

EXPERIMENTAL METHOD:

Methodology:

Paper Strip Cut Out:

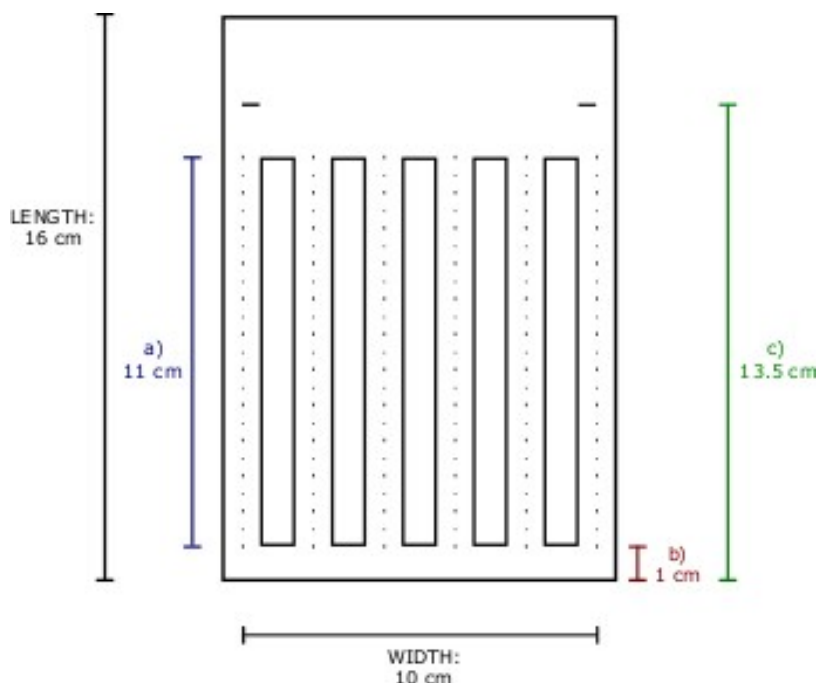


Figure S1: Paper strip cut-out prepared on CorelDRAW X6. a) The total height of the strip. In the middle of each strip are markings 5 mm apart. These are used to measure the elution height up each strip. b) The bottom section of the cut-out. This was included to hold the strips in place during elution. c) The height up the cut-out that the rod was attached to. This is indicated by the horizontal slits on either side of the cut-out.

Test Conditions:

Table S1: Conditions for hydrophobicity and viscosity testing

Experiment	Relevant Figure	Conditions	
Effect of hydrophobicity on different surfaces	Figure 4a	Cellulose	0.25 % w/v cellulose 0 U/mL Thrombin 0 g/L Fibrinogen
		Cellulose & Fibrinogen	0.25 % w/v cellulose 0 U/mL Thrombin 2 g/L Fibrinogen
		Cellulose & Thrombin	0.25 % w/v cellulose 10 U/mL Thrombin 0 g/L Fibrinogen
		Cellulose & Fibrin	0.25 % w/v cellulose 10 U/mL Thrombin 2 g/L Fibrinogen

Effect of Glycerol Viscosity on Elution Height	Figure 4b	Paper Type	Tissue 1
		Strip Width	3 mm
		Position of Glycerol from Bottom of Strip (cm)	1 cm
		Glycerol-Water Mixture Volume	6 μ L
		Glycerol Wicking Time	60 sec
		Elution Time	3 min

Table SII: Conditions for variable-sensitivity testing

Experiment	Relevant Figure	Conditions	
Effect of Paper Structure and Fibrinogen Concentration on Elution Height	Figure 5	Strip Width	5 mm
		Thrombin Solution Volume	3 μ L
		Thrombin Concentration	30 U/mL
		FXIIIa Concentration	0 U/mL
		Fibrinogen Solution Volume	20 μ L
		Reaction Time	60 sec
		Elution Time	5 min
Effect of Strip Width and Fibrinogen Concentration on Elution Height	Figure S8	Paper Type	Tissue 2
		Thrombin Solution Volume (3 mm/5 mm)	2/3 μ L
		Thrombin Concentration	30 U/mL
		FXIIIa Concentration	0 U/mL
		Fibrinogen Solution Volume (3 mm/5 mm)	12/20 μ L
		Reaction Time	60 sec
		Elution Time	5 min
Effect of Fibrinogen Solution Volume and Fibrinogen Concentration on Elution Height	Figure 6(a)	Paper Type	Tissue 2
		Strip Width	3 mm
		Thrombin Solution Volume	2 μ L
		Thrombin Concentration	30 U/mL
		FXIIIa Concentration	0 U/mL
		Reaction Time	60 sec
		Elution Time	5 min
Effect of Thrombin Concentration and Fibrinogen Concentration on Elution Height	Figure 6(b)	Paper Type	Tissue 2
		Strip Width	3 mm
		Thrombin Solution Volume	2 μ L
		FXIIIa Concentration	0 U/mL
		Fibrinogen Solution Volume	12 μ L
		Reaction Time	60 sec
		Elution Time	5 min
Effect of FXIIIa Concentration and Fibrinogen Concentration on Elution Height	Figure S7	Paper Type	Tissue 2
		Strip Width	3 mm
		Thrombin Solution Volume	2 μ L
		Thrombin Concentration	30 U/mL
		Fibrinogen Solution Volume	24 μ L
		Reaction Time	60 sec
		Elution Time	5 min
Effect of Reaction Time and Fibrinogen Concentration on Elution Height	Figure 7(a)	Paper Type	Tissue 2
		Strip Width	3 mm
		Thrombin Solution Volume	2 μ L
		Thrombin Concentration	60 U/mL
		FXIIIa Concentration	0 U/mL

