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Evaluation of the Aptima HCV Quant Dx Assay for Qualitative and Quantitative RNA Detection Using Dried Blood Spots

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Hepatitis C virus (HCV) Molecular Testing in the Bloodborne Virus Lab (BVL)

15 Years

- HCV Genotyping with Phylogenetic Analysis
  - Outbreak Investigations
  - Surveillance

10 Years

- Confirmatory HCV RNA Testing
- HCV Viral Load Testing

3 Years

- Dried blood spots (DBS) for Confirmatory HCV RNA Testing

This Year

- Seeking Approval to use DBS for HCV Viral Load Testing

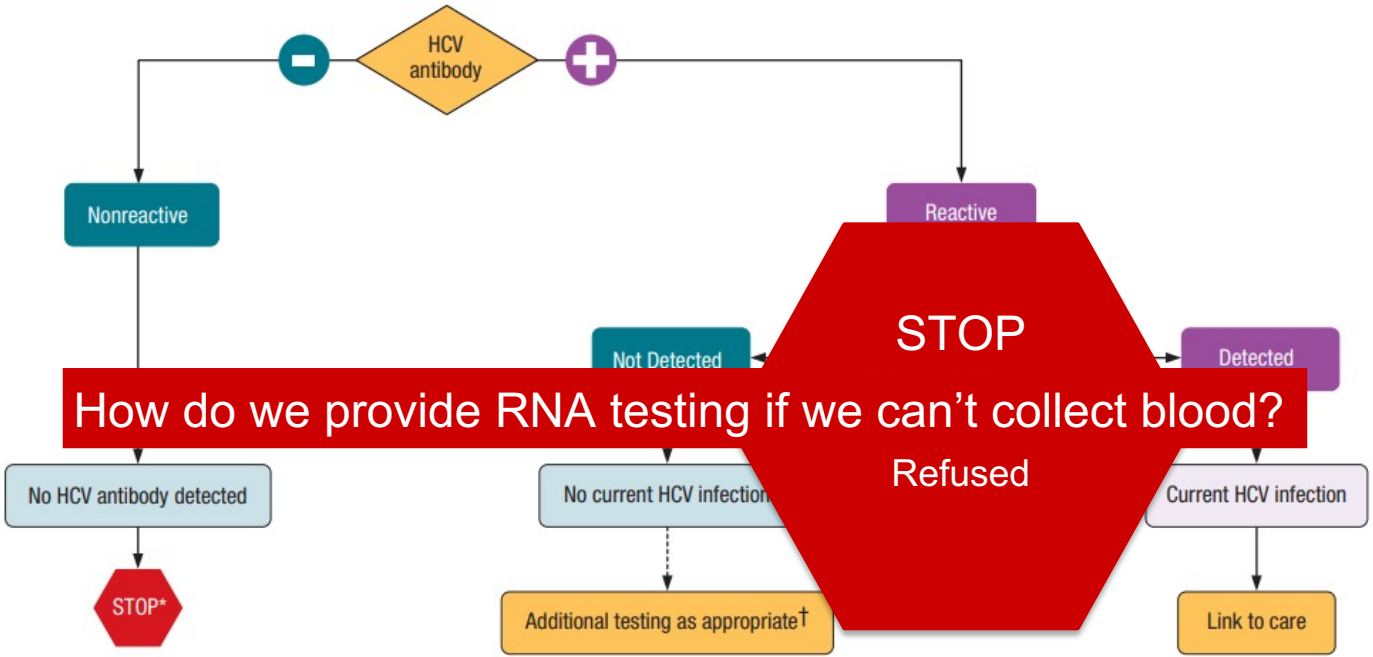
✓Random Access
✓ High Throughput
✓ Test Results in 3 Hours

Hologic's Aptima HCV Quant DX Assay is FDA Approved

- Real-Time Transcription Mediated Amplification Test
- Fully Automated Panther System
- Limit of Detection (LOD) of 3.9 IU/mL
- Lower Limit of Quantitation (LLOQ) of 10 IU/mL
- Fresh or Frozen Plasma or Serum Specimens

Recommended Testing Sequence for Identifying Current Hepatitis C Virus (HCV) Infection

U.S. Department of Health and Human Services
Centers for Disease Control and Prevention



STOP

How do we provide RNA testing if we can't collect blood?

Refused

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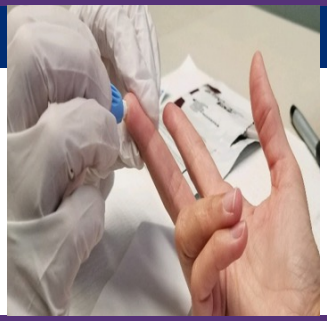

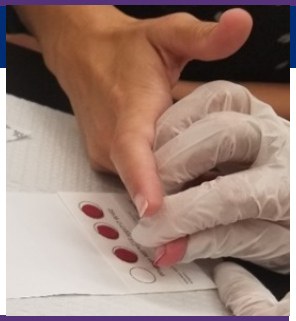

DBS: An Alternative to Plasma

They are simply collected by fingerstick onto filter paper.

