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Hepatitis B Potency Testing Standardization

Report of a Meeting
WHO/SEARO, New Delhi, 9- 11 April 2001

WHO Project: ICP VAB 001

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Annexes

1. INTRODUCTION

This is the first time that a regional WHO meeting on Standardization of Potency Testing of Hepatitis B Vaccines (HBV) was held in SEARO, New Delhi. A unique feature of the meeting was that representatives from the manufacturers of HBV from all the producing countries (having in place an established National Regulatory Authority (NRA) for vaccines) and their respective National Control Lab (NCL) staff participated.

A total of 26 participants from four Member Countries of the Region attended the meeting. For programme of the meeting see Annex 1. The list of participants and faculty members is at Annex 2.

Five manufacturers from India, one manufacturer each from Indonesia and Myanmar and the NCL staff from all the four vaccine-producing countries including Thailand participated in the meeting.

2. OBJECTIVES

The objectives of the meeting were:

(1) To discuss all issues related to the potency testing of Hepatitis B vaccines, and

(2) To standardize Hep B potency testing methods and standards used by the manufacturers and National Control Laboratories.

3. INAUGURATION

Welcoming the participants, Dr Palitha Abeykoon, Director, Department of Health Technology and Pharmaceuticals, WHO Regional Office for South-East Asia said that, presently four out of the ten Member Countries in the Region, namely Bhutan, Indonesia, Maldives and Thailand had already included HBV in the EPI schedule and under the initiative of GAVI, more countries in the Region were likely to introduce it shortly. India was likely to introduce it as an EPI vaccine gradually and in a phased manner.

Dr (Ms) Nora Dellepiane, WHO-Headquarters was nominated the Chairperson and Dr (Ms) Jaspal Sokhey, WHO-SEARO the Rapporteur.

4. BACKGROUND

Dr Nora Dellepiane, WHO-HQ said that during the recent inspection of vaccine manufacturers for their prequalification to supply vaccines to UN Agencies, the problems faced by the manufacturers and the NCLs in the standardization of potency testing of HB vaccines by *in vivo* method and, in changing from *in vivo* method of testing to the *in vitro* method came to light.

The WHO procedure as described in WHO/VSQ/97.06 for assessing the acceptability, in principle, of vaccines for purchase by UN Agencies or the prequalification of manufacturers to supply vaccines to UN Agencies was explained in detail by Dr Nora Dellepiane. It was stated that, WHO had recently introduced an additional requirement in the initial evaluation procedure for "Prequalification of manufacturers to supply vaccines to UN Agencies" of full evaluation of the NRA of the country against the indicators of the six NCA functions. However, for the reassessment evaluation (which is done every two years), the requirement for site visit to the manufacturer jointly with the NRA could be waived if a set of specified criteria are met.

5. *IN VIVO* VS *IN VITRO* METHODS OF POTENCY TESTING

5.1 Background

Following the two very exhaustive talks on "Hepatitis B Vaccine Standardization: A Historical Perspective" and "Establishment and Replacement of Standards for Use in the Control of Hepatitis B Vaccines" delivered by Dr Morag Ferguson, all the participants actively participated in the discussions, and clarified their doubts. It was realized by all that, it was important that the clinical trials are conducted by the manufacturers with their respective Primary Working Standard (WS) for evaluating the safety and efficacy of the vaccine. The clinical trial data is to be submitted by the manufacturer to the NRA and WS is to be shared by the manufacturer with the NCL for the potency testing of the final vaccine. At the time of replacement of the Primary WS with the Secondary WS, it was agreed that, the *in vivo* method of potency test be retained.

There are two methods available for the potency testing of HBV i.e. the *in vivo* and *in vitro* methods. To obtain comparable *in vitro* potency results between the manufacturer and the NCL, it is important that both adopt the same procedure and method of testing.

