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RESEARCH ARTICLE

INCLINATION TOWARDS CHEMILUMINESCENT IMMUNOASSAYS IN COMPARISON TO COLORIMETRIC IMMUNOASSAYS FOR DIAGNOSIS OF HBV, HCV AND HIV

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ABSTRACT

The objective of this study was to reveal the shif of various diagnostic centres towards chemiluminescent immunoassays from colorimetric immunoassays for diagnosis of HBV, HCV and HIV. National Institute of Biologicals (NIB) carries out quality evaluation of enzyme linked immunoassays intended to be used for diagnosis of HBV, HCV and HIV. The batches of these assays are forwarded to NIB by drugs regulatory authority of India. The number of batches of both types of immunoassays received from the year 2011 to October 2015, clearly showed that the demand for chemiluminescent immunoassays have increased and these assays are occupying the space of traditional colorimetric immunoassays. In the year 2011, 2012, 2013, 2014 and 2015 (till October 2015) the number of batches of colorimetric immunoassays received were 174, 89, 112, 163 and 112 respectively, while the number of batches of chemiluminescent based enzyme immunoassays received were 0, 4, 39, 48 and 89 respectively. The reason for this shift may be their higher sensitivity, uniform linear range and need of lesser sample.

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INTRODUCTION

An immunoassay is a biochemical test that measures the presence or concentration of a macromolecule in a solution through the use of an antibody or immunoglobulin. The macromolecule detected by the immunoassay is often referred to as an "analyte" and is in many cases a protein. Analytes in biological liquids such as serum are frequently measured using immunoassays for medical and research purposes. First immunoassays were developed by Rosalyn Sussman Yalow and Solomon Berson in the 1950s and Yalow was awarded the Nobel Prize for her work in immunoassays in 1977 (Berson *et al*., 1990). Immunoassays became simpler to perform and popular when techniques to link enzymes to antibodies were developed in the late 1960s (Lequin *et al*., 2005). In early 1980s radioactive iodine used in immunoassays was replaced with a chemiluminescent molecule called "acridinium ester". This type of immunoassay is used in around 100 million clinical tests every year worldwide, enabling clinicians to detect pathogens, measure a wide range of proteinsand other molecules in blood samples (NPS Focus, Royal Society of Chemistry, 2003).

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By 2012, the commercial immunoassay industry earned US$17 billion and expected to have annual growth in the 2 to 3 percent range (Carlson, 2014).

Principle of Immunoassays

Immunoassays are based on the property of an antibody to recognize and bind to a specific macromolecule in mixture and population of macromolecules. The macromolecule recognized and bound by an antibody is called as an antigen and the area on an antigen to which the antibody binds is called an epitope. In some immunoassays an antigen is used to detect for the presence of antibodies, which recognize that antigen in a solution. In other words, in these immunoassays, the analyte may be an antibody rather than an antigen.

In addition to the binding of an antibody to its antigen, the other property of all immunoassays is to produce a measurable signal in response to the binding. Most of the immunoassays uses detectable label linked antibodies or antigens. A variety of labels are available in modern immunoassays and the activity of binding of an antibody to antigen is measured through these labels by different means. Many labels are detectable because they either emit radiation, produce a colour change in a solution, fluoresce under light, or can be induced to emit light.

Colorimetric Immunoassay

In colorimetric immunoassays the chemically linked label produce colour change in the solution as a measure of antigen and antibody binding. This technique has been well established and considered as the technology of choice for a wide variety of applications in diagnostics, research, food testing, process quality assurance and quality control, and environmental testing. The most commonly used colorimetric immunoassay is based on colorimetric reactions of chromogenic substrates, (such as TMB) and label enzymes.

