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ANATOMIC PATHOLOGY

The Role of Pathologists in the Era of Personalized Medicine

Eric E. Walk

Although the field of oncology has made significant progress in improving the duration and quality of life for patients with cancer, response rates to traditional chemotherapeutic agents remain unacceptably low when compared with response rates in other disease areas. This situation, combined with the disturbing trend of decreasing annual new drug applications despite increasing drug development spending, has accelerated the adoption of the personalized medicine concept in several areas of medicine but most significantly in oncology. In this field, in the past 5 to 10 years, the terms *personalized medicine*, *biomarkers*, and *translational medicine* have gone from being hypothetical concepts to being widespread actualities in drug development, basic and clinical research, and clinical practice. Once obscure points of discussion at cancer conferences, these topics now dominate many congresses and have spawned dozens of biomarker meetings around the world each year.

In its simplest form, personalized medicine seeks to do away with the cancer treatment paradigms of “one drug fits all” and “trial and error,” replacing them with a model centered on the pairing of molecularly classified cancer subgroups with new generations of chemotherapeutic molecules targeting the specific pathogenic mechanisms that drive the respective neoplastic processes in each subgroup. The objective is to administer the right drug to the right patient at the right time and at the right dose. This model is entirely dependent, however, on a new generation of cancer tests that accurately and comprehensively characterize patients' tumors at the DNA, RNA, and protein levels, thereby allowing physicians to identify likely responders *before* beginning treatment.

These tests have been designated *companion diagnostics* to acknowledge that, in the personalized medicine model, the pharmaceutical compound and the diagnostic assay become inexorably linked. That is, without the drug, there is no need for the companion diagnostic; and perhaps more conceptually provocative, without the companion diagnostic, there may be no utility for the drug. Thus, while targeted therapies often demonstrate dramatic responses in diagnostic-driven patient subsets, the therapy's efficacy in unselected patient cohorts is typically too low for regulatory approval. The US Food and Drug Administration has published a guidance document in draft form on the co development of drugs and diagnostics. A major point of emphasis in that document is the need to begin development of the diagnostic assay early in the drug development process, ideally in the preclinical stage, to best ensure that a robust assay can be approved by the US Food and Drug Administration and be commercialized in conjunction with the pharmaceutical compound.

If one considers the large and growing number of molecularly targeted therapies that are either available commercially or in development, as well as the enormous amount of ongoing translational research in oncology, it becomes clear that the personalized medicine concept is not

going to be a short-lived trend but rather a true paradigm shift and a new way of diagnosing and treating cancer as a disease.

At the same time, the personalized medicine concept faces a number of significant challenges before its potential can be fully realized. At the scientific level, although our knowledge surrounding the molecular underpinnings of cancer continues to expand at a rapid pace, we are a long way from having a complete understanding of how cancer cells are driven, how they evolve over time, how they interact with the host microenvironment and how they react to therapeutic intervention. In drug development, we face the challenges of developing therapies against targets we do not completely understand and of selecting optimal trial designs, clinical endpoints, and biomarker strategies. In the clinic, we need to deal with the reality of acquired drug resistance, even in patients who have had dramatic initial responses, and with a previously underestimated degree of inpatient and intratumoral heterogeneity. Although the combination of targeted therapies appears to be one solution to the latter challenges, clinical trials that include adaptive designs and combinatorial approaches have only recently been initiated, and it remains to be seen whether toxicity will be a limiting factor. At the logistic level, sample collection, especially tissue collection, continues to be challenging in the context of clinical trials. This is due to cost, patient enrollment impact, preanalytic variables, and lack of standardization. Given that the entire concept of personalized medicine is predicated on the interdependence of drugs and diagnostics, how will each of these critical components be valued, priced, and reimbursed, so they are widely available and best serve the needs of patients?

The solutions to these issues will depend on a coordinated approach from a variety of disciplines, including oncology, molecular biology, and certainly, pathology.

The Role of Pathologists in Personalized Medicine: The Need for Change

As personalized medicine takes hold and revolutionizes the way we diagnose, characterize, and treat cancer, there is a tremendous opportunity for pathologists to make major contributions to this emerging new paradigm and to dramatically improve the care of patients with cancer. Some significant changes, however, need to occur within the field of pathology for this to become a reality. Pathology, as a field, has been occupied primarily with the diagnosis, classification, and sub classification of disease. Certainly, pathologists also provide prognostic information in the form of histological grade, and in a small number of cases, they provide information, such as *HER2* status, that is directly related to therapy. For the most part, however, pathologists have not played an active role in driving forward personalized medicine. Pathology, as a field, needs to move beyond the realm of diagnosis and mere classification/sub classification of diseases, to become the field responsible for providing personalized medicine information. Pathologists need to be the experts in the medical system who answer the following questions for patients and their treating physicians:

- Does the patient have an indolent disease or one that threatens his or her life?
- If the patient has a malignancy, how aggressive is it? What is the expected patient survival rate in the absence of therapy?
- Which specific genes are dysregulated and are responsible for driving the tumor? How many signaling pathways are involved, either directly or as compensatory or parallel

mechanisms? Based on this information, which targeted therapy or targeted therapy combinations will be effective in the patient?

- If the patient becomes resistant to initial therapy, what additional pathways need to be shut down?

If pathologists do not embrace personalized medicine, other fields and various nonpathology biotechnology companies will provide answers to these questions, but they will do so in the absence of morphologic data, ultimately generating information that will be inherently of less value. In a recent article, Thomas Giordano, MD, outlines what he calls *personalized predictive pathology* and appropriately asks, “Will molecular profiling become part of the routine pathologic assessment of cancers performed by surgical pathologists, or will it become the domain of subspecialty labs, either academic or commercial, with surgical pathologists observing from the sidelines?” He adds, “Prediction of therapeutic response by molecular profiling is the logical and natural extension of the work of surgical pathologists.”

Pathologists are the only professionals in the health care/scientific system that can interpret genomic, gene expression, and proteomic data in the context of tumor morphology. Only the pathologist can comment on the presence, absence, and differential expression of biomarkers in tumor cells versus normal cells, in in situ tumors versus invasive tumors, and in different grades and patterns of a tumor present within one sample. Only the pathologist can provide a comprehensive assessment of companion diagnostics and other biomarkers on a per patient, per clone, and per cell basis. The correlation of biomarker data with traditional histomorphology lies exclusively in the realm of the anatomic surgical pathologist and holds the promise of unlocking crucial insights into the biology of tumors and, therefore, into the most rational and effective therapeutic strategies.

