

**Title: PPM procedure**

- Cross References: PPM Competency Assessment
- PPM Quiz
- PPM Urine Sediment Quiz
- PPM Fern Test Patient Result Log
- PPM KOH and Wet Prep Patient Test Log
- PPM Urine Sediment Patient Result Log
- MGH POCT Inter-Laboratory Correlation Procedure
- MGH POCT QC Storage Ordering and Documentation Guide



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Date: 11/5/12

## Purpose

This document outlines policies and procedures pertaining to the tests described. In an effort to be concise, some information from the manufacturer's recommended procedure may be excluded. It is recommended that operators familiarize themselves with the manufacturer's product information that accompanies each package and their manual, if one exists.

## Scope

Level of Personnel: All MD's, NP's and certified Nurse Midwives who have successfully completed training and maintained annual competency.

Testing Site: All sites approved and on file with the Pathology Services POCT Division

Approved Tests: 10% KOH with or without Swartz Enhancement, Vaginal Wet Prep, Fern Test, and Urine Sediment

## Policy and Procedure Statement

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

The formed elements suspended in the urine are precipitated by centrifugation and analyzed under the microscope. Cellular elements and casts often give valuable information as to the pathology of urinary tract disease, and the detection of metabolic or systemic diseases not directly related to the kidney.

Examination of vaginal secretions, skin, hair, and nails with a 10% KOH (Potassium Hydroxide) or KOH w/Swartz-enhanced solution is utilized as an aid in diagnosis and classification of various yeast and fungal infections. As a strong alkali, 10% KOH digests the keratin surrounding the fungi so that the hyphae and spores can be seen.

Wet mount preparations are used to detect the presence of bacterial, fungal, or parasitic organisms, and other cellular elements indicative of pathological conditions (i.e. clue cells and polymorphonuclear leukocytes).

Examination of vaginal secretions for the presence of amniotic fluid is known as the fern test. In conjunction with patient history and vaginal fluid pH, the fern test is used to determine if the amniotic sac had ruptured. Due to the protein and sodium chloride content, amniotic fluid crystallizes to form a fern pattern when allowed to air-dry on a microscope slide.

## Proficiency Testing

The College of American Pathologists (CAP) and the WSLH sends unknown samples to the laboratory for analysis several times per year. Results are submitted to the PT agency within 10 days of survey receipt. If a site fails 2 out of 3 events or two consecutive events according to federal law, it may be required to discontinue testing.

- ◆ All Survey results are to be handled and reported in the same manner as clinical results following the directions on the survey package insert. The samples are not to be analyzed in duplicate unless clinical specimens are analyzed in duplicate. Actions or decisions must be documented.
- ◆ Participation must be random and not assigned to specific individuals. Successful participation may be used as demonstrating successful competency for that year.
- ◆ Upon receiving the survey:
  - The POCT program will contact Vincent's OB/GYN regarding the survey and the timeline for submitting the results.
  - The departments must be available within the period identified by POCT.
  - The Key operators must make sure of the following:
    - Instruments (if applicable) are in good working order.
    - Randomly select staff to participate, but ensure that subsequent surveys are rotated among different staff (e.g. document in a log)

- ◆ Once results are obtained, they should be given to the POCT Coordinators who will send them to the PT provider via mail, fax or electronic entry on the PT provider's website.
- ◆ Site Director and CLIA certificate Director or designees shall review survey results to assess performance and ensure compliance with the standard and comment.
- ◆ After the survey results are received by the POCT Program, the survey is then sent to all other sites performing PPM as an alternate proficiency challenge through medtraining.org.
- ◆ Scores of 100% minimally requires documentation of review by the Director or designee.
- ◆ Scores between 100% and 80% requires a comprehensive investigation and remedial action documented of unsuccessful challenges.
- ◆ Scores less than 80% requires a comprehensive investigation and documentation of remedial action of unsuccessful challenges. Scores of less than 80 percent may jeopardize a site's ability to continue to perform testing.
- ◆ Should a site fail proficiency, they will be required to immediately perform a comprehensive investigation and document remedial action. Operator re-training may be required.
- ◆ In order to avoid cessation of testing, a site failing a challenge will be expected to develop and implement a more aggressive plan for performance improvement.
- ◆ Each site is responsible for completing survey challenges when they arrive.

