A SYSTEMATIC REVIEW OF MONOVALENT HEPATITIS B VACCINE THERMOSTABILITY

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ABSTRACT

Introduction: In many resource-poor countries, a substantial percentage of births may occur outside of health care facilities. Lack of access to vaccine in cold storage may reduce birth-dose hepatitis B vaccine (HBV) coverage and thus place infants at risk of perinatal transmission. One mechanism to address this issue would be to allow vaccine to be out of the cold chain at the point of delivery, but few manufacturers have pursued an on-label indication for storage at >8^{o}C (known as the extended controlled temperature chain [ECTC]), including the World Health Organization (WHO) CTC programmatic approach allowing for vaccine to be stored at 40^{o}C for three days.

Methods: Thermostability data was obtained from eight of nine monovalent WHO prequalified HBV manufacturers. A systematic literature review was conducted to identify studies in which HBV was stored outside the cold chain.

Results: Seven manufacturers provided in-vitro potency results following storage at 37^{o}C for four weeks, and all met minimum lot release specifications, with an average decrease in potency of 16%. Four manufacturers assessed in-vitro potency after 1 to 4 weeks storage at 45^{o}C, and five assessed in-vivo potency after storage at 37-45^{o}C and all met minimum specifications as well. The systematic literature review identified four controlled field studies that evaluated an out-of-the-cold-chain approach; no differences were seen in GMTs or seroconversion between children who received vaccine in intervention versus non-intervention communities. Similarly, two experimental studies in humans and three in animals supported HBV thermostability over a four-week period.

Conclusions: Access to HBV birth dose is hampered for deliveries that occur at home, and in some settings home births constitute a large proportion of all births. The current review found that most HBVs are heat stable based on in-vivo and in-vitro testing at temperatures up to 45^{o}C for one week and up to 40^{o}C for several weeks. These data support manufacturers' pursuit of an on-label indication for storage outside the cold chain (ECTC). While this process is concluded, WHO's Strategic Advisory Group of Experts could facilitate the expanded delivery of HBV by recommending that where appropriate countries use an off-label out-of-the-cold-chain approach. In addition, field experience suggests that there may be programmatic advantages to keeping HBV at ambient temperature (37-45°C for 1-4 weeks) before vaccination at service delivery points, especially as a strategy for reaching home births.

SUMMARY SENTENCES

The published literature does not provide comprehensive data on hepatitis B vaccine (HBV) thermostability.

Data from eight manufacturers support HBV thermostability at 37-45^{o}C for 1-4 weeks that could support out of the cold chain delivery of monovalent HBV.

Field trials have found similar human immune responses following vaccine storage in and outside the cold chain.

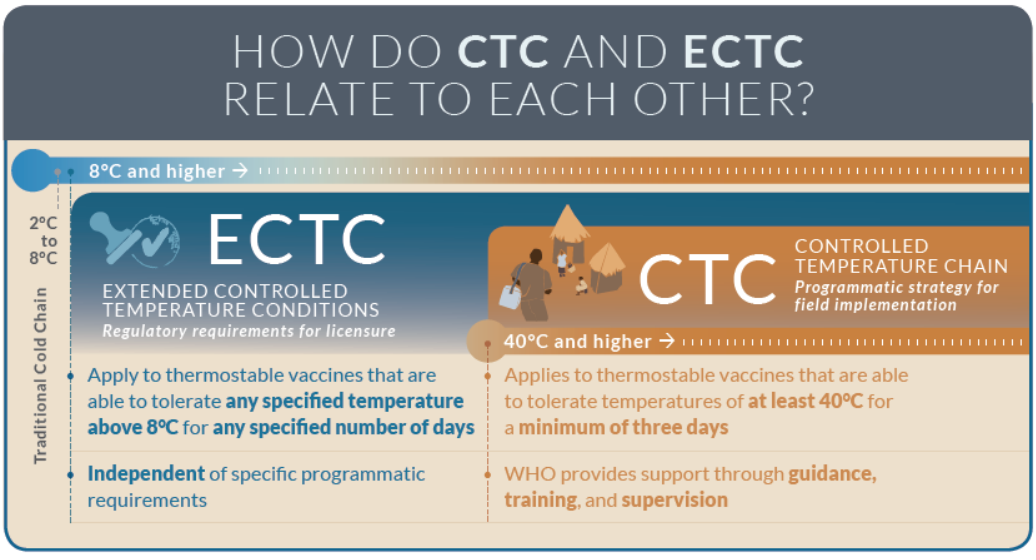
Results support a recommendation to allow vaccine storage at up to 40^{o}C for three days in line with CTC programmatic requirements.

INTRODUCTION

Routine infant hepatitis B vaccination in national immunization programs has been implemented in 184 countries through 2015 and the World Health Organization (WHO) has estimated coverage with three doses at 83%. By contrast, only 96 countries have introduced a birth dose (i.e., within 24 hours of life) of hepatitis B vaccine (HBV) with estimated coverage of 39% (website: http://www.who.int/mediacentre/factsheets/fs378/en/; last accessed 24 July 2016). Lack of a birth dose places infants at risk of perinatal transmission, particularly in areas with high maternal hepatitis B surface antigen positivity (44,45).

One limitation for achieving high birth dose coverage in resource poor settings is that many births occur at home or in other locations without access to HBV. Even if national immunization programs had an interest in facilitating vaccine delivery via lay midwives or other community health workers, this would be difficult to implement due to lack of cold storage facilities and an on-label indication requiring storage at 2-8^{o}C. Consequently, an on-label indication for storing HBV outside of the cold chain at the site of delivery for some period of time likely will improve birth dose coverage.

The introduction of an out-of-the-cold-chain (OCC) strategy for heat stable vaccines has the potential for increasing immunization coverage by allowing short-term transport of these vaccines OCC. While CTC is well defined, OCC does not have a clear definition or monitoring standards and is considered "off-label" vaccine use.
Two terms are used to describe the regulatory perspective of the CTC approach (Figure 1):



1) Extended controlled temperature chain (ECTC) refers to regulatory requirements for licensure, and on on-label indication, for any temperatures >8^{o}C. Vaccines licensed for

use under ECTC are required to have sufficient information on the approved conditions (such as maximum temperature and time) on the package insert.

2) Controlled temperature chain (CTC) is a subset of ECTC and refers to a WHO-developed regulatory framework for the stability evaluation of vaccines under a CTC that requires a vaccine exhibit stability following a single exposure to at least 40°C for a minimum of 3 days just prior to administration, while remaining compliant with the approved vaccine specifications. (46). Additionally, the program requires that the CTC provision be included in licensure by the relevant NRA and by WHO prequalification. The CTC approach was successfully used in Africa for the serogroup A meningococcal conjugate vaccine (MCV-A) (49,50).

Information on the stability evaluation of vaccines for use under extended controlled temperature conditions takes the form of WHO Guidelines rather than Recommendations because vaccines represent a heterogeneous class of agents and the stability testing program will need to be adapted to suit the product in question. WHO Guidelines allow greater flexibility than Recommendations with respect to specific issues related to particular vaccines. Within this context, WHO has not made a recommendation on off-label HBV storage outside of the cold chain. However, the WHO HBV position paper (51) acknowledged evidence for HBV thermostability and the benefits of additional vaccine management systems:

> "Although the vaccine has been exposed to temperatures of up to 45 °C for 1 week and temperatures up to 37 °C for 1 month without change in immunogenicity or reactogenicity, exposure to hot environmental temperatures should be minimized."
>
> "Expanding vaccine management systems and innovative outreach to provide vaccine for home births will ensure that hepatitis vaccine is available in settings where births take place."

Several demonstration projects in Asia have reported encouraging results from field evaluations of OCC approaches (20,22,24,26). Additionally, some manufacturers have adopted the ECTC approach and provided an on-label indication for temperatures above 8°C. Product inserts for 11 monovalent HBVs with a pediatric indication, including seven that were WHO pre-qualified (website: https://extranet.who.int/gavi/PQ_Web/, last accessed 23 July 2016) (Table 1), indicated that all HBV vaccines had a recommended storage temperature of 2-8°C and a specific warning not to freeze the vaccine (5-15). Two inserts, including one for a prequalified vaccine, indicated that vaccine was stable for 1 month at 37°C and one week at 45°C, thus meeting the programmatic criteria for CTC.

The goal of the current study was to review and summarize available data on monovalent HBV thermostability, with a view towards making recommendations on whether sufficient data exist for CTC programmatic recommendation or an ECTC on-label indication for storage outside the cold chain. A previous review of thermostability data was published during 2006 (23). Consequently, the current review included data identified in this previous review supplemented with data published from 2006 to the present.

METHODS

Manufacturers' thermostability data

To address potential publication bias, WHO staff requested and received thermostability data from all seven manufacturers of prequalified monovalent HBV; WHO staff also requested data from three manufacturers of non-prequalified monovalent HBV, among whom one responded. Data requested included the minimum release potency; end of shelf-life lower potency limit; product on-label storage recommendations and shelf-life, in-vitro and in-vivo potency by batch number, week, and storage temperature evaluated for all test results available; test results related to vaccine appearance, sterility, and pH at higher temperatures; and any results from modeling thermostability data to predict whether vaccine could be used after exposure to particular temperatures and lengths of time. Only results for products that were tested at 37^{o}C or higher were included in this analysis.

Project Optimize Reports

The World Health Organization, as a partner with PATH in Project Optimize (website: http://www.path.org/projects/project-optimize.php, last accessed 24 July 2016) provided reports of two evaluations conducted on HBV stability (47,48) that were not otherwise available in the online literature (see below).

Published literature search

Using PRISMA guidelines (1), a systematic literature review was performed to identify studies in animals or humans of HBV heat thermostability. The following PICO framework was used:

- Population = all animals or humans in which experimental or introduction studies were conducted;
- Intervention = monovalent hepatitis B vaccine;
- Comparison = vaccine stored at 2-8^{o}C versus vaccine stored at 37^{o}C or higher;
- Outcome = in-vitro relative potency, with secondary outcome = in-vivo potency.

No attempt was made to assess stability at freezing temperatures (less than 0^{o}C) or to assess vaccine stability during multiple freeze-thaw cycles.

Databases searched included PubMed, World of Science and CINAHL; however, as all relevant abstracts from CINAHL were identified in the other two databases, it was not considered further. Because a previous literature search had been done that included references through 2005 (2), publications were searched from January 1, 2006 through July 11, 2016. Publications identified in the previous review were included. No language restrictions were employed; however, no manuscripts were identified in a language other than English, and so no translation was required.

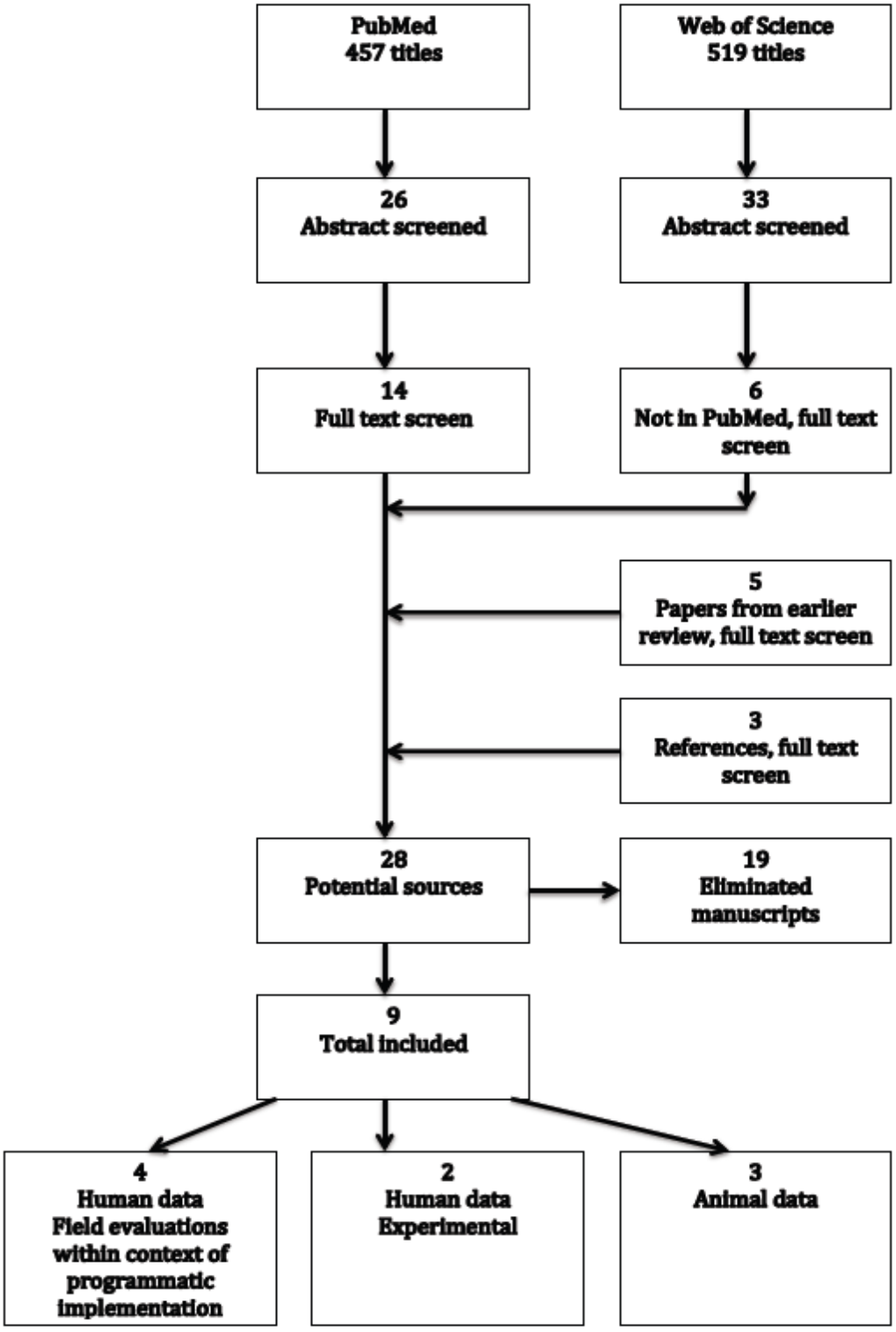
The final search strategy was developed through an iterative process to reduce the likelihood of missing relevant publications. The search strategy for all databases was as follows: ("hepatitis B" OR "HBsAg") AND ("vaccine" OR "immunization") AND

("heat" OR "thermostability" OR "thermostable" OR "exposure" OR "stability" OR "cold chain" or "controlled temperature chain"). References of all reviewed full-length manuscripts were examined to identify additional potential relevant publications.
All studies regardless of design were included that reported data for hepatitis B vaccine thermostability at temperatures higher than the recommended 2-8^{o}C. Acceptable data included vaccine potency, seroconversion, and geometric mean titers (GMTs). Studies were excluded if they did not include original data, or if they included only programmatic data on hepatitis B vaccine use outside of the cold chain. After deleting duplicates, sequentially titles, abstracts and the full-texts were screened to identify manuscripts included in analysis (Figure 2). Due to funding and time limitations a single person performed all aspects of the study, including screening titles, abstracts and manuscripts; abstracting data, and analyzing data.