The Need for New Tools

Pathologists will certainly require novel tools and technologies to enable this promise. For decades, pathologists have had the ability to correlate the expression of proteins with histomorphologic patterns by using immunohistochemical methods. Recently, because of the advent of light microscopy-based in situ hybridization methods, gene amplification has been added to the brightfield repertoire of the surgical pathologist, allowing pathologists to visualize individual copies of genes and amplification states in the context of morphology. Unlike fluorescence in situ hybridization, these technologies use traditional counter stains and generate high-quality cellular morphology, allowing for distinction between different aspects of the specimen, such as invasive and in situ tumor components. This type of technology is a critical advance for the field of pathology because, for the first time, pathologists are able to generate genomic profiles of patients' tumors that incorporate histological pattern information. For example, in a breast cancer case that contains both an invasive ductal and an invasive lobular component, pathologists can use these novel technologies to observe separately the *HER2* gene status of the 2 components. Several studies have investigated the frequency and extent of heterogeneity in breast cancer, and it will be important for pathologists to continue to further this type of work by correlating intratumoral biomarker heterogeneity with differential clinical responses to targeted therapies.

Although the visualization of amplification status in the context of histomorphology represents a major advance in the field of pathology, it is only the first of many types of molecular genomic technologies that pathologists will need to acquire. As we expand our knowledge of the differences between cancer cells and their normal counterparts at the DNA, RNA, and protein levels, it is becoming clear that the characterization of tumors as part of the personalized medicine paradigm will need to occur in a comprehensive manner and will not be possible with single markers or technologies. The evolving picture of personalized medicine for non-small cell lung cancer is a recent example supporting this notion.

To varying degrees, numerous studies have demonstrated the usefulness of epidermal growth factor receptor (EGFR) mutations, copy number/amplification status, and to a lesser extent, protein expression by immunohistochemistry to predict responses to EGFR-specific therapies, such as erlotinib (Tarceva, Genentech, South San Francisco, California and OSI Pharmaceuticals, Melville, New York) and gefitinib (Iressa, AstraZeneca Pharmaceuticals, Wilmington, Delaware), in patients with advanced non-small cell lung cancer. Although not perfect, these markers can be used to enrich patient cohorts for responders, and in some cases, the clinical responses in patients with metastatic disease have been dramatic. One notable patient subset that is immediately resistant to this approach (ie, primary resistance) is that of patients harboring mutations in the *KRAS* gene. Activating mutations in this parallel pathway serves to bypass EGFR inhibition and to maintain downstream signaling through growth and proliferation pathways.

Unfortunately, even the group of patients with non-small cell lung cancer who initially respond to EGFR inhibitors inevitably relapse after a period of 2 to 3 years because of drug resistance. The mechanisms underlying this secondary or acquired resistance are being elucidated and are providing insight into the future of personalized medicine and the types of diagnostic and therapeutic approaches that will be necessary to overcome them. One mechanism of acquired resistance in this setting is the emergence of second-site mutations in the *EGFR* gene, such as the T790M point mutation, which renders first-generation EGFR tyrosine-kinase inhibitors less effective competitors for adenosine triphosphate. A second mechanism involves the amplification of the *MET* gene, which along with *ERBB3/HER3*, serves to bypass the inhibition of EGFR via the phosphatidylinositol-3-kinase pathway. Finally, a third mechanism of acquired resistance to EGFR tyrosine kinase inhibitors elucidated in A431 cell lines is the activation of the IGF1R pathway via IRS-1.

This example of acquired resistance via multiple mechanisms is being replicated with other targeted therapies in other indications, and suggests that therapeutic strategies incorporating combinations of pathway-specific targeted molecules will be required to overcome the redundancy and parallel nature of aberrant growth signaling in cancer. At the same time, if pathologists are to have a significant role in personalized medicine, they will need to have access to in situ and tissue-based diagnostic tools that span the gamut of genetic derangements in cancer. In addition to protein expression and gene copy number, pathologists may require tools that allow them to visualize point mutations, insertions, deletions, translocations, mRNA expression, methylation, and other epigenetic events, to name a few. The in situ detection of micro-RNAs has already been demonstrated and represents a positive development in the expansion of the pathologist's tool kit.

In the era of personalized medicine, the practice of pathology will need to undergo some changes to remain aligned with the needs of oncologists and their patients. Traditional histomorphology and morphologic diagnosis will not disappear but, rather, will take on more importance as pathologists expand their ability to comprehensively profile patients' tumors with next-generation, in situ methods. The pathologist's role will be to integrate the data from these diverse technologies into a coherent stream of information for patients and clinicians (Figure 1). The pathology report of the future will contain not only diagnostic and prognostic information but also critical predictive information pertaining to which drugs will be effective in a particular patient. An interesting opportunity for pathologists that emerges from these changes is that of direct patient interaction. As the amount and complexity of data that directly affect the care of the patient increasingly falls under the purview of pathologists, patients are more frequently seeking the expertise of pathologists to best understand their diagnoses, tumor profiles, and resulting implications for treatment. Rather than infringing on the responsibilities of oncologists, pathologists who take on this patient-facing role can be seen as providing information that is complementary to, and synergistic with, the discussions that oncologists have with patients. Some pathology departments have begun to experiment with making pathologists available to patients a few hours a week for consultations with very positive results. Other pathologists have organized educational programs for cancer patients that focus on the interpretation of their surgical pathology reports.

Implications for Pathology-Training Programs

Pathology-training programs also need to change to ensure that pathology residents and fellows are aware of the developments taking place in personalized medicine. As the links between drugs and diagnostics continue to strengthen, it becomes increasingly important for pathologists to be knowledgeable about, or at least exposed to, developments in clinical oncology, molecular biology, and translational science. Developments in these areas have direct implications for the diagnosis and molecular profiling of cancer and, therefore, for pathology. According to Dr Giordano, "Most pathology training programs are not adequately preparing their trainees in molecular profiling." This deficiency needs to be addressed if we are to ensure that new pathologists are equipped with the skills and knowledge necessary for them to be active participants in the era of personalized medicine. There are existing activities in which pathology residents (and practicing pathologists) can participate to increase their exposure to these topics. Besides attending the annual conferences organized by the United States and Canadian International Academy of Pathology, College of American Pathologists, and International Academy of Pathology, pathology trainees can attend meetings organized by the American Association for Cancer Research and the American Society of Clinical Oncology, where the latest developments in cancer research and translational science are presented and discussed. Also recommended is this Futurescape of Pathology conference, an annual meeting focusing on technical and conceptual innovations that are transforming the field of pathology. Pathology trainees can also follow the latest discoveries and research relevant to personalized medicine by reading journals, such as *Science*, *Nature*, *New England Journal of Medicine*, *Journal of Clinical Oncology*, and *Clinical Cancer Research*.

The Role of Pathologists in Industry

Pathologists have important contributions to make in the practice of personalized medicine, including its key components, oncology therapeutics and companion diagnostics (Figure 2). Anatomic and clinical pathologists are increasingly being hired by pharmaceutical and diagnostic companies because these industries have recognized the importance of having pathologists' input in the development of oncology drugs and companion diagnostics (Figure 3).

Pathologists in the Pharmaceutical Industry

In the pharmaceutical industry, pathologists are finding roles in all phases of drug development, including preclinical research, translational medicine, and clinical drug development. The most common roles for pathologists are in the areas of research and translational medicine.

