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Posted Date: 2 August 2023

doi: 10.20944/preprints202201.0226.v3

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Review

# Laboratory Methods in Liver Pathology Diagnosis

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**Abstract:** The study aimed to manage and to analyse the results of the laboratory tests, routinely practice for hepatitis C diagnosis, using blood tests. Comparison of ELISA method results (Enzyme-Linked Immunosorbent Assay) and chemiluminescence methods results. Statistical analysis of this study results, was performed using the laboratory informatic system. The results of the study are substantial and intricate referring to comparison between two methods concretly ELISA method (Enzyme-Linked Immunosorbent Assay) and chemiluminescence methods and their results. For this purpose, the results of preliminary ECL screening method of patients at risk for HCV who took part in the study, are presented in specific tables. More than good to mention that the correlations of results were used in order to identify a possible relationships between indicators of ELISA method and ECL index. In addition, correlations antibodies detected in electrochemiluminescence, ECL and Enzyme-Linked Immunosorbent Assay ELISA are pointed out. ECL and ELISA method results, are relevant for screening and for diagnostic confirmation in HCV risk patients. Unfortunately in the present study, were impossible to conclude about false-negative results. Good to know our opinion that RT-PCR technique, it is considered proper for the diagnosis of HCV.

**Keywords:** blood tests; hepatitis C virus; liver; laboratory techniques; diagnosis

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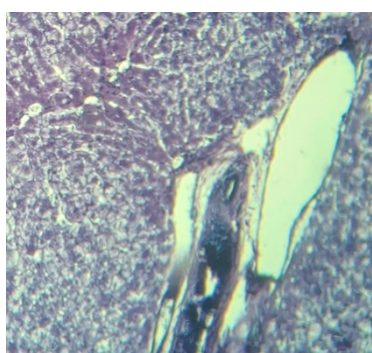
## Introduction

Actually, HCV infection could be consider as one of bad results after percutaneous blood administration to ill patients. Also good to mention that HCV infection ist is known as one of the most commonly through injection drug use. [1] The first and most important step in the care cascade is testing for HCV. Nowadays is an enlarge number of expansion of populations eligible for testing for HCV prevention. [1-3]

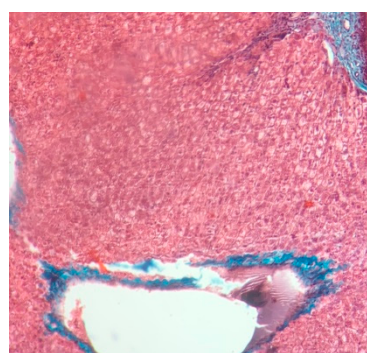
For this medical purpose, HCV prevention efforts are important. It is know about *care cascade*, including multiple key points in diagnosis HCV infection. An HCV *care cascade* is consider as a model for identifying opportunities and barriers in order to improve laboratory tests, linkage to care, and proper treatment access. [4,5]

Relatively recently, there have been increases in HCV detection among women of childbearing age. [6,7] Reinfection with HCV after curative therapy in illness status in different patients, is a concern for population and for medical team leders, in generally. [8-10] Actually, laboratory diagnosis of chronic HCV infection is requires the use of two types of tests. Proper laboratory techniques include immunoglobulin (Ig) G antibody enzyme immunoassays (anti-HCV) and nucleic acid tests (NAT), as modern methods, beside previously knows screen blood tests.[11] In case that need to distinguish between two directions such as true or false positivity of the anti-HCV

antibody result, previously mentioned tests, may be done with a second FDA-approved HCV antibody assay that is different from previously used for testing [11,12] Morphologically, HCV is an enveloped, positive-sense, single-stranded RNA virus of the *Flaviviridae* family.[13-15] The great key point knowing as a start of the direct-acting antiviral (DAA) era was in 2011. The important role in this direction was the introduction of two NS3/4A protease inhibitors. Both previously mentioned were used in combination with interferon-based regimens for chronic HCV treatment to ill patients diagnosed. Also this two NS3/4A protease inhibitors marked the start of the direct-acting antiviral (DAA) era.[16] Results of studies show that the HCV replication process is error prone. Finally results practically could be observe in variant viruses knowing as quasispecies.[17,18] Nowadays there are 7 genotypes of HCV. So there are known 6 major genotypes and the recent addition of genotype 7. This last 7 genotip has been found only in a few cases diagnosed to HCV positive patients. In addition, are mentioned as many as 100 subtypes identified by lower-case letters. [19] Hepatitis C virus (HCV) infection is a great cause of various liver diseases as cirrhosis and hepatocellular carcinoma. Following promising news, significant scientific discovering things remain in attention for reducing morbidity and mortality, associated to HCV. [20,21] Understanding the properties of hepatitis C virus (HCV) viral RNA and proteins facilitates the development of diagnosis methods and also a proper treatment, including antivirals.[22-24] In addition we can mention that HCV genotyping assays approved for *in vitro* diagnostic use are commercially available[25,26] The Linear Array HCV Genotyping Test (Roche Molecular Systems) targets the 5'UTR is currently use. This assay is based on conventional PCR amplification followed by reverse hybridization onto membrane strips containing specific probes. The finally result, refer to obtained a band pattern which can be visually interpreted or read by a specific scanner. Assays targeting other regions in addition to the 5'UTR have been nowasays developed for the proper discriminate between subtypes 1a and 1b. [27] From another scientific perspective, the Abbott RealTime HCV Genotype II (Abbott Molecular) targets the 5'UTR and NS5B regions. This assay is based on a single-step real-time RT-PCR with labeled genotype-/subtype-specific probes with a god end referring to the minimizes contamination but with amplified products.[28,29] There are scientific informations about four steps that require specific activities and which also play a role for deliberate interventions, in order to achieve the elimination of diseases and the eradication of HCV infection, Important key points for cure HCV refers to the control of liver disease, to the elimination of liver disease, to the elimination of HCV infection and to the eradication of HCV infection.[30] The HCV eradication can be achieved only if prevention methods and the proper treatment are implemented.[31,32]



Liver x20. Heideinhain stein



Liver x20. Goldner Szekely stein

## Material and methods

For this study, investigations were done in the Olymp Clinical Diagnostic Laboratory, Karaganda, from October 2016 to December 2016.

Cobas 6000 automatic modular analyzer, Roche Diagnostics model, were used for electrochemiluminescence ECL analysis.

A Bio RAD immunoassay analyzer, were used for ELISA method. (Enzyme-Linked Immunosorbent Assay). In this laboratory investigation, there are using a set of reagents Vector-

BEST (Russia) for detecting total antibodies to each of the 4 antigens of the HCV, concretely the core and non-structural proteins NS3, NS4, NS5. Interpretation for conformation: tests with a positive result for the core antigen, or with two or three non-structural proteins NS3, NS4 or NS5 were considered as positive.

Enzyme-Linked Immunosorbent Assay technique results, were evaluated by optical density (OD) using a microplate spectrophotometer (BioRAD). Critical optical density (COD) is equal to the half-sum of optical density values of two negative control samples plus a correction factor.

## Results

**Statistical Analysis** was performed using the laboratory information system LIS (Moscow, Russia) and the online calculator medstatistic.ru was used to calculate median (Me), lower (Q25) and upper (Q75) quartiles. A non-parametric Spearman correlation coefficient was used to determine data correlation. The characteristics for this method are shown in Table 1.

**Table 1.** Statistical basis for Spearman correlation coefficient (Adapted from Dencey and Reidy 2011).

Sperman (r)	Correlation
$\geq 0,70$	Strong relationship
4-6	Moderate relationship
1-3	Weak relationship

The statistical significance of the correlation coefficient was estimated by Student's criterion. Level of significance was at  $p < 0.05$ .

ECL analysis was performed using a Cobas 6000 automatic modular analyzer, manufactured by Roche Diagnostics. Anti-HCV-total test system includes 3 recombinant antigens: c22-3, c200 and NS-5 to determine total antibodies (IgG + IgM).

### Procedure steps:

1. Pipetting of the sample, reagent and microparticles.
2. The reaction mixture is supplied for measurement.
3. Each cycle is performed within 42 seconds.
4. The number of pipetting steps and preparation of the reaction mixture depend on the test.

Some tests require dilution with a diluent, which increases the number of pipetting steps. The incubation time at 37 °C ranges from 4.5 to 9 minutes, depending on the test.

**Calibration and quality control in ECL.** Calculation of the calibration curve was carried out at the time of production of the reagent and it was encoded in a 2-dimensional bar code of the corresponding set with the reagent. This information is then read by the analyzer. Monitoring the operation of the analyzer was conducted with 2 levels of controls, normal and pathological. Calibration and quality control were performed.

Results for the ECL assay were expressed as a signal to cut-off (s/co) ratio; a s/co ratio  $< 0.9$  was a negative and s/co ratio  $\geq 1.0$  indicated positive result. A s/co ratio within 0.9 and  $< 1.0$  was considered to fall into "a gray zone" and these serum samples were considered negative for calculations this study.

Interpretation for conformation: tests with a positive result for the core antigen, or with two or three non-structural proteins NS3, NS4 or NS5 were considered as positive.

The results of preliminary ECL screening of 1369 patients at risk for HCV are presented in Table 1.

**Table 1.** Analysis of ECL results.

s/co ratio < 1,0		s/co ratio ≥ 1,0		Total	
n	%	n	%	n	%
1164	85,06	205	14,94	1369	100

The table indicates that 205 (14,94%) samples were positive and 1164 (85,06%) negative, but S / CO ratio values ranged widely. The data of the positive S / CO ratios are summarized in Table 2. Of the 205 samples with a positive result in the ECL test, 176 were confirmed in the ELISA. Of the 176 samples confirmed in the ELISA, 174 had an ECL index higher than 4.0 in only two samples S / CO ratio was 1,0 and 3,0.

**Table 2.** Values of S / CO ratio in positive samples.

S/CO ratio	1-4	5-10	11-20	21-30	31-40	41-50	51-60	61-100	100 >	Total
n	17	19	35	48	29	22	16	15	4	205
%	8,29	9,27	17,07	23,42	14,15	10,73	7,81	7,31	1,95	100

We noted the nonparametric distribution of the values of S / CO at Me30,02 (Q75-Q25 49,14-16,89). 205 positive blood samples were sent for confirmation in the ELISA test.

**Table 3.** The significance of differences of the antibodies to non-structural antigens in ELISA in patients at risk for HCV.

A-body	n	p%	m%	Significance of differences					
				NS3 and NS4		NS4 and NS5		NS3 and NS5	
				z1-2	p-level	z2-3	p-level	z1-3	p-level
NS3	154	75,12	3,48	4,97	0,0000*	1,57	0,119	6,44	0,0000*
NS4	90	43,9	5,23						
NS5	65	31,7	5,77						

As a specific table nr. 3, show us that the correlations antibodies detected in ECL and ELISA are outlined. More than, previously mentioned correlations were used to identify any possible relationships between ECL index and indicators of ELISA, as set out in another specific below table. The correlations between the ECL ratio and core antibody were moderate ( $r=0,54$ ), and no relationships between ECL ratio antibodies to nonstructural NS3, NS4, NS5 antigens. The correlations between core antibody and NS3, NS4, NS5 antibodies varied from moderate to weak ( $r=0,48$ ;  $0,42$ ;  $0,32$ ) respectively. The largest correlations is between NS3 and NS4 ( $r=0,67$ ), also between NS5 and NS3, NS4 ( $r=0,63$ ).

## Discussion

Knowing the data collated in 2016, we can conclude after results of the study, that the incidence of the HCV, is higher than in the one of the previous period studied, respectively, 2004-2009. Electrochemiluminescence method and Enzyme-Linked Immunosorbent Assay method, are not able to show unclear results as false negative or false positive, in HCV diagnostic. It is important to test vein blood using RT-PCR technique (Reverse transcription polymerase chain reaction), in order to exclude false results. Antibodies detected in blood samples and HCV core antigen play a significant role in a diagnostic for HCV infection[16]. In this context antibodies to NS3 are specific for diagnosis

the early stages of hepatitis C. Also NS3 is considered as an independent diagnostic marker of the HCV acute process. The number of positive samples with NS4 and NS5 antibodies significantly less in comparison with NS3, show us in this study that a higher number of patients were in the acute form of HCV. The cronicisation process of the liver distruction could be relevant studing in the laboratory, exactly, anti-NS4 and anti-NS5. The study show also differents correlative results between the ECL ratio and antigens as NS3, NS4, NS5.

## Conclusions

The study showed that ECL and third generation of ELISA are important for screening and for conformation HCV risk patients.

Antibodies defined in the ECL test correlated with core antibodies ELISA are also good to know for a proper diagnostic.

The highest correlation were among antibodies to non-structural antigens. NS3, NS4, NS5 antibodies had an independent value for the differentiation of an acute or chronic process.

The study cannot conclude about false negative and false positive in this study. So unclear to find the presumptive enlarged "gray zone", including false results.

RT-PCR technique is consider one of the modern and with high potential for the diagnosis of hepatitis C patients, in order to confirm or to exclude this disease.

