**POTENCY TEST REFERENCES**

Relevant regulations and guidance documents:

Title 9, Code of Federal Regulations (9CFR 101.5, 113.8, 113.XX series containing Standard Requirements

Veterinary Services Memorandum (VSM): 800.90 Guidelines for Veterinary Biological Relative Potency Assays and Reference Preparations Based on ELISA Antigen Quantification

VSM 800.112 Guidelines for Validation of In Vitro Potency Assays

VSM 800.118 Live Master References

VSM 800.211 Guidelines for Master Reference Qualification and Requalification

Reviewers manual

4_4_3_MasterReferenceQualificationRequalificationandUnderstandingVSM800211

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1. **Master Reference Description**

The Master Reference (MR) is a preparation of a biological product, made by the licensee, and derived from the Master Seed. The potency is correlated directly or indirectly to host animal immunogenicity (efficacy). The MR is used to evaluate the potency of serials of inactivated products by comparing the response of the serial to that of the MR in the potency test method. A MR may be fully formulated product (a bacterin, vaccine, antiserum, toxoid, immunomodulator), a purified preparation of the immunogen or antigen, or a non-adjuvanted harvested culture of microorganisms (live (see VSM 800.118) or inactivated) prepared according to the filed Outline of Production (9 CFR 101.5). In contrast, inactivated products with Standard Requirements (e.g. 9CFR 113.120) that describe a Standard Reference refer to a preparation provided by CVB that has been assessed for potency usually by in-house animal testing.

The potency test methods may be analytical or comparative (VSM 800.112 Appendix III) hence they may be quantitative or relative (response based). Regardless, the MR is treated the same.

The MR must be identified in the Outline of Production (not the Special Outline) by lot number and assigned an expiration date. The dating period begins on the date of the initiation of the efficacy study (date of first vaccination rounded to the end of that month) while the duration is set by one of several methods.

In the past, the dating period for a MR was the same as the dating of the product or if the MR was frozen and the firm demonstrated the freezing and subsequent thawing did not affect the potency estimation (dose-response curves superimposable and magnitude same for never frozen and frozen thawed samples) the MR was given 5 years dating (9CFR 113.8(d)(2)). This view of MR expiration dating is being supplanted by the guidelines in VSM 800.211 which permits the expiry period to be as much as 15 years depending on several factors such date of licensure, validation of the potency assay and approval of the stability monitoring program (see VSM

800.211, 800.112 appendix III, and the reviewers manual 4.4.3. for the details). Note that the terms of use and requirements for requalification of the MR for products licensed before January 1, 2011, (Previously Licensed Products or Legacy Products see VSM 800.211) are treated different than those licensed after that date.

The MR may be a concentrate [redacted] which are diluted in the appropriate matrix for use in the potency test method.

MR's *ideally* would represent the minimum protective dose (MPD) of the product, but in practice they represent a dose shown to be protective in studies conducted by the firm and may be greater than the true minimum needed for protection. Thus, relative potency measurements should not be interpreted as direct measures of efficacy because two different antigen preparations may be equally effective in the immunogenicity trial but different in protective antigen content. In this scenario the animal challenge studies cannot evaluate the stability of the MR and only indicate that its current potency is at least the MPD. The initial potency of a MR may have been much greater than the true MPD, and the amount of degradation is not detected in the immunogenicity study because the "potency" is at least the MPD.

Note that not all inactivated products are tested by comparison with a MR. Some products are tested for potency *in vivo* and rely on clinical or serologic outcomes without a reference or they follow a Standard Requirement as mentioned above.

In addition to the MR, other biological preparations are usually run concurrently with a test serial for purposes of determining if the particular assay run is valid (i.e. not a "No Test"). These are usually called "positive controls" instead of references, but sometime firms will use these terms (as well as "standard") interchangeably. Evaluate the context used and encourage clear terminology. Positive and negative controls or validity standards are not subject to the same scrutiny as MR but they should be lot controlled and have criteria for acceptance identified in the Outline of Production.

> Note
> In relative potency (RP) assays the release and throughout dating level has been an RP≥1.0 and 1.0 was defined as in the range 0.95-1.05. This is inconsistent with the treatment of live products which require an overage for assay variability, production variability and stability. In addition it is not consistent with our approach to assay validation which requires assessment of precision, accuracy, and ruggedness of the testing method. Draft documents addressing this are in process and will very likely modify the release and throughout dating value for relative potency assays as well as the quantitative in vitro assays for inactivated products.

