COMPARISON OF TWO CHEMILUMINESCENT AUTOMATED SYSTEMS FOR THE SCREENING OF HBsAg, HCV, HIV

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BACKGROUND

In the past years, HBsAg, HCV and HIV screening testing was always required for all patients at the time of hospitalization. This was done mainly as a preventive measure for health care workers against the biological risk of accidental exposure and as a way to check the status of the people at first hospital admission.
Today, in these years of spending review and due to an increased attention towards the cost-benefit analysis, there is the need to give more attention to the testing requests, with a main focus to the patients that really will take an advantage from the diagnostic tests, as well as to the use of those tests for at-risks patients, and for the screening of the blood, organs and tissues donors.
Universal and suitable precautions should be adopted for all patients regardless the suspect of bearing an infection. In this context, the clinical laboratory could be active not only as the structure devoted to the testing delivery, but also as a promoter of the culture of the most appropriateness of diagnostic requests. At the same time, the availability of diagnostic tests with high level of sensitivity, specificity, robustness and reliability, combined with automation and traceability of the results to improve the turnaround time of tests execution, can allow to reduce the overall costs, with the reduction of additional tests and investigations, cost of hospitalization and personnel costs.

AIM of this study was to evaluate the performance and compare the results obtained with Chemiluminescent assays for the screening of HBsAg, HCV and HIV Ag/Ab, available on two automated platforms with high throughput: LIAISON® XL (DiaSorin, Saluggia, Italy) and Advia Centaur XP (Siemens Healthcare Diagnostics, USA). All discordant and problematic samples have been tested with additional methods.

MATERIALS AND METHODS

334, 353 and 306 serum samples taken from the daily routine of the serology lab, have been tested with HBsAg, HCV and HIV Ag/Ab respectively. Among them, 43 samples were taken from our serum bank, stored at -20° C and were from problematic patients and /or showing low level of reactivity against at least one marker. The results of these samples were analyzed together with those from the routine. In addition one panel of "rec DNA HBsAg Mutants 2010 "provided by DiaSorin and the 1st WHO International Reference Panel for Hepatitis B Virus (HBV) Genotypes for Hepatitis B Surface Antigen (HBsAg) Assays, PEI code 6100/09, have been evaluated for HBsAg.

TESTS PERFORMED ON ADVIA CENTAUR XP (SIEMENS):

HBsAg II: chemiluminescent enzyme immunoassay for the qualitative detection of hepatitis B surface antigen (HBsAg). The test utilizes monoclonal antibodies to capture anti-HBs. The chemiluminescent reaction is directly proportional to the amount of HBsAg in the sample. The results are expressed as an Index. Samples with an Index <1 are classified as negative, while samples with an Index ≥ 1 and <50 are positive but require a confirmatory assay (HBsAg Confirmatory). Samples with an Index ≥ 50 are positive.
HCV: chemiluminescent enzyme immunoassay for the qualitative detection of IgG antibodies to HCV. The test employs two recombinant HCV antigens of NS3, NS4, NS5 regions (c200 and NS5) and a synthetic peptide of the core (c22). The chemiluminescent reaction is directly proportional to the amount of anti-HCV present in the sample. The results are expressed as an Index. Samples with an index value <0.8 are negative. Samples with an Index value ≥0.8 and <1 are equivocal. Samples with an Index value ≥ 1 are positive.
HIV Ag/Ab Combo: chemiluminescent enzyme immunoassay for the simultaneous qualitative detection of HIV p24 antigen and antibodies to HIV type 1, including the group "O", and / or type 2. The assay employs as a solid-phase recombinant antigens of the ENV HIV-1 protein (gp41 / gp120), a protein of the ENV HIV-2 (gp36), a synthetic peptide for the detection of HIV group "O" antibodies and two monoclonal antibodies specific to detect the HIV p24 antigen. The chemiluminescent reaction is directly proportional to the amount of HIV antibody and / or p24 antigen present in the sample. The results are expressed as an Index. Samples with an Index value <1 are negative. Samples with an Index value ≥ 1 are positive.

