

Evaluation of diagnostic tests for the detection of HBsAg in sample DBS in children born to HIV-Positive mother to HIV-I in senegal (Determine AgHBs® vs Microscreen ELISA and HBsAg® Architect HBsAg Qualitative II®)

Abstract

Introduction: According to the World Health Organization, Senegal is the country presenting one of the highest prevalence of HBV ($\geq 8\%$) in the world. However, the diagnosis of this disease remains inaccessible in some areas of the country. We hypothesized that the use of Dried Blood Spots (DBS) may be potentiated as in the cases of HIV through the use of rapid diagnostic tests to facilitate decentralization of the biology of HBV.

Objective: The aim of our study was to evaluate and to compare the sensibility, specificity of three new diagnostic tests with the gold standard test Architect HBsAg Qualitative II® for the diagnosis of HBsAg from DBS samples. .

Method: This is a retrospective and comparative study of DBS samples collected from 930 patients between July 2007 and November 2012 in the decentralized sites of Mother-Child HIV Transmission Programme in Senegal. These samples were submitted to hepatitis B antigen detection using three independent tests: the test kit Determine HBsAg®, ELISA Kit Microscreen HBsAg® and ELISA kit Architect HBsAg Qualitative II ®.

Results: Patients were predominantly male with 520 (56.0%). Use of HBsAg ® Determine kit showed no reactivity to HBsAg suggesting zero sensitivity. However, we found a sensitivity of 43, 48% [23.22 -63.74], a specificity of 99.30% [98.34 to 100.27], a PPV of 83.33% and a VPV of 95.64% for the ELISA Microscreen AgHBs® overlooked ELISA Qualitative Architect II HBsAg ®. Inversely to the sensitivity of the Architect II Qualitative HBsAg® vis-à-vis Microscreen HBsAg ELISA® is 83.33 % [62.25 to 104.42], specificity was 95.64% [93.32 to 97.96] the positive predictive value was 43.48 (PPV), and predictive negative value (NPV) is 99.30%.

Conclusion: ELISA Microscreen AgHBs® is the only new test that shows interesting results on the detection of HBsAg. These data and could glimpse its usefulness in the diagnosis of this disease on DBS.

Keywords: HBV, DBS, HIV, Rapid diagnostic tests, Senegal

Volume 2 Issue 5 - 2015

Mintsa S,¹ Diop Ndiaye H,¹ Aminata D,¹ Guindo I,^{1,2} Sockna BN,¹ Mboup S,¹ Kane CT¹

¹Laboratory of Bacteriology-Virology, Université Cheikh Anta Diop, Senegal

²Laboratory of Bacteriology-Virology, INRSP, Mali

Correspondence: Mintsa Sandrine, Laboratory Bacteriology-Virology of the Hospital Aristide on Dantec CHU in Dakar, Faculty of Medicine and Odontology of the University Tcheik Anta Diop in Dakar, Senegal, and Faculty of Medicine of Libreville, Gabon. BP: 9016 Libreville-Gabon /7325 Dakar-Senegal, Tel +241 02469277, Email mintsasandrine@yahoo.com

Received: May 04, 2015 | **Published:** May 27, 2015

Abbreviations: HBV, Hepatitis B Virus; MTCT, Mother-Child Transmission Programme; DBS, Dried Blood Spots; VPV, Negative Predictive Value; PPV, Positive Predictive Value

Introduction

Infection with Hepatitis B virus (HBV) is a major public health problem worldwide. In 2010, the World Health Organization (WHO) estimates that over 2 billion people have been in contact with 350 million of those suffering from chronic HBV infection. More than one million of them die each year from complications related this infection, particularly cirrhosis and hepatocellular carcinoma, making HBV the second major cause of cancer after smoking. The risk of chronicity is 90% in children under one year, 25-30% in children aged 1 to 5 years in the context of family transmission.¹⁻⁴

