Peterson's Press

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UP FRONT

DIAGNOSIS OF ONYCHOMYCOSIS BY SPECIAL STAIN

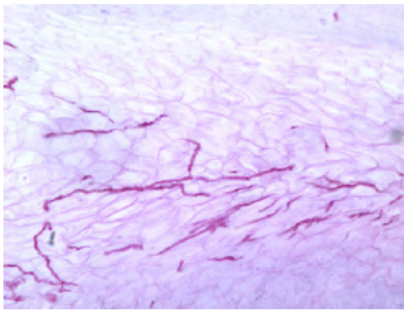
Peterson Laboratory Services has been performing special staining (PAS and the equivalent-GMS) to assist in the diagnosis of onychomycosis since 1965. To order, submit the specimen in a formalin container accompanied by a pathology requisition marked "special stain." Turn-around time for special stain diagnosis is 24 hours, compared to a fungal culture turn-around time of 4-6 weeks. Both PAS and GMS stains are also highly specific, as detailed in the following article and abstract.

USE OF PERIODIC ACID-SHIFF (PAS) REACTION FOR THE DIAGNOSIS OF DERMATOPHYTOSIS

Bradley Bakotic, DPM, DO
Institute for Podiatric Pathology

PAS reaction is not a recently developed laboratory test. Rather it is a test that has been used for many decades to aid in the diagnosis of diseases as disparate as lupus erythematosis, collagenous colitis, and acute erythrocytic leukemia. PAS reaction is performed by exposing tissue and various substrates to a series of oxidation-reduction reactions. As an end result, positive elements, such as carbohydrates, basement membrane material, and mucosubstances, become candy-apple red. These PAS-positive components contrast sharply against the dull pink-blue background. As the result of recent research boasting its effectiveness, PAS reaction has become an increasingly common test for the diagnosis of dermatophytosis. Its utility in this regard is based upon the ability of the PAS reaction to highlight the carbohydrate-rich cell wall of dermatophytes and various other fungi. This allows for the identification of even rare fungal elements within tissue. Because dermatophytes cannot be visualized with routine histopathologic stains (H&E), the performance of such a test is imperative. PAS reaction is the ideal adjunctive test in that it allows for both histopathologic analysis and fungal identification

How does PAS reaction compare to other testing methods?
Sensitivity: For decades, the most common test for the diagnosis of onychomycosis has been culture. This test is commonly performed on either a dermatophyte testing medium (DTM) or on Sabourauds dextrose agar. With the implementation of Clinical Laboratory Improvement Amendments (CLIA) regulations of the last decade, the use of a DTM has fallen off sharply; however, commercial laboratory-based culture remains an extremely common means of diagnosing onychomycosis. In recent years, investigators have compared the sensitivity of culture with that of potassium hydroxide (KOH) and PAS reaction. In a report published in the Journal of the American Podiatric Medical Association (JAPMA) in 2001, several colleagues and I noted that PAS reaction was significantly more sensitive than both KOH and culture. In a comparison of 50 cases of clinically suspected onychomycosis, we found that PAS reaction was, by far, the most sensitive test, yielding a p-value of 0.01. Even more dramatic results were published in the Journal of the American Academy of Dermatology by Weinberg, et al. (abstract follows). In this study of 105 cases, investigators found PAS to be profoundly more sensitive than culture with a p-value of 0.00002 (2). The reported sensitivity of culture was 58% versus PAS reaction at 92%. This means that fewer than six of every ten cases of bona fide onychomycosis resulted in a positive culture (2).



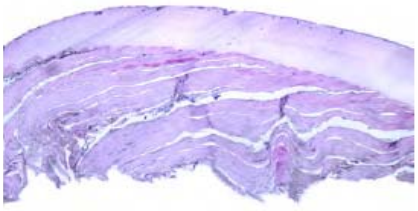
(Fig. 1 PAS reaction demonstrating bright red fungal hyphae)

Why is PAS reaction so much more sensitive than culture? This may be attributed, in part, to the fact that large numbers of living fungi are required to yield a positive culture. In contrast, PAS reaction will allow for the identification of rare fungal elements, even when no longer living.

NEWS AND NOTES

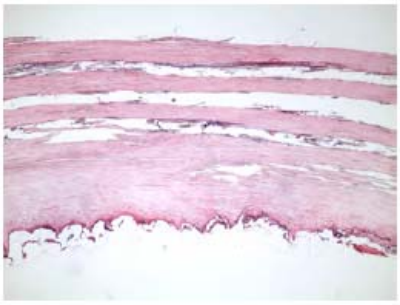
Specificity: One of the purported benefits of culture has been its ability to identify the genus and species of the organism in question. Some pathology laboratories go a step further by recommending a "drug of choice" based upon the reported sensitivity of the identified organism. Unfortunately, the high specificity attributed to culture is based upon a false premise -- that the organism that is grown from the submitted tissue is, in fact, the offending organism. Just as cultures grown from samples derived from diabetic ulcers may be entirely misleading, so, too, may biopsies taken from the nail unit. Of particular concern is the high number of false-positive cultures for contaminant saprophytic molds, as this may lead to inappropriate medical management.

