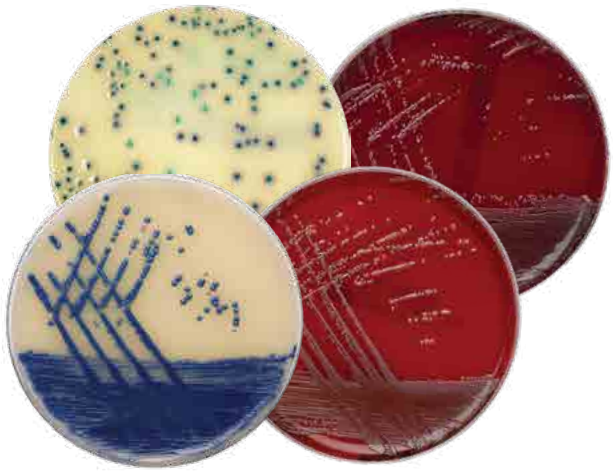
Guidelines for the Evaluation of Commercially Prepared Culture Media

Principle

Evaluation of each medium may require different procedures. For primary media, a variety of microorganisms and specimens may be evaluated to determine if the medium will support the isolation and differentiation of the microorganisms. Media may also be designed to inhibit the growth of various bacteria, which can be evaluated by inoculation with representative strains expected to be inhibited. Concurrent with testing the media with laboratory cultures, it is also important to test media using actual specimens to verify its ability to grow and inhibit the resident microorganism flora. When evaluating different brands of prepared media, the laboratory inoculates the same specimens in parallel for each commercial source.



For secondary media, it may be sufficient to validate the medium only for the specific purpose and for the specific microorganisms for which the medium will be used. This is especially important for a medium that does not require user quality control.

It is recommended that you should check with your accrediting body for guidance regarding the evaluation required for your laboratory when switching to a different commercial manufacturer. Other considerations to evaluate include the delivery speed and reliability, order fill rates, and diversity of product range.

The following procedure is a guideline suggestion intended to be modified as required by your laboratory requirements.

Material for testing

Primary media

Generally, the medium formulations evaluated will be those most commonly used to inoculate specimens. When evaluating media from different manufacturers, obtain the media from the manufacturer on the same day. Inoculate at least two plates per medium with each organism as required. Suggested media to evaluate are listed below.

- TSA w/5% Sheep Blood (BAP)
- Chocolate Agar (CHOC)
- MacConkey Agar (MAC) or EMB Levine Agar (EMB)
- Columbia CNA Agar w/5% Sheep Blood (CNA)
- Thayer-Martin Improved or Modified (TM)
- Non-selective Anaerobic Medium (ANA BA) (includes CDC, Reducible, or Brucella formulations)

Secondary media

1. Test media
    - Media can be evaluated by inoculating well-characterized laboratory strains and quality control organisms as listed in the Clinical Microbiology Procedure Handbook (CMPH) quality control chapter.[1]
    - As part of the evaluation, each technologist performing the test should blindly test organisms expected to give a positive and negative reaction with the product.
    - For these tests, it is not necessary to evaluate each vendor simultaneously.
2. Susceptibility Test Media
    - Evaluate the media as required by your accreditation body using applicable CLSI procedures.

Testing materials

1. ATCC® quality control microorganisms
2. Fresh clinical isolates of microorganisms
3. Specimens to supply the needed variety of microorganisms for testing media
    - Sputum and wound specimens
    - Rectal and throat swabs
4. Other supplies
    - Pasteur pipets with graduations for 1 mL and 0.1 mL (2 drops)
    - Sterile swabs and sticks
    - Inoculating loops
    - Tubes of broth and saline (9 mL and 10 mL) for dilutions
5. Equipment
    - Biological safety cabinet
    - Incubators (35-37°C; both 5% CO_2 and ambient air)
    - Anaerobic gas-generating system

Quality control

Each medium is controlled with a positive and a negative reacting organism per the CLSI document M22-A3.[2]

For biochemical tests not listed in the CLSI M22-A3 document, use the recommended microorganisms found in CMPH.[3]

Evaluation procedures

Refer to the Thermo Fisher Scientific™ Excel® file, 'Evaluation of Commercially Prepared Culture Media Report Form' (which can be supplied by your local representative or via Technical Support). This document should be customized for your laboratory to contain the media, ATCC® and clinical strains, and testing criteria as advised by your accrediting body. You may also wish to add additional testing that is not required but helpful for your laboratory in training staff regarding the differences between the media from one manufacturer versus the other.