TESTS PERFORMED ON THE LIAISON® XL (DIASORIN):

HBsAg Quant: chemiluminescent immunoassay for the quantitative determination of HBV surface antigen. The test uses monoclonal antibodies directed against epitopes of the external and highly conserved internal and transmembrane regions of HBsAg with a balanced reactivity against subtypes ad and ay, which ensures a high sensitivity for the detection of mutants and different genotypes. The instrument automatically calculates the concentrations of HBsAg expressed in IU/ml using a stored curve obtained on the basis of dose calibrators calibrated against the NIBSC standard (WHO code 00/588). The threshold value which distinguishes between the presence and absence of HBsAg is 0.05 IU/ml, therefore values <0.05 IU/ml are negative; values ≥ 0.05 IU/ml are positive
HCV Ab: chemiluminescent immunoassay for the qualitative determination of specific antibodies against the virus of hepatitis C. The solid phase consists of two recombinant antigens (core and NS4) and a third antigen (NS3). The presence of antibodies in the samples is determined by comparing the signal of the chemiluminescent reaction with the threshold value (cut-off). The analyzer calculates the results: the samples with S / CO <1 are negative; Samples with S / CO ≥ 1 are positive.
HIV Ag/Ab: chemiluminescent immunoassay for the qualitative detection of HIV-1 combined p24 antigen and antibodies to HIV-1 (group M and group O) and / or HIV-2. The test uses two different integrals of reagents, one for the detection of antibodies to HIV and one for the detection of p24 antigen, and uses recombinant antigens gp41 of HIV-1 (group M and group O) and gp35 of HIV-2. The presence of p24 antigen and / or of anti-HIV antibodies is determined by comparing the signal of the chemiluminescent reaction with the threshold value (cut-off) as provided by each specific assay. The analyzer calculates the combined results: Samples with S / CO <1 are negative; Samples with S / CO ≥ 1 are positive.

ADDITIONAL TESTS FOR DISCORDANT RESULTS AND "PROBLEMATIC" SAMPLES WITH WEAK REACTIVITY:

- Abbott Architect HBsAg quantitative: chemiluminescent immunoassay performed on i2000SR Architect for the quantitative detection of HBV surface antigen. Values <0.05 IU/ml are negative; values ≥ 0.05 IU/ml are positive.
- Abbott Architect HBsAg qualitative II: chemiluminescent immunoassay for the detection of HBV surface antigen, values <1 S/CO are negative; values ≥ 1 S/CO are positive.
- Abbott Architect anti-HCV: chemiluminescent immunoassay for the qualitative detection of antibodies to structural and non-structural proteins of HCV (core, NS3 and NS4). Values <1 S/CO are negative; values ≥ 1 S/CO are positive.
- INNO-LIA™ HCV Score Innogenetics (now Fujirebio): 3rd generation Immunoblot for the evaluation of antibodies against antigens of structural and non-structural HCV regions (core, E2, NS3, NS4A, NS4B, NS5A). For the interpretation of the results the software LiRAS™ have been used according to the criteria proposed by Innogenetics.
- Abbott Architect HIV Ag/Ab Combo: chemiluminescent immunoassay for the simultaneously qualitative detection of HIV p24 antigen and antibodies against recombinant antigens and synthetic peptides from sequences of the viral transmembrane protein of HIV-1 and 2. Values <1 S/CO are negative; values ≥ 1 S/CO are positive.
- Siemens Advia Centaur XP HIV Ab 3rd generation: chemiluminescent enzyme immunoassay for the qualitative detection of antibodies to HIV (type 1, type 2 and subtype O) directed against recombinant antigens of the ENV and the core. Values <1 index are negative; values ≥ 1 index are positive.
- bioMérieux VIDAS HIV p24 II: quantitative test for HIV p24 antigen detection using ELFA technology (Enzyme Linked Fluorescent Assay). Values <3 pg/ml are negative; values ≥ 3 and <5 pg/ml are equivocal; values ≥ 5 pg/ml are positive.