**Anticipated Survey Periods:**

<b>Provider Performed Microscopy (PM)</b>	
<b>Product Receipt</b>	<b>Evaluation receipt</b>
January	February
July	August

**Regulatory Requirements**

I. All test results must be maintained in patient records with all required information for four years.

Required information:

1. Patient's name
2. Medical Record Number
3. Patient's gender
4. Patient's age or date of birth
5. Date & time test collected, performed and reported
6. Ordering Physician
7. Responsible physician (if not 6)
8. Reference or Target Range
9. Test Performed
10. Test units
11. Lab name

II. Additional information that must be retained for four years:

1. Testing personnel records
2. Product information (i.e. serial number, lot numbers, expiration dates, etc.) and any remedial action
3. Microscope maintenance sheets and reference ranges

III. Other

1. Universal precautions must be observed when handling any patient specimen.
2. A physician's order or policy statement detailing standard of care is required prior to performing test.
3. The Hospital Hand Hygiene policy must be adhered to at all times.

## Competency Assessment

All operators must read the procedure manual. Competency assessment is done bi-annually during the first year, and annually thereafter.

Competency is assessed using the four applicable methods listed below:

1. Review of proficiency testing  
Review of alternate PT assigned through medtraining.org
2. Problem solving skills as appropriate to the job – written quiz
3. Monitoring, recording and reporting of test results - Transcription review of patient results
4. Direct observation of instrument function – peer observation of microscope use, specimen collection and labeling of slides.

## Test Kit/Supplies/Equipment

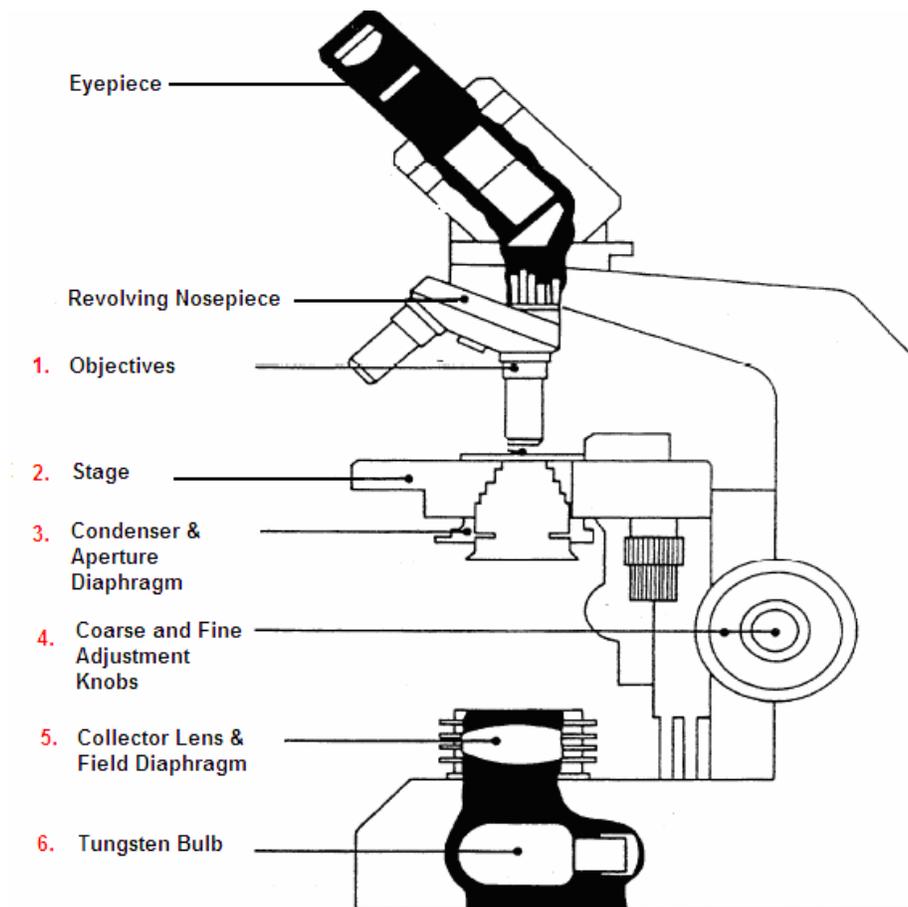
Product	Vendor	Manufacturer #	PeopleSoft#	Comments
Physiologic Saline (NaCl 0.9%), sterile				
75mm X 25mm plain glass slides, non-sterile.	Friedrich & Dimmock, Inc.	BMS-005-05-030-50	142042	
22mm X 40mm glass coverslip glass.	Electron Microscopy Sciences	70466-30	148899	.
KOH 10% Dropper bottle	Fisher or MGH Pharmacy			Store at room temp.
10% KOH (Swartz Enhanced Potassium Hydroxide) solution.	MGH Pharmacy			Store at room temp.
Sterile Swabs	Becton-Dickinson	4360210		
Grave Speculum	EM Adams	EM Adams	132789	
Conical urinalysis centrifuge tube with caps				
Plastic pipettes				
Centrifuge with locking lid	Contact the POCT Program for recommendations			Maintenance required at least annually
Microscope with a direct light source.	Contact the POCT Program for recommendations			Maintenance required at least annually

