PharmacologyOnLine

Archives • 2021 • vol.3 • 509-517

Review article

Nucleic acid test for detection of hepatitis B infection among blood donors –Is it a cost-effective screening tool?

Pai Divya[1], Chaitra d[2*]

NITTE (Deemed to be University), Deralakatte, Mangalore, Karnataka, India[*]

[2*]chaitrad@nitte.edu.in

Abstract:

Objective of the study is to compare two techniques, routinely used ELISA (HBsAg) versus nucleic acid test (NAT), in terms of their sensitivity and specificity as screening tests in detecting hepatitis B infection among blood donors as well as to compare their cost effectiveness. We also aim to find out the prevalence of hepatitis B infection among blood donors. One thousand voluntary blood donors will be screened both by ELISA and NAT, sensitivity and specificity of both as screening tests will be compared. Subjects with ELISA results negative, but NAT positive will be followed up weekly with ELISA test, till it becomes positive. The number of extra positive cases detected by NAT and weeks by which the window period may probably shortened in them will be calculated. Quality adjusted life year (QALY) gained will be calculated from the scores of qualities of life (QOL) questionnaire and shortened window period (lead time) for NAT. CEA will be done by calculating ICER and ACER for both the tests. The prevalence of hepatitis B infection will also be calculated among blood donors.

The primary benefit of NAT is the ability to reduce residual risk of infectious window period donations. The major constraint for implementing NAT as a routine screening technique in India appears to be its high cost per test. There is a need to evaluate the technique and its cost-effectiveness as compared to routine screening test, ELISA in Indian setting as there is a scarcity of literature in this area.

Key words: *Hepatitis B, ELISA, Nucleic acid test*

Introduction :

Hepatitis B virus (HBV) infection is one of the significant global health problems. World Health Organization (WHO) estimates suggest that more than 2 billion people worldwide have been infected with HBV. Of these, approximately 240 million individuals have chronic liver infections and at risk of serious illness and death, mainly from liver cirrhosis and hepatocellular carcinom. More than 780 000 people die every year due to the acute or chronic consequences of hepatitis B[1-4].

Different areas of the world are classified as having high (8%), intermediate (2–7%) or low (<2%) HBV endemicity, based on the prevalence of Hepatitis B surface Antigen (HBsAg) . South-East Asia, China, most of Africa, most of Pacific Islands, the Amazon basin and parts of the Middle East have high endemicity. South Asia, Eastern and Southern Europe, Russia and Central and South America have intermediate endemicity. United States, Western Europe and Australia have low endemicity [5].

Prevalence of Hepatitis B in India

As India has one-fifth of the world's population, it accounts for a large proportion of the worldwide HBV burden. India harbors 10–15% of the entire pool of HBV carriers of the world[6]. It has been estimated that India has around 40 million HBV carriers. About 15–25% of HBsAg carriers are likely to suffer from cirrhosis and liver cancer and may die prematurely. Infections occurring during infancy and childhood have the greatest risk of becoming chronic. Of the 2.6 Crore (26 million) infants born every year in India, approximately 10 Lakhs (1 million) run the life-time risk of developing chronic HBV infection. The overall rate of HBsAg positivity has been reported to range between 2% and 8% in most studies [7-10]. Many of these studies were based on data from blood bank donors, including professional blood donors who are known to have a higher prevalence of HBV infection. Results of a systematic review by Lodha et al concluded that the true prevalence of hepatitis B in India was 1–2%[11]. Many of the blood banks show HBsAg prevalence was 0.2–4%, most of which have prevalence much lower than that of the commonly quoted prevalence data [12-16].

Transfusion of Blood & Blood Products and Need for a sensitive screening technique

Transfusion-transmitted infection is a major challenge to the transfusion services. The prevalence of HBV infection reported by various authors from India ranges from 2 to 69.2%[17-20]. An earlier report of 1995 had shown that 69.2% of thalassemic patients had HBV infection[17]. However, subsequent reports have however shown a lower prevalence of HBV infection in thalassemics. Vidja et al have shown that only 2% of 200 multi-transfused patients of beta thalassemia major had HBV infection [20]. The decrease in seropositivity may be because of implementation of measures such as donor education, strict standards for donor selection criteria, improved serological screening protocols and improved blood collection and transfusion techniques.

