Quality Control in Clinical Microbiology Laboratory

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Introduction

The clinician and the microbiologist must actively work together to maximize the clinical value of diagnostic microbiology testing. Unfortunately, the current trend to consolidate laboratory services and to move them off-site has made timely communication between laboratory personnel and patient care providers more difficult.

Introduction

In addition, these changes potentially increase the time between specimen collection and laboratory processing, compromising the specimen integrity and delaying the availability of critical test results. These changes, along with other cost-cutting measures, make it even more important that the infectious diseases physician and the microbiologist work together. The following expectations regarding the microbiologist's and the clinician's responsibilities should be recognized and addressed.

Microbiologist Responsibility

1- Provide an appropriate, comprehensive test menu that is responsive to physician

needs

2-Establish a relationship with an external laboratory for performance of infrequent, expensive, or sophisticated testing that cannot be performed on site.

3-Provide cut-off times for processing test requests and turnaround times for reporting results

4-Provide guidelines for specimen collection and transport

Microbiologist Responsibility

5-Maintain an effective computerized system for acknowledging receipt of specimens, testing in-progress, and reporting of results; immediate notification to the physician of critical results should be a component of an overall communication system

6-Periodic publication of antimicrobial susceptibility patterns for the most commonly isolated bacteria in the institution

Microbiologist Responsibility

7-Maintain a program of quality control that ensures the accuracy of all offered tests

8-Establish a laboratory that conforms to regulatory standards

9-A system of short-term storage of all specimens and long-term storage of important isolates should be established to facilitate additional testing if required

Clinician's Responsibilities

1-Maintain knowledge of the laboratory test menu and specimen collection and transport guidelines

2-Alert the laboratory when a specific organism is sought (e.g., a fastidious or highly pathogenic organism)

2-Prioritize test requests when a limited quantity of specimen can be collected

Establish an open communication with the laboratory director when testing needs are not satisfied by the available test menu or special handling of a specimen is required

Quality Control

- Continual monitoring of working practices, equipment & reagents so as to detecting & correcting defects

- => Maintains **reliable / timely** analytical
- performance (result / outcome)
- => More patient-care-oriented approach

Stages of laboratory activities

- The QC program must ensure optimum patient specimens and result integrity throughout the 3 stages processes:

- 1. Pre-analytical
- 2. Analytical
- 3. Post-analytical

Three stages of activities

Table 1- Three stages of activities that affect outcome of laboratory testing:

Stage	Activities
Preanalytical	Test ordering
	Order transcription
	Patient preparation
	Specimen collection
	Specimen identification
	Specimen transport
Analytical	Sample testing (ID & ST)
Post-analytical	Result transcription
	Result intrepretation
	Action taken on basis of result

A quality outcome can be interrupted or destroyed at any point in the process.

Elements of QA

- *Specimen Collection and Transport*
- *Standard Operating Procedure (SOP)*
- *Personnel*
- *Proficiency Testing*
- *Performance checks*
- *Reporting of Results*

Continued..

- ***Quality Control of Bacteriology:***
  - ***Media***
  - ***Stains***
  - ***Reagents***
  - ***Antisera***
  - ***Antimicrobial Susceptibility Testing***
  - ***Equipment's & instruments***
  - ***Maintenance of QC Records***
  - ***And etc***

Standard Operation Procedures (SOP)

A SOP is a set of fixed instructions to carry out a routine activity or process. An example of a procedure would be a step-by-step description of how to perform a disk diffusion antimicrobial susceptibility test on a bacterial isolate. SOPs should have a consistent format and should be clear to all users so that the task being described is consistently performed.

Need of SOP s in laboratory work

Standard operating procedures in a microbiology laboratory are needed for following reasons:

- To improve and maintain the quality of laboratory service to patients and identify problems associated with poor work performance;
- · To provide laboratory staff with written instructions on how to perform test consistently to an "acceptable standard" in the laboratory;
- To provide written standardized techniques for use in the training of laboratory personnel;
- To facilitate the preparation of a list of essential reagents,
- chemicals and equipment's;
- *To promote safe laboratory practice.*

Important features of SOP

An individual SOPs must be:

- Applicable and achievable in the laboratory in which they will be used;· Clearly written and easy to understand and follow; and · Kept up-to-date using appropriate technologies.

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Preparation of SOP

The procedure manual, a reference for standardization and organization of all tests and functions, is the most important document in the microbiology laboratory. It is written for new inexperienced personnel but also serves as a reference for experienced laboratory personnel.6 For that reason, SOPs must be written and implemented by qualified experienced laboratory officers, and followed exactly by all members of staff. Each SOP must be given a title and identification number, and be dated and signed by an authorized person.

Types of SOP

Standard operating procedures can be described under two

broad headlines:

One of them is 'standard procedure for general laboratory practices' which include: (a) request form, (b) collection and transportation of clinical specimens, (c) specimen acceptability and criteria for rejection, (d) procedure for processing specimens, (e) systematic descriptions of the tests performed, (f)antimicrobial susceptibility testing, (g) safety in laboratory and (h)quality assurance.

Stages of SOP

Total activities performed by laboratory personnel in order to provide accurate diagnosis from a clinical specimen can be divided into three stages. These are :

Pre-Analytical stage- primarily deals with the general concepts for specimen collection and handling

Analytical stage- deals with testing the specimens, .

Post-Analytical stage- comprises of reporting and interpreting test results.

Specimen Selection ,collection and transport

A variety of pathogens are usually considered, which increases the types of specimens that must be collected and the number of tests ordered. Although this may not pose a problem if the quantity of specimen is sufficient (e.g., blood, urine, stool, expectorated sputum), this can present a significant problem if the quantity is limited or the specimen difficult to obtain (e.g., aspirated fluids, tissue biopsy).

Continue…

Thus, the physician has the responsibility to consider carefully potential pathogens and the diagnostic tests that should be ordered. The physician also has the responsibility to ensure specimens representative of the site of infection are collected in the appropriate container and transported to the laboratory in a timely fashion.

Continue…

- Delays while specimens remain in the patient care area adversely affect diagnostic testing. The microbiologist has the responsibility to provide appropriate, readily available instructions and materials for collection and transport of the specimens.

Continue…

Selection of the appropriate specimens depends on multiple factors. If the diagnostic test is isolation of the organism in culture, then the specimen must contain viable organisms; in addition, care should be taken to avoid organisms that may either suppress or overgrow the pathogen or confound the interpretation of the culture

Continue…

Ideally, the specimens should be collected before administration of antimicrobials. An adequate volume of specimen should be collected to maximize recovery of the pathogen, and an adequate number of specimens should be collected if the organism is transiently present (i.e., blood cultures for detection of bacteremia or fungemia)

Continue…

- Microscopy is a rapid but typically insensitive and nonspecific method for detection of pathogens. Thus, if the quantity of specimen is limited, the value of microscopy must be balanced with the need for other diagnostic tests. If microscopy is the primary diagnostic test (e.g., examination of peripheral blood smears for plasmodia, *Babesia, Borrelia*), then multiple specimens may need to be examined by using specialized stains or microscopy techniques.