Sub-Saharan Africa is a high endemic area for HBV, with a porting prevalence of HBsAg exceeding 8%. Transmission of HBV

from mother to infant occurs mainly during the perinatal period. It is particularly high (70-90%) when the mother carries HBV replication markers. In the absence of active replication, the risk of transmission is only 10 to 40%.⁵ The prevalence of HBsAg was 12.7% in a cohort of patients living with HIV in Burkina Faso,⁶ 15.4% in Niger donors⁷ and 13.9% in Mali among blood donors respectively. Diarra et al.⁸ in North Africa, the prevalence of HBsAg seem low, with only 4% in pregnant women, in Tunisia⁹ and 1.6% among volunteer in the general population in Morocco.¹⁰ In Senegal, according to the national program the fight against hepatitis (PNLH), 85% of the population have at least one marker of HBV and porting of chronic infection in the general population is 17%; mortality from this virus is not assessed but seems high [www.hepatites.sn/le-pnlh]. However, safe and effective vaccines against hepatitis B are available.¹¹⁻¹³ In many countries, mass immunization campaigns, as recommended by WHO, has led to significant reductions of the incidence of HBV infection in infants, children and adolescents.¹⁴

In Senegal, the vaccination is carried out from 6 to 8 weeks with the hexavalent vaccine (*Diphtheria, Tetanus, Pertussis, Polio, Haemophilus influenzae b* and *hepatitis B* in a complete scheme of 3 injections, the first two a month of Meanwhile, the third at 6 months after the date of the second injection.^{1,13,15} The markers of HBV are numerous and their prescription differs depending on the context. Several strategies defined for the screening of this infection exist. In Senegal, HBsAg is the marker used to screen HBV infection. The reference specimen for research of this marker is serum/plasma.^{4,16,17} Dried Blood Spots (DBS), widely used for HIV serology and neonatal diagnosis of HIV infection^{18,19} have also been evaluated for the detection of viral and serologic markers of HBV.^{20,21} There is quick and accessible testing for HIV DBS.²² The use of DBS may be potentiated, as in the case of HIV, through the use of rapid diagnostic tests to further facilitate the decentralization of the biology of HBV. This proposal nationwide will have to go through the evaluation of their performance on the media.²³ However, to date, no assessment data such tests have been published in the African context. In this situation, this work has been undertaken and aims to evaluate diagnostic tests for the detection of HBsAg: Determine the AgHBs® (Abbott Diagnostics Japan), the ELISA kit Microscreen HBsAg® (Span Diagnostics Ltd.) and the ELISA kit Architect HBsAg Qualitative II collected in different health centers and hospitals in Senegal. ®(Abbott Diagnostics Japan) from DBS.

Methodology

Population

This is a retrospective and comparative study of DBS samples collected between July 2007 and November 2012 in decentralized sites of Mother- Child Transmission Program (MCTP) of HIV in Senegal. All DBS valid collected from patient's age from 2 weeks to 15 years old and born to HIV-positive mothers were included. DBS invalid, serum child out of PMTCT are excluded.

Confection and DBS management

DBS was made by direct recollection drop blood on filter paper after bite with vaccine style heel for children under 10 kg and in the toes or fingers for children over 10kg. DBS were dried at room temperature in a sealable plastic bag containing desiccant and a humidity indicator are used in many closed envelope. The moisture content is checked every two days. Desiccants and indicator are changed if the indicator in the bag reaches 30% humidity. They are sent to the laboratory of reference along with their profile and their transmission analysis report, avoiding sunlight and moisture during transport. In the laboratory of reference, the sampling of Compliance is checked, then the DBS is retained for immediate or further processing, stored at room temperature or at - 80° C. An analysis report is attached to each DBS and shall contain in addition to the request sampling date, patient ID, sex, age, serological profile of the mother, the treatment regimen and duration of followed if the patient is on ART.

Neonatal HIV diagnosis

HIV status of children was determined by molecular diagnostics using the Cobas Amplicor platforms Cobas TaqMan, NucliSens, Biocentric® PCR or "house" HIV-2. Detection of HBsAg. Two HBV diagnostic tests were evaluated from elution made with PBS at 10%. Each DBS, 3 discs were punched in a saturated zone of blood and a volume of 200µl of PBS 10% was added by DBS disc. The mixture is left overnight at 4° C and the eluate was tested immediately or stored at -20°C until use. ELISA Architect HBsAg Qualitative II ® (Abbott Diagnostics Japan) was the gold standard in our study. This

is an automated, closed system, which detects the genotypes A to F and H, with an average analytical sensitivity of between 0.019 and 0.020 U/ml for plasma. It was designed to improve the detection of mutants and sero conversion samples. All samples were tested by this method and the positive cases were retested two times according to the procedure specified by the confirmation test manufacturer.

