Public Health Agency of Canada | Agence de la santé publique du Canada

Canad

Characterization and data assessment of NGS-based genotyping using VQA HIVDR proficiency panels

Hezhao Ji / Emma R Lee

National HIV and Retrovirology Laboratories
National Microbiology Laboratory at JC Wilt Infectious Diseases Research Centre
Public Health Agency of Canada

IDRW 2018, Johannesburg

October 23, 2018

OTECTING AND EMPOWERING CANADIANS
O IMPROVE THEIR HEALTH

NGS vs Sanger for genotypic HIVDR testing

- ✓ ⬆ Sensitivity for LADRVs
- ✓ ⬆ Resolution for HIV quasispecies
- ✓ ⬆ Data throughput
- ✓ **Trending new "standard" for genotypic HIVDR testing**

“Standardization” of NGS HIVDR assay

To “standardize” NGS HIVDR assays, one would need:

- Fully validated lab SOPs for quality NGS data generation.
- NGS proficiency panels for assay QA purposes.
- Well-defined NGS HIVDR data processing strategies.
- User-friendly, automated, customizable NGS HIVDR pipelines/tools.
- Assay performance assessment guidelines suitable for NGS assays.
- External quality assurance program.

Objective

- **To explore assay characterization and data assessment strategies that may help to assess the value of the existing VQA panels for external quality assurance (EQA) of NGS HIVDR assays.**

Methods

- **7** previously characterized EQA panel specimens were distributed to **6** HIVDR labs in Canad USA, Mexico and Spain.
- NGS HIVDR typing was performed in the labs using their respective protocols/platforms.
- Raw NGS data (FASTQ files) was processed using HyDRA pipeline *(http://hydra.canada.ca).*
- Only DRMs detected by **≥ 4** out of the 6 labs at median frequency of **≥ 5%** were considered f subsequent performance assessment.

reviously proposed NGS HIVDR assay assessment syste

Performance Characteristics	Definitions Specific to NGS HIV DR Assay	Recommendation
Limit of Detection	The lowest actual percentage of a DRM that can be consistently detected with acceptable precision, sensitivity and specificity.	**≥1%**
Linear Range	The percentile range of actual DRM frequencies within which linear correlation is achievable accurately between the expected and observed values.	**1%~100%**
Precision	The extent to which repeated testing on identical samples renders comparable results with acceptable intra-run repeatability and inter-run reproducibility.	**Combined %CV≤25%**
Accuracy	The extent to which the detected DRM frequency is in agreement with reference materials.	**%CV≤20%**
System Error	The compounding error from all experimental procedures and data processing.	**≤0.4%**
Analytical Sensitivity	The probability that the assay detects known DRM (measured as 1- False Negative Rate).	**≥99%**
Analytical Specificity	The probability that the assay does NOT detect a DRM when it is absent (measured as 1- False Positive Rate).	**≥95%**
Limit of Viral Load	The lowest viral load level at which the test can positively identify all known DRMs from a sample at a defined input volume.	**≥1000cp/mL**
Robustness	The capability of the assay to reliably genotype clinical samples comprised of any major HIV subtypes.	**All major subtypes**

-- Liang D, et al. presented at the 25th International HIV DR Workshop., 201

Assessment Parameters

- **Linear range**
- **Analytical sensitivity**
- **Analytical specificity**
- **Variation of detected DRM frequencies**
- Concordance between NGS consensus and matching Sanger sequence ***(Parkin's talk, #3***

Linear Range

efinition:

e percentile range of actual DRM frequencies
thin which linear correlation is achievable
tween the expected and observed values.

sting Method:

mparing DRM frequency (%) readouts with
pected frequencies (the group median).