Continue…

Selection of the appropriate specimen for detection of microbial antigens is determined by the suspected pathogen. For example, cerebrospinal fluid (CSF) would be appropriate for diagnosis of cryptococcal meningitis, but both CSF and urine should be collected for diagnosis of pneumococcal meningitis. Likewise, the optimum specimens for diagnosis of *Legionella* pneumonia are bronchial lavage for culture and urine for antigen testing

Continue…

Serologic tests are useful if the collection of blood is properly timed to coincide with the peak level of antibodies or to demonstrate a significant rise in antibodies. Because these levels can vary for different pathogens, collections of samples should be coordinated carefully to optimize their usefulness and not as an afterthought when confronted with initial negative tests.

Continue…

- Transport of specimens to the microbiology laboratory must be done in a timely fashion, which is particularly important in the handling of urgent requests. The laboratory should determine in what manner and how often specimens should be delivered and should periodically monitor this process to make sure that delivery to the laboratory is occurring as expected.

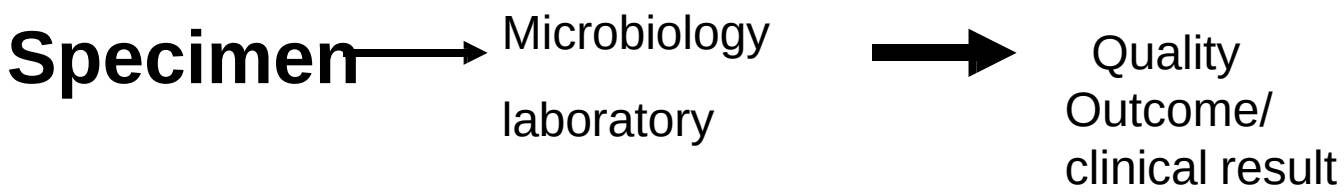
Continue…

- It is important to include, with the time required to transport a specimen to the laboratory, the delays between collection of the specimen and shipment to the laboratory and the time between receipt of the specimen in the laboratory and laboratory processing. Efforts to minimize transit time (e.g., use of pneumatic tube systems) are wasted if there are significant delays after collection or receipt in the laboratory.

Continue…

- The physician has the responsibility to minimize the former and the microbiologist the latter. These transit times should be monitored systematically as part of a diagnostic quality assurance program. It is also important to time the collection and transport of specimens to ensure they arrive in the laboratory before an established cutoff time for tests that are performed once per day or less often.

Quality of the specimen



- Health care value of the information provided by clinical microbiology lab is being significantly compromised by **inappropriate specimens**

Quality of the specimen

- Inappropriate specimens:
  - Submission of contaminated specimens
  - Delay in specimen delivery
  - Viral culture without transport media
  - Collection of specimens from inappropriate body sites
- =>Collection of microbiology specimens is generally not under direct control of lab.

Rejection criteria for improper samples:

Criteria should be developed by a laboratory on the basis of which the processing may not be done by the laboratory. The following are some examples of unacceptable specimens:

1-Missing or inadequate identification;

2-Insufficient quantity;

3-The specimen has been transported at the improper temperature in improper medium (e.g., specimen for anaerobic bacteria submitted in aerobic transports

Rejection criteria for improper samples

4- Specimen collected in an inappropriate container;

5- Contamination suspected;

6- Unknown time delay;

7- Leaking container or open mouthed container;

6- Specimen is dried up;· Saliva instead of sputum; and · Inappropriate request, e.g., Folley's catheter tip, oral swab, etc.

Rejection criteria for improper samples

- It is an important rule to talk to the requesting physician before discarding unacceptable specimens. In some cases, such as mislabeling of a specimen or requisition, the person who collected the specimen or requisition can come to the laboratory and correct the problem. Correction of a mislabeled specimen or requisition should not be done over the telephone.

Personnel

- Active participation by everyone working in the system is required to meet quality standards & continuously improve performance
- Assign responsibility / duties

Personnel

Personnel	Function
Quality Improvement Executive Committee	-Reviews and approves policies and procedures required to achieve quality improvement goals - Fosters interdisciplinary communication, facilitates problem solving and documents results of QA activities.
Microbiologist	-Participates in monitoring and evaluating laboratory services. Provides recommendations for improving services; for example; The microbiologist must make many clinical decisions regarding collection and transmission of specimens. The extent of his/her role in the interpretation and utilization of microbiological information must also be considered
Technologists & Technician	Implements procedures and manages data in accordance with QA goals. Provides recommendations to director for improving services

Personnel

- **The employee's personnel records contain:**
  - Qualification & experience
  - The tasks & procedures that the employee is authorized to perform (dates of received training & competence tests)
  - Continuing education activities (attend some training program or workshop)
  - Regular meeting to keep staff informed of changes & to solicit their suggestion for improving the lab. service

Culture Media

- Definitions of culture media
- Types of culture media
- Significant culture media used within Microbiology QC
- Approaches to the QC testing of media
- Selecting microorganisms
- Supplier audits

Definition of Culture medium

- Microbiological culture medium
- A substance which encourages the growth, support and survival of micro-organisms.
  - Contains nutrients, growth promoting factors, energy sources, buffer salts, minerals, metals and gelling agents (for solid media).

