BACKGROUND

- The WHO has developed a global laboratory network to support HIV drug resistance genotyping in resource-limited countries.
- An external QA program is being implemented to ensure the reliability of genotyping data generated by the various laboratories.
- Three proficiency panels were developed in collaboration with Viagena and WHO and sent to 174 network members or candidate laboratories in Europe, North America, Asia, Africa and the Caribbean during 2007-2009.
- Appropriate and widely accepted evaluation criteria have not been described previously.

METHODS

- Each panel was composed of 5 clinical samples:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Lab</th>
<th>Outcome</th>
<th>Mix</th>
<th>Concordance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>30</td>
<td>Yes</td>
<td>Mixed in consensus</td>
<td>99.9%</td>
</tr>
<tr>
<td>Sample 2</td>
<td>30</td>
<td>No</td>
<td>Consensus</td>
<td>99.9%</td>
</tr>
<tr>
<td>Sample 3</td>
<td>30</td>
<td>No</td>
<td>Consensus</td>
<td>99.9%</td>
</tr>
<tr>
<td>Sample 4</td>
<td>30</td>
<td>No</td>
<td>Consensus</td>
<td>99.9%</td>
</tr>
<tr>
<td>Sample 5</td>
<td>30</td>
<td>No</td>
<td>Consensus</td>
<td>99.9%</td>
</tr>
</tbody>
</table>

- Consensus sequences (PLA codons 10 to 99 and RT: 30 to 1144) for each sample were generated based on 90% concordance of results from >30 labs and all platforms.
- Individual test results were compared to that sample consensus sequence. If >90% concordance is not achieved at any position, an “N” is inserted in the consensus sequence and is not considered in the scoring.
- An overall sequence identity score as well as concordance at Drug Resistance Mutation (DRM) codons (as defined by IAS-USA) were used to assess lab performance.
- Overall, 25% of labs did not report the DRM site score for all samples.
- These evaluation methods and criteria differ from those used by the VQA.

RESULTS

- Overall, 72 of 93 submissions passed (78%).
- Success rates were higher with panel 1 (83%) than panel 2 (64%).
- Scoring criteria were adjusted for panel 3 (14% success rate)
- Specific scores for failure include:
  - Editing errors leading to frameshifts
  - Multiple sequence (primer design, subtemplate)
  - Amplification failure (primer design, subtemplate)
  - Cross contamination or pass-proliferating the saving errors
- Low concordance rate in positions with mixed bases in the consensus.
- Samples with naturally-occurring mixtures were more challenging than others (Table 7 & 8).
- Also obtained a nucleotide alignment score (%N for sample 4 to panel 1 (this sample’s consensus sequence had the most mismatches in Table 7 and 8). For sample 4 to panel 2, only 19 of 31 labs reported sequence with >99% concordance, compared to 26 to 30 out of 31 for the other 4 samples.
- Based on comparison of chromatogram data from several labs (Figures 9 and data not shown), subjectivity in base-calling and PCR amplification losses (‘framer effect’) can contribute to lower reproducibility when mixtures are present.

CONCLUSIONS

- The use of a single and stringent criteria (e.g. <99%) for evaluation of sequence-based assays may be unrealistic when using clinical samples containing mixed bases.
- Acceptance criteria may need to be relaxed for such samples, or be flexible and based on the number of mixed positions in each sample.
- These observations led to revised assessment criteria for 2009 proficiency panels:

- Discrepancies to be counted in the DRM site score:
  - Mixed: if mutant in consensus, lab report mutant
  - Consensus: <97% RT –<75% ACT
  - Mixed in consensus: report the compatible base, no change in amino acid
  - Consensus: <97% RT –<75% ACT
- Unmixed in consensus, lab report a mixture containing the consensus base, no change in amino acid
  - Consensus: <97% RT –<75% ACT
  - Acceptance criteria: DRM and sequence alignment score (%N), considering only positions mismatched in consensus, >99%.

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