Chemiluminescent Immunoassays

Luminescent immunoassays are variations of the standard Enzyme-linked immunosorbent assays (ELISA). An enzyme converts a substrate into a reaction product that emits photons of light instead of developing a visible colour or coloured product. Luminescence is described as the emission of light from a substance as it returns from an electronically excited state to ground state. The various forms of luminescence (bioluminescence, chemiluminescence, photoluminescence) differ in the way the excited state is reached. Photoluminescence is simply fluorescence; the excitation is initiated by light at a particular wavelength. Bioluminescence is characterized by the use of a bioluminescent compound, such as luciferin and firefly luciferase. Chemiluminescence is light produced by a chemical reaction. The chemiluminescent substance is excited by the oxidation and catalysis forming intermediates. When the excited intermediates return back to their stable ground state, a photon is released, which is detected by luminometer. These assays are very sensitive and have a wide dynamic range. It is believed that luminescence is the most sensitive detection method currently in use due to the ability of signal multiplication and amplification. Luminescent reactions are measured in relative light units (RLU) that are typically proportionate to the amount of analyte present in a sample.

Colorimetric Immunoassay Vs Chemiluminescent Immunoassays

Chemiluminescent immunoassay has been shown to be more sensitive than the conventional colorimetric method(s), and does not require long incubations or the addition of stopping reagents, as is the case in some colorimetric assays. Among various enzyme assays that employ light-emitting reactions, one of the most successful assays is the enhanced chemiluminescent immunoassay involving a horseradish peroxidase (HRP) labeled antibody or antigen and a mixture of chemiluminescent substrate, hydrogen peroxide, and enhancers. Chemiluminescence Immunoassay (CLIA) detection using Microplateluminometers provides a sensitive, high throughput, and economical alternative to conventional colorimetric methodologies.

MATERIAL AND METHODS

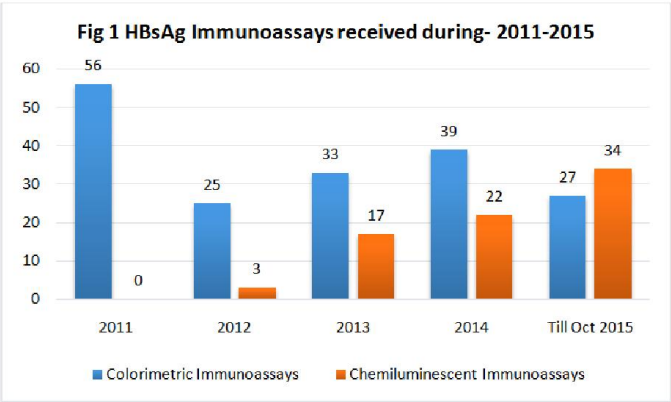
The data of batches of immunoassays received during five years from 2011 to October 2015 was analysed. The enzyme immunoassays used for diagnosis of HBV, HCV and HIV were taken into account. Based on the technology used in these immunoassays, the two categories (i) colorimetric (ii) chemiluminescent, were formed.

RESULTS

National Institute of Biologicals (NIB) is an autonomous institute under Ministry of Health and Family Welfare, Government of India. One of the mandate of this institute is quality evaluation of enzyme linked immunoassays used for diagnosis of HBV, HCV and HIV. As per Drugs and Cosmetics Act (3rd amendment 2001), Govt. of India, all blood units must be tested for HIV antibodies using 3rd generation enzyme linked immunoassay (Malik, 2003). The batches ofthese assays are forwarded by drugs control authority of India to NIB for performance evaluation. During last few years it has been observed that the number of batches of colorimetric based enzyme immunoassays received for evaluation have gradually and significantly decreased; and number of batches of chemiluminescent based enzyme immunoassays have increased. Therefore, this study was planned to reveal the exact trend of these two different categories of immunoassays with respect to HBV (HBsAg), HCV and HIV. The data of five years from 2011 to October 2015 was referred to reach the conclusion.

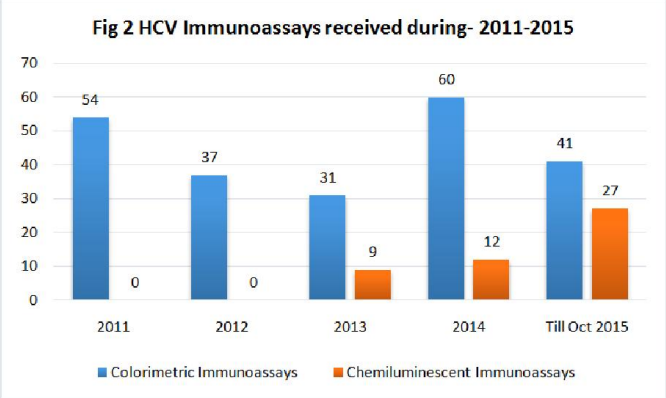
HBsAg Immunoassaydata (Figure: 1)

