Laboratory Support of Global Health Research

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Laboratory generated data are used in support of several types of global health research. Routinely obtained clinical diagnostic data are used for disease surveillance, epidemiologic analysis of frequencies and trends, health outcomes research, and sponsored research projects. Clinical data from research laboratories is also collected in support of sponsored research projects. Whether the initial purpose of the testing is in support of research protocols or the data are retrospectively reviewed, the quality of the laboratory data is essential to drawing correct conclusions. The types and use of data generated by on-site, routine diagnostic, research diagnostic and basic science laboratories will be described, with a focus on quality-related issues. Full integration of laboratory management as a partner is essential to successful research planning and execution.

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During the last century, the development of medical laboratory services has resulted in substantial improvements in clinical care by identifying sources of infection quickly and accurately, measuring safety and effectiveness of treatments, and establishing biomarkers of chronic disease. Exponential advances in technology over the last two decades have furthered the ability of laboratories to be integral partners in high quality medical care. In addition to the direct impact of laboratory science on patient care, data generated by laboratories have been used for many epidemiologic and program assessment purposes.[1,2] Data used for monitoring case rates for most notifiable diseases are generated and reported to public health agencies by routine clinical laboratories. Changes in inpatient isolation practices are evaluated for success using laboratory data regarding nosocomial infections. Thus, health outcomes research relies heavily on clinical laboratory data. However, prior to being used as routine diagnostic assays in a clinical laboratory, new diagnostic methods are developed through a process that involves basic science laboratories and research-oriented diagnostic laboratories. The latter are often reference laboratories that perform specialized assays that are not yet suitable for application in routine clinical laboratory settings.

Of the numerous types of laboratories that exist, this paper will be restricted to a discussion of biomedical laboratories, and will still fall short of covering all of the possible settings and situations that may be found around the world. While the HIV pandemic has been devastating on a global level, and particularly so in resource constrained settings, one of the positive outcomes of the response to this disease has been a global trend in improved laboratory quality and access for traditionally underserved populations. This natural, and in the case of HIV, accelerated, developmental continuum of laboratory capacity is the focus of this paper. The description presented here focuses on four basic laboratory types, although it will quickly become apparent that there is significant overlap and blurred distinctions among these laboratory types. The work performed is often inter-related, and often contained in separate sections within one large laboratory; the highest success results from recognizing the unique strengths of each of the laboratories to achieve the common goal of improving the health of the population. Whether this work is performed under the auspices of routine care or research becomes somewhat irrelevant when all patients benefit from the process.

The four basic laboratories discussed here are 1) Site Labs, 2) Clinical Diagnostic Labs, 3) Research Clinical Labs, and 4) Basic Science Labs. Any of these except the site labs may be remotely located and centralized to serve a greater area than the site lab, which by definition is located on the clinical premises. Site labs routinely perform phlebotomy and any point-of-care (POC) tests for which the resources are available. These laboratories often process and store samples for transport to centralized laboratories for additional testing. Clinical diagnostic laboratories, which may be located on the site of the clinical services (e.g. within a hospital) or may be remotely located (e.g. at a larger hospital), have access to more analytic equipment and resources, such as culture capacity. Research clinical labs are situated as reference laboratories that provide some routine clinical services, as well as cutting edge diagnostics that are not available elsewhere (e.g. molecular diagnostics or strain typing). Basic science laboratories are routinely housed in academic or governmental research institutions.

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These laboratories utilize highly specialized equipment to perform experiments that will eventually result in the development of assays with diagnostic applications. Each of these laboratory types will be described in greater detail below and the role that they play in global health research will be elucidated. References provide additional examples of the concepts that are described here. Good Laboratory Practice (GLP) is a process that has been widely disseminated in many resource constrained settings and has had a positive impact on quality improvement and the development of quality management systems.[1] A general theme of the discussion presented here is the quality of the laboratory output: why quality matters and how to maximize it.

SITE LABORATORIES

The site laboratory is often overlooked in the context of planning laboratory capacity building. However, the site laboratory may be one of the most important features of overall laboratory support. This laboratory has the responsibility for sample collection, initial processing if required, storage, and shipment of specimens that will be sent to a central or reference laboratory. The quality of the laboratory test result is only as good as the quality of the specimen and this is controlled by the site lab. The central laboratory may be required to reject samples due to improper labeling, insufficient blood-draw volume, improper storage, or breakage/leakage during transport. Specimen rejections ultimately result in missing laboratory data for patients, study participants, or retrospective data analyses. Often this requires an additional clinic visit for patients, which may cause serious hardship in many settings. These issues are avoidable when site laboratories are well trained in GLP and standard operating procedures (SOPs) are in place and adhered to in all areas of the site lab.