## 2. Working Reference Description

The Working Reference (WR) is defined in 9CFR 101.5(p) "is the reference preparation that is used in the in vitro test for the release of serials of product. Working References may be: MR or serials of product that have been prepared and qualified, in a manner acceptable to APHIS for use as reference preparations." The WR is used in place of the MR and has a potency that is equal to or slightly greater than the MR. In terms of characteristics it is almost always product like and the dose response curves are parallel to the MR otherwise it cannot be used. The WR is used instead of the MR because it can be replenished reducing the depletion rate for the MR

stocks. The WR has an expiry period identical to the product, regardless of the dating for the MR. The WR reference is determined to be suitable for use by comparing it to the MR in the approved potency assay. Hence the working reference is "qualified" by comparison to the MR in the potency assay. This means the MR must be unexpired and therefore qualified (at some point in time) by testing in an immunogenicity study.

The WR used in relative potency assays usually has an RP-value >1.0 however it represents the MR and is evaluated as if it has an RP=1.0. No correction is permitted. This is in contrast to WR for quantitative assays where the WR is used to prepare a standard curve for extrapolation of the values for a serial. In this case the actual value of the WR is used.

**3. Qualifying Serial Description**

The Qualifying Serial (QS) is defined in 9CFR 101.5(q) and is a serial of the product that has a relative potency ≤ 1.0. This is used to qualify (in an immunogenicity study) the MR usually when the MR is not product like.

4. **Internal control-see 800.112 App. I**

5. **Reference-see 800.112 App. I**

6. **Standard-see 800.112 App. I**

7. **Reagent Blank-see 800.112 App. I**

8. **Unknown-see 800.112 App. I**

9. **Live Master Reference-see 800.118**

10. **Reference Standard-see 800.90**

11. **Relationship between Master References, Working References, and Qualifying Serials**

a. **MR is the QS and the WR**

The MR is a fully formulated serial of product ( "product-like Master Reference"). It is used to qualify itself and therefore is the QS and it is used in the potency assay for serial release so it is also the WR.

**b. MR is not product like**

The MR is either a purified or semi-purified preparation, concentrate or non-adjuvanted harvest fluids (*e.g.* Live Master Reference or inactivated fluids) that is not suitable for use in a qualification study because it is not product-like. In order to qualify this type of MR the firm prepares a QS that has a potency less than or equal to the MR or a designated dilution of the MR. The QS is evaluated in an efficacy study and if satisfactory the MR is qualified.

- The MR may be used as the WR at the dilution used to measure the potency of the QS.

- The MR at the use dilution may be used to evaluate another preparation for use as a WR
- Technically the QS may also be used as the WR however the expiry period is limited to the same as product dating and typically the firm does not set aside a large number of vials of the QS unless the intention is to use it as a MR.

The potency of the WR regardless of the source is set at an RP=1.0 (traditionally CVB has recognized an RP of 0.95 to 1.05 as the equivalent of 1.0). If the potency test method is quantitative (*e.g.* expressed as µg Protein/dose) the MR may be used as a WR that is prepared to cover the dynamic range of the test method hence it does not need to be exactly equivalent to the QS because the test method uses extrapolation of the value from a series of dilution of the WR.

New *in vitro* assays must be validated (VSM 800.211 and 800.112), and representative serials of each product code affected must be shown to have dose-response curves that are parallel to the Master and Working Reference. Adjuvant and other antigens may affect the parallelism that is critical for the typical *in vitro* potency tests. Relative potency methods require that the Master or Working Reference and the serials have parallel dose-response curves throughout the entire curve because relative potency measurements are based on the relative (horizontal) shift of the unknown curve compared to the reference curve. Thus for the estimate to be interpretable as the RP the curves must be parallel.

Quantitative assays have to be validated and show that representative serials and the MR have parallel dose-response curves over the dynamic range of the test method.