No phlebotomist, centrifuges or cold chain needed.

*Photos from the New York State Department of Health's HCV Rapid Testing Program's Training for HCV RNA Testing via Dried Blood Spot

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All DBS for validation were made in the BVL
Not from fingerstick

2 Types:

- From Venipuncture
  HCV positive EDTA-whole blood
  Spotted prior to separating plasma

- Contrived
  HCV negative whole blood
  OR red blood cells spiked
  with HCV positive plasma

Clinical Laboratory Evaluation Program (CLEP) Modified Specimen Type for FDA Approved Assay Guidelines- Validation MUST Include:

1. Limit of Detection (LOD)
2. Lower Limit of Quantitation (LLOQ)
3. Upper Limit of Quantitation (ULOQ)
4. Stability at room temperature and 37°C, within 1 Log of LLOQ
5. Intra-Assay Reproducibility
6. Inter-Assay Reproducibility
7. Accuracy

Note: For 2-7 All viral load results must be compared to plasma

How can we compare 2 6-mm punches of a DBS to 700 uL of plasma?

Determining DBS Conversion Factor

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Volume 112, March 2019, Pages 40-44

Performance evaluation of the Hologic Aptima HCV Quant Dx assay for detection of HCV RNA from dried blood spots

Beth Catlett [a, b], Alex Carrera [a], Mitchell Starr [a], Tanya L Applegate [b], Peter Lowe [c], Jason Grebely [b, 1], H. Philip Cunningham [a, 1]

- *volume of ATM (µl) / [DBS volume (µl), x (1 – .45)]*
  - *(1000/70x.55=25.97)*

Volume of Buffer/ DBS Volume x 1-Hematocrit

Volume of (DBS Elution Buffer)= 750 uL
DBS Volume=2 x 10 uL Punches= 20 uL
1-Hemarocrit= 1-0.45= 0.55

Determining OUR DBS Conversion Factor

750 (µl) / [20 (µl), x 0.55]

750 (µl) / 11= 68.2

Limit of Detection (LOD) Goals:

✓ Duplicate testing on multiple runs

✓ ≥95% detected (qualitative)

DBS LOD Shown as percent detected for two 6-mm Punches

IU/mL	# Replicates tested	# Detected	% Detected
1000	7	7	100%
500	7	7	100%
250	7	7	100%
125	7	1	14%
62.5	7	3	43%
31.25	7	0	0%

Result: We can detect down to
2.4 Log_{10} IU/mL (250 IU/mL)
This is our LOD

Lower Limit of Quantitation (LLOQ) Goals:

✓ 20 replicates on multiple runs

✓ ≥95% quantitated within 1 Log of plasma

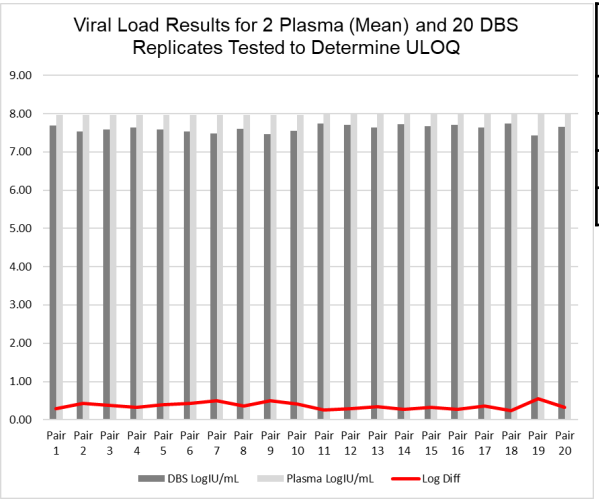
Dilution →

Log_{10} IU/mL	3.12	3.14	3.26	3.34	3.42	3.57	3.61
# tested	20	20	20	20	20	20	20
# quantitated	19	20	20	19	20	20	20
% quantitated	95	100	100	95	100	100	100
% with VL diff ≤ 1	0	0	0	0	0	0	0
Log difference range	-0.35-0.13	-0.38-0.03	-0.25-0.42	-0.31-0.39	-0.10-0.44	-0.15-0.42	-0.21-0.41

Result: We can quantitate down to
3.12 Log_{10} IU/mL (1304 IU/mL)
This is our LLOQ

Upper Limit of Quantitation (ULOQ) Goals:

- ✓Quantitated result within 1 log of plasma
- ✓ 20 replicates on multiple runs
- ✓ Low SD between samples



Run	N	[Mean] IU/mL	[Mean] log_{10} IU/mL	Intra Run SD
1	10	37,033,041	7.61	0.06
2	7	44,855,939	7.65	0.07
3	3	49,457,526	7.69	0.05
Total	20		Inter Run SD (Total SD)	0.08

7.65 Log_{10} IU/ml

The highest viral load we can accurately quantitate

Stability Question: At what condition does the quantitative value become unreliable?

