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Quality Assurance in Medical Microbiology Laboratory

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Abstract

Quality control (QC) in microbiology plays an important role to ensure that the result is valid. The QC should include instruments like autoclave, incubator, refrigerator, freezer, dry oven, media, reagents, antimicrobial susceptibility testing, sterility testing. Autoclave should be controlled with Bacillus stearothermophilus spores. Refrigerator, freezer and dry oven, the temperature should be recorded every day. Media and reagents being tested with positive and negative bacteria. Antimicrobial susceptibility testing should be controlled with E. coli ATCC 25922, S. aureus ATCC 25923 and Pseudomonas aeruginosa ATCC 27853. Media sterility can be checked by overnight incubation before using it. Mueller Hinton media's depth should also be checked every time.

Keywords: Quality control, microbiology laboratory

INTRODUCTION

Quality assurance (QA) is a wide ranging concept covering all matters that individually or collectively influence the quality of a product. It denotes a system for continuously improving reliability, efficiency and utilization of products and services. In the context of quality assurance, 2 important definitions need to be clearly understood [1]:

1. Internal Quality Control (IQC) which denotes a set of procedures undertaken by the staff of medical microbiology laboratory for continuously and concurrently assessing laboratory work so that good quality results are produced by the laboratory
2. External Quality Assessment (EQA): is a system of objectively assessing the medical microbiology performance by an outside agency. This assessment is retrospective and periodic but is aimed at improving the internal control.

IQC and EQA are complementary in ensuring the reliability of the procedures, results, and quality of the product. QA programs are required for the following reasons [1]:

- To generate reliable, reproducible results
- To establish inter-laboratory comparability in laboratory testing
- To establish the credibility of the laboratory among doctors and the public at large
- To motivate the staff for further improvement
- To prevent of legal complications which may follow poor quality results

- To improve the quality of health care

INTERNAL QUALITY CONTROL

It is commonly believed that the quality of laboratory results solely depends upon the laboratory undertaking the analysis. However, there are many pre-analytical and post-analytical factors which influence the quality of the end results to a very significant extent. The principle of 'GIGO' – 'Garbage In Garbage Out' very well applies to the laboratory test also. Some of the important factors influencing quality are listed here [1]:

(1) **Specimen**. This is the single most important factor. Selection of the right sample, collection in a right manner, adequate quantity, proper transportation to the laboratory, and processing of the sample before testing, are crucial factors.

(2) **Personnel** : The quality of the laboratory results generated is directly proportional to the training, commitment and motivation of the technical staff.

(3) **Environmental factors**. Inadequate lighting, workspace or ventilation or unsafe working conditions may influence the laboratory results.

(4) **Analytical factors**. The quality of reagents, chemicals, glassware, stains, culture media, use of standard procedures and reliable equipment all influence laboratory results. Failure to examine a sufficient number of microscope fields can lead to false negative results.

(5) **Post analytical factors**. Transcription errors, incomplete reports, and improper interpretation can adversely influence the laboratory results.

INTERNAL QUALITY CONTROL IS THE MAINSTAY OF QUALITY ASSURANCE

The backbone of a good quality assurance programme is a good Internal quality control. Intermediate and peripheral laboratories must put in place various IQC procedures and may participate in any External Quality Assurance Scheme that is in operation [1].

Requirements of internal Quality Control [1].

Several requirements of Internal quality control are :

- Comprehensive – over all steps from collection of sample to reporting
- Regular - continuous monitoring
- Rational – focus more on critical factors
- Practical – should not attempt to evaluate everything
- Economical – should be cost-effective and within the provided budget

Each laboratory should have Standard Operating Procedure Manuals (SOPMs) which should include the following information about the infrastructure of a laboratory [1]

- Biosafety precautions
- Disposal of infectious waste
- Collection, transport and storage of specimens
- Criteria of rejection of samples

- Processing of specimens
- Maintenance of equipment
- Recording of results
- Reporting of results
- Procedure of quality control
- Referral system

SPOMs should be periodically reviewed an revised and religiously followed in the laboratories.

MAINTENANCE OF EQUIPMENT

A preventive maintenance program to ensure proper functioning of all electrical and mechanical equipment should be established in all microbiology laboratories. Equipment should be checked at prescribed time intervals; certain working parts should be replaced after a specified period of use, even though they may not appear worn. A brief list of some of the equipment, the monitoring procedures to be carried out and the frequency and tolerance limit is shown in **Table 1**. Assignments should be made among laboratory personnel to ensure that all inspections are carried out and all data are recorded accurately onto charts or in maintenance manuals. It is important to detect upward or downward trends immediately, so appropriate corrective action can be taken before errors result. The temperature of incubators, refrigerators, freezers, water baths and heating blocks must be determined and recorded daily with a thermometer calibrated by the Bureau of Standards or with one that has been checked against a calibrated thermometer. The concentration of CO2 in all CO2 incubators must also be determined daily. For any reading that falls outside of the established quality control range, the cause must be determined and the defect quickly corrected [2].

