Application of a Sanger-Based External Quality Assurance Strategy for the Transition of HIV-1 Drug Resistance Assays to Next Generation Sequencing

Presented By

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Background

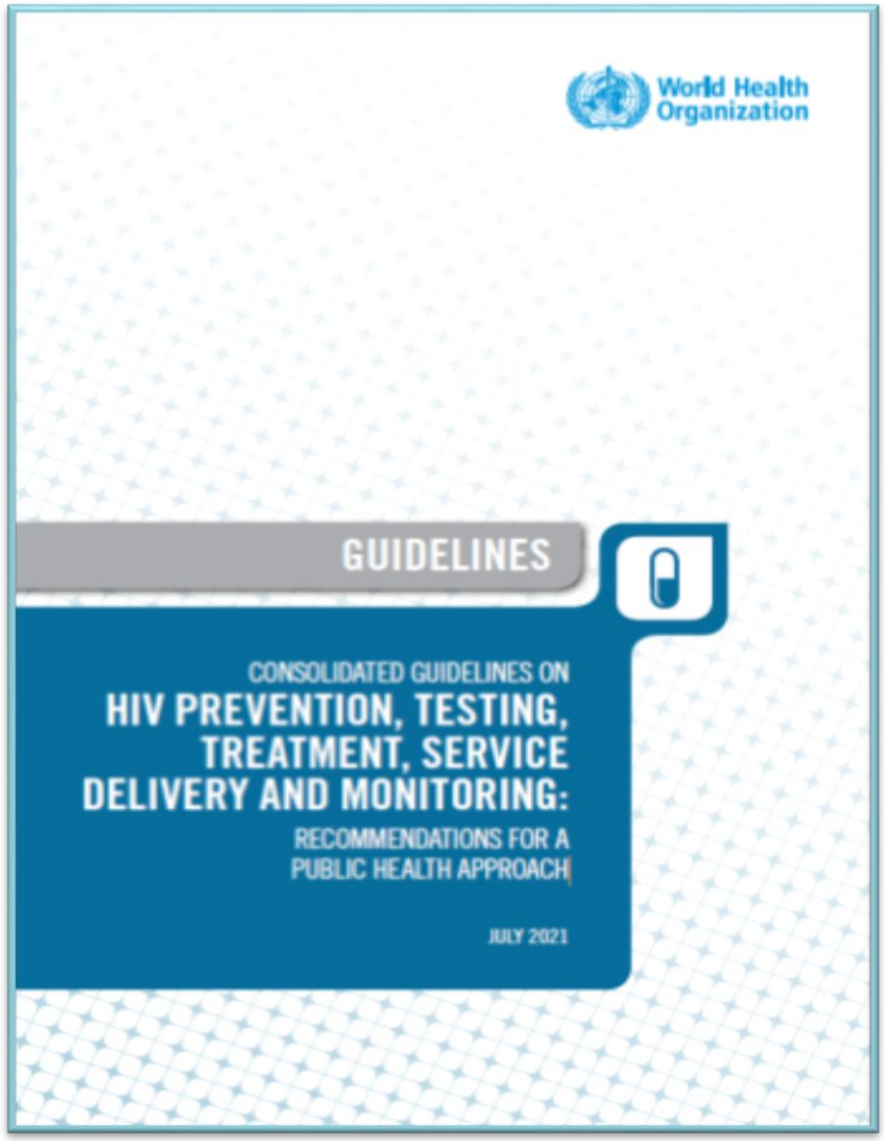
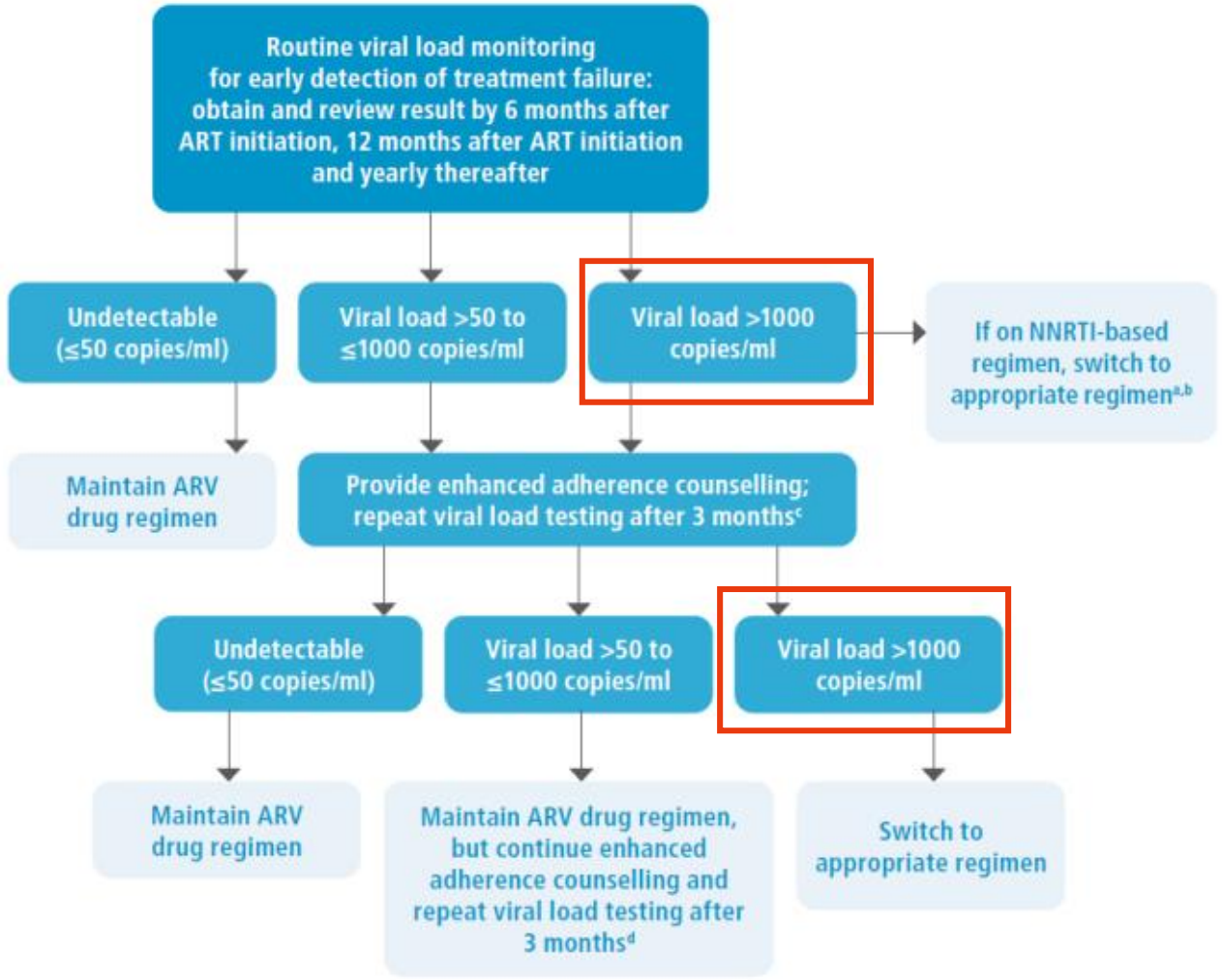