# The Microscope

Achieving an accurate result with microscopic techniques requires an understanding of the operating characteristics and limitations of the equipment used. Therefore, it is recommended that the manufacturers' instructions are read prior to using the microscope.

## I. Parts of the Microscope

1. Objective Lenses: Used to magnify the specimen a defined amount ( e.g. 10X, 40X, 100X)
2. Stage: Horizontal platform on which the specimen slide is placed
3. Condenser:
  - Mounted under the stage to concentrate and focus light from the Tungsten bulb
  - Raising or lowering it adjusts the amount of contrast and resolution
  - Contains an *aperture diaphragm*, which can be opened or closed to control the amount of light striking the specimen
4. Coarse and Fine Adjustment Knobs: Used to bring the slide into focus by moving the stage toward or away from the objectives. The coarse adjustment moves the stage in large increments and the fine adjustment causes subtle movement.
5. Collector Lens: Better microscopes have a *field diaphragm* on the collector lens. The adjustment of this diaphragm increases or decreases the circle of light in the viewing field
6. Tungsten Bulb: A built-in light source that can be changed by the operator when it burns out. The light intensity can be controlled with the dimmer.



## II. How to use the Microscope:

- a. Place a properly labeled specimen slide (i.e. 2 patient identifiers) on the stage (2).
- b. Lower the condenser and turn the lamp to low. Open both the aperture diaphragm (3) and the field diaphragm (5).
- c. With the 10X objective in place and while observing from the *side of the slide and not through the eyepiece*, use the coarse adjustment knob (4) to slowly raise the stage until the slide comes close to the objective.
- d. Next, look through the eyepiece and use the coarse and/or fine adjustment until the image is sharpest.
- e. Close the field diaphragm (5) almost completely and raise the condenser until the edges of the diaphragm are sharply focused (the condenser is usually at its highest position). Then, open the field diaphragm slowly, stopping just as it disappears from view.
- f. Open and close the aperture diaphragm to optimize contrast. Contrast is increased by closing the aperture. If more light is needed, turn up the lamp.

**The high (40X) objective:**

- a. Focus and center the specimen with the 10X objective, and rotate the nosepiece slowly to bring the 40X objective into the light path.
- b. Use the FINE adjustment knob to bring the specimen into focus.

**NOTE:** Never raise the stage with the coarse adjustment knob when using the 40X lens. Doing so may cause the lens to hit the slide and break.

**The 100X oil immersion objective:**

- a. Focus and center the specimen with the 10X objective, followed by the 40X objective; lower the stage using the coarse adjustment, with the 40X objective in the light path.
- b. Place a drop of immersion oil on the coverslip of the slide, directly over the light path and beneath the objective. Rotate the nosepiece until the 100X oil immersion objective comes into the light path.
- c. Look at the stage from the side and not through the eyepiece. Slowly raise the stage until the objective makes contact with the oil drop. Look through the eyepiece and use the fine adjustment knob to bring the specimen into focus
- d. When finished, lower the stage and rotate the nosepiece to the 10X objective to prevent oil from inadvertently touching the 40x objective. Clean the 100x objective with a lens paper dampened with lens cleaner.

## Maintenance

Microscope maintenance is performed annually by contract with: DSC Optical Services  
PO Box 67204  
Chestnut Hill, MA. 67204  
(Tel): 617-332-8200  
(Fax): 617-332-8201

In addition, microscopes should be inspected, cleaned, and checked a minimum of once per week.

**NOTE:**

- Cover the microscope when not in use and leave the 10X objective in position
- Remove excess lotions, powders, or emollients from hands before use.

## Vaginal Wet Prep

A vaginal wet mount (sometimes called a vaginal smear) is a test to find the cause of vaginitis, or inflammation of the vagina and the area around the vagina.

## Specimen Collection

Collect a sampling of vaginal material with a sterile cotton swab passed through and along the area of concern. Place the swab in a clean glass test tube labeled with two patient identifiers (e.g. EPIC label) and containing approximately 0.5 ml sterile saline. The appearance of the saline solution prior to the addition of the specimen should be clear and colorless. If not, obtain a fresh sample. Swirl the swab vigorously in the saline to dislodge any particulates.

## Testing Procedure

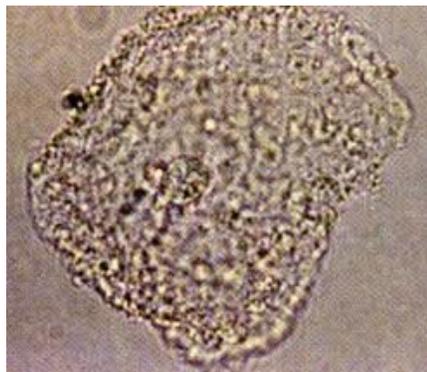
Perform hand hygiene and put on gloves.

1. Place a drop of patient specimen mixed with saline on a clean glass slide labeled with two patient identifiers. Cover with coverslip.
2. The microscope condenser must be lowered prior to reading all specimens.
3. Examine the slide carefully for epithelial cells completely covered with bacteria (Clue cells) and for the presence of hyphae, budding yeast, or mobile trichomonads.

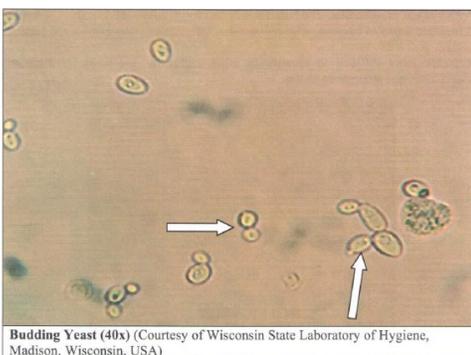
**Microscope slides must be labeled with two patient identifiers (e.g. EPIC label) per hospital policy.**



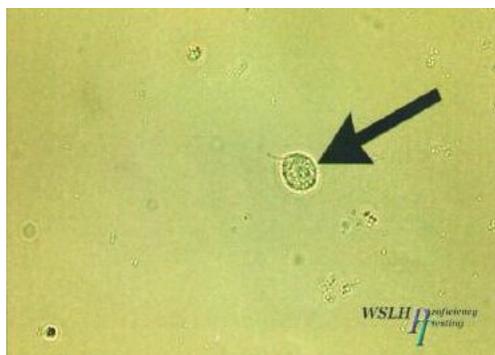
**Normal Epithelial Cells**



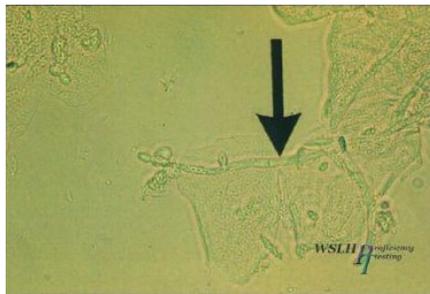
**Clue Cell**



**Budding Yeast**



**Trichomonas vaginalis**



**Hyphae on Epithelial cells**

## Reporting results

Report as presence or absence of clue cells/ hyphae or yeast/ trichomonads:

- in the patient's record
- on a patient log or
- on another approved permanent record

## Limitations

- Non-motile trichomonads could be mistaken for white blood cells. To preserve the motility of the organisms, the specimen should not be refrigerated but examined immediately.
- Failure to vigorously swirl the swab in the saline to dislodge particulates may lead to erroneous results.
- Oil droplets from intravaginal medications may be mistaken for yeast. Oil droplets show great variation in size and are highly refractile. Yeast cells often show budding.

## Quality Control Monitoring

Traditional quality control for the Vaginal Wet Prep is not required. Typical result examples are available for reference.

## References

1. CLSI Physician and Nonphysician Provider-Performed Microscopy Testing; Approved Guideline-Second Edition. POCT10-A2 ; 2011
2. Wisconsin State Laboratory of Hygiene, Proficiency Test Photos, 2012

## KOH with or without Swartz Enhancement

10% KOH is a strong alkali used to digest cellular elements and other contaminants that would otherwise obscure the presence of fungal elements (e.g. pseudohyphae and yeast). KOH with Swartz enhancement improves viewing of the negative space and light refraction.

