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HIGH-THROUGHPUT HIV-1 GENOTYPING IN THE ERA OF RECOMBINATION: A SECOND-GENERATION MULTI-REGION HYBRIDIZATION ASSAY FOR SOUTHEAST ASIA

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INTRODUCTION: Southeast Asian HIV-1 epidemics are rapidly evolving and increasing in complexity. In Thailand, subtype B, CRF01_AE, CRF15_01B, and unique B/E recombinants are found. Complex epidemics from neighboring countries, where subtypes B, C, CRF01_AE, CRF07_BC, CRF08_BC, and unique recombinant forms (URFs) co-circulate, are also likely to have an impact. The analysis of large cohorts is crucial to define this genetic heterogeneity. However, full-genome sequencing (FGS) cannot be practically applied to large sample sets. Here we present the development and field test of a second-generation multi-region hybridization assay (MHA) for Southeast Asia: MHAbce_v2.

METHODS: MHAbce_v2 is the latest member of the MHA family, assays with specific geographic areas of application: MHAacd for East Africa, MHAcrf02 for West/West Central Africa, and MHAbf for South America. A previous version of MHAbce was revisited to accommodate for the increasing complexity of epidemics in the region. MHAbce_v2 can use primary PBMC or plasma/serum as starting materials and is based on the amplification of 8 HIV-1 genomic regions and subsequent genotyping with clade-specific TaqMan probes in real-time PCRs. Robotic nucleic-acid extraction and the use of 384-well plates allow for the processing of 100 samples/week.

RESULTS: MHAbce_v2 was validated on a panel of sequenced full-genome HIV-1 amplicons (45 CRF01_AE, 11 subtype B, 5 subtype C, 1 CRF08_BC, 4 CRF15_01B, 12 B/E URFs, 1 B/C URF, and 1 E/C URF). The assay performed with high sensitivity and specificity, discriminating the different recombinant forms. MHAbce_v2 was then field-tested with plasma samples from a mother-to-child transmission study (Lampang, Thailand, years 1996-98). HIV-1 from 179 mothers was analyzed, and 100% could be genotyped. 171 samples were CRF01_AE, 4 subtype B, 3 B/E URFs, and 1 BCE URF. Non-CRF01_AE samples were confirmed by FGS.

CONCLUSIONS: MHAbce_v2 is a sensitive and specific tool, suitable for studying large HIV-1 cohorts in Southeast Asia, allowing for the real-time monitoring of dynamic epidemics.

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