Roles in preclinical research are best suited to pathologists who have either a doctor of medicine or doctor of philosophy degree or an interest in basic science work. This work typically involves the use of cell line or xenograft models to evaluate the effectiveness of novel compound candidates, the discovery and evaluation of putative biomarkers, as well as experiments to understand the molecular epidemiology of therapeutic targets and, therefore, potential clinical indications. These roles typically are laboratory-based and include oversight of a laboratory.

Frequently, pathologists will also find roles in translational groups within the pharmaceutical industry. Besides the traditional research and development groups that have always existed at pharmaceutical companies, having a group or groups that work at the interface of research and development has now become standard practice. These groups are given various names, such as Translational Medicine, Experimental Medicine, and Molecular Medicine, and commonly have a pathologist as either a team member or the group leader. The term *translational medicine* is meant to convey the idea of rapidly translating novel basic science discoveries and scientific knowledge into clinical applications, going quickly from bench to bedside. Pharmaceutical companies now realize that it is no longer acceptable or feasible for basic science researchers to work in isolation from their clinical development colleagues. Generating a compound in the laboratory and “throwing it over the wall” to clinical teams for clinical trials and development is not an approach that will work in the era of targeted therapy and personalized medicine. Instead, research and development must work together from the very beginning of the drug discovery process, and in many pharmaceutical companies, translational medicine groups are bridging this gap. Pathologists who work in these types of groups will typically work with both research scientists and clinical development teams in developing and implementing biomarker strategies for several compounds or across a portfolio of compounds. For example, with early stage (ie, phase 0 or 1) compounds, this may include creating an immunohistochemical assay to measure a phosphorylated protein and to measure pharmacodynamic effects. For a later-stage compound, the objective may be to develop an assay panel that is predictive of drug response (ie, a companion diagnostic). These tasks may include working with in-house colleagues as well as outsourcing work to central laboratories and diagnostic companies.

Pathologists may also assist in writing the clinical trial study protocol or the study laboratory manual, especially for written sections that deal with sample collection and biomarker analysis and scoring. The collection of tissue samples, as a necessary component of biomarker development, is becoming commonplace in both early and late-stage clinical trials. In various

clinical trial scenarios, the pathologist contributes critical expertise regarding the histopathologic sub classification of tumors, familiarity with histological practices, and the feasibility of collecting different types of samples (eg, needle core biopsies vs. archival formalin-fixed, paraffin-embedded blocks vs. unstained slides). Inadequate, poorly planned or poorly executed sample collection can have a significant effect on the quality and robustness of clinical trial data. The collection of high-quality tissue biopsy samples has proven to be very challenging in large late phase clinical trials. For example, in the phase 3 registration trial for the drug erlotinib (Genentech and OSI Pharmaceuticals), only 44% of patients had usable slides for immunohistochemistry, and only 31% of patients had usable tissue available for sequencing or fluorescence in situ hybridization analysis.

Although positions in the clinical development phases of drug development are not typically available to pathologists without prior experience, once a pathologist has worked in one of the aforementioned roles, if interested, he or she may feasibly become a clinical project leader, overseeing the overall development of early or late-stage therapeutic compounds.

Pathologists in the Diagnostic Industry

In the diagnostics industry, pathologists can contribute to the entire life cycle of a diagnostic product, including discovery, development, technical validation, clinical validation, regulatory approval, commercial launch, marketing, sales, and customer support. The specific opportunities available to a pathologist will depend on the focus of the diagnostic company, with the greatest number of opportunities being at those companies that have tissue-based diagnostic products. Specific job titles could include staff pathologist, laboratory medical director, and chief medical officer.

In the discovery phase, a pathologist may be responsible for identifying, evaluating, and prioritizing the medical and scientific value of diagnostic opportunities. The pathologist may also design or participate in experiments to assess various novel or existing technologies or the technical feasibility of a novel assay concept. Pathologists can provide critical input during this phase to ensure that these 2 processes occur in the correct order. That is, new diagnostic products should begin with the identification of a medical value scenario or an area of unmet medical need, which in turn drives the search for appropriate enabling technologies. Reversing this order can lead to inefficiency, loss of focus, and ultimately, regulatory and/or commercial failure.

During the development phase, pathologists can contribute to the verification and validation of assays and diagnostic testing platforms. During this phase, experiments are conducted to ensure that the diagnostic product meets the required technical specifications and customer needs and demonstrates an acceptable level of intrarun, interrune, interinstrument, and interlaboratory reproducibility. The validation phase typically includes external validation studies during which the pathologist may interact with outside pathologists or other physicians who are serving as the primary investigators for these studies.

Pathologists can also play an important role in the regulatory approval of a diagnostic product, contributing to or overseeing the medical affairs process of designing and executing clinical diagnostic trials that will serve as the basis for approval by the US Food and Drug

Administration and other regulatory agencies worldwide. In certain situations, pathologists can play a valuable role during and after the launch of the diagnostic product by contributing to the marketing campaign, serving as a consultant and expert to the sales and marketing teams, and meeting with pathologists and other physicians to assess market reaction to a product. Pathologists may also be responsible for initiating and maintaining collaborations with thought leaders to better understand the potential medical value of assays that are launched as research-use only products or to understand the medical need for new products. This type of role can be attractive to pathologists who want to maintain academic activities, including publishing and presenting data from their collaborations.

Careers in Industry versus Traditional Pathology Practice

Compared with traditional practices in a community, academic, or reference laboratory, the role of a pathologist in either the pharmaceutical or diagnostic industry is different in focus, culture, working environment, and time frames. Rather than conducting macroscopic and microscopic evaluations of individual patient cases, a pathologist in industry contributes to the development of a therapeutic compound, a diagnostic/companion diagnostic assay, or both. Rather than working as an individual in a department, a pathologist in industry generally has a position that requires close collaboration with colleagues in a multidisciplinary team and often in a “matrixed” environment. Rather than being the sole authority figure in a laboratory, pathologists in industry need to become accustomed to working in a collaborative and hierarchical environment in which they will have a direct manager. Instead of completing cases in days or perhaps weeks, pathologists in industry work on projects that typically take months or years to complete, and in some cases, they will work on projects that ultimately do not make it to market. Positions in industry frequently require domestic and international travel, 30% to 40% of the time or more in extreme cases. Financial compensation for pathologists in industry is typically in the form of a base salary, an annual bonus with both company and individual performance multipliers, and an equity component that may include stock or stock options. Physicians typically experience an initial drop in salary when they enter a corporate environment; however, the long-term financial potential in industry can exceed that of typical clinical practice environments. Although some pathologists may perceive these differences as advantages, others may perceive them as disadvantages. These differences certainly should be considered before a pathologist entertains the idea of a career in industry. Pathologists who have made the transition from clinical practice to a career in industry universally describe a reduction in stress related to the absence of threat of medical malpractice liability.