An example of the impact of the site lab on the quality of testing that occurs frequently is the use of syringes to draw blood and then transfer of the sample to a vacutainer tube. This practice can result in shearing, which destroys cells in the sample. However, on receipt in a remote central laboratory, there is no external evidence that the sample may be inadequate. As a result, the specimen is tested and the results entered into the patient record without any indication that it may be a poor quality result. In settings with a high level of engagement with the site laboratory, vacutainer blood draw systems have been provided and SOPs describing appropriate blood-draw procedures have been instituted. These activities have often been prompted by the requirements of a research protocol, but long-term adherence to these procedures provides improved quality of laboratory testing for all patients that utilize these site clinics. This is an example of the unintended, but very real benefits of research projects to the larger population in the area.

In addition to specimen collection and handling, the site laboratory often performs POC testing such as rapid HIV, tuberculosis (TB) smears, pregnancy tests, hemoglobin levels, etc.[3,4] POC tests are designed for use in settings where a full laboratory is not available, and in fact may be used by non-laboratory personnel such as clinicians and counselors. The data generated by these tests are part of the patient medical record and, in the case of research projects, become part of the study documents. Therefore, the accuracy of results is critical for patient management, but also for future use in monitoring trends in test results.

Diseases that have multiple methods of diagnostics are particularly relevant for consideration of this issue. For example, a retrospective review of the frequency of TB smear positive patients will rely on data generated at site labs and may be used to inform program and policy decisions at local, national or international levels. However, the quality of the smear results depends on the quality of the staining, both reagents and adherence to SOPs, and the quality of the microscopy. The former can, and should be assessed via external quality audits of the site labs. The quality of the microscopy can be assured by engagement in training and in subsequent review of slides to confirm the accuracy of the results obtained by the microscopist. A review of 10 % of the slides read at a site lab can provide feedback that can be used to improved diagnostic quality. Finally, sputum samples can be sent to a reference lab for testing using improved technologies such as MGIT culture or molecular testing for TB. In Eldoret, Kenya, the AMPATH Reference Laboratory (ARL) has accepted the charge to provide external review and quality assurance oversight for site labs in the region that provide data to the national TB control program. Thus, technologies that were instituted for use in support of research projects have been instrumental in improving the quality of the data that are used by the national TB control programs regarding the distribution of this disease.

In many research protocols, the results of POC tests may be used to determine participant inclusion into a specific study. For example, a study may be recruiting only HIV-negative participants for longitudinal follow-up to assess HIV incidence in an at-risk population. Inaccurate results may lead to inappropriate enrollment (in the case of false negative results) or lost opportunities to identify potential participants (in the event of false positive results). In summary, the quality of site lab data generated for patient care, in support of research protocols, and utilized for epidemiologic and policy purposes is as critical as any testing performed in a central laboratory. Inclusion of site lab personnel as active partners in research and in all GLP training is an essential component of continuous quality improvement that will benefit both patients and researchers.

CLINICAL DIAGNOSTIC LABORATORIES

Samples requiring testing beyond the capacity of the site labs will be transported to a central laboratory. There are two possible types of laboratory that may serve in this role:

the clinical diagnostic laboratory and the reference (or research) laboratory. These may be entirely separate facilities or separate sections within a large central laboratory. While the distinction may not be apparent to the end user, these laboratories serve quite different functions and their roles regarding global health research are distinct as well. The clinical diagnostic laboratory performs tests for routine healthcare needs and does not deviate from routine. All SOPs are followed regardless of the status of the patient as a patient or a research participant. Adherence to GLP is the responsibility of the parent organization (e.g. hospital or governmental laboratories) and systems in place are not generally amenable to change in response to needs of research investigators. This laboratory uses only recognized diagnostic methodologies and assays; those with appropriate regulatory approval (e.g. FDA approval in the US) where possible. For example, a routine clinical laboratory may perform flow cytometry for CD4 analysis, while specialized staining for markers of lymphatic illnesses would use the same instrumentation, but would be performed in a research laboratory. Additionally, if a research protocol required CD8 and CD3, although these are commercially available tests with regulatory approval, if they are not routinely offered by the clinical diagnostic lab, they would need to be performed in a research laboratory.

