Original Article

Quality in Diagnostic Microbiology: Experiential Note to Emphasize Value of Internal Control Programs

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Abstract

Background: Quality control (QC) in diagnostic microbiology is a matter of effective, efficient, accurate reporting in the expected turnaround time. Major stages of the analytical "standard operational procedures" where QC could be easily affected include organism identification and antibacterial susceptibility testing. **Aim:** The objective of this experiential technical note is to provide an evidence base to highlight the value of internal QC program in evaluating the effectiveness and efficiency of a laboratory's standard operational procedures; and the competences of individual scientific/technical staff. **Materials and Methods:** This report is based on four different scenarios requiring internal QC, including cases that are not reported within the turnaround time of standard operational procedures. Small-scale evaluations of (i) internal QC program, (ii) ciprofloxacin vs. moxifloxacin susceptibilities, and (iii) calibrated dichotomous susceptibility vs. directed susceptibility testing were performed. **Results:** The internal QC program identified sources of discrepancies in laboratory results. Evidence base for decision on new methodology and antibiotic testing were developed. For instance, it is observed that calibrated dichotomous susceptibility gives greater annular radius than directed susceptibility ($P < 0.01$). **Conclusions:** Internal QC program continues to be valuable means of identifying discrepancies, and vetting new ideas. This report presents evidence base to reaffirm that the need for internal QC is ever present.

Keywords: Internal quality control, Standard operational procedures, Turnaround time

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Introduction

Quality in diagnostic pathology comprises quality assurance (QA) and quality control (QC). While QA mainly involves external activities that check the final specific results that may impact on patient outcomes, QC involves internal activities that insure diagnostic accuracy. QA is associated with reduced turnaround time for diagnosis of infection, which in turn influences cost/length of stay of patient in the hospital as well as the clinician and patient satisfaction.[1]

QA in clinical microbiology is expected to be comprehensive, covering all aspects of the preanalytical, analytical, and postanalytical phases. For instance, any clerical errors at pre- or postanalytical phase can ultimately lead to the generation of a faulty report.[2] However, external QA may not decipher between personnel or standard operational procedure (SOP) of a registered facility, but internal QC does.

QC is the bedrock of QA, because much of the timely reporting of accurate test results depends on the practice of rigorous QC.[3] The two major factors that influence QA and QC in the analytical phase of diagnostic microbiology are (1) personnel and (2) the process, materials, and methods used in the laboratory. The first refers to the competency or dexterity of individual scientific/technical staff and the second refers to the SOPs.

The author has been privileged to work in three microbiology laboratories from across three different countries and continents. Based on the hands-on experience and interaction with colleagues, it can be said

with certainty that most laboratory facilities have SOPs for their tests. It is also certain that external QA programs are now running in many countries. However, the same cannot be said about internal QC *per se*. Indeed, a survey had reported that many laboratories neither have a QC program, nor an SOP for the tests they performed.[4] This experiential technical note presents case reports on four different scenarios requiring internal QC. The objective is to provide evidence base to highlight the value of internal QC program in evaluating the effectiveness and efficiency of the processes, materials, and methods used in the laboratory and the individual scientific/technical staff.

The issue of microbiology turnaround time as part of quality with obvious benefits for clinicians and patients is not new. This is hallmarked by the development of technologies such as (i) assorted chromogenic media to enable identification of candidiasis, *Escherichia coli*, *group-B Streptococcus*, *MRSA*, and *VRE*, possibly within 24 hours; (ii) automated flow cytometry system for urine culture;[5,6] and (iii) polymerase chain reaction methods for many organisms, including *Chlamydia trachomatis, Mycobacterium tuberculosis, and Neisseria gonorrhoea*, among others.[7]

