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INCREASING POSITIVITY FOR NON-PLASMA SAMPLES USING THE ABBOTT REALTIME HIV-1 ASSAY

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BACKGROUND:

Measuring HIV-1 RNA levels in biological compartments of infected patients provides insight to viral shedding, transmission, reservoirs, and pathological manifestations. Commercial HIV-1 RNA quantitative assays approved by the FDA for plasma samples are often used to quantify HIV-1 RNA in non-plasma specimens. Endocervical swabs, for example, contain bacterial and human byproducts which may affect PCR amplification of HIV-1 and subsequently generate false negative results.

AIM:

A retrospective study on the archived tested results by the Abbott Real*T*ime HIV-1 assay in 2015

MATERIALS & METHODS:

Abbott RealTime HIV-1 assay

- RNA extraction from plasma samples
- HIV-1 RNA amplification, detection and quantification

The default interpretive criteria for plasma samples

- MaxRatio (MR): The incremental change of florescence intensity during PCR amplification. The maximal value of incremental changes for each sample
- MR ≥ 0.070: HIV-1 RNA detected
  MR < 0.070: HIV-1 RNA not detected
- Samples w/ MR ≥ 0.070 → determined the cycle number (CN) where the amplification curve crossed the threshold →HIV-1 RNA copies/mL

Materials

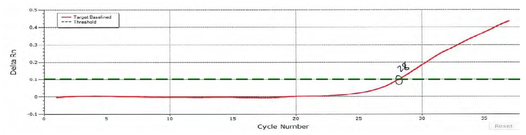
- Abbott m2000rt archived tested results of
  - **148 plasma** samples from UWMC
  - **434 plasma** samples collected from Kenya/Uganda from 2008 to 2012 as part of the Partners PrEP Study
  - **762 endocervical swab** samples collected from HIV-1 infected female individuals within the first year of ART in Kenya and Uganda as part of the Partners PrEP Study
- Archived data for tested samples: Sample ID, HIV-1 MR, HIV-1 CN, HIV-1 RNA result, Internal control (IC) CN, IC MR and the amplification graph

Methods

- Examined **the amplification graph** for each default **"not detected"** sample where the internal control was acceptable.
  - If the sample's amplification **curve crossed the default HIV-1 threshold** (Δ **Rn=0.100**), then a CN was identified and recorded to estimate HIV-1 RNA copies/swab.
  - If the sample's amplification curve did not cross the threshold, then the sample was assigned CN=38.00 and was interpreted as a negative result.

RESULTS:

Example: an endocervical swab sample with HIV MR<0.070, the amplification curve crossing the threshold (delta Rn=0.1)

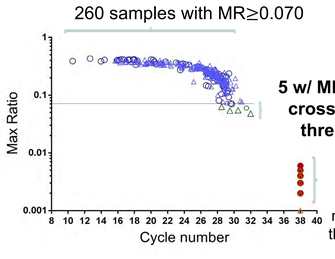


Default interpretation:

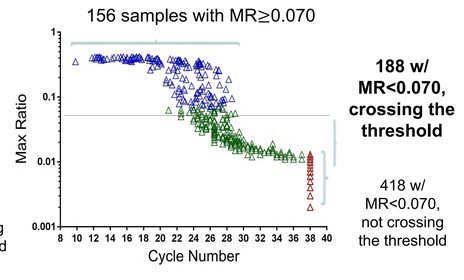
- HIV-1 Result: Not detected
- HIV-1 CN: -1.00
- HIV-1 MR: 0.048
- IC CN: 20.75
- IC MR: 0.206

Sample type	Source	# samples	# default HIV-1 detected	# default HIV-1 not detected	# crossing threshold	# not crossing threshold
Plasma	UWMC	148	63	85	1	84
Plasma	Kenya/ Uganda	434	197	237	4	233
Endo-cervical swab	Kenya/ Uganda	762	156	606	**188**	418

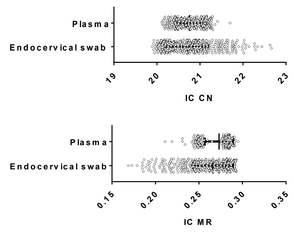
The distribution of HIV MRs for **plasma samples** from Kenya/Uganda (○) and UWMC (△)



The distribution of HIV MRs for **endo-cervical swabs** from Kenya/Uganda



IC CN and MR of plasma and endocervical swab met the default criteria. Variances were significantly higher for endocervical swab than plasma.



Welch's t-test		Plasma	Endo-cervical swab	*P* value for mean	*P* value for variance
Mean ± SEM	IC CN	20.75 ± 0.012	20.71 ± 0.017	0.0194	<0.0001
	IC MR	0.273± 0.000678	0.266± 0.000854	<0.0001	<0.0001

Endocervical swabs' HIV-1 RNA levels

The criterion for HIV-1 positivity: MR ≥0.070		The criterion for HIV-1 positivity: confirmation of the amplification curve crossing the threshold	
MR≥0.070 N=148 with quantifiable results (range: 2.40-7.65 Log_{10} copies/swab) N=8 with results <2.39 Log_{10} copies/swab			
MR<0.070	N=606 with HIV-1 not detected results	**MR range: 0.011-0.068**	**N=64 with quantifiable results (range: 2.49-4.29 Log_{10} copies/swab**
			N=124 with results <2.39 Log_{10} copies/swab
		MR range: 0.002-0.013	**N=418 with HIV-1 not detected results**
HIV-1 RNA positivity	**20% (156/762)**	**HIV-1 RNA positivity**	**45% (344/762)**

Non-plasma sample type: endocervical swab

- PCR interfering factors may be the collection by endocervical swab containing mucus, cervical microbiomes, epithelial cells and lymphocytes (i.e., not the swab material). Increased variances of IC CN and MR indicated the presence of PCR interfering factors.
- Prior testing endocervical swabs from HIV-1 non-infected individuals may be beneficial to evaluate PCR interfering substances.
- Dilution of "positive samples" may reduce PCR interference.
- Alternative extraction protocols may reduce interference for cervical swabs. For example, the Abbott HIV-1 RNA protocol for dried blood spot (DBS), is optimized for DBS.

CONCLUSIONS:

- In addition to HIV RNA results, the Abbott HIV-1 assay provided amplification graphs and MR values useful for examining non-plasma sample results.
- In this study, an additional 188 HIV positive endocervical swabs were identified. HIV RNA positivity significantly increased from **20% to 45%** in swabs collected from infected females after initiation of ART, implying a greater rate of genital shedding than previously reported in some earlier studies(e.g. Mujugira et al. In J Acquir Immune Defic Syndr. 2016)

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