Hepatitis C Virus Testing Technologies

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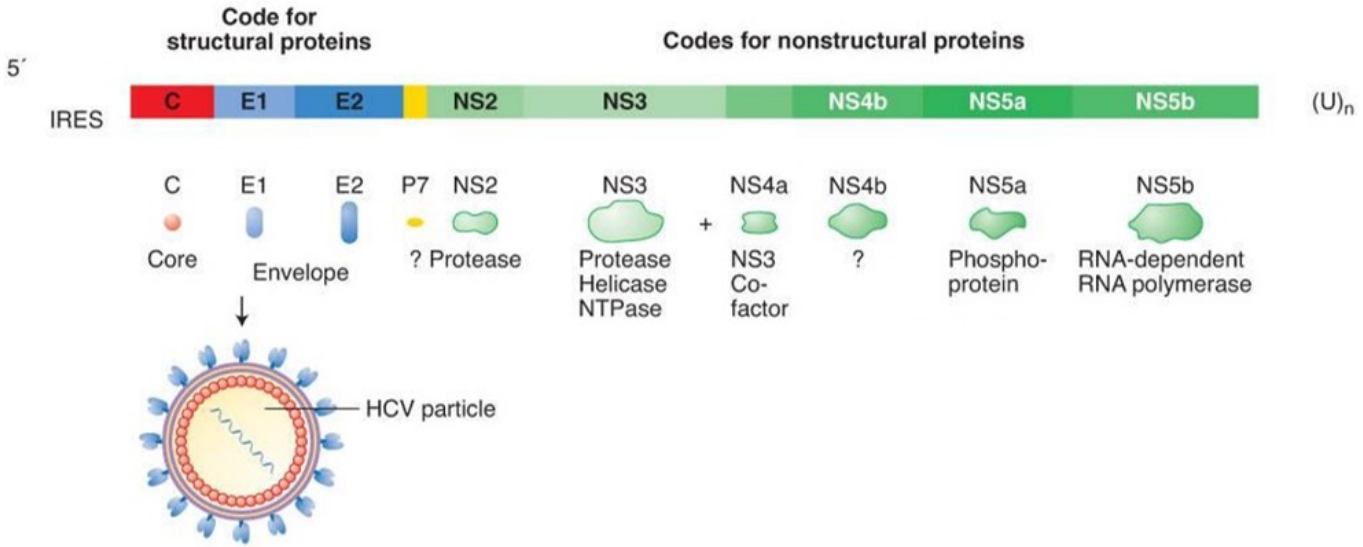
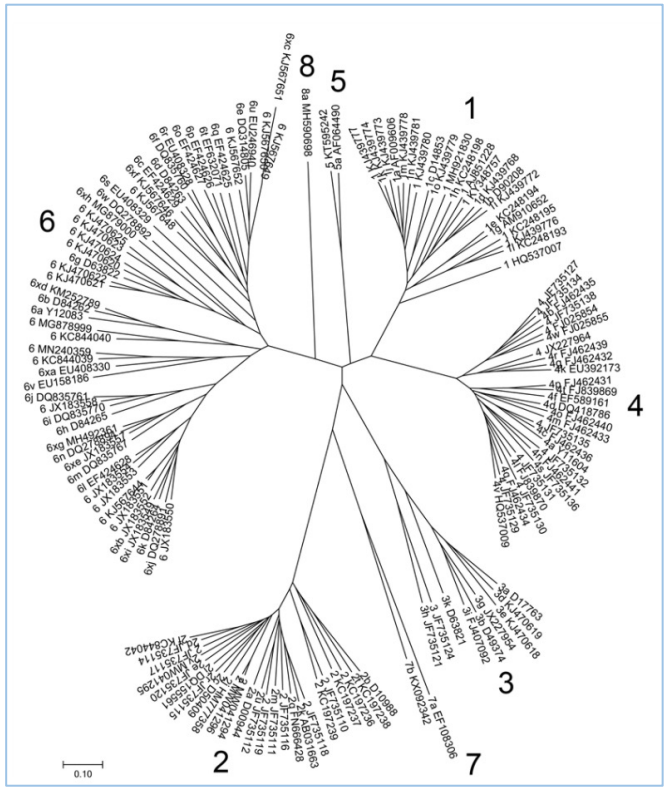
2022 Advancing HIV, STI, and Viral Hepatitis Testing Conference
29 March 2022

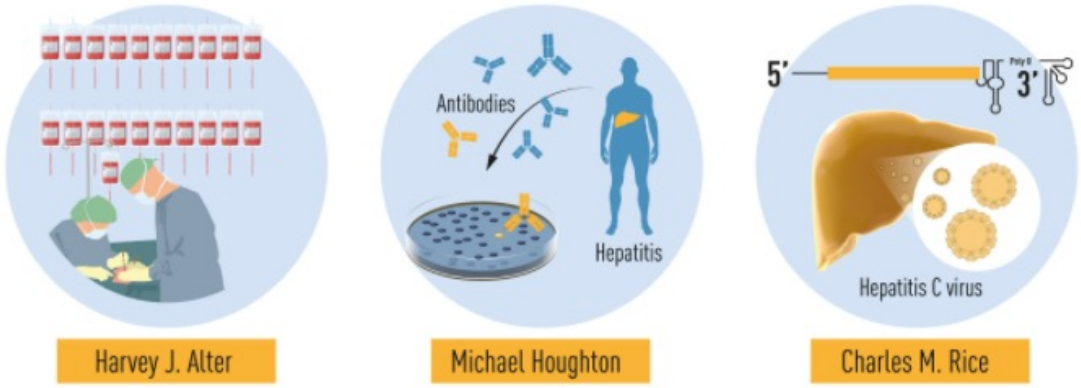
Hepatitis C Virus





- **Identified in 1989**
- **Single, positive-stranded RNA virus**
- **Family *Flaviviridae***
- **7 genotypes with >90 subtypes**