Case Reports and QC Scenarios

Some cases not reported within turnaround time

As always, there are still some cases that could not be decided and reported within the turnaround time. There are Gram-negative rods (GNRs) or Gram-positive cocci (GPC) cultures for identification that can be undecided within the turnaround time. Worthy of mention in this note on quality is a cursory observation of, albeit nondifficult, three cases that could not be reported within the turnaround time. They include *Salmonella* specie mimicking *Escherichia coli* on chromogenic agar, false-positive methicillin-resistant *Staphylococcus aureus* based on color development in the chromogenic agar plate, and GNR from (aerobic and anaerobic) blood culture bottles with inconsistent chromogenic plate presentation. All three cases share one thing (false-positive chromogenic culture growth) in common, which was identified by the SOP that requires all chromogenic cultures should be accompanied by other culture media, or a positive chromogenic culture be subcultured for verification.

Internal lab QC

Six microbiology laboratory units of a large pathology facility performed internal laboratory QC activities between February 2009 and July 2010. A senior microbiology scientist in-charge coordinated the program in an 'interlab' format. A total of ten QC cases were performed, of which four has been discretionally selected for this technical note [Table 1a and b].

Case 1

QC 1/2009 was taken from the sputum culture of a 10-year-old girl with cystic fibrosis. In addition to this isolate there was also *Stenotrophomonas maltophilia* and *Aspergillus fumigatus*. This organism did not readily identify. Because it was not a 'classical' *Pseudomonas aeruginosa*, we set up a Vitek GNI + card. The result was *Pseudomonas aeruginosa* 87%. Although with other characteristics, it appeared to be a mucoid *Pseudomonas aeruginosa*, a 20NE was set up. The 20NE gave a result of *Burkholderia cepacia* 99%. However, this isolate would not produce the typical colonies on Burkholderia agar. Three days later, we received another sputum specimen on the same patient. The second specimen on this patient also yielded the same mucoid organism. This time, the Vitek ID was *Pseudomonas aeruginosa* 99%. Although it appears that the *Shewanella* and *Burkholderia* results produced by other laboratories came from wrong API results, it was pleasing to note complete agreement on antibiotic susceptibility testing on this isolate. Based on the date of dispatch being Monday, February 16 and the receipt of reports being between February 19 and March 2, it was also noted that the turnaround times for this particular QC ranged from 2 to 13 days.

Case 2

QC 2/2009 was atypical *Streptococcus pneumoniae* taken from the eye swab of a 34-year-old woman with conjunctivitis. All laboratories correctly identified this isolate as *Streptococcus pneumoniae*. This isolate presented with poor growth and was not producing the completely typical colonial morphology. All laboratories tested and unanimously reported chloramphenicol as susceptible. Except lab 5, all other laboratories correctly identified the isolate as having reduced susceptibility to penicillin and ampicillin. Also, the reports varied considerably on the antibiotics tested and/or reported. For instance, lab 1 tested cefotaxime as resistant but did not report it; whereas labs 4 and 6 tested and reported it as susceptible. Other three laboratories did not test for cefotaxime susceptibility [Table 1b].

Case 3

QC 1/2010 was isolated at a count of >10^8/liter from mid-stream urine culture of an 81-year-old man with renal failure. All laboratories correctly identified this isolate as Enterococci as well as tested and unanimously reported vancomycin as resistant. However, the results varied a bit in 'specie' level identification and in Nitrofurantoin susceptibility [Table 1]. If antibiotic treatment is required, nitrofurantoin is very much the first option with which to treat VRE in a patient with a

Table 1: Internal lab QC reports from six microbiology units of a laboratory service showing differences in outcomes that indicate concern for quality control.
(a) Results of organism identification 33% inaccuracy

Antibiotic discs	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6
Case 1	*Pse. aer*	Would refer* *Bur. Cepacia*	Would refer* *Pse. aer*	*She. Putrefaciens*	*Pse. aer*	*Pse. aer*
Case 2‡			*Str. Pneumo*			
Case 3‡	VRE	*Ent. Faecium*	*VRE faecium*		*Ent. faecium*	VRE
Case 4†	*Str. Pneumo*		*Str. pneumo Sta. aureus*	*Sta. aureus*	No report	Loss of viability

