Cross-clade HIV-1 DNA detection from Frozen Whole Blood using Droplet Digital PCR

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Conflict of interest: None

University of Washington Retrovirology Laboratory

HIV diagnostic testing laboratory at Harborview Medical Center
Seattle, Washington

Virology Specialty Laboratory for the AIDS Clinical Trials Group

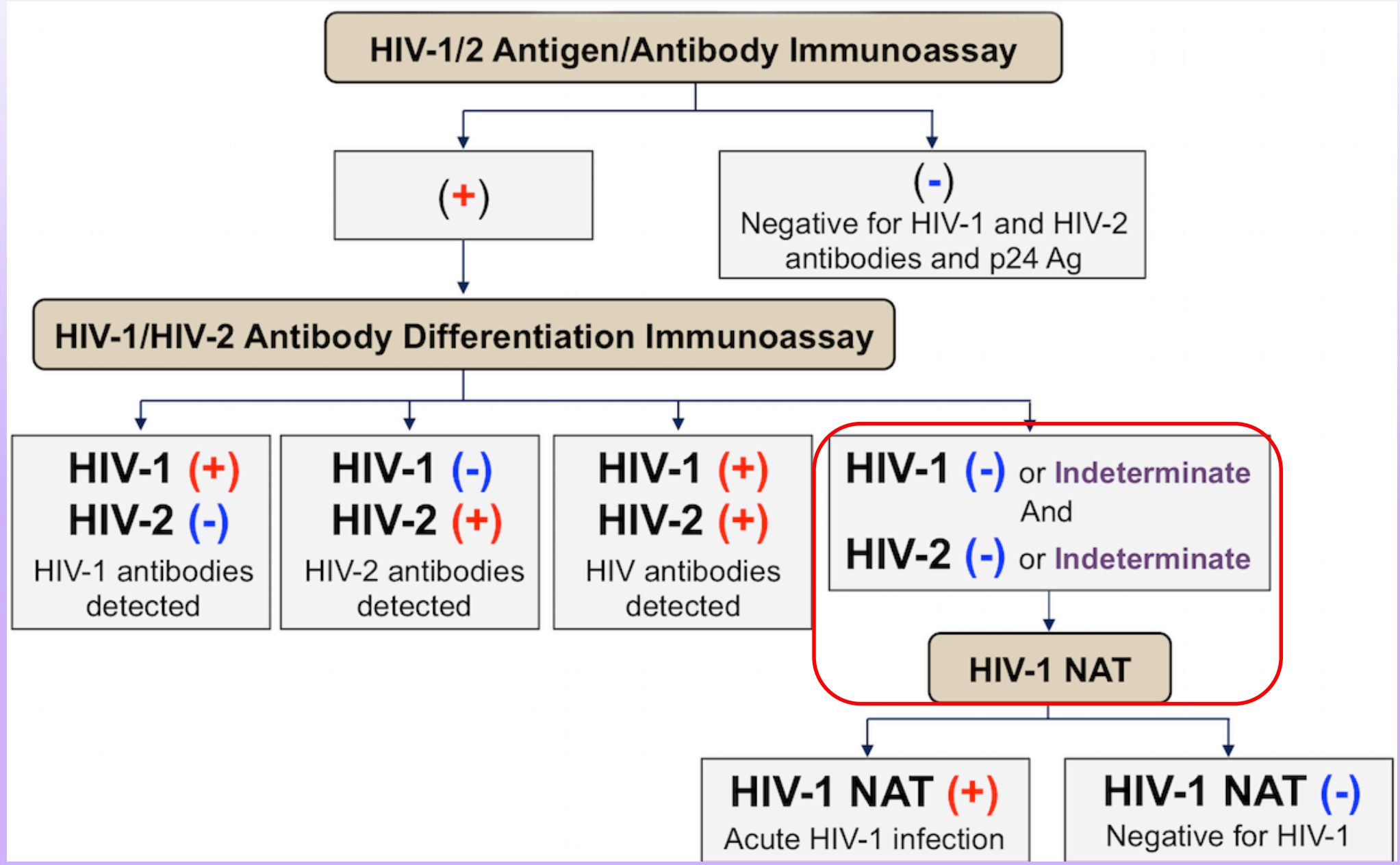
Diagnostic testing laboratory for the HIV Vaccine Trials Network

UW Medicine
LABORATORY MEDICINE
VIROLOGY

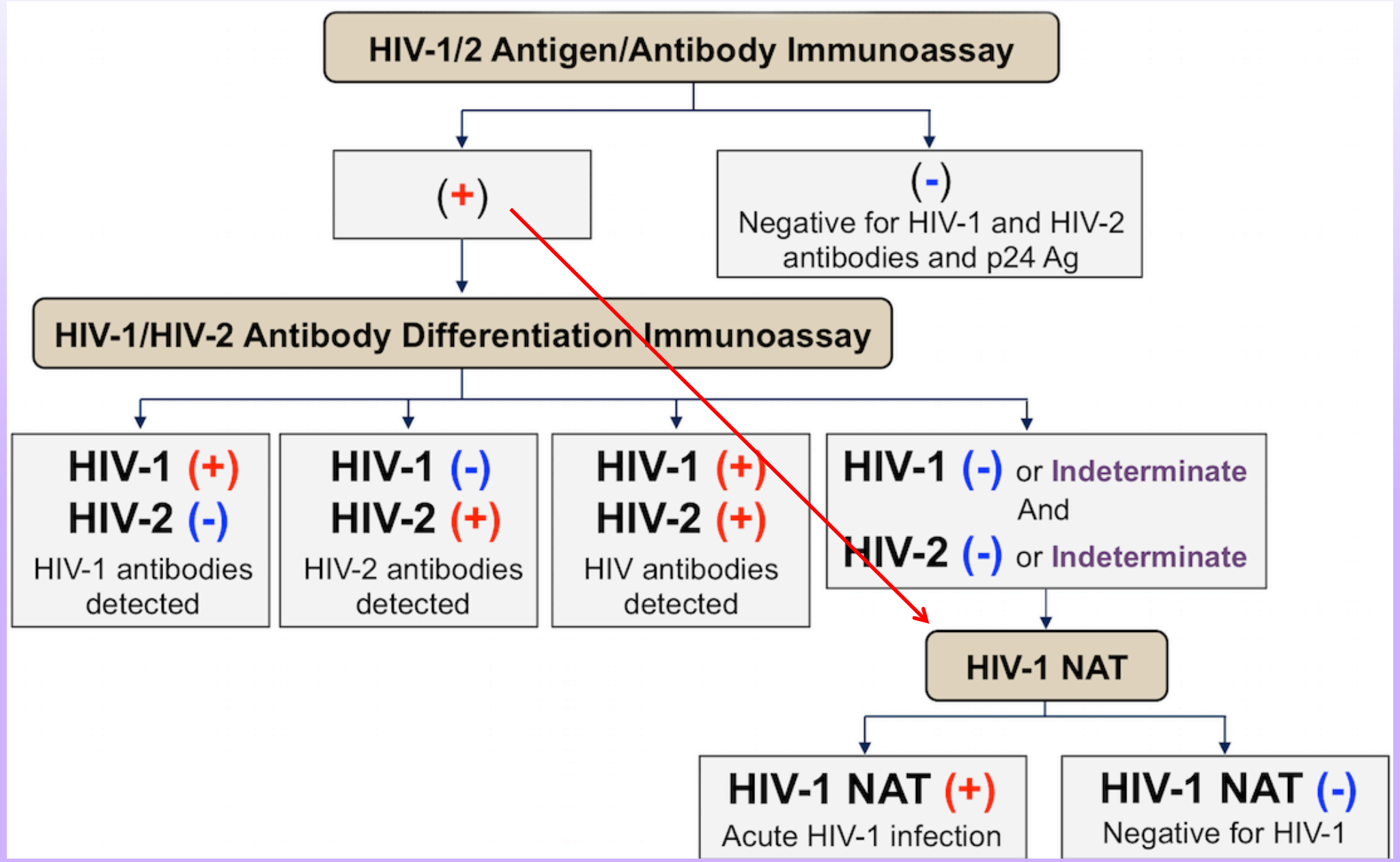
HIV VACCINE
TRIALS NETWORK

ACTG
AIDS CLINICAL TRIALS GROUP

CDC recommended HIV diagnostic algorithm



CDC recommended HIV diagnostic algorithm



What is the need for a cell associated HIV-1 DNA assay?

1. Infants born to sero-positive mothers
   - Maternal antibodies may be detectable for 24 months following birth
   - HIV-1 RNA in plasma may be undetectable

2. Patients taking pre-exposure prophylaxis (PrEP)

How does daily PrEP use change HIV diagnostic testing?