Was achieved by this technique with the kit HBsAg confirmatory qualitative II®. Determine AgHBs® (Abbott Diagnostics Japan) is an immuno-chromatographic test used for all samples. 100 DBS formed by both positive and negative Architect selected at a pitch of 26, were retested for this control method. Microscreen HBsAg® ELISA (Span Diagnostics Ltd.) is a manual direct ELISA that also detects all subtypes of HBsAg with analytical sensitivity of the kit 0,2PEU / ml in serum / plasma. It was used for the selected positive and 300 negative Architect at a pitch of 3.

Statistical analysis of data

Data were analyzed using EPI INFO 7. The performance tests were determined by calculating the sensitivity, specificity, positive predictive value and negative predictive value. The kappa test was used to measure the degree of agreement between the tests with the interpretation grid Landis and Koch.²⁴

Results

Population characteristics

The study population consists of 930 patients followed in decentralized sites supported PMTCT. The overall analysis of this population allowed us to highlight different features below. DBS samples came from more regions of Dakar and Ziguinchor, respectively with 34.4% (320/930) and 14.2% (132/930). Patients are male majority with 520(56.0%) and 409 female (44%) with a sex ratio M/F = 1.27 in favor of males. The average age of our population was 26.5 weeks and the median age of 20 weeks. It varies from 2 weeks to over 48 weeks with a standard deviation of 36 weeks. The most represented age groups that are less than 6 weeks and that between 6 and 12 weeks with 23.9% and 17.9% respectively (Table 1).

Table 1 Characteristic of the study population

Characteristic	Effective	Percentage
Areas		
Dakar	320	34,4
Diourbel	45	4,8
Fatick	36	3,9
Kaffrine	2	0,2
Kaolack	57	6,1
Kédougou	15	1,6
Kolda	50	5,4
Louga	40	4,3
Matam	14	1,5
Saint-Louis	58	6,2
Sédhiou	63	6,8
Tambacounda	44	4,7
Thiès	46	4,9
Ziguinchor	132	14,2
Non précisée	8	0,9
Sex (ratio M/F=1,27)		
Masculine	520	56,0
Féminine	409	43,9
Non precise	1	0,1

Table Continued...

Characteristic	Effective	Percentage
Age brackets (Age median 20 weeks)		
< 6 weeks	218	23,4
6 - 12 weeks	163	17,5
12 - 18 weeks	74	7,9
18 - 24 weeks	109	11,7
24 - 30 weeks	64	6,9
Table 2:T 30 - 36 weeks	101	10,8
36 - 42 weeks	35	3,8
42 - 48 weeks	53	5,7
> 48 weeks	95	10,2
Non précisée	18	2,0
HIV status		
Negative	905	97,3
Positive	24	2,6
Doubtful	1	0,1
Total	930	100,0

Molecular diagnosis of HIV was positive in 24 cases or 2.6% of the population (Table 1). The techniques used were Cobas AmpliCor (563 tests or 60.5%), Cobas TaqMan (181 tests or 19.4%), NucliSens® (163 tests or 17.5%), PCR “house” HIV-2 (16 tests or 1.7%) or Biocentric® (8 tests or 0.9%). The overall analysis of the mothers of the status shows a majority prophylactic use of AZT + 3TC + NVP combination Search HBsAg and performance tests. All DBS of our population were tested ELISA Qualitative Architect II HBsAg® Determine HBsAg and the search for HBsAg. Staffs of 28 DBS was responsive but none were reactive Determine HBsAg®. DBS 310 having positive ELISA Qualitative Architect HBsAg® II, 12 were reactive in ELISA Microscreen HBsAg® (Table 2). HBsAg was found more in the most represented age group (less than 6 weeks) with 11 positive 5.05% (11/218). Slices of 18-24 weeks and 12-18 weeks followed with 5/109 respectively (i.e. 4.59%) and 3/74 (i.e. 4.05%). HBsAg was found slightly among female children than male children respectively girls 15/409, 13/521 3.67% and 2.5% boys. In the study population, 24 cases of HIV and 28 cases of HBsAg were found, but no cases of co-infection (Table 3) Using Determine HBsAg®, we found no reactivity to HBsAg, resulting in zero sensitivity.

