Animal and Plant Health Inspection Service, USDA

§ 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 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:

1 percent tetrazolium chloride (ml) .............. 5.5
1 percent thallium acetate (ml) .................. 25
Penicillin (units) ........................................ 500,000
Inactivated horse serum (ml) ..................... 100

(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>Ingredient</th>
<th>Amount</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>Ingredient</th>
<th>Amount</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>
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</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 3d 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 CO\textsubscript{2} 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.

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


§ 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