The effect of oral pre-exposure prophylaxis on the progression of HIV-1 seroconversion

Donnell D, et al. AIDS 2017, 31:2007–2016

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Findings:

1. Delay in HIV-1 infection diagnosis for participants taking daily PrEP
2. Undetectable HIV-1 RNA in plasma during acute infection
3. HIV-1 DNA assay is recommended to confirm infection in participants taking daily PrEP

Matrix options for cell associated HIV-1 DNA testing

- Whole blood
- Peripheral blood mononuclear cells (PBMCs)
  - PBMCs isolated from whole blood using density gradient centrifugation
- CD4 cells
  - CD4 cells isolated from PBMCs using negative column selection

Matrix options for cell associated HIV-1 DNA testing

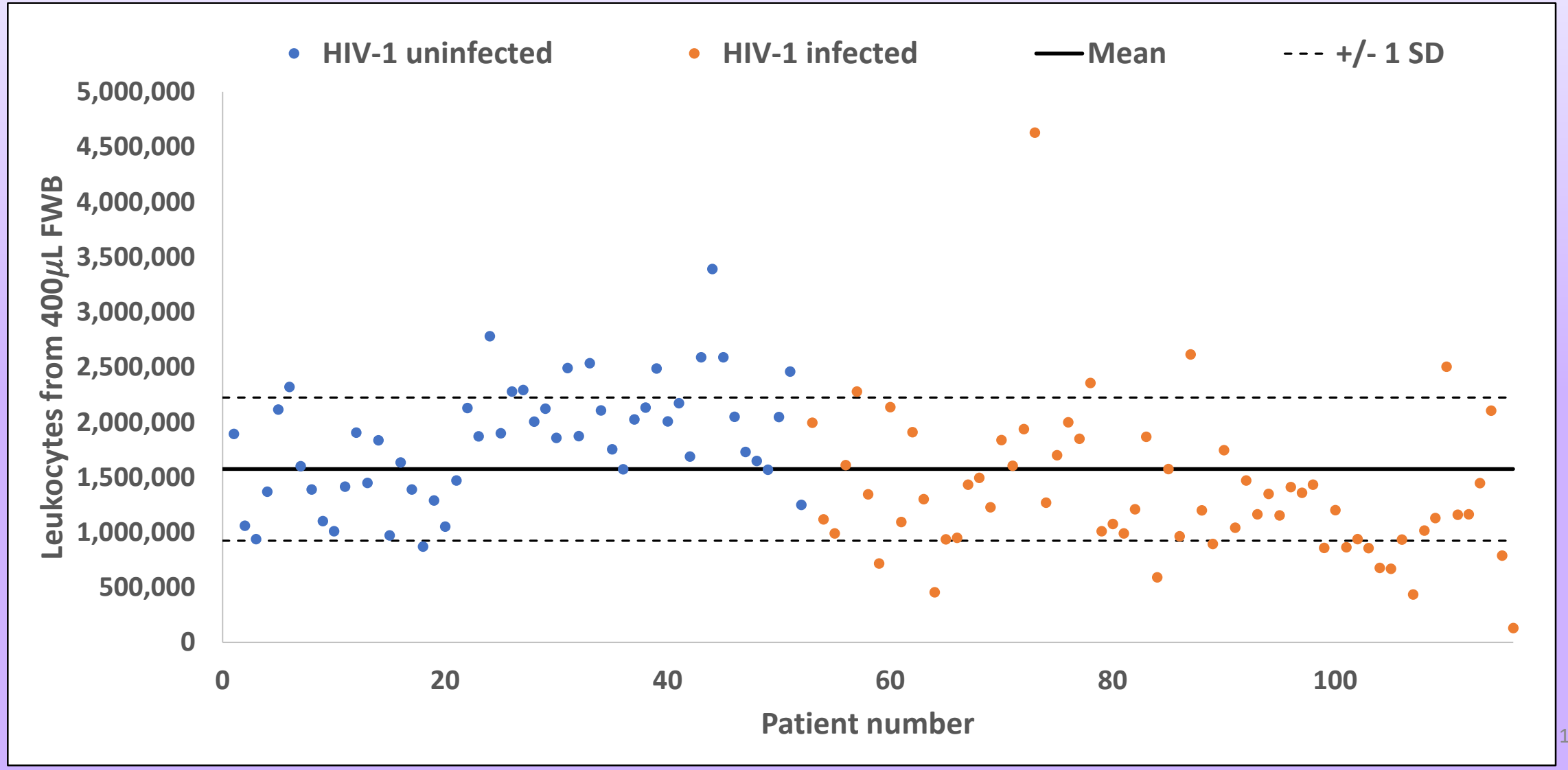
- Whole blood
  - Ambient or refrigerated temperatures for up to 7 days
  - May be frozen and shipped on dry ice
  - Easy and instrument free storage possible for large number of clinical trial participants
  - Small percentage of these samples will be assayed

Whole blood extraction

- Requires 400μL fresh or frozen whole blood
- Modified Qiagen whole blood extraction procedure
- Lab prepared frozen whole blood (FWB) controls and Virology Quality Assurance program (VQA) FWB controls
- Ribonuclease P subunit 30 (RPP30) used to determine the number of leukocytes detected from 400μL FWB
  - 2 copies of RPP30 per leukocyte
  - Non-multiplexed ddPCR assay on same plate using same enzyme as HIV-1

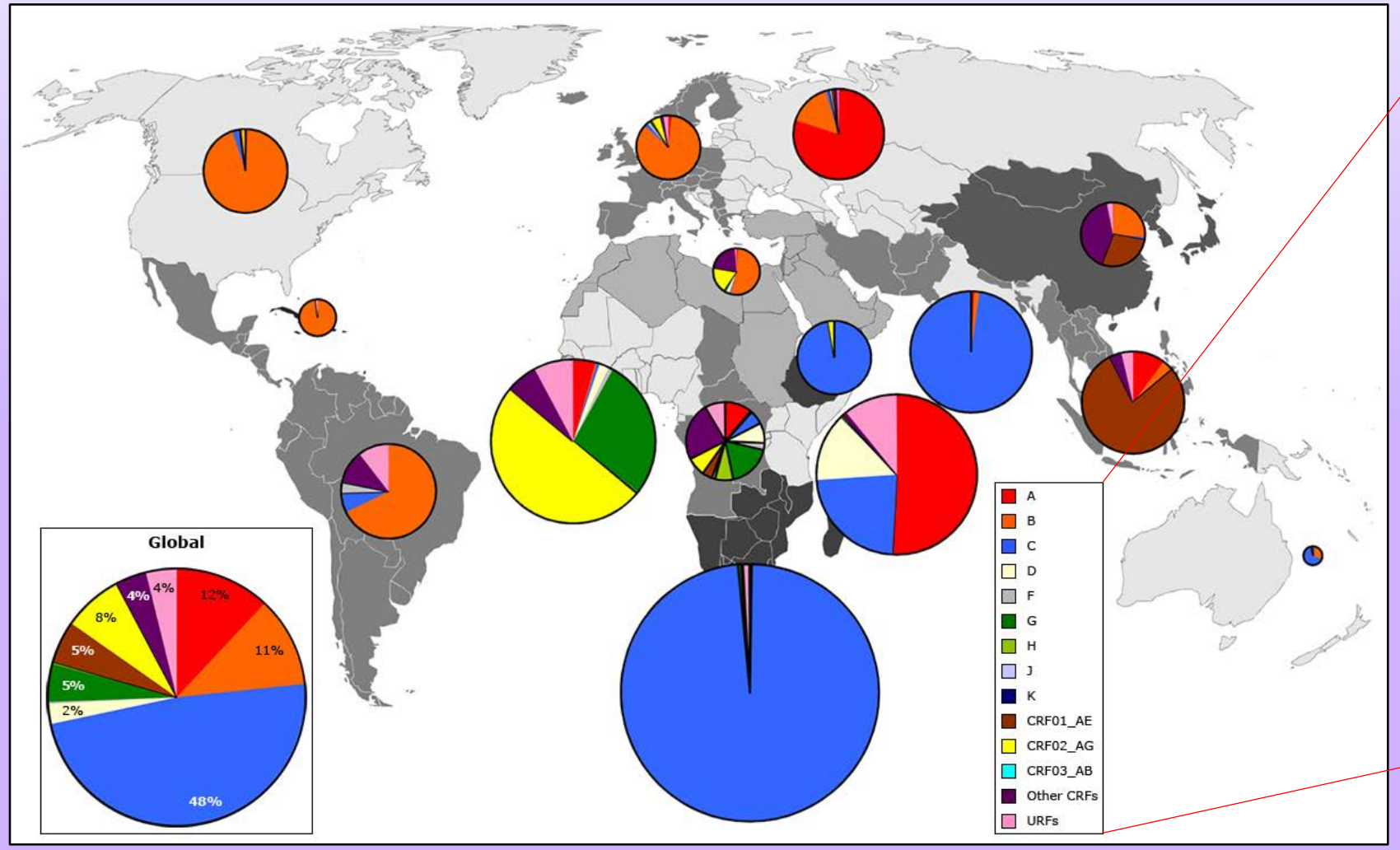
Leukocytes detected from 400μL FWB (n=116)

