PathWAYS
InCyte Pathology News and Information
InCyte Pathology
Diagnostic Excellence
Vol. 7, No. 2, September 2003

From Tissue to Slides: What Happens?

By Stacy Reisenauer, HTL; Bonnie McMahill, HTL; Karen Ireland, M.D.; and Felix Martinez, M.D.

Laboratory turnaround time (TAT) is the interval between the time a tissue sample is received in the laboratory and the final report is issued with the pathologic diagnosis. The industry standard, which is the goal for most laboratories, is 24 to 48 hours. Unfortunately, fast TAT does not provide the best diagnosis for patients if it negatively affects the diagnostic quality of the material being interpreted. A pathologist requires first-rate histologic preparations to provide accurate and specific diagnoses. Specific diagnoses are required for optimal patient care. It is the mission of InCyte Pathology to produce the highest quality of slides with the shortest turnaround time possible.

So what does it take to produce a quality slide, and why does it sometimes take so long for the final diagnosis?

The bulk of production time is for fixation and processing. There is an old saying in Histology, "Junk in; junk out." This means that even the best, most experienced histologist cannot produce a quality slide from tissue that has been fixed inadequately or processed too rapidly. The quality of our work depends first and foremost on the quality of fixation and processing.

Good fixation begins in the physician's office. It is extremely important for tissue to be placed *immediately* in fixative, typically 10% buffered formalin. The tissue can then fix until it is received in the laboratory. Fixation requirements vary from 4-24 hours, depending on the size of the specimen and the density of the tissue. Generally, the longer a tissue fixes, the more efficiently it will process and cut.

After the specimen reaches the laboratory, the next step is the gross evaluation. This includes measuring and describing the specimen, and inking some specimens for orientation. Gross evaluation of most specimens in our laboratory is performed at scheduled intervals. At the InCyte laboratory, gross evaluation occurs in the late afternoon Monday through Friday and in the morning Tuesday through Saturday. Grossing specimens Saturday morning, rather than waiting until Monday morning, speeds up the processing of these late-in-the-week tissues. Very small biopsy specimens can be evaluated in an evening gross session after late arriving courier runs, Monday through Friday.

Following gross evaluation, representative samples of tissue are put into plastic embedding cassettes and placed into a processing machine.

Even the best, most experienced histologist cannot produce a quality slide from tissue that has been fixed inadequately or processed too rapidly.

The processor removes all of the water from the tissue, preparing it to be embedded in paraffin wax. The tissue samples are then triaged into three categories, depending on the size and density of the tissue. Small biopsies from cervix, colon or lung require the shortest processing time of three and one-half hours. Larger, denser and/or fat-rich tissues such as breast needle cores, skin, cervical LEEP biopsies and tonsils require a minimum of six and one-half hours of processing time. Large tissue samples from colon, breast, uterus, placenta, etc., require the longest processing time. These large resection samples require a minimum of ten hours to process adequately.

Is it possible to decrease processing time?

Unfortunately, decreasing the processing time will almost always compromise the quality of the tissue. If processing is not adequate, it is difficult – and sometimes even impossible – to cut a tissue section to place on a slide. Poor tissue sections compromise pathologist interpretation and, ultimately, patient care.

After processing, slide production progresses relatively quickly. The tissue is embedded in paraffin wax and thin sections (3-5 microns thick) are cut and placed on glass slides to dry. The glass slides are then stained routinely with hematoxylin and eosin stains, coverslipped, and sent to the pathologist for diagnosis.

Special staining and additional testing ordered by the pathologist to complete diagnoses may delay the case and require more than one day to make a final diagnosis. Special stains that are ordered in the morning are generally completed and sent to the pathologist for interpretation that afternoon. Immunoperoxidase stains using antibodies are often employed in difficult cases. There are two immunohistochemistry runs daily. Slides on the early morning run will be ready for the courier to take to the hospital pathologist that same afternoon. Slides from the later run are sent out the following morning. Her-2-neu by FISH (for breast cancer) is performed once a week on Wednesday mornings.

To customize our service, courier runs, grossing schedules, and processing schedules have all been carefully and methodically organized to provide the best quality, cost-effective, and timely service for our clients and patients. At InCyte Pathology, we strive for diagnostic excellence. Occasionally, this may result in a longer TAT than anticipated, but our commitment is to provide you and your patients with the highest quality of service available. ▲

Providing correct patient demographics, clinical history, and billing information saves office staff time and helps ensure accurate, reliable, and timely results.

