

## Evaluation of Performance Characteristics of Commercially Available Tests for Diagnosing Hepatitis B Surface Antigenemia

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### ABSTRACT:

#### BACKGROUND:

A number of serologic immunoassays techniques have been developed in diagnostic virology with different degrees of sensitivity and specificity for the detecting hepatitis B virus (HBV) antigens and their relevant antibodies.

#### OBJECTIVE:

This study was designed to apply and assess the sensitivity and specificity of different commercially available laboratory techniques for detecting hepatitis B surface antigen (HBsAg).

#### METHODS:

One hundred and twenty-one sera samples were collected from National Center for Blood transfusion, Gastroenterology and Liver Diseases Hospital, Central Public Health Laboratories and Teaching Laboratories. According to the manufacturer's practical instructions, a group of commercially available laboratory methods for detecting HBsAg were applied, including enzyme linked immunosorbent assay (ELISA), enzyme linked fluorescent assay (ELFA), immunochromatographic assay (ICA), and latex agglutination test (LAT).

#### RESULT:

Among ELISA, ELFA, ICA, and LAT methods for detecting HBsAg, the 3<sup>rd</sup> generation ELISA was proved to have very high specificity (no false negative results) and the least one that has necessitated few confirmatory repetitions. ELFA versus ELISA has showed relatively lower sensitivity (more false negative results). However, similar to ELISA, ELFA showed very high specificity. Immunochromatographic assay (ICA) confidentially appeared to be a good, rapid and simple technique with comparable sensitivity and specificity to ELISA and ELFA techniques. Although LAT was introduced as a rapid, simple and cheap technique for HBsAg screening, it showed frank lower sensitivity and specificity that deranged it from competing with all those tested techniques.

#### CONCLUSION:

The concomitant use of ELFA with ELISA compensates its relatively lower sensitivity in front of ELISA. Latex agglutination test for HBsAg has relatively lower sensitivity and specificity than all other tests. For its comparable performance characteristics to ELISA, the use of ICA is ideally suited for HBsAg screening, in respect to its lower cost, rapidity, simplicity and no need for expensive equipments

**KEY WORDS:** hepatitis b surface antigen, enzyme linked immunosorbent assay, enzyme linked fluorescent assay, immunochromatographic assay, and latex agglutination test.

### INTRODUCTION:

Hepatitis A Virus (HAV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Hepatitis D Virus (HDV) and Hepatitis E Virus (HEV) represent the major viral agents recognized in acute viral hepatitis in

children and in adults<sup>(1,2)</sup>. Many other viruses cannot be ascribed to those known agents, thus their associated disease is designated as non A-E hepatitis<sup>(3,4)</sup>.

Hepatitis B Virus (HBV) infection with its associated sequelae is a disease of major public health importance worldwide<sup>(5)</sup>.

Clinically, HBV infection is indistinguishable from other viral hepatitis. Accordingly, its diagnosis relies on a specific laboratory tests for distinguishing it from such viruses<sup>(6,7)</sup>.

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Hepatitis B surface antigen (HBsAg) is detectable in serum prior to the development of symptoms, and remains detectable during clinical convalescence. The serological presence of HBsAg beyond 6 months defines chronic hepatitis B. However, some people, referred to as "carriers", may have little or no damage to

liver at all, albeit they are continuously making as well as transmitting such viruses for years<sup>(7-11)</sup>. Therefore adequate screening of patients and blood donors for Hepatitis B surface antigenaemia is advocated in order to reduce the transmission of this virus<sup>(5)</sup>.

There is neither seasonal trend for HBV infection nor high predilection for any age group. However, definite high risk groups were identified, among them parenteral drug abusers, institutionalized persons, those dealing with health care (such as surgeons, dentists, nurses, pathologists, and blood bank personnel), multiply transfused patients, organ transplanted and hemodialysed patients<sup>(12, 13)</sup>.

Following Blumberg's discovery of hepatitis B surface antigen (HBsAg), many attempts have been made to develop several in vitro diagnostic techniques for the detection of this antigen and its homologous antibody, including a wide-range of different serological, immunological and molecular-biological techniques<sup>(14)</sup>.

