Truenat® for *Plasmodium* sub-microscopic infections: Miles to go. . .

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Although Shankar and Kumar suggested Truenat® for *Plasmodium* sub-microscopic infections (SMIs)[1] but it is not backed by compelling evidence[2,3] due to certain limitations:

(1) **Single-copy target**: Truenat® relies on detection of *P. falciparum* (Pf) and *P. vivax* (Pv) single-copy Erythrocyte Binding Protein gene which might compromise comparable limit of detection (LoD) with nPCR based on multi-copy 18s rRNA. The chances of false-negative results could be higher with single-copy target due to spontaneous mutations in the target.

(2) **Comparison with nPCR**: Nair and colleagues did not compare Truenat® with nPCR on patients' samples, but rather compared it with microscopy/RDT on febrile patients' samples.[2] They did compare Truenat®-tested samples with nPCR but the reference cited described RDTs instead of nPCR.

(3) **LoD:** Truenat®'s performance was validated using only 10 each of Pf and Pv patients' samples for concurrence with nPCR but its species-specific LoD has not been estimated.[3] Further, the amount of blood/DNA used for nPCR was not mentioned which imparts ambiguity while comparing concurrence.

(4) **Estimation of LoD**: The LoD for *Plasmodium* by PCR can only be determined using accurate count of parasites/microliter of whole blood.[4,5] The published LoD of Truenat® is 4.7 parasites/μL and 10 genome-equivalents per PCR reaction for Pv.[2] It is unclear how exactly were the dilutions prepared, which diluent was used and how the LoD was estimated. Moreover, according to Truenat® pack-insert sheet, the LoD of Pf/Pv was estimated using plasmid DNA. Using WBC-depleted blood/plasmid DNA for LoD and then extrapolating it on patients' blood might be erroneous as the majority of DNA from a malaria patient's blood is derived from the host WBCs rather than the parasite and is therefore, not comparable.

(5) **Mixed infections**: Nair et al., did not estimate the LoD of mixed PfPv using Truenat® and nPCR. Because mixed *Plasmodium* infections pose significant diagnostic challenges, it is critical for any new technique to be at least equi-sensitive to mono- and mixed-infections.[2] Furthermore, Truenat® can only detect Pf and Pv against nPCR that can detect all species.

Truenat® appears to be more sensitive than microscopy/RDT but when compared with nPCR, it seems that Truenat® has miles to go, particularly for screening SMIs. Further, Nair et al. had strong competing interests: 6/10 authors being employees of the technology developer which also funded the study.[2] Therefore, Truenat®'s performance must undergo unbiased evaluation on sufficient number of febrile patients and results replicated in varied malaria settings.

Contributors

ND: Original draft of the manuscript, Data analysis, Interpretation, Editing the manuscript critically; AS: Conceptualization, Data interpretation, Drafting the manuscript and revising it critically.

Declaration of interests

None.

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References

1 Shankar H, Kumar G. Truenat: an affordable and user-friendly option for screening of sub-microscopic *Plasmodium* infections in low resource countries. *Lancet Reg Health - Southeast Asia*. 2022:100032. ISSN 2772–3682.
2 Nair CB, Manjula J, Subramani PA, et al. Differential diagnosis of malaria on Truelab Uno®, a portable, real-time, microPCR device for point-of-care applications. *PLoS One*. 2016;11(1):e0146961.
3 Hussian SS, Ojha VP, Jeena M, et al. Validation of micro-chip based PCR assays for diagnosis of both *Plasmodium* falciparum and *Plasmodium* vivax. *J Vector Borne Dis*. 2022;59(1):57.
4 World Health Organization. Meeting report of the WHO evidence review group on low-density malaria infections. *Malaria policy advisory committee meeting, WHO/HTM/GMP/MOAC/2017/10*. 2018.
5 World Health Organization. Global malaria program. *Policy brief on malaria diagnostics in low-transmission settings*. 2014.

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