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ORIGINAL ARTICLE

Validation of novel point-of-care test for alanine aminotransferase measurement: A pilot cohort study

Jessica Howell[1,2,3] | **Huy Van**[1] | **Minh Pham**[1,3] | **Rohit Sawhney**[2,4,5] | **Fan Li**[1] | **Purnima Bhat**[6] | **John Lubel**[7] | **William Kemp**[7] | **Stephen Bloom**[4,5] | **Avik Majumdar**[8] | **Geoff McCaughan**[8,9] | **Samuel Hall**[2] | **Timothy Spelman**[1] | **Joseph S. Doyle**[1,3,5,10] | **Margaret Hellard**[1,3,5,10] | **Kumar Visvanathan**[2] | **Alexander J. Thompson**[2] | **Heidi E. Drummer**[1,11,12] | **David Anderson**[1,3]

[1]Disease Elimination, Burnet Institute, Melbourne, Australia

[2]Department of Gastroenterology, St Vincent's Hospital and University of Melbourne, Melbourne, Australia

[3]School of Epidemiology and Preventive Medicine, Monash University, Melbourne, Australia

[4]Department of Gastroenterology, Eastern Health, Melbourne, Australia

[5]Department of Medicine, Monash University, Melbourne, Australia

[6]Department of Gastroenterology, Canberra Hospital, Canberra, Australia

[7]Central Clinical School, Monash University, Melbourne, Australia

[8]Australian National Liver Transplant Unit, Royal Prince Alfred Hospital, Sydney, Australia

[9]Liver Injury and Cancer, Centenary Institute, Sydney, Australia

[10]Department of Infectious Diseases, The Alfred, Melbourne, Australia

[11]Department of Microbiology and Immunology, University of Melbourne, Melbourne, Australia

[12]Department of Microbiology, Monash University, Melbourne, Australia

Correspondence
Jessica Howell, Disease Elimination, Burnet Institute, Melbourne, Australia.
Email: jessica.howell@svha.org.au

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INTRODUCTION

Chronic liver disease is a major global public health challenge: without timely diagnosis, untreated chronic liver diseases such as hepatitis B and C can lead to death from cirrhosis and liver cancer.[1,2] Alanine aminotransferase (ALT) is a blood-based marker of acute liver inflammation used to diagnose, evaluate, and monitor chronic liver disease.[3–6] However, conventional ALT measurement poses a significant barrier to timely liver disease management in low resource and remote settings where access to routine pathology testing is limited.[7,8]

We developed a novel, easy-to-use rapid point-of-care (POC) immunoassay lateral flow test that measures the liver-specific ALT1 component of total enzymatic ALT. Using a single droplet of blood or plasma, the POC ALT1 test provides a semi-quantitative visual result for an ALT cutoff of 40 IU/L, or quantitative ALT1 using the Axxin hand-held flow test reader, within 30 minutes. Prototype POC ALT1 tests were provided by Nanjing BioPoint Diagnostics (PR China).

In this pilot study, we demonstrate the diagnostic accuracy of the POC ALT1 test for clinically significant elevations in ALT compared to the laboratory gold standard assay in people with viral hepatitis.

Jessica Howell and Huy Van co- first author.