A survey of blood transfusion practices in India showed that screening for transfusion-transmitted infections is unsatisfactory, often poorly regulated, and enforcement of existing guidelines is poor [21]. A strict audit of blood banking practices is required to prevent transmission of the disease. Use of nucleic acid testing (NAT) has been proposed for preventing transmission of HBV as well as other blood borne pathogens in Indian blood donors [22,23]. While such a strategy would make the blood transfusions safer, this would add to the cost of blood screening and is therefore not routinely recommended.

Nucleic acid testing (NAT) as a screening technique during blood transfusion

Nucleic acid testing is a molecular technique for screening blood donations. This technique reduces the risk of transfusion transmitted infections (TTIs) in the recipients. Thus, it provides an additional layer of blood safety. It was introduced in the developed countries in the late 1990s and early 2000s and presently around 33 countries in the world have implemented NAT for human immunodeficiency virus (HIV) and around 27 countries for hepatitis B virus (HBV) [24]. NAT technique is highly sensitive and specific for viral nucleic acids. It is based on amplification of targeted regions of viral ribonucleic acid or deoxyribonucleic acid (DNA) and detects them earlier than the other screening methods thus, narrowing the window period of HIV, HBV and hepatitis C virus (HCV) infections. NAT also adds the benefit of resolving false reactive donations on serological methods which is very important for donor notification and counseling. In a Malaysian study [25] 1388 donor samples were tested by serology as well as NAT, authors found 1.37% samples reactive on standard serology methods but non-reactive by NAT. These samples were confirmed to be "false reactive" on confirmatory serological tests.

In India, mandatory blood screening for HBV, HIV and HCV is done by serological tests for HBsAg and antibodies to HIV 1/2 and HCV. The screened seronegative donations are still at risk for TTIs because of false negative results. Thus, there is a need for a sensitive screening test to reduce this residual risk. It has been reported that risk of TTIs have been reduced significantly over the last two to three decades in western countries where NAT has been implemented. NAT testing has been started in few centers in India, but it is not a mandatory screening test for TTIs as per Drug and Cosmetics Act, 1940 and the rules therein [26]. Major barriers in implementing routine NAT testing in India is its high cost and lack of technical expertise in most of the blood centers.

In India blood centers are gradually introducing NAT to provide safe blood to their patients. First multicenteric study was done by Makroo *et al.*[27] where a total of 12,224 samples along with their serological results were obtained from eight blood banks in India and were tested individually manually by procleix ultrio assay for HIV 1, HCV and HBV. They observed eight NAT yield cases. According to a study from the western part of India combined NAT yield (NAT reactive/seronegative) for HIV, HCV and HBV was 0.034% (1 in 2972 donations)[28] which is high when compared to studies from developed countries. In another study conducted in north India, 18,354 donors were tested by both ID-NAT and fourth generation enzyme-linked immunosorbent assay (ELISA), 7 were found to be NAT-positive but ELISA-negative (NAT yield) for HBV and HCV. The prevalence of NAT yield cases among routine donors was 1 in 2622 donations tested (0.038%)[29]. This high yield of NAT is due to the high prevalence of TTIs in India, further highlighting the need for NAT in India. In another study from a tertiary care center from north India ID NAT results were compared to serological method for 73,898 samples, 1.49% were reactive by NAT, HIV-1 (0.09%), HCV (0.25%), 1.05% were reactive for HBV only and around 0.08% were HBV-HCV co-infections with a combined yield of 1 in 610 donations (total 121 NAT yields)[30].

NAT is a highly sensitive and advanced technique which has reduced the window period of HBV to 10.34 days[31] but it is highly technically demanding, involving issues of high costs, dedicated infrastructure facility, equipments, consumables and technical expertise. The need for NAT depends on the prevalence and incidence rate of infections in

blood donor population, available resources and the evidence of benefit added with serology tests.

Cost effectiveness analysis

Cost-effectiveness analysis is an important tool to assist clinicians, scientists and policymakers in determining the efficiency of healthcare interventions, guiding societal decision-making on the financing of healthcare services and establishing research priorities. Diverse approaches to synthesize evidence have been considered in biomedical research [32-35], including economic evaluations of healthcare interventions [36-43]. At the same time, decision-making in health care requires an understanding of the state of economic evaluation at a national level, where the completeness of the reporting is generally less well understood but where specific priorities are often set. Cost effectiveness analysis (CEA) compares two diagnostic tests, where the costs are identified in monetary terms and the outcomes in non-monetary terms.

