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Integration of Specialized Research Services into Clinical Laboratory Operations

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INTRODUCTION

There are clear opportunities for clinical laboratories to play a role in the development and implementation of clinical research protocols that go beyond the provision of routine diagnostic laboratory services. This is accomplished most successfully when the clinical laboratory works as a partner in the clinical research mission by translating new concepts into patient applicable testing, developing expertise in areas that complement the special needs of research protocols and maintaining an academic focus. These activities must be balanced against the strong economic pressures facing clinical laboratories.

We will develop the concepts that have been applied within the Department of Laboratory Medicine (DLM) in the NIH Clinical Center that have enabled our group to provide critical laboratory resources that support the clinical research mission of the NIH. The Clinical Center is the hospital component of the NIH intramural research program and supports clinical protocols from each of the

various NIH institutes. The Clinical Center is a 240-bed hospital and patients are admitted exclusively under clinical research protocols. DLM has been in operation since 1955 shortly after the opening of the Clinical Center and operates in many ways like a routine clinical laboratory supporting patient care, but it has a co-equal mission of supporting clinical research. As a consequence of the latter mission, DLM has developed many unique programs to support this second mission. Some of the operational issues related to funding of DLM, which is provided through an annual Congressional budget, may not be applicable to laboratories that charge a fee for service and are mandated to operate with a net profit; however, many of the other issues that we address will be applicable to clinical laboratories in academic medical centers.

One of the major challenges in a clinical laboratory participating as a partner in clinical research is to understand that the distinction between routine service and research activities at times may be blurred. This is particularly evident

when staff members devote professional time and effort not only to the development of new testing methodologies, but also to the critical assessment of currently available techniques applied in unique ways in the clinical research setting. The key to success in collaborative studies with clinical research colleagues, in our operation, has revolved around the capacity to demonstrate an in-depth knowledge of the applications of diagnostic methods, a track record in developing new diagnostic techniques, and the flexibility to integrate research into routine diagnostic testing.

SPECIALIZED LABORATORY SERVICES DEVELOPED FOR CLINICAL RESEARCH

A list of some of the common steps in the delivery of clinical laboratory services is shown in Table 40-1, along with examples on how DLM has created specialized services to support clinical research. The following is a general discussion of how specialized research services can be implemented into a routine clinical laboratory.

Clinical Protocol Development

Like most institutions, the principal investigator at the NIH ultimately is responsible for clinical protocol development, and approval is granted by the institutional review boards within each institute. Members of the senior staff within DLM provide assistance in deciding optimum utilization of existing clinical laboratory tests and the feasibility of developing new diagnostic or other specialized tests to support a research protocol. Guidance often is

offered in the selection of preferred tests when multiple tests could possibly be used. Issues related to the frequency of testing often are discussed also in terms of what is feasible for the clinical laboratory to perform, and also based on the likely rate of change of any given laboratory test. The development of a new test, such as a technique to detect a specific analyte or infectious agent, is determined by the feasibility of having an outside laboratory perform the test versus the technical expertise and resources available within DLM. An advantage of new test development initially focused on a research question is the potential for the future integration of this test into the menu offered for routine patient care. The chief of DLM and/or relevant senior staff members also review all newly approved protocols. This provides an opportunity to determine the impact a new protocol may have on clinical laboratory services and also allows another opportunity for the clinical laboratory to provide advice on laboratory testing in clinical protocols.

Test Ordering

Most laboratory information systems and/or hospital information systems are suitable for providing a pathway for test ordering within clinical research protocols. However, to accommodate the testing of stored samples obtained previously from research subjects, a procedure was developed for the investigator to order tests in this setting with the inclusion of the collection date and generation of sample identifiers. There are many other issues to consider in the context of how to best integrate research samples into the regular workload of patient samples. A major issue is what tests should be offered in the regular test menu. Over 400 tests are available to be ordered from DLM via the standard hospital information system ordering system at the NIH. All clinical laboratory tests used in routine patient care are available as well as many specialized tests, which often are used to support research objectives. Over 90% of standard laboratory test volume is done within DLM, with the remainder sent out to various reference laboratories primarily based on low frequency of test requests. Testing not presently available as part of our menu that is requested based on an emerging need is handled by consultation with a senior staff member of DLM. When the requested test is used for patient care, it is managed and funded by DLM, but when used primarily for research DLM only assists the researchers in finding an appropriate testing site. Alternatively, under these circumstances DLM may utilize its onsite testing resources to develop new tests as part of a research collaboration between one of the DLM senior staff and the principal investigator. This may involve performing a new test that has become available on an existing analyzer in the laboratory (but currently not

