Along with the expanding availability of antiretroviral drugs in Africa, genotypic HIV drug resistance testing (GRT) is highly advisable to monitor for development and transmission of HIV drug resistance. However, GRT is seldom feasible in most African laboratories due to unavailability of the equipment and expertise required to complete the lengthy and relatively complex GRT process.

As part of the activities planned within the EDCTP project “Integrated training activities and IT infrastructures to improve capacities in Eastern African area” (EDCTP JP.2009.10800.002), coordinated by Anders Sonnerborg (Karolinska Institute, Stockholm, Sweden), we evaluated a strategy for splitting GRT between a local and a remote site. The feasibility of such a strategy was directly tested in conjunction with the first laboratory training course scheduled.

METHODS

Two to four trainees for each of the African sites included in the project (Dar Es Salam, Nairobi, Addis Ababa) were selected based on their previous laboratory experience and interest in gaining GRT skills. To tailor the course appropriately, a pre-course questionnaire was filled by each trainee to provide information about her/his past laboratory activities and knowledge in the field of HIV drug resistance.

The course was kindly hosted at the Kenya Medical Research Institute in Nairobi and spanned five full-time days. The activities were facilitated by two trainers, one coordinator and one GRT-qualified laboratory worker, both from the Department of Biotechnology of the University of Siena, Italy. The first day was dedicated to background information about (i) HIV drug resistance, (ii) GRT and (iii) setting up a laboratory for GRT. The other four days were all laboratory intensive sessions, first run by the trainers and next run by the trainees under the supervision of the trainers.

The split GRT design was to run locally the procedure from plasma RNA extraction to generation of the amplified HIV protease/reverse transcriptase segment to be sequenced remotely. Once established, this strategy can be a valid and likely more effective strategy than GRT on dried blood spots (DBS), an approach showed to lack sensitivity in most studies and still requiring shipment of infectious material.

The RT-PCR protocol used seems to be robust and effective in recognizing HIV strains from Kenya. However, since viral load data on the samples tested were not available a specific study must be set up to examine the sensitivity of the method both in terms of detection of divergent HIV strains and in terms of amplification from low-level viremia samples.

RESULTS

The course was attended by ten trainees. Their background experience spanned different areas, mainly including serology and parasitology. Only two trainees had some experience with molecular techniques. The facilities kindly offered at KEMRI included laboratories and laboratories mainly including serology and parasitology. Only two trainees had some experience with molecular techniques. The facilities kindly offered at KEMRI included laboratories and

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In the first and second run, 7/10 and 6/10 samples were successfully amplified. Viral load data for the samples analysed were not available but current treatment information was available. Overall, 7/7 amplification failures vs. only 2/13 successful amplifications were derived from patients under treatment suggesting that most failures could be due to low or undetectable viral load.

Sequencing of the PCR products carried out at Italy at room temperature and stored at +4°C for additional two weeks worked fine. The complete protease region and the reverse transcriptase region coding for amino acids 1-300 were obtained with quality values comparable to the standard procedures run on fresh samples.