Introduction

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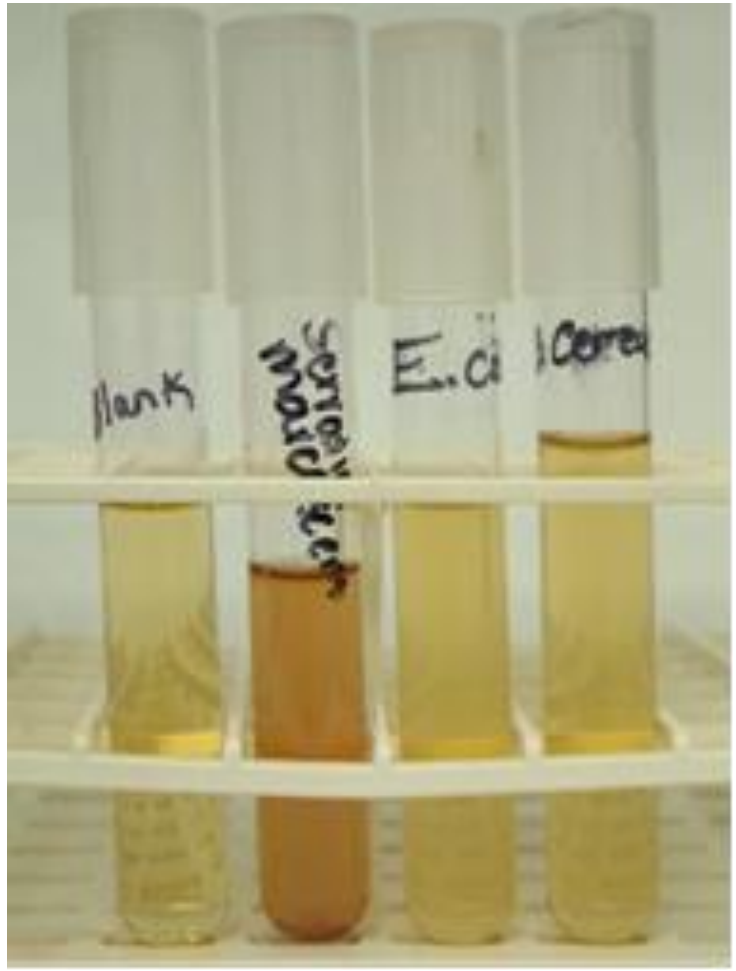
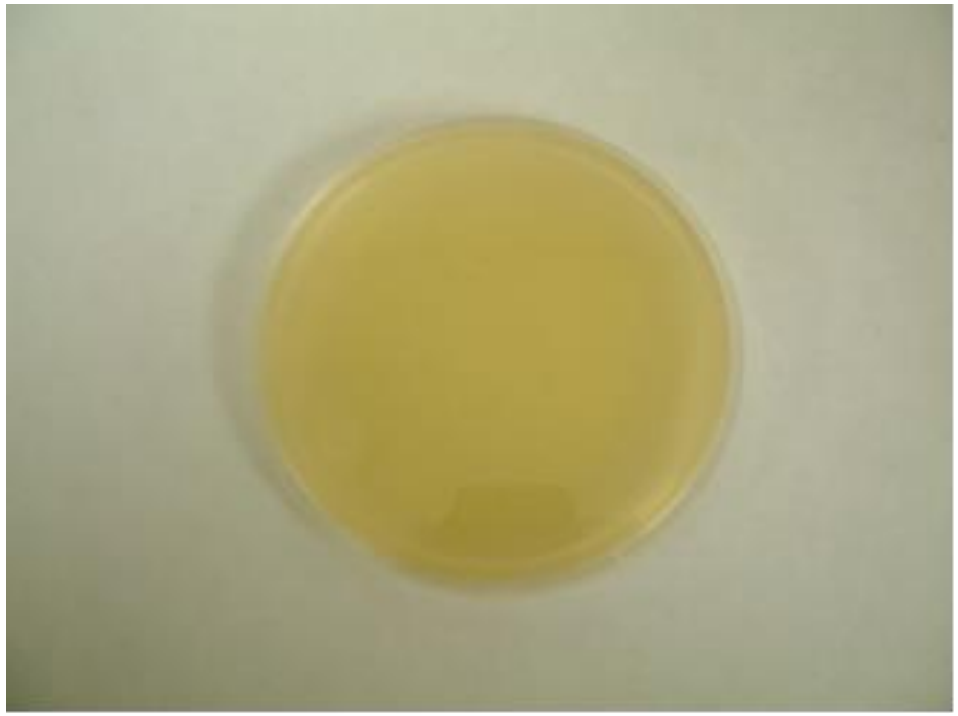
Culture Media: Types

- Culture media is divided into:
  - Liquid culture media. This is commonly called ‘broth’.eg TSB
  - Nutrient broth and



Culture Media: Types

- Solid and semi-solid culture media.
  - Contains a solidifying material which may be agar-agar or gelatine or some other analogous substance.
  - This media is commonly called ‘agar’, irrespective of the type of solidifying agent used.



Culture Media: Types

- Further divisions:
  - Growth media
  - Transport media (e.g. Carry Blair .Stuart …)
  - Preservation media
  - Resuscitation media
  - Enrichment media (e.g. blood agar ,chacholate agar)
  - Selective growth media (e.g. MacConkey agar)
  - Differential media (with some sort of indicator, typically a dye)
  - Minimal media (for ‘wildtypes’)
- Some of the above media may have multiple uses.e.g. Blood agar

Culture media preparation

- Choice of media, selection of brand and formulation
- Calculation and weighing of dehydrated media
- Mixing with water and heating
- Sterilization
- Fine-tuning: Adding supplements, pH verification
- Dispensing of prepared culture media
- Packing and storage
- Sterility check

Quality Assurance

- Quality Assurance
- Those processes before, during and after the manufacture of medical microbiological culture media that verify the adequacy of the media for its intended purpose.
- **Quality Control:**
- The final inspection and testing of the finished product to ensure its compliance with predetermined performance criteria

Quality control requirements for culture media preparation

- ✓ 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 Q
- pH verification.

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 Muellere Hinton agar, the agar depth should be 4 ± 0.5 mm)

Visual inspection

- ✓ Haemolysis of blood-containing media
- ✓ Changes in the expected colour of the media
- ✓ Excessive bubbles or rough surfaces
- ✓ Excessive moisture or dehydration
- ✓ Obvious contamination

Visual inspection

- ✓ *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*

Sterility check

-
- ✓ 48 h incubation at 35±2C (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

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

Troubleshooting: overview of the most common errors and possible causes

- ✓ 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 25C)
- ✓ Overheating: excessive sterilization, heterogeneous mixing, medium kept at 50C 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 50C for too long,
- repeated re-melting or at too high temperature

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 50C
- ✓ 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 50C 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 50C for too long, repeated
- re-melting or at too high temperature of medium

  ✓ incomplete dissolution/mixture
- ✓ Inhibitory substances in water, container or inoculum
- ✓ Wrong pH

- Physical characteristics

Date	Medium	Lot #	Colour	Clarity	Gel	pH	sterility	Sign
2/5/02	Cho	X	Ok	Ok	Ok	Ok	ok	Jo
	MAC	xx	Ok	Ok	Ok	Ok	ok	Jo

Labeling:

Date of preparation_____
Media_____
Lot No._____
Expiration Date_____
QC_____
Storage condition_____
Technologist_____