Measurement of cost effectiveness could be made in two different ways:

1. ACER – Average Cost Effectiveness Ratio
2. ICER – Incremental Cost Effectiveness Ratio

It helps a decision maker to compare one treatment/diagnostic test to other thereby quantifying the opportunity cost of decisions.

Quality adjusted life years (QALY) is used in economic evaluation to assess the value for money which of two medical interventions/diagnostic tests. It is a measure of disease burden which include both quality and quantity of life lived. One QALY equates to one year of perfect health and QALY of a dead person will be zero. QALY can be used to evaluate different intervention, programs and to set priorities for future programs (44).

Methods:

Laboratory Investigations: The blood samples for analysis will be drawn from the recruited patients at the time of screening for blood donation as follows

1. 5 ml plain vial for HBsAg - ELISA
2. 5 ml EDTA sample - NAT

Detection test for HBV surface antigen (HBsAg) by using enzyme-linked immunosorbent assay (ELISA) as well as NAT for DNA of HBV virus will be carried out over a period of two years. At the time of blood donation, about 5 ml each of blood will be collected directly into EDTA vacutainer and plain tubes under strict aseptic conditions. Plasma will be separated and used for testing with RT-PCR assay, the positivity of the sample will be confirmed by amplifying the product. Subjects with NAT positive results will be counseled and referred to the physician for further necessary investigations/treatment. Subjects who will not be on treatment will be followed up weekly till their ELISA test results are positive for HBsAg. Thereby the number of weeks by which window period is shortened will be calculated and used for cost effective analysis. Direct non-medical costs like transport expenses will be refunded from the project fund.

Quantification of hepatitis B virus with Real-time PCR

The plasma HBV DNA will be extracted with a SACACE Ribo-Sorb kit, silica-based technology as mentioned by Boom *et al.*[45] and real-time PCR will be then performed on a Biorad MJ Mini

instrument using a SACACE HBV monitor kit. The PCR will be run in a total volume of 25 µl containing 12.5 µl of template, 11 µl of Premix Ex-Taq (Takara), and 1.5 µl of primers and probe. The amplification performed will be as follows: initial hot start denaturation at 95°C for 900 s, followed by 42 cycles of denaturation at 95 °C for 20 s, annealing and extension at 60 °C for 40 s. The fluorescence will be measured at the annealing stage of each cycle. The quantification of sample was carried out using the internal control co-efficient value and the fluorescent values of both sample and internal control.

Quantification of genomic DNA: The quantification and purity of DNA will be checked by the spectrophotometer (ratio of OD260 / OD280). DNA concentration was calculated using the following formula:

Concentration (µg/ml) of DNA in original solution= Absorbance x 100 x 50 µg/ml.

Cost effective analysis of two screening techniques

Incremental cost effectiveness ratio (ICER) and average cost ratio(ACER) will be calculated as follows:
Incremental cost effectiveness ratio : It compares the incremental cost divided by the incremental effect. This can be described in an incremental cost effectiveness ratio (ICER), and can be expressed in an equation:
ICER = C1 – C2/E1 – E2 where C1 and E1 are the cost and effect due to NAT , and C2 and E2 are the cost and effects of ELISA, effect(E1 & E2) being the number of cases detected.
A high ICER indicates more expenditure for better health outcome while compared to a lower ICER .Hence an intervention with a lower ICER would be preferred.

Average Cost-effectiveness Analysis:Cost-effectiveness ratio of each diagnostic test will be calculated and the two ratios are compared . Specifies the cost of an intervention required to achieve each unit of effect. Average cost-effectiveness ratio (ACER) =

Cost of screening test = Cost per unit of effect

No. of positive cases detected achieved

Validated QOL questionnaire will be administered to all positive cases so as to calculate QOL score. The SF-36 is a set of standard validated questions which is used to judge the quality of life in patient/ subjects . This questionnaire contains 36 items spread over different domains such as physical function, physical role, general health, vitality, body pain, social wellbeing, emotional role and mental health of the individual in the past 4 weeks. The score of each question when entered in the excel sheet will be converted into a number depending on the response to the question. Upon entering the response to all the question, the software will give the consolidated response of physical and mental health domain. The final quality of health of an individual will be calculated by dividing the total SF-36 value by 100. The QOL of a participant/patient range from 0 to 1, where 1 refers to perfect health and 0 refers to worst health (death).
Quality adjusted life year (QALY) gained will be calculated as QOL score multiplied by number of weeks of shortened window period among positive NAT results or number of weeks of extended window period among initial ELISA positive
Cost per QALY gained by NAT will be calculated as follows:
Cost per QALY
= cost of NAT + Cost of ELISA for NAT positive patients
QALY gained