MONITORING CULTURE MEDIA, REAGENTS AND SUPPLIES

All media and reagents must be checked against appropriate controls for the proper reactivity. It has been recognized that many modern commercial media perform with a high degree or reliability. Consensus recommendations has been developed for the necessity of local quality control. The few media with occasional quality control problems (e.g. chocolate agar, media for *Campylobacter jejuni*, and Thayer Martin agar) should be subjected to control tests in each laboratory. Many others need not be tested if the manufacturer of the media provides documentation that the appropriate reactivity has been observed [2].

A list of suggested organisms and acceptable results for the culture media most commonly used in clinical microbiology laboratories is found in **Table 2**. Quality control of selected reagents and media can be found in **Table 3**. Quality control stock organisms may be maintained in the laboratory by subculturing bacterial isolates recovered as part of the routine work. Alternatively, and more conveniently, dried stock organisms may be purchased from culture collections such as ATCC (American Type Culture Collection, 12301 Parklawn Dr., Rockville MD) or from commercial vendors. Each batch of media should be checked for reactivity and for appropriate support of microbial growth, either by the manufacturer or in the local laboratory [2].

Culture tubes, plates of media and reagents must bear a label that clearly indicates the content and the dates of preparation and expiration. "coded' culture tubes plated media and reagents should be referenced in such a way that even non laboratory personnel would be able to interpret the code [2].

QUALITY ASSURANCE IN SUSCEPTIBILITY TEST

Clearly defined quality control rules apply to antimicrobial susceptibility testing. The precision and accuracy of the test are controlled by the parallel use of a set of control strains, with known susceptibility to antimicrobial agents. These quality control strains are tested using exactly the same procedure as for the test organisms. The zone sizes shown by the control organisms should fall within the range of diameters given in **Table 4.** When the results regularly fall outside this range, they should be regarded as evidence that a technical error has been introduced into the test, or that the reagents are at fault. Each reagent and each step in the test should then be investigated until the cause of the error has been found and eliminated [3].

The quality assurance program should use standard reference strains of bacteria that are tested in parallel with the clinical culture. They should preferably be run every week, or with every fifth batch of tests and in addition, every time that a new batch of Mueller Hinton agar or a new batch of discs is used. The standard strains are [3]:

Escherichia coli ATCC 25922

Staphylococcus aureus ATCC 25923

Pseudomonas aeruginosa ATCC 27853

Culture for day-to-day use should be grown on slants of nutrients agar (tryptic soy agar is convenient) and stored in a refrigerator . These should be subcultured onto fresh slants every 2 weeks.

Table 1. Quality Control Surveillance Procedures of Commonly Used Microbiology Equipment

EQUIPMENT	PROCEDURE	SCHEDULE	TOLERANCE LIMITS
Refrigerators	Recording of temperature*	Daily or continuous	2^0C-8^0C
Freezer	Recording of temperature*	Daily or continuous	-8^0C tp -20^0C -60^0C to -75^0C
Incubators	Recording of temperatures*	Daily or continuous	35,5^0C $\pm$ 1^0C
Incubators (CO_2)	Measuring of CO_2 content Use blood gas analyzer of Fyrite** device	Daily or twice daily	5%-10%
Water baths	Recording of temperature*	Daily	36^0C-38^0C 55^0C-57^0C
Heating blocks	Recording of temperature*	Daily	$\pm$ 1^0C of setting
Autoclaves	Test with spore strip (*Bacillus stearothermophilus*)	At least weekly	No growth of spores in subculture indicates sterile run
pH meter	Test with pH-calibrating solutions	With each use	$\pm$0,1 pH units of standard being used
Anaerobic jars	Methylene blue Indicator strip	With each use	Conversion of strip from blue to white indicates low 0_2 tension
Anaerobic gloves box	*Clostridium novyi* type B culture Methylene blue indicator solution	Run periodically Continuously or daily	Growth indicates very low 0_2 tension. It is used only where extremely low 0_2 tension is required Solution remains colorless if 0_2 tension is low
Microbiology rotator	Count revolutions per minute	With each use	180 RPM $\pm$ 10 RPM
Centrifuges	Check revolutions with tachometer	Monthly	Within 5% of dial indicator setting
Safety hoods	Measure air velocity*** across face opening	Semiannually or quarterly	50 ft of airflow per minute $\pm$ 5

			ft/min

*Each monitoring thermometer must be calibrated against a standard thermometer
**Bacharach Instrument Co., Pittsburgh, PA.
***Velometer Jr., Alnor Instrument Co., Chicago, IL.

