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VS Memorandum (VSM) 800.211

Guidance for Master Reference Qualification, Requalification, Dating, and Monitoring

1. Purpose and Background

Many relative potency assays used for serial release testing of veterinary biologics rely on the use of a reference preparation that was qualified, directly or indirectly, in a host-animal vaccination/challenge study. Title 9, *Code of Federal Regulations* (9 CFR), section 113.8 (d) indicates that the lot of reference used to determine relative antigenic content must have an initial dating period equal to the dating of the product or as supported by data acceptable to the Animal and Plant Health Inspection Service (APHIS). The dating period of the Master Reference (MR) and Working Reference (WR) may be extended if data are provided to support that the minimum potency of the Master Reference is adequately above the minimum level needed to provide protection in the host animal.

In 2011, the Center for Veterinary Biologics (CVB) published VSM 800.211, *Guidance for Master Reference Qualification and Requalification*, to revise the policy for determining the dating period for Master References of certain products, historically referred to as "previously licensed products", to reduce required animal testing and lessen the burden of time consuming and resource intensive vaccination/challenge studies. The intention was to limit the time and resources devoted to maintaining assays for licensed products and encourage the development and validation of assays for products licensed thereafter. VSM 800.112, *Guidelines for Validation of In Vitro Potency Assays*, was also updated in 2011 to include Appendix III, *Guidance for Validating ELISA Relative Potency Assays*, which includes guidance on monitoring and Master Reference dating for products licensed after January 1, 2011.

This memorandum describes the expectations for monitoring the stability of MRs for legacy and modern products. This purpose of this document is to preserve the guidance in the 2011 version of VSM 800.211 for what were called "previously licensed products" and additionally, to update and clarify the guidance in VSM 800.112 Appendix III and VSM 800.211 pertinent to dating and stability monitoring of MRs of products licensed since January 1, 2011. This updated memorandum also includes guidance on reference qualification and requalification. This document also includes information from VSM 800.90, Guidelines for Veterinary Biological Relative Potency Assay and Reference Preparations Based on ELISA Antigen Quantification, which was removed from the CVB website in 2016.

2. Document Status

A. Issue Date: 2/8/2023

B. This document replaces VSM 800.211 dated June 28, 2011, which is cancelled.

3. Reason for Reissuance

This memorandum has been updated to incorporate contemporary information regarding dating and reference stability monitoring for all references.

4. Authority and References

A. Authorities

7 CFR 371.4
9 CFR 113.8

B. References

- VSM 800.112, Guidelines for Validation of In Vitro Potency Assays
- VSM 800.206, General Licensing Considerations: Preparing Outlines of Production for Vaccines, Bacterins, Antigens, and Toxoids and Diagnostic Test Kits

C. Definitions

1) 9 CFR 101.5 (o) Master Reference: A reference whose potency is correlated, directly or indirectly, to host animal immunogenicity. The MR may be used as the working reference in *in vitro* tests for relative potency. The MR may also be used to establish the relative potency of a serial of product used in requalification studies and to establish the relative potency of working references. The preparation of an MR as described in a filed Outline of Production (OP) may be:

- A completed serial of vaccine or bacterin prepared in accordance with a filed OP;
- A purified preparation of a protective immunogen or antigen; or
- A nonadjuvanted harvested culture of microorganisms.

2) 9 CFR 101.5 (p) Working Reference: The reference preparation that is used in the *in vitro* test for the release of serials of product. Working References (WRs) may be:

- The Master Reference; or
- A serial of product that has been prepared and qualified, in a manner acceptable to APHIS for use as a reference preparation.

WRs used in relative potency assay methods must have a relative potency ≥ 1.0 when compared to the MR. The geometric mean potency of the WR when compared to the MR must be established in the approved potency assay by five or more independent assays.

3) 9 CFR 101.5 (q) Qualifying Serial (QS): A serial of biological product used to test for immunogenicity when the Master or Working Reference is a purified antigen or nonadjuvanted harvest material. Qualifying serials must:

- Be produced in accordance with the filed OP.
- Be tested for immunogenicity.
- Have a geometric mean relative potency, when compared to the Master Reference, of not greater than 1.0 as established by:
    - Independent parallel line assays with five or more replicates; or
    - Other valid assay methods for determining relative antigen content which demonstrate linearity, specificity, and reproducibility at least equivalent to the parallel line assay and are acceptable to APHIS.
- Be within its permitted dating period.

