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A vaccine measured with a highly variable assay: Rabies

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Abstract

Manufacturers and regulators are challenged when evaluating stability of vaccines when potency is measured using a highly variable assay. Participants in the IABS Workshop on Stability Evaluation of Vaccines, a Life Cycle Approach, were offered a case study from a series of stability studies of a rabies vaccine, using the NIH potency assay. The case study was introduced with a scenario in which a new manufacturer was to formulate, lyophilize and fill the vaccine from bulk supplied by another manufacturer. The regulatory authority requested that data from the new manufacturer be supplied, to supplement that of the original producer. Participants were asked to answer a series of questions posed by the regulator, and critique the study design and data analysis according to principles described during the workshop.

Keywords: Rabies vaccine; Variable assay

1. Introduction

The goal of the IABS Workshop on Stability Evaluation of Vaccines: A Life Cycle Approach, was to elucidate designs and analyses that may be utilized to reveal the stability characteristics of vaccines, and through these implement procedures which help assure adequate quality through shelf life of the product. As part of the workshop, participants were provided several case studies highlighting the unique challenges facing manufacturers and regulators when evaluating vaccine stability. One such challenge is evaluation of stability when product is measured using a highly variable assay.

A rabies case study was presented in which a vaccine was formulated, filled, and lyophilized by a different manufacturer. Stability studies from both manufacturers were offered, and participants were asked to address several questions from the regulator. Along with the data, a statistical evaluation was performed utilizing the methods described throughout the workshop. Responses to the questions were discussed among the groups, and key conclusions were highlighted.

2. Case study

Manufacturer A imported concentrated bulk of rabies vaccine from Manufacturer B to be formulated, filled and lyophilized in the filling plant of Manufacturer A. The formulation, filling, and lyophilization processes had been validated following those of Manufacturer B. During the registration process, Manufacturer A submitted long-term stability data from Manufacturer B (see Table 1) and proposed the same shelf life (24 months) as the rabies vaccine produced by Manufacturer B. However, the National Regulatory Authority requested that a long-term stability study be conducted on the finished product to confirm the shelf life of the rabies vaccine which was formulated, filled, and lyophilized by Manufacture A (see Table 2). The testing parameter used for this stability study was potency.

The potency was measured with a multi-dilution dose NIH method, which has high variability. The end-of-shelf life potency specification is not less than 2.5 IU/SHD.

The assay validity criteria are as following: (i) both test vaccine and reference vaccine should have a 50% protective

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Table 1
Stability data (potency) of rabies vaccines prepared from concentrated bulks stored at 2–8 °C for 6–11 months after preparation.

Batch No.	Thermostability (37 °C 1 month)	T0	6 months	12 months	15 months	18 months
001	2.7	3.0	2.7	1.1	1.4	2.4
002	2.6	2.7	2.8	1.8	2.8	2.2
003	2.9	6.7	3.5	1.6	4.2	3.3

Batch 001, 002 and 003 were derived from different bulks.

dose which falls between the lowest and highest doses given to mice; (ii) the 0.03 ml dose of challenge suspension contains no less than 10 LD_{50}; (iii) the statistical analysis shows a significant slope and no significant deviation from linearity or parallelism of the dose-response profiles; and (iv) the fiducial limits of error ($P = 0.95$) are not less than 25% and not more than 400% of the estimated potency.

3. Workshop exercise

Workshop participants were split into groups of six- to eight-members per group, and were asked to address the following questions. A workshop facilitator was included in each group to answer questions and to help guide the group through the exercise. In addition to the case study description, the groups were offered a statistical evaluation of the data from the two studies.

3.1. Questions

(1) From Table 1; should the manufacture continue testing the potency of vaccine at 24 months?
(2) Is there any significant difference between the stability data in Table 1 compared to those in Table 2?
(3) What is your opinion on the shelf life of concentrated bulks and frequency of testing in Tables 1 and 2?
(4) From Table 2, only two batches were studied. Is it acceptable?

3.2. Statistical evaluation

The potency results from the two studies are summarized in Table 3.

An analysis was performed on data from the individual studies using methods described in Vaccine stability study design and analysis to support product licensure [1]. The analysis was performed on the natural log of potency (ln potency) in order to achieve linearity in the degradation profiles. A regression analysis and lower one-sided 95% confidence bound was calculated, and the shelf life was determined numerically as the last time interval (month) that the confidence bound falls above the minimum requirement (bold value in Table 4).

Table 2
Stability data of rabies vaccines prepared from concentrated bulks stored at 2–8 °C for 1 month after preparation.