5.2 Manufacturers' Data on Characterization of the Vaccine

Presentations were made by all the seven manufacturers of HBV outlining the characterization of the vaccines produced by them in the Region. Annex 3 gives the comparison of the source of vaccine, yeast, adjuvant, shelf life, clinical trial data and other details as presented by the manufacturers. As can be seen, all manufacturers from the countries represented are producing the recombinant HBV except in Myanmar, where presently plasma-derived HBV is produced. But within the next 2-4 years, Myanmar is likely to start the manufacture of recombinant HBV with transfer of technology from the Republic of Korea.

5.3 Conclusions

It was agreed that for switching over to the *in vitro* method of potency testing on the final vaccine, it (*in vitro* method) should be properly validated and parallel testing done by both the methods (*in vivo* and *in vitro*) initially to gain experience and confidence in obtaining comparable results.

At least four dilutions of the test vaccine and WS be used in the performance of the *in vitro* test against the one or two dilutions as presently used by some manufacturers and the NCLs.

6. ISSUES FOR CONSIDERATION ON *IN VITRO* AND *IN VIVO* POTENCY TESTS

The participants were divided in two working groups (A & B) to discuss at length the issues for consideration on *in vivo* and *in vitro* potency tests, given in Annex 4. In group A were participants from M/s Shantha Biotech, M/s Bharat Biotech and Panacea Biotech and group B included participants from M/s Wockardt, Serum Institute of India, Biofarma and Myanmar. Both groups were assisted by the facilitators during the discussions on the procedure of potency testing of the vaccine by *in vivo* and *in vitro* methods, validation of the test methods, WS, replacement of the WS by the secondary WS and clinical trial material etc.

6.1 Working Standards

It was unanimously agreed that the WS of the manufacturer is part of the same batch of HBV which has been used as clinical trial material demonstrating the safety and efficacy of the vaccine with known potency value. It is useful and economical to produce a large size batch of the vaccine for such activities and also to conduct the stability studies for assigning the shelf life of the vaccine. More information will be collected and provided by the manufacturers to WHO experts on assigning the shelf life (if any) to the WS. It is important that the Primary WS be replaced with the Secondary WS by calibrating the latter with the Primary WS and using the *in vivo* method of testing. It was emphasized that the *in vivo* test be retained and performed when the Primary WS is replaced with the Secondary WS.

6.2 International Reference Reagent

The International Reference Reagent (IRR) should not be used as WS for batch release, as no specification is established for the individual recombinant vaccines. There is no single Reference Standard available for HB vaccines for the simple reason that each vaccine produced is a specific product of the manufacturer. Hence, the use of homologous WS is absolutely critical in the potency testing of the vaccine.

6.3 International Standard 80/549

The International Standard (IS) 80/549 which is non-adjuvanted and is a dilution of partially purified plasma-derived HBsAg in PBS/BSA is not suitable for use in *in vitro* potency testing of adjuvanted vaccines.

6.4 Conclusions

(1) Each manufacturer's product is a specific product and the NCL should receive the WS from the manufacturer for testing the product of the respective manufacturer.

(2) Primary WS could be replaced with the Secondary WS provided the latter's method of preparation, validation and testing is the same as that of the Primary WS. However, the conduct of additional clinical trials is not required for the Secondary WS if it is calibrated against the Primary

WS (clinical trials conducted) using the same method of testing i.e. the *in vivo* method. It is imperative that the WSs are always tested by *in vivo* test.

(3) *In vitro* method of potency testing of the vaccines could be adopted only after it has been proper validated with the *in vivo* method.

7. STANDARDIZATION OF r HBV

Points to consider for the standardization of recombinant HBV included in the monograph on recombinant Hep B were discussed in great detail (see Annex 5). Some changes in the text of this Annex and addition of more details are likely to be included by Dr Morag Ferguson and Dr Nora Dellepiane after having discussions with their colleagues both at NIBSC, London and WHO-HQ. A revised version of the Annex will be circulated later on to all the participants for information and review.

