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Review

Culture media for clinical bacteriology in low- and middle-income countries: challenges, best practices for preparation and recommendations for improved access

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A B S T R A C T

Background: Culture media are fundamental in clinical microbiology. In laboratories in low- and middle-income countries (LMICs), they are mostly prepared in-house, which is challenging.
Objectives: This narrative review describes challenges related to culture media in LMICs, compiles best practices for in-house media preparation, gives recommendations to improve access to quality-assured culture media products in LMICs and formulates outstanding questions for further research.
Sources: Scientific literature was searched using PubMed and predefined MeSH terms. In addition, grey literature was screened, including manufacturer's websites and manuals as well as microbiology textbooks.
Content: Bacteriology laboratories in LMICs often face challenges at multiple levels: lack of clean water and uninterrupted power supply, high environmental temperatures and humidity, dust, inexperienced and poorly trained staff, and a variable supply of consumables (often of poor quality). To deal with this at a base level, one should be very careful in selecting culture media. It is recommended to look for products supported by the national reference laboratory that are being distributed by an in-country supplier. Correct storage is key, as is appropriate preparation and waste management. Centralized media acquisition has been advocated for LMICs, a role that can be taken up by the national reference laboratories, next to guidance and support of the local laboratories. In addition, there is an important role in tropicalization and customization of culture media formulations for private *in vitro* diagnostic manufacturers, who are often still unfamiliar with the LMIC market and the plethora of bacteriology products.
Implication: The present narrative review will assist clinical microbiology laboratories in LMICs to establish best practices for handling culture media by defining quality, regulatory and research paths.
Jeanne Orekan, Clin Microbiol Infect 2021;27:1400

Introduction

Low- and middle-income countries (LMICs) are seriously hit by the worldwide antimicrobial resistance crisis [1,2]. Clinical bacteriology contributes to patient care and antimicrobial resistance surveillance and links to antibiotic stewardship and infection prevention and control, thereby tackling three out of the five domains

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of the WHO action plan to contain antimicrobial resistance [3]. Pending the arrival of new-generation diagnostics, culture-based techniques remain the standard for clinical bacteriology in LMICs and should be included in the services of LMIC first-level referral hospitals according to the WHO Model List of Essential *In Vitro* Diagnostics (WHO EDL), launched in 2018 [4,5].

Culture media are fundamental in clinical bacteriology. They are complex products based on a buffered solution of biological nutrients (animal-, plant- or yeast-based). They comprise peptones and extracts (N source) as well as (poly)saccharides (C source), mineral salts, growth factors and vitamins. To obtain solid media, a gelling agent is added, mostly bacteriological agar [6]. Given the biological components, variations in media composition may occur [7].

For many decades, laboratories in high-income countries have been procuring pre-poured ready-to-use culture media from specialized manufacturers, a practice that is integrated in the laboratory quality management system [8]. In contrast, LMIC laboratories mostly buy dehydrated culture media and prepare them in-house [3]. This is not without its challenges: the organizers of the 2011–2016 WHO external quality assessment of African Public Health Laboratories noted in-house preparation of culture media as a major problem [9]. Worldwide, the dehydrated culture media segment represents the biggest market share (44%) of the booming culture media market (next to prepared media (31%) and chromogenic culture media (25%)). A further growth is expected in the coming years (8.1% compound annual growth rate 2020–2027; with highest growth in the Asia Pacific region) [10].

Scope

This narrative review describes challenges related to culture media in LMICs, compiles best practices for in-house media preparation, gives recommendations to improve access to quality-assured culture media products in LMICs and formulates outstanding questions for future research.

Search strategy and source documents used

PubMed was searched using the MeSH terms 'microbiological techniques' AND 'bacteria/growth and development' AND 'culture media'. Grey literature was also screened, including manufacturer's websites and manuals [7,11–13], as well as microbiology textbooks.

Challenges related to culture media in low- and middle-income countries

The complexity of culture media and their preparation presents a challenge for quality [14]. Adding to the complexity are the plethora of culture media used, the requirements for product stability and—in view of long shipment delays—the need for extended shelf life [3,4]. As an example, numerous variants of MacConkey agar exist with different levels of selectivity complicating selection (see Supplementary material, Document S1). Moreover, similar product names may convey substantial differences in composition, which may lead to confusion (see Supplementary material, Fig. S1). Furthermore, information on the product labels is often printed in small font sizes hampering readability. Instructions for preparations may be difficult to understand, particularly by non-native English- or French-speaking staff.

Challenges at the laboratory level: infrastructure, environment, staff

Laboratories in LMIC referral hospitals are basically equipped, staffed by laboratory technicians with little expertise in microbiology and have a poorly implemented quality management system [3,15]. Clean water and uninterrupted power supply are not assured. High environmental temperature, humidity and dust challenge the storage of dehydrated media and poured plates [3,4]. Using human blood as a substitute for sheep or horse blood is commonplace [16,17]. Laboratory orders are frequently processed by the hospital procurement service, which prioritizes low price over quality and consistency. Media preparation is frequently carried out in the 'laboratory kitchen', close to autoclaves and sinks (high temperature, high humidity), by in-house-trained and poorly supervised auxiliary staff (authors' personal observations).

Challenges at the national level: market factors, regulation

Diagnostic manufacturers concentrate on high-income markets. Despite the predicted increase of the *in vitro* diagnostic (IVD) market and more specifically the culture media market [10,18], manufacturers are reluctant to engage in LMICs, and local production of IVDs is rare [1,4]. Most manufacturers of culture media have a low to moderate presence in Africa and Latin America, but are better represented in the Asia Pacific region [10]. Brazil, India and China, as well as other emerging market economies, could fill this gap. In the absence of effective regulation, product quality is variable. Country demands are inconsistent and price setting is non-transparent [18–20]. Unlike malaria, human immunodeficiency virus (HIV) and tuberculosis, clinical bacteriology is not supported by national control programmes. There are no supranational alliances (e.g. Roll Back Malaria) or international donors with bulk procurement expertise [18]. In terms of international regulations for IVDs, most culture media are considered as 'low risk' (Class A) IVDs with consequent minimal regulation and higher variations in product quality [3,18,21,22].

