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Quality Assurance (QA) of Qualitative serological Tests in the Clinical Microbiology Laboratory: Limitations and Solutions

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Abstract

In a clinical microbiology laboratory, accurate and reliable results can only be achieved by adhering to quality assurance and quality control protocols. This process starts from patient's sample collection, receiving, processing and final reporting. Method validation of each component is essential, and a breach may lead to faulty result. Due to high prevalence and rapid emergence of infectious diseases, the importance of rapid and reliable qualitative serological tests has increased. However, ignorance and noncompliance to quality assurance process by the laboratories, especially in low resource settings leads to inappropriate diagnosis which leads to wrong interpretation and hence inappropriate treatment and ultimately poor prognosis.

Key words
Method validation, quality assurance, quality control

The paradigm shift of laboratory medicine's role from specimen-centered clinical testing toward patient-centered clinical decision making has helped significantly in improving patient's outcome. The claim that laboratory diagnoses the contribute in clinical assessment can only be reliable if it is appropriately ordered, conducted, returned with results on a timely basis, correctly interpreted and affect a decision for further diagnosis and treatment. Medical laboratory Quality assurance (QA) plan ensures that the entire processes of any test are monitored at every step and the results generated are accurate, reliable, timely and reproducible. Results of In-vitro diagnostic (IVD) could be unreliable in the absence or deviance from QA plan. This could have negative consequences including unnecessary treatment, treatment complications, failure to provide the proper treatment, delay in correct diagnosis, additional and unnecessary diagnostic testing. As IVD cost has influence on health care expenditures, the issues consequently cause increased cost, time and personnel effort, and often in poor patient outcomes.

Communicable disease has emerged as significant cause of morbidity and mortality globally.[1] Research and development in IVD for reliable diagnosis of infectious diseases, has started its journey from conventional microbial culture along with serological techniques and now complex molecular methods.[2]

In diagnostic laboratories the infectious disease serological techniques are typically use for non-cultureable microorganism e.g. viruses, difficult to cultivate bacteria like *Treponema pallidum*, *Helicobacter pylori* and parasites. Some time it also helps in therapeutic monitoring. As a rule of thumb, serological methods also require vigilant quality assurance processes for generating reliable laboratory results. Those clinical laboratories which are perform infectious disease serological tests have questionable reliability due to their noncompliance with the quality practices. Quality assurance recommendations, e.g. College of American Pathologist and ISO 15189 are followed by few laboratories in Pakistan.[3,4]

The non-conformities with the quality standards are as follows:

1. Laboratories do not update the test methodologies in view with the updated recommended diagnostic guidelines.
2. The prefer to use low cost diagnostic kits which are generally not approved any regulatory authority (e.g. FDA, WHO, CE mark)
3. Knowledge gaps and limited application of total quality assurance process by technical staff.
4. Considering quality control as financial liability, generally the laboratory managements do not show commitment to put these extra monitory inputs.

Infectious diseases serological tests are qualitative and quantitative. Before introducing any qualitative tests in the laboratory, a vigorous quality assurance process allows a complete evaluation of the kit and the related method. The components of quality assurance process are as follows that must be followed prior to initiate qualitative serological tests.

Quality Assurance Process:[5-7]

A. **Pre-analytical component**

1. Sample collection: Appropriate container according to the required test is essential.
2. Sample transportation: It must be abided by recommended

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condition i.e. temperature. In case of delay, addition of preservative or refrigeration according to the manufacturer's recommendations.

3. Sample processing: Before performing the test, the quality of specimen must be assessed visually for hemolysis and turbidity.

B. **Analytical component**

Following are the analytical components

1. Method validation
2. Quality control
3. Equipment maintenance
4. Lot to lot verification
5. External quality assessment

1. Method validation

Validation and verification of process is key process in analytical component.

- **Validation**

Process of proving that a procedure, process, system, equipment, or method used works as expected and achieves the intended results. It includes calculation of accuracy, precision, specificity, sensitivity, positive and negative predictive value. These processes must repeat in case of change in method or manufacturer.

- **Verification**

Process of confirmation by examination and provision of objective evidence that specified requirements have been fulfilled.[8]

Following points must be considered before starting validation process:

- Thorough literature review and market search for authentic method and kit
- Select kits of reliable manufacturer approved by FDA or CE mark.
- Avoid kits which are intended for research purposes only.