Average 1.57 million (SD 0.65 million)



Assay design rationale

- Genetic diversity of HIV-1



Clade

Assay design rationale

- Genetic diversity of HIV-1

- Referenced the Los Alamos National Laboratory (LANL) 2018 HIV-1 sequence compendium
  - Developed a primer/probe set within a highly conserved region in the Pol integrase gene

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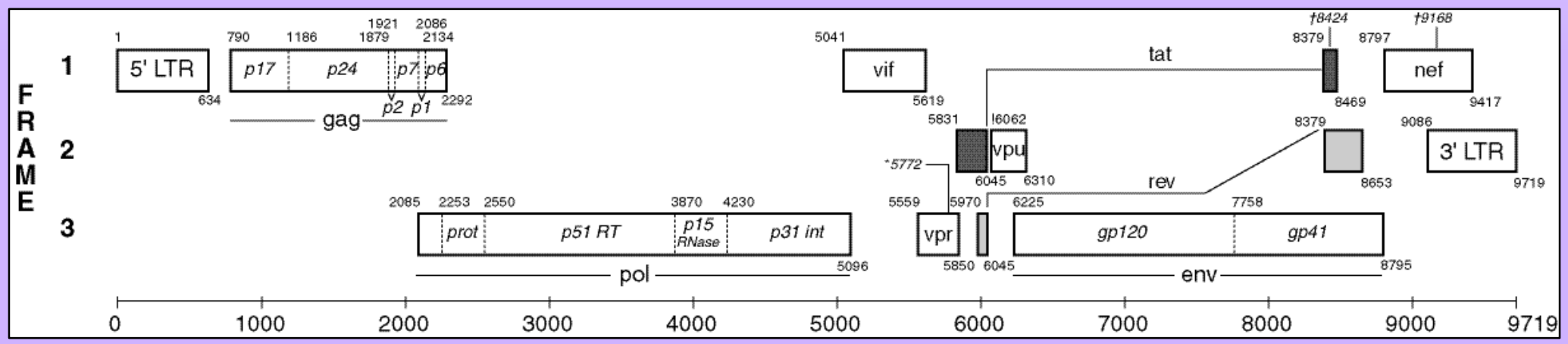

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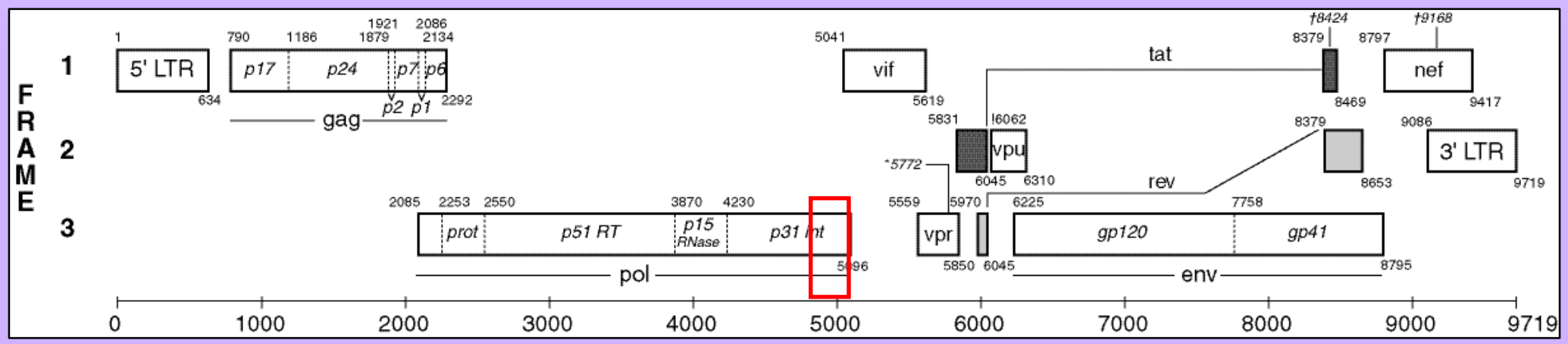

HXB2 reference sequence 4900 to 5071

Droplet Digital PCR: Bio-Rad QX200 ddPCR system

- TaqMan PCR master mix

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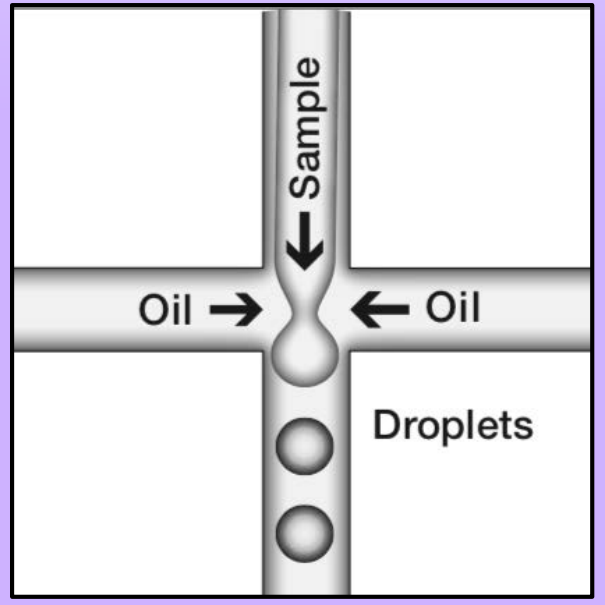
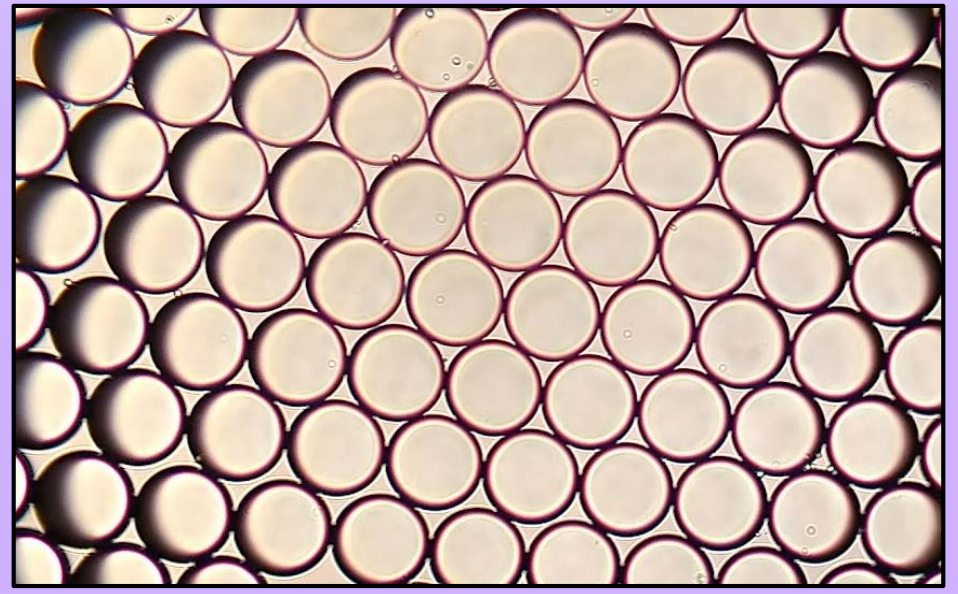
- Each well is partitioned into ~ 20,000 nanoliter sized droplets

  → 20,000 individual PCR reactions per well





Droplet Digital PCR

- Droplets are thermal cycled to endpoint, amplifying target nucleic acid within each droplet

