**LRGHealthcare**

**LABORATORY PROCEDURE**

**Unless otherwise noted, this policy applies to Lakes Region General Hospital and Franklin Regional Hospital.**

**Title:** KOH and Wet Prep Testing Provider Performed Microscopy (PPM)

**Effective Date**: 9/13  **Date Last Revised**:

**Approved by:**

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**Distribution:** Point of Care Testing (POCT) – Provider Performed Microscopy (PPM)

**Policy:** To provide instructions for KOH and Wet Prep testing.

**Principle**:

Microscopic examination of unfixed “wet mounts” of clinical specimens may be useful for the rapid detection of the presence of bacterial, fungal, and parasitic organisms. A number of well-recognized pathological conditions may be identified. Fungi are usually larger than bacteria and in material from skin, hair or nails, they can be seen by direct microscopy, provided the material is first softened and cleared with a strong alkali such as 10% (w/v) potassium hydroxide (KOH). The purpose of the alkali is to digest the keratin surrounding the fungi so the hyphae and conidia (spores) can be seen.

**Specimen type**: Swabs of the vaginal mucosa and vaginal pool, skin scrapings, hair clippings, scrapings or plucking.

**Supplies**:

Glass slides

Cover slips

Cotton-tipped swabs

Capped sterile tubes

pH paper

Specimen container

70% ethanol

Microscope

Sterile saline

KOH 10%: NOTE: highly corrosive. Use appropriate PPE including gloves and eye/face protection.

**Specimen collection**:

*Skin Scraping*:

1. Remove all powders, ointment and creams using 70% ethanol.
2. Allow the area to dry.
3. With a blade scrape the skin area:
	1. Scales on the area bordering the “normal” skin.
	2. Warty knots with black dots should be scraped at and around the dot.
	3. Blisters should have their lids removed and the contents of the blister as well as the scraping collected.
4. Scrape into a clean vessel for transport or onto clean colored paper if the area’s shape will not allow direct collection into a vessel. Scraping should occur until the dermis is exposed and bleeding begins.
5. If paper was used in the collection, transfer the contents of the paper into a clean vessel.
6. Securely cap the vessel.
7. Label the vessel with the patient’s name, date of birth and date and time of collection.

*Nail Clipping and Scraping*

1. Using clippers, remove the nail to the bed.
2. Using a blade or clippers, clip or shave the nail and nail bed at any separation site or white patch.
3. Place clippings and shavings of the nail and/or the nail bed into a clean vessel.
4. Label the vessel with the patient’s name, date of birth, date and time of collection.

*Hair collection*

1. Using a clean hemostat, grasp 30-50 hairs in the region most affected or if bald spots are occurring, the areas most adjacent to the bald spot.
2. If the hair bulb is required, using the hemostat with a quick sharp tug, pluck the hairs from the head.
3. If the bulb is not required, with a clean blade, scrape the skin and cut the hairs.
4. Place the hemostat and hairs in a clean vessel for transport.
5. Securely cover the vessel.
6. Label the vessel with the patient’s name, date of birth, date and time of collection.

*Vaginal*

1. Collect a sampling of vaginal material with two sterile cotton swabs passed through and along the area of concern.
2. Place the swabs in a clean glass test tube containing 0.5 mL of normal saline (0.9%) labeled with the patient’s name, date of birth, date and time of collection.
3. Gently swish the swabs in saline to dispel material into the solution.

**Specimen handling:**

Vaginal:

Do not refrigerate and examine within ½ hour of collection.

Non-Vaginal KOH procedure:

1. If appropriate transfer the specimen to a labeled clean glass slide.
2. Place one drop of 10% KOH on the specimen.
3. Check for a “fishy” amine odor. “Fishy” odor indicates anaerobic bacterial growth.
4. Allow the slide preparation to rest for 5 -30 minutes to allow cellular tissue, skin, and other debris to dissolve. Maintain the slide in a moist environment to prevent drying of the specimen.
5. Cover with a cover slip.
6. Examine the slide with the 10x objective for epithelial cells and any budding yeast of psuedohyphae. Under high power (40x) look for smaller blastospores.
7. Heating will speed the dissolving process, especially for skins and nail scrapings. Any gentle heat source is usable. Nail clippings may require overnight soaking in 10% KOH to completely clear.

Vaginal Procedure:

1. Prepare two clean glass slides labeled with the patient’s name, date of birth, collector’s initials and date and time of collection.
2. Place one drop of specimen on each slide.
3. To one slide add one drop of 10% KOH solution.
4. Check the KOH slide for a “fishy” odor.
5. Allow the KOH slide preparation to rest for up to five minutes to allow cellular tissue and other debris to dissolve. Maintain the slide in a moist environment to prevent drying of the specimen. Heating will speed the dissolving process, especially for skins and nail scrapings. Any gentle heat source is usable.
6. Cover both slides with a cover slip.
7. Examine the saline slide with the 10x objective for epithelial cells and any budding yeast or psuedohyphae.
8. Examine the saline wet mount with the 40x objective and quantify organisms and cells per HPF using the following:

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| Quantification, Direct Exams |
| Rare | Less than 10 organisms or cells/slide |
| 1+ | Less than 1 organisms or cell/HPF |
| 2+ | 1 to 5 organisms or cells/HPF |
| 3+ | 6 to 30 organisms or cells/HPF |
| 4+ | Greater than 30 organisms or cells/HPF |

1. Examine the slide with the 10x objective for epithelial cells and any budding yeast of psuedohyphae. Under high power (40x) look for smaller blastospores.

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|  | Bacterial Vaginosis | Vaginal Candidiasis | Trichomonas Vaginitis | Desquamative Inflammatory Vaginitis | Reference Values |
| WBC | Rare or absent | 3+ to 4+ | 2+ to 4+ | 3+ to 4+ | 2+ |
| Lactobacilli | Rare or absent | Present | Present/absent | Reduced/absent | Absent |
| “clue cells” | >20% | Absent | Absent/present |  | Absent |
| Other Cells |  | Large clumps of epithelial cells |  | Occasional parabasal/basal cells | Absent (except red cells during menses) |
| Other organisms | Increase in small curved bacilli, coccobacilli and pleomorphic bacilli | Budding yeast psuedohypahe | Trichomonas | 2+ bacteria | Other lactobacilli subgroups occasional yeast |
| Other findings | pH>4.5 “Fishy” odor present, homogenous vaginal discharge |  | pH >4.5. | pH >4.5 Vaginal erythema | pH <4.5 |

**Reference:**

CLSI: Physician and Non-physician Provider Performed Microscopy Testing; Approved Guideline, 2nd Edition

Date Originated: 09/13

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Date Revised: