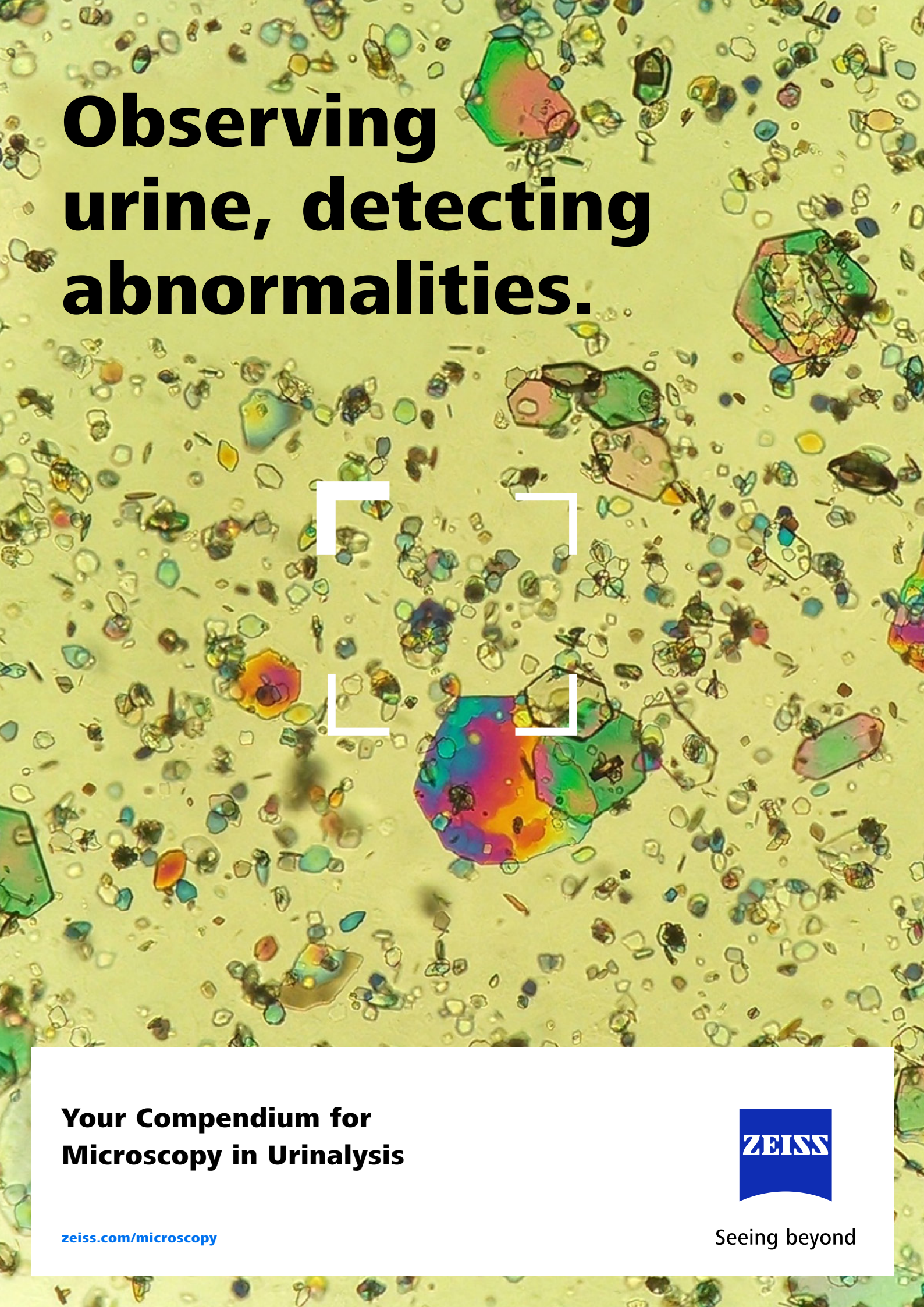

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About the author:
Graduated in Pharmacy-Biochemistry at Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS) in 2005. Worked from 2005 to 2019 in the Urinalysis Section of the Carlos Franco Voegeli Clinical Analysis Laboratory at Santa Casa de Misericórdia de Porto Alegre. In 2014 obtained a Masters Degree and in 2019 a Doctoral Degree, both in Health Sciences at Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA). From 2015 to 2022 worked as Assistant Professor of the Health School at Universidade do Vale do Rio dos Sinos (Unisinos). Urinalysis Consultant at Controllab since 2014 and Educational Manager at Controllab since 2022.

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Content

1. Introduction 5
2. Urine, Kidneys and the Urinary Tract System 6
3. Urinalysis Procedure 8
    - Urine Collection 8
    - Sample Preparation and Analysis 8
    - Reporting Results 10
4. Elements Observed in the Urine Sediment 12
    - Cells 12
    - Casts 25
    - Crystals 32
    - Microorganisms 46
    - Others 53
5. Microscope Equipment 62
    - Köhler Illumination 64
    - Brightfield Microscopy 65
    - Phase Contrast Microscopy 65
    - Polarized Light Microscopy 66
6. Clinical Profiles 68
    - The Urinary Tract Infection Profile 68
    - The Tubular Injury Profile 70
    - Tubular injury (Ischemic/nephrotoxic) Ex.: Drug toxicity 70
    - Tubular injury (associated to crystals) Ex.: Primary hyperoxaluria 70
    - Tubular injury (associated to direct bilirubin) Ex.: alcoholic cirrhosis and hepatitis C infection 70
    - Tubular injury (associated to hemoglobin) Ex.: hemolytic anemia 70
    - Tubular injury (associated to myoglobin) Ex.: rhabdomyolysis 76
    - Nephrotic profile (Nephrotic syndrome) Ex.: Diabetic nephropathy, amyloidosis 76
    - Nephritic profile (Nephritic sediment) Ex.: acute post-infectious glomerulonephritis, Schönlein-Henoch purpura nephritis, cryoglobulinemic glomerulonephritis 76
    - Nephrotic and nephritic profile (Proliferative glomerulonephritis) Ex.: IgA nephropathy, class IV lupus nephritis 81

1. Introduction

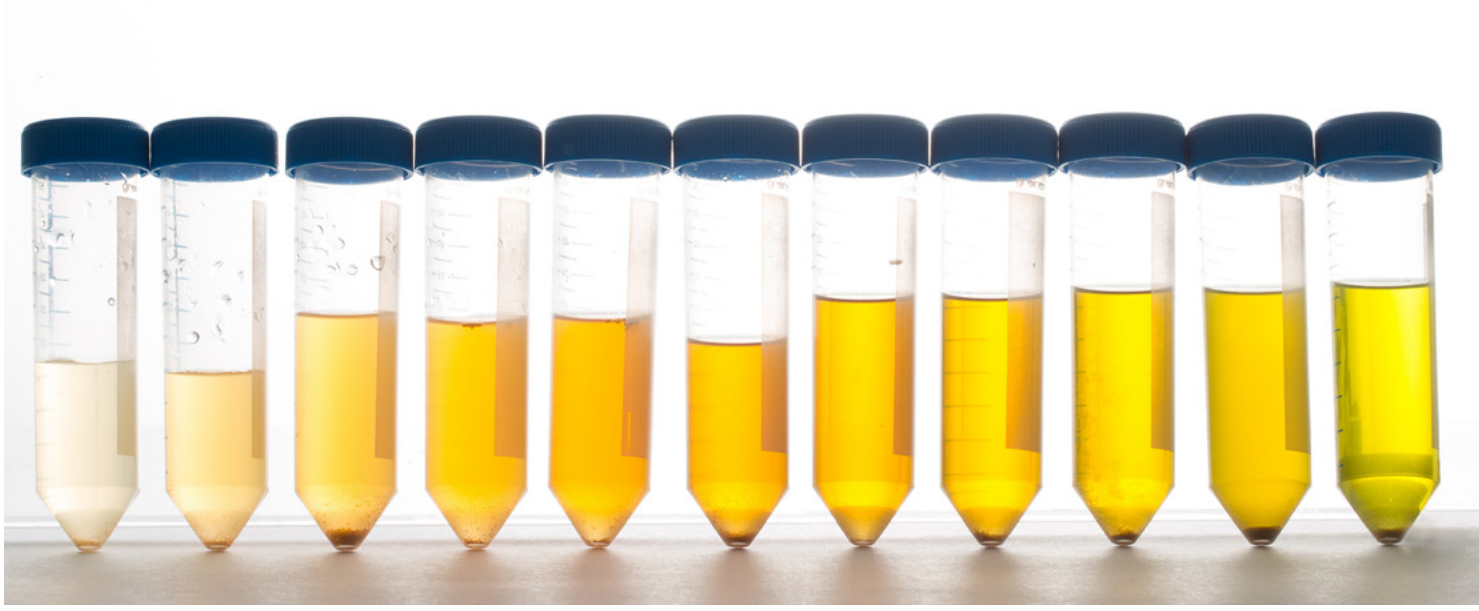
When we talk about urinalysis, we are talking about one of the oldest diagnostic methods used by human beings. A urine sample is easily obtained and it comes from inside the body, thus making relevant information—the presence of blood, for example—easily identifiable. That's why this biological material has been widely used as a means of clinical diagnosis since the dawn of humanity.

In clinical laboratories, the urine sample is extensively analyzed to evidence changes caused by diseases of the kidneys and urinary tract as well as for carbohydrate metabolism disorders, and liver and hemolytic disorders.

Dipstick tests coupled with microscopic analysis of the urine sediment are among the most requested examinations in clinical laboratories worldwide. In recent years many technological improvements have enabled the implementation of technologies that can do the work—both dipstick tests and urinary particle counts—almost completely through automated systems. Yet microscopic analysis of urine sediment continues to be the gold standard method for urine particle counts and identification.
This book is for professionals who evaluate human urine samples as part of their daily routine. It focuses specifically on the use of a microscope for identifying urine particles, their clinical significance and the findings that can be made depending on the disease affecting the patient.

While urinalysis may be considered an "easy" test, it is the total opposite in practice. To properly explore the wide range of information urine specimens can give us about the clinical picture of the patient, the professional operating the microscope needs knowledge of a number of disciplines in the laboratory medicine area—among them biochemistry, cytology, hematology, microbiology, virology, mycology, parasitology, pathology and immunology.

There is still a lot to be discovered and also much more to be clarified about urinary findings and their relationship with diseases. So let's start our journey into the world of urinalysis!

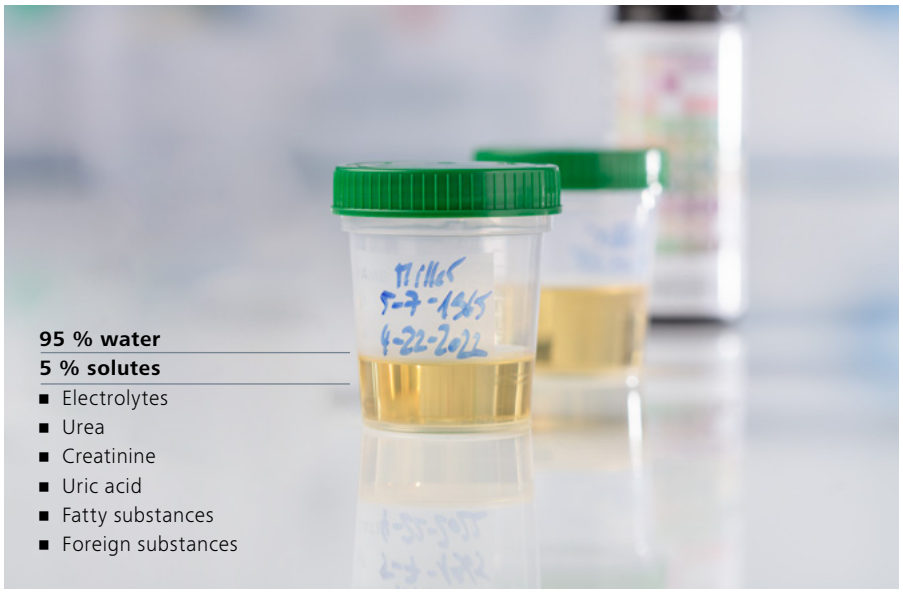


2. Urine, Kidneys and the Urinary Tract System

Urine is a mostly yellowish liquid that the body regularly excretes through the urinary tract. Urobilinogen is responsible for the yellowish color. This is a breakdown product of the bile pigment bilirubin. The more of it there is in the urine, the yellower the liquid becomes. Urine is about 95 % water. The remaining 5 % are made up of substances that have previously been filtered out of the blood by the kidneys.

Kidneys produce urine continuously by blood filtration, secretion and reabsorption. These processes are realized by the nephrons. In general, urine is composed of urea and other organic and inorganic chemicals dissolved in water. Urine may also contain formed elements such as cells, casts, crystals, mucus and bacteria, and when these formed elements are observed in increased amounts they can be indicative of disease [1].

The kidney and urinary tract system consists of the kidneys, ureters, bladder and urethra.



Kidneys: The two kidneys are positioned in the retroperitoneum, one in the left side and the other in the right. Each kidney has between one and two million nephrons, located mainly in the córtex of the kidneys' parenchyma. The medulla comprises parts of renal tubules and collecting ducts. The renal tubules are made up of a single layer of renal tubular epithelial cells. The renal pelvis, bladder and parts of the urethra are covered by urothelial cells.

Ureter: The ureter is a long narrow tube. After emerging from the renal pelvis, the left and right ureters descend and open separately at the fundus of the bladder. The ureter has a mechanism that prevents urine from the bladder backflowing into it.

Bladder: The bladder is located anterior to the rectum in men and anterior to the uterus and vagina in women. At the fundus of the bladder, the ureters open on the left and right sides from the posterior side. The urethra starts from the anterior part (internal urethral orifice). Most parts of the bladder mucosa consist of urothelial epithelial cells, but the mucosa of the trigone of the bladder consists of simple columnar epithelial cells that secrete mucus.

Urethra: In males, the urethra is a long tube that starts from the internal urethral orifice. The prostatic urethra consists of urothelial epithelial cells and ejaculatory ducts open into this region from both sides. The membranous portion of the urethra comprises pseudostratified columnar epithelial cells and the ducts of a pair of bulbourethral glands (Cowper's glands) that open into the initial part of the urethra. The area from the fossa

navicularis to the external urethral orifice comprises stratified squamous epithelial cells. In females, the urethra is comparatively short, starting from the internal urethral orifice. The membranous urethra passes through an area anterior to the vagina and opens at the external urethral orifice in the vaginal vestibule. Women have greater vestibular glands (Bartholin's glands) which correspond to the bulbourethral glands of men [2].

Always keep in mind that elements and chemical compounds from virtually any part of the kidneys and urinary tract system can be found in urine. Knowing the possible findings is crucial to being able to identify information of clinical interest during the test.

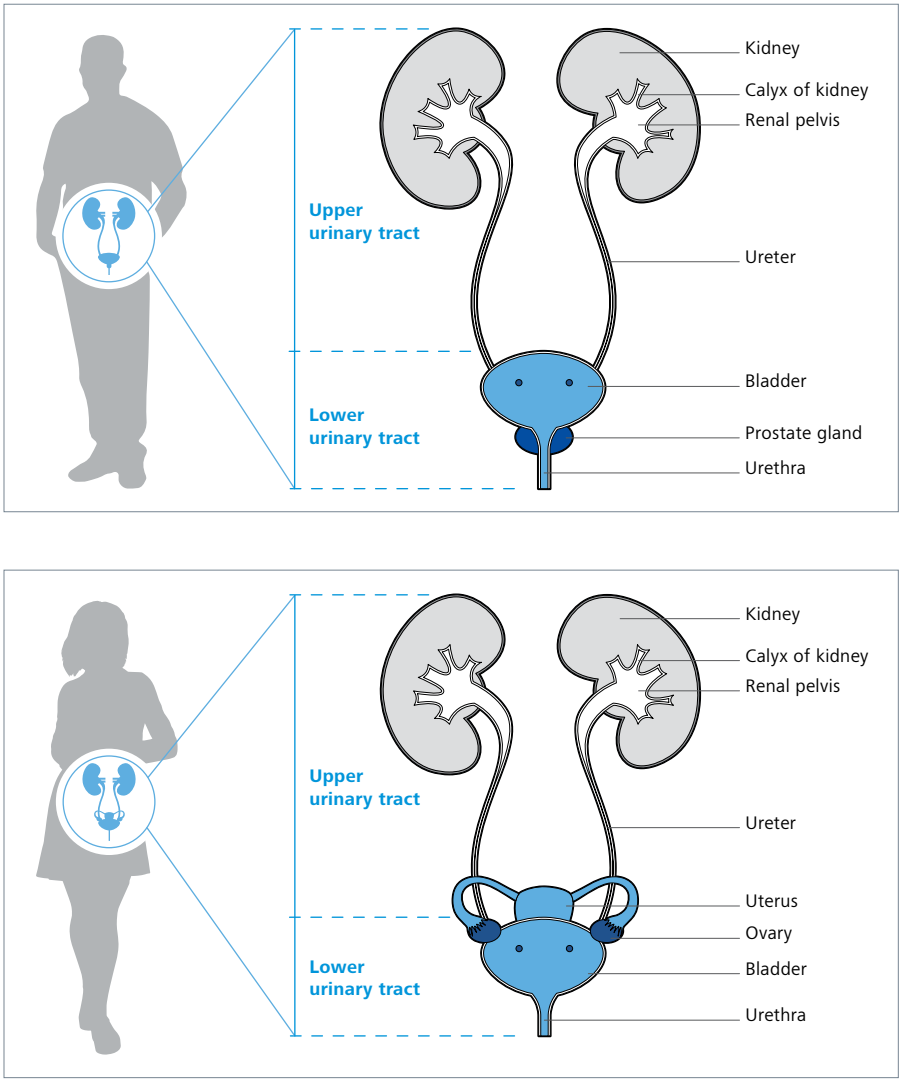


Figure 2.1: Kidney and urinary tract system

References

[1] Strasinger, S.K. & Schaub-DiLorenzo, M. (2008) *Urinalysis and body fluids*, 5th edition, photography by B. Wang *et al.*, illustration by S. Bonomelli. Philadelphia, PA, F.A. Davis.

[2] Itoh, Kiichi (2014) *Atlas of urinary sediment*, Japanese Association of Medical Technologists.

3. Urinalysis Procedure

Urine Collection

Urine collection plays a critical role in the quality of the urinalysis results. The procedure depends on the patient's comprehension and proper execution of the "clean catch" sample collection. It is mandatory to give the patient clear instructions on hygiene of the genital area and the steps of sample collection.

The first morning urine, collected soon after the patient wakes up, is the urine usually evaluated. However, the second morning urine, collected within two hours after the first, can also be analyzed.

The steps presented in Figure 3.1 comprise the clean catch procedure and need to be properly addressed with the patient.

Other types of sample collection in daily practice include catheterization, suprapubic aspiration and use of a collecting bag. These procedures are applied when the clean catch procedure cannot be performed by the patient and they require well trained and experienced professionals to carry them out.

Sample Preparation and Analysis

Standardized procedures are mandatory and you are strongly advised to follow the local, regional, [national] or continental guideline on urinalysis to prepare the sample according to the standardized procedure recommended by your relevant health organization. However, any method you choose will have its limitations [2].

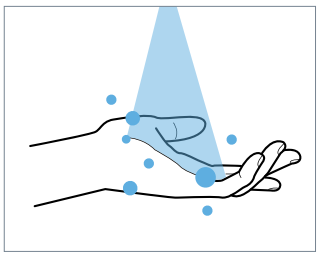
The literature contains very good documents / guidelines, among them:

- European Urinalysis Guideline [1]
- Clinical and Laboratory Standards Institute (CLSI) Urinalysis Approved Guideline [3]
- Japanese Committee for Clinical Laboratory Standards Urinary Sediment Examination Standardization Committee [4].

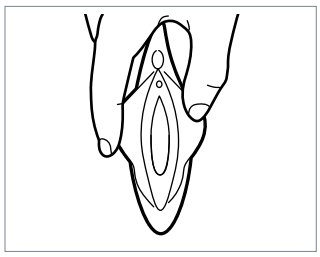
It is beyond the scope of this book to recommend an analysis protocol for urinalysis. The guidelines cited here have [their own] standardized protocols to guide professionals in dealing with urine samples, but there are some common steps described on urinalysis protocols / documents:

a. First, homogenize the urine sample.
b. Then transfer a standardized volume of urine to the conical tube (e.g., 10 mL*).
c. Perform the urinalysis test strip analysis prior to sample centrifugation.
d. Centrifuge the sample at a standardized time and speed (e.g., 5 minutes; 400 xg*).
e. Carefully remove the supernatant.
f. Let a standardized volume of urine into the conical tube to resuspend the urine sediment (e.g., 0.5 mL*).
g. Carefully resuspend the urine sediment using a Pasteur pipette.
h. Transfer a standardized volume of the resuspended urine sediment to a slide (e.g., 20 µL*).
i. Cover the urine sediment on the slide with a standardized coverslip (Ex.: 22 × 22 mm*).
j. Place the slide on the microscope to perform the microscopic analysis.
k. Evaluate a number of high power microscopic fields (Ex.: 10 microscopic fields) and calculate the medium number of particles (epithelial cells, leukocytes and erythrocytes) observed per field (e.g., 5 Leukocytes / HPF**).

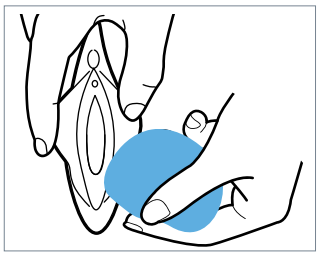
Urine Collection: Female Patients



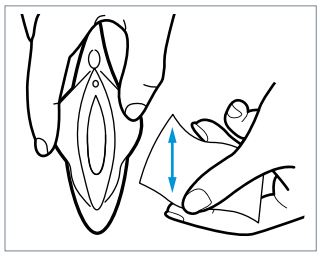
a. Wash your hands.



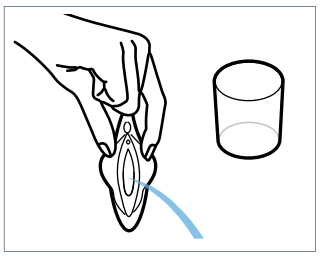
b. Open your vaginal lips.



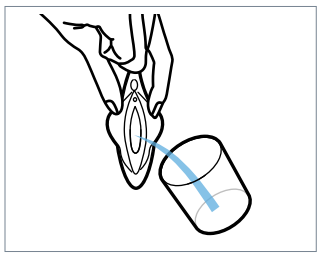
c. Wash your external genitals.



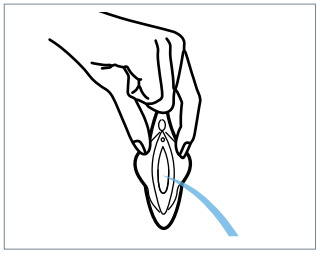
d. Dry with a paper towel, using downward movements.



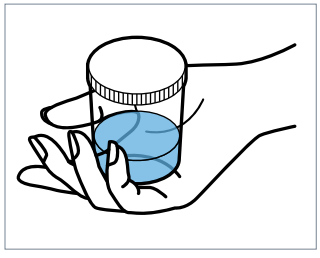
e. With the vaginal lips apart, start urination and eliminate the first part of the urine into the toilet.



f. Without interrupting urination, collect the midstream of urine, filling the collection bottle to approximately half its capacity. Avoid touching the inside of the collection bottle.

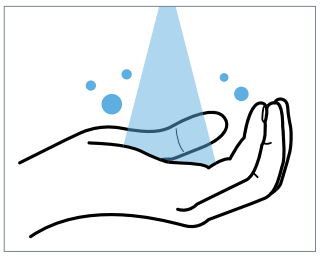


g. Eliminate any excess urine into the toilet.

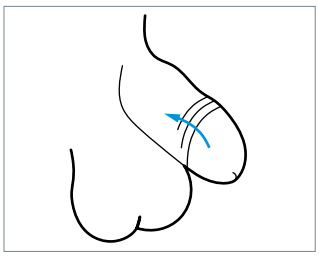


h. Deliver the sample to a laboratory professional. [1]

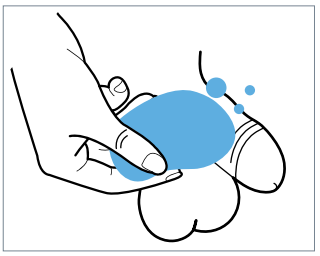
Urine Collection: Male Patients



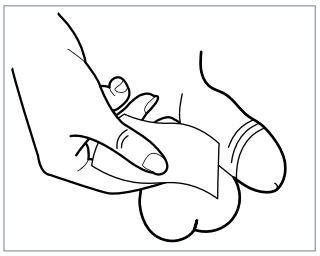
a. Wash your hands.



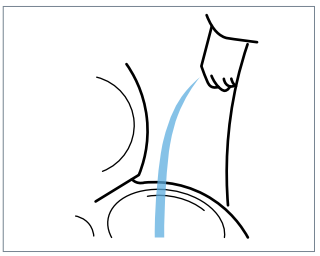
b. Pull back the foreskin from the penis.



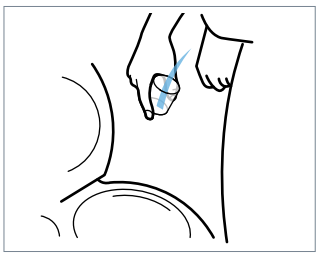
c. Wash the glans penis.



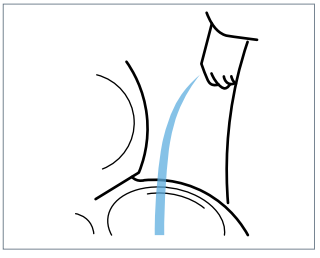
d. Dry with a paper towel.



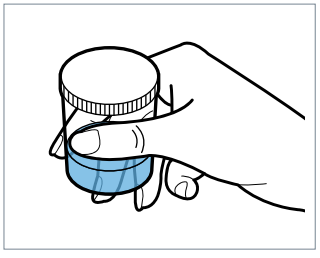
e. Start urination, eliminating the first part of urine (first jet) into the toilet.



f. Without interrupting urination, collect the midstream of urine, filling the collection bottle to approximately half its capacity. Avoid touching the inside of the collection bottle.



g. Eliminate any excess urine into the toilet.



h. Deliver the sample to a laboratory professional.

Figure 3.1: Description of the procedures to the clean catch technique for both male and female patients.

Keep in mind that urinalysis guidelines usually recommend reporting the urine particle count per volume unit so you will need to use a calculation to convert the count/field result to a count/volume unit. The calculation will depend on several factors and you are well advised to follow the procedure described on your local/regional/national/continental guideline.

l. Evaluate a number of low power microscopic fields (e.g., 10 microscopic fields) and calculate the medium number of casts observed per field (e.g., 2 casts/LPF***).
m. Note the cast subtypes and the presence of any clinically-relevant urinary particles (such as yeasts or crystals).

References

[1] European Confederation of Laboratory Medicine (2000) European urinalysis guidelines. Scand J Clin Lab Invest Suppl. 2000;231:1-86. PMID: 12647764.
[2] Fogazzi G.B., Ponticelli C. & Ritz E. (2010) The Urinary Sediment: An Integrated View. Milan, Elsevier Masson.
[3] Clinical and Laboratory Standards Institute (2009) Urinalysis; Approved Guideline, Third Edition. CLSI document GP16-A3. Wayne, PA, CLSI.
[4] Japanese Committee for Clinical Laboratory Standards (JCCLS) Urinary Sediment Examination Standardization Committee (2011) Urinary Sediment Examination Procedures GP1-P4, 7–9, Examination of Urinary Sediment 2010, Japanese Association of Medical Technologists, 2011.

*Values presented on these steps are just for the purpose of giving examples. Follow your local/regional/national/continental guideline for a proper standardized procedure.
**High Power Field (HPF) = 400× magnification.
***Low Power Field (LPF) = 100× magnification.

Reporting Results

The urinalysis report must contain information on both the dipstick test analysis results and the microscopic analysis of the urine sediment. A report could take this form, for example:

DATE: . . . . . .
PATIENT NAME: . . . . . .
DATE OF BIRTH: . . . . . .

DIPSTICK TEST RESULTS:
PH: . . . . . .
SPECIFIC GRAVITY: . . . . . .
NITRITE: . . . . . .
LEUKOCYTE ESTERASE: . . . . . .
PROTEIN (ALBUMIN): . . . . . .
HEMOGLOBIN: . . . . . .
BILIRUBIN: . . . . . .
UROBILINOGEN: . . . . . .
GLUCOSE: . . . . . .
KETONE BODIES: . . . . . .

MICROSCOPIC ANALYSIS RESULTS:
400×
EPITHELIAL CELLS: . . . . . .
LEUKOCYTES: . . . . . .
ERYTHROCYTES: . . . . . .
100×
CASTS: . . . . . .
TYPES: . . . . . .

OBSERVATIONS . . . . . .
. . . . . .

COMMENTS: . . . . . .
. . . . . .

. . . . . .
SIGNATURE


(-2)—(+2)
22—26 mmo
>96 %
20 ml/dl
94—98 %
94—98 %
35—45 mmHg
7,36—7,44
75—100 mmHg

4. Elements Observed in the Urine Sediment

This chapter describes the urinary particles that can be observed during microscopic analysis of urine sediment. To be of use to professionals, we will present these elements – whenever possible – in brightfield and phase contrast microscopy. When necessary, polarized light will also be used to show the characteristics of the structures that are identifiable by using this microscopic resource. In rare cases, the structure is presented stained with Sternheimer-Malbin stain.

Cells

Urine samples can contain different types of cells: those derived from the circulation (leukocytes and erythrocytes) and those from the epithelia that covers the urinary system. Some types are of clinical interest while others can be observed but have no [known] clinical significance.

Squamous Epithelial Cells

Squamous epithelial cells or SECs (Figure 4.1) derive mostly from the superficial layers of the mucous membrane around the external urethral orifice, especially the vaginal epithelium (Figure 4.2 – right). Frequently they are observed folded or aggregated, and when observed in large numbers they are a sign of an improper collection procedure (Figure 4.3) [1,2]. When coupled with *Döderlein bacilli*-free nuclei (Figure 4.4) can be present as a result of cytolysis. This kind of situation can cause inexperienced professionals to misidentify the free nuclei as leukocytes and *Döderlein bacilli* as pathogenic bacteria, thus causing disagreement between urinalysis findings and urine culture—an important issue in the investigation of urinary tract infections. SECs are a type of cell that has no clinical significance when observed in urine sediment.

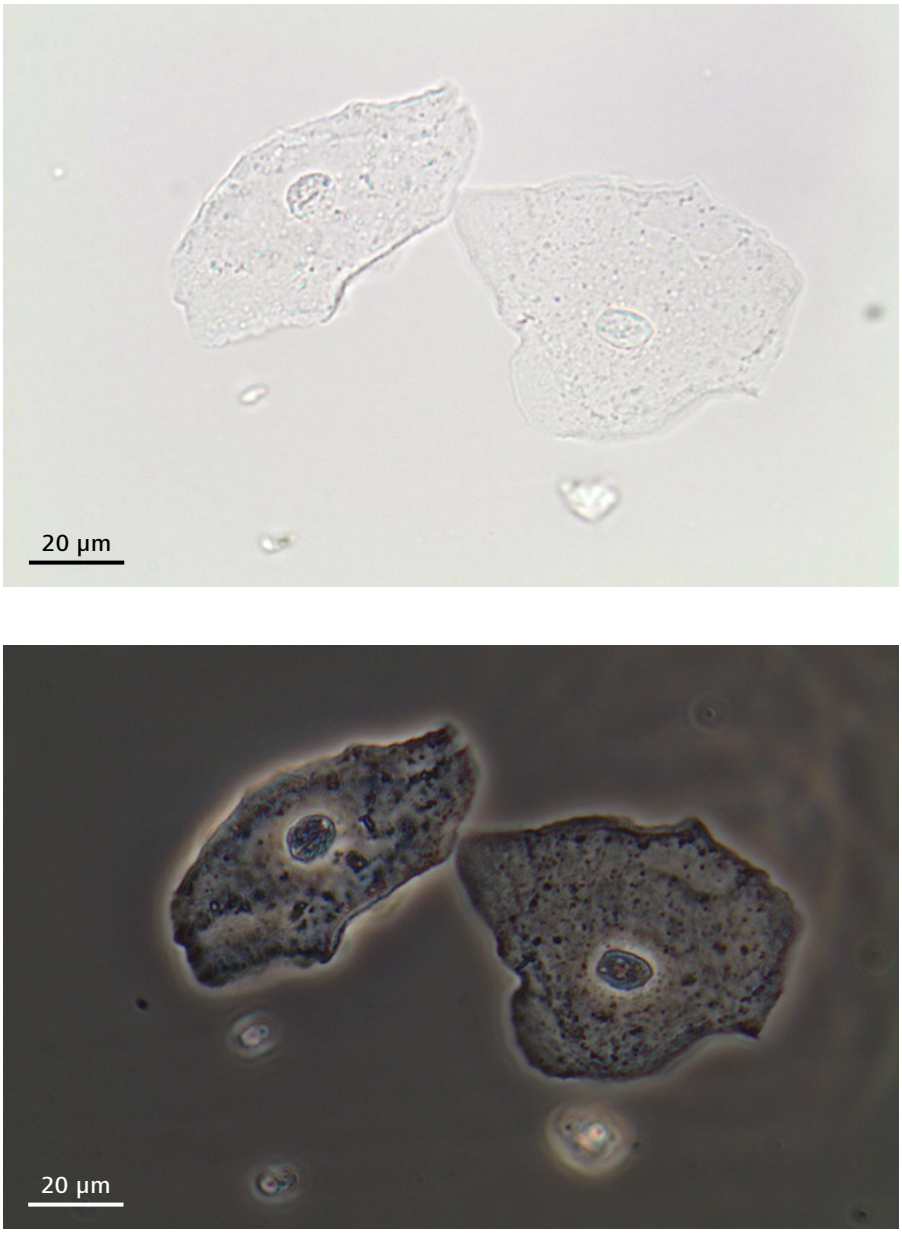


Figure 4.1: Squamous epithelial cells. Fresh and unstained urine sediment. Brightfield (upper); Phase contrast (lower). Original magnification 400×. Courtesy of Controllab.

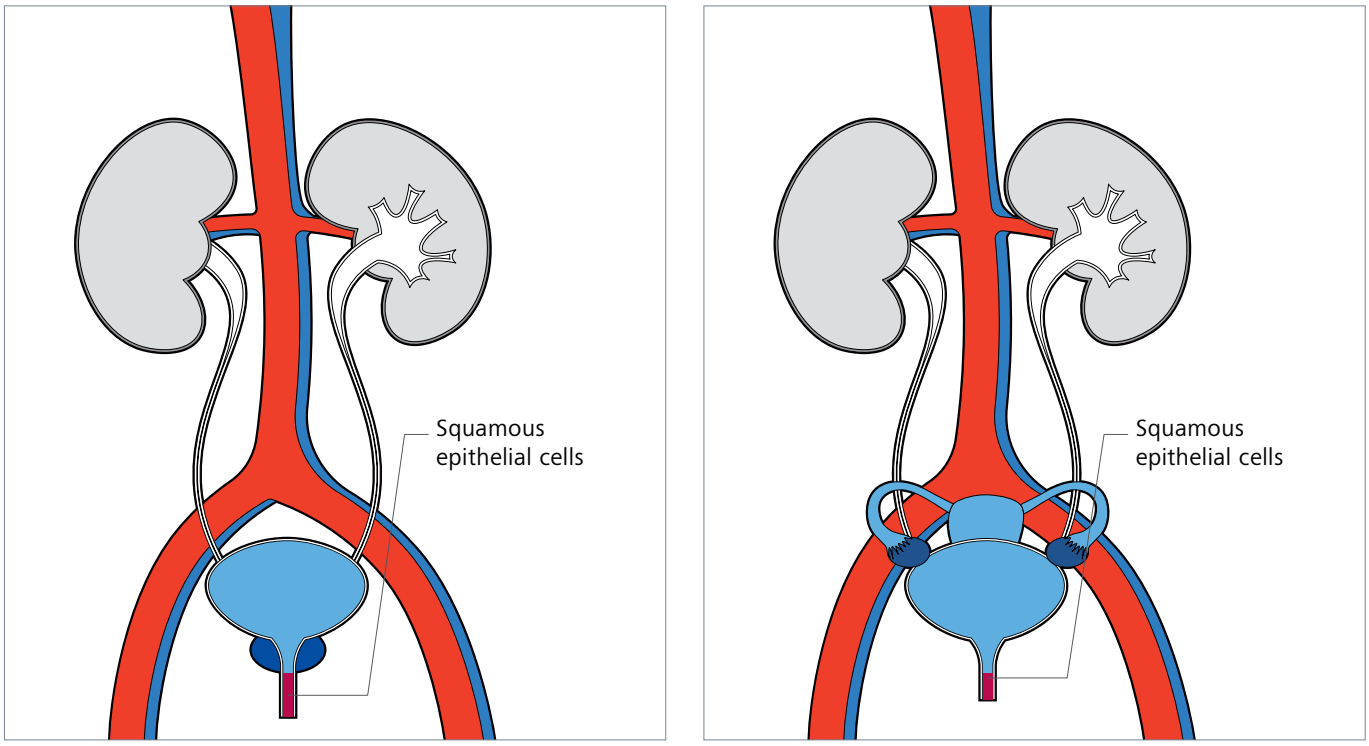


Figure 4.2: Squamous epithelium; left – male; right – female

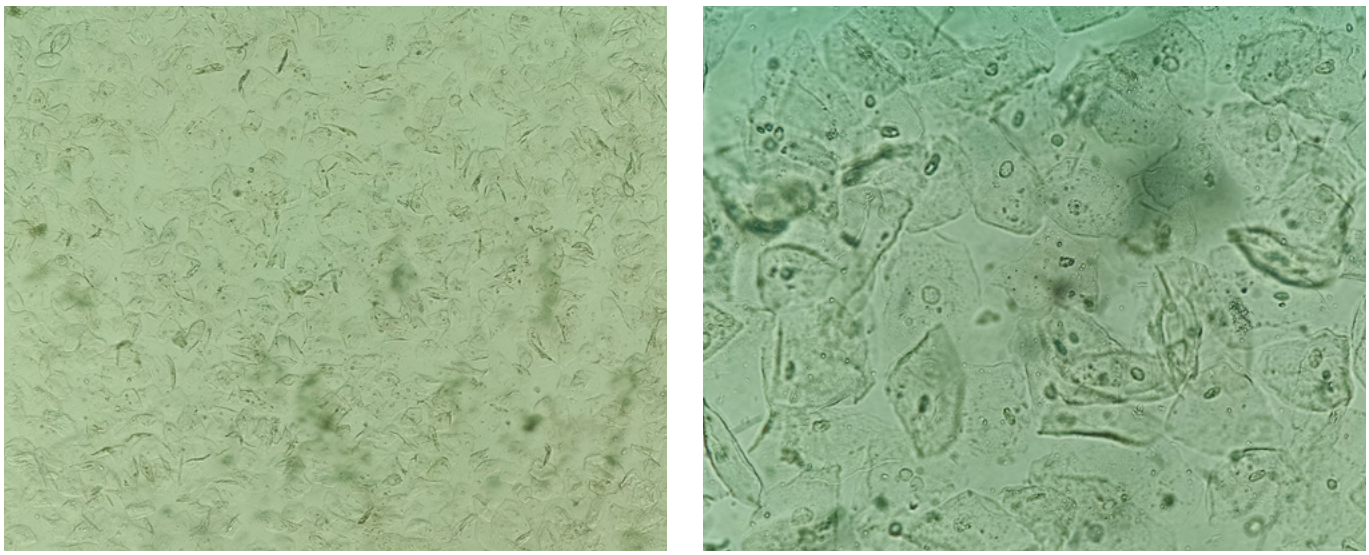


Figure 4.3: Large amount of squamous epithelial cells. Fresh and unstained urine sediment. Brightfield. Original magnification 100× (left); 400× (right). Courtesy of J. A. T. Poloni