*The reports indicated organism is queried and would be referred for confirmation; ‡Difference in antimicrobial susceptibility result; †Note that identification and reporting Staphylococcus aureus changes the picture for the doctor completely–the essence of quality in laboratory testing

Table 1: (b) Antimicrobial susceptibility result indicating disparities† in advice on antibiotic

QC case	Case 2		Case 3
Organism	*Str. Pneumonia*		*Ent. faecium* (VRE)
Antibiotic	Amp and pen	CTX	Nitrofurantoin
Lab 1	S	R‡	S
Lab 2		NT	S
Lab 3	S	NT	R
Lab 4	S	S	R
Lab 5	R	NT	S
Lab 6	S	S	R

†Note 50% disparity in Nitrofurantoin susceptibility and all six lab units failed to produce exactly the same report for any one antibiotic–the essence of quality in laboratory testing; ‡Not included in the final report to clinician; NT: Not tested

urinary tract infection. Therefore, the variation in results indicated obvious difference in effectiveness or quality regarding choice of nitrofurantoin with the strain of vancomycin-resistant *Enterococcus faecium*.

Case 4

QC 2/2010 was a *Streptococcus pneumoniae* isolate grown from the ear swab received from a 66-year-old man. The history indicated "otitis media with perforated ear drum." The susceptibility reports on *Str. pneumoniae* were accurate. However, there was considerable variation in the identification report [Table 1]. There was found to be contaminating *S. aureus* in this QC dispatch. The subcultures were prepared with a single mucoid *Str. pneumoniae* colony from a primary plate, but later realized there was *S. aureus* underneath the colony. There was also a viability problem with this isolate.

CDS vs. CD comparison

One of the reasons for performing antimicrobial susceptibility testing for patient care is to detect new antimicrobial resistance strains or trends. Therefore, generating accurate and reproducible antimicrobial susceptibility test data requires a QC program.[3] For quite some time now, the SOP on urine "culture and sens" bench has involved performing a direct susceptibility (DS) testing for any pure growth of GNR. At some point in 2010, the method of calibrated dichotomous susceptibility (CDS) was to be implemented. To determine quality, the QA officer authorized that the pathology service must perform a comparative evaluation of CDS results against the established DS protocol.

The report of this evaluation has been used to show proof of correlation and QC during the re-accreditation visit of NATA in 2011. This report being included here is to highlight the need for such evaluation, with or without any pending accreditation agenda. Overall annular radius from the DS is statistically significantly greater than in CDS ($P < 0.01$); although with the exception of ciprofloxacin and norfloxacin, the differences observed in other antibiotics did not achieve statistical significance [Table 2].

Ciprofloxacin vs. Moxifloxacin comparison

Moxifloxacin (MXF) is a third-generation synthetic fluoroquinolone chemotherapeutic agent developed by Bayer AG (initially called BAY 12-8039).[8] It is marketed worldwide as Avalox, Avelon, or Avelox for oral administration. It is also available in parenteral form for intravenous infusion as well as in an ophthalmic solution (eye drops) under the brand name Vigamox for the treatment of conjunctivitis. Of particular interest here is that it was marketed as the heir apparent to ciprofloxacin (CIP).[9]

Specifically, MXF is attributed to have a broad-spectrum antibiotic activity against GNR and GPC. Due to the notion that it is Bayer's heir apparent to CIP, there is the attendant opinion that MXF is superior to CIP both in susceptibility and in toxicity. In our laboratory at SWPS, MXF susceptibility neither has been tested nor has advice regarding it been sought by clinicians. On advice to start stocking MXF discs, permission to run a brief evaluation was verbally granted on request.

