

The National Laboratory for HIV Reference Services, Ottawa, ON



NATIONAL HIV RESISTANCE TESTING EXTERNAL QUALITY ASSESSMENT PROGRAM

Report on Testing

Panel A-02

(May, 2003)



British Columbia
Centre *for* Excellence
in HIV/AIDS

- **Introduction**

The objective of the NLHRS HIV-1 Resistance Testing External Quality Assessment Program is to enable clinical, reference and research laboratories to monitor performance and competence of HIV genotypic resistance testing. The goal of the program is to ensure a high degree of quality assurance across testing sites, and to standardize the outcome of testing within laboratories.

In November of 2002, panel A-02 was sent out to laboratories for testing by resident genotypic methods. The nucleotide and amino acid sequences returned by the laboratories are compared in this report. The report will allow participants to compare their performance with other laboratories.

- **Methods**

Ten laboratory sites tested EQA Panel A-02: nine in Canada (BC, AB, ON, Que) and one in the US. Four sites used “in-house” methods, three sites used TRUGENE HIV-1 kits (Visible Genetics Inc./Bayer Toronto), one site used both in-house and TRUGENE, and one site used the ViroSeq HIV-1 kit (ABI/Abbott). EQA Panel A-02 was composed of five undiluted, unadulterated plasma samples: 4 plasma samples collected from ARV-experienced patients, plus 1 sample from a non-infected patient used as a negative control.

Each laboratory was requested to:

- Sequence the reverse transcriptase (RT) and protease (PR) regions of the genome, in both directions, using the primers and protocols that the laboratory routinely follows for HIV genotyping.
- Analyze and assemble the final text sequences using the laboratory’s standard protocol.

Each laboratory was requested to submit simple text files containing the nucleotide sequence of each sample for analysis to the BC Centre for Excellence in HIV/AIDS. A consensus reference sequence (CRS) for PR codons 4-99 and RT codons 38-247 (918 total nucleotide bases) was assembled for each isolate based on >50% agreement between all sites at each position. IUPAC symbols were used to identify mixtures of nucleotides at one position: W=A+T, R=A+G, Y=C+T, M=A+C, K=G+T, S=G+C.

2. Sequence Analysis

Sequences were analyzed to determine the level of concordance between laboratories at all: (a) nucleotide and (b) amino acid positions. Note that the association between amino acid discordance and key-resistance associated mutations was not examined in this panel. Furthermore the interpretative criterias

currently in use were also not examined. These parameters will be examined in future panel sendouts.

3. Results

3.1 Nucleotide and Amino Acid Sequence Analysis

All laboratories successfully sequenced the 4 isolates from HIV-infected, ARV experienced patients. All labs correctly identified the negative sample (DR02). Most laboratories determined the complete sequence of the PR gene (codons 1-99). Laboratory site **DR-08a** reported PR sequences of variable length, with the beginning codon ranging between codon 4 and codon 10. Laboratories reported different lengths of RT sequence. Although the analysis of concordance was conducted on the RT sequence that was reported by all laboratories (codon 38-247), laboratory site **DR-10** reported RT sequences that finished between codon 242 and codon 247. These omissions were not considered as discrepancies.

The nucleotide consensus sequences generated by the group for the protease and reverse transcriptase genes can be found in Appendices 2 and 3 respectively

3.1.1 Nucleotide Concordance

Results are summarized in Table 1 by individual samples and as a group.

Overall, of the total of 36,665 nucleotides compared (4 samples, 918 bases per sample, 10 sites, minus sequence gaps as noted in 3.1) there were 357 discrepant nucleotide results reported, for a concordance of 99.0%.

Discrepant nucleotides are either “partial” or “complete”. A partial discrepancy is when a laboratory or the CRS reports a mixture of nucleotides at one position and others report only one of the nucleotides present in the mixture, eg Y ©+T) vs C. A complete discrepancy is when a laboratory reports a nucleotide that is completely different from the CRS, eg A vs G. Of the 357 discrepant nucleotides, 311 (87.1%) were partial discrepancies and 46 (12.9%) were complete. Only three laboratory sites reported complete discrepancies: site **DR-12** reported 24 complete discrepancies, site **DR-10** reported 21, and site **DR-08a** reported 1. All laboratory sites reported partial discrepancies with the consensus sequence.