TABLE 40-1 Common Steps in Clinical Laboratory Services and Accommodations for Clinical Research Studies

Step	Accommodation
Clinical protocol development	Review and provide guidance
Test ordering	Ordering pathway for stored samples, special approval pathway for unusual and/or emerging tests
Sample collection and processing	Collection and delivery of research samples, system for monitoring total blood volume drawn for clinical and research testing
Test analysis	Offer wide variety of testing, maintaining parity of testing, alternative pathways for performing research testing
Test reporting	Data management software, data repository, website to disseminate test information

offered) or in some cases this may require the development of a new test method within DLM. Tracking specialized test development for research protocols is an important aspect in identifying how the translational research resources of the clinical laboratory are being utilized in support of specific clinical research protocols among various institutes of the NIH.

Sample Collection and Processing

Sample collection at the NIH for specimens to be run in the clinical laboratory is done by a trained phlebotomy team, as well as clinical care personnel including nurses and physicians. The unique aspects of sample collection and processing for clinical research relates to proper management (specific collection devices, temperature management, etc.) and distribution, as well as tracking blood volume associated with research testing. Although this process generally follows the normal pathway of sample collection, a specialized HIS ordering process has been developed specifically for research testing. This system includes options for type and number of collection vessels, specific instructions in terms of handling the sample(s) and contact information for sample pick-up. Because of workload and space constraints, DLM does not provide processing of patient samples obtained exclusively for research lab testing and also does not provide long-term storage for these samples, which is typically managed by the principal investigator. The exception to this approach is found in the microbiology group that manages long-term storage of selected organisms for later use, and this has proven to be an extraordinarily powerful tool in translational projects within microbiology. DLM does have an IRB-approved protocol that allows anonymized leftover samples (i.e., those that are beyond our routine seven-day holding period) to be accessed by the laboratory for new test development or validation, and by the principal investigator for the protocol under which the patient was admitted.

The maximum amount of blood drawn per unit time from each patient is stipulated in each clinical protocol. With the development of the specialized research lab test ordering program in the HIS, it is now possible to accurately track the total blood withdrawn based on the samples obtained for both clinical and research testing during the defined time unit.

TESTING APPROACHES USED IN SUPPORT OF CLINICAL RESEARCH

The major impact of clinical research on the clinical laboratory at the NIH relates to the wide variety of testing necessary to support the research mission, and the specialized services that may be unique to individual

protocols. In the examples below, we discuss general issues related to test analysis.

The Use of Routine Instrumentation to Support Clinical Research

Instruments that are used in clinical laboratories for patient-based diagnostic testing, including common clinical chemistry and hematology tests, readily can perform the majority of testing in support of clinical research. Immunoassay tests for a wide variety of hormones, drugs, and tumor markers often are needed also, but because of advances in this area, it can now be readily done with just a limited number of analyzers. Generally most of the research samples are analyzed along with the routine clinical samples, because it is the least disruptive for the operation of the laboratory. Occasionally, the laboratory will process samples in a batch when these have been previously collected and stored, and or when an investigator wants to reduce inter-assay variability.

One issue that often arises in the use of routine diagnostic tests is the parity between results of different assays. Problems can arise when patients are admitted to a protocol based on an assay result done by a different clinical laboratory that show poor correlation with the result performed at the study institution. The lack of harmonization of clinical laboratory results is one of the major problems in the field of diagnostic testing.¹ Efforts by several international organizations to develop gold standard reference methods and well-characterized standards for calibration will improve this problem in the future. The current situation makes it necessary for clinical laboratorians to understand the strengths and limitations of the various assays they offer and provide guidance to clinical researchers in the interpretation of the results.

