www.sciencetranslationalmedicine.org/cgi/content/full/4/152/152ra129/DC1

Supplementary Materials for

A Paper-Based Multiplexed Transaminase Test for Low-Cost, Point-of-Care Liver Function Testing

Nira R. Pollock,* Jason P. Rolland,* Shailendra Kumar, Patrick D. Beattie, Sidhartha Jain, Farzad Noubary, Vicki L. Wong, Rebecca A. Pohlmann, Una S. Ryan, George M. Whitesides

*To whom correspondence should be addressed. E-mail: jrolland@dfa.org (J.P.R.); npollock@bidmc.harvard.edu (N.R.P.)

Published 19 September 2012, *Sci. Transl. Med.* **4**, 152ra129 (2012)
DOI: 10.1126/scitranslmed.3003981

The PDF file includes:

Supplementary Methods:

Reagents for ALT assay

Alanine solution: A solution containing 1 M L-alanine (Sigma Aldrich), 30 mM α–ketoglutaric acid (Sigma Aldrich), 2 mM KH_2PO_4 (Sigma Aldrich), 20 mM $MgCl_2$ (Sigma Aldrich), 2 mM thiamine pyrophosphate (MP Biosciences), 2 mM of 4-aminoantipyrine (Sigma Aldrich), and 25 U/ml (0.1 mg/ml) horseradish peroxidase (HRP) (Sigma Aldrich) was prepared in 200 mM Tris buffer (pH 7.4).

Dimethylaminobenzoic acid (DABA) solution: A solution containing 10% (w/v) Poly(ethylene glycol) (PEG) (MW = 35,000 g/mol, Sigma Aldrich) and 10 mM dimethylaminobenzoic acid was prepared in DI water.

Pyruvate oxidase: A solution containing 100 U/ml of Pyruvate Oxidase (MP Biosciences, EMD) was prepared in 200 mM Tris buffer, pH 7.4.

PEG solution: A solution containing 5% (w/v) PEG (MW = 35,000 g/mol, Sigma Aldrich) was prepared in DI water.

Reagents for AST assay

AST dye solution: A solution containing 0.5% (w/v) Methyl Green, 0.05% (w/v) Rhodamine B, 0.025% (w/v) Triton X 100, and 1% (w/v) poly(vinyl alcohol) (13,000-23,000 g/mol) was prepared in DI water.

Cysteine sulfonic acid (CSA) solution: A solution containing 171.1 mg CSA (Sigma Aldrich), 14.6 mg α–ketoglutaric acid, and 10 µL of 200 mM EDTA solution was prepared in 1 ml of 40 mM phosphate buffer, and the pH was adjusted to 8.0.

AST positive control solution: 5 % (w/v) PEG (MW = 35,000 g/mol, Sigma Aldrich) and 6.17 µl AST (5177 U/ml, MP Biosciences) were added to 1X PBS to make 200 kU/l AST solution.

Artificial blood plasma buffer

A solution containing 84% (w/v) NaCl, 4% (w/v) $NaHCO_3$, 2% (w/v) KCl, 2% (w/v) $Na_2HPO_4 \cdot 3H_2O$, 3% (w/v) $MgCl_2 \cdot 6H_2O$, 3% (w/v) $CaCl_2$, and 1% (w/v) Na_2SO_4 was prepared in DI water and the pH was adjusted to 7.4.

Device performance

Linearity. Measured amounts of AST and ALT (Lee Bio) were added to fresh whole blood (drawn by venipuncture, baseline AST/ALT 24/22 U/l) to generate final concentrations of 40, 60, 80, 100, 120, 150, 180, 200, 250, 300, and 400 U/l. Thirty µl of sample were added to each of 3 devices for all concentrations. After 15 minutes, the devices were scanned using a desktop scanner (Canon). Experiments were performed at room temperature (25°C). Images were analyzed using ImageJ software (NIH) to obtain color intensity values for each zone. ALT data were quantified using the green channel and final values were normalized by subtracting from 255 (255 is the maximum color intensity value in the RGB color model and this normalization allows for a positive correlation between ALT values and color intensity in the green channel). AST data were quantified using the red channel without normalization, and 95% prediction intervals were calculated using an excel macro, PredInt.xls, version 8.3.

Limit of detection. Measured amounts of AST and ALT (Lee Bio) were added to artificial blood plasma buffer to generate final concentrations of 0.1, 1, 5, 10, 20, 40, 80, 120, 160, 200, 400, 1000, and 10,000 U/l. Thirty µl of sample were added to each of 7 devices for all concentrations. After 15 minutes, the devices were scanned using a desktop scanner (Canon). Experiments were performed at room temperature (25°C). Images were analyzed using ImageJ software (NIH) to obtain color intensity values for each zone. ALT data were quantified using the green channel and final values were normalized by subtracting from 255. AST data were quantified using the red channel without normalization. LOD curves were generated for the AST and ALT assays and fit to the Hill equation by nonlinear regression (*14*).

Repeatability. Commercial serum standards containing known (as measured by the vendor) AST/ALT values were purchased from Pointe Scientific. Two levels were used for these studies: Level I standards contained 69/56 U/l of AST/ALT, respectively, and Level II standards contained 244/128 U/l of AST/ALT, respectively. Whole blood samples were also prepared by adding known amounts of AST and ALT (Lee Bio) to fresh whole blood (drawn by venipuncture, baseline AST/ALT 24/22 U/l) to generate final concentrations of 40 U/l (Level I) and 200 U/l (Level II). A total of four samples were prepared for testing (Level I/II serum samples and Level I/II whole blood samples). Thirty µl of sample were added to each of 10 devices for all concentrations. Experiments were performed at room temperature (25°C). After 15 minutes, the devices were scanned using a desktop scanner (Canon). Images were analyzed using ImageJ software (NIH) to obtain color intensity values for each zone. ALT data were quantified using the green channel and final values were normalized by subtracting from 255. AST data were quantified using the red channel without normalization.