		Fibrinogen Solution Volume	12 μ L
		Elution Time	5 min
Effect of Elution Time and Fibrinogen Concentration on Elution Height	Figure 7(b)	Paper Type	Tissue 2
		Strip Width	3 mm
		Thrombin Solution Volume	2 μ L
		Thrombin Concentration	60 U/mL
		FXIIIa Concentration	0 U/mL
		Fibrinogen Solution Volume	12 μ L
		Reaction Time	30 sec
Effect of BSA Concentration and Fibrinogen Concentration on Elution Height.	Figure 8	Paper Type	Tissue 3
		Strip Width	3 mm
		Thrombin Solution Volume	2 μ L
		Thrombin Concentration	60 U/mL
		FXIIIa Concentration	0 U/mL
		Fibrinogen Solution Volume	12 μ L
		Reaction Time	30 sec
		Elution Time	7 min

Table SIII: Structural Properties of Paper Substrates

	Grammage (GSM)	Thickness (mm)	Density (kg/m³)
Tissue 1	25	0.14	180
Tissue 2	30	0.16	190
Tissue 3	41	0.24	180
Filter	91	0.23	400

Table SIV: Positionings of fibrinogen and thrombin solution up paper strips for each test condition in **Figure 5**. Each condition was reacted for 60 seconds and eluted for 5 minutes.

	Position of Thrombin Solution from Bottom of Strip (cm)	Position of Fibrinogen Solution from Bottom of Strip (cm)
Tissue 1	2.5	3
Tissue 2	2	2.5
Tissue 3	1.5	2
Filter Paper	1.5	2

Table SV: Positionings of fibrinogen and thrombin solution up paper strips for each test condition in **Figure 6a**. Each condition was reacted for 60 seconds and eluted for 5 minutes.

	Position of Thrombin Solution from Bottom of Strip (cm)	Position of Fibrinogen Solution from Bottom of Strip (cm)
3 μL Fibrinogen Solution	0.5	1
6 μL Fibrinogen Solution	1	1.5
12 μL Fibrinogen Solution	2	2.5
18 μL Fibrinogen Solution	3	3.5
24 μL Fibrinogen Solution	4	4.5

Table SVI: Positionings of fibrinogen and thrombin solution up paper strips for each test condition in **Figure 6b**, **7**, **S7** and **S8**. Each condition except **Figure 7** was reacted for 60 seconds and eluted for 5 minutes.

	Position of Thrombin Solution from Bottom of Strip (cm)	Position of Fibrinogen Solution from Bottom of Strip (cm)
All Conditions	2	2.5

Table SVII: Positionings of fibrinogen and thrombin solution up paper strips for each test condition in **Figure 8**.
Each condition was reacted for 30 seconds and eluted for 7 minutes.

	Position of Thrombin Solution from Bottom of Strip (cm)	Position of Fibrinogen Solution from Bottom of Strip (cm)
0 g/L BSA	1.5	2
80 g/L BSA	1	1.5

Table SVIII: Optimal Test Conditions in **Figure 9**.

Optimal Test Conditions	Figure 9	Paper Type	Tissue 3
		Strip Width	3 mm
		Thrombin Solution Volume	2 μ L
		Thrombin Concentration	60 U/mL
		FXIIIa Concentration	0 U/mL
		Fibrinogen Solution Volume	12 μ L
		Reaction Time	30 sec
		Elution Time	7 min
		Position of Thrombin Solution from Bottom of Strip (cm)	1
		Position of Fibrinogen Solution from Bottom of Strip (cm)	1.5

Table SIX: Conditions for Serum vs Plasma testing in **Figure 3**.

Elution of Serum vs Plasma	Figure 3	Paper Type	Tissue 2
		Strip Width	5 mm
		Thrombin Solution Volume	3 μ L
		Thrombin Concentration	30 U/mL
		FXIIIa Concentration	0 U/mL
		Serum/Plasma Volume	20 μ L
		Reaction Time	60 sec
		Elution Time	5 min
		Position of Thrombin Solution from Bottom of Strip (cm)	1.5
		Position of Fibrinogen Solution from Bottom of Strip (cm)	2

RESULTS:

Raw Data:

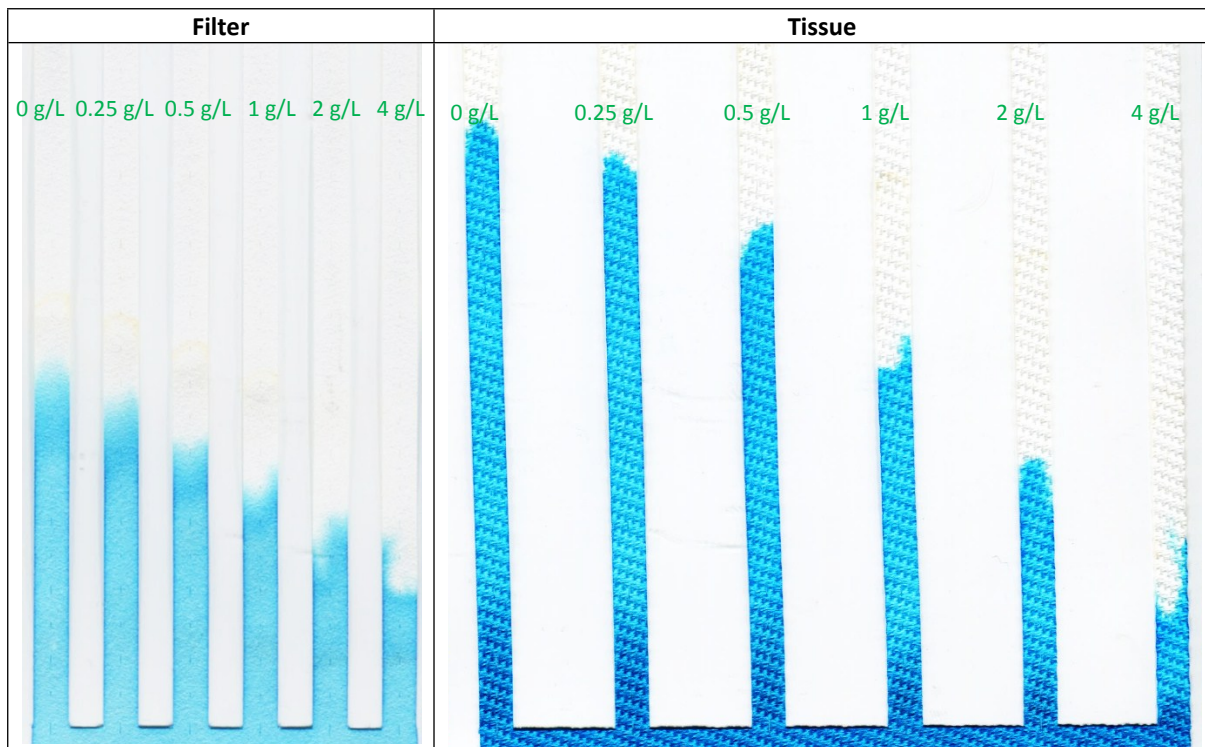


Figure S2: Elution of Filter (High Density) Paper vs Tissue (Low Density) Paper. Tissue Paper elutes a lot faster than Filter Paper. Therefore, it is far more sensitive at differentiating between different fibrinogen concentrations. Green numbers indicate fibrinogen solution added to each strip.

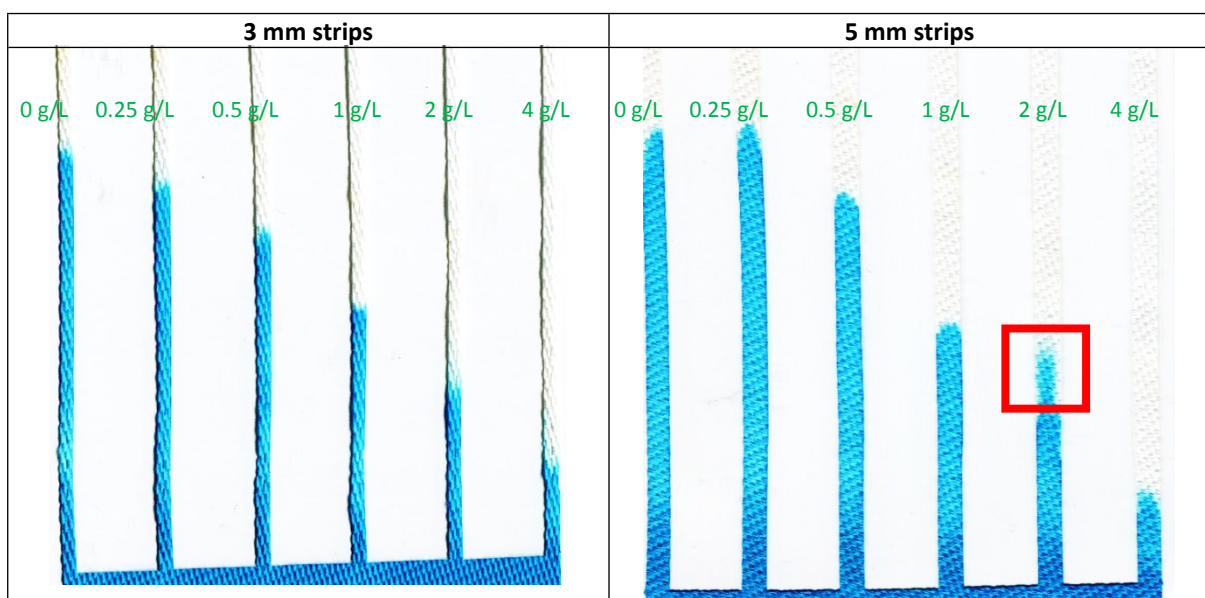


Figure S3: Elution of 3 mm strips vs 5 mm strips. 3 mm strips produces greater accuracy than 5 mm strips. This is due to the lower frequency of non-homogeneous elution. Non-homogenous elution (indicated by the red square) is attributed to the inconsistencies in paper pore structure and causes the aqueous dye to elute further than expected. Green numbers indicate fibrinogen solution added to each strip.