2020 Nobel Prize for the Discovery of HCV



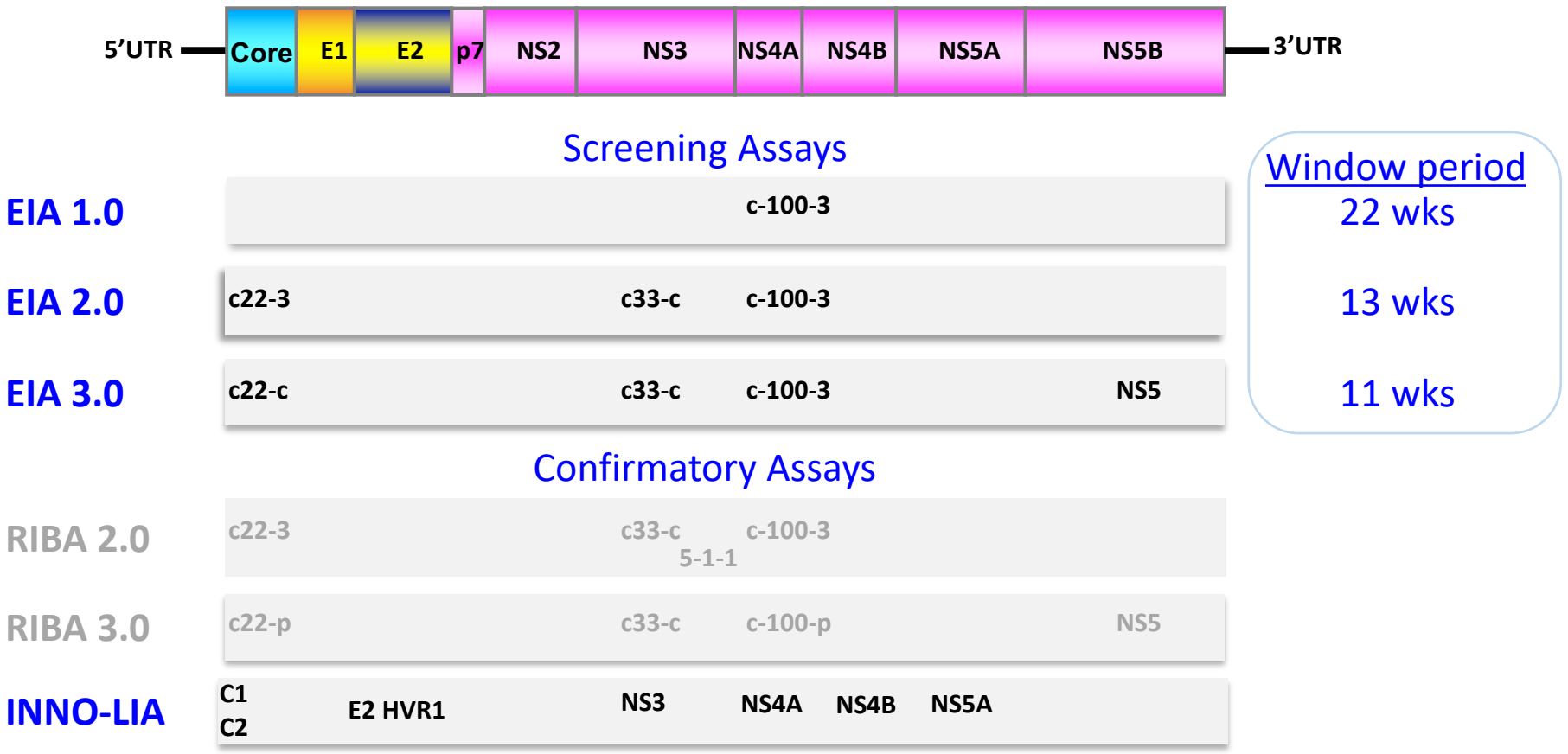
Charles Rice Michael Houghton Harvey Alter

Identification of Hepatitis C virus

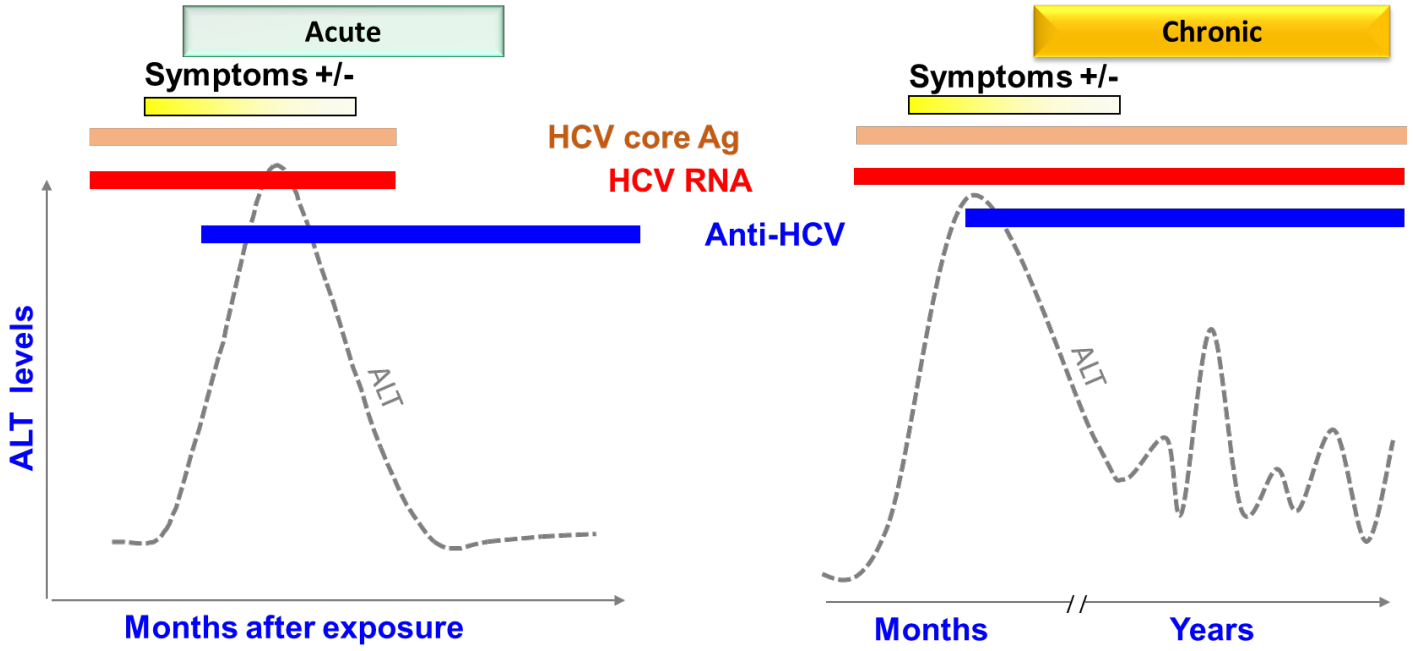
Identification of the novel virus was now a high priority. All the traditional techniques for virus hunting were put to use but, in spite of this, the virus eluded isolation for over a decade. Michael Houghton, working for the pharmaceutical firm Chiron, undertook the arduous work needed to isolate the genetic sequence of the virus. Houghton and his co-workers created a collection of DNA fragments from nucleic acids found in the blood of an infected chimpanzee. The majority of these fragments came from the genome of the chimpanzee itself, but the researchers predicted that some would be derived from the unknown virus. On the assumption that antibodies against the virus would be present in blood taken from hepatitis patients, the investigators used patient sera to identify cloned viral DNA fragments encoding viral proteins. Following a comprehensive search, one positive clone was found. Further work showed that this clone was derived from a novel RNA virus belonging to the *Flavivirus* family and it was named Hepatitis C virus. The presence of antibodies in chronic hepatitis patients strongly implicated this virus as the missing agent.