### Specimen Collection

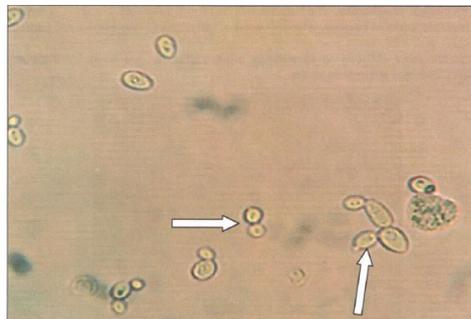
Vaginal specimens: Collect a sampling of vaginal material with a sterile swab passed through and along the area of concern. Place the swab in a properly labeled (i.e. 2 patient identifiers or EPIC label) clean glass test tube containing approximately 0.5 ml sterile saline and twirl it vigorously in the saline to dislodge any particulates.

Non-vaginal specimens: Specimens should be placed immediately on a clean, glass slide labeled with two patient identifiers. Cotton swabs should not be used to prepare the slides because cotton strands may resemble fungi.

**Microscope slides must be labeled with two patient identifiers per hospital policy.**

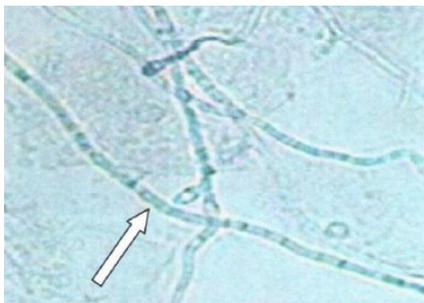
### Testing Procedure

1. Perform hand hygiene and put on gloves.
2. Place one drop of specimen to a clean glass slide labeled with two patient identifiers.
3. Add one drop of KOH solution (standard or enhanced) to the specimen.
4. Check the slide immediately for a "fishy," amine odor and note presence or absence. (The odor indicates anaerobic bacteria overgrowth.)
5. Allow the slide preparation to rest for up to 5 minutes for vaginal specimens and up to 30 minutes for other specimens to allow cellular tissue and other debris to dissolve. Gentle heating may aid in clearing the mount.
6. Cover with a coverslip.
7. Scan under low power (10X) for budding yeast and pseudohyphae and under 40X (high power) to look for smaller fungal elements.



**Budding Yeast (40x)** (Courtesy of Wisconsin State Laboratory of Hygiene, Madison, Wisconsin, USA)

#### Budding Yeast



**Pseudohyphae in Wet Prep (400x)** (Courtesy of Wisconsin State Laboratory of Hygiene, Madison, Wisconsin, USA)

#### Pseudohyphae (w/o KOH)



**Pseudohyphae on KOH Prep (66x)** (Courtesy of the College of American Pathologists, Northfield, Illinois, USA)

#### Pseudohyphae with KOH

## Reporting results

Report out presence or absence of either yeast or hyphal elements:

- in the patient's record
- on a patient log or
- on another approved permanent record

## Limitations

- KOH can grow fungus if stored beyond the expiration date. Do not use if solution appears cloudy.
- Cotton strands from the swab may resemble fungal elements.

## Quality Control Monitoring

Traditional quality control for the KOH test is not required. Typical result examples are available for reference.

## References

1. CLSI Physician and Nonphysician Provider-Performed Microscopy Testing; Approved Guideline-Second Edition. POCT10-A2 2011

## Fern Test Examination of Amniotic Fluid

Amniotic fluid will crystallize when allowed to dry on a glass slide and will form a fern pattern. Vaginal secretions that do not contain amniotic fluid will not show a fern pattern.

## Specimen Collection

1. Perform hand hygiene and put on gloves.
2. Collect vaginal secretion from the posterior vaginal pool with a sterile swab. Avoid the use of any lubricants or antiseptics, because these may interfere with the test.
3. Do not touch the mucus plug in the cervix.
4. After collection, immediately roll the saturated swab tip across the slide while applying slight pressure to express the fluid. Spread the specimen to create a very thin smear.
5. Allow slide to air dry for 5 to 7 minutes. Do not blow air or heat the slide to shorten dry time. Do not apply a coverslip.

