Validation of Viral Load Assays for Diagnostics: New York State's Experience

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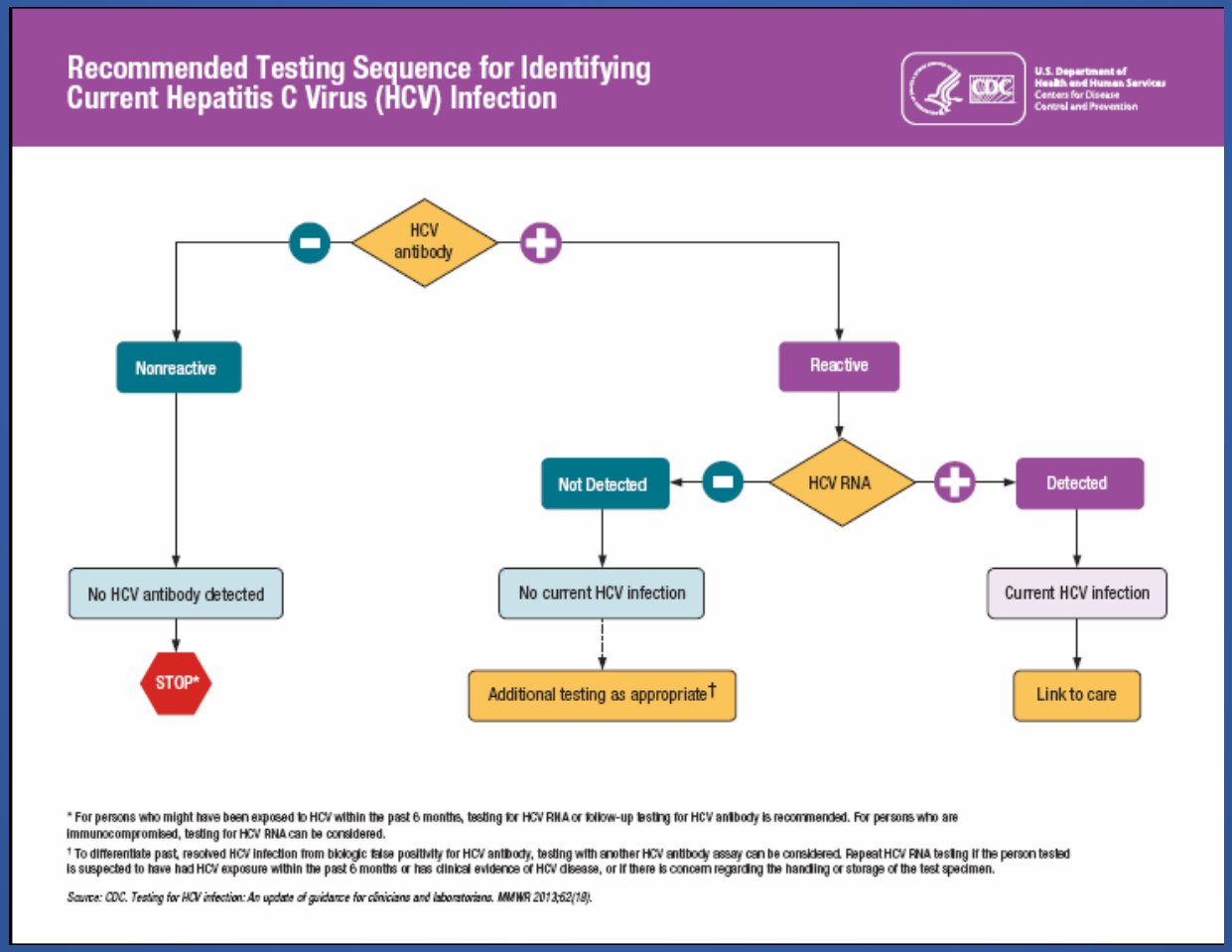
Reflexing to an HCV RNA test after a reactive antibody test is the recommended practice for laboratories



Several FDA-approved HCV RNA tests are available

	Test Name	Manufacturer	Intended Use	LOD/LLOQ	Specimen Type
Qualitative	**VERSANT HCV RNA Qualitative Assay/APTIMA HCV RNA Qualitative Assay**	Gen-Probe	Diagnostic	7.5 IU/mL (gt 1) 9.6 IU/mL overall	Serum or plasma
	COBAS Amplicor HCV v2.0 *and* **COBAS AmpliPrep/COBAS Amplicor HCV, v2.0**	Roche		100 IU/mL	Serum or plasma
	AMPLICOR HCV Test, v2.0	Roche		50 IU/ml	Serum or plasma
Quantitative	**Abbott RealTi*me* HCV**	Abbott	Aid in the management of HCV-infected patients undergoing antiviral therapy	12/12 IU/mL	Serum or plasma
	COBAS AmpliPrep/ COBAS Taqman HCV Test	Roche		15/15 IU/mL	Serum or plasma
	COBAS TaqMan HCV Test For Use w/ High Pure System	Roche		20/25 IU/mL	Serum or plasma
	VERSANT HCV RNA 3.0 bDNA	Siemens		LOD 988/1100 IU/mL (340/440 system) Detection Cutoff 615 IU/mL	Serum or plasma