Conclusion

In light of an ongoing revolution in the understanding of cancer and the approaches necessary to treat molecularly defined patient subsets, the field of pathology finds itself at a crossroads. For the field of pathology to remain relevant in an era of personalized medicine, pathologists need to become the providers of personalized-medicine information, delivering critical answers to questions relating not only to diagnosis but also to prognosis and response prediction. By driving the clinical practice of molecular predictive pathology and taking on new roles in pharmaceutical and diagnostics development, pathologists have the opportunity to create and adopt innovative tools that allow the in situ characterization of patient tumors in a comprehensive way that

directly informs specific treatment decisions. By embracing these concepts and delivering molecular genomic data in the context of traditional histomorphology, pathologists will play a leadership role in the era of personalized medicine and will directly improve the lives of patients with cancer.

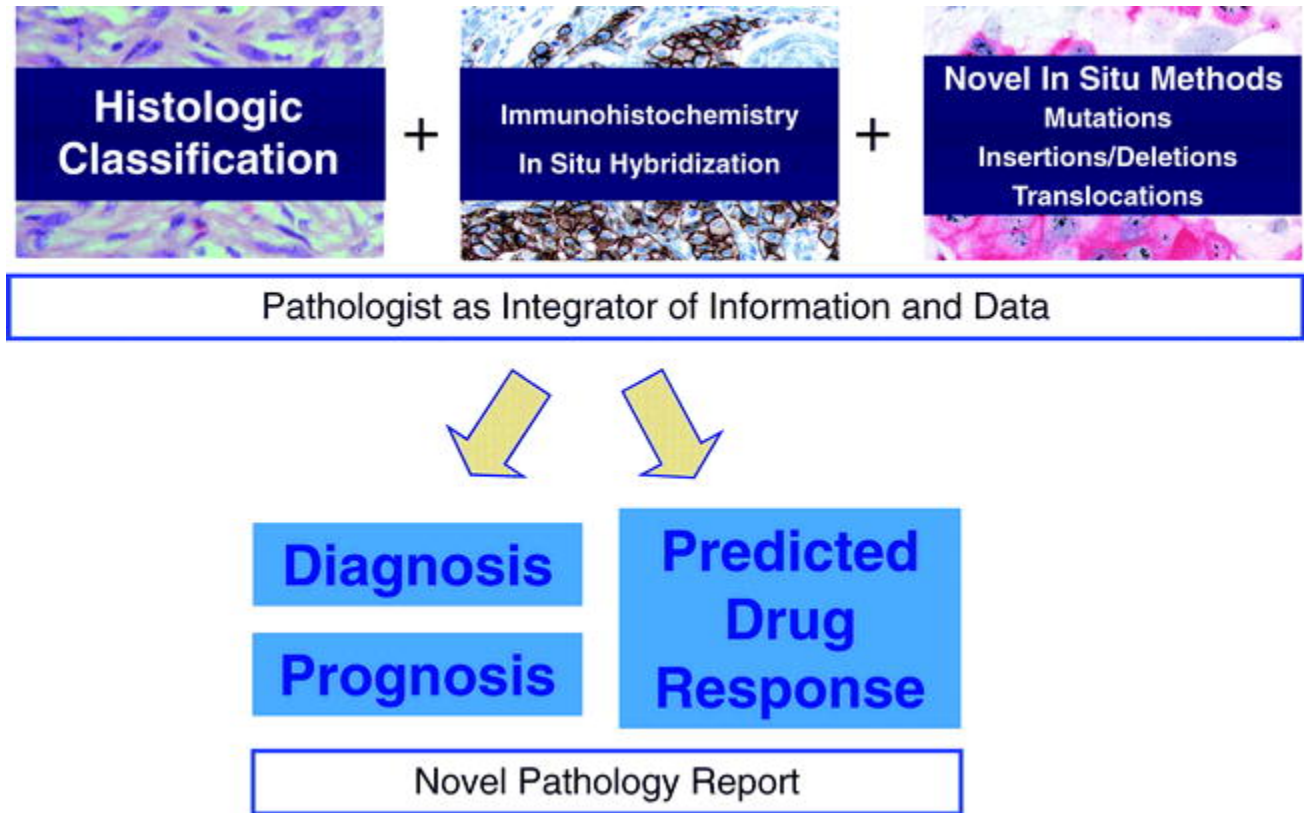


Figure 1. Concept for the role of the pathologist in the era of personalized medicine. Pathologists will integrate traditional histomorphology with data from existing and next-generation molecular assays to provide patients and clinicians with diagnostic, prognostic, and predictive information

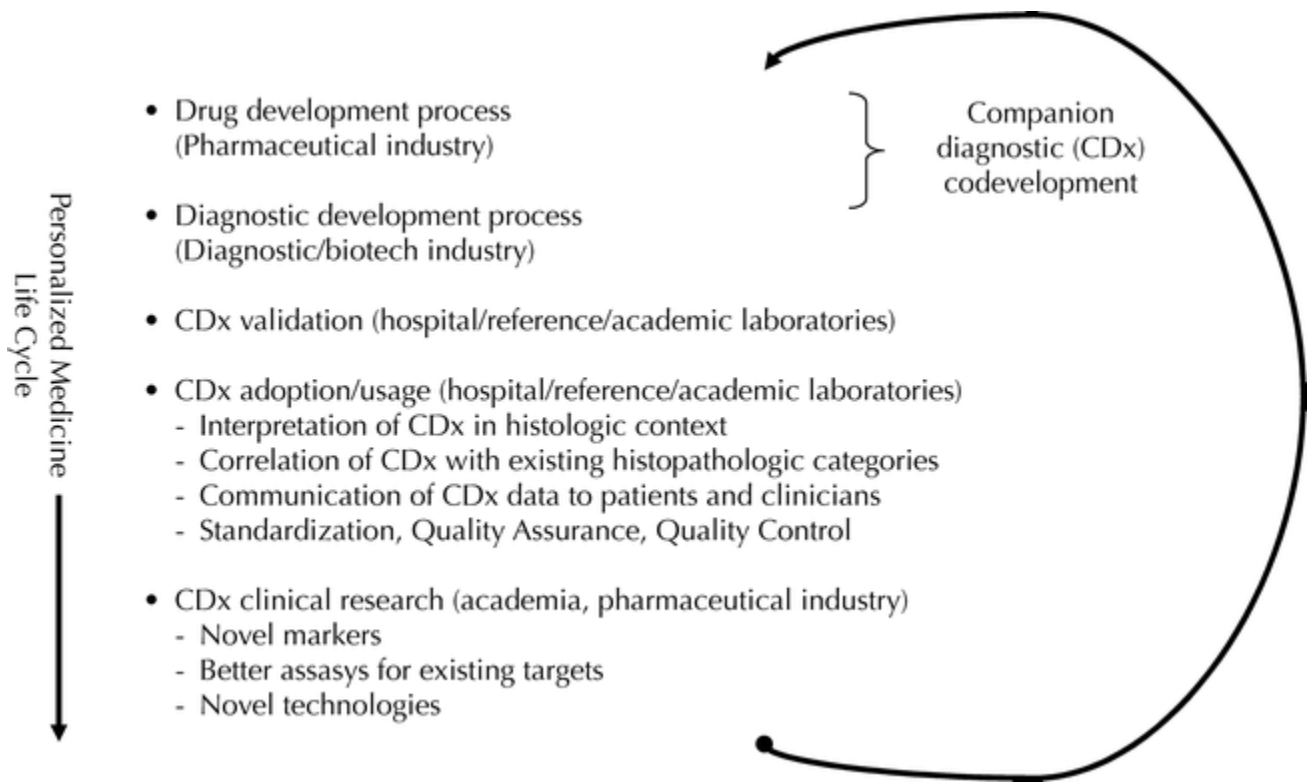


Figure 2. Among the important contributions pathologists make during the entire personalized medicine life cycle are the development of targeted therapies and companion diagnostics, the validation and adoption of diagnostics in clinical practice, and clinical research that paves the way for novel biomarkers

