

RESEARCH NOTE

Open Access



Verification of dried blood spot as a sample type for HIV viral load and early infant diagnosis on Hologic Panther in Zambia

Precious Simushi^{1*}, Mukoshya Nchima Kalunga¹, Tuku Mwakyoma¹, Mulenga Mwewa¹, Lweendo Muchaili¹, Nchimunya Hazeemba¹, Chileshe Mulenga¹, Patience Mwewa¹, Kaseya O. R. Chiyenu¹, John Kachimba¹, Powell Choonga², Aaron Shibemba³, Benison M. Hamooya⁴, Mowa Zambwe⁵, Peter J. Chipimo⁶ and Lackson Kasonka⁷

Abstract

Objective Zambia has embarked on improving the diagnostic capacity by setting up high throughput and accurate machines in the testing process and introduction of dried blood spot (DBS) as a sample type. This was a cross sectional study to verify dried blood spot as a sample type for HIV viral load and early infant diagnosis (EID) on Hologic Panther platform and Evaluate the analytical performance (precision, linearity and measurement of uncertainty) of the Hologic Panther.

Results The specificity and sensitivity of EID performance of Aptima Quant Dx assay on Hologic panther machine against the gold standard machine COBAS Taqman (CAP/CTM) was 100% with an overall agreement of 100%. The quantitative HIV Viral Load (VL) accuracy had a positive correlation of (0.96) obtained against the gold standard (plasma samples) run on COBAS4800 platform. Analytical performance of the Hologic panther machine was evaluated; Precision low positive repeatability 3.50154 and within lab 2.268915 at mean 2.88 concentration and precision high positive repeatability 1.116955 and within lab 2.010677 at mean 5.09 concentration were obtained confirming manufacturers claims. Uncertainty of measurement for this study was found to be ± 71 copies/ml. Linearity studies were determined and all points were within acceptable limits. We therefore recommend DBS as a sample type alternative to plasma for the estimation of HIV-1 viral load and EID diagnosis on the Hologic panther machine.

Keywords Dried blood spot, Early infant diagnosis, HIV viral load, Hologic Panther

*Correspondence:

Precious Simushi
precioussimushi.ps@gmail.com

¹ Livingstone University Teaching Hospital Laboratory, Livingstone, Zambia

² Ministry of Health, Ndeke House, Lusaka, Zambia

³ University Teaching Hospital, Pathology, Lusaka, Zambia

⁴ Mulungushi University, Livingstone, Zambia

⁵ Benefits Department, Workers Compensation Fund Control Board, Lusaka, Zambia

⁶ World Health Organization, Lusaka, Zambia

⁷ Ministry of Health, Lusaka, Zambia

Introduction

Zambia is part of the Joint United Nations Programme on HIV/AIDS (UNAIDS) targets of 95–95–95 which means: 95% of all HIV-positive individuals should be diagnosed, provide antiretroviral therapy (ART) for 95% of those diagnosed and achieve viral suppression for 95% of those treated [1]. However, sub Saharan countries have had challenges in achieving the third 95 goal partly as a result of Covid-19 pandemic [2]. In order to achieve this goal, Zambia has embarked on improving the diagnostic capacity by setting up high throughput and accurate machines in the



© The Author(s) 2023. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

testing process and by introducing dried blood spot (DBS) as a sample type on different PCR platforms across the country although DBS as a sample type has been already in use for early infant diagnosis (EID) in most resource limited countries [3] and it has been observed that the use of DBS can be a practical tool to mitigate challenges to deal with transport of blood specimens, trained phlebotomist, centrifugation and ultimately a well functional cold chain [4].

World Health Organization (WHO) consolidated guidelines for HIV care and treatment indicate that viral load testing is a preferred option for monitoring clients on antiretroviral therapy [5]. Prompt testing ensures timely monitoring, treatment adherence and efficacy, and minimizing treatment failure [6]. It is recommended that routine monitoring of antiretroviral therapy (ART) clients using viral load (VL) plasma testing be done at 6 months and every 12 months [7]. However, in Zambia, there is low coverage of viral load testing [8]. This is because of hard to reach areas, inadequate resources for immediate transportation of the specimens, storage and maintaining sample integrity as in the case of plasma, equipment breakdown, and stock outs of reagents at testing sites [7, 8, 8–13]. Therefore, the introduction of DBS as a sample type for VL and EID on a new platform Hologic Panther might increase the service delivery and mitigate some of these issues to do with costs, storage and transport requirements.