Stability. Approximately 100 devices were fabricated and stored in foil-lined pouches (Plastic Bags For You), 10 tests to a pouch, each containing one packet of silica desiccant (Sigma). The pouches were sealed using a Hualian heat sealer. The pouches were stored at 25°C and were tested at 0, 4, 8, 14, 21, 33, 42, 63, and 77 days using buffer standards prepared in artificial blood plasma buffer. Buffer standards were generated by adding measured amounts of AST and ALT (Lee Bio) to artificial blood plasma buffer to generate final concentrations of 40 and 200 U/l. Thirty µl of sample were added to each device for all concentrations. After 15 minutes, the devices were scanned using a desktop scanner (Canon). Experiments were performed at room temperature (25°C). Images were analyzed using ImageJ software (NIH) to obtain color intensity values for each zone. ALT data were quantified using the green channel and final values were normalized by subtracting from 255. AST data were quantified using the red channel without normalization.

Bland–Altman analysis

Bland–Altman analysis was conducted for values ranging from 0–275 U/l for each data set (ALT whole blood, ALT serum, AST whole blood, AST serum). Values greater than 275 U/l were removed from this analysis because the test signal saturates above this threshold. Such values resulted in artificially high 95% limits of agreement. Furthermore, the clinical action is the same for all values in this bin. For example, a value of 2000 U/l (as measured by the Roche Analyzer) would saturate the paper test signal and yield a visual value of 400 U/l. The D value (Equation 1) for this data point would be –1600 U/l despite correctly identifying the sample in the >5X ULN bin.

Bland–Altman analysis was conducted by calculating the following:

$$D_i = (P_i – R_i) \quad \text{(Equation 1)}$$

where D_i is the difference between a value measured by the Paper transaminase test (P_i) and the Gold–Standard Roche Instrument (R_i). A scatter plot was generated using Microsoft Excel consisting of (M_i, D_i) for each data point in the series, where M_i is the average of P_i and R_i. The mean difference (U) was calculated:

$$U = \frac{1}{n}\sum_{i=1}^{n} D_i \quad \text{(Equation 2)}$$

where U is the average difference, D_i is the difference between the methods for a given point, and n is the number of samples. U was then plotted as a straight line on the scatter plot as the equation $Y=U$. The standard deviation (S_d) of D_i was calculated:

$$S_d = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(D_i - U)^2} \quad \text{(Equation 3)}$$

Ninety-five percent limits of agreement were calculated according to the equations, where L_1 is the upper limit of agreement, where L_2 is the lower limit of agreement, U is the mean difference, and S_d is the standard deviation of D_i:

$$L_1 = U + (1.96)S_d \quad \text{(Equation 4)}$$

$$L_2 = U – (1.96)S_d \quad \text{(Equation 5)}$$

L_1 and L_2 were then plotted as straight lines on the scatter plot as the equations $Y=L_1$ and $Y=L_2$.The line of equality was plotted as straight line according to the equation (Y=0).

Log transformation was applied according to the methods described by Bland and Altman (*25*). Briefly, y-axis values were obtained by taking the natural logarithm (ln) of the paper test and Roche values and subtracting ln Roche from ln Paper test. X-axis values were obtained by averaging ln paper test and ln Roche values. Ninety-five percent limits of agreement were

calculated as described above only using the log-transformed values. Limits of agreement were then back transformed with an antilog function (e^x).

Supplementary Figures:

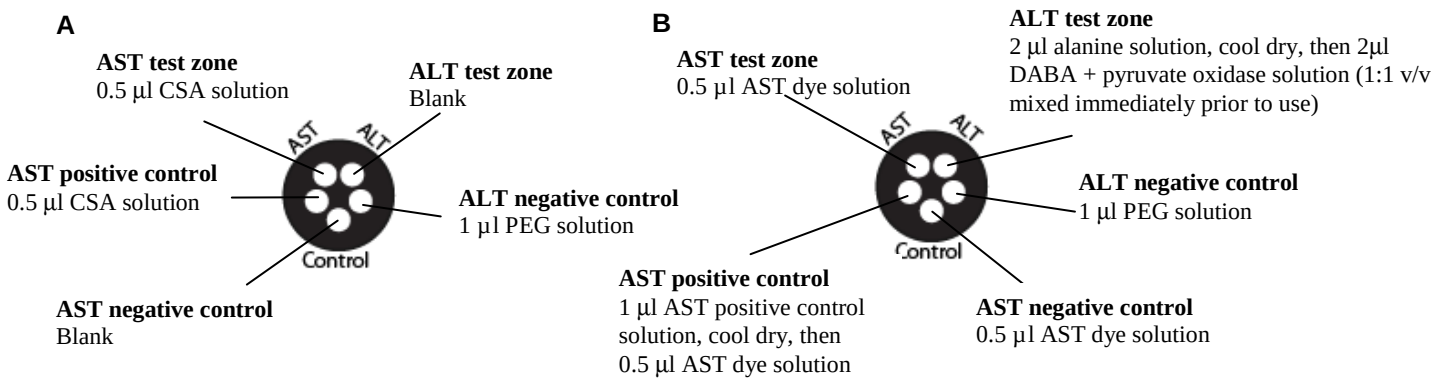
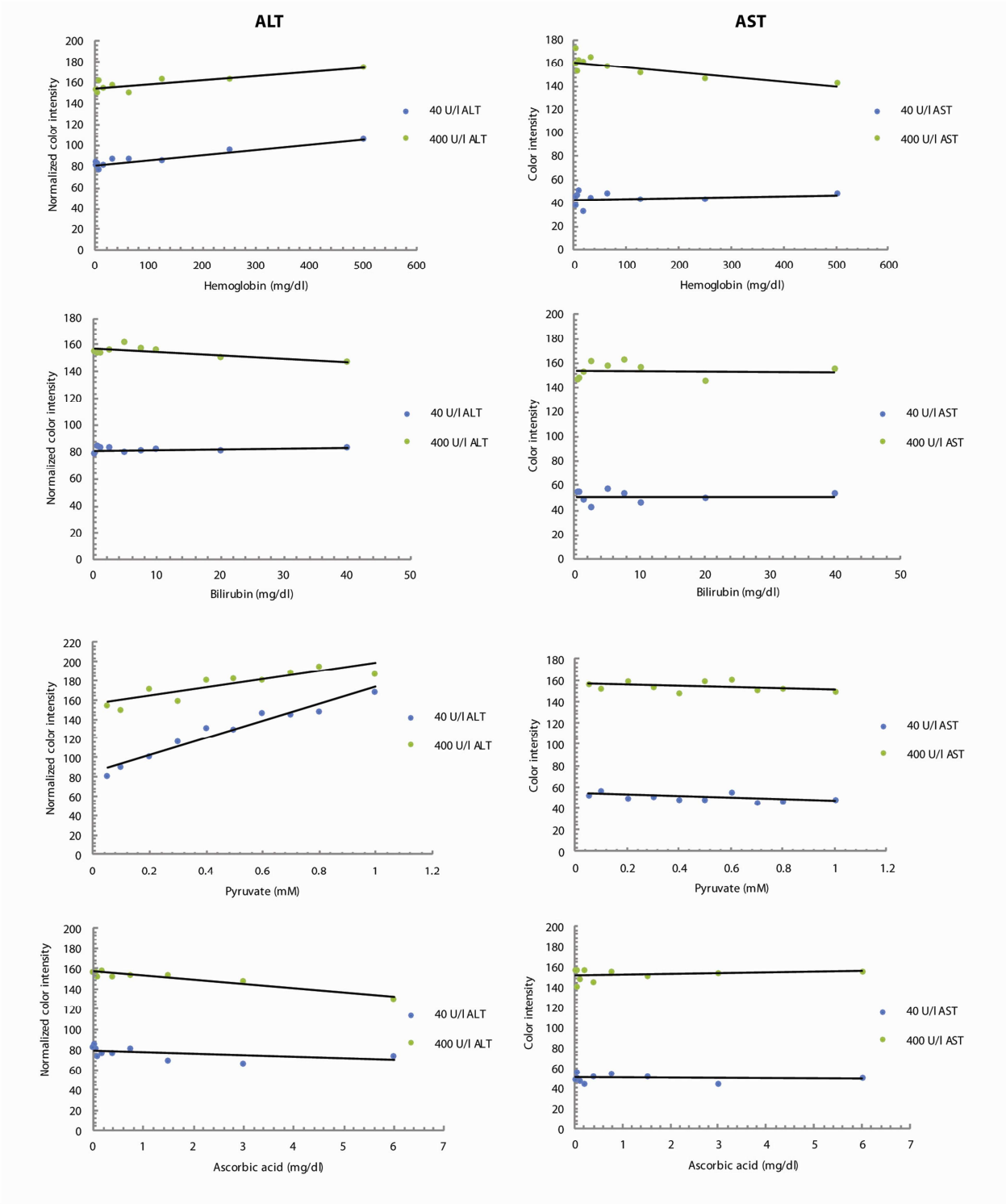


Figure S1. Locations of spotted reagent solutions for ALT and AST assays. (**A** and **B**) Two layers of patterned paper are used in the paper-based transaminase test, one in which the back side is directly in contact with the filter membrane (A), and one from which the results are read (B).


ALT
AST
Normalized color intensity
Color intensity
Hemoglobin (mg/dl)
Bilirubin (mg/dl)
Pyruvate (mM)
Ascorbic acid (mg/dl)
40 U/l ALT
400 U/l ALT
40 U/l AST
400 U/l AST