For studies included in analysis, data were abstracted directly into a pre-developed electronic database, with a focus on the primary outcomes of vaccine potency, seroconversion, and GMTs. Quality assessment of studies in human populations was conducted using the Newcastle-Ottawa Scale for non-randomized studies (3) and the Cochrane Collaboration risk of bias tool for randomized studies.

Nine studies were identified, of which three were in animals and six in humans (Figure 1). Studies differed with respect to age of study participants (infants to adults), study design (community randomized, individually randomized, observational), duration of heat exposure, temperature of heat exposure, vaccine formulation, outcomes reported, and other features. Consequently, no meta-analysis was conducted.

Figure 2 Flowchart



RESULTS

Manufacturers data

All eight manufacturers provided data from in-house testing on thermostability at 37°C or higher, with temperatures ranging from 37-70°C and with follow-up periods up to 12 months (Table 2). Other than potency, manufacturers provided different levels of detail on different aspects of stability but regardless of the available data, vaccines from all manufacturers at all temperatures and for all lots were stable until the end of the testing period. Most manufacturers did not provide data for short-term storage at 2-8°C and so this information could not be included.

Seven of eight manufacturers provided data on in-vitro potency and five on in-vivo potency (Figures 3a to 10b). For in-vitro potency, one manufacturer did not provide details on the test method used, five used an in-house test method, and one used the commercial Murex ELISA test. Exposure to 37°C for 4 weeks (considered equivalent to 1 month or 30 days) resulted in an average loss of in-vitro potency compared to baseline of 16%, with variation from 8% to 40% depending on the manufacturer (Figure 11a) (eight data points from seven manufacturers). Exposure to 37°C for 4 weeks resulted in an average increase in in-vivo potency compared to baseline of 7% with variation from a decrease of 23% to an increase of 36% (four data points from four manufacturers). HBV from all seven manufacturers met potency and other stability specifications at 37°C for at least 4 weeks.

Five of eight manufacturers provided data on exposure to 40°C or higher and thus could be assessed for compliance with the CTC programmatic requirements (Figure 11b). In all cases, potency was retained for one week or greater (including in-vitro potency from all five manufacturers and in-vivo potency from four manufacturers) and thus CTC standards were met. Manufacturer B provided the most extensive data. Of particular interest is a study that showed that vaccine met minimum potency requirements following exposure to 40°C for up to 4 weeks regardless of whether the batch tested was fresh, mid-shelf life (1.5 years old), or near expiry (3 years old).

A single manufacturer (manufacturer H) provided data from human clinical studies. For one vaccine lot stored at 4°C, seroconversion was 99.6% among 44 volunteers with a geometric mean antibody titer (GMT) of 648 IU/L. For three lots stored at 37°C for 15 days, 107 volunteers had seroconversion of 99.6-100% and GMT of 416 to 772 IU/L. For three lots stored at 37°C for 30 days, 125 volunteers had seroconversion of 99.6-100% and GMT of 495 to 781 IU/L. For three lots stored at 45°C for 7 days, 118 volunteers had seroconversion of 99.6-100% and GMT of 493 to 647 IU/L.

Some manufacturers indicated that they will pursue an ECTC on-label indication. For example, one manufacturer indicated that they will add “Keep the vaccine at 40°C up to 3 days immediately previous to administration”; will further propose similar language to include in the WHO and Unicef information label noting that this is not a recommendation for storage but rather to guide decision-making when exposure to higher temperatures is planned; and include a recommendation that after opening the vaccine

vial should be stored at 2-8^{o}C for a maximum of 4 weeks. Other manufacturers indicated that they will seek an ECTC on-label indication following the completion of additional studies.

Review of Project Optimize data

Study 1

Project Optimize conducted an evaluation of three WHO prequalified monovalent HBVs with exposure to 4^{o}C or 37^{o}C for eight weeks (cycling between the two temperatures was also evaluated but this is not reported here) (47). Brand names and manufacturers were not identified. Potency was assessed using an enzyme immunoassay kit from Bio-Rad Laboratories, Inc. and the Murex HBsAg Version 3 Kit distributed by DiaSorin, Italy.

All three evaluated HBVs maintained potency throughout the study when stored at 4^{o}C. At 37^{o}C, potency decreased by 44% or greater over the first week and at a slower rate thereafter. Expressed as a percentage of initial potency, vaccine potencies at 8 weeks were 23%, 25%, and 26% using the Bio-Rad test (Figure 12). A comparison of the Bio-Rad and Murex assays for one vaccine stored for one week at 37^{o}C found that the Murex assay indicated no potency loss while the Bio-Rad assay indicated 40% potency loss.

No vaccines were tested at greater than 37^{o}C and thus could not be assessed for meeting CTC criteria. Additionally, potency specifications for tested vaccines were not reported, so could not be assessed for any ECTC criteria.

Study 2

A second Project Optimize study assessed thermostability for monovalent HBVs from five manufacturers. Vaccines were held at 37^{o}C and 45^{o}C until VVMs on the vaccines held at 45^{o}C had expired (ranging from 3 to 8 days). In-vitro potency tests were done by the manufacturers, the national control laboratories of Korea and Indonesia, and the National Institute of Biological Standards and Control (NIBSC) using two different assays. Three vaccine lots were tested for each HBV and for each assay method; within lot and assay method two or three tests were done and values averaged.

In-vitro potency varied by manufacturer, assay, and lot (Figures 13a-e). The assay used by NIBSC tended to have the most variation and gave the highest levels while the manufacturer's assay had the least variation. Minimum lot release specification standards for in-vitro potency were met at both 37^{o}C and 45^{o}C for vaccines from manufacturers 1 and 5 and - except for a single test - manufacturer 3, and thus these vaccines met CTC programmatic criteria. Depending on the assay and batch, vaccines from manufacturers 2 and 4 often did not meet specification standards at both 37^{o}C and 45^{o}C, and thus did not meet CTC criteria at these temperatures.

Systematic literature review

Overview

There were 457 titles identified in PubMed and 519 in Web of Science. Of the 26 relevant abstracts in PubMed, 14 full-length manuscripts were selected for full-text evaluation (16-29). Of 33 relevant abstracts in Web of Science, six not previously

identified in PubMed were selected for full-text evaluation (30-35). Finally, for the period before 2006, five manuscripts analyzed in the earlier review were selected for full-text evaluation (36-39,43) (the sixth manuscript in this review was published during 2006 and identified in the PubMed search). Among these 25 manuscripts, reference review led to three additional manuscripts included in full-text review (40-42). Nine manuscripts had original data on hepatitis B vaccine thermostability at elevated temperature and were included in analysis; of these, five were derived from the PubMed search (16-20) and four from the previous review (36-38,43). The primary reason for exclusion was the absence of data on thermostability and a focus on other, usually programmatic, aspects of HBV use in the CTC.

Human trials

Within the six trials involving humans, four used an OCC approach in the field, comparing HBV thermostability in intervention and non-intervention areas; this included two studies of plasma-derived (18,37), one recombinant (20), and one unknown type of vaccine (43). Of these, three allocated vaccine by community (18,20,37) and one did not include information on allocation (43). The temperature at which vaccines were stored outside the cold chain varied from 2^{o}C to <49^{o}C and duration at elevated temperature varied from 1 day to 1 month (Table 3a). No differences were seen in GMTs or seroconversion between children who received vaccine in intervention versus non-intervention communities (Table 3b; figures 14a and 14b).

Two experimental studies, both using recombinant HBV, were done among small groups of adults to measure response after vaccine exposure to either 37^{o}C or 45^{o}C for 1 week to 1 month (36,38). No substantial differences were found in GMTs or seroconversion regardless of whether vaccine was kept at 4^{o}C, 37^{o}C, or 45^{o}C for 1 week or 1 month and thus the vaccines used in these studies met CTC criteria.

Study quality generally was low (Table 3c). One study contained almost no information on methods (43). Another indicated that children in the OCC areas could have received vaccine kept entirely in the cold chain if they were born in the hospital but no data reported the proportion in which this occurred, making interpretation difficult (20). Most of the studies did not employ blinding, did not describe the method of randomization, and experienced dropout of participants without explaining why this occurred.

Animal trials

Three studies used in-vitro and in –vivo testing in mice to compare standard HBV (recombinant Shanvac-B from Shantha in all cases) to new vaccine formulations designed to improve thermostability (16,17,19) (Table 4a). Two of the studies had samples sizes of eight mice per group, while one did not report this information. All studies compared vaccine at 4^{o}C versus 37^{o}C and one study also included data for vaccine kept at 45^{o}C. None of the studies were designed to assess a CTC approach based on exposure to elevated temperatures for days or weeks, but rather to look at improvements in stability following exposure over months.

Studies found consistent results for in-vitro potency using the Auszyme test, showing little loss of potency for standard or modified vaccine stored at 4°C or for modified vaccine stored at 37°C or 45°C (16,17,19) (Figure 15) (Table 4b). However, standard vaccine stored at either higher temperature demonstrated potency loss of 20-45% after one month and 70% or greater after 6 months. Because minimum release potency specifications for Shanvac-B require a relative potency compared to baseline of 0.8, vaccine did not meet CTC criteria, although no data were presented on loss of potency over the shorter time periods more relevant for CTC.

In-vivo evaluations varied substantially. One study (16) reported a 100-fold lower GMT for the standard vaccine stored at 37°C at 12 months (the only time period evaluated) compared to the other three study groups. A second study (17) found a 40-fold lower GMT following a single HBV dose that had been stored at 37°C for 15 months compared to baseline; similarly, seroconversion was 12.5% for standard vaccine stored at 37°C for 15 months. After a booster dose, there was a 4-fold lower GMT following storage at 37°C for 15 months compared to baseline, but 100% seroconversion. The final study (19) found relative in-vivo potencies of unmodified Shanvac-B of 1.13 and 0.86 following 6 months of storage at 4°C and 37°C, respectively, compared to the modified vaccine stored at 4°C.

DISCUSSION

Data collected from eight manufacturers and a systematic literature review support the thermostability of monovalent HBVs, particularly over periods of one to four weeks. All eight WHO pre-qualified HBVs for which manufacturers provided data retained in-vitro potency and met other stability criteria after 4-6 weeks exposure to 37°C and all four with data available reported stability after short-term exposure to 45°C. Additionally, among four community-based and two experimental studies that assessed seroconversion and GMTs in humans, no difference was found for vaccines maintained at 2-8°C and those maintained at higher temperatures.

These data provide strong scientific support for manufacturers to pursue an on-label indication for the ECTC approach, including specifically for the CTC programmatic approach. However, manufacturers may determine that the potential benefits of an on-label indication do not justify the necessary investments to achieve this goal. In the meantime, the assembled data indicate that WHO could support an off-label recommendation for an OCC approach consistent with CTC programmatic requirements. Where appropriate, such a recommendation would help support a country's decision to use the CTC approach to improve birth dose HBV coverage and timeliness.

Beyond the first few weeks, results varied. Three published studies of different modifications of Shanvac-B designed to improve thermostability demonstrated that the original vaccine formulation had substantial declines in in-vitro potency, but this did not always correlate to declines in seroconversion. These studies also reported in-vivo data only following at least 6 months of increased heat exposure. In addition, a study by

PATH found that all three tested vaccines had substantial potency loss after just one week when tested with the Bio-Rad assay.

Factors other than heat exposure may alter in-vitro potency test results. The Project Optimize study 1 demonstrated that Bio-Rad identified more in-vitro potency loss than Murex. This was confirmed by the second Project Optimize, which showed variation in results for four different assay methods. All studies found some lot-to-lot variation in potency, and in some cases this was substantial. For example, for manufacturer 2 in this study, some lots passed minimum release specification and others did not after heat exposure.

The current review could not assess the degree to which changes in potency correlate with changes in human immune response. This was because no studies had a vaccine fail to pass in-vitro potency specifications and reported in-vivo results, either for humans or animals. Nevertheless, it is reassuring that all studies that evaluated immunogenicity in human populations found acceptable responses when vaccine was stored outside of the cold chain.

The main reason for pursuing HBV storage out of the cold chain is to facilitate vaccine delivery, and increased coverage, in settings where a substantial number of women deliver outside of the hospital and thus where maintenance of the cold chain at the delivery point would be challenging. Some data support this hypothesis. In Indonesia, a collection of interventions, including an OCC approach, led to increases in HBV birth coverage (22). In China, compared to baseline, the percentage that received a birth dose increased from 83-89% to 99% in all groups regardless of cold chain requirements; however, the percentage receiving the first dose during the first 24 hours was better among those living in the OCC communities (20). In Laos, an OCC policy led to a 27% increase in HBV birth dose coverage compared with no change in control districts (24).

In many low resource areas, an OCC approach based on CTC requirements will imply accepting and training lay vaccinators, such as traditional birth attendants (22). Furthermore, such an approach may work better when introduced as part of a comprehensive strategy to increase birth dose coverage that might include adapted delivery devices such as the Uniject compact, prefilled, autodisable device (20,22); integrated perinatal and immunization services; and increasing the number of births that occur at health centers equipped to store and deliver vaccine.

An OCC approach, such as that specified by CTC, increases the possibility not only of extended exposure to elevated temperatures but also lower temperatures. For example, when storing vaccine out of the cold chain in rural China, freezing temperatures were documented for all study sites and vaccines were exposure to temperatures of $<0^{o}$C for 2.9-12.9% of their time outside of the cold chain (26). However, this is not only a concern for vaccines stored out of the cold chain since studies also have documented refrigerator temperatures of $<0^{o}$C (20).

In summary, data for HBVs strongly support their thermostability over periods of less than a week at temperatures up to 45^{o}C and for several weeks at temperatures up to 40^{o}C. When evaluated, potency was retained when older vaccines were exposed to higher temperatures or when vaccines were exposed to higher temperatures and then put back at 2-8^{o}C for up to two years. Additionally, access to HBV birth dose is hampered for deliveries that occur at home, and in some settings home births constitute a large proportion of all births. Field data suggest that timely access to a birth dose of HBV is enhanced when an OCC approach is allowed. Despite this, currently only one WHO pre-qualified monovalent HBV has an on-label indication for storage outside of the standard 2-8^{o}C. Having additional vaccines with an on-label indication should increase acceptance of an OCC approach, including one that adheres to CTC approach programmatic requirements. Meanwhile, WHO support of an off-label recommendation for HBV use out of the cold chain may provide sufficient interim support for countries to integrate this process into an integrated strategy for improving newborn HBV coverage and timeliness. Remaining research questions include the impact of different potency tests on test results and the correlation between these results and human immune responses. Lastly, before implementing an OCC approach for HBV, countries should assess the likelihood that vaccine will be exposed to freezing temperatures.