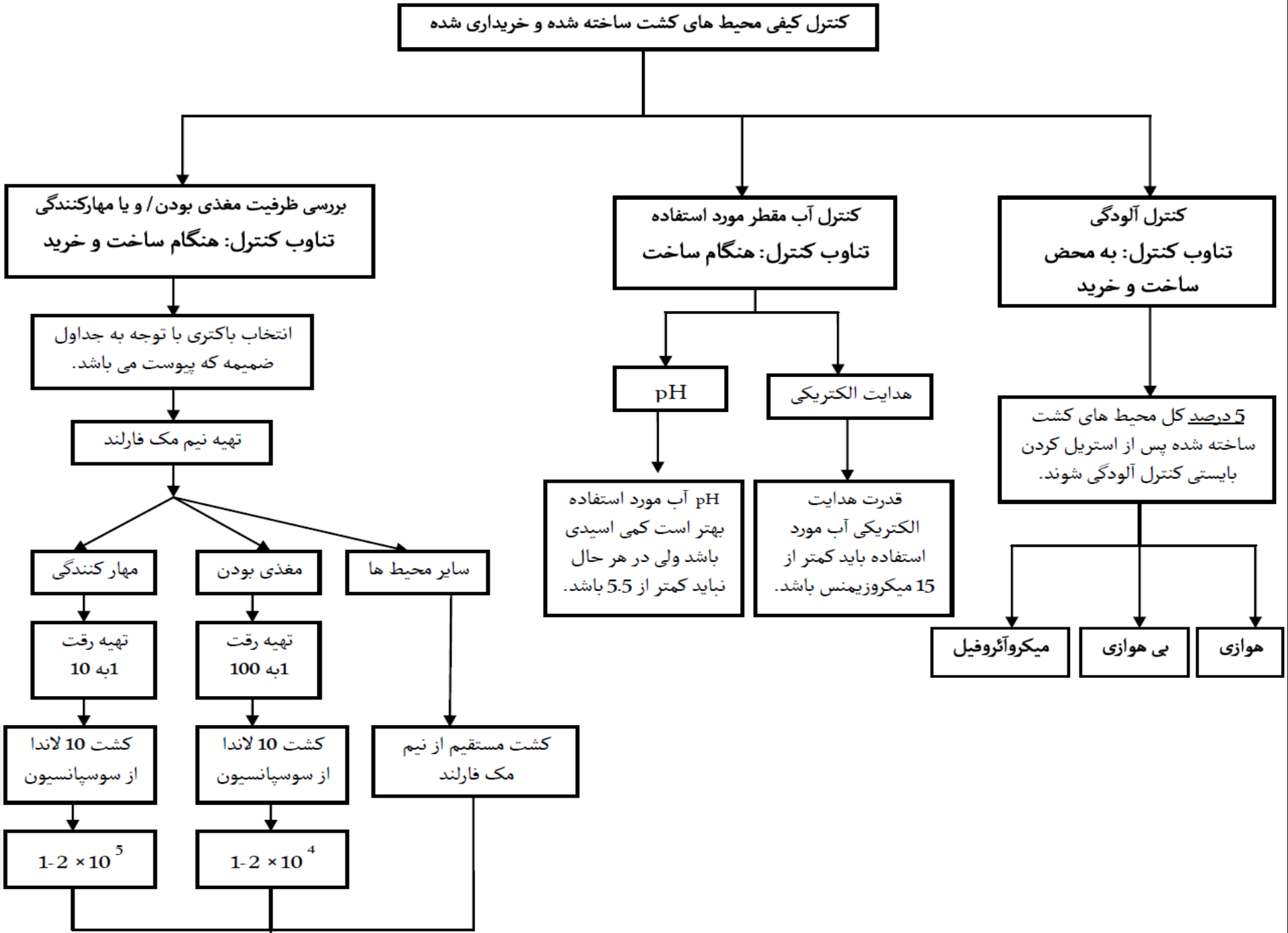

کنترل کیفی محیط های کشت ساخته شده و خریداری شده
کنترل آلودگی
تناوب کنترل: به محض ساخت و خرید
5 درصد کل محیط های کشت ساخته شده پس از استریل کردن بایستی کنترل آلودگی شوند.
هوازی
بی هوازی
میکروآئروفیل
کنترل آب مقطر مورد استفاده
تناوب کنترل: هنگام ساخت
هدایت الکتریکی
قدرت هدایت الکتریکی آب مورد استفاده باید کمتر از 15 میکروزیمنس باشد.
pH
pH آب مورد استفاده بهتر است کمی اسیدی باشد ولی در هر حال نباید کمتر از 5.5 باشد.
بررسی ظرفیت مغذی بودن/ و یا مهارکنندگی
تناوب کنترل: هنگام ساخت و خرید
انتخاب باکتری با توجه به جداول ضمیمه که پیوست می باشد.
تهیه نیم مک فارلند
مهار کنندگی
مغذی بودن
سایر محیط ها
تهیه رقت 1به 10
تهیه رقت 1به 100
کشت مستقیم از نیم مک فارلند
کشت 10 لاندا از سوسپانسیون
کشت 10 لاندا از سوسپانسیون
1-2 ×10^5
1-2 ×10^4

Table 1-26 Quality Control of Commonly Used Media: Suggested Control Organisms and Expected Reactions

MEDIUM	CONTROL ORGANISMS	EXPECTED REACTIONS
Blood agar	Group A *Streptococcus*	Good growth, β-hemolysis
	S. pneumoniae	Good growth, α-hemolysis
Bile–esculin agar	*Enterococcus* species	Good growth, black
	α-Hemolytic	No growth; no discoloration of media
	Streptococcus, not group D	
Chocolate agar	*Haemophilus influenzae*	Good growth
	Neisseria gonorrhoeae	Good growth
Christensen urea agar	*Proteus mirabilis*	Pink throughout (positive)
	Klebsiella pneumoniae	Pink slant (partial positive)
	Escherichia coli	Yellow (negative)
Simmons citrate agar	*K. pneumoniae*	Growth or blue color (positive)
	E. coli	No growth, remains green (negative)
Cystine trypticase (CTA) agar	*N. gonorrhoeae*	Yellow (positive)
Dextrose	*Branhamella catarrhalis*	No color change (negative)
Sucrose	*Escherichia coli*	Yellow (positive)
	N. gonorrhoeae	No color change (negative)
Maltose	*Salmonella* species, or *N. meningitidis*	Yellow (positive)
	N. gonorrhoeae	No color change (negative)
Lactose	*N. lactamicus*	Yellow (positive)
	N. gonorrhoeae	No color change (negative)