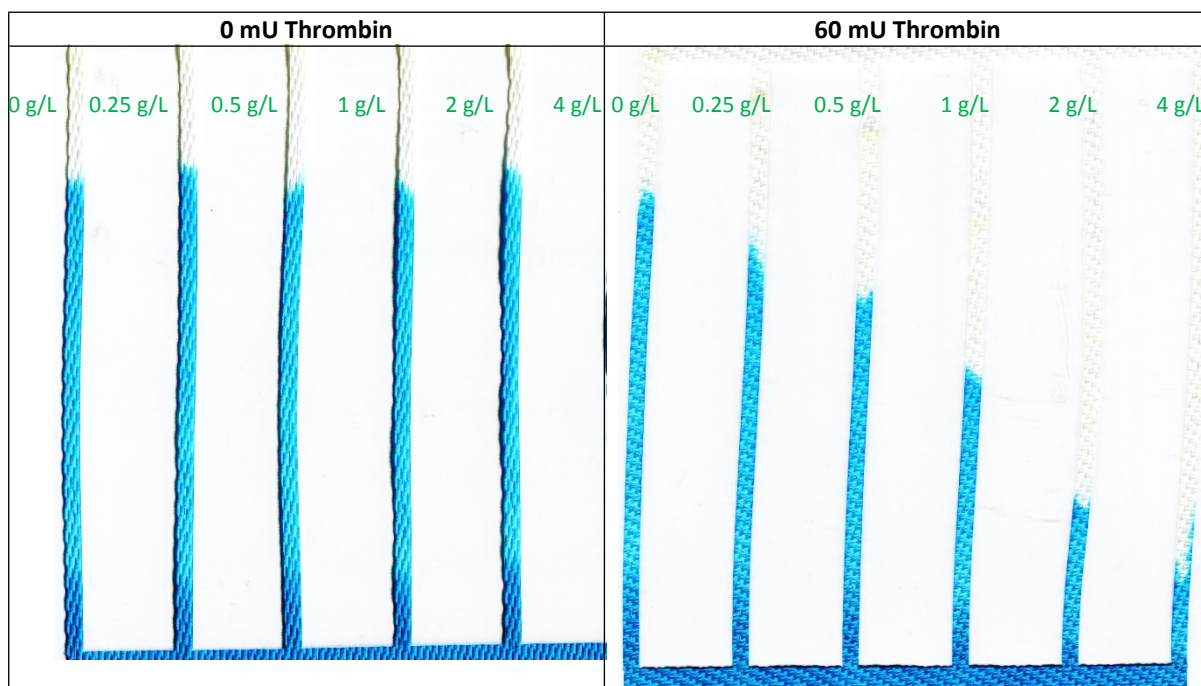


Figure S4: Elution of 0 mU Thrombin vs 60 mU Thrombin. Thrombin is essential for this test. 0 mU thrombin shows negligible elution height differences between different fibrinogen concentrations. 60 mU thrombin however strongly differentiates between different fibrinogen concentrations. Green numbers indicate fibrinogen solution added to each strip.

