CONTENTS

RESEARCH

Correlation between TSH, T3, T4 and Histological Types of Thyroid Carcinoma
Hilda Fitriyani, T. Ibnu Alferraly, Lidya Imelda Laksmi .......................................................... 201-204

Interferon Gamma Expression Cd8+ -T Lymphocyte with Esat-6-CFP-10 Fusion Antigen Stimulation between Active Tuberculosis, Latent Tuberculosis and Healthy People
Holland Lydia Marpaung, Betty Agustina, Jusak Nugraha, Fransiska ...................................... 205-209

Platelet Indexes for Bacterial Sepsis Severity Assessment
Michelle Hendriani Djuang, Fransiscus Ginting, Herman Hariman ......................................... 210-213

Hypercoagulability in Patients with Lung Cancer Undergoing Chemotherapy
Mariani, Herman Hariman, Noni Sari Soeroso ......................................................................... 214-218

Correlation between Platelet to Lymphocyte Ratio and Coronary Artery Narrowing
Enny Marziah, Adi Koesoema Aman, Andre Pasha Ketaren ...................................................... 219-222

The Role of Carcinoembryonic Antigen in Assessing the Success of Surgical Treatment in Colorectal Cancer Based on Staging
Anindya Widyasari, Betty Agustina Tambunan, Vicky S. Budipramana ..................................... 223-227

Comparison of Glycated Hemoglobin and Glycated Albumin in Type 2 DM Patients with and without CAD
Andini Triasti Siregar, Nizam Zikri Akbar, Burhanuddin Nasution ............................................. 228-230

Correlation between Level of Soluble Fas and Degree of Sepsis Severity Based on Apache II Score
Pauline Hadisiswoyo, Endang Retnowati, Erwin Astha Triyono .................................................. 231-234

The Differences Value of P-LCR the B-Thromboglobulin Level, the Fibrin Degradation Products Level in Pre and Post Hemodialysis
Like RN Purwanto AP, Dian W ..................................................................................................... 235-239

Leukocyte Esterase in Ascites Fluid for Detecting Spontaneous Bacterial Peritonitis in Liver Cirrhosis
Mawar Afrida, Ricke Loesnihari, Juwita Sembiring ................................................................. 240-243

The Correlation of Obesity Index and the Level of Triglyceride in Villagers
Fenty, Lucia Wiwid Wijayanti, Aris Widayati ............................................................................. 244-246
The Association between Asymptomatic Bacteriuria and Glycemic Control in Type 2 Diabetes Mellitus
Reni Marlina, Ricke Loesnihari, Santi Syafril ................................................................. 247-250

Determination of Reactive HBsAg Cut-Off That Need Confirmatory Test
Sherly Purnamawaty, Irdal Handayani, Asvin Nurulita, Uleng Bahrun .................................. 251-254

Analysis of LDL-C Measurement Using Direct and Friedewald Formula in Type 2 Diabetes Mellitus Patients
Liong Boy Kurniawan, Windarwati, Budi Mulyono ................................................................ 255-257

Evaluation of Blood Glucose Testing Using Contour® Plus Glucometer
Venny Beauty, Ninik Sukartini ............................................................................................... 258-261

Differences of Asymmetric Dimethyl Arginine Level in Patients with Diabetic Nephropathy and Non Diabetic Nephropathy
Nita Elvina Wisudawati, Coriejati Rita, Leni Lismayanti, Adhi Kristianto Sugianli .................... 262-265

Differences of Liver Function Tests in Type 2 Diabetes Mellitus Patients with and without Coronary Artery Disease
Hendra Saputra, Burhanuddin Nasution, Santi Syafril ............................................................ 266-268

Comparison of HbA1c Level Using Turbidimetry Inhibition Immunoassay, Latex Agglutination Inhibition Method and HPLC Method
Salmon Sutandra, Asvin Nurulita, Mansyur Arif .................................................................... 269-271