8. VACCINE VIAL MONITORS (VVMS)

So far, the manufacturers of HBV in the Region have no experience with VVMs. However, the specifications for VVMs available for various vaccines were discussed. It is important that the appropriate VVM is selected and used for the vaccine by the manufacturer.

9. RECOMMENDATIONS

(1) The NCL should obtain the WS from the manufacturer for testing the potency of the final vaccine.

(2) The manufacturer should replace the Primary WS with the Secondary WS only after the latter has been calibrated against the Primary WS that has been earlier used (as clinical trial material) in the clinical trials.

(3) *In vivo* test to be retained by the manufacturer for the potency testing of the Secondary WS.WHO-HQ will propose revision of Hepatitis B vaccine monograph to the Expert Committee on Biological Standardization.

(4) NIBSC, UK will review the use of IRR.

Annex 1

PROGRAMME

Monday, 9 April 2001

9:00-9:30 hrs	Opening of workshop HTP, WHO/SEARO- Dr Palitha Abeykoon	
9:30- 10:15 hrs	Aims of the workshop Overview of the pre-qualification process - Dr Nora Dellepiane- WHO/HQ	
10:15-10:30 hrs	Discussion	
11:00-11:45 hrs	Hepatitis B vaccine standardization: A historical perspective - Dr Morag Ferguson- Consultant SEARO	
11:45-12:00 hrs	Discussion	
13:00-14:00 hrs	Establishment and replacement of standards for use in the control of hepatitis B vaccines - Dr Morag Ferguson- Consultant SEARO	
14:00-15:00 hrs	Presentations by manufactures on in vivo potency tests and the choice, standardization and use of reference vaccine, correlation with clinical efficacy, selection of the antigen concentration in an adult dose	
	- Shantha Biotech - Bharat Biotech	
	- Panecea Biotech	
	- Wockhardt	
	- Serum Institute of India	Manufacturers
15:30-16:00 hrs	Continuation of presentations by manufacturers Serum Institute of India	Manufacturers
16:00-17:00 hrs	General discussion, input of National Regulatory Authorities from the Region	

Tuesday, 10 April 2001

9:00-9:30 hrs	Recap: Dr Jaspal Sokhey	SEARO

9:30-10:30 hrs	Development and validation of in vitro potency tests for hepatitis B vaccines Dr Morag Ferguson - Consultant SEAROS
11:00-12:00 hrs	General discussions on the development of in vitro potency tests
13:00-14:00 hrs	Presentation by manufacturers on their experience in establishment of an in vitro potency assay
14:00-16:30 hrs	Working groups The participants in three working groups to review the data presented by manufacturers (both for in vivo and in vitro) and make comments on aspects correctly addressed and gaps in: • Correlation between the in vivo test and clinical efficacy. • Potency testing in vivo and in vitro. • Establishment and replacement of standards Facilitators: Dr. Ferguson, Sokhey and Dellepiane
16:30-17:00 hrs	VVMs on all vaccines: Aspects for consideration. Dr Nora Dellepiane - WHO/HQ
17:00-17:15 hrs	Discussion

Wednesday, 11 April 2001

9:00- 10:00 hrs	Presentations from manufacturers on how the meeting has helped them address issues. What they have learned and how to approach the standardization of hepatitis B vaccines
10:30-12:00 hrs	Review of Draft Points to consider - All participants
13:00-15:00 hrs	Finalization of Points to consider- All participants
15:30-16:00 hrs	General discussion on issues to consider for the production of DTP-Hep B combination vaccines in the region.
16:00 hrs	Closure