https://www.nobelprize.org/prizes/medicine/2020/press-release/

Anti-HCV IgG Assays: Antigenic Composition

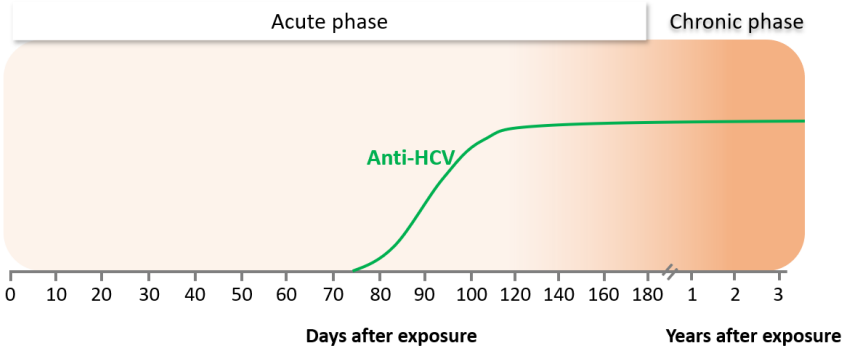


Natural History of HCV Infection



Antibodies to HCV

- Indicates exposure to HCV
- Present throughout acute, resolved and chronic phases of infection
- Laboratory tests
  - Enzyme immunoassays (EIAs) and Chemiluminescence assays (CIAs)
    - Serum, plasma, dried blood spot
  - Rapid diagnostic assays (RDTs)
    - Whole blood, serum, plasma
  - Confirmatory Immunoblot assays
    - Serum, plasma



FDA-approved Anti-HCV Assays

Manufacturer	Assay	% Sensitivity [95% CI]	% Specificity [95% CI]
OraSure Technologies	**OraQuick HCV Rapid Antibody Test**	**99.5** [98.4-99.9]	**99.0** [98.0-99.6]
Roche	Elecsys Anti-HCV II	**99.6** [98.7-99.96]	**98.8** (98.2-99.3)
	Elecsys Anti-HCV	**99.6** [98.5-99.5]	**96.9** [95.9-97.7]
	Elecsys Anti-HCV	**99.6** [98.5-99.95]	**97.1** [96.2-97.9]
	Elecsys Anti-HCV	**99.4** [98.1-99.9]	**97.2** [96.3-98.0]
Abbott Laboratories	ARCHITECT anti-HCV	**99.53** [97.4-99.99]	**97.6** [96.5-99.8]
Siemens Healthcare	ADVIA Centaur HCV	**99.9** [99.5-100]	**97.5** [96.4-98.3]
Ortho Clinical Diagnostics	Vitros Anti-HCV	**99.5** [98.7-99.9]	**98.2** [97.5-98.8]

Courtesy: Maria Garcia (FDA)

Anti-HCV Detection in a Multiplex Format

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IMMUNOASSAYS

Development of a Bead-Based Multiplex Assay for Use in Multianalyte Screening and Surveillance of HIV, Viral Hepatitis, Syphilis, and Herpes

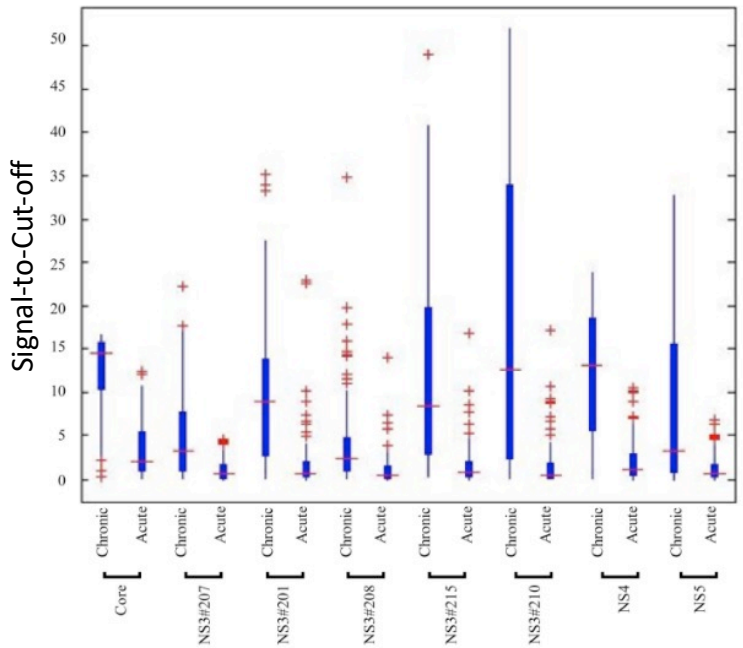
Ernest L. Yufenyuy,[a] Shanmugam Vedapuri,[a] Amy Zheng,[a,b] Gretchen Cooley,[c] Damien Danavall,[d] Shukla Mayur,[d] Maja Kodani,[e] Cheng Chen,[d] Ye Tun,[f] Yetunde F. Fakile,[d] Diana Martin,[c] Saleem Kamili,[e] Kevin Karem,[f] Bharat S. Parekh[a]

TABLE 4 Performance parameters of the 10-plex assay[a]

