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Center for Veterinary Biologics
Testing Protocol

SAM 502

Supplemental Assay Method for the Determination of Residual Moisture in Veterinary Biologics Products

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1. Introduction

This Supplemental Assay Method (SAM) describes a procedure for determination of residual moisture testing in veterinary vaccines, as prescribed in title 9, *Code of Federal Regulations* (9 CFR), part 113.29.

Freeze-dried veterinary vaccines always contain some water, commonly known as residual moisture (RM). It is important to determine the level of RM in final products, since a satisfactory test gives assurance of an adequate shelf life and that the manufacturer's freeze-dry cycle was properly controlled. The RM test should confirm that moisture level is consistently within the manufacturer's specification.

Residual moisture is determined by the gravimetric method as follows: Residual moisture is driven from the test product by heating under vacuum. The residual moisture content (as per cent) of the test product is calculated based on the product weight loss during the drying cycle.

2. Materials

2.1 Cylindrical weighing bottles – individually numbered with airtight glass stoppers

2.2 Vacuum oven – equipped with validated thermometer and thermostat. A suitable air-drying device must be attached to the inlet valve.

2.3 Balance – capable of readability to 0.1 mg (rated precision ± 0.1 mg)

2.4 Desiccator – with phosphorus pentoxide, silica gel or equivalent

3. Preparation for the Test

3.1 Personnel qualifications/training

Technical personnel must have experience or training in this protocol. This includes working knowledge of the use of general laboratory equipment and glassware; and specific training in the operation of the laboratory equipment listed in **Section 2**.

3.2 Environment

Conduct all operations in an environment with a relative humidity less than 45%.

3.3 Weighing bottles

3.3.1 Label one weighing bottle per sample with a unique sample identifier (i.e., submitted sample identification, Laboratory Information Management System (LIMS) assigned sample number or accession number).

3.3.2 Thoroughly clean all weighing bottles.

3.3.3 Place stopper at an angle on top of bottle and dry for a minimum of 30 minutes at 60°± 3°C under vacuum (< 2.5 kPa).

3.3.4 While hot, immediately transfer bottles and stoppers into a desiccator. Allow to cool to room temperature, close stopper, weigh and record the weight as "*Sample ID*$_A$".

3.3.5 Return bottles to desiccator.

3.4 Equipment

All equipment must be operated according to manufacturers' recommendations and monitored in compliance with applicable standard operating procedures.

3.5 Preparation of the sample

Retain sample, in original airtight containers at room temperature until use. Do not break the seal until ready to proceed.

4. Performance of the Test

4.1 Break sample container seal. Using a spatula, break up desiccated product and rapidly transfer (minimum of 100 mg or the amount required for a precise determination at the lower limit, use more than one vial for single dose products if needed) to a previously weighed bottle. Close stopper and immediately weigh. Record the weight as "*Sample ID*$_B$".

4.2 Place the bottle with the stopper at an angle in the vacuum oven. Set vacuum to < 2.5 kPa and the temperature to 60°± 3°C.

4.3 After a minimum of 3 hours, turn off the vacuum pump and bleed dry air into the oven until the pressure inside of the oven is equalized with the atmosphere.

4.4 While the bottle is still warm, stopper bottle and transfer to desiccator, and allow to cool to room temperature (for a minimum of two hours or a time validated to yield a constant weight). Weigh, and record the weight as "*Sample ID*$_C$".

5. Interpretation of the Test Results

Calculate the residual moisture (%) as:

$$\%\ moisture = \left(\frac{Sample\ ID_B - Sample\ ID_C}{Sample\ ID_B - Sample\ ID_A}\right) * 100$$

Sample ID$_A$ is tare weight of bottle.
(*Sample ID*$_B$ – *Sample ID*$_A$**)** is weight of sample before assay.
(*Sample ID*$_B$ – *Sample ID*$_C$**)** is weight equivalent to residual moisture of sample.

6. Report of Test Results

Report results of the test as described by standard operating procedures.

7. References

7.1 Title 9, *Code of Federal Regulations*, part 113.29, U.S. Printing Office, Washington, DC.

7.2 Testing of Residual Moisture, VICH GL26, Final, April, 2002.

8. Summary of Revisions

Version .04

- Updated Contact information.

Version .03

- The Contact information has been updated.
- **3.1:** Personnel qualifications/training have been updated.
- The entire document has been revised to reflect current practices.

Version .02

- The document number has been changed from TCSAM0502 to SAM 502.
- The Contact has been changed from P. Frank Ross to Debra Owens.