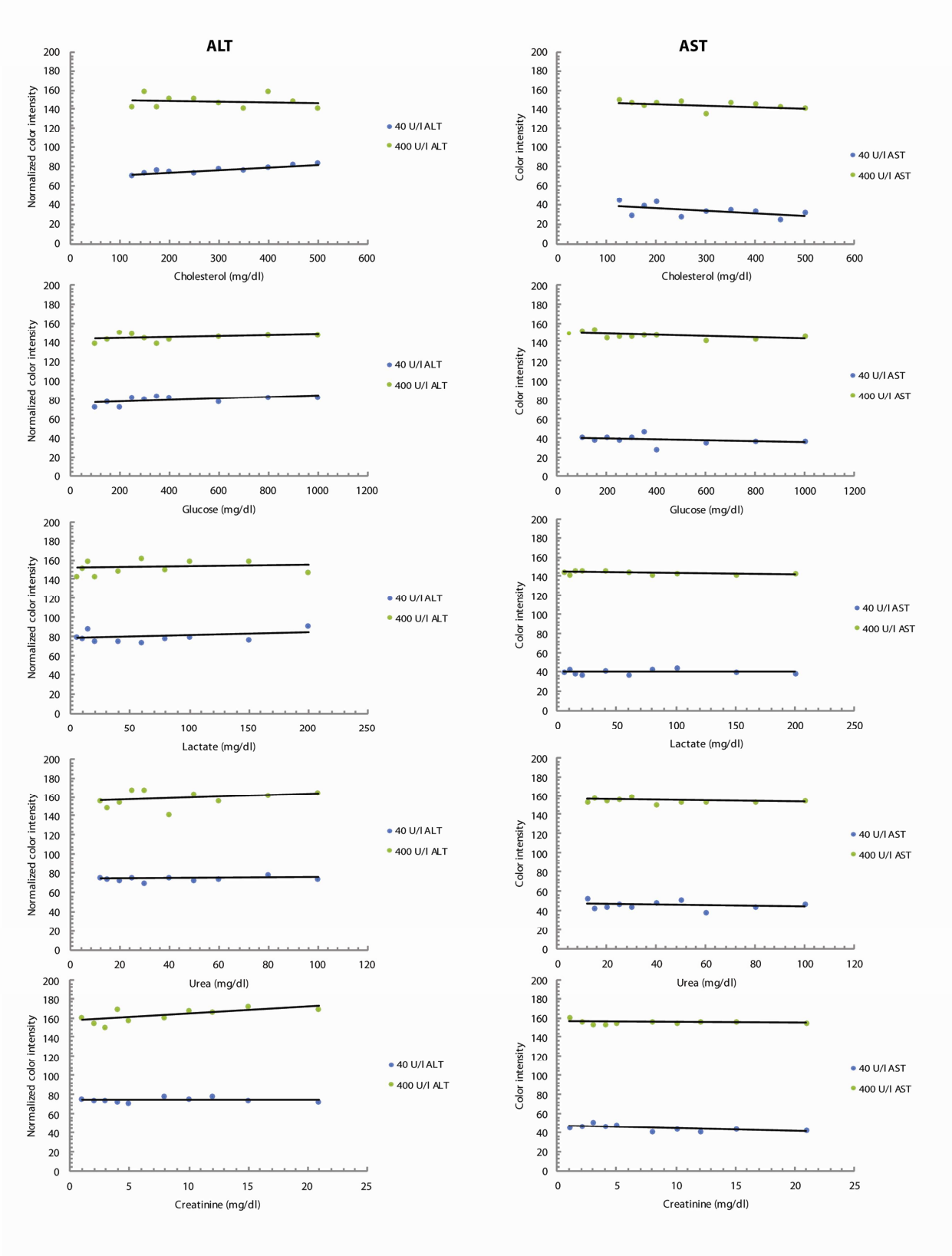

ALT
AST
Normalized color intensity
Color intensity
Cholesterol (mg/dl)
Glucose (mg/dl)
Lactate (mg/dl)
Urea (mg/dl)
Creatinine (mg/dl)
40 U/l ALT
400 U/l ALT
40 U/l AST
400 U/l AST

Fig. S2. Evaluation of potential interfering factors. Color intensity was measured for two enzyme levels, 40 U/l and 400 U/l across a range of interfering agent concentrations with the both. ALT values are normalized by subtracting each point from 255 so that higher color intensity values correspond to higher ALT concentrations. Data are shown for various agents at physiologically relevant concentrations: hemoglobin (0-500 mg/dl)**,** bilirubin (0-40 mg/dl), pyruvate (0.05-1 mM), ascorbic acid (0-6 mg/dl), cholesterol (125-500 mg/dl), glucose (100-1000 mg/dl), lactate (5-200 mg/dl), urea (12-100 mg/dl), creatinine (1-21 mg/dl). Data are N = 1 for each assay condition.

.

23 C		10 min		12 min		15 min		17 min		20 min	
AST	ALT	AST	ALT	AST	ALT	AST	ALT	AST	ALT	AST	ALT
24	22	40	40	40	40	40	40	40	40	80	40
104	70	40	40	40	40	60	40	80	40	80	40
127	118	80	40	80	60	90	60	110	60	120	100
241	221	80	100	100	120	100	120	120	120	150	150
400	400	100	200	120	250	200	250	300	250	400	250

25 C		10 min		12 min		15 min		17 min		20 min	
AST	ALT	AST	ALT	AST	ALT	AST	ALT	AST	ALT	AST	ALT
24	22	40	40	40	40	40	40	60	40	100	40
104	70	40	40	40	40	60	60	90	40	100	80
127	118	60	80	100	120	110	120	180	120	200	120
241	221	100	120	100	150	140	180	200	200	250	200
400	400	120	200	180	250	350	300	400	300	400	300

30 C		10 min		12 min		15 min		17 min		20 min	
AST	ALT	AST	ALT	AST	ALT	AST	ALT	AST	ALT	AST	ALT
24	22	40	40	40	40	60	40	80	40	70	40
104	70	80	60	100	60	120	80	110	80	150	80
127	118	80	80	110	110	150	110	160	120	250	120
241	221	80	110	120	180	180	180	200	180	300	180
400	400	150	180	250	200	400	300	400	300	400	300

34 C		10 min		12 min		15 min		17 min		20 min	
AST	ALT	AST	ALT	AST	ALT	AST	ALT	AST	ALT	AST	ALT
24	22	40	40	60	40	60	40	80	40	100	40
104	70	100	40	100	40	140	40	180	40	250	80
127	118	120	110	140	120	200	120	250	120	400	120
241	221	120	150	180	200	250	200	300	200	400	200
400	400	350	250	400	300	400	400	400	400	400	400

37 C		10 min		12 min		15 min		17 min		20 min	
AST	ALT	AST	ALT	AST	ALT	AST	ALT	AST	ALT	AST	ALT
24	22	80	40	80	40	100	40	120	60	120	60
104	70	100	80	100	80	120	100	150	100	200	110
127	118	120	120	120	120	120	120	150	150	200	150
241	221	180	150	200	180	400	200	400	200	400	200
400	400	400	250	400	250	400	300	400	300	400	300

	Over-read
	Within range
	Under-read

Figure S3. Paper assay results at various temperatures and read times. Results for the gold-standard method (Cholestech LDX) are shown in the first two columns on the left (white cells). Values on the paper test that were read >40 U/l higher were noted as over-reads (red cells); similarly, >40 U/l lower were noted as under-reads (blue cells) and within range were noted as correct reads (green cells). Samples with values measuring 400 U/l by the gold-standard method were considered "correct" if read as >200 U/l on the paper test.

