Supporting Information

Paper Microfluidics Based Fluorescent Lateral Flow Immunoassay for Point-of-care Diagnostics of Non-Communicable Diseases

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Figure S1: The concentration-time curves of cTnI sample at 1000 ng/ml. V_R of the Test and control line were measured by each minute for 25 min after the addition of the sample (50 µl).



Figure S2. The graph depicts the Error percentage for the cTnI plotted against the Error percent in the depicted cTnI.



The error percentage of the assay. Both the blank solutions and spiked sample containing the different concentration of the analyte were prepared for the by lateral flow immunoassay. The acceptable level of accuracy of the calibration (CoV) is 6% for the Troponin I. Percentage of readings predicted within acceptable % error limits 68%. The maximum limits of the error observed -7 % to +7 %. The average error observed 0.6 ± 5.56 % as shown in the (Fig S2 and Table S3)

Figure S3. The graph shows the Background value vs. the cTnI value from the Immunoanalyser



Background characterisation. For the background characterisation, the triangular section beneath the straight line fit in between the two end points of the peak is considered. The Background Value BG1 is the highest values of the P1 (Peak) is 352 and the Background Value BG2 is the highest values of the P2 (Peak) is 327. Both the background values come under the lower limit of the BG value. These results showed the cTnI developed has the less background which simultaneously has the high peak value as shown in the (**Fig S3 and Table S4**)

Figure S4. The graph depicts the peak amplitude of the two lines vs. the cTn I concerntration



Peak characterisation. The signal captured by from each assay kit mainly composed of two strong peaks correlated with the assay reaction occurred at the test and control lines. The peak value for the Test line increases from 925 to 4125. The maximum peak value of 4200 is achieved for the 1000 ng/ml lead to saturation of the Troponin I value. The peak value for the Control line starts from 1006 and reached the 1259. The graph shows the linearity in the peak value for the Test line as shown in the (**Fig S4 and Table S5**)

Figure S5. The graph depicts the values of the Relative peak of two lines vs. the cTnI concerntrations.



Relative peak characterisation. The value indicates the peak value for the Test and control line with regard to the background of their respective lines. The test line minimum value is 1.9717 and the control line minimum value is 2.26. This result shows the both the T and C line relative peak value are more related with less background as shown in the (**Fig S5 and Table S6**)

Table S1: Calibration curve data

Ratio of Calculations		Value Ratio
Calibration curve type		
Calibration Constants	C0	0
	C1	0.0342
	C2	2.1181
R value		0.9618
Ratio Value	Ratio_Mini	0.9
	Ratio_Max	5.43
Minimum Value of Test Parameter	Rmin	0.1
Maximum Value of Test Parameter	Rmax	1000

Table S2: Co-efficient of variation data

Parameters	Range 4 to 16 % cTnI
Acceptable CoV (%) (from standards)	6%
Percentage of readings predicted within acceptable CoV error limits	88%
Max CoV (%) observed	11.18%
Average CoV (%) observed	3.3 +/- 2.12 %

Table S3: Error percentage data value

Parameters	Range 4 to 16 % cTnI
Acceptable CoV (%) (from standards)	6%
Percentage of readings predicted within acceptable CoV error limits	68%
Max error (%) observed	-7 % to +7 %
Average error (%) observed	-7 % to +7 %

 Table S4: Background value

BG Level	BG1_max	327	Max value of Background acceptable in a valid test
	BG2_max	352	Max value of Background acceptable in a valid test

Table S5: Peak amplitude values

Parameter		Value
Min amplitude of control line	C min	1006
Max amplitude of control line	C max	1259
Min amplitude of test line1	T1min	945
Max amplitude of test line1	T1max	4125

Table S6: Relative background value

Parameter	Value
T1_rel_min	0.896
C1_rel_min	4.273
T1_rel_th	0.72
C1_rel_th	3.4