Fig. 4.2 Treatment monitoring algorithm updated in 2021

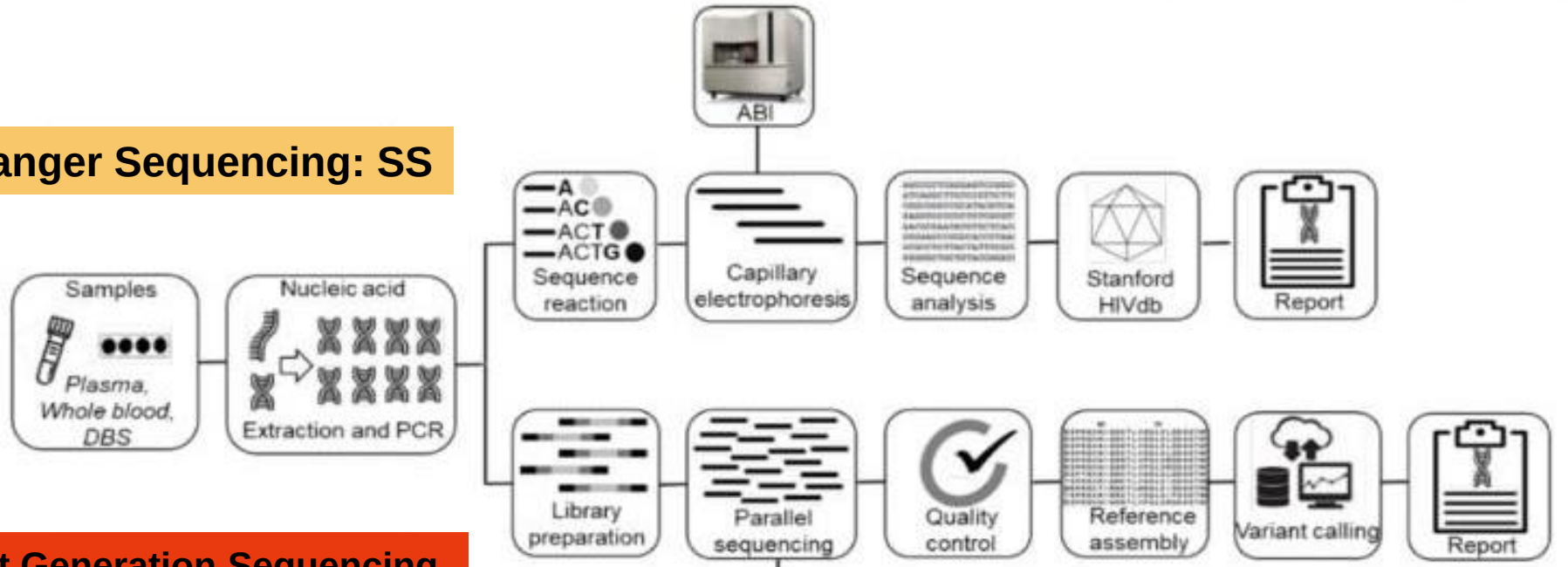


Background

Pre-analytical

Analytical

Sanger Sequencing: SS



Next Generation Sequencing

NGS

illumina®

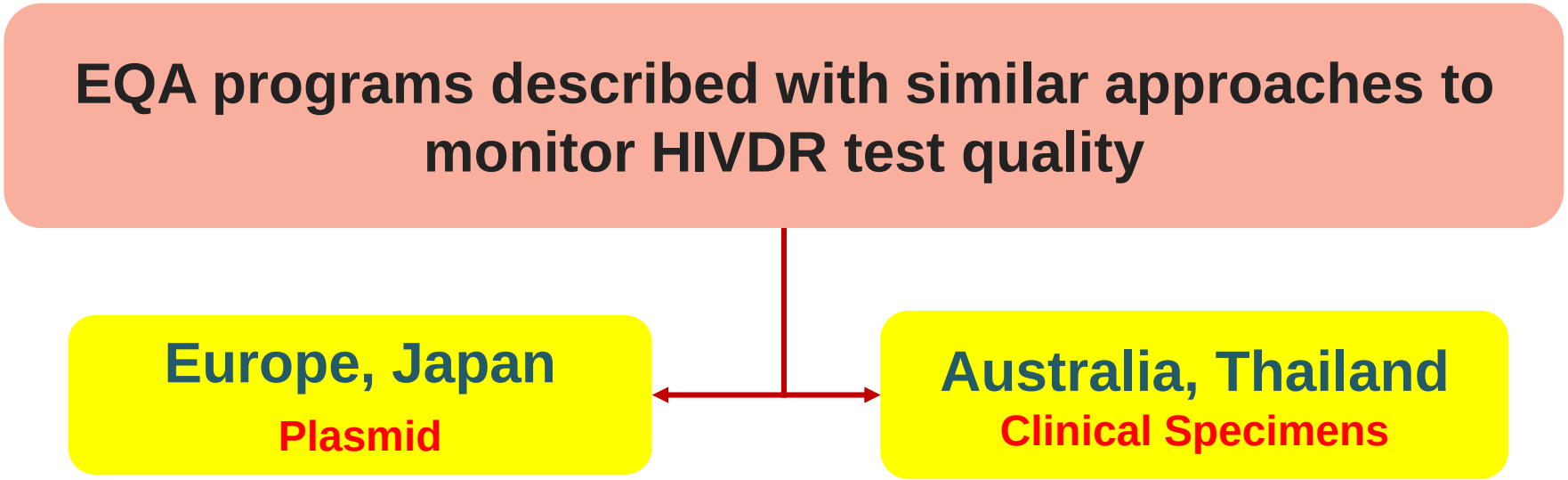
Oxford NANOPORE Technologies

Post-analytical

Introduction

Objective of an EQA program

- **Evaluate tests**
- **Determine laboratory performance**
- **Monitor effectiveness of the laboratory's quality management**