As with data generated by the site lab, data generated by the clinical diagnostic laboratory is essential to surveillance, basic epidemiology and health outcomes research. However, it is important to recognize that these data may not be comparable across sites or over time due to advancements in diagnostic methodologies and changes in reporting practices such as use of standardized units. Many examples of the effect of different technologies exist, including CD4 testing. The CD4 value is used to determine when to start, or potentially change, therapy for HIV-positive patients. These data are often collected from multiple sites to compare health outcomes of persons living with HIV. However, data obtained from the FACSCount system will consistently vary from data obtained using the FACSCaliber system. This difference should be noted during analysis, but this is possible only when the data captured include the methodology used to generate the CD4 values.

Another common change in data includes lab test names (e.g. the change from serum glutamic-oxaloacetic transaminase (SGOT) to aspartate transaminase (AST)). While this change is well recognized by laboratorians and clinicians, and the terms are often used interchangeably on request forms, it is critical that the data management group of the organization be kept informed of changes such as this. Analysis of longitudinal data will require that data previously labeled as SGOT be merged with data now labeled as AST. This minor nominal change exemplifies the need for continuous interaction among clinicians, laboratorians and data management groups. This is particularly relevant for data obtained from routine clinical labs who may not think to inform outside research groups of any internal changes in their routine practices.

It is also important to recognize that even if names or units do not change, increases in test accuracy may influence epidemiological analyses of trends over time. An example of this is easily seen with HIV viral load data. Below detectable limit (BDL) used to indicate a value less than 400 copies/ml, but with improved assays, this is now used for samples with less than 50 copies/ml and actual numeric values between 51 and 399 copies/ml may be recorded. Thus over time, the meaning of BDL has changed so that an evaluation of the proportion of patients on anti-retroviral therapy (ART) who are not BDL, a poor clinical outcome, may actually increase even though patient outcomes remain stable over time. Such artifactual changes in epidemiologic measures occur in all settings; however, the rapid increase in technologic capacity and exponential improvement in laboratory services that has resulted from the response to the HIV pandemic accelerates the frequency of this issue. Health outcomes researchers and surveillance monitors must work closely with laboratories from which they receive data in order to successfully track and account for technology changes that impact case finding or other health parameters.

The clinical diagnostic laboratory serves a critical function in routine healthcare and generates data that may be used in support of research protocols. The current challenge for clinical diagnostic laboratories in resource constrained settings, and for anyone who utilizes these data for research, is to manage the quality of the testing and data being generated.[5] Too few of these laboratories receive sufficient support to empower them to fully adopt GLP. This support requires institutional will as manifested by financial, manpower, training and technical resources. As will be described below, research laboratories often have access to international resources for training, proficiency testing panels and access to external quality assurance programs. However, engagement in quality management activities is often cost prohibitive for routine clinical diagnostic laboratories. The current "trickle-down" philosophy suggesting that improving the quality of the research laboratory will enhance the quality of the clinical diagnostic laboratory has not proven to be a successful strategy. Given the critical role of the laboratories in patient care decisions and in generating data used for health outcomes research and surveillance, additional effort and resources should be directed to supporting the work of these essential laboratories by engaging high-level institutional management. Engagement with hospital administrators and Ministry of Health officials to achieve consensus support is essential to the redirection of resources needed to ensure the highest quality laboratory testing possible.

RESEARCH CLINICAL LABORATORIES

The research clinical laboratory, or reference laboratory, may be a section within a clinical diagnostic laboratory or a

stand-alone laboratory under the management of a research group or non-governmental organization (NGO) rather than part of a hospital. These laboratories often perform tests in support of specific research protocols that are commercially available but may not be in routine use for patient care in resource constrained settings as mentioned with the CD3/CD8 example above. Additional examples include Cryptococcus culture, Hepatitis C antibody testing, and molecular diagnostics for sexually transmitted infections such as *Chlamydia trachomatis* or Human Papilloma Virus. In many cases, these assays are adopted in support of specific protocols, but demonstration of their utility in the local patient population may result in adoption of these assays in the routine diagnostic lab.[6–8] Thus, over time, the lab responsible for performance of certain assays will shift from the research laboratory to the clinical diagnostic lab.[9] The shift in responsibility for assay performance may also result in a shift in the data management or data capture systems and researchers utilizing data must be aware of these possible changes.