Batch No.	Thermostability (37 °C 1 month)	T0	12 months	20 months	24 months
111	4.4	4.7	2.9	4.2	3.4
112	6.0	5.3	3.6	6.5	6.5

Batch 111 and 112 were derived from different bulks.

Table 3
Potency results for combined studies (minimum requirement = 2.5 IU/dose).

Month	Study 1			Study 2	
	Lot 1	Lot 2	Lot 3	Lot 111	Lot 112
0	3	2.7	6.7	4.7	5.3
6	2.7	2.8	3.5		
12	1.1	1.8	1.6	2.9	3.6
15	1.4	2.8	4.2		
18	2.4	2.2	3.3		
20				4.2	6.5
24				3.4	6.5

The lower bound on the regression line for study 1 falls below the minimum potency throughout the study period, and thus a shelf life could not be determined using the method described during the workshop. The lower bound on the regression line for study 2 last remains above the minimum potency at 27 months, thus supporting a 27-month shelf life for the rabies vaccine produced by Manufacturer A.

Further analysis showed that the degradation rates from the two studies were poolable. Using the pooled slope ($b = -0.012$ ln potency/month), the standard error of the pooled slope ($SE_b = 0.009935$), and the residual variability from the analysis ($s = 0.358$ ln potency), a minimum release potency was determined using methods described in Vaccine stability study design and analysis to support product licensure for various shelf lives.

Thus, for example, the minimum release potency to support a 24-month shelf life is 7.0 IU/dose (see Table 5).

A sensitivity analysis was performed to determine which component of the release calculation has the most impact on the minimum release value. It was determined that release assay variability has a major impact. The minimum release was recalculated for scenarios in which duplicate ($n = 2$) and triplicate ($n = 3$) runs of the potency assay are performed at release (see Tables 6 and 7 respectively).

It's observed that increasing the number of runs to $n = 2$ has a significant impact on the minimum release potency (reduced from 7.0 to 5.6 IU/dose for a 24-month shelf life), while there is less reduction in performing three-runs at release (reduced to 5.3 IU/dose for a 24-month shelf life).

4. Discussion

The discussion highlighted the conclusion that even (and especially) with highly variable assays, statistical modeling of data enhances understanding of the stability results. In this case, stability tests were performed in two groups, one on bulk material manufactured by Manufacturer A, but that had previously been stored for 6–11 months before formulation by

Table 4
Evaluation of shelf life from individual studies of rabies vaccine.

Month	Study 1				Study 2			
	Predicted (ln potency)	LCB (ln potency)	Predicted (potency)	LCB (potency)	Predicted (ln potency)	LCB (ln potency)	Predicted (potency)	LCB (potency)
0	1.06	0.77	2.89	2.16	1.66	1.26	5.28	3.53
3	1.03	0.79	2.79	2.21	1.63	1.28	5.09	3.61
6	0.99	0.81	2.69	2.25	1.59	1.30	4.91	3.67
9	0.95	0.80	2.59	2.23	1.56	1.30	4.74	3.69
12	0.92	0.76	2.50	2.15	1.52	1.29	4.57	3.65
15	0.88	0.69	2.41	2.00	1.48	1.26	4.41	3.53
18	0.85	0.60	2.33	1.83	1.45	1.21	4.25	3.34
21	0.81	0.50	2.25	1.65	1.41	1.13	4.10	3.11
24	0.77	0.40	2.17	1.49	1.38	1.05	3.96	2.86
27	0.74	0.29	2.09	1.34	**1.34**	**0.96**	**3.82**	**2.60**
30	0.70	0.19	2.02	1.21	1.30	0.86	3.68	2.36
33	0.67	0.08	1.95	1.08	1.27	0.76	3.55	2.14
36	0.63	−0.03	1.88	0.97	1.23	0.66	3.43	1.93

Manufacturer B, and the other on bulk from Manufacturer A that had previously been stored for 1 month before formulation. Calculation of the potency decay rates in these separate scenarios suggested a decline in potency for the batches in Table 1, and even a modest increase in potency over time for the batches in Table 2. However, assay variability was quite high, making it difficult to draw firm conclusions from any individual test. Statistical examination of the data indicated that, although at first glance different, the results were sufficiently similar that data in Tables 1 and 2 could be pooled to arrive at an aggregate potency slope estimate Tables 5 and 6,7.

Several important points were made by audience participants that did not directly address the presented questions. Several participants pointed out that contemporaneously performed tests were not truly independent of one another, and pooling information from non-independent tests could falsely result in lower assessments of variability, providing a false sense of security in interpreting the data.