Table 2 Distribution of population according to HBsAg tests Research

Research HBsAg test	Effective	Percentage
ELISA Architect HBsAg		
Reactive	28	3,0
Non reactive	902	97,0
Total1	930	100,0
ELISA Microscreen HBsAg®		
Reactive	12	3,9
Non reactive	298	96,1
Total2	310	100,0
Determine Ag HBs		
Reactive	00	00,0
Non reactive	930	100,0
Total3	930	100,0

The sensitivity of the ELISA Microscreen AgHBs® vis-à-vis of ELISA Qualitative Architect HBsAg® II is 43 48% [23.22 -63.74], the specificity of 99.30% [98.34 to 100, 27]; the positive predictive value of 83.33% (PPV), and negative predictive value (NPV) of 95.64%. The value of kappa 0.548 watches an average degree of agreement between the two tests (Table 4). Inversely, the sensitivity of the Architect II Qualitative HBsAg® vis-à-vis of Microscreen HBsAg

ELISA® is 83.33 % [62.25 to 104.42], specificity was 95.64% [93.32 to 97.96] the positive predictive value was 43.48 (PPV), and negative predictive value (NPV) is 99.30%. The kappa value of 0.548 shows an average level of agreement between the two tests (Table 5).

Table 3 Distribution ded the results of HBsAg testing based on Characteristic of the population

Characteristic	AgHBs	Total
Age brackets (n=912) p=0,415		
	Reactive Age brackets	Non reactive
< 6weeks	11	207
6 - 12 weeks	2	161
12 - 18 weeks	3	71
18 - 24 weeks	5	104
24 - 30 weeks	1	63
30 - 36 weeks	2	99
36 - 42 weeks	0	35
42 - 48 weeks	2	51
> 48 weeks	2	93
Sex (n=929) p=0,302		
Masculine	13	507
Feminine	15	394
Statut VIH (n=930) p=0,671		
Negative	28	877
Positive	0	24
Doubtful	0	1

Discussion

We performed the evaluation of two diagnostic tests: ELISA kits Architect HBsAg Qualitative II ® and Microscreen HBsAg®, in order to implement them as new tools for the diagnosis of HBV. This evaluation was based on the comparison of the sensitivity and the specificity of these two tests by those of the HBsAg Determine® which is the gold standard for this disease. The sample used for this purpose was the DBS. The detection of HBsAg for the diagnosis of HBV-related disease is based on the use of serological tests from serum or plasma.^{4,25} DBS, which is widely used for molecular diagnosis and neo-natal diagnosis of HIV infection,¹¹ have been evaluated for the detection of viral and serologic markers of HBV.^{15,21} In our study, we aim to transpose the use of DBS for the detection of HBV by rapid diagnostic tests, which in turn may be potentiated as in the case of HIV, to further facilitate the decentralization of the biology of HBV.

In 1981, Villa et al.²⁴ in order to identify a new epidemiological tool on markers of HBV, have made a comparison study using DBS / serum storage conditions and DBS different elution. They showed allow sensitivity of antibodies with radio immunoassay method and a better detection of HBsAg DBS.²⁵ More recently, the DBS has been used on enzyme immunoassay techniques to study the prevalence of HBV infection in endemic areas and risk groups such as drug users in Spain.^{26,27} The same method was use in a study of Komar et al.²⁸ in which they determined the prevalence of HBV markers in a cohort of students in Bangui, Central African Republic.²⁸ Mahfoud et al.²⁹ in the same year reported the prevalence of HBsAg among Lebanon prisoners as well.²⁹ Architect HBsAg Qualitative II® as gold standard. Architect HBsAg is an automated, closed system, which detect genotypes A to F and H, with an average analytical sensitivity of between 0.019 and 0.020 U / ml for plasma.

It was designed to improve the detection of mutants and sero-conversion samples. Microscreen HBsAg ® ELISA is a direct manual technique also allowing the detection of all AgHBs subtypes with

an analytical sensitivity of the kit 0,2PEU / ml in serum / plasma. The comparative study serum/DBS by Ross et al.³⁰ conducted in Germany in 2013 showed a good sensitivity Architect for the use of DBS. Among 1762 on paired samples of serum/DBS they got a specificity of 100% and an analytical sensitivity of 98.6% DBS vis-à-

vis of the serum. These data which comforts our position in the use of Architect as the gold standard in our series of evaluation. In fact, their results indicate that it is possible to reliably detect the HBs antigen by analytical systems as the Abbott Architect with DBS samples.

