Urine sediment preparation and microscopy technique

Andrej Škoberne
Department of nephrology
University Medical Centre Ljubljana
Urine sediment preparation and examination

- Urine sample collection
- Sample centrifugation and slide preparation
- Examination under the microscope

European Urinalysis Guidelines.
Urine sample collection

• Patient preparation

• Timing of the sample collection

• Manner of the sample collection

• Sample preservation and transport
Patient preparation

• Patient should receive instructions on the following:
  – Refrain from strenuous physical exercise approximately 48 hours prior to sample collection
  – Refrain from sexual intercourse approximately 24 hours prior to sample collection
  – Not to give urine samples during their period (women)
  – Refrain from drinking too much on the morning of the collection and the night before (approximately 200 ml of fluid after 22.00 and no more until sample collection)
Timing of the sample collection

• First morning urine: voided immediately after an overnight bed-rest. Voided after an 8 h period of recumbency and after not less than 4 h of bladder storage time.

• Second morning urine: voided 2-4 h after the first morning urine.

• Random specimen

• Timed collections – 24 h urine sample
Manner of the sample collection

• Washing the hands
• Spreading the labia or withdrawing the glans of the penis
• Cleaning the urethra orifice
• Catching the mid-stream urine in the correct container (at least 50-100 ml, opening of at least 5 cm diameter)
Collection of mid-stream urine, females

1. Wash your hands.
2. Spread your labia.
3. Wash your outer genital organs with 3-4 clean wet towelettes wiping downwards only once with each of them.
4. Dry-wipe with a paper towel downwards.
5. Let the first portion pass into the toilet.
6. Collect about 50 ml urine into the container without breaking the stream.
7. Leave the specimen at the reception desk.
8. Avoid touching the inside.

Collection of mid-stream urine, males

1. Wash your hands.
2. Withdraw your foreskin.
3. Wash the end of your penis with a clean wet towelette.
4. Dry-wipe with a paper towel.
5. Let the first portion pass into the toilet.
6. Allow any excess urine to pass again into the toilet.
7. Collect about 50 ml urine into the container without breaking the stream. Avoid touching the inside.
8. Leave the specimen at the reception desk.

Zero visibility urine
Manner of the sample collection

• Urine catheters
  – Single catheterizations are discouraged
  – If taking samples from indwelling catheters never take the sample from the collecting bag
  – It is preferable to block the passage of urine for approximately 1 hour and then aspirate the sample from the catheter

• Bag urine – carefully wash the genitalia before applying the bag

• Suprapubic aspiration
Sample preservation and transport

• Ideally the sample should be examined within 1 hour of voiding
• If a delay is expected the sample should be refrigerated
• Fixatives – not routinely used
Urine sample centrifugation

1. How much sample is used?
2. What test tube is used?
3. How is the sample transferred to the test tube?
4. What are the centrifugation parameters?
5. How much of the supernatant is discarded and how?
6. How is the sediment resuspended?
7. How much of the resuspended sediment is transferred to the slide?
8. What is the size of the coverslip used?
Urine sample centrifugation

• How much sample is used – usually 10 ml
• What test tube is used?
Urine sample centrifugation

• How is the sample transferred to the test tube?
  – Simply pouring it out of the container
  – Using automatic pipettes

• What are the centrifugation parameters?
  – 400 g for 5 minutes
  – g? – relative centrifugal force (RCF)
  – RPM? (rotations per minute)

  \[ \text{RCF} = 1,118 \times 10^{-5} \times r \times \text{RPM}^2 \]

  \( r = \text{radius in cm from the center of the spindle to the bottom of the tube} \)
What type of centrifuge?
Urine sample centrifugation

• How much of the supernatant is discarded and how?
  – Usually 9.5 ml
  – Very important that this amount is accurate
  – 10 ml to 0.5 ml – concentrated 20x
  – 10 ml to 0.4 ml – concentrated 25x
  – 10 ml to 0.6 ml – concentrated 17x
Urine sample centrifugation

• How is the sediment resuspended?
  – Shaking
  – Pasteur pipette

• How much of the resuspended sediment is transferred to the slide?
  – Usually 30 – 50 µl

• What is the size of the coverslip used?
  – Usually 18 x 18 mm or 22 x 22 mm or 24 x 32 mm
Examination under the microscope

• Examination under low power field (100x – 200x) – 30 fields: enumeration of casts, mucus and squamous epithelial cells

• Examination under high power field (400x) – 20 fields: enumeration of all the other particles, assessment of the type of casts
Examination under the microscope

Middle of the coverslip

Edge of the coverslip
Examination under the microscope

• Enumeration of particles
  – Number of particles per HPF – erythrocytes, leukocytes, tubular epithelial cells...
  – Semi-quantitative enumeration (0 - 4+): bacteria, fungi, mucus...
  – Erythrocyte dysmorphism – counting 100 erythrocytes – percentage of dysmorphic erythrocytes
  – Important to compare the results with the dipstick test
Examination under the microscope

• Bright field microscopy

• Phase contrast microscopy

• Polarized light microscopy
Examination under the microscope

Phase contrast

Bright field
Phase contrast microscopy

FIGURE 1.6 Schema of the phase contrast microscope. A = condenser; B = annular diaphragm of the condenser; C = hollow cone of light; D = objective; E = phase ring with the layer of translucent silver (dotted grey area); \[\rightarrow\] = direct light; \[\leftarrow\] = diffracted light.
Phase contrast microscopy
Phase contrast microscopy
Polarised light microscopy

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Polarised light microscopy
Polarised light microscopy

• Identifies structures that polarise light
  – Crystals
  – Lipids

• Not done routinely on every sample, only for crystal identification or in samples suspected of having lipiduria
Polarised light microscopy
Polarised light microscopy
Summary

Urine sample preparation is boring, but must be done the right way and the exact same way every single time – much like brushing your teeth.