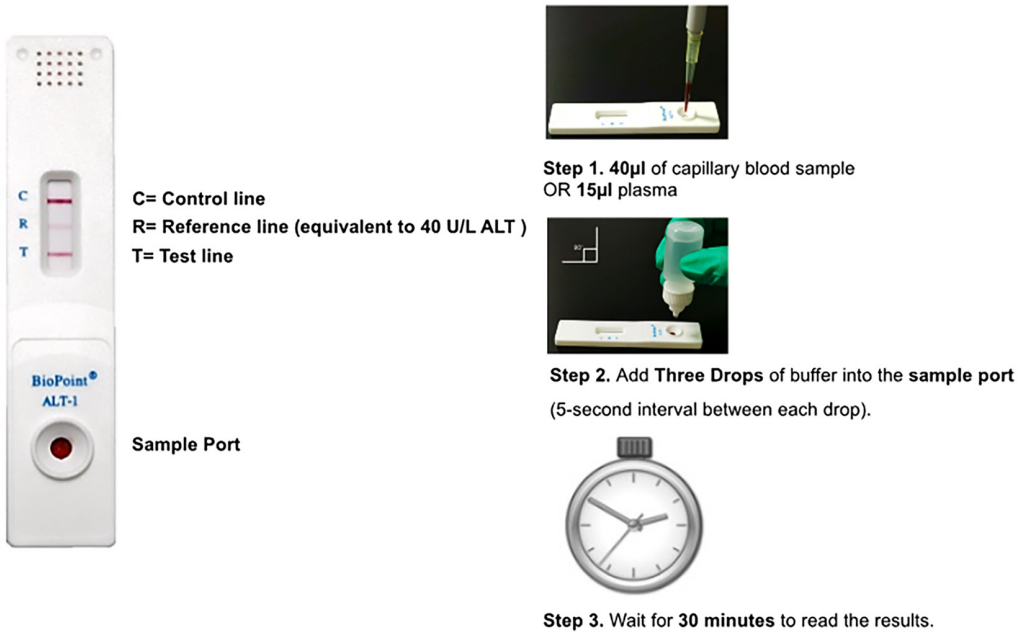

FIGURE 1 Schematic diagram of POC ALT1 ratio test and methods.

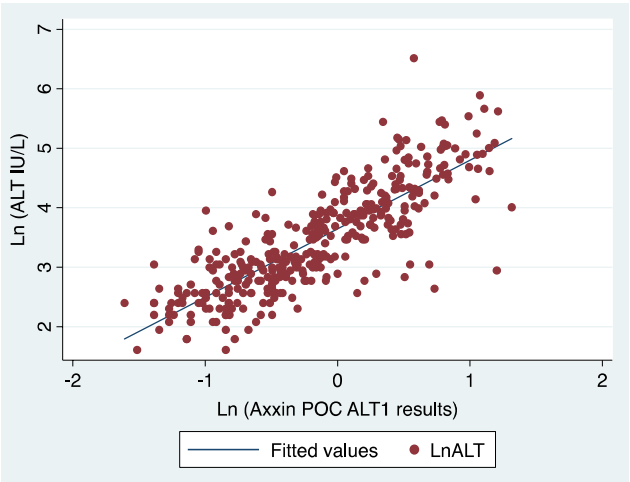

FIGURE 2 Linear regression plot of the association between baseline ln (ALT) and ln (POC ALT1) values (Adj R^2 = 0.68, p < 0.0001; n = 240).

TABLE 1 Distribution of clinical factors in the cohort overall and compared between Test and Validation cohorts (n = 731)

Variable	Test cohort (n = 240)	Validation cohort (n = 491)
Male	168 (71%)	246 (50%)
Female	70 (29%)	245 (50%)
Mean age ± SD	39 ± 8 years	48 ± 13 years
Hepatitis B	74 (30%)	416 (85%)
Hepatitis C	166 (69%)	31 (6%)
Cirrhosis	19 (8%)	85 (17%)
Median ALT IU/L (IQR)	32 IU/L (19, 60)	23 IU/L (18, 35)

Abbreviation: IQR, interquartile range.

METHODS

This cross-sectional cohort study was performed between January 1, 2020 and December 31, 2021. Matched plasma samples and demographic and clinical data from adults aged over 18 years with chronic liver disease were sequentially and non-randomly assigned to Test or Validation cohorts. The Test cohort comprised participants from hepatitis B and hepatitis C studies and was used to determine the diagnostic performance of the POC ALT1 test. We then validated the POC ALT1 test performance in a larger cohort of individuals (Validation cohort); due to ALT being a key treatment criterion in hepatitis B, the Validation cohort included a higher proportion of participants with hepatitis B. All participants were consented in accordance with institutional ethics requirements. Variables collected at the time of sample collection included age, biological sex, aetiology of liver disease, and ALT level.

A total of 15 µL of thawed plasma samples were added to the POC ALT1 device, followed by three drops of running buffer. After 30 minutes, results were visually compared to the reference line; the test cassette was also inserted into an Axxin handheld reader to obtain a quantitative ratio of test to calibrator (Axxin units). Quantitative POC ALT1 results were compared to laboratory ALT using Spearman correlation. Accuracy of POC ALT1 to detect ALT > 40 IU/L was measured by receiver operating characteristics curve (ROC) analysis;

sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were determined by error matrix. All analyses were done with STATA version 14 (Statacorp, Texas USA) (Figure 1).

RESULTS

There were 240 individuals in the Test cohort and 491 individuals in the Validation cohort. Clinical and demographic features of both cohorts are shown in Table 1. A total of 42% of the Test cohort and 19% of the Validation cohort had ALT > 40 IU/L.