This consideration was related to the desire to reduce animal testing and to preserve mice in a rabies potency assay, because independent assays would require increased numbers of mice for controls. Laboratories should consult a statistician for a proper analysis of stability data in cases where the assays are not truly independent.

The implications of attempting to set specifications and shelf life based on highly variable assays were again discussed in the final session of the meeting. The traditional, non-statistical methods were very appealing to some audience members in this setting, because they have a history of assuring efficacious vaccine. Paradoxically, the traditional and statistical approaches yield similar results when assay variability is low—and it is precisely when assay variability is high (such as for rabies vaccines) that the improved understanding of the product and its stability that is provided by the statistical model provides improved assurance of product efficacy. Moreover, the point was raised that even data collected and previously used in the traditional approach is amenable to statistical analysis—which will permit improved understanding of actual product potency throughout the dating period.

1. From Table 1; should the manufacture continue testing the potency of vaccine at 24 months?

While the experiment in Table 1 was terminated early, due to arrival at potencies below the limit considered necessary for efficacy, discussion participants agreed that additional data in this experiment almost certainly would have helped to arrive at more robust estimates of stability.

2. Is there any significant difference between the stability data in Table 1 compared to those in Table 2?

The amount of data presented was insufficient to show a clear difference in loss rates between materials formulated after bulk storage for 6–11 months vs. those formulated after bulk storage for 1 month. However, the inability to show a difference in loss rates does not necessarily mean that no difference exists. If these data were submitted in support of an application to store bulk at 6–11 months, the data in Table 2 would not be applicable due to the clear difference in potency levels between lots formulated from bulks stored 6–11 months and lots formulated from fresher bulks. While the modest increase in potency over time suggested by the data in Table 2 was almost certainly due to assay variability, an increase in

Table 5
Minimum release potency for various shelf lives.

Shelf life	ln (minimum)	Minimum
18	1.83	6.2
24	1.95	7.0
36	2.23	9.3

Table 6
Minimum release potency for various shelf lives using duplicate runs of the potency assay.

Shelf life	ln (minimum)	Minimum
18	1.57	4.8
24	1.72	5.6
36	2.04	7.7

Table 7
Minimum release potency for various shelf lives using triplicate runs of the potency assay.

Shelf life	ln (minimum)	Minimum
18	1.51	4.5
24	1.67	5.3
36	2.00	7.4

potency over time would be biologically implausible and thus, many reviewers of this data would use a slope of 0 in interpreting Table 2, rather than a positive slope which would imply an increase in potency over time. An alternative approach, which was taken in the statistical evaluation is to pool the slopes from the two studies, which resulted in a biologically plausible negative slope.

In the presented data, it appeared that bulks were assessed for potency at the time of manufacture, which permitted the bulks that had been stored for longer periods of time to decline further in potency prior to formulation. Information was not provided regarding the filling model, and the way in which bulk potency information was used to determine dilution factors. If the bulk potencies were less accurate at the time of formulation and filling (as would have been the case if the release potencies for the older bulks were used), then this could lead to lower potencies at release, as well.

One additional consideration was the relative stability of bulks versus final container material—if the bulk stability were lower than that of final product, this could also influence the final outcome.

3. What is your opinion on the shelf life of concentrated bulks and frequency of testing in Tables 1 and 2?

With regard to the frequency of testing, it was pointed out that in general, more data points at the beginning and end of a study would yield more accurate decay rate estimates. Thus, as indicated in the answer to question 1, many participants would have preferred to see a 24 month testing time point in Table 1.

4. From Table 2, only two batches were studied. Is it acceptable?

Several questions (1, 3, and 4) addressed the issue of how much data are needed to understand the stability of a product. In this particular case, it seems unusual that Manufacturer A would only have stability data on two lots for a licensed product. The WHO stability guidance document recommends that at least three batches be tested. The degree of independence of each test is also an important consideration. Overall, increased amounts of information are obtained by performing a greater number of tests at a greater number of time points. This increased amount of information yields increasingly precise stability estimates, so for many products, the question of how much data to obtain is answered by the amount of precision that is required in the stability estimate, in order to assure product safety and efficacy through the dating period.

In this case, the two lots described in Table 2 would have provided stability estimates precise enough to allow evaluation in this context, especially when augmented by the data from the three lots of Table 1. However, it was pointed out that there are additional reasons to request collection of data on a larger number of lots, including the need to be certain that the decay kinetics are first order.

Reference

[1] Schofield TL. Vaccine stability study and analysis to support product licensure. Biologicals 2009;37:387–96.