Issues in the laboratory diagnosis and monitoring of viral hepatitis

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Hepatitis A diagnostic methods

anti-HAV IgM antibodies (blood)

HAV RNA (blood, stool)

anti-HAV IgG antibodies (blood)
Hepatitis A outbreaks 2018-2019

Developing countries

BUT ALSO

Homeless individuals

Intravenous drug users

Men who have sex with men (MSM)
Distribution of hepatitis A cases by gender and male-to-female ratio, January 2012 to August 2018, as of 7 September 2018, EU/EEA* (n=4,475)
Distribution of hepatitis A outbreak-confirmed cases by month of onset and genetic sequence, June 2016 to August 2018, as of 7 September 2018, EU/EEA (n=4,475)
EASL Clinical Practice Guidelines on hepatitis E virus infection

European Association for the Study of the Liver

J Hepatol 2018; 68: 1256-1271
Hepatitis E diagnostic methods

- anti-HEV IgG antibodies (blood)
- anti-HEV IgM antibodies (blood)
- HEV RNA (blood, stool, CSF)
- HEV Ag (blood)
Role of HEV Antigen Detection in HEV-Related Acute Viral Hepatitis and Acute Liver Failure

Saurabh Mishra, Jayanta Borkakoti, Suresh Kumar, and Premashis Kar
Department of Medicine, Maulana Azad Medical College, University of Delhi, New Delhi, India

Performance of an antigen assay for diagnosing acute hepatitis E virus genotype 3 infection

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c CHU Toulouse, Hôpital Purpan, Département de Gastroentérologie, F-31300, France
d CHU Toulouse, Hôpital Purpan, Service de Médecine Interne, F-31300, France
e CHU Toulouse, Hôpital Rangueil, Département de Néphrologie, Dialyse et Transplantation multi-organes, F-31300, France

8 genotypes

GT1 and GT2

- only infect humans
- faecal-oral spread via contaminated water
- large outbreaks
- brief, self-limiting
- never chronic
- high mortality in pregnancy (25%)

GT3 and GT4

- endemic in animal species; e.g pigs and wild boar
- zoonotic infections in humans
- high-income countries
- chronic hepatitis E in immunocompromised individuals
species *Orthohepevirus C* (HEV-C); circulates in rats; highly divergent from HEV-A

HEV-C RNA detected in multiple clinical samples; HEV-C antigen detected in the liver

HEV-C isolate = 93.7% nt similarity to HEV-C strain previously detected in Vietnam

the patient had pre-existing HEV antibodies not protective against HEV-C infection
EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection

European Association for the Study of the Liver

J Hepatol 2017; 67: 370-398
Hepatitis B - “serology markers”

- HBsAg
- anti-HBs
- anti-HBc total antibodies
- anti-HBc IgM
- HBeAg
- anti-HBe
Automated immunoassay analysers

- highly standardised
- reliable
- quick
- random access
- for low-, mid-, high-volume demand

Roche
Siemens
Abbott
Ortho / Johnson & Johnson
An ultra-sensitive Abbott ARCHITECT® assay for the detection of hepatitis B virus surface antigen (HBsAg)

Sheng Lou, Russell Taylor, Sandra Pearce, Mary Kuhns, Thomas Leary

Diagnostics Research, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064, USA

ARTICLE INFO

Keywords:
HBsAg
HBV genotypes
Immunoassay
Nucleic acid test (NAT)

ABSTRACT

Background: Critical to the identification of HBV infection and the prevention of transfusion transmitted disease is the sensitive and accurate detection of Hepatitis B virus surface antigen (HBsAg). Improvements in HBsAg assay sensitivity approaching the performance of nucleic acid testing (NAT) are essential to further reduce the detection window for acute HBV infection in regions where NAT is not widely available.

Objectives and study design: An improved HBsAg assay on the fully-automated Abbott ARCHITECT® platform was developed to improve sensitivity, mutant and genotype detection.