Table 1-26 *Continued*

MEDIUM	CONTROL ORGANISMS	EXPECTED REACTIONS
	Shigella flexneri	Purple slant, yellow deep
	P. mirabilis	Red slant, yellow deep
MacConkey agar	*E. coli*	Pink colonies (lactose positive)
	P. mirabilis	Colorless colonies, no spreading
	Enterococcus species	No growth
Malonate	*E. coli*	No growth
	K. pneumoniae	Good growth, blue (positive)
Motility (semisolid agar)	*P. mirabilis*	Media cloudy (positive)
	K. pneumoniae	No feather edge on streak line (negative)
Nitrate broth or agar	*E. coli*	Red on adding reagents
	Acinetobacter lwoffi	No red (negative)
Phenylethyl alcohol blood agar	*Streptococcus* species	Good growth
	E. coli	No growth
o-Nitrophenol-β-D-galactopyranoside (ONPG)	*Serratia marcescens*	Yellow (positive)
	Salmonella typhimurium	Colorless (negative)
Phenylalanine deaminase	*P. mirabilis*	Green (add 10% $FeCl_3$)
	E. coli	No green (negative)
Salmonella–Shigella (SS) agar	*S. typhimurium*	Colorless colonies, black centers
	E. coli	No growth
Voges–Proskauer	*K. pneumoniae*	Red (add reagents)
	E. coli	No development (negative)
Xylose–lysine–dextrose (XLD) agar	*Salmonella* species	Red colonies (positive lysine)
	E. coli	Yellow colonies (positive sugars)
	Shigella species	Transparent colonies (negative)

* *From Microbiology Checklist, College of American Pathologists, Revised October 14, 2003.*

Table 1-26 *Continued*

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چک لیست مربوط به ارزیابی کنترل کیفی محیط های کشت

نام فرد کنترل کننده:

تاریخ :

ردیف	نام محیط کشت اولیه	نام کارخانه سازنده	شرایط نگهداری					تاریخ انقضا
			در بسته	خشک و خنک	حرارت زیر 25 ° c	دور از نور و رطوبت	دور از منابع حرارتی مانند اتوکلاو و فور	سایر موارد با توجه به دستور العمل کارخانه سازنده

آزمایش ظرفیت مغذی بودن محیط های کشت

Nitritional activity)

تاریخ	نام محیط کشت	Lot Num محیط کشت	تاریخ انقضا محیط کشت	تعداد کلونی های حاصل از کشت 10میکرولیتر از سوسپانسیون 1به 100رقیق شده کدورت نیم مک فارلند از باکتری مورد نظر (محدوده استاندارد $1-2\times10^4$)	نتیجه

آزمایش ظرفیت مهار کنندگی محیط کشت

(Inhibitory Activity)

تاریخ	نام محیط کشت	Lot Num محیط کشت	تاریخ انقضا محیط کشت	تعداد کلونی های حاصل از کشت 10میکرولیتر از سوسپانسیون 1به 10رقیق شده کدورت نیم مک فارلند از باکتری مورد نظر (محدوده استاندارد $1-2\times10^5$)	نتیجه

Use of Quality Control Strains

- Control strains should be cultures that exhibit typical microscopic, macroscopic and biochemical characteristics of the species, and must be cultures that have been verified and validated and whose lineage is documented .

Quality Control Strains

- The cultures used for Quality Control Testing of media have been selected because of growth attributes or biochemical characteristics. Over an extended period, it is expected that these cultures will be consistent in their phenotypic properties. Cultures when received from a culture collection or other recognized (authorised) source should be preserved.

Use of Quality Control Strains

It is desirable to minimize the number of transfers between the master culture and the working culture such that there is limited population or genetic change. The most effective system for managing the culture collection is the hierarchical or tiered system that includes Master, Stock and Working cultures.

Use of Quality Control Strains

When a culture is first received by a laboratory it should be activated and tested for purity and identity. If pure, growth from this plate is used to prepare freeze dried ampoules, frozen glycerol broths or beads, or some equivalent system which minimises change but allows long term viability of the micro-organism.

Use of Quality Control Strains

In addition to the purity check, and at the same time of preservation, the identity of the culture should be verified including the particular characteristics utilized for media growth performance checks. The preserved culture generated by this process is termed MASTER culture and should not be accessed frequently.

External Quality Assessment (EQA)

- The term external quality assessment (EQA) is used to describe a method that allows for comparison of a laboratory's testing to a source outside the laboratory.
- This comparison can be made to the performance of a peer group of laboratories or to the performance of a reference laboratory.
- The term EQA is sometimes used interchangeably with proficiency testing;however, EQA can also be carried out using other processes.

Types of EQA

Several EQA methods or processes are commonly used. These include:

1. Proficiency testing external provider sends unknown samples for testing to a set of laboratories, and the results of all laboratories are analyzed, compared, and reported to the laboratories.

2. Rechecking or retesting slides that have been read are rechecked by a reference laboratory; samples that have been analyzed are retested, allowing for inter-laboratory comparison

EQA benefit

Participation in an external quality assessment program provides valuable data and information which:

1- Allows comparison of performance and results among different test sites

2- provides early warning for systematic problems associated with kits or operations.

3-provides objective evidence of testing quality;

4-Indicates areas that need improvement;

5- Identifies training needs.

EQA benefit

EQA helps to assure customers, such as physicians, patients, and health authorities, that the laboratory can produce reliable results. Individual laboratories can use EQA to identify problems in laboratory practices, allowing for appropriate corrective action. EQA articipation will help to evaluate reliability of methods, materials, and equipment, and to evaluate and monitor training impact.For laboratories performing public health-related testing, EQA can help to assure that results from different laboratories during surveillance activities are comparable

EQA benefit

- EQA participation is usually required for accreditation. Also, EQA participation creates a network for communication, and can be a good tool for enhancing notational
- laboratory network. Samples received for EQA testing, as well as the information shared by the EQA provider, are useful for conducting continuing education activities.

Principal characteristics of an EQA scheme

EQA programs vary but principal characteristics include the following:

1-EQA programs can either be free-of-charge or require a fee. Free EQA programs include those offered by a manufacturer to ensure equipment is working correctly and those organized by a regional or national program for quality improvement

Principal characteristics of an EQA scheme

Some EQA programs are obligatory, either required by an accrediting body or by law. Others are voluntary, and the quality manager may choose to voluntarily participate in an EQA program in order to achieve improvement in the quality of the laboratory's performance.

The EQA program can be organized at different levels: regional, national, or international.

Continue…

- Individual laboratory results are kept confidential, and generally are only known by the participating laboratory and the EQA provider. A summary is generally provided and allows comparison to the overall group
- Some EQA schemes may address a single disease, for example the EQA program for tuberculosis. Others may address many kinds of laboratory tests, looking at the overall testing performance for microbiology. An example of this multi-disease or test program is the national microbiology EQA in France, which is obligatory.