4) 9 CFR 101.5 (r) Immunogenicity: The ability of a biological product to elicit an immune response in animals as determined by test methods or procedures APHIS finds acceptable.

   In this document immunogenicity typically refers to the demonstration of efficacy by means of a host animal vaccination/challenge study.

5) Internal control (IC): A preparation included in an assay to serve as an independent measure of the assay's performance, but which does not serve as an assay validity control. Internal controls may be crude preparations, semi-purified or purified fractions containing the target analyte, or other materials that respond similarly to the reference in the assay. ICs may be used as surrogates to monitor the stability of a reference.

6) Serial release assay: The test method used for determining the potency of a test serial as described in Section V. C. of the Outline of Production.

7) Relative potency assay (RPA): A serial release assay for which the potency of a test serial is compared to that of a reference preparation.

8) Reference stability monitoring (RSM) assay: A test method used to monitor the stability of some aspect of the reference, qualitative and/or quantitative. RSM assays are used to monitor the quantitative and qualitative aspects of a reference preparation, not a serial of product. RSM assays are different than the serial release assay.

9) **Major manufacturing change:** A change to the production of a biological product administered to animals that would require a new efficacy and/or field safety study prior to approval of the change.

10) **Analytical principle**: The physical/biological/chemical/biochemical approach for measuring a target of interest and the metric used. Examples include:

- A serological test measuring an antibody response to vaccination and the titer used to express the amount of antibody present; or
- A sandwich ELISA measuring the potency of OspA relative to a *Borrelia burgdorferi* reference.

11) **Legacy Products:** A category of veterinary biological products (not to include diagnostic test kits) licensed or permitted before January 1, 2011, provided that no major changes in the manufacturing process or serial release assay have been implemented. Historically these were termed "Previously licensed products."

12) **Modern Products:** This is a category of veterinary biological products (not to include diagnostic test kits) licensed or permitted on or after January 1, 2011. This category also includes legacy products that have been reclassified due to a major manufacturing change as defined above, or a change to the analytical principle of the relative potency assay (RPA) that occurred on or after January 1, 2011. These products were previously termed "Newly licensed products."

13) **Time Zero:** Time zero refers to the date animals are first vaccinated in a study to qualify a MR (i.e., demonstrate the efficacy of a proposed reference).

14) **Forced degradation by freeze-thaw study (FDFT):** A study using a specified freeze-thaw regimen (described in Section 6. I. below) to determine if the MR has degraded resulting from stress induced by repeated freezing and thawing.

5. Audience

VS employees and members of the biologics industry.

6. Guidance

The following sections provide specific details regarding dating, monitoring, and qualification/requalification of MRs and WRs.

A. Identifying the Master and Working References

Identify both the MR and the WR including storage conditions in the filed OP. See VSM 800.206 for detailed guidance.

B. Storage Conditions of MRs

Store the MR under constant defined conditions. A Standard Operating Procedure (SOP) must describe a system for recording the storage conditions and verifying that they do not deviate from the specific limits defined in the SOP. Use calibrated equipment to record the temperature at regular intervals by automated or manual means. Store data electronically and make it available for inspection. Promptly notify CVB of any deviation in the allowable range of storage conditions.

C. Master Reference Qualification and Requalification via Host Animal Immunogenicity Study

Qualification of a MR for use in an *in vitro* relative RPA is either the initial establishment of a MR or the establishment of a new MR to replace an existing MR. The MR may be directly qualified in animals when it is a non-frozen product reference. If the MR is 1) a frozen product reference, 2) a stabilized and frozen preparation, or 3) a purified preparation, a QS will be needed for MR qualification or requalification.

When a QS must be used, test the MR and the QS in a minimum of five independent replicate serial release assays to establish the RP of the QS or to determine the dilution of the MR that would result in the QS having an RP of 1.0.

Prior to its expiration or any time there is an indication of lack of stability, a MR must be requalified, or a new MR must be qualified by conducting a host animal immunogenicity study unless qualification *in vitro* is allowed (Section 6. D.). The same animal model and efficacy study design that supported initial licensure or the most recent reference qualification may be used. A similar outcome to either study will be sufficient to qualify or requalify an MR. If the licensee or permittee elects to propose improvements to the study design used previously, the protocol should explain and justify the proposed changes. A new FDFT study (Section 6. I.) must be conducted for a modern product frozen MR every time a MR is qualified or requalified.