In a landmark study recently submitted for publication by Dr. William Scherer, nail unit biopsies were split and sent to competing podiatric pathology laboratories, where KOH and culture were performed. Shockingly, he noted that KOH and culture results derived from the split samples were completely concordant in only 27% of the cases. Taken a step further, the podiatric pathology laboratories agreed on the appropriate medication in only 47% of the cases (3).



(Fig. 2 Nail dystrophy as the result of psoriasis)

These data seem to confirm that the high specificity attributed to culture is fallacious. PAS reaction does not allow for speciation in most cases; however, histopathologic observations may provide clues with regard to the causative organism. For instance, when delicate boring hyphae overwhelmingly predominate, the offending organism is a dermatophyte in nearly all instances. Alternate histopathologic features may favor a saprophytic mold, or Candida specia. Apart from its high sensitivity, possibly the chief benefit of PAS reaction pertains to cases that are *PAS-negative*. In roughly 25-30% of the cases of clinically suspected onychomycosis, fungal elements are not seen by PAS-reaction. PAS reaction is the only commonly used diagnostic technique that allows a dermatopathologist to directly examine the tissue in question and make an assessment regarding possible nonfungal causes of nail dystrophy. Conditions such as psoriasis (Fig. 2), onychoschizia (Fig. 3), spongiotic dermatitis, and onycholysis may be suggested based solely on histopathologic findings. In addition, traumatic nail dystrophy is not unusual in runners and persons with long second digits or varus fifth digits. We have



(Fig. 3 Pathologic splitting of the nail plate; onychoschizia)

seen verruca, molluscum contagiosum, Bowens's disease (squamous cell carcinoma in situ), and melanoma mimic onychomycosis.

How does one best obtain a sample for histopathologic analysis and PAS reaction?

It is imperative for clinicians to be aware that simply obtaining a small fragment of distal nail plate is not an appropriately harvested sample. Within the nail unit, just as elsewhere on the body, dermatophytes exhibit an active border of growth and tend to burn out in their wake. For this reason, simply removing a small fragment of distal nail will result in low sensitivity. Clinicians should obtain generous amounts of tissue, sampling as proximally as possible. Because, in many cases, fungi are most numerous within the hyperkeratotic debris of the superficial nail bed, this should also be sampled, using a curette when necessary. Areas of persistent inflammation, such as the nail plate in or around a paronychia, should not be sampled in isolation, as fungal growth may be limited due to the associated inflammatory response.

References:

1 Borkowski, P., M. Williams, J. Holewinski, and B. W. Bakotic. 2001. Onychomycosis: An analysis of 50 cases and a comparison of diagnostic techniques. *Journal of the American Podiatric Medical Association* 91:351-55.

2 Weinberg, J. M., E. K. Koestenblatt, and L. Najarian. 2003. Comparison of diagnostic methods in the evaluation of onychomycosis. *American Academy of Dermatology* 49:193-97.

3 Scherer, W. P., and M. D. Scherer. In press. A comparison of results from two mycology laboratories for the diagnosis of onychomycosis. *Journal of the American Podiatric Medical Association*.

Editors note: The abstract of the Weinburg article cited by Dr. Bakotic appears below.

COMPARISON OF DIAGNOSTIC METHODS IN THE EVALUATION OF ONYCHOMYCOSIS (Abstract)

Weinberg JM, Koestenblatt EK, Tutrone WD, Tishler HR, Najarian L.

J Am Acad Dermatol. 2003 Aug;49(2):193-7

BACKGROUND: Onychomycosis is a common problem seen in clinical practice. Given the differential diagnosis of dystrophic nails, it is helpful to obtain a definitive diagnosis of dermatophyte infection before the initiation of antifungal therapy. Potassium hydroxide (KOH) preparation and fungal culture, which are typically used in the diagnosis of these infections, often yield false-negative results. Recent reports have suggested that nail plate biopsy using periodic acid-Schiff (PAS) (Bx/PAS) stain may be a very sensitive technique for the diagnosis of onychomycosis.

OBJECTIVE: The purpose of this study was to compare KOH preparation, culture, Bx/PAS stain, and calcofluor white (CW) stain in the diagnosis of onychomycosis and to determine their sensitivity and specificity.