Best practices for media procurement and preparation in resource-constrained settings

Fig. 1 depicts the different steps of media preparation starting from dehydrated media. Table 1 lists the best practices for culture media preparation. Supplementary Figures S2–S5 depict examples of best practices and common errors. Table 2 lists the quality control requirements. Table 3 gives an overview of the most common errors and their possible causes (trouble-shooting guide). Supplementary Table S1 lists basic equipment and materials needed for culture media preparation in LMIC.

Choice of media, selection of brand and formulation

When selecting culture media, define a small panel of well-described media that is supported by the national reference laboratory and look for products from reputable manufacturers [23]. In case of changing to another brand, carefully check the candidate product's name and ingredients, as compositions may differ. Look for humidity-proof packaging (screw cap, peel-off seal) [11,23]. Verify the Safety Data Sheet, instructions for use and the shelf life.

The clinical microbiologist can give guidance on the selection of appropriate culture media taking into account the prioritization of clinical specimens. At a minimum, next to basic nutrient media, selective media for Gram-positive and Gram-negative bacteria should be available. Stool cultures are generally not recommended for clinical care in first level referral laboratories, but—depending on the setting—Thiosulphate Citrate Bile salts Sucrose agar can be kept in stock in view of epidemic preparedness. Note that different types of primary culture media are available with similar applications (e.g. MacConkey versus Eosin Methylene Blue agar) and their use can even be culturally dependent (e.g. French-speaking versus English-speaking countries).

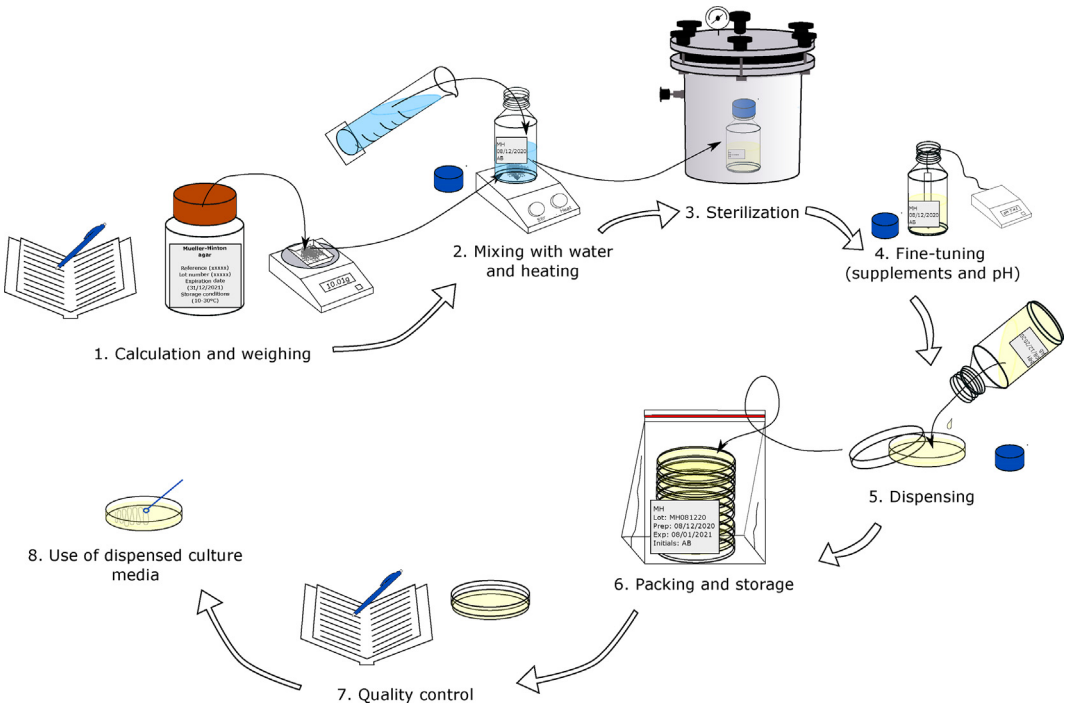

Fig. 1. Preparation of a non-supplemented dehydrated agar medium. Figure inspired by Barry and Fay [14]. Note that the media preparation can only be interrupted at the following steps: (a) for non-selective agar media without supplements, the flask can be stored as solidified agar after autoclaving and re-melted later; (b) the dispensed agar plates can be left closed on the bench to dry overnight (provided there are no insects in the laboratory) and packed and stored the day after.

Procurement, reception, storage and stock management

Select an in-country supplier, providing after-sale support and advice, as well as a back-up supplier; do not rely on non-professional vendors offering occasional sales. Check and record, upon reception, the ordering processing time, correctness of the order delivery, product name and lot number, quantity received, package integrity, expiry date and remaining shelf life [24].

Store dehydrated culture media in a cool, dry place, protected from light and dust [24]. At all times, check the manufacturer's instructions because in rare cases (e.g. Urea Broth powder) storage at 2–8°C is recommended. Monitor the storage area's temperature and humidity. Organize the stock according to a first-in, first-out system, rank media in a logical order and separate opened from closed containers (two-shelf system).

The shelf life of dehydrated culture media is usually long (several years). However, dehydrated media are hygroscopic and quickly deteriorate when exposed to moisture [23]. Therefore, record the date of opening on the container and use the product for maximum 6 months after opening unless otherwise specified by the manufacturer [13]. After this period, visual and performance checks are required [14]. When not in use, keep containers tightly closed and seal the caps with adhesive tape [23]. Discard products when expired, when the powder is clumped, discoloured or not free flowing, or in case of quality issues; record product details and reasons for discarding [24].

Waste management

For disposal of used culture media, follow the in-country guidelines. They are mostly based on WHO guidelines, which recommend inactivation and subsequent incineration before disposal. Inactivation (so-called 'destruction' or Level III inactivation) should be done in the laboratory, in preference by autoclaving [25]. Details about autoclave and incinerator types, validation and controls are described elsewhere [26]. The African Society of Laboratory Medicine recommends, in cases where an autoclave is not available, an overnight treatment with 2% chlorine solution [27]; however, this practice is less effective compared with autoclaving [26].