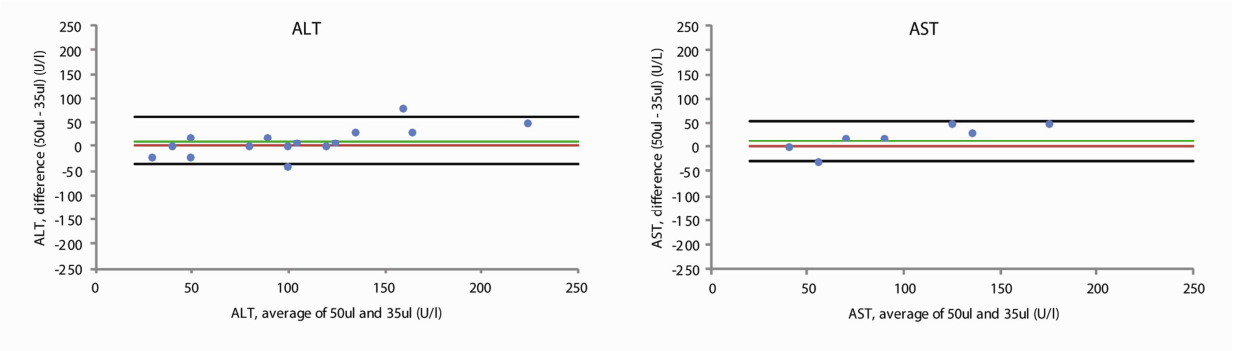


Figure S4. Bland-Altman plots of visual transaminase measurements from two different sample volumes. Two sample volumes (35 µl and 50 µl) were evaluated for both the ALT assay and AST assay on the paper-based test**.** The black lines represent the 95% limits of agreement. The red line is the line of equality and the green line is the average difference (bias) between the volumes (50 µl – 35 µl). Data are derived from the difference between the paper test value using 50 µl of sample (n = 1) and the paper test value using 35 µl of sample (n = 1) (see Bland Altman analysis equations above).

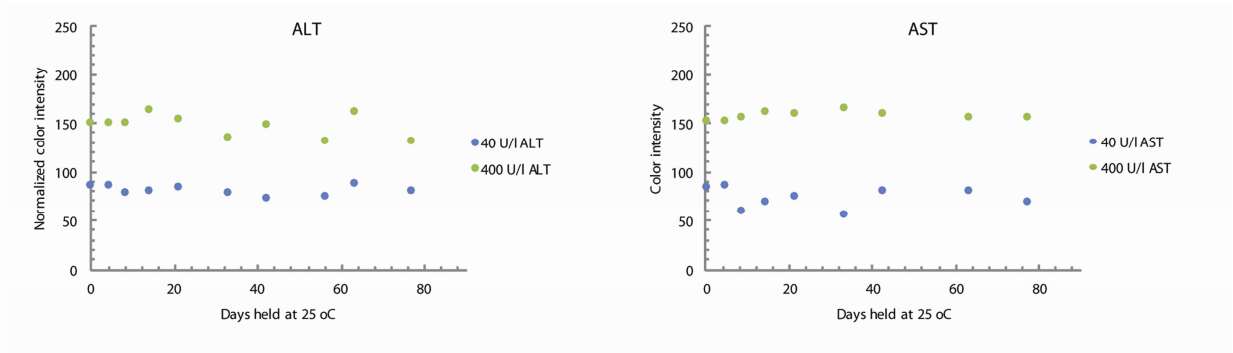


Figure S5. Room temperature stability of the paper-based transaminase test. Stability at room temperature (25˚C) was measured over an 80-day period for the ALT assay and the AST assay on the paper-based test. ALT values are normalized by subtracting each point from 255 so that higher color intensity values correspond to higher ALT concentrations. Two concentrations of each enzyme were evaluated: 40 U/l (blue circles), and 400 U/l (green circles). Each datum represents one measurement collected using a device from a freshly opened pouch at the corresponding time point.

Supplementary Tables:

Table S1. ALT whole-blood raw data. Values are visual reads (*n* = 1 per reader) from 3 independent operators which were then averaged (fifth column). Values were obtained 15 minutes after sample addition (35 µl) using the read guide (Fig. 1).

Roche Analyzer value (U/l)	Paper test value reader 1 (U/l)	Paper test value reader 2 (U/l)	Paper test value reader 3 (U/l)	Paper test value average (U/l)
6	40	40	20	33
8	40	40	25	35
8	80	80	80	80
9	100	110	110	107
12	40	40	40	40
14	50	60	40	50
15	90	70	90	83
16	60	40	60	53
17	50	60	40	50
18	60	80	60	67
19	40	40	60	47
21	80	60	80	73
21	100	100	110	103
24	100	120	110	110
26	60	60	80	67
28	60	60	80	67
28	70	100	80	83
29	60	40	60	53
30	40	40	40	40
32	40	40	60	47
33	80	60	100	80
37	60	40	60	53
38	80	80	80	80
39	70	40	80	63
40	80	100	110	97
40	100	100	100	100
42	40	20	60	40
42	60	40	80	60
42	80	80	80	80
46	120	120	150	130
47	60	60	60	60
47	60	80	60	67
50	60	50	80	63
50	100	80	60	80

51	120	100	120	113
53	40	40	40	40
53	110	110	100	107
55	100	90	110	100
56	60	40	80	60
56	60	80	80	73
58	120	110	130	120
65	80	80	110	90
66	100	80	100	93
68	100	100	100	100
68	120	100	100	107
70	80	80	90	83
74	100	100	110	103
74	100	120	140	120
75	40	20	40	33
78	100	80	100	93
79	150	150	150	150
80	60	80	100	80
84	60	60	100	73
84	110	120	100	110
85	100	110	120	110
99	120	100	130	117
101	60	60	60	60
104	120	120	160	133
105	60	70	110	80
105	130	130	160	140
106	60	60	60	60
107	40	40	40	40
108	40	40	60	47
108	100	100	110	103
110	130	120	150	133
115	150	160	130	147
117	150	160	180	163
118	200	250	190	213
124	250	200	200	217
129	130	140	130	133
147	200	220	250	223
156	250	250	250	250
170	180	200	210	197
171	150	160	160	157
188	160	160	160	160
210	200	220	200	207
220	180	150	120	150
232	250	250	250	250
249	250	250	250	250
266	180	160	200	180
350	400	400	400	400
361	250	250	300	267

398	400	400	400	400
400	400	400	400	400
450	400	400	400	400
470	400	400	400	400

Table S2. AST whole-blood raw data. Values are visual reads (*n* = 1 per reader) from 3 independent operators which were then averaged (fifth column). Values were obtained 15 minutes after sample addition (35 µl) using the read guide (Fig. 1).