DBS viral load measurement has proven to be a better alternative to plasma specimens, more especially in remote settings with no laboratories [14–19]. The monitoring of clients on ART with HIV VL as recommended in developed countries has proven to be a challenge in resource limited countries this can be attributed to high costs and stringent storage and transport requirements that comes with plasma as a sample type [15]. The use of DBS for both HIV VL and EID of HIV-1 exposed infants enables easy access to treatment, timely initiation of antiretroviral therapy (ART), thereby allowing early diagnosis and treatment to slow disease progression and reduce mortality [4, 20–22]. It also simplifies virological monitoring of ART clients in resource limited settings [15]. The addition of a high throughput machine Hologic Panther platform and use of the Aptima Quant Dx HIV-1 assay for both diagnosis and quantification to the testing services will improve Turn-around time, mitigating the low to medium throughput technology which can pose as a hindrance to early treatment [23].

Main text

Methods

Study design and setting

We conducted a retrospective laboratory based cross-sectional study at the Livingstone University Teaching

Hospital laboratory (LUTH). The LUTH laboratory is located in Livingstone town, southern part of Zambia. It is a main referral center for specialized testing for HIV VL and EID diagnosis servicing 13 districts in the province.

Sample size

- Qualitative:

120 samples (60 positives and 60 negatives) of less than or equal to 18 months were used (as per manufacturer recommendation)

- Quantitative:

HIV VL 154 samples

Open-Epi sample size calculator was used with 11.3% HIV prevalence in Zambia at 5% CI

EID DBS cards collected from different facilities that had been received at Livingstone laboratory for routine testing were conveniently sampled after being confirmed reactive and non-reactive on current platform COBAS Taqman (CAP/CTM) from infants of age ≤ 18 months. A sample size of 60 DBS cards with at least 2 spots (as per manufacturer recommendation) were retrieved from storage within a retention period of less than 18 months. For HIV VL, we conveniently sampled 154 HIV VL stored samples comprising of three levels; Target not detected, below titer and above titer. These were in EDTA containers collected within 24 h and spotted on DBS cards and left to dry for 24 h before processing on the Hologic Panther machine. We used results generated by COBAS 4800 to assign the levels.

Test methods

The COBAS Taqman (CAP/CTM) HIV-1 qual test is a qualitative nucleic acid amplification test for the detection of HIV type 1 RNA and proviral DNA in plasma, anti-coagulated fresh whole blood and DBS. The test is based on four major processes; Sample preparation and incubation, Sample preparation to isolate HIV-1 target nucleic acids-both processes utilizing the magnetic glass particle technology, Reverse transcription of the target RNA to generate complimentary DNA(cDNA) and Detection of cleaved dual labelled oligonucleotide detection probe specific to the target.

The COBAS4800 HIV-1 is based on fully automated sample preparation followed by PCR amplification and detection. The cobas[®] 4800 System consists of the cobas \times 480 sample preparation instrument and the cobas z 480 real-time PCR analyzer. Real-time detection and discrimination of PCR products is accomplished by

measuring the fluorescence of the released reporter dyes for the viral targets and RNA QS, respectively.

The Aptima HIV-1 Quant Dx assay involves three main steps, which all take place in a single tube on the Panther system: these include target capture, target amplification by transcription-mediated amplification (TMA), and detection of the amplification products (amplicon) by the fluorescent labeled probes (torches).

Statistical analysis

The collected data were entered in excel, thereafter exported to Stata version 15 for analysis. Chi square test was used for qualitative data to compare results from machine 1 COBAS Taqman (CAP/CTM) and machine 2 (Hologic panther). Pearson correlation test was to determine the relationship between quantitative data generated from COBAS4800 (machine 1) and Hologic panther (machine 2).