As a hypothetical base, literature indicated that MXF could be more effective than CIP against Gram-positive bacteria, but less effective against Gram-negative

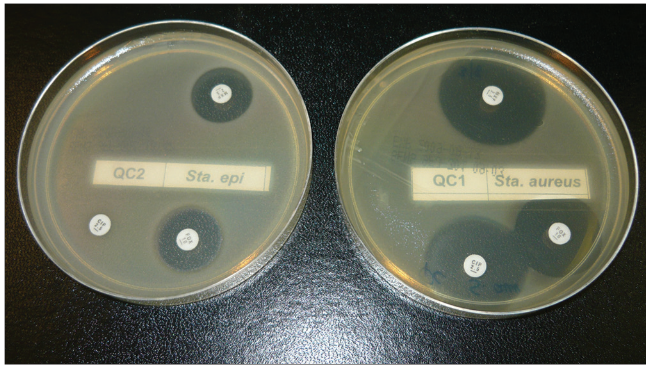

Figure 1: Susceptibility cultures showing ciprofloxacin vs. moxifloxacin comparison on different *Staph species*

bacteria;[10] although mecA-positive *Staphylococcus* species could be equally resistant to CIP and MXF.[11] On this basis, QC cultures of *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. epidermidis* were used for the evaluation. Wild strains (patients' isolates) of *Klebsiella oxythoca*, MR_CoNS, MRSA, and MRSACA were used as well [Figure 1].

On average, the comparative annular radius of CIP and MXF observed were MRSA–wild (MXF 2.0 mm, CIP 0.0 mm); and MRSACA–wild (MXF 12.3 mm, CIP 9.9 mm). Others were QC *S. aureus* (MXF 13.0 mm, CIP 11.0 mm), QC *S. epidermidis* (MXF 6.0 mm, CIP < 6.0 mm), QC *P. aeruginosa* (MXF 9.3 mm, CIP 13.1 mm), QC *E. coli* (MXF 11.7 mm, CIP 13.5 mm), and wild *K. oxythoca* (MXF 8.4 mm, CIP 10.3 mm).

Table 2: Calibrated dichotomous susceptibility vs. direct susceptibility comparative testing‡ on urinary *E. coli*– measures† of annular radius of various antibiotic discs

Antibiotic discs	Mean*	SD	Median*	DS–CDS
Ampicillin				
DS	5.08	4.56	7.50	0.37
CDS	4.71	4.24	7.40	
Augmentin				
DS	7.38	3.26	7.92	0.70
CDS	6.68	3.14	7.00	
Ceftriaxone				
DS	7.49	3.35	8.93	0.81
CDS	6.68	3.07	7.22	
Trimethoprim†				
DS	9.69	3.47	15.54	0.97
CDS	8.71	3.24	9.73	
Nitrofurantoin				
DS	7.09	2.63	8.11	0.75
CDS	6.34	2.51	6.82	
Gentamycin†				
DS	7.52	0.66	7.55	0.69
CDS	6.84	0.89	6.85	
Cefotaxime				
DS	10.80	2.69	11.44	0.91
CDS	9.85	2.64	10.11	
Norfloxacin				
DS	11.10	1.26	11.22	1.25
CDS	9.85	2.56	10.32	
Ciprofloxacin				
DS	12.40	1.35	12.31	1.33
CDS	11.10	1.58	11.12	
Sulphafurazole				
DS	8.15	3.27	8.81	1.08
CDS	7.07	2.73	8.10	
Imipenem				
DS	10.80	1.35	10.90	0.62
CDS	10.20	1.61	10.23	

‡For each case, the same urine specimen was set up for antibiotics susceptibility testing by the two separate methods. On the following day, any case that turned out to be pure *E. coli* was selected and measures of annular radius taken. †Statistical significance levels based on t-test: Two-Sample Assuming Unequal Variances: Overall: $P<0.01$; Ciprofloxacin: $P<0.005$; Norfloxacin: $P<0.05$; others: $P>0.05$.*Annular radius (mm); †'Mean–SD'>6.0 for DS and could be reported as susceptible, but not for CDS