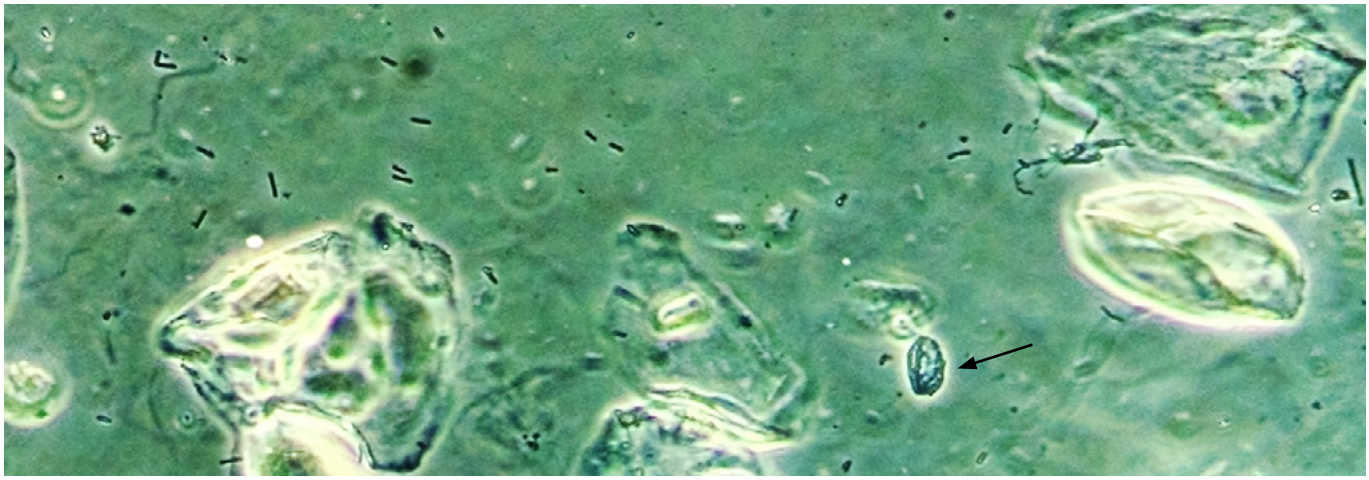
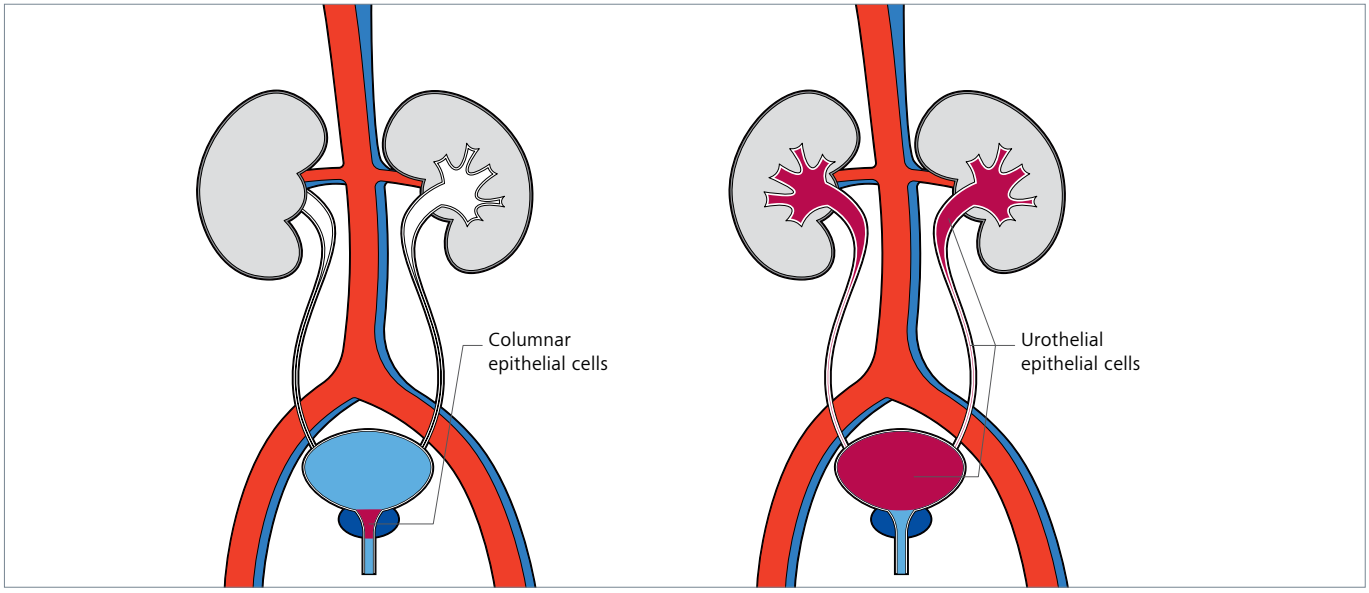


Figure 4.4: Free nuclei of squamous epithelial cells. Fresh and unstained urine sediment. Phase contrast. Original magnification 400×. Courtesy of J. A. T. Poloni



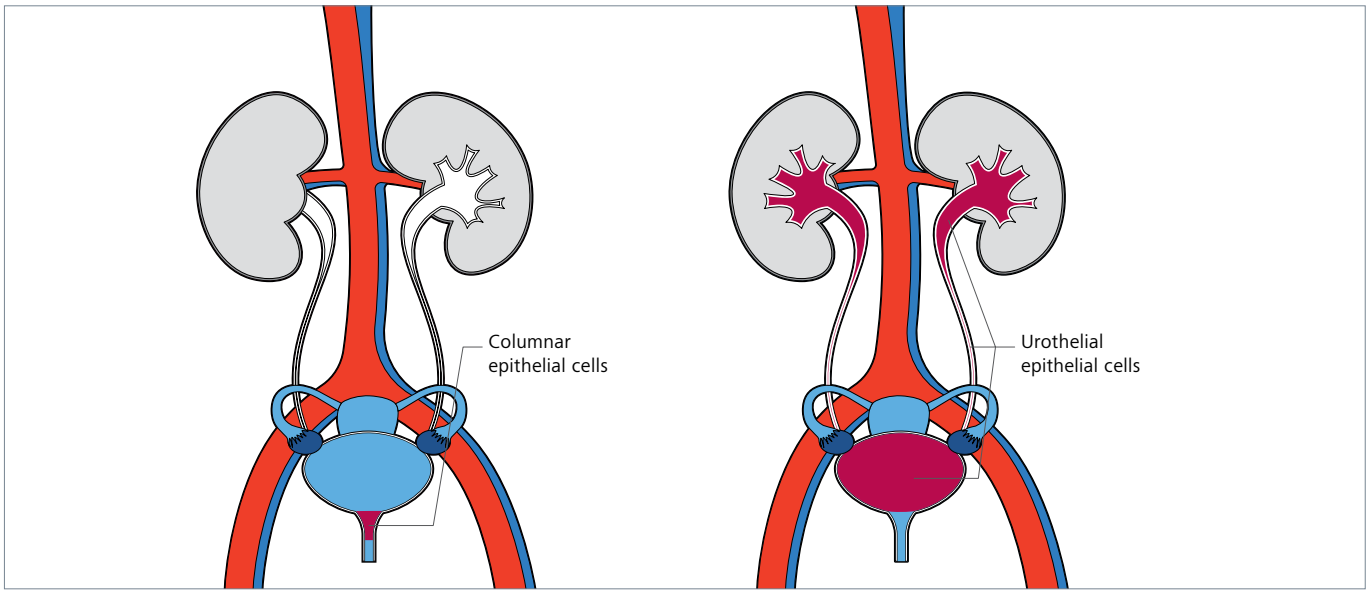


Figure 4.5: Transitional epithelial cells derive from the uroepithelium (top – male, bottom – female)

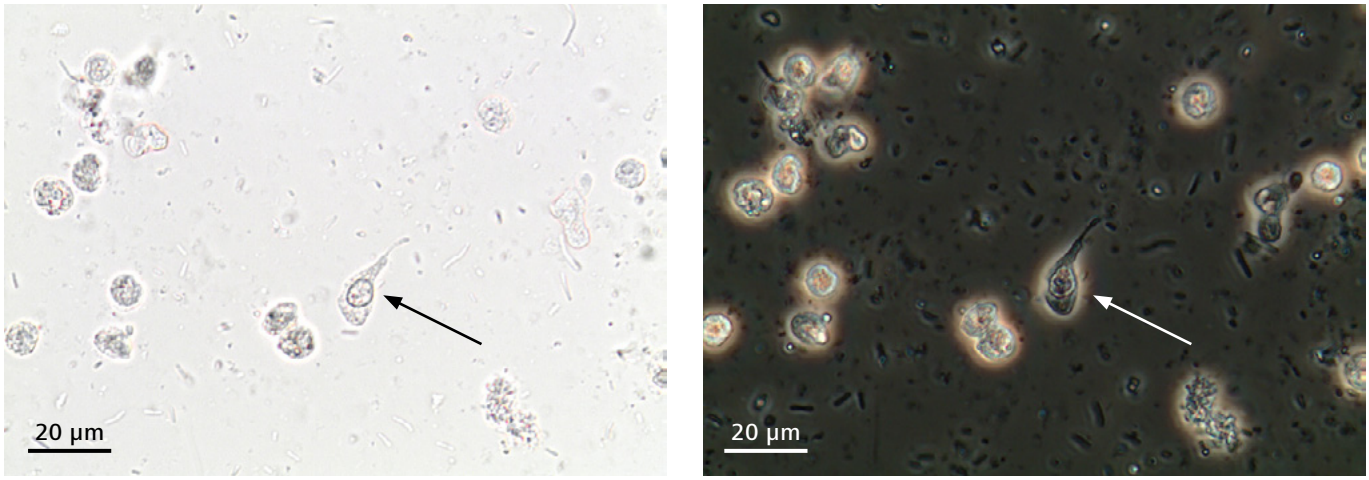


Figure 4.6: Deep transitional cells. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (right). Original magnification 400×. Courtesy of Controllab.

Transitional Epithelial Cells

Transitional epithelial cells derive from the uroepithelium (Figure 4.5). This is an epithelium that consists of several types of cells organized in layers. Cells from all layers, both deep and superficial, can be observed during the sample analysis with the cells from the superficial layers more commonly seen.

Deep transitional cells (Figure 4.6) can be observed in large numbers in conditions that cause damage to the deep layers of the uroepithelium—for example, urolithiais and bladder carcinoma. They are also frequently found in the urine of patients with ureteric stents or bladder catheters (Figure 4.7).

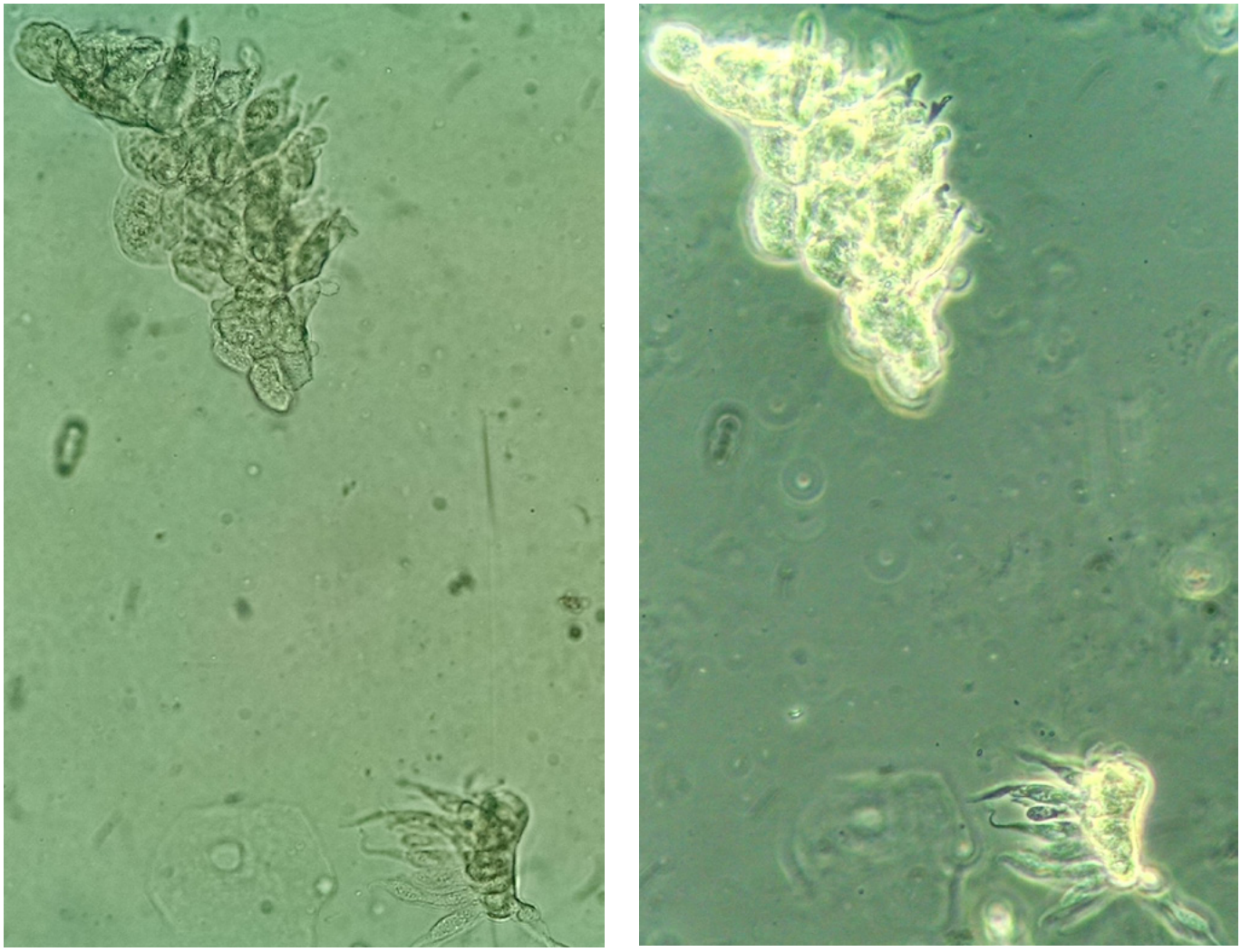


Figure 4.7: Deep transitional cells. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (right). Original magnification 400×. Courtesy of J. A. T. Poloni.

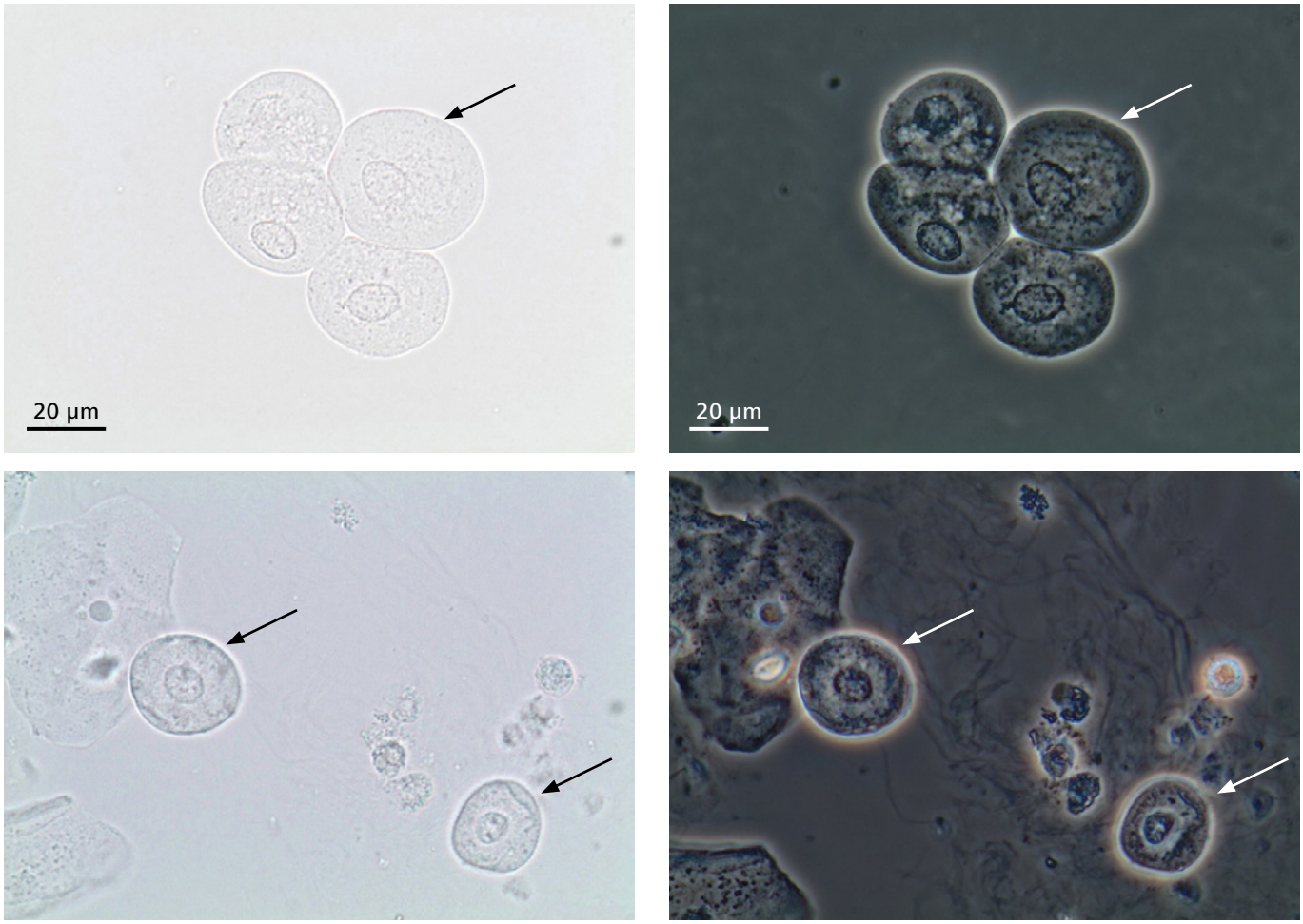


Figure 4.8: Superficial transitional cells. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (right). Original magnification 400×. Courtesy: Controllab.

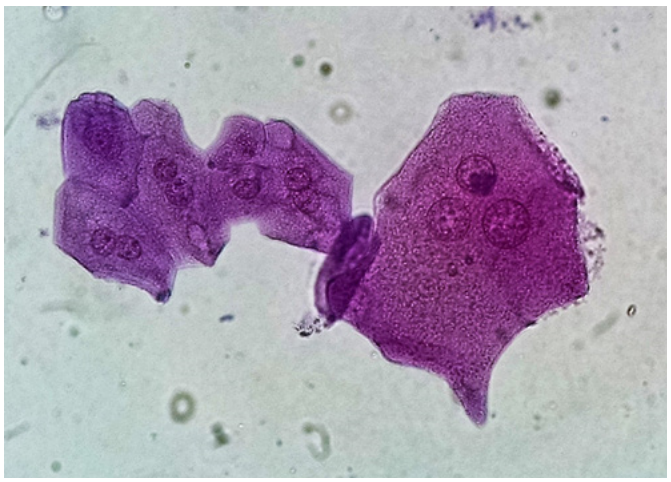


Figure 4.9: Umbrella cells. Fresh urine sediment stained with Sternheimer-Malbin stain. Brightfield. Original magnification 400×. Courtesy: J. A. T. Poloni

Superficial transitional cells (Figure 4.8) are observed more frequently than the cells from the deep layers since they are exfoliated in any grade of injury of the uroepithelium. Cystitis is the clinical condition in which they are more commonly observed [1].

Umbrella cells (Figure 4.9) are another type of superficial transitional cell that can be observed during routine urinalysis, usually presenting with binucleation, but multinucleated umbrella cells are also a possible finding.

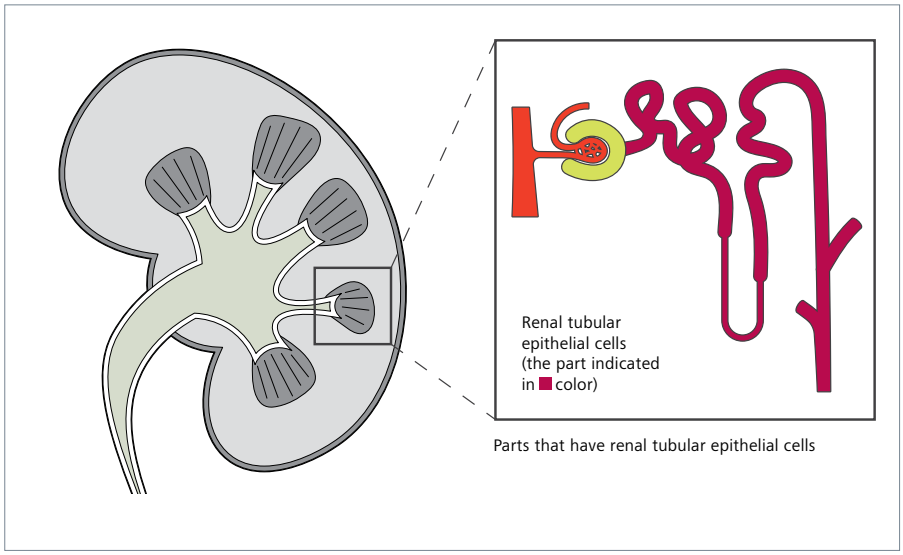


Figure 4.10: Renal tubular epithelial cells

Renal Tubular Epithelial Cells

Renal tubular epithelial cells (RTECs) can be observed in the urine sediment as a result of injury to the tubular system (Figure 4.10). As they are only observed in pathological situations they are always of clinical interest. These cells can vary in shape, contour, cytoplasmic organelles and location of the nucleus, depending on where they are placed in the tubular system.

The most common RTECs observed in urine are probably from the proximal segments (Figure 4.11). Usually these cells are round-to-oval or rectangular in shape, with a large central or eccentric nucleus containing one or two nucleoli and a granular cytoplasm showing abundant organelles.

It is important to remember that RTECs can show degenerative changes or appear in aggregates. As mentioned previously, these cells can be observed within urinary casts (Figure 4.12) that are called epithelial casts or RTEC casts.

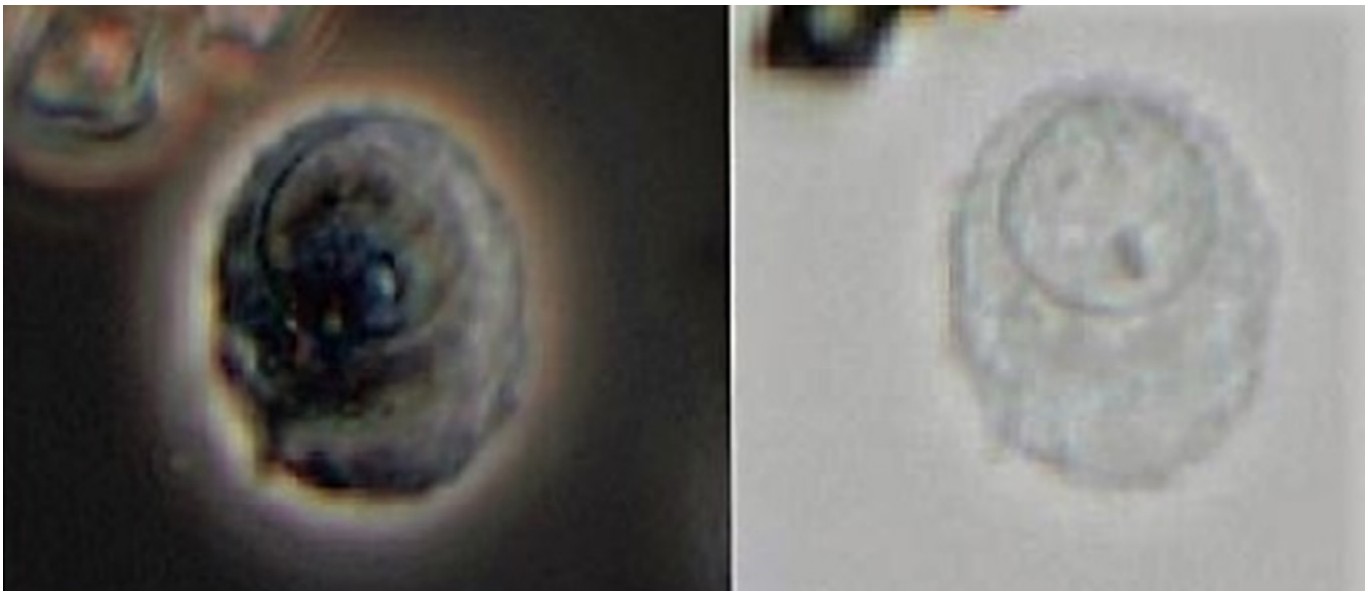


Figure 4.11: Renal tubular epithelial cell (RTEC) (black and White arrows). Cast containing RETCs (red arrows). Fresh and unstained urine sediment. Phase contrast (left); Brightfield (right). Original magnification 400×. Courtesy of Controllab.

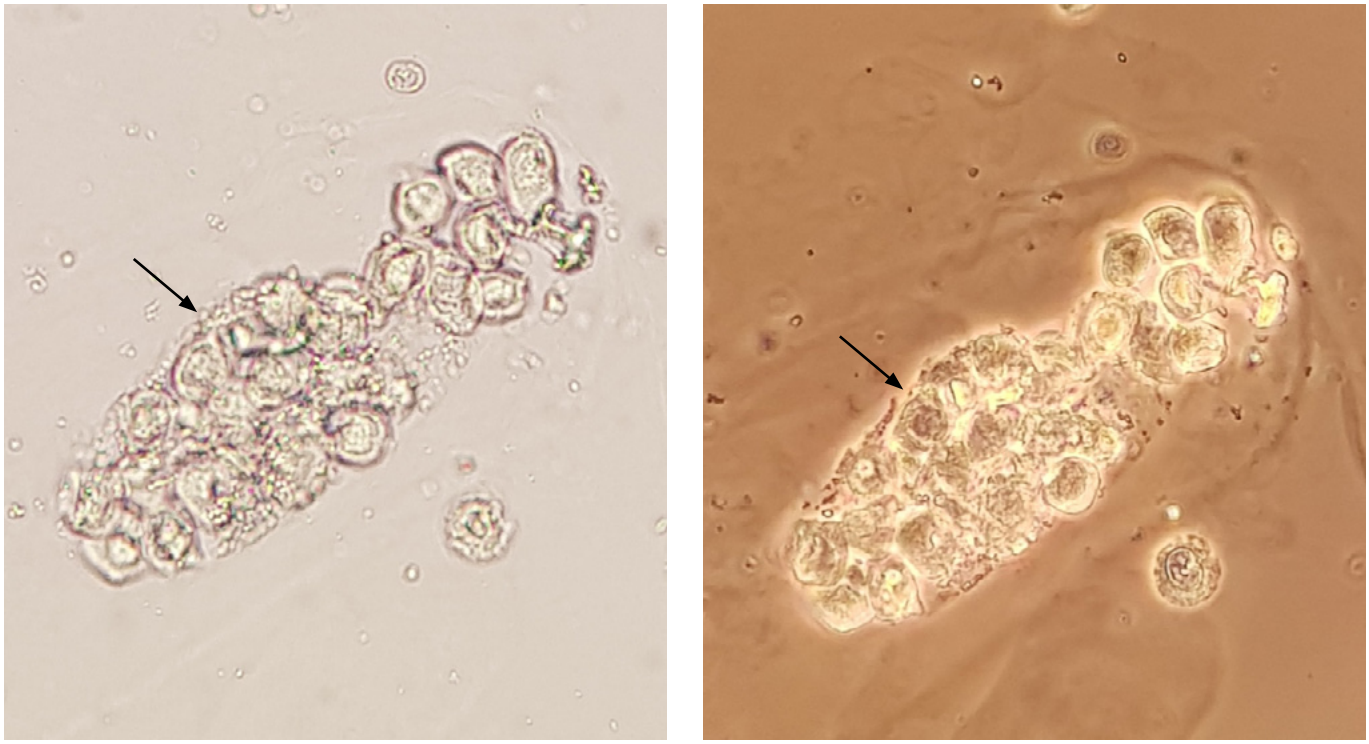


Figure 4.12: Cast containing RTECs (also called Epithelial cast). Fresh and unstained urine sediment. Brightfield (left); Phase contrast (right). Original magnification 400×. Courtesy of A. P. G. Franz.

RTECs can sometimes be difficult to differentiate from deep transitional epithelial cells. To make identification easier, it is very important to look at the clinical picture: RTECs are usually observed together with findings of renal origin such as proteinuria, casts containing RTECs, dysmorphic erythrocytes and other findings from the kidneys. On the other hand, deep transitional epithelial cells are usually observed on a urologic clinical picture without evidence to findings originated in the kidneys. So this in a tip to help differentiate of this type of cell.
While RTECs are usually observed in disorders that primarily involve the tubules–for example, acute tubular necrosis, acute interstitial nephritis or acute rejection of the kidney allograft—they are also usually observed in patients with glomerular disorders. That means RTECs can be found in patients with conditions affecting both the tubular system and the glomerulus [1 – 5].

Atypical / Malignant Cells

Any type of epithelial cell presenting morphological modifications due to viral infections, degenerative changes or malignancies will require the attention of the microscopist. The following are some examples of atypical or malignant cells.

Decoy Cells

Decoy cells or DCs (Figure 4.13) are RTECs or urothelial cells with morphological changes promoted by the intranuclear proliferation of *Polyomavirus BK* (BKV). The classical description of the morphology of this type of cell consists of nuclear enlargement, which confers a ground glass appearance and displacement of the nucleus toward the periphery of the cell as if the nucleus were escaping from the cell. In addition, chromatin margination, which is chromatin clumping along the nuclear membrane, can also be observed along with abnormal chromatin patterns, cytoplasmic vesicles and a single nuclear inclusion body surrounded by a peripheral halo, which gives a bird's eye appearance to the cells [6, 7]. When DCs are observed in the urine sediment they are a sign of BKV reactivation in the urinary system. This is especially relevant in the population of kidney allograft recipients because BKV reactivation can lead to BKV-associated nephropathy (BKVAN), a clinical condition that can cause graft loss. Finding DCs in the urine sediment, however, is not a sign of BKVAN since these cells can originate in any part of the genitourinary system. Finding DCs in urinary casts (Figure 4.14) furnishes a strong clue to the intrarenal reactivation of the BKV, but BKVAN can only be properly identified by a kidney biopsy.

Despite these limitations, the possibility of identifying the DC during routine urinalysis can be useful in the early diagnosis of BKV reactivation, an important cause of graft loss on the large population of kidney allograft recipients [7].

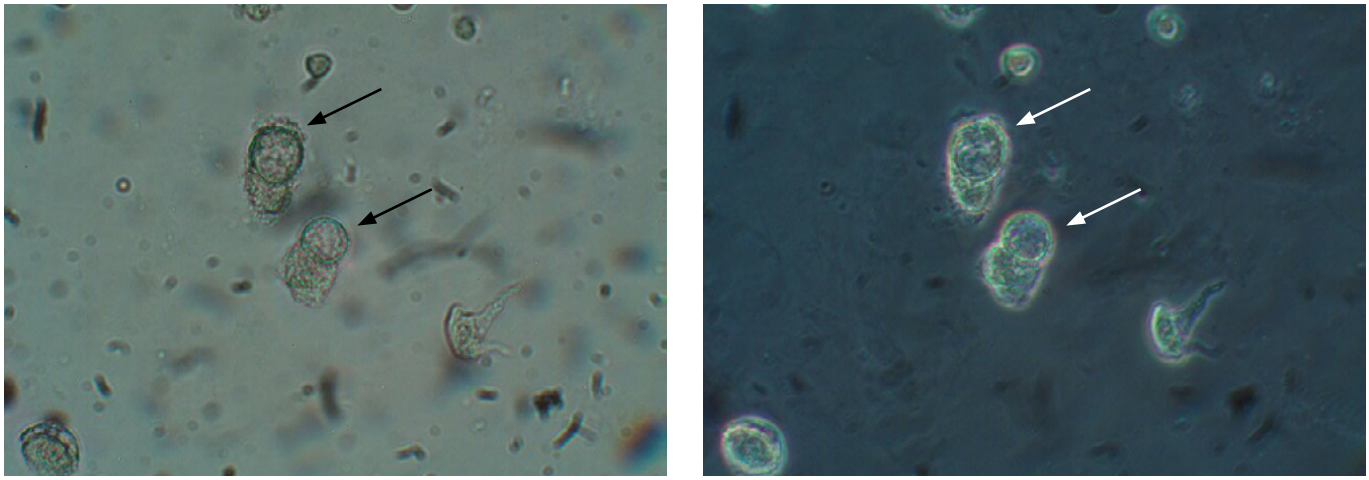


Figure 4.13: Representative image of decoy cells. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (right). Original magnification 400×. Courtesy of J. A. T. Poloni.

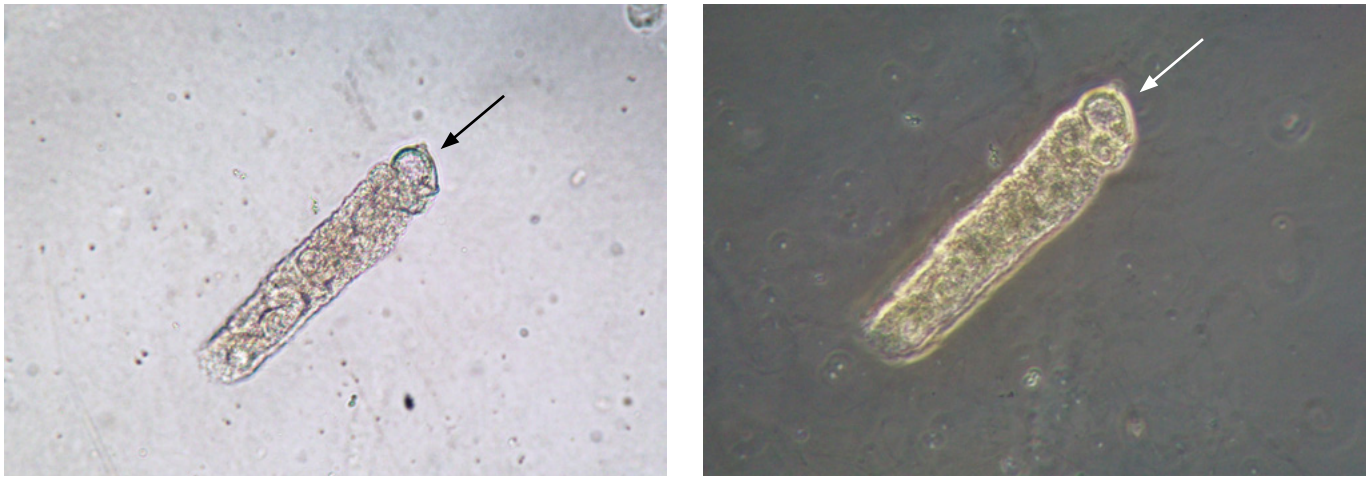


Figure 4.14: Representative image of cast containing decoy cells. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (right). Original magnification 400×. Courtesy of J. A. T. Poloni.

Koilocytes

Koilocytes (Figure 4.15) are the hallmark of human papillomavirus (HPV) infection and can be observed during routine cytology tests when stained by Papanicolaou. However, this kind of cell modification can also be observed during routine urinalysis. This is clinically-relevant information since the morphological changes in epithelial cells are produced by the HPV infection and can be associated with malignancies [8].

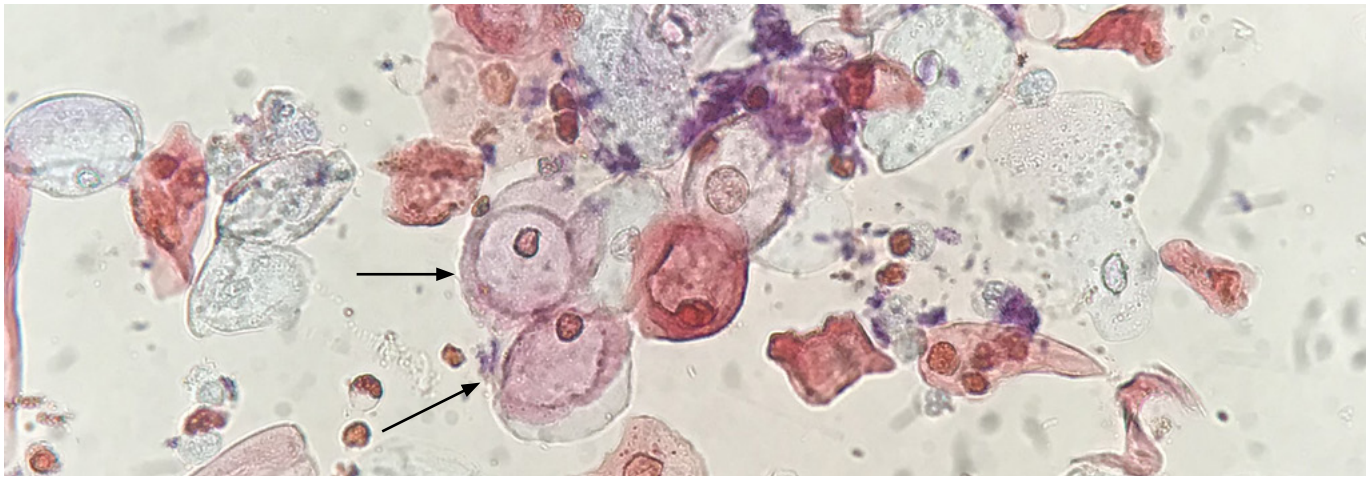


Figure 4.15: Representative image of Koilocytes. Fresh urine sediment stained with Sternheimer-Malbin stain. Brightfield. Original magnification 400×. Courtesy of J. A. T. Poloni.

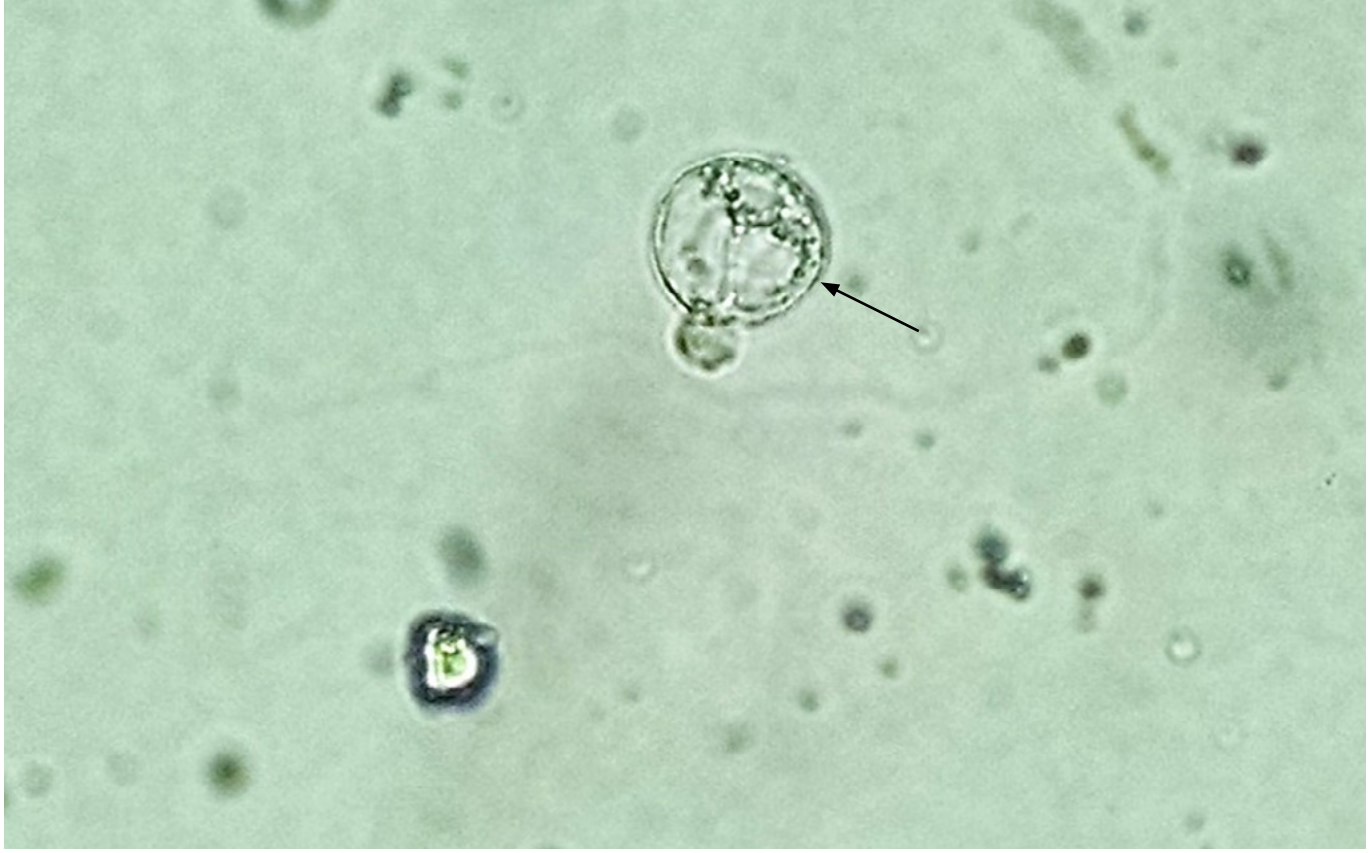


Figure 4.16: Bubble cells. Fresh and unstained urine sediment. Brightfield. Original magnification 400×. Courtesy of J. A. T. Poloni.