In addition to commercially available diagnostics, the research laboratory may also perform testing that has more recently been developed and is currently considered for research use only. It is common for the research laboratories to be involved in evaluations of these new technologies compared to existing assays, or clinical diagnosis, in order to provide clinical evidence of the utility of the new assays. This type of research is an essential step in the process of making newer diagnostics available to clinical diagnostic laboratories and can only be performed in settings with sufficient prevalence of disease to allow comparison of the two diagnostic methods. Again, the long-term strategy is to demonstrate the performance characteristics of the new assays and to eventually make them available to all patients through the clinical diagnostic laboratory.[10]

As a result of their direct role in support of research projects, the research diagnostic laboratories are expected to adhere to international standards of laboratory quality management. The research protocols bring the resources necessary to enable the laboratory to participate in training programs (often international trainings), proficiency testing, external quality assurance programs and laboratory accreditation programs. The International Standards Organization (ISO) has created criteria [ISO-15189] against which laboratories are measured to determine the level of adherence to GLP (see http://global.ihs.com/ for additional information). However, meeting the criteria requires commitment of personnel and substantial expenditures on preventative maintenance, equipment calibration, and quality controls that are often prohibitive in the absence of external support by research groups or NGOs. Research laboratories with which I have worked in Kenya, Uganda and Zimbabwe have all achieved this high standard of performance, but at the cost of having approximately 10 % of their total FTE dedicated to quality management and performing no bench work. This represents a substantial outlay in salary support that is only available in settings with financial support from research projects. Thus, the research diagnostic laboratory must become a resource for clinical diagnostic laboratories and site laboratories providing training and access to materials and equipment. The research diagnostic laboratory must also become engaged in collection and distribution of materials to be used for external quality assurance to laboratories that cannot access these materials from commercial sources.

The ARL in Eldoret, Kenya, as the only Kenyan laboratory to have ISO 15189 accreditation, has been asked by the Kenyan Ministry of Health to assist the governmental effort of overall laboratory quality improvement at every level. ARL serves as a reference laboratory to provide high complexity testing for sites with less capacity. They also serve as a training site bringing in laboratory staff from the region to sessions teaching GLP and quality improvement to lab technologists who can then server as local trainers with the supporting documents provided by ARL. Finally, they are providing regional laboratories with panels of characterized samples to be used for verification of new assays and for external quality assessment. When these activities are fully embraced by funders and donors, the resulting improvement in quality of laboratory results at every level provides, in addition to improved patient management, an improvement in the data that are used for health outcomes research and epidemiologic surveillance.

BASIC SCIENCE LABORATORIES

The last type of laboratory to be discussed, the basic science lab, may seem incongruous with diagnostic laboratories, but in fact, all diagnostic laboratory assays are based on advancement in basic science labs. These laboratories are critical to further development of diagnostic sciences and an improved understanding of the role these labs play in global health research is useful. Basic science laboratories encompass a wide range of activities and the discussion here is related to those labs engaged in translational research that is intended to improve our understanding of pathogens or disease processes in order to eventually improve our ability to manage these diseases. Basic science laboratories that focus on identifying proteins or genes that are expressed by organisms or human hosts as part of a disease process may identify targets for new diagnostics, therapies or vaccines.

An example of the development of testing beginning in basic science labs is ART resistance testing in HIV strains. Basic science laboratories identified sequences in the HIV genome that are related to ART resistance (genotyping) and described the in vitro culture characteristics of virus that are resistant to various treatments (phenotyping). As more basic science research has been performed, certain co-receptors have been identified that affect disease outcomes (tissue tropism). Once identified and clearly demonstrated to be reliable biomarkers of future health outcomes, tests to determine the viral genotype, phenotype and co-receptor tropism were developed. The

process of demonstrating the reliability of the biomarkers necessarily begins in the basic science lab and requires samples obtained from patients with known clinical characteristics. Thus, the basic science lab often utilizes clinical samples to demonstrate proof-of-concept and feasibility. Assays may remain as laboratory developed assays (LDAs), or they may be developed by industry and obtain regulatory approval. LDAs or highly technical assays with regulatory approval such as these HIV resistance assays are then adopted by the research diagnostic lab, where they are used in support of research projects that require advanced technologies. As assays or technology advances and the level of technical expertise required to perform testing reliably improves, the diagnostic testing moves to the clinical diagnostic laboratory and becomes available for all patients.[11] This process can clearly be demonstrated with the advance in diagnostic technologies related to CD4 testing. Thirty years ago, flow cytometry using fluorescent antibodies was a technically complex, specialized research tool that was only available in reference laboratories that had adopted the technology from basic science researchers. Today, this testing can be performed routinely in clinical diagnostic laboratories. The technology is quickly advancing to the point where this will soon become a POC test that can be offered in any clinical setting with immediate and accurate results.

