§ 113.28 Detection of mycoplasma contamination.

The heart infusion test, using heart infusion broth and heart infusion agar, provided in this section shall be conducted when a test for mycoplasma contamination is prescribed in an applicable Standard Requirement or in

(i) If growth is found in any test vessel of the final test, the lot of Master Seed Virus is unsatisfactory.

(d) Master Seed Bacteria. Not less than 4 ml of each lot of Master Seed Bacteria shall be tested. Frozen liquid Master Seed Bacteria shall be thawed, and desiccated Master Seed Bacteria shall be rehydrated with sterile purified water immediately prior to starting the test.

(1) To test for extraneous bacteria, place 0.2 ml of the sample of Master Seed Bacteria into 10 individual vessels each containing at least 40 ml of Fluid Thioglycollate Medium. Incubation shall be at 30 °C to 35 °C for 14 days.

(2) To test for extraneous fungi, place 0.2 ml of the sample of Master Seed Bacteria into 10 individual vessels each containing at least 40 ml of Soybean Casein Digest Medium. Incubation shall be at 20 °C to 25 °C for 14 days.

(3) Examine the contents of all test vessels macroscopically for atypical microbial growth at the end of the incubation period. If growth of extraneous microorganisms cannot be reliably determined by visual examination, judgment shall be confirmed by subcultures, microscopic examination, or both.

(4) For each set of test vessels representing a lot of Master Seed Bacteria tested according to these procedures, the following rules shall apply:

(i) If extraneous growth is found in any test vessel of the initial test, one retest to rule out faulty technique may be conducted using a new sample of Master Seed Bacteria.

(ii) If extraneous growth is found in any test vessel of the final test, the lot of Master Seed Bacteria is unsatisfactory.

(e) Live viral vaccines of chicken embryo origin recommended for administration other than by parenteral injection, which were not tested or have not been found free of bacteria and fungi by the procedures prescribed in paragraph (a) of this section, may be tested according to the procedures prescribed in this paragraph.

(1) Brain Heart Infusion Agar shall be used with 500 Kinetic (Kersey) units of penicillinase per ml of medium added just prior to pouring the plates.

(2) Ten final containers from each serial and each subserial shall be tested.

(3) Immediately prior to starting the test, frozen liquid vaccine shall be thawed, and lyophilized vaccine shall be rehydrated to the quantity recommended on the label using the accompanying sterile diluent or sterile purified water. Product recommended for mass vaccination shall be rehydrated at the rate of 30 ml sterile purified water per 1,000 doses.

(4) From each container sample, each of 2 plates shall be inoculated with vaccine equal to 10 doses if the vaccine is recommended for poultry or 1 dose if the vaccine is recommended for other animals. Twenty ml of medium shall be added to each plate. One plate shall be incubated at 30 °C to 35 °C for 7 days and the other plate shall be incubated at 20 °C to 25 °C for 14 days.

(5) Colony counts shall be made for each plate at the end of the incubation period. An average colony count for the 10 samples representing the serial or subserial shall be made for each incubation condition.

(6) For each set of test vessels representing a serial or subserial tested according to these procedures, the following rules shall apply:

(i) If the average count at either incubation condition exceeds 1 colony per dose for vaccines recommended for poultry, or 10 colonies per dose for vaccines recommended for other animals in the initial test, 1 retest to rule out faulty technique may be conducted using 20 unopened final containers.

(ii) If the average count at either incubation condition of the final test for a serial or subserial exceeds 1 colony per dose for vaccines recommended for poultry, or 10 colonies per dose for vaccines recommended for other animals, the serial or subserial is unsatisfactory.
the filed Outline of Production for the product.

(a) Media additives provided in this paragraph shall be prepared as follows:

(1) DPN-Cysteine Solution:

(i) Use Nicotinamide adenine dinucleotide (oxidized) and L-Cysteine hydrochloride.

(ii) Prepare 1 gram/100 milliliters (ml) purified water (1 percent solution) of each. Mix the solutions together; the cysteine reduces the DPN. Filter sterilize, dispense in appropriate amounts and store frozen at −20 degrees centigrade.

(2) Inactivated horse serum—horse serum which has been inactivated at 56 °C for 30 minutes.

