

HIV drug resistance genotyping external quality assurance (EQA) for laboratories in the World Health Organization (WHO) ResNet during 2007-2009.

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BACKGROUND

The WHO has developed a global laboratory network to support HIV drug resistance genotyping in resource-limited countries. A QA program is being implemented to ensure the reliability of genotyping data generated by the various laboratories. Proficiency panels were developed in collaboration with NIH and sent to 58 network member laboratories in Europe, North America, Asia, Africa and the Caribbean during 2007-09. Appropriate and widely accepted evaluation criteria have not been described previously.

METHODS

Each of 3 panels was composed of 5 samples (including subtypes A, B, C, D, F, CRF01_AE, CRF02_AG) with viral load between 3500-57,000 copies/ml, and was distributed to 21-26 labs each year. Consensus sequences (PR codons 10-99 and RT 38-240) for each sample were generated based on >80% concordance across participating labs; individual test results were compared to that sample's consensus sequence. An overall sequence identity score as well as concordance at drug resistance-associated mutation (DRM) codons were used to assess lab performance.

RESULTS

Overall, 41 of 58 submissions from 2007-08 passed using the 99% criteria. Specific reasons for failure included: editing errors leading to frameshifts, missing sequence, amplification failure, and a low concordance rate in codons with mixed bases in the consensus. Amplification failure was often related to primer-target sequence differences related to viral subtype. Samples with naturally-occurring mixtures at DRM sites were more challenging than others. For example, no lab obtained >99% concordance for sample 4 in panel 1 used in 2007 (median 98.6%); for sample 4 in panel 2 used in 2008, (subtype B, DRMs for all 3 drug classes) only 18 of 33 labs reported sequence with >99% concordance, compared to 26 to 30 labs for the other 4 samples. Results from 2009 panel 3 will also be reported.

CONCLUSIONS

The use of a single and stringent criteria (such as 99%) for evaluation of sequence-based assays may be unrealistic when using clinical samples containing mixed bases at several positions. Acceptance criteria may need to be relaxed for such samples (e.g. 98% identity), or be flexible and based on the number of mixed positions in each sample.