Rechecking process

- This method is most commonly used for acid-fast smears; the slides that were read in the original laboratory are "rechecked" in a central or reference laboratory. This allows for the accuracy of the original report to be evaluated, and also allows for the assessment of the quality of the slide preparation and staining.

Rechecking process

- **The following principles are important when performing recheck procedures**.

1- The slides for re-examination must be collected randomly. Every effort should be made to avoid systematic sampling bias.

2- Rechecking must be based upon statistical considerations. A common method is for the central laboratory to recheck 10% of negative and 100% of positive slides.

3- When discrepancies occur, there should be procedures in place to resolve them.

4- The outcome of rechecking must be analyzed for effective and timely feedback.

On-site evaluation

- A periodic visit by evaluators for on-site laboratory assessment is a type of EQA that has been used when other methods of EQA are not feasible or effective. Again, this method has most frequently been employed for assessment of sites performing AFB smears, and those performing HIV rapid testing.

On-site evaluation can be a valuable tool to

1-obtain a realistic picture of laboratory practices by observing the laboratory under routine conditions in order to check that it is meeting quality requirement

2- provide information for internal process improvement; measure gaps or deficiencies—learn “where we are”; assist the laboratory in collecting information for planning and implementation of training, monitoring, and corrective actions.

On-site evaluation for the purpose of EQA may be conducted by a central reference laboratory or other health authorities. On-site evaluation can be used together with retesting and rechecking schemes to provide more

Managing EQA in the Laboratory

- Participation in EQA
- All laboratories should participate in EQA challenges, and this should include EQA for all testing procedures performed in the laboratory, if possible. The benefits of this participation are considerable, and EQA provides the only means available to a laboratory to ensure that its performance is comparable to that of other laboratories

Managing EQA in the Laboratory

• For laboratories that are accredited, or that plan to seek accreditation, EQA participation is essential. ISO 15189 addresses EQA requirements for laboratories

as follows.

• There is a requirement that the laboratory participate in interlaboratory comparisons.•

Where an established EQA scheme is not available, an alternate EQA mechanism will have to be considered for interlaboratory comparison such as exchange of samples with other laboratories.

The laboratory management shall monitor the results of EQA and participate in the implementation of corrective action

- When participating in EQA programs, the laboratory needs to develop a process
- for the management of the process. A primary objective is to assure that all EQA
- samples are treated in the same manner as other samples tested.
- Procedures should be developed.
- • Handling of samples—These will need to be logged, processed properly, and
- stored as needed for future use.
- • Analyses of samples—Consider whether EQA samples can be tested so that
- staff do not recognize them as different from patient samples–blinded testing.
-

- • Appropriate record keeping—Records of all EQA testing reporting should be
- maintained over a period of time, so that performance improvement can be
- measured.
- • Investigation of any deficiencies—For any challenges where performance is
- not acceptable.
- • Taking corrective action when performance is not acceptable—The purpose of
- EQA is to allow for detection of problems in the laboratory, and to, therefore,
- provide an opportunity for improvement.
- • Communication of outcomes to all laboratory staff and to management.

External Quality Assessment problems

- Pre The sample may have been compromised during preparation, shipping, or after receipt in the laboratory by improper storage or handling. The sample may have been processed or labeled improperly in the laboratory.- examination

Examination

- Pre-examination
- The sample may have been compromised during preparation, shipping, or after receipt in the laboratory by improper storage or handling. • The sample may have been processed or labeled improperly in the laboratory

Examination

• The EQA challenge materials may exhibit a matrix effect in the examination system used by the participating laboratory.•

Possible sources of analytical problems include reagents, instruments, test methods, calibrations, and calculations. Analytical problems should be investigated to determine whether error is random or systemic.

• Competency of staff will need to be considered and evaluated.

Examination

- The EQA challenge materials may exhibit a matrix effect in the examination system used by the participating laboratory.• Possible sources of analytical problems include reagents, instruments, test
- methods, calibrations, and calculations. Analytical problems should be investigated to determine whether error is random or systemic.
- • Competency of staff will need to be considered and evaluated

Post-examination

- The report format can be confusing.
- Interpretation of results can be incorrect.
- Clerical or transcription errors can be sources of error

Guidelines for Susceptibility testing

1-Clinical laboratory Standards Institutes (CLSI) Formerly CLSI 2006

2- European Committee on Antimicrobial Susceptibility Testing – EUCAST

In Our country nearly all microbiology Laboratories use CLSI guidelines.

Critical Challenges in AST

1. Pure isolate
2. Culture media: Muller-Hinton
3. Reagents: disks
4. Size of the inoculums
5. Incubation condition
6. Control with reference strains
7. Reading inhibition diameters (accurate measurement)
8. Knowledge of staff

Quality control strains

- Each QC strain should be obtained from a recognized source (eg, ATCC®). All CLSI-recommended QC strains appropriate for the antimicrobial agent and reference method should be evaluated and produce results within the expected ranges listed in M100 that were established according to the procedures described in CLSI document

Quality Control Reference Strains

- • Escherichia coli ATCC® 25922;
- • Escherichia coli ATCC® 35218; / B-ihibitor.co
- • Haemophilus influenzae ATCC® 49247;
- • Haemophilus influenzae ATCC® 49766;
- • Klebsiella pneumoniae ATCC® 700603; /ESBL
- • Neisseria gonorrhoeae ATCC® 49226;
- • Pseudomonas aeruginosa ATCC® 27853;
- • Staphylococcus aureus ATCC® 25923;
- • Streptococcus pneumoniae ATCC® 49619.

Quality Control Reference Strains

- *Enterococcus faecalis* ATCC® 29212 (or alternatively

  E. faecalis ATCC® 33186) is recommended for monitoring Mueller-Hinton agar for unacceptable levels of thymidine when trimethoprim or sulfonamides are tested.
- *E.faecalis* ATCC® 29212 is also used for control of high-content aminoglycoside disks
- *H. influenzae* ATCC® 49247 is an ampicillin-resistant, B-lactamase-negative organism.
- *H. influenzae* ATCC® 49766 is an ampicillin-susceptible organism that is more reproducible than *H. influenzae* ATCC® 49247 for controlling selected B-lactams.

Storing Quality Control Strains

- Cultures may be maintained at -20 ºC or below (preferably at -60 ºC or below or in liquid nitrogen) in a suitable stabilizer (e.g., 50% fetal calf serum in broth, 10 to 15% glycerol in tryptic soy broth, defibrinated sheep blood, or skim milk), or in a freeze-dried state without significant risk of altering their antimicrobial susceptibility.