Roche Analyzer value (U/l)	Paper test value reader 1 (U/l)	Paper test value reader 2 (U/l)	Paper test value reader 3 (U/l)	Paper test value average (U/l)
11	40	40	60	47
14	40	40	40	40
16	40	60	40	47
17	40	70	50	53
21	40	40	60	47
21	40	40	40	40
24	60	60	60	60
28	40	40	40	40
28	40	60	40	47
28	80	40	40	53
29	40	40	60	47
30	80	80	80	80
34	60	40	60	53
34	40	40	30	37
34	40	50	40	43
36	40	60	70	57
37	60	60	40	53
40	40	40	40	40
40	40	40	40	40
41	60	90	90	80
41	40	60	40	47
42	60	80	80	73
44	40	40	40	40
44	40	40	60	47
45	60	60	60	60
46	80	60	40	60
47	40	40	40	40
47	100	100	90	97
48	90	80	60	77
49	60	40	40	47
49	70	40	80	63
49	80	60	60	67
49	80	80	100	87
53	60	40	80	60
53	60	30	90	60
54	80	70	60	70
54	100	100	95	98
55	40	80	60	60
56	40	40	60	47
58	80	80	90	83
59	60	60	40	53

61	60	40	90	63
61	80	90	80	83
63	60	60	60	60
66	120	100	120	113
66	80	80	60	73
68	80	40	60	60
70	100	100	100	100
70	90	80	110	93
71	60	80	60	67
74	40	40	40	40
76	90	90	100	93
79	60	80	80	73
80	90	90	90	90
80	80	90	90	87
82	90	80	100	90
90	180	150	150	160
96	110	90	100	100
97	60	80	80	73
100	80	60	90	77
101	80	60	50	63
102	80	90	60	77
103	60	80	70	70
103	100	100	110	103
111	110	120	120	117
112	110	120	100	110
114	100	90	90	93
117	60	60	80	67
119	100	90	100	97
120	150	150	130	143
123	80	80	60	73
125	100	100	80	93
133	150	130	120	133
134	100	100	100	100
138	100	100	70	90
147	130	200	180	170
155	180	140	110	143
155	150	120	140	137
160	180	180	130	163
163	180	200	180	187
166	130	140	130	133
166	120	100	90	103
168	180	250	180	203
173	120	120	120	120
176	130	130	140	133
181	130	150	130	137
194	300	250	300	283
204	200	300	200	233
218	300	250	300	283

226	180	150	180	170
350	400	400	400	400
396	400	400	300	367
450	400	400	400	400
490	400	400	400	400
510	400	400	400	400

Table S3. ALT serum raw data. R1, R2, and R3 refer to Reader 1, Reader 2, and Reader 3, respectively. Paper tests A to D refer to up to 4 independent tests used to measure the same sample. Blank cells indicate that an insufficient volume of serum was available for all repeats. "Inv" indicates result was invalid due to sample hemolysis. Values were obtained 15 minutes after sample addition (35 μl) using the read guide (Fig. 1).

	Paper test A (U/l)			**Paper test B (U/l)**			**Paper test C (U/l)**			**Paper test D (U/l)**			
Roche Analyzer value (U/l)	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	**Average paper test value (U/l)**
6	40	40	40										40
8	40	40	40										40
9	40	40	40										40
12	30	40	40	40	40	40	40	40	60	40	40	60	43
14	20	40	40	40	40	40	40	40	40	40	40	40	38
15	40	40	40										40
16	60	50	60										57
17	40	40	40										40
18	40	40	40	40	40	60	40	40	60	40	60	60	47
19	40	40	40	40	40	60	40	40	60	40	40	60	45
21	60	40	40										47
21	40	40	40										40
23	80	90	80	80	100	80	100	100	100	inv	inv	inv	90
24	40	40	40	40	40	60	40	60	60	40	60	60	48
24	40	40	40	40	40	40	40	40	40	40	40	40	40
24	40	20	40										33
26	40	40	40										40
27	40	10	40										30
27	60	40	60	60	40	60	60	40	60				53
28	40	40	40	40	40	40	40	40	40	40	40	40	40
28	60	60	60										60
28	80	60	80										73
29	40	40	40										40
31	40	20	40	60	80	80	60	80	80	60	80	80	63
32	40	40	40										40
32	80	60	80										73
33	40	40	40	40	40	60	40	60	60	40	60	60	48
33	40	40	60	40	40	60	40	40	60				46
34	40	60	40										47
34	40	40	40	40	40	40	40	40	40				40
37	40	60	60	40	40	60	40	40	60	40	40	60	48
38	20	40	40	40	40	40	40	40	40	40	40	40	38
39	80	60	60										67
40	40	40	40										40
40	60	40	60										53
40	60	80	80										73