WHO and national health organizations have a great project. WHO project purpose, is to eradicate HCV infection in the next coming 10 years. The global elimination of HCV before 2030 can be held if global and national health organizations build proper strategies.

**Author Contributions:** Conceptualization, A.M. and G.A.; methodology, G.A.; software, B.K.; validation, S.A.; formal analysis, A.C.; investigation, A.M; resources, A.M.; data curation, G.A.; writing—original draft preparation, A.C., T.S; writing—review and editing, A.C. T.S; visualization, G.A.;

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. AASLD-IDSA. HCV Guidance: Recommendations for Testing, Managing, and Treating Hepatitis C 2016. [Available from: [http://hcvguidelines.org/sites/default/files/HCV-Guidance\\_October\\_2016\\_a.pdf](http://hcvguidelines.org/sites/default/files/HCV-Guidance_October_2016_a.pdf). Guidance produced by the American Association for the Study of Liver Disease and the Infectious Disease Society of America (AASLD-IDSA) covering recommendations for hepatitis C testing, clinical management and treatment.
2. Recommendations for prevention and control of hepatitis C virus (HCV) infection and HCV-related chronic disease. Centers for Disease Control and Prevention. *MMWR Recomm Rep*. 1998;47(Rr-19):1–39. Recommendations from the Centers for the Disease Control and Prevention defining populations for risk-based testing of hepatitis C.
3. Moyer VA. Screening for hepatitis C virus infection in adults: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med*. 2013;159(5):349–57. Hepatitis C testing recommendations for adults from the United States Preventive Services Task Force including risk-based testing and testing for adults born from 1945–1965.
4. Holmberg SD, Spradling PR, Moorman AC, Denniston MM. Hepatitis C in the United States. *N Engl J Med*. 2013;368(20):1859–61. Description of a care cascade integrating data from the Chronic Hepatitis Cohort Study and the National Health and Nutrition Examination Survey.
5. Viner K, Kuncio D, Newbern EC, Johnson CC. The continuum of hepatitis C testing and care. *Hepatology*. 2015;61(3):783–9. Description of an HCV continuum of care in a US urban center highlighting important areas where patients become lost at each stage.
6. Koneru A, Nelson N, Hariri S, Canary L, Sanders KJ, Maxwell JF, et al. Increased Hepatitis C Virus (HCV) Detection in Women of Childbearing Age and Potential Risk for Vertical Transmission - United States and

- Kentucky, 2011–2014. *MMWR Morb Mortal Wkly Rep.* 2016;65(28):705–10. Analysis finding that the proportion of infants born to HCV-infected mothers increased 68% nationally and 124% in Kentucky.
7. Kuncio DE, Newbern EC, Johnson CC, Viner KM. Failure to Test and Identify Perinatally Infected Children Born to Hepatitis C Virus-Infected Women. *Clin Infect Dis.* 2016;62(8):980–5. Analysis of surveillance data from the Philadelphia Department of Public Health finding a significant number of women giving birth in Philadelphia tested positive for HCV but that most of their children were not tested for HCV.
  8. Lambers FA, Prins M, Thomas X, Molenkamp R, Kwa D, Brinkman K, et al. Alarming incidence of hepatitis C virus re-infection after treatment of sexually acquired acute hepatitis C virus infection in HIV-infected MSM. *AIDS.* 2011;25(17):F21–7.
  9. Martin TC, Martin NK, Hickman M, Vickerman P, Page EE, Everett R, et al. Hepatitis C virus reinfection incidence and treatment outcome among HIV-positive MSM. *AIDS (London, England)* 2013;27(16):2551–7.
  10. Ingiliz P, Martin TC, Rodger A, Stellbrink HJ, Mauss S, Boesecke C, et al. HCV reinfection incidence and spontaneous clearance rates in HIV-positive men who have sex with men in Western Europe. *J Hepatol.* 2016.
  11. Testing for HCV infection: an update of guidance for clinicians and laboratorians. *MMWR Morb Mortal Wkly Rep.* 2013;62(18):362–5. Updated guidance for clinicians and laboratorians from the Centers for Disease Control and Prevention on diagnostic testing procedures for hepatitis C.
  12. Moyer VA. Screening for hepatitis C virus infection in adults: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med.* 2013;159(5):349–57. Hepatitis C testing recommendations for adults from the United States Preventive Services Task Force including risk-based testing and testing for adults born from 1945–1965.
  13. Thomas DL, Seeff LB. Natural history of hepatitis C. *Clin Liver Dis.* 2005;9(3):383–98. vi.
  14. Mack CL, Gonzalez-Peralta RP, Gupta N, Leung D, Narkewicz MR, Roberts EA, et al. NASPGHAN practice guidelines: Diagnosis and management of hepatitis C infection in infants, children, and adolescents. *J Pediatr Gastroenterol Nutr.* 2012;54(6):838–55.
  15. Thein HH, Yi Q, Dore GJ, Krahn MD. Estimation of stage-specific fibrosis progression rates in chronic hepatitis C virus infection: a meta-analysis and meta-regression. *Hepatology.* 2008;48(2):418–31.
  16. Conteduca V, Sansonno D, Russi S, Pavone F, Dammacco F. Therapy of chronic hepatitis C virus infection in the era of direct-acting and host-targeting antiviral agents. *J Infect.* 2014;68(1):1–20.
  17. Gomez J, Martell M, Quer J, Cabot B, Esteban JI. Hepatitis C viral quasispecies. *J Viral Hepat.* 1999;6(1):3–16.
  18. Duffy S, Shackelton LA, Holmes EC. Rates of evolutionary change in viruses: patterns and determinants. *Nat Rev Genet.* 2008;9(4):267–76.
  19. Bukh J. The history of hepatitis C virus (HCV): Basic research reveals unique features in phylogeny, evolution and the viral life cycle with new perspectives for epidemic control. *J Hepatol.* 2016;65(1 Suppl):S2–s21.
  20. Ly KN, Hughes EM, Jiles RB, Holmberg SD. Rising Mortality Associated With Hepatitis C Virus in the United States, 2003–2013. *Clin Infect Dis.* 2016;62(10):1287–8. Describes rising HCV-associated mortality in the United States from 2003–2013. During that time period, HCV-associated deaths surpassed 60 other nationally notifiable infectious conditions combined.
  21. Allison RD, Tong X, Moorman AC, Ly KN, Rupp L, Xu F, et al. Increased incidence of cancer and cancer-related mortality among persons with chronic hepatitis C infection, 2006–2010. *J Hepatol.* 2015;63(4):822–8. Analysis of a cohort of HCV-infected persons that found the the incidence of liver cancer and many types of non-liver cancers were higher, and age at diagnosis and death younger, in patients with chronic HCV infection compared to the general population.
  22. Alberti A, Chemello L, Benvegnù L. Natural history of hepatitis C. *J Hepatol.* 1999;31 Suppl 1:17–24.
  23. Hoofnagle JH. Course and outcome of hepatitis C. *Hepatology.* 2002;36:S21–S29.
  24. Chen SL, Morgan TR. The natural history of hepatitis C virus (HCV) infection. *Int J Med Sci.* 2006;3:47–52.
  25. Chevaliez S, Pawlotsky JM. Virology of hepatitis C virus infection. *Best Pract Res Clin Gastroenterol.* 2012;26:381–389.
  26. Saludes V, González V, Planas R, Matas L, Ausina V, Martró E. Tools for the diagnosis of hepatitis C virus infection and hepatic fibrosis staging. *World J Gastroenterol.* 2014;20:3431–3442.
  27. Lam TH, Cheng RS, Lai ST, Tsang TY, Cheng VC, Ho SL, Yam WC. Evaluation of in-house and commercial genotyping assays for molecular typing of hepatitis C virus in Hong Kong. *Br J Biomed Sci.* 2010;67:82–8.
  28. Shinol RC, Gale HB, Kan VL. Performance of the Abbott RealTime HCV Genotype II RUO assay. *J Clin Microbiol.* 2012;50:3099–3101.
  29. Chevaliez S, Bouvier-Alias M, Brillet R, Pawlotsky JM. Hepatitis C virus (HCV) genotype 1 subtype identification in new HCV drug development and future clinical practice. *PLoS One.* 2009;4:e8209.
  30. De Quadros C.A. In: *Considerations for Viral Disease Eradication: Lessons Learned and Future Strategies: Workshop Summary.* Dowdle W.R., Hopkins D.R., editors. John Wiley & Sons; New York, NY, USA: 1998.

31. Calvaruso V., Petta S., Craxì A. Is global elimination of HCV realistic? *Liver Int.* 2018;**38**((Suppl. S1)):40–46. doi: 10.1111/liv.13668.
32. Lombardi A., Mondelli M.U., ESCMID Study Group for Viral Hepatitis (ESGVH) Hepatitis C: Is eradication possible? *Liver Int.* 2019;**39**:416–426. doi: 10.1111/liv.14011.

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