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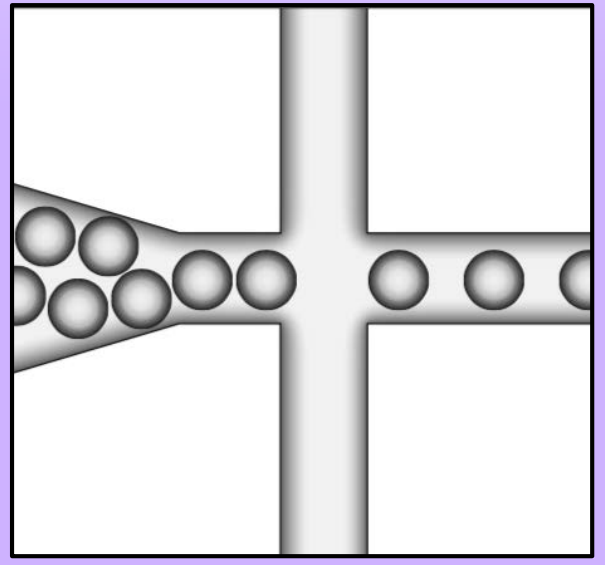
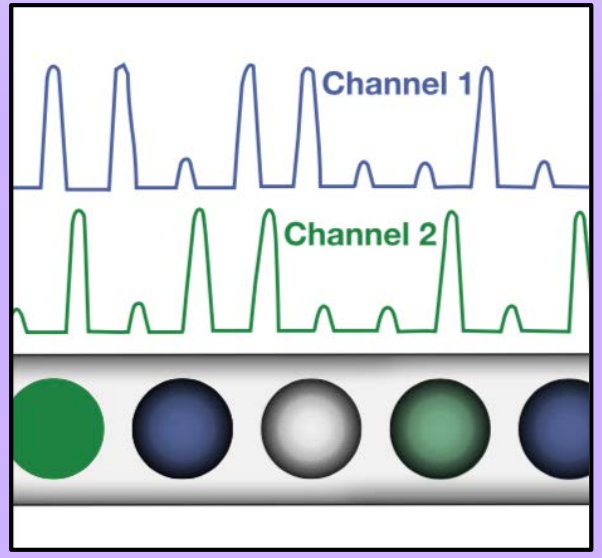
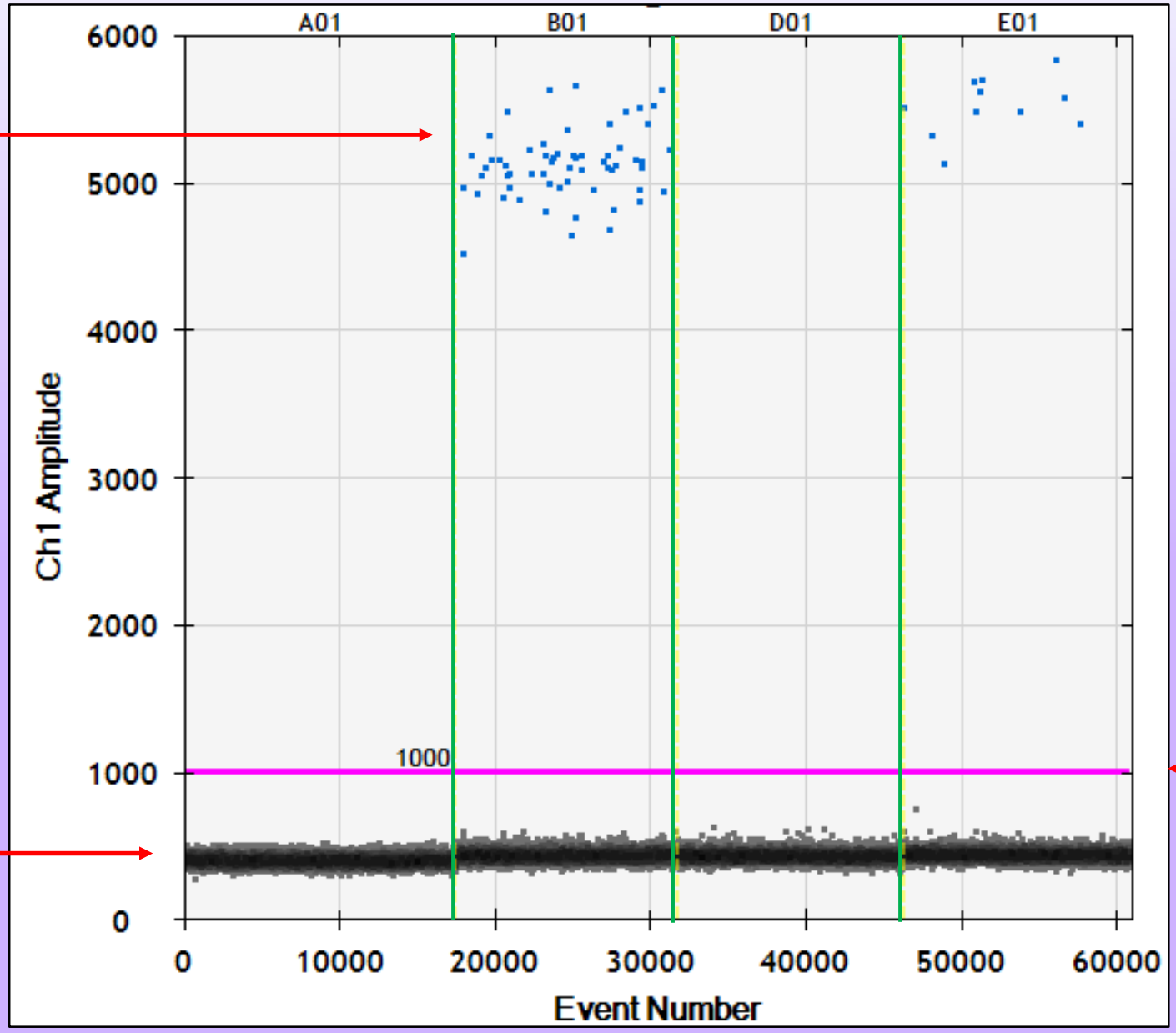
- Droplets are analyzed for fluorescence detection





