

Guthrie Medical Group
POLICY & PROCEDURE

SECTION: Department	SUBJECT: PPM Procedure – KOH Non-Vaginal Specimens	DEPT. Point-of-Care Testing Regional Labs
EFFECTIVE: 7/27/2016		POLICY# GMG-700-2022
SUPERCEDES: 3/04		PAGE # 1 of 4
DISTRIBUTION: PPM permitted offices		

I. INTENDED USE:

Provider-performed microscopy (PPM) is a testing modality that requires the use of a microscope and is performed by physicians and/or nonphysician practitioners during the patient's visit. Microscopic observation of clinical specimens allows for rapid detection of the presence of bacterial, fungal and parasitic organisms.

KOH preparation detect the presence or absence of fungi in specimens containing keratinous material such as skin, hair and nails. KOH digests the cellular components of the host cells and other contaminants leaving the fungal cell wall intact allowing the fungal elements to be clearly observed. Fungal infections of the skin, hair and nails are commonly caused by *Tinea species*, *Trichophyton rubrum* and *Candida albicans*.

II. EQUIPMENT, REAGENTS and SUPPLIES

Product	Epro #	Vendor	Manufacturer
Glass Slides, Frosted	11611	Cardinal Health	Cardinal
Glass Coverslip (glasses) 22x22	12236	Cardinal Health	Cardinal
KOH 10% Dropper	19780	Cardinal Health	Remel
70% Alcohol Wipe	1389	Kendall Health	Cardinal
Gloves			
Sharps Container			
Microscope	Contact the Regional Office Lab Coordinator		
Scalpel blade			

III. QUALITY CONTROL:

External quality control materials are not available for PPM testing. Staff competency assessment will be used to verify reliability of patient test results.

IV. SPECIMEN COLLECTION

Prior to specimen collection, perform hand hygiene and put on gloves.

A. Skin Scrapings

1. Using a 70% alcohol pad, clean the affected area to remove surface bacterial contaminants.
2. Using a scalpel blade, scrape the active border (usually the periphery) for small flakes of skin. The affected area is often erythematous. Be careful not to contaminate scrapings with blood.
3. Place the skin scrapings between two clean glass slides or in a sterile container.

B. Hair

1. Collect at least 10-12 affected hairs with the base of the shaft intact.
2. Place in a sterile container.

C. Nail Scrapings

1. Collect a nail clipping.
2. Place nail clipping in a sterile container.
3. Cut nail clipping into small fragments.
4. Scrape the excess keratin procedure under the nail.

V. KOH PROCEDURE

1. Label a clean microscope slide with patient information
2. Place the specimen on the slide and add one drop of 10% KOH directly on top of the specimen.
3. Do not use KOH beyond expiration.
4. Wait up to 30 minutes to allow cellular tissue and debris to dissolve. Note: The exact time needed will depend on the thickness of the specimen fragments.
5. Immediately put a coverslip over the specimen for examination.
6. Examine the slide to verify that it is not overfilled and leaking once the coverslip is in place.

VI. KOH MICROSCOPE FINDINGS and INTERPRETATION

Examination

1. Place slide on microscope for examination and focus using low power (10X) and low light.
2. Scan the entire slide.
3. Identify objects using high power (40X).
4. Read at least 10 fields using an "S" shaped viewing pattern.
5. Record findings as the presence or absence of yeast or hyphae.
6. Dispose of the slide into an appropriate sharps container.

VII. INTERPRETATION: KOH Preparation Microscopic Findings

1. The superficial dermatophyte fungi of the genera *Microsporum*, *Trichophyton*, and *Epidermophyton* most commonly cause itching, scaling lesions, known as tinea or ringworm. Infected skin scrapings show small and regular hyphae 2-3u in diameter, some branching. Rectangular arthrospores are sometimes seen.
2. Scaling or crusting lesions confined to the moist intertriginous areas of the skin (between finger, toes, or beneath breasts) suggest yeast infections with *Candida* species. *Candida* shows as a colorless, ovoid, thick-walled cell, approx. 5-7u. A cell with a single bud is characteristic. *Candida* species also form elongated cells (pseudohyphae) with distinct points of constriction simulating link sausages.

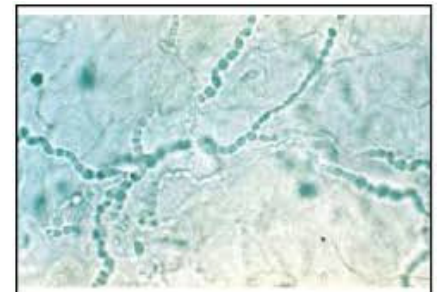
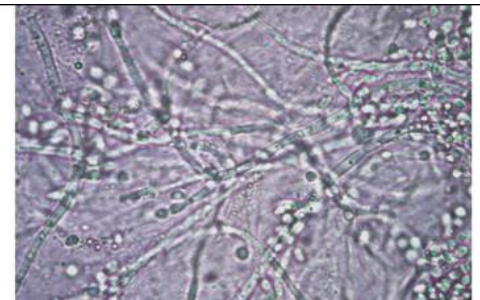
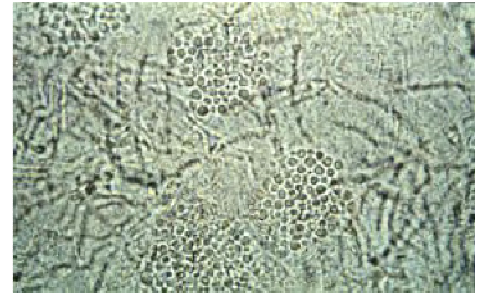