Bizarre Morphology

As the name suggests, bizarre morphology cells present morphological modifications that do not necessarily follow any standard pattern that we can see on the decoy cells or koilocytes. Bizarre by name, bizarre by nature.

Bubble Cells

Bubble cells (Figure 4.16) have only been described in the literature on one occasion: "These cells were bizarre, large cells with a single nucleus, which appeared to contain one or more fluid-filled vesicles. Bubble cells were most prevalent in the sediment of patients with acute tubular necrosis but were also seen in a variety of other renal diseases. In most patients with acute tubular necrosis, the sediment also contained 'normal'-appearing renal tubular cells, muddy brown casts and oval fat bodies which were indistinguishable from those seen in the nephrotic syndrome. By electron microscopy, the bubble cells appeared to be vacuolated renal tubular epithelial cells, which had characteristics of viable cells" [9].

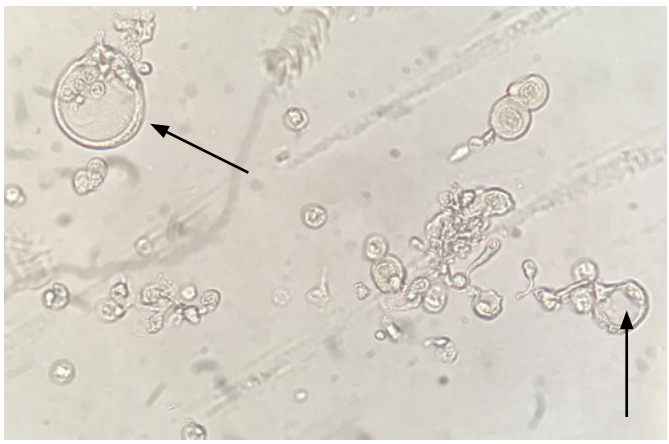


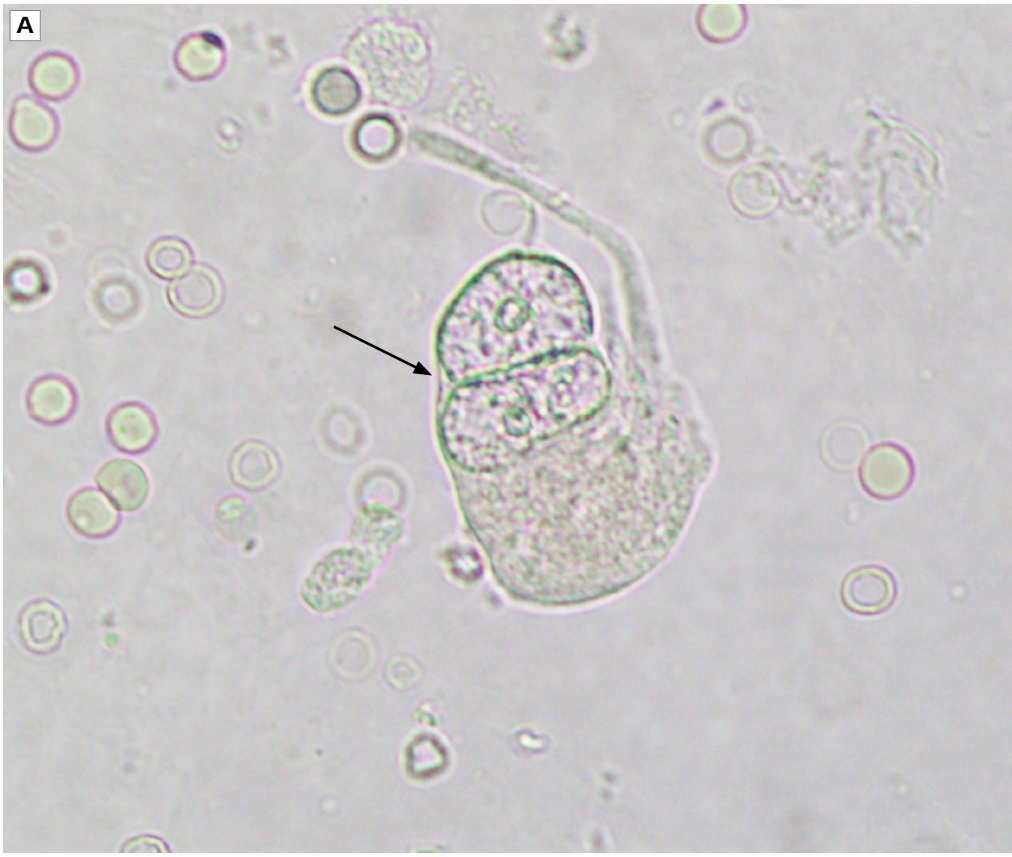
Figure 4.17: Representative image of signet ring carcinoma cells. Fresh and unstained urine sediment. Brightfield. Original magnification 400×. Courtesy of J. A. T. Poloni.

Malignant Cells

Malignant cells can be seen in a variety of morphologies. Signet ring carcinoma is an exceedingly rare and aggressive variant of primary bladder carcinoma [10]. The cells of this tumor (Figure 4.17) can be seen in urine, but cytomorphologic features in urinary specimens have not been well characterized. These cells can be easily missed or misinterpreted due to their rarity and singly dispersed nature.

Morphological modifications of the nucleus and of the cytoplasm of the cells, intranuclear inclusions and intra-cytoplasmic inclusions (Figure 4.18 A) can help with the diagnosis of important clinical conditions such as malignancy or tubular necrosis.

Tadpole cells or snake cells (Figure 4.18 B) are elongated cells where the nucleus stays visible on one side of the cell structure and a long tail-like structure is seen on the other. This kind of cell can be observed in the urine sediment of patients with malignant keratinizing squamous cells originating from a tumor in the urinary system [11]. These very different types of cells are expected to be observed coupled with leukocytes and urologic hematuria in the setting of tumors in the urinary tract. While fresh and unstained urine sediment analysis does not replace urine cytology in the diagnosis of tumors, it can be a tool to aid diagnosis or prompt further investigation [12].



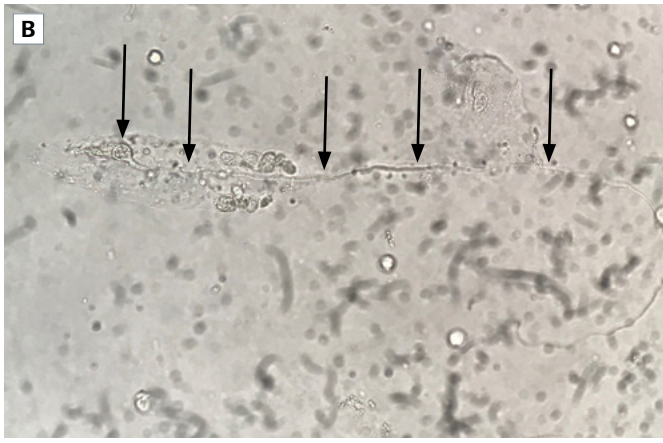


Figure 4.18: Atypical cells: Bizarre morphology (A) and Tadpole or Snake cell (B). Fresh and unstained urine sediment. Brightfield. Original magnification 400×. Courtesy of J. A. T. Poloni.

Leukocytes

Leukocytes (Figure 4.19) are one of the key elements of urinary tract infections (UTIs). Usually present in large numbers in these cases, they are an indicator of the response of the organism to the presence of any pathogenic agent. Most of the leukocytes observed in the urinary sediment during UTIs is composed of neutrophils, observed in the fresh examination as round cells having a granular appearance. However, it may not be just neutrophils present, but also eosinophils, lymphocytes and monocytes (which enter the urinary system and are converted into macrophages). Indeed, basically any leukocyte can be observed in urine samples, but it is impossible to distinguish which subtypes they are without staining. Eventually, especially under low sample concentration conditions (specific gravity <1.010), they can become swollen (Figure 4.20) or even burst. When swollen the nuclei can be seen and their organelles can also be observed in motion, Brownian movements being, at this point, nominated as “glitter cells”. Another type of change that can be observed is an elongation in the cell structure (Figure 4.21). There is, however, no clarification in the literature of how this process originated.

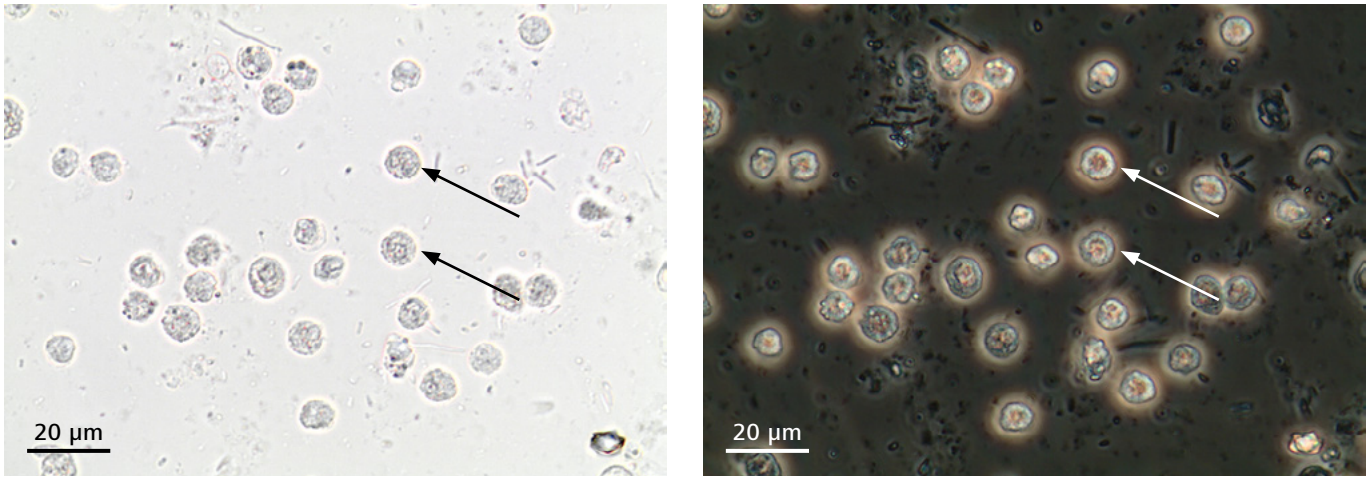


Figure 4.19: Leukocytes (some of them pointed by arrows). Fresh and unstained urine sediment. Brightfield (left); Phase contrast (right). Original magnification 400×. Courtesy of Controllab.

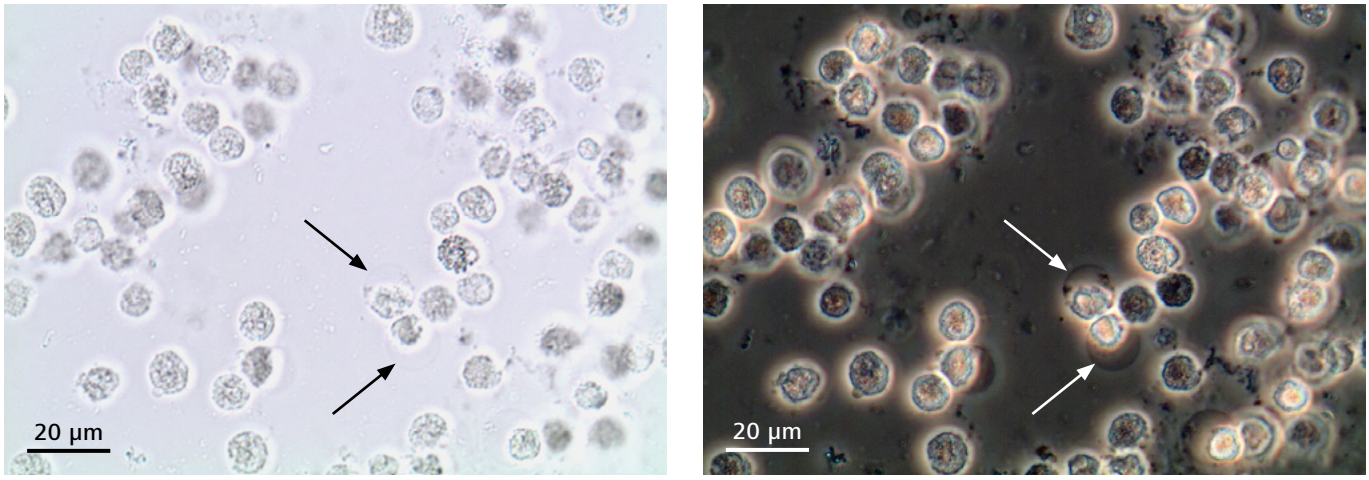


Figure 4.20: Leukocytes (swollen) (some of them pointed by arrows). Fresh and unstained urine sediment. Brightfield (left); Phase contrast (right). Original magnification 400×. Courtesy of Controllab.

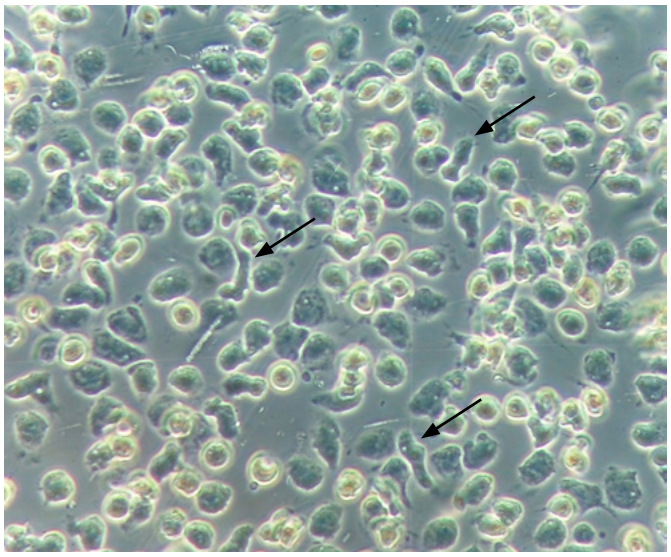


Figure 4.21: Leukocytes (elongated). Fresh and unstained urine sediment. Phase contrast. Original magnification 400×. Courtesy of J. A. T. Poloni.

It is commonly believed that where there are leukocytes, there must be bacteria in the sample, but that is not true. Leukocyturia is a sign of inflammation in the urogenital system and it is usually caused by infectious processes of bacterial origin. However, these findings are not necessarily associated. We can find leukocyturia, for example, in infections in the urinary tract caused by *Mycobacterium tuberculosis*, a bacterium that is not observed in fresh microscopy and that also does not grow in commonly used culture media for uroculture. We may also, for example, find leukocyturia associated with inflammatory processes from a tumor developing in the urinary tract. So yes, leukocytes and bacteria are usually but not necessarily associated. The fundamental thing to remember is that leukocyturia is an important sign of an inflammatory process in the urinary tract, but it is not necessarily of infectious origin [1, 13].

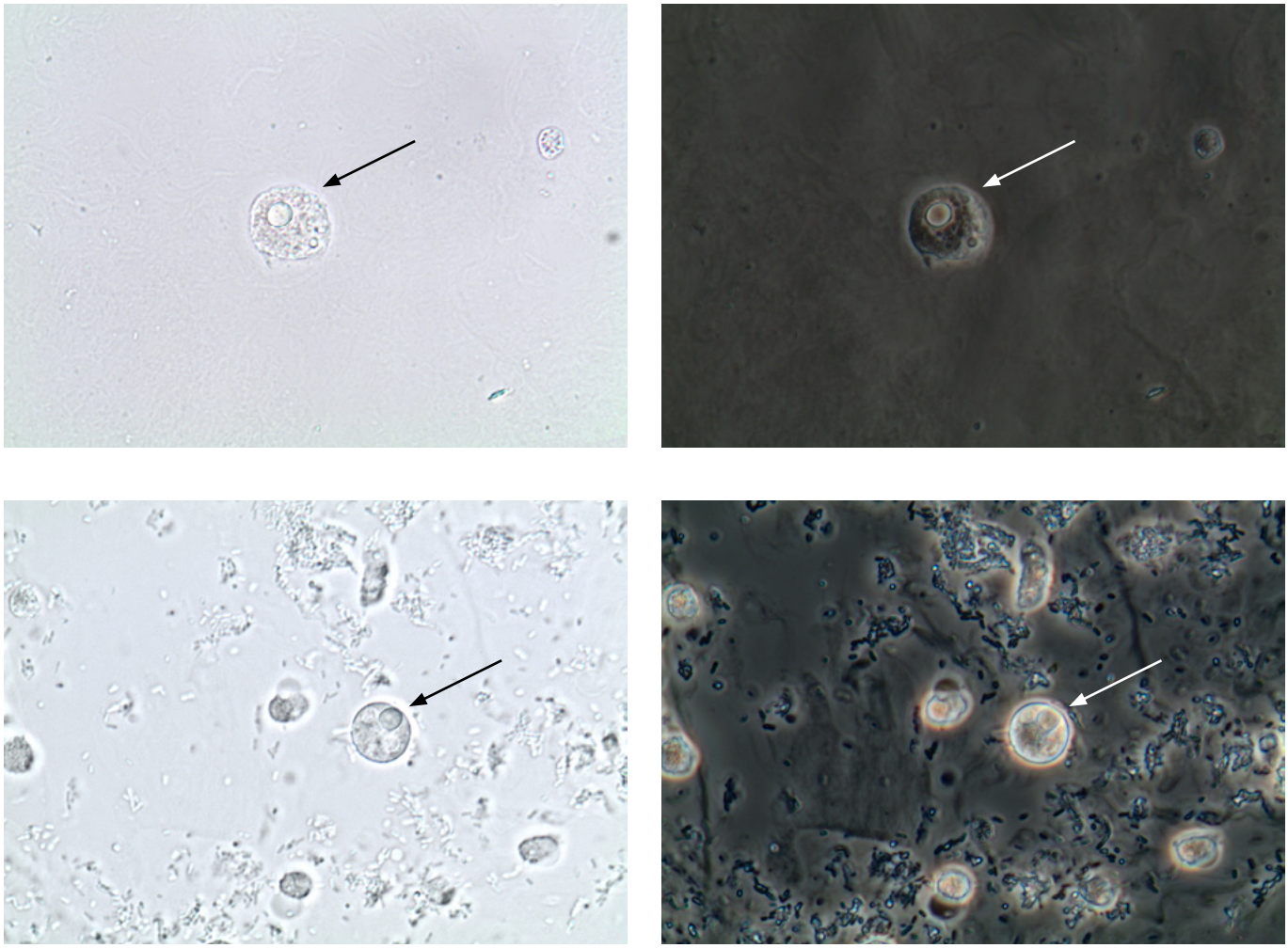


Figure 4.22: Macrophages. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (right). Original magnification 400×. Courtesy of Controllab.

Macrophages

Macrophages (Figure 4.22) are observed when there is migration of monocytes from the blood to some region of the urogenital tract. As monocytes leave the blood, they convert into macrophages and are seen as large-sized cells—in general larger than the leukocytes that are usually observed in the samples—and presenting one or more nuclei in addition to phagocytosed elements in their cytoplasm. It is not possible to identify which region of the urogenital tract they come from. The microscopist should become familiar with their morphology as they are often similar to renal tubular epithelial cells and an inexperienced observer can confuse these elements. There is a single context in which it is common to observe macrophages associated with one element in particular: that is the precise clinical context of a patient with BKV reactivation who is exhibiting decoy cells. Macrophages are almost always found in patients who have decoy cells in their urinary sediment. This is explained by the body's combat mechanism that fights viral infection by infiltrating mononuclear cells, many of which are monocytes that convert into macrophages in BKV-infected tissues [1, 7].

Erythrocytes

The presence of erythrocytes in the urine sediment indicates aggression to some part of the epithelium of the urogenital tract. The morphology of red blood cells contributes to identifying the origin of the hematuria (loss of blood in the urine), whether glomerular or non-glomerular. For example, having a bladder tumor or passing a kidney stone through the ureter promotes aggressions characterized by the presence of hematuria of non-glomerular origin—that is, urological hematuria, composed of red blood cells with normal morphology (Figure 4.23).

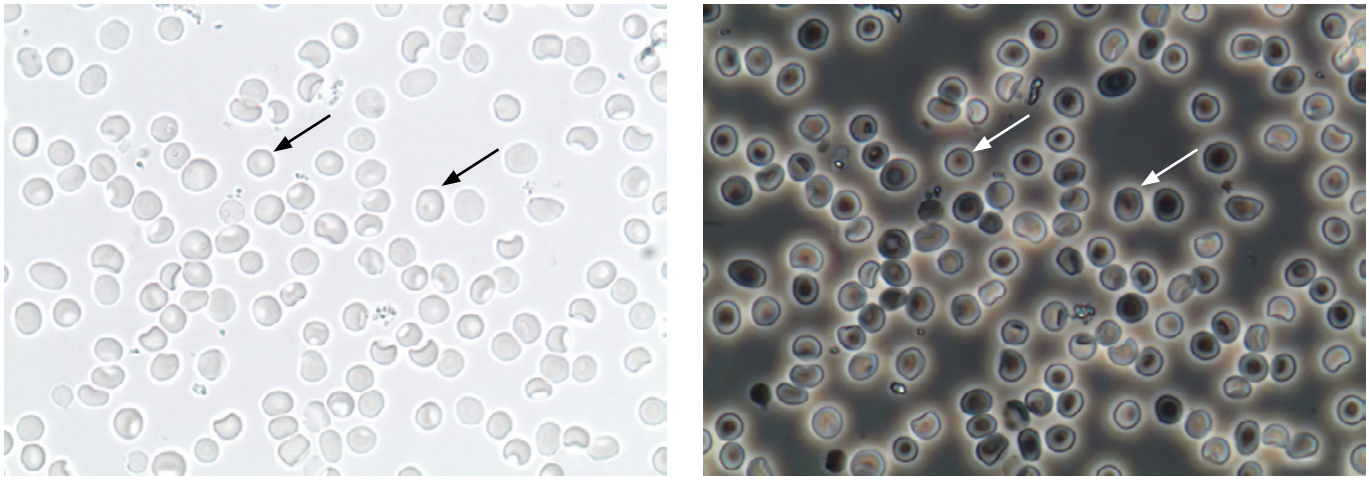


Figure 4.23: Erythrocytes (some of them pointed by arrows). Fresh and unstained urine sediment. Brightfield (left); Phase contrast (right). Original magnification 400×. Courtesy of Controllab.

In cases where there is a glomerular condition (aggression to the membrane of the glomerular capillaries), with the formation of cracks in these capillaries allowing the passage of erythrocytes, we can observe this type of cell with modifications in its structure called "erythrocyte dysmorphism".

Dysmorphic erythrocytes (Figure 4.24) are one of the hallmarks of glomerular hematuria. A special type of dysmorphic erythrocyte presents one or several membrane vesicles called "acanthocytes" or "G1" cells (Figure 4.25). This particular type of dysmorphic erythrocyte presents a higher sensitivity and specificity to identify glomerular hematuria than the previous example [1, 13].

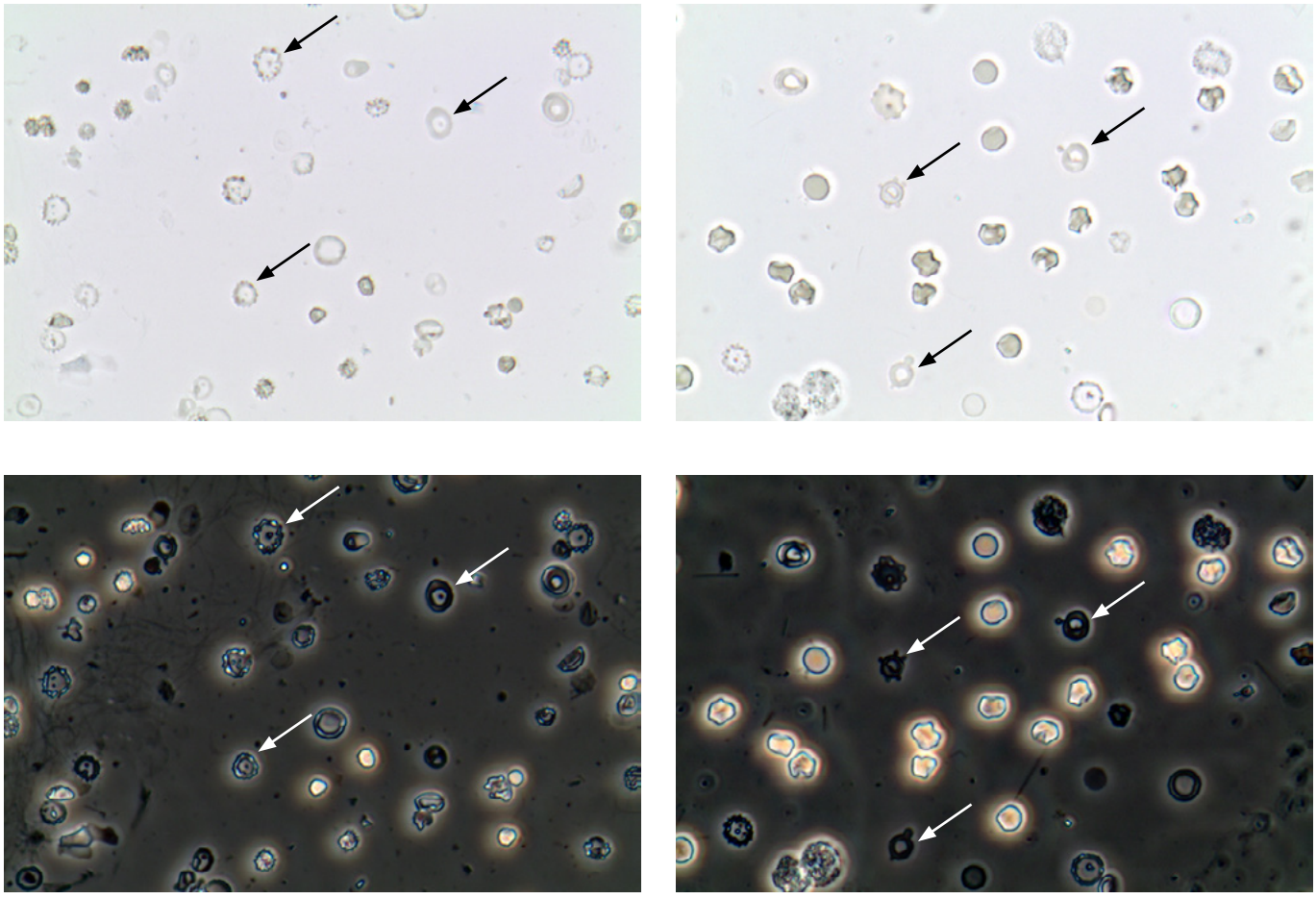


Figure 4.24: Erythrocytes (dysmorphic) (some of them pointed by arrows). Fresh and unstained urine sediment. Brightfield (left top); Phase contrast (left bottom). Original magnification 400×. Courtesy of Controllab

Figure 4.25: Erythrocytes (acanthocytes or G1 cells – a subtype of dysmorphic erythrocyte). Fresh and unstained urine sediment. Brightfield (right top); Phase contrast (right bottom). Original magnification 400×. Courtesy of Controllab.

Other types of erythrocytes can be found in the urine sediment. Sickled erythrocytes (Figure 4.26), elliptocytes, dacriocytes, anisocytes and poikilocytes have already been reported in the literature [14]. Erythrocytes in rouleaux can be found in urine as well [15]. These features known from hematology books are also of clinical utility when observed during routine urinalysis, being linked to hematological clinical conditions.

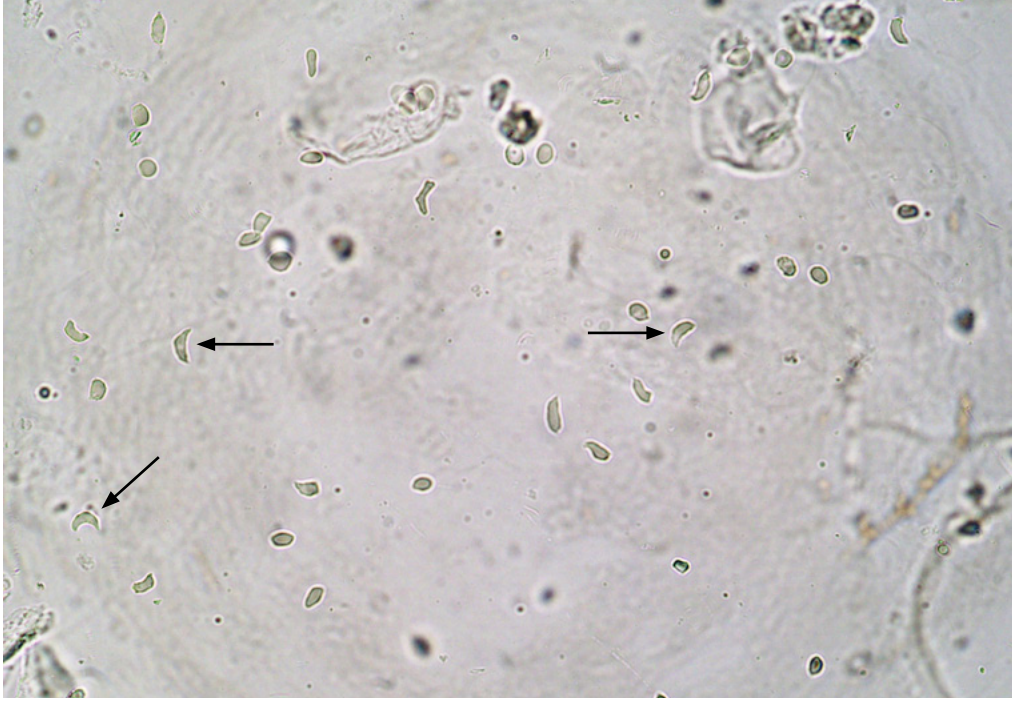


Figure 4.26: Sickled erythrocytes or drepanocytes (some of them pointed by arrows). Fresh and unstained urine sediment. Brightfield microscopy. Original magnification 400×. Courtesy of J. A. T. Poloni.

Casts

Urinary casts are particles that can be observed during routine urinalysis. They can help identify conditions affecting the kidneys since these structures are formed in the tubular lumens and can have elements of clinical interest attached to their proteinaceous matrix.

Factors like increased intratubular concentration of proteins, low intratubular pH and high osmolality favor the aggregation of Tamm-Horsfall glycoprotein (THG) fibers originating a structure with cylindrical shape (the urinary cast). Since THG is secreted by the tubular cells of the ascending limb of Henle's loop, cast formation usually occurs in the ascending limb of Henle's loop as well, and also in the distal tubules and the collecting ducts of the kidneys.

Casts come in a wide range of shapes, sizes, morphologies and particles attached to their matrix. They are classified according to the characteristics of both the matrix and the structures observed within it [1-5, 13, 16]. According to the cast matrix, there are basically two different classifications: hyaline or waxy.

Hyaline Casts

Hyaline casts (Figure 4.27) are composed exclusively of THG. Because of their low refractive index, phase contrast microscopy is the preferred technique for their identification. Hyaline is the only type of urinary cast that's not necessarily linked to pathological conditions [13]. They can be observed in normal subjects—for example, after strenuous physical exercise—and in non-renal disorders such as fever, dehydration or acute congestive heart failure, or in association with the use of Henle's loop diuretics. They can also be observed in patients with kidney diseases such as glomerulonephritis and acute interstitial nephritis [16–18].

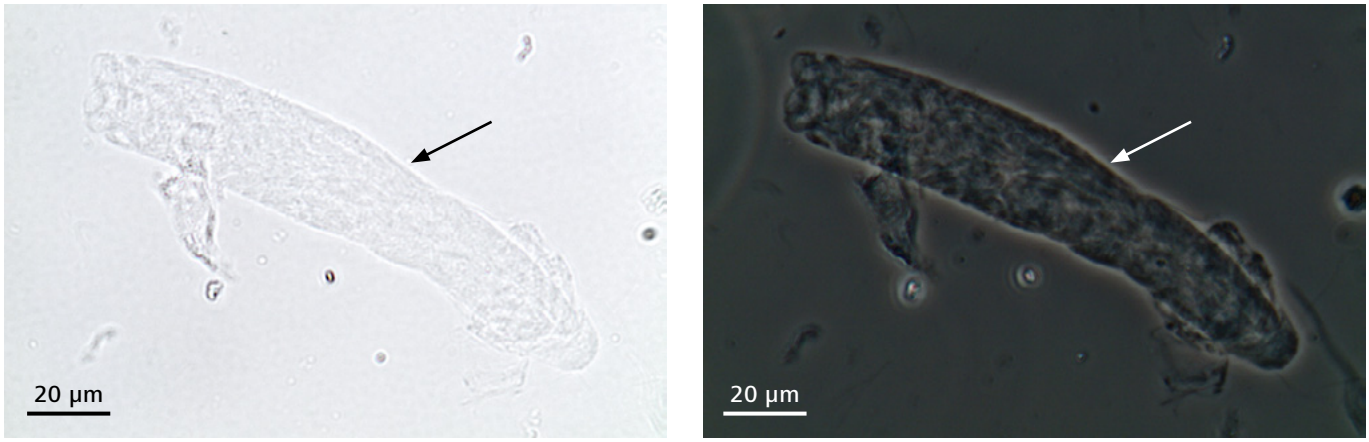


Figure 4.27: Hyaline cast. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (right). Original magnification 400×. Courtesy of Controllab.

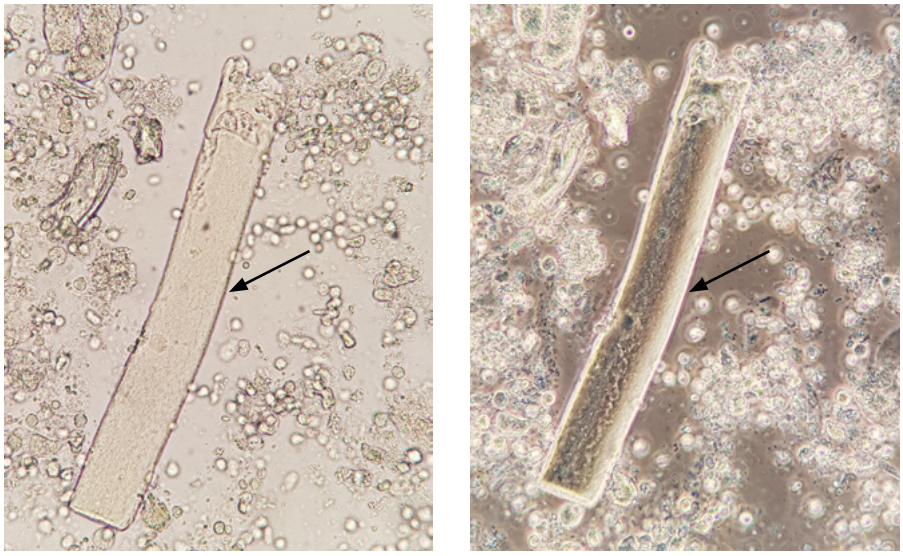


Figure 4.28: Waxy cast. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (right). Original magnification 400×. Courtesy of A. P. G. Franz.

Waxy Casts

In contrast to hyaline casts, waxy casts (Figure 4.28) present a high refractive index and are dark with indented, cracked edges [16]. This type of cast is usually large and, when present in significant numbers, they suggest advanced kidney disease [13]. Again unlike the hyaline cast with its matrix composed of THG, the composition of the waxy cast's matrix remains unknown. However, one important fact was revealed in a study [19] of patients with glomerulonephritis: no THG was found in waxy casts.

According to the structures attached to or embedded in the cast matrix, there are several classifications used with different clinical significances:

Granular Casts

Granular casts (Figure 4.29), as the name implies, present a granular surface which derives from the degeneration of cellular elements like leukocytes, erythrocytes and RTECs that are present in the tubular lumen during cast formation. Granular casts are a clear sign of kidney injury and when observed together with free RTECs and casts containing RTECs, they are a sensitive marker of acute tubular necrosis. Since the granules can come from different cellular sources, it is always important to evaluate these casts along with other urinalysis findings and clinical information [13,16].

Casts Containing RTECs

Casts containing (renal tubular epithelial cells, RTEC casts or epithelial casts (Figure 4.30) are observed within the context of tubular epithelium injury with RTECs shedding into the lumen and their attachment to the cast matrix. Usually this scenario is an indication of tubular necrosis. However, casts containing RTECs are also observed in glomerular diseases since associated tubular damage is usual in both nephrotic and nephritic conditions [13].

Pigmented Casts

Pigmented casts can be observed as a result of different sources of pigments. They can originate from both endogenous (e.g., bilirubin, hemoglobin or myoglobin) and exogenous (e.g., phenazopyridine) pigments.

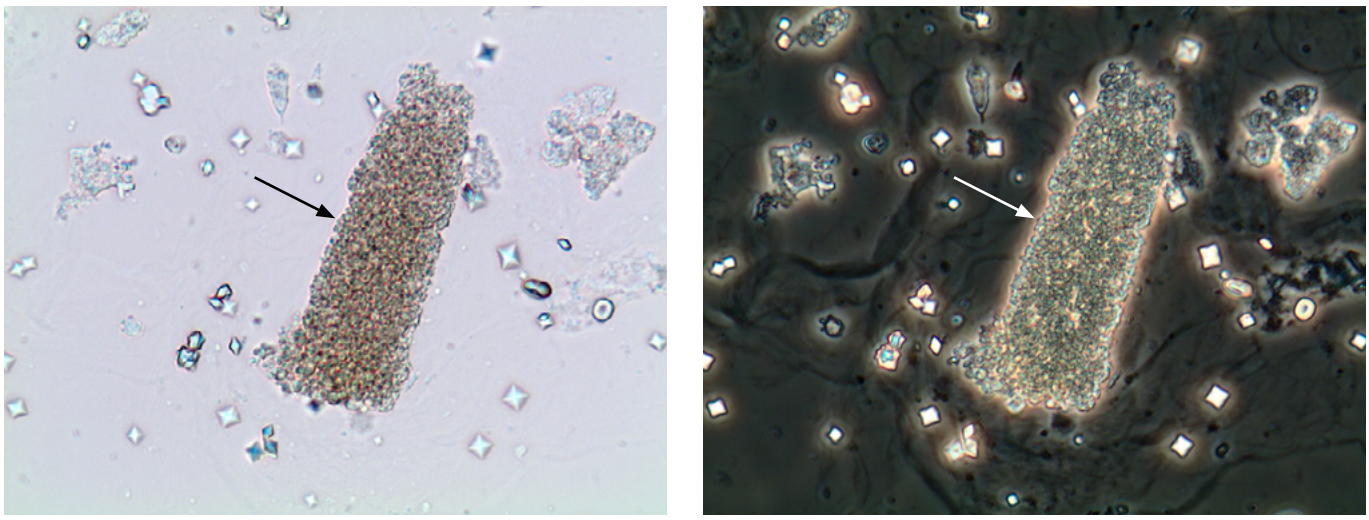


Figure 4.29: Granular cast. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (right). Original magnification 400×. Courtesy of Controllab.