Recommendations to improve access to high-quality culture media in LMICs

Centralized local production of media allows for harmonization and bulk procurement

In high-income countries, commercially prepared media have been shown to be beneficial for quality [28]. In line with this, centralized media acquisition has been advocated for LMICs [29]. Centralized production has the advantage of dedicated media processing areas (controlled for dust and humidity), cost-efficiency, standardization (single lot productions) and validation of shelf-life, shipment and storage conditions [28,29]. An example is the Central Media-Making Laboratory in Phnom Penh, Cambodia, hosted by a governmental tertiary-level medical university [30]. The Central Media-Making Laboratory is ISO9001 certified and currently produces 23 types of culture media delivered to 28 clients in Cambodia.

If overseen by the National Reference Laboratory or the Ministry of Health, the centralized media production can also contribute to harmonization. Harmonization will lead to a more predictable demand and allows for bulk purchasing and supply and pricing arrangements [18,23,29]. Besides pricing agreements, selected pooled procurement mechanisms may contribute to a viable and dynamic market, as has been shown for HIV diagnostics: offering multi-year contracts with committed annual product volumes and sourcing to different manufacturers has fostered innovation and competition [31,32].

Table 1
Best practices for culture media preparation in resource-constrained settings

1. Calculation and weighing of dehydrated media

a. *Location and conditions:*
- ✓ Damp-free environment (low humidity), if available a laboratory fume hood
- ✓ Table, balance & materials: clean, dust-free and powder-free

b. *Balance:*
- ✓ Top loading (precision) balance
- ✓ Positioned on a level, stable, vibration-free table
- ✓ Calibrated at regular intervals (e.g. annually)
- ✓ Verify control weights before use or on a daily basis

c. *Materials and PPE:*
- ✓ To avoid inhalation and skin contact: use a safety mask and gloves
- ✓ Wash hands before and after working with the dehydrated culture media
- ✓ If required (hazard warnings on the MSDS sheets or labels, particularly 'skin and eye irritation'): wear safety goggles (e.g. for sodium deoxycholate)

d. *Calculate:*
- ✓ Follow the manufacturer's instructions
- ✓ Prepare a table with pre-defined weights and volumes for a fixed number of plates: to generate solid medium with a depth of 4 mm in a Petri plate of 9-cm diameter, a volume of ~20–25 mL is needed
- ✓ Record product name, lot number, preparation date, weight and volume of water, balance used and operator identification in logbook

e. *Weighing:*
- ✓ To facilitate weighing: use materials with pre-defined volume
- ✓ Open the container and take the required amount of powder
- ✓ Close the container immediately to protect it from humidity
- ✓ Do not put excess powder back into the bottle!

2. Mixing with water and heating

a. *Water:*
- ✓ Fresh water prepared by distillation, deionization or reverse osmosis. No tap water (affects selectivity and pH). Store in clean, sealed glass container.
- ✓ Verify purity before use (no bacterial growth!)
- ✓ Optionally, verify the quality before use (recommended by some references):
  - ○ pH: 5.5–7.7 (Note: to measure pH, add 0.3 mL of saturated potassium chloride to 100 mL of water)
  - ○ Conductivity: <15 microSiemens

b. *Materials:*
- ✓ Heater-stirrer, heating mantle (for large volumes) or bain-marie
- ✓ Heat-resistant gloves
- ✓ Glassware: borosilicate, resistant to chemicals and heat
  - ○ Rinse with distilled/deionized water after cleaning to avoid detergent residue
  - ○ Store in cupboard. Discard broken, etched or chipped glassware
  - ○ Use Erlenmeyers (wide base for uniform heating, small top to avoid evaporation), bottles/flasks/tubes with screw caps, graduated cylinder to measure water
  - ○ If required (e.g. glass Petri plates), sterilize at 160°C for 2 hours in hot air oven. Allow oven to cool to 50°C before opening (to avoid cracking of glassware)

c. *Mixing with water (preparation):*
- ✓ Pre-heating the water to 50–60°C may facilitate dissolution
- ✓ Fill to max ½ flask capacity (to avoid spills), max 1 L per flask (for mixing)

d. *Procedure for dissolving powder in water:*
- ✓ Pour half of the required water in the flask, and add the pre-weighed powder
- ✓ Stir or rotate for a few minutes (do not shake!)
- ✓ Pour the rest of the water down the sides to dissolve any excess powder sticking to the flask walls (dry powder may not be sterilized in the autoclave)

e. *Heating (required for agar-containing media):*
- ✓ Do not close flasks tightly
- ✓ Heat up to boiling, with frequent stirring, until solution becomes clear
- ✓ Avoid boiling, overheating and foaming, scorching and burning, clumping and inconsistent mixing
- ✓ Only autoclave when completely dissolved. Dissolution during sterilization can cause uneven dissolution, pH drifts and browning

3. Sterilization

a. *Autoclaving:* (usually at 121 ± 2°C for 15 ± 1 minutes)
- ✓ Note that manufacturer's instructions refer to volumes of up to 1 L
- ✓ Some media cannot be autoclaved (e.g. SS agar, Cary Blair agar)
- ✓ For liquid media in tubes or bottles: first dispense in tubes/bottles, then autoclave!

b. *Avoid:*
- ✓ Over-sterilization causing precipitation, pH change, component destruction
- ✓ Under-sterilization resulting in contaminated medium
- ✓ Direct contact with water or autoclave and water accumulation in recipients

c. *Ensure:*
- ✓ The wrapping allows for steam penetration
- ✓ The lids/caps are not completely tightened
- ✓ There is enough space between the items to allow steam circulation

d. *Identification of sterilized items:*
- ✓ Use indicator tape to label the flask (medium name/code, preparation date, initials)

e. *Verification of autoclave cycles:*
- ✓ Chemical indicators with each cycle (e.g. time–steam–temperature strips)
- ✓ Biological indicators on interval basis

f. *After sterilization:*
- ✓ Use a 'cool down program' or wait until the pressure drops sufficiently before opening (~70–80°C) to avoid a fast pressure drop (liquid can boil over, caps can be blown off)
- ✓ Do not wait too long before unloading the autoclave, overheating may destroy media ingredients

4. Fine-tuning: Adding supplements, pH verification

a. *Adding supplements:*
- ✓ Let the media cool down to 45–50°C before adding heat-labile supplements
- ✓ Filter-sterilize heat-labile supplements (not in the case of blood)
- ✓ Let supplements come to room temperature before adding
- ✓ Add sterile supplements aseptically
- ✓ Ensure adequate mixing

b. *Verification of pH after sterilization (not before):*
- ✓ Verify pH (usually at 25°C) with pH meter before dispensing
- ✓ Take 20 mL out of the flask, let cool down and measure pH. Keep flask in water bath
- ✓ Compare to the pH range specified in the manufacturer's instructions. If pH is ok, continue with dispensing
- ✓ The pH of ready-made dehydrated media should not require adjustment. In case of deviation, verify water, dehydrated medium, glassware, procedure and start again

