

Hepatitis B monovalent vaccines produced by different manufacturers: comparative study on the quality of vaccine in period before and after the shelf life

Vacinas de Hepatite B monovalente produzidas por diferentes fabricantes: estudo comparativo da qualidade das vacinas no período pré e após o prazo de validade

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ABSTRACT

For over 20 years, the hepatitis B (HB) vaccine has been produced by the expression of the viral gene encoding the hepatitis B surface antigen (HBsAg) in yeast. According to the data from WHO, the hepatitis B vaccines are generally stable for up to three years when stored at 2 °C to 8 °C. The purpose of this study was to evaluate whether the hepatitis B vaccine, at the time of their release, the quality criteria of this product were maintained seven years after the expiration date. Vaccine vials in multi-dose (10 and 05 doses) and three lots from each manufacturer (A, B and C) were analyzed. All batches were assayed for visual appearance, potency, bacterial endotoxin, thiomersal amount, aluminum hydroxyde contents and pH by means of validated tests. The nine lots evaluated seven years after the expiration date showed similar concentrations when compared to those demonstrated at the time of batches release by the National Institute for Quality Control in Health (INCQS). No significant change in the quality of the hepatitis B vaccine after the expiration date was confirmed. These data might be useful to subsidize a future evaluation for reviewing an extension of the vaccines shelf life.

Keywords. hepatitis B vaccine, quality control, shelf life, potency, stability.

RESUMO

As vacinas contra a hepatite B são produzidas pela expressão do gene viral codificado para o antígeno de superfície do vírus da hepatite B (HBsAg) em levedura, há mais de 20 anos. De acordo com os dados da OMS, a vacina de hepatite B tem até três anos de estabilidade quando armazenada entre 2 °C e 8 °C. O objetivo deste estudo foi de avaliar se, no momento da liberação, os critérios de qualidade da vacina de hepatite B foram mantidos após sete anos da data de validade. Foram analisados frascos de vacinas multi-dose (10 e 5 doses), sendo três lotes de cada produtor (A, B e C). Todos os lotes foram avaliados quanto às características de aparência visual, potência, endotoxina bacteriana, presença de timerosal, conteúdo de hidróxido de alumínio e pH por meio de testes validados. Os nove lotes avaliados sete anos após a data de expiração tiveram resultados similares quando comparados às concentrações na época de liberação dos lotes, realizada pelo Instituto Nacional de Controle de Qualidade em Saúde (INCQS). Os estudos confirmaram a manutenção da qualidade da vacina após o período de expiração. Estes dados podem subsidiar uma futura avaliação para extensão do prazo de validade das vacinas.

Palavras-chave. vacina contra hepatite B, controle de qualidade, prazo de validade, potência, estabilidade.

INTRODUCTION

Hepatitis B viruses (42 nm spherical particles) is classified into the family *Hepadnaviridae*, presenting DNA involved by an icosahedral capsid and finally by a lipoproteic envelope, that consist of three proteins composing the hepatitis B virus surface antigen (HBsAg). These viruses cause an infectious life-threatening liver disease and leading frequently to chronic liver disease, which causes high risk of death from liver cirrhosis and liver cancer¹. Prevention of infection through thorough vaccination is the most effective strategy to reduce global morbidity and mortality². Hepatitis B vaccine is 95 % effective in preventing HBV infection and its chronic consequences, and is the first vaccine against a major human cancer. The vaccine has an outstanding record of safety and effectiveness. Recombinant hepatitis B vaccines have been produced by the expression of the viral gene coding for hepatitis B surface antigen (HBsAg) in yeast for over 20 years^{3,4}.

Recombinant hepatitis B vaccines are available as monovalent products or included in combined vaccines together with other antigens such diphtheria toxoid, tetanus toxoid, whole-cell or acellular pertussis components, *Haemophilus influenza* type B conjugated antigen and inactivated poliomyelitis viruses⁵⁻⁸.

Hepatitis B vaccine is a liquid suspension consisting of purified hepatitis B surface antigen (HBsAg), which is adsorbed on aluminum salt, and additional components such as adjuvants and stabilizers. Antimicrobial preservatives are used in multidose presentations.

The quality of vaccines is guaranteed by the use of robust and reproducible production processes. Quality control of hepatitis B vaccines is based on World Health Organization's (WHO) recommendations^{9,10}, the European Pharmacopoeia monograph¹¹ and guideline for lots release^{12,13}. The quality control on release of final vaccine lots is performed both by the manufacturer and the national control laboratory of the country where a particular vaccine batch will be used.