The other problem that the lack of test harmonization creates is the difficulty of changing assays before the completion of a protocol. A clinical laboratory may make changes in their assays for a variety of reasons, including replacement of a discontinued assay or improvement in the accuracy, cost effectiveness, or turnaround time with a new assay. The impact of these changes can be minimized, if the substitute assay is chosen so that it closely matches the results of the previous assay. It also is useful to run both assays in parallel for a short period of time to establish a regression equation relating the two assays. Using such an equation, the investigator can convert the results of one assay to the other, to reduce the increased variance from the multiple assays. It also is important to carefully establish the reference interval of the new assay and to interpret the results in the context of this new range when the two assays differ significantly. In the case of tumor markers, which can often show substantial differences between assays, it may be necessary to offer both assays in parallel for an extended

period of time until all the patients are “re-baselined” with the new assay. It is critically important to communicate with the primary investigators in the setting of an assay that is undergoing a change so they understand why the assay is being changed and the potential impact of this on their protocols. Finally, when the clinical laboratory supports a Phase I, II or III clinical trial, it has to establish at the outset that the assays it provides meet FDA recommendations.

Specialized Instrumentation Used to Support Clinical Research

Depending on the specific needs of a clinical protocol, specialized instrumentation may be necessary to support any given study. Because of their flexibility, the following types of assays and/or instruments are frequently useful for many different types of clinical research studies: Enzyme Linked Immunoassays (ELISA), High Performance Liquid Chromatography (HPLC), Liquid Chromatography-Mass Spectrometry (LC-MS), and Multiplex Immunoassays.

Although most immunoassay analyzers used in clinical laboratories offer a wide variety of tests, often more than 50 different assays per instrument, they are primarily focused on assays that are useful diagnostically. In contrast, ELISA kits are available for a much wider variety of tests and thus this is often the preferred format for developing a new test. It may be necessary, however, to have an automated ELISA workstation that is an open platform, if ELISA testing processes a large number of samples. HPLC is also a versatile technique, particularly for small molecules, such as vitamins and drugs, for which there are no commercial assays. Perhaps the most versatile platform is LC-MS, which can be applied for an even wider variety of tests, because of its superb sensitivity and specificity. It also can be used for the measurement of proteins and peptides, without the need for developing an antibody.² In the last few years, several types of instruments have been developed for multiplex immunoassays,³ such as the MesoScale electrochemiluminescent plate reader and the Luminex bead-based technology, which can simultaneously measure from a few to a large number of different protein analytes on a relatively small sample volume. These types of assays are particularly well suited for clinical protocols that require the measurement of a large number of different cytokines.

The presence of specialized instrumentation also can be an advantage in the operation of the routine diagnostic laboratory. For example, a matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometer in DLM was used to generate a routine assay for the identification of bacteria, mycobacteria, and fungi.^{4,5} Finally, LC-MS now also is widely used in many academic clinical laboratories for the routine measurement of vitamins, drugs, and hormones.

TEST REPORTING

We also have made accommodations to the last step in clinical laboratory testing, namely, test reporting, to facilitate clinical research. Similar to the process of requesting a test, the reporting of test results for clinical research provided through the DLM also can be accomplished easily with standard laboratory and hospital information systems. We have developed, however, a special system to enable investigators to extract clinical laboratory data into electronic worksheets for further manipulation. We also have established a long-term data repository of all clinical data, which goes back more than three decades. A common request of investigators when preparing data for publication is details specific to the assay used and its performance characteristics including precision, reference range, and sensitivity. For assays that are no longer being performed, this type of information must be saved. All of this relevant information regarding current assays and historical reference ranges is posted on the DLM website to facilitate easy access by the investigator.

EXAMPLES OF SPECIALIZED LABORATORY SERVICES DEVELOPED FOR CLINICAL RESEARCH

In [Table 40-2](#), we list some of the specialized services for clinical research offered by DLM. Following are some specific examples.