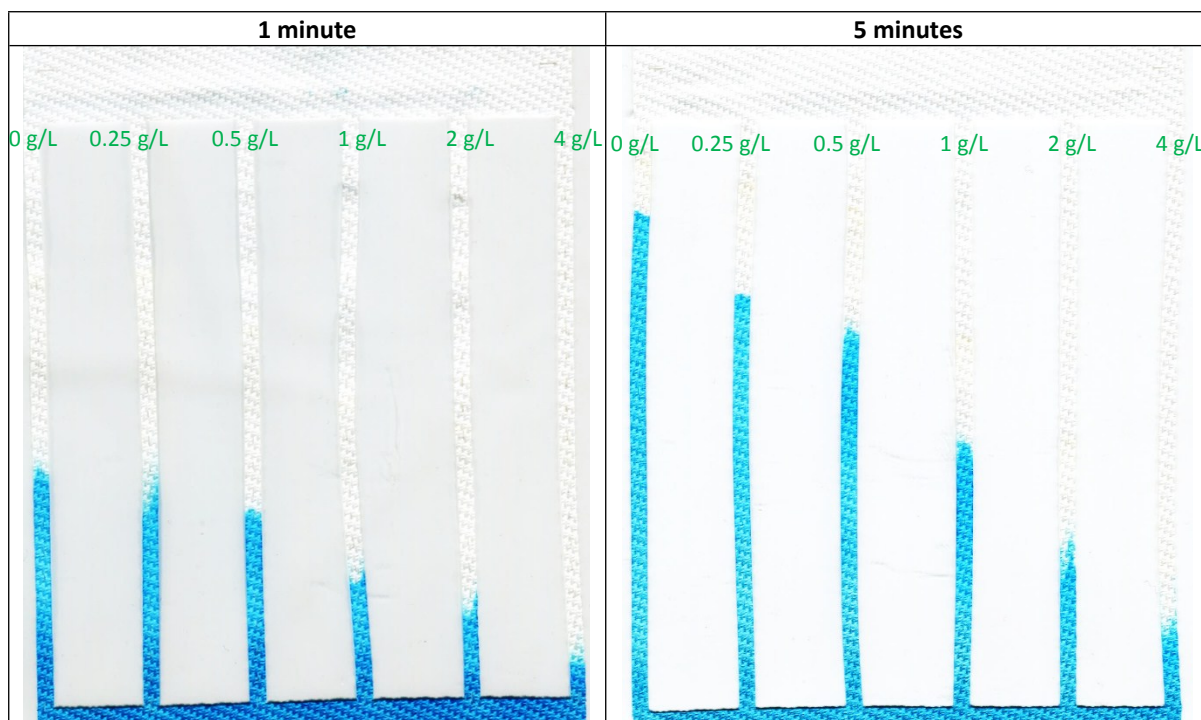


Figure S5: 1 minute elution time vs 5 minutes elution time. Elution Time plays a large impact on sensitivity. At 1 minute separation between different fibrinogen concentrations was poor. However, by 5 minutes, separation was clear between different fibrinogen concentrations. Green numbers indicate fibrinogen solution added to each strip.

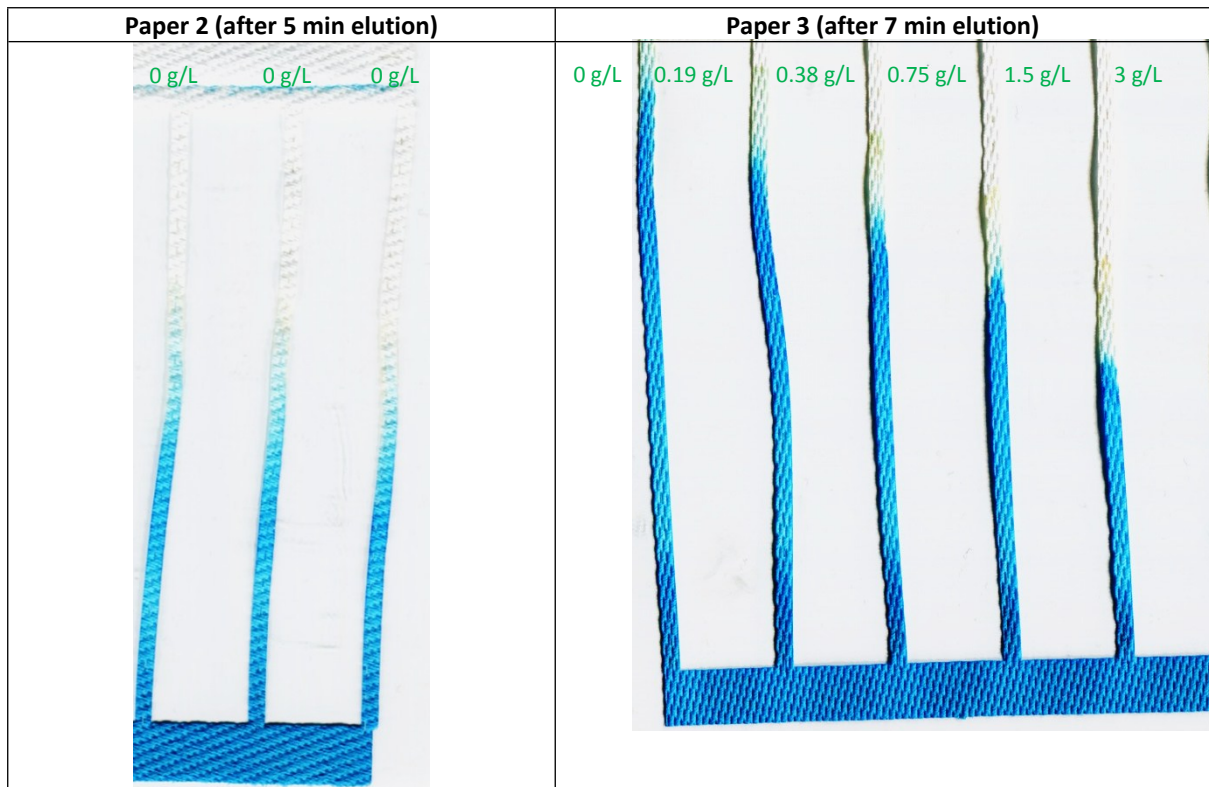


Figure S6: Paper 2 (0.16 mm thickness) vs Paper 3 (0.24 mm thickness). Paper Thickness impacts on the visibility of the elution front for Fibrinogen Solutions in 80 g/L BSA. Paper 2 produces an elution front gradient, making results difficult to read. Paper 3 however produces a much sharper elution front making results easier to read.

Effect of FXIIIa concentration:

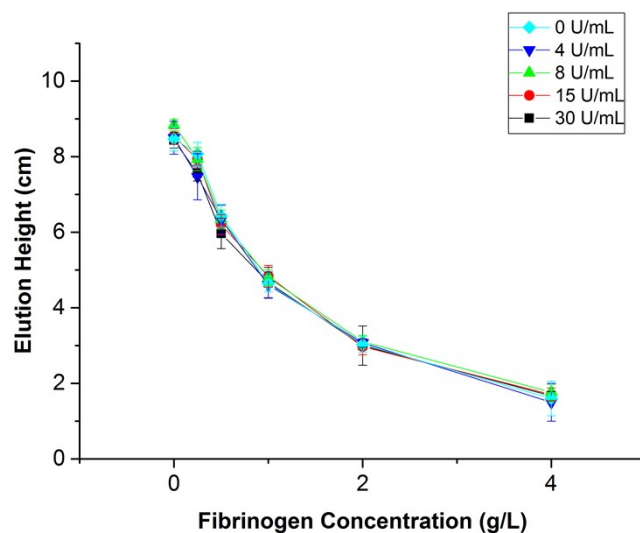


Figure S7: Effect of FXIIIa Concentration and Fibrinogen Concentration on Elution Height. Test conditions can be found in **Table SII** and **SVI**. Each test was performed in quadruplet and the average and standard deviations are reported.