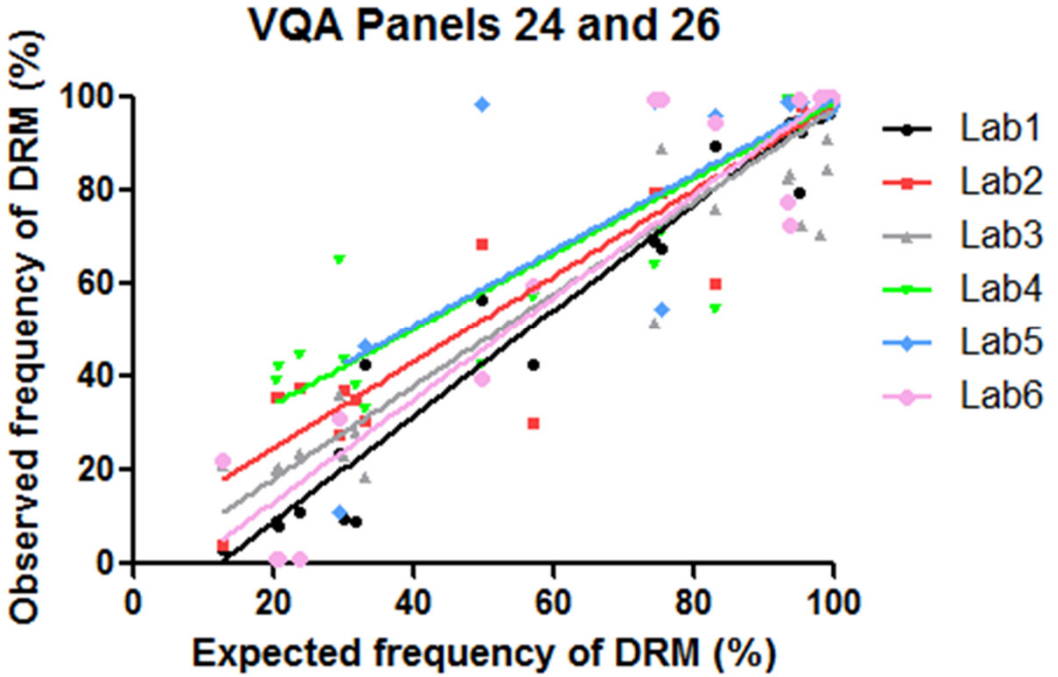
nalysis Method:

near regression analysis.

Identify all DRM frequencies between 5~100%;

Expected frequencies are determined by using the group median.

Linear regression analysis/plot using expected % and the frequency readouts from individual labs.



	Lab1	Lab2	Lab3	Lab4	Lab5	Lab6
Slope	1.13 ± 0.03	0.92 ± 0.04	0.99 ± 0.04	0.81 ± 0.04	0.81 ± 0.09	1.10 ± 0
r^2	0.98	0.94	0.93	0.9	0.67	0

Analytical Sensitivity & Specificity

inition:

sitivity: The probability that the assay detects a wn DRM when it is present.

cificity: The probability that the assay does NOT ect a DRM when it is absent.

ting Method:

sitivity= 1-False Negative Rate (#DRM **missing**/total)x100

cificity = 1-False Positive Rate (# **extra** DRM/total) x 100

alysis Method:

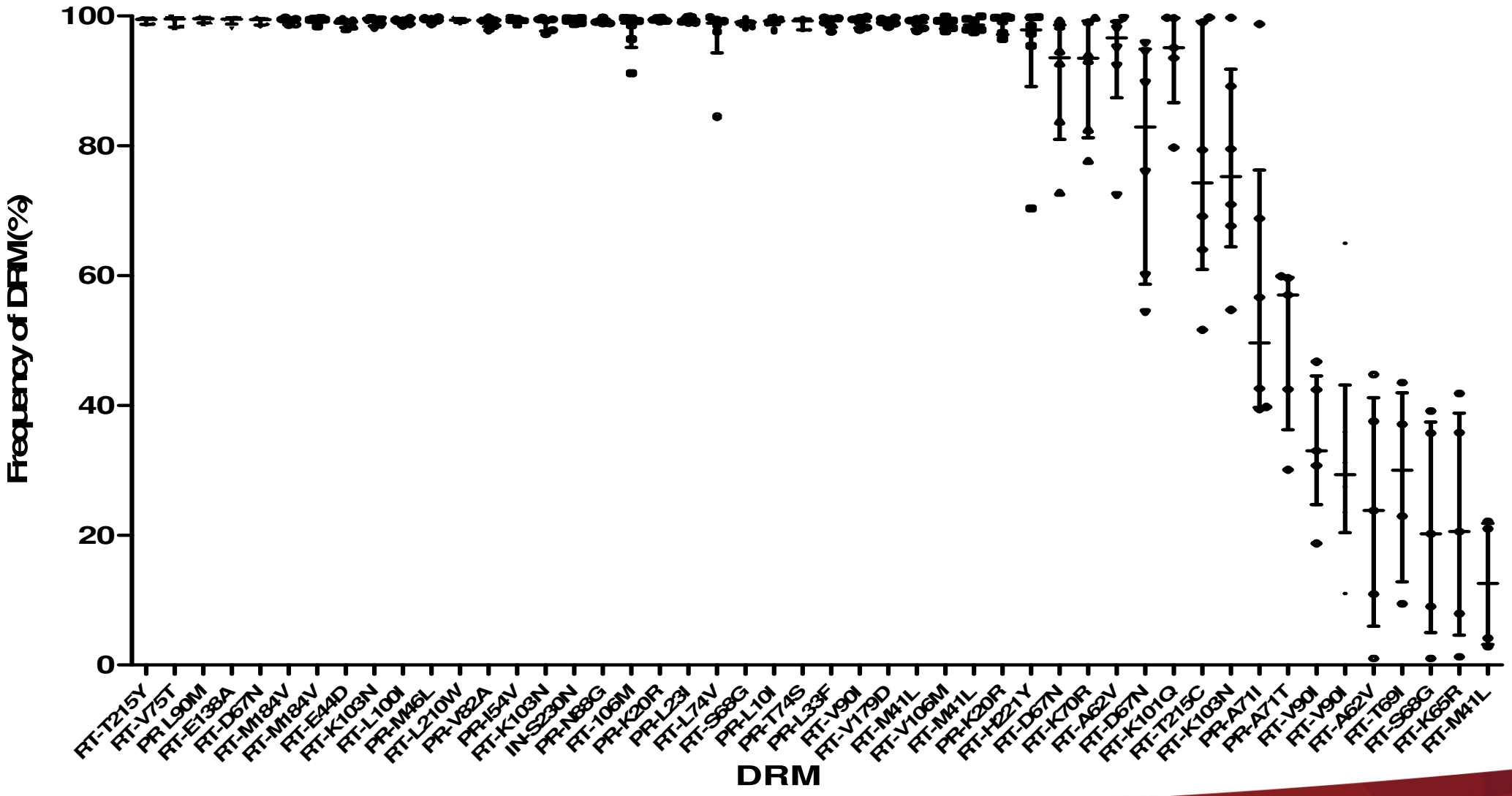
ch and count the expected and unexpected DRMs.

Calculate # of all reportable DRMs from the panel.
Match & count expected/unexpected DRMs of each lab.
Calculate the sensitivity and specificity

N. DRMs	Lab1	Lab2	Lab3	Lab4	Lab5
≥20%	49	49	49	49	42
Sensitivity at ≥20%	100	100	100	100	85.7
Specificity at ≥20%	97.6	100	100	100	100
≥5%	51	51	50	50	43
Sensitivity at ≥5%	100	100	98	98	84.3
Specificity at ≥5%	90.2	92.2	92.2	92.2	100

Average sensitivity ≥5% = 95.4% (range: 84.3-100%
Average specificity ≥5% = 93.8% (range: 90.2-100%

Variation of DRM Frequencies



Conclusions

The applied strategies are applicable for NGS HIVDR data assessment.

- *Linear Range*
- *Sensitivity*
- *Specificity*
- *Variation of DRM frequencies check to identify outliers.*

The EQA panels used for assessing Sanger-based testing can be applied to NGS HIVDR assa

Such analysis may complement the results from using NGS consensus for HIVDR analysis.

Future directions

- Further research to properly address the inconsistency of frequency measurement of DRM present at <20% among different protocols.
- Comparison of varied NGS HIVDR data assessment pipelines (HyDRA, MiCall, PASeq, Hivmmer, DeepGen) *(abstract submitted to CROI 2019).*
- Use additional EQA panels or well-characterized virus stocks to create new controls that would permit replication testing at a broader range of viral load levels and with wider range DRM frequencies.

Acknowledgements

NML@JCWilt/PHAC

Paul Sandstrom
Rupert Capina

NML Bioinformatics/PHAC

Gary Van Domselaar
Eric Enns
Eric Marinier

UBC/BC-CfE

Richard Harrigan
Chanson Brumme

IrsiCaixa:

Roger Paredes
Marc Noguera-Julian
Maria Casadellà

VQA/NIH team

Cheryl Jennings
Joe Fitzgibbon
Keith Crawford
James Bremer
Daniel Zaccaro

Brown University

Rami Kantor
Mark Howison

Data First Consulting

Neil Parkin

CWRU

Miguel E. Quiñones-Mateu

CIENI

Santiago Avila Rios

PAHO

Giovanni Ravasi

- **Federal Initiative to Address HIV/AIDS in Canada.**
- **Genomic Research & Development Initiatives (GRDI).**
- **Funding supports to all participating institutes/ programs.**