Table 4 Microscreen ELISA HBsAg® performance vis-à-vis de ELISA Architect Qualitative II HBsAg®

Distribution of samples tested by Microscreen ELISA HBsAg® et ELISA Architect Qualitative II HBsAg® (P=0,000 et Kappa=0,548)

		Architect HBsAg		Total
		Reactive	Non reactive	
Microscreen	Reactive	10	2	12
	Non reactive	13	285	298
		23	287	310

Microscreen ELISA HBsAg® performance vis-à-vis de ELISA Architect Qualitative II HBsAg®

Sensibility	43,48% [23,22 – 63,74]
Specificity	99,30% [98,34 – 100,27]
VPP	83,33
VPN	95,64

Table 5 Architect Qualitative II HBsAg® performance vis-à-vis de Microscreen ELISA HBsAg®

Distribution of samples tested by ELISA Architect Qualitative II HBsAg® et Microscreen ELISA HBsAg® (P=0,000 et Kappa=0,548)

		Microscreen		Total
		Reactive	Non reactive	
Architect HBsAg	Reactive	10	13	23
	Non réactive	2	285	287
Total		12	298	310

Performance de ELISA Architect Qualitative II HBsAg® vis-à-vis de Microscreen ELISA HBsAg®

Sensibility	83,33% [62,25 – 104,42]
Spécificity	95,64% [93,32 – 97,96]
VPP	43,48
VPN	99,30

Performance testing

The fact that no positive HBsAg was detected by Determine® gives zero sensitivity vis-à-vis of the technique Architect HBsAg Qualitative II®. Among 23 positive samples detected in triplicate by Architect HBsAg, only 10 were processed by Microscreen AgHBs® ELISA (Table 4) Compared to Architect Qualitative ELISA II HBsAg®, to low sensitivity of 43.48% [23.22 -63.74], a specificity of 99.30% [98.34 to 100.27], to positive predictive value of 83.33% (PPV) and negative predictive value (NPV) of 95.64% were found with this test.

The kappa value (0, 548) shows a moderate degree of agreement between the two tests. These results seem to corroborate those found by Maity et al.³¹ stating that this test has a low performance in the serum; According to their study which compares diagnostic kits available on trade to assess their effectiveness in detecting HIV infection, HBV, HCV serum HBsAg Microscreen (Span Diagnostics Ltd.) Microscreen AgHBs® had not revealed the expected results³¹ this is an argument against the use and explains its low vis-à-vis sensitivity Architect®. Furthermore, Forbi et al.³² showed that another ELISA micro plate (Shan test TM HBsAg ELISA) showed low performance DBS vis-à-vis the serum with a sensitivity of 78.6% and a specificity of 88.6% nevertheless higher than ours; the poor performance of the ELISA micro plate would thus resulting in a reduced ability of these techniques to detect HBsAg DBS.³² Given the results of the tests for determining performance in our study, we find that Determine® HBsAg has zero sensitivity vis-à-vis the Architect HBsAg Qualitative and Microscreen® HBsAg ELISA on DBS. Which is not the case in

the work performed on serum Rajaonatahina et al.³³ in Madagascar who evaluated Biotin and Determine with as gold standard Genscreen HBsAg manual ELISA test (BioRad France).

The evaluation tests with 76 positive and 74 will be negative for HBsAg Determine® and 40 positive sera and 49 negative sera for SD Biotin® HBsAg showed characteristics Determine® HBsAg (sensitivity = 96.1%, specificity = 93.2%, PPV = 93.6%, NPV = 95.8%) higher than SD Biotin® HBsAg but close. The results obtained show that the optimal performance of the rapid tests on serum is reached for the detection of HBsAg in their study.³³ Their results raise several research questions: HBsAg is it therefore not detectable by the DBS Determine® HBsAg? Or common intrinsic characteristics of our study population - such as HIV-positive mothers, all children under 15 years prophylactic treatment, storage conditions DBS - have they had an impact on the result of this test?