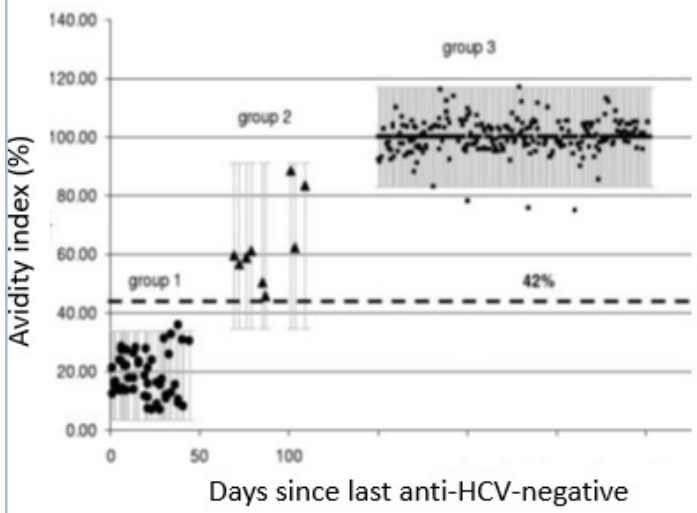
Analyte	Antigen(s)	No. of results					% sensitivity	95% CI of % sensitivity	% specificity	95% CI of % specificity	% agreement	95% CI of % agreement	Kappa score	95% CI of kappa score
		TP	TN	FP	FN	Sum								
HIV-1[b]	p24-gp41	70	347	0	0	417	100.0	94.9, 100.0	100.0	98.9, 100.0	100.0	99.1, 100.0	1.00	1.00
HIV-2[c]	HIV-2 IDR, rIDR-M	5	412	0	0	417	100.0	47.8, 100.0	100.0	99.1, 100.0	100.0	99.1, 100.0	1.00	1.00
T. pallidum[d]	rp17-βgal	46	358	5	8	417	85.2	72.9, 93.4	98.6	96.8, 99.5	96.9	94.7, 98.3	0.88	0.79, 0.93
	TmpA-βgal	40	348	15	14	417	74.1	60.4, 85.0	95.9	93.3, 97.7	93.0	90.2, 95.3	0.69	0.59, 0.80
HBV[e]	HBV-270	72	331	6	6	415[g]	92.3	84.0, 97.1	98.2	96.2, 99.3	97.1	95.0, 98.5	0.91	0.85, 0.96
HCV[e]	HCV-11	48	347	1	19	415[g]	71.6	59.3, 82.0	99.7	98.4, 100.0	95.2	92.7, 97.0	0.80	0.71, 0.88
	HCV-239	46	338	10	21	415[g]	68.7	56.2, 79.4	97.1	94.8, 98.6	92.5	89.6, 94.9	0.71	0.61, 0.80
HSV-1[f]	rHSVgG1	273	129	5	10	417	96.5	93.6, 98.3	96.3	91.5, 98.8	96.4	94.1, 98.0	0.92	0.88, 0.96
HSV-2[f]	rHSVgG2	199	197	9	12	417	94.3	90.3, 97.0	95.6	91.9, 98.0	95.0	92.4, 96.9	0.90	0.86, 0.94

[a]Shown are parameters of antibody binding to different antigens in the Luminex 10-plex IgG assay compared to standard diagnostic tests. TP, true positive; TN, true negative; FP, false positive; FN, false negative.
[b]ELISAs and Western blot assays were the standard tests used for the reference data.
[c]The Multispot assay was the standard test used for the reference data.
[d]The rapid plasma reagin, TP-PA, and Trep-Sure enzyme immunoassay were the standard tests used for the reference data.
[e]The Vitros Diagnostics assay was the standard test used for the reference data.
[f]The Final Focus and Trinity Biotech assays were the standard tests used for the reference data.
[g]Some specimens were depleted, and the sample volume was not large enough to run the assay.

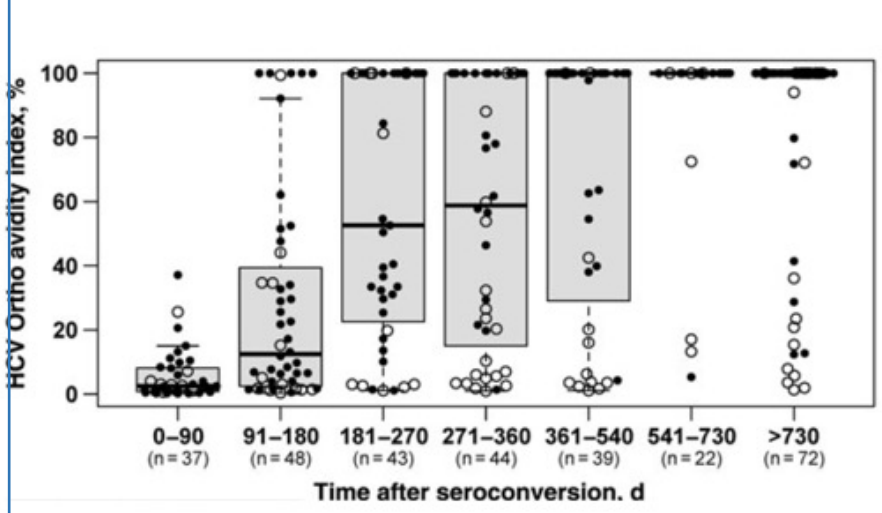
Anti-HCV Tests for Diagnosing Recent Infection



Araujo, AC. J. Clin. Micro. 2011.



Klimashevskaya, S. J. Clin. Micro. 2007.



Patel, EU. J. Inf. Dis. 2016.

Development of an avidity-based HCV recency assay

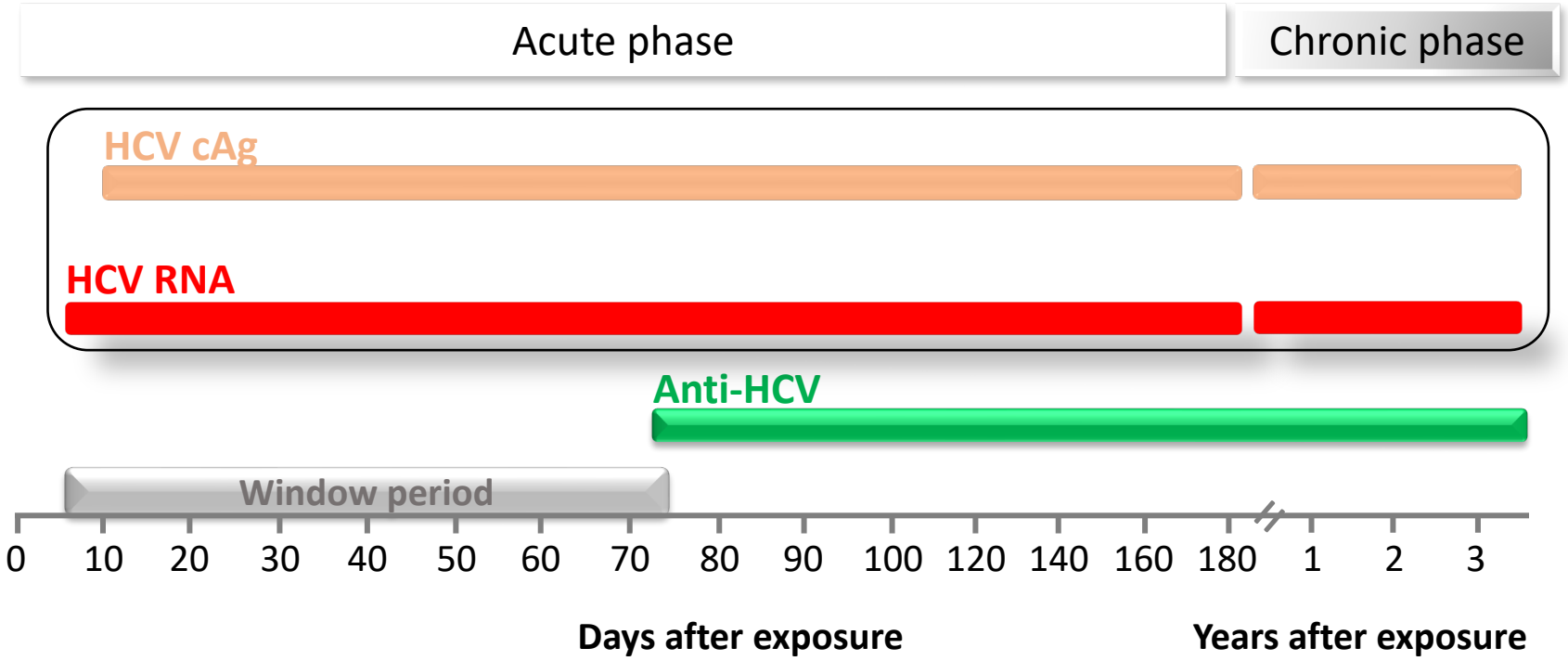
- The antibody targets included in the assay affect how long after seroconversion a sample is classified as recent.

Antibodies targeting	Days since estimated seroconversion
C+NS3+NS4+NS5	58 (50-79)
C+NS4+NS5	116 (82-180)
Fusion HCV antigen	193 (137-384)

- Could allow for tuning a recency assay to certain populations or purposes.

Pauly M - unpublished

Viral Detection Markers of HCV Infection



FDA-approved HCV RNA Tests

Manufacturer	Assay	LoD[a] serum IU/mL	% Sensitivity [95% CI]	% Specificity [95% CI]
Hologic	Aptima HCV Quant Dx Assay	**3.4**	**98.8** [96.7-99.6]	**100** [95.4-100]
Roche Molecular	COBA-HCV	**13.7**	**100.0** [97.5-100]	**98.8** [93.3-99.8]
Abbott Molecular	Abbott RealTime HCV[b]	**12**	**N/A[b]**	**N/A[b]**
Roche Molecular	COBAS AmpliPrep/ COBAS TaqMan HCV Test	**18**	**100** [97.3-100]	**100** [95.5-100]
Hologic (Gen-Probe)	VERSANT TM HCV RNA Qualitative Assay	**7.5**	**99.7 [d]**	**97.9 [d]**

[a] LoD as assessed with the WHO IS for HCV RNA (Genotype 1a); LoDs can vary by genotype
[b] Performance metrics were related to the ability of the test result to predict SRV (PPV/NPV)
[c] Diagnostic claim added to P060030 per PMA supplement
[d] Performance against another PCR assay

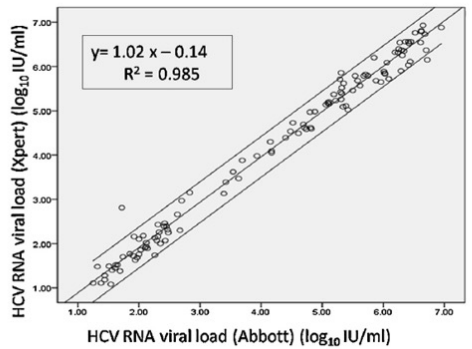
Courtesy: Silke Schlottmann (FDA)

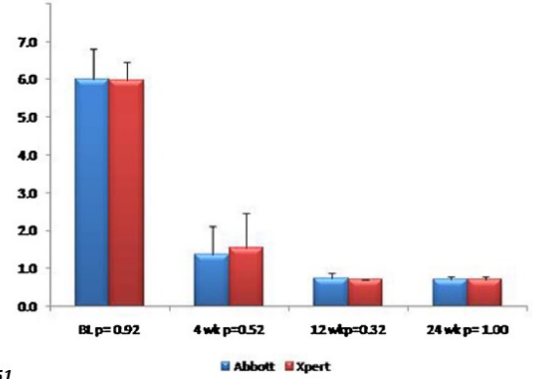
POC HCV RNA Test

- GeneXpert® HCV VL (Cepheid): automated qRT-PCR
- LOD: 4.91 IU/mL
- Dynamic range: 10-100,000,000 IU/mL
- Laboratory set-up: minimal
- Time to get results: >2 hours
- Price: >$20 per cartridge

- WHO prequalified, CE-marked

Performance Characteristics of POC HCV RNA Test





Journal of Clinical Virology 88 (2017) 46–51

Lancet Gastroenterol Hepatol. 2017 Jul;2(7):514-520. doi: 10.1016/S2468-1253(17)30075-4. Epub 2017 Apr 22.

Evaluation of the Xpert HCV Viral Load point-of-care assay from venepuncture-collected and finger-stick capillary whole-blood samples: a cohort study.

Grebely J[1], Lamoury FMJ[2], Hajarizadeh B[2], Mowat Y[2], Marshall AD[2], Bajis S[2], Marks P[2], Amin J[3], Smith J[4], Edwards M[5], Gorton C[6], Ezard N[7], Persing D[8], Kleman M[9], Cunningham P[10], Catlett B[10], Dore GJ[2], Applegate TL[2]; LiveRLife Study Group.

Time to Detection of Hepatitis C Virus Infection With the Xpert HCV Viral Load Fingerstick Point-of-Care Assay: Facilitating a More Rapid Time to Diagnosis

Jason Grebely,[1] Beth Catlett,[1,2] Indika Jayasinghe,[1] Heather Valerio,[1] Behzad Hajarizadeh,[1] Andrey Verich,[1] Philip Cunningham,[1,2] Marianne Martinello,[1] Shane Tillakeratne,[1] David Silk,[1] Gregory J. Dore,[1] and Tanya L. Applegate[1]; on behalf of the ETHOS II Study Group

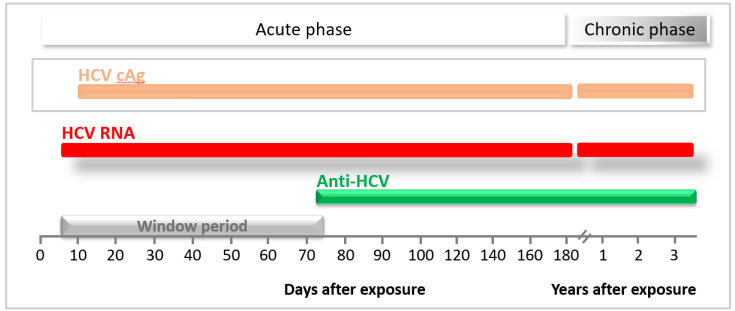
[1]The Kirby Institute, UNSW, Sydney, Australia, and [2]St Vincent's Centre for Applied Medical Research, Sydney, Australia

JID 2020:221 (15 June) • 2043

HCV Core Antigen

- Detectable within 1-2 weeks after exposure to HCV
- Broad linear range of detection
- Test samples
  - Serum, plasma, dried blood spots
  - Lower sample volume than NAT
  - No pristine sample needed

- Undetectable when HCV RNA <2000 IU/ml
- Requires Abbott ARCHITECT i2000 platform