D. Master Reference Qualification *In Vitro*

If a MR is monitored from time zero for stability using validated RSM assays in an approved RSM plan, a new MR may be qualified *in vitro* using the RSM assays upon inventory depletion or degradation of the existing MR. Most MRs qualified prior to the date of this memorandum will require one additional host animal immunogenicity study to qualify a MR that is tested via an approved RSM plan from time zero. An allowance for *in vitro* qualification will be made if a newly approved monitoring plan is implemented and time zero testing is conducted within 2 years of the host animal immunogenicity study used to qualify the MR.

To be eligible for *in vitro* qualification of a MR, establish a baseline at time zero. Test 20 vials of the MR at time zero in the quantitative RSM assay to establish the Baseline 1 discussed in Section 6. H. Estimate the mean and 90 percent confidence interval from testing the 20 vials in the quantitative RSM assay. The lower bound of this 90 percent confidence interval sets the baseline for *in vitro* qualification in the future (Baseline 1 in Section 6. H.). CVB will consider proposals for establishing the baseline for *in vitro* qualification using alternative methods. This baseline is treated as a non-inferiority margin for the statistical analysis when qualifying a new MR using the *in vitro* testing approach. To qualify a new MR using the quantitative RSM assay, test a sufficient number of vials and estimate the mean and 90 percent confidence interval. If the lower bound of this confidence interval remains above the margin (baseline) established at time zero, the new MR will be qualified. The precision analysis from the RSM assay validation can be used to help determine a sufficient number of vials to test (Section 6. J.).

Test the MR using the qualitative assay to demonstrate no unexpected results.

In vitro MR qualification will not be an option when the MR stability is monitored using an IC in the serial release assay. RSM assays for the IC are required to ensure the IC is not degrading at the same rate as the MR, which would not necessarily be detected in the serial release assay. The MR is not tested using RSM assays and therefore cannot be qualified using an *in vitro* approach.

E. Working Reference Qualification

When a separate WR is prepared, demonstrate parallelism between the MR and WR in the serial release assay.[1] Establish the geometric mean potency of the WR when compared to the MR in the approved serial release assay by five or more independent test runs. The WR must have a geometric mean RP no less than 1.0 when compared to the approved MR.

[1] Demonstrating similarity between the MR and WR may be necessary for other types of assay systems.

F. Dating & Associated Monitoring Requirements for Master References

1) *Dating for Legacy Product MR.* MRs for legacy products may be used continuously for serial release (i.e., as a WR) or to establish new WRs for up to 15 years from the date of initial qualification (or requalification) via host animal immunogenicity study if there are no obvious changes in the behavior of the MR in the serial release assay (Section 6. G.). MRs exhibiting an obvious change in behavior in the serial release assay at any time must be replaced. No dating beyond 15 years will be considered under this scenario unless the MR is requalified in a host animal immunogenicity study (Section 6. C.).

   Licensees and permittees may elect but are not required to use procedures described in Section 6. H. to monitor MRs for legacy products, which would allow longer dating periods for MRs of legacy products without new host animal immunogenicity studies and allows for the possibility of *in vitro* qualification.

2) *Dating for Modern Product Frozen MR.* Evaluate all modern product frozen MRs in a FDFT study (Section 6. I.). The results of this study will determine if, in addition to serial release trending data, monitoring the MR by RSM assays is necessary.

   a. MRs of modern products that are stored frozen and pass the FDFT study can be used for a period of 15 years. If the licensee or permittee chooses to monitor using only serial release trending data, then the MR would not be eligible for dating beyond the 15 years without requalifying the MR or qualifying a new MR in a host animal immunogenicity study. If the licensee or permittee chooses to monitor using RSM assays, they should follow the same time frames provided in Section 6. F. 2) b. If time zero testing using the RSM assays is conducted within 2 years of qualification, the MR will be eligible for dating beyond 15 years with the possibility of *in vitro* qualification.

   b. MRs of modern products that are stored frozen and do not pass the FDFT study will initially be granted 5 years dating. The dating can be increased in 5-year increments to a total of 15 years, contingent upon submission of serial release trending data at 2 ½ year intervals and the milestones described below. Prior to the final expiration, qualify a new MR or requalify the current MR in a host animal immunogenicity study (Section 6. C.). This step is necessary so that the qualified/requalified MRs can be tested according to the approved RSM plan with validated RSM assays from time zero.

      1. Submit a conceptual RSM plan for review within the first 2 ½ years (Section 6. H.). Revise as necessary based on feedback from CVB.