Fig-2: KOH preparation of skin scraping shows septate branching mycelium with arthroconidia

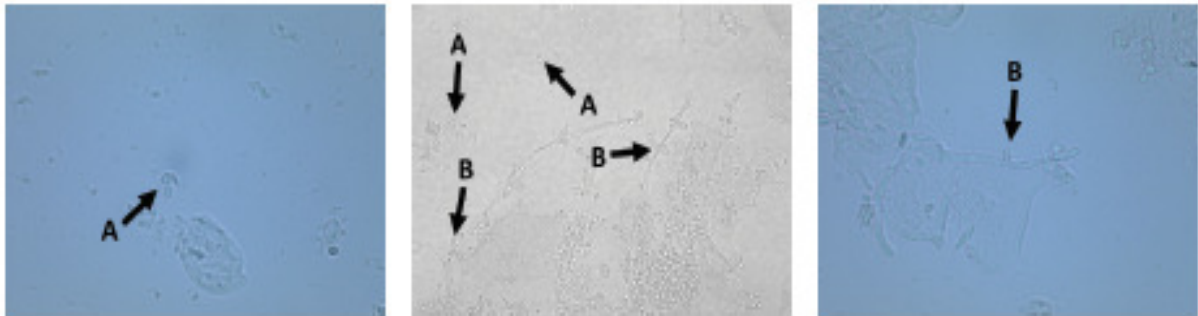


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3. Superficial scaling lesions, varying in size, shape and color, of the back suggest tinea versicolor. The slide preparation shows presence of hyphal elements 2.5-4u in diameter and of variable length, along with oval or round cells 3-7u in diameter.



4. Yeasts are unicellular fungi that appear commonly in vaginal discharge wet mounts. Yeasts are larger than bacteria, approximately the size and shape of the nuclei of epithelial cells. Individual cells of yeast propagate by budding out similar cells from their surface. In PPM observations, yeasts are indicated by a loose arrangement of budding cells. In many fungi, the budding cells remain attached to the parent cell resulting in an elongated filament-like string of attached cells, pseudohyphae, with a cell budding off the tubular structure. In the images below, the arrows indicated by "A" are examples of yeast and the arrows indicated by "B" are examples of pseudohyphae.



VIII. REPORTING RESULTS:

Record finding the patient's electronic medical record using the enter / edit Epic activity. Refer to procedure GMG-700-2030. The test should be reported as:

- Negative – No fungal elements observed.
- Positive, presumptive for dermatophytic fungi.
- Positive, presumptive for *Candida* species.
- Positive, presumptive for tinea versicolor.
- Positive for fungus – additional specimen referred to Clinic Laboratory for testing.

IX. NOTES:

Although screening for fungus in skin scrapings may yield useful, diagnostic information, it is essential that the limitations of its practice be acknowledged and understood.

1. *Candida* species "other than *albicans*" *Saccharomyces* species, *Rhodotorula* species, and *Trichosporon*, all yeasts have been implicated in infections involving the skin, especially in immunocompromised hosts. Due to often "very slight" morphologic differences among them, definitive identification should be done by a microbiologist, usually in conjunction with a culture. The provider must decide when the situation warrants going further than "presence of budding yeast, probable *Candida*."

2. Other opportunistic fungi as Aspergillus, Penicillium, Fusarium, members of the Class Phycomycetes, may also be seen in scrapings. From a superficial lesion that has been properly cleansed, its clinical significance is unlikely. If the patient has an underlying immunocompromising condition however, it should be referred for additional testing.
3. Due to seriousness of fungal infections in specimens “other than skin” and the difficulty in recognizing and differentiating subtle differences in all the human pathogens, all other specimens (sputum, eyes, lesions, etc.) should be processed by the Microbiology Dept.

X. REFERENCES:

1. CLSI Physician and Nonphysician Provider-Performed Microscopy Testing: Approved Guideline-Second Edition POCT 10-A2 ; 2011
2. Wisconsin State Laboratory of Hygiene, Proficiency Test Photos, 2012
3. Bauer, John M.D. Clinical Laboratory Methods, 8th ed. C.V. Mosby Company, St. Louis, 1974: 50-60.
4. Todd, Sanford and Davidsohn, Clinical Diagnosis and Management by Laboratory Methods, 16th ed. W.B. Saunders Co. Philadelphia, 1985: 559-629
5. OnFocus, internet site, accessed 8/4/2014 <http://onfocus-laboratories.software.informer.com/>
6. CMS Provider Performed Microscopy Procedures, A Focus on Quality Practices, Feb. 2016.

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Date: 3/3/2004, revised images 8/4/2014, 6/10/2016, 6/30/2016
Revised Procedure Tonya Wilhelm, MT 7/26/2016

Key Contact: POCT Coordinator, Laboratory Medical Director

Reviewed and Approved by: _____
Laboratory Medical Director* Date

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*Original signed document is available in the Regional Laboratory Coordinator’s Office