Annex 2

LIST OF PARTICIPANTS

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Annex 3

CHARACTERIZATION OF VACCINES PRODUCED IN SEAR

	Shanta Shanvac B	Bharat	Panacea Enivac	Wockardt Biovac B	Serum Institute of India Genevac B	Biopharma	Myanmar
Source of vaccine construct	Shanta	Bharat	Filling of adjuvanted bulks from CIGB Cuba	Rhein Biotech, Germany	Rhein Biotech, Germany	Formulation and Filling of bulks produced by KGC (Rhein Biotech, Germany) Final bulk formulated at Biopharma	NY Blood Centre (plasma derived). Transfer of technology for rHBV from Republic of Korea
Yeast species	Pichia pastoris	Sacc.cerevisiae	Pichia pastoris	Hansenula	Hansenula	Hansenula	Plasma (Proposed Hansenula from Republic of Korea
Formulation	Based on protien	HBsAg internal standard, target 20µg HBsAg	20 ug	Protien	20 ug	20 ug	10 ug
Adjuvant	Aluminium hydroxide	Aluminium hydroxide	Aluminium hydroxide	Aluminium hydroxide	Aluminium hydroxide	Aluminium hydroxide	Aluminium hydroxide
Thiomersal	0.05 mg/ml	0.05 mg/ml	0.05 mg/ml	0.05 mg/ml	0.05 mg/ml	0.05 mg/ml	0.05 mg/ml

	Shanta Shanvac B	Bharat	Panacea Enivac	Wockardt Biovac B	Serum Institute of India Genevac B	Biopharma	Myanmar
Dose (adult/ paediatric)	20µg/10µg Pediatric up to 10 yrs	20µg/10µg Pediatric up to 10 yrs	Pediatric up to 10 yrs (CIGB data support up to 19 yrs, submitted to DCGI) 20µg/10µg	20µg/10µg up to 10 yrs	20µg/10µg up to 10 yrs	20µg/10µg up to 10 yrs	10/5 ug up to 10 yrs
Immunisation schedule	0,1,2 and 12 months or 0,1 6 months	0,1,2 months or 0,1 6 months	0,1 2 and 12 months, 0,1,6	0,1,6	0,1,6 and 0,1,2 and 12 months	2, 3, 4 months 0, 2, 3 months	0, 1, 2
Shelf life	3 years	3 years	3 years	2 years	(non-adjuvanted bulks 2 years) vaccine 2 years	2 years	3 years
Clinical trials	Highly immunogenic, 100 % seroconversion	Tested in CT. At least 98% seroconversion	95% seroconversion in infants, 100% in healthy young adults. GMT>cf Engerix.	98.3 % seroconversion	95.8% to 98.4 % seroprotection in different studies (i.e. >10 mIU)	Infants newborn or 2 months. 0,2,3 96% seroconversion, both in vials and in uniject	500 volunteers, 90-94 % seroconversion
In vivo potency test specification	Upper confidence interval>1.0	Mean relative potency>1.0	potency upper confidence limit >1.0 (set by CIGB)	Upper confidence interval>1.0	Upper confidence interval>1.0	Upper confidence interval>1.0	Mean relative potency of 1

	Shanta Shanvac B	Bharat	Panacea Enivac	Wockardt Biovac B	Serum Institute of India Genevac B	Biopharma	Myanmar
Mouse strain/sex	Balb C 5week old females	Balb C	Balb C, 5 weeks, females	Balb C, 14-16g, 4-5wk, both sexes	Balb C, 4-5 weeks, 14-16 g, both sexes	Balb C, both sexes, 4-5 weeks	ICR, 5 weeks both sexes
Number per dilution/ dilutions	At beginning 20; now 10 1:32,1:64, 1:128, 1:256	5 male, 5 females doubling 1/16 to 1/128	10 mice, 1:16,1:64, 1:256, 1:1024	12, 125-1000ng	10 male, 10 females; 1:16,1:32, 1:64,1:128,	20 mice, 1:4,1:16,1:64, 1:256,	15 mice, four fold dilutions
Diluent	Containing $Al(OH)_3$	Containing $Al(OH)_3$	Containing $Al(OH)_3$	Containing $Al(OH)_3$	Containing $Al(OH)_3$	Containing $Al(OH)_3$	Containing Al(OH)3
Cut-off	Based on mean response of control mice + 2sd	10mIU, (Abbott quantitation panel)	Based on mean response of control mice + 2 sd	Based on mean response of control mice+ 2sd	Based on mean response of control mice+2sd	Based on mean response of control mice	Based on mean response of control mice + 2 sd
Antibody assay kit used	AUSAB	AUSAB	AUSAB	AUSAB	AUSAB	AUSAB	AUSAB
Reference used	IRR but now product specific	IRR but now product specific	CIGB standards New Ref prep.received. Tested in clinical trials? Specifications?	Initial clinical trial batch, then new batch compared with previous standard in 10 in vitro and 7 in vivo potency tests	Tested in clinical trials	KGC references	IRR