2. The RSM plan must be tentatively approved within the first 5 years. Upon tentative approval, the MR expiration will be extended for an additional 5 years (for a total of 10 years from time zero). Final approval is subject to successful validation of the RSM assays.

3. Validate the RSM assays and obtain CVB approval before the end of the current (10-year) expiration. Upon approval of the RSM assays, the expiration of the MR will be extended 5 years (for a total of 15 years).

3) *Dating for Modern Product Non-Frozen MR.* MRs of modern products that are not stored frozen will be given product dating prior to the approval of the RSM plan and validation of the RSM assays. There is no option to extend dating beyond product dating based on the development of the RSM plan and assay validation efforts. Once a non-frozen MR can be tested from time zero using validated RSM assays, such MRs may be eligible for indefinite dating with demonstration of stability.

4) *Dating for WRs.* If a WR is serial of product, stored according to the label, it will receive product dating. See Section 6. E. for details on qualifying new WRs.

G. Monitoring Master Reference Stability Using Serial Release Trending Data

The only monitoring requirement for any legacy MR to be eligible for 15-year dating is using serial release testing data from the time of initial qualification. The monitoring of any modern MR should include serial release testing as well, which may be all that is required for monitoring (Section 6. F. 2. a.) or may be just one component of the monitoring (Section 6. F. 2. b.).

Reports summarizing all serial release data from time zero must be submitted to CVB Policy, Evaluation and Licensing (CVB-PEL) at 2 ½ year intervals. A submission should be made to CVB-PEL including all raw data from the MR, the WR (if different than the MR), test serials, and controls in an electronic format consistent with the CVB Data Guide, available on CVB's website. Include all data from the initial reference qualification through the most recent serial release test. The report should include graphical and/or tabular summaries of the data as appropriate and a summary statement of the storage conditions and temperature monitoring procedure for the MR (and WR if different than the MR). Test the MR a minimum of once every quarter in the serial release assay. This will be necessary when the WR is different than the MR or if no serials are tested for serial release during such time.

The raw data sets should be complete. For example, ELISA raw data would include the optical density for every well on each plate, along with the related information, such as plate layout and dilution sequences. For serological assays, include serum titers of each animal and for laboratory animal challenge tests, include the response of every animal, including those used for back titration of the challenge material.

Where appropriate, data summaries should include plots of each individual test. This is important, for example, with test methods using dilution sequences, such as ELISAs or animal vaccination-challenge tests. The plot should include the reference and serials. In many situations, such as ELISAs, it may also be useful to plot the parameter estimates from regression models fit to individual preparations on each test, as well as the ratio of parameter estimates comparing those from the serials to those from the reference.

H. Master Reference Monitoring Using Reference Stability Monitoring Assays

To monitor MRs via RSM assays, whether required or optional, the licensee or permittee must establish an acceptable RSM plan with validated RSM assays. The licensee or permittee is encouraged to submit a plan before submitting the assays. The plan should include details of the proposed assays, frequency of testing, MR storage conditions, and method of monitoring the storage conditions. There is no expectation that the assays will be validated at the time the plan is submitted, but CVB review at this time will allow preliminary feedback and preliminary acceptance if the plan appears reasonable.

The assays in a RSM plan must monitor the quantitative and qualitative characteristics of the MR. Some assay methods might be capable of fulfilling both objectives, but multiple assay methods may be necessary. See Section 6. L. for additional considerations for RSM assays. All RSM assays must be validated prior to final CVB approval and implementation of the RSM plan.

In addition to typical validation parameters (see VSM 800.112), RSM assays must be evaluated for their ability to detect degradation of the specific MR they are intended to monitor. For a product in which potency is measured by a RPA, the assumption is that any preparation with the same or more antigen (protective antigen targeted in the assay) will be efficacious in the host animal when prepared in a manner consistent with the QS. At the time the pivotal efficacy study is conducted (time zero), the amount of antigen considered to be efficacious is established, and a benchmark is established regarding the “signal” produced in the RPA. The goal of using RSM assays is to assess whether the benchmark has changed.[2] Specifically, the objective is to

[2] It is not possible to observe the real quantity of interest, namely the reference at any point in time compared to itself at time zero tested in the same run of the assay.

assure that the signal of the reference in the RPA does not change over time, thus assuring the potency of a serial is consistently measured. If the signal does change, it is assumed the reference is changing. Therefore, two key baselines must be established:

- Baseline 1: Performance specifications for the MR at time zero which will serve as a non-inferiority margin for future *in vitro* qualification.
- Baseline 2: Performance specifications when the MR has degraded to the extent that it is no longer acceptable for use in serial release testing.