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Figure 3a. In-vitro relative potency of manufacturer A monovalent hepatitis B vaccine, exposed to different temperatures for different time periods. Minimum release and end of shelf-life relative potency >=0.45. Data provided by manufacturer and results based on Murex test kit (Diasorin).

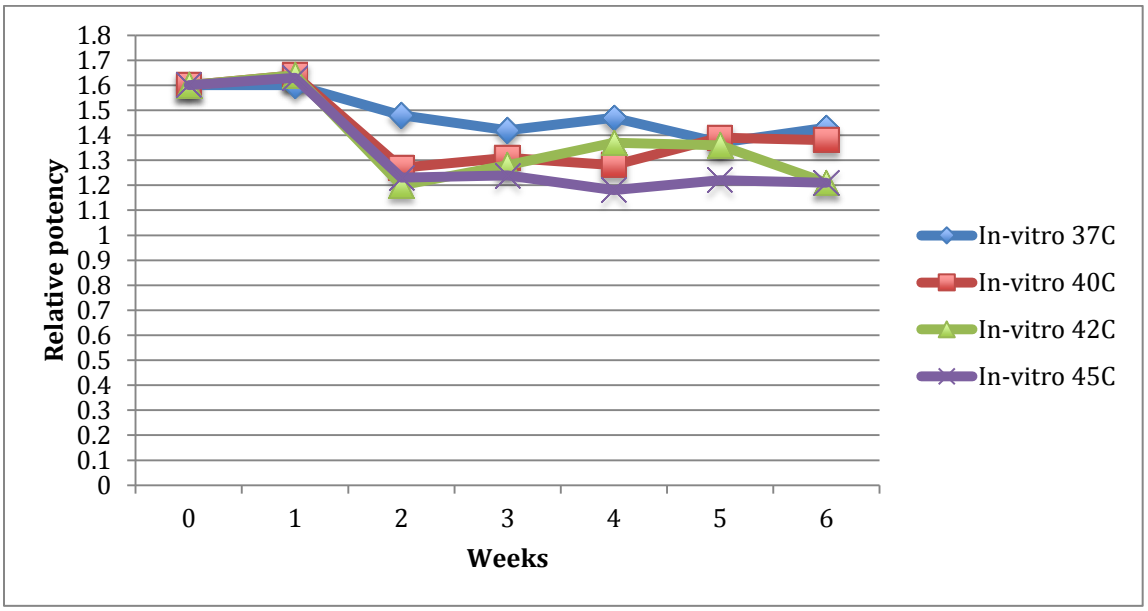

Figure 3b. In-vivo relative potency of manufacturer A monovalent hepatitis B vaccine, exposed to different temperatures for different time periods. Minimum release and end of shelf-life relative potency upper 95% confidence limit of estimated relative potency >=1.0. Data provided by manufacturer.

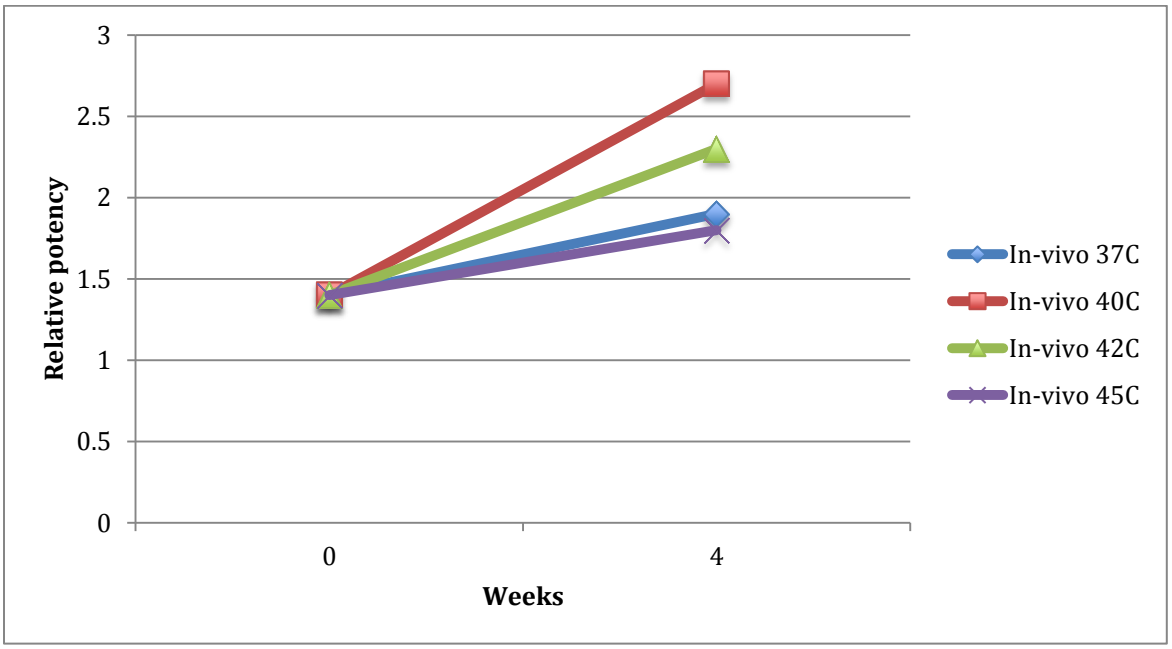

Figure 4a. In-vitro relative potency of manufacturer B monovalent hepatitis B vaccine, exposed to 37°C for 4 weeks. Minimum release and end of shelf-life relative potency >= 20ug/ml. Data provided by manufacturer and based on in-house potency test. Values represent averages of two batches.

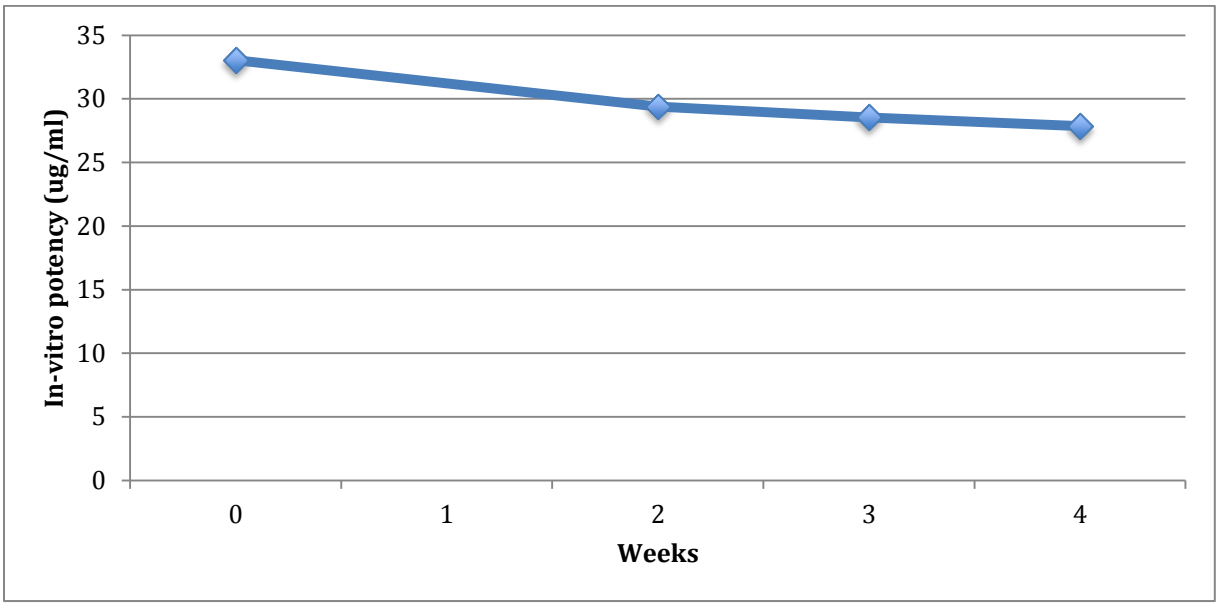

Figure 4b. In-vitro relative potency of manufacturer B monovalent hepatitis B vaccine, exposed to 40°C for 4 weeks., using vaccine batches that were fresh, mid-shelf life, or near expiry. Minimum release and end of shelf-life relative potency >= 20ug/ml. Data provided by manufacturer and based on in-house potency test.

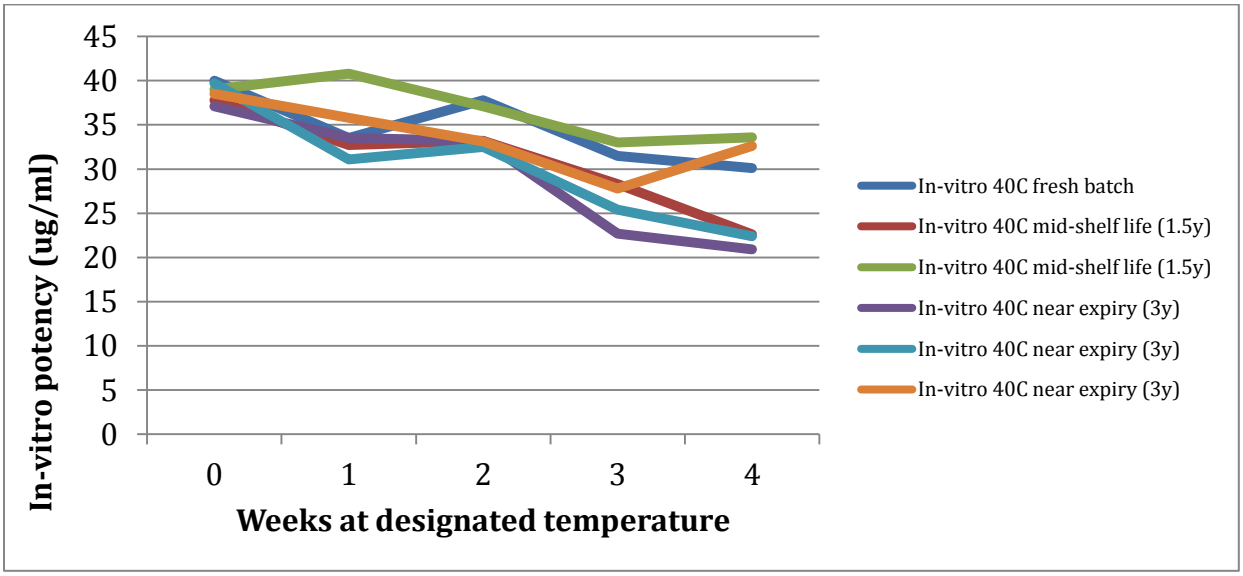

Figure 4c. In-vitro relative potency of manufacturer B monovalent hepatitis B vaccine, exposed to different temperatures and times. Minimum release and end of shelf-life relative potency >=20 ug/ml. Data provided by manufacturer and based on in-house potency test. Values represent averages of three different lots and error bars show maximum and minimum values.

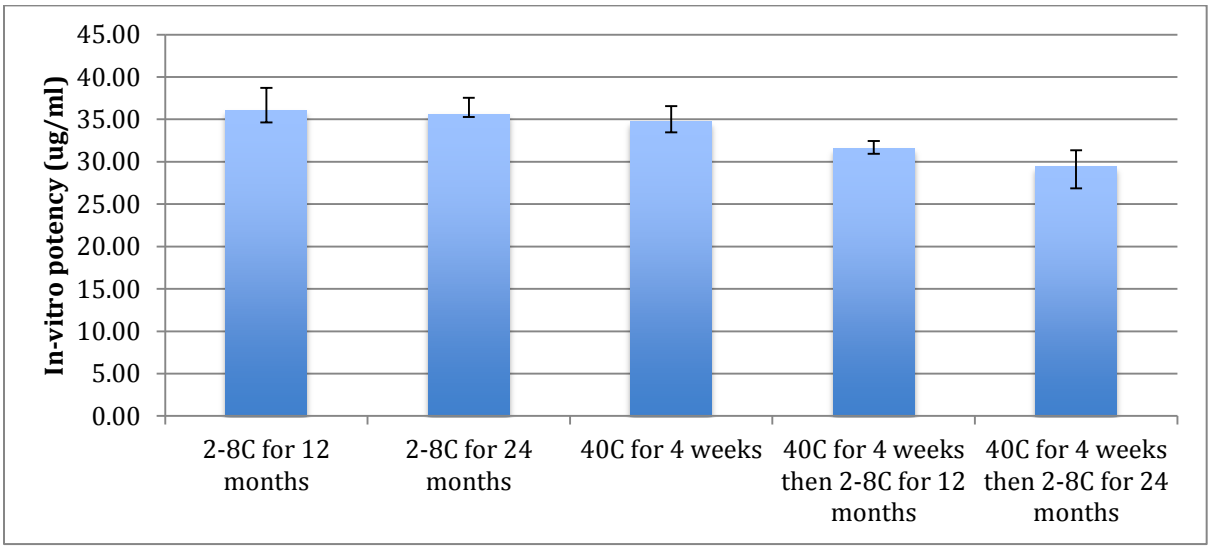

Figure 4c. In-vivo relative potency of manufacturer B monovalent hepatitis B vaccine, exposed to different temperatures and time periods. Minimum release and end of shelf-life relative potency upper 95% confidence limit of estimated relative potency >=1.0. Data provided by manufacturer and based on in-house potency test. Values represent averages of two different lots.

