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WHO International Standards/Reference Reagents

Submission to ECBS of post-establishment stability evaluation

1st IS for Inactivated Hepatitis A Vaccine

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Code 95/500

Project leader	Established by Dr David Wood. Current NIBSC custodian Gillian Cooper
Date of evaluation	May 2011
Date of establishment	1999
Principle application(s)	The role of the standard is for calibration of secondary reference materials to assign immunogenicity and antigen content in International Units. The assigned unitage of 95/500 is 100 IU/ampoule for both immunogenicity and antigen content The WHO report of this study is BS/99.1914
Summary of Stability data obtained at establishment	Stability samples were laid down at the time of production but no evaluation of stability was performed for the establishment report. The stability evaluation for this report only covers antigen content.
ECBS notes/follow-up recommendations at establishment	The ECBS in 1999 recommended ongoing stability monitoring as this preparation was a liquid form and as predictions of long-term stability were not available because of the inherent variability of the assays.
Summary of post establishment stability evaluation	Samples tested for antigen content by comparison with Frozen baselines stored continuously at -150 °C Accelerated degradation samples tested :- -70 °C continual storage for 16 years. -20 °C stored for 10 years followed by storage at -70 °C. + 4 °C stored for 10 years followed by storage at -70 °C. +37 °C for 1 week followed by storage at -70 °C. No evidence of significant loss of stability over the 16 years of storage at -70 °C
Recommendation for ECBS endorsement	Extended studies confirm appropriate stability for WHO International Standards.
Proposed date of further evaluation	It is proposed to replace this standard as soon as possible.

Report

Introduction

The 95/500 International Standard for Hepatitis A vaccine was established in 1999 and has been widely used by OMCLs and in a recent EDQM collaborative study without issues. However, an issue was reported with the loss of potency (complete loss of antigenic activity) of 95/500 in one laboratory. This was investigated and attributed to the presence of a low level bacterial contaminant in a small percentage of samples. For this reason it was considered useful to conduct a post-establishment stability evaluation of 95/500. Stability samples were laid down at the time of production but no evaluation of stability was performed for the establishment report. The stability evaluation only included data on vaccine antigen. Although this standard was also assigned immunogenicity IUs, no evaluation of the stability of 95/500 has been performed in vivo. It was thought advisable not to perform this due to the presence of a low level bacterial contaminant.

Sample Evaluation and Results

As described in the table below the potency (antigen content) of samples of 95/500 stored for extended periods of time at -70 °C, -20 °C, +4 °C and +37 °C was determined against frozen baseline samples of 95/500 stored at -150 °C. Due to the limited number of -150 °C frozen baseline ampoules, few ampoules were opened, aliquoted and stored at -20 °C in the laboratory freezer. This practise is widely used by laboratories. The +37 °C samples were tested against -150 °C samples stored in this manner. The antigen content was determined with a standard ELISA method using the Enzygnost Anti-HAV kit following manufacturer's instructions. As shown in Tables 1 and 2, the potency values of most samples in the study were comparable. One data point showed unexpected low value for one -20 °C sample. The mean values were calculated with and without this data point, so as not to bias the final result. It is unclear whether this sample was affected by the low level contaminant or whether there were issues with the titration of the sample in the assay.

There was some variability between assays and this is attributed to a change in the manufacturer of the Enzygnost Anti Hav kit used for the assays. This issue was raised in a recent EDQM collaborative study to establish a working reference for Hepatitis A. A common method is now under investigation to address this problem.

Data from the 3 assays of +37 °C samples show that there is no difference in the results obtained for 95/500 when a freeze thaw cycle is introduced into the storage pattern.

Discussion

Overall the antigen data supports the continuous storage of this preparation at -70 °C. The potency of 95/500 should be evaluated annually to confirm that continues to be fit for purpose

Stability studies of 95/500**Table 1 Raw data for antigen ELISA performed on 95/500**

Temperature and storage location	-70°C 6039	-70°C SPD B	-70°C SPD Y	-20°C 6039	+4°C 6039	-150°C SPD	+37°C SPD	Reference used for evaluation
20/05/11	91.5	87.4	-	55.6	-	-	-	-150
20/05/11	-	96.2	-	-	-	-	-	-150
24/05/11	-	85.1	96.8	98.5	84.9	83.2	-	-150
24/05/11	-	-	-	92.5	-	-	-	-150
24/05/11	-	101.8	115.9	117.9	101.6	119.2	-	-150
24/05/11	-	-	-	110.7	-	-	-	-150
03/06/11	-	95.1	110.8	-	91.2	-	92.9	-150 *
03/06/11	-	-	-	-	-	-	102.2	-150 *
03/06/11	-	-	-	-	-	-	99.0	-150 *
Mean	87.8	93.1	99.4	95.3 101.9**	92.6	101.2	98.0	N/A
Stdev	4.4	6.8	18.7	19.8	8.4	25.4	4.7	N/A
% CV	5	7.3	9.1	20.7	9	25.2	4.8	N/A

*Reference undergone 1 freeze thaw.

** Mean excluding value 55.6

SPD: Standards Processing Department.

SPD B refers to samples over labelled after establishment.

SPD Y labelled at filling

6039 Refers to Laboratory storage in trended freezers.

Table 2

Combined data expressed in international unit per ampoule for antigen content of 95/500 with continuous storage at -70 °C for 16 years

Temperature	Mean	Std	%CV
-70 °C SPD (B+Y)*	98.6	10.6	10.8

* See Table 1.

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