Conclusion

Our study focused on the evaluation of three new diagnostic tests, Determine® HBsAg, ELISA and ELISA Microscreen HBsAg® Architect Qualitative II HBsAg® for detection of HBsAg on Dried Blood Spots (DBS), the Laboratory of Bacteriology -Virology the Aristide Le Dantec hospital in Dakar. The sample was collected from the DBS decentralized PMTCT sites in Senegal in children born to HIV-positive mothers. The performance tests were determined by calculating the sensitivity, specificity, positive predictive value and negative predictive value, and the degree of agreement between the tests by measuring kappa. The study population was comprised of 930

children, predominantly male with an average age of 26.5 weeks and a median age of 20 weeks. We used ELISA Qualitative Architect II HBsAg® as gold standard and ranked ELISA Microscreen HBsAg® and Determine® AgHBs. Determine® HBsAg was reactive with any sample DBS, which gives it a zero sensitivity. By contrast, among 23 DBS reagents ELISA Qualitative Architect HBsAg® II, only 10 were detected by ELISA Microscreen HBsAg® with a low sensitivity (43, 48% [23.22 -63.74]) Microscreen ELISA AgHBs® vis-à-vis Architect Qualitative ELISA II HBsAg® significant and moderate degree of agreement between the two tests ($\kappa=0,548$). The Determine® HBsAg cannot be used with DBS to date to facilitate decentralization of the biology of HBV like HIV. But would be on Architect HBsAg Qualitative II in triplicate and Microscreen AgHBs® gave us the results suggesting its usefulness in the diagnosis of HBV. The Following recommendations are based on our study: - HBV screening by Determine HBsAg from DBS samples would not be appropriate for now but would be on Architect HBsAg Qualitative II in triplicate. - Have samples of the mother-child couples to better appreciate the rate of transmission of HBV infection.

Outlook

In order to achieve more substantial results, further work is required and must include samples of the mother-child couples to better appreciate the rate of transmission of HBV infection, the reevaluation of DBS storage conditions for search for HBsAg and HBsAg on Determine® conducting research with paired samples DBS / Serum.

Acknowledgments

None.

Conflicts of interest

None.