	Shanta Shanvac B	Bharat	Panacea Enivac	Wockardt Biovac B	Serum Institute of India Genevac B	Biopharma	Myanmar
Characterisation	Tested in clinical trials Potency of clinical lots to be checked	Tested in clinical trials Working std included in CT. Data to be circulated	Tested in clinical trials Lots produced in new facility?			Tested in clinical trials, both in vials and in uniject	
Clinical trials	Highly immunogenic, 100% seroconversion		95% seroconversion in infants, 100% in healthy young adults. GMT> cf Engerix.		95% seroprotection (ie >10mIU), GMT9200	Infants newborn or 2months. 0,1,2 96% seroconversion	500 volunteers, 90-94% seroconversion
In vitro potency test - assay kit							
Pre-treatment step							
Specification							

Annex 4

ISSUES FOR CONSIDERATION

In vivo test

- Demonstrate consistency of products against the product-specific working standard
- Show that the existing specification is meaningful either by using existing or new data (as example upper confidence limit). This is basically a retrospective validation.
- Develop a working standard checked in clinical trials, or a secondary standard calibrated against a standard checked in clinical trials.
- Need to go through immunogenicity studies as characterization of the vaccine and the standard and use it always when a new standard is being set.
- Consider review of cut-off if criterion used is different from negative control mice OD

In vitro test

- Is it validated? Is it suitable for your specific product?
- Changes in Abbott kit
- Correlation against immunogenicity test.

Annex 5

POINTS TO CONSIDER IN THE STANDARDIZATION OF HEPATITIS B VACCINES

Recombinant hepatitis B vaccines were licensed in the mid-1980s. WHO held two meetings to review progress in their development and formulate requirements. WHO requirements for hepatitis B vaccines made by recombinant techniques in yeast were published in 1987 and revised in 1989 to include all vaccines produced by recombinant DNA techniques. Although these requirements provide guidance on the characterization and tests to be undertaken, it is the responsibility of the NRA to approve many aspects of production and characterization of the antigen and also several quality control test methods and specifications.

Recombinant hepatitis B vaccines produced by different manufacturers must be considered as different products. Both manufacturers and NRAs have queried many aspects of the characterization and quality control tests to be undertaken on individual vaccines. This document has been produced to highlight points that must be considered in evaluating the immunogenicity and efficacy of a recombinant hepatitis B vaccine, testing vaccines for potency and the establishment of suitable reference preparations.

Evaluation of vaccines in clinical trials

According to the WHO requirements for recombinant hepatitis B vaccines, a vaccine will 'reliably induce antibody responses to HBsAg in human recipients and the frequency and titre should be at least equivalent to those induced by plasma derived vaccines which meet WHO requirement'. The NRA is required to approve the data showing that the vaccine produces and adequate antibody response (titre, duration and quality) in human beings.

There is no minimum requirement for antigen content of a hepatitis B vaccine and based on the results of clinical trials, manufacturers should establish the protein /antigen content of their vaccine.