Modified from APHL's "Testing For Hepatitis C Viral Infections: Frequently Asked Questions"

Labs must adhere to the FDA-approved package insert

- A modification constitutes off-label use of an FDA-approved device
- Before an HCV viral load result can be reported for diagnostic use, a full validation is required
- The laboratory is responsible for conducting the validation studies

Many questions emerge:

- What type of validation? How many samples? What criteria must be met?

Why is NYS involved in this process?

Wadsworth Center is charged with oversight of clinical labs in NYS

- NYS Public Health Law: NYSDOH has regulatory authority over clinical laboratories and blood banks performing testing on specimens originating in NYS
- *The department shall operate a reference system and prescribe standards for the proper operation of clinical laboratories and blood banks*
- *The department may review and approve testing methods developed or modified by clinical laboratories and blood banks prior to the testing methods being offered in this state*

Scope of the NYS Clinical Laboratory Reference System

- On site surveys of laboratory facilities
- Operation of Proficiency Testing Program
- Review of laboratory personnel qualifications
- Review and approval of assays not expressly approved (PMA), cleared (510k), or exempted by the FDA
- What types of tests need to undergo review and approval for being used clinically?

Tests that require NYS review and approval

- Commercialized test kits variously labeled as
  - Research Use Only (RUO) or
  - Investigational Use Only (IUO)

- Laboratory Developed Tests (LDTs) that include the use of reagents prepared by the lab and/or analyte-specific reagents (ASRs)

- FDA cleared/approved tests that have been modified from their intended use

NYS has a formal approval process for LDTs & modified FDA-approved tests

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COMPREHENSIVE TEST APPROVAL POLICY AND SUBMISSION GUIDELINES

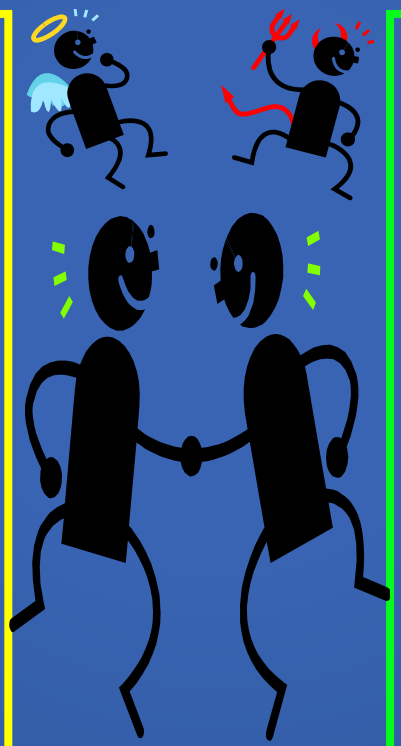
Subsection 58-1.10(g) of Part 58 of Title 10 (Health) of the Official Compilation of Codes, Rules and Regulations of the State of New York states that *all technical procedures employed in a laboratory shall be of proven reliability and generally accepted by leading authorities in the specialties of laboratory medicine and/or approved by the Department*. A laboratory can perform only those assays for which the performance characteristics have been *established and validated*, or if

Submission Guidelines available for:

- Cellular Immunology
- Forensic Identity
- Genetic Testing-Molecular
- *Molecular Microbiology*
- Oncology-Molecular
- Toxicology
- Trace Elements

Wadsworth scientists are responsible for reviewing applications

Clinical Laboratory



Regulatory Program

Submission guidelines were developed to standardize the process

- Molecular Microbiology NAAT Checklist and detailed guidelines available on NYSDOH website*
- Components of a Full Validation Package include:
  - Standard operating procedure manual (SOPM)
  - Test requisitions and reports
  - Pertinent literature references
  - Plans for carrying out proficiency testing
  - Data demonstrating the analytical and clinical validity of the assay

*http://www.wadsworth.org/labcert/TestApproval/forms/Microbiology_NAAT_Checklist.pdf

NYS established validation guidelines for lab-developed microbiology NATs

- Specificity/Cross-reactivity
  - Include organisms that are genetically-related; produce similar symptoms; occur in the same specimen type
- Sensitivity/LOD
  - Methods that determine LOD with 95% confidence
  - Dilution series of ≥ 3 separate samples, in duplicate
- Reproducibility
  - ≥ 3 authentic or spiked clinical samples on 3 different days including ≥ 1 at or near the LOD, or
  - A single positive control run on ≥ 15 days
- Precision
  - ≥ 3 authentic or spiked clinical samples in triplicate on the same run, including ≥ 1 at or near the LOD
  - Repeat for different instruments, operators etc.