References

1. Antona D. Aspects épidémiologiques et stratégies vaccinales. 24 journée nationale de formation continue en hépato-gastroentérologie 2006. *Bulletin Epidémiologique Hebdomadaire*. 2007;51:425,428.
2. Degos F. Hepatitis B vaccine after the 2003 consensus conference. *Gastroenterol Clin Biol*. 2005;29(4):388,392.
3. Marcellin P, Lada O Managing resistance to analogue antiviral drugs in the treatment of chronic hepatitis B. *Gastroenterol Clin Biol*. 2006;30(10 Pt 2):3S5,3S8.
4. Mast E WC, Cindy MW, Anthony EF et al. A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep*. 2008;54(RR16):1,31.
5. Mackie CO, Buxton JA, Tadwalkar S et al. Hepatitis B immunization strategies: timing is everything. *CMAJ*. 2009;180(2):196,202.
6. Bado G, Penot P, N'Diaye M D et al. Hepatitis B seroprevalence in HIV-infected patients consulting in a public day care unit in Bobo Dioulasso, Burkina Faso. *Med Mal Infect*. 2013;43(5):202,207.
7. Mayaki Z, Dardenne N, Kabo R et al. Seroprevalence of infectious markers among blood donors in Niamey (Niger). *Rev Epidemiol Sante Publique*. 2013;61(3):233,240.
8. Diarra A, Kouriba B, Baby M, Murphy E, Lefrere JJ et al. HIV, HCV, HBV and syphilis rate of positive donations among blood donations in Mali: lower rates among volunteer blood donors. *Transfus Clin Biol*. 2009;16(5,6):444,447.
9. Hannachi N, Bahri O, Mhalla S et al. Hepatitis B virus infection in Tunisian pregnant women: risk factors and viral DNA levels in HBe antigen negative women. *Pathol Biol*. 2009;57(3):e43,e47.
10. Sbai A, Baha W, Ougabrai H et al. Hepatitis B prevalence and risk factors in Morocco. *Pathologie,biologie*. 2012;60(5):e65,69.
11. Marcellin P, Asselah T, Boyer N Treatment of chronic hepatitis B. *Rev Prat*. 2005;55(6):624,632.
12. Montesano R Preventing primary liver cancer: the HBV vaccination project in the Gambia (West Africa). *Environ Health 10 Suppl*. 2011;1:S6.
13. vaccines OHB Hépatites B vaccines *Weekly epidemiological record* 84. 2012
14. AL Eine icterus epidemic. (In German). *BerlKlin Wochenschr* 1885;22:20,23.
15. Hui CK, Lau GK Peginterferon, alpha2a (40 kDa) (Pegasys) for hepatitis B. *Expert Rev Anti Infect Ther* 2005;3(4):495,504.
16. Beasley RP, Trepo C, Stevens CE, Szmuness W The e antigen and vertical transmission of hepatitis B surface antigen. *Am J Epidemiol*. 1977;105(2):94,98.
17. Lorient MA, Marcellin P, Walker F et al. Persistence of hepatitis B virus DNA in serum and liver from patients with chronic hepatitis B after loss of HBsAg. *J Hepatol*. 1997;27(2):251,258.
18. Lee CE, Sri Ponnampalavanar S, Syed Omar SF et al. Evaluation of the dried blood spot (DBS) collection method as a tool for detection of HIV Ag/Ab, HBsAg, anti-HBs and anti-HCV in a Malaysian tertiary referral hospital. *Ann Acad Med Singapore*. 2011;40(10):448,453.
19. Marcellin P Advances in therapy for chronic hepatitis B. *Semin Liver Dis*. 2002;22(S1):33,36.
20. Goumba CM, Yandoko, Nakoune ER, Komas NP A fatal case of acute hepatitis E among pregnant women, Central African Republic. *BMC Res Notes*. 2010;3:103.
21. Kiire CF The epidemiology and prophylaxis of hepatitis B in sub-Saharan Africa: a view from tropical and subtropical Africa. *Gut*. 1996;38(S2):S5,S12.
22. Gani A Rino ADH, Reny Mulatsih, Lesmana LA, Ali Sulaiman, Nurul Akbar Genotyping of hepatitis B virus from dried and stored serum on filter paper. 14. 2005
23. Gonzalez MP, Klautau GB, Prestes DP et al. Vitamin D and cholesterol serum levels are inversely associated in HCV, HIV and HIV/HCV coinfecting patients. *Journal of hepatology*. 2012 ;56:S61,S69.
24. Villa E, Cartolari R, Bellentani S et al. Hepatitis B virus markers on dried Blood spots. A new tool for epidemiological Research. *J Clin Pathol* 1981;34(7):809,812.
25. Leelawiwat W, Young NL, Chaowanachan T et al. Dried blood spots for the diagnosis and quantitation of HIV,1: stability studies and evaluation of sensitivity and specificity for the diagnosis of infant HIV,1 infection in Thailand. *J Virol Methods* 2009;155(2):109,117.
26. Vallejo F, Toro C, de la Fuente L et al. (Hepatitis B vaccination: an unmet challenge in the era of harm reduction programs. *J Subst Abuse Treat*. 2009;34(4):398,406.
27. Brugal MT, Pulido J, Toro C et al. Injecting, sexual risk behaviors and HIV infection in young cocaine and heroin users in Spain. *Eur Addict Res*. 2009;15(3):171,178.
28. Komas NP, Bai, Sepou S, Manirakiza A et al. The prevalence of hepatitis B virus markers in a cohort of students in Bangui, Central African Republic. *BMC Infect Dis*. 2010;10:226.
29. Mahfoud Z, Kassak K, Kreidieh K et al. Prevalence of antibodies to human immunodeficiency virus (HIV), hepatitis B and hepatitis C and risk factors in prisoners in Lebanon. *J Infect Dev Ctries*. 2012;4(3):144,149.

30. Ross RS, Stambouli O, Grüner N et al. Detection of infections with hepatitis B virus, hepatitis C virus, and human immunodeficiency virus by analyses of dried blood spots, performance characteristics of the ARCHITECT system and two commercial assays for nucleic acid amplification. *Virology*. 2013;10:72.
31. Maity S, Nandi S, Biswas S et al. Performance and diagnostic usefulness of commercially available enzyme linked immunosorbent assay and rapid kits for detection of HIV, HBV and HCV in India. *Virology* 2012;9:290.
32. Forbi JC, Obagu JO, Gyar SD et al. Application of dried blood spot in the sero, diagnosis of hepatitis B infection (HBV) in an HBV hyper, endemic nation. *Annals of African medicine* 2010;9(1):44,45.
33. Rajaonatahina DH, Randriamanantany ZA, Rakotomalala R et al. Evaluation of two rapid diagnostic testing for detecting hepatitis B surface AgHBs at the unit of Immunology at the CHUA, JRA Ampefiloha, Antananarivo, Madagascar. *Rev Méd Madag*. 2011;1(1):20,23.