Electronic Supplementary Material (ESI) for Analyst.

Paper microfluidics device based colorimetric sensor for the detection and discrimination of elapid versus viper envenomation

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Supplementary information

Table ST1

S. No.	Methodology of detection	Viper species	Range of detection	Detection time (in min)	References
1	Enzyme-linked Immunosorbent assay (ELISA)	Saw-scaled viper	5 ng ml^{-1}	240	[1]
2	Enzyme-linked Immunosorbent assay (Fluorescence-based)	Russell's viper	0.1 pg ml^{-1}	240	[2]
3	Enzyme-linked Immunosorbent assay	Russell's viper	2.4 ng ml^{-1}	300	[3]
4	Enzyme-linked Immunosorbent assay (ELISA)	Russell's viper Saw-scaled viper	0.1 ng ml^{-1}	25	[4]
5	Radioimmunoassay (RIA)	Russell's viper	0.1 ng ml^{-1}	1440	[5]
6	Radioimmunoassay (RIA)	Russell's viper	4 ng ml^{-1}	60	[6]
7	Antibody based LFA (Later flow assay)	Russell's viper	5 ng ml^{-1}	10	[7]
8	Immunochromatographic strip Gold nanoparticles – Antibody Later flow assay	Russell's viper	10 ng ml^{-1}	25	[8]
9	**Paper microfluidics immobilised with gelatin nanoparticles**	**All viper species**	**3.125 ng for RVV 6.25 ng for SSV**	**25**	**This work**

Table ST1: Detection of viper's venom using various technique (in the presence of buffer and spiked sera

LOD and LOQ calculation

LOD is the point at which the corresponding lower concentration absorbance is more that can be reliably detected

For Buffer Mean of control + 3 X S.D. of control

Mean of control: 0.045

S.D of control: 0.001

LOD: 0.045 + 3 X 0.001

LOD: 0.0480

For Serum Mean of control + 3 X S.D. of control

Mean of control: 0.057

S.D of control: 0.00529

LOD: 0.057 + 3 X 0.00529

LOD: 0.0728

Hence, the lowest amount of venom, whose absorbance is more than 0.0728 can be considered as LOD

LOQ is point of concentration that can be measured with a defined precision and accuracy

For Buffer Mean of control + 10 X S.D. of control

Mean of control: 0.045

S.D of control: 0.001

LOD: 0.045 + 10 X 0.001

LOD: 0.055

For Serum Mean of control + 10 X S.D. of control

Mean of control: 0.057

S.D of control: 0.00529

LOD: 0.057 + 10 X 0.00529

LOD: 0.1099

Hence, the lowest amount of venom, whose absorbance is more than 0.1099 can be considered as LOQ

Table ST2

Table ST2. Spiked recovery test for detection of protease activity of viper venom in human serum (1 to 6 for Russell's viper and 7 to 13 for Saw-scaled viper)

Si.no	Amount of venom (ng)	Recovery (%)	Average Recovery (%)
	Russell's viper		
1	200	86	
2	100	87	
3	50	80	**89.4**
4	25	76	
5	12.5	83	
6	6.25	122	
	Saw-scaled viper		
7	200	92	
8	100	81	**89.5**
9	50	90	
10	25	89	
11	12.5	103	
12	6.25	76	
13	3.125	91	