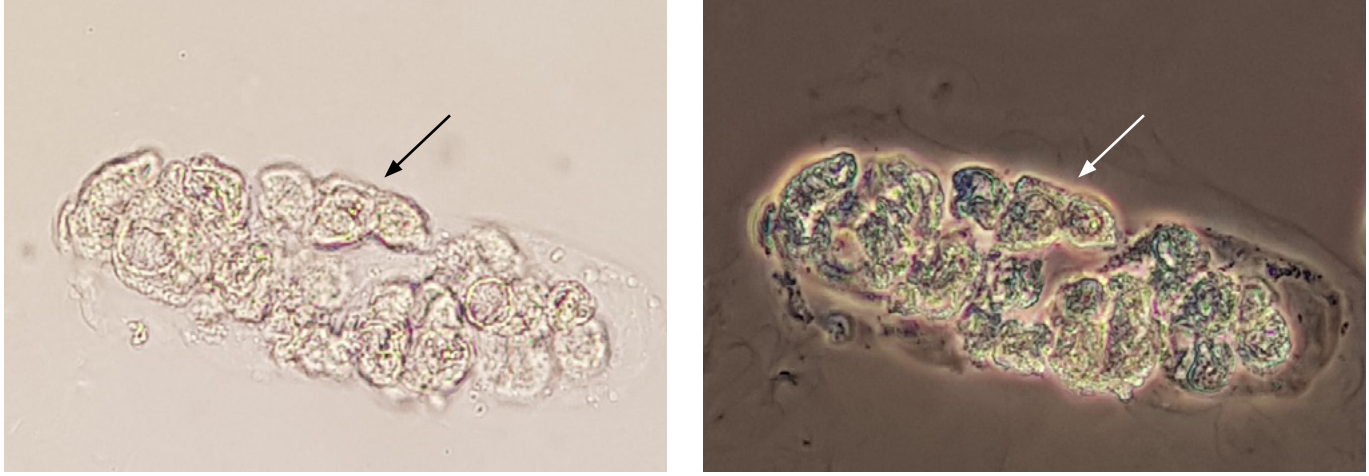


Figure 4.30: Cast containing renal tubular epithelial cells (RTECs), aso called RTEC cast or Epithelial cast. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (right). Original magnification 400×. Courtesy of A. P. G. Franz.

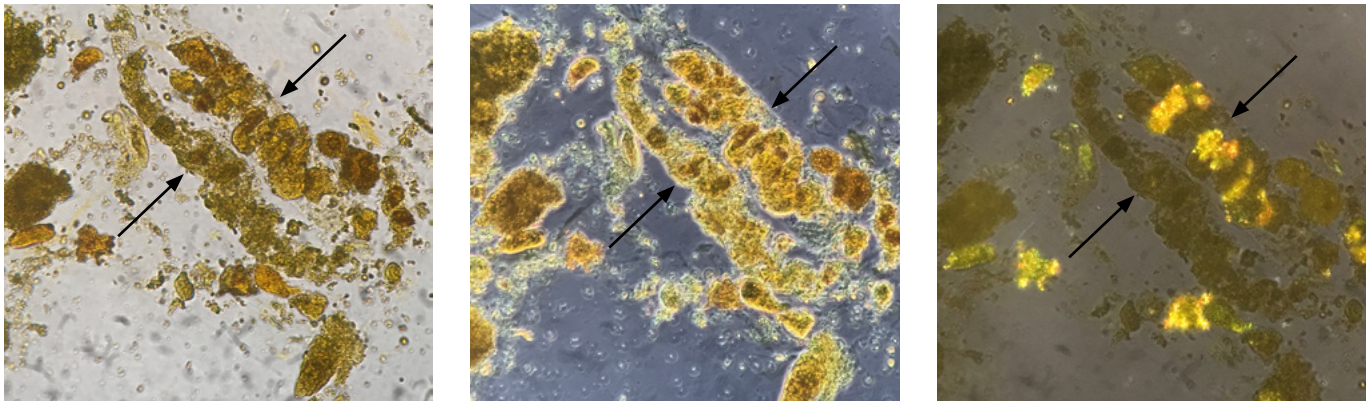


Figure 4.31: Bilirubin casts (granular and containing RTECs – under polarized light also it is possible to see that there are crystals). Fresh and unstained urine sediment. Brightfield (left); Phase contrast (center); Polarized light (right). Original magnification 400×. Courtesy of J. A. T. Poloni.

Bilirubin Casts

Bilirubin casts (Figure 4.31) are a type of pigmented cast observed in patients with high levels of direct bilirubin in the blood and urine. These casts are usually stained with a typical yellow-brown color, and both granular casts and casts containing RTECs can be found pigmented by bilirubin [16,20]. Waxy casts can also be stained by this endogenous pigment (Figure 4.32).

Hemoglobin Casts

Hemoglobin casts can come from two different sources—those caused by glomerular hematuria (Figure 4.33) and those from hemoglobinuria caused by hemolytic anemia (Figure 4.34). Their color ranges between brown and red. To properly identify the condition that is causing the observation of hemoglobin casts, it is useful to create a clinical picture of the patient. Consider, for example, hemoglobin and protein positive tests at dipstick analysis; the presence of dysmorphic erythrocytes and casts containing erythrocytes that favor the identification of hemoglobin casts due to glomerular hematuria; a positive test for hemoglobin and elevated urobilinogen at dipstick analysis; the absence of erythrocytes in the sediment; the presence of RTCs or casts containing RTECs' and granular casts favoring the identification of hemoglobin casts due to acute tubular necrosis in hemolytic anemia 16,21].

Figure 4.32: Bilirubin cast (waxy). Fresh and unstained urine sediment. Brightfield. Original magnification 400×. Courtesy of J. A. T. Poloni.

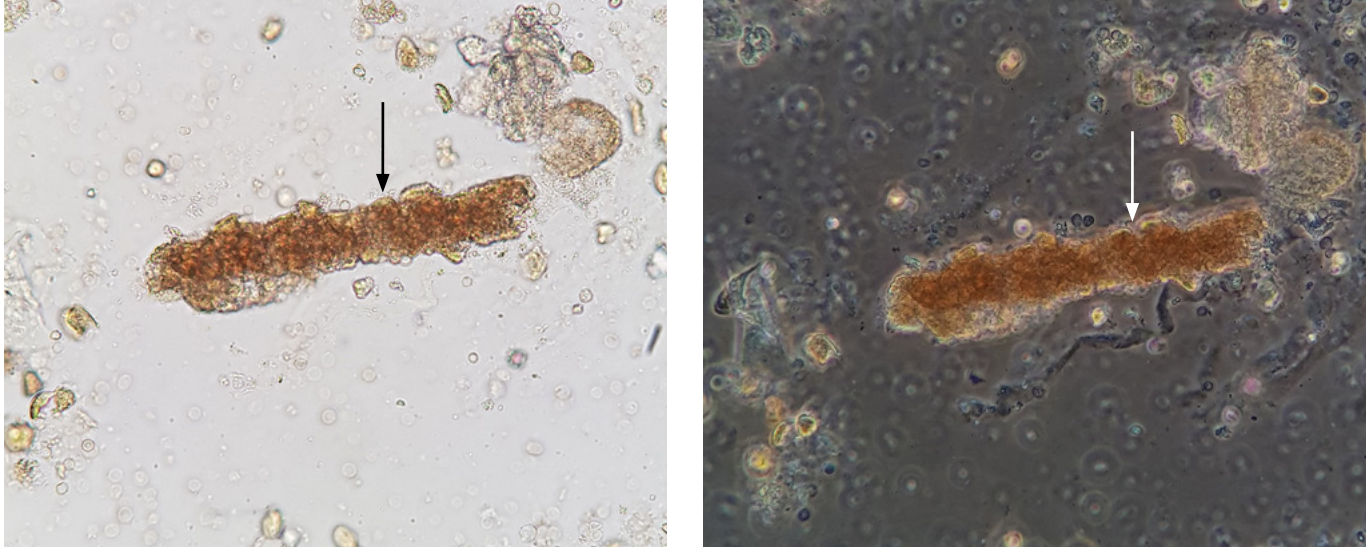


Figure 4.33: Hemoglobin cast (granular cast) in the fresh and unstained urine sediment of a patient with glomerular hematuria. Brightfield (left); Phase contrast (right). Original magnification 400×. Courtesy of A. P. G. Franz.

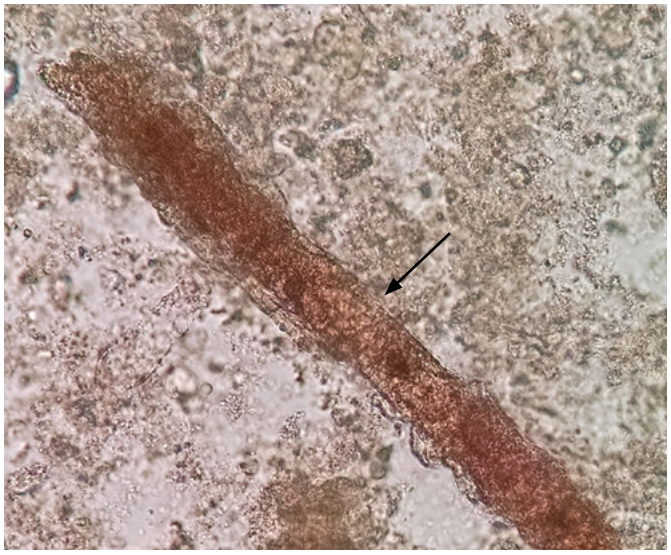


Figure 4.34: Hemoglobin cast (granular cast) in the fresh and unstained urine sediment of a patient with hemolytic anemia. Brightfield. Original magnification 400×. Courtesy of J. A. T. Poloni

Myoglobin Casts

Myoglobin casts (Figure 4.35) also present a brown-red color but, unlike hemoglobin casts, they are not observed within the context of glomerular hematuria or hemolytic anemia. Myoglobin casts are found in patients with severe muscle damage leading to acute kidney injury associated with rhabdomyolysis. Usually myoglobin casts are observed in acute tubular necrosis (free RTECs associated to casts containing RTECs and granular casts) [16,22].

Phenazopyridine Casts

Phenazopyridine casts (Figure 4.36) can be observed in patients using medications that contain phenazopyridine. These casts present an intense orange color and both granular casts and casts containing RTECs can be observed within this context. Since excessive ingestion of phenazopyridine can cause acute renal failure [23], finding this type of cast can be of clinical interest.

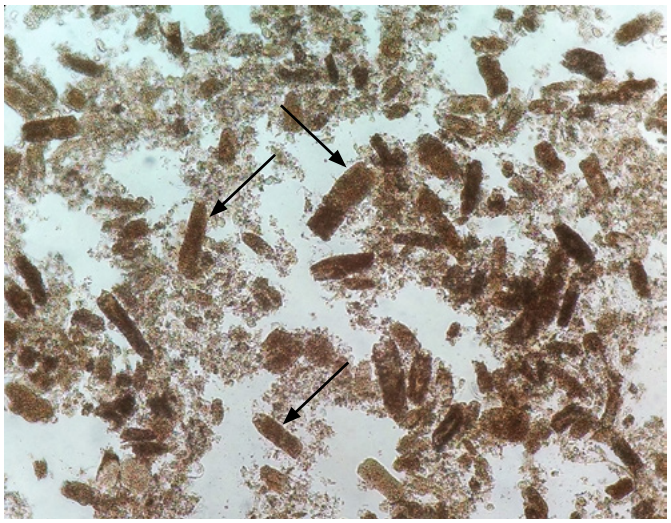


Figure 4.35: Representative image of Myoglobin casts (granular casts) in the fresh and unstained urine sediment of a patient with rhabdomyolysis. Brightfield. Original magnification 100×. Courtesy of J. A. T. Poloni

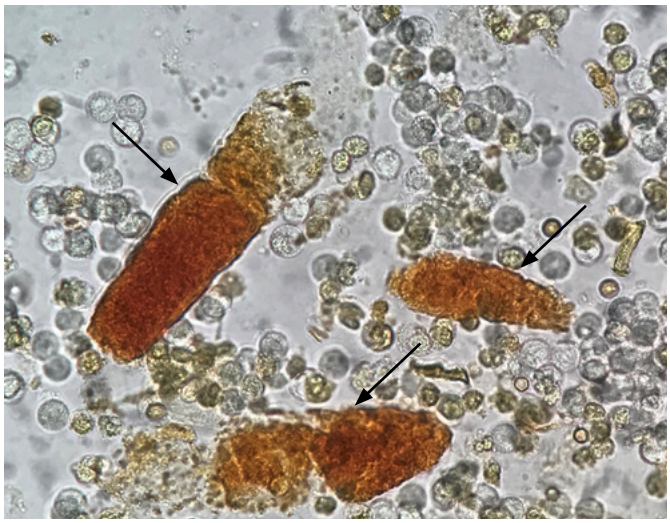


Figure 4.36: Phenazopyridine casts (granular casts). Fresh and unstained urine sediment. Brightfield. Original magnification 400×. Courtesy of J. A. T. Poloni.

Casts Containing Leukocytes

When an inflammatory condition is affecting the kidneys, particularly the tubular system, casts can be observed containing leukocytes (Figure 4.37). Differentiating leukocytes in casts from RTECs can be very challenging because it is common to see the structures in a degenerated state in the urinary casts. When leukocytic casts are observed within the context of urinary tract infection they are a clue for differentiating pyelonephritis from cystitis as the leukocytic cast favors the identification of pyelonephritis. Other clinical conditions characterized by intrarenal inflammation—for example, acute interstitial nephritis and glomerular disorders—can also have leukocytic casts between their findings [16].

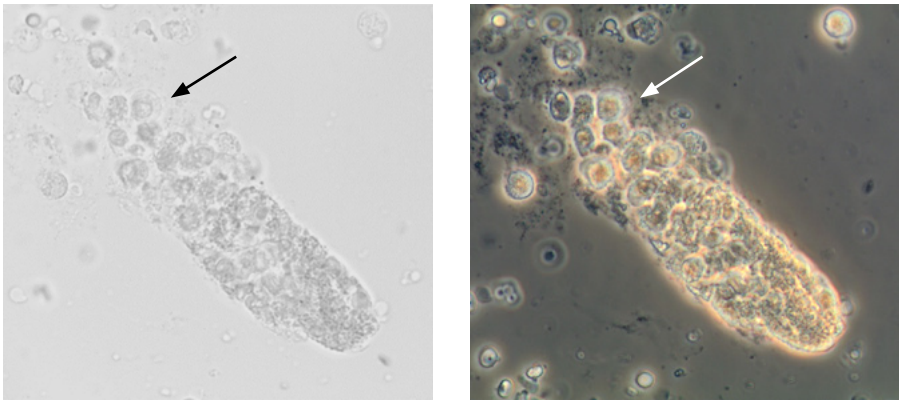


Figure 4.37: Cast containing leukocytes, also called Leukocytic cast or WBC cast. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (right). Original magnification 400×. Courtesy of Controllab.

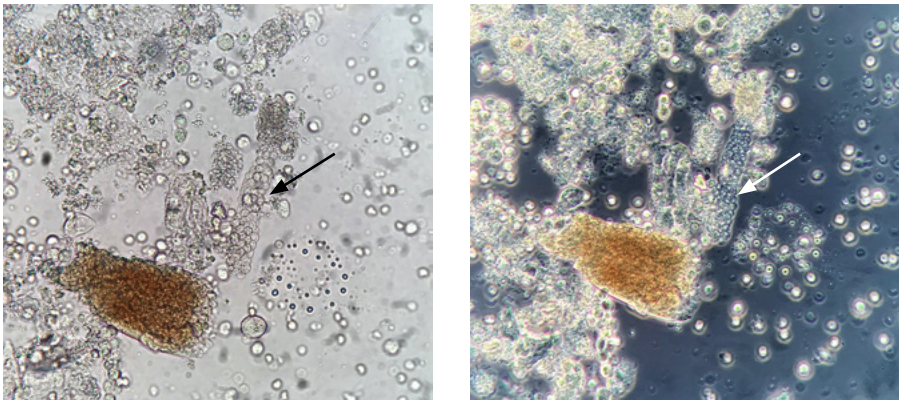


Figure 4.38: Cast containing erythrocytes, also called Erythrocytic cast or RBC cast. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (right). Original magnification 400×. Courtesy of José A. T. Poloni.

Casts Containing Erythrocytes

Casts containing erythrocytes (Figure 4.38) are a well-established marker of glomerular hematuria. Their clinical significance remains the same, regardless of the number of erythrocytes in the urinary cast. They are usually observed together with dysmorphic erythrocytes (the other known marker of glomerular hematuria) [16].

Fatty Casts

The term "fatty casts" refers to casts containing lipid droplets (Figure 4.39), and / or oval fat bodies and / or cholesterol crystals (Figure 4.40).

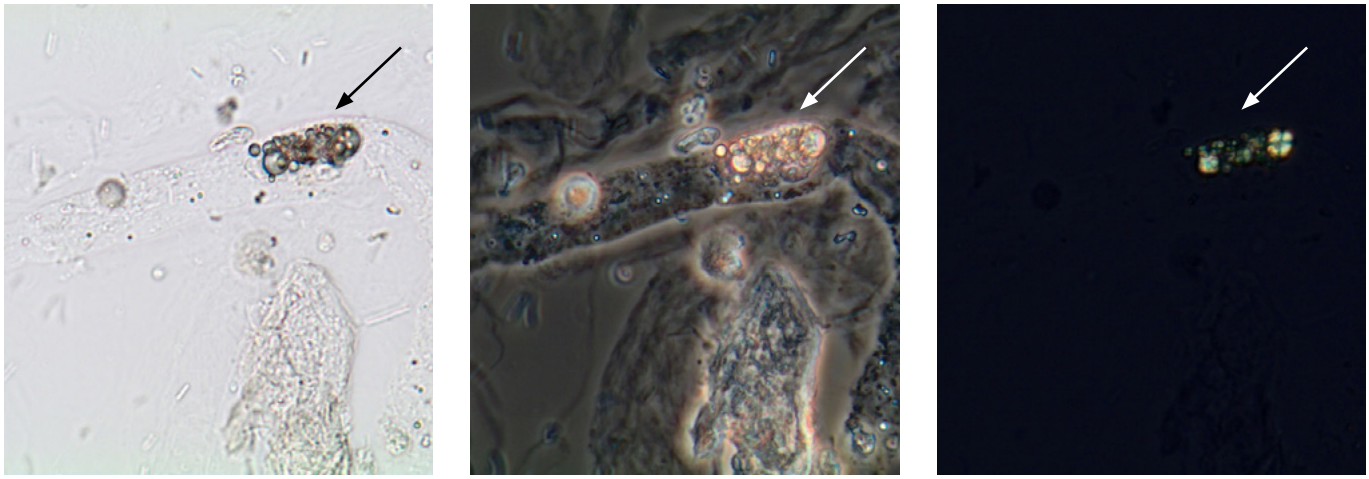


Figure 4.39: Cast containing lipid droplets (Fatty cast). Fresh and unstained urine sediment. Brightfield (left); Phase contrast (center); Polarized light microscopy (right). Original magnification 400×. Courtesy of Controllab.

Proper identification of fatty particles requires polarized light microscopy to observe the Maltese cross-like patterns produced by cholesterol esters when observed using this kind of microscopic resource. Identification of cholesterol crystals also benefits from the use of the polarized filters since these crystals usually present a monochromatic pattern under this microscope. Fatty casts usually are observed in patients with heavy proteinuria (the nephrotic range proteinuria) [16].

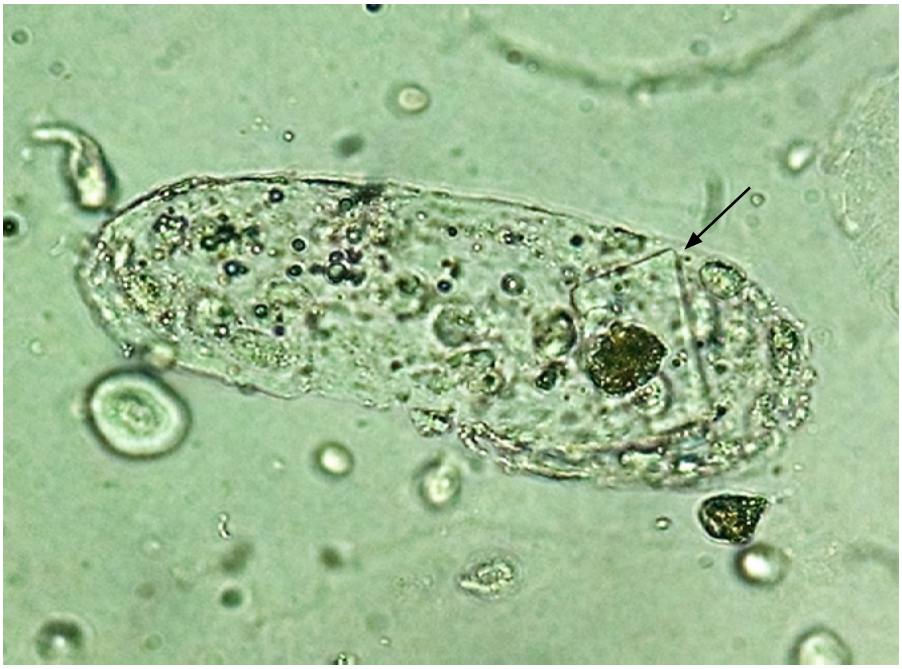


Figure 4.40: Cast containing cholesterol crystals (Fatty cast). Fresh and unstained urine sediment. Brightfield. Original magnification 400×. Courtesy of J. A. T. Poloni.

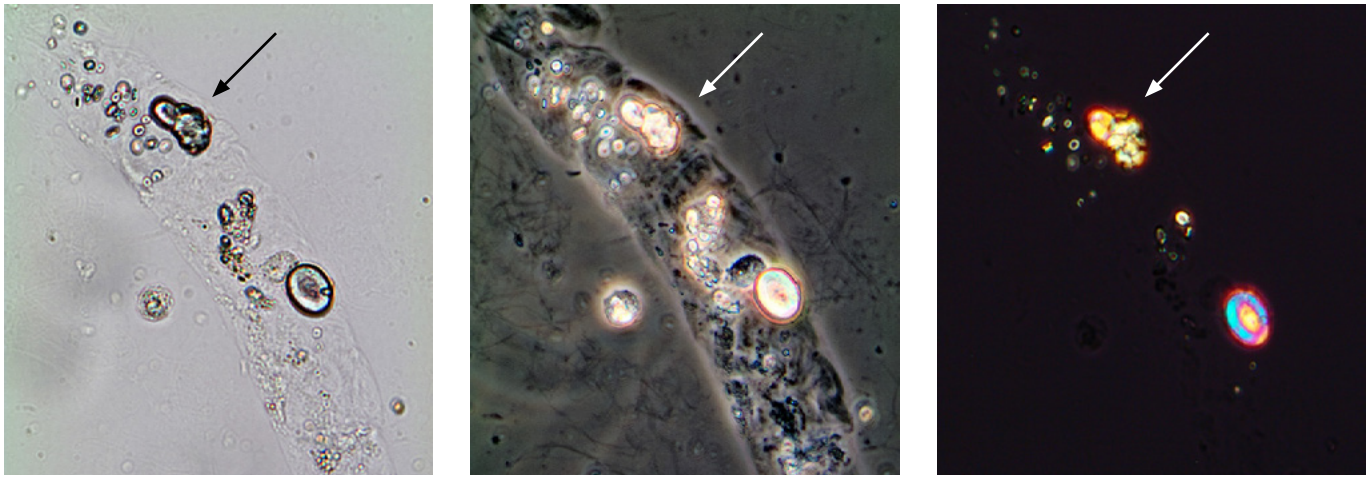


Figure 4.41: Cast containing calcium oxalate monohydrate crystals. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (center); Polarized light (right). Original magnification 400×. Courtesy of Controllab.

Casts Containing Crystals

Casts containing crystals are a sign of intratubular precipitation of crystals. Virtually any kind of crystal can precipitate in the urinary tubular system and, consequently, become entrapped in the cast matrix. The most common types of crystals observed in casts are calcium oxalate monohydrate (Figure 4.41) and bi-hydrate. Polarized light microscopy is very helpful for making a proper identification. [16, 24].

Casts Containing Microorganisms

Casts containing microorganisms are a sign of infection in the kidney tissue. Both bacteria (Figure 4.42) and fungi can be observed in the cast matrix, and this is of clinical interest. When *Candida sp.* or *Cryptococcus sp.* (Figure 4.43) is observed, it suggests systemic infection [16, 25, 26].

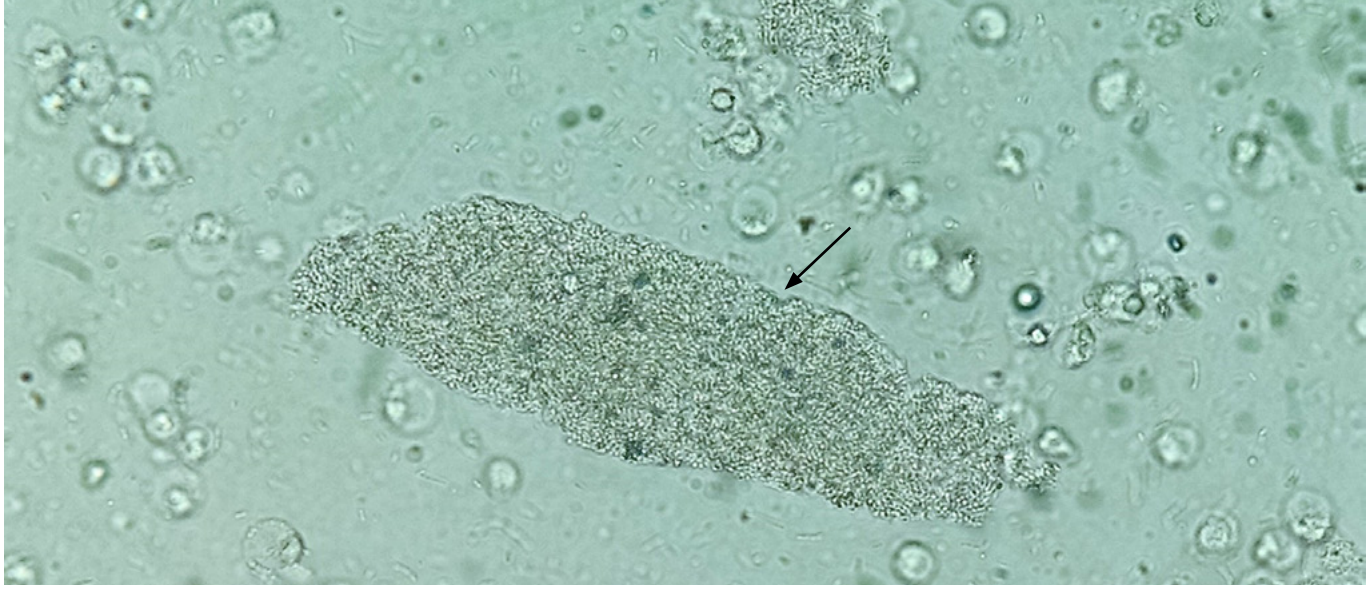


Figure 4.42: Cast containing bacteria. Fresh and unstained urine sediment. Brightfield. Original magnification 400×. Courtesy of José A. T. Poloni.

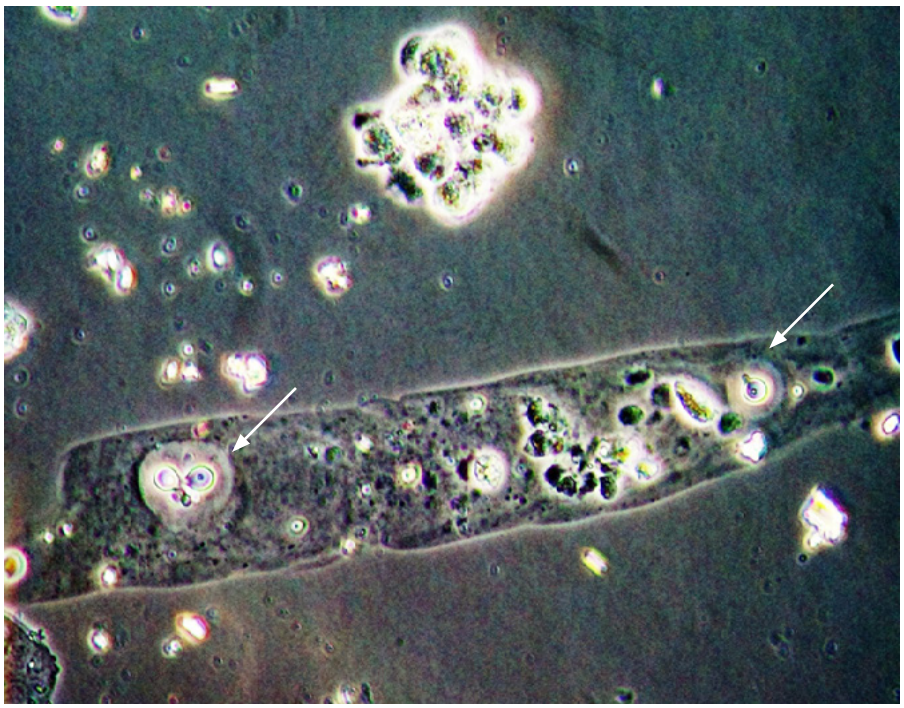


Figure 4.43: Cast containing Cryptococcus sp. encapsulated yeast. Fresh and unstained urine sediment. Phase contrast. Original magnification 400×. Courtesy of J. A. T. Poloni.

Vacuolated Casts

Vacuolated casts (Figure 4.44) are a distinct type of cast that can be found in specimens of patients with advanced proteinuric glomerulopathy. This type of cast was described in the literature only recently and very little is known about it [27]. The specific origin and composition of these casts remain unknown and require further study.

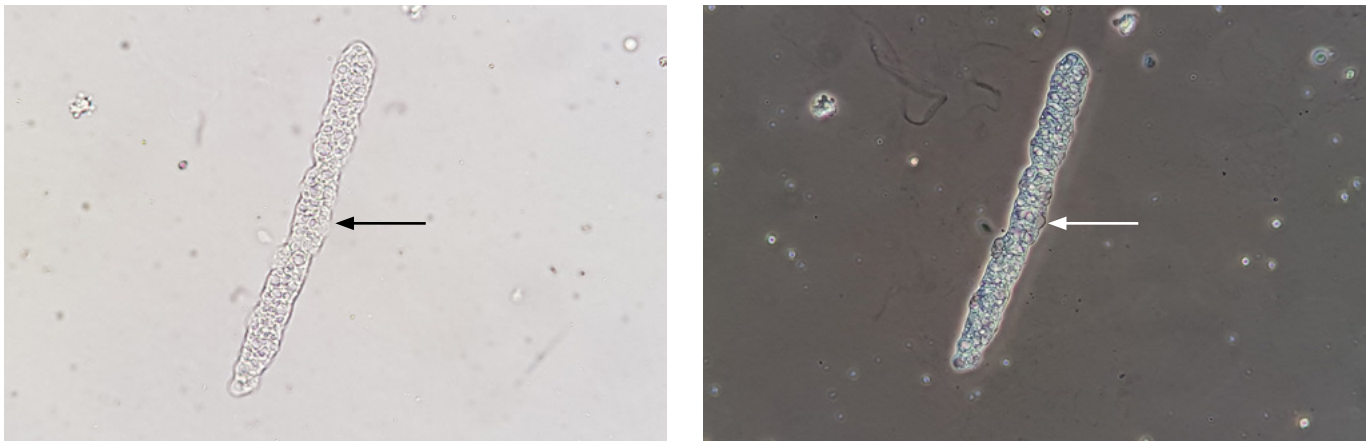


Figure 4.44: Vacuolated cast. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (right). Original magnification 400×. Courtesy of A. P. G. Franz.

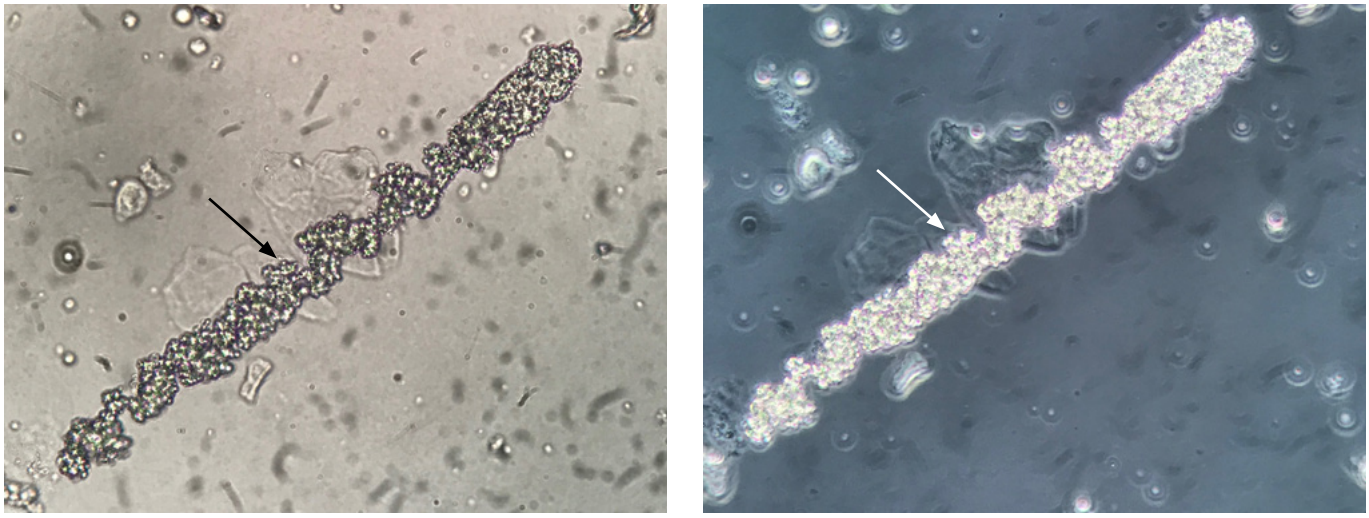


Figure 4.45: Light chain crystal cast. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (right). Original magnification 400×. Courtesy of J. A. T. Poloni.

Light Chain Crystal Casts

Light chain crystal casts (Figure 4.45) have been reported in patients with multiple myeloma complicated by acute kidney injury. Identifying these crystalline casts in the urine offers a non-invasive way of recognizing light chain-associated kidney injury (cast nephropathy, light chain proximal tubulopathy). This type of cast was also described only recently and limited information is available about it in the literature [28–30].

Mixed casts

A large variety of mixed casts can be found in the urine, the most frequent being hyaline-granular (Figure 4.46). Among others, these include erythrocytic-epithelial, granular-cellular, granular-fatty, waxy-granular (Figure 4.47) and waxy-cellular [16]. Since these casts are made by at least two different compounds, their clinical significance will be related to the components that are present in the mixed cast.

Cylindroids

A morphological variant of casts, cylindroids (Figure 4.48) are associated with casts and can have the same appearance. Their surface contains THG [1].

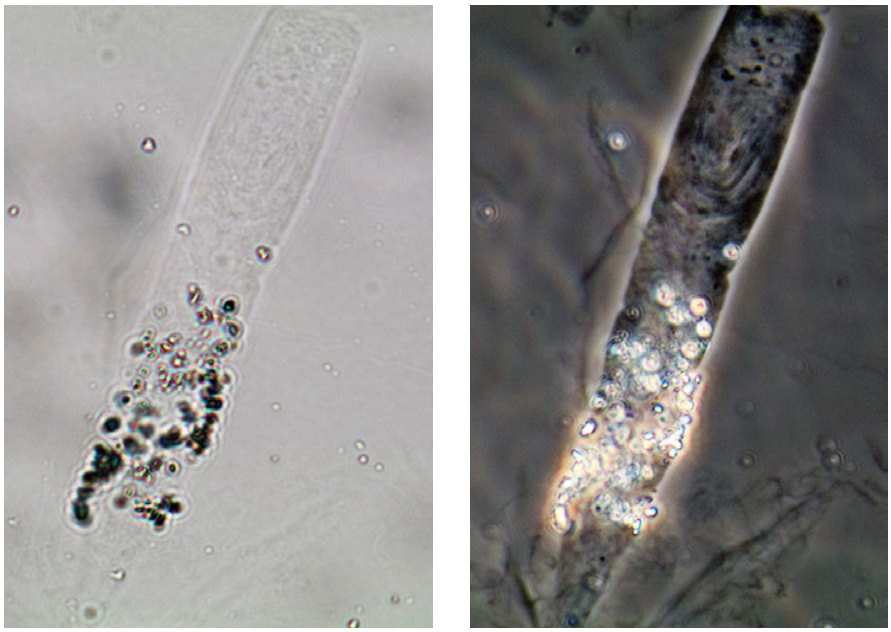
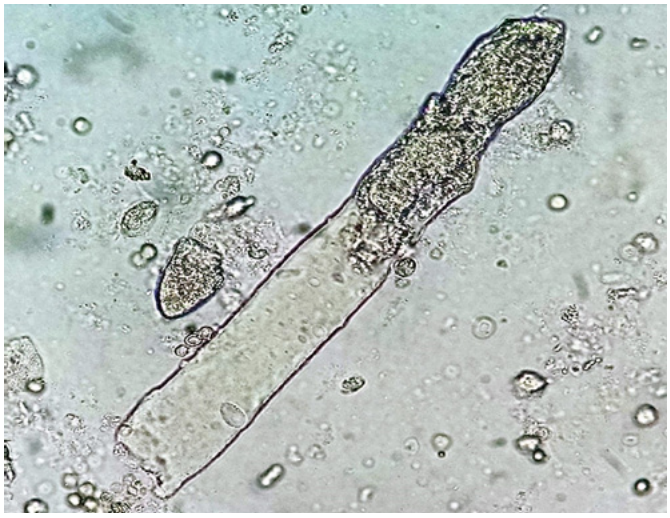


Figure 4.46: Mixed cast (Hyaline-granular cast). Fresh and unstained urine sediment. Brightfield (left); Phase contrast (right). Original magnification 400×.Courtesy of Controllab.

Crystals

Crystals in urine (crystalluria) is a common finding in both normal individuals and urolithiasis patients. Knowledge and proper identification of crystals in urine are useful skills when, for example, diagnosing lithogenic diseases such as primary hyperoxaluria and identifying acute kidney injury or chronic kidney disease due to medication.

To properly identify urinary crystals, it is useful to know the urine pH and have details of the medication in use. Virtually any type of crystal observed in urine can be identified, given this information coupled with knowledge of the most typical morphologies of the crystals and the right microscopic resources (phase contrast and polarized light). However, crystals are probably the most challenging structures that a professional



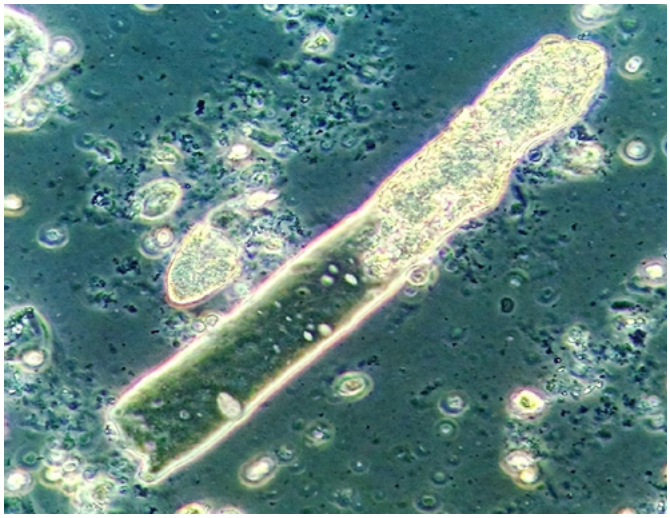
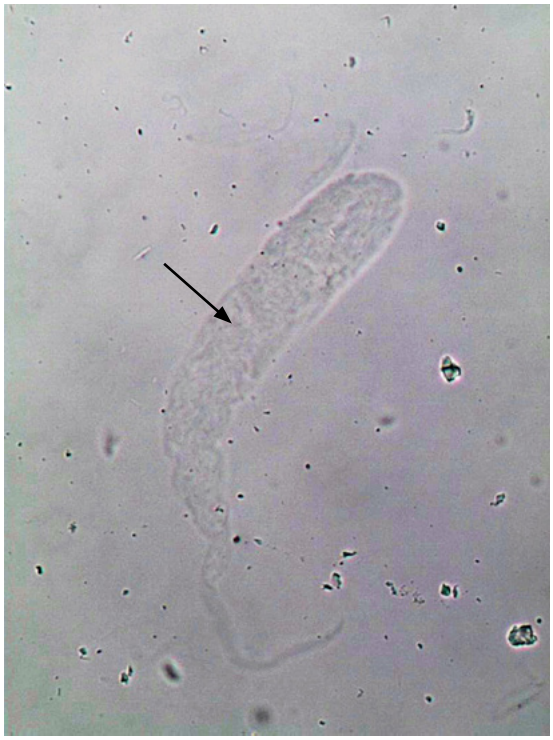


Figure 4.47: Mixed cast (Waxy-granular cast). Fresh and unstained urine sediment. Brightfield (top); Phase contrast (bottom). Original magnification 400×. Courtesy of José A. T. Poloni.

performing urine microscopy deals with due to the wide range of morphologies, shapes and sizes they can present—and indeed, some types of crystals can be observed in several different forms [1, 13, 31].