Evaluation of media

1. Perform tests using the ATCC® strains or clinical isolates as required by your accrediting body and others as you require for staff training for each medium to determine that the medium is able to support the growth and/or inhibit the growth intended, as well as showing the appropriate morphology.
2. Using a standardized suspension of the quality control organisms is a better challenge of media performance than direct inoculation. A standardized suspension also allows comparison of quality control results between users. A more concentrated suspension tests the ability of selective media to successfully inhibit certain organisms. A lighter suspension challenges the ability of the nonselective media to adequately support growth. Refer to the Flow Chart (Figure 1).

Plate media

(See Figure 1)

- Prepare a suspension in a sterile, non-bacteriostatic saline (0.85% w/v NaCl) or broth to match a 0.5 McFarland standard. Use an 18-24 hour culture to prepare the suspension.
- Dilute the suspension 1:10 in sterile broth or non-bacteriostatic saline. Inoculate each medium with 10 µL (0.01 mL) of the suspension. Streak for isolation. If the 1:10 dilution inoculum proves too dense, use a 1:100 dilution to produce isolated colonies.

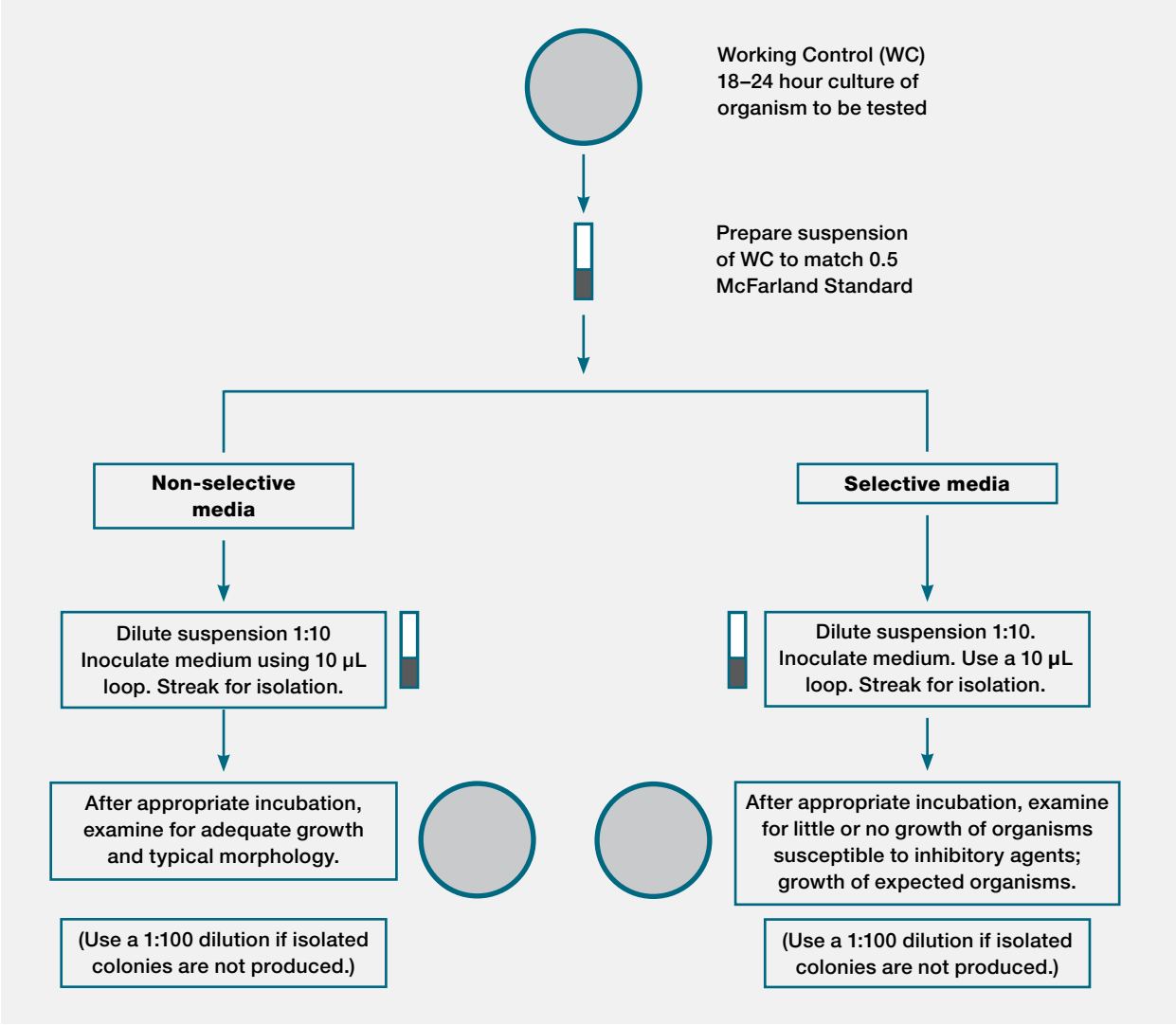
Tubed media

- Inoculate with 10 µL of the undiluted 0.5 McFarland standard suspension.

Specimens

1. Process specimen(s) types indicated in each Worksheet in the Evaluation of Commercially Prepared Culture Media Report Form, per usual laboratory procedures. If swab specimens are being used, alternate which manufacturer's medium is inoculated first each time.
2. Determine if the results of growth from each brand of medium agree. Place the medium side by side to compare.
3. Generally, three to five specimen comparisons are sufficient.
4. For biochemical tests, determine that appropriate reactions are achieved using a variety of strains. Generally, five positive- and five negative-reacting strains are sufficient.

Figure 1. Flow chart for evaluating media



Other evaluation factors

Evaluation of Prepared Media requires the performance of the media, delivery, packaging, expiration dating, moisture content, and other factors.

1. **Delivery Time –** Does the delivery time from placement of the order to receipt of order meet your laboratory's needs?
2. **Complete Order –** Is the order complete? Do the products delivered match the products listed on the packing slip?