As commonly practiced in other proficiency testing programs, labs displaying a higher than expected level of discordance are contacted during the data analysis and report preparation stages. Labs DR-10, DR-11 and DR-12 were independently contacted regarding their data submissions. Further inquiry and reanalysis of only their data revealed a significant improvement in their individual level of concordance. Not unexpectedly this also resulted in an improvement in the overall group concordance level as shown in Table 3 of the Appendix.

Table 1: EQA A-02 SAMPLE nucleotide concordance to consensus sequences, by site.

SAMPLE DR01

Laboratory Site	Total Discordance	Complete Discordance	Partial Discordance	Site Concordance
DR-03	4	0	4	99.6
DR-06	4	0	4	99.6
DR-07	2	0	2	99.8
DR-08a	5	0	5	99.5
DR-08b	2	0	2	99.8
DR-09	16	0	16	98.3
DR-10	28	8	20	96.9
DR-11	11	0	11	98.8
DR-12	38	19	19	95.9
DR-13	1	0	1	99.9
TOTAL	111	27	84	
MEAN	11.1	2.7	8.4	98.8

SAMPLE DR03

Laboratory Site	Total Discordance	Complete Discordance	Partial Discordance	Site Concordance
DR-03	2	0	2	99.8
DR-06	3	0	3	99.7
DR-07	5	0	5	99.5
DR-08a	6	0	6	99.3
DR-08b	1	0	1	99.9
DR-09	3	0	3	99.7
DR-10	13	1	12	98.6
DR-11	57	0	57	93.8
DR-12	10	1	9	98.9
DR-13	5	0	5	99.5
TOTAL	105	2	103	
MEAN	10.5	0.2	10.3	98.9

SAMPLE DR04

Laboratory Site	Total Discordance	Complete Discordance	Partial Discordance	Site Concordance
DR-03	0	0	0	100.0
DR-06	0	0	0	100.0
DR-07	1	0	1	99.9
DR-08a	3	1	2	99.7
DR-08b	1	0	1	99.9
DR-09	2	0	2	99.8
DR-10	5	1	4	99.5
DR-11	3	0	3	99.7
DR-12	4	1	3	99.6
DR-13	2	0	2	99.8
TOTAL	21	3	18	
MEAN	2.1	0.3	1.8	99.8

SAMPLE DR05

Laboratory Site	Total Discordance	Complete Discordance	Partial Discordance	Site Concordance
DR-03	2	0	2	99.8
DR-06	10	0	10	98.9
DR-07	0	0	0	100.0
DR-08a	5	0	5	99.5
DR-08b	3	0	3	99.7
DR-09	2	0	2	99.8
DR-10	32	14	18	96.5
DR-11	55	0	55	94.0
DR-12	9	0	9	99.0
DR-13	2	0	2	99.8
TOTAL	120	14	106	
MEAN	12	1.4	10.6	98.7

Total Nucleotide Concordance

Laboratory Site	Total Discordance	Complete Discordance	Partial Discordance	Site Concordance
DR-03	8	0	8	99.8
DR-06	17	0	17	99.5
DR-07	8	0	8	99.8
DR-08a	19	1	18	99.5
DR-08b	7	0	7	99.8
DR-09	23	0	23	99.4
DR-10	78	24	54	97.9
DR-11	126	0	126	96.6
DR-12	61	21	40	98.3
DR-13	10	0	10	99.7
TOTAL	357	46	311	
MEAN	35.7	4.6	31.1	99.0

- a VGI TRUGENE method, at site DR-08
- b In-house method, at site DR-08.

Values that differ significantly from the mean are shown in red.

Three laboratory sites reported a combined total of 46 complete discordant nucleotide calls, while the remaining sites reported no discrepancies. Site DR-12 reported 19 single nucleotide gaps in sample DR01, which were scored as complete discordance.

