SHORT COMMUNICATION

Comparative Study of Two ELISA Kits for Estimation of Antibodies to Hepatitis B Virus in Human Normal Immunoglobulin and Specific Immunoglobulin Intended for Intravenous or Intramuscular Use

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Received: 26 November 2015 / Accepted: 19 June 2016 / Published online: 29 June 2016
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Abstract Current study is conducted to evaluate method verification of two locally available kits manufactured by DSI & BIORAD for quantitative estimation of Hepatitis B virus antibodies in human normal immunoglobulin by using International standard of National Institute of Biological Standards and Control. Four analyst perform five sets of test in duplicate analysing accuracy, precision, and limit of detection, sensitivity and specificity. Our results suggest that both DSI and BIORAD kits fulfil the validation criteria and are sensitive to detect up to 10 mIU concentration precisely and accurately. DSI kit is more precise at concentration 100 mIU and economically 4–5 times cheaper in local market; on the other hand, BIORAD kits provide larger detection range up to 1000 mIU.

Keywords Immunoglobulin · Hepatitis B virus · Limit of detection · Sensitivity · Specificity · Precision · Accuracy

Introduction

Human normal immunoglobulin is human pooled plasma derived therapeutic preparation which contains mainly human immunoglobulin IgG intended to use for the treatment of primary/secondary immune deficiencies [1], autoimmune diseases [2], various haematological, neurological disorder and acute infections. These commercially available human normal immunoglobulin (HNI) and specific immunoglobulin (SI) intravenous (IV) or

intramuscular (IM) preparation leads to neutralization of a wide range of antigens including pathogens and super antigens [3]. Almost all the plasma derived products like albumin, blood coagulation factors, immunoglobulin are prepared by Cohn cold ethanol fractionation method or combination with liquid chromatography [4, 5] Frozen plasma is thawed at 4 °C and cryoprecipitate is first extracted from plasma for preparation of factor VIII concentrate and the supernatant of the cryoprecipitate contains fractions FI, FII and FIII used for the production of IgG (ref) IV or IM administration. In addition, ‘specific’ immunoglobulin are preparations that prepared from high titres plasma of particular antibodies, e.g. anti-D, anti-tetanus, etc. [6, 7]. To avoid any adverse effects in recipients of these products, quality control of these plasma derived products as per Pharmacopial monographs by regulatory laboratory is mandatory. Antibodies to HBs Ag is one of the critical parameter for HNI IV/IM and SI must contain at least 0.5 IU/gm or more antibodies to HBs Ag to provide protection against any possible chance of Hepatitis B virus (HBV) infection. Any individual having serum levels of Anti-HBs Ag level ≥ 10 mIU/mL is considered immune against HBV and less than that of anti-HBs Ag more susceptibility to HBV infection. During immune deficient stage, hospitalised patients are more prone to HBV infection and for avoiding such condition, human indented preparation of HNI and SI are prescribed to contain the stated value of antibodies to HBV. Currently available Anti-HBs ELISA kits are meant for in vitro quantitative detection of antibodies to hepatitis B virus surface antigen (anti-HBsAg) in clinical samples mainly for assessing antibody response levels to HBsAg-vaccine but currently no kit is available which is specific for estimation of antibodies against HBV in HNI and SI preparation. So current study is conducted in our laboratory to evaluate

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Table 1 Showing comparative performance study between DSI (DS-EIA-Anti HBs AG) and BIO RAD (MONALISA Anti HBs Plus) ELISA kits using 2nd International Standard for anti-hepatitis B surface antigen (anti-HBs) Immunoglobulin, human, and NIBSC code: 07/164

Parameters	DSI (DS-EIA-Anti HBs AG)			BIO RAD (MONALISA Anti HBs Plus)		
	Mean	SD	% CV	Mean	SD	% CV
Correlation coefficient	0.999	0.001	0.103	0.995**	0.002**	0.219**
10 mIU spike recovery %	98.421	17.405	17.684	98.947 ^{ns}	17.605 ^{ns}	17.792 ^{ns}
25 mIU	25.234	2.697	10.690	25.974 ^{ns}	2.783 ^{ns}	10.714 ^{ns}
50 mIU	49.457	2.904	5.873	49.340 ^{ns}	3.826 ^{ns}	7.754 ^{ns}
100 mIU	98.419	3.214	3.266	101.145**	6.841**	6.763**
125 mIU	123.364	6.127	4.967	127.665 ^{ns}	8.898 ^{ns}	6.970 ^{ns}
150 mIU	149.455	6.506	4.353	150.605 ^{ns}	6.720 ^{ns}	4.462 ^{ns}

Values are shown in mean, SD and coefficient of variation (CV) for n = 20. Unpaired t test. ** Significantly different (P < 0.05) and ns non-significant

performance verification of two kits available with local vender DSI (DS-EIA-Anti HBs AG) and BIO RAD (MONALISA Anti HBs Plus) anti-HBsAg IgG/Ig.