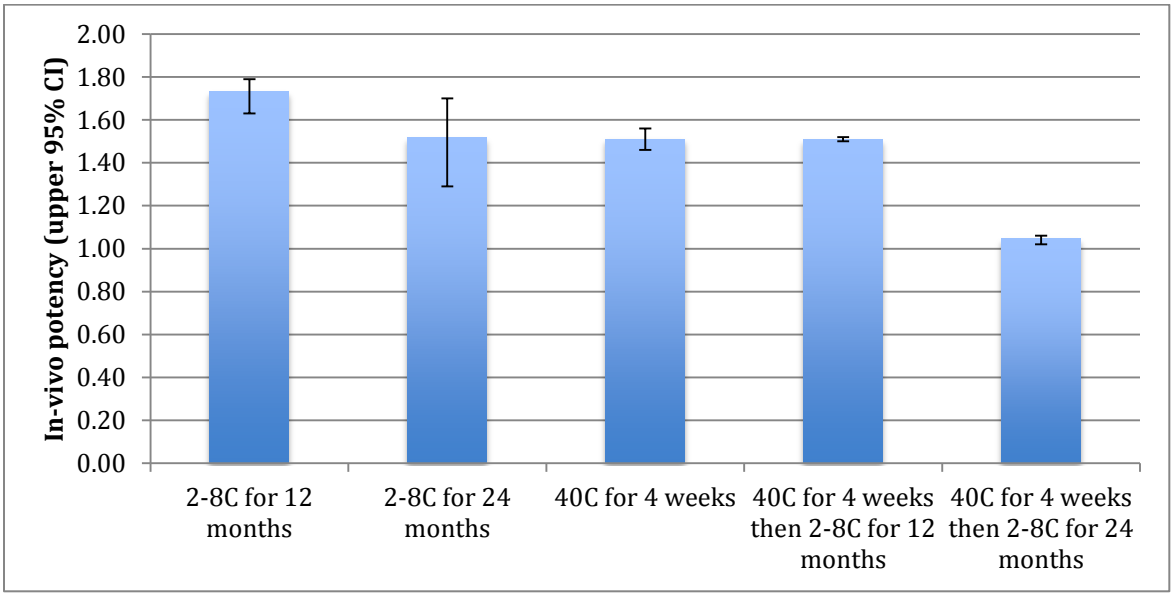

Figure 5a. Study 1: in-vitro relative potency of manufacturer C monovalent hepatitis B vaccine, exposed to different temperatures for different time periods. Minimum release and end of shelf-life relative potency >=0.50. Data provided by manufacturer. Values represent averages of two different lots.

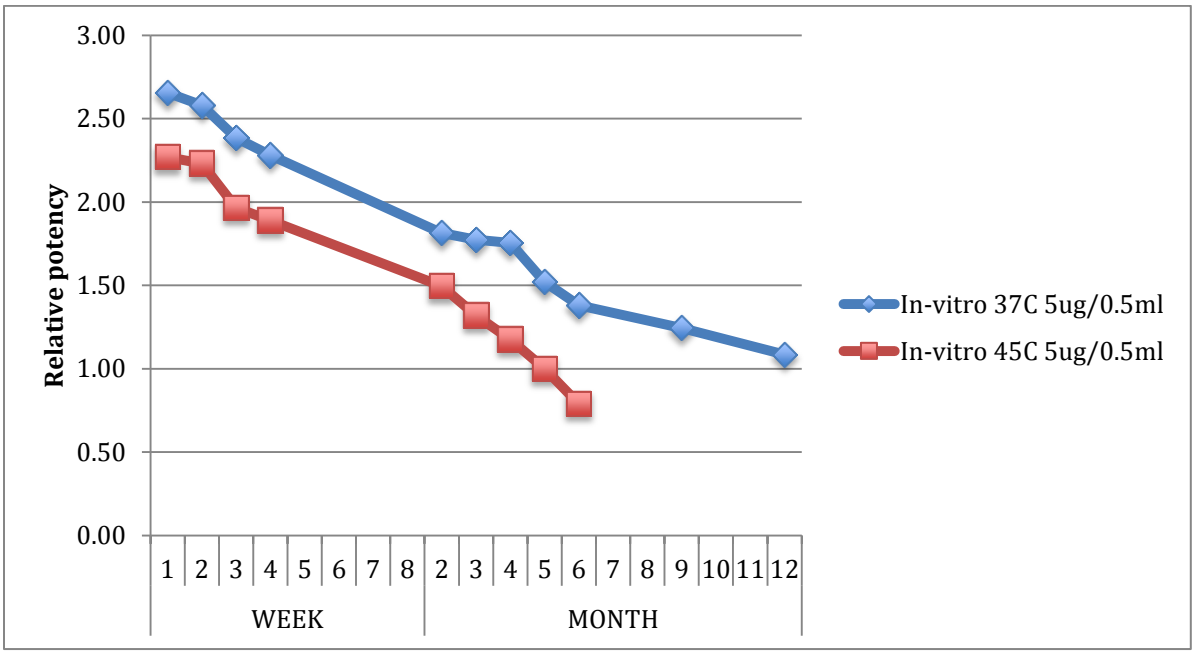

Figure 5b. Study 2: in-vitro relative potency of manufacturer C monovalent hepatitis B vaccine, exposed to different temperatures for different time periods. Minimum release and end of shelf-life relative potency >=0.50. Data provided by manufacturer. Values represent averages of three different lots.

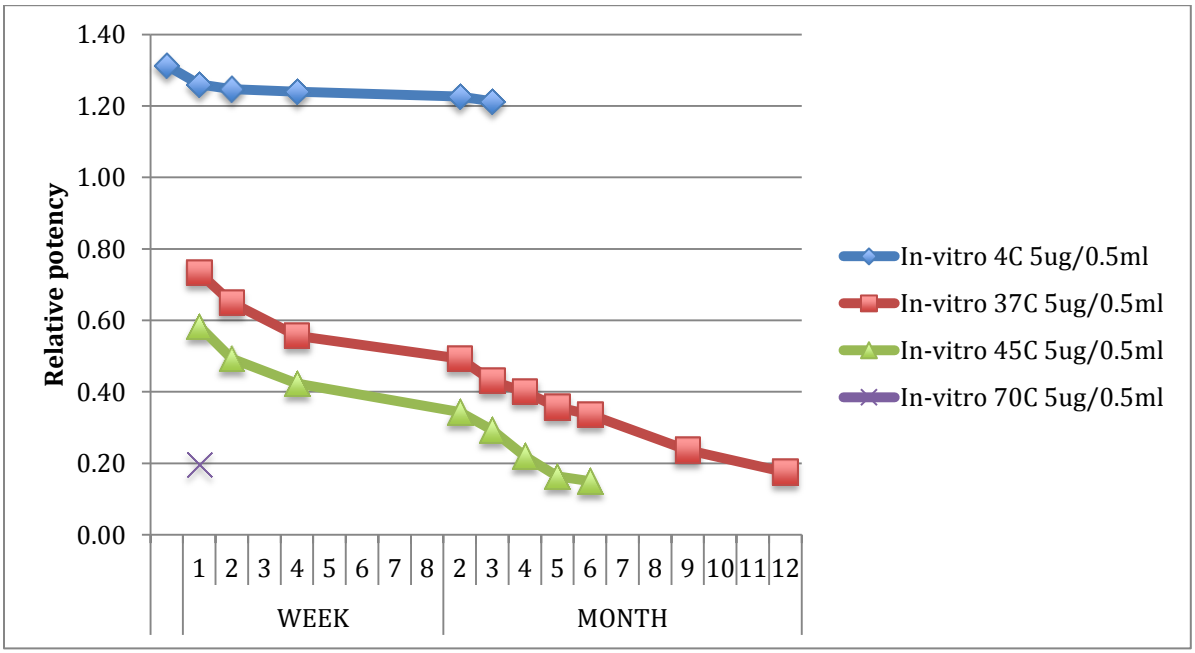

Figure 6a. In-vitro relative potency of manufacturer D monovalent hepatitis B vaccine, exposed to 37°C for 5 weeks. Minimum release and end of shelf-life relative potency >=0.80. Data provided by manufacturer and based on in-house potency test. Values represent averages of three different lots.

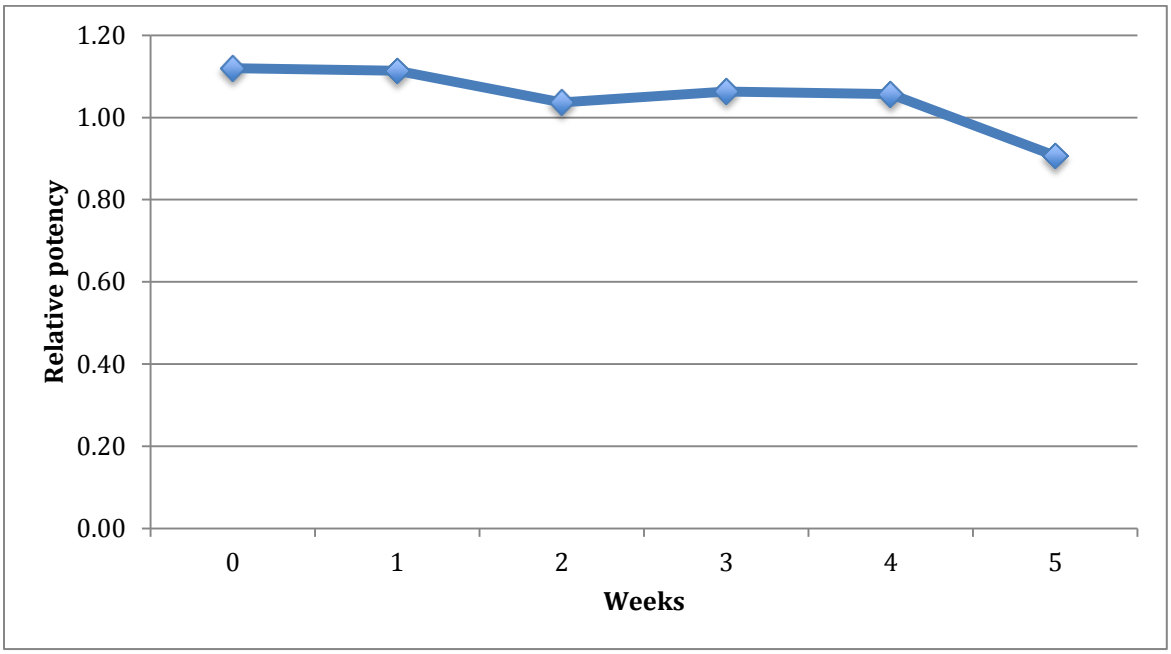

Figure 6b. In-vivo relative potency of manufacturer D monovalent hepatitis B vaccine, exposed to 37°C for 5 weeks. Minimum release and end of shelf-life relative potency upper 95% confidence limit of estimated relative potency >=1.0. Data provided by manufacturer and based on in-house potency test. Values represent averages of three different lots.

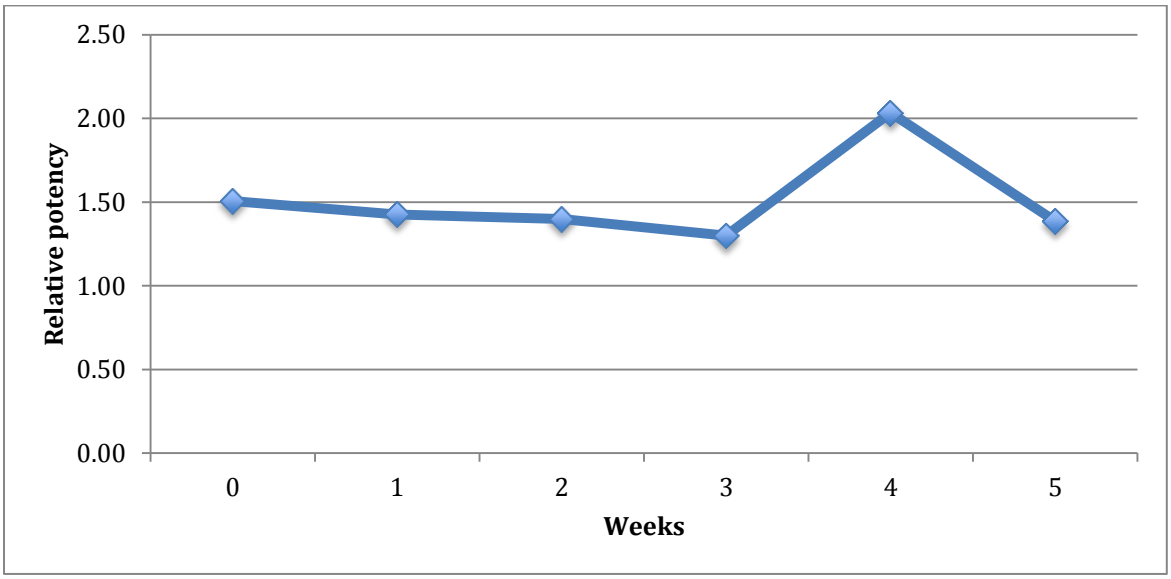

Figure 7a. In-vitro relative potency of manufacturer E monovalent hepatitis B vaccine, exposed to 37°C for 4 weeks and 45 °C for 1 week. Minimum release and end of shelf-life relative potency 15-25 ug/ml. Data provided by manufacturer and based on in-house potency test. Values represent averages of four different lots at 37°C and three lots at 45 °C.

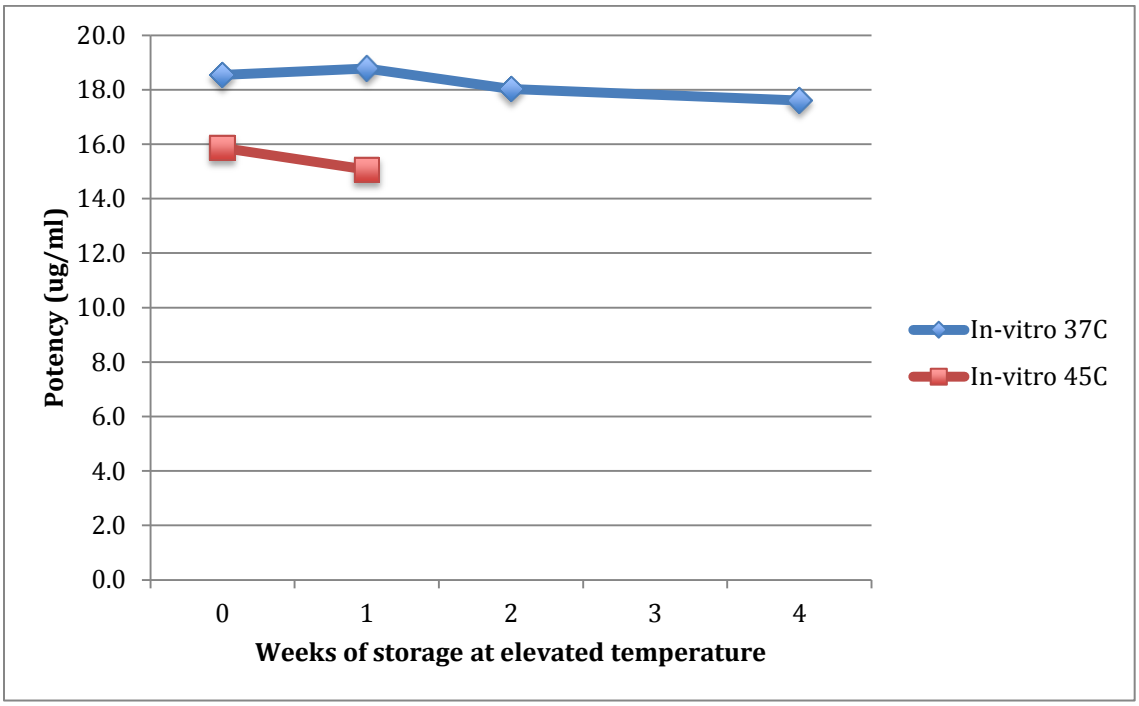

Figure 7b. In-vivo relative potency of manufacturer E monovalent hepatitis B vaccine, exposed to 45°C for 1 week. Minimum release and end of shelf-life relative potency upper 95% confidence limit of estimated relative potency >=1.0. Data provided by manufacturer and based on in-house potency test. Values represent averages of three different lots.

