for each stage shall be used. The criteria used for judging the specific response in the controls and vaccinates shall be in accordance with the test protocol used in the Master Seed immunogenicity test.

(2) If at least 80 percent of the controls do not show specific responses to challenge, the test is inconclusive and may be repeated. If a vaccinate shows the specific responses to challenge expected in the controls, the vaccinate shall be listed as a failure.

(3) The results of the testing shall be evaluated according to the following table:

<table>
<thead>
<tr>
<th>Stage</th>
<th>Number of animals</th>
<th>Failures for satisfactory serials</th>
<th>Failures for unsatisfactory serials</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>1 or less</td>
<td>3 or more.</td>
</tr>
<tr>
<td>2 (or 1)</td>
<td>20</td>
<td>4 or less</td>
<td>5 or more.</td>
</tr>
</tbody>
</table>

(4) When a serial has been found unsatisfactory for potency by the test provided in paragraphs (e)(1), (2), and (3) of this section, the serial shall be withheld from the market and the following actions taken:

(i) The Administrator shall require that at least two additional serials prepared with the same Master Seed be subjected to similar animal potency tests by Animal and Plant Health Inspection Service or the licensee or both.

(ii) If another serial is found unsatisfactory for potency, the product shall be removed from the market while a reevaluation of the product is made and the problem is resolved.

§ 113.25 Culture media for detection of bacteria and fungi.

(a) Ingredients for which standards are prescribed in the United States Pharmacopeia, or elsewhere in this part, shall conform to such standards. In lieu of preparing the media from the individual ingredients, they may be made from dehydrated mixtures which, when rehydrated with purified water, have the same or equivalent composition as such media and have growth-promoting buffering, and oxygen tension-controlling properties equal to or better than such media. The formulas for the composition of the culture media prescribed in §§113.26 and 113.27 are set forth in the United States Pharmacopeia, 19th Edition.

(b) The licensee shall test each quantity of medium prepared at one time from individual ingredients and the first quantity prepared from each lot of commercial dehydrated medium for

§ 113.9 New potency test.

A potency test written into the filed Outline of Production for a product shall be considered confidential information by Animal and Plant Health Inspection Service until at least two additional product licenses are issued for the product or unless use of the test is authorized by the licensee, in which case, such potency test may be published as part of the Standard Requirement for the product.
§ 113.26 Detection of viable bacteria and fungi except in live vaccine.

Each serial and subserial of biological product except live vaccines shall be tested as prescribed in this section unless otherwise specified by the Administrator. When cell lines, primary cells, or ingredients of animal origin used in the preparation of a biological product are required to be free of viable bacteria and fungi, they shall also be tested as prescribed in this section.

(a) The media to be used shall be as follows:

1. Fluid Thioglycollate Medium with 0.5 percent beef extract shall be used to test for bacteria in biological products other than clostridial toxoids, bacterins, and bacterin-toxoids.

2. Fluid Thioglycollate Medium with or without 0.5 percent beef extract shall be used to test for bacteria in biological products other than clostridial toxoids, bacterins, and bacterin-toxoids.

3. Soybean-Casein Digest Medium shall be used to test biological products for fungi; provided, that Fluid Thioglycollate Medium without beef extract shall be substituted when testing biological products containing mercurial preservatives.

(b) Test procedure:

1. Ten test vessels shall be used for each of two media selected in accordance with paragraph (a)(1), (a)(2), or (a)(3) of this section. Each test vessel shall contain sufficient medium to negate the bacteriostatic or fungistatic activity in the inoculum as determined in §113.25(d).

2. Inoculum:

   i. When completed product is tested, 10 final container samples from each serial and each subserial shall be tested. One ml from each sample shall be inoculated into a corresponding individual test vessel of culture medium: Provided, That, if each final container sample contains less than 2 ml, one-half of the contents shall be used as inoculum for each test vessel.

   ii. When cell lines, primary cells, or ingredients of animal origin are tested, at least a 20 ml test sample from each lot shall be tested. One ml shall be inoculated into each test vessel of medium.

3. Incubation shall be for an observation period of 14 days at 30 °C to 35 °C. to test for bacteria and 14 days at 20 °C to 25 °C. to test for fungi.

4. If the inoculum renders the medium turbid so that the absence of growth cannot be determined by visual examination, subcultures shall be made on the seventh to eleventh day from biological products prepared from clostridial toxoids, bacterins, and bacterin-toxoids and the third to seventh day for other biological products. Portions of the turbid medium in amounts of not less than 1.0 ml. shall be transferred to 20 to 25 ml. of fresh medium, and incubated the balance of the 14-day period.

(c) Examine the contents of all test vessels for macroscopic microbial growth during the incubation period.