PROGRAMME DESCRIPTION:
HIV-1 GENOTYPIC DRUG RESISTANCE EXTERNAL QUALITY ASSESSMENT SCHEME

PROGRAMME CODE: HIVG425
The NRL is a:
- NATA-accredited proficiency testing provider, complying with ILAC-G13:2000
- World Health Organisation (WHO) Collaborating Centre for Diagnostics and Laboratory Support for HIV and AIDS and Other Blood-borne Infections

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1. INTRODUCTION

The evolution of HIV drug resistant strains within an individual depends on the genetic variation of HIV-1 which drives selection of antiretroviral (ARV)-resistant variants during therapy. Certain mutations, alone or in combinations in the viral genome, reduce ARV susceptibility compared with that of wild-type viruses.

HIV Genotypic Resistance Testing (HIV genotyping) is a complex, multi-layered test involving genetic sequencing of the viral genome, editing of the genetic sequence, the detection of drug resistance mutations (DRMs) that confer ARV resistance, and the interpretation of drug resistance to a list of ARV drugs. It is not uncommon for participants to use a variety of commercial or in-house, assembled methods to generate genetic sequence and different software programs to edit the genetic sequence (Huang, 2003). Furthermore, the edited genetic sequence generated by software programs may often require manual, subjective assessment by laboratory personnel when sequence quality is poor, or when two or more nucleotide bases are present at one position, indicating a mixed population of wild type and mutant virus. Frequently this is the case when drug resistance has emerged in the setting of decreased access to drug or diminished adherence. The correct identification of nucleotide mixtures that occur at positions associated with ARV resistance is important because these reflect the evolution of resistant virus under the pressure of ARV therapy or conversely the re-emergence of sensitive virus when drug pressure is removed. Participants use different systems to interpret ARV drug resistance from the genotypic data and, in addition, may amend the level of drug resistance from that generated by the interpretation system in the light of other evidence they consider significant (Huang, 2003).

The complicated nature of the assay, the variety of testing procedures between participants, and the possibility of subjective components in the testing process combine to create the potential for intra- and inter-laboratory variation in the reporting of HIV genotyping (Erali, 2001; Galli, 2003; Sayer, 2003; Schuurman, 1999 & 2002; Schafer, 2001).

External quality assessment schemes (EQAS) are designed to assess the accuracy and reliability of test outcome, detect problems in participants’ testing procedures, and to identify, and subsequently minimize, discrepancies between participants’ results (Fulisaki, 2007; Quint, 1995; Schirm, 2002). Participation in EQAS involves participants processing a panel of samples using their standard protocols and reporting results for analysis to the EQAS provider. Regular participation in EQAS can maintain and, if necessary, improve the quality of laboratory diagnosis by detecting testing problems, and deficiencies and disparities in and between participants’ processes (Evans, 1987; Salkin, 1997; Valentine-Thon, 2001; Word, 1997). Thereby, on-going
participation in EQAS facilitates standardisation of test outcomes across participating laboratories.

2. METHODS

Panels in the National Serology Reference Laboratory, Australia’s (NRL’s) HIV-1 Drug Resistance EQAS are shipped twice a year to participants. Participants report results of testing the panel for comparative analysis.

The analysis includes:

- alignment of edited nucleotide sequences returned by participants against a consensus (target genotype) sequence; and

Assessment of:

- inter-laboratory agreement at the level of nucleotide sequencing and editing;
- the ability of participants to detect nucleotide mixtures (NMs) and drug resistance mutations (DRMs) considered to have been associated with drug resistance in the HIV-1 protease (PR) and reverse transcriptase (RT) genes;
- the reporting of ARV susceptibility and
- the participant’s ability to perform HIV genotyping on non-B subtypes.

Panels consist of five samples of either HIV-1 virus or plasma sourced from HIV-1-infected, ARV-treated or -naive individuals, sets of electropherograms derived from clinical material that span the PR and RT regions of the HIV genome, or raw nucleotide sequences and/or edited nucleotide sequences derived from clinical material.

2.1 Nucleotide Sequence Alignments

Participants’ nucleotide sequences are aligned to see if and where differences occur. The alignments can be accessed in the Members’ section of the NRL Web site (www.nrl.gov.au).

2.2 Sequence analysis

The edited nucleotide sequences submitted by the participants are analysed to determine the:

- agreement of each edited nucleotide sequence across the entire region sequenced by comparing it with the target genotypes (TGs) [see definition below]. The analysis takes into account the differences in the length of nucleotide sequence reported by participants and considers partial and complete differences. Partial differences predominantly occur when the participant reports a single nucleotide at a position that is defined as a NM (2 or more nucleotides existing at the same position) in the TG. Partial differences also occur when the participant reports a NM that is different from that defined in the TG, e.g. the TG
is a mixture of C and T, and the participant reports a mixture of A and T. Complete differences occur when the participant reports a nucleotide that is different from that defined in the TG, e.g. the participant reports G and the TG is C

- DRMs, as determined by comparison with the TGs, identified and
- ability of each participant to detect NMs of wild type and mutant when compared with those deduced in the TGs.