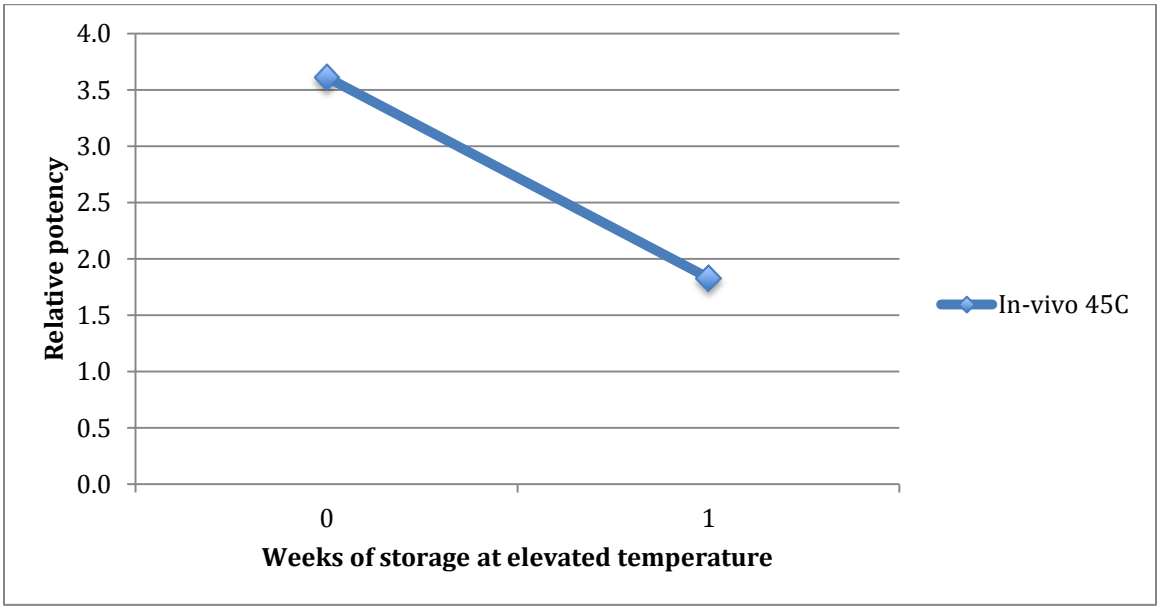

Figure 8. In-vivo relative potency of manufacturer F monovalent hepatitis B vaccine, exposed to 37°C for 4 weeks. Minimum release and end of shelf-life relative potency upper 95% confidence limit of estimated relative potency >=1.0. Data provided by manufacturer and based on in-house potency test. Values represent averages of three different lots.

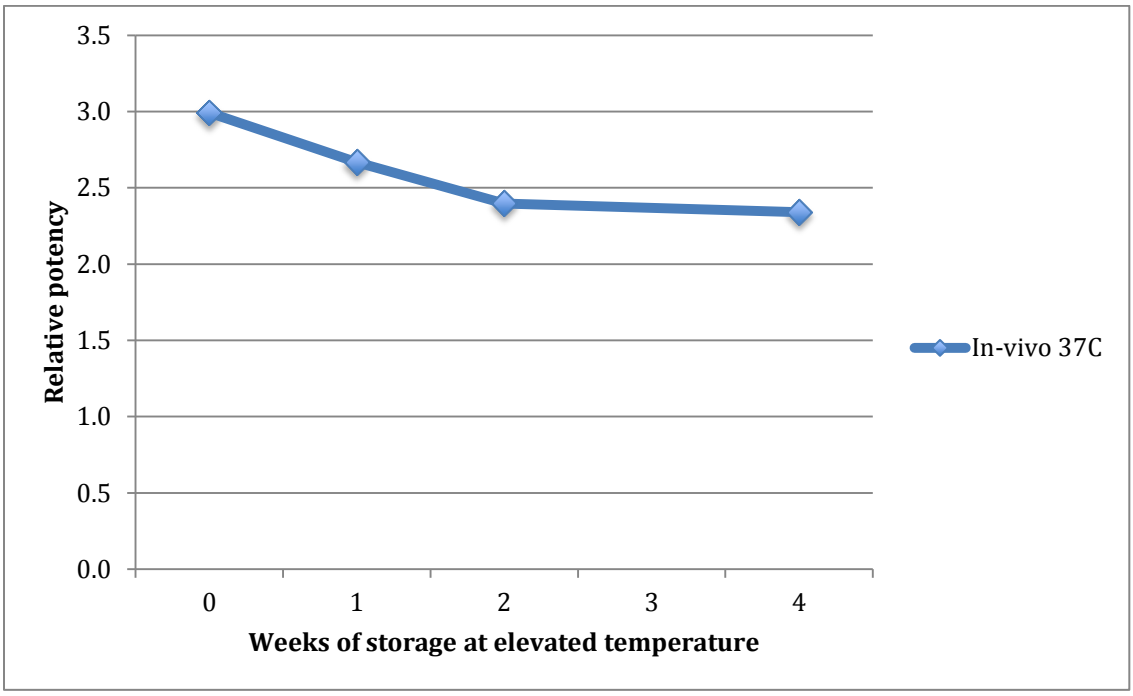

Figure 9. In-vivo relative potency of manufacturer G monovalent hepatitis B vaccine, exposed to 37°C for 5 weeks. Minimum release and end of shelf-life relative potency >=0.56. Data provided by manufacturer and based on in-house potency test. Individual batch values are presented to demonstrate variation in meeting minimum specification.

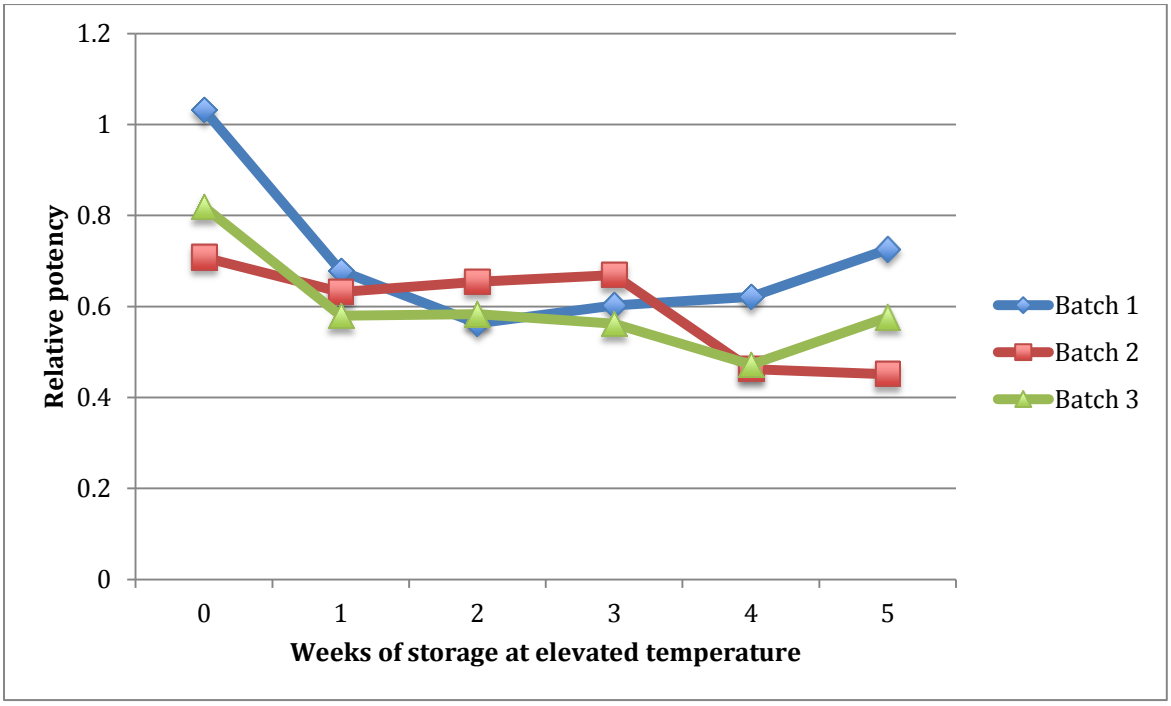

Figure 10a. In-vitro relative potency of manufacturer H monovalent hepatitis B vaccine, exposed to 37°C for 6 months (average value for testing of 10 lots), 45°C for 2 weeks (average value of 2 lots), and 60°C for 2 weeks (average value of 2 lots). Minimum release and end of shelf-life relative potency 15 ug/ml. Data provided by manufacturer.

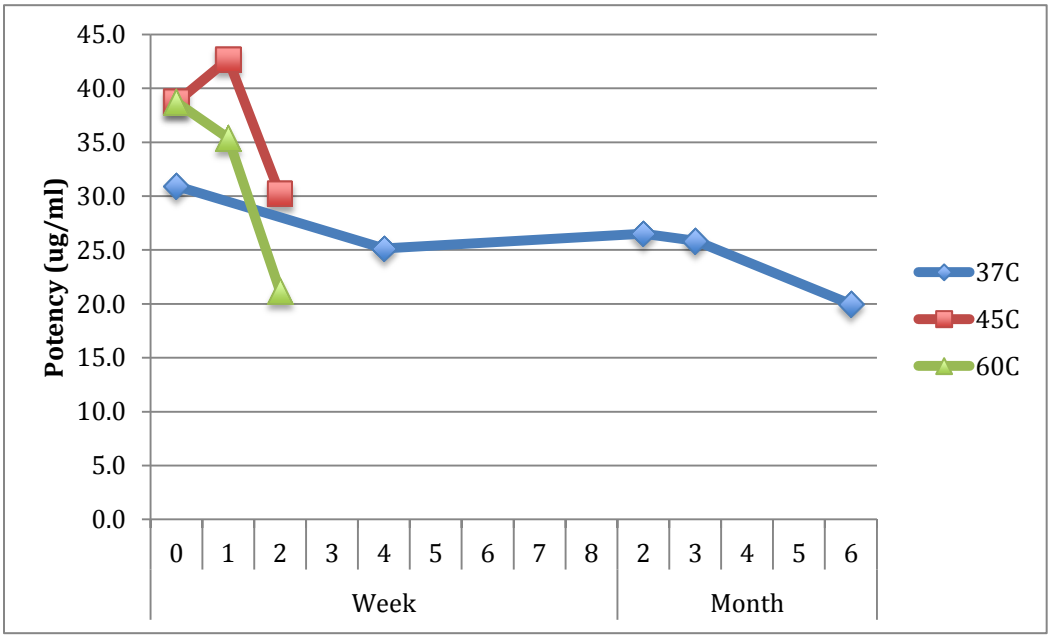

Figure 10b. In-vivo relative potency of manufacturer H monovalent hepatitis B vaccine, exposed to 37°C for 6 months (average value for testing of 7 lots) and 45°C for 2 weeks (average value of 3 lots),. Minimum release and end of shelf-life relative potency upper 95% confidence limit of estimated relative potency >=1.0. Data provided by manufacturer.

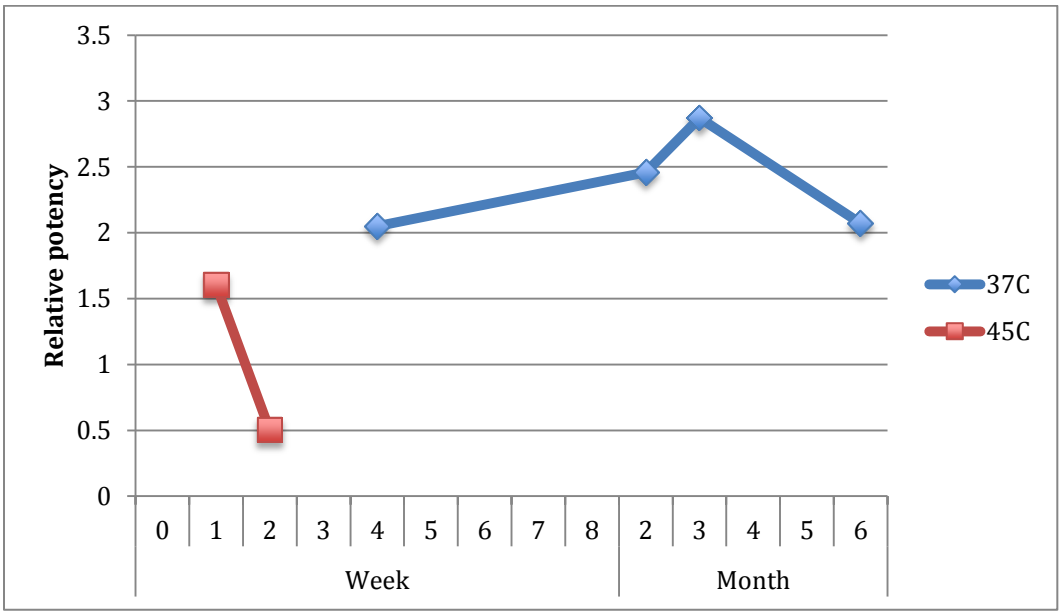

Figure 11a. Change in in-vitro potency relative to baseline (1 week for manufacturer C and at time 0 for other manufacturers) for monovalent hepatitis B vaccine exposed to 37°C for 4 weeks.