RESULTS

The correlation between the tests performed on LIAISON® XL and Advia Centaur XP was 100% for HBsAg, 97.4% for anti HCV and 97.7% for HIV Ag / Ab (see Tables 1, 2, 3).
Diagnostic sensitivity of LIAISON® XL HBsAg Quant was 100%, and diagnostic specificity was 100%. Diagnostic sensitivity of Advia Centaur XP HBsAg was 100%, and diagnostic specificity was 100%.
The 9 discordant results for HCV (5 positive with LIAISON® XL and 4 positive with Advia Centaur XP) all showed a weak positive results (see Table 4). The test run on Architect agreed with the positivity of a single positive sample with LIAISON® XL. Out of 5 samples positive with LIAISON® XL and negative with Advia Centaur XP, the INNO-LIA confirmed the positivity of 2 samples (true positive for LIAISON® XL and false negative with Advia Centaur XP), while the 3 other samples were classified as negative (false positive with LIAISON® XL). The INNO-LIA did not confirm the 4 samples positive with Advia Centaur XP and negative with LIAISON® XL (false positive with Advia Centaur XP). The molecular biology test of Real Time PCR, performed on the 3 samples available, gave a result below the sensitivity threshold of the method used (<15 IU/ml). Diagnostic sensitivity of LIAISON® XL HCV was 100%, and diagnostic specificity was 99%.
Diagnostic sensitivity of Advia Centaur XP HCV was 94.7%, and diagnostic specificity was 98.7%. The 7 discordant results for HIV Ag/Ab (3 positive with LIAISON® XL and 4 positive with Advia Centaur XP) showed a weak reactivity with both systems (see Table 5): all were negative with the 4th generation test performed on Architect for the evaluation of Ag/Ab, with the 3rd generation test run on Centaur for the evaluation of anti-HIV antibodies only, and with the test for the HIV p24 antigen alone performed on Vidas.
Diagnostic sensitivity of LIAISON® XL HIV Ab/Ag was 100%, and diagnostic specificity was 99%.
Diagnostic sensitivity of Advia Centaur XP HIV Ag/Ab was 100%, and diagnostic specificity was 98.6%.
The sensitivity of the HBsAg Quantitative test on LIAISON® XL on the panel of mutants was excellent (see Table 6).
Both HBsAg assays showed a good sensitivity across the different genotypes, when using the 1st WHO International Reference Panel for Hepatitis B Virus (HBV) Genotypes for Hepatitis B Surface Antigen (HBsAg) Assays, PEI code 6100/09 (see Table 7).

Table 1

HBsAg	ADVIA CENTAUR XP		
LIAISON® XL	neg	pos	tot
neg	321	0	321
pos	0	13	13
tot	321	13	334

Table 2

HCV Ab	ADVIA CENTAUR XP		
LIAISON® XL	neg	pos	tot
neg	310	4	314
pos	5	34	39
tot	315	38	353

Table 3

HIV Ag/Ab	ADVIA CENTAUR XP		
LIAISON® XL	neg	pos	tot
neg	292	4	296
pos	3	7	10
tot	295	11	306

Table 4

Sample ID	LIAISON® XL HCV S/CO	Advia Centaur XP HCV Index	Architect HCV S/CO	Innogenetics Immunoblot HCV Score	Roche Real time PCR HCV RNA
31s	1.60	Neg	4.62	Pos	
Tbl	1.40	Neg	Neg	Pos	
9605	1.00	Neg	Neg	Neg	
Psr	1.60	Neg	Neg	Neg	
R001	1.20	Neg	Neg	Neg	< 15 IU/mL
18s	Neg	1.26	Neg	Neg	
23s	Neg	1.10	Neg	Neg	
R002	Neg	1.34	Neg	Neg	< 15 IU/mL
R004	Neg	3.60	Neg	Neg	< 15 IU/mL