FWB-Neg FWB-Pos VQA 0 VQA 50

Positive droplets

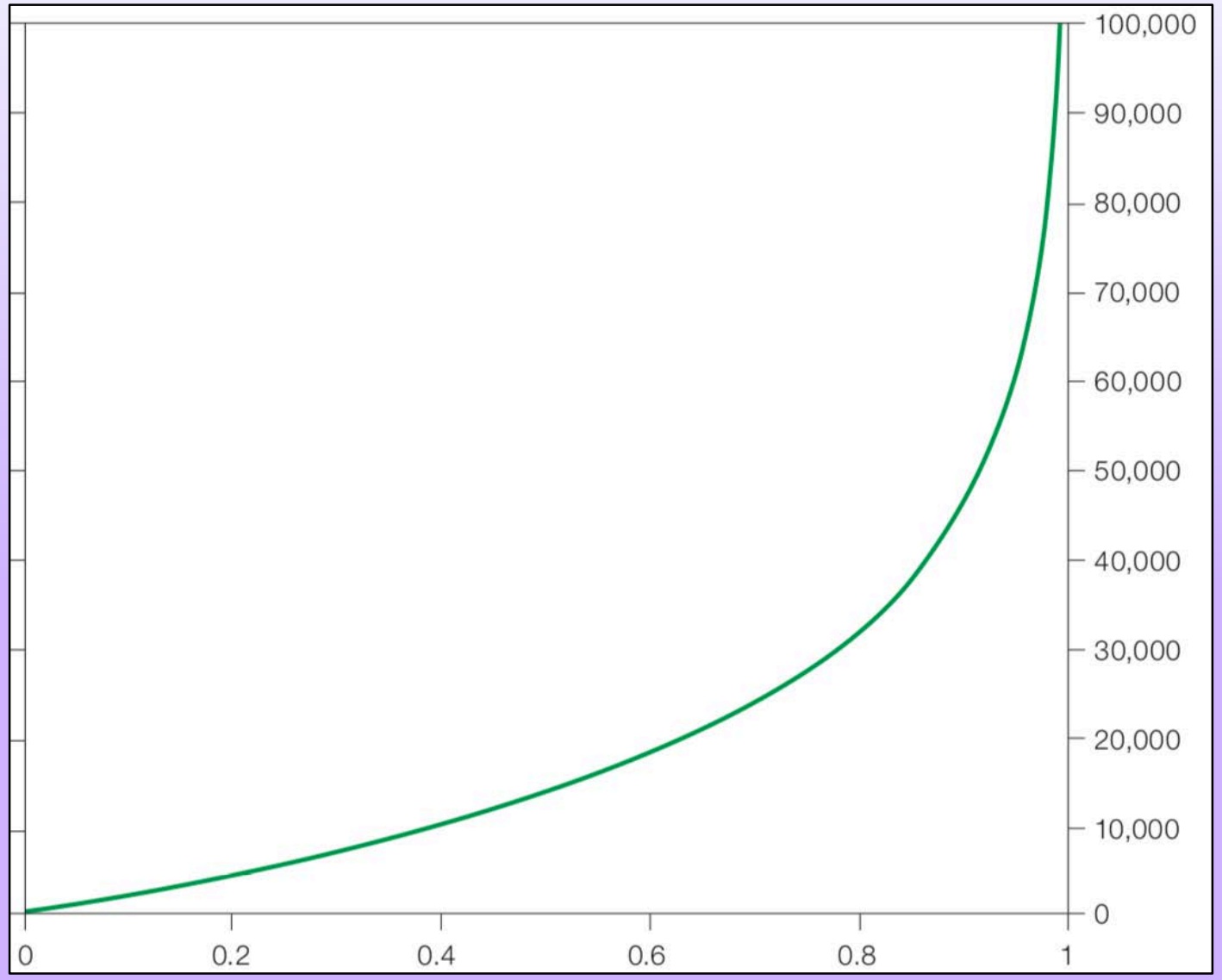


Threshold

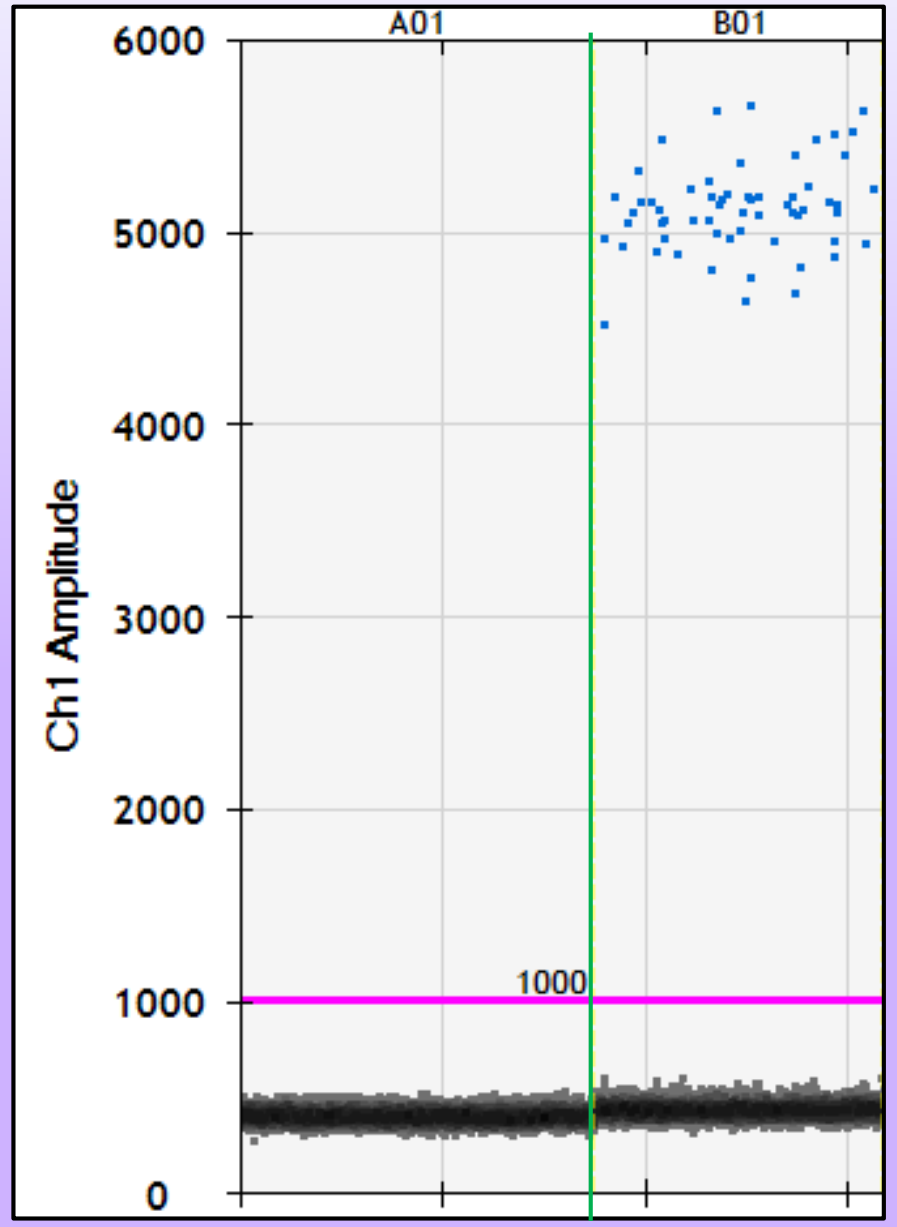
Negative droplets

Poisson Distribution for Absolute Quantification

Absolute quantification of target nucleic acid based on the fraction of positive droplets