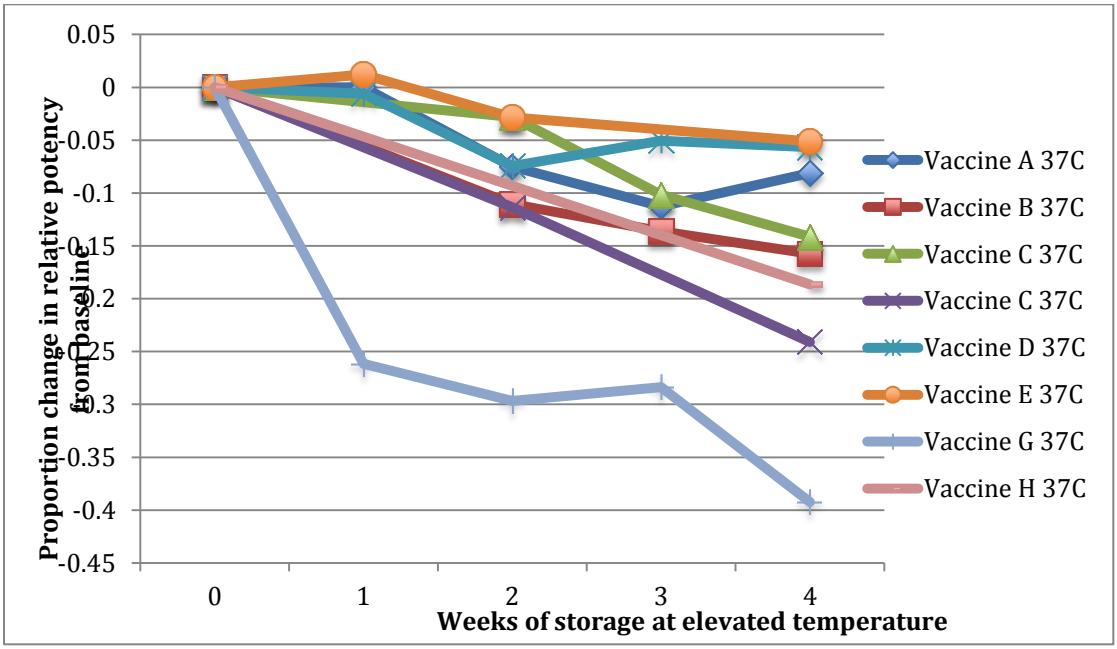

Figure 11b. Change in in-vitro potency relative to baseline (1 week for manufacturer C and time 0 for other manufacturers) for monovalent hepatitis B vaccine exposed to at least 40°C for 1 or more weeks.

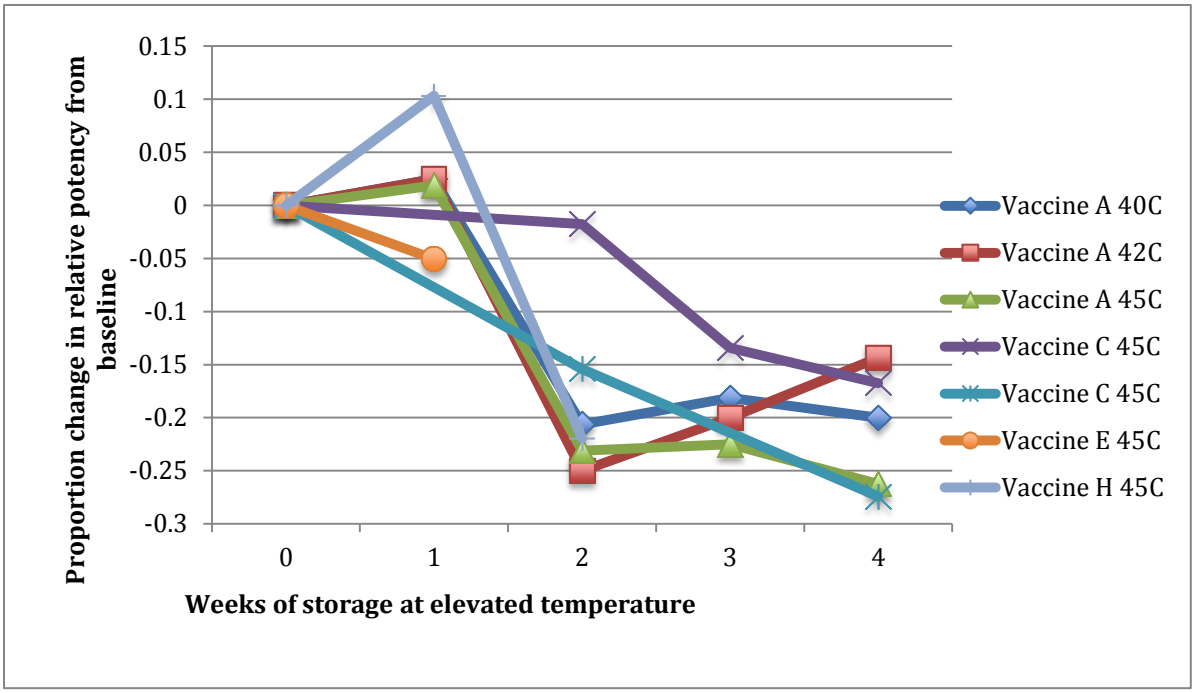

Figure 12. In-vitro potency evaluation of three monovalent hepatitis B vaccines exposed for 8 weeks to 4°C or 37°C. Values at time 0 and 8 weeks were reported while values at other time points were estimated from visualization of published graphs (47).

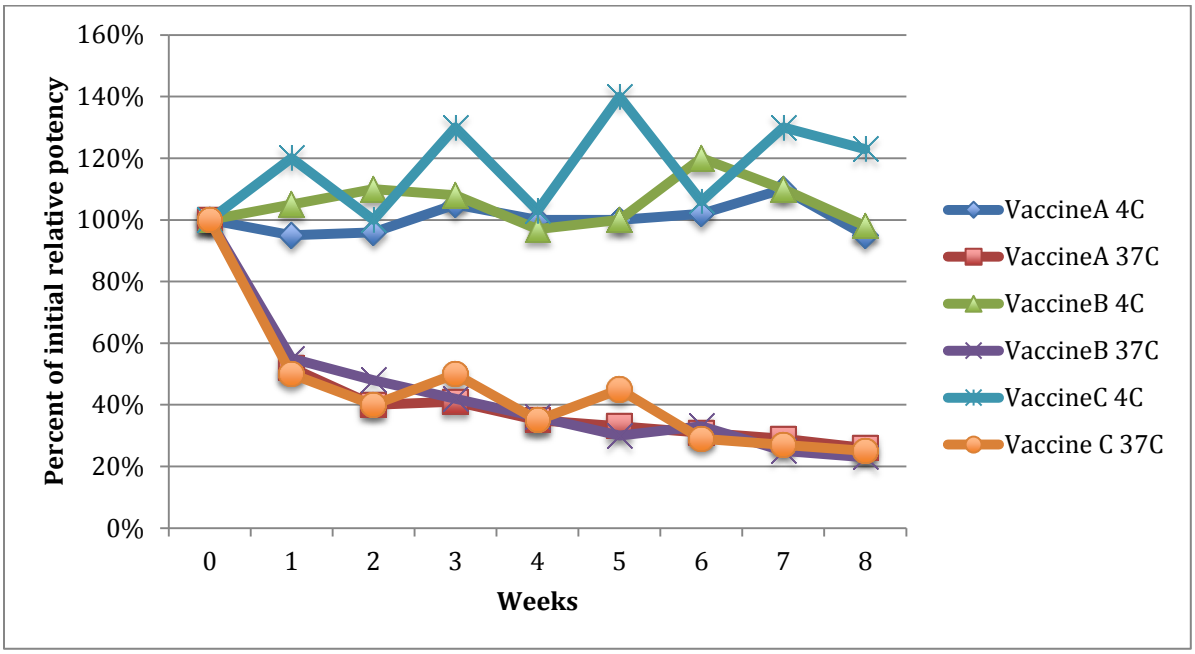

Figure 13a. Relative potency of monovalent hepatitis B vaccine from manufacturer 1 using 4 different assays. Minimum release potency >=0.45 (0.65 when assay 2 from National Institute of Biological Standards and Control (NIBSC) was used). NCL = National Control Laboratory. Error bars show range of values.

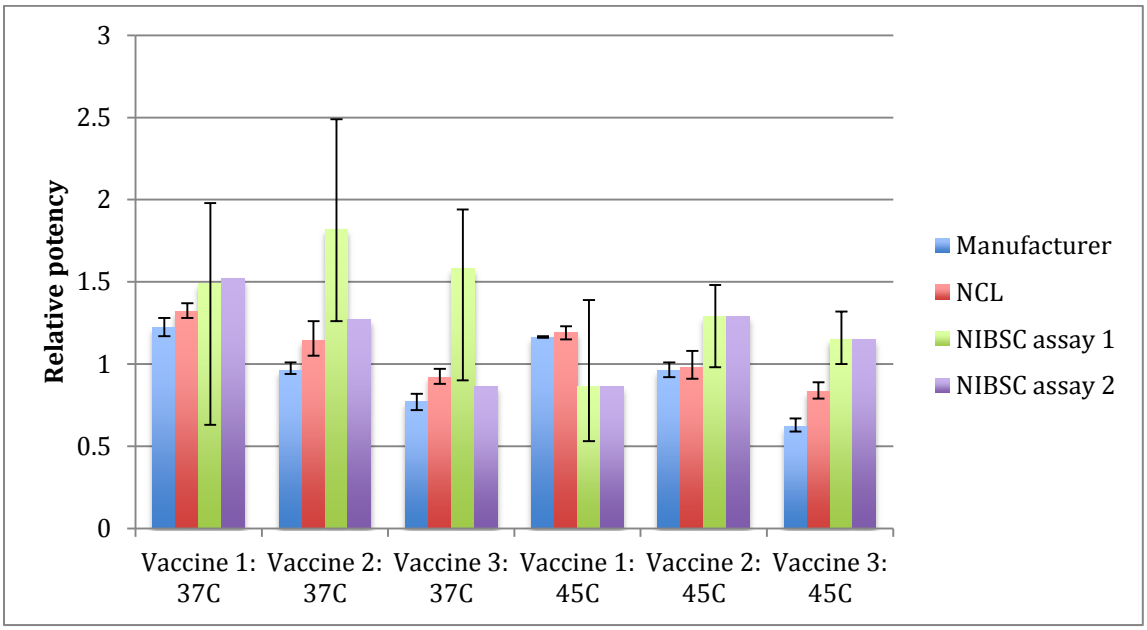

Figure 13b. Relative potency of monovalent hepatitis B vaccine from manufacturer 2 using four different assays after vaccine exposed to two elevated temperatures. Minimum release potency >=0.56. NIBSC = National Institute for Biological Standards and Control; NCL = National Control Laboratory. Error bars show range of values.

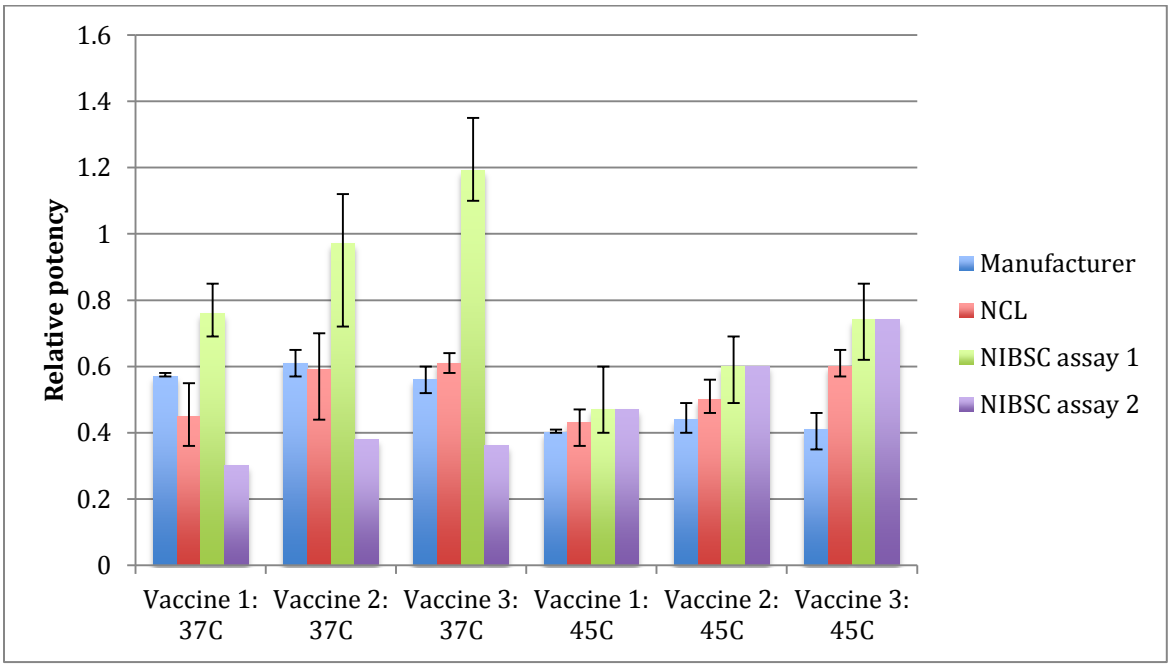

Figure 13c. Relative potency of monovalent hepatitis B vaccine from manufacturer 3 using four different assays. Minimum release potency >=0.5. NIBSC = National Institute for Biological Standards and Control; NCL = National Control Laboratory. Error bars show range of values.

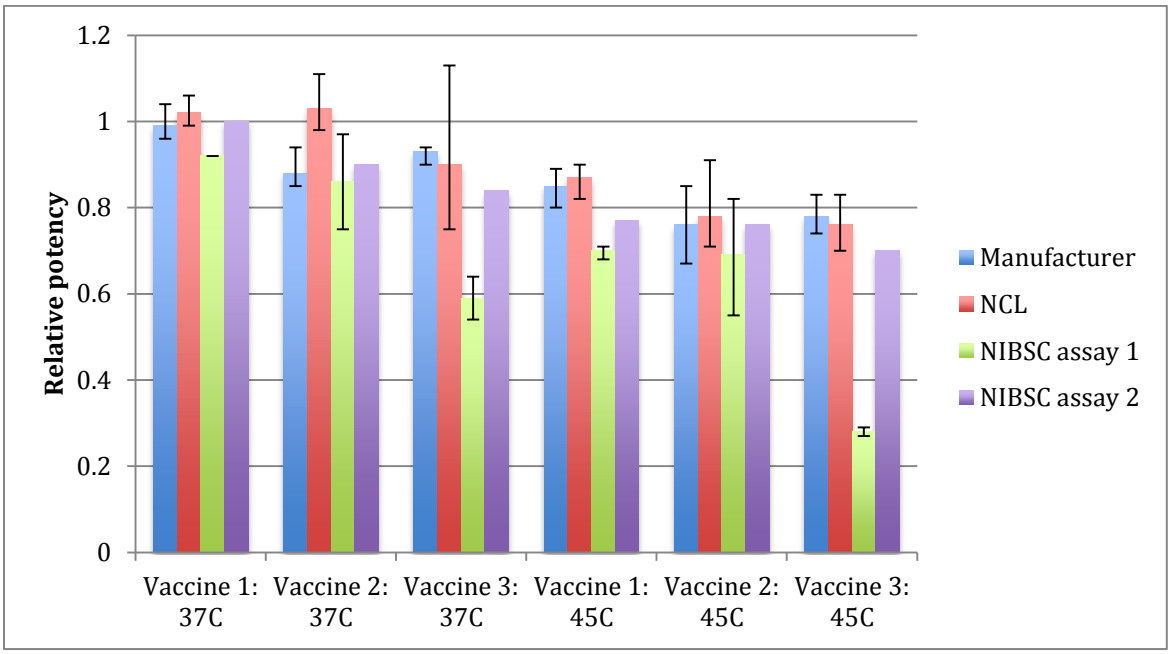

Figure 13d. Relative potency of monovalent hepatitis B vaccine from manufacturer 4 using three different assays. Minimum release potency 95% upper confidence limit >=1.0. NIBSC = National Institute for Biological Standards and Control. Error bars show range of values.