Activated Partial Thromboplastin Time and Fibrinogen in Pediatric Nephrotic Syndrome During Relapse and Remission
Trianita Tarigan, Adi Koesoema Aman, Oke Rina Ramayani .................................................... 272-275

Comparison of HPV Detection Using HC-II Method with Pap Smear Screening in Commercial Sex Workers in Kediri
Erawati, Puspa Wardhani, Aryati ............................................................................................. 276-280

LITERATURE REVIEW
Galectin-3, MMP-9 and ST-2: Biochemical Markers in Cardiovascular Diseases
Anak Agung Wiradewi Lestari ................................................................................................. 281-286

CASE REPORT
Chronic Myeloid Leukemia in Pregnancy
Rosa Dwi Wahyuni, Agus Alim Abdullah, Mansyur Arif ............................................................ 287-291
DETERMINATION OF REACTIVE HBSAG CUT-OFF THAT NEED CONFIRMATORY TEST

Sherly Purnamawaty, Irdha Handayani, Asvin Nurulita, Uleng Bahrun

Department of Clinical Pathology, Faculty of Medicine, Hasanuddin University/Dr.Wahidin Sudirohusodo Hospital Makassar, Indonesia. E-mail: sherlypurnamawaty@gmail.com

ABSTRACT

Hepatitis B surface antigen (HBsAg) is the earliest and most important serological marker for the diagnosis of HBV infection. The availability of new methods with a high sensitivity to detect HBsAg results in the increase of false reactive results so that a confirmatory test is needed. Analysis of the ROC curve obtained HBsAg cut-off value that need confirmatory test. Total samples were 80 with 51 (63.8%) confirmed reactive and 29 (36.2%) non-reactive. There was a statistically significant difference between HBsAg that confirmed reactive (median 2.76 COI) and non-reactive (median 0.32 COI) (p<0.001). ROC curve showed an AUC of 0.805 which meant a good diagnostic performance for HBsAg test based on a confirmatory test. The specificity of 89.66% and sensitivity 64.71% were obtained from the cut-off 1.08 COI and considered the best cut-off. Some possible causes of false reactive results were Hepatitis B vaccine, G-CSF therapy and limitation of the HBsAg methods. HBsAg cut-off with ELFA method that need HBsAg confirmatory test was <1.08 COI. The researchers suggests further studies with different sampling methods so a better data distribution can be obtained.

Key words: HBsAg, HBsAg confirmatory test, reactive cut-off

INTRODUCTION

Hepatitis B is an inflammation of hepatocytes caused by hepatitis B virus (HBV) infection. It is estimated that 240 million people around the world are having chronic hepatitis B infection and more than 686,000 people die every year due to hepatitis B complication. Hepatitis B virus is a deoxyribonucleic acid (DNA) virus belonging to the hepadnaviridae family, size 42 nm that consists of 27 nm core nucleocapsids surrounded by a lipoprotein layer (envelope). HBV envelope contains surface antigen termed hepatitis B surface antigen (HBsAg) which is secreted into the bloodstream. Serological markers for HBV infection consist of HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc IgM and IgG. The identification of serological markers allows identifying patients with HBV infection, to elucidate the natural course of Chronic Hepatitis B (CHB), to assess the clinical phases of infection and to monitor antiviral therapy. HBsAg is the earliest serological marker of HBV infection and plays an essential role in diagnosis. Accurate detection of HBsAg is crucial in early diagnosis and therapy. After acute exposure to HBV, HBsAg appears in the serum within one to ten weeks. Persistence of this marker for more than six months implies chronic HBV infection. The availability of new methods with a high sensitivity to detect HBsAg results in increased false reactive results. HBsAg screening assays are generally supported by confirmatory tests, which are used to confirm repeatable reactive (positive) results. Typically the confirmatory test involves neutralization of the HBsAg in the sample by >50% using a human anti-iHBs antibody. In Western Europe and North America, the standard procedure for diagnosing HBV infection is to repeat and confirm test results in specimens with borderline levels of HBsAg and those with reactive results in initial testing.