FWB-Neg FWB-Pos

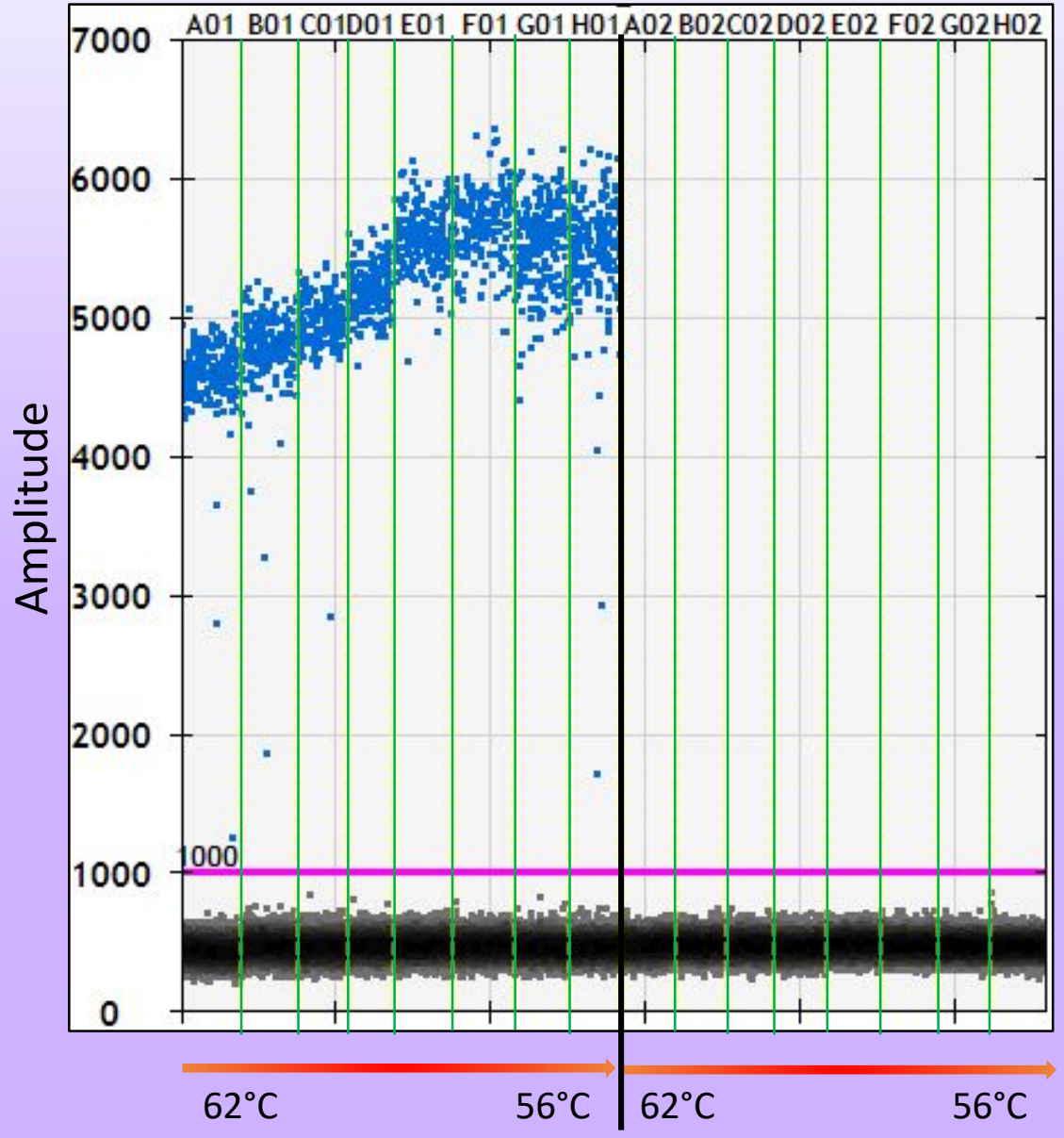


A01: 0 positive droplets
17,531 negative droplets
→ 0 copies/reaction

B01: 62 positive droplets
14,248 negative droplets
→ 102 copies/reaction

← Events

Annealing temperature gradient



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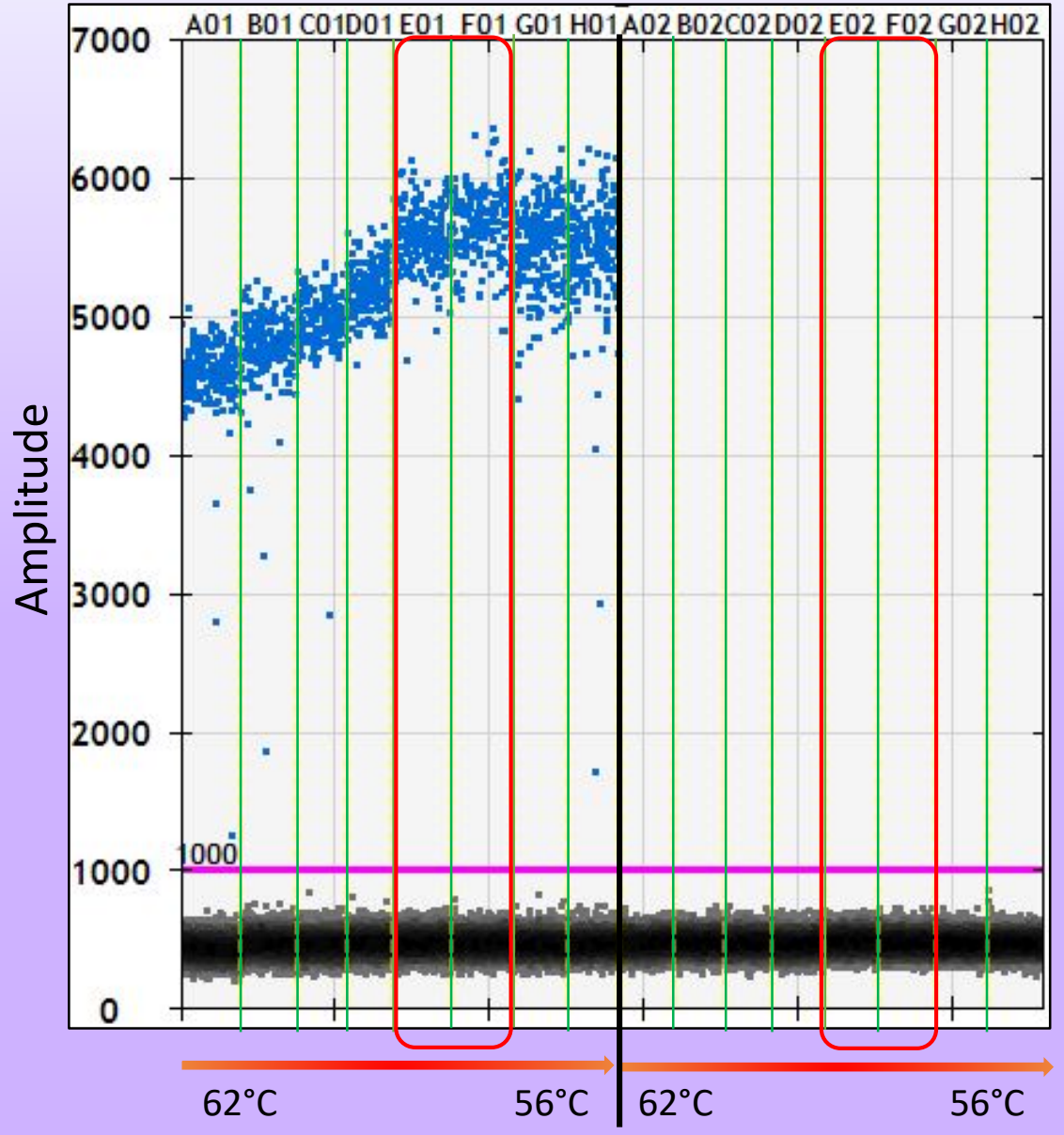


Optimal annealing temperature is 58°C

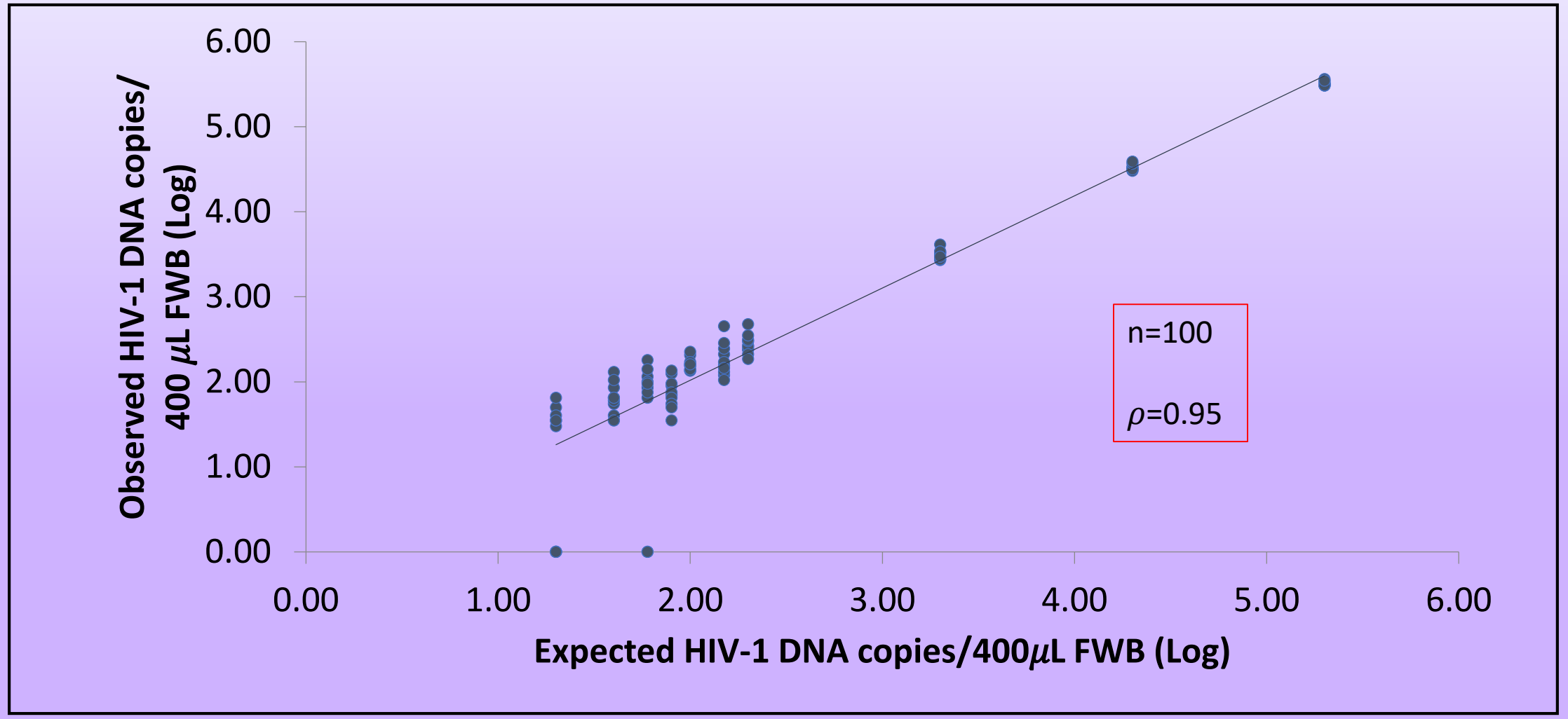
Limit of detection (LOD) panel

- Prepared using U1 cells and HIV-1 negative whole blood
  - HIV-1 cell line containing 2 DNA copies per cell

- 10 LOD levels: 10 replicates each
  - extracted/analyzed over 5 days

HIV-1 DNA copies/400 μL FWB:
200,000
20,000
2,000
200
150
100
80
60
40
20

Limit of detection panel results



Limit of detection panel results

- Probit analysis for failures
  - 95% Detection: 66 HIV-1 DNA copies/400µL FWB
  - 90% Detection: 53 HIV-1 DNA copies/400µL FWB
  - 80% Detection: 36 HIV-1 DNA copies/400µL FWB

- **Limit of Detection (LOD) = 66 HIV-1 DNA copies/400µL FWB**

Limit of detection panel results

HIV-1 DNA copies/400µL FWB (Log) expected from U1 cell content	Mean of 10 replicates HIV-1 DNA copies/400µL FWB (Log)	Coefficient of Variation 10 replicates
5.30	5.51	0.47%
4.30	4.52	0.74%
3.30	3.50	1.57%
2.30	2.44	4.79%
2.18	2.26	8.59%
2.00	2.17	5.91%
1.90	1.87	9.68%
1.78	1.96 (n=8)	8.13% (n=8)
1.60	1.79	10.82%
1.30	1.61 (n=7)	7.61% (n=7)

LOD

Diagnostic sensitivity and specificity

- FWB from HIV clinical trial participants (n=50) and VQA prepared FWB panels (n=24)
  - 43 are known HIV-1 infected
  - 31 are known HIV-1 uninfected

HIV-1 DNA from FWB	Diagnostic Sensitivity and Specificity		Total
	Detected	Not Detected	
Known Infected	43	0	43
Known Uninfected	0	31	31
Total	43	31	74

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No cross reactivity with HIV-2, Hepatitis B, Hepatitis C or *P. falciparum*

Potential inhibiting substances in whole blood

AcroMetrix™ Inhibition Panel:

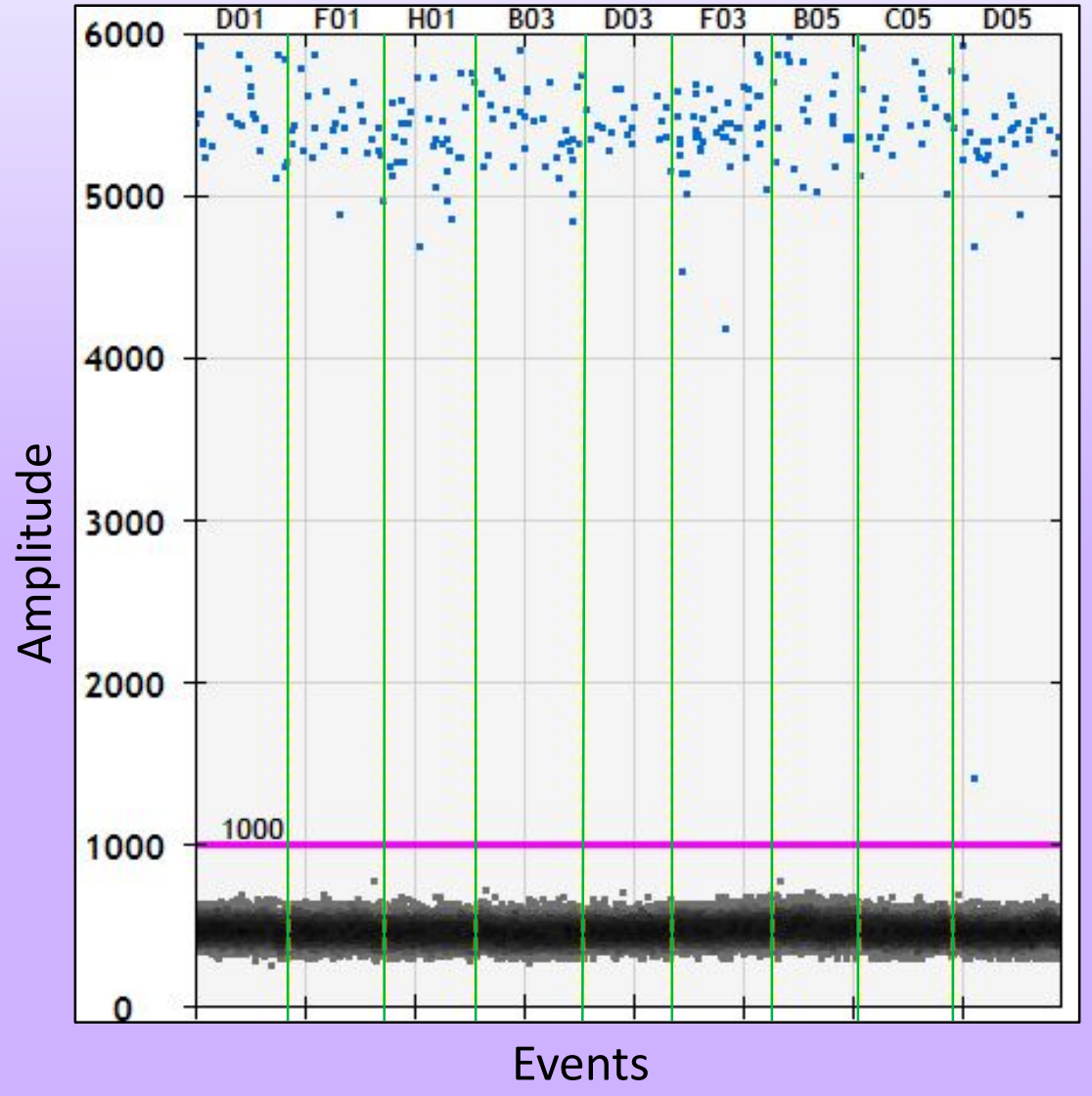
Substance
EDTA plasma
Hemolyzed Low
Hemolyzed Mid
Hemolyzed High
Heparin plasma
Lipemic
Icteric
No inhibitor A (control)
No inhibitor B (control)

Potential inhibiting substances in whole blood

AcroMetrix™ Inhibition Panel:

Substance	well
EDTA plasma	D01
Hemolyzed Low	F01
Hemolyzed Mid	H01
Hemolyzed High	B03
Heparin plasma	D03
Lipemic	F03
Icteric	B05
No inhibitor A (control)	C05
No inhibitor B (control)	D05

No significant change in droplet amplitudes or quantitation of HIV-1 DNA



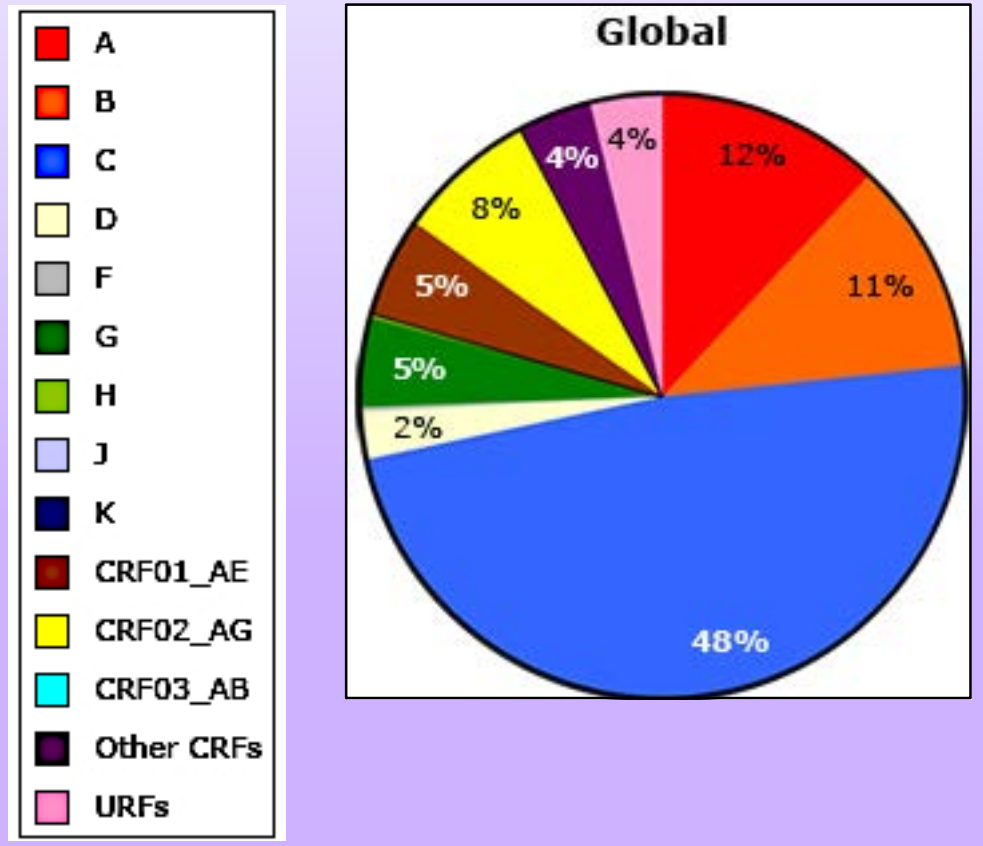
HIV-1 genetic diversity panel

- External Quality Assurance Program Oversight Laboratory (EQAPOL) at Duke Human Vaccine Institute

- Comprised of 50 circulating strains of HIV from around the world
  - Viral isolates – performed RT-ddPCR

EQAPOL HIV-1 genetic diversity panel

Subtype	Number of Unique Sequences Evaluated
A	2
B	15
C	5
D	3
F	2
G	1
BF	2
AD	1
ADG	1



