29.92	30.04	0.11	63.37264276	63372.64276	58462.75069	4608.446356	58.46%
3	7	Room Temp	30.15	30.04	0.11	54.23086974	54230.86974			
3	7	Room Temp	30.06	30.04	0.11	57.78473956	57784.73956			
3	7	Room Temp	29.90	29.97	0.14	64.39489175	64394.89175	61390.64171	5588.614191	61.39%
3	7	Room Temp	30.13	29.97	0.14	54.9424607	54942.4607			
3	7	Room Temp	29.89	29.97	0.14	64.83457269	64834.57269			
3	14	Room Temp	30.26	30.12	0.14	50.36461444	50364.61444	55422.81974	5279.785266	55.42%
3	14	Room Temp	30.13	30.12	0.14	55.00454189	55004.54189			
3	14	Room Temp	29.98	30.12	0.14	60.89930289	60899.30289			
3	14	Room Temp	30.26	30.42	0.17	50.15166351	50151.66351	45172.89574	5310.769715	45.17%
3	14	Room Temp	30.40	30.42	0.17	45.78402448	45784.02448			
3	14	Room Temp	30.61	30.42	0.17	39.58299922	39582.99922			
3	1	37C	30.11	30.05	0.12	55.68337012	55683.37012	58372.10367	4974.642586	58.37%
3	1	37C	29.91	30.05	0.12	64.11250306	64112.50306			
3	1	37C	30.12	30.05	0.12	55.32043783	55320.43783			
3	1	37C	29.64	29.58	0.09	77.17159571	77171.59571	80700.3073	5001.993725	80.70%
3	1	37C	29.47	29.58	0.09	86.42459834	86424.59834			
3	1	37C	29.61	29.58	0.09	78.50472785	78504.72785			
3	7	37C	30.16	30.37	0.19	53.89603617	53896.03617	46735.01869	6206.233844	46.74%
3	7	37C	30.47	30.37	0.19	43.39369599	43393.69599			
3	7	37C	30.49	30.37	0.19	42.9153239	42915.3239			
3	7	37C	30.20	30.19	0.05	52.31304161	52313.04161	52824.38655	1839.975585	52.82%
3	7	37C	30.23	30.19	0.05	51.2941685	51294.1685			
3	7	37C	30.13	30.19	0.05	54.86594952	54865.94952			
3	14	37C	29.70	29.99	0.25	73.93026367	73930.26367	61324.49127	11066.35569	61.32%
3	14	37C	30.08	29.99	0.25	56.83409091	56834.09091			
3	14	37C	30.18	29.99	0.25	53.20911924	53209.11924			
3	14	37C	30.46	30.63	0.23	43.74360522	43743.60522	39364.49139	5846.476407	39.36%
3	14	37C	30.53	30.63	0.23	41.62451962	41624.51962			
3	14	37C	30.88	30.63	0.23	32.72534933	32725.34933			

