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Challenges with meeting stringent specimen requirements

Discussion Panel:
Using a molecular test as the second test in the HIV diagnostic algorithm

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Financial conflicts of interest:
None to report

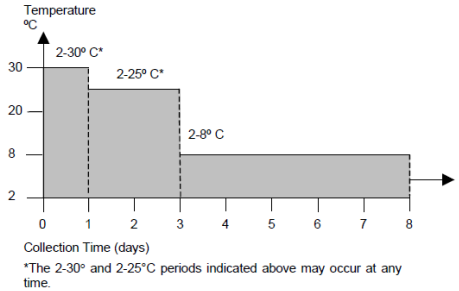
What are the challenges?

- Submitters may not be able to centrifuge blood tubes
  - e.g. Community-based HIV testing programs
- It is not always possible for blood specimens to arrive and be processed in the lab within a specified timeframe
- Labs are bound to follow specimen requirements in the package insert of an FDA-approved test
  - Although modifications may be feasible, the lab assumes all responsibility and expense

Comparison of specimen storage conditions

	Aptima HIV-1 RNA Qualitative (Manual)	Aptima HIV-1 RNA Quant Dx (Automated)
Whole blood	2°C to 25°C for ≤ 72 hrs, includes ≤ 24 hrs at up to 30°C	2°C to 30°C for ≤ 24 hrs
Separated plasma or serum*	2°C to 25°C for ≤ 72 hrs, includes ≤ 24 hrs at up to 30°C	2°C to 30°C for ≤ 24 hrs
Separated plasma or serum*	additional five days at 2°C to 8°C	2°C to 8°C ≤ 3 days

*Note: Only qualitative results can be reported for serum using the Aptima HIV-1 RNA Quant Dx assay



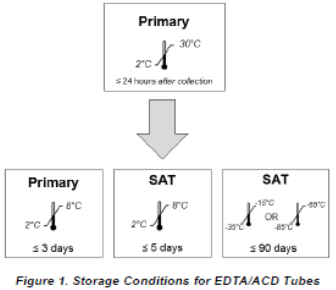


Figure 1. Storage Conditions for EDTA/ACD Tubes

Source: Package inserts (Hologic, Inc.)

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Wadsworth Validation Study

Objective: Validate the Aptima HIV-1 RNA Quant Dx assay for *qualitative* detection using EDTA-whole blood collected under the conditions allowed by the Aptima HIV-1 RNA Qualitative Assay

- Made serial dilutions using RNA+ plasma of known viral load
- Used plasma to reconstitute blood cells to create mock whole blood samples
- Stored samples at room temp (~23-25°C) for 24 hrs and 72 hrs
- Separated plasma and stored at 20°C until tested
- Validation studies:
  - Limit of Detection (LOD)
  - Reproducibility: Intra-assay (same day) and inter-assay (different days)

Analytical Sensitivity-Wadsworth

	24 hrs		72 hrs-1			72 hrs-2			72 hrs-3			72 hr	
Sample	copies/ml	Log_{10}	copies/ml	Log_{10}	ΔLog_{10}	copies/ml	Log_{10}	ΔLog_{10}	copies/ml	Log_{10}	ΔLog_{10}	Mean Log_{10}	ΔLog_{10}
1	6,587	3.82	5532	3.74	-0.08	5007	3.70	-0.12	not done	n/a	n/a	3.72	-0.10
2	2,642	3.42	2596	3.41	-0.01	2587	3.41	-0.01	not done	n/a	n/a	3.41	-0.01
3	1,484	3.17	1727	3.24	0.07	1184	3.07	-0.10	not done	n/a	n/a	3.16	-0.01
4	902	2.96	626	2.80	-0.16	730	2.86	-0.10	785	2.89	-0.07	2.85	-0.11
5	480	2.68	404	2.61	-0.07	316	2.50	-0.18	412	2.61	-0.07	2.57	-0.11
6	284	2.45	170	2.23	-0.22	200	2.30	-0.15	332	2.52	0.07	2.35	-0.10
7	134	2.13	93	1.97	-0.16	40	1.60	-0.53	115	2.06	-0.07	1.88	-0.25
8	77	1.89	75	1.87	-0.02	<30 detected	<1.47	n/a	93	1.97	0.08	1.92	0.03
9	30	1.48	46	1.66	0.18	<30 detected	<1.47	n/a	<30 detected	<1.47	n/a	n/a	n/a
10	54*	1.73	<30 detected	<1.47	n/a	<30 detected	<1.47	n/a	<30 detected	<1.47	n/a	n/a	n/a

*expected 20 copies/ml

Whole blood at 72 hrs:

- Qualitative detection of RNA at all dilutions
- Quantitation drops off at about 2 logs (100 copies/ml)

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Precision/Reproducibility-Wadsworth

Intra-run Precision

Sample	Average c/ml	SD	%CV
4-72	635	148.52	23.4
5-72	382	30.50	8.0
6-72	249	79.36	31.9
7-72	86	27.15	31.5
8-72	50	38.37	77.3

3 samples at each dilution tested on same day

Inter-assay Reproducibility

Sample	Average c/ml	SD	%CV
4-72	663	58.39	8.8
5-72	367	45.80	12.5
6-72	203	35.12	17.3
7-72	72	28.16	39.1
8-72	44	28.29	64.8

1 sample at each dilution* tested on 3 separate days

*Median of 3 results from precision run used for day 3 result

Variation increases at lower viral loads, approaching lower limit of quantitation

Points for Discussion

- EDTA-whole blood stored at 20-25°C for 72 hrs maintains acceptable performance for qualitative detection.
- LLOQ is higher at 72 hrs than for 24 hrs, but quantitative results are accurate
- Additional validation would be needed to determine the reliability of quantitative results under modified conditions
- Could more flexibility in specimen conditions be considered for qualitative detection in qual/quant assays?