Validation and verification process must start after reliable kit selection

Validation Process

It requires positive and negative control samples. In case of non-availability of controls, proficiency tests samples and well characterized positive patient sample can be used for this purpose. Well characterized samples are those that correlate with patients' clinical details and must be verified by reference laboratory. Twenty positive and negative samples can be used for FDA approved kits. However, for unapproved kits large sample size is required (e.g. > 100).[9]

2. Quality Control

Frequency and sample for quality control is very important. Commercial controls are available however positive patient sample can use as in-house control. These controls must run daily before start testing. Kits with internal controls should also

Summary of Quality assurance steps for laboratory bases tests (9)

Components	Definition	Calculation
Accuracy	How much is the given method accurate to generate the result	*A= No. of correct results/ total no. of results x 100*
Precision	Can we get the same result upon repeat testing on same sample numerous times at different days by different individual under the same operating conditions	*P= No. of repeated results in agreement/ total no. of results x 100*
Specificity	Ability of method to detect only the analyte in the presence of other factors	*Sp = No. of true negative results/ (No. of true negative results + No. of false positive results) x 100*
Sensitivity	Ability of method to detect smallest quantity of analyte	Se = No. of true positive results / (No. of true positive results + No. of false negative results) x 100
Positive Predictive Value	Check probability of the presence of an analyte in a specimen	*PPV=No. of true positive results / (No. of true positive results + No. of false positive results) x 100*
Negative Predictive Value	Check probability of the absence of an analyte in a specimen.	*NPV=No. of true negative results / (No. of true negative results + No. of false negative results) x 100*

be monitored vigilantly with each test.

Results should be documented in designated forms and must review daily by bench in charges and monthly by laboratory manager.

In case of erroneous quality control results, do not hold patient testing. Repeat with alternate kit (same manufacturer and lot or different lot). If problem persists, perform root cause analysis. Check the temperature and conditions of the storage area. The storage condition of the associated reagents must also be evaluated.

3. Equipment maintenance

Equipment maintenance schedule as daily, monthly, annually. Check manufacture's recommendation also. Yearly maintenance schedule for each instrument with frequency and designated person must maintain by manager.

Instrument calibration should perform before initiating clinical sample testing.

Coordinate with biomedical department for any erroneous instruments. For placed equipment, there should be clear documentation in contract regarding maintenance responsibilities.

4. Lot-to-Lot verification

New lot of kit must be verified before testing patient sample. New shipment requires verification

5. External assessment

External Quality assurance scheme is an important quality assessment tool.[10]

C. Post-analytical

1. Review of results

Results must review and correlate with other tests before final result.

2. Audits

Internal and external audits are performed at regular intervals to ensure compliance

3. Reference ranges

Authentic and recent reference ranges must be provided for result interpretation.

Conclusion

Adherence to pre analytical, analytical and post analytical component is essential for reliable result. Laboratories must develop their quality assurance plan; collaborate with laboratories that follow authentic quality assurance protocol

References

1. World Health Organisation. Cause-specific mortality and morbidity, Table 2 2009. Available from: http://www.who.int/whosis/whostat/EN_WHS09_Table2.pdf.
2. Caliendo AM, Gilbert DN, Ginocchio CC, Hanson KE, May L, Quinn TC, *et al*. Better tests, better care: improved diagnostics for infectious diseases. *Clin Infec Dis* 2013;57(suppl_3):S139-S70.
3. Council PNA. Medical Labs Accreditation 2016. Available from: http://pnac.org.pk/medical-laboratories-accreditation-iso-15189/.
4. Pathologist COA. 2018. Available from: http://www.cap.org/apps/cap.portal?_nfpb=true&ALSearch_3_1_actionOverride=%2Fportlets%2Faccrlabsearch%2Faccrlabsearchpgflow%2Fprocess_searchcriteria&_windowLabel=ALSearch_3_1&_pageLabel=accrlabsearch_page.
5. Westgard J. Basic Method Validation 2009. Available from: https://www.westgard.com/basic-method-validation.htm.
6. Garrett PE, Lasky FD, Meier KL. User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline: CLSI; 2008.
7. Tholen D, Berte L, Cooper W, Gun-Munro J, Noble M. Using proficiency testing to improve the clinical laboratory: Approved guideline—. Clinical Laboratory Standards Institute. 2007;27:1-41.
8. Horowitz GL, Altaie S, Boyd JC. Defining, establishing, and verifying reference intervals in the clinical laboratory; approved guideline: CLSI; 2010.
9. Klee GG, Westgard JO. 7 Quality Management. Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics-E-Book. 2014:90.
10. UK NEQAS. International quality expertise microbiology 2013. Available from: http://www.ukneqasmicro.org.uk/.