Statistical comparisons are made using Chi squared and Pearson’s Correlation Coefficient.

### 2.2.1 Target genotype

To compare the edited nucleotide sequences generated by participants, a TG was generated for each sample. The TG represented the most likely consensus sequence for each sample. The TG was deduced by aligning the edited nucleotide sequence reported by each participant and choosing the majority result as described in Table 1 (Sayer, 2003).

**Table 1. An example of how a target genotype is deduced.**

<table>
<thead>
<tr>
<th>Laboratory ID</th>
<th>Nucleotide at positions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>A</td>
</tr>
<tr>
<td>2</td>
<td>A</td>
</tr>
<tr>
<td>3</td>
<td>A</td>
</tr>
<tr>
<td>4</td>
<td>A</td>
</tr>
<tr>
<td>5</td>
<td>A</td>
</tr>
<tr>
<td>6</td>
<td>A</td>
</tr>
<tr>
<td>7</td>
<td>A</td>
</tr>
<tr>
<td><strong>Target genotype</strong></td>
<td>A</td>
</tr>
</tbody>
</table>

1 If a single participant sequenced a different nucleotide from that sequenced by the other participants, either as a partial or as a complete difference, then the target genotype (TG) is the consensus nucleotide sequenced by the other participants (e.g. Table 1 positions 2 and 3: TG = AA). If two or more participants reported an identical nucleotide which was different from the nucleotide reported by the other participants, the TG is a mixture of the two different nucleotide sequences reported (e.g. Table 1 positions 4 and 5: TG = RY). If two participants reported nucleotides different from those reported by the other participants and different from those reported by each other, the TG is the sequence reported by the majority (e.g. Table 1 position 6: TG = T). However, if two participants reported sequences different from those reported by the majority and one is a mixture that is present and contains the sequence reported by the majority and the other participant, the TG is the mixture (e.g. Table 1 position 7; TG = W).

### 2.3 Comparison of DRMs and ARV susceptibility profiles

The DRMs reported by each participant to have contributed to ARV resistance and the participant’s interpretation of ARV susceptibility to the given list of drugs are compared.
2.4 Methods used by participants to interpret ARV susceptibility from genotypic data

Participants may use different interpretation methods to infer ARV susceptibility from the genetic sequence.

The Stanford Database (SD) (http://hivdb.stanford.edu/pages/algs/HIVdb.html) and other systems are available on the Internet. Drug resistance to ARV drugs can be inferred using rules hyperlinked to data within the database. When using this facility participants submit nucleotide sequences online and a list of DRMs and the associated ARV susceptibility profile is generated.

Other systems are available. Commercial assays have been developed for HIV genotyping and they include software that generates a list of DRMs and an interpretation of susceptibility to a list of ARV drugs; for example Trugene HIV-1 Genotyping System (Siemens) and ViroSeq HIV-1 genotyping kit (Abbott).

3. RESULTS

Reports of the analyses of the EQAS results are available on the NRL Web site (www.nrl.gov.au).

Participants should inspect the nucleotide sequence alignments to see if their alignments differ from those of other participants. The alignments can be accessed in the Members’ section of the NRL Web site (www.nrl.gov.au). Interpretation of edited nucleotide sequences from the original chromatograms should be reviewed where differences are noted.

In the reports the DRMs and interpretation of ARV drug susceptibility returned by the participants are presented in Appendix A. The analysis of these data is presented in Appendix B. In addition participants are provided with an individualised summary of their test results. These are compared with those reported by the other participants, including the detection of DRMs and interpretation of ARV susceptibility.

Participants should examine the report and note differences or deficiencies in their laboratory’s performance. They should also inspect the nucleotide sequence alignments to determine if their alignments differed from those of other participants. The alignments can be accessed in the Members’ section of the NRL Web site (www.nrl.gov.au).
4. ACKNOWLEDGEMENTS

The NRL thanks the participants of this HIV-1 Genotypic Drug Resistance EQAS and hopes the programme is a useful tool for participants in assessing the performance of their testing process. We thank Dr David Sayer, (Conexio Genomics, Fremantle, Australia) for providing on-going expert analysis for this EQAS. The NRL welcomes your feedback. Comments or problems should be directed to Sally Land on 03 9418 1105, email: sally@nrl.gov.au.

5. REFERENCES