METHODS: We evaluated 105 patients with suspected onychomycosis using 4 diagnostic methods: KOH preparation, culture, Bx/PAS, and CW stain. CW stain binds to cellulose and chitin, and fluoresces when exposed to UV radiation. It is a highly sensitive and specific technique for the detection of dermatophytes. To determine the clinical usefulness and performance characteristics of each test, CW was chosen as the gold standard for statistical analysis.

RESULTS: Of the patients, 93 had at least 1 of the 4 diagnostic methods positive for the presence of organisms. The following were calculated for each test: sensitivity; specificity; positive predictive value; and negative predictive value. The sensitivities of each of the techniques were as follows: KOH 80%; Bx/PAS 92%; and culture 59%. Both KOH and Bx/PAS methods were more sensitive than culture (P =.00002). Bx/PAS was also more sensitive than KOH (P =.03). The specificities were as follows: KOH 72%; Bx/

PAS 72%; and culture 82%. The positive predictive value calculated for the different techniques were: KOH 88%; Bx/PAS 89.7%; and culture 90%. In terms of negative predictive value, the results were: KOH 58%; Bx/PAS 77%; and culture 43%.

CONCLUSION: Bx/PAS is the most sensitive method for the diagnosis of onychomycosis. It is also superior to the other methods in its negative predictive value. It is indicated if other methods are negative and clinical suspicion is high, and potentially is the single method of choice for the evaluation of onychomycosis.

CMS TECHNICAL CLARIFICATION REGARDING ICD-9 UPDATE

On June 26, CMS published a clarification to Transmittal 1531, Change Request (CR) 6084, "Laboratory NCD Edit Software Changes for July, 2008."

CR 6084 includes the addition and/or deletion of more than 70 ICD-9 CM codes and affects nine of the 23 laboratory NCDs:

- HIV Testing
- Blood Counts
- Prothrombin Time
- Serum Iron Studies
- Glycated Hemoglobin/Glycated Protein
- Thyroid Testing
- Gamma Glutamyl Transferase
- Hepatitis Panel/Acute Hepatitis Panel
- Fecal Occult Blood Test

The implementation date was July 7, 2008. To read the transmittal, please visit http://www.cms.hhs.gov/transmittals/downloads/R1531CP.pdf

UPCOMING INFECTIOUS DISEASE TELECONFERENCES

How do laboratory managers find the time to keep themselves and their staffs up-to-date on all the advances in the field? We recognize that busy schedules and lean staffing make it difficult to obtain new information. PLS regularly offers live teleconferences on current laboratory advances. Scheduled in the near future are:

Advance Understanding of Hepatitis Laboratory Testing: Thursday, August 21, Bella Mercy Restaurante 1, MRHC, 1823 College Ave, Manhattan, KS

Topics will include: atypical results and/or unusual patterns for acute and chronic hepatitis B and C, including case studies, an introduction to other hepatitis viruses, such as hepatitis D and hepatitis E, co-infections, flares, reactivations, monitoring therapy, and follow-up laboratory testing for chronic hepatitis patients.

2008 Influenza Update: Tuesday, November 18, 2008; Location TBA

Overview of influenza and available diagnostic methods. The significance as a public health issue, expectations for the coming season and the status of novel strains worldwide will also be described.

Pre-registration information will be sent directly to your facility. For now, please mark your calendar to join us!

HISTOLOGY QUALITY VARIANCE REPORT

Patient safety and result accuracy guide our daily practices. CLIA regulations require that we document ordering and/or labeling errors, as well as how those errors were resolved.

The requisition form must contain patient name, date of birth, date and time specimen collected, ordering physician name, and specimen source (site/side of body).

The specimen label must contain patient name, specimen source (site and side of body, physician name and date.

If there is missing or conflicting information, we are required to return the specimen to you noting the discrepancy, accompanied by a "Histology Quality Variance Report.

Please complete the report as required and return with the specimen for processing. We cannot process the specimen without the completed form.

The discrepancy on original specimen receipt, as well as how the discrepancy was resolved, by whom and on what date becomes part of the result report.

SPECIMEN SUBMISSION REMINDER

The window of time for accurate and complete pathology diagnosis is very short in the absence of appropriate tissue fixative. Without appropriate fixative, tissue begins very quickly to autolyze (decay).

Please ensure that tissue specimens are preserved in the appropriate fixative (normally formalin) at a ratio of 10:1, or at least covered. Additionally, rinse large specimens with water. Excess blood or other body fluids dilute formalin's fixative properties.

Page four of the Surgical Pathology Reference Manual provides additional packaging guidelines. As always don't hesitate to give us a call if you have questions.