The effect of Factor XIIIa concentration on elution height for different fibrinogen concentrations is investigated (**Figure S8**). Factor XIII has no effect on the sensitivity of the test.

Effect of Strip Width:

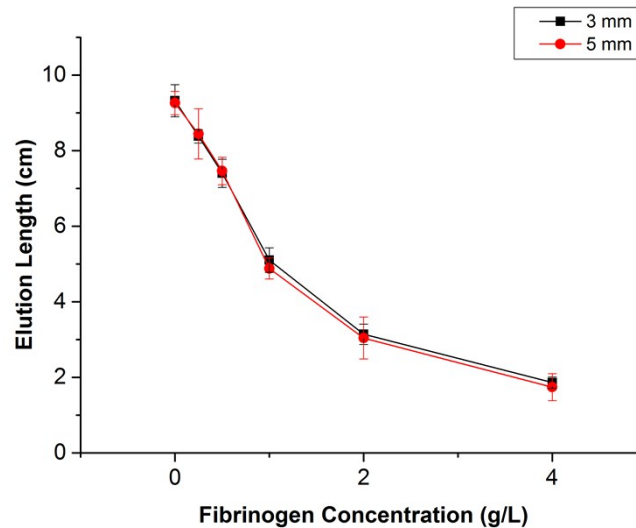
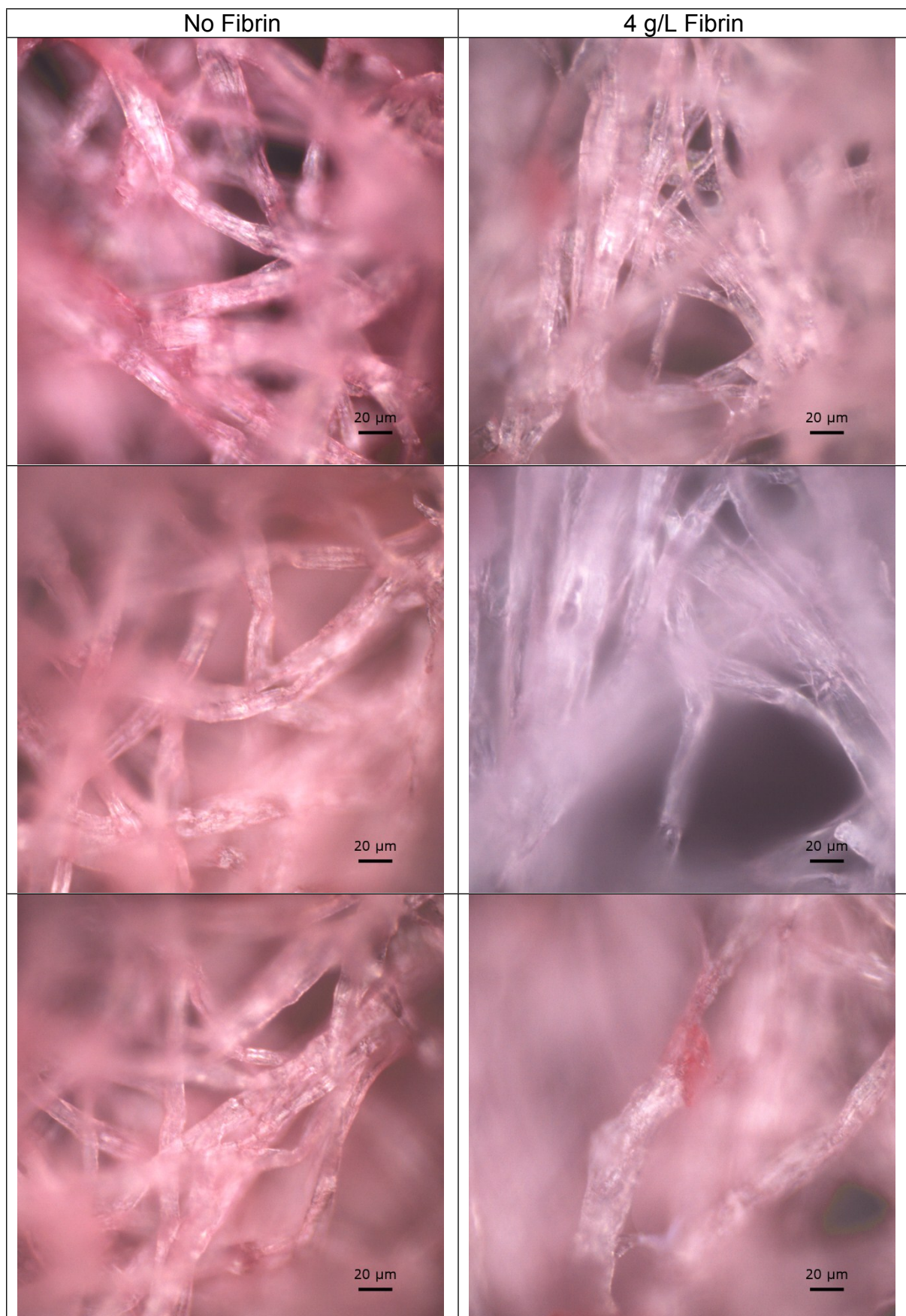


Figure S8: Effect of Strip Width and Fibrinogen Concentration on Elution Height. Test conditions can be found in **Table SII** and **SVI**. Each test was performed in quadruplet and the average and standard deviations are reported.