According to the financial facilities as well as technical feasibility (i.e. trained personnel and the availability of advanced laboratory equipments) one should have the choice to select and apply one or more of these laboratory tests<sup>(15)</sup>. However, many companies insert instructions and promotion leaflets within their commercial diagnostic kits that are deranged from those practically obtained results. Such observations raised the need for intra-laboratory and inter-laboratory quality control measures to justify utilization of those kits to satisfy the dependable criteria of highly sensitive, specific and reproducible tests.

### **MATERIALS AND METHODS:**

During the period from August 2006 till January 2007, a total number of one hundred and twenty-one (121) HBsAg-positive sera were collected from patients & control individuals attending to the following medical centers:

- 1- Gastroenterology and Liver Diseases Hospital
- 2- Teaching Laboratories / Virology Department.
- 3- Hemodialysis and Artificial Kidney Unit / Baghdad Teaching Hospital.
- 4- National Blood Transfusion Center.
- 5- Central Public health Laboratories / Hepatitis Viruses Unit.

### **MATERIALS:**

The following ready-to-use commercial diagnostic kits were used in this work (table 1).

**Table1: Commercial Diagnostic kits for HBsAg Detection.**

NO.	Trading Name of Kit	Manufacturing Company	The Country
1-	Hepanostika HBsAg Uniform(ELISA)	Organon Teknika	Holland
2-	HBs Ag ( Mini VIDAS)	Bio Merieux	France
3-	HBs (Rapid ICA Device)	Atlas Medical	England
4-	HBs (Rapid Latex)	Atlas Medical	England

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### METHODS:

The following laboratory techniques were done in the Virology Unit / Teaching Laboratories /Medical City Complex .They were applied according to the instructions of the manufacturing companies.

1. Enzyme Linked Immunosorbent Assay (ELISA).
2. Enzyme Linked Fluorescent Assay (ELFA).
3. Immunochromatographic Assay (ICA).
4. Latex Agglutination Test (LAT).

### Statistical Analysis:

The suitable statistical methods (16) were used including the followings:

#### 1- Descriptive statistics:

- A) Statistical tables including observed frequencies with their percentages.
- B) Statistics of the readings distribution (mean, SD, S.E, minimum & maximum).
- C) Graphical presentation by ROC curve.

#### 2 – Inferential statistics:

These were used to accept or reject the statistical hypotheses, they include the followings:

- A) Binomial test.
- B) Kruskal Wallis test.
- C) Student test (t- test).
- D) Mann-Whitney U test.
- E) Validity tests they include the followings: Sensitivity, Specificity, Positive predictive value (PPV.), negative predictive value (NPV.) & Accuracy.

The comparisons of significant (P-value) in any test were:

S= Significant difference (P<0.05).

HS= Highly Significant difference (P<0.01).

NS= Non Significant difference (P>0.05).

### 3-Computer & programs:

Pentium-4 was used to perform both SPSS (version 10) and Excel programs.

### RESULTS:

A. The validity of Enzyme Linked Fluorescent Assay for detecting HBsAg as compared to Enzyme Linked Immunosorbent Assay:-

The present study included (91) HBsAg- positive sera samples, repeatedly proved to be positive by criteria of 3rd generation ELISA test, to be tested by ELFA using Minividas apparatus.

As shown in table(2) and figure (1), it was found that out of these (91) ELISA -positive sera samples ,only (69) samples showed positive- ELFA reactions, whereas the rest (22) samples have given HBsAg-negative ELFA results.

Statistically, ELFA had showed (75.8 %) sensitivity when compared to ELISA technique in detecting HBsAg-positive sera samples. However, ELFA technique showed (100 %) specificity in detecting ELISA - negative samples. The positive predictive value (PPV) of this technique was found to be (100 %) whereas its negative predictive value (NPV) was (57.9 %). Therefore, the accuracy of ELFA technique compared to ELISA was found to be (81.8 %).