5. Dispensing of prepared culture media

a. *Cool down media in water bath (45–50°C) before dispensing to minimize condensation:*
- ✓ Dispensing at too high temperature → excessive evaporation
- ✓ If media stay too long in water bath → precipitation (reheat, but do not overheat)
- ✓ Do not use cold water to cool down agar media → flakes/cloud formation

b. *Dispense the media aseptically:*
- ✓ Work close to a flame or in a biological safety cabinet
- ✓ Flame sterilize the neck of the flask before and between pouring

d. *Drying of plates:*
- ✓ Dry for several hours at room temperature (up to 24 hours) to remove condensation
- ✓ Selective media: ±30 minutes with lid ajar. If contamination risk: keep lids closed
- ✓ Dry before packing to prevent condensation on the lids. Avoid over-drying (cracks)

e. *Dispensing of agar or liquid media in tubes:*
- ✓ Use tubes with lids that allow ventilation (e.g. screw caps), do not tighten completely

(*continued on next page*)

Table 1 (*continued*)

- ✓ Dispense in a draught-free room (with closed windows), avoid fans or climate control

c. *Dispensing of agar media in plates:*
- ✓ Mix gently by rotating the flask before dispensing
- ✓ For antimicrobial susceptibility testing, agar depth should be 4 ± 0.5 mm:
  - ◦ Circular Petri plate of 90 mm = ~25 mL
  - ◦ Use sterile, graduated pipettes or media distribution syringe/pump
- ✓ Avoid air bubbles (flame surface or use heated loop to remove them)
- ✓ Dispense on a level surface
- ✓ Put tubes in an autoclavable rack
- ✓ Mix gently by rotating the flask before dispensing
- ✓ Dispense the correct volume per tube. Use sterile, graduated pipettes or a media distribution syringe/pump
- ✓ Close screw caps tightly after autoclaving
- ✓ Drying depends on the type of media:
  - ◦ For agar slants, let dry in a sloped position to give a butt of 2.5–3 cm deep and a slope of 2–2.5 cm long. Use a standardized and validated rack
  - ◦ For agar, semi-solid tubes, liquid media: let dry in rack (vertical position)

6. Packing and storage

a. *Packing and storage of plates:*
- ✓ Label individual plates: name prepared medium/code, preparation date, lot number
- ✓ Wrap plates in sealed, labelled, plastic bags, maximum ten plates/bag (to avoid moisture)
- ✓ Store upside down, at 2–8°C, in the dark
- ✓ Close packaging again after opening to take out some plates
- ✓ Shelf life [13]:
  - ◦ Blood agar: 7 days; with unstable additives: 2–5 days
  - ◦ Most selective media: 5–7 days
  - ◦ Nutrient agar without blood: 2–4 weeks

b. *Storage of prepared but not dispensed agar (solidified):*
- ✓ Only for autoclaved, non-selective media (without heat-labile components)
- ✓ In tightly closed flasks, at room temperature or 2–8°C, in the dark
- ✓ Shelf life up to 6 months
- ✓ Add supplements after re-melting

c. *Packing and storage of tubes:*
- ✓ Store the tubes in a labelled rack in the dark, following manufacturer's instructions
- ✓ Ensure that the screw caps are tightly closed
- ✓ Shelf life [13]:
  - ◦ Simple, non-selective broths and agars: 6 months
  - ◦ Selective media: 3 weeks (2–8°C)
  - ◦ Selenite broth: 2–3 months

d. *Labelling of outer packaging of plates, racks of tubes and prepared agar:*
- ✓ Medium name (abbreviated)/code
- ✓ Lot number—simple with preparation date included (e.g. MC110220—a/b/c in case of multiple lots for the same medium)
- ✓ Date of preparation
- ✓ Date of expiration
- ✓ Initials

7. Quality control (refer to Table 2)

8. Use of dispensed culture media

a. *Bring to room temperature before use*
- ✓ No visible drops of water on the agar surface or inside the lid
- ✓ Do not shake off condensation water from the lid!
- ✓ Dry plates for 20–30 minutes at 35–37°C with agar plates upside down, agar base resting at an angle on the lid
- ✓ If necessary, dry plates at 20–25°C overnight
- ✓ Do not over-dry plates (cracks in surface, surface wrinkled)

b. *Visual sterility check before use*
- ✓ Check the plates for contamination/growth of colonies

According to references [11–14,23,24,29,56]. The focus is put on dehydrated agar-containing solid media. These best practices are complementary to the manufacturer's instructions and the laboratory Quality Management System. For some practices, different options were found according to different references (e.g. stacking plates after pouring, leaving them on the bench with the lid open, etc.). In these cases, we selected the option that is most adapted to settings in low- and middle-income countries.
Abbreviations: MSDS, material safety data sheet; PPE, personal protective equipment.

Table 2
Quality control requirements for culture media preparation

Quality control

a. *Quality control (QC):*
- ✓ Should be based on a pragmatic, risk-based approach
- ✓ Perform QC for each newly prepared batch
- ✓ Quarantine newly prepared and dispensed media until they pass QC

b. *pH verification* (refer to *Table 1*)

c. *Visual inspection*
- ✓ Cracked or damaged plates
- ✓ Agar detached from the plates
- ✓ Frozen or melted agar
- ✓ Unequal filling of the plates
- ✓ Insufficient amount of agar (<3 mm)
  (Note: For Mueller–Hinton agar, the agar depth should be 4 ± 0.5 mm)
- ✓ Haemolysis of blood-containing media
- ✓ Changes in the expected colour of the media
- ✓ Excessive bubbles or rough surfaces
- ✓ Excessive moisture or dehydration
- ✓ Obvious contamination
- ✓ Presence of precipitates
- ✓ Integrity of the packaging
- ✓ Presence of broken or cracked Petri plates or tubes
- ✓ Presence of leakage from the Petri plates or tubes
- ✓ Accuracy of the labelling