NYS validation guidelines cont'd: Accuracy verification for LDTs

- Compare results to those of a gold standard or FDA or NYS-CLEP approved assay
- With a minimum of 30 positive and 10 negative samples for each specimen matrix
  - at least 10 of the 30 positives should be weak positives, i
- Clinically relevant subtypes or genotypes must be represented in the study, when applicable
- Study should be conducted in blinded manner
- Any discrepant results need to be explained

NYS checklist includes guidelines for modified FDA or NYS-approved assays

Full validation is required for:

- Any major modification (e.g. change in primer, probe or genomic target region)
- A change in intended use such as using a quantitative assay (prognostic) for qualitative detection (diagnostic)
  - Specificity/Cross-reactivity
  - Sensitivity/LOD
  - Reproducibility and Precision

Does this make sense in all cases?

- It depends on the performance characteristics and the validation data in the package insert
  - Older FDA-approved tests may not have the same type of data as newer ones
- Some data collected by the manufacturer for a quant assay may suffice for a qual/diagnostic assay
  - Provided that no changes are being made to the FDA-approved procedure
- Need to review each assay's data and determine acceptance criteria

NYS guidance for validation of HCV quantitative assays for diagnostic use

- Wadsworth Bloodborne Viruses section reviewed HCV testing recommendations, package inserts, and current NYS test validation guidelines
- Draft guidance was presented to the Wadsworth Method Validation Workgroup
  - Workgroup comments incorporated
- Draft guidance will go out to CMS and NYS permitted laboratories for comment
- What will labs be expected to do for approval?

Standard Operating Procedures

- Lab must submit their SOPs describing use of the HCV RNA test for diagnostic purposes
- The SOPs must include:
  - Specimen collection and storage requirements
  - Testing procedures
  - Quality control
  - Interpretation and reporting of results
- Guidance presented here is based on adhering to all steps in the package insert
- Any modifications to the manufacturer's instructions will require additional validation

Specimen collection and storage requirements can be a challenge for reflex testing

	Test Name	Intended Use	Whole blood storage	Serum/Plasma storage*
Qualitative	**APTIMA HCV RNA Qualitative Assay**	Diagnostic	≤ 24 hr at room temp	2-8°C ≤ 48 hr
Qualitative	**COBAS Amplicor HCV v2.0**	Diagnostic	≤ 6 hr at 2-25°C	2-8°C ≤ 72 hr
Quantitative	**Abbott RealTi*me* HCV**	Management	≤ 6 hr at 2-30°C	15 -30 °C ≤ 24 hr 2-8°C ≤ 72 hr
Quantitative	**COBAS AmpliPrep/ COBAS Taqman HCV Test**	Management	≤ 6 hr at 2-25°C	2-8°C ≤ 72 hr

*Serum/plasma may be stored at -20 and -70 for variable lengths of time

NYS draft guidance for validation of HCV RNA quantitative assays for diagnostic use

Sensitivity/LOD

- The LOD must be equivalent or better than the FDA-approved diagnostic assays
  1. Abbott RealTime HCV: 12 IU/mL
  2. Roche COBAS AmpliPrep/COBAS TaqMan HCV: 15, 20 IU/mL
  3. VERSANT RNA 3.0 HCV bDNA: 988 IU/mL
- Only assays 1 and 2 will be considered for diagnostic use
- Lab needs to verify the LOD in each specimen matrix
  - LOD should be verified for at least 3 different genotypes
  - For each sample, perform test on a dilution series, including one dilution below the LOD
  - Quantitated clinical samples or spiked virus in appropriate matrix may be used

Specificity/Cross-reactivity

- Were validation studies of similar scope conducted?
- Are results comparable to those of FDA-approved diagnostic RNA detection assays?
- Primers and probes are sufficiently specific; no additional specificity/cross-reactivity data are required

Quantitative/Management

Table 11: Cross Reactivity Specimens

Viruses	Non-HCV Flavivirus
Adenovirus type 2	West Nile Virus
Cytomegalovirus	St. Louis Encephalitis Virus
Epstein-Barr virus	Murray Valley Encephalitis Virus
Human Herpes Virus type 6	Dengue Virus Type 1
Herpes simplex virus type 1	Dengue Virus Type 2
Herpes simplex virus type 2	Dengue Virus Type 3
Human T-Cell Lymphotropic virus type 1	Dengue Virus Type 4
Human T-cell Lymphotronic virus type 2	Yellow Fever Virus
Influenza A	Zika Virus
Hepatitis A virus	Banzi Virus
Hepatitis B virus	Ilheus
Human Immunodeficiency Virus Type 1B	FSME Virus
	Heptatis G Virus (GBV-C)
Bacteria	**Yeast**
Staphylococcus aureus	Candida albicans
Propionibacterium acnes	