There was good correlation between quantitative POC ALT1 test results and laboratory ALT results in the Test cohort, with an adjusted R^2 coefficient of 0.68 (Figure 2).

Diagnostic performance of the POC ALT1 test is shown in Table 2. Quantitative POC ALT1 test results (Axxin reader) had excellent diagnostic accuracy for ALT > 40 IU/L, with an area under the ROC (AUROC) of 0.93 (95% confidence interval [CI] 0.89–0.96, $p < 0.001$) in the Test cohort and 0.92 in the Validation cohort (95% CI 0.86–0.95; Figure 3). An optimized POC ALT1 ratio cutoff of 0.8 provided 97% sensitivity and 71% specificity for ALT > 40 IU/L in the Test cohort and moderate PPV (77%) and high NPV (93%) in the Validation cohort (Table 2). Semi-quantitative visual read results were comparable to quantitative results for diagnosis of ALT > 40 IU/L (77% sensitivity and 93% specificity).

TABLE 2 Accuracy of the POC ALT1 test for laboratory ALT ($n = 731$)

	AUROC (95% CI)	Sensitivity	Specificity	PPV	NPV
Test cohort (n = 240)					
Quantitative ALT results (Axxin reader), POC ALT1 ratio cutoff 0.8					
ALT > 40 IU/L	0.84 (95% CI 0.80–0.88)	97%	71%	71%	97%
Visual semi-quantitative POC ALT result (>40 IU/L)					
ALT > 40 IU/L	0.85 (95% CI 0.80–0.89)	80%	82%	73%	87%
Validation cohort (n = 491)					
Quantitative ALT results (Axxin reader), POC ALT1 ratio cutoff 0.8					
ALT > 40 IU/L	0.92 (95% CI 0.88–0.95)	68%	95%	77%	93%
Visual semi-quantitative POC ALT result (>40 IU/L)					
ALT > 40 IU/L	0.85 (95% CI 0.81–0.90)	77%	93%	74%	94%

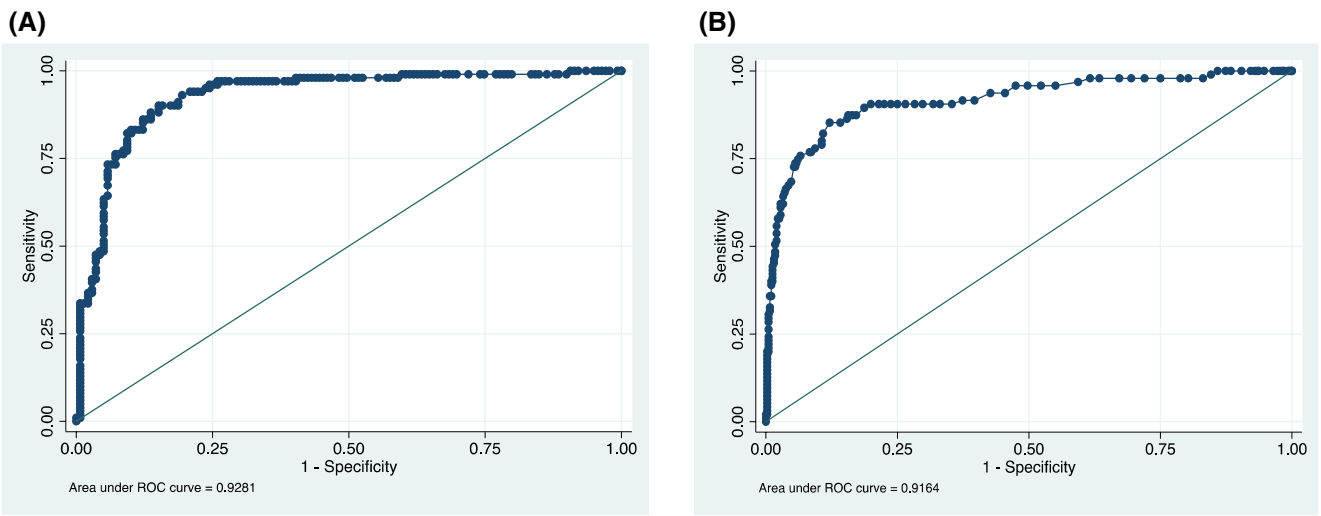

FIGURE 3 (A) Test cohort: AUROC plot of the diagnostic accuracy of quantitative POC ALT1 ratio (Axxin reader) for laboratory ALT value > 40 IU/L (AUROC 0.928, 95% CI 0.893–0.962). (B) Validation cohort: AUROC 0.92 (95% CI 0.88–0.95).