(b) Heart infusion broth shall be prepared as provided in this paragraph.

(1) Dissolve in 970 ml of purified water, 25 grams of heart infusion broth, 10 grams of proteose peptone No. 3, and either 5 grams of yeast autolysate or 5 ml of fresh yeast extract.

(2) Add the following:

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 percent tetrazolium chloride (ml)</td>
<td>5.5</td>
</tr>
<tr>
<td>1 percent thallium acetate (ml)</td>
<td>25</td>
</tr>
<tr>
<td>Penicillin (units)</td>
<td>500,000</td>
</tr>
<tr>
<td>Inactivated horse serum (ml)</td>
<td>100</td>
</tr>
</tbody>
</table>

(3) Adjust pH to 7.9 with NaOH, filter sterilize, and dispense 100 ml aliquots into 125 ml flasks and store until needed.

(4) Add 2 ml of DPN-Cysteine solution to each 100 ml of broth on day of use.

(c) Heart Infusion Agar shall be prepared as provided in this paragraph.

(1) Dissolve in 900 ml of purified water by boiling the following:

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart infusion agar (g)</td>
<td>25</td>
</tr>
<tr>
<td>Heart-infusion broth (g)</td>
<td>10</td>
</tr>
<tr>
<td>Proteose peptone No. 3 (g)</td>
<td>10</td>
</tr>
<tr>
<td>1 pct thallium acetate (ml)</td>
<td>25</td>
</tr>
</tbody>
</table>

(2) Cool the medium and adjust pH to 7.9 with NaOH.

(3) Autoclave the medium.

(4) Cool the medium 30 minutes in a 56 °C waterbath.

(5) Dissolve 5 grams of yeast autolysate in 100 ml of distilled water, filter sterilize, and add to the medium.

(6) Add to the medium:

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>126 ml of inactivated horse serum</td>
<td></td>
</tr>
<tr>
<td>21 ml of DPN-Cysteine solution</td>
<td></td>
</tr>
<tr>
<td>525,000 units of Penicillin</td>
<td></td>
</tr>
</tbody>
</table>

Dispense 10 ml aliquots into 60×15 mm disposable culture dishes or petri dishes.

(d) The test procedure provided in this paragraph shall be followed when conducting the mycoplasma detection test.

(1) Preparation of inoculum. Immediately prior to starting the test, frozen liquid vaccine shall be thawed, and lyophilized vaccine shall be rehydrated to the volume recommended on the label with mycoplasma medium. In the case of a lyophilized biological product, e.g., 1,000 dose vial of poultry vaccine to be administered via the drinking water, the vaccine shall be rehydrated to 30 ml with mycoplasma medium. In the case of a cell line or a sample of primary cells, the inoculum shall consist of the resuspended cells. Control tests shall be established as provided in paragraph (d)(4) of this section.

(2) Inoculation of plate. Plate 0.1 ml of inoculum on an agar plate and make a short, continuous streak across the plate with a pipet. Tilt the plate to allow the inoculum to flow over the surface.

(3) Inoculation of flask of medium. Transfer 1 ml of the inoculum into a flask containing 100 ml mycoplasma medium and mix thoroughly. Incubate the flask at 33 to 37 °C for 14 days during which time, one of four agar plates shall be streaked with 0.1 ml of material from the incubating flask of inoculated medium on the 3rd day, one on the 7th day, one on the 10th day, and one on the 14th day post-inoculation.

(4) Control tests shall be conducted simultaneously with the detection test using techniques provided in paragraphs (d)(2) and (3) of this section, except the inoculum for the positive control test shall be selected mycoplasma cultures and the negative control test shall be uninoculated medium from the same lot used in the detection test.

(5) All plates shall be incubated in a high humidity, 4–6 percent CO₂ atmosphere at 33 °C to 37 °C for 10-14 days and examined with a stereoscopic microscope at 35x to 100x or with a regular microscope at 100x.

(e) Interpretation of test results.

(1) If growth appears on at least one of the plates in the positive control test and does not appear on any of the plates in the negative control test, the test is valid.
§ 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 ±0.01 mg).
(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.

(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:

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

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.

§ 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...