Despite the fact that stones formed in a patient's kidneys or urinary tract are composed of crystals that can be observed in urine microscopy (for example, calcium oxalate monohydrate), finding crystals in urine is not necessarily linked to stone formation.

The main types of urinary crystals are summarized in the following pages.



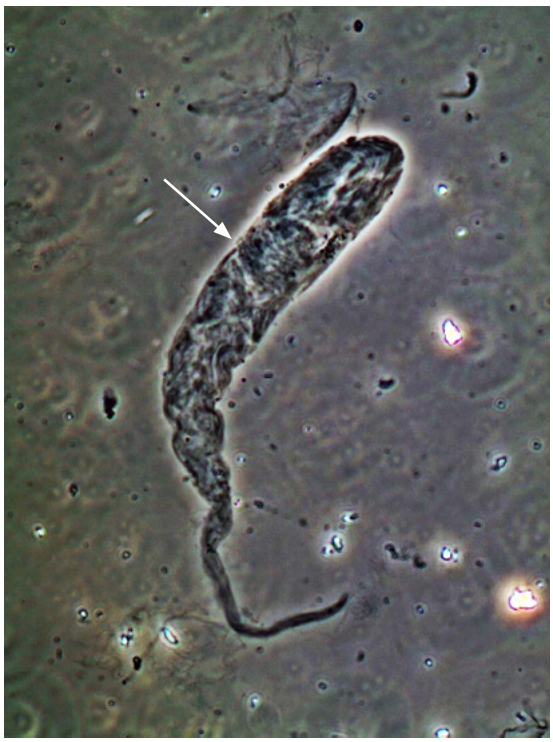


Figure 4.48: Cylindroid. Fresh and unstained urine sediment. Brightfield (top); Phase contrast (bottom). Original magnification 400×. Courtesy of Controllab.

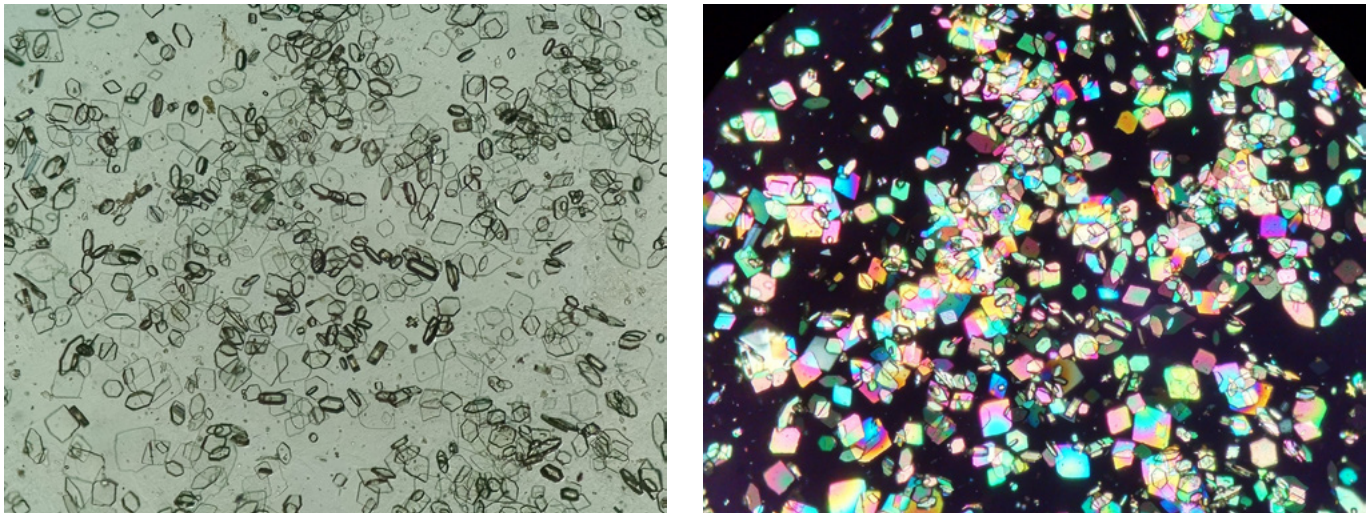


Figure 4.49: Uric acid crystals. Fresh and unstained urine sediment. Brightfield (left); Polarized light (right). Original magnification 400×. Courtesy of J. A. T. Poloni.

Uric Acid Crystals

Uric acid crystals (observed in urines with acidic pH, usually pH <5.4–5.8) come in several different forms and sizes (Figures 4.49–4.53). This is considered a common type of crystal and when observed under polarized light microscopy, the crystals show a strong birefringence, very frequently polychromatic.

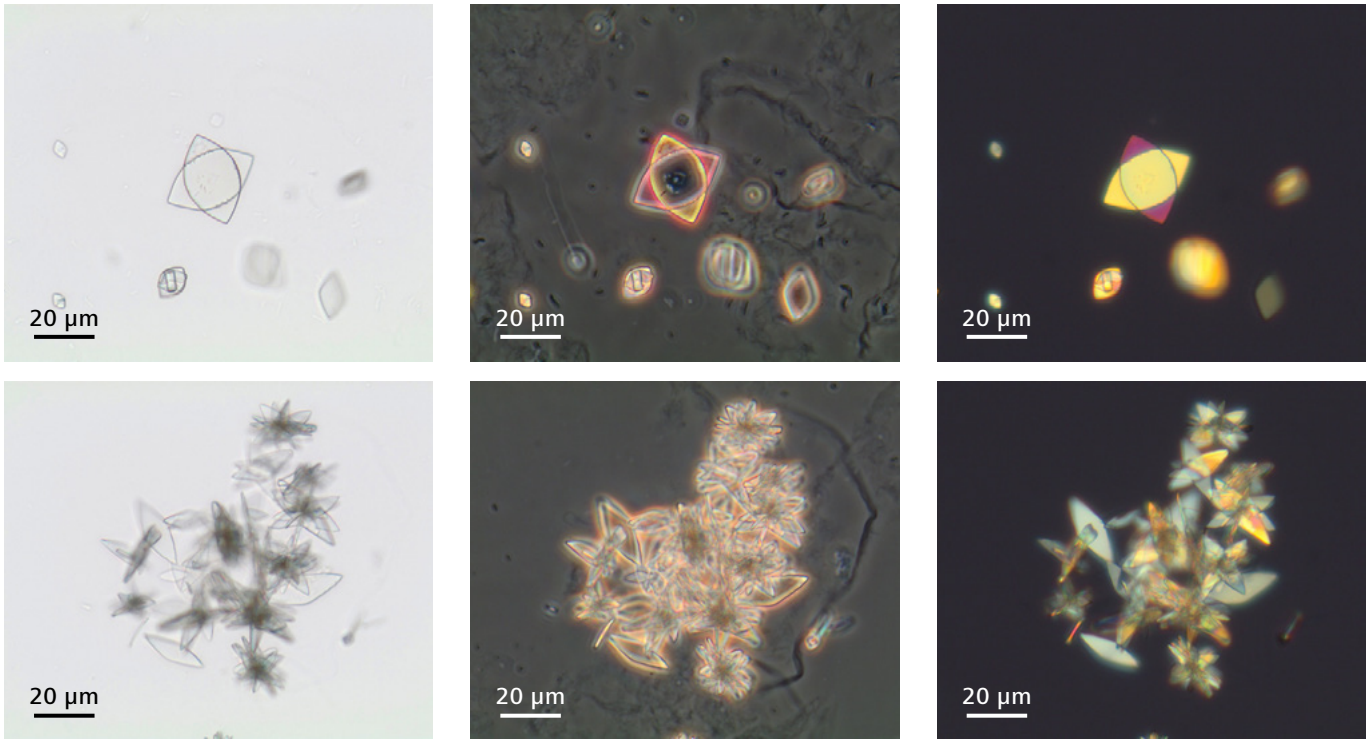


Figure 4.50: Uric acid crystals. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (center); Polarized light (right). Original magnification 400×. Courtesy of Controllab.

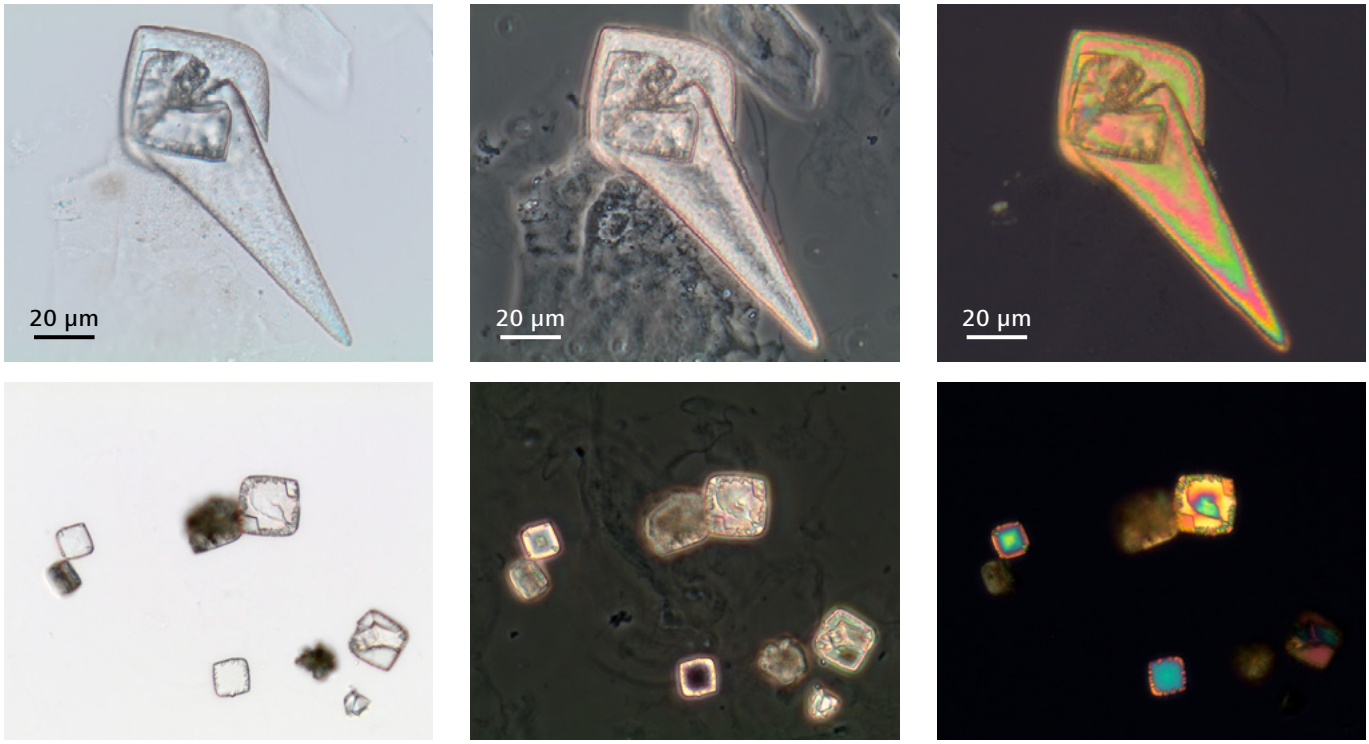


Figure 4.51: Uric acid crystals. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (center); Polarized light (right). Original magnification 400×. Courtesy of Controllab.

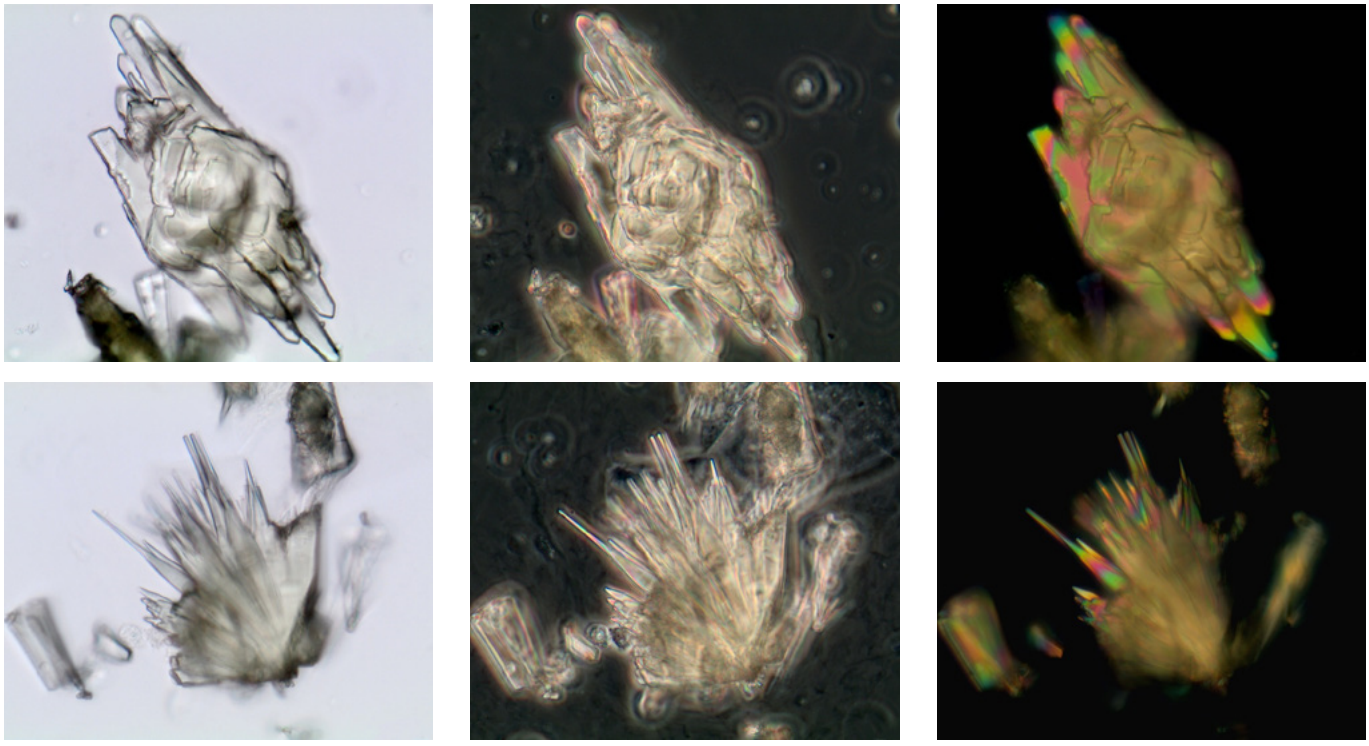


Figure 4.52: Uric acid crystals. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (center); Polarized light (right). Original magnification 400×. Courtesy of Controllab.

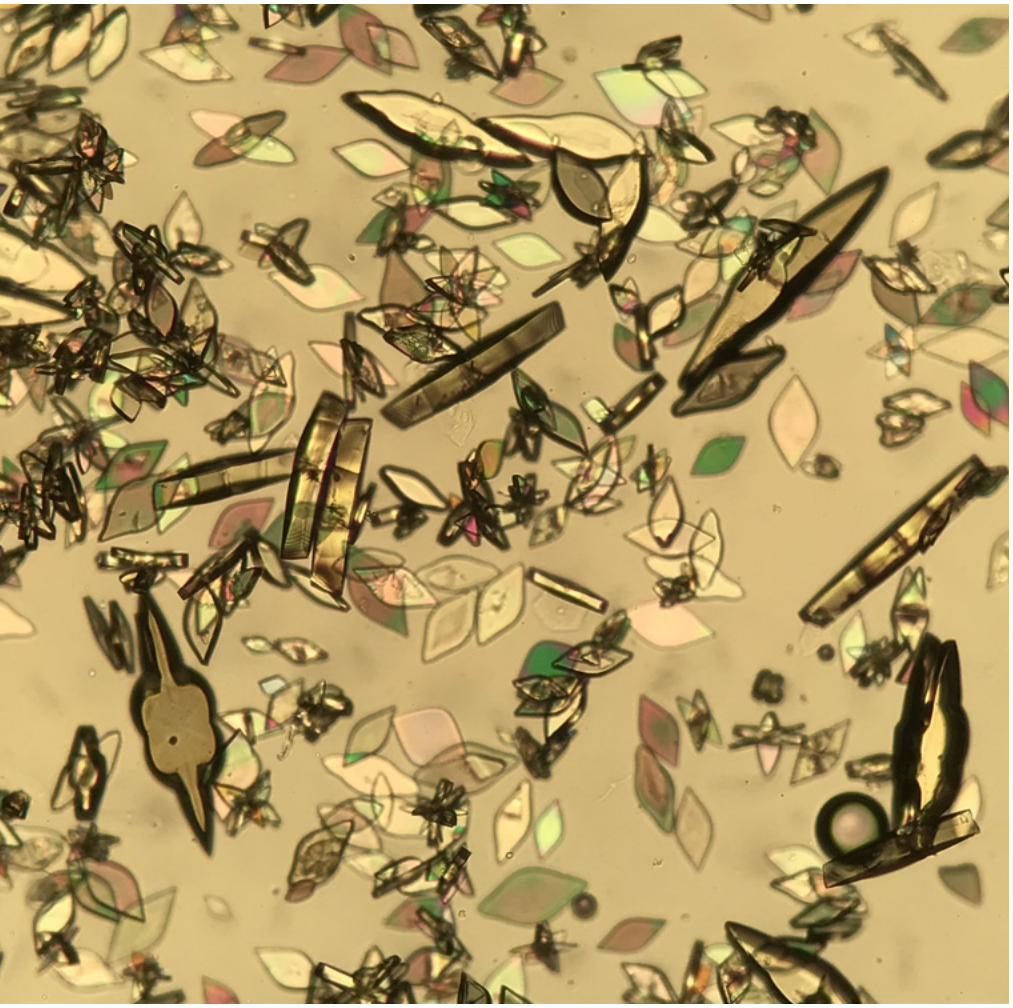


Figure 4.53: Uric acid crystals. Fresh and unstained urine sediment. Polarized light (filter partially aligned). Original magnification 400×. Courtesy of José A. T. Poloni.

Uric acid crystals can be found in normal subjects but also in patients with urolithiasis caused by uric acid. Patients with lymphoproliferative disorders or solid tumors can develop severe hyperuricemia as a consequence of tumor lysis (tumor lysis syndrome), both spontaneously or induced by chemotherapy, and this condition can lead the patient to uric acid nephropathy [1].

Calcium Oxalate Crystals

Calcium oxalate crystals (observed in urines with acidic pH, usually pH <5.4−6.7) are considered a common type of crystal and can be divided into two types: monohydrate and bi-hydrated [1].

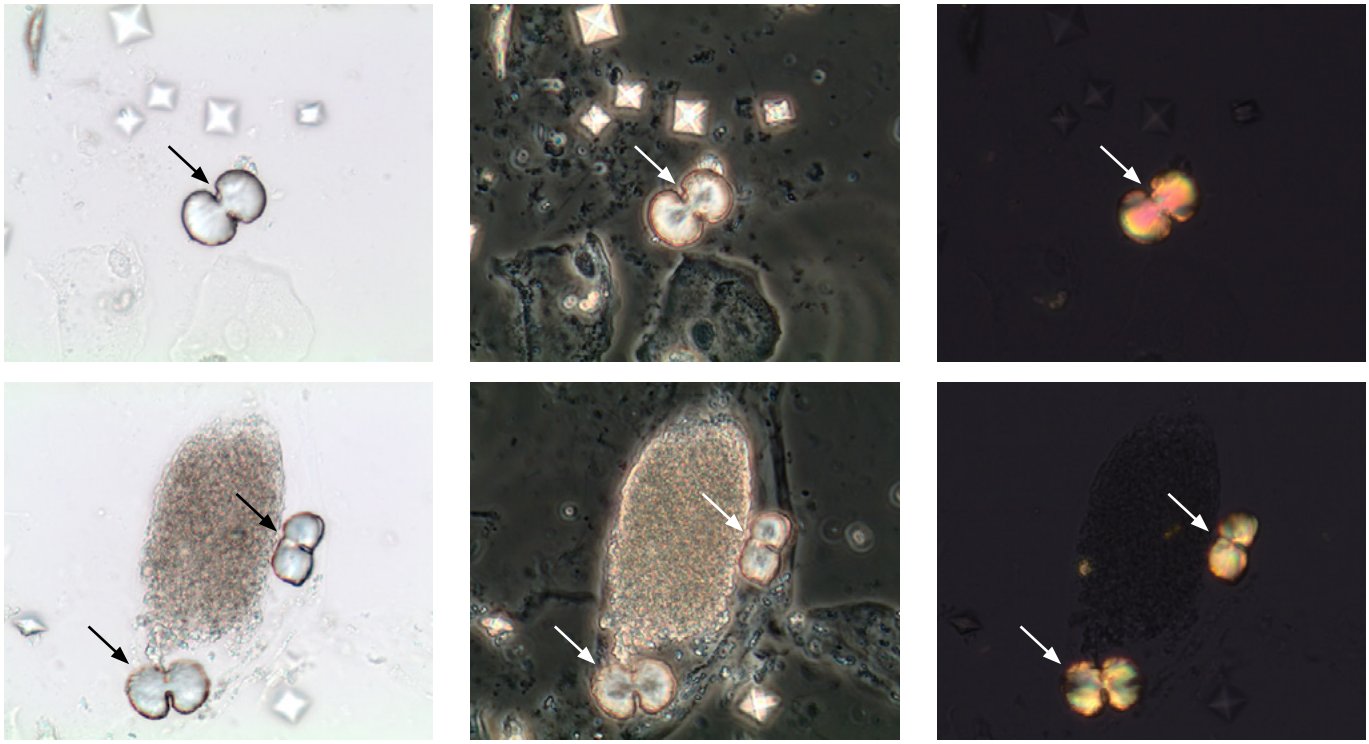


Figure 4.54: Calcium oxalate monohydrate crystals. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (center); Polarized light (right). Original magnification 400×. Courtesy of Controllab.

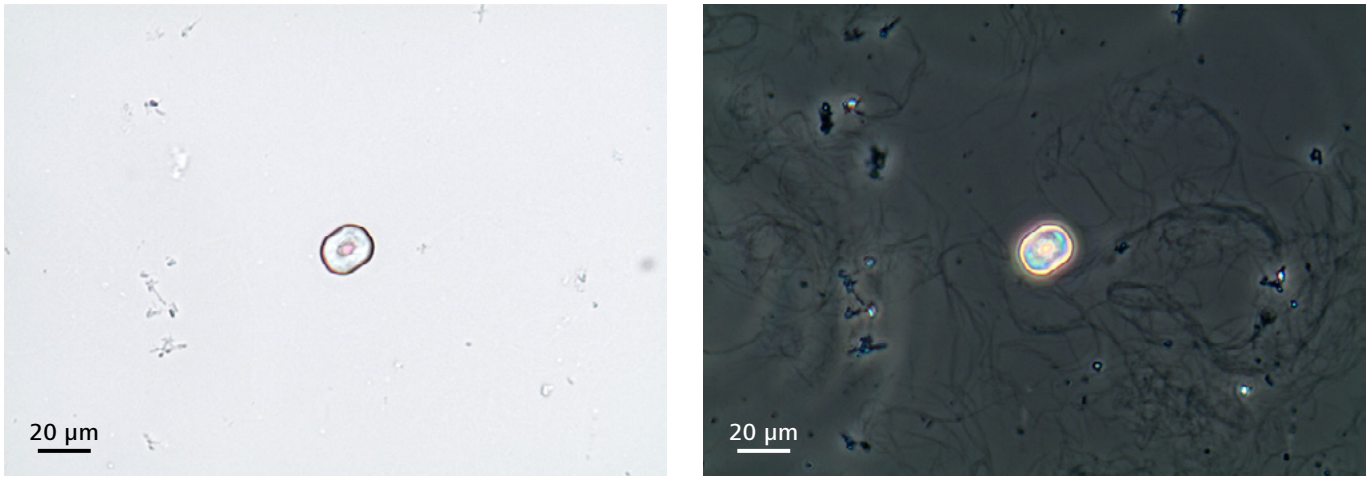


Figure 4.55: Calcium oxalate monohydrate crystals. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (top right); Polarized light (bottom right). Original magnification 400×. Courtesy of Controllab.

Calcium oxalate monohydrate (Figures 4.54 and 4.55) is associated with high oxalate concentration in the presence of normal or low calcium [31, 32]. This type of crystal is colorless and pleomorphic, and most commonly seen as ovoid structures, biconcave disks, dumbbells and rods. Under polarized light they are strongly birefringent and usually polychromatic.
Calcium oxalate bi-hydrate (Figure 4.56) is usually associated with hypercalcuria [31, 32]. It appears as bipyramidal colorless structures in a wide range of sizes. Usually, this type of crystal does not present birefringence under polarized light. Large crystals can be birefringent with a monochromatic pattern [1].

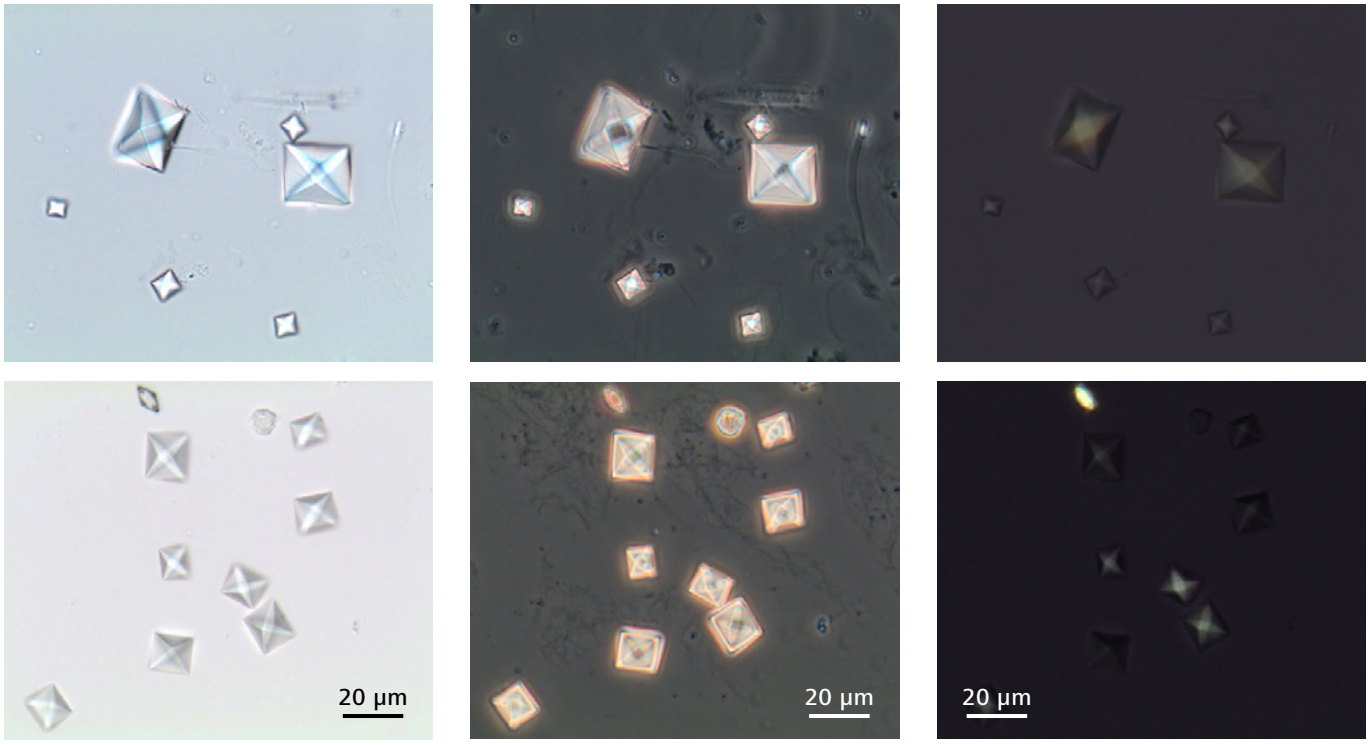


Figure 4.56: Calcium oxalate bi-hydrated crystals. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (center); Polarized light (right). Original magnification 400×. Courtesy of Controllab.

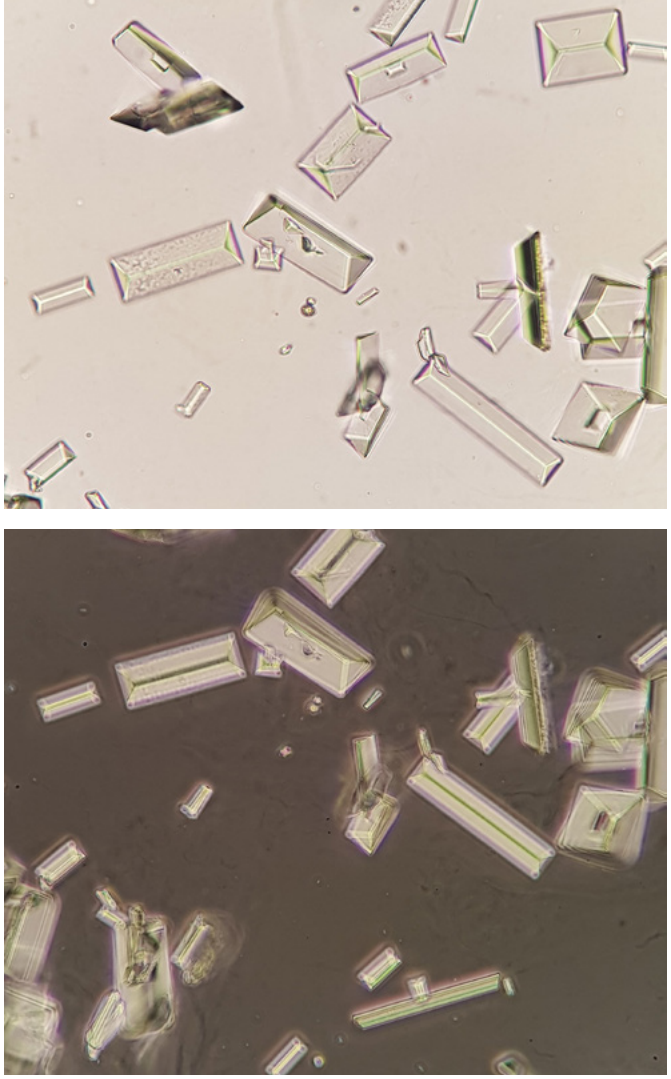


Figure 4.57: Triple phosphate crystals. Fresh and unstained urine sediment. Brightfield (top); Phase contrast (bottom). Original magnification 400×. Courtesy of A. P. G. Franz.

Triple Phosphate Crystals

Triple phosphate crystals (observed in urines with alkaline pH, usually pH 6.2 – > 7) are considered a common type and contain magnesium ammonium phosphate. They can be found in several forms, but the most common is the "coffin-lid" morphology (Figures 4.57 – 4.61). Under polarized light they usually present a weak monochromatic birefringence. This type of crystal is usually observed in the presence of urea-split microorganisms such as *Proteus mirabilis, Klebsiella pneumoniae* and *Pseudomonas* aeruginosa [1].

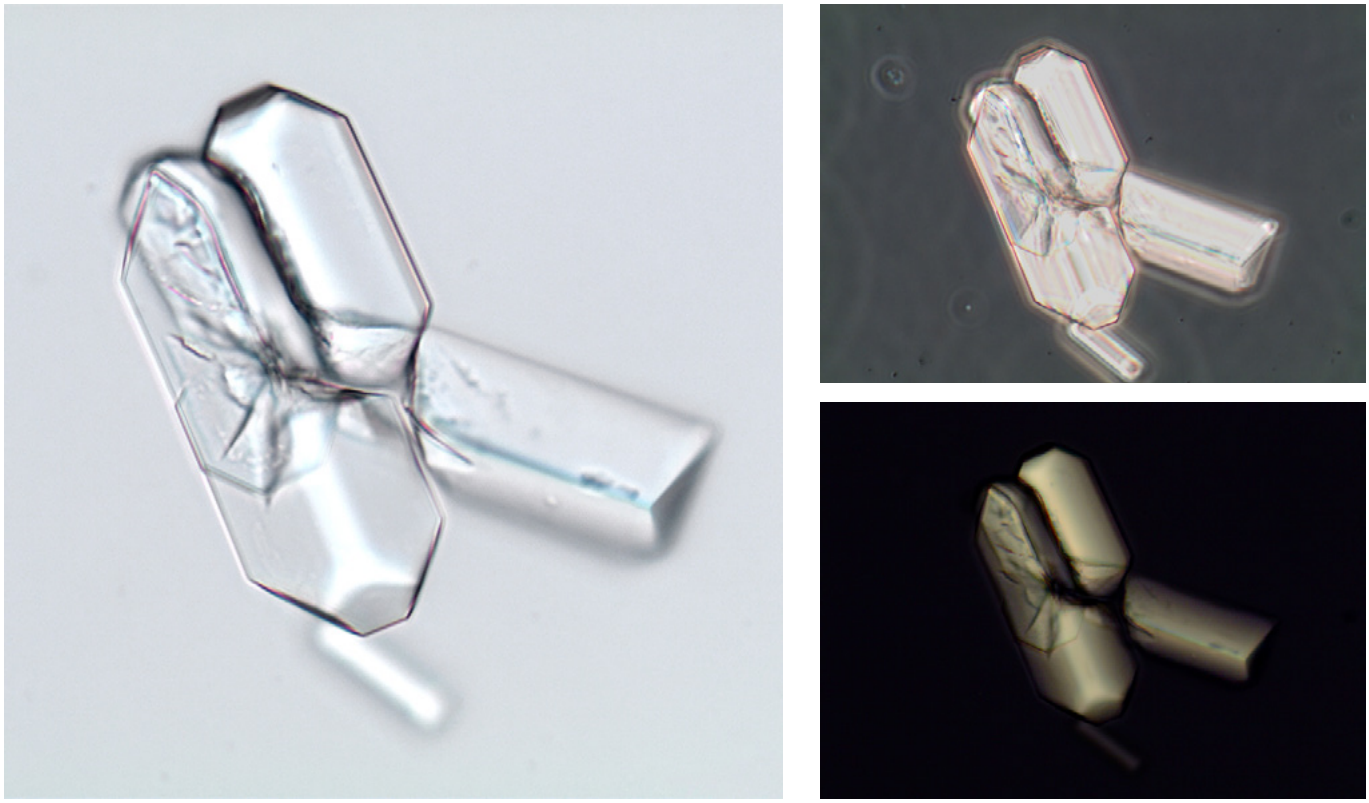


Figure 4.58: Triple phosphate crystals. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (top right); Polarized light (bottom right). Original magnification 400×. Courtesy of Controllab.

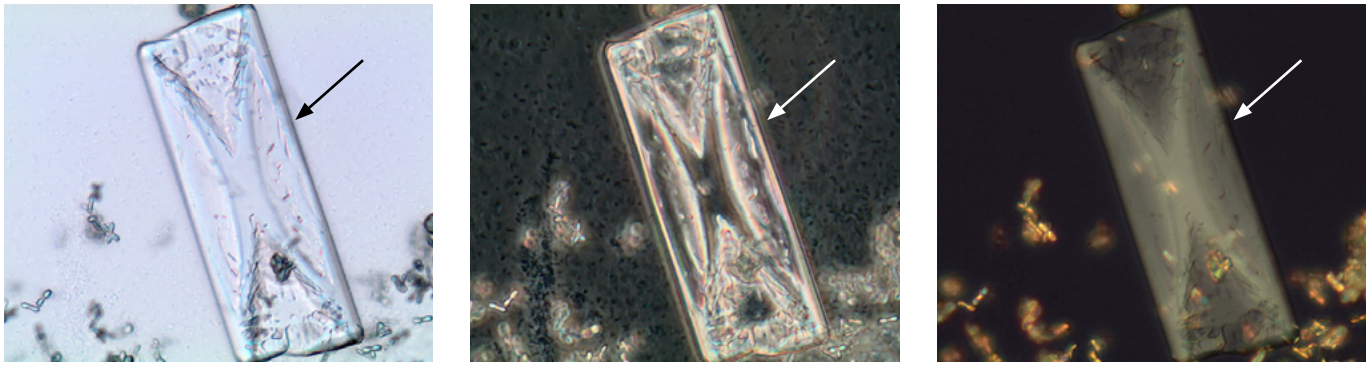


Figure 4.59: Triple phosphate crystals. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (center); Polarized light (right). Original magnification 400×. Courtesy of Controllab.

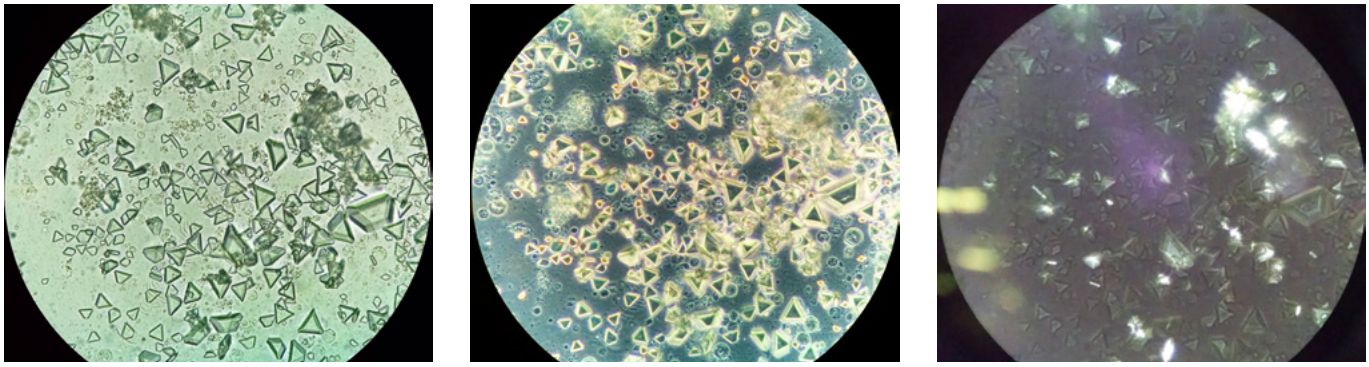


Figure 4.60: Triple phosphate crystals. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (center); Polarized light (right). Original magnification 100×. Courtesy of J. A. T. Poloni.