DISCUSSION

Lack of access to diagnostics, including for assessment of liver inflammation, poses a major barrier to community-based liver disease screening and treatment programs in low resource and remote settings.[7,9,10] In this study, we have shown that our POC ALT1 test can measure ALT using a single droplet of blood or plasma, is easy to use, and has good diagnostic accuracy for clinically significant ALT elevations. These data show the POC ALT1 test has the exciting potential to expand access to affordable liver disease screening and monitoring in low resource and remote settings by improving coverage of ALT test access and facilitating rapid, same-day liver disease assessment and treatment in community settings. The POC ALT1 test had lower sensitivity in the Validation cohort than the Test cohort, which may reflect greater proportion of patients with hepatitis B compared with hepatitis C. Median ALT level was lower in the Validation cohort and our POC ALT1 test may have lower accuracy at lower ALT levels. Interestingly, the visual POC ALT1 test results were more sensitive for elevated ALT in the Validation cohort than those determined by the Axxin reader (77% versus 69% sensitivity), suggesting the POC ALT1 visual read test has good clinical utility for low resource settings. Importantly, no ALT levels greater than twice the upper limit of normal were missed by either the visual read or Axxin read POC ALT1 test.

Our study has limitations, including its cross-sectional design and use of stored samples from a predominantly hospital derived patient population, meaning the findings may not be generalizable to other populations. Strengths of this study include the large sample size, inclusion of diverse etiologies of liver disease, ethnicities, and detailed matched clinical and demographic data for each sample. Future work evaluating the accuracy of the POC ALT test in fingerstick whole blood is underway.

CONCLUSION

The novel POC ALT1 test had good correlation with laboratory assay ALT and good accuracy for identifying people with elevated ALT levels. Further validation of the POC ALT1 test in prospective clinical trials is warranted.

CONFLICT OF INTEREST

DA and HV hold a patent on the POC ALT1 test. JH has received investigator-initiated grant funding and speaker fees from Gilead Sciences and Eisai. The Burnet Institute was a shareholder in Nanjing BioPoint. JD's and MH's institution has received investigator-initiated funding for research from AbbVie and Gilead Sciences and consultancies for education from AbbVie.

REFERENCES

1. Akinyemiju T, Abera S, Ahmed M, Alam N, Alemayohu MA, Allen C, et al. The Burden of Primary Liver Cancer and Underlying Etiologies From 1990 to 2015 at the Global, Regional, and National Level: Results From the Global Burden of Disease Study 2015. JAMA Oncol. 2017;3:1683–91.
2. Global burden of disease collaborators. The global, regional, and national burden of cirrhosis by cause in 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Gastroenterol Hepatol. 2020;5(3):245–66.
3. Younossi Z, Tacke F, Arrese M, Chander Sharma B, Mostafa I, Bugianesi E, et al. Global perspectives on nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. Hepatology. 2019;69(6):2672–82.
4. European Association for the Study of the Liver. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. J Hepatol. 2017;67(2):370–98.
5. European Association for the Study of the Liver. EASL clinical practice guidelines: management of alcohol-related liver disease. J Hepatol. 2018;69(1):154–81.
6. American Association for the Study of Liver Disease. Hepatitis C guidance: AASLD-IDSA recommendations for testing, managing, and treating adults infected with hepatitis C virus. Hepatology. 2015;62(3):932–54.
7. Cooke GS, Andrieux-Meyer I, Applegate TL, Atun R, Burry JR, Cheinquer H, et al. Accelerating the elimination of viral hepatitis: a Lancet Gastroenterology & Hepatology Commission. Lancet Gastroenterol Hepatol. 2019;4:135–84.
8. Howell J, Pedrana A, Schroeder SE, Scott N, Aufegger L, Atun R, et al. A global investment framework for the elimination of hepatitis B. J Hepatol. 2021;74:535–49.
9. Pedrana AHJ, Scott N, Schroeder S, Kuschel C, Lazarus JV, Atun R, et al. Global hepatitis C elimination: an investment framework. Lancet Gastroenterol Hepatol. 2020;5(10):927–39.
10. World Health Organisation. Global Hepatitis Report 2017. [cited January 4, 2022]. Available from: http://appswhoint/iris/bitstream/handle/10665/255016/9789241565455-engpdf;jsessionid=9DECA1FF83BC4A8C41B74E3BE2649662?sequence=1

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