Discussion

Some pathology management may argue that participation in external QA program (QAP) is sufficient to measure the quality of performance of the laboratory's facility, especially considering costs; but it has been opposed as untrue. For instance, one of the counter-arguments that quickly comes to mind is that QAP is a form of proficiency survey that "merely provides a snapshot in time and should not be used as a substitute for a daily QC" with an internal laboratory QC program.[12]

In the context of effectiveness and efficiency, it is common knowledge that accurate clinical microbiology report that reaches the GP late may be effective but not efficient. Imagine the "case 1" of internal lab QC. It was pleasing to note accuracy on antibiotic susceptibility testing, but the differences in turnaround times ranged from 2 to 13 days. It was determined that some labs do put QAP cases on the back burner to attend to urgent patients' cases. Thus, it must be acknowledged that external QAP report quite often shows a turnaround time that is not representative of standard SOP; and especially, the administrators of the external QAP are not evaluating turnaround time. This goes on to reaffirm that external QAP should not be used as a substitute for a daily QC whereby a laboratory can perform a self-evaluation of its effectiveness regarding turnaround times.

Further, it is pertinent to note that excessive consciousness of turnaround time without QC could be counterproductive. In this report, three albeit nondifficult cases that could not be reported within the turnaround time are mentioned. All three cases

presented with false-positive chromogenic reactions based on which inaccurate reports could have been made within the turnaround time if there were no QC measures in the SOPs. Besides cases requiring microscopic examination, these cases of cultures had "preliminary reports" such as (1) Gram-negative rods (GNRs) or Gram-positive cocci (GPC) isolated–identification and susceptibility to follow.

Cases 2-4 of the internal lab QC provide opportunities that require improvement in the analytical SOP. Of particular relevance to customer/patient outcome, for instance, conjunctivitis is often treated topically and the antibiotic used most commonly is chloramphenicol eye drops. There is a problem if the patient cannot tolerate chloramphenicol. Hence a viable alternative recommendation is good practice. Considering the internal lab QC for 'Case 3', there was a real difference in the SOPs, whereby some laboratory units read <6.0 mm annular radius for nitrofurantoin as resistant, whereas some others use 4.0 mm as cut-off. Bearing in mind that whether the specimen is urine, in which there are large amounts of the antibiotic, or swabs (respiratory, skin, vaginal, etc.,) in which certain normal flora could be considered as significant pathogen; there needs to be consistency in reports from different laboratory units going to a GP within a community. Thus, there is a need for internal lab or interlab QC program to monitor accuracy or proficiency of the SOPs.

From the observation of statistically significant difference in annular radius between DS and CDS ($P < 0.01$), the QC evaluation has at least informed the laboratory that there could be discrepancy in susceptibility test results depending on whether CDS or DS method is used. For instance, a critical evaluation of the results presented show that where 6.00 mm is the cut-off, the lower limits of trimethoprim (TMP) is susceptible by CDS (6.22 mm), but resistant or intermediate by DS (5.47 mm). Similarly, the lower limits of gentamicin (GM) is susceptible by CDS (6.86 mm), but resistant or intermediate by DS (5.95 mm) [Table 2].

In the comparison of CIP vs. MXF, it is observed that multiresistant methicillin *Staph aureus* (MRSA) were resistant to both CIP and MXF, while nonmultiresistant methicillin *Staph aureus* (MRSACA) and the tested GNRs were susceptible. Difference was observed in the annular radius with CIP presenting greater diffusion than MXF. Further difference was observed in the susceptibility of *Staphylococcus epidermidis*, which was resistant to CIP but susceptible to MXF [Figure 1]. The observations are quite in agreement with those of Duggirala *et al.*;[10] that MXF could be more effective against Gram-positive bacteria and less effective against Gram-negative bacteria relative to CIP.

Conclusion

This experiential note emphasizes the need for laboratory's self-evaluation to checkmate relative complacency and to ensure evidence-based confidence in everyday quality. It is a reminder to those microbiology laboratories that have neither internal QC programs, nor SOP for the tests they performed to rethink quality in their diagnostic practice.

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