Flow Cytometry

Flow cytometry testing based on individualized protocols of multicolor testing has been applied to a variety of research protocols. In each case this has evolved from direct

TABLE 40-2 Specialized Laboratory Services Supporting Clinical Research Offered by DLM

Laboratory section	Specialized service
Chemistry	Animal testing, endotoxin testing of therapeutics, high-performance liquid chromatography and mass spectroscopy section
Hematology	Chimerism testing, animal testing, specialized coagulation testing
Immunology	Flow cytometry, molecular diagnostics
Microbiology	High containment Biosafety Level 3 (BSL-3) facility, molecular diagnostics, microbe identification using mass spectroscopy, sterility testing for therapeutics

interaction between the principal investigator of the specific protocol and the staff of the flow cytometry laboratory. The individual study is developed to focus on the questions being asked by the protocol. In addition, specialized testing beyond extensive immunophenotyping panels has been developed within DLM to address other issues related to one or more research protocols. For example, our laboratory clinically validated a method for evaluating oxidase activity in granulocytes originally described in research publications that now is the standard diagnostic screening test for chronic granulomatous disease.⁶ Another example is the development of a flow cytometry-based assay to evaluate intracellular protein phosphorylation following cytokine stimulation as part of a complete assessment of patients with defects in a specific cytokine receptor.⁷ As the capacity of flow cytometry evolved in the area of intracellular protein detection, the laboratory developed a number of intracellular protein assays linked to research protocols in which this information provided important links to possible pathogenic mechanisms in the disease process.

Microbiology Core Laboratory

An expansion of specialized testing and test facilities is the creation of a core laboratory facility to support one or more research programs. In 2005 the DLM Microbiology Service created a core laboratory to support a multicenter epidemiology study of transmission of methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE) in intensive care units. Specimens were shipped on a weekly basis from 18 national ICUs to the NIH where highly sensitive cultures and molecular assays were performed to detect carriers of these antibiotic-resistant bacteria. Specialized testing methods, a computerized reporting system, and an organism repository were developed for the study. The clinical laboratory benefited from the study by implementation of new, more sensitive assays that could be introduced into routine diagnostic testing, and the researchers benefited by the development of a cost-effective, accurate means for accomplishing their research goals. Additionally, the repository of organisms centralized in one facility has enabled additional research studies to be performed.⁸ A second study performed in 2008 using the same core facility studied MRSA colonization of marine officer candidates during their field basic training.

The decision to develop a core facility versus use of existing clinical or research laboratory facilities is determined by a number of factors. If highly specialized testing or equipment is required and it is anticipated that this can be used for multiple research studies, then the core laboratory would be a logical consideration. Likewise, consolidation of specialized testing for multicenter studies in a core facility would reduce the cost of testing by elimination of redundancy in equipment and technical expertise.

This cost saving must be balanced by the increased costs and delays associated with specimen transport to a distant laboratory and the development of information systems to track specimens and report test results.

Molecular Diagnostic Testing

A number of genomic-based molecular assays have been developed in the last few years that have limited value for routine diagnostic purposes but have been valuable for the support of research initiatives. For example, the Microbiology Service has developed polymerase chain reaction (PCR) assays for the direct detection and identification of a number of bacterial, viral, and parasitic pathogens (e.g., *Legionella pneumophila*, *Chlamydomphila pneumoniae*, human herpesviruses 6 and 7, orthopoxvirus, JC virus, SARS coronavirus, avian and swine influenza viruses, *Leishmania* species, *Brugia malayi*, *Loa loa*, *Onchocerca volvulus*, and *Wuchereria bancrofti*). Although the diversity of these tests is influenced by the research-oriented nature of the NIH clinical research program, the model for the development of these assays could be applied to any clinical laboratory having a research laboratory partnership. In most of these examples, the research laboratory identified the gene target, the clinical laboratory refined the assay for integration into the diagnostic lab menu of tests, and then validated the assay using clinical specimens provided by the researcher. The end result of this work is an expanded menu of clinical tests and assays that directly benefit the researchers.

Another example of how DLM was able to support the research program while at the same time expanding the diagnostic capacity of the clinical service is in the area of gene sequencing for microbial identification. Although this technique is more commonly used in research laboratories, expansion of genomic identification of bacteria, mycobacteria and fungi has led to the discovery of novel organisms such as the bacterium *Granulobacter bethesdensis*,⁹ the mycobacteria *M. massiliense* and *M. bolletii*,¹⁰ and the fungi *Neosartorya udagawae* and *Aspergillus viridinutans*.¹¹ In each example these organisms had not been identified previously and their discovery led to new avenues of research in the pathogenesis of disease.