Performance Characteristics of HCV cAg Test

Annals of Internal Medicine REVIEW

Hepatitis C Core Antigen Testing for Diagnosis of Hepatitis C Virus Infection

A Systematic Review and Meta-analysis

J. Morgan Freiman, MD; Trang M. Tran, BA; Samuel G. Schumacher, MSc, PhD; Laura F. White, PhD; Stefano Ongarello, PhD; Jennifer Cohn, MD, MPH; Philippa J. Easterbrook, MD, MPH; Benjamin P. Linas, MD, MPH; and Claudia M. Denkinger, MD, PhD

Annals of Internal Medicine • Vol. 165 No. 5 • 6 September 2016

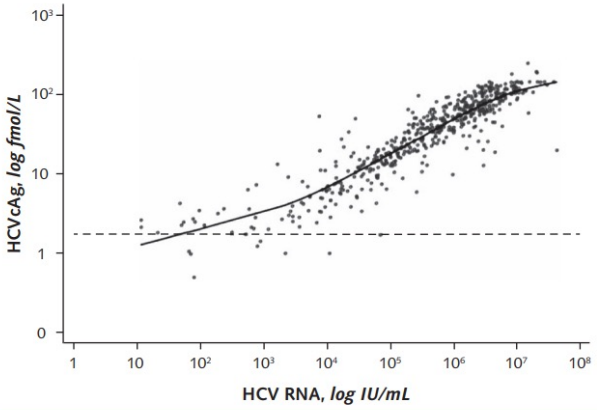
Performance of ARCHITECT HCV core antigen test with specimens from US plasma donors and injecting drug users

Tonya Mixson-Hayden[a], George J. Dawson[b], Eyasu Teshale[a], Thao Le[a], Kevin Cheng[b], Jan Drobeniuc[a], John Ward[a], Saleem Kamili[a,*]

[a] Centers for Disease Control and Prevention, Division of Viral Hepatitis, Atlanta, GA, USA
[b] Abbott Laboratories, Abbott Park, IL, USA

T. Mixson-Hayden et al. / Journal of Clinical Virology 66 (2015) 15–18

Figure 2. Nonparametric regression smoother of pooled quantitative data assessing the correlation between Abbott ARCHITECT and HCV RNA.



The dashed line indicates the positivity threshold of the HCVcAg index test, corresponding to 3 fmol/L. Abbott ARCHITECT = Abbott ARCHITECT HCV Ag assay; Ag = antigen; HCVcAg = hepatitis C virus core antigen; HCV = hepatitis C virus.

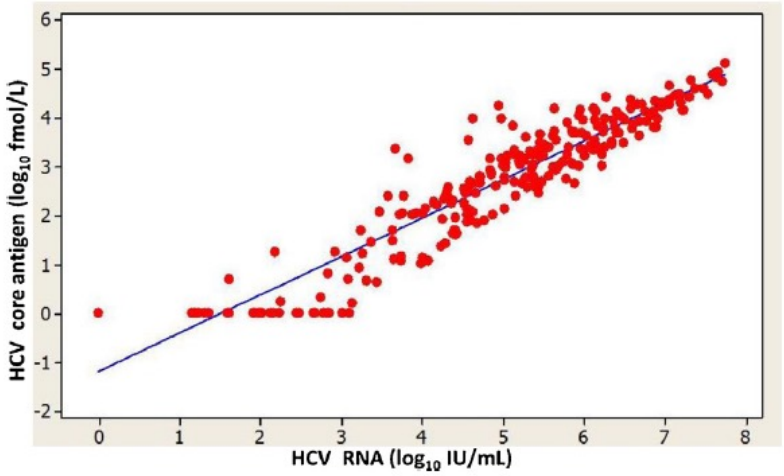


Fig. 1. Correlation between HCV core antigen and HCV RNA levels.

Dried Blood Spots for detection of HCV Markers

The Author(s) *BMC Infectious Diseases* 2017, **17**(Suppl 1):700
DOI 10.1186/s12879-017-2777-y

BMC Infectious Diseases

RESEARCH Open Access

Diagnostic accuracy of serological diagnosis of hepatitis C and B using dried blood spot samples (DBS): two systematic reviews and meta-analyses

Berit Lange[1,2*], Jennifer Cohn[3], Teri Roberts[4], Johannes Camp[1], Jeanne Chauffour[5], Nina Gummadi[6], Azumi Ishizaki[7], Anupriya Nagarathnam[8], Edouard Tuaillon[9,10], Philippe van de Perre[9,10], Christine Pichler[11], Philippa Easterbrook[7] and Claudia M. Denkinger[4]

The Author(s) *BMC Infectious Diseases* 2017, **17**(Suppl 1):693
DOI 10.1186/s12879-017-2776-z

BMC Infectious Diseases

RESEARCH Open Access

Diagnostic accuracy of detection and quantification of HBV-DNA and HCV-RNA using dried blood spot (DBS) samples – a systematic review and meta-analysis

Berit Lange[1,2*], Teri Roberts[3], Jennifer Cohn[4], Jamie Greenman[4], Johannes Camp[1], Azumi Ishizaki[5], Luke Messac[4], Edouard Tuaillon[6,7], Philippe van de Perre[6,7], Christine Pichler[1], Claudia M. Denkinger[3] and Philippa Easterbrook[5]

Pooled estimates

Anti-HCV

Sensitivity: 98% (CI95%:95–99)

Specificity: 99% (CI95%:98–100)

HCV RNA

Sensitivity: 98% (95% CI:95–99)

Specificity: 98% (95% CI:95–99.0)

Journal of Virological Methods 212 (2015) 66–70

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

Disparate detection outcomes for anti-HCV IgG and HCV RNA in dried blood spots

Alexandra Tejada-Strop[a], Jan Drobeniuc[a,*], Tonya Mixson-Hayden[a], Joseph C. Forbi[a], Ngoc-Thao Le[c], Lixia Li[a], Joanne Mei[a], Norah Terrault[b], Saleem Kamili[a]

[a] Centers for Disease Control and Prevention, 1600 Clifton Rd, Atlanta, GA 30329, USA
[b] University of California San Francisco Medical Center, 513 Parnassus Ave., San Francisco, CA 94143, USA
[c] Alaska Health and Social Services, 350 Main Street, Juneau, AK 99811, USA