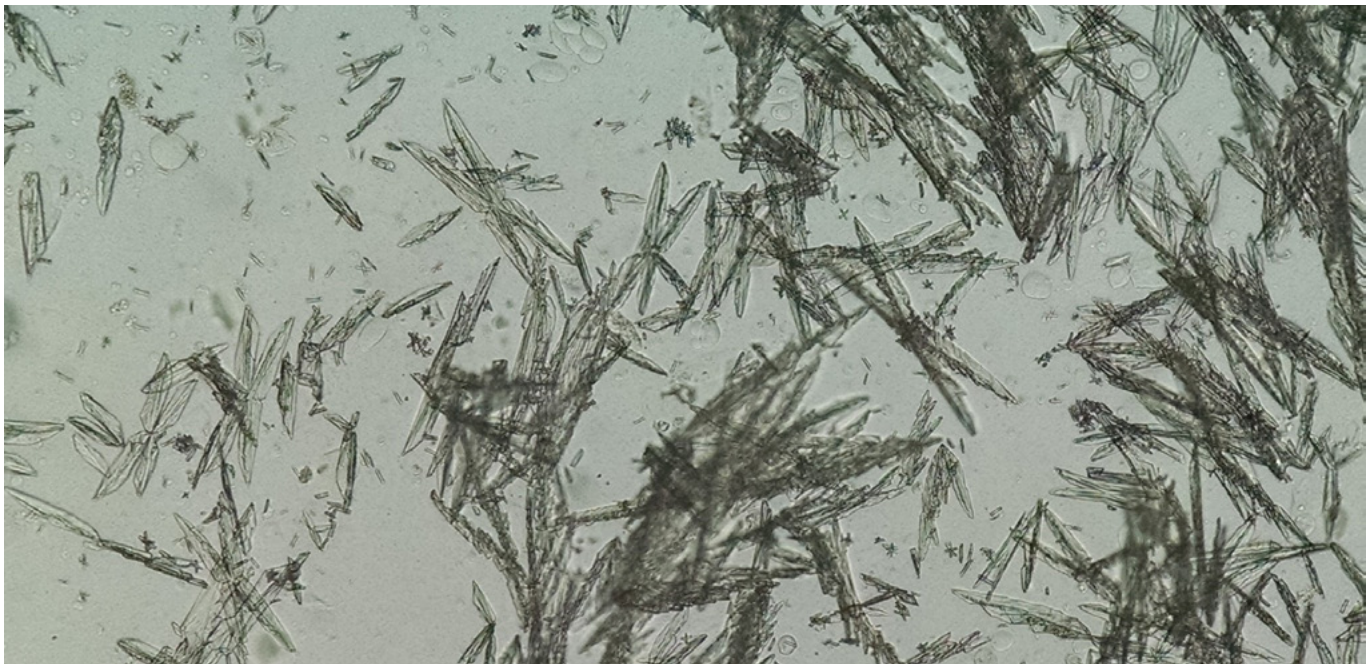


Figure 4.61: Triple phosphate crystals. Fresh and unstained urine sediment. Brightfield. Original magnification 400×. Courtesy of José A. T. Poloni.

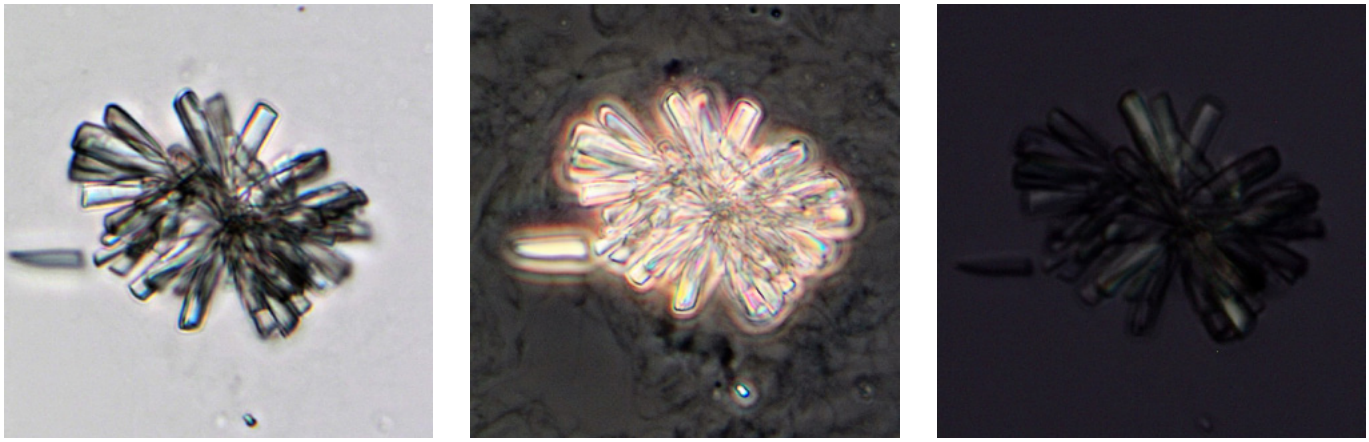


Figure 4.62: Calcium phosphate crystals. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (center); Polarized light (right). Original magnification 400×. Courtesy of Controllab.

Calcium Phosphate Crystals

Calcium phosphate crystals (observed in urine with alkaline pH, usually pH >7) are considered a common type of crystal. They can be found in a wide spectrum of morphologies, including prisms, stars, rosettes, sticks or needles, and can appear isolated or in aggregates (Figure 4.62). They can also be found as plates. Under polarized light they can present a weak monochromatic birefringence [1].

Amorphous Granule Crystals

Amorphous granules (both types) are common types of crystals. They can be seen as tiny granules of irregular shape, both singly and in aggregates. Refrigerating the urine (2–8°C) before examination can cause precipitation. Large amounts of amorphous granules can make sample analysis difficult by masking the presence of other particles which may be in the urine and they can be confused with bacteria (cocci especially) and cell debris [1]. Amorphous granules may be difficult to differentiate from each other, but they present some distinctive features:

- Amorphous urates (observed in urine with acidic pH, usually pH <5.4–5.8) are birefringent under polarized light microscopy (Figure 4.63 – upper panel). Also, when found in large amounts in the sample, they can be seen macroscopically forming a precipitate of pink-to-reddish color [1].
- Amorphous phosphates (observed in urine with alkaline pH, usually pH 6.2 → 7) are not birefringent under polarized light microscopy (Figure 4.63 – lower panel). When found in large numbers in the sample, they can be seen forming a precipitate of white-to-beige color [1].

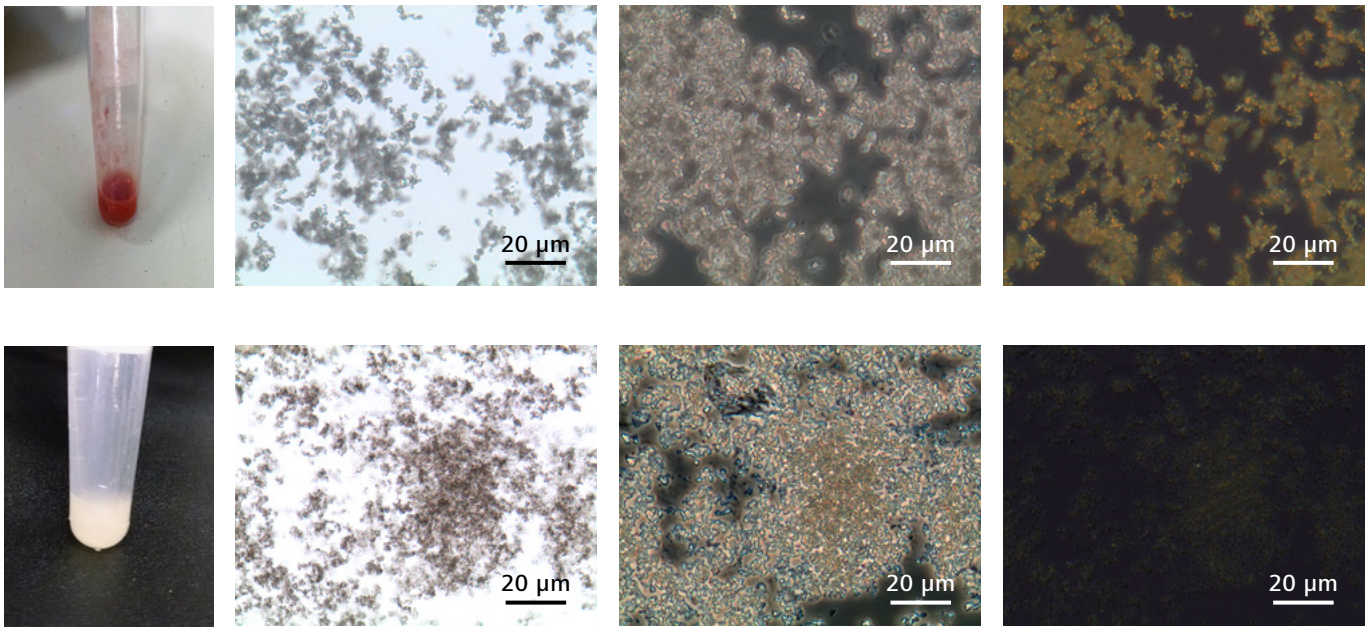


Figure 4.63: Amorphous granules. Urate (Upper panel). Phosphate (lower panel). Fresh and unstained urine sediment. Brightfield (left); Phase contrast (center); Polarized light (right). Original magnification 400×. Courtesy of Controllab.

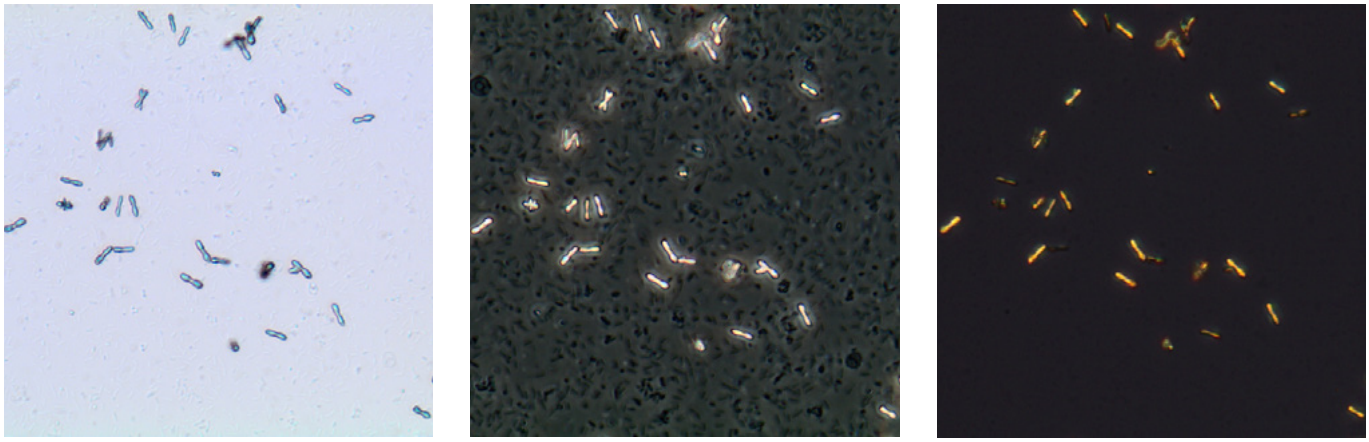


Figure 4.64: Calcium carbonate crystals. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (center); Polarized light (right). Original magnification 400×. Courtesy of Controllab.

Calcium Carbonate Crystals

Calcium carbonate crystals (usually observed in urine with alkaline pH) are a less common type. They are most often observed in the shape of "dumbbells" and they are strongly birefringent under polarized light microscopy (Figure 4.64). Their clinical meaning is not known, but they can be associated with the ingestion of large amounts of vegetables [1, 33].

Ammonium Biurate Crystals

Ammonium biurate crystals (usually observed in urine with neutral or alkaline pH) are also a less common type. They appear as yellow-brown spheres with spikes or "thorn apples" (Figures 4.65 and 4.66) and present a strong birefringence under polarized light microscopy (Figure 4.66) [1]. Their presence most often indicates inadequate hydration in the patient [5]. Usually they are observed in urine rich in ammonia, most commonly caused by urea split bacteria [1].

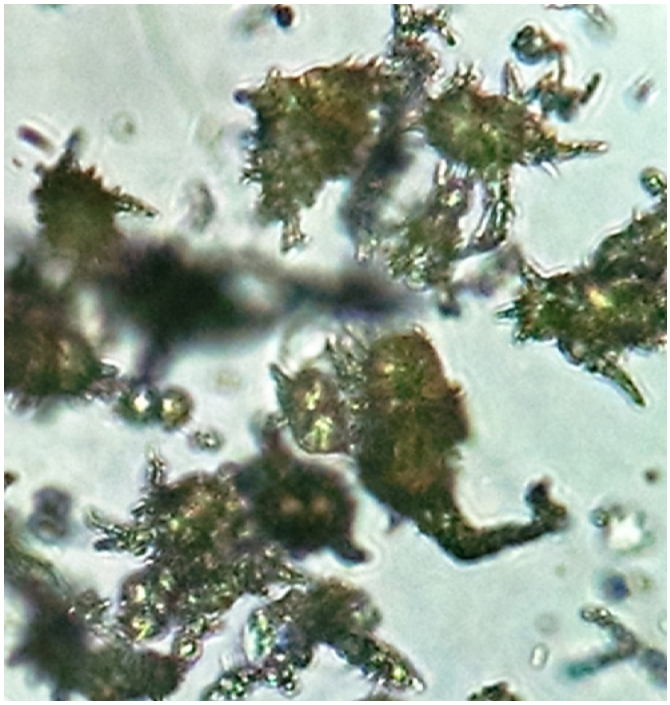


Figure 4.65: Ammonium biurate crystals. Fresh and unstained urine sediment. Brightfield. Original magnification 400×. Courtesy of José A. T. Poloni.

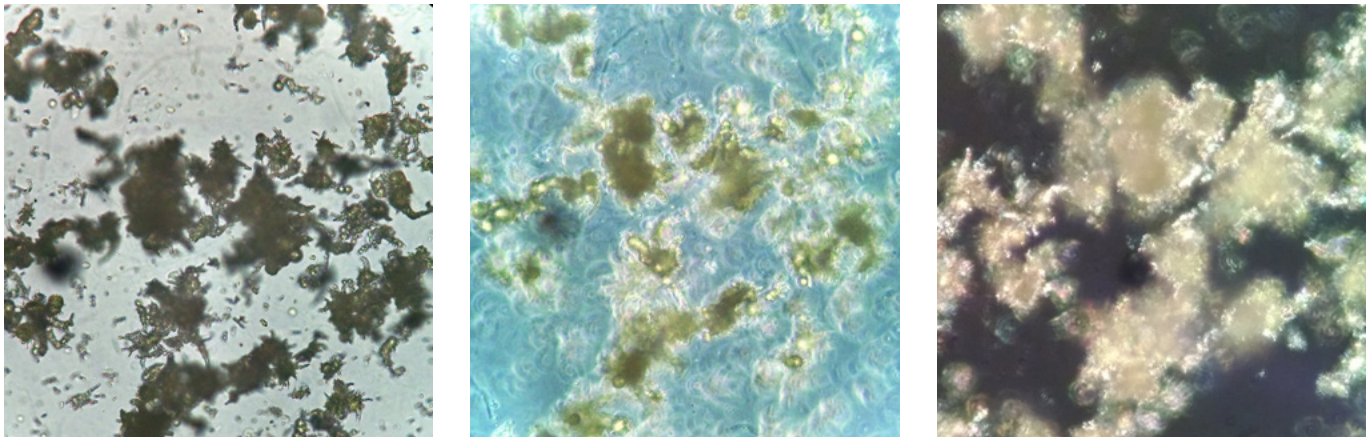


Figure 4.66: Ammonium biurate crystals. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (center); Polarized light (right). Original magnification 400×. Courtesy of José A. T. Poloni.

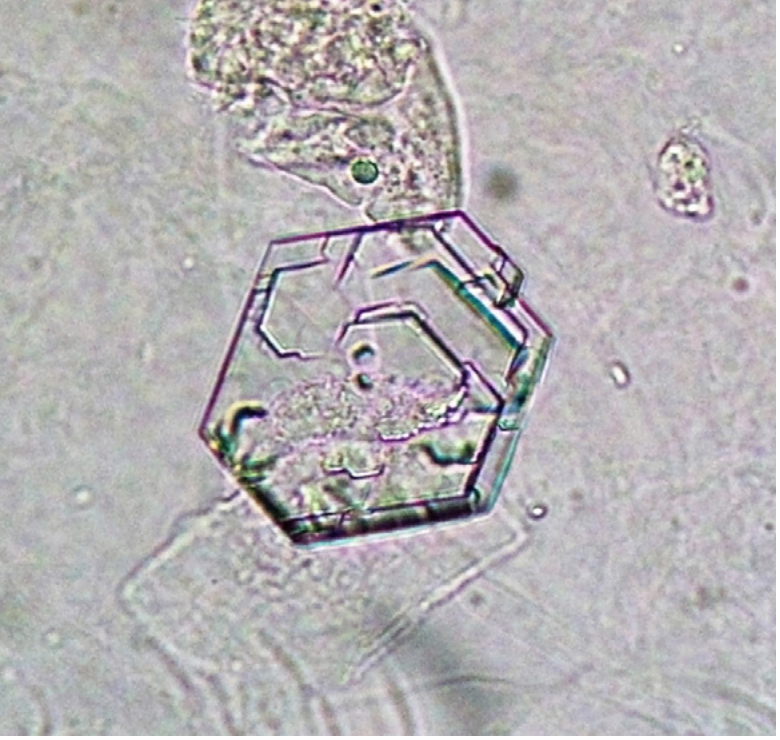


Figure 4.67: Cystine crystals. Fresh and unstained urine sediment. Brightfield. Original magnification 400×. Courtesy of José A. T. Poloni.

Cystine Crystals

Cystine crystals (usually observed in urine with acidic pH) are a rare pathologic type of crystal. They are observed as thin hexagonal colorless plates with irregular sides (Figures 4.67 and 4.68) [1,5,13]. Under polarized light microscopy they present a monochromatic birefringence (Figure 4.68). Cystine crystals can only be observed in patients with cystinuria, a genetic disease characterized by the deficient absorption of cystine, lysine, arginine and ornithine in the renal tubules. Patients with cystinuria usually present with urolithiasis and obstructive uropathy [1].

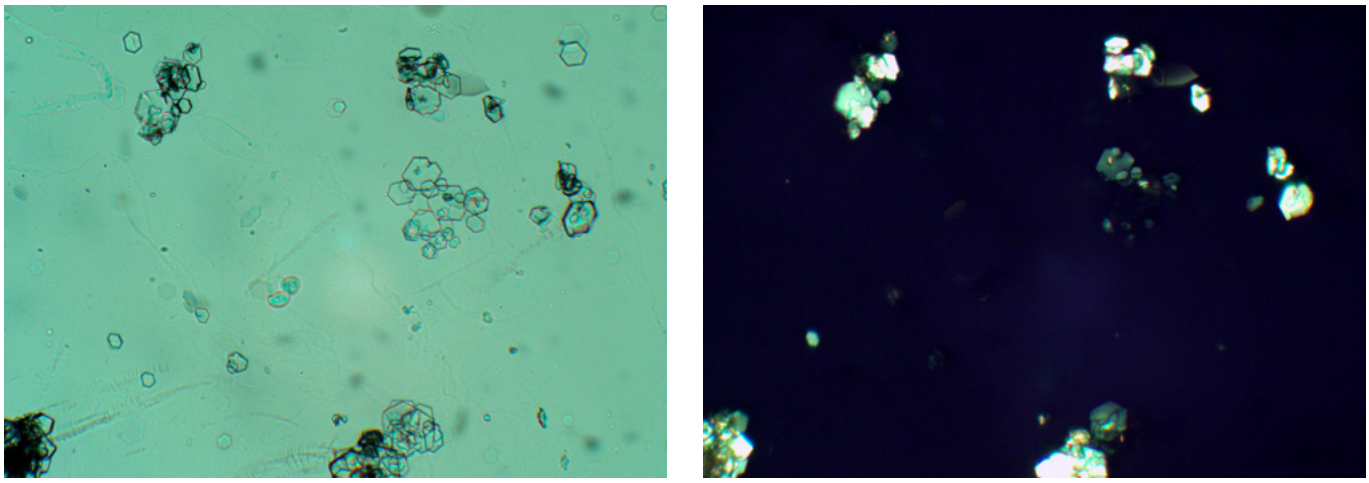


Figure 4.68: Cystine crystals. Fresh and unstained urine sediment. Brightfield (left); Polarized light (right). Original magnification 400×. Courtesy of J. A. T. Poloni.

Tyrosine Crystals

Tyrosine crystals (usually observed in urine with acidic pH) are a rare pathologic type of crystal. They appear as fine needles, often in aggregates, and there is basically nothing in the literature about the characteristics of these crystals under polarized light microscopy. In the case pictured here (Figure 4.69), we can see a strong birefringence using this microscopic resource. They are usually observed in patients with liver failure [1.5].

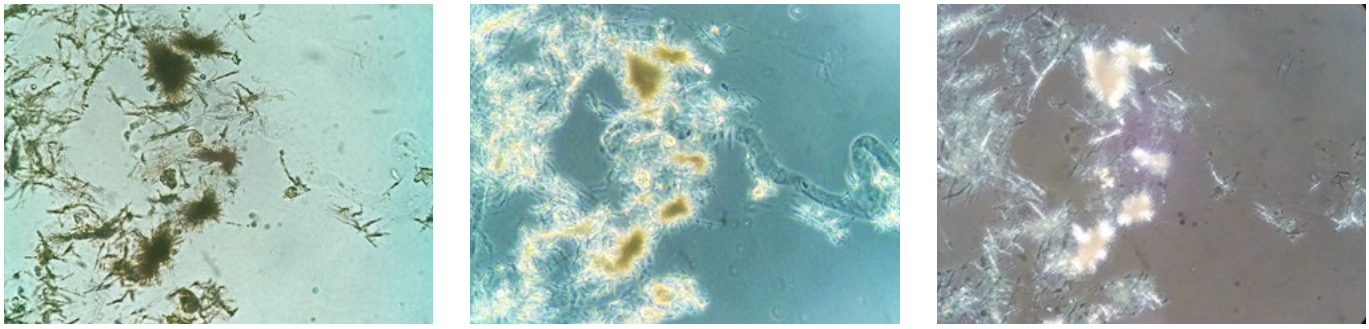


Figure 4.69: Tyrosine crystals. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (center); Polarized light (right). Original magnification 400×. Courtesy of J. A. T. Poloni.

Leucine Crystals

Leucine crystals (usually observed in urine with acidic pH) are a rare pathologic type of crystal that is also observed in patients with liver failure. The crystals appear as yellow-brown spheres with concentric striations. When observed under polarized light microscopy they form birefringent yellow pseudo-Maltese crosses (Figure 4.70) [1, 5].

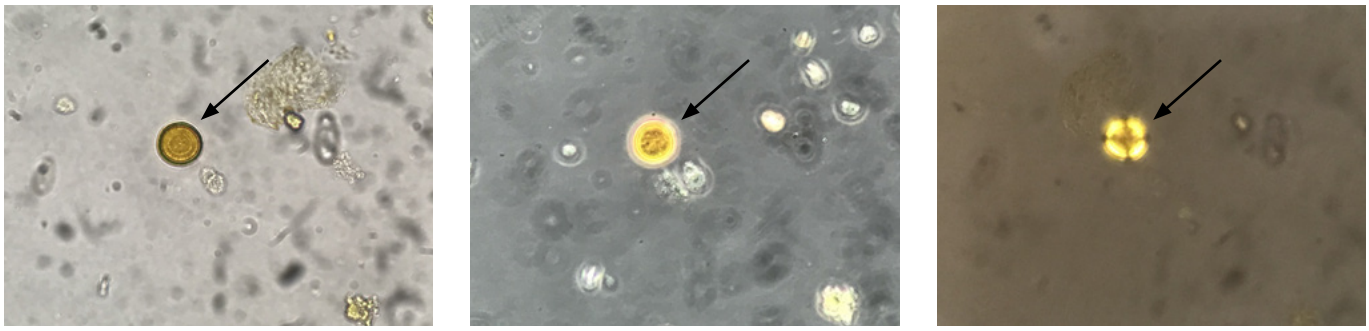


Figure 4.70: Leucine crystals. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (center); Polarized light (right). Original magnification 400×. Courtesy of J. A. T. Poloni.

Bilirubin Crystals

Bilirubin crystals (usually observed in urine with acidic pH) are a rare pathologic type of crystal that appears as small clusters of fine needles (Figure 4.71), although granules have also been observed. Both needles and granules can be seen free or in leukocytes or RTECs (also RTECs in urinary casts) (Figure 4.72). Finding bilirubin crystals is associated with bilirubinuria in patients with liver disease or obstruction [5].

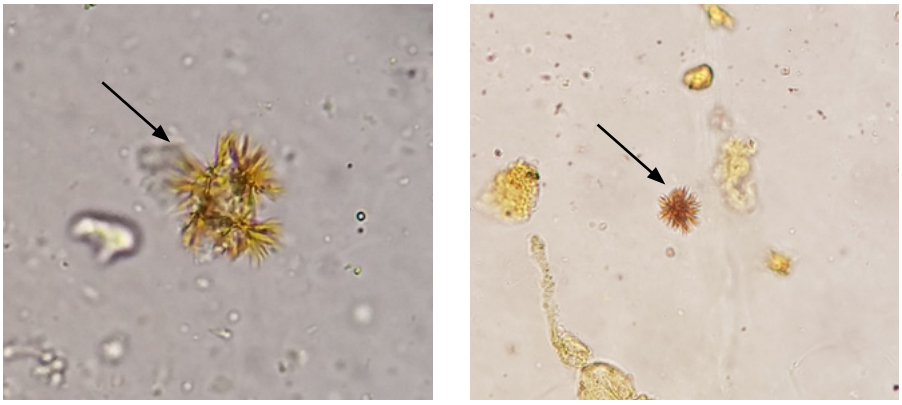


Figure 4.71: Bilirubin crystals. Fresh and unstained urine sediment. Brightfield. Original magnification 400×. Courtesy of A. P. G. Franz.

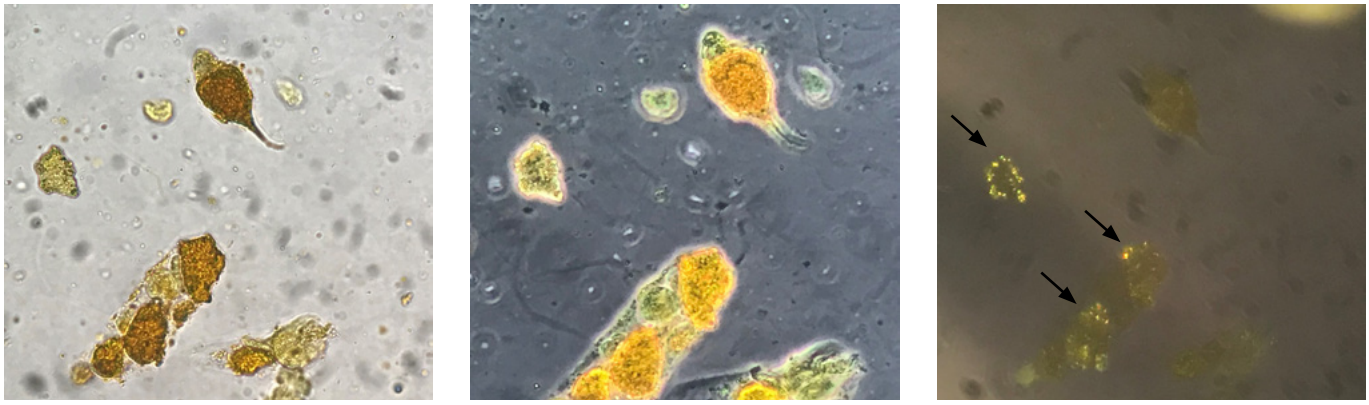


Figure 4.72: Bilirubin crystals within RTECs. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (center); Polarized light (right). Original magnification 400×. Courtesy of J. A. T. Poloni.

Cholesterol Crystals

Cholesterol crystals (usually observed in urine with acidic pH) are a rare pathologic type of crystal that appears as thin, transparent plates, commonly heaped one upon another. Under polarized light microscopy they can show a weak monochromatic birefringence (Figure 4.73). They can appear both free and in urinary casts (Figure 4.40). Cholesterol crystals are urine particles usually associated with other lipid structures and can be found in patients with intense proteinuria—for example, patients with nephrotic syndrome [1].

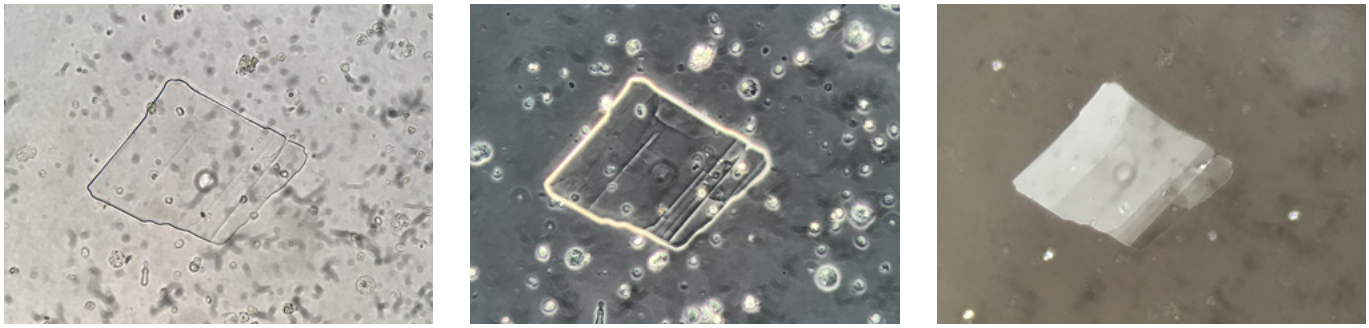


Figure 4.73: Cholesterol crystals. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (center); Polarized light (right). Original magnification 400×. Courtesy of J. A. T. Poloni.

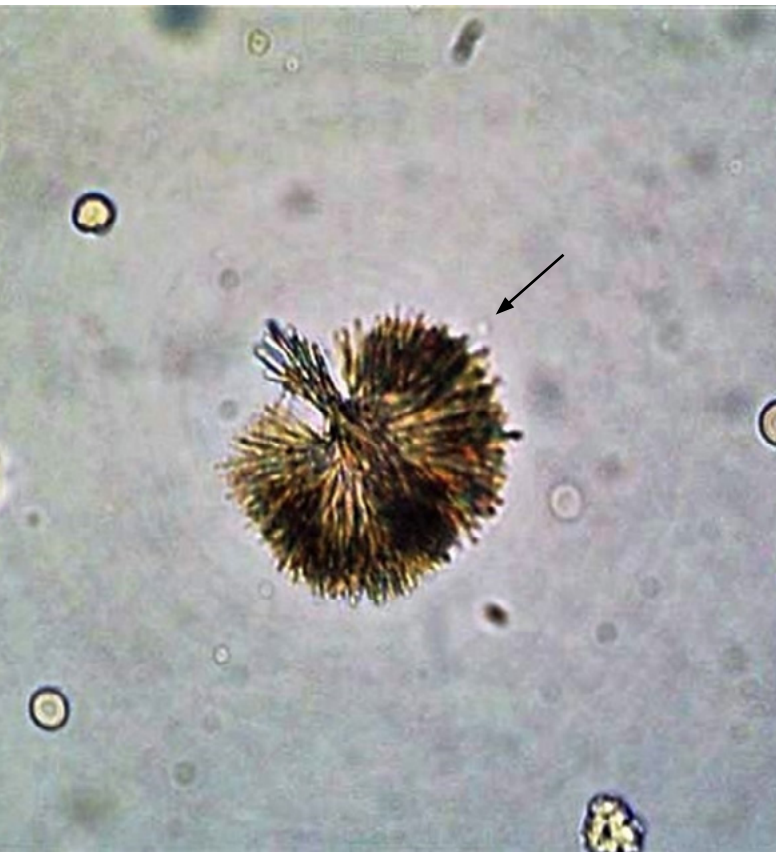


Figure 4.74: Sulfadiazine crystals. Fresh and unstained urine sediment. Brightfield. Original magnification 400×. Courtesy of José A. T. Poloni.

2,8-Dihydroxyadenine Crystals

2,8-dihydroxyadenine (DHA) crystals (observed in both acidic and alkaline urines) are a rare type of pathologic crystal that appears in individuals with adenine phosphoribosyltransferase deficiency. These crystals have a characteristic appearance and polarization pattern. Under brightfield microscopy they are round and reddish-brown with a dark outline and central spicules. Under polarized light microscopy they appear yellow with a central Maltese cross pattern [1, 34].

Crystals Caused by Drugs

Several different medications can lead to developing crystalluria. The crystals can be isolated or observed in conjunction with other urinary particles and can be linked to a wide spectrum of clinical implications including acute renal failure [1].

While the majority of cases occur as a consequence of supersaturation of a low volume urine with the drug, it develops in other cases as a result of drug insolubility in alkaline or acidic urine [13].

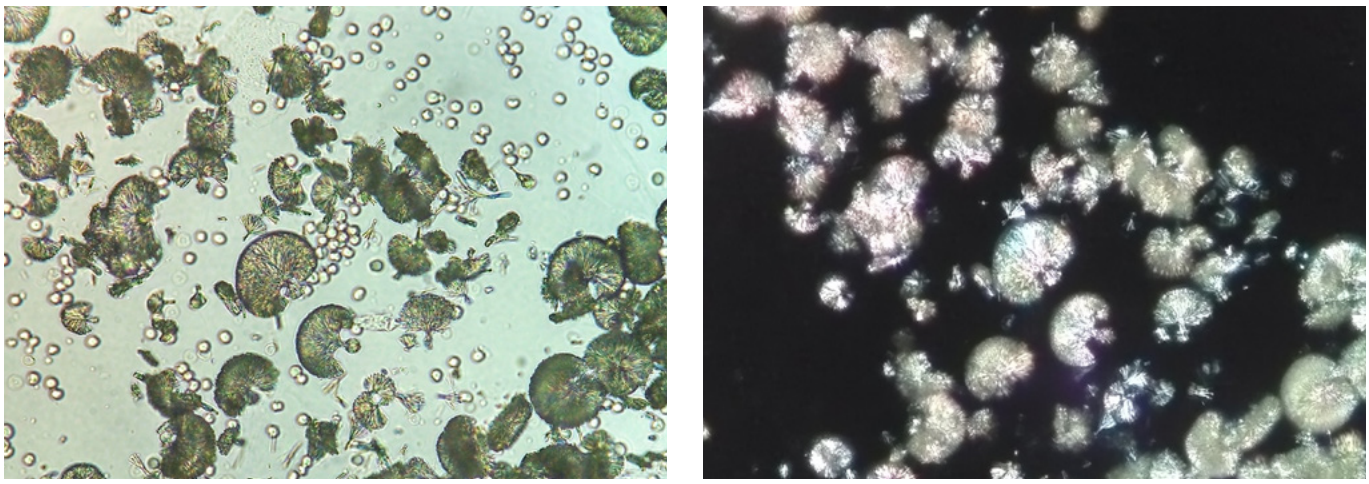


Figure 4.75: Sulfadiazine crystals. Fresh and unstained urine sediment. Brightfield (left); Polarized light (right). Original magnification 400×. Courtesy of J. A. T. Poloni.

Crystals resulting from drugs in urine are a major challenge during routine work and it is mandatory to have information on the medication in use, urine pH, and the morphology of the crystals and their characteristics under polarized light microscopy.

Among the drugs described as causing crystalluria are sulfadiazine (Figures 4.74 and 4.75), amoxicillin (Figure 4.76), piperacillin (Figure 4.77), sulfamethoxazole (Figure 4.78), ciprofloxacin, acyclovir (Figure 4.79), indinavir, triamterene, piridoxylate, primidone, naftidrofuryl oxalate, vitamin C, orlistat and felbamate.

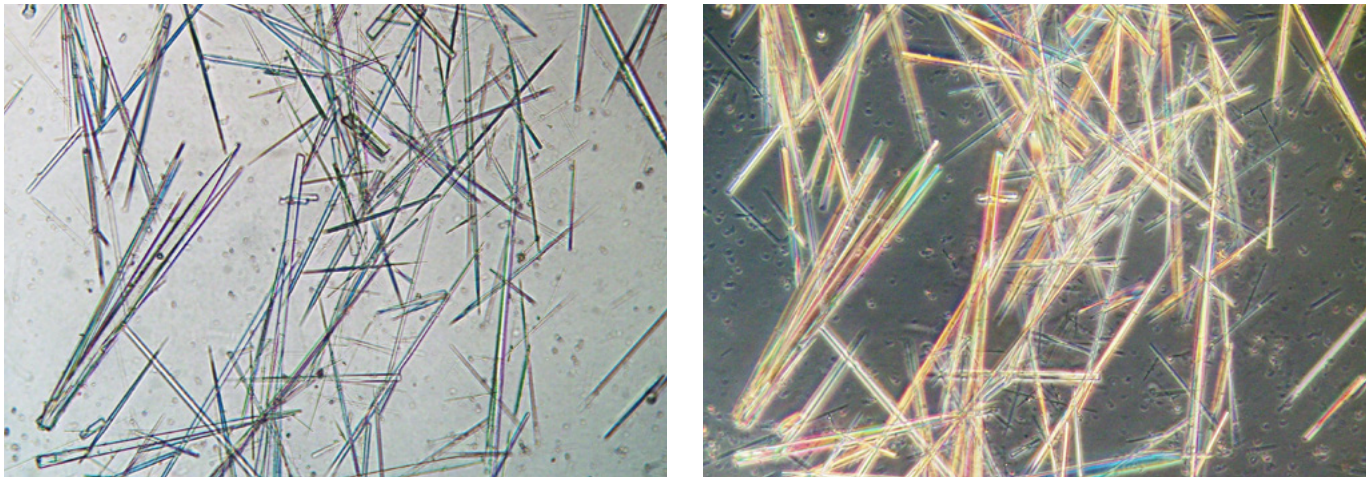


Figure 4.76: Amoxicilin crystals. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (right). Original magnification 400×. Courtesy of J. A. T. Poloni.

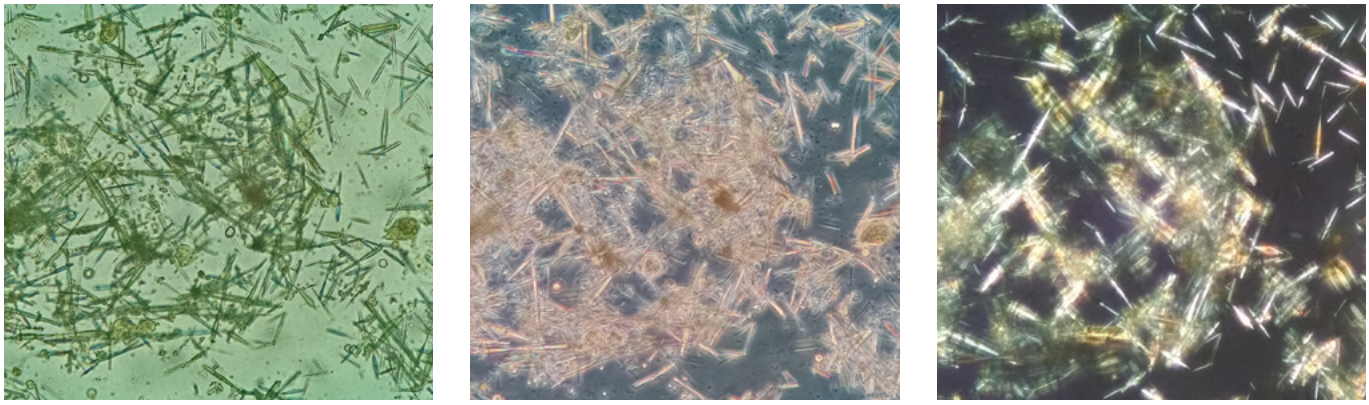


Figure 4.77: Piperacilin crystals. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (center); Polarized light (right). Original magnification 400×. Courtesy of J. A. T. Poloni.