41	40	40	30										37
42	40	40	40	40	40	40	40	40	40	40	40	40	40
42	40	40	40	40	40	40	40	40	40	40	40	40	40
42	40	60	60	50	40	40	40	40	40	60	40	60	48
45	60	40	40	60	60	60	60	40	60				53
46	60	80	80										73
47	60	60	80	40	60	60	40	60	60	40	60	60	57
47	40	40	40										40
47	40	40	60										47
50	40	80	80	60	60	60	60	40	60	60	40	60	58
50	110	90	100										100
51	100	90	100										97
53	40	60	100	40	40	60	40	40	60	40	40	60	52
53	20	40	40										33
55	100	100	100										100
56	40	60	40	40	40	60	60	60	80	50	40	60	53
56	40	60	40	40	40	40	40	60	40	40	40	40	43
58	100	80	90										90
60	80	80	80										80
65	40	20	40	80	80	60	80	80	60	70	80	60	63
66	80	80	80	60	40	60	60	60	80	60	60	80	67
68	20	40	40	60	40	60	60	40	60	60	40	60	48
68	60	80	80										73
70	40	70	60										57
74	90	60	80	80	80	80	80	60	80	80	40	60	73
74	100	100	160										120
75	40	20	40	40	20	40	40	20	40	40	20	40	33
78	60	60	60	60	60	60	60	40	60	60	40	60	57
79	100	110	120	60	80	80	60	80	80	60	80	60	81
79	60	60	60	60	60	70	60	60	80				63
80	110	60	80	40	20	40	60	40	40	60	60	60	56
80	40	40	40	40	40	40	40	40	40				40
81	80	70	110	90	120	110	100	130	120	90	120	110	104
84	80	100	100	80	100	100	80	100	100	60	100	100	92
84	80	80	100										87
84	60	60	100										73
85	80	80	120	80	130	120	90	130	120	80	130	120	107
85	120	80	80	100	80	80	100	80	80				88
89	70	100	80	70	90	80	70	80	80				80
99	130	80	110	100	130	120	100	120	100	80	120	100	108
101	60	60	60	40	20	40	40	20	40	40	20	40	40
104	80	60	inv	70	80	100	70	80	100	70	80	100	81
104	40	40	40	40	40	40	40	40	40				40
105	60	80	100	60	100	100	80	100	100	80	100	80	87
105	120	130	160	80	100	100	100	100	100	100	100	100	108
106	20	40	60										40
107	40	40	60	40	40	40	40	40	60	50	40	60	46
107	100	90	130	100	100	130	100	80	130				106

108	60	80	100	80	100	120	40	40	120	60	100	120	85
108	40	40	30										37
110	90	90	130	80	100	100	60	80	100	60	80	100	89
115	40	50	80	80	120	120	100	130	120	100	130	120	99
117	120	120	160										133
118	180	140	180	120	180	160	150	180	180	100	160	150	157
122	110	100	110	120	100	120	120	100	120				111
122	100	100	110	120	100	120	100	100	100				105
124	200	180	200										193
124	120	120	100	120	120	100	120	120	120				115
128	120	120	100	120	110	100	100	100	80				105
129	110	100	110										107
147	120	120	120	60	80	80	120	150	120	120	120	120	111
156	110	130	160										133
170	200	180	180										187
171	100	100	130	100	120	120	80	120	110	inv	inv	inv	109
179	100	140	130	100	130	130	100	130	130				121
188	130.00	120.00	130.00										127
210	250	250	200										233
220	150	150	200	180	200	200	180	200	200	250	200	200	193
232	400	400	250										350
241	120	120	130	120	120	150	120	120	130				125
249	250	250	200	180	200	200	200	200	200	250	200	200	211
255	130	130	170	120	180	200	100	160	160	150	200	200	158
266	120	110	180	250	250	250	250	250	200	inv	inv	inv	207
316	200	200	180	180	190	150	200	190	180				185
316	250	200	190	250	200	200	250	180	190				212
318	200	200	140	250	250	160	200	200	140				193
361	250	250	300										267
398	250	250	250										250
400	400	400	400	250	200	250	300	250	250	300	250	250	292
419	200	250	250	250	250	250	250	250	250				244
423	300	250	200	250	200	200	300	300	250				250
425	400	400	400	400	400	400	400	400	400	inv	inv	inv	400
450	400	400	400	250	250	250	250	250	250	200	250	250	283
450	400	400	250	250	400	400	200	400	200	250	400	400	329
470	400	400	400										400

Table S4. AST serum raw data. R1, R2, and R3 refer to Reader 1, Reader 2, and Reader 3, respectively. Paper tests A to D refer to up to 4 independent tests used to measure the same sample. Blank cells indicate that an insufficient volume of serum was available for all repeats. Inv = result was invalid due to sample hemolysis. Values were obtained 15 minutes after sample addition (35 µl) using the read guide (Fig. 1).

	Paper test A (U/l)			Paper test B (U/l)			Paper test C (U/l)			Paper test D (U/l)			
Roche Analyzer value (U/l)	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	Average paper test value (U/l)
11	40	70	60										57
14	30	40	40	40	40	60	40	40	40	40	40	40	41
16	70	80	60										70
17	40	40	40										40
21	40	40	40										40
21	40	40	40										40
23	60	80	60	60	40	40	60	40	40	inv	inv	inv	53
24	60	40	60	40	40	40	40	40	40	40	40	40	43
25	40	40	40	40	40	40	60	40	40				42
26	40	40	40	40	40	40	40	40	80				44
28	60	60	40	40	40	40	40	40	40	40	40	40	43
28	40	40	40	40	40	40	40	40	40	40	40	40	40
28	40	40	40	60	60	60	60	60	60	60	60	60	55
29	40	40	40	40	40	40	40	40	40	40	40	40	40
30	40	40	40										40
30	40	40	60	40	40	60	60	40	80				51
34	100	90	100										97
34	90	60	60										70
36	150	200	100										150
37	60	40	40	40	40	40	40	40	40	40	40	40	42
37	50	40	40										43
40	40	80	60	40	80	60	40	80	60	40	80	60	60
40	40	40	40										40
41	90	100	80										90
41	40	40	60										47
42	40	80	150										90
44	50	60	40	40	40	40	40	40	40	40	40	40	43
44	90	100	90	40	40	60	40	40	60	40	40	60	58
44	40	40	60										47
44	40	80	60	40	80	40	60	80	60				60
44	60	40	60	60	40	60	80	40	80				58
45	50	50	40	40	40	40	40	40	40	40	40	40	42
46	40	40	40	40	40	60	40	40	60	40	40	60	45
46	40	80	60	40	80	60	40	80	60				60