Storing Quality Control Strains

- Working control cultures should be stored on tryptic soy agar (nonfastidious strains) or on enriched chocolate agar (fastidious strains) slants at 2 to 8 ºC and subcultured each week for no more than three successive weeks. New working cultures should be prepared at least monthly from frozen,freeze-dried, or commercial cultures.

The 15-Replicate (3 × 5 Day) Plan

- Test three replicates of each applicable QC strain using individual inoculum preparations for five consecutive test days and document results.

The 15-Replicate (3 × 5 Day) Plan

- Upon successful completion of the 15-replicate (3 × 5 day) plan, it is acceptable to go to weekly QC testing.
- If completion of the 15-replicate (3 × 5 day) plan is unsuccessful, take corrective action as appropriate, and continue daily QC testing.

Antimicrobial Agent	Disk Content	Disk Diffusion QC Ranges, mm		
		Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 25923
in	30 µg	19-26	20-26	20-26
lin	10 µg	15-22	-	27-35
mycin	15 µg	-	-	21-26
in	75 µg	-	24-30	-
nam	30 µg	28-36	23-29	-
icillin	100 µg	23-29	18-24	-
r	30 µg	23-27	-	27-31
ndole	30 µg	26-32	-	26-34
in	30 µg	21-27	-	29-35
r	5 µg	24-28	-	25-32
ren	5 µg	22-28	-	20-28
ne	30 µg	31-37	25-31	23-29
net	10 µg	24-29	-	-
ocol	30 µg	25-31	22-31	-
e	5 µg	20-26	-	-
azole	30 µg	26-32	-	25-34
id	30 µg	25-29	-	22-28
razone	75 µg	28-34	23-29	24-33
ime	30 µg	29-35	18-22	25-31
an	30 µg	28-34	-	17-23
in	30 µg	23-29	-	23-29
oxime	10 µg	23-28	-	19-25
il	30 µg	21-27	-	27-33
line	30 µg	26-34	-	26-35
dime	30 µg	25-32	22-29	16-20
ten	30 µg	27-35	-	-
xime	30 µg	30-36	12-17	27-35
prole	5 µg	25-31	-	20-27
xone	30 µg	29-35	17-23	22-28

Table 6. 15-Replicate (3- ×5-Day) Plan: Acceptance Criteria and Recommended Action*

Number Out of Range With Initial Testing (Based on 15 Replicates)	Conclusion From Initial Testing (Based on 15 Replicates)	Number Out of Range After Repeat Testing (Based on All 30 Replicates)	Conclusion After Repeat Testing
0–1	Plan is successful. Convert to weekly QC testing.	N/A	N/A
2–3	Test another 3 replicates for 5 days.	2–3	Plan is successful. Convert to weekly QC testing.
≥4	Plan fails. Investigate and take corrective action as appropriate. Continue QC each test day.	≥4	Plan fails. Investigate and take corrective action as appropriate. Continue QC each test day.

* Each QC strain and antimicrobial agent combination is assessed separately.

Abbreviations: N/A, not applicable; QC, quality control.

Implementing Weekly Quality Control Testing

Perform quality control testing once per week and

whenever any reagent component of the test (e.g., A new lot of agar or a new lot of disks from the Same or a different manufacturer) is changed.If any of the weekly quality control results is out Of the acceptable range, corrective action is required..

معرف	ارگانیسم کنترل	شماره ATCC	نتیجه مورد انتظار	دفعات انجام آزمایش QC
Bacitracin disk	*Strep.pyogenes* *Strep. agalactiae*	19615 12386	هاله عدم رشد (بزرگتر از 10mm) عدم هاله یا کمتر از 10mm	هر سری ساخت و سپس هر ۳ ماه
Coagulase (لام با آب مقطر و زیر ده ثانیه)	*Staph.aureus* *Staph.epidermidis*	25923 12228	مثبت (ایجاد لخته) منفی (عدم ایجاد لخته)	هر سری ساخت و سپس با هر بار آزمایش
Ferric chloride (10%)	*Proteus vulgaris* *Escherichia coli*	33420 25922	مثبت (سبز رنگ) منفی (بدون تغییر رنگ)	هر سری ساخت و سپس هر ۳ ماه
Methyl red	*E. coli* *K. pneumoniae*	25922 13883	مثبت (قرمز رنگ) منفی (بدون تغییر رنگ)	هر سری ساخت و سپس هر ۳ ماه
ONPG	*E. coli* *Proteus mirabilis*	25922 29245	مثبت (زرد رنگ) منفی (بدون تغییر رنگ)	هر سری ساخت و سپس هر ۳ ماه
Voges-Proskauer	*Enterobacter cloacae* *E. coli*	13047 25922	مثبت (قرمز رنگ) منفی (بدون تغییر رنگ)	هر سری ساخت و سپس هر ۳ ماه
Indole (Kovacs)	*E. coli* *P. aeruginosa*	25922 27853	مثبت (ارغوانی رنگ) منفی (بدون تغییر رنگ)	هر سری ساخت و سپس هر ۳ ماه
Optochin (در حضور CO2)	*Strep. pneumoniae* *Strep. pyogenes*	6305 19615	حساس مقاوم	هر سری ساخت و سپس هر ۳ ماه

CATALASE - 3% HYDROGEN PEROXIDE (H202)

ORGANISMS:

Streptococcus pyogenes ATCC 8668
Staphylococcus aureus ATCC 25923

Date	Lot #	Company	*Streptococcus pyogenes* ATCC 8668 **(Negative)**	*Staphylococcus aureus* ATCC 25923 **(Positive)**	Commemts

REFERENCES:1. Isenberg HD. ed. Clinical Microbiology Procedures Handbook. Washington DC: American Society for Microbiology, 1992.

ONPG DISK

ORGANISMS:

Escherichia coli ATCC 25922
Klebsiella pneumoniae ATCC 10031

Date	Lot #	Company	*Escherichia coli* ATCC 25922 **(Positive)**	*Klebsiella pneumoniae* ATCC 10031 **(Negative)**	Commemts

REFERENCES:COWAN AND STEEL'S Manual for identification of medical bacteria,. G, I, BARROW,. R, K, A, FELTHAM. 2003. CAMBRIDGE UNIVERSITY PRESS.

OPTOCHIN DISKS

ORGANISMS:

Streptococcus pneumoniae ATCC 33400
Streptococcus pyogenes ATCC 8668

Date	Lot #	Company	*Streptococcus pneumoniae* ATCC 33400 **(Sensitive)**	*Streptococcus pyogenes* ATCC 8668 **(Resistant)**	Commemts

REFERENCES:COWAN AND STEEL'S Manual for identification of medical bacteria,. G, I, BARROW,. R, K, A, FELTHAM. 2003. CAMBRIDGE UNIVERSITY PRESS.