Additional guidance regarding these benchmarks is covered in Sections 6. D. and 6. J, respectively. Once CVB accepts the validation reports for the RSM assays described in the RSM plan, you may implement the RSM plan. Submit reports to CVB every 2 ½ years along with the serial release trending data.

I. Forced Degradation by Freeze-Thaw

At the time of qualification of a modern product frozen MR perform an FDFT study using a validated serial release assay. Evaluate the data for a change in the shape of the curve and a decrease in the relative potency. This is only necessary for MRs qualified in host animal immunogenicity studies.

Subject 10 vials of the MR to the following freeze-thaw regimen (henceforth referred to as “treated reference”):

- Vial volumes should represent how the reference is stored, ideally in small aliquots (e.g., 1 mL).
- Freeze at normal storage conditions (e.g., -70 to -80 °C) for at least 6 hours to ensure complete freezing.
- Thaw at 20-25 °C until the vial is fully thawed or at least 60 minutes, whichever is longer.
- Complete 10 cycles of freezing and thawing as specified above.
- After the final freezing cycle, conduct 10 independent serial release potency tests, where one vial of treated reference and one vial of untreated reference are tested in each run of the assay.

Evaluate the data for a change in the shape of the dose-response curve and a decrease in the relative potency. For relative potency ELISA assays, assess parallelism in a manner consistent to assay validation (see VSM 800.112 Appendix III Section 2.2.5 and Parallelism Analysis in Assay Validation (STATWI0006)). If the treated reference passes the parallelism assessment, evaluate the change in potency and compare the lower limit of a 90 percent confidence interval estimate of the relative potency of the treated reference to a value of 0.8. An MR is determined to pass the FDFT study if the treated reference passes the parallelism assessment and the lower

limit of the confidence interval for the RP remains above 0.8. MRs that do not do not pass the FDFT study are dated according to Section 6. F. 2. b.

J. Ability of a Reference Stability Monitoring Assay to Detect MR Degradation

A critical step in validating assays for an RSM plan is demonstrating the assay's ability to detect degradation in the MR prior to unacceptable signal loss in the serial release test. This requires testing MRs at various degrees or levels of degradation during RSM assay validation.

Licensees or permittees may "age" an MR artificially by means other than time. If repeated freezing and thawing (Section 6. I.) can degrade the reference (which may require more than 10 cycles), use this method as it is the most likely type of stressor to which a frozen reference will be exposed. If not, harsher methods such as heat or chemical treatment may be proposed. Diluting the reference is not an acceptable surrogate for degradation. Describe in the RSM assay validation protocol how the selected method of forced degradation will mimic natural degradation over time under the proposed storage conditions. Vials will be tested after different levels of degradation, such as different numbers of freeze and thaw cycles.

Test a panel of MR vials degraded to various degrees in each proposed RSM assay and in the serial release assay. MR degradation may manifest itself in various ways: Quantitative, qualitative, or both.

The simplest scenario is where the detected change is quantitative, and the relationship between the response in the quantitative RSM assay and the response from the serial release assay is linear. When this is the case, establish a threshold/baseline (on the fitted line) corresponding to the response of the degraded MR that produces an expected RP of 0.8 in the serial release assay (baseline 2 from Section 6. H.). If degradation manifests in other ways or the relationship between the response from the quantitative RSM assay and the serial release assay is not linear, alternative metrics are necessary (such as parameter ratios) and other cutoff test values may be used. Licensee or permittee must take responsive action, such as qualifying a new MR prior to the MR producing a value below the threshold/baseline in the RSM assay.

When establishing the dose-response relationship, evaluate precision by testing multiple vials of the degraded MR in both the serial release assay and the RSM assays. The precision of the RSM assay should be considered when determining the number of vials of the MR to test at future time points.

K. Using an Internal Control to Monitor Reference Stability

There may be instances in which an IC is used as a surrogate to monitor stability of the MR. In these instances, the IC is tested with the RSM assays and the relationship between the IC and the MR in the serial release assay is monitored. Two baselines are necessary in this instance, one regarding the IC's response in the RSM assays and one related to the relationship between the IC and the MR in the serial release assay.