Calculation of prevalence: Using the below formula, prevalence will be calculated.

$$\text{Prevalence} = \frac{\text{no. of cases detected by NAT}}{\text{No. of blood donations}}$$

Statistical Analysis

Quantitative data like NAT and ELISA results, yield, cost of the tests, number of NAT positive individuals followed up till ELISA positivity, will be expressed in mean and standard deviation. Qualitative data such as Quality of life using SF-36 questionnaire of individuals detected to have Hepatitis B by both methods will be expressed using percentages and proportions.

Data thus collected will be coded, validated and entered into Microsoft Excel version 2010 and analyzed using SPSS (Statistical package for Social Sciences) version 21. Prevalence of Hepatitis B will be calculated separately using both the tests as a proportion of individuals testing positive with each of the tests from the total number of donors. Sensitivity, specificity, Positive predictive values given the prevalence among donors will be computed. Comparison of sensitivity and specificity of NAT and ELISA test will be carried out using McNemar's test. Receiver operating curves will be generated separately to assess the utility of the NAT and ELISA test and Area under the curve (AUC) will be determined. The cut offs for the sensitivity without significant decrease in specificity will be chosen for each of the test. QALY will be calculated as described in methodology section.

Significance of the study:

The use of Nucleic acid test reduces the risk of HBV transfusion which is seen over the past few decades. In the high endemic countries, anti-HBc-testing done without compromising blood availability and thus HBsAg testing in association with nucleic acid test would be preferred. Therefore, by using several non-exclusive changes in the standard procedure nucleic acid test sensitivity can be enhanced.

Conclusion:

The blood transfusion is the most important and is a part of treatment in the medical field. Along with the blood transfusion there is also risk of transfusion transmitted infections. If there is effective screening, then the transfusion transmitted infections can be minimized. Nucleic acid test is one such technology that helps in screening these harmful pathogens at the time of blood transfusion. Thereby reducing the rate of transfusion transmitted infections.

Refernces:

1. World Health Organization. Hepatitis B. World Health Organization Fact Sheet No 204 (Updated July 2014) [cited 2014 Dec 07]. Available at: http://www.who.int/mediacentre/factsheets/fs204/en/.
2. Lavanchy D. (2004). Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. *Journal of viral hepatitis*, *11*(2), 97–107.
3. Lok AS. (2002). Chronic hepatitis B. N Engl J Med, 346:1682–1683.
4. Goldstein, S.T., Zhou, F., Hadler, S.C., Bell, B.P., Mast, E.E., Margolis, H.S. (2005). A mathematical model to estimate global hepatitis B disease burden and vaccination impact. Int J Epidemiol, 34, 1329–1339.
5. Te, H. S., & Jensen, D. M. (2010). Epidemiology of hepatitis B and C viruses: a global overview. *Clinics in liver disease*, *14*(1), 1–6.
6. Datta S. (2008). An overview of molecular epidemiology of hepatitis B virus (HBV) in India. *Virology journal*, *5*, 156.