- Design of clinical and companion diagnostic studies
 - Biomarker strategy and concept
 - Collaborations with oncologists and molecular biologists

- Sample collection practices
 - Approach to sample procurement
 - Sample handling procedures
 - Control of preanalytic variables

- Analytic phase
 - Selection of reagents (primary antibodies and probes) and controls
 - Assessment of biomarkers in the context of morphology
 - Selection of slide – scoring methodology

- Data analysis phase
 - Correlation of biomarker data with clinical data
 - Determination of cut points

Figure 3. The many ways in which pathologists contribute to targeted therapy and companion diagnostic development are listed

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Histology without Xylene

Buesa RJ, Peshkov MV.

After the hazardous effects of xylene became indisputable in the 1970s, many potential substitutes became available, some with as many if not more hazards. This article discusses the inadequacy of 5 vegetable oils as substitutes, as well as the characteristics of 22 D-limonene-based substitutes, all less effective in their chemical role, some capable of inducing health problems, and costing more than twice as much as xylene. Some of the 35 alkane-based substitutes discussed are effective for tissue processing, less toxic, with a cost about the same as xylene, but are not very effective for dewaxing and other staining tasks. Isopropanol (2-propanol) alone or mixed with molten paraffin is a technically acceptable and cost-effective substitute for xylene for tissue processing, but in this study, we demonstrate that the best clearing agents from the sectioning quality and diagnostic value point of view, with automated or manual protocols, are mixtures of 5:1 and 2:1 isopropanol and mineral oil, followed by undiluted mineral oil, all at 50 degrees C, making them a safer and cheaper substitute than xylene. Using a 1.7% dishwasher soap aqueous solution at 90 degrees C to dewax before staining and oven drying the stained sections before coverslipping will eliminate xylene from the staining tasks. Tissue processors retorts and conduits can be dewaxed with a 2% solution of a strong glassware laboratory detergent. These 4 methodologies will make the histology laboratory xylene-free but, due to the natural resistance to change, many histotechs will be reluctant to adopt them if they think that their technical expertise could be jeopardized, and the only way these changes will succeed is if the pathologists, as stewards of the histology laboratory, commit to their implementation.

Ann Diagn Pathol. 2009 Aug;13(4):246-56. Epub 2009 Feb 5.

A New Grossing Knife with Two Parallel Blades for Preparing Uniform Thickness Gross Tissue Sections

Yang, J; Chen, X; Su, B; Zhao, S; Qiang, L

Grossing, a term that refers to examination and dissection of surgical specimens, along with preparation of sections from those tissues requiring processing, is the initial step in surgical pathology dissection. According to the textbooks and manuals of surgical pathology dissection, the thickness of gross tissue section is 2–4 mm. Every pathologist is familiar with the frustrations of trying to submit uniform thickness tissue sections. Through grossing is a simple job, the process is typically a time-consuming, hands-on process and is all too often not carried out appropriately.

The hollow structure and cystic specimens (eg, oesophagus, stomach, bowel, appendix and gallbladder) are the more frequently encountered specimens in the surgical pathology laboratory. Although the specimens are all anatomically simple hollow or saccular structures, they consist of several layers of different structures (outer surface and inner surface, and muscular wall or soft tissues wall), which often slide over each other during sampling. In practice, it is difficult to obtain representative full-thickness tissue sections of specimens (such as stomach mucosa, wall and serosa) through the full thickness of the lesion and the underlying wall by using a traditional cutter.

In order to resolve this problem, the authors have designed a new grossing knife (or dissection knife) for grossing (Fig. 1), which allows standardisation and greatly facilitates easy and rapid preparation of optimal uniform thickness tissue sections. The grossing knife is composed of a handle and a head with two parallel slots for supporting two essentially parallel blades. The gap between the two parallel blades is predetermined at 3 mm to form a tissue-receiving gap.

The sampling steps are as follows:

- According to surgical pathological dissection principles, after the specimen has been opened, the lesion areas to be sampled are detected.
- While sliding the grossing knife along the surface through the lesion areas from one end of the specimen to the other, gross desired representative uniform thickness tissue sections (3 mm thickness) are produced through the tissue-receiving gap.
- The last step is to hand-cut the representative slice sample to fit into the embedding mould; maximum area will be about 22 mm.

The knife has been used for a long time for sampling sundry specimens. By using it, gross desired uniform thickness tissue sections (3 mm in thickness) are produced through the tissue-receiving gap easily and quickly. The knife can not only be used for sampling uniform thickness tissue sections from hollow structures and cystic specimens, but also from solid organs, by changing different length of blades.

In summary, the grossing knife is an accurate, reliable, user-friendly instrument for sampling uniform-thickness slices of tissue, especially for full-thickness tissue sections from hollow or

cystic structural specimens. It allows standardisation and greatly facilitates grossing by providing tissue sections consistently uniform in thickness, and can be used in all surgical pathology laboratories by staff pathologists, pathologists' assistants, histotechnologists, residents, etc.