Figure S1

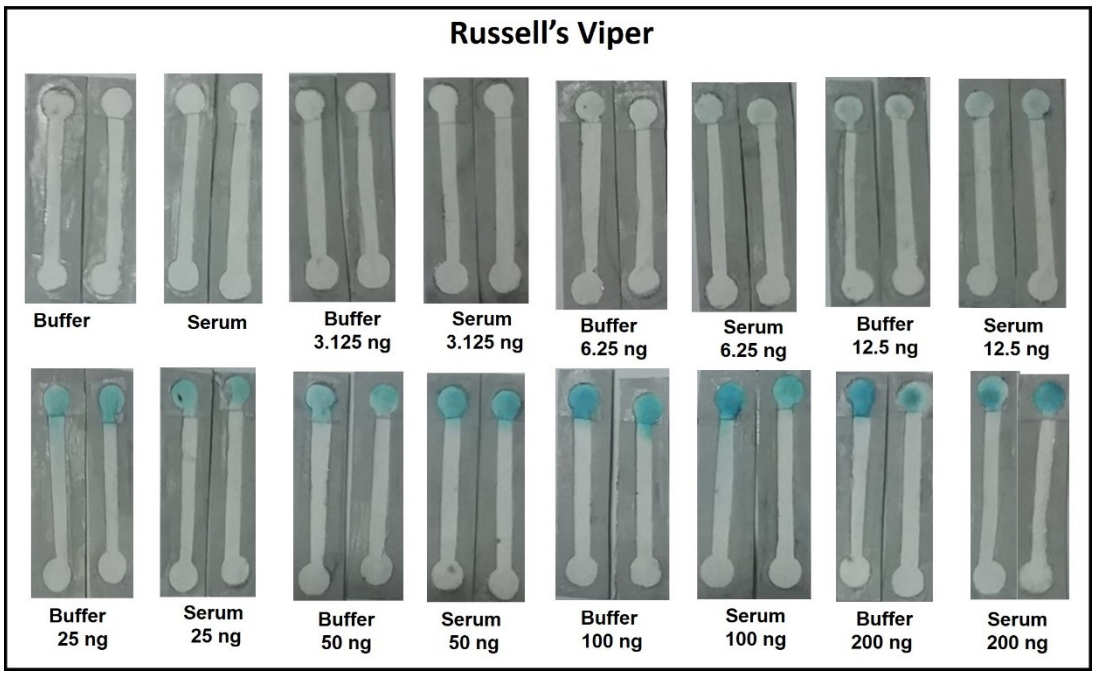

Figure S1: Detection of Russell's viper venom using paper microfluidics incubated with various amount of venom (3.125, 6.25, 12.5, 25, 50, 100, 200 ng) in the presence of buffer and spiked sera

Note: First two images labelled Buffer and Serum refers to control buffer and unspiked serum respectively.

Figure S2

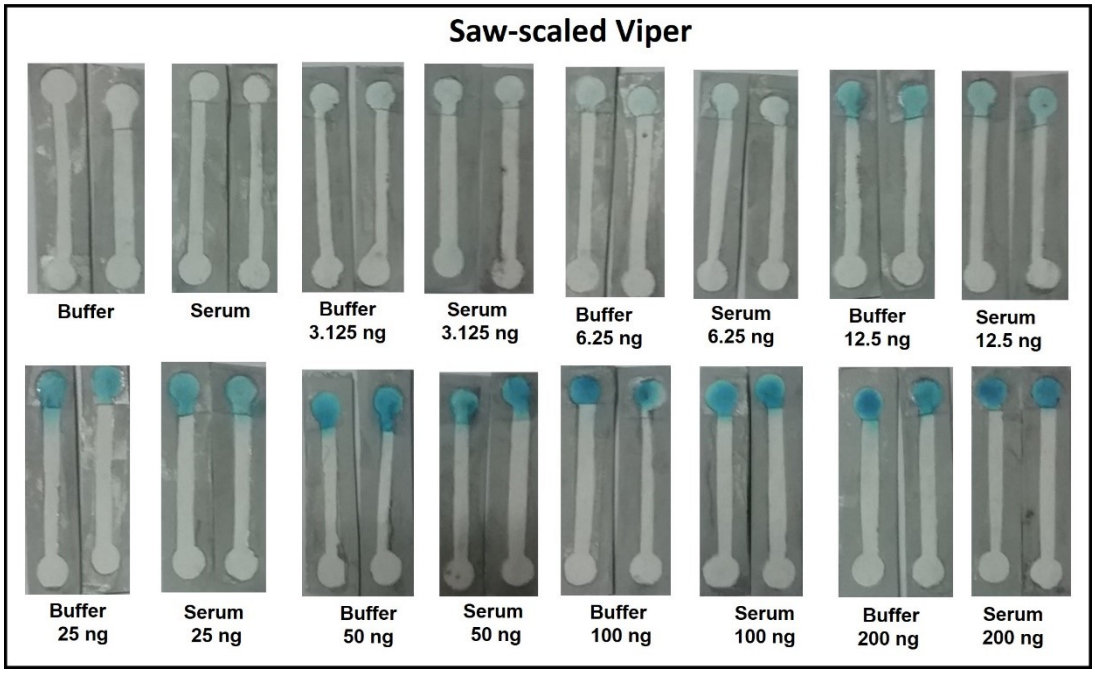

Figure S2: Detection of Saw-scaled viper venom using paper microfluidics incubated with various amount of venom (3.125, 6.25, 12.5, 25, 50, 100, 200 ng) in the presence of buffer and spiked sera
Note: First two images labelled Buffer and Serum refers to control buffer and unspiked serum respectively.