Confirmatory tests can be done using HBsAg Ultra Confirmation reagent to confirm a reactive HBsAg result from other standard methods. This confirmatory test is based on an antibody neutralization assay. All available commercial HBsAg test kits emphasize the importance of confirmatory test for reactive HBsAg results. Utilization of the neutralization test instead of HBV DNA test provides a cost saving for patients. The use of the neutralization test as a validation test when the HBsAg titer is less than or equal to a set limit will significantly reduce the cost of the test without the need for HBV DNA test.

A study by Latuconsina determined the gray zone range that need a confirmatory analysis which was 0.13 – 0.17 COI. HBsAg value of more than 0.17 COI will be interpreted as reactive. All HBsAg values of more than 0.17 COI should be confirmed by a confirmatory test. However, if all the results have to be confirmed, it will greatly increase the overall cost so a cut-off for reactive HBsAg value that needs a confirmatory test is needed to make the use of this confirmatory test more efficient.

Determination of Reactive HBsAg – Purnamawaty, et al
Based on those backgrounds, this study was conducted to determine a reactive HBsAg cut-off using Enzyme Linked Fluorescent Assay (ELFA) that need a confirmatory test so it can be applied in the Clinical Pathology Laboratory of the Dr. Wahidin Sudirohusodo Hospital Makassar as well as other laboratories using the same methods.

METHODS
This study was cross-sectional which studied all specimens undergoing HBsAg initial test with ELFA methods in the Clinical Pathology Laboratory of the Dr. Wahidin Sudirohusodo Makasar from November 2015 to April 2017. The study samples included all specimens with an HBsAg initial test value >0.17 COI.

The 0.17 COI cut-off was used based on the cut-off applied in the Clinical Pathology Laboratory of the Dr. Wahidin Sudirohusodo Makasar. HBsAg detection used serum specimens, and sandwich ELFA methods (Vidas® HBsAg Ultra). All samples with HBsAg initial result >0.17 COI was subsequently confirmed by HBsAg confirmatory test. HBsAg confirmatory

Two measurements were done simultaneously as a confirmatory assay. The first measurement was done without addition of a confirmatory reagent containing anti-HBs while the second measurement was done with addition of a confirmatory reagent.

The reduction of the signal from the first measurement to the second measurement was calculated and expressed in percentage. HBsAg was considered as reactive if the signal reduction was more than or equal to 50%, while recognized as non-reactive if the reduction was less than 50%. Ethical clearance was obtained from the Commission of Medical Research Ethics, Faculty of Medicine, Hasanuddin University/Dr. Wahidin Sudirohusodo Hospital Makassar.

The HBsAg value difference between confirmed reactive and a non-reactive group was analyzed statistically using the Mann-Whitney test. An HBsAg cut-off that needed a confirmatory test was determined by Receiver Operating Characteristic (ROC) curve analysis. The results were presented in tables and graphs. Differences were considered statistically significant if the p-value was <0.05.

Table 1. Sample characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>n=80</th>
<th>HBsAg (COI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Median (min-max)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 – 39</td>
<td>11</td>
<td>(13.75%) 0.93 (0.18 – 27.18)</td>
</tr>
<tr>
<td>40 – 59</td>
<td>38</td>
<td>(47.5%) 0.75 (0.18 – 26.11)</td>
</tr>
<tr>
<td>≥ 60</td>
<td>31</td>
<td>(38.75%) 0.79 (0.18 – 29.38)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>50</td>
<td>(62.5%) 0.79 (0.18 – 29.38)</td>
</tr>
<tr>
<td>Female</td>
<td>30</td>
<td>(37.5%) 0.77 (0.18 – 26.11)</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION
Total specimens with HBsAg value >0.17 COI and confirmed with HBsAg confirmatory test was 80. The number of specimens from subjects aged 20-39 years was 11 (13.75%), 40-59 years was 38 (47.5%) and ≥ 60 years was 31 (38.75%). These results were consistent