A total of 87.1% (311/357) of the discrepancies were the result of mixtures. Sites DR-10, DR-11 reported significantly more mixtures in samples DR01, DR03, DR05 than any other site.

3.1.2 Amino Acid Concordance

Since not all nucleotide discrepancies result in amino acid changes, Table 2 summarizes the discordant amino acids reported by each site. Note that discordant amino acids leading to differences in key resistance associated mutations are not presented as this was not the intent for this first panel. Future panels will examine the interpretive criterias used to assign resistance associated mutations.

Table 2: EQA A-02 amino acid concordance to consensus sequences, by site.

Laboratory Site	Total aa Discordance	Complete Discordance	Partial discordance	Site Concordance
DR-03	4	0	4	99.7%
DR-06	6	0	6	99.5%
DR-07	3	0	3	99.8%
DR-08a	6	1	5	99.6%
DR-08b	4	0	4	99.7%
DR-09	9	0	9	99.3%
DR-10	29	13	16	97.6%
DR-11	74	0	74	93.9%
DR-12	27	19	8	97.8%
DR-13	5	0	5	99.6%
Total	167	33	134	
Mean	16.7	3.3	13.4	98.6%

As with nucleotides, most of the discrepancies were partial (80.2%, 134/167). Overall site concordance was 98.6% (range 93.9% - 99.8%). Sites **DR-10** and **DR-12** reported 32 out of the total of 33 complete amino acid discordances. **DR-12** reported sequence gaps, which may be an indication of problems encountered in data export rather than with final sequence alignment.) Sites **DR-10** and **DR-11** reported significantly more amino acid mixture discrepancies than other sites. All 19 complete discrepancies reported by site **DR-12** resulted from nucleotide gaps in the submitted sequence for one sample.

Conclusions

- Laboratories use different assay methods and report on different sequence lengths for the RT gene.
- There was generally high concordance in nucleotide sequences reported by the different laboratory sites. Mean 99.0% [96.6-99.8 %]. The majority of differences were due to partial discordances and may reflect differences in the criteria used by the different DNA sequencing technologies in assigning bases in the presence of mixtures.
- Three laboratory sites initially reported higher than average discrepancies. After reanalysis of data from these three labs was performed, it became clear that problems with data management and export was a major factor underlying the higher-than-expected discordance initially seen in these labs. Total discordance was reduced to 18.1 [7-45] after reanalysis which was attributable to reductions in complete, 0.3 [0-2] and partial, 17.8 [7-43] discordance. Overall site concordance would have improved to 99.5 % (vs 99.0%) after this reanalysis (Table 3 of the Appendix)

Acknowledgments

This report was in large part prepared by the British Columbia Center for Excellence in HIV/AIDS in conjunction with the National Lab for HIV Reference Services of Health Canada. In particular the contributions of Rick Galli, Brian Wynhoven and Dr. Richard Harrigan are acknowledged and appreciated.

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Appendix-1

**Table 3. EQA A-02 SAMPLE nucleotide concordance to
consensus sequences, by site
(REVISED)**

Laboratory Site	Total Discordance	Complete Discordance	Partial Discordance	Site Concordance
DR-03	8	0	8	99.8
DR-06	17	0	17	99.5
DR-07	8	0	8	99.8
DR-08a	19	1	18	99.5
DR-08b	7	0	7	99.8
DR-09	23	0	23	99.4
DR-10	(45) 78	(2) 24	(43) 54	(98.8) 97.9
DR-11	(22) 126	(0) 0	(22) 126	(99.4) 96.6
DR-12	(22) 61	(0) 21	(22) 40	(99.4) 98.3
DR-13	10	0	10	99.7
TOTAL	(181) 357	(3) 46	(178) 311	
MEAN	(18.1) 35.7	(0.3) 4.6	(17.8) 31.1	(99.5) 99.0

* (N) = data and calculation after reanalysis of data submission from labs DR-10, DR-11 and DR-12