3. **Lot Number – Package and Media –** Does the lot number on the packing slip, media box, and plates or tubes match? Are there multiple lots of the same medium?
4. **Days to Expiration –** Does the expiration dating on the media at time of receipt meet the manufacturer's minimum expiration guarantee?
5. **Appropriate Containers –** Does the media box offer protection of the product during shipment? Are media containing dyes shielded from light? Does the box have the required storage temperature, lot number, and expiration date?
6. **Moisture Content –** Is there excessive moisture in the package? Is there drying-out of the media in the package?
7. **Fill Volume –** Is the fill volume of plated media as expected?
8. **Contamination –** Are the media contaminated? For the purposes of an evaluation, five plates or tubes of each medium should be incubated to check for contamination.
9. **Condition of Plates –** Are they clean? Are labels readable?

10. **Condition of Media –** Are the media in good condition? Media should be checked for the following:
    - Cracked or dried medium
    - Medium detached from the plates
    - Frozen or melted medium
    - Unequal fill, and correct depth
    - Change in color from what is expected
    - Excessive bubbles or irregular surfaces
    - Excessive moisture
    - Contamination
    - Cracked/broken plates, tubes or plate/tube defects
11. **Packing slip conforms to CLSI requirements –** Does the packing slip contain lot numbers, expiration dates, product numbers, descriptions of medium, space to record discrepancies, and a statement that the media conform to CLSI standards?

Reporting

Rate each medium on the criteria required by your accrediting body and any other important points that you wish to document for staff training. Record results on the Evaluation of Commercially Prepared Culture Media Report Form.

References

1. Clinical Microbiology Procedures Handbook (CMPH)--Fourth Edition Editor: Amy L. Leber, [Print ISBN: 9781555818807, e-ISBN: 9781555818814, DOI: 10.1128/9781555818814].
2. CLSI (Formerly NCCLS). *Quality Control for Commercially Prepared Microbiological Culture Media; Approved Standard—Third Edition*. NCCLS document M22-A3 [ISBN 1-56238-536-4]. NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2004.
3. Jenkens, S. and Sewell, D. *Quality Control*, pp. 14.2.1 – 14.2.34. Clinical Microbiology Procedure Handbook, Ed. Henry D. Isenberg. 2003. ASM Press, Washington D.C.

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