Results: The analytical sensitivity of the improved prototype assay is 5.2 mAU/mL, which is 3.86- to 14.54-fold more sensitive than comparator assays based on the WHO International Reference Standard. The enhanced sensitivity was also demonstrated with 27 HBV seroconversion panels, detecting more panel members (191 of 364) vs. the ARCHITECT® Qual I (144), Qual II (160) and PRISM® (158) HBsAg assays. Further, the assay detected 7 of 12 HBV DNA positive/HBsAg negative samples, and detected all evaluated mutants and genotypes with higher sensitivity than the comparator assays. The improvement in sensitivity did not diminish assay specificity, attaining 100% (95% CI, 99.97-100%) on 10,653 blood donors.

Conclusions: An Abbott ARCHITECT® HBsAg assay with clinical performance approaching that of mini-pool NAT (approximately 100 copies/mL) was developed. The assay has superior HBsAg mutant and genotype detection and specificity, all of which are important for the diagnosis and management of HBV infection.
quantitative measurement of HBV DNA

HBV DNA viral load

pg/ml

copies/ml

IU/ml
anti-HB$_c$ - alone status

HBV DNA !!!!!
HBsAg-positive; HBeAg-negative patient

HBeAg-negative chronic HBV infection
(“inactive HBsAg carrier”)

HBeAg-negative chronic hepatitis B
HBsAg concentration

IU/ml
HBV DNA and HBsAg production are controlled by different pathways.

HBV DNA and qHBsAg provide different but complementary information.
The REVEAL-HBV study (Taiwan)

3,500 HBsAg-positive participants, 85% HBeAg-negative, average follow-up 11 years on

**HBV DNA 2,000 IU/mL or less vs. HBV DNA more than 2,000 IU/mL**

- RR cirrhosis = 2.5 (95% CI 1.6-3.8)
- RR hepatocellular carcinoma = 2.7 (95% CI 1.3-5.6)

further increased with incremental augmentations of HBV DNA concentration

**HBV DNA less than 2,000 IU/mL, HBsAg 1,000 IU/mL or more vs. less than 1,000 IU/mL**

- RR hepatocellular carcinoma = 13.7 (95% CI 4.8-39.9)

Ghany MG, Doo EC.
Antiviral resistance and hepatitis B therapy.
Cumulative incidence of HBV resistance to NAs in pivotal trials in NA-naïve patients with chronic hepatitis B†

- LAM: 24% (1 year), 38% (3 years), 49% (4 years), 67% (5 years)
- ADV: 0% (1 year), 3% (2 years), 11% (3 years), 18% (4 years), 29% (5 years)
- TBV: 4% (1 year), 3% (2 years), 4% (3 years), 17% (4 years)
- ETV: 0% (1 year), 3% (2 years), 1.2% (3 years)
- TDF†: 0% (1 year), 0% (2 years), 0% (3 years)
- TAF: 0% (1 year), 0% (2 years), 0% (3 years)
Alignment of patient RT-gene sequence to Consensus D

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rtM204V
rtMet204Val

substitution of methionine with valine at position 204 in rt
HBV genotypic drug resistance testing (i)

Sanger sequencing

- discrimination power: 15-20%

Next-generation sequencing

- discrimination power: 0.1%
Detection of Anti-Hepatitis B Virus Drug Resistance Mutations Based on Multicolor Melting Curve Analysis

Yi Mou, a,b Muhammad Ammar Athar, a,b Yuzhen Wu, a,b Ye Xu, a,b Jianhua Wu, c Zhenxing Xu, c Zulfiqar Hayder, d Saeed Khan, e Muhammad Idrees, f Muhammad Israr Nasir, g Yiqun Liao, a,h Qingge Li a,b

![Graphs showing melting curve analysis results for different reactions.](image)

**FIG 1** Readout for a wild-type plasmid and 24 mutant plasmids using the MMCA method. (Top) Typical readout for reaction A; (bottom) typical readout for reaction B. Black lines, melting curve of wild-type plasmid; gray lines, melting curves of 24 mutant plasmids. WT, wild type; -dF/dt, negative derivative of fluorescence over temperature; TET, tetrachloro-6-carboxyfluorescein.
New markers for management of chronic hepatitis B

- ultrasensitive HBsAg
- HBcrAg
- anti-HBc quantitative
- HBV RNA viral load
- new markers for innovative (yet not approved) drugs

HBcrAg is a combined measure of three proteins encoded by the precore/core region of the cccDNA: HBcAg, HBeAg and a 22 kDa precore protein (p22cr)
182 children; enrollment median age 10.6 years; median follow-up of 19.8 (11.9–21.9) years; spontaneous HBeAg seroconversion in 85 children (46.7%)

a baseline anti-HBc titer of > 500 IU/mL (hazard ratio= 2.81), HBV genotype B and B+C (hazard ratio= 3.46), and a baseline hepatitis B surface antigen titer of ≤ 4.8 log10 IU/mL (hazard ratio= 3.09) were predictive of spontaneous HBeAg seroconversion
Serum HBV RNA as a Predictor of Peginterferon Alfa-2a Response in Patients With HBeAg-Positive Chronic Hepatitis B

Florian van Bömmel,1 Alena van Bömmel,2 Alexander Krauel,1 Cynthia Wat,3 Vedran Pavlovic,3 Lei Yang,3 Danilo Deichsel,1 Thomas Berg,1 and Stephan Böhm1,5

1Hepatology Section, Department of Gastroenterology and Rheumatology, University Hospital Leipzig, Germany; 2Max Planck Institute for Molecular Genetics, Berlin, Germany; 3Roche Products, Welwyn Garden City, United Kingdom; 4Roche China Holdings, Shanghai, China; and 5Max von Pettenkofer-Institute, Ludwig-Maximilians-University, Munich, Germany

Background. Hepatitis B virus (HBV) RNA is a novel serum biomarker that has the potential to predict treatment response in patients with chronic hepatitis B. We explored whether HBV RNA serum levels can predict hepatitis B e antigen (HBeAg) seroconversion in patients treated with peginterferon alfa-2a.

Methods. Serum samples from HBeAg-positive patients previously treated with peginterferon alfa-2a in 2 large randomized controlled trials were retrospectively analyzed. HBV RNA levels were measured using a real-time polymerase chain reaction assay. Ability of individual biomarkers to predict HBeAg seroconversion at 24 weeks posttreatment was evaluated using receiver operating characteristics (ROC) analyses.

Results. The study included 131 subjects (70% male, 96% Asians, 35% HBV genotypes B and 61% C), 76 treated with peginterferon alfa-2a alone and 55 in combination with lamivudine. Median HBV RNA levels were significantly lower, at all timepoints, in patients achieving HBeAg seroconversion. Levels of HBV RNA at treatment weeks 12 and 24 showed good ability to predict HBeAg seroconversion (area under ROC scores >0.75, \( P < .001 \)). A HBV RNA cutoff of \( >5.5 \log_{10} \text{copies/mL} \) identified 30% of nonresponders at week 12 (negative predictive value >90%).

Conclusion. Serum HBV RNA is an early predictor of HBeAg seroconversion in patients treated with peginterferon alfa-2a.
Hepatitis D diagnostic methods

HBsAg + anti-HDV total antibodies

- HBsAg +ve
- anti-HDV total antibodies +ve
- HDV RNA or HDV Ag (+/- anti-HDV IgM) +ve
- Liver biopsy

Additional tests:
- HDV RNA
- HDV Ag
- anti-HDV IgM antibodies
Early phase of HCV infection

Transfusion settings

High-risk groups
(IVDU, hemodialysis patients, HIV positive individuals, organ donors)
Hepatitis C?

anti-HCV

+ 

HCV RNA
anti-HCV + HCV RNA?

suspected acute hepatitis C
immunocompromised patients
persons at risk of reinfection after previous viral clearance
Short communication

Twenty-four mini-pool HCV RNA screening outside a blood transfusion setting: Results of a 2-year prospective study

Katja Seme, Tina Močilnik, Kristina Fujs, Dunja Z. Babič, Aleksandra Todorović, Tamara Fras-Stefan, Mario Poljak*

Short communication

Twenty-four mini-pool HCV RNA screening in a routine clinical virology laboratory setting: A six-year prospective study

Katja Seme, Tina Močilnik, Mario Poljak*
24 mini-pool HCV RNA screening
routine diagnostic laboratory setting

86,309 anti-HCV negative specimens (4,060 mini-pools) tested by HCV RNA 24 mini-pool screening strategy between 1 June 2004 and 31 May 2019

100 (1 : 863) anti-HCV negative/HCV RNA positive samples detected

57 anti-HCV negative/HCV RNA positive patients detected

anti-HCV-negative, PCR-positive blood donors Slovenia, 2008-2017 = 1 : 924,087
How to proceed after anti-HCV positive result?

No RIBA necessary anymore!

A positive HCV antibody screening test should be followed by reflex RNA testing!
HCV core antigen is a surrogate marker of HCV replication and can be used instead of HCV RNA to diagnose acute or chronic infection when HCV RNA assays are not available or not affordable (core antigen assays are slightly less sensitive than HCV RNA assays for detection of viral replication) (A1).
HCV core antigen as an alternative to HCV RNA testing in the era of direct-acting antivirals: retrospective screening and diagnostic cohort studies


retrospective screening cohort study in Ontario, Canada

75/80 HCV RNA positive samples positive for HCV core antigen
- sensitivity 94% (95% CI 86–98)

0/993 HCV RNA negative samples tested positive for HCV core antigen
- specificity 100% (95% CI 94-100)

HCV core antigen testing can be used instead of HCV RNA testing for diagnosis and documentation of treatment adherence; inferior for SVR determination

lower costs, improved access to care, particularly in low- and middle-income countries
Research paper

An improved gold nanoparticle probe-based assay for HCV core antigen ultrasensitive detection

Hui-qiong Yin\textsuperscript{a}, Chang-fu Ji\textsuperscript{a,1}, Xi-qin Yang\textsuperscript{b}, Rui Wang\textsuperscript{a}, Shu Yang\textsuperscript{a}, He-qiou Zhang\textsuperscript{b}, Jin-gang Zhang\textsuperscript{a,}\textsuperscript{*}

\textsuperscript{a}\ Beijing Institute of Transfusion Medicine, Beijing 100850, China
\textsuperscript{b}\ Beijing Institute of Basic Medical Science, Beijing 100850, China

\textbf{ABSTRACT}

A gold nanoparticle probe-based assay (GNPA) was developed for ultrasensitive detection of Hepatitis C virus (HCV) core antigen. In the GNPA, after anti-HCV core antigen polyclonal antibodies and single-stranded barcode signal DNA were labeled on gold nanoparticle probe (NP), DNA enzyme was used to degrade the unbound barcode DNAs. The anti-HCV core antigen monoclonal antibodies were coated on magnetic microparticles probe (MMP). Then the NP-HCV core antigen-MMP sandwich immuno-complex was formed when the target antigen protein was added and captured. Magnetically separated, the immuno-complex containing the single-stranded barcode signal DNA was characterized by TaqMan probe based real-time fluorescence PCR. A detection limit of 1 fg/ml was determined for the HCV core antigen which is magnitude greater than that of ELISA (2 ng/ml). The coefficients of variation (CV) of intra-assay and inter-assay respectively ranged from 0.22–2.62% and 1.92–3.01%. The improved GNPA decreased the interference of unbound barcode DNAs and may be an new way for HCV core antigen detection.

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Rapid point-of-care tests using serum, plasma, fingerstick whole blood or crevicular fluid (saliva) as matrices can be used instead of classical enzyme immunoassays to facilitate anti-HCV antibody screening and improve access to care (A1).
Rapid point-of-care HCV RNA tests?
Evaluation of the Xpert HCV Viral Load point-of-care assay from venepuncture-collected and finger-stick capillary whole-blood samples: a cohort study

Jason Grebely, Francois M J Lamoury, Behzad Hajarizadeh, Yasmin Mowat, Alison D Marshall, Sahar Bajis, Philippa Marks, Janaki Amin, Julie Smith, Michael Edwards, Carla Gorton, Nadine Ezard, David Persing, Marika Klement, Philip Cunningham, Beth Catlett, Gregory J Dore, Tanya L Applegate, on behalf of the LiveRLife Study Group

Lancet Gastroenterol Hepatol 2017;2:514-520

Background Point-of-care hepatitis C virus (HCV) RNA testing offers an advantage over antibody testing (which only indicates previous exposure), enabling diagnosis of active infection in a single visit. In this study, we evaluated the performance of the Xpert HCV Viral Load assay with venepuncture and finger-stick capillary whole-blood samples.

Methods Plasma and finger-stick capillary whole-blood samples were collected from participants in an observational cohort enrolled at five sites in Australia (three drug and alcohol clinics, one homelessness service, and one needle and syringe programme). We compared the sensitivity and specificity of the Xpert HCV Viral Load test for HCV RNA detection by venepuncture and finger-stick collection with the Abbott RealTime HCV Viral Load assay (gold standard).

Findings Of 210 participants enrolled between Feb 8, 2016, and July 27, 2016, 150 participants had viral load testing results for the three assays tested. HCV RNA was detected in 45 (30% [95% CI 23–38]) of 150 participants based on Abbott RealTime. Sensitivity of the Xpert HCV Viral Load assay for HCV RNA detection in plasma collected by venepuncture was 100·0% (95% CI 92·0–100·0) and specificity was 99·1% (95% CI 94·9–99·0). Sensitivity of the Xpert HCV Viral Load assay for HCV RNA detection in samples collected by finger-stick was 95·5% (95% CI 84·5–99·4) and specificity was 98·1% (95% CI 93·4–99·8). No adverse events caused by the index test or the reference standard were observed.

Implications The Xpert HCV Viral Load test can detect active infection from a finger-stick sample, which represents an advance over antibody-based tests that only indicate past or previous exposure.
Single-use, electricity-free amplification device for detection of HIV-1

Kelly A. Curtis\textsuperscript{a,}\textsuperscript{*}, Donna L. Rudolph\textsuperscript{a}, Daphne Morrison\textsuperscript{a}, Dylan Guelig\textsuperscript{b}, Steven Diesburg\textsuperscript{b}, David McAdams\textsuperscript{b}, Robert A. Burton\textsuperscript{b}, Paul LaBarre\textsuperscript{b}, Michele Owen\textsuperscript{a}

\textsuperscript{a} Laboratory Branch, Division of HIV/AIDS Prevention, National Center for HIV/AIDS, Hepatitis, STD, and TB Prevention, Centers for Disease Control and Prevention, 1600 Clifton Road NE, Atlanta, GA 30329, USA
\textsuperscript{b} PATH, 2201 Westlake Avenue, Suite 200, Seattle, WA 98121, USA


non-instrumented nucleic acid amplification, single-used disposable (NINA-SUD) devices for the detection of HIV-1 in whole blood using reverse-transcription, loop-mediated isothermal amplification (RT-LAMP) with lyophilized reagents

NINA-SUD heating device harnesses the heat from an exothermic chemical reaction initiated by the addition of saline to magnesium iron powder

Lyophilized HIV-1 RT-LAMP reagents stable at 30°C for up to one month
Separation of Plasma from Whole Blood by Use of the cobas Plasma Separation Card: a Compelling Alternative to Dried Blood Spots for Quantification of HIV-1 Viral Load

Sergio Carmona, a,b Britta Seiverth, c Dieketseng Magubane, b Lucia Hans, a,b Matthias Hoppler c

J Clin Microbiol 2019;57:e01336-18

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HCV resistance: new drugs, no problems?
high rates of sustained virologic response among patients across HCV genotypes in whom treatment with a DAA regimen had previously failed. The presence of baseline RASs had no impact on SVR12.
Clinically relevant RASs which when detected at baseline by means of either population sequencing or deep sequencing with a cut-off of 15%, may influence the choice of first-line treatment regimen.

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Identification of 19 Novel Hepatitis C Virus Subtypes—Further Expanding HCV Classification

Charlotte Hedskog, Bandita Parhy, Silvia Chang, Stefan Zeuzem, Christophe Moreno, Stephen D. Shafran, Sergio M. Borgia, Tarik Asselah, Laurent Aflric, Armand Abergel, Jyh-Jou Chen, Jane Collier, Dharmesh Kapoor, Robert H. Hyland, Peter Simmonds, Hongmei Ma, and Evguenia S. Svarovskaia

Highly Diverse Hepatitis C Strains Detected in Sub-Saharan Africa Have Unknown Susceptibility to Direct-Acting Antiviral Treatments

Chris Davis, George S. Mgomella, Ana da Silva Filipe, Eric H. Frost, Genevieve Giroux, Joseph Hughes, Catherine Hogan, Pontiano Kaleebu, Gershim Asiki, John McLauchlan, Marc Niebel, Ponsiano Ocama, Cristina Pomila, Oliver G. Pybus, Jacques Pépin, Peter Simmonds, Joshua B. Singer, Vattipally B. Sreenu, Clara Wekesa, Elizabeth H. Young, Donald G. Murphy, Manj Sandhu, and Emma C. Thomson
Identification of a Novel Hepatitis C Virus Genotype From Punjab, India: Expanding Classification of Hepatitis C Virus Into 8 Genotypes

Sergio M. Borgia,1* Charlotte Hedskog,2 Bandita Parhy,2 Robert H. Hyland,2 Luisa M. Stamm,2 Diana M. Brainard,2 Mani G. Subramanian,2 John G. McHutchison,2 Hongmei Mo,2 Evgenia Svarovskaia,2 and Stephen D. Shafran3*

1William Osler Health System, Brampton Civic Hospital, Ontario, Canada; 2Gilead Sciences, Foster City, California; 3University of Alberta, Edmonton, Canada

Background. Hepatitis C virus (HCV) exhibits great genetic diversity and is classified into 7 genotypes (GTs), with varied geographic prevalence. Until the recent development of pangenotypic direct-acting antiviral regimens, the determination of HCV GT was necessary to inform optimal treatment.

Methods. Plasma samples with unresolved GT using standard commercial genotyping methods were subjected to HCV full-genome sequencing, and phylogenetic analysis was performed to assign GT.

Results. Four patients, previously classified as GT5 by LiPA or Abbott RealTime polymerase chain reaction assays, were identified as infected with a novel HCV GT. This novel HCV GT, GT8, is genetically distinct from previously identified HCV GT1–7 with >30% nucleotide sequence divergence to the established HCV subtypes. All 4 patients were originally from Punjab, India, but now reside in Canada and are epidemiologically unlinked. Despite presence of baseline resistance-associated substitutions within the GT8 virus of all 4 patients (NS3: V36L, Q80K/R; NS5A: Q30S, Y93S), all patients achieved a sustained virologic response; 2 treated with sofosbuvir/velpatasvir/voxilaprevir for 8 weeks, 1 with sofosbuvir/ledipasvir plus ribavirin for 24 weeks and 1 with sofosbuvir plus daclatasvir for 12 weeks.

Conclusions. The discovery of a novel HCV GT8 confirms the circulation of this newly identified lineage in the human population.
HCV Sanger based sequencing

Due to high genetic diversity of HCV Sanger based sequencing requires amplification of target genes (NS3, NS5A or NS5B) using primers that are genotype and subtype specific.

Genotypes: 1a, 1b, 3a
Region: NS3, NS5A, NS5B

⇒ 3×3×2 = 18 PCR primers

Low viral load + high genetic diversity = nested PCR
⇒ 18×2 = 36 primers

Long NS5B PCR amplicons ⇒ seq primers
⇒ 36+3×2 = 42 primers

⇒ endless optimization
NGS provides a rapid method for generating the whole HCV genome to accurately and simultaneously determine HCV genotypes/subtypes, RASs, and quasispecies diversity and to allow comprehensive viral strain analysis.
Worldwide deaths from chronic viral hepatitis as compared with deaths from tuberculosis, human immunodeficiency virus infection and malaria.
**Global Elimination of Chronic Hepatitis**

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**Global continuum of care for HCV infection and 2030 WHO elimination targets**

**Global continuum of care for HBV infection and 2030 WHO elimination targets**
Elaborating hepatitis C

WHO has ambitious global targets, made feasible by new highly effective treatments. But drugs alone are not enough. Talha Burki reports.

Egypt has the highest prevalence of HCV in the world since 2015, Egypt has treated 2.4 million infected individuals, most of whom have been cured

Egypt intends to have screened its entire adult population by September, 2019, and to treat 2.5-3 million people within 6 months of diagnosis

Egypt cut the cost of each anti-HCV antibody test to around $0.50
Analytical performance of newly developed rapid point-of-care test for the simultaneous detection of hepatitis A, B, and C viruses in serum samples

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Elimination of Viral Hepatitis: Are We Ready?

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