The hepatitis B vaccine is used as a routine by the Brazilian National Immunization Program

(PNI) from the Ministry of Health for people under 49 years of age and to the priority groups with high exposition risk or high susceptibility, even out of age range¹⁴. As any other immunobiological used by PNI, the hepatitis B vaccine is sent to the National Institute for Quality Control in Health (INCQS) – Oswaldo Cruz Foundation (Fiocruz) and submitted to laboratory and documentary analysis before releasing the batches for human use. All batches are evaluated for visual appearance, in vitro potency and identity, bacterial endotoxin, thiomersal, aluminum hidroxyde contents and pH by validated tests¹⁵⁻¹⁹.

WHO recommendations for production, quality control and evaluation of vaccines feature stability as an important element. Data suggest that hepatitis B vaccines are apparently stable at elevated temperatures: for month at 20 °C to 25 °C, for one week at 37 °C and three days at 45 °C and for up to three years of shelf life when stored at 2 °C to 8 °C²⁰⁻²². The purpose of this study was to evaluate if the hepatitis B vaccine quality criteria at the time of release were maintained seven years after the expiration date.

MATERIAL AND METHODS

Sample vaccine

Hepatitis B monovalent vaccine samples expired after seven years at the recommended storage temperature at 4 °C were used for the study. The manufacturers were named as producer A, B and C (these codes were designated according to the policy). These samples were produced by the expression of hepatitis B surface *antigen* (HBsAg) using *Saccharomyces cerevisiae* (producer B) and *Hansenula polymorpha* (producers A and C) yeasts. The vaccines were in a multi-dose (10 and 05 doses) vial, randomly selected and three lots for each manufacturer were tested.

Product-specific reference vaccine

For comparison of vaccines coming from various types of production, all lots were determined *versus* the corresponding homologous reference vaccine^{23, 24}. The standard reference used by each manufacturer contains 20 µg/mL of HBsAg as evaluated by the Lowry protein assay⁹.

Evaluation of retrospective data at time of batches release

Batches of hepatitis B vaccine from three different manufacturers were evaluated for the visual appearance, determination of the *in vitro* potency, endotoxin, thiomersal, aluminum hydroxide and pH.

Visual appearance of the vaccine

Color and opacity of suspensions were monitored and reported for the vaccine-specific in its final container.

***In vitro* potency and identity determination**

The potency and identity of lots of hepatitis B monovalent vaccines, before and after shelf life, were evaluated by their HBsAg content in triplicates at concentrations 0.25, 0.5, 1.0 and 1.5 ng/mL using a commercial kit, Murex immune assay (Abott Laboratories, Abbott Park, IL, USA), according to the producer instructions. The antigen potency of each vaccine batch was calculated *versus* its homologous reference using a parallel-line model²⁵ and expressed in µg/mL.

Bacterial endotoxin content

The *Limulus* amoebocyte lysate test (test LAL) is accepted by the test for bacterial endotoxins²⁶. The LAL test was performed in-house according to the European Pharmacopoeia monograph using the semi-quantitative gel clot method (Lonza Walkersville, Inc, USA). Since one International Unit (IU) of endotoxin is equal to one endotoxin unit (EU), the values were expressed in EU/mL. The specification for the bacterial endotoxin content in the vaccines is ≤ 10 EU/mL²⁷.

Thiomersal content

The electroanalytical methodology for the determination of thiomersal content, in each final lot vaccines was developed utilizing the differential pulse voltammetry system of Metrohm model 757 VA Computrace coupled to an electrochemical cell composed by three electrodes²⁸. The specification for the thiomersal content in the vaccines is ≤ 200 ppm²⁷.

Aluminum hydroxide content

Each final lot was assayed for the aluminum hydroxide content. The method used and the permitted concentration was determined according to the European Pharmacopoeia monograph¹¹. The amount of aluminum permitted in the recommended single human dose of a product should not be more than 1.25 mg/mL^{27,29,30}.

pH

The pH values of the lots of vaccines were measured using a pHmeter of a glass electrode (InoLab pH Level 2, WTW). The specification for the vaccines pH is from 5.5 to 7.4²⁷.