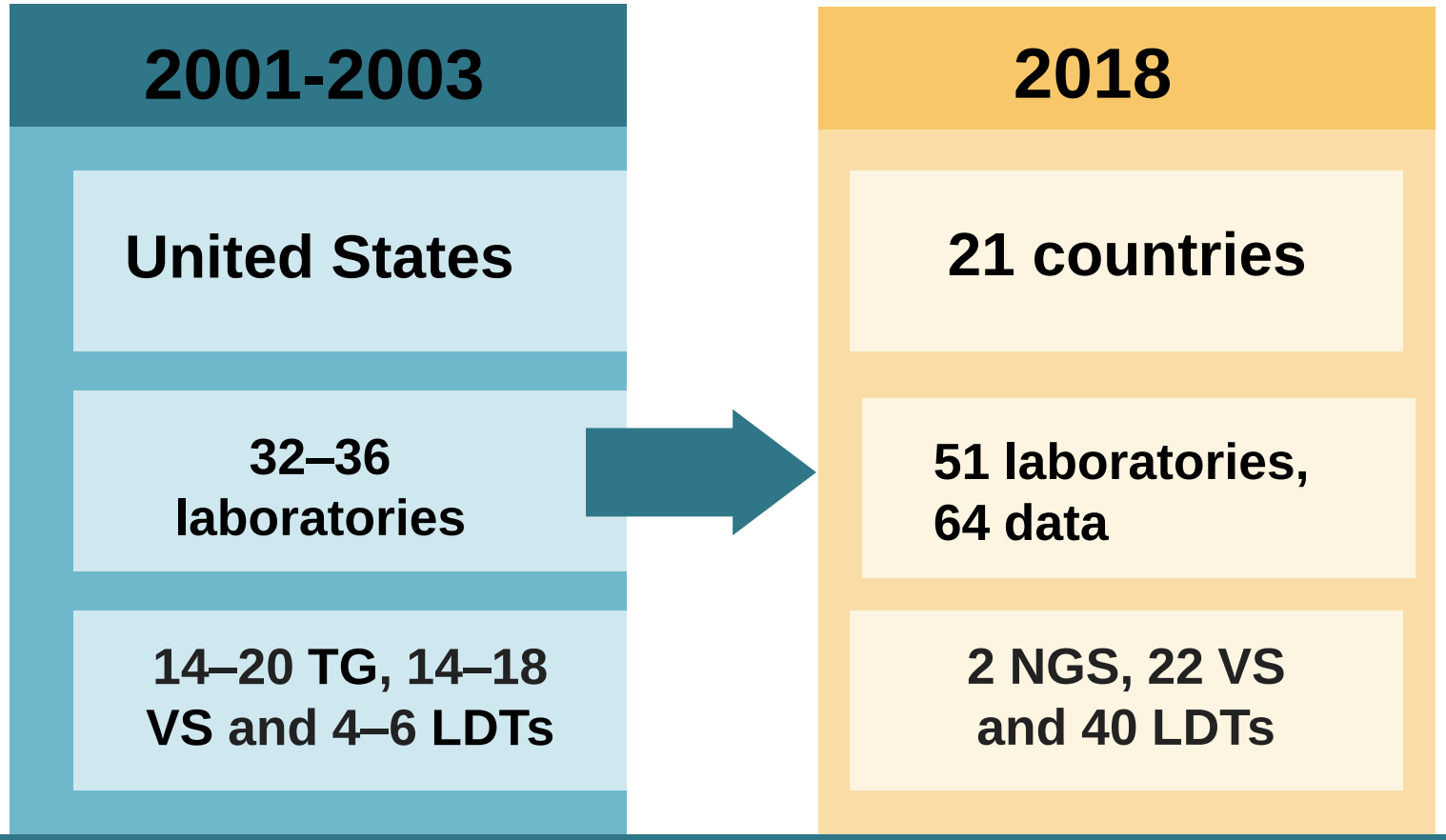


Introduction

VQA
Virology Quality Assurance
An NIH, NIAD, DIAIDS Program

In 2001

- The National Institute of Allergy and Infectious Diseases (NIAID) Virology Quality Assurance (VQA) established proficiency testing program for Sanger sequencing (SS)-based HIV-1 drug resistance (HIVDR)



Introduction

Virology Quality Assurance
An NIH, NIAD, DIAIDS Program

2 panels/year

Plasma specimens

Subtypes
A, CRF02_AG, AE, B, C, D, F, and G

Viral Load
2,000–100,000 copies/mL

Watch Regions

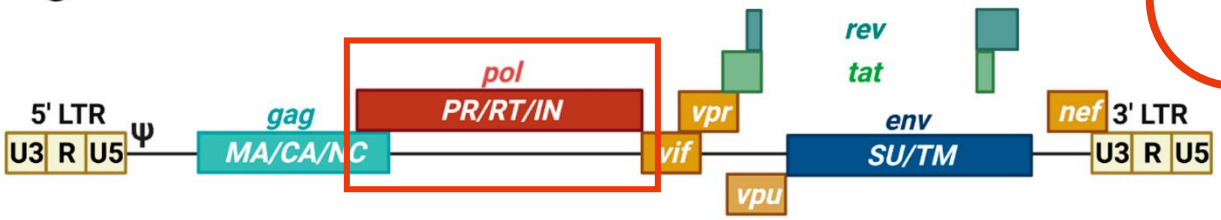
Amino Acids positions

- 4–99 for protease (PR)
- 38–247 for reverse transcriptase (RT)
- 50–200 for integrase (INT)

Sequence Analysis

- Aligned 7 **Amino Acids** data sets to generate consensus sequence
- 80% sequence agreement at any position
- If any position has > 80% agreement indicates “**non-consensus**”

HIV-1 genome



Introduction

VQA
Virology Quality Assurance
An NIH, NIAD, DIAIDS Program

In 2017

- The Rush VQA, in collaboration with the WHO Global HIV-1 Drug Resistance laboratory network and the Public Health Agency of Canada , invited laboratories that had **developed HIVDR testing using NGS to participate in a pilot study**.

- **Using Sanger sequencing (SS)-based EQA scoring** strategies as a transitional approach for switching to NGS technologies.

What Have We Learned from **Sanger-Based EQA?**

- EQA program is to **ensure the quality of testing**, which may include the distribution of well-characterized quality control material to verify the **performance specifications of a new assay,** to verify the **run performance** or **new reagent** or **kit lots** of an existing assay, or to verify **ongoing performance through proficiency testing.**

- **Human-derived** QCMs is **not** knowing what **DRMs** they contain and the exact abundance of each variant within the specimen.

- PT should include specimens with a **range of viral loads** that would be **expected in routine testing.**

Objective :
Can We Use Existing **SS EQA Programs** to Evaluate **NGS Data?**

- To **applied the scoring criteria** used for assessing Sanger sequencing (SS) HIVDR testing.
- To determine whether NGS data can be used to **generate Sanger-like data** and can be assessed for evaluating NGS assay performance.
- To evaluate NGS consensus sequences generated with **a range of detection thresholds (5%, 10%, and 20%)** and compare to the data from Sanger-based consensus sequences

Figure 1. **Comparison Nucleotide alignment**

panel 26 g

- Alignment segment (451–540, RT amino acids 188–217)
- Yellow highlighted bases differ from Sanger sequencing (SS) consensus.
- An “n” in the consensus (first) row indicates that no consensus was achieved in the original analysis.