**Microscope slides must be labeled with two patient identifiers (e.g. EPIC label) per hospital policy**

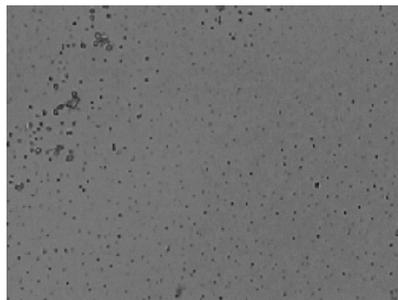
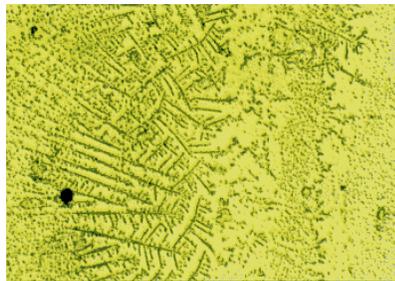
## Testing Procedure

1. Examine the dried smear under low power (10X) without a cover slip.
2. If present, the amniotic fluid crystallizes to form a fern-like pattern due to the relative concentrations of sodium chloride, proteins, and carbohydrates in the fluid. Examine all fields on the slide thoroughly.
3. If uncertain about the ferning pattern, examine under high power (40/45X).
4. Dispose of slide into an appropriate sharps container.

**Positive Ferning**



**Positive Ferning**



**Negative for Ferning**

## Reporting results

Record results in one of the following:

- in the patient's record
- on a patient log or
- on another approved permanent record

## Limitations

- The Fern Test should be performed in conjunction with a pH test. (See MGH Phenazine pH Procedure)
- If the pH test and Fern Test are both positive, probable membrane rupture has occurred.
- If the pH test is negative but the Fern Test is positive, there is probable rupture of the membranes due to the Fern Test's greater specificity.
- If the pH Test is positive but the Fern Test is negative, a second specimen should be collected and tested.
- False positive results may occur from specimens contaminated with blood, urine, or cervical mucus, which also "ferns" but usually in a coarser pattern.
- False negative results may occur from prolonged rupture of the membranes (longer than 24 hours).
- False negative results may occur if only a small volume of fluid has leaked.

## Quality Control Monitoring

No commercially prepared controls are available for the fern test. Typical result examples are available for reference.

## References

1. Addison, Lois Anne. *Laboratory Medicine*, July, 1999, Vol 30, No.7, p451.
2. CLSI Physician and Nonphysician Provider-Performed Microscopy Testing; Approved Guideline-Second Edition. POCT10-A2 ; 2011
3. Vincent Memorial Obstetrics Service, Fern Test Procedure

## Examination of Urine Sediment

The microscopic examination of urine is the most common testing procedure used for the detection of renal and/or urinary tract disease.

### Specimen Collection

Collect a FRESH urine specimen in a clean, dry container and labeled with two patient identifiers (e.g. EPIC label) per hospital policy. Specimens that are held unrefrigerated for more than 2 hours should be rejected. Casts, RBC's, and WBC's are susceptible to lysis when the specific gravity is <1.010 and in alkaline urine (pH>7.0).

### Test Procedure

1. Perform hand hygiene and put on gloves
2. Pour 12 mL of well-mixed urine into a properly labeled (i.e. EPIC label) centrifuge tube.
3. Place centrifuge tube in the centrifuge and spin at 400 RCF (1500-2200 rpm) for 5 minutes. (Refer to the operator's manual for specific RPM equivalent to 400 RCF).  
**NOTE:** Higher RCF and longer centrifugation times, although useful in recovering cells, will break up cellular casts.
4. Carefully decant the supernatant into a biohazard-designated sink or receptacle.
5. Gently resuspend the sediment in the remaining supernatant.
6. Using a transfer pipette transfer a drop of sediment onto a properly labeled slide, then cover with a cover slip. Allow urine to settle for 30-60 seconds before examining.
7. Examine the urine sediment on low power for crystals, casts, and epithelial cells. Count 10 fields and report the average number per low-power field. Lower light intensity is required to see hyaline casts and hyphal forms.
8. Examine the sediment on high power for RBC's, WBC's and renal tubular cells. Count 10 fields and report the average number of cells per high-power field. Note the presence of bacteria, yeast, trichomonads and mucus.

### Elements to be reported:

**WBC:** None, 0-2, 3-6, 4-8, 5-10, 10-20, 20-40, 30-60, >100 Packed (per HPF). Also report WBC clumps.

**RBC:** None, 0-2, 3-6, 4-8, 5-10, 10-20, 20-40, 30-60, >100 Packed (per HPF). Grossly bloody samples should be forwarded to the Clinical Laboratory evaluation.