Table 2. HBsAg value based on HBsAg confirmatory test results

<table>
<thead>
<tr>
<th>HBsAg confirmatory test</th>
<th>n = 80</th>
<th>HBsAg (COI)</th>
<th>Median (min-max)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive</td>
<td>51</td>
<td>2.76 (0.18 – 29.38)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Non-reactive</td>
<td>29</td>
<td>0.32 (0.18 – 3.57)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Mann-Whitney test

Figure 1. Box plot of HBsAg based on HBsAg confirmatory test results

Figure 2. ROC curve of HBsAg value based on HBsAg confirmatory test results
with a report from Muljono stating that the highest prevalence of hepatitis B in Indonesia is in the age group 45-49 years. Specimens from male subjects were 50 (62.5%) while from female subjects were 30 (37.5%). The highest number of samples was in the age group of 40-59 years and in the male group. The median of HBsAg value of different age and sex groups were not significantly different.

Table 2 showed the median of HBsAg value based on HBsAg confirmatory test results. The number of specimens confirmed reactive was 51 (63.8%) and non-reactive 29 (36.2%).

Figure 2 was the ROC curve of HBsAg value based on HBsAg confirmatory test results. Area Under Curve (AUC) of the ROC curve was 0.805 which meant a good diagnostic performance for HBsAg test. Sensitivity and specificity with various cut-off values were determined using ROC curve analysis (Table 3).

The highest specificity, of 100%, was obtained from the cut-off of 4.095 COI with a sensitivity of 47.06%. It meant that all HBsAg results ≥ 4.095 COI would be confirmed as reactive and no false reactive results. However, the sensitivity at this cut-off was very low. The specificity of 89.66% with a sensitivity of 64.71% was obtained from the cut-off of 1.075 COI (= 1.08 COI). The researcher chose 1.08 COI as the HBsAg cut-off that needed a confirmatory test by considering the specificity and sensitivity obtained from ROC analysis. This cut-off determination was also considered the highest cost if we chose the cut-off with the highest specificity, which meant that more samples needed to be confirmed.

The HBV encodes the three proteins of the HBsAg, which form the viral envelope, small (SHBsAg), middle (MHBsAg) and large (LHBsAg). All three envelope proteins have a glycosylated form responsible for the secretion of viral particles. Serum HBsAg level could possibly reflect the amount and transcriptional activity of Covalently Closed Circular (CCC) DNA inside the hepatocytes. Detection of HBsAg is not only crucial for diagnosis but also for monitoring.

Availability of various new methods with a high sensitivity to detect HBsAg, results in increased false reactive results. Our study also showed a high false reactive rate which is 36.2% of all reactive results. It leads to the importance of confirmatory test for reactive HBsAg results, and a reactive cut-off is required so that confirmatory test will be more efficient. The researcher chose a reactive value of 1.08 COI as the reactive value that needed to be confirmed, with an expectation that it will give more accurate results with reasonable cost.

A study from Fletcher et al. stated that HBsAg values that need to be confirmed are weakly reactive values whereas highly reactive HBsAg values are not important for confirmatory testing. No literature set HBsAg with ELFA methods cut-off for weakly reactive and highly reactive values so that each laboratory has to set its own HBsAg value cut-off that needs to be confirmed in order to reduce the possibility of false reactivity. Our results showed that a cut-off of 1.08 COI had the best specificity and sensitivity. It was consistent with the results of Fletcher et al. study which stated that confirmatory tests are only required for weakly reactive results. Although there was no weak reactivity cut-off with the ELFA method, but the value of 1.08 COI could be classified as weakly reactive given that this value was in the low-value group of all the data obtained (0.18 - 29.38).