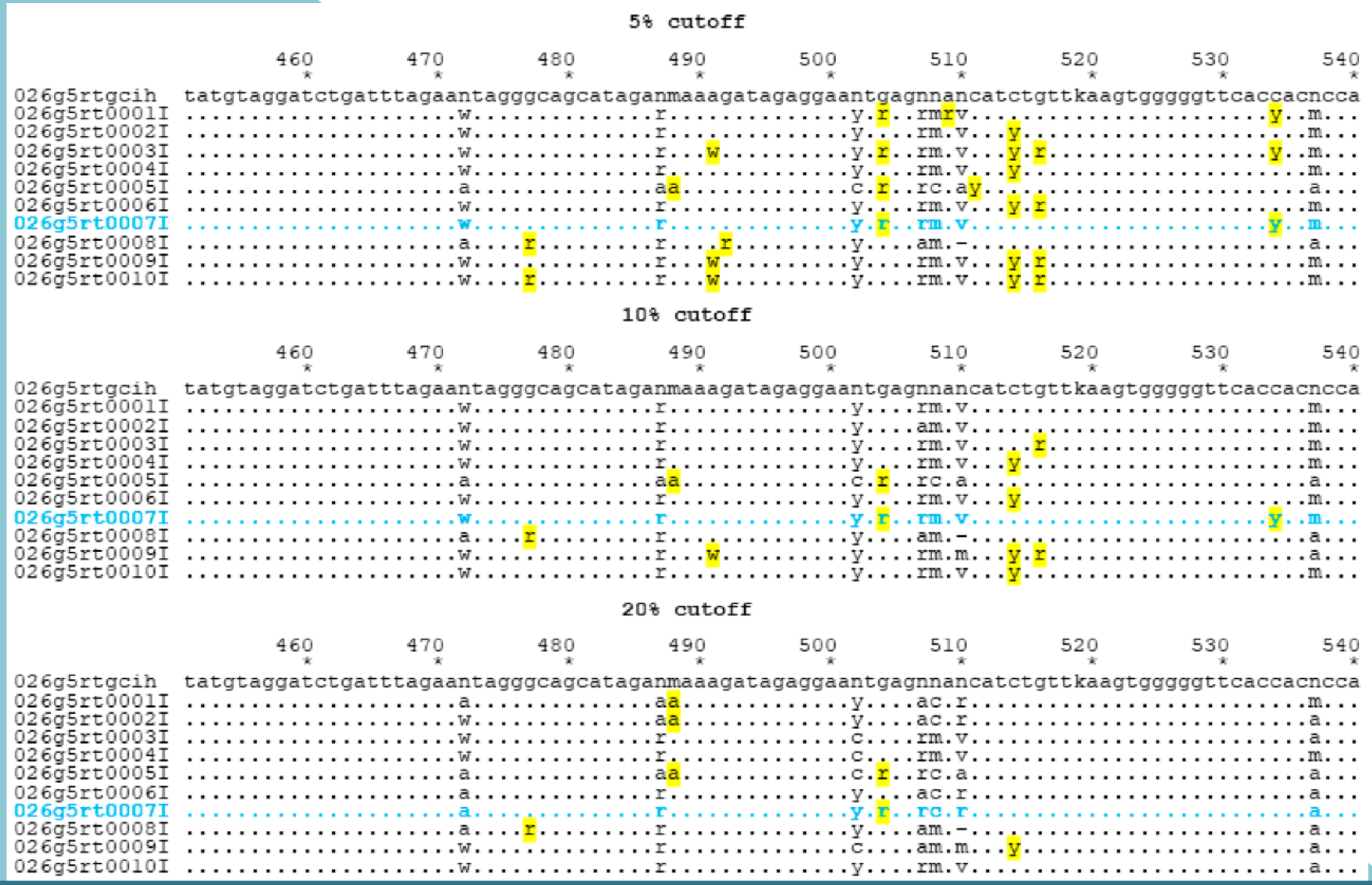


Table 1. errors (total errors, partial mismatches, complete mismatches) in reverse transcriptase (RT) from panel 26 g