**Table2: Validity of ELFA for HBsAg detection as compared to ELISA.**

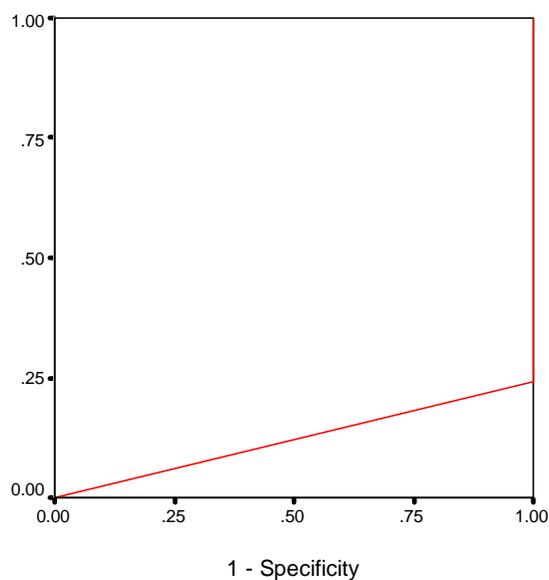
Validity		ELISA test		Total
		Positive	Negative	
ELFA test	Positive	69	0	69
	Negative	22	30	52
Total		91	30	121

Sensitivity = 75.82 %. Specificity = 100 %.

PPV = 100 %. NPV = 57.96 %.

Accuracy = 81.81 %.

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**Figure1: ROC Curve for validity of ELFA for HBsAg detection as compared to ELISA.**

**B.** The validity of Immunochromatographic assay for HBsAg detection as compared to ELISA:-

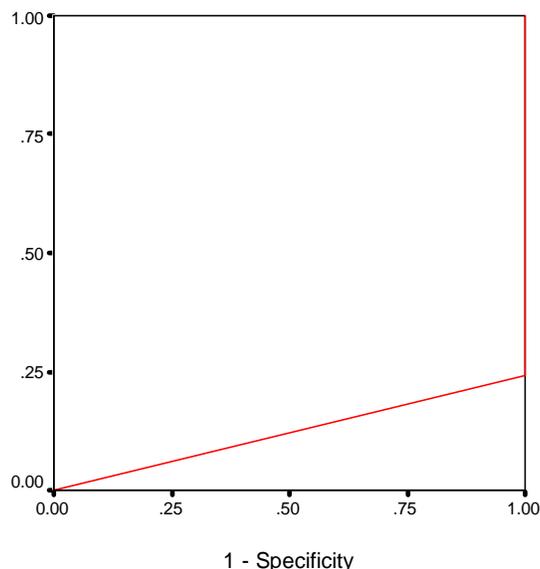
On applying a rapid chromatographic immunoassay device for HBsAg on a total number of (91) sera, that were positive by using 3<sup>rd</sup> generation ELISA kit for HBsAg, it was found that (22) out of these (91) sera samples showed negative results; whereas the rest (69) sera samples were compatible to the results of ELISA- positive tested samples. In addition, all the

30 HBsAg -negative sera by ELISA also showed negative results for HBsAg on applying immunochromatographic assay (table 3 and figure 2). This technique versus ELISA has a sensitivity of (75.8 %), specificity of (100 %) and accuracy of (81.8 %). This technique was completely compatible to ELISA technique regarding positive and negative predictive values (i.e. 100 % and 57.9 %, respectively).

**Table3: Validity of rapid- HBsAg detection by immunochromatographic assay as compared to ELISA.**

Validity		ELISA test		Total
		Positive	Negative	
Rapid chromatographic assay test	Positive	69	0	69
	Negative	22	30	52
Total		91	30	121

Sensitivity = 75.82 %. Specificity = 100 %. PPV = 100 %.  
NPV = 57.96 %. Accuracy = 81.81 %.