Availability of various new methods with a high sensitivity to detect HBsAg, results in increased false reactive results. Our study also showed a high false reactive rate which is 36.2% of all reactive results. It leads to the importance of confirmatory test for reactive HBsAg results, and a reactive cut-off is required so that confirmatory test will be more efficient. The researcher chose a reactive value of 1.08 COI as the reactive value that needed to be confirmed, with an expectation that it will give more accurate results with reasonable cost.

A study from Fletcher et al. stated that HBsAg values that need to be confirmed are weakly reactive values whereas highly reactive HBsAg values are not important for confirmatory testing. No literature set HBsAg with ELFA methods cut-off for weakly reactive and highly reactive values so that each laboratory has to set its own HBsAg value cut-off that needs to be confirmed in order to reduce the possibility of false reactivity. Our results showed that a cut-off of 1.08 COI had the best specificity and sensitivity. It was consistent with the results of Fletcher et al. study which stated that confirmatory tests are only required for weakly reactive results. Although there was no weak reactivity cut-off with the ELFA method, but the value of 1.08 COI could be classified as weakly reactive given that this value was in the low-value group of all the data obtained (0.18 - 29.38).

A study from Fletcher et al. stated that HBsAg values that need to be confirmed are weakly reactive values whereas highly reactive HBsAg values are not important for confirmatory testing. No literature set HBsAg with ELFA methods cut-off for weakly reactive and highly reactive values so that each laboratory has to set its own HBsAg value cut-off that needs to be confirmed in order to reduce the possibility of false reactivity. Our results showed that a cut-off of 1.08 COI had the best specificity and sensitivity. It was consistent with the results of Fletcher et al. study which stated that confirmatory tests are only required for weakly reactive results. Although there was no weak reactivity cut-off with the ELFA method, but the value of 1.08 COI could be classified as weakly reactive given that this value was in the low-value group of all the data obtained (0.18 - 29.38).

Table 3. Specificity and sensitivity with various cut-off value

<table>
<thead>
<tr>
<th>HBsAg cut-off</th>
<th>Specificity</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.770</td>
<td>75.86</td>
<td>66.67</td>
</tr>
<tr>
<td>0.880</td>
<td>86.21</td>
<td>64.71</td>
</tr>
<tr>
<td>1.075</td>
<td>89.66</td>
<td>64.71</td>
</tr>
<tr>
<td>1.275</td>
<td>89.66</td>
<td>62.75</td>
</tr>
<tr>
<td>1.400</td>
<td>89.66</td>
<td>60.78</td>
</tr>
<tr>
<td>1.485</td>
<td>89.66</td>
<td>58.82</td>
</tr>
<tr>
<td>1.915</td>
<td>93.10</td>
<td>56.86</td>
</tr>
<tr>
<td>2.345</td>
<td>96.55</td>
<td>56.86</td>
</tr>
<tr>
<td>3.260</td>
<td>96.55</td>
<td>47.06</td>
</tr>
<tr>
<td>4.095</td>
<td>100.00</td>
<td>47.06</td>
</tr>
</tbody>
</table>

Rysgaard et al. concluded that one of the causes of false reactive HBsAg results was hepatitis B vaccination, although it generally only gave false reactive results up to 14 days post-vaccination. The study also concluded that weakly reactive HBsAg results often do not imply true
Determination of Reactive HBsAg

Indonesian Journal of Clinical Pathology and Medical Laboratory, 2018 July; 24 (3) : 251-254

CONCLUSION AND SUGGESTION

Based on the results of this study, the cut-off value of HBsAg with ELFA methods that need HBsAg confirmatory test was <1.08 COI. HBsAg value of more than or equal to 1.08 COI is not necessary to be confirmed due to the high possibility to be confirmed reactive so that the test time and cost can be saved. The researcher suggests further studies with different sampling methods so a better data distribution can be obtained.

REFERENCES