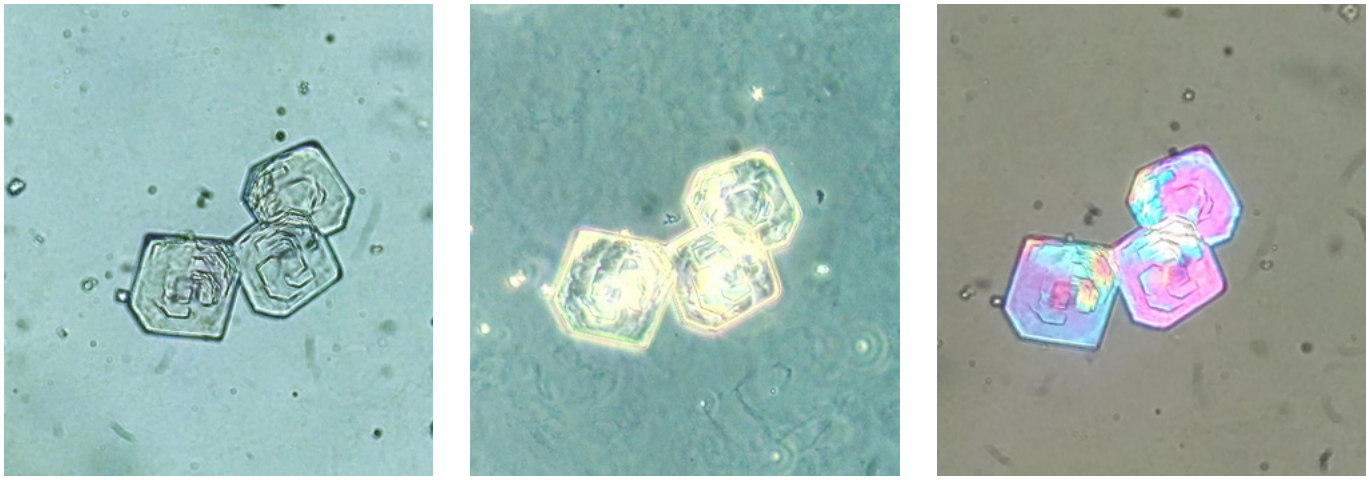
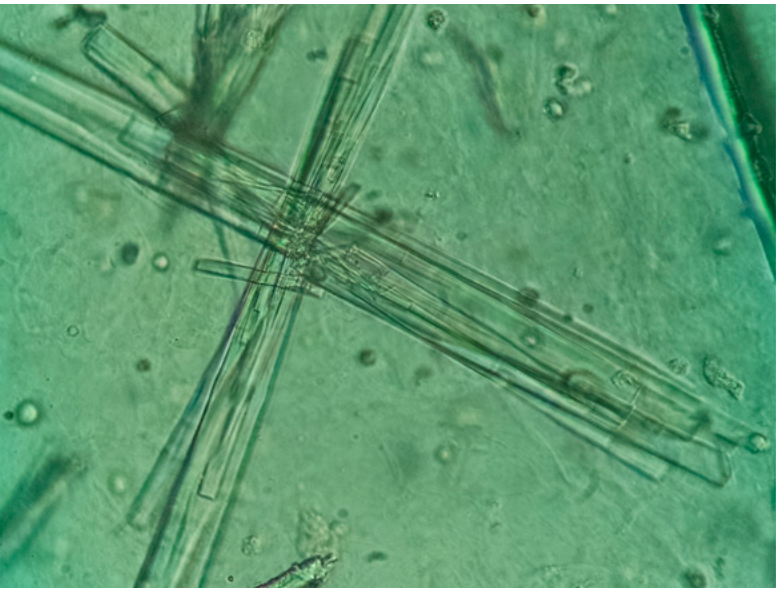


Figure 4.78: Sulfamethoxazole crystals. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (center); Polarized light (right). Original magnification 400×. Courtesy of J. A. T. Poloni.



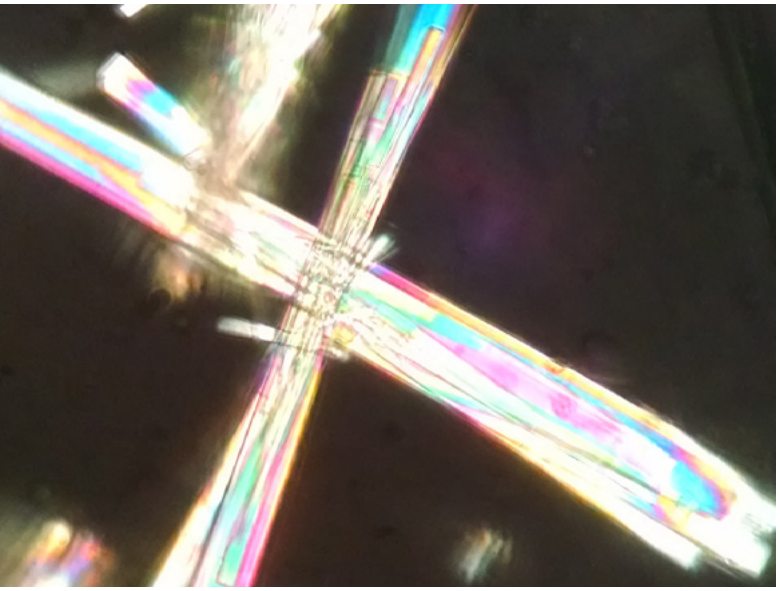


Figure 4.79: Acyclovir crystals. Fresh and unstained urine sediment. Brightfield (top); Polarized light (bottom). Original magnification 400×. Courtesy of J. A. T. Poloni.

Related to crystal morphology, sulfamethoxazole can be observed as hexagonal crystals, making it difficult to differentiate them from cystine crystals. Polarized light microscopy can help differentiate between these crystals since cystine is monochromatic (Figure 4.68) and sulfamethoxazole is polychromatic (Figure 4.78) under polarized light [35].

Keep this in mind: finding numerous crystals of uncommon and pleomorphic appearance should give rise to the hypothesis of drug crystalluria. Your next step would be to check for the medication in use by the patient. Once you know that, you can check the literature for images to help identify the doubtful crystals. These steps will let you identify the crystals successfully.

Microorganisms

Several microorganisms can be observed in urine sediment. These can be caused by infectious processes or sample contamination. The following discussion focuses on the different types of microorganisms.

Parasites

Basically two main types of parasites can be observed as causing infection in the human urinary tract. In addition, contamination of the sample with parasites from the intestinal tract is another possible way to explain their presence.

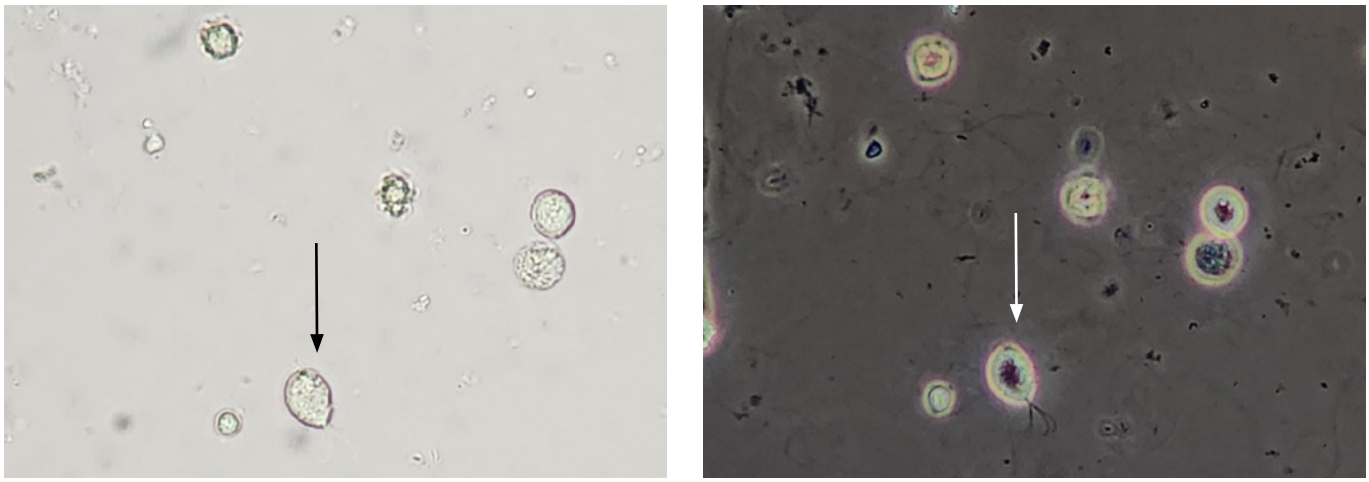


Figure 4.80: Representative image of Trichomonas vaginalis. *Fresh and unstained urine sediment. Brightfield (left); Phase contrast (right). Original magnification 400×. Courtesy of A.P.G. Franz.*

Trichomonas vaginalis

Trichomonas vaginalis (Figure 4.80) is the predominant infectious parasite in the urogenital tract. This protozoan is easily identified in a fresh examination of the urinary sediment when its movement, produced by its flagella and undulating membrane, can be seen as intense and distinctive.

Infections are most commonly seen in women, but both men and women can be infected. Because they generally cause infections in the lower genital tract, particularly vaginitis, these parasites are often observed as an occasional finding in a sample that was collected without proper hygiene or without using the midstream collection technique [1, 36].

Schistosoma haematobium

Schistosoma haematobium is a trematode that infects the genital tract, lodging mainly in the bladder. Its eggs – and sometimes the miracidia that hatch from the eggs – can be observed during the analysis of the urinary sediment. Usually, the infectious process presents itself in association with urological hematuria [1].

Intestinal parasites

Intestinal parasites can be observed in urine mainly as a contamination that happened during sample collection. *Enterobius vermicularis* (Figure 4.81) is the most commonly found due to its behavior of migrating outside the intestine through the anus and perianal skin, making it more likely to be found than the other types of parasites.

Other intestinal parasites—for instance, *Strongyloides stercoralis* (Figure 4.82) [37] and Balantidium coli [38]—were already reported in the urine sediment.

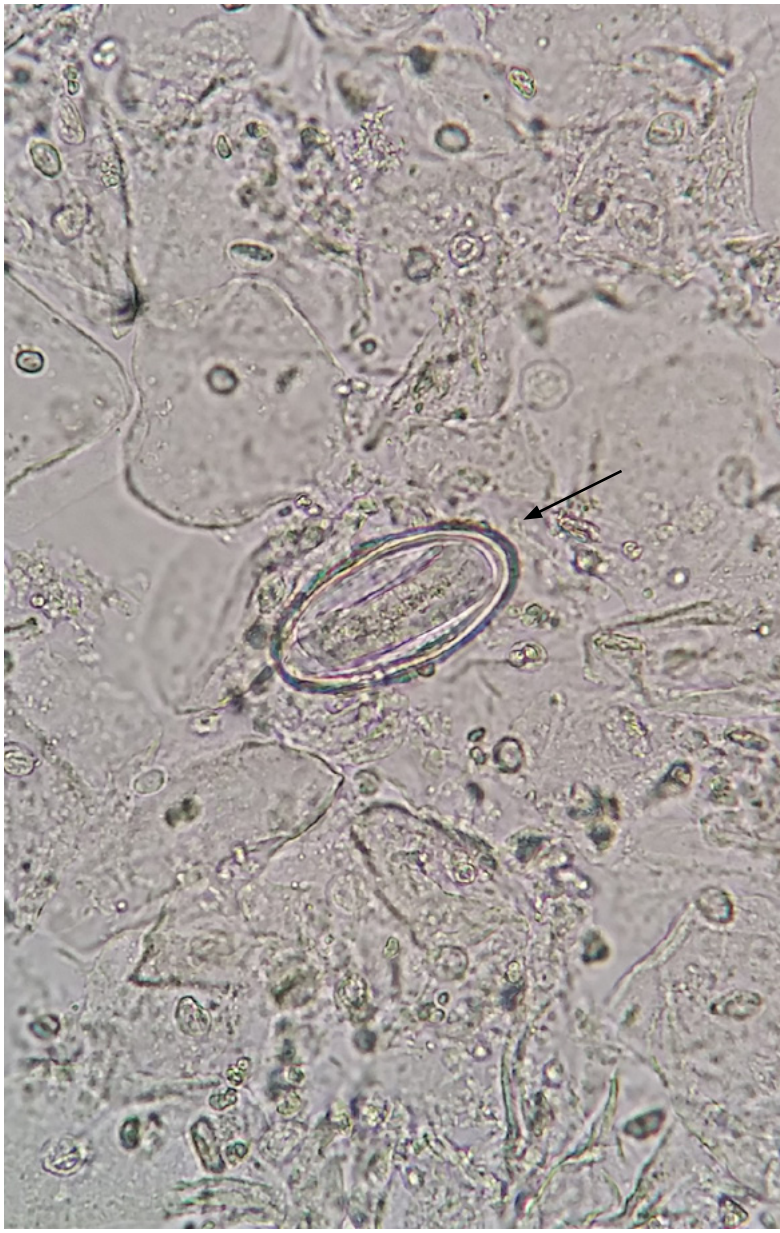
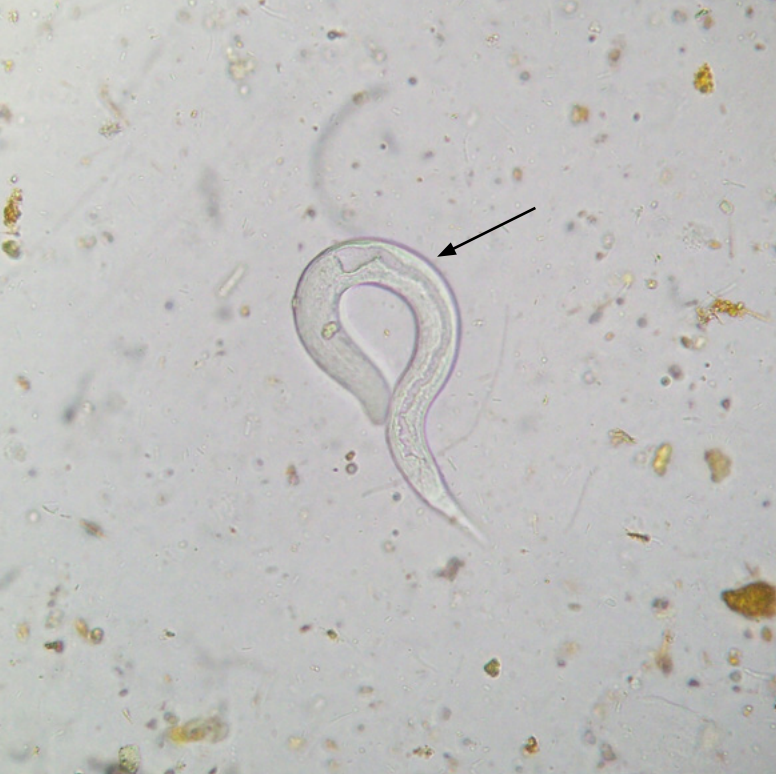


Figure 4.81: Enterobius vermicularis *eggs. Fresh and unstained urine sediment. Brightfield. Original magnification 400×. Courtesy of A. P. G. Franz.*



As mentioned previously, intestinal parasites are usually a sign of sample contamination with fecal material and are not expected to be found in urine samples. However, any kind of infectious process is possible in immunossuppressed / immunocompromised patients. The professional performing urine microscopy needs to be aware of the possibility of finding these unusual particles.

Figure 4.82: Strongyloides stercoralis *larvae. Fresh and unstained urine sediment. Brightfield. Original magnification 400×. Courtesy of J. A. T. Poloni.*

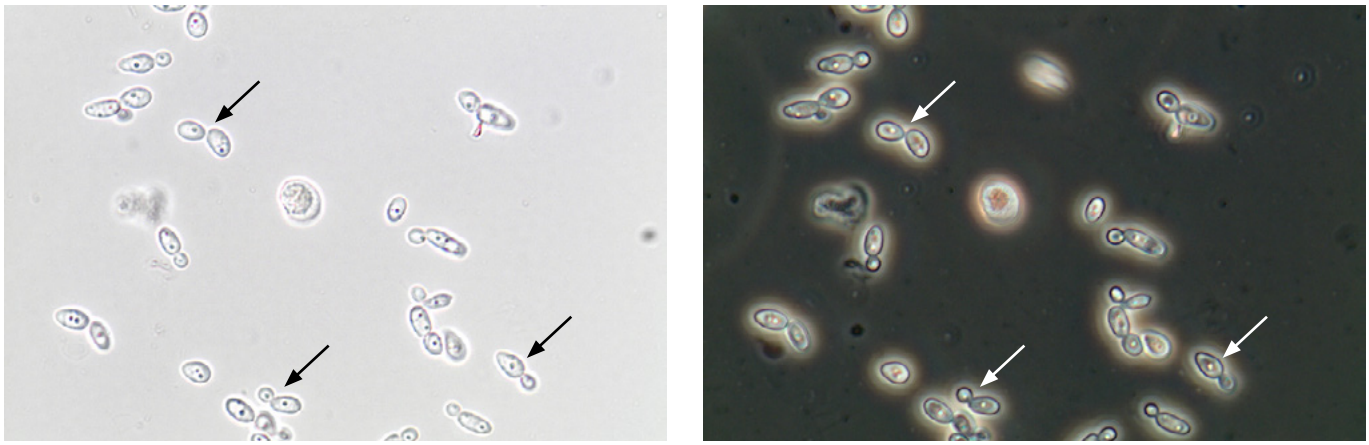


Figure 4.83: Yeasts (Blastoconidia). Fresh and unstained urine sediment. Brightfield (left); Phase contrast (right). Original magnification 400×. Courtesy of Controllab.

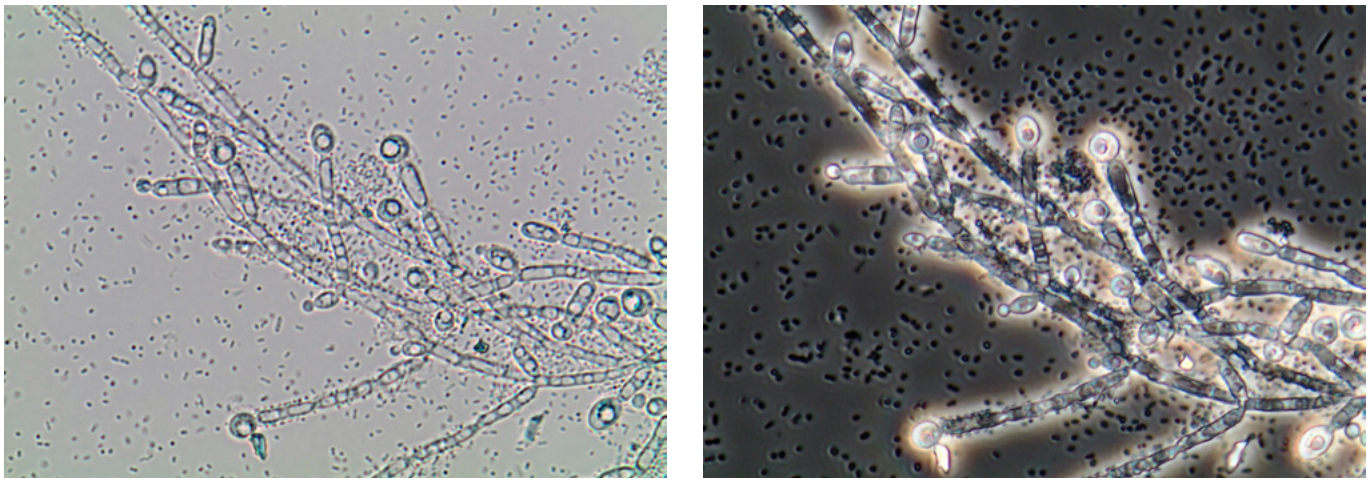


Figure 4.84: Yeasts (Pseudohyphae). Fresh and unstained urine sediment. Brightfield (left); Phase contrast (right). Original magnification 400×. Courtesy of Controllab.

Fungi (Yeasts / Pseudohyphae)

These microorganisms are often found in urinary sediment, both as the cause of urinary tract infection and as collection contaminants. Their observation does not necessarily indicate the presence of a fungal infection of the urinary tract. That will require confirmation in culture media or other specific diagnostic methods and, of course, evaluation of the patient's clinical context to clarify whether the finding actually has clinical relevance [1, 13].

Yeasts can be observed both in the form of blastoconidia (Figure 4.83) and as pseudohyphae (Figure 4.84), being *Candida spp.*, the main microorganism of this type to be observed in urine samples.

Rare findings of fungal origin

(*Cryptococcus spp., Histoplasma capsulatum, Fusarium spp., Coccidioides immitis, Curvalaria*)

Other types of yeasts / fungi—such as those listed here—can eventually be found in urine samples. It is essential for the analyst to bear in mind that the population of immunodeficient patients (immunosuppressed / immunocompromised) is increasing, including those who are HIV-infected and those using immunosuppressants or glucocorticoids. These patients are more susceptible to infectious processes than the general population and require the full attention of the clinical analyst to identify a possible infection that may be present.

It is certainly not unheard of to come across infections by little-known microorganisms in the daily life of the lab. Usually this will be a microorganism that poses no danger to the general population. However, patients with a weak immune system can develop severe diseases. As an example, *Cryptococcus spp.* (Figure 4.85), which is the main causative agent of fungal meningitis in HIV-positive patients, can be observed in the urine. *Cryptococcus spp.* is an encapsulated yeast, requiring the use of China ink to see the capsule properly and identify the fungal particle. Finding this in urinary sediment can help detect an important infectious agent which may be infecting not only the patient's urinary tract, but also other sites such as the central nervous system [13, 25, 39-41].

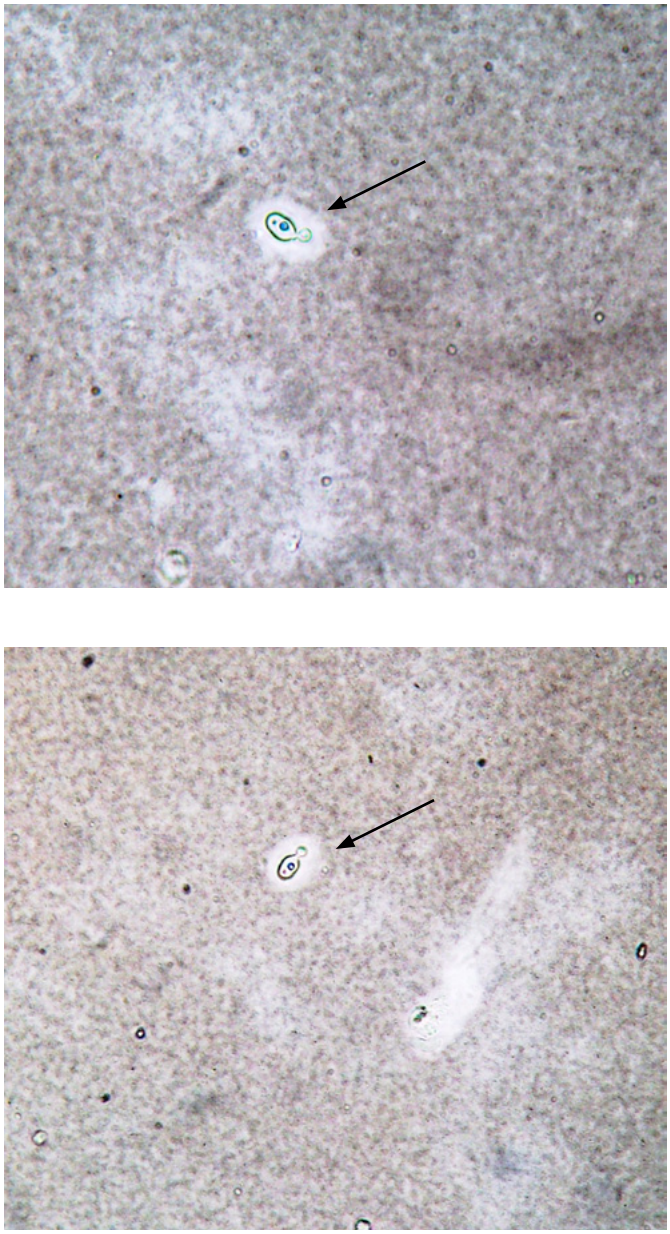


Figure 4.85: Cryptococcus spp. *encapsulated yeasts. Fresh urine sediment stained with China ink. Brightfield. Original magnification 400×. Courtesy of J. A. T. Poloni.*

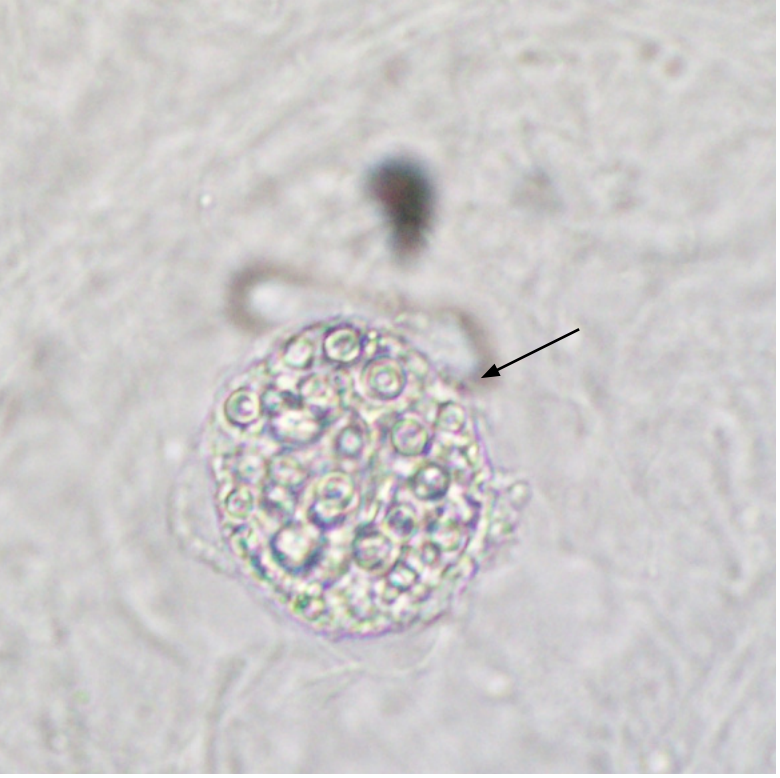


Figure 4.86: Cryptococcus spp. *yeasts within a macrophage. Fresh and unstained urine sediment. Brightfield. Original magnification 400×. Courtesy of J. A. T. Poloni*

Fungi can also be observed in urinary casts and in macrophages (Figure 4.86) [42]. Its observation in macrophages is a clear sign of the immune system trying to eliminate this pathogen from the body of the host.

In fact, the immune system also shows another particular finding in the urine sediment. The observation of neutrophils firmly attached to pseudohyphae (Figure 4.87) was already reported. It is reasonable to assume that they are trying to phagocytose the fungal structures that are too large for this type of cell [43, 44].

Bacteria

Bacteria are the main microorganisms causing infectious processes in the genitourinary tract. When they are observed in large numbers in urine that has been recently and properly collected, and they are associated with large numbers of leukocytes, bacteria are a good indicator of urinary tract infection [1]. Observing bacteria in a fresh analysis of the urinary sediment, however, does not necessarily indicate the presence of infection: the cause may be sample contamination. Neither, on the other hand, does the absence of bacteria in a fresh analysis of the sediment rule out the presence of these microorganisms.

Urine culture is considered the gold standard for detecting bacteria in urine. This test will accurately tell whether there are bacteria in the sample. Observing bacteria in a fresh analysis must always be evaluated with caution by the analyst and then by the clinician who receives the test report. Different types of bacteria can cause urinary tract infection in humans—both cocci (Figures 4.88 and 4.89) and bacilli (Figure 4.90) may be associated with infectious processes. However, far more than other species of bacteria, the main microorganisms causing urinary tract infections are *Escherichia coli* followed by *Klebsiella pneumoniae* [45].

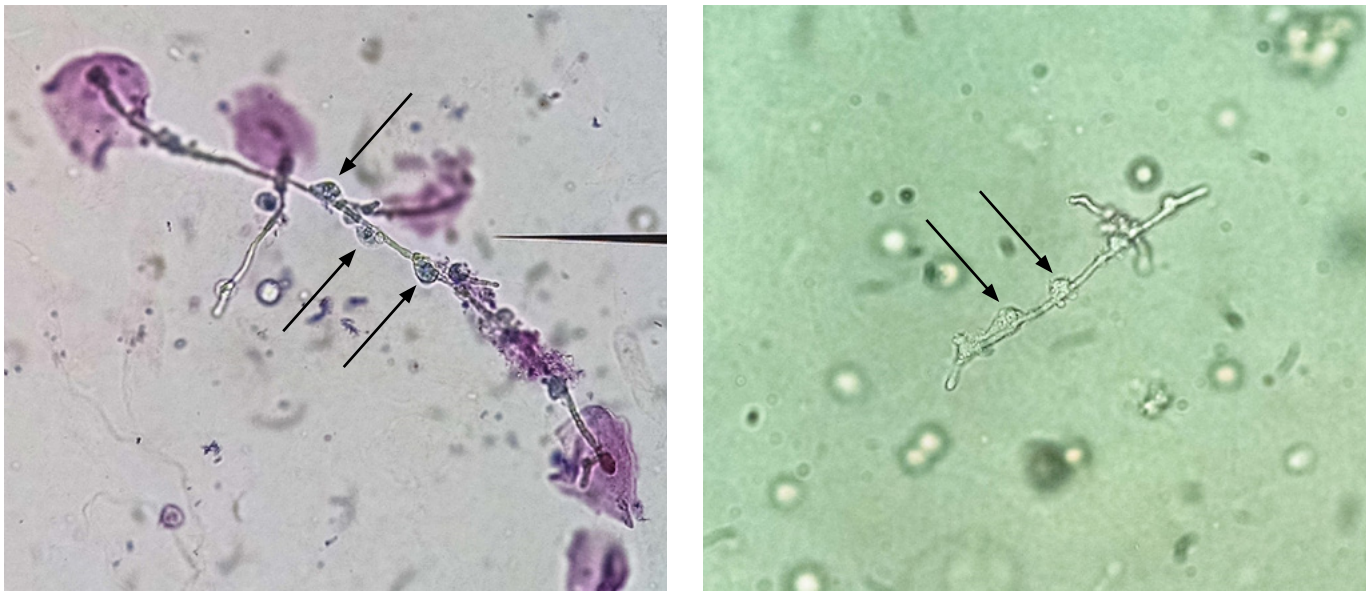


Figure 4.87: Leukocytes (neutrophils) attached to pseudohyphaes. Fresh urine sediment stained with Sternheimer-Malbin stain (left); Fresh and unstained urine sediment (right). Brightfield. Original magnification 400×. Courtesy of J. A. T. Poloni.

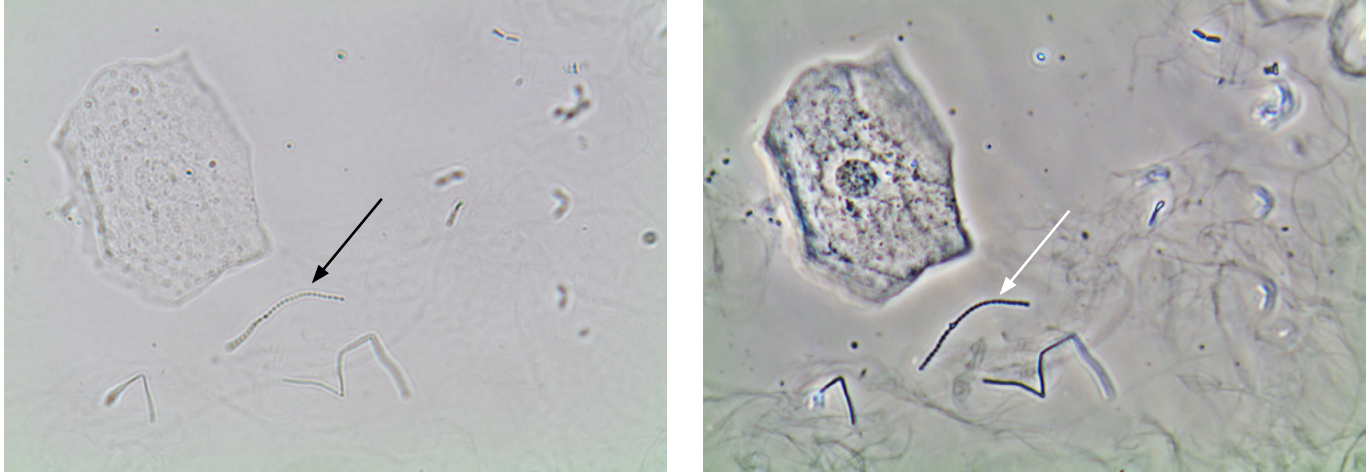


Figure 4.88: Bacteria (cocci). Fresh and unstained urine sediment. Brightfield (left); Phase contrast (right). Original magnification 400×. Courtesy of Controllab.

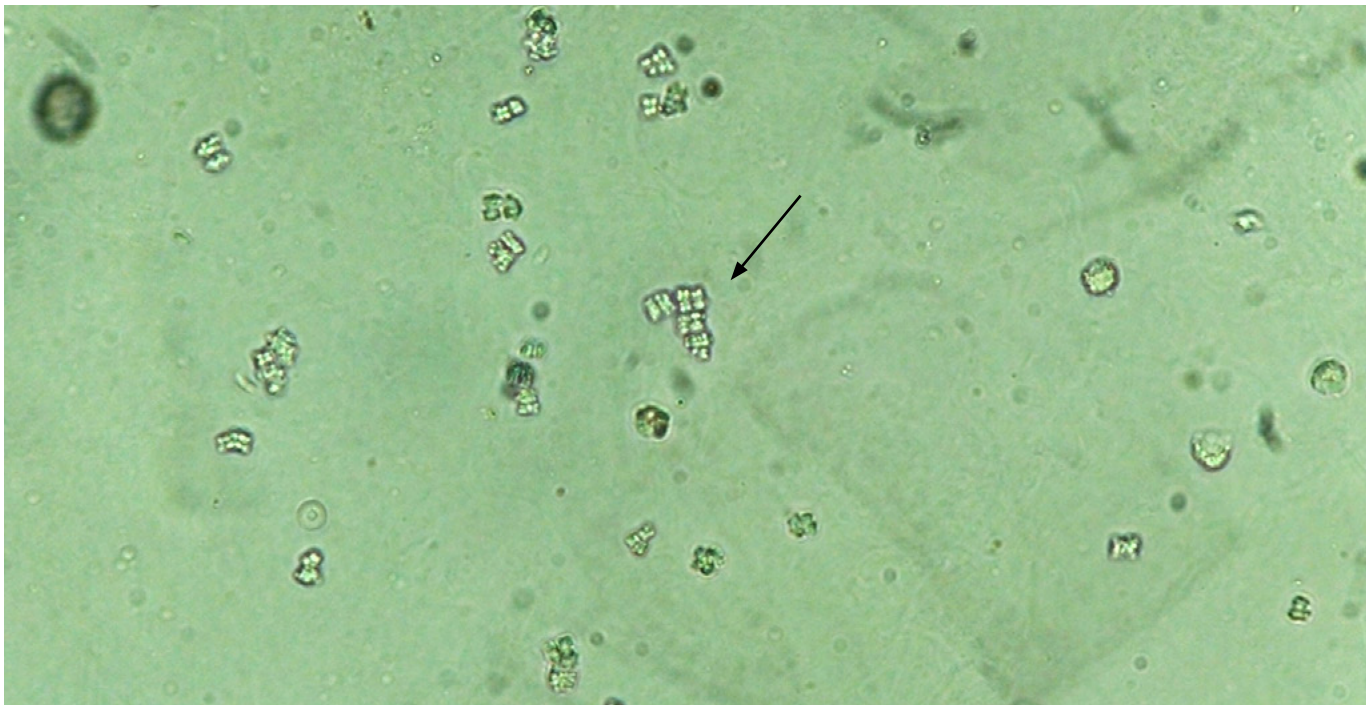


Figure 4.89: Bacteria (cocci). Fresh and unstained urine sediment. Brightfield. Original magnification 400×. Courtesy of A. P. G. Franz.

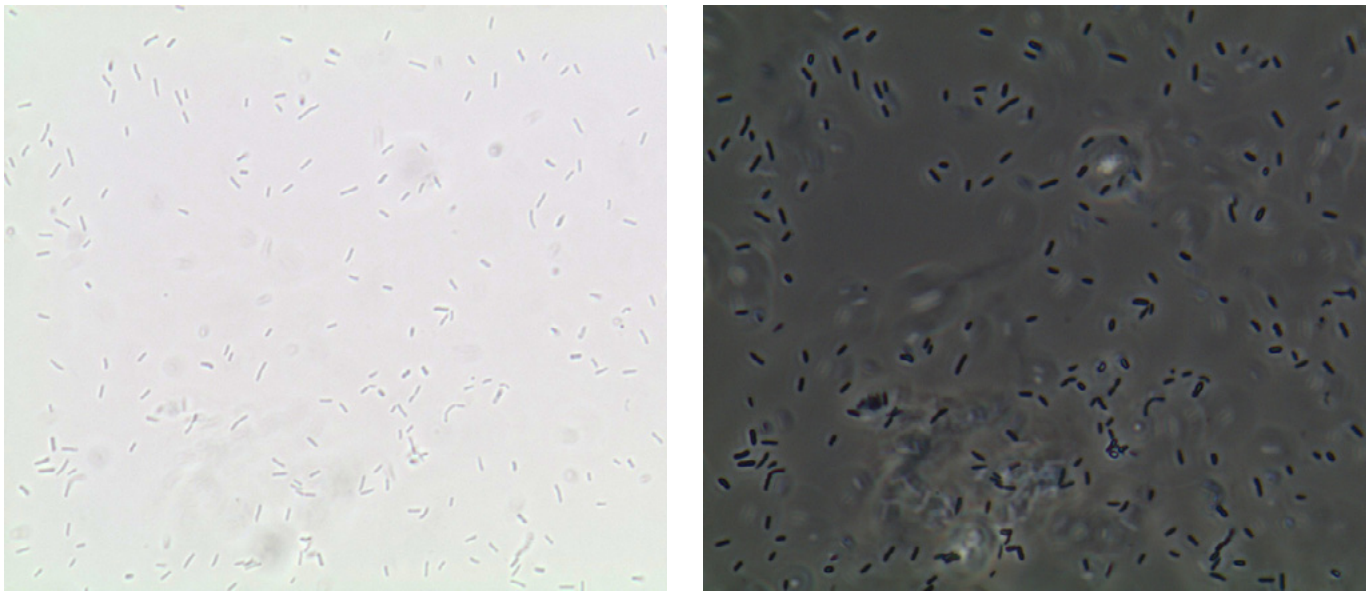


Figure 4.90: Bacteria (Bacilli). Fresh and unstained urine sediment. Brightfield (left); Phase contrast (right). Original magnification 400×. Courtesy of Controllab.

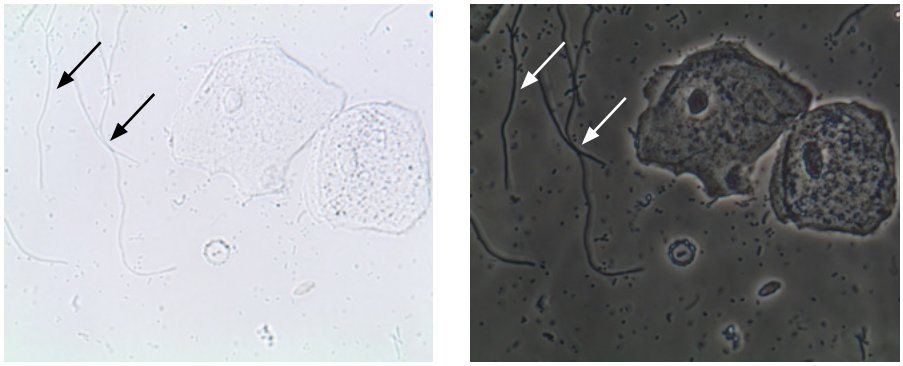


Figure 4.91: Bacteria (Gram-negative bacilli with filamentous forms). Fresh and unstained urine sediment. Brightfield (left); Phase contrast (right). Original magnification 400×. Courtesy of Controllab.

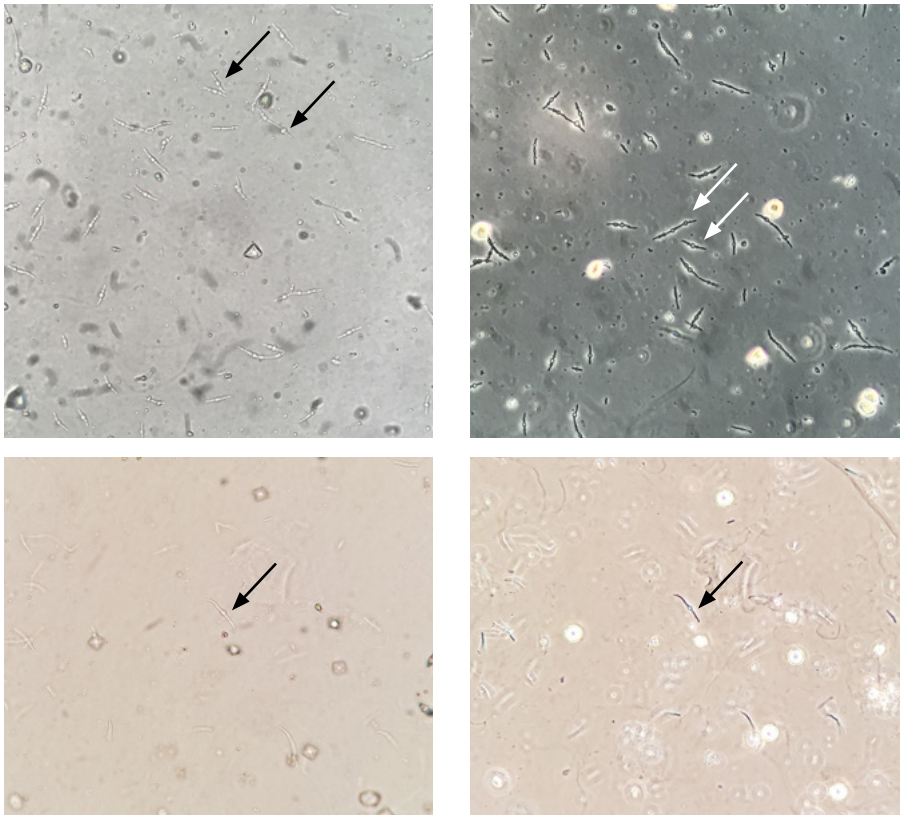


Figure 4.92: Bacteria (Spheroplasts). Fresh and unstained urine sediment. Brightfield (left); Phase contrast (right). Original magnification 400×. Courtesy of J. A. T. Poloni (Upper panel); A. P. G. Franz (Lower panel).