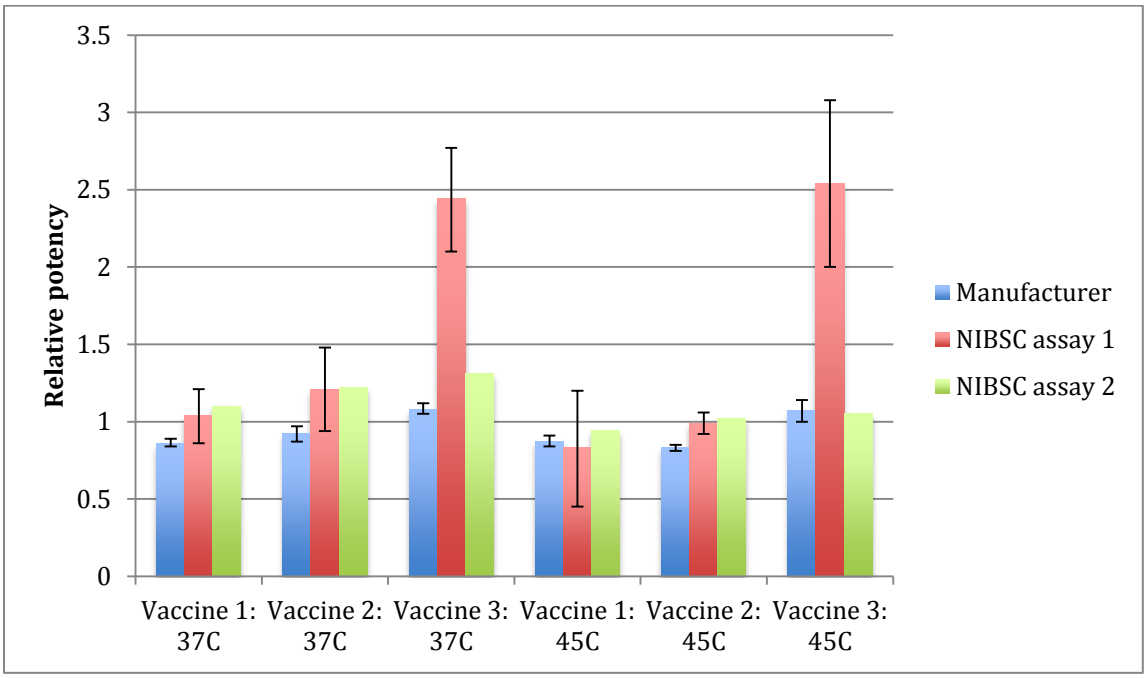

Figure 13e. Relative potency of monovalent hepatitis B vaccine from manufacturer 5 using three different assays. Minimum release potency >=20 ug/ml. NIBSC = National Institute for Biological Standards and Control. Error bars show range of values.

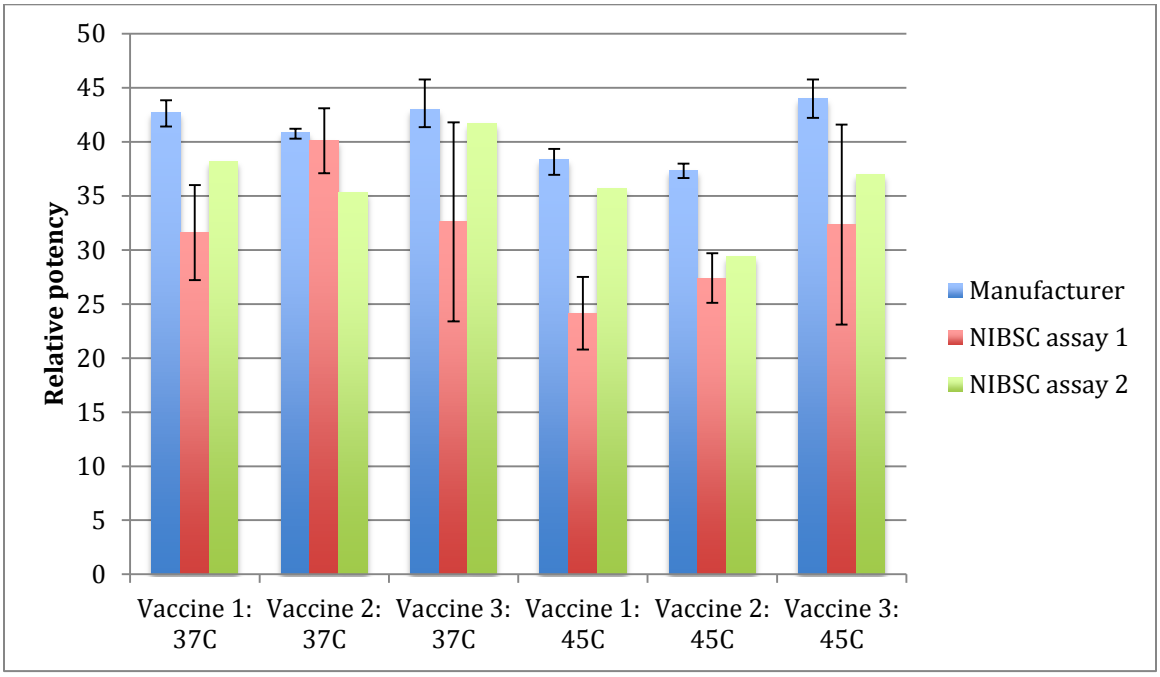

Figure 14a. Average geometric mean titres from four community-based studies that delivered childhood hepatitis B vaccine after storage inside or outside the cold chain. Error bars show range of values.

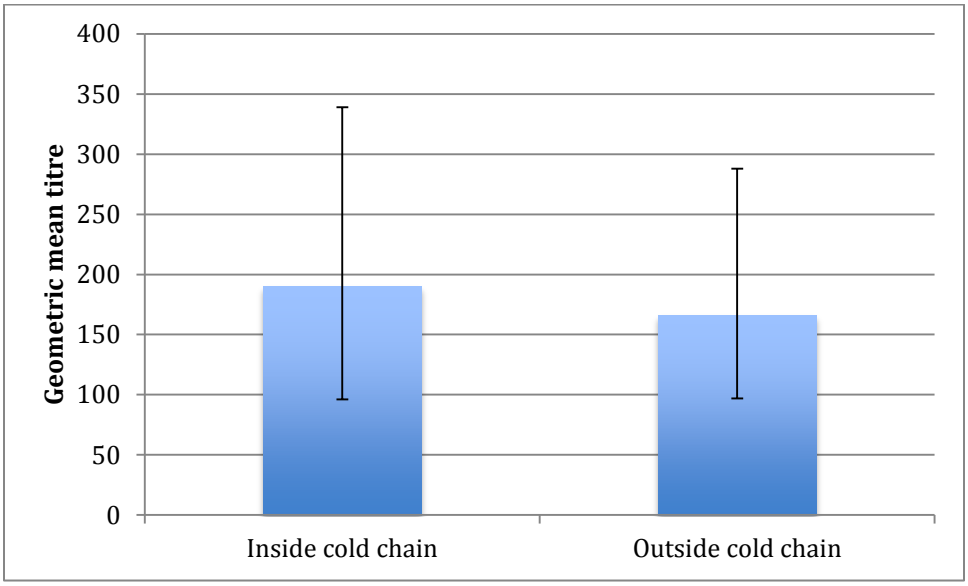

Figure 14b. Average seroconverson from four community-based studies that delivered childhood hepatitis B vaccine after storage inside or outside the cold chain. Error bars show range of values.

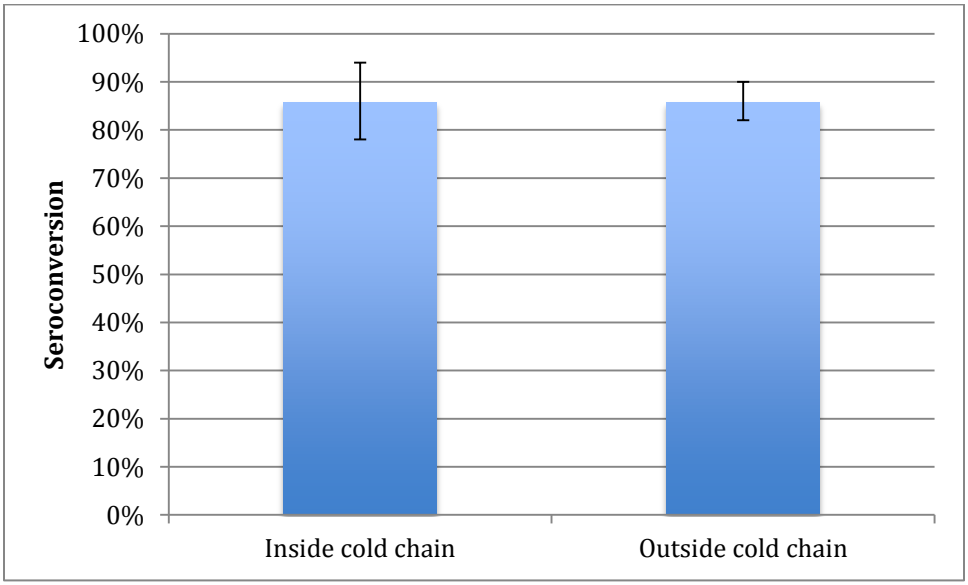

Figure 15. Changes in in-vitro potency of standard and modified (to improve heat thermostability) Shanvac-B hepatitis B vaccine following 1, 3, or 6 months of exposure to 4°C, 37°C, or 45°C.

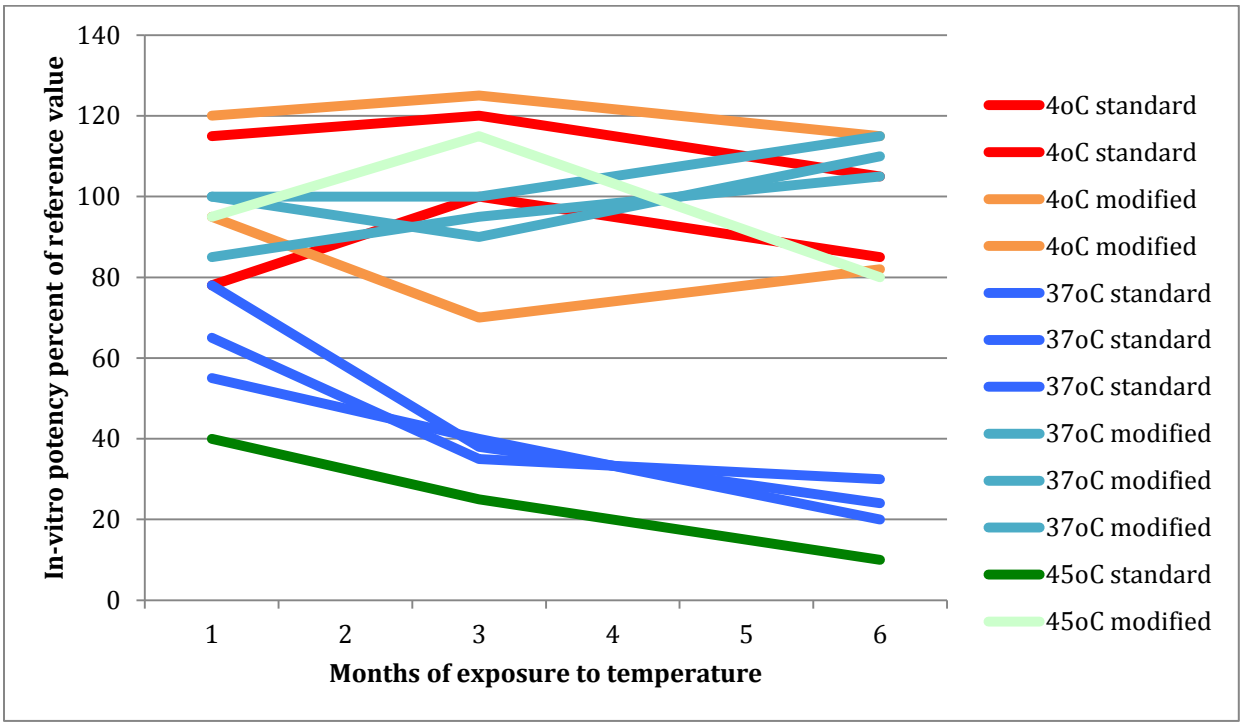

Table 1. Product insert information for monovalent hepatitis B vaccines with an indication during infancy; NR = not recorded and VVM = vaccine vial monitor; all vaccines supplied as 0.5 ml/dose, and all vaccines are recombinant.

Product name (reference)	**Company**	**Hb content mcg**	**Thiomersal**	**VVM**	**Rec. storage temp**	**Specific no freeze statement**	**Shelf life at 2-8°C**	**Heat stability**
Elovac-B*	Indian Immunologicals Ltd	10	0.025 mg		2-8°C	Yes	NR	NR
Energix-B	Glaxo-Smith-Kline	10	NR		2-8°C	Yes	NR	NR
Enivac HB*	Panacea	10	0.025 mg		2-8°C	Yes	36 m	NR
Euvax-B	LG Life Sciences Ltd	10	Yes, in multidose		2-8°C	Yes	36 m	NR
Genevac-B	Serum Institute of India	10	0.005%		2-8°C	Yes	36 m	NR
Heberbiovac HB	Centro de Ingenieria Genetica y Biotecnologia (CIBG)	10	0.025 mg	Yes	2-8°C	Yes	48 m	37°C: 1 m 45°C: 1 w
Hepavax-Gene TF	Janssen Vaccines Corp./Berna Biotech Korea Corp.	10	None		2-8°C	Yes	36 m	NR
Recombivax HB*	Merck	5	NR		2-8°C	Yes	NR	NR

Product name (reference)	**Company**	**Hb content mcg**	**Thiomersal**	**VVM**	**Rec. storage temp**	**Specific no freeze statement**	**Shelf life at 2-8°C**	**Heat stability**
Revac-Bmcf*	Bharat Biotech International	10	None		2-8°C	Yes	36 m	37°C: 1 m 45°C: 1 w
Shanvac-B	Shantha	10	0.025 mg	Yes	2-8°C	Yes	36 m	NR
Vaksin Hepatitis B Rekombinan	Biofarma	10	0.01% wgt/vol	Yes	2-8°C	Yes	NR	NR

*Not World Health Organization pre-qualified

Table 2. For different manufacturers, stability tests performed – other than potency data – during evaluation of monovalent hepatitis B vaccines exposed to temperatures of 37°C or higher. All results are from the end of the testing period. Values in parentheses are specification values, where these were provided.