Obviously, the basic science laboratory is essential to this developmental process. However, it should be noted that basic science laboratories are not subject to the same regulatory oversight as diagnostic laboratories and as a result, the quality management may be significantly reduced. In a basic science laboratory, the goal is to provide a definitive proof-of-concept and test a hypothesis regarding the utility of the assay. The objective should never be to generate data that is suitable for patient management decisions. This distinction is critical and often difficult to make during the phase of transfer from proof-of-concept to clinical evaluation. However, the control of all aspects of testing from sample collection, adherence to routine SOPs, preventative maintenance and calibration, to data management is critical for maintaining patient safety and quality assurance. This level of control, if rarely possible in a basic laboratory setting as a result of the infrastructure required to ensure that quality management, is in place at all times. Researchers interested in assessing new technologies that are currently only available in basic science laboratories should do so in partnership with the staff of both the basic science and research diagnostic laboratories to facilitate the eventual hand-off from one group to the other.

SUMMARY

Different laboratory types, or in some settings sections, all play an essential role in generating data that are used for a variety of types of global health research. The unique function and features of each of these laboratories should be recognized and understood by any researcher who plans to use these data. Reliable analysis must depend on quality data, some of which is generated specifically for research projects and can be carefully controlled and some of which is generated in the process of routine healthcare. Researchers must understand the structure of the laboratory data and factors that may affect that structure over time. As laboratory technologies and capacity improve globally, the impact on data structure will continue to be seen in changing trends in case finding and prevalence rates.

We have an opportunity now to reflect on the challenges and the vast potential for growth that await us in the near future. Laboratories in resource constrained settings will continue to develop and adopt automation and electronic data handling solutions to routine laboratory diagnostics and the capacity for high complexity testing in reference laboratories will expand as well. We should use this opportunity to plan strategically in terms of the best method for electronically sharing data generated in the future. By laying the groundwork and establishing common data sharing methods, we can increase the capacity for epidemiologic monitoring of the diseases we are currently monitoring as well as emerging issues as they develop.

The example of HIV viral loads mentioned above provides an excellent study in how laboratory results change in response to changing technology, how we share those results outside of the local setting and how we can plan for future changes. As more sensitive methods of detecting viral load, once the exclusive purview of the research clinical lab, became routinely available in the clinical diagnostic laboratory, there has been a shift in the meaning of the term "below detectable limits" of approximately 1 log order of magnitude. Should electronic medical records be changed to reflect that the new cut-off is much lower than the previous value? Is there clinical relevance to the change? Should we be using copies/mL or standard units (log_{10}/ml) so we can share our data internationally? If a person was tested 2 years ago and had a BDL result, but now has a result of 100 copies/mL, is a change in clinical outcomes? The answer to the latter question is likely "No"; they are probably identical results. What will be the detectable limit 2 years from now? All of these questions illustrate the need for teams composed of basic and clinical research scientists (who may be able to help us see what is in development), laboratory management (from all types of diagnostic labs), informaticians and data managers, and physicians to work continuously to anticipate and adapt to the changing landscape of laboratory technology.

Now and into the future, the overall quality of all diagnostic laboratory results should be of primary concern not only as it affects specific research projects, but, more

importantly, as it affects patient care and epidemiologic analyses of trends across locations or over time. The resources necessary to focus on quality management in resource-constrained settings often come from participation in international research projects that are attempting to reduce between site variations. We must actively take advantage of the opportunities provided by engagement in these projects to not only improve the quality of our research diagnostic laboratories, but also to develop their capacity to serve as resources for local laboratories that do not have access to research funding streams.[12,13] Fully integrating laboratory management as a full partner in the research process provides the opportunity to coordinate these improvement efforts for all laboratories in a region. As a result, all patients throughout the region have the potential to benefit from research activities even if they are not study participants, and we have the opportunity to leave a benefit for the entire population that lasts beyond the duration of any single research protocol.

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