7. Abraham P. (2012). Viral hepatitis in India. *Clinics in laboratory medicine*, *32*(2), 159–174.
8. Thyagarajan, S.P., Jayaram, S., Mohanavalli, B. (1996). Prevalence of HBV in general population of India. In: Sarin SK, Singal AK, eds. Hepatitis B in India: Problems and Prevention. New Delhi: CBS Publishersand Distributors, 5–16.
9. Prevention of Hepatitis B in India – An Overview. New Delhi: World Health Organization South-East Asia Regional Office; 2002.
10. Puri P. (2014). Tackling the Hepatitis B Disease Burden in India. *Journal of clinical and experimental hepatology*, *4*(4), 312–319.
11. Lodha, R., Jain, Y., Anand, K., Kabra, S. K., & Pandav, C. S. (2001). Hepatitis B in India: a review of disease epidemiology. *Indian pediatrics*, *38*(4), 349–371.
12. Singh, K., Bhat, S., & Shastry, S. (2009). Trend in seroprevalence of Hepatitis B virus infection among blood donors of coastal Karnataka, India. *Journal of infection in developing countries*, *3*(5), 376–379.
13. Chandra, T., Rizvi, S.N.F. and Agarwal, D. (2014) Decreasing Prevalence of Transfusion Transmitted Infection in Indian Scenario. The Scientific World Journal, 2014, 1-4.
14. Arora, D., Arora, B., & Khetarpal, A. (2010). Seroprevalence of HIV, HBV, HCV and syphilis in blood donors in Southern Haryana. *Indian journal of pathology & microbiology*, *53*(2), 308–309.
15. Das, B.K., Gayen, B.K., Aditya, S., Chakrovorty, S.K., Datta, P.K., Joseph, A. (2011). Seroprevalence of hepatitis B, hepatitis C and human immunodeficiency virus among healthy voluntary first-time blood donor in Kolkata. Ann Trop Med Public Health [Serial Online],4, 86–90.
16. Pahuja, S., Sharma, M., Baitha, B., & Jain, M. (2007). Prevalence and trends of markers of hepatitis C virus, hepatitis B virus and human immunodeficiency virus in Delhi blood donors: a hospital based study. *Japanese journal of infectious diseases*, *60*(6), 389–391.
17. Choudhury, N., Saraswat, S., & Naveed, M. (1998). Serological monitoring of thalassaemia major patients for transfusion associated viral infections. *The Indian journal of medical research*, *107*, 263–268.
18. De, M., Banerjee, D., Chandra, S., & Bhattacharya, D. K. (1990). HBV & HIV seropositivity in multi-transfused haemophilics & thalassaemics in eastern India. *The Indian journal of medical research*, *91*, 63–66.
19. Singh, H., Pradhan, M., Singh, R. L., Phadke, S., Naik, S. R., Aggarwal, R., & Naik, S. (2003). High frequency of hepatitis B virus infection in patients with beta-thalassemia receiving multiple transfusions. *Vox sanguinis*, *84*(4), 292-299.
20. Vidja, P. J., Vachhani, J. H., Sheikh, S. S., & Santwani, P. M. (2011). Blood transfusion transmitted infections in multiple blood transfused patients of Beta thalassaemia. *Indian journal of hematology & blood transfusion : an official journal of Indian Society of Hematology and Blood Transfusion*, *27*(2), 65–69.
21. Kapoor, D., Saxena, R., Sood, B., & Sarin, S. K. (2000). Blood transfusion practices in India: results of a national survey. *Indian journal of gastroenterology : official journal of the Indian Society of Gastroenterology*, *19*(2), 64–67.

22. Makroo, R.N., Choudhury, N., Jagannathan, L.(2008). Multicenter evaluation of individual donor nucleic acid testing (NAT) for simultaneous detection of human immunodeficiency virus -1 & hepatitis B & C viruses in Indian blood donors. Indian J Med Res. 127, 140–147.
23. Chatterjee, K., Coshic, P., Borgohain, M., Premchand, Thapliyal, R. M., Chakroborty, S., & Sunder, S. (2012). Individual donor nucleic acid testing for blood safety against HIV-1 and hepatitis B and C viruses in a tertiary care hospital. *The National medical journal of India*, 25(4), 207–209.
24. Roth, W.K., Busch, M.P., Schuller, A., Ismay, S., Cheng, A., Seed, C.R. (2012).International survey on NAT testing of blood donations: Expanding implementation and yield from 1999 to 2009. Vox Sang.102,82–90
25. Yaseen, S. G., Ahmed, S. A., Johan, M. F., Kiron, R., & Daher, A. M. (2013). Evaluation of serological transfusion-transmitted viral diseases and mutliplex nucleic acid testing in malaysian blood donors. *Transfusion and apheresis science : official journal of the World Apheresis Association : official journal of the European Society for Haemapheresis*, 49(3), 647–651.
26. Part XIIB. Department of Health Schedule F. The Drugs and Cosmetics Act, 1940 and the Drugs and Cosmetics Rules, 1945, as amended up to 30th June; 2005. Government of India. Ministry of Health and Family Welfare; p. 326. Available from: http://www.cdsco.nic.in/DrugsandCosmeticAct.pdf .
27. Makroo, R.N., Choudhury, N., Jagannathan, L., Parihar-Malhotra, M., Raina, V., Chaudhary, R.K. (2008). Multicenter evaluation of individual donor nucleic acid testing (NAT) for simultaneous detection of human immunodeficiency virus -1 & hepatitis B & C viruses in Indian blood donors. Indian J Med Res.127,140–7.
28. Jain, R., Aggarwal, P., & Gupta, G. N. (2012). Need for nucleic Acid testing in countries with high prevalence of transfusion-transmitted infections. *ISRN hematology*, *2012*, 718671.
29. Chatterjee, K., Coshic, P., Borgohain, M., Premchand, Thapliyal, R. M., Chakroborty, S., & Sunder, S. (2012). Individual donor nucleic acid testing for blood safety against HIV-1 and hepatitis B and C viruses in a tertiary care hospital. *The National medical journal of India*, 25(4), 207–209.
30. Agarwal, N., Chatterjee, K., Coshic, P., & Borgohain, M. (2013). Nucleic acid testing for blood banks: an experience from a tertiary care centre in New Delhi, India. *Transfusion and apheresis science : official journal of the World Apheresis Association : official journal of the European Society for Haemapheresis*, 49(3), 482–484.
31. Weusten, J., Vermeulen, M., van Drimmelen, H., & Lelie, N. (2011). Refinement of a viral transmission risk model for blood donations in seroconversion window phase screened by nucleic acid testing in different pool sizes and repeat test algorithms. *Transfusion*, *51*(1), 203–215.
32. Chan, A. W., & Altman, D. G. (2005). Epidemiology and reporting of randomised trials published in PubMed journals. *Lancet (London, England)*, *365*(9465), 1159–1162.
33. Hopewell, S., Dutton, S., Yu, L,M., Chan, A.W., Altman, D.G. (2010). The quality of reports of randomised trials in 2000 and