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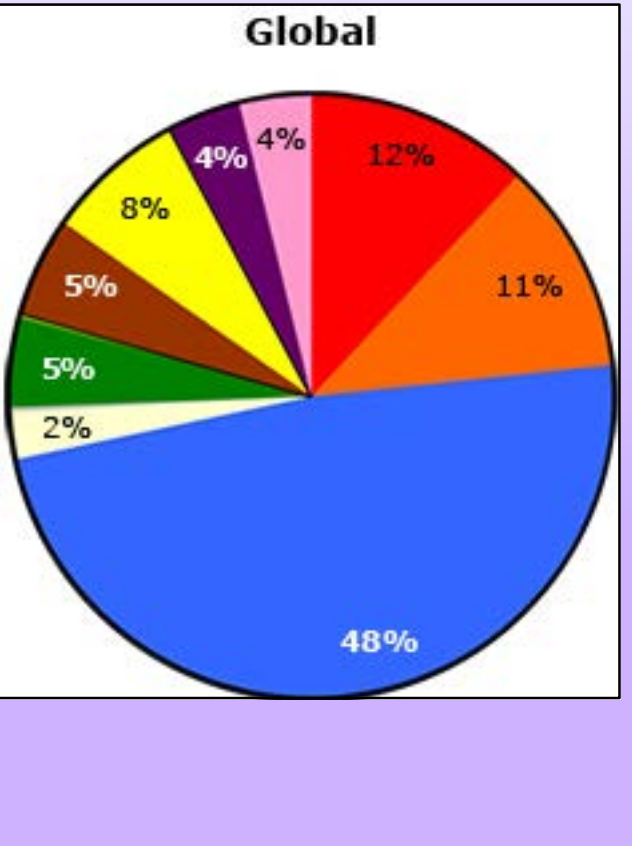
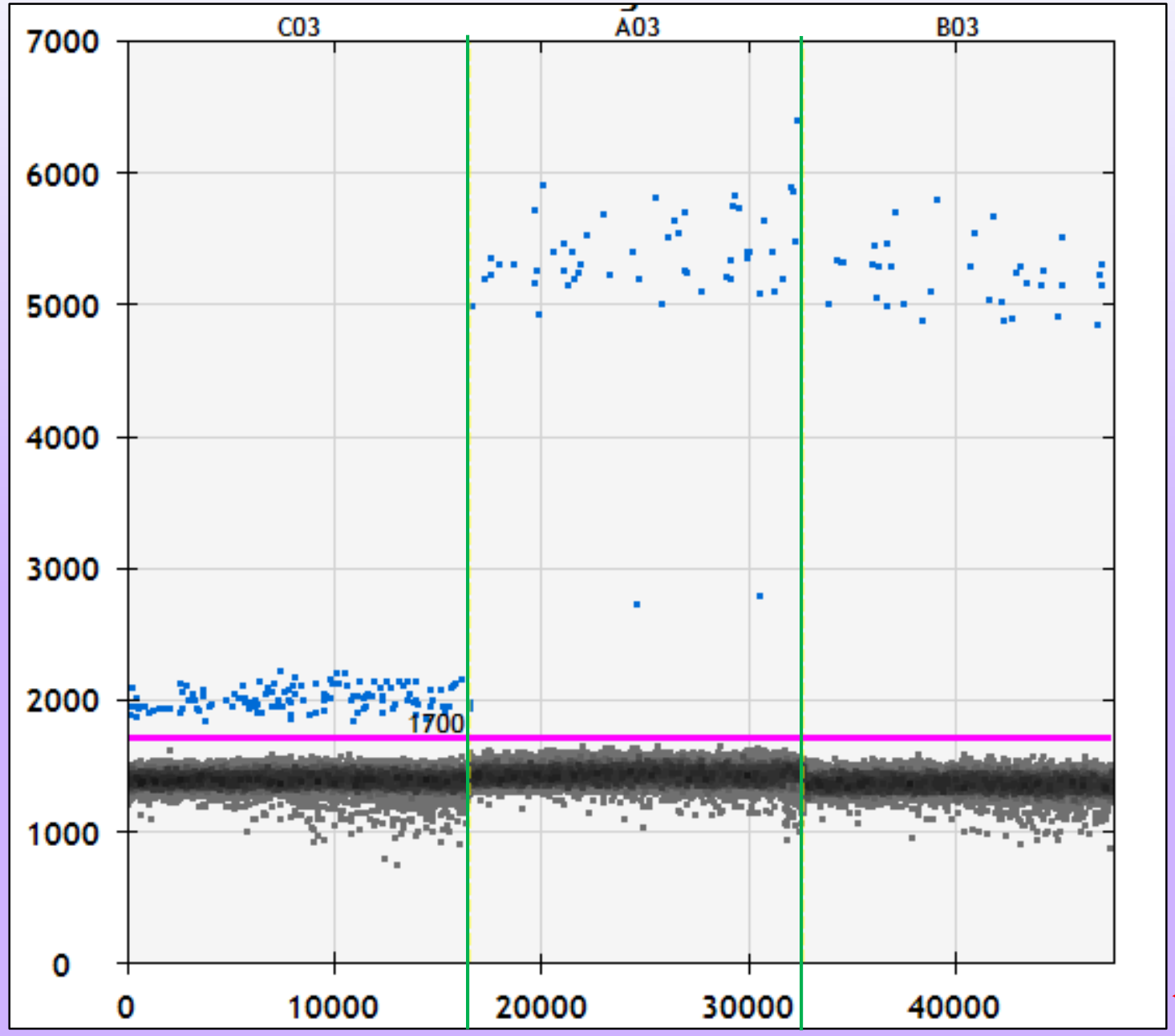
Recombinant Forms Evaluated
CRF01_AE
CRF02_AG
CRF14_BG
CRF24_BG
CRF47_BF
CRF04_CPX
URF_A1B
URF_BC



EQAPOL HIV-1 genetic diversity panel results



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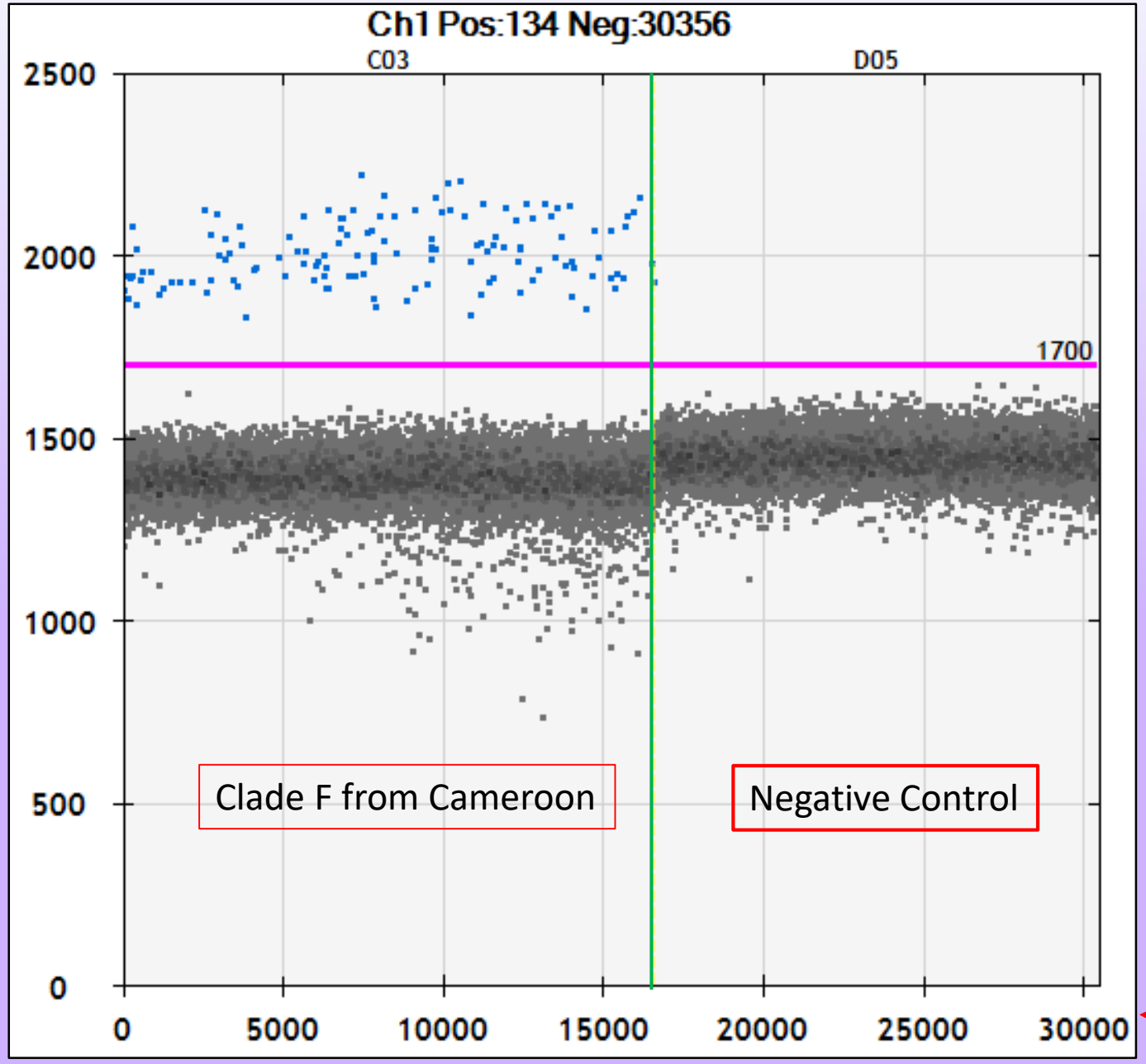

Simple Cost Analysis

	10 samples (half plate)	24 samples (full plate)
Whole Blood Extraction	$177	$340
ddPCR	$256	$475
Labor/Admin (UW fixed)	$548	$822
Total cost per run	$980	$1,637
Total cost per sample	$98	$68

4 controls extracted/analyzed per run, HIV-1 DNA ddPCR run in duplicate, RPP30 ddPCR run in singleton. Consumable costs based on pricing negotiated by UW. Labor, administrative and repeat costs fixed per UW.

In Summary

- Whole blood matrix
- LOD: 66 HIV-1 DNA copies/400µL FWB
- Absolute quantification – no standard curve comparison required
- Cross-clade detection ability
- Reasonably priced nucleic acid test

Acknowledgements

ddPCR Team

* Emily Degli-Angeli
  Glenda Daza

Everyone in the UW Retrovirology Laboratory

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Retrovirology Staff

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