| Title: Manual Urine Microscopic Exam |
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| SOP# UA-SOP-3.2 | Related Procedures: UA-SOP-1; UA-SOP-2 |
| File Name: S:\P&LMS\Greenville HCC Lab\6. Urinalysis\UA-SOP-3.2 Manual Urine Microscopic Procedure.docx | Effective Date:8/30/14 |

**SOP HISTORICAL RECORD**

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| DATE | WRITTEN/REVISED BY | SUPERVISOR REVIEW | MD/PhDAPPROVAL/REVIEW |
| May 12, 2014 | Lauren Ashley | Heather Duncan | Dr. Elizabeth Boswell (8/30/14) |
|  6 November 2014 |  Heather Duncan |  |  |
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| REVISION DATE | REVISION DESCRIPTION |
|  11/6/14 | Add detail to interpretation and result reporting  |
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**Manual Urine Microscopic Exam**

1. **Principle and Significance:** This procedure is to provide standardized instructions to perform manual urine sediment microscopic examination. Urine is centrifuged and the sediment is examined for insoluble materials and structures such as cells, casts, crystals, and bacteria that are present.

The type and number of formed elements in urinary sediment may give valuable diagnostic information about the presence, type and localization of pathological processes within the urinary tract.

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| **Element** | **Clinical Significance** |
| **RBCs** | Increased numbers may indicate renal hematuria. |
| **WBCs** | Increased numbers may indicate renal disease or acute infection. |
| **Epithelial cells** | A few epithelial cells are normal and indicate normal sloughing off of aging cells. Increased numbers may indicate renal disease, urinary tract infection, or poor technique in specimen collection. |
| **Bacteria** | A few bacteria are normally seen due to poor technique in collection of the specimen. Increased numbers may indicate kidney, bladder, or urinary tract infection. |
| **Mucus** | Has no clinical significance. Mucus is a protein material produced by the glands and epithelial cells of the lower genitourinary tract, and is more frequently seen in female specimens. |
| **Spermatozoa** | Increased numbers are found post ejaculation; usually not pathological. |
| **Trichomonas** | Normally not found in urine. Presence of Trichomonas is abnormal and may indicate infection. |
| **Yeast** | Increased numbers indicates infection. |
| **Hyaline casts** | Increased numbers are seen with renal diseases and transiently with exercise, fever, congestive heart failure, and diuretic therapy. |
| **Waxy casts** | Waxy casts are commonly associated with tubular inflammation and chronic renal failure. They are also found during acute or chronic renal allograft rejection. |
| **Granular casts** | Granular castsare semitransparent cylinders containing small (fine) or large (coarse) granules. These granules represent plasma protein aggregates. Granular casts appear with glomerular or tubular diseases. |
| **Fatty casts** | These casts consist of semitransparent or granular cylinders containing large highly refractive vacuoles or droplets. Visible fat droplets are triglycerides or cholesterol esters. These are commonly seen with heavy proteinuria and are a feature of nephrotic syndrome. |
| **Crystal Casts** | Crystal casts consist of crystalline inclusions in a semitransparent or granular cylinder. These casts indicate disposition of crystals in the tubule or collecting duct.  |
| **Hemoglobin (Blood) Casts** | These casts are associated with glomerular disease. |
| **Myoglobin casts** | These casts are red-brown in color and occur with myoglobinuria following acute muscle damage. |
| **Bilirubin and other drug casts** | Bilirubin is seen in urine when there is obstructive jaundice, and will color casts as deep yellow brown. Drugs such as phenazopyridine (Pyridium) cause a bright yellow to orange color in acid urine and will color casts and cells. |
| **Erythrocyte (Red Blood Cell) casts** | Disorders associated with the presence of erythrocyte casts in the sediment may include acute glomerulonephritis, IgA nephropathy, lupus nephritis, sub acute bacterial endocarditis, and renal infarction. |
| **Cystine Crystals** | Associated with inherited metabolic disorder (cystinuria). |
| **Tyrosine Crystals** | Associated with liver disorders. |
| **Leucine Crystals** |
| **Bilirubin Crystals** |
| **Cholesterol Crystals** | Associated with disorders that produce lipiduria, such as nephrotic syndrome. |
| **Sulfonamide (Sulfadiazine) Crystals** | Associated with sulfonamide medication use. Usually forms when patient is inadequately hydrated. |