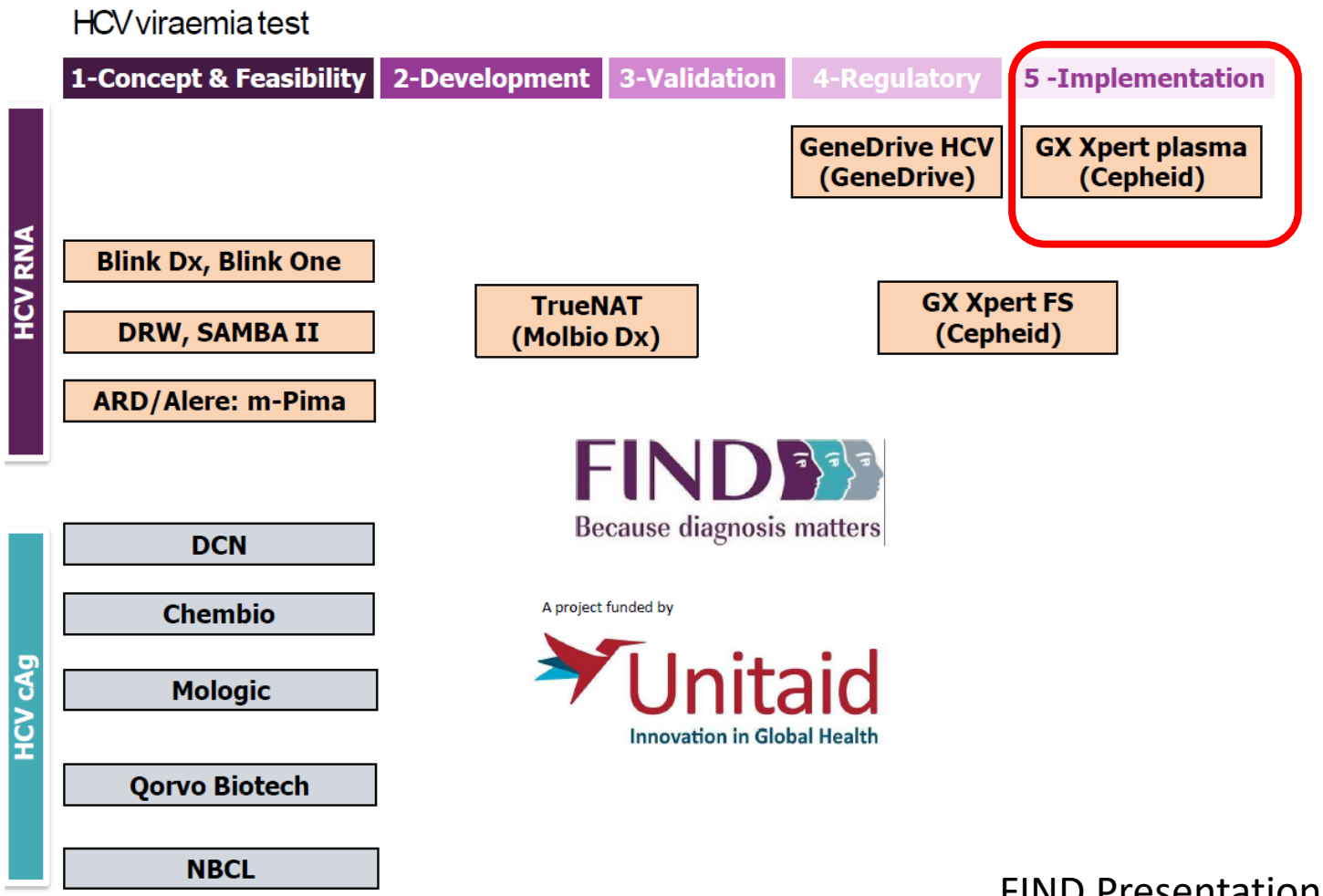
Sequencing of HCV Genome

- Genotyping
- Outbreak investigations
- Transmission studies
- Resistance-associated variants
- Recombinants
- Evolutionary studies
- Phylodynamics/phylogeography

- Technologies
  - Sanger
    - Whole genome
    - Amplicon
    - clones
  - NGS
    - Ion Torrent
    - MiSeq
    - PacBio

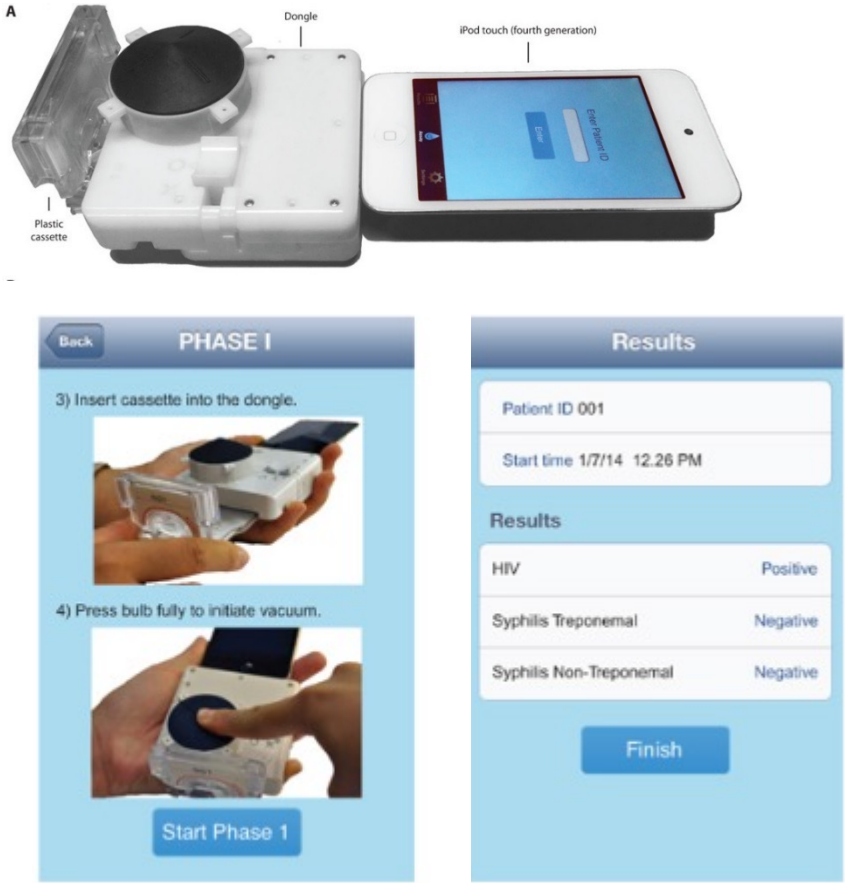
Global Hepatitis Outbreak and Surveillance Technology (GHOST)