## 12. Master Reference Qualification & Requalification

**Qualification**

The MR must be qualified in an immunogenicity study in order to be used in a potency assay for serial release. Guidance for this is in 9 CFR 113.8 and VSMs 800.90 and 800.92. (Also consult VSM 800.211, section 1. above and Chapter 4.4.3. of this manual for the timing and necessity of this action.) The process of confirming that a prospective reference material is acceptable for use as a MR is called "reference qualification" or "qualifying a reference". This usually requires host animal challenge to demonstrate that the proposed reference or the QS is efficacious.

**Requalification**

When a reference expires, it may be "requalified" for an additional dating period equal to the first dating period, or it may be replaced by a new reference that has been recently qualified. The terms "qualify" and "requalify" have specific meaning in the CVB vernacular but sometimes firms use them interchangeably, so make sure the meaning is clear when discussing or corresponding with the firm. VS Memo 800.90 offers the possibility of using *in vitro* means of requalifying a reference so firms are not absolutely required to go back into host animals if they can come up with an acceptable alternative means of monitoring reference stability and demonstrating the reference has remained unchanged over time. [redacted]

[redacted]

**Potency reference database**
A database to track Master References and their expiration dates is available. CVB-IC will not release serials that are tested with expired MRs. CVB-PEL reviewers must notify of this change by CC'ing the LIE of the change whenever they approve a change to MR ID or dating.

**Potency Testing of Modified Live Products and Other Inactivated Products**
The potency of live products is determined by viable counts while inactivated products that are not potency tested by *in vitro* methods, or not covered by a Standard Requirement are tested by *in vivo* methods. MRs are not required for either one. The reason for this is mostly historical. As a general principle every test method needs a reference or standard for comparison and performance monitoring, and each test method should be validated. This is the direction CVB is moving but is not a requirement.

### 13. MR expiration dating

Guidance for setting the expiration dating of MR is in VSM 800.211 and an explanation of this memorandum is in Chapter 4.4.3. of this Manual.

### 14. Temporary Reference Dating Extensions

**Introduction**
The need for extensions of the expiration date of MR has been largely superseded by VSM 800.211, Some MR's may continue to have the shorter expiry periods than the ones permitted by that VSM for a number of reasons (failure to meet the minimum requirements of the VSM; firm logistics).

VSM 800.90 provides guidance for this as follows:

> "Temporary extensions of dating may be granted by the CVB-LPD reviewer provided that the licensed manufacturer provides evidence of a protocol, plan, and continuing progress for accomplishing requalification. In general, such extensions shall not exceed 12 months and shall be supported by protocols and data acceptable and approved by APHIS. Such requests should be the exception and not routine practice."

**Guidance**
1. Plan. For "evidence of a protocol, plan and continuing progress," requests for reference dating extensions should be accompanied by the following.
    a. A plan and timeline for completion of the qualification of a reference.
    b. A protocol describing a study to qualify a reference

c. A summary of the firm's efforts to qualify a reference. The summary should be provided when the firm is requesting an extension because of difficulties associated with conducting a study. What you as a reviewer have to determine is whether or not the firm is making a good faith effort or not. The firm must provide a summary of their efforts and address how they intend to solve the problem(s) encountered.

2. Data. For "data acceptable and approved by APHIS," firms have typically provided historical data, usually OD's of the reference and/or plate controls, over the lifetime of the reference. If complete plate data were provided (including serial OD's) such data would be useful as a crude tool to monitor the stability of the assay system as a whole (note this is allowed in VSM 800.211 for previously licensed products). Note, however, that almost none of the currently approved relative potency tests are designed to provide a sensitive assessment of the stability of the reference. Rigorous evaluation of the stability of a reference depends on tests that measure its qualitative and quantitative characteristics independently of the potency test. OD values or parameter estimates from the potency test are not sufficient by themselves to determine if the reference is stable.

   Independent testing may be performed on either the reference or an internal control as long as the internal control is assayed concurrently in the potency test. The independent testing needs to be performed with validated assays that measure one quantitative and qualitative characteristic other than the one(s) measured in the potency test. Monitoring these characteristics over time should provide sufficient information to evaluate if the reference and the potency test are performing satisfactorily.

   For example, if the ELISA potency test is an indirect antigen capture sandwich assay using a monoclonal detecting antibody, the independent assays could be SDS-PAGE and Western Blotting where molecular size, antibody binding, and quantity (densitometry or precision dilution to extinction) are assessed. But note that because of the differences in products there is not a simple answer or one-size fits all approach. Consult with others with expertise in the product and testing methods.