PROFILE

Robert J. Achterberg, MD

Board-certified in oral and maxillofacial pathology and oral medicine, Dr. Robert J. Achterberg joined InCyte Pathology in August. He received his DDS degree from Indiana University School of Dentistry and then completed a Dental General Practice Residency at Andrews AFB, Maryland. Dr. Achterberg later earned an MS degree in oral and maxillofacial pathology from Indiana University, followed by an additional residency at the Armed Forces Institute of Pathology in Washington, DC. Dr. Achterberg comes to InCyte from Creighton University School of Dentistry in Omaha, Nebraska, where he was an associate professor.

With no children and no pets to tie them down, Dr. Achterberg and his wife, Nancy, intend to take advantage of the resources of the Pacific Northwest. Both enjoy hiking and biking, and Dr. Achterberg plans to try his hand at fishing, if Dr. Martinez will be his mentor.

InCyte is excited to have Dr. Achterberg on its professional staff. His proficiency in oral pathology will support InCyte's mission to provide a wide variety of subspecialty expertise to our clients and their patients.

Why Still Report "Reactive" Pap Findings?

By: Felix Martinez, MD

Dating back to the original Papanicolaou class system of reporting, enlargement of nuclei in cells of a Pap smear which are not clearly neoplastic has been reported using a variety of terms (see Table 1).

TABLE 1

Terminology Used To Describe Non-Neoplastic Nuclear Enlargement Within A Pap Smear

Papanicolaou Class II	Non-specific Reactive Changes
Atypia	Benign Cellular Changes
Benign Atypia	Benign Nuclear Enlargement
Inflammatory Atypia	Non-specific Nuclear Enlargement
Reactive Atypia	Proplasia
Reactive Cellular Changes	

Arguments in Favor of the Term "Reactive"

Despite criticism for lack of specificity, the word "reactive" in cytology generally is used to describe alterations in benign cells which are the result of exogenous or endogenous stimuli. The alterations could occur as a result of inflammation, metaplasia, re-growth, radiation, or defects in normal, non-neoplastic maturation. Often, the cause of reactive change remains obscure to the pathologist's eye. Reactive changes may be observed in either glandular or squamous epithelium.

In Papanicolaou smear interpretation, the term "reactive epithelial changes" is often a sign of benign cellular alterations which exceed the appearances of normal epithelium. Reactive changes can be misinterpreted as neoplastic. Since the distinction of neoplastic from non-neoplastic is a basic function of a pathologist, terms to describe such distinction are important.

The Bethesda Classification Was Helpful in Standardizing Reporting Terminology

One of the greatest accomplishments of the Bethesda Conferences for Standardization of Nomenclature in1988 and 1991 was to eliminate the terms "atypia" and "atypical" used to describe reactive cellular changes, reserving these terms only for cases in which there would be concern about a neoplastic or pre-neoplastic lesion.

Bethesda '88 created a general category of "Benign Cellular Changes" (BCC) to encompass reactive cellular changes, the presence of organisms, and other non-neoplastic conditions. The BCC category, however, was confusing because Pap tests falling into the category due to reactive cellular changes were not abnormal enough to be "Atypical" (a category created by Bethesda '88), but were not normal enough to qualify for "Within Normal Limits," either.

In addition to this somewhat ambiguous classification, there have never been national guidelines issued for management of patients with reactive changes in a Pap smear. Therefore, when the option was presented at Bethesda 2001 to combine BCC and "Within Normal Limits" into one negative category, there was almost a unanimous agreement among practitioners and pathologists attending the conference to adopt this proposal.

We feel it important, however, to recognize and document cases which have been clearly separated from the category of "Atypical Squamous Cells of Undetermined Significance." After Bethesda '91, many laboratories began reporting cases formerly termed "reactive" under the category of ASCUS, an alarming misuse of the published criteria set forth to distinguish between the two diagnostic categories.

The Current Approach at InCyte Pathology

In our laboratory, we continued to use the diagnostic category of "reactive changes" for the following reasons:

❶ Reactive terminology was useful in our laboratory as a triage tool and as documentation to satisfy CLIA '88 regulations that stipulated referral of reactive/reparative changes to a pathologist for review.

❷ As a concept and as a reporting vocabulary, reactive terminology establishes a discipline in applying cytologic criteria during primary screening and sign-out.

❸ Documentation of observances of both cytotechnologists and pathologists can be helpful in review of false negative Pap tests resulting from interpretive discrepancy.

❹ Studies have reported a slightly increased incidence of SIL in patients with reactive Pap smears over those diagnosed as "within normal limits." Documentation of reactive cellular changes in the report may allow both laboratory and clinical office to spot trends in a series of Pap smears from one woman.

❺ In retrospective series, the laboratory may be able to identify non-neoplastic cellular changes that do have clinical significance and should be recognized even though there are no current management guidelines.