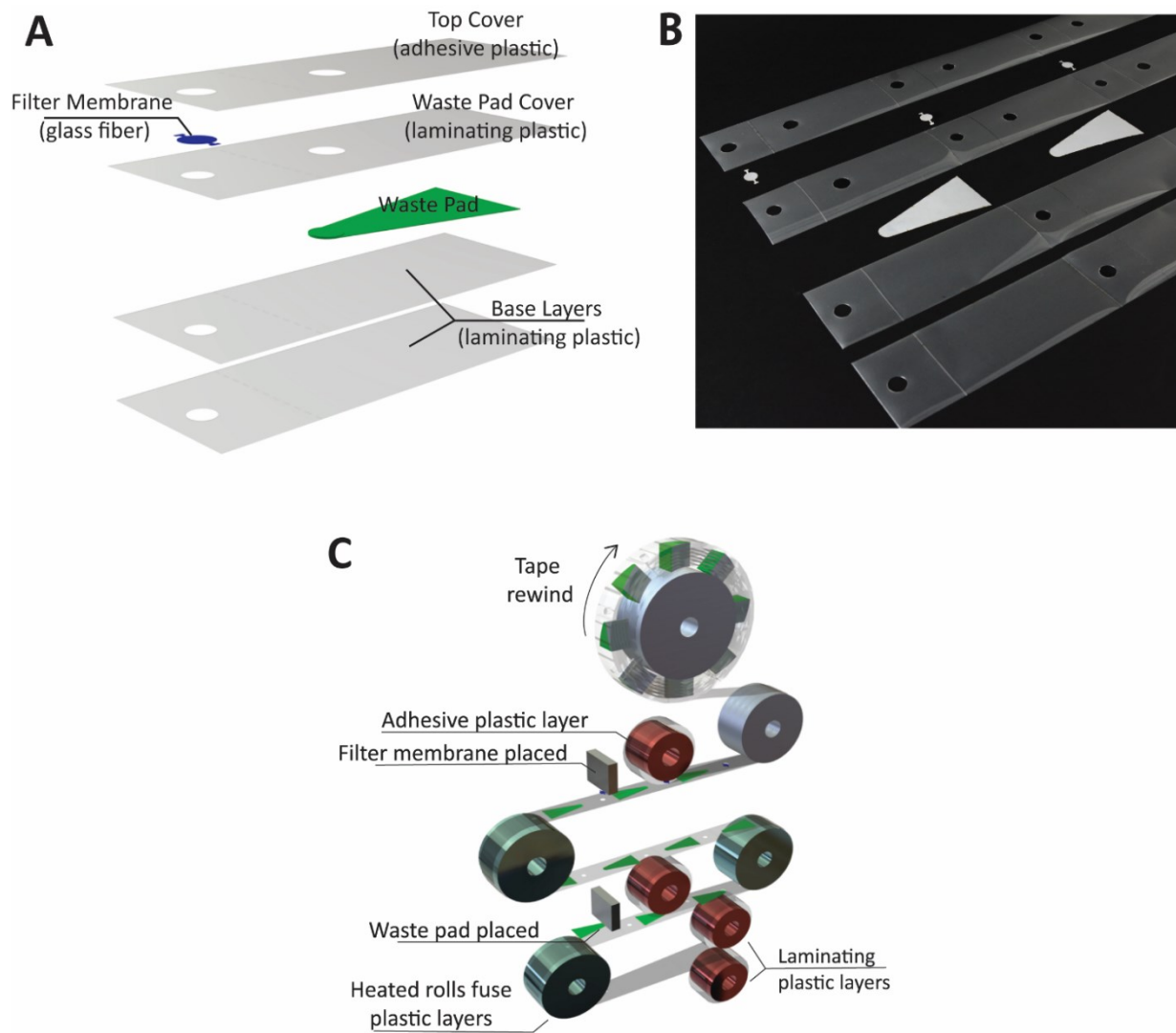


Figure S3: A) SNAPflex layers. (B) All components of the device can be manufactured in configurations suitable for roll-to-roll manufacturing. (C) Proposed roll-to-roll manufacturing schematic.

	1:2 Blood:Lysis Buffer			1:1.5 Blood:Lysis Buffer		
Sample (uL)	100	200	250	100	200	250
Total volume	1133.6	1562.1	1776.4	1062.1	1419.3	1597.9