1. **Sample Requirements:**

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| **Sample Type****(Urine)** | **Tube Type** | **Minimum Volume**  | **Optimal Volume** | **Storage Temperature** | **Stability** |
| * Clean Catch
* Catheter Collection
* Random
* Suprapubic aspiration
 | Non-Additive (Sterile Urine Container) | 1 mL | 12 mL | Ambient | 2hrs |
| 2ºC to 8ºC | 24hrs |

**Criteria for Rejection**

* Specimens received > 2 hours after collection (if not refrigerated)
* Improperly labelled specimen (must have two complete unique patient identifiers)
* Visible signs of contamination (such as fecal contamination)
* Samples containing preservatives or additives
* Quantity not sufficient for analysis
* Specimens refrigerated >24 hours
1. **Materials:**

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| **Materials**  |
| Urine Sample | Slides |
| Urine tubes  | Pipette  |
| Caps | Centrifuge |
| Cover slips |  |

1. **Safety:** All patient specimens should be considered potentially infectious and must be handled with precautions used for human bodily fluids, as described by CDC recommendations and in compliance with the Federal OSHA Blood borne Pathogen Standard, 29CFR part 1910.1030. Follow specimen handling as outlined by laboratory safety policy.
* Wear gloves and a lab coat.
* Wear safety glasses if there is a risk of splashing.
* Do not ingest reagents.
* Refer to SDS for specific handling instructions for reagents in the event of exposure.
1. **Procedure:**

**Manual Urine Microscopic Procedure**

* Use appropriate image and literature resources to assist with identification when needed.

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| **Step** | **Action** |
| **1** | Process any required chemical screen testing before centrifuging the urine. * Refrigeration may cause precipitation of amorphous urates, amorphous phosphates, and other non-pathological crystals that can obscure other elements in the urine sediment.
 |
| **If** | **Then** |
| Excessive crystals are present (Sample will be very cloudy) | Sample may be warmed to 37°C prior to centrifuging.* This may dissolve excessive crystal formed due to refrigeration of the sample.
 |
| **2** | Place a capped tube containing the urine specimen in the centrifuge and spin for 5 minutes at 1500-1800 rpm. |
| **3** | Remove the tube from the centrifuge, being careful not to shake the tube.  |
| **4** | Decant the tube by pouring off the supernate with a single quick motion. **Note**: As a general guideline, 0.5 to 1.0 mL of sample (including sediment) should remain in the bottom of the tube. |
| **5** | Let the remainder drain down the tube and re-suspend the sediment by shaking/flicking the tube with the finger. The sediment can also be mixed with a transfer pipette. |
| **6** | Place a drop of mixed sediment on a slide. **Note:** Ensure that the drop is not overly large. With very large drop size, the coverslip will float when added to the slide. |
| **7** | Place a coverslip on the drop. |
| **8** | Observe a minimum of 10 phase low power (10x) fields to obtain the average number of the following elements per low power field:* Casts
* Mucus
* Epithelium
* Crystals
* Amorphous Materials

**Notes:** Using the dark field on the microscope is useful in finding hyaline casts. Casts are often found around the edge of the cover slip. |
| **9** | Observe a minimum of 10 high power (40x) fields to obtain the average number of the following elements per high power field:* RBC ( If TNTC report as >100)
* WBC ( If TNTC report as >100)
* Squamous and Transitional Epithelial cells
* Renal tubular epithelial cells
* Crystals
* Bacteria
* Yeast
* Other elements
 |
| **10** | **If** | **Then** |
| Seldom-seen abnormal crystals are observed (tyrosine, leucine, bilirubin, cholesterol) | * Ask another technologist to confirm your observations.
* Consult supervisor if consensus is not reached.
* Notify provider if these crystals are present.
 |
| Questionable elements are observed | * Ask at least one other technologist to review the slide.
* Consult supervisor if consensus is not reached.
* Arrive at a consensus before reporting the results.
 |
| **11** | Report observations in VistA. |

