



VS Memorandum 800.124

Guidelines for Potency Specifications of Biological Products Administered to Animals

A. Purpose and Background

This Memorandum provides guidance for setting potency specifications for the serial release of biological products administered to animals. This guidance also provides recommendations for setting potency specifications based on experimental data and provides a means of reducing the potency specifications by increasing the amount of testing.

Title 9, *Code of Federal Regulations* (9 CFR), [part 113.5 \(a\)](#) requires all serials of biological products to satisfactorily complete potency testing before they are released for marketing. Part [113.8\(a\)\(3\)](#) discusses two of the common types of potency tests, titers/counts of viable organisms and relative potency immunoassays. For titers/counts, it calls for an overage to account for adverse conditions and test error. For relative potency immunoassays, it calls for reproducibility in a manner acceptable to the Animal and Plant Health Inspection Service. These examples indicate that serial release potency specifications must reflect the variability of the testing method and of the manufacturing process by including adequate increments over the observed potency of the serial used in the pivotal efficacy study. Historically, such increments were set by adding constant amounts related to the nature of the product and the potency assay.

This guidance applies to all biological products whose pivotal efficacy study is initiated after July 1, 2021, with standardized dosing regimens. It does not apply to products that are administered to animals in doses tailored to the individual recipient (e.g., a product that is dosed based on weight). It does not apply to immunodiagnostic products that are not administered to animals. Licensees may, but are not required to, apply these guidelines to currently licensed products whose dating has been previously approved, those with codified potency tests, or those with codified potency specifications. Licensees may also request exemptions to codified potency specifications. The Center for Veterinary Biologics (CVB) may apply this guidance to a licensed product if the licensee requests significant changes to the nature of the product, the manufacturing process, or the potency test. These guidelines will not be applied retroactively to licensed products with confirmed dating if no significant changes are made to the product or the potency test.

Pursuant to the Congressional Review Act (5 U.S.C. § 801 et seq.), the Office of Information and Regulatory Affairs designated this rule as a non-major rule, as defined by 5 U.S.C. § 804(2).

B. Document Status

1. Issue date: 10/02/2020.



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2. This guidance document provides a data-driven, consistent approach to setting potency specification for all types of *in vitro* potency assays and products covered under the Purpose and Background section.

C. Authorities and References

1. Authorities (*Code of Federal Regulations* (CFR)):

- [7 CFR 371.4](#)
- [9 CFR part 113](#)
- [9 CFR part 114](#)

2. References:

- [CVB-WI-5224](#); Technical Notes Regarding Establishing Control Chart Parameters
- [VS Memorandum 800.112](#); Guidelines for Validation of In Vitro Potency Assays
- [VS Memorandum 800.202](#); General Licensing Considerations: Efficacy Studies

D. Audience

VS employees and members of the biologics industry.

E. Guidance

1. Introduction

All serials of biological products must satisfactorily complete potency testing before they are released to the market (9 CFR [113.5 \(a\)](#)). The regulations at 9 CFR [114.13](#) define stability criteria as the specifications for potency at release, potency throughout the dating period, and the length of the dating period. This memo discusses setting the release and throughout-dating potency specifications. The guidelines rely on the premise that a market serial¹ emulates the pivotal efficacy (PE) serial.

2. Setting Potency Specifications for Serial Release

Initial potency specifications are set prior to licensure and are reviewed and possibly modified with data obtained after licensure. The confirmation of dating (COD) study is the initial stability study conducted and is necessary to determine the mean potency loss (MPL) during the dating period of the product.

2.1 Elements of potency specifications

¹ A market serial is one that has been made in production and passed all Section V of the Outline of Production testing, including pre-license serials. Production serial and market serial will be used synonymously.



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- 2.1.1 Pivotal potency (PP). The potency of the PE serial or the potency of the serial used to establish the duration of immunity (DOI) if it is greater than the potency of PE serial (see [VSM 800.202](#), Section 8.7.2).
 - 2.1.2 Practical standard deviation (PSD). The PSD is intended as a measure of the variability of the potency due to manufacture and testing.
 - 2.1.3 Mean potency loss (MPL). The MPL is the expected average potency loss over the dating period. The MPL is added to the throughout-dating specification to set the release specification.
- 2.2 Potency Specifications²
- 2.2.1 Throughout dating. The specification(s) that must be met when tested at any time during the dating period. Options one (1) through three (3) will have throughout-dating specifications for both the mean of all vials and each individual vial.
 - 2.2.2 Release. The potency specification(s) that must be met for release to the market. Options one (1) through three (3) will have release specifications for both the mean of all vials and each individual vial. Potency specifications for both the mean potency and potency of the individual vials is no less than:

$$\textit{Throughout-dating} + \textit{MPL}$$

3. Foundations

This section provides the foundations underlying the guidance. Details regarding implementation, including provisions when the ideal is not achievable, will follow in later sections.

- 3.1.1 The PE study. An acceptable PE study is one that has satisfied the requirements of the Virus Serum Toxin Act and the regulations and guidelines.
- 3.1.2 The PE serial. The basis for serial release potency testing is an implicit comparison of the market serial to the PE serial. The market serial is considered exchangeable with the PE serial if it emulates the PE serial in formulation and use.
- 3.1.3 Antigen content. Ideally, a market serial will contain the least amount of antigen that will provide acceptable efficacy throughout the dating period. To ensure there is not unnecessary excessive antigen in a vaccine serial

² In this document's treatment, the addition sign does not imply universal additivity. For example, it is often appropriate to work with logarithms, where addition is multiplication on the original scale.



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that could create safety concerns, use potency assays that are well-optimized, accurate, precise, and validated according to VSM [800.112](#).