Roche COBAS Ampliprep/COBAS TaqMan HCV

Qualitative/Diagnostic

Microorganism Exclusivity

Specificity of the COBAS AMPLICOR HCV Test, v2.0 was evaluated by testing for potential cross-reactivity with, or interference by, pathogenic microorganisms and normal epidermal microflora that could be present in specimens. Twenty-nine specimens that contained virus (25) or bacteria (4) yielded Negative HCV and Positive HCV IC results (Table 9). These results indicate that the COBAS AMPLICOR HCV Test, v2.0 did not cross-react with a variety of viruses and bacteria that could be present in specimens and that these microorganisms did not interfere with amplification of HCV IC RNA.

Roche COBAS Amplicor HCV v2.0

Reproducibility and Precision

- Were criteria for inter-assay, intra-assay, operator-to-operator reproducibility met for low, mid and high range samples?
- Reproducibility and precision data of the real-time PCR assays are robust; no additional validation required

Quantitative/Management

Qualitative/Diagnostic

Table 8
Abbott RealTime HCV Clinical Reproducibility
(Log IU/mL)

Panel Member	Genotype	n	Mean Concentration (Log IU/mL)	Within-Run Component SD*	Between-Run Component SD*	Between-Lot Component SD*	Between-Site Component SD*	Total SD*[,b]
1	1a	270	7.98	0.04	0.00	0.02	0.08	0.09
2	1a	269[c]	5.15	0.05	0.03	0.00	0.05	0.09
3	1a	270	3.17	0.07	0.02	0.00	0.02	0.08
4	1a	269[d]	1.07[i]	0.21	0.00	0.03	0.04	0.22
5	1a	242[e]	0.61[j]	0.25	0.03	0.05	0.07	0.27
6	3	266[f]	6.96	0.06	0.02	0.02	0.06	0.09
7	3a	270	4.51	0.06	0.03	0.01	0.04	0.08
8	3a	270	2.61	0.07	0.02	0.01	0.03	0.08
9	3a	270[g]	1.34	0.17	0.00	0.04	0.07	0.19
10	3a	252[c,h,i]	0.79[k]	0.29	0.05	0.08	0.00	0.31

* Standard deviations are in log IU/mL.

Abbott RealTime HCV Assay

Table 8. Overall Reproducibility for EDTA Plasma Panel Members

	HCV RNA				
Genotype	IU/mL	Copies/mL	N	% Valid Agreement	95% C.I.
1	9.6	50	216	96.8	93.5–98.4
1	96	500	107	100	96.6–100
2b	57.6	300	219	96.8	93.6–98.4
2b	192	1,000	106	97.2	92.0–99.4
2b	577	3,000	107	99.1	94.9–100
Negative Serum	0	0	213	98.1	95.3–99.3
Negative Serum	0	0	221	99.5	97.5–99.9
Negative Serum	0	0	108	98.1	93.5–99.8

APTIMA HCV RNA Qualitative Assay

Accuracy Verification

- Verify against an FDA-approved, diagnostic RNA detection assay
- Use archived samples previously tested in-house or by another laboratory, or
- Prospectively, split samples for testing on the approved diagnostic assay and the assay being validated
- Blinded testing of 30-50 HCV RNA-positive and 30 HCV RNA-negative specimens is recommended
- Include an appropriate number of genotypes/subtypes among the positives
- Resolve any discrepancies between assay and comparator method

Interpretation and Reporting

- Example reports must be submitted for review
- Results should be reported using language consistent with the CDC recommendations (MMWR, May 7, 2013,Vol. 62)

TEST OUTCOME	INTERPRETATION	FURTHER ACTIONS
HCV antibody nonreactive	No HCV antibody detected	Sample can be reported as nonreactive for HCV antibody. No further action required. If recent exposure in person tested is suspected, test for HCV RNA.*
HCV antibody reactive	Presumptive HCV infection	A repeatedly reactive result is consistent with current HCV infection, or past HCV infection that has resolved, or biologic false positivity for HCV antibody. Test for HCV RNA to identify current infection.
HCV antibody reactive, HCV RNA detected	Current HCV infection	Provide person tested with appropriate counseling and link person tested to care and treatment.†
HCV antibody reactive, HCV RNA not detected	No current HCV infection	No further action required in most cases. If distinction between true positivity and biologic false positivity for HCV antibody is desired, and if sample is repeatedly reactive in the initial test, test with another HCV antibody assay. In certain situations,§ follow up with HCV RNA testing and appropriate counseling.

Comments are welcome

- Are the NYS draft guidelines too demanding, too lenient, just right?
- Will labs be willing and able to conduct this type of validation?
- Other thoughts on the topic?

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