Appendix-2
HIV-1 Protease Consensus Sequences

>Sample DR 01-PR

**ACTCTTTGGCAGCGACCCCTCGTCACAATAAAGGTAGGGGGACAATAAA
GGAAGCCCTATTAGATACAGGAGCAGATGATACAGTACTAGAAGATATAA
ATTTGCCAGGAAAATGGAAACCAAAAATGATAGGGGGAATTGGAGGTTTT
ATCAAAGTAAGACAGTATGATCAARTACCCATAGACATCTGTGGGCATAA
AGCTGTAGGTACAGTATTAGTAGGACCTACACCKGTCAACATAATTGGAA
GRAATTTGTTGACTCAGATTGGTTGCACTTTAAATTTT**

>Sample DR 03-PR

**ACTCTTTGGCAACGACCCCTCGTCACAATAAAGATAGGGGGGCAAATAAA
GGAAGCYCTATTAGATACAGGGGCAGATGATACAGTATTAGAAGAGATGA
ATTTACCAGGAAGATGGAAACCAAAAATGATAGGGGGAATTGGAGGTTTT
ATCAAAGTAAGACAGTATGATCAGATACCCATAGAAATCTGTGGACATAA
AGCTATAGGTACAGTATTAGTAGGACCTACTCCTGTCAACATAATTGGAAG
AAATCTGTTGACTAAGATTGGTTGCACTTTAAATTTT**

>Sample DR 04-PR

**ACTCTTTGGCAACGACCCCTTGTCAACAATAAAAATAGGGGGGCAGCTAAA
GGAAGCTCTATTAGATACAGGAGCAGATGATACAGTATTAGAAGAAATGA
ATTTGCCAGGAAGGTGGAAACCAAAAATGATAGGGGGAATTGGAGGCTTT
ATTAAAGTAAGACAGTATGATCAGRTACCCATAGAAATCTGTGGACATAAA
GCTATAGGTACAGTATTAATAGGACCTACACCTGTCAACATAATTGGAAGA
AATCTGTTGACTCAGCTTGGTTGCACTTTAAATTTT**

>Sample DR 05-PR

**ACTCTTTGGCAACGACCCCTCGTCACAATAAAGATAGGGGGGCAACTAAA
GGAAGCTTTATTAGATACAGGAGCAGATGATACAGTGTTAGAAGACATCG
ATTTACCAGGAAGATGGAAGCAAAAATGATAGGGGGAATTGGAGGTTTT
ATCAAAGTAAGACAGTATGAGCAGGTGCTCGTAGAAATCTGYGGGCATAA
AGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCAACATAATTGGAAG
AAATCTGTTGACTCAGATTGGTTGCACTTTAAATTTT**

Appendix-3
HIV-1 Reverse Transcriptase Consensus Sequences

>Sample DR 01-RT

TGTACAGAAWTGGAAAAGGARGGAAAAATTTCAAAAATTGGGCCTGAAAA
TCCATACAATACTCCAGTATTTGCWATAAAGAAAAAAGACAGTACTAAATG
GAGAAAASTAGTAGATTTTCAGAGARCTTAATAARAGAACTCAAGATTTYTG
GGAAGTTCAATTAGGAATACCACATCCCGCAGGGTTAAAAAAGAACAAT
CAGTAACAGTACTAGATGTGGGTGACGCATATTTCTCAGTTCCCTTAGATG
AARACTTCAGGAAGTATACTGCGTTTACCATACCTAGTATAAACAATGAGA
CACCAGGRATTAGATATCAGTACAATGTGCTTCCACAGGGATGGAAAGGA
TCACCAGCAATATTCCAAAGTAGCATGACAARAATCTTAGAGCCTTTTGA
AAACAAAATCCAGACATGGTKATYTRTCAATACATGGATGATTTGTATGTA
GGATCTGAYTTAGAAATAGRGCAACATAGAACAAAAATAGAGGAACTGAG
ACAACAYCTGTKGAAGTGGGGRTTWMCACACCAGACAAAAAACATCAG
AAAGAACCYCCATTTCKTTGGATGGGTATGAACTCCATCCTGATAAATGG
ACAGTACAGCCTATAGTGCTGCCA