Plasma VL 3.61	Storage Condition	
Sample	Ambient	37°C
3dayRep 1	3.41	3.57
3dayRep 2	3.49	3.44
3dayRep 3	3.4	3.62
6dayRep 1	3.49	3.38
6dayRep 2	3.51	3.25
6dayRep 3	3.52	3.47
8dayRep 1	3.51	3.45
8dayRep 2	3.71	3.4
8dayRep 3	3.27	3.4
14dayRep 1	3.44	3.66
14dayRep 2	Not done	3.53
14dayRep 3	3.33	3.38
16dayRep 1	3.54	3.31
16dayRep 2	3.62	3.52
16dayRep 3	3.75	3.75
Mean	**3.5**	**3.47**
SD	**0.13**	**0.14**
% CV	**3.81**	**3.9**

We still don't know the answer

We do know that: Little variability exists between our conditions across all timepoints and when compared to plasma

Reproducibility:
Intra-Assay: 3 replicates of 5 specimens
Inter-Assay: 3 runs of 4 triplicate specimens

Sample	Dilution 1	Dilution 2	Dilution 3	Dilution 4	Dilution 5
Intra-Assay Reproducibility Run 1					
Mean	3.35	3.83	4.27	4.48	4.71
SD	0.12	0.09	0.07	0.09	0.04
% CV	3.59	2.27	1.54	2.07	0.90
Intra-Assay Reproducibility Run 2					
Mean	3.28	3.71	4.12	4.49	4.85
SD	0.10	0.10	0.04	0.04	0.10
% CV	3.07	2.71	0.95	0.84	2.11
Intra-Assay Reproducibility Run 3					
Mean	Sample Exhausted	3.78	4.11	4.32	4.72
SD		0.10	0.05	0.06	0.06
% CV		2.63	1.22	1.44	1.18
Inter-Assay Reproducibility Run 1- Run 3					
Mean	Not Done	3.77	4.17	4.43	4.76
SD		0.06	0.09	0.10	0.08
% CV		1.55	2.07	2.17	1.58

[SD Range] 0.04-0.12
[%CV] 0.84- 3.59

Little variability within runs and between runs

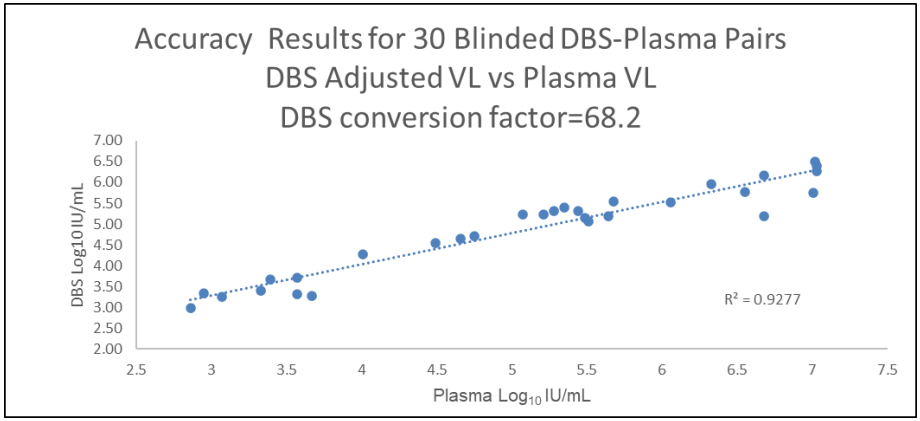
Blinded Accuracy

One plasma sample viral load (VL)=15 (<DBS LOD)

	Quantitated Plasma	Negative Plasma	Total
Quantitated DBS	29	0	29
Negative DBS	1	11	12
Total	30	11	41