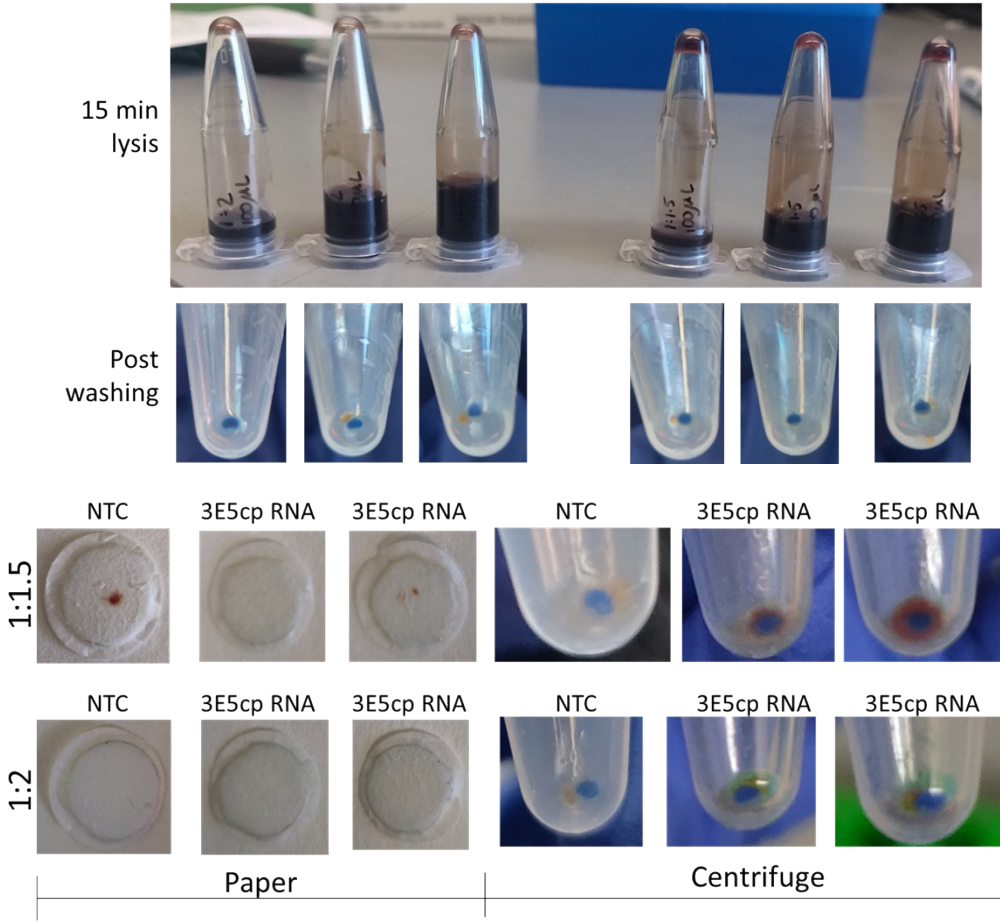
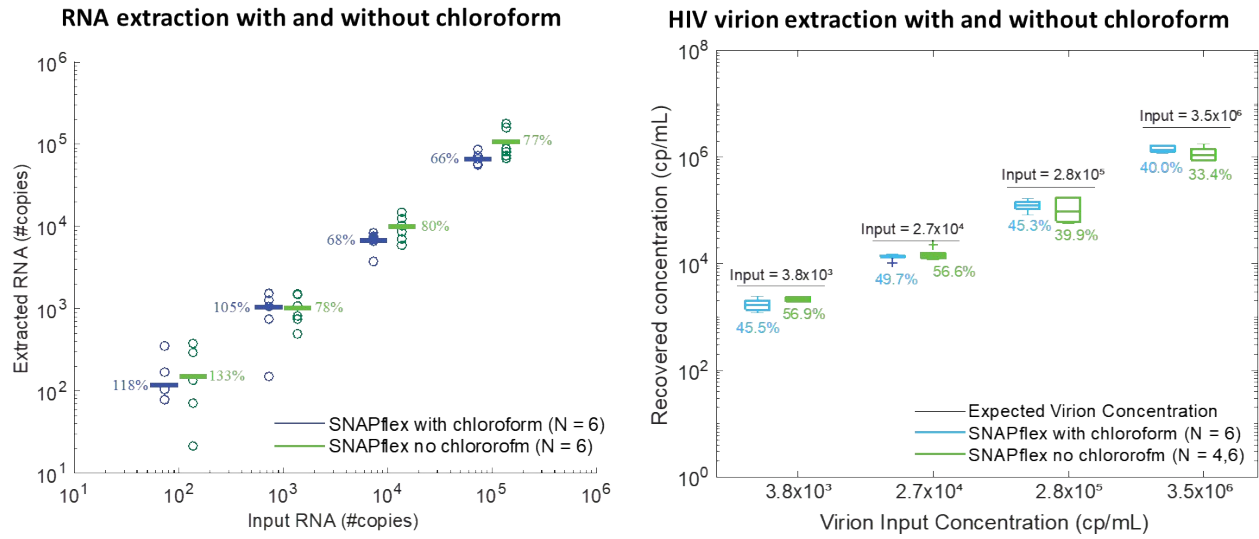


Figure S4: Investigation of sample: lysis buffer ratio. A final ratio of 1:2 (sample :lysis buffer) enabled sufficient lysis of blood components. Complete lysis of whole blood components enabled complete removal of lysed components from the surface of the sample membrane and in centrifugation samples as well.