In response to an expanded NIH program focused on primary immunodeficiencies, DLM developed a mutation analysis laboratory focused on identifying genetic defects associated with these disorders in a collaboration between the principal investigators and members of the DLM senior staff. Likewise, an expanded non-myeloablative allogeneic stem cell transplantation program at the NIH prompted DLM to develop lineage-specific chimerism testing to meet the need of monitoring the level of donor engraftment among various hematopoietic elements. This has been further refined to meet the specific need of evaluating donor chimerism at the level of cell subpopulations as well as the

requirement to monitor donor chimerism in the setting of hematopoietic stem cell transplantation based on two different donor cord bloods administered to one recipient.

Development of a High Containment Biosafety Level 3 (BSL-3) Laboratory for Emerging Agents

The Microbiology Service has a new BSL-3 facility generated with assistance from the National Institute of Allergy and Infectious Diseases (NIAID). The need for this facility was first appreciated with the anthrax contamination in Washington, DC in 2001, and then subsequent domestic and international outbreaks of infections with West Nile virus, SARS coronavirus, and avian influenza virus. This specialized laboratory is not likely to be available in most clinical laboratory settings. However, with the unique mission of the NIH along with specialized research currently ongoing in the area of bioterrorism being conducted locally at the NIH, military laboratories and Homeland Security laboratories, this facility serves a very specialized need in our setting. It also has a dual purpose in view of emerging agents that are highly contagious and potentially of high risk to the general population following natural exposure (e.g., SARS, influenza virus) as well as researchers working with these agents.

This BSL-3 facility complements a primary BSL-3 laboratory in the Microbiology Service that is used routinely for diagnostic mycobacteriology and mycology testing. This laboratory still is critical to the research mission of DLM. Intramural scientists from the Clinical Center and NIAID have active research programs studying mycobacterial infections in HIV-infected patients in a number of foreign countries including Mali, Kenya, and South Africa. Diagnostic testing to support these programs, as well as training of technologists and medical researchers from these countries, is performed in this facility. Additionally, this laboratory is used for the development, validation and technical training in new technologies such as the detection and identification of mycobacteria in clinical specimens using nucleic acid amplification assays¹² and molecular typing of recovered *Mycobacterium tuberculosis* strains by spoligotyping, a polymerase chain reaction-based method for simultaneous detection and typing of *Mycobacterium tuberculosis* strains.¹³

Clinical Laboratory Service for Animal Samples

The Clinical Chemistry and Hematology sections of DLM offer clinical laboratory services for animal specimens. By offering this service, DLM has been able not only to provide a convenient source for such testing to serve the NIH research community, but also to reduce the overall cost

compared to using an outside veterinary laboratory. Blood and occasionally urine on a wide variety of species are analyzed with the majority of samples coming from mice, based on the significant focus on murine models of disease. Most routine general chemistry tests designed for human specimens will also work on animal samples.¹⁴ The same is true for automated complete blood counting, although an adjustment has to be made for some species.¹⁴ Immunoassays designed for human samples, however, frequently do not work well on animal samples, a problem that is particularly true for protein immunoassays.

A consistent problem with managing animal samples is the very limited sample size available from small animals like mice. Specialized tubes designed for pediatric samples often are used in these cases, and it is important to choose an analyzer that has a relatively small dead space requirement for the proper aspiration of samples. Because of the wide variety of species and strains of animals used, no reference ranges are typically reported with animal samples.

SUMMARY

With a number of relatively minor modifications in operating procedure, most clinical laboratories should be able to accommodate, at least to some degree, samples for clinical research studies. It can require, however, a considerable financial investment particularly to offer some of the specialized clinical laboratory services as performed by DLM. Many of these services also require personnel with more advanced training. In many cases, the introduction of a significant number of research samples also will negatively impact on the overall efficiency of the clinical laboratory operation, particularly due to the extra effort and diligence that is necessary to process samples of these types. However, when compared to creating a separate stand-alone laboratory specifically for managing research samples, integrating these samples into the workload of a routine clinical laboratory is generally the most efficient approach. This usually also will lead to better quality results due to the major emphasis that clinical laboratories place on quality control and assurance.

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