**Figure2: ROC Curve for validity of rapid-HBsAg detection by immunochromatographic assay as compared to ELISA**

C. Comparative assessment of HBsAg detection by latex agglutination test as compared to ELISA: Table 4 and figure 3 show the results of using latex kit for HBsAg testing on(91)sera specimens, proved to be positive for HBsAg by ELISA criteria where only (68) specimens showed positive- HBsAg latex agglutination reaction, whereas (23) specimens denied to show any positivism and then evaluated as HBsAg -negative sera. On subjecting the (30) sera samples that were HBsAg- negative by ELISA criteria for testing by

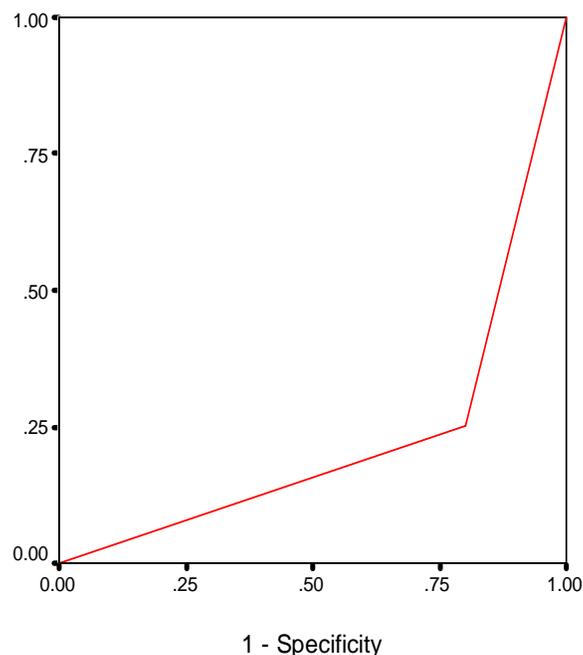
latex agglutination test, only (6) of them showed positive- HBsAg by latex agglutination and the remaining (24) specimens showed negative reactions ,and as such are compatible to their (24) HBsAg-negative sera counterparts by ELISA test. The latex agglutination test in front of ELISA technique had statistically showed sensitivity of (74.7 %); specificity of (80 %); high positive predictive value (91.9 %) versus (51 %) negative predictive value and therefore accuracy of (76 %).

**Table4: Validity of Latex agglutination test for HBsAg detection as compared to ELISA.**

Validity		ELISA test		Total
		Positive	Negative	
Latex agglutination test	Positive	68	6	74
	Negative	23	24	47
Total		91	30	121

Sensitivity = 74.72 %. Specificity = 80 %. PPV = 91.89 %.  
 NPV = 51.06 %. Accuracy = 76.03 %.

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**Figure3: ROC Curve for validity of Latex agglutination test as compared to ELISA.**

### D. Validity of immunochromatographic assay for HBsAg detection as compared to ELFA:-

To evaluate ELFA properties in front of the properties of ICA technique, (121) samples were introduced for that evaluation. Out of (69) HBsAg-positive sera by ELFA , (68) sera showed positive reactions by immunochromatographic assay and only one serum had deviated and negatively reacted in this rapid test device (table 5 and figure 4).

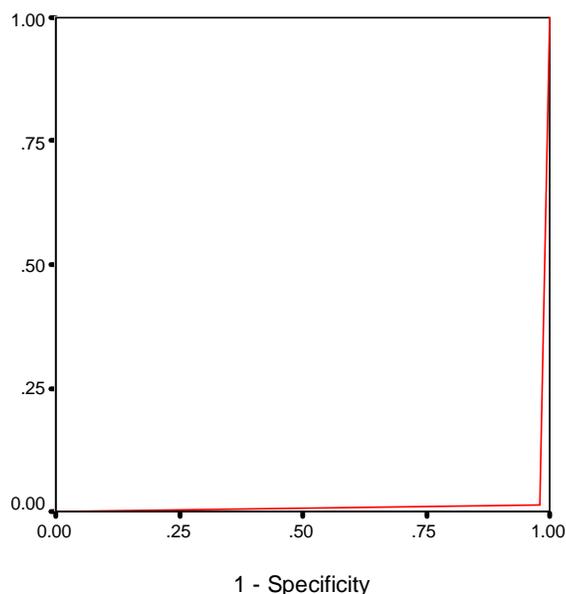
On subjection of (52) HBsAg- negative sera according to criteria of ELFA test, it was found that only one serum deviated to the positivism side where it was evaluated as HBsAg- positive serum by criteria of immunochromatographic assay. The immunochromatographic technique had statistically showed accuracy of (98.3%) with (98.5 %) sensitivity, (98 %) specificity; (98.5 %) positive predictive value and (98 %) negative predictive value.