The effect of fibrinogen concentration on elution height for different strip widths is investigated (**Figure S8**). 2 mm strips were too fragile for proper diagnostic manipulation. 3 mm and 5 mm wide strips show no significant difference in elution sensitivity. However, the thinner strips show better reproducibility between different fibrinogen concentrations due to an elution front that is clearer and easier to read (see **Figure S3**). Therefore, 3 mm strips were selected for their more reliable performance.

Paper Pore Blockage Analysis:



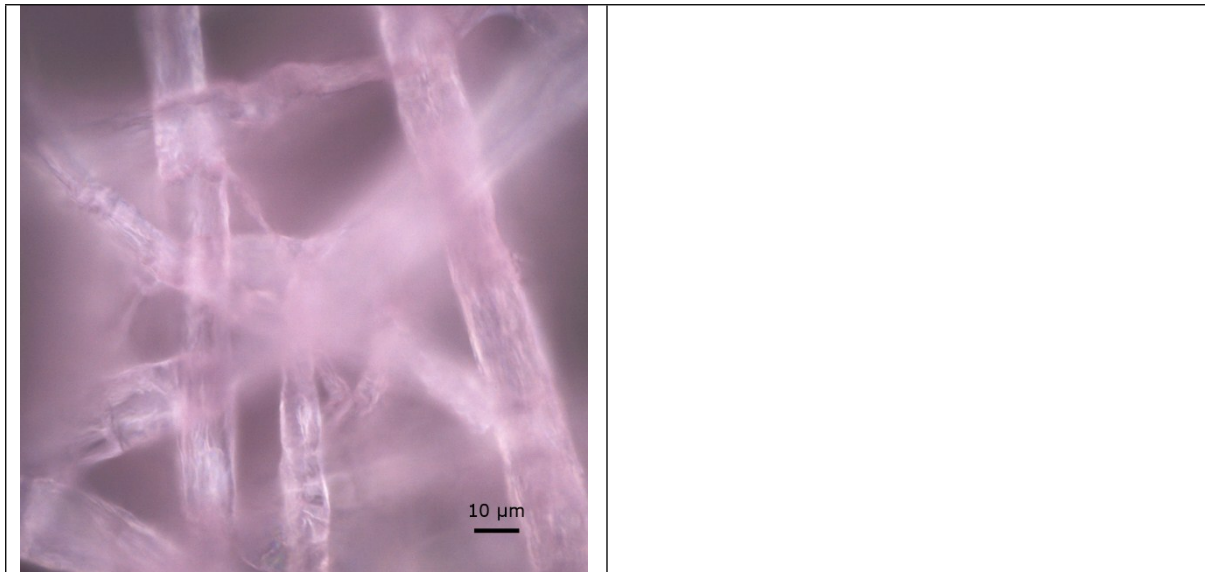


Figure S9: Images of Tissue Paper 3 coated with and without fibrin as captured under a laser microscope. Paper squares 1 X 1 cm² were cut on Epilog Laser Cutter. Thrombin vials were reconstituted with 0.3% Ponceau 4R (from Queens Red Food Dye) diluted in water. 2 μL of Thrombin was quickly mixed with 24 μL of Fibrinogen solutions (0 g/L and 4 g/L in PBS) in a PCR tube before being pipetted onto the paper. They were then imaged under a LEXT 3D Measuring Laser Microscope OLS5000 at 50X and 100X.