Figure S3

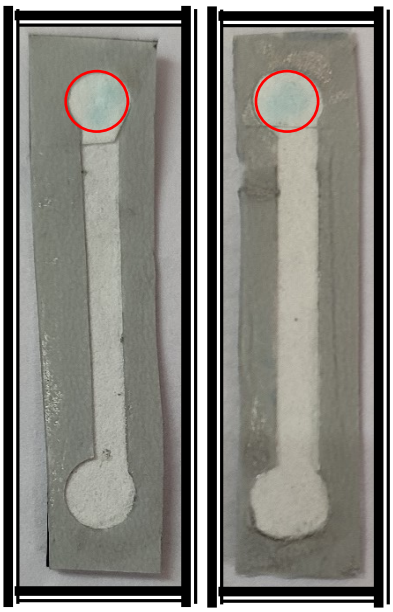

Figure S3: Low-end detection limit of gelatinase activity determined using paper microfluidics (red circles illustrate colour change (blue) at detection zone). First panel represent sensor response in buffer and second one in venom spiked human serum.

Figure S4

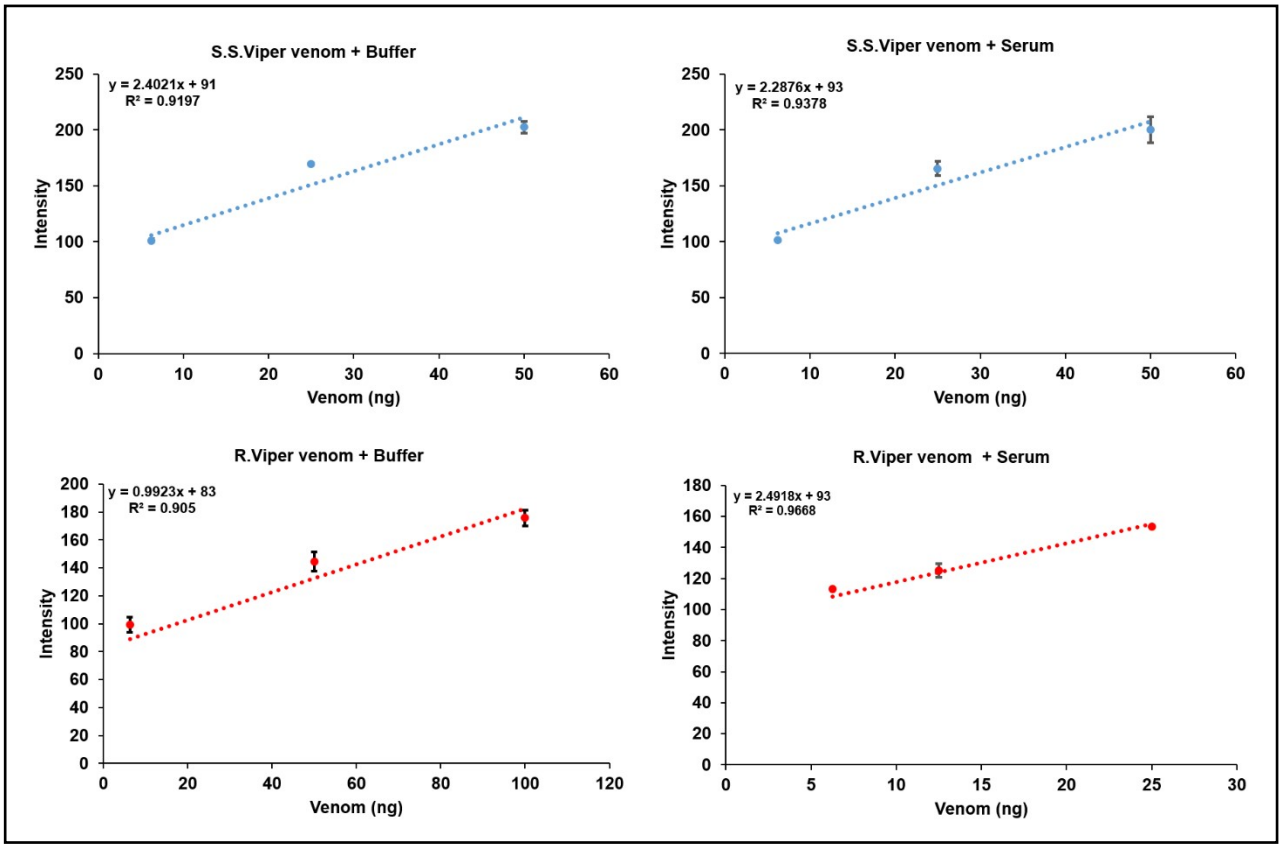