POCTs for HCV RNA and HCV core Antigen

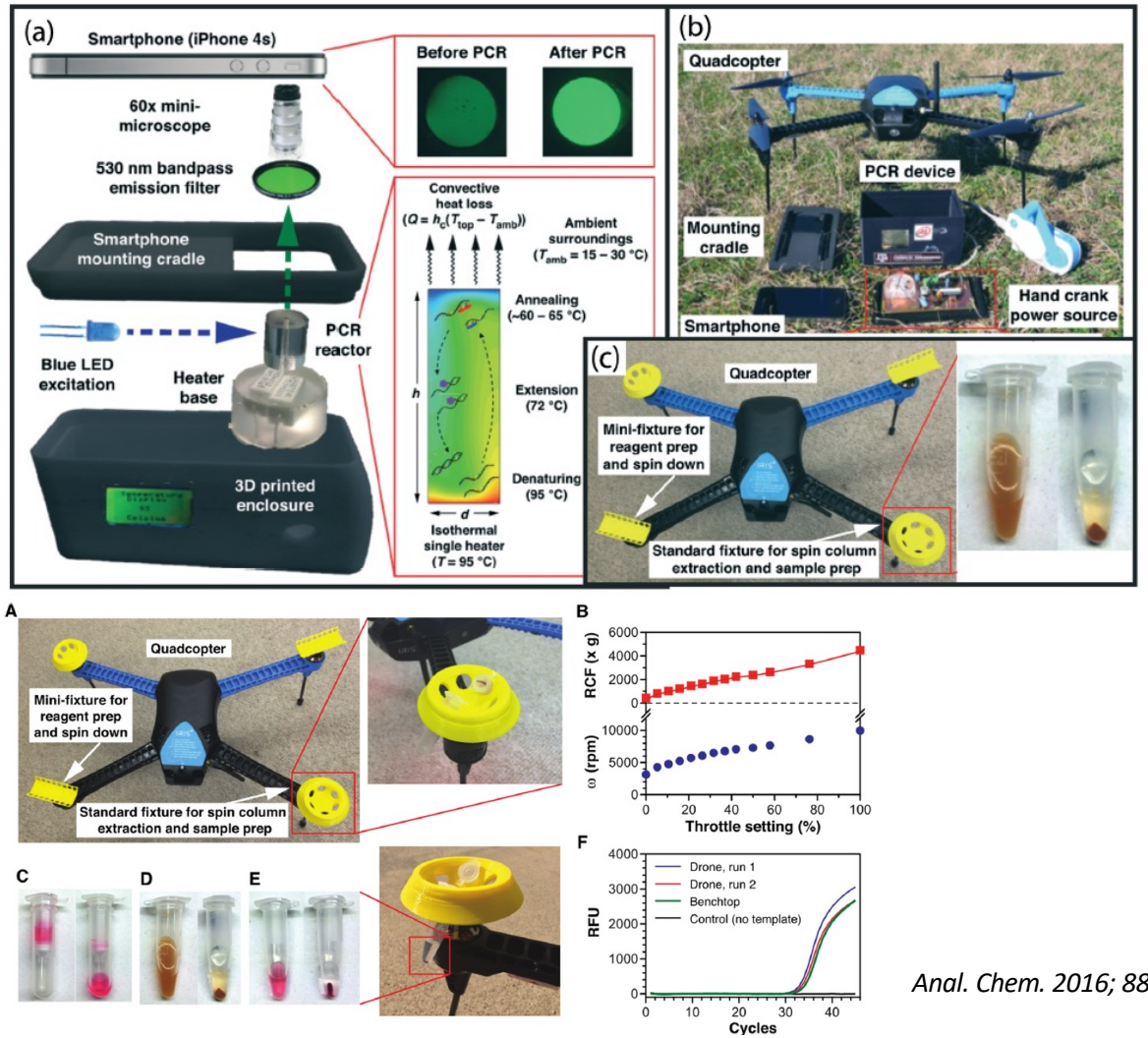


FIND Presentation at EASL 2019, Vienna

Future Technologies in Diagnostics

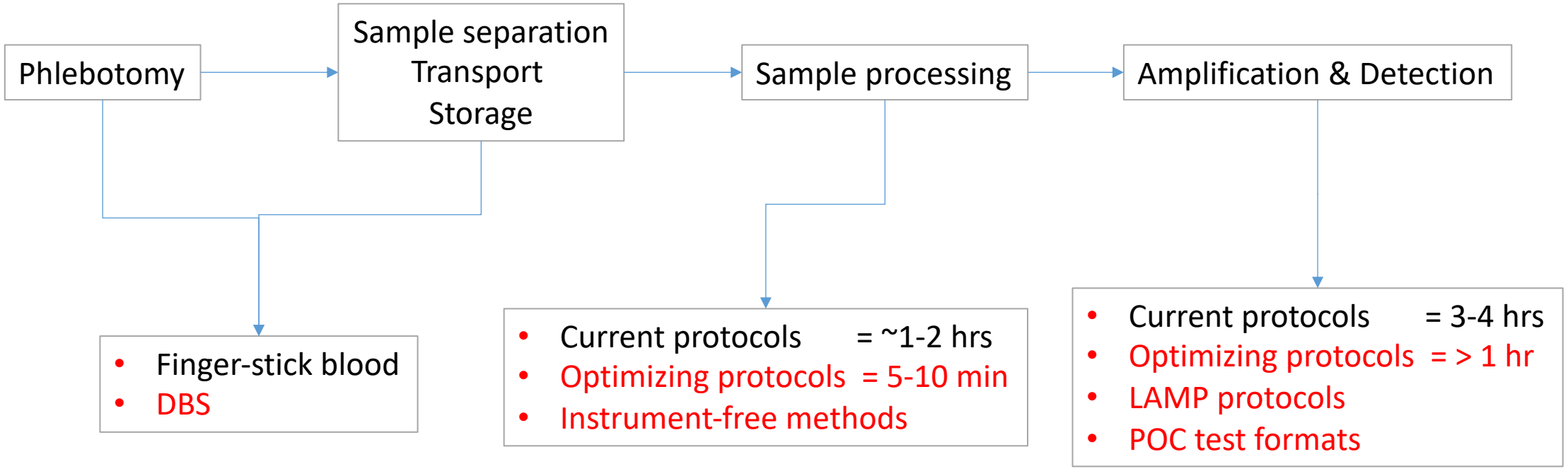


Tassaneewan L et al., Sci Transl Med 2015;7:273re1



Anal. Chem. 2016; 88:4651−4660

On-going Projects to Improve HCV RNA Detection



Explore CRISPER technology for detection of HCV RNA

Summary

- The diagnostic landscape of hepatitis C is limited to just anti-HCV and HCV RNA; HCV core antigen
- Anti-HCV antibodies are present through acute, chronic, and resolved phases of infection
- HCV RNA (and HCV core antigen) remain(s) the only marker/s for diagnosing current infection
- NGS technologies provide unique opportunities for outbreak investigations, transmission studies, and molecular surveillance of HCV
- **A**ccurate, **A**ffordable and **A**ccessible viral detection assays for diagnosing current infection key to HCV elimination programs

For more information, contact CDC
1-800-CDC-INFO (232-4636)
TTY: 1-888-232-6348 www.cdc.gov

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.