Statistical analysis

The statistic tests Shapiro-Wilk (normality), paired t-Student (parametric) and Wilcoxon (non parametric) were used to the comparative evaluation for the results obtained during the time of release and seven years following the expiration date³¹.

RESULTS

Visual appearance of the products

Products were translucent and showed uniform appearance, without development of agglomerates even after vigorous shaking.

Comparative analysis for the potency at time of batch release and after the expiration period

The vaccines potency of all evaluated lots at the time of release were (21.28, 20.77 and 18.37 µg/mL) for manufacturer A; (20.82, 26.80 and 20.0 µg/mL) for manufacturer B and (21.29, 19.58 and 20.0 µg/mL) for manufacturer C, respectively. The potency for the same lots of vaccine after the expiration date were (22.07, 24.53 and 21.77 µg/mL) for manufacturer A; (21.52, 28.70 and 19.87 µg/mL) for manufacturer B and C (18.14, 21.10 and 17.73 µg/mL) for manufacturer C, respectively. The comparative analysis obtained from all nine tested lots showed good concentration of antigen, with no statistically significant differences on the tested vaccines expired after seven years of their designated shelf life (Figure 1).

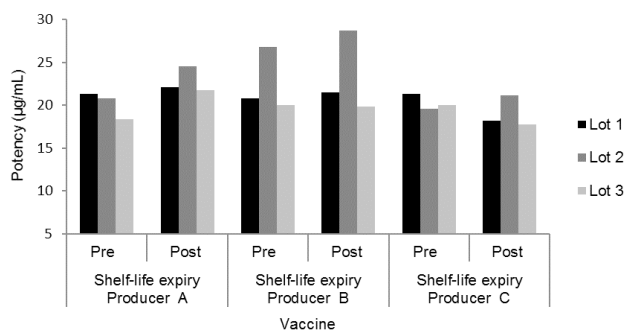


Figure 1. Vaccine potency data for pre and post shelf-life expiration date for hepatitis B vaccine stored at 2 °C - 8 °C. The statistical analysis comparing before and after resulted in *p*-value 0.1053, 0.2971 and 0.4599 for producer A, B and C, respectively

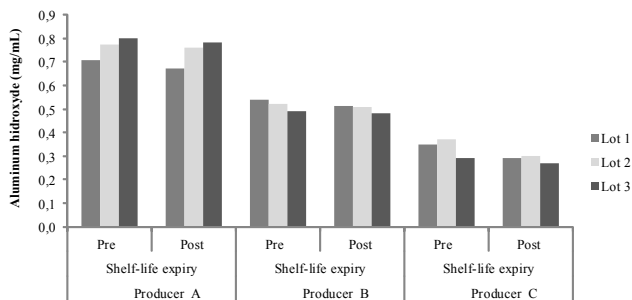


Figure 2. Graphical representation of Aluminum hydroxide values obtained in pre and post shelf-life for A, B and C producers. The statistical analysis comparing before and after resulted in *p*-value 0.0730, 0.0655 and 0.0663 for producer A, B and C, respectively.

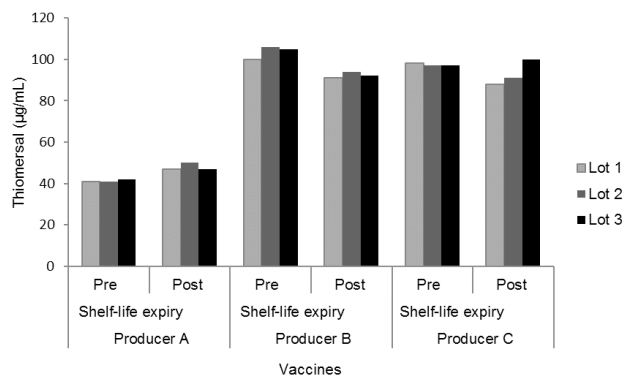


Figure 3. Comparison of thiomersal contents for hepatitis B vaccines at the time of release and seven years after the expiration date. The statistical analysis comparing before and after resulted in *p*-value 0.0898, 0.1797 and 0.3273 for producer A, B and C, respectively

Aluminum hydroxide content

Lots of vaccines containing aluminum hydroxide after expiration date were evaluated among different manufacturers. In general, the content varies among each vaccine manufacturer A, B and C. The comparative analysis after expiration date showed results similar to observed at the time of the batch release (Figure 2).