**Table5: Validity of immunochromatographic-HBsAg assay as compared to ELFA- counterpart assay.**

Validity		ELFA		Total
		Positive	Negative	
Rapid chromatographic immunoassay test	Positive	68	1	69
	Negative	1	51	52
Total		69	52	121

Sensitivity = 98.55 %. Specificity = 98.07 %. PPV = 98.55 %.  
 NPV = 98.07 %. Accuracy = 98.34 %.

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**Figure 4: ROC Curve for validity of rapid chromatographic immunoassay as compared to ELFA test.**

### E. Validity of latex agglutination test for HBsAg detection as compared to ELFA:

When comparing the latex agglutination with ELFA technique for HBsAg detection, it was found that (7) out of (69) sera, proved to be positive for HBsAg by ELFA technique showed negative reactions on application of latex agglutination test.

Fifty-two (52) HBsAg- negative sera by criteria of ELFA were subjected to HBsAg- latex test where

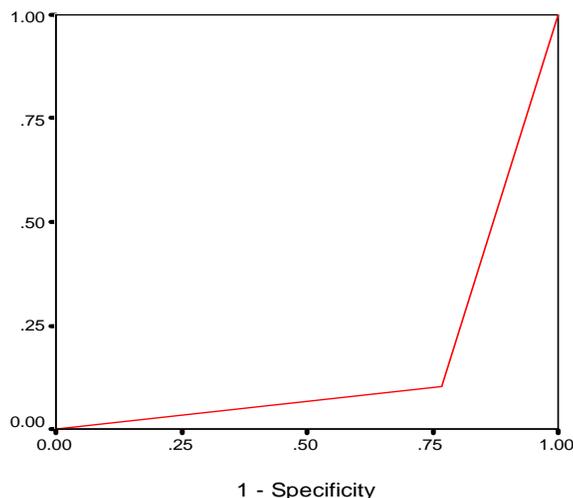
only (40) of them sustained HBsAg- negative evaluation whereas the rest (12) sera were relationally deviated and gave HBsAg- false positive results (table 6 and figure 5).

The statistical analysis of the latex agglutination testing, in comparison to ELFA , showed (89.8 %) sensitivity; (76.9 %) specificity; (83.8 %) positive predictive value; (85 %) negative predictive value and over all accuracy rate of this technique as referred to ELFA was (84.3 %).

**Table6: Validity of Latex agglutination test for HBsAg detection as compared to ELFA.**

Validity		ELFA		Total
		Positive	Negative	
Latex agglutination test	Positive	62	12	74
	Negative	7	40	47
Total		69	52	121

Sensitivity = 89.85 %. Specificity = 76.92 %. PPV = 83.78 %.  
 NPV = 85.1 %. Accuracy = 84.29 %.