Figure S5: Linear range plot of colour intensity in paper microfluidics measured using ImageJ program with different amount of viper venom (Russell's viper, Saw-scaled viper) in buffer and spiked serum

Figure S5

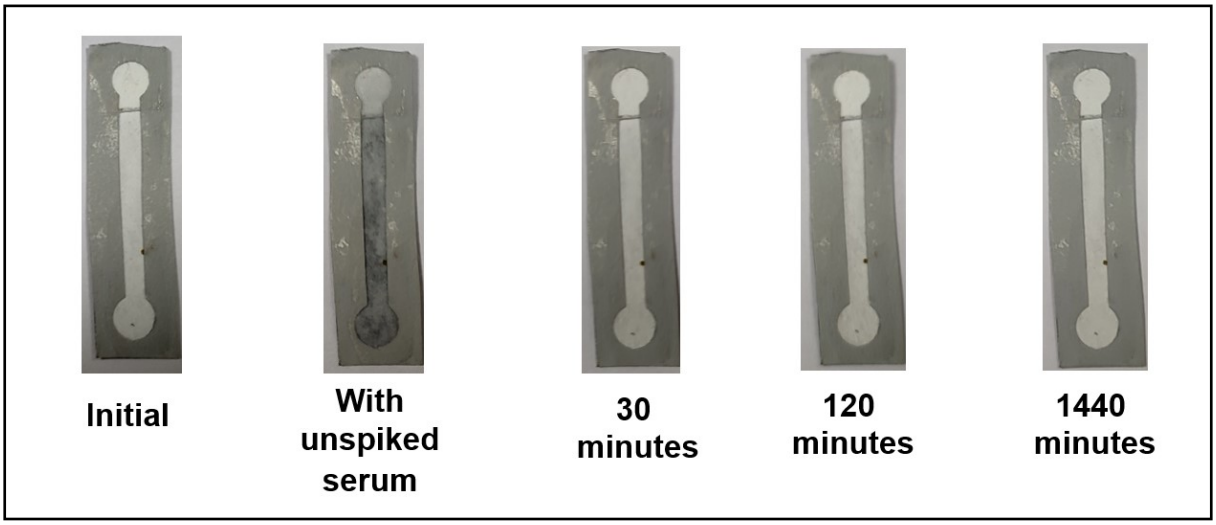

Figure S4: Stability of paper microfluidics incubated with the presence of unspiked serum (no venom added) at various time points