2006: comparative study of articles indexed in PubMed. BMJ. 340:c723.
34. Moher, D., Tetzlaff, J., Tricco, A.C., Sampson, M., Altman, D.G. (2007). Epidemiology and reporting characteristics of systematic reviews. PLoS Med.4, e78.
35. Hutton, B., Salanti, G., Chaimani, A., Caldwell, D.M., Schmid, C., Thorlund, K. (2014). The quality of reporting methods and results in network meta-analyses: an overview of reviews and suggestions for improvement. PLoS One. 9:e92508.
36. Elixhauser, A., Luce, B. R., Taylor, W. R., & Reblando, J. (1993). Health care CBA/CEA: an update on the growth and composition of the literature. *Medical care*, *31*(7 Suppl), JS1–JS149.
37. Elixhauser, A., Halpern, M., Schmier, J., & Luce, B. R. (1998). Health care CBA and CEA from 1991 to 1996: an updated bibliography. *Medical care*, *36*(5 Suppl), MS1–MS147.
38. Neumann, P. J., Stone, P. W., Chapman, R. H., Sandberg, E. A., & Bell, C. M. (2000). The quality of reporting in published cost-utility analyses, 1976-1997. *Annals of internal medicine*, *132*(12), 964–972.
39. Neumann, P. J., Greenberg, D., Olchanski, N. V., Stone, P. W., & Rosen, A. B. (2005). Growth and quality of the cost-utility literature, 1976-2001. *Value in health : the journal of the International Society for Pharmacoeconomics and Outcomes Research*, *8*(1), 3–9.
40. Rosen, A. B., Greenberg, D., Stone, P. W., Olchanski, N. V., & Neumann, P. J. (2005). Quality of abstracts of papers reporting original cost-effectiveness analyses. *Medical decision making : an international journal of the Society for Medical Decision Making*, *25*(4), 424–428.
41. Neumann, P. J., Fang, C. H., & Cohen, J. T. (2009). 30 years of pharmaceutical cost-utility analyses: growth, diversity and methodological improvement. *PharmacoEconomics*, *27*(10), 861–872.
42. Jefferson, T., Demicheli, V., & Vale, L. (2002). Quality of systematic reviews of economic evaluations in health care. *JAMA*, *287*(21), 2809–2812.
43. Hutter, M. F., Rodríguez-Ibeas, R., & Antonanzas, F. (2014). Methodological reviews of economic evaluations in health care: what do they target?. *The European journal of health economics : HEPAC : health economics in prevention and care*, *15*(8), 829–840.
44. Weinstein, M.C.,Torrance, G., McGuire, A. (2009). QALYs:The basics.Value in health.12, S5-S9.
45. Boom, R., Sol, C. J., Heijtink, R., Wertheim-van Dillen, P. M., & van der Noordaa, J. (1991). Rapid purification of hepatitis B virus DNA from serum. *Journal of clinical microbiology*, *29*(9), 1804–1811.