**Figure5: ROC Curve for validity of Latex agglutination test as compared to ELFA.**

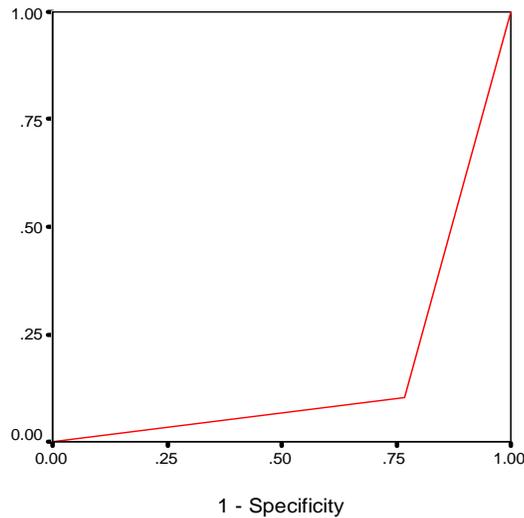
**F. Comparison of two rapid HBsAg tests; Latex agglutination versus Immunochromatographic assay.** In the present study, among (69) HBsAg- positive sera samples that were tested by rapid chromatographic immunoassay, (62) samples were found to be HBsAg- positive when evaluated by latex agglutination test and the rest seven sera samples were found falsely negative (table7 and figure 6). To evaluate of the specificity of latex agglutination test, (52) HBsAg- negative sera (by criteria of chromatographic immunoassay) were included in this study and we found out that (12) of them deviated

and gave positive HBsAg results; whereas the remaining (40) samples of sera sustained their criteria of negativity (i.e. true negatives). Therefore, this technique has (89.9 %) sensitivity as compared to chromatographic immunoassay. In addition, on statistical analysis, latex agglutination test has showed (76.9 %) specificity when compared to that technique as well as it showed negative predictive value of (85.1 %) of the true negative results expressed by rapid chromatographic test. The accuracy of latex test versus chromatographic test (as 2 rapid HBsAg- test devices) was (84.3 %).

**Table7: Validity of Latex agglutination test as compared to Immunochromatographic assay**

Validity		Rapid immunochromatographic assay		Total
		Positive	Negative	
Latex agglutination test	Positive	62	12	74
	Negative	7	40	47
Total		69	52	121

Sensitivity = 89.85 %. Specificity = 76.92 %. PPV. = 83.78 %.  
 NPV. = 85.1 %. Accuracy = 84.29 %.



**Figure 6: ROC Curve for validity of Latex agglutination Test as compared to Immunochromatographic assay for HBsAg detection.**

**DISCUSSION:**

The studies have specified the third generation of ELISA technique for testing blood donors and viral hepatitis patients for its high sensitivity and specificity in detecting HBsAg- positive and HBsAg-negative sera <sup>(8, 17)</sup>.

In addition, a new enzyme-linked fluorescence assay (ELFA) suitable for use with peroxidase-antibody conjugates was described. The fluorescence is stable and unaffected by light and the measurement of this fluorescence is automated. Thus, ELFA is an ELISA with a final detection of fluorescence albeit the assay compared favorably with a standard ELISA. However, the sensitivity of the test was approximately 50 times higher than that of a commercial radioimmunoassay. ELFA technique was introduced to detect many microorganisms including many viruses such as HBV in a reference to its high sensitivity and specificity in detecting the wide-ranged HBV serological markers, including HBsAg (18-20).

Our validity investigations clearly indicated that there was significant difference between ELISA and ELFA tests in detecting HBsAg.

Statistically, Enzyme-linked fluorescent assay (ELFA) had showed (75.8 %) sensitivity when compared to ELISA technique in detecting ELISA positive HBsAg sera samples. Diagnostic EIA products, that are licensed to detect HBsAg, follow a generic protocol with a number of minor variations

that not significantly alters the sensitivity or the specificity of the assay. However, procedures that have protocols with a shortened periods of incubation for the different steps of test could significantly affect the sensitivity of this technique; ELFA is one of these techniques that markedly shortens the time of achieving a test for HBs Ag (in comparison to the old as well as new ELISA generations) <sup>(8, 20)</sup>.

The VIDAS- HBsAg test is an enzyme-linked fluorescent immunoassay which was performed in the automated VIDAS system (according to the VIDAS HBsAg ULTRA booklet, operators' manual, 2006) where this assay can be performed according to two protocols: HBL long protocol (90 minutes) and HBS short protocol (60 minutes). In our study, we used the HBS short protocol, but using HBL long protocol could, to some extent, increase the sensitivity of this test by increasing the percentage of detection of HBsAg- positive sera samples <sup>(20)</sup>.