Reporting guidelines

We used the CLSI guidelines, EP15-A3 in our reporting and interpretation of the findings.

Results

Qualitative study

Total number of DBS samples retrieved and rerun on COBAS Taqman (CAP/CTM) were 60 of which 30 were negative and 30 were positive. These results were comparable when run on the Hologic Panther machine in which

30 were negative and 30 were positive giving a specificity and sensitivity of 100% and an overall agreement of 100%.

Quantitative study

Accuracy/method correlation

The findings in this study were a positive correlation of 0.96 demonstrating that DBS HIV VL and plasma values correlates well as shown in Fig. 1

Precision results

Repeatability and within lab mean results for samples of High and low concentrations respectively had CV claims lower than that of the manufactures, indicating the precision claims of the manufacture are true as indicated in Tables 1 and 2.

Linearity results

All points were within acceptable limits as shown in Fig. 2

Discussion

Qualitative studies showed the specificity and sensitivity of EID performance of Aptima Quant Dx assay on Hologic panther machine against the gold standard machine COBAS Taqman (CAP/CTM) was 100% with an overall agreement of 100%.

In quantitative studies, correlation studies of the existing testing platforms with Hologic panther machine using the HIV-1 Quant Dx assay were conducted. This was achieved by determining accuracy/method correlation

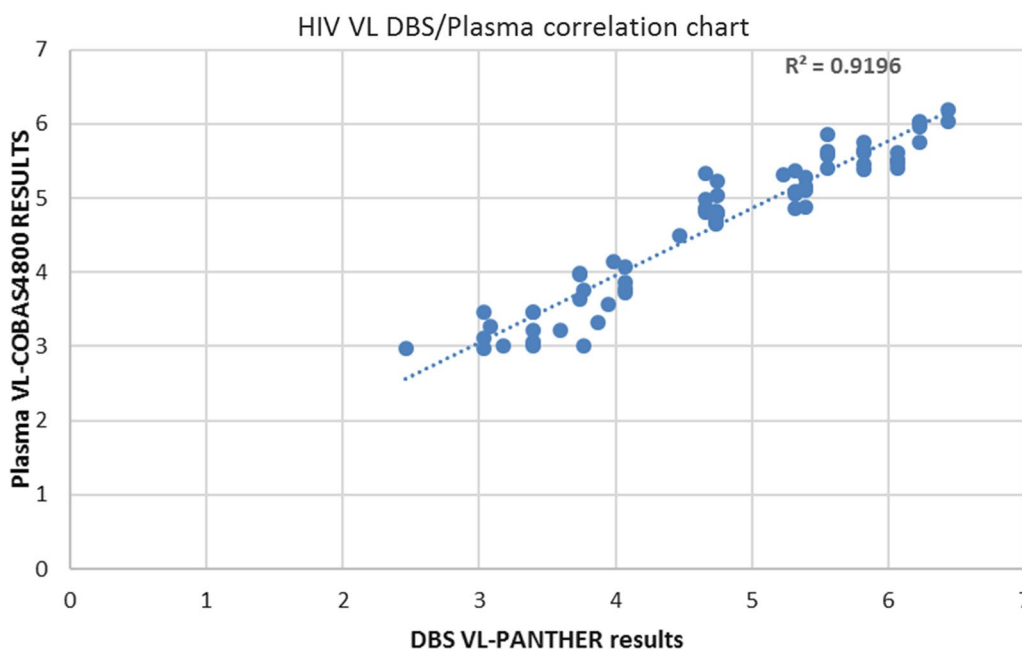


Fig. 1 HIV VL DBS/plasma correlation chart

Table 1 High positive precision

Precision studies					
Analyte	Test type	Precision high positive			Acceptability
		Mean	CV (%) claim	LGHL obtained CV (%)	
Viral load (VL) High positive	Repeatability	5.09	2.30	1.116955	Acceptable
	Within Lab		2.30	2.016077	Acceptable