***Note: It is important to focus up and down as the slide is multi-planar and this will allow one to see the depth of the element as well as the different focal planes. The use of the phase contrast on the microscope can aid in the identification of urinary elements. Subdued light is suggested to provide adequate contrast.***

1. **Interpretation:**

**Result Correlation**

* Review the chemical analysis and correlate microscopic sediment findings with macroscopic results. Test results that do not correlate must be repeated. Any discrepancy must be resolved before results can be reported. It may be appropriate to ask for a recollection.
* Crystal formation is formed by precipitation of urine solutes. Factors such as pH, temperature, and concentration impact the quantity and type of crystal formation. Crystals may be seen in normal or abnormal urine.

The following positive chemistries generally correlate with microscopic findings:

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| **Chemical Analysis** | **Microscopic Analysis** |
| Blood | RBCs |
| Protein | Microorganisms, Cast |
| Nitrite | Bacteria |
| Leukocyte esterase | WBCs, casts |
| Acidic pH | Crystals |
| Normal Crystals | Abnormal Crystals |
| Amorphous UratesUric AcidCalcium Oxalate | Cystine Tyrosine Leucine Sulfonamide (Sulfadiazine)Cholesterol |
| Alkaline pH | Bacteria, crystals |
| Normal Crystals | Abnormal Crystals |
| Amorphous PhosphatesTriple PhosphatesAmmonium BiuratesCalcium Phosphates | None |

**Result Quantification**

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| **Test** | **Result** |
| * RBC
* WBC
* Squamous and Transitional Epithelial cells
* Renal tubular epithelial cells
* Oval Fat Bodies
 | Reported as the average number/HPF* For WBC and RBC, report cells too numerous to count as >100
 |
| Casts | Reported as the average number/LPF |
| Bacteria | **Quantity Observed** | **Report As** |
| No Bacteria Seen | None Observed |
| 1-2 organisms/HPF | Few |
| 2-5 organisms/HPF | 1+ |
| 5-10 organisms/HPF | 2+ |
| >10 organisms/HPF | 3+ |
| WBC Clumps (containing large quantities of WBC) | **Quantity Observed** | **Report As** |
| 1-2 clumps/HPF | Few |
| 2-5 clumps/HPF | 1+ |
| 5-10 clumps/HPF | 2+ |
| >10 clumps/HPF | 3+ |
| Budding YeastHyphenated Yeast | **Quantity Observed** | **Report As** |
| No Yeast Seen | None Observed |
| 1-2 yeast/HPF | Rare/Occasional |
| 2-5 yeast/HPF | Few |
| 5-10 yeast/HPF | Moderate |
| >10 yeast/HPF | Abundant |
| Trichomonas | **Quantity Observed** | **Report As** |
| 1-2 trich/HPF | Few |
| 2-5 trich/HPF | 1+ |
| 5-10 trich/HPF | 2+ |
| >10 trich/HPF | 3+ |
| Amorphous Phosphate CrystalsAmorphous Urate CrystalsCystine Crystals | **Quantity Observed** | **Report As** |
| 1-2 crystals/HPF | Rare |
| 2-5 crystals/HPF | Few |
| 5-10 crystals/HPF | Moderate |
| >10 crystals/HPF | Abundant |
| Amorphous CrystalsUric Acid CrystalsCalcium Oxylate CrystalsTriple Phosphate Crystals | **Quantity Observed** | **Report As** |
| 1-2 crystals/HPF | Few |
| 2-5 crystals/HPF | 1+ |
| 5-10 crystals/HPF | 2+ |
| >10 crystals/HPF | 3+ |
| Other Crystals not in VISTA (tyrosine, leucine, sulfonamide, cholesterol, ammonium biurates, calcium phosphates, etc.) | Free text quantity and identity into the comments section under the report |
| Sperm* Report only for male patients
 | Free text quantity and identity into the comments section under the report using the interpretation guide below: |
| **Quantity Observed** | **Report As** |
| 1-2 sperm/HPF | Rare |
| 2-5 sperm/HPF | Few |
| 5-10 sperm/HPF | Moderate |
| >10 sperm/HPF | Abundant |