Thiomersal content

All lots of vaccine after expiration date showed thiomersal contents similar to observed at the time of the batch release (Figure 3).

Bacterial endotoxin content

The endotoxin contents determined seven years after expiration date showed vaccines from manufacturer A contained > 2.5 and < 5.0 EU/mL, those from manufacturer B contained > 0.1 and < 2.5 EU/mL, while those from manufacturer C contained > 1.25 and < 5.0 EU/mL (Table 1).

pH

The pH value of each final vaccine lot tested showed results within the range from values 6.0 to 7.0 after and before expiration period (Table 2).

Statistical analysis

The comparative analysis for the obtained results, using vaccine samples before and after expiration date, for potency determination and aluminium hydroxide content (t-Student) and thiomersal content (Wilcoxon) has presented a *p*-value greater than the 0.05 significance level (*p* > 0.05). It indicates that is not possible to prove statistically that the samples before and after expiration date have different results.

DISCUSSION

A comparative study for the vaccines quality was conducted with samples vaccines from three manufacturers. The potency of the lots of vaccines was evaluated by using the *in vitro* potency with product-specific reference preparation³². All nine lots hepatitis B vaccines evaluated seven years of their

Table 1. Endotoxin content from different manufacturers of hepatitis B vaccines seven years after the expiration date

Lots	Manufacturers of hepatitis B vaccines		
	Producer A	Producer B	Producer C
	Post shelf-life expiry	Post shelf-life expiry	Post shelf-life expiry
Lot 1	> 2.5 and < 5 EU/mL	> 1.25 and < 2.5 EU/mL	≤ 2.5 EU/mL
Lot 2	≤ 2.5 EU/mL	> 1.25 and < 2.5 EU/mL	> 1.25 and < 2.5 EU/mL
Lot 3	> 1.25 and < 2,5 EU/mL	> 0.125 and < 1.25 EU/mL	> 2.5 and < 5 EU/mL

licensed expiration date had similar potency concentrations when compared to potency at time of batches release. All lots were within the specifications even after several years following the expiration date of their designated shelf life. The samples were stored at 2 °C to 8 °C, which is probably responsible for maintaining the stability after seven years of the end of validity. The comparative analysis obtained from all nine tested lots showed good concentration of antigen, with no statistically significant differences on the tested vaccines after seven years following the expiration date³³.

The vaccine stability evaluation is defined as a science-driven procedure based on clearly defined quality criteria: the ability of a vaccine to retain its chemical, physical, microbiological and biological properties within specified limits throughout its shelf life. This study showed that stability remained constant^{34,35}, no significant differences ($p > 0.05$) were observed for the lots tested before and after shelf life.

The vaccines differed only in potency among manufacturers A, B and C probably because of the diversity in the reactivity of vaccines produced by different manufacturing techniques used for formulation³⁶. WHO recommendations for production, quality control and evaluation of vaccines feature stability as an important element. Data from the WHO shows hepatitis B vaccines as generally stable for up to three years of shelf life when stored at 2 °C to 8 °C²².

Comparative analysis of bacterial endotoxin was not made because pyrogen assays were determined at the time of release. The levels of endotoxin were very consistent within product license specifications required ($\leq 10\text{EU/mL}$). The LAL test showed that the vaccine vials tested were pyrogen free, as expected. All evaluated lots were satisfactory in all parameters of quality control. Results from the study suggested there was no impact on vaccines quality after shelf life.

Table 2. Comparison of pH for batches of hepatitis B vaccines at the time of release and seven years after the expiration date

Lots	Manufacturers of hepatitis B vaccines					
	Producer A		Producer B		Producer C	
	Shelf-life expiry		Shelf-life expiry		Shelf-life expiry	
	Pre	Post	Pre	Post	Pre	Post
Lot 1	7.0	7.0	6.0	6.0	7.0	7.0
Lot 2	7.0	7.0	6.0	6.0	7.0	7.0
Lot 3	7.0	7.0	6.0	6.0	7.0	7.0

CONCLUSION

The hepatitis B vaccine presented an excellent stability, all batches were in accordance to the requirements described in the official compendium. The importance of proper storing showed that the quality of the vaccines produced by three different manufactures has remained stable after years following the expiration date. This study may contribute to a future extension of the validity period of the vaccines stored at the recommended temperature (2 °C to 8 °C). Currently the validity period is three years.

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