Points to consider:

- Assay of antibody responses to HBsAg in human recipients
- Cut-off value for sero-conversion
- Standard included in assays
- Titre and sero-conversion rates

Formulation

According to the WHO requirements for recombinant hepatitis B vaccines, final adjuvanted vaccine bulks should be produced from aqueous bulks, which meet the specifications of all QC tests undertaken. Antigen purified by different methods and produced on different substrates may react differently when assayed using different HBsAg detection kits. The International Standard for HBsAg (80/549) was established for use in the determination of the sensitivity of assay kits for the detection of HBsAg. It is assigned a unitage in International Units, and although 1 IU can be considered as approximately equal to 1ng, this standard is not ideal for evaluating the HBsAg content of a vaccine in ng. Manufacturers should use a highly purified preparation of known protein content for this purpose and establish an in-house standard against which the protein content of future batches could be determined.

The HBsAg content of the aqueous bulk should be determined by appropriate methods and by the lower limit of the ratio of HBsAg to total protein approved by the NRA.

Points to consider:

- Assay of the antigen content of non-adjuvanted bulks – standard used.
- Formulation of their vaccine - is this based on the protein content of the vaccine or on the results from assays of HBsAg
- Monitoring of consistency of production of non-adjuvanted bulks through the protein/antigen ratio

Potency testing

When WHO requirements for recombinant hepatitis B vaccines were published, it was considered that assays of antigenicity of adjuvanted vaccines

would be difficult to standardize. It was proposed that the content of pure HBsAg in the product should be used as the basis for comparing immunogenicity in mice and responses in human subjects. The relationship between antigenicity of the product in *in vitro* tests, in mouse immunogenicity tests and in human beings should therefore be established. The potency requirement stated 'that an appropriate quantitative test for antigen content and an immunogenicity assay be performed on samples representative of the final filling lot. The vaccine potency should be compared with that of a reference preparation and the NRA shall determine the lower limit of potency'.

Several manufacturers have since developed and validated *in vitro* potency tests which are suitable for use with their individual vaccines. Mouse potency tests are no longer being performed by these manufacturers on every final lot. Since the vaccines in question were well established and had been used in millions of individuals, the WHO requirements for recombinant vaccines were therefore modified by ECBS in October 1997 to permit the use of an *in vitro* test that has been validated in correlation with immune response in humans or with the results obtained in mouse immunogenicity tests for which an appropriate specification has been set by a manufacturer.

The requirement for potency testing now reads: 'The vaccine shall be identified as envelope antigens of hepatitis B virus by appropriate methods. An appropriate quantitative test for potency by *an in vitro* or *in vivo* method shall be performed on samples representative of the final filling lots. Any in vitro method shall be appropriately validated and the test method approved by the NRA. The vaccine shall be compared with a reference vaccine and the NRA shall establish limits of potency'.

Because of the diversity in the reactivity of vaccines containing HBsAg produced by different manufacturing processes and to which adjuvants have been added by different methods, it was considered unlikely that a single international standard would be suitable for the standardization of vaccines in both *in vivo* and *in vitro* assays. Manufacturers were encouraged to consider establishing a product-specific reference which could be related to the immunogenicity of the vaccine in humans. This vaccine will serve as a working standard and be included in all potency tests.

The International Reference Reagent for plasma-derived vaccine was shown in a collaborative study to be suitable for use as reference preparation in mouse immunogenicity tests on recombinant vaccines. This reference reagent has no assigned unitage and although the potency of successive production batches of a given product should give consistent potencies

relative to this material, it has always been emphasized that hepatitis B vaccines which are suitable for use in man need not be qualitatively equivalent to this reference reagent. In addition, there is not necessarily a constant relationship between the immunogenicity in animal tests of hepatitis B vaccines prepared by different procedures and their immunological potential in man.

In vivo potency tests

A suggested test in mice is described in the WHO requirements. This test involves the immunization of groups of mice with dilutions of test and reference vaccines and bleeding them out four weeks later. Individual sera are assayed for anti-HBs and the mice scored positive or negative for anti-HBs. The potency of the test vaccine relative to the reference is calculated by statistical analysis.