In the year 2011, 56 batches of colorimetric immunoassays used for diagnosis of HBV by detection of HBsAg were received for evaluation and no batch of chemiluminescent based immunoassay was received. In the year 2012, 2013, 2014 and 2015 (till October 2015) the number of batches of HBsAg colorimetric immunoassays received were 25, 33, 39 and 27 respectively. The number of batches of HBs Agchemiluminescent based enzyme immunoassays received in year 2012, 2013, 2014 and 2015 (till October 2015) were 3, 17, 22 and 34 respectively. During these five years a significant increase in the number of batches of chemiluminescent based immunoassays was observed and in year 2015 number of batches of these assays surpassed the number of batches of colorimetric assays received. Interestingly, total number of batches of both assays received in year 2014 and 2015 were 61, but out of 61 batches in year 2014 the major proportion belonged to colorimetric assays while in 2015, out of 61 batches major proportion belonged to chemiluminescent immunoassays.



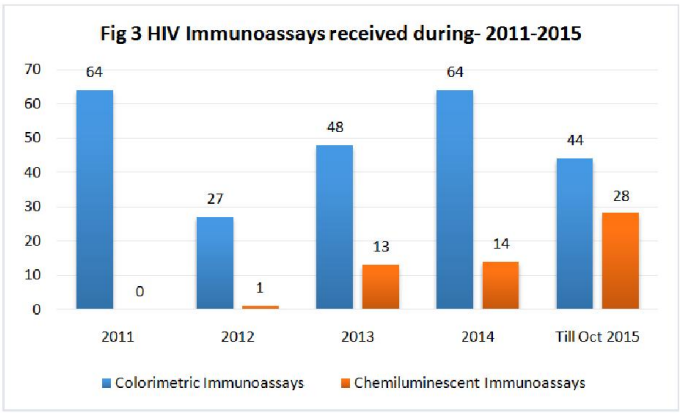
HCV Immunoassay data **(Figure 2)**

Fifty four batches of colorimetric immunoassays used for diagnosis of HCV were received in year 2011 for evaluation while no batch of chemiluminescent based immunoassay was received. In the year 2012, 2013, 2014 and 2015 (till October 2015) the number of batches of HCV colorimetric immunoassays received were 37, 31, 60 and 41 respectively, while the number of batches of HCV chemiluminescent based enzyme immunoassays received were 0, 9, 12 and 27 respectively. Therefore a significant increase in the number of batches of chemiluminescent based immunoassays was observed during these years and in year 2015 number of batches of these assays were more that 50% of the number of batches of colorimetric assays received.



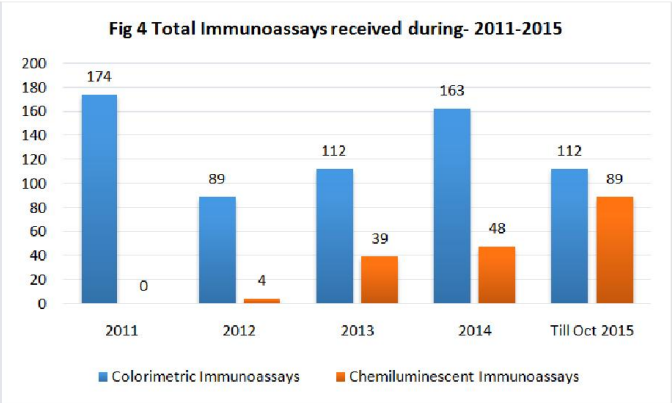
HIV Immunoassay data **(Figure: 3)**

In the year 2011, 64 batches of colorimetric immunoassays used for diagnosis of HIV were received for evaluation and no batch of chemiluminescent based immunoassay was received.In the year 2012, 2013, 2014 and 2015 (till October 2015) the number of batches of HIV colorimetric immunoassays received were 27, 48, 64 and 44 respectively. The number of batches of HIVchemiluminescent based enzyme immunoassays received in year 2012, 2013, 2014 and 2015 (till October 2015) were 1, 13, 14 and 28 respectively. A sizable increase in the number of batches of chemiluminescent based immunoassays was observed during these five years.