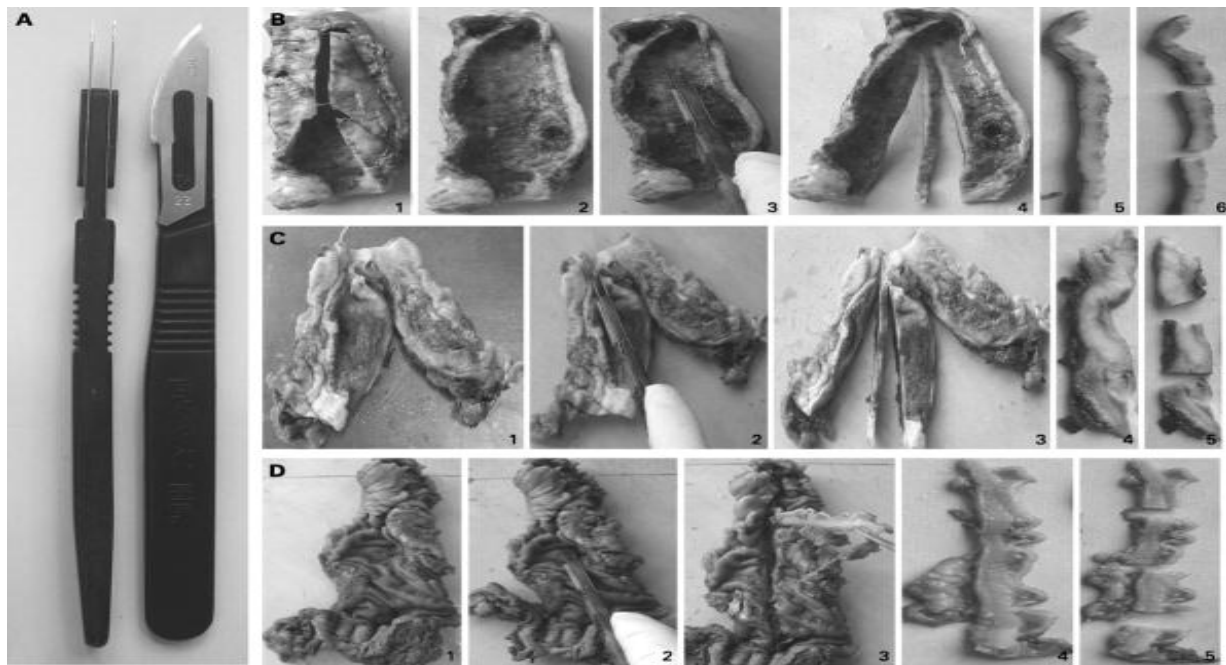


Figure 1. Structure and application of the grossing knife.

(A) The grossing knife is composed of a handle and a head with two parallel slots for supporting two essentially parallel array blades to form a 3 mm tissue-receiving gap. While sliding the knife through the surface of the specimen, gross desired uniform full-thickness tissue 3 mm thickness tissue sections are produced through the tissue-receiving gap. (B) Gallbladder specimen. (C) Oesophagus specimen. (D) Rectum specimen.

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CLINICAL PATHOLOGY

New Pathology Tests Double Sensitivity to Detect Bile Duct, Pancreatic Cancers

ROCHESTER, Minn -- June 1, 2009 -- Researchers have developed new pathology tests that double the ability to detect bile duct and pancreatic cancers. The results appear in the June issue of the journal *Gastroenterology*.

Pancreatic cancer and bile duct cancer are difficult to diagnose and often fatal because they are discovered in the advanced stages of the disease.

In the study, 498 patients with pancreatobiliary duct narrowing underwent an endoscopic procedure, and cell brushings were taken. Brushings were then analysed by routine cytology, digital image analysis and fluorescence in situ hybridization (FISH) to determine the various tests' effectiveness and sensitivity in detecting and diagnosing cancer.

While traditional cytology analysis relies on identifying abnormally shaped cells, the FISH test detects malignant cells using coloured probes visible with a fluorescence microscope. Since cancer cells have an abnormal amount of DNA, by FISH these cells show extra copies of the probes compared with normal cells.

Lewis Roberts, PhD, Mayo Clinic, Rochester, Minnesota, and colleagues found that the combination of cytology and FISH raised the detection rate of bile duct and pancreatic cancer from 20% to 43%.

"We were very pleased to see that the combination of FISH and cytology significantly improved our chances of diagnosing patients reliably," said Dr. Roberts. "The earlier we can diagnose a patient, the better the types of treatment we can offer and the more likely they are to have long-term survival after treatment."

SOURCE: Mayo Clinic

CYTOPATHOLOGY

Fine Needle Aspiration of the Thyroid: A Pathologist's Perspective

Oertel, Yolanda C

I have been aspirating palpable “lumps and bumps” for over 30 years and also interpreting aspirates performed by radiologists, endocrinologists, and other physicians; the last 9 years performing mostly thyroidal aspirates. What follows is based on my reflections and on the insight developed into the particular problem of thyroid nodules. We have to understand the limitations of fine needle aspiration (FNA) of thyroidal lesions. FNA is a diagnostic tool. Tools only work if they are handled properly. The five most important things I have learned in all these years are: (1) the role of the aspirator is crucial, (2) the technique is deceptively simple, (3) you have to have a team, (4) communication with the referring physician is essential, and (5) you have to persist.

THE ROLE OF THE ASPIRATOR IS CRUCIAL

I cannot overemphasise this premise. I believe that this still is overlooked in the literature. Frequently I read about how important it is to have an experienced cytopathologist interpreting the smears. The cytopathologist is only as good as the sample he obtains or receives. You can have the best cytopathologist in the world and he will not be able to diagnose your patient's nodule accurately if you only aspirated blood. Remember, that to acquire experience you must perform a sufficient number of aspirations. How many is enough? Only you will know. Also, you have to be self critical, and you must be a life-long learner. After 30 years I am still tinkering with my technique and getting better. In the last 9 years at the Washington Hospital Center I have aspirated over 10 000 patients (92% thyroids). There is a positive relation between “procedural experience” and outcomes. This is not restricted to FNA.

THE TECHNIQUE IS DECEPTIVELY SIMPLE

Again, I want to reiterate that not many “aspirators” realise that it is not only a matter of inserting a needle. The backbone of a cytological diagnosis is an adequate sample. What I see most frequently on submitted smears are haemodiluted samples. I believe that this is due to excessive suction. When dealing with thyroid lesions, you have to be an extremely gentle aspirator; even more gentle if the patient is hyperthyroid, or pregnant, or taking anticoagulants. I believe this technical problem can be overcome easily.

Remember that most patients are nervous, anxious and afraid (either of the procedure or about the results of the test), and their blood pressure will go up (“white coat syndrome”). This will contribute to easy bleeding. Hence, it is essential to learn how to get the patient to relax. Using an ice pack, performing the FNA with the patient sitting upright (rather than lying down), and applying pressure at the site of aspiration will prevent haematomas.

YOU HAVE TO HAVE A TEAM

You must realise that a team effort is required: the physicians involved in obtaining the samples, the technicians and technologists handling them (making the smears and staining them), and the cytopathologists interpreting the aspirates must cooperate with each other, communicate, be willing to follow instructions, and pay attention to detail.

COMMUNICATE WITH REFERRING PHYSICIANS

It is essential that you communicate with the referring physician to achieve optimal management of the patient. The main means of communication for the pathologist is the cytopathology report. We are aware that there is dissatisfaction with our reports; that they are frequently “nebulous.” We should strive constantly to provide a clear and definite diagnosis, in a timely manner, and using standardised nomenclature.

YOU HAVE TO PERSIST

In 1976, when I started performing FNAs at the George Washington University Medical Center, there was reluctance to accept this diagnostic tool. Now there is general agreement that it is the most valuable single test in assessing the nature of a mass in the thyroid gland. I want to share the following with you: Octavio Paz (Nobel laureate in literature) quotes the Spaniard B Pérez Galdós from *La Segunda Casaca*, 1883, “We see the instant triumph of the true idea over the false one, in the realm of thought, and we believe that just as quickly an idea can triumph over customs (or habits). Time has made customs so slowly and with so much patience, just as it made mountains; and only time, working every day (day in and day out) can destroy them. You cannot tear down a mountain with a bayonet.” So it will take us longer using a needle.