Points to consider

- Strain and sex of mice used must give a suitable dose response to the reference and test antigen
- Number of mice per dilution
- Diluent
- Number of dilutions
- The concentrations of vaccine tested should be selected to permit the calculation of the dilution giving 50% seroconversion ie ED_{50}
- Assay of sera
- Calculation of the cut-off value
- Statistical analysis
- Interpretation of results - ED_{50}/relative potency
- Specification

In vitro potency tests

In vitro potency tests should be able to distinguish vaccines of low potency, which may affect the immune response in man. Several factors must be considered when validating an assay for an individual vaccine, as vaccines

contain antigen manufactured by different processes and different forms of adjuvant. These include the need for a pre-treatment step to disaggregate the antigen, eg with detergent, to ensure consistent responses in vaccines of all ages, the HBsAg test kit used, the diluent in which dilutions of the vaccine are prepared and the reference preparation.

Test and reference vaccines should be tested concurrently in an assay designed to produce dose-response curves suitable for quantitative analysis by an appropriate statistical method (eg parallel line analysis). An assessment of statistical validity of the assay, an estimate of the potency of the test vaccine relative to the reference and a measure of the assay precision (confidence interval) should be produced.

Points to consider

Test method

- Documentation of test method
- Selection of test kits and how to use for quantitation
- Diluent
- Need for pre-treatment to disaggregate adjuvanted antigen
- Statistical analysis
- Assessment of statistical validity of the assay
- Precision (confidence intervals) of assay

Establishment of test specification based on correlation with mouse potency data or response in man or on assay results from a series of batches, which pass the mouse immunogenicity test

Demonstration that a batch which fails *in vivo* test would also fail the in vitro test

Validation of in vitro potency tests

- Linearity /Parallelism/Specificity/Robustness
- Linearity - 3 lots /6 independent runs
- Parallelism - acceptable parallelism to standard
- Reproducibility- 3 lots /6 independent runs/Multiple operators

Expect to be within ?+/- 15% of mean potency and for each dilution, expect errors in the preparation of dilutions to be ?+/- 10% response for each dilution

Standard

As per WHO requirements, vaccine potency assayed in *in vivo* and *in vitro* assays should be compared with a reference preparation. As indicated above, an International Reference Preparation has been established, but no unitage is assigned to this material and it cannot be used for the establishment and calibration of secondary standards. There is no requirement that vaccines, which are suitable for use in man, need be qualitatively equivalent to this reference reagent. Although the potency of successive production batches of a given product should give consistent potencies relative to the International Reference Reagent, manufacturers should consider establishing a product-specific reference which could be related to the immunogenicity of the vaccine in humans. This vaccine will serve as a working standard and be included in all potency tests.

Points to consider

- Source
- Characterization
- Immunogenicity in man
- Stability studies

Replacement of standard

The standard should be replaced before it starts showing loss of activity. The shelf-life of standard could be longer than the shelf life of vaccine, if data to demonstrate stability of an individual vaccine for this period are available. The shelf life should be established under defined storage conditions and maintenance of sterility. Real time studies on vaccines should be supported by accelerated degradation studies

A replacement working standard should be a typical batch of vaccine preferably of similar potency to the previous standard. If not, an adjustment factor may have to be incorporated into potency calculations if the specification is based on relative potency; or the specification may have to be amended. For example, if current reference has an assigned potency of 1.0

and the replacement a potency of 1.2 relative to these standard, vaccines will be of lower potency relative to the replacement

Points to consider

- Documentation of the procedure for replacing standards
- Evaluation of stability of standards and establishment of shelf-life
- Identification of loss of potency e.g. lower responses in potency tests changes in dose response curves.
- Definition of acceptable potency loss (e.g. mean initial potency minus 3 standard deviations)
- Full characterization available in summary batch protocol
- Calibration Vs previous standard in *in vivo* and *in vitro* tests

References

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