Laboratory	5% Cutoff		10% Cutoff			20% Cutoff		
	% Homology *	Stage 1 Errors	Poisson *p*-Value	% Homology *	Stage 1 Errors	Poisson *p*-Value	% Homology *	Stage 1 Errors	Poisson *p*-Value
1	99.0	5		99.6	2		99.4	3	
2	98.3	10	0.021	99.1	5		99.1	5	
3	97.6	14	0.0004	99.0	6		100	0	
4	98.1	11	0.009	99.3	4		99.8	1	
5	97.2	16	<0.0001	97.2	16	<0.0001	97.4	15	0.00011
6	97.9	12	0.003	99.3	4		100	0	
7	98.3	10	0.021	99.1	5		99.5	3	
8	98.1	11	0.009	98.3	10	0.021	98.3	10	0.02138
9	98.1	11	0.009	98.1	11	0.009	98.3	10	0.02138
10	97.4	15	0.0001	99.1	6		100	0	

* % Homology was calculated by dividing the number of bases submitted that matched the group consensus sequence by the number of bases in the group consensus sequence after excluding non-consensus positions (Ns). Missing bases (89) for laboratory 1 are not included in the homology calculation and are not considered errors for Stage 1 (Poisson) error point calculations.

Table 2. EQA scoring outputs for NGS consensus sequences using different thresholds

Lab	24 g Total Points, Score						26 g Total Points, Score					
	5%		10%		20%		5%		10%		20%	
	Points	Score	Points	Score	Points	Score	Points	Score	Points	Score	Points	Score
1	13	PC	4	C	2	C	9	PC	1	C	2	C
2	8	PC	0	C	0	C	14	PC	2	C	2	C
3	6	C	2	C	0	C	6	C	3	C	0	C
4	9	PC	2	C	5	C	8	PC	0	C	0	C
5	9	PC	6	C	3	C	9	PC	9	PC	8	PC
6	6	C	2	C	0	C	12	PC	6	C	3	C
7	3	C	2	C	0	C	3	C	2	C	0	C
8	3	C	3	C	4	C	4	C	3	C	3	C
9	9	PC	5	C	1	C	12	PC	8	PC	7	C
10	10	PC	1	C	0	C	9	PC	1	C	0	C

Total errors (stage 1 and 2) for all fives specimens for protease (PR) and RT combined within the panel are tallied by the laboratory, gene, % thresholds for NGS consensus generation and external quality assurance (EQA) scoring of the laboratory performance when NGS consensus at various thresholds was applied. Missing data were not included in the error counts because some data sets did not include sequences for the entire examined region. PC = provisionally certified (shaded cells; scores of 8–14; problems noted) and C = certified (scores of 0–7; no major problems noted).

C: Certified scores = 0-7

PC: Provisionally certified scores = 8-14

Discussion

- **Clinical specimens** are **better for monitoring** laboratory performance.
- The use of standardized **electronic reporting** for the collection of EQA data helps to **facilitate statistical analyses and minimize errors** associated with manual transcription.
- **Replicate specimens** within and between panels that include a range of viral loads can help to generate temporal trend data for evaluations of **sensitivity, precision, and accuracy** of mixture reporting.
- NGS consensus FASTA files created with **thresholds of 20%** yielded the **best scores** when compared to Sanger-based data.
- **Three laboratories** received **passing scores for all panels & thresholds** when compared to SS data.

Conclusion

- **SS-based EQA strategy may serve as a transitional solution for evaluating the performance of a laboratory conducting NGS-based HIV-DR assays.**

- **Additional studies are needed to better characterize a NGS HIV-DR test and fully define the performance expectations for such assays, especially concerning sensitivity and the quantitative detection of low abundance DRMs.**


THANK YOU