4. Formulation, Use, and Potency of the PE Serial

The link between a market serial and the PE serial requires similarity in formulation and use. In practice that link may not be perfect due to pragmatic issues, but certain features are essential and cannot be omitted.

4.1 Formulation

- 4.1.1 Prepare the PE serial. The PE serial must be prepared in accordance with the Outline of Production. If an Outline has not yet been filed at the time the experimental product is made for the pivotal study, ensure the study report contains sufficient detail on the manufacture of the experimental product to support a subsequently filed Outline.
- 4.1.2 Fill into final containers of the same material that will be used for market. Containers may be smaller than those used in production serials. Containers may be short filled.
- 4.1.3 Complete all finishing steps, such as lyophilization or freezing.

4.2 Vaccination

- 4.2.1 Determine the number of vials³ needed for the PE study, related concurrent testing, and retention. Select the vials needed for vaccination, concurrent testing, and retention using a well-defined sampling process (e.g., random sampling). Describe the selection of vials in the protocol and final efficacy study report.
- 4.2.2 Administer the product to animals directly from a minimum of five (5) vials or a pool prepared from a minimum of five (5) vials.
- 4.2.3 Administer a single dose to each subject animal.
 - 4.2.3.1 Before vaccination each container or the vaccine pool may be diluted by a pre-determined amount. Determine the dilution factor, if necessary, from a vaccine pool prepared from the same vials of the same serial/lot that will be administered to the animals, or another set of vials of equal number.
 - 4.2.3.2 For vaccines requiring a second dose, preparation of the material to be used for vaccination should be reflective of end-use. For instance, if a

³ For simplicity, the rest of this document uses the term “vial” to mean any type of final container.



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new vial is required, use a new set of vials or create a new vaccine pool.

4.3 Estimating pivotal potency

4.3.1 PP is determined at the time of the PE study by testing the PE serial with a validated potency test. If the potency test is not validated prior to the PE study, it should be developed and optimized, and the testing procedure used to determine pivotal potency should be consistent with the procedure that will be validated.

4.3.1.1 Timing of testing. Initiate the potency tests no later than one (1) month from the day of administration to animals.

4.3.1.2 Number of tests. If animals are administered a dose from a vaccine pool, test the pool used or a representative pool no less than five (5) times. If animals are administered a dose from a vial, test no fewer than five (5) representative vials.

5. Estimating the Practical Standard Deviation (PSD)

The PSD is intended to capture the variability within a typical market serial and the assay variability when used in a manner consistent with future serial release. Ideally, the variability of the serial administered to animals in the PE serial would be the same as that of future market serials administered to animals in the field. In practice, it is difficult to achieve perfect identity and hence, the estimate of the PSD will be obtained from all suitable data available from testing the PE serial, market serials tested in studies transferring the potency test from one laboratory to another (e.g., Research and Development to Quality Control), and assay validation studies. Include sources of variability associated with the potency assay and vial-to-vial in determining the PSD. Use available appropriate sources of data, such as assay validation, testing of pre-license serials, studies to scale up production, and technology transfer studies to support the PSD. Submit a report and electronic data describing each study the licensee wishes to include in determining the PSD and to support the potency specifications provided in the Outline of Production at the time of licensure.

An assessment of the appropriateness of the PSD will be made using the data from the COD study, although the PSD will not necessarily be changed.

6. Setting the MPL

6.1 Preliminary MPL. Before the COD is completed, a preliminary MPL must be used to set the release specification. The value used should be based on current regulatory requirements, available knowledge about the type of product, the expected potency loss during the proposed dating period, and the potency assay.

6.2 Final MPL. The final MPL is estimated from the COD study.



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7. Options for Setting Potency Specifications and Serial Release Potency Tests

Options are presented for serial release testing. The options are designed so that increased testing will enable lower potency specifications. Each option provides release and throughout-dating specifications for both the mean potency of all vials tested as well as the potency of each individual vial.

- 7.1.1 Option 0 – The serial release potency test is conducted on a single vial. Since the mean is the same as the individual result, only one criterion is necessary.
- 7.1.2 Option 1 – The potency test of a serial consists of the mean and individual results of independent tests conducted on three (3) vials.
- 7.1.3 Option 2 – The potency test of a serial consists of the mean and individual results of independent tests conducted on six (6) vials.
- 7.1.4 Option 3 – Option 2 conducted at more than one (1) time point.

Conduct release testing twice, one (1) month (three (3) to five (5) weeks) apart. Increase the dating period by one (1) month to allow a full marketing period after the second set of tests. Request release of the serial after a satisfactory second potency test. Test the serial again at expiration.

Monitor all serials according to the control chart methods described in Technical Notes Regarding Establishing Control Chart Parameters ([CVB-WI-5224](#)). The control chart limits will be set using the data from the confirmation of dating study. Include the limits in the Outline of Production.

- 7.1.5 Table 1 provides a comparison between the options in terms of the number of vials used for serial release, the throughout-dating potency specification for the mean potency of all vials tested, and the potency specification for each individual vial.

Table 1 – Comparing Options for Setting Throughout-Dating Potency Specifications**

<i>Option</i>	<i>Number of Vials</i>	<i>Mean</i>	<i>Individual*</i>
0	1	---	PP + 3PSD
1	3	PP + 2PSD	PP – PSD
2	6	PP + PSD	PP – 2PSD
3	6 tested twice; 1 month apart	PP	PP – 3PSD

*The individual vial specification for Options One (1) through Three (3) is equivalent to the mean specification – 3PSD.

** The Mean Potency Specifications can be determined by adding MPL to the Throughout-Dating Potency Specifications.