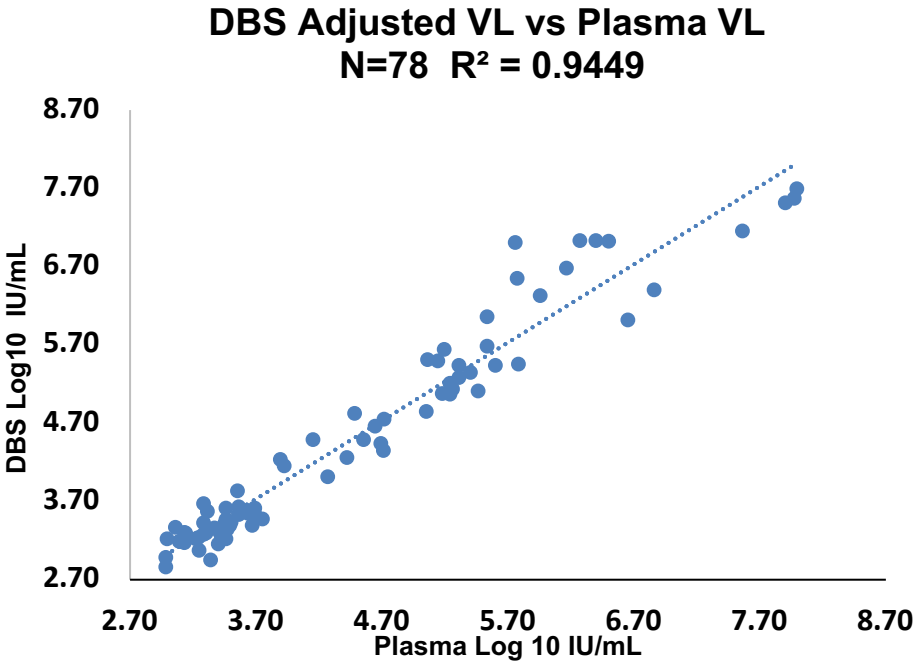
R^2 is acceptable

The lowest quantitated DBS VL= 748



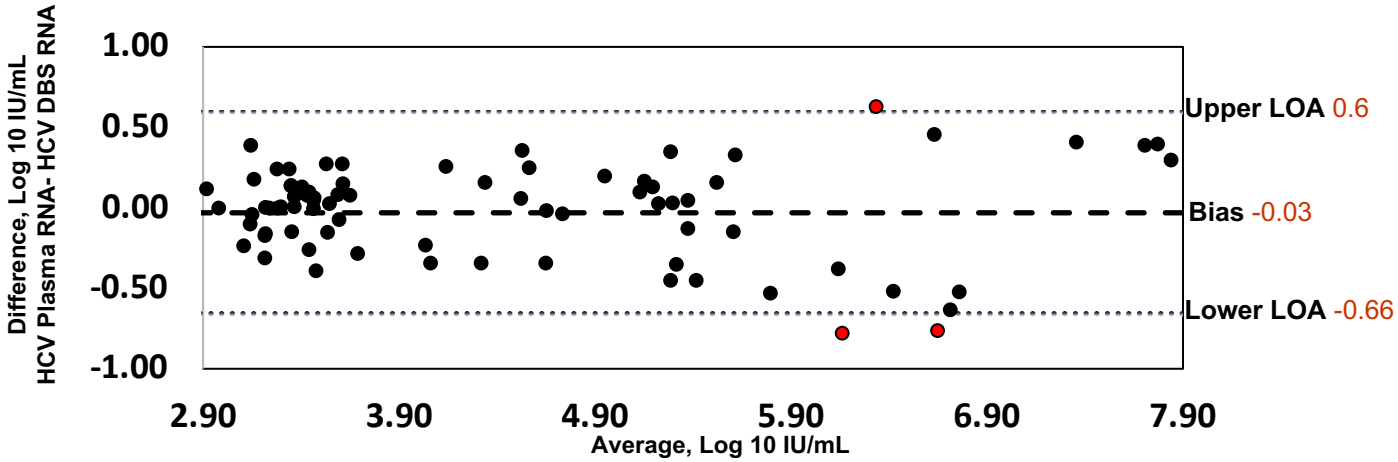
There is a strong correlation between plasma and DBS

Linear Regression



Predicted strong, positive correlation between plasma and DBS holds true

Bland Altman Analysis: 78 Paired Specimens



Ideally, bias should be close to 0 if producing similar results: -0.03
≥95% of points should be between the Limits of Agreement (LOA): 96.9%

There is a high degree of agreement between plasma and DBS

The assay is accurate and highly reproducible when using the DBS conversion factor of 68.2 for DBS:

✓stored up to 15 days between room temperature and 37°C
✓viral loads between 3.12 and 7.98 Log_{10} IU/mL

Should we use DBS for viral load testing?

Yes If venipuncture is not an option

Acknowledgements

- The New York State Department of Health's AIDS Institute
- The AIDS Institute's HCV Rapid Testing Program
- New York State Rapid Testing Sites
- The Wadsworth Center's Bloodborne Virus Laboratory

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