>Sample DR 03-RT

TGTACAGAAATGGAAAAGGAAGGGAAAATTTCAAAAATTGGGCCTGAAAA
TCCATACAATACTCCAGTATTTGCCATAAAGAAAAAAGACRGTACTAAATG
GAGAAAATTAGTAGATTTTCAGAGA ACTTAATAAGAGA ACTCAAGACTTCTG
GGAAGTTCAATTAGGAATACCACATCCGGCAGGGTTAAAAAAGAAAAAAT
CAGTAACAGTACTGGATGTGGGTGATGCATATTTTTTCAGTTCCYTTAGATA
AAGACTTYAGGAAGTATACTGCATTTACCATACCTAGTACAAACAATGAGA
CACCAGGGATTAGGTATCAGTACAATGTGCTTCCACAGGGATGGAAAGGA
TCACCAGCAATATTCCAAAGTAGCATGACAAGAATCTTAGAGCCTTTTGA
AAACAAAATCCAGACATAGTTATYTA YCAATACATGGATGATTTGTATGTA
GGATCTGACTTAGAAATAGGGCARCATAGAACAAAAATAGARGAACTGAG
ACAACATCTGTTGAGRTGGGGTTTACCACACCAGACAAAAAACATCAGA
AAGAACCCTCCATTYCTTTGGATGGGTATGAACTCCATCCTGAYAAATGGA
CAGTACAGCCTATAGAGCTGCCA

>Sample DR 04-RT

TGTACAGAATTGGAAAAGGAAGGGAAAATTTCAAAAATTGGGCCTGAAAA
TCCATACAATACTCCAGTATTTGCCATAAAGAAAAAAGATGGTACTAAGTG
GAGAAAATTGGTAGATTTTCAGAGA ACTTAATAAGAGA ACTCAAGACTTCTG
GGAAGTTCAATTAGGAATACCACATCCCGCAGGGTTAAAAAAGAAAAAAT
CAGTAACAGTGCTGGATGTGGGTGATGCATATTTTTTCAGTTCCCTTAGATA
AAGACTTCAGGAAGTATACTGCATTTACCATACCTAGTATAAACAATGAGA
CACCAGGGATTAGATATCAGTACAATGTGCTTCCACAGGGATGGAAAGGA
TCACCAGCAATATTCCAAAGTAGCATGACGAAAATTTTAGATCCTTTTGA
AAGCAAAAATCCAGRCATAGTTATCTATCAATACATGGATGATTTGTATGTA
GGATCTGACTTAGAAATAGAGCAGCATAGARCAAAAATAGAAGAATTGAG
ACAACATCTGTGGAGGTGGGGATTTGACACACCAGACAAAAAACATCAGA

AAGAACTOCATTOCTTTGGATGGGTTATGAACTOCATCTGATAAATGGACAGTACAGCTATAGTGCTGCCA

>Sample DR 05-RT

TGTACAGAAATGGAAAAGGAAGGGAAAATTTCAAAAATTGGRCCTGARAA
TCCATACAATACTCCAGTATTTGCTATAAAGAAAAAGACAGTACTAAATG
GAGAAAATTAGTAGATTTTCAGAGAACTTAATAAAAAGGACTCAAGACTTCTG
GGAAGTCCARTTAGGRATACCACATCCTGCAGGGTTAAAAAAGAAAAAAT
CAGTAACAGTACTGGATGTGGGTGATGCATATTTTTTCAGTTCCCTTAGATG
AAGACTTCAGGAAGTATACTGCATTACCATACCTAGCAYAAACAATGAG
ACACCAGGGATTAGATATCAGTACAATGTGCTTCCACAGGGATGGAAAGG
ATCACCAGCAATATTCCAAAGTAGCATGACAAAAATCTTAGAGCCCTTTAG
AAARCAATATCCAGACATAGTTATCTATCAATACATGGATGATTTGTATGTA
GGATCTGACTTAGAAATAGAGCAGCATAGAGCAAAAATAGAGGAACTGAG
ACAACATCTGTTGAAGTGGGGATTTACCACACCAGACAAAAARCATCAGA
AAGAACCTCCATTCTTTGGATGGGTTATGAACTCCATCCTGATAAATGGA
CAGTACARCCTATAGTRCTGCCA