	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
Temperatures (degrees C)	37, 40, 42, 45	37	37, 45, 70	37	37,45	37	37	37, 45, 60
Exposure time points	1- 6 wks	2-4 wks	1-4 wks 2, 3, 4, 5, 6, 9, 12 mos.	1-5 wks	1, 2, 4 wks (37C); 1 wk (45C)	1, 2, 4 wks	1-5 wks	1, 2, 3, 6 mos (37C); 1, 2 wks (45C, 60C)
Lots tested	2	2	3	3	4 (37C); 3 (45C)	3	3	10 (37C); 2 (45C and 60C)
Appearance	Pass for all temps/lots	Complies for all lots	Not provided	Complies for all lots	Pass for all temps/lots	Not provided	Pass for all lots	Complies for all lots
Particulate matter	Pass for all temps/lots	Not provided	Not provided	Not provided	Not provided	Not provided	Not provided	Not provided
pH	7.0-7.1 (5.4-7.4)	6.8-6.9	5.9-6.3 (5.5-7.0)	7.2 (6.4-7.2)	6.8-6.9 (6.4-7.4)	Not provided	6.9-7.1 (5.4-7.4)	6.55-6.97
Thiomersal content	Thiomersal free	0.0042 % w/v for both	Thiomersal free	0.049-0.051 (0.0425-0.575	<25ng/20ug protein (<25 ng/20	Not provided	0.0096%-0.011%(<=0.012%)	0.030-0.060 mg/20 ug

	A	B	C	D	E	F	G	H
		lots		mg/ml)	ug protein)			HBsAg
Formaldehyde	0.00 for all temps/lots (<=0.1 w/v%)	Not provided	Not provided	Not provided	Not provided	Not provided	<0.0001% for all lots (<=0.01%)	Not provided
Protein	22-23 (<=40ug/ml)	Not provided	Not provided	Not provided	Not provided	Not provided	28.3-31.1 (20.0-31.5 ug/ml)	13-30 ug/ml
Aluminum	0.48-0.49 (<=1.25)	0.65-0.67	Not provided	Not provided	0.48-0.53 mg/ml (0.35 to 0.62mg per ml)	Not provided	0.50-0.58 (<=1.25 ug/ml)	0.38-0.64 mg/20 ug HBsAg
Identity	Identified at all temps	Not provided	Not provided	Not provided	Positive for all lots at 45C	Not provided	Not provided	Identified for all lots
Sterility	Sterile for all temps/lots	Not provided	Not provided	Sterile for all temps	Sterile for all lots	Not provided	Sterile for all lots	Complies for all lots
Bacterial endotoxins (EU/ml)	Not provided	Not provided	Not provided	2.35-4.08 (<=30 EU/ml)	<6.25 EU/ml (<30.0 EU/ml)	Not provided	Not provided	Not provided

	A	B	C	D	E	F	G	H
Pyrogen	Pass for all temps/lots	Complies for both lots	Not provided	Not provided	Not provided	Not provided	Pass for all lots	Complies for all lots
Abn. toxicity: guinea-pig	Pass for all temps/lots	Not provided	Not provided	NA	Pass for all lots at 45C	Not provided	Not provided	Not provided
Abn. toxicity: mouse	Pass for all temps/lots	Not provided	Not provided	NA	Pass for all lots at 45C	Not provided	Not provided	Not provided
Abn. toxicity: animal not spec.	NA	Not provided	Not provided	Complies in all lots	NA	Not provided	Not provided	Not provided
Vaccine vial monitor	Pass at all temps	Not provided	Not provided	Not provided	Not provided	Not provided	Not provided	Not provided
Adsorption rate	99-100% (>=95%)	Not provided	Not provided	98% (>=95%)	Not provided	Not provided	Not provided	98-100%

Table 3a. Characteristics of studies identified in the systematic literature review that provided data on humans.

Reference	**Vaccine**	**Design**	**Age/schedule**	**Days vaccine at ↑ temp**	**Temperature comparison**	**Age/timing testing done**
Hipgrave et al (18)	Plasma-derived; National Institute of Hygiene and Epidemiology, Vietnam; 2.5 mcg HbAg	Non-randomized community allocation with field implementation	Birth, 2m, 3m	Group 1: 1 to 14 days Group 2: 15 to 31 days	Inside cold chain: 4-8°C Outside cold chain: 20-35°C	Age 9-18m, only if all 3 doses received
Wang et al (20)	Recombinant; Beijing Tiantan Biological products; 20 mcg HbAg	Community randomized with field implementation	Birth, 1m, 6m	1m or until VVM indicated discard	Inside cold chain: not stated (occ. <0°C) Outside cold chain: 2-30°C (avg: 16 °C)	Age 7-11m, only if all 3 doses received
Otto et al (37)	Plasma-derived; Korean Green Cross; 5 mcg HbAg	Community randomized with field implementation	Birth, 2m, 4m	Up to 1 month	Inside cold chain: 2-8°C Outside cold chain: <49°C	Age 5m, 28 days after dose 3
Anonymous (43)	Not reported	Not reported	Age 10-20 months; number of doses not reported	Not reported	Refrigerated: no temp. reported Not refrigerated: no temp. reported	Not reported

Reference	Vaccine	Design	Age/schedule	Days vaccine at ↑ temp	Temperature comparison	Age/timing testing done
Just et al (36)	Recombinant; Energix B, GSK; 20 mcg HbAG	Individually randomized, experimental	Avg age 23 yrs; vaccine at 0-1-6m	1 week	4°C 37°C	2-6-7m after study start
Van Damme et al (38)	Recombinant; Energix B, GSK; 20 mcg HbAG	Individually randomized, experimental	Age 18 to 30 yrs; vaccine at 0-1-6m	1 month	4°C 37°C 45°C	2-7-12m after study start

Table 3b. Results of studies identified in the systematic literature review that provided data on humans for vaccine inside the cold chain (ICC) compared to outside the cold chain (OCC) or comparing a fixed standard refrigeration temperature to a higher temperature.

Reference	**Group**	**Enrolled/tested**	**Geometric mean titers (mIU/ml)**	**% seroconversion**	**Adverse events /reactogenicity**
Hipgrave et al (18)	1: ICC	358/unknown	135	78%	Not reported
	2: OCC for 1-14 days	748 total groups 2 and 3/unknown	111	84%	
	3: OCC for 15-31 days		115	83%	
Wang et al (20)	1: ICC ampule	401/random sample of 200	96	89%	Passive assessment; no reported adverse events
	2: OCC ampule	391/random sample of 200	93	91%	
	3: OCC Uniject	410/random sample of 200	102	89%	
Otto et al (37)	1: ICC syringe	66/57 for seroconv., 55 for GMT	376	95%	Not reported
	2: ICC Uniject	98/83 for seroconv., 75	312	93%	

Reference	Group	Enrolled/tested	Geometric mean titers (mIU/ml)	% seroconversion	Adverse events /reactogenicity
		for GMT			
	3: OCC Uniject	103/93 for seroconv., 87 for GMT	288	88%	
Anonymous (43)	1: ICC	Not reported/232		82%	Not reported
	2. OCC	Not reported/358		82%	
Just et al (36)	1: 4°C	31/31	21/54/2054†	100%	Not reported
	2: 37°C	27/27	29/71/3392†	96%	
Van Damme et al (38)	1: 4°C	Unknown; 45/33/36 at 2/7/12 months‡	65/10359/2018‡	100%/100%/100%‡	Any: 68%; soreness: 32%; induration: 6%
	2: 37°C	Unknown; 44/37/36 at 2/7/12 months‡	48/5937/1527‡	93%/100%/100%‡	Any: 67%; soreness: 32%; induration: 12%
	3: 45°C	Unknown; 46/39/37 at 2/7/12 months‡	44/6813/1043‡	87%/95%/97%‡	Any: 58%; soreness: 31%; induration: 9%

*Seroconversion based on having anti-HBs ≥10mIU/ml except reference 43 (not reported) and reference 38 (≥1mIU/ml)

†GMTs at 2 months (1 month after dose 2); 6 months (just before dose 3); 7 months (1 month after dose 3)

‡Results at 2 months (1 month after dose 2); 7 months (1 month after dose 3); 12 months (6 months after dose 3)

Table 3c. Quality assessment of studies identified in the systematic literature review.

Reference	**Assessment tool**	**Score**	**Comments**
Hipgrave et al (18)	Newcastle-Ottawa Scale	6 of 9	• Study groups not drawn from same communities. • No information collected or adjustments done to account for potential differences between groups that could affect antibody response.
Wang et al (20)	Cochrane Collaboration	High risk of bias	• Unknown method of randomization • Blinding not done • Unknown if laboratory staff blinded • Serology done only for those with 3 doses, who may differ from other children • Children in outside the cold chain group could have received only inside the cold chain vaccine if born in hospital and no data reporting the proportion in which this occurred
Otto et al (37)	Cochrane Collaboration	Moderate risk of bias	• Unknown method of randomization • Blinding not done • Unknown if laboratory staff blinded • Moderate drop-out (up to 77% depending on group and analysis) with no explanation of reasons for drop-out
Anonymous (43)	Newcastle-Ottawa Scale	0 of 9	Little information on methodology presented.
Just et al (36)	Cochrane Collaboration	Low-moderate risk of bias	• Unknown method of randomization • Blinding not done • Unknown if laboratory staff blinded
Van Damme et al (38)	Cochrane Collaboration	Moderate risk of bias	• Unknown method of randomization • Blinding not done • Unknown if laboratory staff blinded • Moderate drop-out (up to 73% depending on group and analysis) with no explanation of reasons for drop-out

Table 4a. Characteristics of studies identified in the systematic literature review that provided data on animals.

Reference	**Vaccine**	**Temperature comparison and sample size**	**Design**	**Days vaccine at ↑ temp**	**Age/schedule for in-vivo testing**	**Timing in-vivo testing done**
Braun et al (16)	Recombinant; ShanvacB Shantha; 20 mcg HbAg for in-vitro testing and 2 mcg for in-vivo testing; experimental vaccine with 20% propylene glycol, 40mM phosphate, 40mM histidine	4°C vs. 37°C ShanvacB standard and modified for in-vivo	In-vitro potency In-vivo potency in mice (8 randomized per group)	12m for in-vivo 1-2-3-6-12m for in-vitro	5-7 week old mice; vaccine at 0 and 28 days	Day 42 after vaccine dose 1
Chen et al (17)	Recombinant; ShanvacB Shantha; 20 mcg HbAg for in-vitro testing and 2 mcg for in-vivo testing; experimental vaccine spray-dried with trehalose, mannitol, and NaH_2PO_4	4°C vs. 37°C ShanvacB standard and modified for in-vivo	In-vitro potency In-vivo potency in mice (8 randomized per group)	15m for in-vivo 1-2-3-6-18m for in-vitro	5-7 week old mice; vaccine at 1, 28 days	Day 28, 42 days after vaccine dose 1
Jezek et al (19)	Recombinant; ShanvacB Shantha; 20 mcg HbAg for in-vitro testing and unknown concentration for in-vivo testing; experimental vaccine with 40mM phosphate, 40mM histidine, at pH 5.2	4°C vs. 37°C ShanvacB standard and modified for in-vivo 37°C vs. 45°C ShanvacB standard and modified for in-vitro	In-vitro potency In-vivo potency in mice (randomization and size not reported)	6m for in-vivo 1-3-6m for in-vitro	Age of mice not reported; vaccine schedule not reported	Not reported

Table 4b. Results from studies identified in the systematic literature review that provided data on animals; all data are estimated from presentation on graphs in original manuscripts except reference 17 seroconversion and reference 19 relative in-vivo potency

Reference	**Group**	**Relative in-vitro potency***	**GMT (ref 16 and 17) or relative in-vivo potency (ref 19)**	**Seroconv.**	**Adverse events/ reactogenicity**
Braun et al (16) †	1: 4°C ShanvacB standard	115%/115%/120%/105%/100%	10000		No sig. local or systemic symptoms in rabbits
	2: 37°C ShanvacB standard	115%/115%/125%/110%/105%	100		
	3: 4°C ShanvacB modified	120%/110%/125%/115%/115%	12000		
	4: 37°C ShanvacB modified	100%/125%/100%/115%/80%	9000		
Chen et al (17)‡	1: 4°C ShanvacB standard	78%/90%/100%/85%/115%/100%	100,000	88% post dose 1; 100% post dose 2	No data
	2: 37°C ShanvacB standard	78%/72%/38%/24%	60,000	13% post dose 1; 100% post dose 2	
	3: 4°C ShanvacB			75% post dose 1;	

Reference	Group	Relative in-vitro potency*	GMT (ref 16 and 17) or relative in-vivo potency (ref 19)	Seroconv.	Adverse events/ reactogenicity
	modified 4: 37°C ShanvacB modified	95%/100%/70%/82%/100%/80% 100%/118%/90%/110%/78%/100%	200,000 90,000	100% post dose 2 100% post dose 1; 100% post dose 2	
Jezek et al (19)§	1: 4°C ShanvacB standard 2: 37°C ShanvacB standard 3: 45°C ShanvacB standard 4: 4°C ShanvacB modified 5: 37°C ShanvacB modified 6: 45°C ShanvacB modified	65%/35%/30% 40%/25%/10% 85%/95%/105%	113% 86% 100% 111%		No data

Reference	Group	Relative in-vitro potency*	GMT (ref 16 and 17) or relative in-vivo potency (ref 19)	Seroconv.	Adverse events/ reactogenicity
		95%/115%/80%			

*References 16 and 17 report relative potency compared to baseline, standard vaccine at 4°C; reference 19 reports relative loss of potency for each vaccine formulation and temperature. potency compared to modified vaccine at 4°C after all vaccines stored for 6 months at indicated temperature.

†In-vitro potency measured at 1, 2, 3, 6, 12 months of temp. exposure; GMT measured at 12 months temp. exposure and 42 days post-vaccination

‡In-vitro potency measured at 1, 2, 3, 6, 18, and 24 months of temp. exposure (group 2 only had first 4 measurements); GMT measured at 15 months temp. exposure and 28 and 42 days post-vaccination

§In-vitro potency measured at 1, 3, 6 months of temp. exposures; relative in-vivo potency measured at 6 months temp. exposure