47	80	40	90	40	80	60	40	80	60	40	80	60	63
47	90	90	80										87
48	40	50	40										43
49	80	80	90	40	40	40	40	40	40	40	40	40	51
49	60	40	40	40	40	40	40	40	40	40	40	40	42
49	80	70	80	60	60	40	60	60	40	60	60	40	59
49	80	80	90										83
50	40	40	40										40
51	60	80	40										60
53	80	90	40	40	40	40	40	40	40	40	40	40	48
53	60	40	40	40	40	40	40	40	40	40	40	40	42
53	60	80	60										67
54	100	90	70	40	40	40	40	40	40	40	40	40	52
54	40	40	30										37
55	100	100	110	40	90	60	60	90	80	40	90	60	77
56	60	60	40	40	40	40	40	40	40	40	40	40	43
56	60	90	40	60	100	60	60	100	60	80	100	60	73
58	80	80	90										83
59	40	40	80										53
61	110	110	110	40	80	60	40	80	60	40	80	60	73
61	100	40	100	40	40	40	40	40	40	40	40	40	50
63	60	50	60	80	90	80	80	90	80	80	90	80	77
66	110	100	60										90
66	80	90	90										87
68	80	80	60	40	80	60	40	80	60	40	80	60	63
68	100	100	100										100
70	100	100	100										100
70	40	60	40										47
71	40	60	40										47
74	60	80	90	40	40	60	40	40	60	40	40	60	54
75	100	90	80										90
76	120	110	110	120	150	110	60	80	60	110	100	90	102
79	60	40	40										47
80	120	100	100	90	100	90	90	100	100	90	100	100	98
80	90	100	80	100	100	90	120	100	110	80	60	80	93
82	90	40	80										70
83	110	100	90	120	120	110	120	100	100				108
90	60	80	80	40	80	60	40	80	60	40	80	60	63
90	120	120	120										120
96	150	130	150	100	100	100	120	100	120	inv	inv	inv	119
97	80	100	80	80	100	80	60	100	60	60	100	80	82
99				80	100	80	100	100	100	80	100	100	93
100	100	100	90										97
101	80	70	70										73
102	90	90	60										80
103	100	100	100	80	80	80	120	80	120	80	90	80	93
103	100	100	100										100
103	180	200	180										187

105	180	180	180	180	180	180	150	150	150				170
111	120	120	100	180	200	180	120	120	100	150	180	150	143
112	100	100	80	100	100	80	80	90	40	60	90	40	80
114	120	110	120	100	100	90	150	150	150	120	100	120	119
117	120	120	100										113
119	120	100	120										113
120	150	160	150										153
123	100	100	100										100
125	120	180	150	120	120	150	100	100	120	100	120	120	125
125	120	120	150	120	150	150	120	120	150				133
126	100	100	80	100	100	100	100	100	80				96
133	150	150	180										160
134	90	90	80	110	120	150	150	300	180	250	300	200	168
136	100	100	90	100	100	90	100	100	100				98
138	200	210	200										203
147	200	250	200	120	120	120	180	180	180	180	200	180	176
152	150	180	200	120	120	150	120	180	180				156
155	200	250	200	300	300	200	180	200	120	300	400	200	238
155	220	190	180										197
158	120	120	120	150	150	130	150	120	150				134
160	180	160	150										163
162	150	180	180	150	180	150	150	180	180				167
163	200	210	200										203
166	150	150	180	200	180	150	200	180	150	200	300	300	195
166	250	190	180										207
168	200	300	300	400	400	400	400	400	400	400	400	300	358
173	180	400	200	400	400	400	400	400	300	400	400	300	348
174	400	300	300	400	300	400	300	300	300				333
176	300	400	300	400	400	300	400	400	300	400	400	200	350
181	220	210	180										203
194	180	250	300	400	400	200	400	400	200	400	400	300	319
196	300	250	300	300	300	200	300	250	300				278
204	180	200	180	180	180	180	120	120	120	250	300	200	184
218	400	400	400	300	300	200	300	300	300	300	300	300	317
226	300	300	200										267
278	400	400	300	300	400	400	300	400	300				356
293	300	400	400	400	400	400	300	400	400				378
376	400	400	400	300	400	300	400	400	300				367
383	400	400	300	400	400	300	400	400	400				378
396	400	400	400										400
400	400	400	400	400	400	400	400	400	400	400	400	400	400
425	400	400	400	400	400	400	400	400	400	400	400	400	400
450	400	400	400	400	400	400	400	400	400	400	400	400	400
463	400	300	400	400	300	400	400	300	300				356
470	400	400	400										400
490	400	400	400										400

Table S5. Cost per device estimate for the paper-based transaminase test. Manufacturing costs are based on estimated infrastructure and personnel costs for India (costs for similar manufacturing in the US would be 2-3–fold higher). Consumable pricing is based on cost of materials currently being incurred at the research level and adjusted for lower prices based on volume discount. Infrastructure costs include building, setting up manufacturing line, and approximate labor costs.

Total Manufacturing Equipment & Personnel		**$0.0049**
Consumable		
Whatman chromatography paper		$0.0025
Wax for Xerox wax printer		$0.0008
Fellowes laminate sheets		$0.0005
Adhesive (Unitak 131)		$0.0004
Pall vivid GX filter membrane		$0.0250
Foil-lined pouch (100 devices/pack)		$0.0075
Desiccant (1 pack/100 devices)		$0.0075
Reagents (enzymes, chemicals, etc.)		$0.0225
Total consumables		**$0.0667**
Total cost		**$0.0715**