Table 2 Low positive precision

Analyte	Test type	Low positive precision			Acceptability
		Mean sample conc.	CV (%) Manf. claim	LCHL obtained CV (%)	
Viral load (VL) Low positive	Repeatability	2.88	4.83	3.50154	Acceptable
	Within Lab		4.83	2.268915	Acceptable

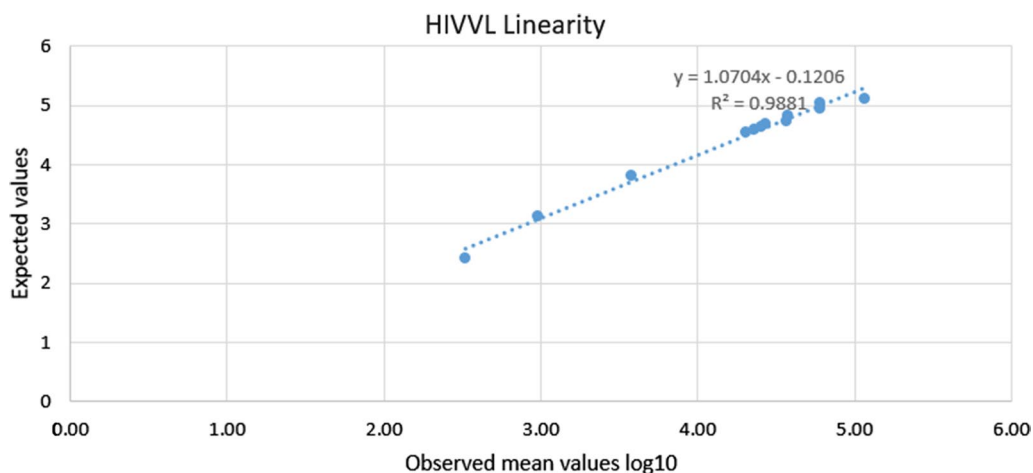


Fig. 2 HIVVL linearity chart

at three levels; Target not detected, below titer/log < 2.95 and log > 2.95. TND and log < 2.95 levels were comparable between COBAS4800 and Hologic panther machine. The method correlation was obtained using the correlation coefficient of ± 1 , the data obtained from the COBAS C4800 was compared to data generated from the Hologic Panther machine to estimate relationship. Findings in this study correlates with results in a study done in Kenya in which HIV-1 viral load results using DBS samples processed on the Hologic panther were compared with HIV-1 viral load results using plasma samples (from venous blood), showed a positive correlation indicating as plasma values were correlating with the DBS values obtained on the Hologic panther machine [23].

Precision, linearity and uncertainty of measurement were characteristic performance that were analyzed using

control material. For precision, it was observed that the manufacturers total CV was greater than the CV repeatability and CV within laboratory obtained by the LUTH during the verification process, thereby being acceptable for both the high positive and low positive controls as shown in Tables 1 and 2.

Linearity studies were determined using a sample with a high concentration and making serial dilutions with reduced concentration and the linearity was at 0.99 regression and all data points were within acceptable range as shown in Fig. 2. Uncertainty of measurement was calculated by using the CLSI guidelines; this was done by using 25 IQC results and using an MU excel calculator to calculate the total MU and the uncertainty of measurement total obtained in this study was at ± 71 copies/ml.

Hologic Panther is an instrument that can be used for running both plasma and DBS samples for viral load testing simultaneously, it is a high throughput machine with random access and runs on a fully automated system. In addition, the Aptima Quant Dx assay used on the Hologic panther is used for both HIV diagnosis and quantification thereby a cost effective option.

Conclusion

The Hologic Panther machine using Aptima HIV-1 Quant Dx assay with DBS sample protocol can be used for both EID DBS and HIV VL DBS. Findings demonstrated a high sensitive, specific and precise and correlates well with plasma values. Therefore, DBS as a sample type can be used as an alternative for the estimation of HIV-1 viral load and EID diagnosis on the Hologic panther machine.

Limitations

The use of this assay is limited to personnel who have been trained in the use of the Hologic Panther operation procedure. This assay has been validated for use with LabMate EID DBS collection Bundle K50SUKKID.