However, ELISA HBsAg 3<sup>rd</sup> generation test is known to show false-positive results, and this requires doing another ELISA test in duplicate as well as confirming these results via the use of a confirmatory HBsAg test to exclude false positive results of EIA <sup>(17, 21)</sup>.

As this study included plasma from patients who had hemodialysis and who are given heparin prior to dialysis, it is possible that some of heparin appeared in the blood of such patients where such partially-

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heparinized plasma can cause low level of false positive- HBsAg reactions by using 3<sup>rd</sup> generation of ELISA<sup>(8)</sup>.

However, ELFA technique in this study showed 100 % specificity ( in detecting ELISA HBsAg- negative samples) and 100% positive predictive value and 57.96 % negative predictive value. The present results agree with the results of<sup>(22)</sup> regarding the specificity but show little bit lower sensitivity than their results. This is for ELFA has very high specificity in detecting HBsAg where in a study done by Jolivet-Reyanaud et. al. (2001) on 367 HBsAg-negative sera that were tested by using both the short and long protocols of VIDAS system, they were also found to be negative by using EIA technique<sup>(23)</sup>.

On evaluation of the clinical laboratory utility of this new HBsAg-detection system, that follow the principle of chromatographic immunoassay, the validity analysis of application of such rapid ICA versus ELISA showed a sensitivity of (75.8 %); (100 %) specificity and accuracy of (81.8 %). In this study, ICA technique was relatively compatible to the criteria of ELISA technique regarding positive and negative predictive values (i.e. 100 % and 57.9 %, respectively). The present results are compatible with those of Verstraeten and Keya(1997) , Shin et al (2001) and Cha et al (2006)(24-26) but incompatible with Sato et al (2006)<sup>(27)</sup> regarding sensitivity albeit consistent with them regarding specificity and vice versa regarding incompatibility of<sup>(28)</sup> results with ours where they found both very high sensitivity and specificity .

Immunochromatographic assays (ICAs) are also referred to as rapid tests, since they are simple and the results can be obtained within minutes after manually loading a few drops of a sample into each sample well of the test device<sup>(25)</sup>. In this study, it was also revealed that their reactions are completed in as little as (10-15) minutes and the advantage of examining a single sample was simply achieved by cutting the chromatographic strip as required. In addition, the cost of ICA is four times less than that of EIA and the system doesn't require any specific instrumentation. Furthermore, this test requires only a small amount of samples (25 µl) and can be readily performed.

The ICA kits are as sensitive as EIA for the detection of anti-HBs antibodies, but are less sensitive than EIA for HBsAg. The ICA kits for the rapid detection of HBsAg was recommended for a limited use in the

clinical laboratory<sup>(26)</sup>. Although the sensitivity of ICA was slightly lower than that of EIA at an incubation time of (10-15 minutes), it is possible to improve the sensitivity to a level equal to that of EIA by extending the incubation time to 60 minutes. Accordingly, and after the aforementioned modification this rapid test is ideally suited for HBsAg screening, with respect to its cost, speed, simplicity, flexibility and no need for availability of expensive equipments and could be recommend the use of ICA for many institutions for the purpose of HBsAg screening.

Qualitative and semi-quantitative enzyme immunoassay (EIA) methods are considered to be the most sensitive tests and are widely recommended to be used at well-equipped reference centers or central blood bank. On other hand, rapid tests are intended for qualitative detection of HBsAg in human serum or plasma wherever EIA methods are impractical or can't be sustained. The majority of rapid tests are based on agglutination or immunochromatographic principles<sup>(29)</sup>.