d. *Sterility check*
- ✓ 48 h incubation at 35–37°C (varying from 24 h to 5 days depending on the reference)
- ✓ Batch with <100 units: 2% sample; batch with >100 units: ten random units
- ✓ In case of contamination, reject the batch and prepare a new one
- ✓ Do not re-use the plates used for sterility check
- ✓ Note that an additional visual check should always be done right before use

e. *Performance check:*
- ✓ Use QC organisms:
  - ◦ Choose appropriate QC organisms based on standards, guidelines or manufacturer's instructions
  - ◦ Type culture collection organisms (e.g. ATCC) are recommended but previously characterized clinical organisms or EQA strains shown to be phenotypically stable (documentation required) are also accepted
  - ◦ QC organisms should be correctly maintained and stored (refer to CLSI M22-A3 [8])
- ✓ Use a standardized suspension to inoculate the culture media
- ✓ Check and record growth (colony size and morphology), selectivity and differentiation:
  - ◦ Support of target organism: Use at least one organism, record growth and biochemical reaction (e.g. colour on the MacConkey)
  - ◦ Selective media: Use at least one organism that is expected to grow and at least one organism that is not expected to grow
  - ◦ Differential medium: Use organisms which will display the intended growth and reactions (e.g. MacConkey: *Shigella flexneri*, *Escherichia coli* and *Staphylococcus aureus*)
  - ◦ Biochemical media (e.g. urease-test): Use at least one organism that will produce a negative reaction and one organism that will produce a positive reaction
- ✓ Perform disc diffusion to check Mueller–Hinton agar plates

According to references [8,13,23,24,29,56–59].
Abbreviations: ATCC, American Type Culture Collection; EQA, external quality assessment; QC, quality control.

Table 3
Troubleshooting: overview of the most common errors and possible causes

Error	Possible causes
Clumping of dehydrated culture media	✓ Humidity too high during storage ✓ Container left open for too long ✓ Container not tightly closed after opening ✓ Dehydrated culture medium beyond shelf life
Wrong pH	✓ pH meter not calibrated ✓ pH verification done on too hot medium (generally to be done at 25°C) ✓ Overheating: excessive sterilization, heterogeneous mixing, medium kept at 50°C for too long, repeated re-melting or at too high temperature ✓ Use of poor-quality water or container ✓ Use of chemically contaminated containers ✓ Incomplete dissolution/mixture of medium ✓ Dehydrated medium stored incorrectly (e.g. not tightly closed) or beyond shelf life
Incomplete solubility	✓ Use of inadequate water ✓ Inadequate heating/inadequate timing for dissolution ✓ Insufficient soaking or incomplete mixing ✓ Flask too small to allow adequate mixing and/or convection
Darkening, caramelization	✓ Overheating: excessive sterilization, heterogeneous mixing, medium kept at 50°C for too long, repeated re-melting or at too high temperature ✓ Incomplete dissolution of medium
Incomplete gelling or soft agar	✓ Incorrect proportions of product to water: error in weighing or over-dilution ✓ Agar not properly dissolved: poor mixing, prolonged storage at 50°C ✓ Overheating of culture medium, possibly at low pH ✓ Repeated re-melting causing overheating
Turbidity, precipitation	✓ Poor quality of dehydrated media ✓ Use of poor quality of water or container ✓ Overheating: excessive sterilization, heterogeneous mixing, medium kept at 50°C for too long, repeated re-melting or at too high temperature ✓ Wrong pH ✓ Incomplete dissolution/mixture of medium ✓ Loss of water of the prepared culture medium due to evaporation
Poor growth or loss of differential properties	✓ Incorrect or improperly maintained QC organisms used ✓ Overheating: excessive sterilization, heterogeneous mixing, medium kept at 50°C for too long, repeated re-melting or at too high temperature ✓ Incomplete dissolution/mixture of medium ✓ Inhibitory substances in water, container or inoculum ✓ Wrong pH

According to references [11–13,60].
Abbreviation: QC, quality control.

Anticoagulated hair sheep blood as a substitute for defibrinated wool sheep or horse blood

Citrated blood of hair sheep (the short-haired sheep resistant to tropical climates) is a practical alternative for defibrinated wool sheep and horse blood [16,33]. It may be obtained in partnership with a local farmer, slaughterhouse or veterinary school.

Role of the national reference laboratories and clinical microbiologists

As part of their cardinal role in the antimicrobial resistance surveillance, National Reference Laboratories provide guidance, technical support, training and supervision to the tiered national laboratory network [15,34]. For culture media, this comprises guidance in selection and supply, procedures for storage and preparation, quality control (including lot-to-lot variation) and post-market surveillance of culture media. In addition, services include distribution of strains for quality control and antibiotic susceptibility testing for those organisms requiring more complex media (e.g. fastidious organisms) [23,29].

The pivotal role of the clinical microbiologist in the day-to-day working of a clinical bacteriology laboratory is apparent. Clinical microbiologists give advice on prioritization of specimens, selection of culture media and diagnostic tests, choice of antibiotics for susceptibility testing, validation of results and safeguarding quality. Moreover, they play a central role in the strengthening of diagnostic capacity in LMICs [35]. In many LMICs, however, equivalents to clinical microbiologists are rare and ill-defined and most clinical bacteriology laboratories are overseen by managers with little expertise in clinical bacteriology [3]. There is an urgent need for more qualified professionals in LMICs to fill this gap.

The WHO essential diagnostics list

The WHO EDL promotes and prioritizes access to IVDs. It is modelled on and complementary to the WHO List of Essential Medicines [36], which has been crucial for access to affordable treatment in LMICs [35]. The WHO EDL creates political will and calls for manufacturers' commitment [18]. WHO member states are invited to adapt the WHO EDL to their local disease burden. As an example, the Cambodian National Essential Medicine List included a list of essential culture media and diagnostic testing devices even before the WHO EDL launch [37]. Through the WHO EDL and their member state adaptations, manufacturers are informed that listed products (including culture media) are considered as essential and so must be quality assured [18].