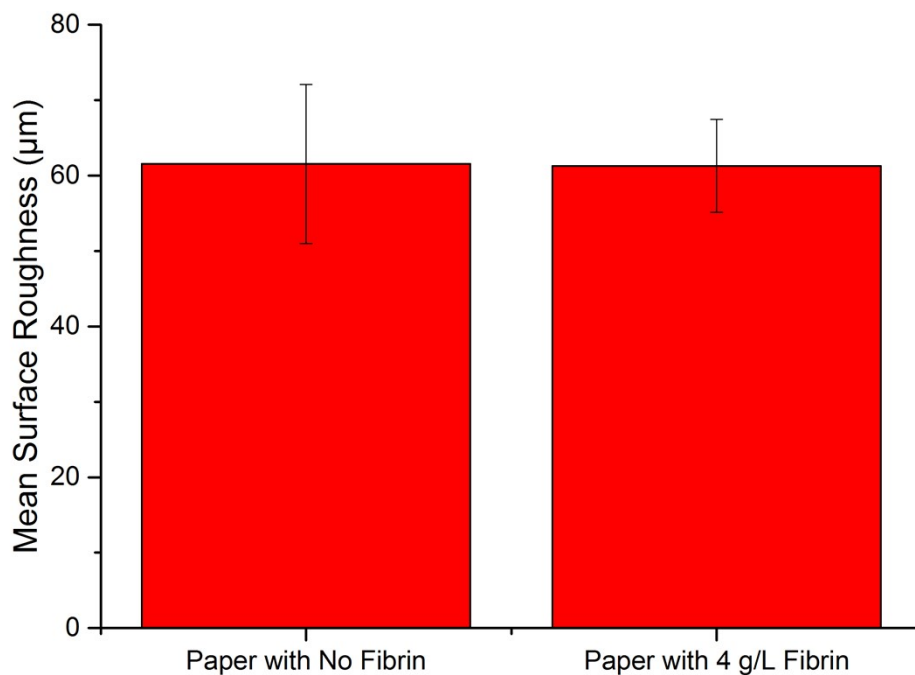


Figure S10: Mean surface roughness of Tissue Paper 3 coated with and without fibrin as measured under a profilometer. Thrombin vials were reconstituted with water. 2 μL of Thrombin was quickly mixed with 24 μL of Fibrinogen solutions (0 g/L and 4 g/L in PBS) in a PCR tube before being pipetted onto the paper. The mean surface roughness was measured using a LEXT 3D Measuring Laser Microscope in triplicate.

Figure S9 and **S10** verifies wherever the physical blockage of the tissue paper pores occurred due to fibrin formation. Laser microscope images are shown in **Figure S9**. 2 observations are noticed: 1) The paper pores are very large and can exceed 100 μm . 2) There is no observed decrease in pore size due to the formation of fibrin. Mean surface roughness measurements are also shown in **Figure S10**. If fibrin did fill the voids of the paper pores, then the paper surface would smoothen and the measured mean surface roughness would decrease. However, that was not the case as the paper surface roughness was similar regardless of the formation of fibrin. This means that the physical blockage of the pores is not a driving mechanism of the diagnostic.

Washburn Flow Kinetic Analysis:

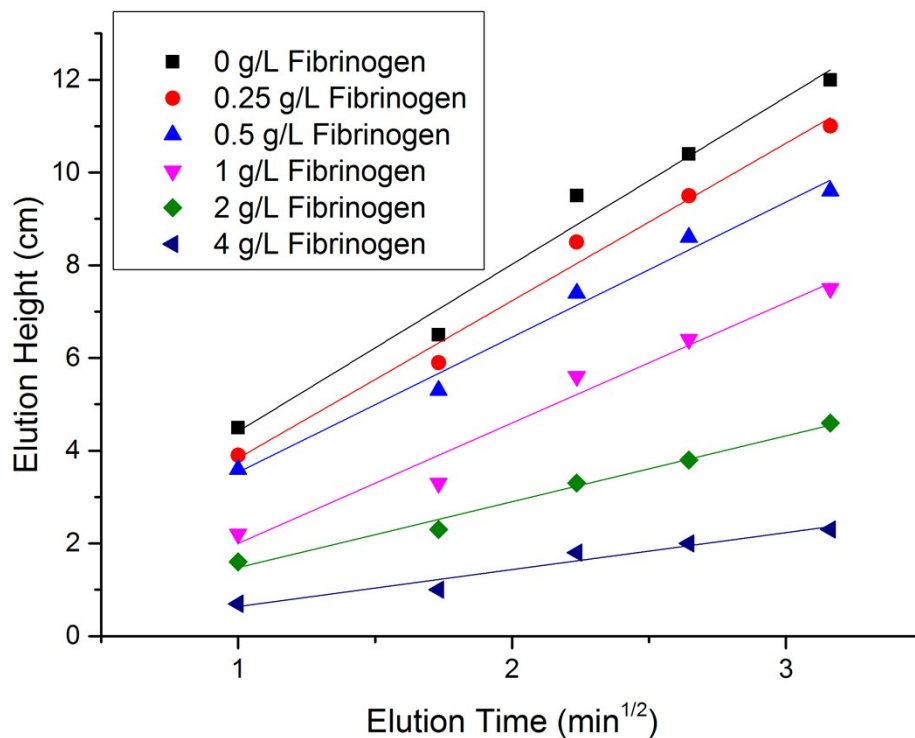


Figure S11: Washburn kinetics analysis derived from the data of **Figure 7b**. Each test was performed in quadruplet and the linear line of best fit through each set of data is given.

Fibrinogen Concentration (g/L)	Coefficient of Determination (R^2)
0	0.98
0.25	0.99
0.5	0.99
1	0.97
2	0.99
4	0.95

Table SX: Coefficient of Determination calculations corresponding to the linear lines of best fits in **Figure S11**.

Figure S11 and **Table SX** verifies wherever the polymerisation of fibrinogen in the test causes elution to deviate away from Washburn Kinetics. The Lucas-Washburn Equation is summarised below:

$$L = \sqrt{\frac{\gamma r t \cos \theta}{2\eta}}$$

Where L is the elution height γ is the apparent γ is the air-blue dye surface tension, r is the capillary radius, θ is the water-capillary surface contact angle t is the elution time and η is the blue dye viscosity.

Curves that follow Washburn Kinetics will show a linear trend when L is plotted against $t^{\frac{1}{2}}$.

In **Figure S11**, all fibrinogen concentrations give linear trend-lines. In **Table SX**, all trend-lines have an R^2 value above 0.95. This means that the formation of fibrin in the test does not cause elution to deviate away from Washburn kinetics.