Latex test, which do not require elaborate instrumentation and are relatively inexpensive is a technique available for HBsAg screening in our country. In addition, these rapid test kits usually contain all the supplies and reagents needed to be performed. For these, clinical private laboratories used latex test kits for screening patients for HBsAg. Accordingly, in this study, the sensitivity and specificity of these rapid and low- cost latex agglutination test kits for HBsAg detection were tested for their performance characteristics as compared to their EIA counterparts. Statistically, the HBsAg-latex agglutination test in front of ELISA technique had showed sensitivity of (74.7 %); specificity of (80 %); high positive predictive value (91.9 %) versus (51 %) negative predictive value and therefore accuracy of (76 %). The sensitivity and specificity results of this study are consistent with the results of Finny and co-workers (1996) and Verstraeten and Keya (1997)<sup>(24, 28)</sup>. In view of their low sensitivity and specificity, these rapid tests are not suitable for screening blood in major / district hospitals and even it is practicable to be used at the provincial or peripheral health care level. It was revealed that appropriate and compulsory screening of blood donors using sensitive methods, must be ensured to prevent post transfusion hepatitis<sup>(30)</sup>.

The new ELFA technique was introduced to detect HBsAg of HBV with a reference to its lower time

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requirement (in comparison to ELISA) as well as to its comparable high sensitivity and specificity in detecting HBsAg in sera samples<sup>(18,19)</sup>.

This ICA technique when applied in this research work had statistically showed accuracy of (98.3%) as a result of statistical validity analysis of (98.5 %) sensitivity, (98 %) specificity; (98.5 %) positive predictive value and (98 %) negative predictive value. In these respects, the results of ICA reveal high compatibility to ELFA. In addition, the very short achieving time of ICA test kits (i.e.10-15 minutes with reference to the ELFA short 60 minutes and long 90 minutes protocols), cheapness and their no need for the expensive Minividas apparatus and its relevant kits<sup>(31)</sup>.we suggest for the confidential use of ICA to detect HBsAg, at least, for peripheral clinical laboratory units.

The statistical analysis for the latex agglutination testing in comparison to ELFA testing showed (89.8 %) sensitivity; (76.9 %) specificity; (83.8 %) positive predictive value; (85 %) negative predictive value and over-all accuracy rate of this technique ,as referred to ELFA ,was (84.3 %).

The LAT is used as screening test for its speed and simplicity in detecting HBsAg as well as it doesn't require complex instrumentation<sup>(31)</sup>.On contrary, ELFA technique requires such complex instrumentation as well as achieving time for this assay is relatively longer; HBL long protocol (90 minutes) and HBS short protocol (60 minutes) (according to VIDAS HBsAg ULTRA booklet, 2006). In spite of all above advantages of latex agglutination test, the observed properties of ELFA in detecting HBsAg in this study are more preferable than those of latex agglutination test for such purpose.

Comparison assessment of two rapid HBsAg tests has showed that the LAT technique has (89.9 %) sensitivity as compared to chromatographic immunoassay. In addition, on statistical analysis, LAT has showed (76.9 %) specificity when compared to that technique as well as it showed negative predictive value of (85.1 %) of the true negative results expressed by rapid chromatographic test. The accuracy of latex test versus chromatographic test (as 2 rapid HBsAg test device) was (84.3 %).These results are compatible with the results of<sup>(24)</sup>.

The two rapid tests, LAT and ICA, are characterized by their low cost and they don't require complex instrumentation. These tests can be recommended for

use in routine screening, especially for emergency use, in the clinical laboratory<sup>(28)</sup>. Our results showed that the sensitivity and specificity of the latex agglutination test for HBsAg is relatively lower than those of ICA -HBsAg test. This could be related to the fact that the latex test is based on latex particles coated with anti-HBs specific antibodies and that this test is depends on frank and heavy clumping of Ag-Ab reaction. However, it is subjectively dependent on the technician for the weakly- positive clumps that may be invisible so as to give such negative result; where as The ICA test depends on the membrane chromatographic capillary action to react and it depends on band reactions (31-33).

### CONCLUSION:

- 1-The ELFA test in front of ELISA showed more false negative results (i.e. relatively lower sensitivity). However, similar to ELISA test, ELFA showed very high specificity.
- 2-Confidentially, ICA appeared as a good, rapid and simple test with comparable sensitivity and specificity to ELISA and ELFA techniques for HBsAg detection.
- 3-Although it was introduced as rapid, simple and cheap technique for HBsAg detection, latex agglutination test had shown frank lower sensitivity and specificity that deranged it from competing with all available techniques in this study.

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