To establish the baseline in the RSM assays, test the degraded IC (at various levels) in the RSM assays and against the MR in the serial release assay. Establish the relationship between the response in the RSM assays and the metrics used to monitor the MR in the serial release assay. Establish a baseline in a similar manner to the description provided in Section 6. J.

The second baseline will be pre-determined and related to the response in the serial release assay (relationship between the IC and the MR). For an ELISA, that baseline may be related to a ratio (such as upper asymptote or scale parameters) or to a difference in location parameter estimates. Propose a baseline in a protocol and justify its value. Conduct a study to demonstrate the baseline value may be generated (or spanned) using various degrees of a degraded MR.

L. Other Considerations when selecting the RSM assays

1) The monitoring assays must be able to detect a signal change in the serial release assay, whether that manifests only as a loss in relative potency or in other ways, such as a change in the shape of the dose-response curve.

2) Ideally, but not necessarily, the RSM assays will incorporate the antibody used in the serial release assay. This will allow an assessment of the antigen-antibody interaction. A measurement of total protein will not be considered as a quantitative metric for RSM.

3) If the RSM assays rely on the use of another preparation, such as a standard, consider the source of the standard and the frequency and method of replacement. Standards that are commercially available with a certificate of analysis and defined expirations will be more appropriate than an in-house created preparation that could be degrading at a similar rate as the MR.

M. Legacy Status for Combination Products and Major Manufacturing Changes

Major changes in the manufacturing procedure will result in a product licensed prior to January 1, 2011, being classified as a modern product for the purposes of this guidance. An example would be changes that require a new product code and a new efficacy and/or safety study before approval of the change.

Combination products containing a legacy product and a modern product will be treated as a modern product for the new antigens, and as a legacy product for those antigens licensed prior to January 1, 2011, if the combination of fractions does not impact the potency tests for the legacy licensed fractions. Multivalent products combining antigens of legacy products will be evaluated on a case-by-case basis.

Improvements to the serial release potency assays of legacy products will not cause a legacy product to be reclassified if such changes do not alter the analytical principle of the test method. Modifications that improve the performance of a legacy product's test method will not require complete revalidation of the assay.

N. Summary of Dating, Monitoring, Reference Qualification

This section is intended to provide highlights on legacy and modern product MRs regarding monitoring methods, dating, and reference qualification. CVB intends the information here to help simplify and clarify guidance; it does not intend it to be all inclusive.

1) Scenario 1

- Legacy product MRs
- Monitoring via serial release data only, submitted every 2 ½ years
- 15-year dating maximum
- Host animal immunogenicity study required to qualify a new MR or requalify the current MR

2) Scenario 2

- Modern product MRs stored frozen
- Passes FDFT regimen
- Monitoring via serial release data only, submitted every 2 ½ years
- 15-year dating maximum
- Host animal immunogenicity study required to qualify a new MR or requalify the current MR

3) Scenario 3

- Modern product MRs stored frozen
- Passes FDFT regimen
- Monitoring via RSM assay and serial release data, submitted every 2 ½ years
- 15-year initial dating during which RSM plan must be approved and RSM assays validated

- Reference qualification/requalification via host animal immunogenicity study if time zero testing not conducted within 2 years of original MR qualification

4) Scenario 4

- Modern product MRs stored frozen
- Fails FDFT regimen
- Monitoring via RSM assays and serial release data
- Dating assigned in 5-year increments based on process toward approval of RSM plan and RSM assays validation – Maximum 15 years
- Reference qualification/requalification via host animal immunogenicity study required

5) Scenario 5

- Modern product frozen MRs
- Monitoring via RSM assays and serial release data, submitted every 2 ½ years
- Required when FDFT regimen fails or can be optionally chosen for the benefits of dating and reference qualification *in vitro*
- RSM plan approved, RSM assays validated, time zero testing conducted within 2 years of MR qualification in host animals
- Indefinite dating possible

6) Scenario 6

- Modern product MR not frozen, product storage
- Monitoring via RSM assays and serial release data, submitted every 2 ½ years
- Product dating prior to approved RSM plan and validating RSM assays
- Reference qualification/requalification host animal immunogenicity study required prior to monitoring using RSM assays from time zero

7) Scenario 7

- Modern product MR not frozen, product storage
- Monitoring via RSM assays and serial release data, submitted every 2 ½ years
- Approved RSM plan and validating RSM assays, testing initiated at time zero
- Reference qualification *in vitro*

7. Implementation/Applicability

Updated policy in this memorandum is effective immediately.