

§ 113.29

9 CFR Ch. I (1-1-13 Edition)

(2) If mycoplasma colonies are found on any of the plates inoculated with material being tested, the results are positive for mycoplasma contamination.

[38 FR 29887, Oct. 30, 1973, as amended at 41 FR 6752, Feb. 13, 1976; 41 FR 32882, Aug. 6, 1976]

§ 113.29 Determination of moisture content in desiccated biological products.

Methods provided in this section must be used when a determination of moisture content in desiccated biological products is prescribed in an applicable Standard Requirement or in the filed Outline of Production for the product. Firms currently using methods other than those provided in this section for determining the moisture content in desiccated biological products have until November 5, 2004 to update their Outlines of Production to be in compliance with this requirement.

(a) Final container samples of completed product shall be tested. The weight loss of the sample due to drying in a vacuum oven shall be determined. All procedures should be performed in an environment with a relative humidity less than 45 percent. The equipment necessary to perform the test is as follows:

- (1) Cylindrical weighing bottles with airtight glass stoppers.
- (2) Vacuum oven equipped with validated thermometer and thermostat. A suitable air-drying device should be attached to the inlet valve.
- (3) Balance, accurate to 0.1 mg (rated precision ±0.01mg).
- (4) Desiccator jar equipped with phosphorous pentoxide, silica gel, or equivalent.
- (5) Desiccated vaccine in original sealed vial. Sample and control should be kept at room temperature in their original airtight containers until use.

(b) Test procedure:

- (1) Thoroughly cleaned and labeled sample-weighing bottles with stoppers should be allowed to dry at 60 ±3 °C under vacuum at less than 2.5 kPa.
  - (i) Transfer hot bottles and stoppers into the desiccator and allow to cool to room temperature.

(ii) After bottles have cooled, insert stoppers and weigh and record the weights of the bottles as "A."

(iii) Return weighing bottles to the desiccator.

(2) Remove the sample container seal.

(i) Using a spatula, break up the sample plug and transfer the required amount of sample to the previously tared weighing bottle.

(ii) Insert the stopper and weigh and record the weights of the weighing bottles as "B."

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

(4) After a minimum of 3 hours of drying time, turn off the vacuum pump and allow dry air to bleed into the oven until the pressure inside the oven is equalized with the prevailing atmospheric pressure.

(5) While the bottle is still warm, replace the stopper in its normal position and transfer the weighing bottle to the desiccator.

(i) Allow a minimum of 2 hours for the weighing bottle to cool to room temperature or for its weight to reach equilibrium.

(ii) Weigh, and record the weight as "C."

(6) Calculate the percentage of moisture in the original sample as follows:

$$(B - C) / (B - A) \times (100) = \text{Percentage of residual moisture, where:}$$

- A = tare weight of weighing bottle
- B - A = weight of sample before drying
- B - C = weight of sample after drying

(7) The results are considered satisfactory if the percentage of residual moisture is less than or equal to the manufacturer's specification.

[68 FR 57608, Oct. 6, 2003]

§ 113.30 Detection of Salmonella contamination.

The test for detection of Salmonella contamination provided in this section shall be conducted when such a test is prescribed in an applicable Standard Requirement or in the filed Outline of Production for the product.

(a) Samples shall be collected from the bulk suspension before bacteriostatic or bactericidal agents

have been added. When tissue culture products are to be tested, 1 ml of tissue extract used as the source of cells or 1 ml of the minced tissue per se shall be tested.

(b) Five ml of the liquid vaccine suspension shall be used to inoculate each 100 ml of liquid broth medium (tryptose and either selenite F or tetrathionate). The inoculated media shall be incubated 18–24 hours at 35–37 °C.

(c) Transfers shall be made to either MacConkey agar or Salmonella-Shigella agar, incubated for 18–24 hours and examined.

(d) If no growth typical of Salmonella is noted, the plates shall be incubated an additional 18–24 hours and again examined.

(e) If suspicious colonies are observed, further subculture on suitable media shall be made for positive identification. If Salmonella is found, the bulk suspension is unsatisfactory.

[38 FR 29888, Oct. 30, 1973]

#### § 113.31 Detection of avian lymphoid leukosis.

The complement-fixation test for detection of avian lymphoid leukosis provided in this section shall be conducted on all biological products containing virus which has been propagated in substrates of chicken origin: *Provided*, An inactivated viral product shall be exempt from this requirement if the licensee can demonstrate to Animal and Plant Health Inspection Service that the agent used to inactivate the vaccine virus would also inactivate lymphoid leukosis virus.

(a) Propagation of contaminating lymphoid leukosis viruses, if present, shall be done in chick embryo cell cultures.

(1) Each vaccine virus, cytopathic to chick embryo fibroblast cells, shall be effectively neutralized, inactivated, or separated so that minimal amounts of lymphoid leukosis virus can be propagated on cell culture during the 21-day growth period. If a vaccine virus cannot be effectively neutralized, inactivated, or separated, a sample of another vaccine prepared the same week from material harvested from each source flock (or other sampling procedure acceptable to Animal and Plant Health Inspection Service) used for the

preparation of the questionable vaccine virus that cannot be neutralized, inactivated, or separated shall be tested each week during the preparation of such questionable vaccine.

(2) When cell cultures are tested, 5 ml of the final cell suspension as prepared for seeding of production cell cultures shall be used as inoculum. When vaccines are tested, the equivalent of 200 doses of Newcastle disease vaccine or 500 doses of other vaccines for use in poultry, or one dose of vaccine for use in other animals shall be used as inoculum. Control cultures shall be prepared from the same cell suspension as the cultures for testing the vaccine.

(3) Uninoculated chick embryo fibroblast cell cultures shall act as negative controls. One set of chick fibroblast cultures inoculated with subgroup A virus and another set inoculated with subgroup B virus shall act as positive controls, A and B respectively.

(4) The cell cultures shall be propagated at 35–37 °C for at least 21 days. They shall be passed when necessary to maintain viability and samples harvested from each passage shall be tested for group specific antigen.

(b) The microtiter complement-fixation test shall be performed using either the 50 percent or the 100 percent hemolytic end point technique to determine complement unitage. Five 50 percent hemolytic units or two 100 percent hemolytic units of complement shall be used for each test.

(1) All test materials, including positive and negative controls, shall be stored at –60 °C or colder until used in the test. Before use, each sample shall be thawed and frozen three times to disrupt intact cells and release the group specific antigen.

(2) The antiserum used in the microtiter complement-fixation test shall be a standard reagent supplied or approved by the Animal and Plant Health Inspection Service. Four units of antiserum shall be used for each test.

(3) Presence of complement-fixing activity in the harvested samples (from passages) at the 1:4 or higher dilution, in the absence of anticomplementary activity, shall be considered a positive test unless the activity can definitely