Table 2. Quality Control of Commonly Used Media: Suggested Control Organisms and Expected Reactions

MEDIUM	CONTROL ORGANISMS	EXPECTED REACTION
Blood agar	Group A *Streptococcus* *S.pneumoniae*	Good growth, β-hemolysis Good growth, -hemolysis
Chocolate agar	*Haemophilus influenzae* *Neisseria gonorrhoeae*	Good growth Good growth
Christensen urea agar	*Proteus mirabilis* *Klebsiella pneumoniae* *Escherichia coli*	Pink throughout (positive) Pink slant (partial positive) Yellow (negative)
Simmons citrate agar	*K. pneumoniae* *E. coli*	Growth or blue color (positive) No growth, remains green (negative)
Decarboxylases Lysine	*K. pneumoniae* *Enterobacter sakazakii*	Bluish (positive) Yellow (negative)
Arginine (dihydrolase)	*E. cloacae* *Proteus mirabilis*	Bluish (positive) Yellow (negative)
Ornithine	*P. mirabilis* *K. pneumoniae*	Bluish (positive) Yellow (negative)
Deoxyribonuclease (DNase)	*Serratia marcescens* *E.cloacae*	Zone of clearing (add 1 N HCL) No zone of clearing
Indole (Kovac's)	*E. coli* *K. pneumoniae*	Red (positive) No red color (negative)
Kligler iron agar	*E. coli* *Shigella flexneri* *Pseudomonas aeruginosa* *Salmonella typhimurium*	Acid slant/ acid deep Alkaline slant/acid deep Alkaline slant/alkaline deep Alkaline slant/black deep
Lysine iron agar	*S. typhimurium* *Shigella flexneri* *P.mirabilis*	Purple deep and slant, + H_2S Purple slant, yellow deep Red slant, yellow deep
MacConkey agar	*E. coli* *P. mirabilis* *Enterococcus spesies*	Pink colonies (lactose positive) Colorless colonies, no spreading No growth
Motility (semisolid agar)	*P. mirabilis* *K. pneumoniae*	Media cloudy (positive) No feather edge on streak line (negative)
Phenylalanine deaminase	*P. mirabilis* *E. coli*	Green (add 10% $FeCl_3$) No green (negative)
Salmonella-Shigella (SS) agar	*S. typhimurium* *E. coli*	Colorless colonies, black centers No growth
Voges-Proskauer	*K. pneumoniae* *E. coli*	Red (add reagents) No development (negative)

Table 3. Quality Control of Selected Reagents and Media

MEDIA OR REAGENTS	FREQUENCY	CONTROLS
Gram's stain	Each new batch of stain and at least weekly	Gram-positive and Gram-negative organism
Other nonimmunologic, nonfluorescent stains	Each day of use and each new batch, lot number, and shipment	Appropriate reactivity
Fluorescent stains	Each time of use	Appropriate reactivity
Catalase, coagulase, oxidase, bacitracin, optochin, ONPG, X or V or XV disks, identification systems	Each new batch, lot number, or shipment	Positive and negative controls
Antisera (Salmonella and Shigella)	Each new batch, lot number, and shipment when prepared or opened and once very 6 months thereafter	Positive and negative controls
β-lactamase (ther than Nitrocefin)	Each day of use	Positive and negative controls
β-lactamase (Nitrocefin)	Each new batch, lot number, and shipment	Positive and negative controls

MEDIA OR REAGENTS	FREQUENCY	CONTROLS
Nucleic acid probes	Each day of use	Positive and negative controls
AFB stains	Each day of use	Positive and negative controls
Antimicrobial susceptibility tests	Daily or weekly if criteria met	Appropriate organism

Table 4. Disk Diffusion Testing – Acceptable Limits (mm) for Quality Control Strains Used to Monitor Accuracy; Nonfastidious Organisms Using Mueller-Hinton Medium Without Blood or Other Supplements [4]