Abbreviations

EID	Early infant diagnosis
DBS	Dried blood spot
LUTH	Livingstone university teaching hospital
CLSI	Clinical laboratory standards institute
HIV	Human immunodeficiency virus
VL	Viral load
MU	Measurement of uncertainty

Acknowledgements

We are very grateful to all Laboratory personnel research team and the office of the Senior Medical Superintendent at Livingstone university teaching hospital for their support and enthusiasm for research projects.

Author contributions

PS and MNK conceived the study. TM, MM, LM, NH, CM and PM conducted the data collection. MNK, PS and BMH contributed to data analysis and led the writing of the manuscript. CK, JK, PC, AS, BMH, MZ, PJC and LK reviewed. All authors read and approved the final manuscript.

Reporting guidelines

All methods were carried out in accordance the CLSI guidelines, EP15-A3.

Funding

The verification process was funded by Medysis Ltd Zambia.

Availability of data and materials

All data generated or analyzed during this study are included in this published Article. For other data, these may be requested through the corresponding Author.

Availability of data and materials

All data generated or analyzed during this study are included in this published Article. For other data, these may be requested through the corresponding author.

Declarations

Ethics approval and consent to participate

This study was approved and granted ethical waiver by the National Health Research Authority, Zambia within the research ambit of "Laboratory Quality Improvement Research in Ministry of Health Laboratories" (NHRA000004/16/11/2021). The ethical waiver was obtained on the basis of anonymity given that solely aggregate facility-level data was used for this study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 17 September 2022 Accepted: 28 April 2023

Published online: 11 May 2023

References

1. Health K, Levi J, Hill A. The Joint United Nations Programme on HIV/AIDS 95–95–95 targets: worldwide clinical and cost benefits of generic manufacture. *AIDS*. 2021;35(Supplement 2):S197.
2. Jewell BL, Mudimu E, Stover J, ten Brink D, Phillips AN, Smith JA, et al. Potential effects of disruption to HIV programmes in sub-Saharan Africa caused by COVID-19: results from multiple mathematical models. *The Lancet HIV*. 2020;7(9):e629–40.
3. Zhang G, DeVos J, Medina-Moreno S, Wagar N, Diallo K, Beard RS, et al. Utilization of dried blood spot specimens can expedite nationwide surveillance of HIV drug resistance in resource-limited settings. *PLoS One* [Internet]. 2018;13(9):e0203296.
4. Johannessen A. Dried blood spots in HIV monitoring: applications in resource-limited settings. *Bioanalysis*. 2010;2(11):1893–908. <https://doi.org/10.4155/bio.10.120>.
5. Consolidated guidelines on HIV prevention, testing, treatment, service delivery and monitoring: recommendations for a public health approach. Geneva, Switzerland: World Health Organization; 2021.
6. Sigaloff KCE, Hamers RL, Wallis CL, Kityo C, Siwale M, Iwe P, et al. Unnecessary antiretroviral treatment switches and accumulation of HIV resistance mutations; two arguments for viral load monitoring in Africa. *J Acquir Immune Defic Syndr*. 2011;58(1):23–31.
7. Roberts T, Cohn J, Bonner K, Hargreaves S. Scale-up of routine viral load testing in resource-poor settings: current and future implementation challenges. *Clin Infect Dis*. 2016;62(8):1043–8.
8. Nichols BE, Girdwood SJ, Crompton T, Stewart-Isherwood L, Berrie L, Chimhamhiwa D, et al. Impact of a borderless sample transport network for scaling up viral load monitoring: results of a geospatial optimization model for Zambia. *J Intern AIDS Soc*. 2018;21(12). <https://doi.org/10.1002/jia2.25206>.
9. Girdwood SJ, Nichols BE, Moyo C, Crompton T, Chimhamhiwa D, Rosen S. Optimizing viral load testing access for the last mile: geospatial cost model for point of care instrument placement. *PLoS ONE*. 2019;14(8):e0221586. <https://doi.org/10.1371/journal.pone.0221586>.
10. Nichols BE, Girdwood SJ, Crompton T, Stewart-Isherwood L, Berrie L, Chimhamhiwa D, et al. Monitoring viral load for the last mile: what will it cost? *J Int AIDS Soc*. 2019;22(9):e25337. <https://doi.org/10.1002/jia2.25337>.
11. Nichols BE, Girdwood SJ, Shibemba A, Sikota S, Gill CJ, Mwananyanda L, et al. Cost and impact of dried blood spot versus plasma separation card for scale-up of viral load testing in resource-limited settings. *Clin Infect Dis*. 2020;70(6):1014–20. <https://doi.org/10.1093/cid/ciz338>.
12. Qiao S, Zhang Y, Li X, Menon JA. Facilitators and barriers for HIV-testing in Zambia: A systematic review of multi-level factors. *PLoS ONE*. 2018;13(2):e0192327. <https://doi.org/10.1371/journal.pone.0192327>.
13. Laboratory Tests|HIV Testing|HIV/AIDS|CDC [Internet]. 2020 [cited 2022 Aug 27]. Available from: <https://www.cdc.gov/hiv/testing/laboratorytests.html>.