Total Immunoassay data **(Figure: 4)**

One hundered seventy four batches of colorimetric immunoassays used for diagnosis of HBV, HCV and HIV were received in year 2011 for evaluation while no batch of chemiluminescent based immunoassay was received. The number of batches of chemiluminescent based enzyme immunoassays increased from 0 in the year 2011 to 89 in the year 2015, while the number of batches of colorimetric immunoassays during this period decreased from 174 in the year 2011 to 112 in the year 2015.



DISCUSSION

Selection of the appropriate immunoassay test kits is an essential prerequisite for accuracy and reproducibility of test results and good quality management. A large number of assays are available commercially each offering essential as well as attractive performance characteristics namely sensitivity, specificity, efficiency and predictive values. However, the performance of the test will depend upon the technology of the assay, technical expertise, population being tested etc. New and improved kits are also being developed continuously and these also need evaluation in the same way as the established kits (Guidelines for HIV testing, NACO, 2007).

Evaluation of the performance characteristics of the HIV test kits by the designated statutory agency should be done and is mandatory prior to the test kit being available for diagnostic testing. Situations under which these kits may have to be evaluated are (i) Imported kits – to be evaluated with local serum panel (ii) New kits developed/manufactured indigenously - to assess efficacy (iii) To resolve controversies regarding discordant results or deterioration of components and reagents. This may be due to break in cold chain during transit and/or storage of in-use kits. In India, Drugs Controller General of India (DCGI) directs the manufacturer to submit the representative sample of kits for evaluation, using a locally made and well characterized serum panel by the Institutes identified for this purpose. The National Institute of Biologicals is the statutory body that has been assigned the responsibility of evaluating and approving these kits prior to their distribution in the Government and Private sector.

Each lot of kits has also to undergo evaluation by the National Reference Laboratories after obtaining NIB approval and prior to the lot being released for use in government testing labs. The evaluations must be performed under identical conditions. The parameters to be evaluated to assess the performance characteristics of the kits include: Sensitivity and Specificity. The parameter's values should be within those recommended

by the technical expert committee, Government of India on this subject. The final approval of the test kit is given by DCG (I) on the basis of the evaluation reports submitted by the identified evaluation centres (Guidelines for HIV testing, NACO, 2007).

The trend of number of batches of immunoassays received for evaluation at NIB showed that there is a clear inclination towards chemiluminescent based immunoassays in comparison to colorimetric immunoassays used for diagnosis of HBV, HCV and HIV. The total number of batches of immunoassays received during 2011 to 2015, did not show much increase in volume but number of batches of chemiluminescent based immunoassays increased up to more than 50% of the colorimetric immunoassays and in case of HBsAg the number surpassed the colorimetric immunoassays.

It indicates that slowly and gradually chemiluminescent based immunoassays are replacing colorimetric immunoassays. Chemiluminescent immunoassays generally use chemilumines cent indicator to label antigen or antibody directly (Weeks *et al*., 1983; Wu (Ed.), 2000). This technology has improved the analytical sensitivity of immunoassays. Direct labeling of chemiluminescent indicator was limited by a relatively short duration of light output. Consequently, chemiluminescence enzyme immunoassay (CLEIA) is developed based on enzyme–antibody conjugates using a chemiluminescent substrate, and luminometer is used for measurements. In this way, these assays with improved duration of light output has been developed in recent years.

Further to improve the performance, separating agent and solid phase with higher sensitivity and larger detection linear range have been introduced (Zhang *et al*., 2009; Pamme, 2006; JE (Ed.), 1996). The reports show that chemiluminescent immunoassays has obvious advantages over colorimetric immunoassays in terms of the performance and mechanism. These assays have been shown to have higher sensitivity, better linear range and requires lesser sample volume that colorimetric assays (Zhang *et al*., 2012; Hatch and Scalarone, 2009). Therefore, diagnostic experts are preferring and moving towards chemiluminescent immunoassays for efficient diagnosis of HBV, HCV and HIV.

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