**Epithelial cells:** Few, Moderate, or Many (per HPF).

**Crystals:** Amorphous, uric acid, calcium oxalate, triple phosphate, tyrosine, cystine, unidentified. In amounts: Few, Moderate, or Many (per LPF).

**Bacteria:** Few, Moderate, or Many (per HPF).

**Casts** (read under low power): Hyaline, Granular, WBC, Epithelial, RBC, Hgb, Broad, Waxy, or Mixed.: 0-5, 5-10, 10-20, 20-50, 50-100, >100 per LPF).

**Yeast:** Few, Moderate, or Many.

**Mucus:** If present

**Other:** Report any additional information

### Reference Ranges

**WBC:** Negative. Occasional (<5/hpf)

**RBC:** Negative. Occasional (<5/hpf)

**Epithelial:** Negative. Few may be present in urine from males, larger numbers in urine from females.

**Crystals:** Negative. Some crystals precipitate after the sample cools. Crystals are of little clinical significance except for cystine, leucine, tyrosine and cholesterol. The type of crystal depends largely upon the pH of the freshly voided urine.

- Bacteria:** Negative. Bacteria are of little significance except in fresh or catheterized specimens. Gram stain and culture may be utilized for identification.
- Casts:** Negative.
- Yeast:** Negative.

## Reporting Results

Record results in one of the following:

- in the patient's record
- on a patient log or
- on another approved permanent record

## Limitations

An inaccurate reading may be caused by one or several of the following errors made in specimen collection or technique:

- Specimen not obtained by "clean catch" method and thus contains elements from sources other than the urinary tract (e.g., vaginal discharge, penile discharge)
- Specimens that are held unrefrigerated for more than 2 hours
- Specimen not centrifuged long enough or longer than 5 minutes
- Urine extremely dilute so no sediment obtained, or not enough elements available in amount of urine tested.
- Specimen not examined with proper lighting or focusing
- Microscope not functioning properly, e.g., lens dirty
- Examiner fails to recognize the elements on the slide

## Centrifuge Maintenance

Refer to the manufacturer's operator's manual for care and maintenance schedules specific to the particular centrifuge in use. As a general guideline, the following steps should be taken for all centrifuges:

1. Unplug the centrifuge from its electrical source before carrying out preventative maintenance or cleaning.
2. Daily disinfect the outside of the centrifuge with a hospital approved disinfectant.
3. At least monthly clean the interior chamber with the manufacturer-recommended cleaner. Rinse thoroughly to prevent residue from causing corrosion.
4. After thorough cleaning, run the centrifuge at varying speeds and check the braking mechanism to ensure a smooth gradual stop.
5. Provide adequate ventilation by placing the centrifuge on a hard smooth surface.
6. Always spin a balanced load
7. The rotor, buckets and shields should be examined for signs of mechanical stress (e.g. cracks, corrosion)
8. The accuracy of the timer, verification of speed control settings, and verification of RPM are critical to satisfactory operation. Consult the Operators Manual for the frequency for checking these parameters.

Centrifuge service providers currently in use at MGH are:

1. Precise Instruments
2. Northeast Instrument Service
3. Wilkinson Associates

## References

1. Bauer, John M.D. Clinical Laboratory Methods, 8<sup>th</sup> ed. C.V. Mosby Company, St. Louis, 1974: 50-60.

2. Davstar Industries Ltd. One Newport Place, 1301 Dove Street, Suite 360, Newport Beach, CA.
3. Todd, Sanford and Davidsohn. Clinical Diagnosis and Management by Laboratory Methods, 16<sup>th</sup> ed. W.B. Saunders Co, Philadelphia, 1985: 559-629
4. CLSI Physician and Nonphysician Provider-Performed Microscopy Testing; Approved Guideline-Second Edition. POCT10-A2; 2011
5. Riddle ,Peter. Centrifugation: a guide to equipment use and maintenance. The Biomedical Scientist, February 2013.
6. Centra-CL2 Instruction Manual
7. Fisher Healthcare Laboratory Centrifuge, Model 614B, Operator's Manual
8. CLSI Laboratory Instrument Implementation, Verification, and Maintenance; Approved Guidelines. GP31A, April 2009
9. LW Scientific C5 Instruction Manual