Professional societies in clinical microbiology and academia

Professional societies issue evidence-based guidelines and algorithms about IVDs and some of them are very rationalized practices in LMICs. As an example, for quality control of culture media outside antibiotic susceptibility testing, the CLSI M22-A3 guideline authorizes the use of well-characterized clinical organisms [8]. This pragmatic recommendation allows the National Reference Laboratories to biobank and distribute phenotypically stable quality control strains instead of procuring expensive culture collection strains. Even more impact can be expected from independent product evaluations such as those recently carried out by the European Committee on Antimicrobial Susceptibility Testing for Mueller–Hinton media and antibiotic discs [38,39]. Apart from guidance, independent evaluation studies create awareness among users and manufacturers and incite product improvement [40], as was demonstrated for the antibiotic discs [39].

A more stringent and harmonized regulation of in vitro diagnostics

The Mueller–Hinton and antibiotic disc evaluation studies performed by the European Committee on Antimicrobial Susceptibility Testing highlighted the need for more stringent regulatory criteria [39]. In the absence of implemented regulations in some LMICs, laboratories frequently refer to regulatory approval in high-income countries, for example the CE (*Conformité Européenne*) mark of the European Union. The current EU IVD 98/79 Directive will be superseded by the more stringent EU Regulation 2017/746, which will be effective from 2022 onwards, but the exact place of culture media is still unclear [41]. The Pan-African Harmonization Working Party is working on a harmonized IVD regulatory framework, with emphasis on a common registration file, clinical evidence and post-marketing [42,43].

In vitro diagnostic manufacturers

The landscape of diagnostic manufacturers in LMICs is changing, as manufacturers from booming economies are entering [4,10,18]. However, for emerging economies to be competitive, a higher demand from LMICs, North–South technology transfer and an improved diagnostics production and supply chain are required [18]. In addition, diagnostic manufacturers must become familiar with the aspects of clinical bacteriology and the multitude and diversity of consumables [3]. Public–Private Partnerships or similar can be explored to enable laboratories or networks of laboratories to strengthen media production, including pooled procurement and training [44]. An example in the context of HIV and tuberculosis is the Public–Private Partnership between the US President's Emergency Plan for AIDS Relief (PEPFAR) and Becton Dickinson to strengthen laboratory systems in several African countries, which resulted in improved specimen referral systems in Ethiopia [45]. To be successful, clear communication and explicit division of the roles and responsibilities of each partner must be ensured, and the autonomy and flexibility of the laboratory must be safeguarded [3,45].

Further research to be done

Rational use of resources and culture media

Cost-effective use of culture media is key to building sustainable clinical microbiology services. A tiered identification system with basic (genus or group level) identification on site and referral to reference laboratories for species identification can reduce costs and complexity [4]. Further, adapted culture media should be studied in a clinical setting. Examples are glucose-topped MacConkey agar for urine cultures as well as chromogenic media (provided it is affordable and stable) [46,47]. The high investment needed to obtain sheep blood in remote settings calls for alternatives, such as blood-free media for large-colony haemolytic streptococci [48]. Re-usable culture tubes and Petri plates made of borosilicate glass can reduce costs as well as biplates, which are cost-effective, but may be difficult to find and are more complex to prepare. The clinical microbiologist can give recommendations after a risk- and cost-based evaluation.

Bacteriological agar is obtained from sea algae at only a few harvesting sites and its increasing use may imply higher cost and shortage. In addition, it is expensive (estimated at US$500/kg in 2012) and its cost represents more than 90% of the cost of common solid media [49]. In food and water microbiology, ready-to-use absorbent pads impregnated with culture media are used but they have not yet been adapted to the need for subculture in clinical bacteriology [50]. Alternatives for bacteriological agar are underway but need further research; they include low-cost food-grade agar [49], cellulose produced by engineered bacteria [51] and alternative gelling agents [6,52].

Tropicalization and customization of culture media formulations

Marketed products typically display stability at temperatures up to 25°C or 30°C but actual storage temperatures in LMICs may be much higher [53]. Therefore, research also needs to address the temperature stability of culture media and the humidity resistance of their packaging. Examples of low-tech low-cost formulations are pre-weighed sealed packages (e.g. ready-to-use for 1 litre) offered by several suppliers and prepared media stored in bottles that can be re-melted and poured. Further, granulated formulations of dehydrated media may have, in addition to their other advantages (less dust, longer shelf life), the potential of better temperature and humidity resistance [12]. More demanding questions are about extending the shelf life of poured products (e.g. by vacuum-sealed gas-flushed packaging) and the implementation of biodegradable plastic materials [54,55].

Transparency declaration

The authors have no conflicts of interest to declare.

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Author contributions

JO, SO, J-BR and JL contributed to the methodology, formal analysis, and to reviewing and editing; BB contributed to the

methodology, formal analysis, writing the original draft, and to reviewing and editing; JJ contributed to the conceptualization, methodology, formal analysis, writing the original draft, reviewing and editing, and supervision; DA contributed to the conceptualization, reviewing and editing, and supervision; and LH contributed to the conceptualization, methodology, formal analysis, writing the original draft, reviewing and editing, visualization and supervision.