Materials and Methods

Two ELISA kits are selected and five kits of each with same lot number DSI (DS-EIA-Anti HBs AG) lot number 0871067 (DSI) and BIO RAD (MONALISA Anti HBs Plus) lot no 3F0044 (BIORAD) are procured from local supplier for method verification of testing HBs Ag antibodies in HNI & SI preparations. ELISA tests are conducted as per manufacturer instruction. Study is planned as per institutional Method Validation SOP no NIB/BPL/IG/MV/HBs Ag antibodies in normal immunoglobulin (IV/IM)/02. In brief, four analyst perform five sets of test in duplicate for calculating verification parameters like accuracy and reproducibility studies by using concentration of 25, 50, 100, 125, 150 mIU/ml of 2nd International Standard for anti-hepatitis B surface antigen (anti-HBs) immunoglobulin, human, and NIBSC reference material code: 07/164 prepared by serial dilutions. Sensitivity studies are conducted by spike recovery of 10 mIU/ml NIBSC standard in 10 % HNI preparation.

Statistical Analysis

Statistical analysis was performed by GraphPad Prism 6.0, mean, Standard deviation and percentage coefficient of variance followed by the unpaired t test. Significance was considered at the P < 0.05 level.

Result

All the five sets of tests performed by each analysis are valid as per manufacturers kit insert, different standards absorbance versus stated standard concentration are used to plot

standard graph with the help of Microsoft Excel program, regression coefficient and slope calculated. All the results for each test are analysed by GraphPad Prism 6.0 and results are shown in Table 1. DSI (DS-EIA-Anti HBs AG) kits shows better regression coefficient of 0.999 ± 0.001 SD than BIO RAD (MONALISA Anti HBs Plus) 0.995 ± 0.002 . Further analysis of NIBSC standards concentration of 25, 50, 100, 125 and 150 mIU/ml and spike study of 10 mIU rules out that at concentration 100 mIU levels. DSI (DS-EIA-Anti HBs AG) kits has significant difference with BIO RAD (MONALISA Anti HBs Plus) at P < 0.05. For analysis of accuracy, precision comparative chart of mean and SD is plotted as shown in the Fig. 1.

Discussion

In our current study, results show that DSI kit has better regression coefficient than BIORAD kit and both show significant difference at P < 0.05, this may be due to higher range of calibrators in BIORAD kit system 10, 100, 400, 1000 mIU and in DSI kit is 10, 50, 100, 150 mIU, and same is confirmed recalculating the standard graph of BIORAD kit by eliminating calibrator concentration 1000 mIU in standard curve then mean regression coefficient of twenty tests by BIORAD kit is more than 0.999 ± 0.01 . Further analysis of our data also shows that, both the methods are equally precise and accurate for measuring Human antibodies to HBV antigen in HNI at concentration levels 25, 50, 125 mIU all the results are within ± 2 SD, but at concentration level 100 mIU NIBSC standard, DSI kit shows more precision in analysis than the BIORAD kit all results are within ± 1 SD as shown in Fig. 1. On analysing, the spike recovery study in both the kits show mean % recovery more than 98.0 % and BIORAD kits have edge in accuracy over DSI kit as shown in Table 1, but results are more precise in DSI kit as shown in Fig. 1. Our study shows that both the kits available in market are suitable for quantitative estimation of HBV antibodies in NHI and SI preparation, and can measure up to 10 mIU/ml

Fig. 1 Showing comparative performance study between DSI (DS-EIA-Anti HBs AG) and BIO RAD (MONALISA Anti HBs Plus) ELISA kits using 2nd International Standard for anti-hepatitis B surface antigen (anti-HBs) Immunoglobulin, human, and NIBSC

code: 07/164 at concentration **a** 25 mIU/ml, **b** 50 mIU/ml, **c** 100 mIU/ml, **d** 125 mIU/ml, **e** 150 mIU/ml, **f** showing recovery studies of 10 mIU/ml

accurately and precisely. DSI kit is economical around 4–5 times cheaper in local market than BIORAD but BIORAD kits can measure up to 10–1000 mIU concentration compared to DSI kit which is up to 10–150 mIU.

Conclusion

In comparison of both the locally available kits DSI and BIORAD can effectively be used for measurement of HBs Ag antibodies in normal human immunoglobulin and specific immunoglobulin. DSI kit is more precise and more economical than BIORAD kits. BIORAD kits are suitable for larger range of detection in single run.

Acknowledgments We are thankful to Ministry of Health & Family Welfare, Govt. of India for the funding Assistance and also thankful to Dr. Charu Kamal, Head SRRDU & IDKL for providing BIORAD kits.

Funding This study was funded by Ministry of Health & Family Welfare, India.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human or animal participants performed by any of the authors.

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