A)



B)

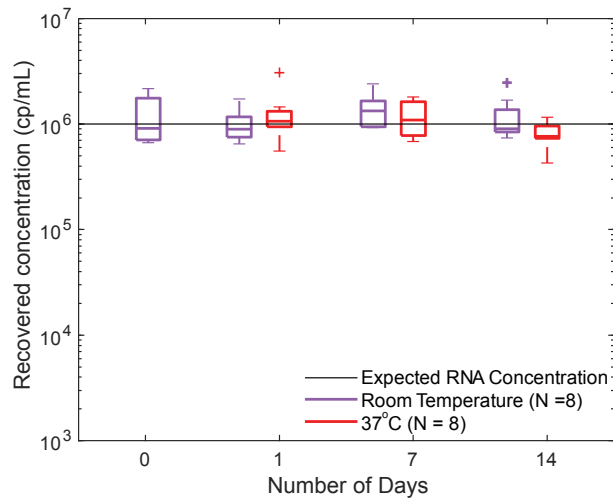


Figure S5: A) Comparison of *in vitro* transcribed RNA recovery and HIV-1 virion RNA recovery with (blue) and without (green) chloroform. B) Recovery of *in vitro* transcribed RNA from whole blood with chloroform included in the precipitation buffer, stabilization at room temperature (purple) and 37°C (red) for 14 days. Including chloroform in the precipitation buffer reduces the necessary volume of precipitation buffer from 35% to 25% but does not significantly change sample recovery.

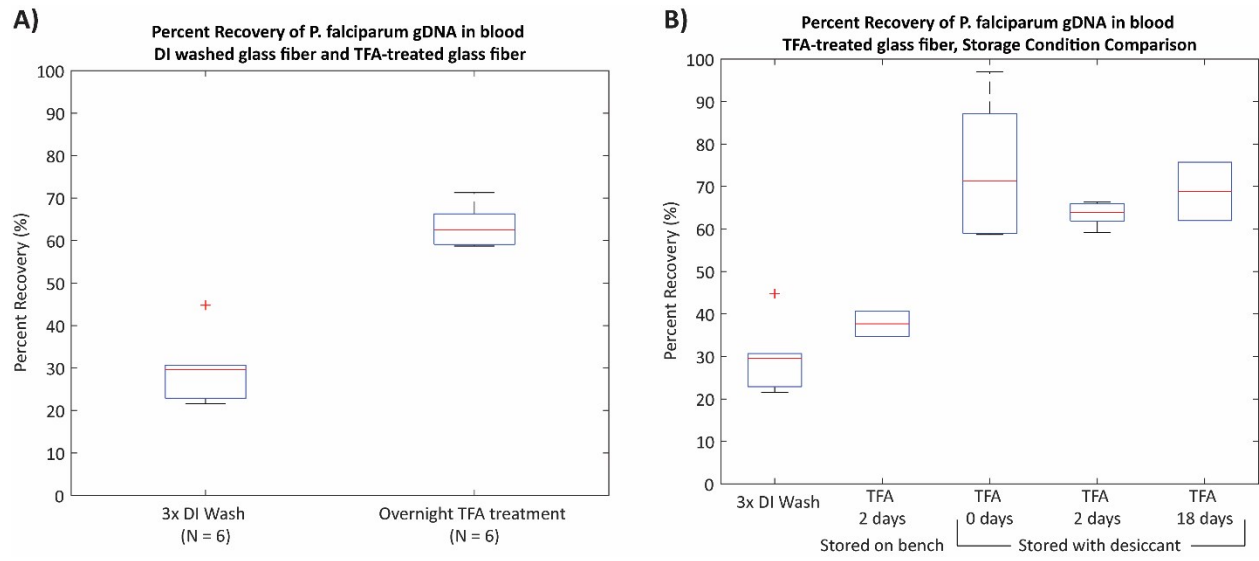


Figure S6: A) Trifluoroacetic acid (TFA) treatment improves DNA recovery from glass fiber membrane, B) TFA-treated membranes must be stored in a dry environment with desiccant is to maintain recovery improved sample recovery.