Appendix A. Supplementary data

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References

[1] Bebell LM, Muiru AN. Antibiotic use and emerging resistance: how can resource-limited countries turn the tide? Glob Heart 2014;9:347.
[2] Founou RC, Founou LL, Essack SY. Clinical and economic impact of antibiotic resistance in developing countries: a systematic review and meta-analysis. PLoS ONE 2017;12.
[3] Jacobs J, Hardy L, Semret M, Lunguya O, Phe T, Affolabi D, et al. Diagnostic bacteriology in district hospitals in sub-Saharan Africa: at the forefront of the containment of antimicrobial resistance. Front Med 2019;6:205.
[4] Ombelet S, Ronat J-B, Walsh T, Yansouni CP, Cox J, Vlieghe E, et al. Clinical bacteriology in low-resource settings: today's solutions. Lancet Infect Dis 2018;18.
[5] World Health Organization. WHO model list of essential in vitro diagnostics. Available at:. 1st ed. Geneva: WHO; 2018. https://www.who.int/medicines/publications/essentialmedicines/18th_EML.pdf. [Accessed 17 December 2020].
[6] Bonnet M, Lagier JC, Raoult D, Khelaifia S. Bacterial culture through selective and non-selective conditions: the evolution of culture media in clinical microbiology. New Microb New Infect 2020;34. https://doi.org/10.1016/j.nmni.2019.100622.
[7] Zimbro MJ, Power DA, Miller SM, Wilson GE, Johnson JA. Difco™ & BBL™ manual. Manual of microbiological culture media. Available at:. 2nd ed. Sparks, MD: Becton Dickinson; 2009. https://www.bd.com/documents/guides/user-guides/DS_CM_Difco-and-BBL-culture-media-manual_UG_EN.pdf. [Accessed 17 December 2020].
[8] Clinical and Laboratory Standards Institute (CLSI). Quality control for commercially prepared microbiological culture media; approved standard. CLSI Document M22-A3. 3rd ed. Wayne, PA: CLSI; 2004.
[9] Perovic O, Yahaya AA, Viljoen C, Ndihokubwayo JB, Smith M, Coulibaly SO, et al. External quality assessment of bacterial identification and antimicrobial susceptibility testing in African national public health laboratories, 2011-2016. Trop Med Infect Dis 2019;4.
[10] Up Market Research. Global culture media market analysis & forecast, 2017–2027. Maharashtra. Up Market Research; 2020.
[11] Valls JS, Nacente RB. Handbook of microbiological culture media, 11th ed. Barcelona. Available at:. 2011. https://www.scharlabmagyarorszag.hu/katalogus/scharlau_mikrobiologiai_kezikonyv.pdf. [Accessed 17 December 2020].
[12] Merck. Microbiology manual, 12th ed. Darmstadt: Merck. Available at: http://www.laboquimia.es/pdf_catalogo/MERCK_Manual_de_microbiologia_12a_edicion.pdf. [Accessed 17 December 2020].
[13] Oxoid Limited. Technical Support—FAQs on culture media, supplements and raw materials. Available at: http://www.oxoid.com/UK/blue/techsupport/its.asp?itsp=faq&faq=tsfaq011&cat=culture+media%2C+supplements+and+raw+materials&lang=EN&c=UK. [Accessed 17 December 2020].
[14] Barry AL, Fay GD. A review of some common sources of error in the preparation of agar media. Am J Med Technol 1972;38:241–5.
[15] Barbé B, Yansouni CP, Affolabi D, Jacobs J. Implementation of quality management for clinical bacteriology in low-resource settings. Clin Microbiol Infect 2017;23:426–33.
[16] Russell FM, Biribo SSN, Selvaraj G, Oppedisano F, Warren S, Seduadua A, et al. As a bacterial culture medium, citrated sheep blood agar is a practical alternative to citrated human blood agar in laboratories of developing countries. J Clin Microbiol 2006;44:3346–51.
[17] Ferguson JK, Joseph J, Kangapu S, Zoleveke H, Townell N, Duke T, et al. Quality microbiological diagnostics and antimicrobial susceptibility testing, an essential component of antimicrobial resistance surveillance and control efforts in Pacific island nations. West Pac Surveill Response J 2020;11:41–6.
[18] Stevenson M. A situation analysis of the state of supply of in vitro diagnostics in Low-Income Countries. Glob Public Health 2020;15:1836–46.
[19] Daily J. Fever diagnostic technology landscape, 1st ed. Vernier. Available at:. 2018. https://unitaid.org/assets/Fever_diagnostic_technology_and_market_landscape.pdf. [Accessed 17 December 2020].
[20] Daily J. Malaria diagnostics technology and market landscape, 3rd ed. Vernier. Available at:. 2016. https://www.ghdonline.org/uploads/Unitaid-Malaria-Dx-Tech-Mkt-Landscape-3rd-Ed-April-2016.pdf. [Accessed 17 December 2020].
[21] International medical device regulators forum (IMDRF). Available at: http://www.imdrf.org/about/about.asp. [Accessed 17 December 2020].
[22] World Health Organization (WHO). WHO global model regulatory framework for medical devices including in vitro diagnostic medical devices. Geneva. Available at:. 2017. https://www.who.int/medical_devices/publications/global_model_regulatory_framework_meddev/en/. [Accessed 17 December 2020].
[23] Cheesbrough M. District laboratory practice in tropical countries. Part 2. 2nd ed. Cambridge: Cambridge University Press; 2006.
[24] Kaur T, Gupte S, Kaur M. Establishment and application of ideal media room in microbiology laboratory. Int J Med Microbiol Trop Dis 2016;2:22–3.
[25] World Health Organization. Laboratory biosafety manual. Available at:. 4th ed. Geneva: WHO; 2020. https://www.who.int/publications/i/item/9789240011311. [Accessed 26 February 2021].
[26] World Health Organization. Safe management of wastes from health-care activities. Available at:. 2nd ed. Geneva: WHO; 2014. https://www.who.int/water_sanitation_health/publications/safe-management-of-wastes-from-healthcare-activities/en/. [Accessed 17 December 2020].
[27] MLO Staff. The fundamental challenge of laboratory associated waste management. Available at: African Association for Laboratory Medicine—Lab Culture; 2018. p. 14–8. https://aslm.org/wp-content/uploads/2018/05/ASLM_LabCulture_Issue_18.pdf?x10839. [Accessed 17 December 2020].
[28] Nagel JG, Kunz LJ. Needless retesting of quality-assured, commercially prepared culture media. Appl Microbiol 1973;26:31–7.
[29] Perilla MJ, Ajello G, Bopp C, Elliott J, Facklam R, Knapp JS, et al. Manual for the laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health importance in the developing world. Atlanta. Available at:. 2003. https://www.who.int/csr/resources/publications/drugresist/WHO_CDS_CSR_RMD_2003_6/en/. [Accessed 17 December 2020].
[30] University of Health Sciences Cambodia—Central Media Making Laboratory. Available at: https://www.uhs.edu.kh/cmml/. [Accessed 17 December 2020].
[31] Unitaid. Market and Technology Landscape. HIV rapid diagnostic tests for self-testing, 3rd ed. Vernier. Available at:. 2017. https://www.who.int/hiv/pub/vct/hiv-self-testing-2017-thirdedition/en/. [Accessed 17 December 2020].
[32] The Global Fund. Market shaping strategy. Available at: https://www.theglobalfund.org/en/sourcing-management/market-shaping-strategy/. [Accessed 17 December 2020].
[33] Yeh E, Pinsky BA, Banaei N, Baron EJ. Hair sheep blood, citrated or defibrinated, fulfills all requirements of blood agar for diagnostic microbiology laboratory tests. PLoS ONE 2009;4.
[34] World Health Organization. GLASS Guidance for national reference laboratories. Available at: Geneva: WHO; 2020. https://www.who.int/publications/i/item/9789240010581. [Accessed 17 December 2020].
[35] Yusuf E, Hamers RL. What the WHO's List of Essential Diagnostics means for clinical microbiology laboratories and antimicrobial stewardship practice worldwide. Clin Microbiol Infect 2019;25:6–9.
[36] Schroeder LF, Guarner J, Amukele TK. Essential diagnostics for the use of world health organization essential medicines. Clin Chem 2018;64:1148–57.
[37] Available at: Essential medicine list. 9th ed. 2018. https://www.ddfcambodia.com/bureau/essential-drug-bureau.html. [Accessed 17 December 2020].
[38] Åhman J, Matuschek E, Kahlmeter G. EUCAST evaluation of 21 brands of Mueller–Hinton dehydrated media for disc diffusion testing. Clin Microbiol Infect 2020;26:1412. e1–1412.e5.
[39] Åhman J, Matuschek E, Kahlmeter G. The quality of antimicrobial discs from nine manufacturers—EUCAST evaluations in 2014 and 2017. Clin Microbiol Infect 2019;25:346–52.
[40] Cunningham J, Jones S, Gatton ML, Barnwell JW, Cheng Q, Chiodini PL, et al. A review of the WHO malaria rapid diagnostic test product testing programme (2008–2018): performance, procurement and policy. Malar J 2019;18:387.
[41] Reinikainen M, Suppo M. Explaining IVD classification issues. London. Available at: https://www.bsigroup.com/globalassets/meddev/localfiles/fr-fr/whitepapers/md-classification_issues_for_ivd.pdf. [Accessed 17 December 2020].
[42] Pan African harmonisation working party. Available at: http://www.pahwp.org/. [Accessed 17 December 2020].
[43] McNerney R, Peeling RW. Regulatory in vitro diagnostics landscape in Africa: update on regional activities. Clin Infect Dis 2015;61:S135–40.
[44] Ravishankar N, Lehmann J. Opportunities abound: public private partnerships for laboratory services in East Africa. Available at:. 2015. https://openknowledge.worldbank.org/handle/10986/22483. [Accessed 17 December 2020].
[45] Nkengasong JN, Mbopi-Keou FX, Peeling RW, Yao K, Zeh CE, Schneidman M, et al. Laboratory medicine in Africa since 2008: then, now, and the future. Lancet Infect Dis 2018;18:e362–7.
[46] Karah N, Rafei R, Elamin W, Ghazy A, Abbara A, Hamze M, et al. Guideline for urine culture and biochemical identification of bacterial urinary pathogens in low-resource settings. Diagnostics 2020;10.
[47] Perry JD. A decade of development of chromogenic culture media for clinical microbiology in an era of molecular diagnostics. Clin Microbiol Rev 2017;30:449–79.

[48] Niederstebruch N, Sixt D. Standard Nutrient Agar 1 as a substitute for blood-supplemented Müller–Hinton agar for antibiograms in developing countries. Eur J Clin Microbiol Infect Dis 2013;32:237–41.
[49] Petrovski S, Tillett D. Back to the kitchen: food-grade agar is a low-cost alternative to bacteriological agar. Anal Biochem 2012;429:140–1.
[50] Liofilchem. Easy dry media. Available at: https://www.liofilchem.com/products/liofilchem-products/category.html?classe=012. [Accessed 17 December 2020].
[51] Yin N, Santos TMA, Auer GK, Crooks JA, Oliver PM, Weibel DB. Bacterial cellulose as a substrate for microbial cell culture. Appl Environ Microbiol 2014;80:1926–32.
[52] Das N, Triparthi N, Basu S, Bose C, Maitra S, Khurana S. Progress in the development of gelling agents for improved culturability of microorganisms. Front Microbiol 2015;6. https://doi.org/10.3389/fmicb.2015.00698.
[53] Jacobs J, Kühne V, Lunguya O, Affolabi D, Hardy L, Vandenberg O. Implementing COVID-19 (SARS-CoV-2) rapid diagnostic tests in sub-Saharan Africa: a review. Front Med 2020;7. https://doi.org/10.3389/fmed.2020.557797.
[54] Choi YW, Rogers J v. Shelf-life assessment of agar plates vacuum-sealed in nylon/polyethylene packaging flushed with nitrogen. J Microbiol Exp 2015;2. https://doi.org/10.15406/jmen.2015.02.00075.
[55] Dittrich S, Tadesse BT, Moussy F, Chua A, Zorzet A, Tängdén T, et al. Target product profile for a diagnostic assay to differentiate between bacterial and non-bacterial infections and reduce antimicrobial overuse in resource-limited settings: an expert consensus. PLoS ONE 2016;11. https://doi.org/10.1371/journal.pone.0161721.
[56] European Committee on Antimicrobial Susceptibility Testing (EUCAST). Media preparation for EUCAST disk diffusion testing and for determination of MIC values by the broth microdilution method. Version 6.0. Available at:. 2020. https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Disk_test_documents/2020_manuals/Media_preparation_v_6.0_EUCAST_AST.pdf. [Accessed 17 December 2020].
[57] Jones RN, Krisher K, Bird DS, College of American Pathologists Microbiology Resource Committee. Results of the survey of the quality assurance for commercially prepared microbiology media. Update from the College of American Pathologists Microbiology Surveys Program (2001). Arch Pathol Lab Med 2003;127(6):661–5. https://doi.org/10.5858/2003-127-661-ROTSOT.
[58] Cantarelli VV, Inamine E, Zenobin Brodt TC, Secchi C, de Souza Pereira F, Amaro MC, et al. Quality control for microbiological culture media. Is it enough to follow the NCCLS M22-A2 procedures? Braz J Microbiol 2003;34:8–10.
[59] The Australian Society for Microbiology. Guidelines for assuring quality of medical microbiological culture media. Available at:. 2nd ed. 2012. https://biomeddiagnostics.com/laravel-filemanager/files/5/Guidelines%20for%20Assuring%20Quality%20of%20Medical%20Microbiological%20Culture%20Media.pdf. [Accessed 17 December 2020].
[60] Basu S, Pal A, Desai PK. Quality control of culture media in a microbiology laboratory. Ind J Med Microbiol 2005;23:159–63. https://doi.org/10.4103/0255-0857.16586.