Who should aspirate?

Repeatedly, it has been reported that better results are obtained when the pathologist performs the FNAs. However, not many pathologists are willing to see patients and perform aspirations. Pathologists have been invisible and hence undervalued by patients. We are perceived as sedentary introverts, thought to be more controlling and less flexible than other physicians. Hence, whoever is willing to perform the procedure and to master it, should be the one to do it. Also, you have to consider the availability of “aspirators” in your geographical area. It is good to have “a mix of aspirators.”

What should the endocrinologist expect from the pathologist?

I believe you and your patient deserve a prompt cytopathology report with a clearly stated diagnosis; an unambiguous report. A timely report is necessary but not sufficient. The report should be concise and “to the point” and should provide you with the information you need or will use for diagnostic and therapeutic decisions.

What do pathologists expect from endocrinologists?

We would like to receive an adequate sample that has been smeared properly. We need more than blood on the smears. Also, we need properly filled requisitions with legible demographics

and relevant clinical information. It makes a difference whether the nodule was 1 cm or 5 cm in diameter. We cannot (and should not) interpret smears in a vacuum. We want endocrinologists to understand that FNA has limitations and is no substitute for clinical judgment. Also, we need follow-up information to keep learning what we are doing right and to correct what we are doing wrong.

What should be aspirated? Why?

Any palpable lesion should be aspirated. This is because FNA will provide a diagnosis most rapidly, most accurately, and at the lowest cost to the patient (by eliminating the need for ordering additional unnecessary tests). Non-palpable lesions should be aspirated under sonographic guidance if the patient has a high-risk history or suspicious ultrasound findings.

Should a multinodular goitre be aspirated?

I believe it should. Concentrate on the firmer nodules (which are usually the smaller ones), but any discrete nodule can be aspirated. However, even if all aspirates are benign, this should not give us a false sense of security. Clinical judgment should dictate the course to follow. If there are symptoms of compression, if the lesion keeps growing, if cosmetically it is a problem, then one should consider surgical excision.

If the patient is going to have surgery, why subject her to an FNA?

The reason for aspirating is to avoid surprises. I have aspirated multinodular goitres and found unsuspected metastatic renal cell carcinoma and also medullary carcinoma; lesions that modified the surgical approach.

Would you aspirate a thyroid nodule incidentally found during CT scanning of the neck?

If I can palpate it, I would aspirate it. I believe that once one becomes aware of the presence of a thyroid nodule (even if it is asymptomatic and less than 1 cm), if it is palpable, it should be aspirated.

Do you recommend ultrasound-guided FNA of palpable lesions?

I do not think ultrasound is particularly helpful in palpable lesions. It has been reported that ultrasound-guided FNA may improve the yield. At those institutions where this happens, I believe it is because the ultrasonographer is more experienced and has a better technique. Hence the success rate depends on the operator rather than on the machine. I believe our insatiable embrace of the image knows no bounds and we use many more ultrasounds than needed. What should be done when the aspirate is reported as unsatisfactory?

Repeat the FNA after a minimum lapse of 2 weeks, otherwise you will get another unsatisfactory specimen. Anytime you perform an aspiration you will cause some bleeding. If the lesion is small, and you repeat the aspiration after only a few days, you will be aspirating a haematoma and that is what the pathologist will see on the slides (cholesterol crystals and haemosiderin-

laden macrophages). However, if the lesion is large and there is no discoloration of the skin overlying it, a repeat FNA may be successful after only a few days. Usually it is best to wait one month.

If you know why you failed on the first aspirate, try to correct the problems. If the lesion bleeds easily or is difficult to aspirate, admit that you need help and refer the patient to an expert (when available).

What should be done when the aspirate is reported as suspicious or inconclusive?

In some of these cases, a repeat FNA after a suppressive trial of thyroid hormone for 6–8 months might be helpful. Also, if no other studies have been done prior to FNA, it would be helpful to perform a I scan. An autonomous nodule with a “suspicious” cytology then may be spared from surgery.

What should be done when the aspirate is reported as benign?

The endocrinologist or clinician will determine whether suppressive therapy is necessary or not. From the pathologist’s point of view, we recommend a repeat FNA no sooner than a year. Why? If we missed a lesion, we will have a second chance to diagnose it. It has been an excellent educational experience to compare the consecutive aspirates.

What should be expected in a FNA report?

An FNA report should be concise and should highlight (emphasise) the important information that requires the referring physician’s attention. Also, it should allow for the collection of accurate and comprehensive data.

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MICROBIOLOGY

Swine influenza H1N1. Is your laboratory prepared?

Sameera Aljohani

Influenza A virus is a member of the family *Orthomyxoviridae*. Influenza viruses are enveloped, with a segmented, single-stranded RNA genome. This family also contains influenza B and C viruses. Point mutations in the envelope protein hemagglutinin (H), referred to as antigenic drift, result in the emergence of new strains of influenza A and B viruses and the resultant annual outbreaks and epidemics. Sub-typing of influenza A virus is based on antigenic characteristics of 2 envelope proteins, H and neuraminidase (N). New influenza A virus subtypes emerge as the result of reassortment of H and N sequences from 2 different subtypes, referred to as antigenic shift. These new subtypes are responsible for influenza pandemics. There are currently 16 recognized H subtypes and 9 recognized N subtypes. Subtypes H1N1, H3N2, H2N2, and H1N2 have circulated, or are currently circulating widely, among humans. If, or when, the virus reassorts; a new genetically different and unique virus will form; and if transmitted from human to human easily, the new virus will likely produce an influenza pandemic with unpredictable mortality rates.

Swine influenza (swine flu) is a respiratory disease of pigs caused by type A influenza viruses, but human infections can, and do happen. Swine flu infection can be serious. In September 1988, a previously healthy 32-year-old pregnant woman in Wisconsin was hospitalized for pneumonia after being infected with swine flu and died 8 days later. A swine flu outbreak in Fort Dix, New Jersey, occurred in 1976 that caused more than 200 cases with serious illness in several people and one death. Swine influenza A (H1N1) virus appears to spread the same way as seasonal flu. Flu viruses are spread from person to person through coughing or sneezing of infected people. Sometimes people can get infected by touching contaminated surfaces or materials with flu viruses on it.¹

I. Special instructions. A laboratory should not perform culture on specimens if H1N1 influenza is suspected, unless performed under enhanced Biosafety Level 3 (BSL-3) laboratory conditions and with very close supervision.

Microbiology laboratories may perform rapid influenza antigen tests and direct fluorescent antibody staining on respiratory specimens from suspected cases, but only under BSL-2 conditions in a Class II biological safety cabinet. However, influenza A H1N1-specific reverse-transcriptase polymerase chain reaction (RT-PCR), is the preferred method because of its high sensitivity.