Table 5

Sample ID	LIAISON® XL HIV Ab S/CO	LIAISON® XL HIV Ag S/CO	LIAISON® XL HIV Ab/Ag S/CO	Advia Centaur XP HIV Ag/Ab Index	Advia Centaur XP HIV Ab (3° gen) Index	Architect HIV Ag/ Ab S/CO	BioMerieux Vidas HIV p24 pg/mL
Prl	4.14	Neg	Pos	Neg	Neg	Neg	Neg
Sen	1.19	Neg	Pos	Neg	Neg	Neg	Neg
9354	Neg	3.48	Pos	Neg	Neg	Neg	Neg
Gls	Neg	Neg	Neg	1.63	Neg	Neg	Neg
2	Neg	Neg	Neg	1.20	Neg	Neg	Neg
5	Neg	Neg	Neg	1.69	Neg	Neg	Neg
7	Neg	Neg	Neg	1.40	Neg	Neg	Neg

Table 6

Sample ID	Mutation	DiaSorin LIAISON® XL HBsAg Quant	Abbott Architect HBsAg Quant	Abbott Architect HBsAg II Qualitative	Siemens Advia Centaur XP HBsAg Qualitative
		CO = 0.05 IU/mL	CO = 0.05 IU/mL	CO = 1.00 Index	CO = 1.00 Index
1	T123N	0.92	0.05	19.98	10.18
2	T123N-T124S	0.19	0.02	9.94	1.06
3	P142L-F/Y143H-D144E-G145R	0.26	0.02	< 0.10	0.90
4	I110R-S117I-G119R-T123N	0.69	0.04	< 0.10	2.67
5	122+DT	1	0.06	42.08	3.58
6	122+DT-G145R	1.4	0.06	< 0.10	4.46
7	G145R	0.39	0.23	5.57	9.13
8	D144A	0.56	0.33	24.01	41.33
9	P142L- G145R	0.49	0.19	7.41	7.96
10	P142S- G145R	0.55	0.22	9.5	9.35
Negative matrix		< 0.030	0.02	< 0.10	0.19

Table 7

Sample ID panel PEI 6100/09	HBV Sub-Genotype	HBV Sub-Genotype	Origin	LIAISON® XL Murex HBsAg Quant	Siemens Advia HBsAg II
1	A1	adw2	South Africa	REACTIVE	REACTIVE
2	A1	adw2	Brazil	REACTIVE	REACTIVE
3	A2	adw2	Germany	REACTIVE	REACTIVE
4	B1	adw2	Japan	REACTIVE	REACTIVE
5	B2	adw2	Japan	REACTIVE	REACTIVE
6	C2	adr	Japan	REACTIVE	REACTIVE
7	C2	adr	Japan	REACTIVE	REACTIVE
8	C2	adr	Russia	REACTIVE	REACTIVE
9	D1	ayw2	Germany	REACTIVE	REACTIVE
10	D2	ayw3	Russia	REACTIVE	REACTIVE
11	D3	ayw2	South Africa	REACTIVE	REACTIVE
12	E	ayw4	West Africa	REACTIVE	REACTIVE
13	F2	adw4	Brazil	REACTIVE	REACTIVE
14	F2	adw4	Brazil	REACTIVE	REACTIVE
15	H	adw4	Germany	REACTIVE	REACTIVE

CONCLUSIONS

Both systems (LIAISON® XL and Advia Centaur XP) demonstrated to have available excellent assays for the evaluation of HBsAg, HCV Ab and HIV Ag/Ab, suitable for routine use and characterized by very high diagnostic sensitivity and specificity. Excellent results were obtained on samples of the panel of mutants "rec HBsAg DNA Mutants 2010", with the HBsAg Quant DiaSorin that has correctly identified all the mutations. Both assays were able to detect the different genotypes of the PEI panel.

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