Figure S6

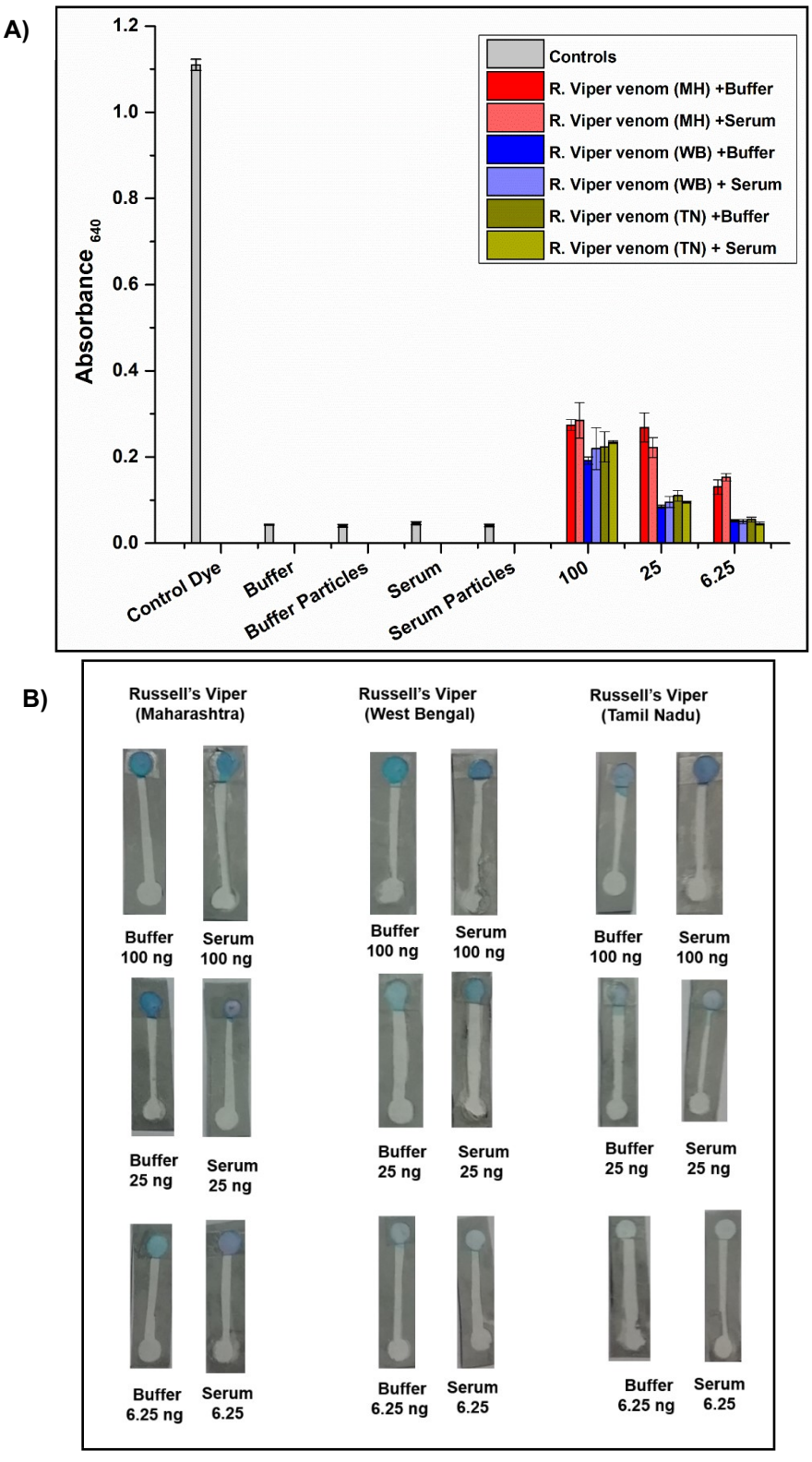

Figure S6: Detection of different Russell's viper venom from geographically distinct regions, based on dye release from GMG nanoparticles due to gelatinase activity of proteases **A)** ELISA **B)** paper microfluidics

Figure S7

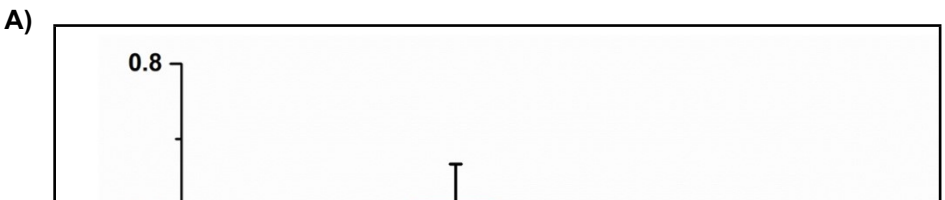

Figure S7: Detection of stability of GMG nanoparticles in presence of enzymes and amino acids mainly urease, human phospholipase A2, malate synthase and casaminoacids both quantitatively ((A) ELISA) and qualitatively ((B) paper microfluidics)

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