II. Recommendations. A. *General*. Virology laboratories should not inoculate specimens suspected of containing influenza A H1N1 virus into cell culture. Only laboratories capable of performing culture under BSL-3 conditions with enhancements should perform culture to evaluate a suspected influenza H1N1 case. If these criteria are met, and culture is performed, consultation with the Ministry of Health laboratory is recommended. The use of rapid antigen tests for influenza is increasing in laboratories and point of care locations. These tests are among

the least reliable for diagnosis of influenza, and should not be used alone to rule out H1N1 influenza in a suspected case, especially during the current pre-pandemic phase, and especially consider its performance for the current H1N1 strain is unknown.

B. Precautions. Culture diagnosis of suspected avian influenza A H1N1 requires enhanced BSL-3 laboratory conditions. Enhancements include the use of respirators, decontamination of all waste (solid and liquid), and showering of personnel before exiting. Molecular and rapid antigen testing can be performed on respiratory specimens under standard BSL-2 conditions in a Class II biological safety cabinet.

C. Specimens. Respiratory specimens. The nasopharyngeal swab (NPS), as well as nasopharyngeal aspirate (NPA) and lower respiratory samples such as bronchoalveolar lavage and tracheal aspirates are the preferred specimens for detection of influenza A virus. But in Avian flu H5N1; throat swab was superior, all respiratory specimens should be acceptable. Nasal swabs and aspirates are acceptable, but may contain lower titers. We recommend 2 respiratory specimens per one patient, first the nasopharyngeal and the second is the throat swab, to be submitted in 2 different virus transport medium (VTM).

Specimen collection and handling. Detection of influenza A H1N1 is optimum if specimens are collected within the first 48 hours of illness. If possible, serial specimens should be collected over several days from the same patient to increase clinical sensitivity. As with seasonal flu, Dacron or rayon tipped swabs should be used for specimen collection, as other materials may inhibit RT-PCR. Swabs placed in viral transport medium are generally suitable for RT-PCR testing. The collection of lower respiratory specimens generates aerosols, and requires infection control precautions for influenza A H1N1, including the use of Personal Protective Equipments.³ Specimens should be stored at refrigerated temperatures 2-4°C. For virus isolation, specimens should be stored at refrigerated temperatures no longer than 2 days, or frozen at -70°C, and shipped on dry ice. Laboratories should follow current regulations for packaging and shipping hazardous materials. Transportations should be carried out under Ministry of Health directives.

E. Available laboratory tests. Rapid antigen tests. As rapid influenza antigen tests provide a result in 30 minutes or less, they significantly impact patient treatment and management. These tests are the Clinical Laboratory Improvement Amendments (CLIA) 88 regulated, and are widely used for diagnosis of influenza in central, point-of-care, and physician office laboratories. Several rapid antigen tests are commercially available, some of which are able to distinguish between influenza A and B types. Rapid antigen tests are less sensitive than culture or RT-PCR. While rapid antigen capture assays may detect influenza subtypes, including H1N1, currently available tests are NOT capable of distinguishing specific influenza A subtypes. Some evidence indicates that rapid antigen tests are extremely insensitive for non-human influenza, and should not be used ALONE to rule out NON-HUMAN influenza in a suspect case, especially during the current pre-pandemic phase. Rapid antigen testing can be performed on respiratory specimens from suspected H1N1 influenza cases under standard BSL-2 conditions in a Class II biological safety cabinet where aerosols cannot be avoided.

Fluorescent antibody staining of antigens (DFA). The staining of influenza antigens with fluorescent antibody is an additional rapid test. This method can provide results in less than an

hour. Fluorescent-labeled antibodies specific for influenza A and B viruses are available. Fluorescent antibody staining is generally considered to be more sensitive than rapid antigen tests. Specificity is high, but needs well-trained, experienced technologists. Fluorescent antibody staining can be performed on respiratory specimens from suspected influenza cases under BSL-2 conditions in a Class II biological safety cabinet.

Nucleic acid amplification. Nucleic acid amplification methods, such as RT-PCR and nucleic acid sequence-based amplification (NASBA), are becoming more commonly used for detection of influenza virus and other respiratory viruses. Test turn around time is around 5 hours. These are the most sensitive methods for detection of influenza viruses in general, including H1N1. Specificity depends on selection of the right primers and probes, optimization of amplification conditions, and interpretation of results. These tests need to have specific and strict protocols to avoid false positive results. The RT-PCR testing only for the H1N1 subtype is not recommended. Specimens from suspect cases should be tested for both influenza A and B, and currently circulating influenza A subtypes in addition to H1N1. Specimen processing should be performed within a biological safety cabinet in a BSL-2 laboratory.

Culture. Culture provides highly specific laboratory diagnosis of influenza, but this is considered to be too slow to impact patient treatment or isolation decisions. Culture is essential for detecting influenza infection missed by rapid testing, confirmation of non-culture results when disease prevalence is low, and to obtain isolates for characterization and surveillance. Do not perform culture on specimens if influenza A H1N1 is suspected outside a level III laboratory.

Serology. Serologic test methods to detect influenza virus-specific antibodies are available. These methods include indirect fluorescent antibody (IFA), complement fixation (CF), hemagglutination inhibition (HI) and neutralization. Serology has limited diagnostic value, as you need to collect both acute and convalescent sera to detect sero-conversion or a 4-fold rise in antibody titer.

F. Interpretation and reporting. Communication is the key to success. When a patient presents with suspected swine influenza, communication between the Microbiology laboratories, Molecular Laboratory, Hospital Infection Control, and the Ministry of Health is essential. Specimens from suspected H1N1 influenza cases should be referred to the local public health laboratory, as per Ministry of Health directives.

Finally, is your laboratory ready? To ensure laboratory readiness and capacity to collect, diagnose, and ship patient's samples, it is not essential to have sophisticated techniques. The most important thing is to ensure appropriateness and effectiveness of simple laboratory safety standards. All laboratories, even community based or private laboratories must ensure availability of Personal Protective Equipment (PPE). The appropriate PPE for these types of rapid tests includes: laboratory coat, gloves, eye protection, and face mask (surgical, dental, medical procedure, isolation, or laser masks). There should also be appropriate packages for transportation of dangerous goods along with trained staff on how to pack and transports such specimens. Laboratory staff, especially those working in virology laboratories, should be fit tested for N95 masks as per National Institute for Occupational Safety and Health (NIOSH) regulations. A first level laboratory should be able to collect and transport specimens to reference

centers. A second level screening laboratory can perform simple tests such as rapid antigen detection and/or DFA, but they must have certified Biological Safety Cabinets, and can ship all positive cases to a reference laboratory. A third level laboratory should be able to perform RT-PCR, using approved primers and probes for H1N1. These tests should be performed and approved by a certified scientist in that field, and verification of the test result should be carried out before reporting the result. The local health authorities should be notified, and they should arrange shipping of samples to Centers for Disease Control and Prevention or WHO as per local protocols.

Finally, a level 4 laboratory, should be able to test the samples and sequence the virus to detect its genomic background. This should be interpreted at an international level, to ensure that all viruses from across the globe have the same genetic characteristics and belong to the same strain responsible for outbreak or even pandemics. This must be controlled by the World Health Organization, and approved collaborative centers across the world.

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