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Engineering a Thermostable Reverse Transcriptase from a Mozambican HIV-1 Isolate for Point-of-Care Viral Load Monitoring in South Africa: A Systematic Review

P, i, e, t, e, r, B, o, t, h, a, ,, L, e, r, a, t, o, N, k, o, s, i, ,, T, h, a, n, d, i, w, e, M, b, e, k, i, ,, J, a, m, e, s, v, a, n, d, e, r, M, e, r, w, e

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Abstract

Viral load monitoring is essential for managing HIV treatment in South Africa, but laboratory-based quantification remains inaccessible in many resource-limited settings. Robust, thermostable enzymes are a core requirement for point-of-care molecular diagnostics. The reverse transcriptase from a Mozambican HIV-1 isolate is a candidate for engineering due to its inherent stability and regional relevance. This systematic review aimed to synthesise and critically appraise published evidence on engineering thermostable reverse transcriptases, particularly from African HIV-1 isolates, for application in point-of-care viral load monitoring devices suitable for South Africa. A systematic search of multiple electronic databases was conducted following a pre-defined protocol. Studies reporting on the genetic engineering, thermostability enhancement, or performance characterisation of HIV-1 reverse transcriptases were included. Data were extracted and the quality of evidence was assessed using appropriate tools for laboratory-based studies. Directed evolution and rational design were the predominant strategies for enhancing reverse transcriptase thermostability. A consistent theme was the observed trade-off between improved thermal stability and enzymatic activity. Specific point mutations, particularly in the connection subdomain, were frequently associated with increased heat tolerance. No studies were identified that specifically engineered a reverse

transcriptase from a Mozambican HIV-1 isolate for point-of-care diagnostics. While significant progress exists in engineering thermostable reverse transcriptases, a targeted research gap remains regarding developing such enzymes from regionally prevalent African HIV-1 clades. The potential of a reverse transcriptase derived from a Mozambican isolate is unexplored. Future research should prioritise the isolation, characterisation, and engineering of the reverse transcriptase from the Mozambican HIV-1 strain. Collaborative efforts between structural biologists and diagnostic engineers are needed to develop a fit-for-purpose enzyme for low-cost, heat-tolerant point-of-care devices in southern Africa. HIV-1, reverse transcriptase, thermostability, protein engineering, point-of-care diagnostics, viral load monitoring, South Africa, Mozambique. This review consolidates the methodological landscape for engineering thermostable reverse transcriptases and identifies a specific gap concerning regionally relevant African isolates, thereby directing future research towards developing context-appropriate diagnostic tools for HIV management.