14. Organization WH. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach. World Health Organization; 2016.
15. Johannessen A, Trøseid M, Calmy A. Dried blood spots can expand access to virological monitoring of HIV treatment in resource-limited settings. *J Antimicrob Chemother.* 2009;64(6):1126–9.
16. Mbida AD, Sosso S, Flori P, Saoudin H, Lawrence P, Monny-Lobé M, et al. Measure of viral load by using the Abbott Real-Time HIV-1 assay on dried blood and plasma spot specimens collected in 2 rural dispensaries in Cameroon. *J Acquir Immune Defic Syndr.* 2009;52(1):9–16.
17. Monleau M, Montavon C, Laurent C, Segondy M, Montes B, Delaporte E, et al. Evaluation of different RNA extraction methods and storage conditions of dried plasma or blood spots for human immunodeficiency virus type 1 RNA quantification and PCR amplification for drug resistance testing. *J Clin Microbiol.* 2009;47(4):1107–18.
18. Rottinghaus EK, Ugbena R, Diallo K, Bassey O, Azeez A, Devos J, et al. Dried blood spot specimens are a suitable alternative sample type for HIV-1 viral load measurement and drug resistance genotyping in patients receiving first-line antiretroviral therapy. *Clin Infect Dis.* 2012;54(8):1187–95.
19. Schmitz ME, Agolory S, Junghee M, Broyles LN, Kimeu M, Ombayo J, et al. Field evaluation of dried blood spots for HIV-1 viral load monitoring in adults and children receiving antiretroviral treatment in Kenya: implications for scale-up in resource-limited settings. *J Acquir Immune Defic Syndr.* 2017;74(4):399–406.
20. Alvarez-Muñoz MT, Zaragoza-Rodríguez S, Rojas-Montes O, Palacios-Saucedo G, Vázquez-Rosales G, Gómez-Delgado A, et al. High correlation of human immunodeficiency virus type-1 viral load measured in dried-blood spot samples and in plasma under different storage conditions. *Arch Med Res.* 2005;36(4):382–6.
21. Andreotti M, Pirillo M, Guidotti G, Ceffa S, Paturzo G, Germano P, et al. Correlation between HIV-1 viral load quantification in plasma, dried blood spots, and dried plasma spots using the Roche COBAS Taqman assay. *J Clin Virol.* 2010;47(1):4–7.
22. Brambilla D, Jennings C, Aldrovandi G, Bremer J, Comeau AM, Cas-sol SA, et al. Multicenter evaluation of use of dried blood and plasma spot specimens in quantitative assays for human immunodeficiency virus RNA: measurement, precision, and RNA stability. *J Clin Microbiol.* 2003;41(5):1888–93.
23. Mwau M, Mbugua J, Danda J, Handa A, Worlock A, Kim HC, et al. Performance evaluation of the Aptima HIV-1 Quant Dx assay for detection of HIV in infants in Kenya. *J Clin Virol.* 2020;125:104289.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