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HISTOLOGY QUALITY VARIANCE REPORT

Date: _____ Accession Number: _____

Patient Last Name: _____ First Name: _____

Date of Birth: _____ Sex: _____ Physician: _____

Facility: _____

Describe the problem and corrective action taken:

_____

_____

_____

_____

Person Spoken to: _____ Time: _____ Signature: _____

Corrective action taken by client (to be filled out by client if specimen has been returned):

_____

_____

_____

Name (please print): _____ Signature: _____

IN THE SPOTLIGHT

L to R: Rachelle Haller, Laboratory/Data Technician; Julie Fitch Quiring, CT (ASCP), Cytotechnologist; Troy Parmely, CT(ASCP), MT(AMT), Cytotechnologist; and Shirley Brungardt, Laboratory Technician

We are proud to introduce to you the skilled and friendly team who process and result more than 23,000 of your genital and non-genital cytology tests each year.

Cytology interpretation is subjective, so the skill and experience of the pathologists, cytotechnologists and laboratory technicians are critical to quality results. Peterson Laboratory pathologists are Board Certified. Read on to learn more about the cytology department member's qualifications:

> *"They are the most client-friendly group to be found — very accommodating! We really like how the reports are laid out, they are clear, easy to read, bold headings, the patient phone number is right there for our follow-up. Turn around is fast and they reflex our abnormals (ASCUS Paps) to HPV without us having to initiate a new order."*
>
> *Lotti Porsi, RN, FHSU Student Health Center, Hays, KS*

Julie Fitch Quiring, CT(ASCP), Cytology Department Supervisor: Julie was raised just a mile north of the Oklahoma border, in Caldwell, KS. Before devoting her career to PLS clients, she worked for her alum - KU Med—and Providence St. Margaret Hospital in Kansas City. Her son is working on an RN degree at Neosha Community College and her daughter is attending the University of Minnesota. Julie was recently recognized for her 25 year career at PLS. Career dedication runs in the family - her husband, Jay, has completed 40 years and counting with Burnett Automotive.

Troy Parmely, CT(ASCP), MT(AMT): In addition to nearly 20 years experience interpreting genital and non-genital cell cytology, Troy brings a wide perspective from the many locations he has worked as a cytotechnologist/medical technologist. Although Lawrence, KS, is his hometown, Troy traveled widely with the US Army after completing his training at Ft. Sam Houston, San Antonio, TX Troy has three children-two sons and a daughter. When not at PLS, you will find Troy on the golf course or rooting for KU sports.

Shirley Brungardt: Using a combination of experience, skill and state-of-the-art instrumentation, Shirley creates slides from genital and non-genital specimens for the cytotechnologists and pathologists to review. Shirley has gifted the lab and clients alike with her abilities and sunny personality for nine years. As a native of Ellis County, KS, she is the "go-to" source for German cooking and recently become a "poster person" for the Ellis County Oktoberfest! Prior to applying her skills and talents here at PLS, she excelled as a certified nurse aid and physical therapy assistant at Hays Regional Health Center. Four children (including twins) and five grandsons enjoy her special attention.

> *"Their attention to clinical detail, reading the Pap smears and maintaining quality control measures have helped them maintain their standard of excellence.*
>
> *Their system of patient recall is also superior to their competitors. Patients who have failed to follow-up in the office are identified at screening intervals of any abnormal Pap smear results, so further action can be taken before more time has elapsed.*
>
> *Cathy Mih-Taylor, M.D., ACOG Mih-Taylor Women's Clinic, Chanute, KS*

Rachelle Haller: Rachelle is the newest member of the cytology department, and much valued for her data management skills. The monthly or annual cytology statistical reports you may have elected to receive are created by Rachelle. In addition to keying all genital and non-genital cytology information from your requisitions into APEasy, Rachelle also assists in processing non-genital specimens and provides morning courier service for local clients! Rachelle was born and raised just outside Randolph with her twin sister and older brother.

Service Highlights: Normal cytology case findings are signed-out by our experienced cytotechnologists. If abnormal cells are found (approximately 15% of the total cases reviewed), the case is then reviewed by a pathologist. Again, an exceptional feature of our quality assurance program is that 100% of all pathology, non-gyn cytology and abnormal genital cytology cases are over-read. In addition, 10-15% of all normal/negative cases are read by two cytotechnologists. Corresponding cytology (Pap) and pathology (curettage, colposcopy/biopsy) slides are reviewed side-by-side for correlation of findings.

The Pap turnaround time of 3-5 days far exceeds the national standard (14 days). Four tests can be performed from a liquid-based Pap test — Pap, HPV, Gonorrhea and Chlamydia.

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Fall 2008, Volume II Issue 3 Editor: Maureen Bruening Jensen, MS