**Note:** If seldom-seen abnormal crystals are observed (tyrosine, leucine, bilirubin, cholesterol), ask another technologist to confirm your observations. Consult supervisor if consensus is not reached and notify the provider if these crystals are present.

1. **Result Reporting**

There are no critical values associated with this test.

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| **Step** | **Action** |
| **1** | Result testing in the VistA computer system. |
| **If** | **Then** |
| If seldom-seen abnormal crystals are observed (tyrosine, leucine, bilirubin, cholesterol) | Notify the provider or designee of the results (if possible) |
| **2** | The VistA software will forward the results to CPRS, which is the patient’s electronic chart. |

* There are multiple microscopes available for use in the lab. In the unlikely event this test system should become inoperable; samples will be sent to the Durham VA Medical Center laboratory for analysis.
1. **Reference Ranges**:

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| WBC’s | 0-3/HPF |
| RBC’s | 0-3/HPF |
| Epithelial | Only Squamous Epithelial |
| Crystals | Few Calcium Oxylate, Amorphous Urates (acid urine), Amorphous Phosphates (alkaline urine) |
| Casts | 0-1/LPF Hyaline Casts |
| Bacteria | Few or <1+ |
| Yeast | Negative |

1. **Test Limitations:**
* Formed elements in the urine disintegrate rapidly. Urine specimens should be examined while fresh when at all possible. Refrigeration within 2 hours of collection is important to preserve the integrity of the sample.
* Refrigeration may cause precipitation of amorphous urates, amorphous phosphates, and other non-pathological crystals that can obscure other elements in the urine sediment.
* Casts tend to float towards the edges of the coverslip. Therefore, low-power scanning of the slide perimeter is recommended when scanning for casts.
* Red blood cells may often appear shrunken, crenated, or as ghost cells due to the centration of the urine.
* Yeast cells, oil droplets, and air bubbles may often be confused for red blood cells. Careful examination for refraction, budding yeast, and size should be used. Addition of acetic acid to the sample will lyse red blood cells if additional aid in identification should be needed. Correlation with chemical analysis (positive for blood) can also aid in identification of red blood cells.
* White blood cells may potentially be confused for renal tubular epithelial cells.
* Amorphous phosphates or urates may be confused for bacteria. Observation of motile bacteria in fresh urine can aid in identification. Warming the sample to 37°C may aid in clearing amorphous crystals.
* Yeast multiply rapidly in unrefrigerated samples, which can create a false elevation in yeast quantification.
* Non-motile Trichomonas may resemble a WBC or epithelial cell.
* Copious mucus content in the urine may resemble hyaline casts.
* Urine artifacts such as starch granules, vegetable fiber, oil droplets, and pollen may be mistaken for clinically significant formed urine elements. Reference to appropriate image and literature resources to assist with identification should be employed when needed.
1. **Maintenance:**

**Microscope(s)**: Objective lenses, eyepiece, and condenser will be cleaned daily with lens paper that has been dampened with an approved lens cleaner. Document daily maintenance using GN-FORM-5, Microscope Daily Maintenance Log

* Annual cleaning, inspection and service will be performed by Biomedical Services or a qualified third party service.
1. **Related Documents**:
* GN-FORM-5, Microscope Daily Maintenance Log
1. **References:**
* *Lorenzo*, M., & Strasinger, S. (2008). *Urinalysis and Body Fluids, Fifth Edition.* Philadelphia, PA: F.A David Company.
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