OXIDASE DISKS

ORGANISMS:

pseudomonas aeruginosa ATCC 27853
Neisseria meningitidis PTCC 1057
Staphylococcus aureus ATCC 25923

Date	Lot #	Company	*pseudomonas aeruginosa* ATCC 27853 **(Positive)**	*Neisseria meningitidis* PTCC 1057 **(Positive)**	*Staphylococcus aureus* ATCC 25923 **(Negative)**	Comment

REFERENCES:

COWAN AND STEEL'S Manual for identification of medical bacteria,. G, I, BARROW,. R, K, A, FELTHAM. 2003. CAMBRIDGE UNIVERSITY PRESS.

NOVOBIOCIN DISKS

ORGANISMS:

Staphylococcus saprophyticus ATCC 15305
Staphylococcus aureus ATCC 25923

Date	Lot #	Company	*Staphylocccuso saprophyticus* ATCC 15305 **(Resistant)**	*Staphylococcus aureus* ATCC 25923 **(Sensitive)**	Commemts
			Reporting policy		

REFERENCES:COWAN AND STEEL'S Manual for identification of medical bacteria,. G, I, BARROW,. R, K, A, FELTHAM. 2003. CAMBRIDGE UNIVERSITY PRESS.

Post analytical stage (Results Reporting)

The result of microbiological examination usually becomes available in stages on successive days. So, the SOP needs to include: (a) reporting and verifying test results, (b)interpreting test reports correctly, and (c) taking appropriate action when a result has serious implications for a patient or public health.

Wording of reports:

- The aim of the clinical microbiologist is to provide clinicians and health officers with reports that are understandable, The laboratory should, therefore, have a carefully constructed policy for the wording of reports and all staff should adhere to the policy.

Reporting policy

The laboratory policy for reports should specify not only the wording of interpretative comments, but also the circumstances in which the different comments are to be made. It should, for instance ·

Lay down the circumstances in which the finding of coagulase-negative Staphylococci in a blood culture to be reported with the comment 'probably a contamination from the skin', and without giving its antibiotic sensitivity report.

Reporting policy

In different circumstances as, in a compromised patient when the finding is to be reported as 'possibly of clinical significance', and the antibiotic sensitivity given.

Reporting policy

A policy is also required for reporting the finding of AFB in different specimens. Thus their finding in sputum might be reported: 'many AFB resembling tubercle bacilli seen in film, culture for Mycobacterium is in progress (or is advised). However, there finding in urine might be reported more cautiously: 'a few AFB seen in film which may be commensal Smegma bacilli, culture for Mycobacterium is in progress (or is advised).

Reporting policy

Particular care must be given to the policy for the wording of negative reports. These should be phrased in such a way as to indicate which pathogens were sought and not found. The uninformed recipient of a report on a throat swab stating 'no pathogen on culture', might well imagined that a search had been made for every kind of respiratory tract pathogen including viruses, Mycoplasmas and Chlamydiae, when the specimen had been cultured only for pyogenic bacteria

Reporting policy

If a throat swab from acute sore throat has been examined the

report might properly read 'mixed upper respiratory organisms present. No *Streptococcus pyogenes* on culture. Not cultured for viruses, mycoplasmas and other pathogens.Similarly, if faeces from acute diarrhea has been examined the report should read 'no Salmonella, Shigella, Vibrio orCampylobacter found.

عات استریل بدن شامل: جنب، آسیت، مفصل، زجاجیه، پریکارد و ...	تمام گروه های سنی	هر نتیجه مثبت	اولین تشخیص با گرم بحرانی تلقی مثبت شدن مجدد نتیجه دیگر بحرا
سی بافت مانند مغز، کبد، کلیه، استخوان و مغز استخوان	تمام گروه های سنی	هر نتیجه مثبت	
نه های بخش جراحی مانند آبسه های مغزی و مایعاتی که در حین جراحی برداشته می شوند	تمام گروه های سنی	هر نتیجه مثبت یا منفی	
تراشه قرنیه	تمام گروه های سنی	هر نتیجه مثبت	

، بافت مانند مغز، کبد، کلیه، استخوان و مغز استخوان	تمام گروه های سنی	هر نتیجه مثبت	
مدفوع	تمام گروه های سنی	نتیجه مثبت برای *ویبریو کلرا*	
	کمتر از ۱۸ سال	نتیجه مثبت برای *E. coli* Enterohemorrhagic مانند *E. coli* O157	
	کمتر از ۱۲ سال	نتیجه مثبت برای *شیگلا*	در بیما
	تمام گروه های سنی	نتیجه مثبت برای *شیگلا دیسانتریه*	در بیماران ب

، بافت مانند مغز، کبد، کلیه، استخوان و مغز استخوان	تمام گروه های سنی	هر نتیجه مثبت	
مدفوع	تمام گروه های سنی	نتیجه مثبت برای *ویبریو کلرا*	
	کمتر از ۱۸ سال	نتیجه مثبت برای *E. coli* Enterohemorrhagic مانند *E. coli* O157	
	کمتر از ۱۲ سال	نتیجه مثبت برای *شیگلا*	در بیما
	تمام گروه های سنی	نتیجه مثبت برای *شیگلا دیسانتریه*	در بیماران ب

ی گروه A جدا شده از فاسیت و یا زخم های جراحی	تمام گروه های سنی	نتیجه مثبت	
لژیونلا	تمام گروه های سنی	نتیجه مثبت	
لپتوسپیرا	تمام گروه های سنی	نتیجه مثبت	
لیستریا	تمام گروه های سنی	نتیجه مثبت	زنان باردار و افراد م سیستم
ت مثبت *مایکوباکتریوم توبرکولوزیس*	تمام گروه های سنی	اولین بار جداسازی و جداسازی سویه های مقاوم (MDR و XDR)	در صورتی که از نمو هفته دوباره باکتری گزارش شفاهی یا
سایر گونه های *مایکوباکتریوم*	تمام گروه های سنی	اولین جواب مثبت	هر نم

تشخیص مورد جدید سیفیلیس	تمام گروه های سنی	نتیجه مثبت	
لپتوسپیرا	تمام گروه های سنی	تیتر با ارزش آنتی بادی	
وکسین A و B *کلستریدیوم دیفیسیل* در نمونه مدفوع	تمام گروه های سنی	نتیجه مثبت	تشخیص اولیه به ع تلقی می شود و جوا طی یک هفته، دیگ بحرانی تلقی
کلستریدیوم بوتولینوم در نمونه ماده غذایی مصرفی	تمام گروه های سنی	نتیجه مثبت	
شیگاتوکسین در نمونه مدفوع	تمام گروه های سنی	نتیجه مثبت	