Antimicrobial Agent	Disk Content	*Escherichia coli* ATCC® 25922[a]	*Staphylococcus aureus* ATCC® 25923	*Pseudomonas aeruginosa* ATCC® 27853	*Escherichia coli* ATCC® 35218[b]
Amikacin	30 µg	19-26	20-26	18-26	-
Amoxicillin-clavulanic acid	20/10 µg	18-24	28-36	-	17-22
Ampicillin	10 µg	16-22	27-35	-	6
Ampicillin-sulbactam	10/10 µg	19-24	29-37	-	13-19
Azithromycin	15 µg	-	21-26	-	-
Azlocillin	75 µg	-	-	24-30	-
Aztreonam	30 µg	28-36	-	23-29	-
Cefazolin	30 µg	21-27	29-35	-	-
Cefepime	30 µg	31-37	23-29	24-30	-
Cefoperazone	75 µg	28-34	24-33	23-29	-
Cefotaxime	30 µg	29-35	25-31	18-22	-
Cefoxitin	30 µg	23-29	23-29	-	-
Ceftobiprole	30 µg	30-36	26-34	24-30	-
Ceftriaxone	30 µg	29-35	22-28	17-23	-
Cefuroxime	30 µg	20-26	27-35	-	-
Cephalothin	30 µg	15-21	29-37	-	-
Chloramphenicol	30 µg	21-27	19-26	-	-
Ciprofloxacin	5 µg	30-40	22-30	25-33	-
Clarithromycin	15 µg	-	26-32	-	-
Clindamycin[c]	2 µg	-	24-30	-	-
Colistin	10 µg	11-17	-	11-17	-
Daptomycin[d]	30 µg	-	18-23	-	-
Dirithromycin	15 µg	-	18-26	-	-
Doripenem	10 µg	28-35	33-42	29-35	-
Doxycycline	30 µg	18-24	23-29	-	-
Ertapenem	10 µg	29-36	24-31	13-21	-

Antimicrobial Agent	Disk Content	*Escherichia coli* ATCC® 25922[a]	*Staphylococcus aureus* ATCC® 25923	*Pseudomonas aeruginosa* ATCC® 27853	*Escherichia coli* ATCC® 35218[b]
Erythromycin[c]	15 µg	-	22-30	-	-
Faropenem	5 µg	20-26	27-34	-	-
Fosfomycin[e]	200 µg	22-30	25-33	-	-
Gentamicin[f]	10 µg	19-26	19-27	16-21	-
Imipenem	10 µg	26-32	-	20-28	-
Kanamycin	30 µg	17-25	19-26	-	-
Levofloxacin	5 µg	29-37	25-30	19-26	-
Linezolid	30 µg	-	25-32	-	-
Meropenem	10 µg	28-34	29-37	27-33	-
Methicillin	5 µg	-	17-22	-	-
Moxifloxacin	5 µg	28-35	28-35	17-25	-
Nalidixic acid	30 µg	22-28	-	-	-
Netilmicin	30 µg	22-30	22-31	17-23	-
Nitrofurantoin	300 µg	20-25	18-22	-	-
Oxacillin	1 µg	-	18-24	-	-
Piperacillin-tazobactam	100/10 µg	24-30	27-36	25-33	24-30
Polymyxin B	300 units	13-19	-	14-18	-
Quinupristin-dalfopristin -	15 µg	-	21-28	-	-
Rifampin	5 µg	8-10	26-34	-	-
Teicoplanin	30 µg	-	15-21	-	-
Telithromycin	15 µg	-	24-30	-	-
Ticarcillin	75 µg	24-30	-	21-27	6
Ticarcillin-clavulanic acid	75/10 µg	24-30	29-37	20-28	21-25
Tigecycline	15 µg	20-27	20-25	9-13	-
Tobramycin	10 µg	18-26	19-29	19-25-	-
Trimethoprim-sulfamethoxazol[g]	1.25/23.75 µg	23-29	24-32	-	-
Vancomycin	30 µg	-	17-21	-	-

Footnotes

a. ATCC is a registered trademark of the American Type Culture Collection.

b. Because this strain may lose its plasmid, careful organism maintenance is required; refer to M02-A10, Section 15.4.

c. When disk approximation tests are performed with erythromycin and clindamycin, *S. aureus* ATCC® BAA-977 (containing inducible *erm*A-mediated resistance) and *S. aureus* ATCC® BAA-976 (containing *msr*A-mediated macrolide-only efflux) are recommended as supplemental QC strains (eg, for training, competency assessment, or test evaluation). *S. aureus* ATCC® BAA-977 should demonstrate inducible clindamycin resistance (ie, a positive D-zone test), while *S. aureus* ATCC® BAA-976 should not demonstrate inducible clindamycin resistance. *S. aureus* ATCC® 25923 should be used for routine QC (eg, weekly or daily) of erythromycin and clindamycin disks using standard MHA.

d. Some lots of MHA are deficient in calcium and give small zones.

e. The 200-µg fosfomycin disk contains 50 µg of glucose-6-phosphate.

f. For control limits of gentamicin 120-µg and streptomycin 300-µg disks, use *Enterococcus faecalis* ATCC® 29212 (gentamicin: 16 mm to 23 mm; streptomycin: 14 - 20 mm).

g. Ulifloxacin is the active metabolite of the prodrug prulifloxacin. Only ulifloxacin should be used for antimicrobial susceptibility testing.
h. These agents can be affected by excess levels of thymidine and thymine. See M02-A10, Section 7.1.3 for guidance should aproblem with QC occur.

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