So, while our laboratory applauds the simplification of reporting accomplished by the merger of "Benign Cellular Changes" and "Within Normal Limits" into one negative category, we chose to exercise our option of including in the report "reactive cytologic changes" if the screening cytotechnologist and reviewing supervisor/pathologist agree that such changes are present. ▲

References

1. Barr Soofer S, Sidway MK. Reactive cellular change; is there an increased risk for squamous intraepithelial lesions? Cancer 1997;81:144-147.
2. Malik SN, Wilkinson EJ, Drew PA, Hardt NS. Benign cellular changes in Pap smears. Causes and significance. ACTA Cytologica 2001;45:5-8.
3. Young, NA. Back to the negative pap test: behind the scenes at Bethesda 2001. Diagnos Cytopathol 2002;26:(4):207-208.

WHO WAS DR. PAP? (Third of Three Parts)

Dr. George Papanicolaou Deserved But Was Never Awarded A Nobel Prize

By Karen M. Ireland, MD and Felix Martinez, MD

Dr. George Papanicolaou was becoming discouraged by the lack of acceptance of his new screening technique. However, he finally collaborated with Dr. Herbert Traut, Jr., a gynecologist at New York Hospital. The results of their studies were published in the *American Journal of Obstetrics and Gynecology* in 1941, "The Diagnostic Value of Vaginal Smears in Carcinoma of the Uterus." Subsequently, Dr. Pap produced an atlas, "Diagnosis of Uterine Cancer by the Vaginal Smear," published in 1943 and supported by the Commonwealth Fund. In 1945, the newly organized American Cancer Society devoted 25% of its budget to promoting the new technique of cervical cytology. The American Cancer Society also sponsored the first national conference on cytology in Boston in 1948. Subsequently, Dr. Pap expanded his technique to the evaluation of sputum, urine, and gastric washings. In his studies of urine specimens, he established extensive morphologic drawings of the cells seen in all aspects of his work, each representing progressive degrees of cytologic abnormality as seen under the microscope. These drawings can still be used today as references for cytologic criteria of malignancy.

In 1960, Dr. Pap was nominated for the Nobel Prize in physiology and medicine. He was ranked third by the Nobel judges and lost out to the inventors of kidney transplantation, Medawar and Burnet. In November 1961, after almost fifty years at Cornell, Dr. Pap accepted the directorship of the Cancer Research Institute at the University of Miami. Unfortunately, on February 19, 1962, Dr. Pap died at Jackson Memorial Hospital of a myocardial infarction. He was buried in Clinton, New Jersey.

In 1995, the government of Greece issued a $10,000 drachma bank note in honor of George Papanicolaou. He was subsequently honored by three different nations by having his visage placed on postage stamps. In 1978, then-First Lady Rosalyn Carter presented a postage stamp issued in the U.S. to Mrs. Papanicolaou at the White House.

A brief acknowledgment must be made to Mary Papanicolaou, Dr. Pap's extremely supportive and long-suffering spouse. She functioned as his unpaid technician for years because Cornell would not allow a husband and wife to be employed in the same department. He often introduced her as "my wife and my victim," acknowledging the innumerable cervical smears he had obtained from her. The American Cancer Society presented her with a special award in March, 1969, in recognition of her numerous contributions. She died October 13, 1982, in Miami Beach.

Thus, from the humble beginnings of an interest in the sex life of sea urchins, the study of genital squamous cells evolved to become a screening test that has saved innumerable women's lives. ▲

References

Vilos, G.A., After Office Hours: The History of the Papanicolaou Smear and the Odyssey of George and Andromache Papanicolaou. Obstet Gynecol 91: 479-483, 1998.

Valente, P.T., The Life of Dr. George M. Papanicolaou and the Origins of Cytology. ASCP, Spring 2002 teleconference series, program # 3165, June 6, 2002.

InCyte Pathology, P.S.
P.O. Box 3405 • Spokane, WA 99220-3405

PathWAYS

InCyte Pathology News and Information

Vol. 7, No. 2, September 2003

PathWays is a periodic publication of

InCyte Pathology, P.S.
P.O. Box 3405 • Spokane, WA 99220-3405
(509) 892·2700 • 1·888·814·6277

© 2003 by InCyte Pathology

This publication may not be reproduced or copied without permission of InCyte Pathology.

InCyte Pathology is an independent professional service company of pathologists that provides interpretation of biopsies and cytology preparations.

Circulation List

provided for your convenience

PathWays has items of interest for office personnel and assistants as well as for physicians, nurse practitioners, nurses and physician assistants. We recommend that, upon completion of circulation, your copy of ***PathWays*** be filed in the InCyte Pathology *Anatomic Pathology Services Manual* for future reference.

❑ 1. ____________________

❑ 2. ____________________

❑ 3. ____________________

❑ 4. ____________________

❑ 5. ____________________

❑ 6. ____________________