Gram-negative bacilli

Gram-negative bacilli with filamentous forms (Figure 4.91) or forming spheroplasts (Figure 4.92) can be seen in urinary sediment although little information related to these bacterial morphological changes is available in the literature. The work on spheroplasts reports that the morphological changes are promoted by exposure of these microorganisms to subinhibitory concentrations of beta-lactam antibiotics. The real clinical meaning of this finding is not yet understood, but it is important to know its morphology to avoid misclassifying it as yeast/fungi, for example [46–48].

To highlight another important observation regarding spheroplasts: automated systems that perform urinalysis may wrongly classify this element as erythrocytes. Thus, it is essential to consider the clinical context and the possibility of visualizing these structures on the screen to avoid providing an inadequate result [49].

Gram-positive bacilli
(Döderlein bacilli)

Döderlein bacilli (Figure 4.93) are microorganisms commonly observed in the urine of women, especially during pregnancy. They are larger than the bacilli usually seen in UTIs. These bacilli are part of the normal microbiota of women and are even beneficial for the female genital tract, promoting acidification of the genital region and contributing to decreased infectious processes by pathogenic bacteria.
An associated phenomenon to the presence of *Döderlein bacilli* is cytolysis (destruction of epithelial cells with release of their nuclei), which this microorganism performs to get access

to intracellular glycogen. The free nuclei of the squamous epithelial cells (Figure 4.93) are morphologically similar to leukocytes and may cause confusion in their identification if the analyst is not experienced with microscopic analysis of urinary sediments.

Usually *Döderlein bacilli* associated with free nuclei of squamous epithelial cells are seen in samples containing large numbers of squamous epithelial cells, an indicator that the sample was not collected properly. In this case the best course of action is to request a new urine sample, collected after hygiene of the genital region, using the clean catch technique and collecting the midstream urine.

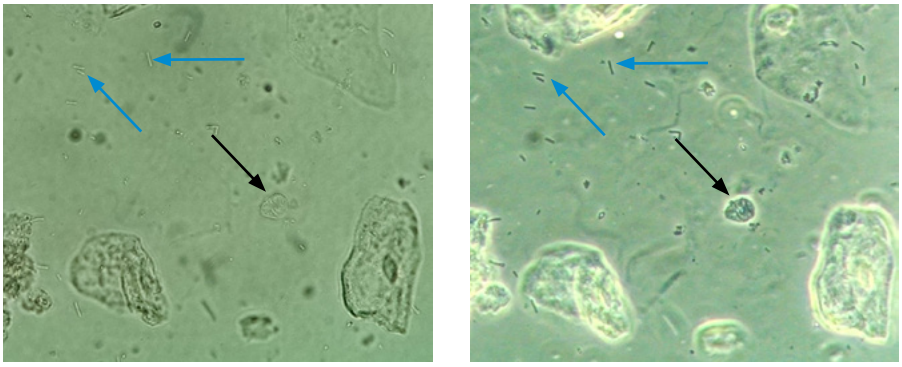


Figure 4.93: Döderlein bacilli *(blue arrows) and free nuclei of squamous epithelial cells (black arrows). Fresh and unstained urine sediment. Brightfield (left); Phase contrast (right). Original magnification 400×. Courtesy of J. A. T. Poloni.*

Others

Lipid Droplets and Oval Fat Bodies

Lipid droplets (Figure 4.94) can be observed free in the urine sediment or entrapped in urinary casts. They can also be found in renal tubular epithelial cells or macrophages that, due to their round / oval morphology and the fact that sometimes they are completely filled with lipid droplets, are called "oval fat bodies" (Figure 4.95).

Both lipid droplets and oval fat bodies are a common finding in patients with nephrotic range proteinuria.

Polarized light microscopy helps identify both, as the lipid droplets are made out of cholesterol esters so this microscopic filter produces a Maltese cross pattern [50].

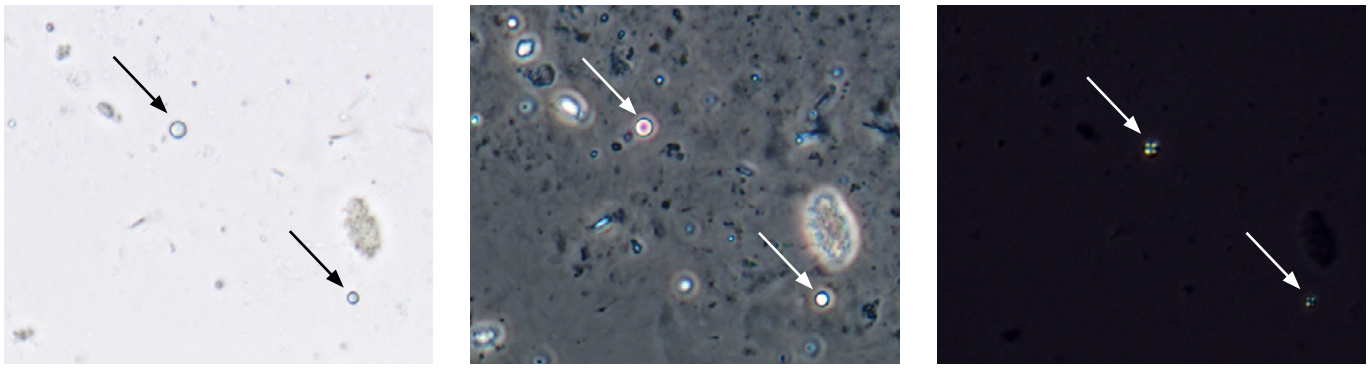


Figure 4.94: Lipid droplets. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (center); Polarized light (right). Original magnification 400×. Courtesy of Controllab.

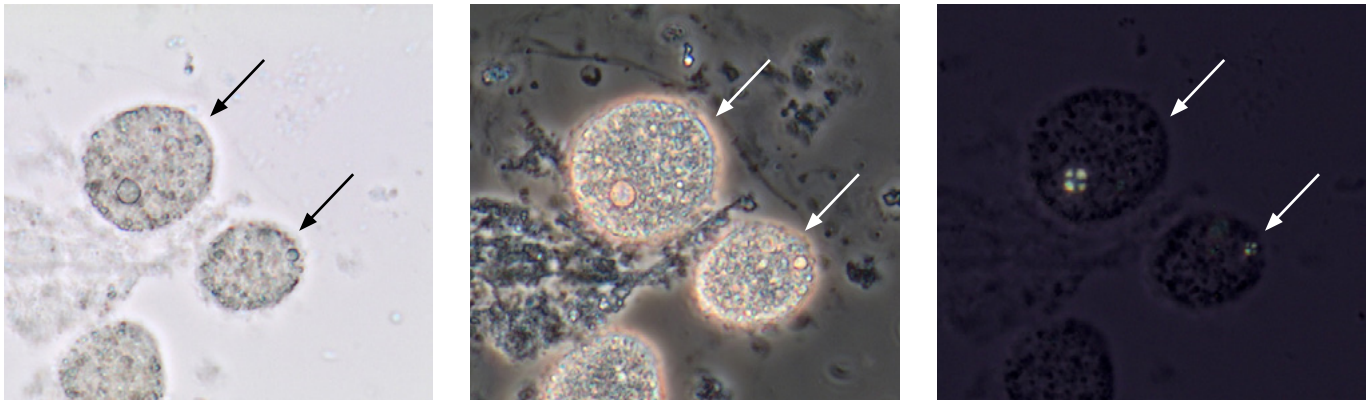


Figure 4.95: Oval fat bodies. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (center); Polarized light (right). Original magnification 400×. Courtesy of Controllab.

Mucus

Mucus (Figure 4.96) is a very common finding in urine sediment. It appears as irregular ribbon-like threads. This substance is derived from the accessory glands being observed in both men and women. Mucus can trap cells, potentially leading to interference in quantification of urinary particles [1].

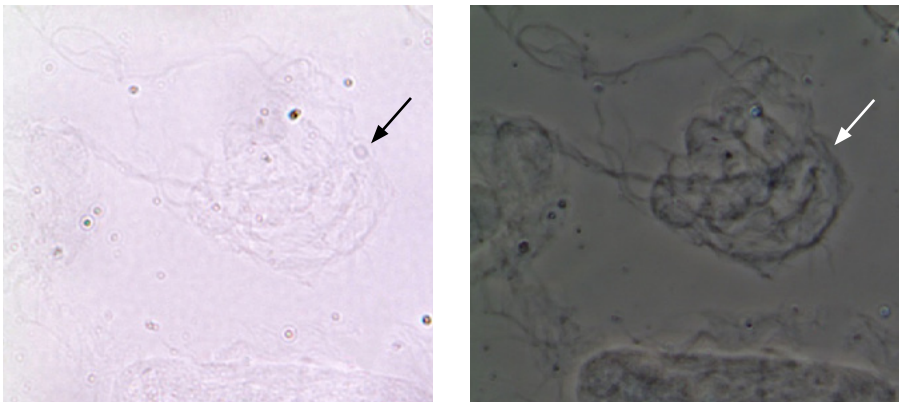


Figure 4.96: Mucus. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (right). Original magnification 400×. Courtesy of Controllab.

Spermatozoa and Sperm Bodies

Spermatozoa (Figure 4.97) can be observed in both men and women if the urine collection is performed after intercourse, and also in men after masturbation. Sperm bodies (Figure 4.98) are phagocytes containing spermatozoa, but the clinical significance of this finding is not clear [1, 51].

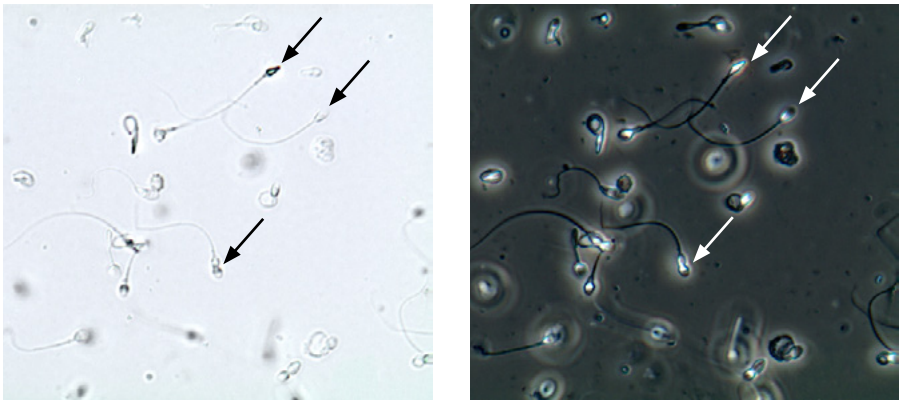


Figure 4.97: Spermatozoa. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (right). Original magnification 400×. Courtesy of Controllab.

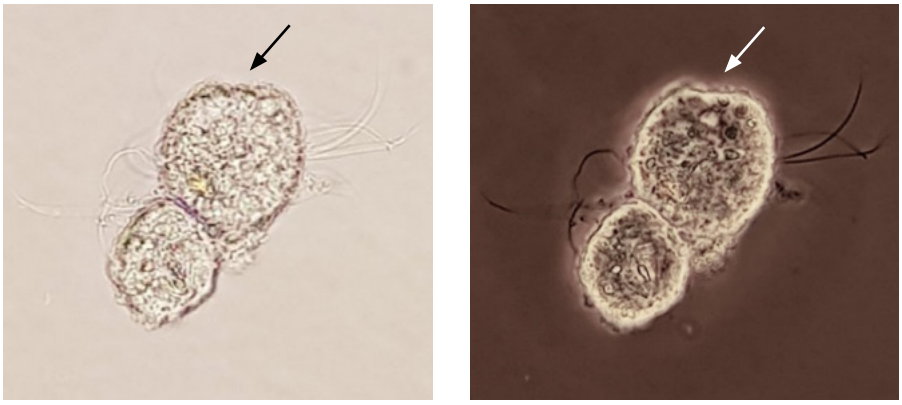


Figure 4.98: Sperm bodies. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (right). Original magnification 400×. Courtesy of A. P. G. Franz.

Artifacts

Several types of structures can contaminate urine samples and be observed during microscopic analysis. It is virtually impossible to list all of the artifacts that can be found. The important thing is to learn about the most common types of contaminants (Figures 4.99–4.102) and be prepared to face some challenges during routine work (Figures 4.103–4.109).

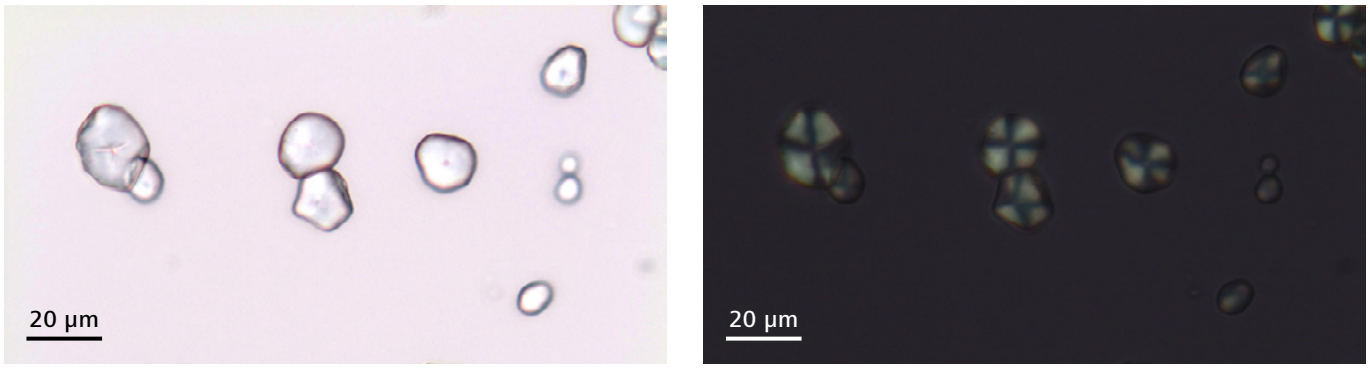


Figure 4.99: Starch. Fresh and unstained urine sediment. Brightfield (left); Polarized light (right). Original magnification 400×. Courtesy of Controllab.

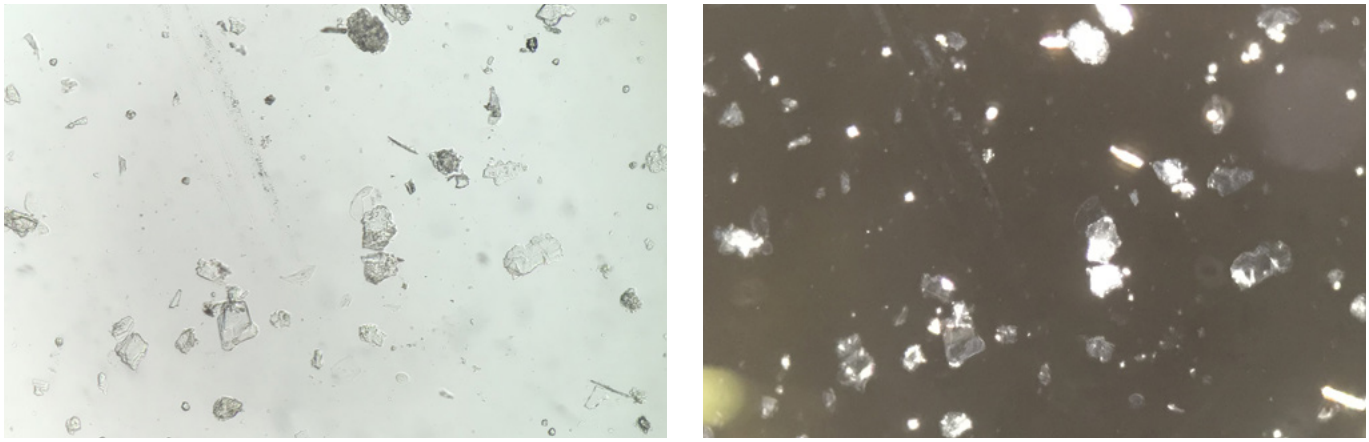


Figure 4.100: Talcum powder. Fresh and unstained urine sediment. Brightfield (left); Polarized light (right). Original magnification 100×. Courtesy of J. A. T. Poloni.

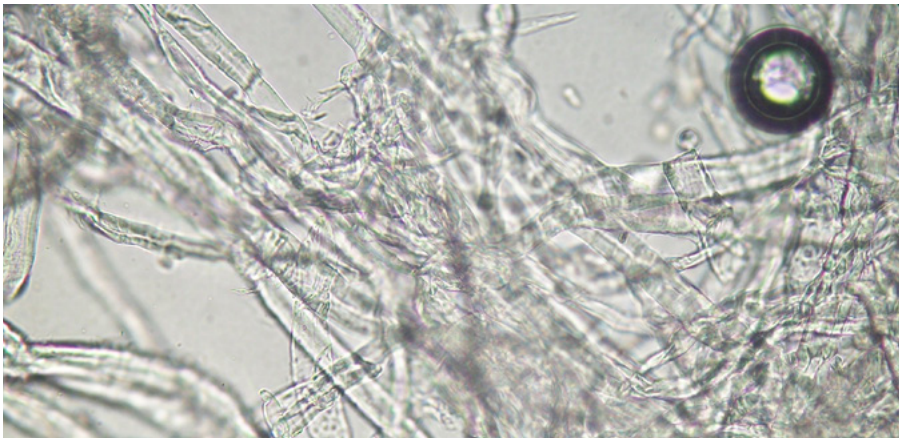


Figure 4.101: Paper fibers and air bubble. Fresh and unstained urine sediment. Brightfield. Original magnification 400×. Courtesy of J. A. T. Poloni.

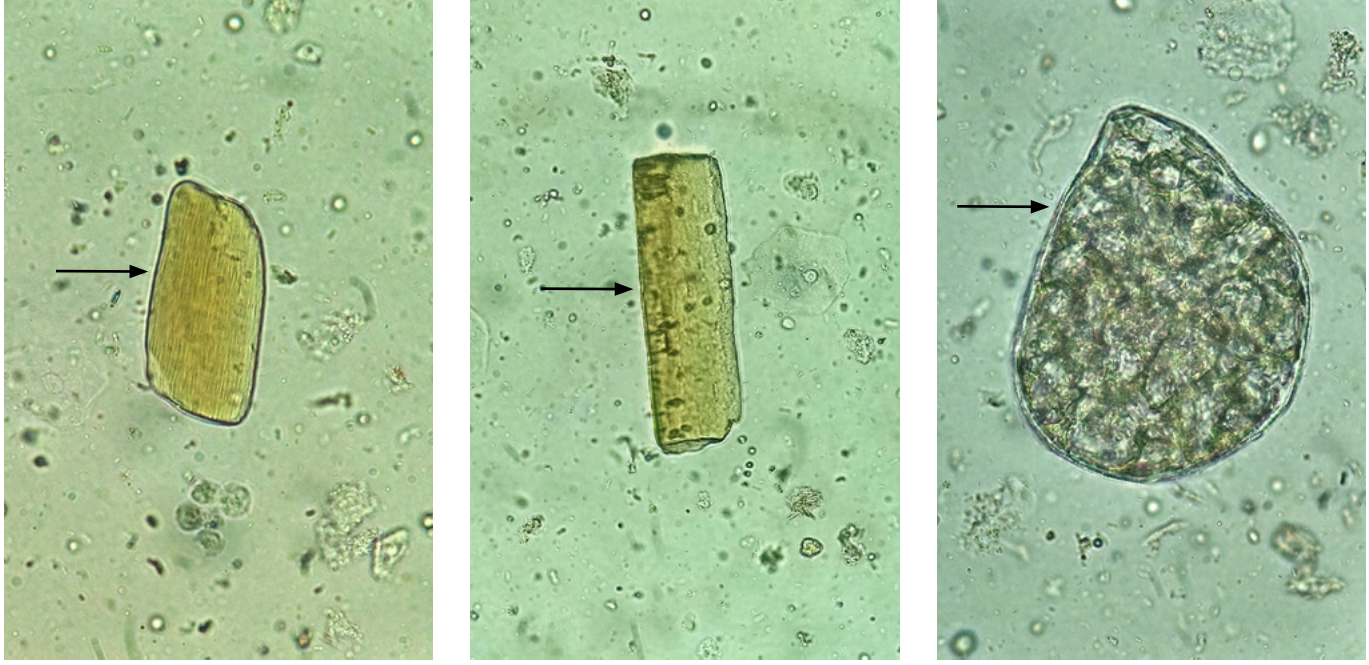
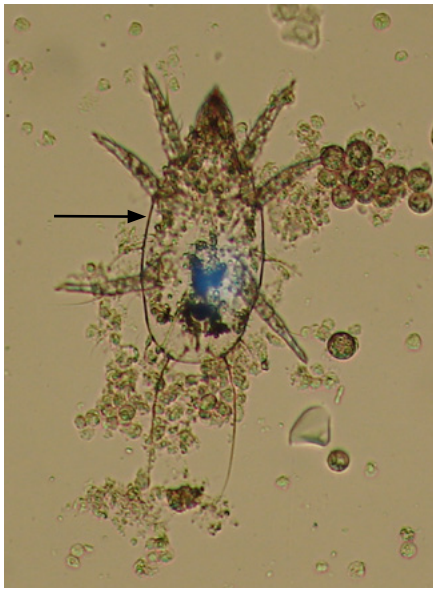


Figure 4.102: Fecal contamination. Fresh and unstained urine sediment. Brightfield. Original magnification 400×. Courtesy of J. A. T. Poloni.



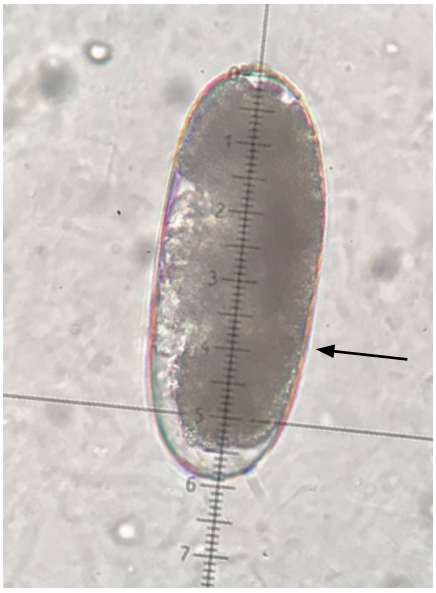
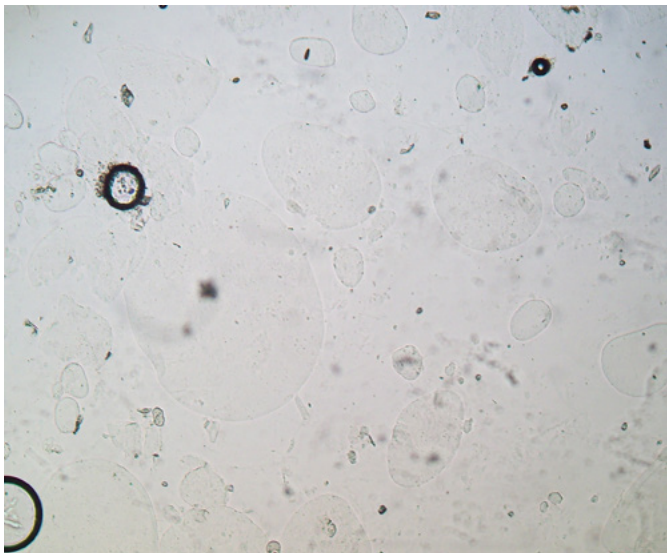


Figure 4.103: Mite (adult – left; egg – right) Fresh and unstained urine sediment. Brightfield. Original magnification 100× (left); 400× (right). Courtesy of J. A. T. Poloni.

Figure 4.104: "Powder" of moth wings. Fresh and unstained urine sediment. Brightfield. Original magnification 400×. Courtesy of J. A. T. Poloni.



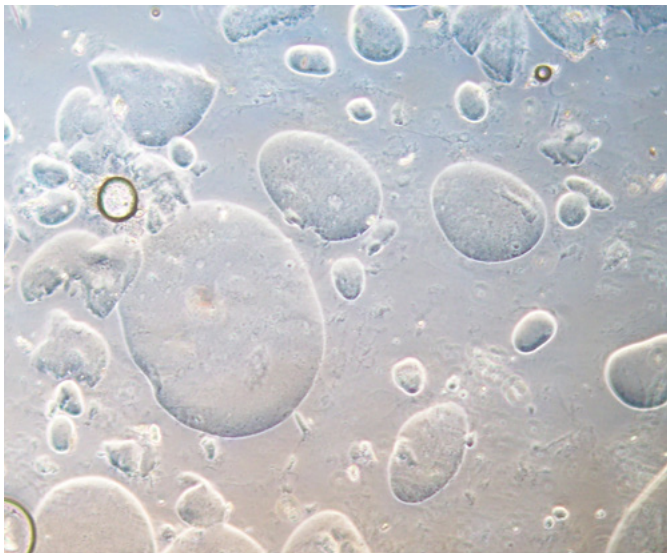


Figure 4.105: Oily particles due to topic medication in use in the genital area. Fresh and unstained urine sediment. Brightfield (left); Phase contrast (right). Original magnification 400×. Courtesy of J. A. T. Poloni.

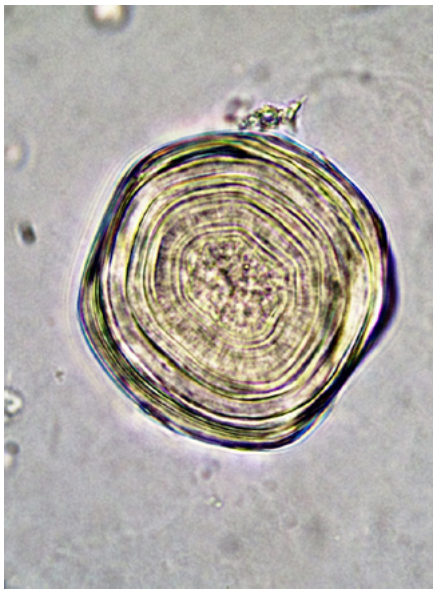


Figure 4.106: Corpora amylacea. Fresh and unstained urine sediment. Brightfield. Original magnification 400×. Courtesy of J. A. T. Poloni.

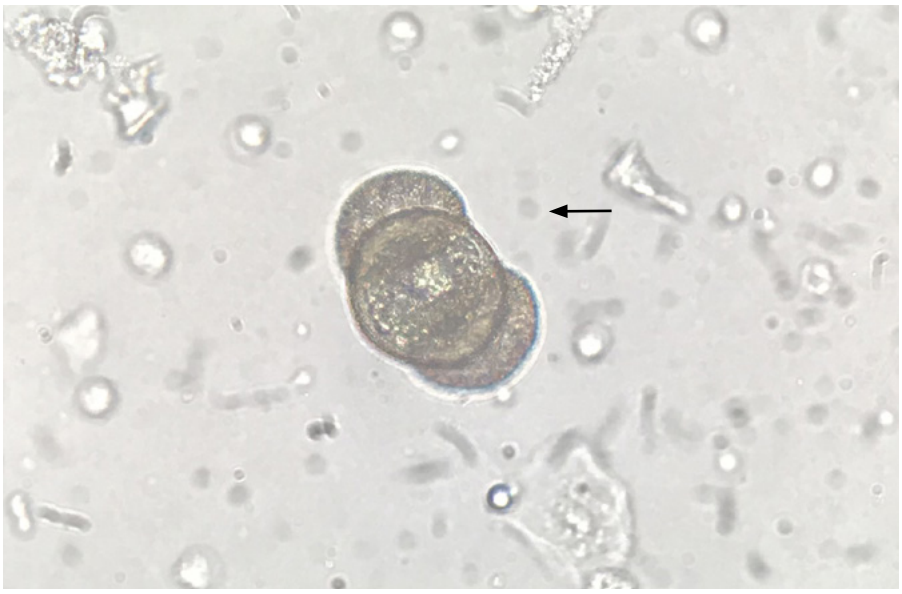


Figure 4.107: Pollen grain. Fresh and unstained urine sediment. Brightfield. Original magnification 400×. Courtesy of J. A. T. Poloni.

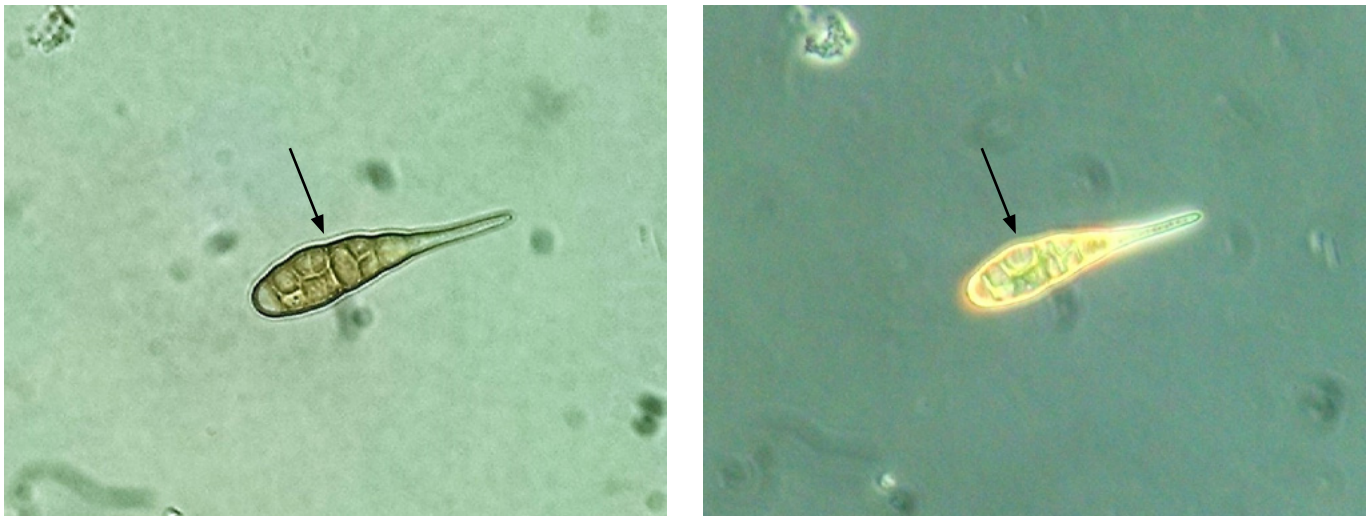


Figure 4.108: Alternaria sp. *Fresh and unstained urine sediment. Brightfield (left); Phase contrast (right). Original magnification 400×. Courtesy of J. A. T. Poloni.*

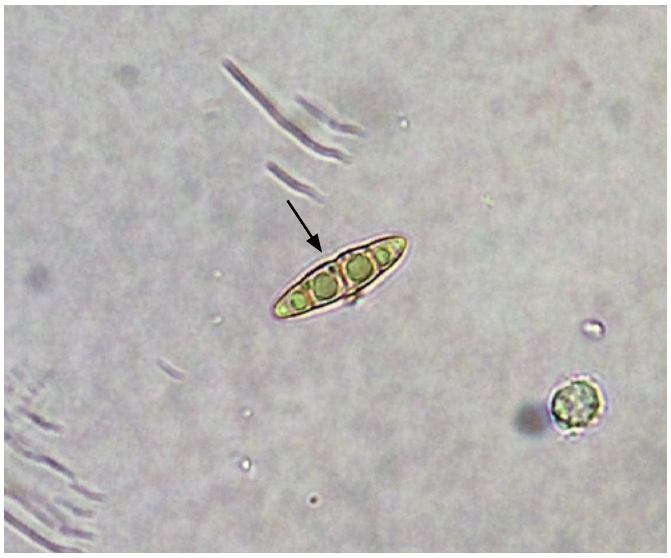


Figure 4.109: Macroconidia. *Fresh and unstained urine sediment. Brightfield. Original magnification 400×. Courtesy of J. A. T. Poloni.*

References

[1] Fogazzi, G.B. (2010) The urinary sediment an integrated view, 3rd edition. San Francisco, Elsevier.

[2] Itoh, K. (2014) Atlas of urinary sediment, Japan.

[3] Strasinger, S.K. & Schaub DiLorenzo, M. (2008) Urinalysis and body fluids, 5th edition, photography by B. Wang et al., illustration by S. Bonomelli. Philadelphia, PA, F.A. Davis.

[4] Mundt, L.A. & Shanahan, K. (2011) Graff's textbook of routine urinalysis and body fluids, 2nd edition. Philadelphia, PA, Lippincott Williams & Wilkins.

[5] Brunzel, N.A. (2013) Fundamentals of urine & body fluid analysis, 3rd edition. USA, Elsevier Saunders.

[6] Singh, H.K.; Bubendorf, L.; Mihatsch, M.J.; Drachemberg, C.B. & Nickeleit, V. (2006) Urine cytology findings of polyomavirus infections. Adv Exp Med Biol 2006;577:201–212.

[7] Poloni, J.A.; Pinto, G.G.; Giordani, M.S.; Keitel, E.; Inocente, N.; Voegeli C.F.; Fogazzi, G.B.; Pasqualotto, A.C. and Rotta. L.N. (2016) Bright field microscopy to detect decoy cells due to BK virus infection in the fresh and unstained urine sediment in kidney allograft recipients. J Clin Lab Anal. 2016 Nov;30(6):1044-1050. doi: 10.1002/jcla.21978. Epub 2016 Apr 28. PMID: 27122351; PMCID: PMC6807007.

[8] Poloni, J.A.T.; Meinerz, G.; Caurio, C.F.B. and Pasqualotto, A.C. (2020) Koilocytes due to HPV in the urine sediment. J Bras Nefrol. 2020 Oct-Dec;42(4):482-483. doi: 10.1590/2175-8239-JBN-2019-0158. PMID: 32353103; PMCID: PMC7860655

[9] Graber, M,; Lane, B.; Lamia, R. and Pastoriza-Munoz, E. (1991) Bubble cells: renal tubular cells in the urinary sediment with characteristics of viability. 1991 Jan;1(7):999-1004. doi: 10.1681/ASN.V17999. PMID: 1883970

[10] Guan, H.; Tatsas, A.D. and Ali, S.Z. (2012) Signet ring cell carcinoma in urine cytology: cytomorphologic findings and differential diagnosis. 2012;56(2):177-82. doi: 10.1159/000335118. Epub 2012 Feb 17. PMID: 22378081.

[11] Caglar, D.; Gagnon, R.F. & Kassouf, W. (2013) Tadpole cells in an unstained urine sediment. . 2013 Apr;83(4):763-4. doi: 10.1038/ki.2012.405. PMID: 23538700.

[12] Fogazzi, G.B.; Pallotti, F. & Garigali, G. (2015) Atypical/malignant urothelial cells in routine urinary sediment: worth knowing and reporting. Clin Chim Acta. 2015 Jan 15;439:107-11. doi: 10.1016/j.cca.2014.10.021. Epub 2014 Oct 17. PMID: 25451946.

[13] Reilly Jr., R.F. & Perazella, M.A. (2014) Nephrology in 30 days, 2nd edition. New York, McGraw Hill.

[14] Tesser Poloni, J.A.; Bosan, I.B.; Garigali, G. & Fogazzi, G.B. (2012) Urinary red blood cells: not only glomerular or nonglomerular. Nephron Clin Pract. 2012;120(1):c36-41; discussion c41. doi: 10.1159/000330286. Epub 2011 Dec 23. PMID: 22205019.

[15] Said, F.R.; Griffith, R.C. & Bayliss, G.P. (2011) An unusual presentation of hematuria. Kidney Int. 2011 Apr;79(8):923. doi: 10.1038/ki.2010.547. PMID: 21451540.

[16] Caleffi, A. & Lippi, G. (2015) Cylindruria. Clin Chem Lab Med. 2015 Nov;53 Suppl 2:s1471-7. doi: 10.1515/cclm-2015-0480. PMID: 26079824.

[17] Fogazzi, G.B,; Saglimbeni, L.; Banfi, G.; Cantú, M.; Moroni, G.; Garigali, G. et al. (2005) Urinary sediment features in proliferative and nonproliferative glomerular diseases. J Nephrol 2005;18:703–10.

[18] Fogazzi, G.B,; Ferrari, B,; Garigali, G.; Simonini, P. & Consonni, D. (2012) Urinary sediment findings in acute interstitial nephritis. Am J Kidney Dis 2012;60:330–2.

[19] Spinelli, D.; Consonni, D.; Garigali, G. & Fogazzi, G.B. (2013) Waxy casts in the urinary sediment of patients with different types of glomerular diseases: results of a prospective study. Clin Chim Acta 2013;424:47–52.

[20] Poloni, J.A.T.; Perazella, M.A.; Keitel, E.; Marroni, C.A; Leite, S.B. & Rotta, L.N. (2019) Utility of a urine sediment score in hyperbilirubinemia/hyperbilirubinuria . Clin Nephrol. 2019 Sep;92(3):141-150. doi: 10.5414/CN109673. PMID: 31198169.

[21] Poloni, J.A.T .and Hickmann, F.H. (2020) AKI in a patient with hemolytic anemia. Kidney 360 Jan 2020, 1 (1) 72-73; doi: 10.34067/KID.0000082019.

[22] Tesser Poloni, J.A. & Perazella, M.A. (2017) A rarely recognized cause of acute kidney injury in rhabdomyolysis (2018) Am J Med Sci. 2018 Sep;356(3):e27. doi: 10.1016/j.amjms.2017.03.028. Epub 2017 Mar 22. PMID: 30049408.

[23] Onder, A.M.; Espinoza, V.; Berho, M.E.; Chandar, J.; Zilleruelo, G. & Abitbol, C. (2006) Acute renal failure due to phenazopyridine (Pyridium) overdose: case report and review of the literature. Pediatr Nephrol. 2006 Nov;21(11):1760-4. doi: 10.1007/s00467-006-0196-1. Epub 2006 Aug 1. PMID: 16897003.

[24] Poloni, J.A.; Garcia, C.D.; Rotta, L.N. & Perazella, M.A. (2016) Calcium oxalate crystalluria points to primary hyperoxaluria type 1. Kidney Int. 2016 Jan;89(1):250. doi: 10.1016/j.kint.2015.11.001. PMID: 26759051.

[25] Poloni, J.A.; Rotta, L.N.; Voegeli, C.F. & Pasqualotto, A.C. (2013) Cryptococcus within a urinary cast. Kidney Int. 2013 Jul;84(1):218. doi: 10.1038/ki.2012.474. PMID: 23812371.

[26] Poloni, J.A.T. and Rotta, L.N. (2020) Urine Sediment findings and the immune response to pathologies in fungal urinary tract infections caused by candida spp. J Fungi (Basel). 2020 Oct 23;6(4):245. doi: 10.3390/jof6040245. PMID: 33114117; PMCID: PMC7711825.

[27] Martínez-Figueroa, C.; Cortés-Sarabia, K.; Catalán-Nájera, H.G.; Martínez-Alarcón, M.A. and Molina-Avilés, E.A. (2021) Cilindro desnaturalizado vacuolar, un elemento poco conocido del sedimento urinario. Nefrologia. 2021;41:365–366.

[28] Luciano, R.L.; Castano, E.; Fogazzi, G.B. and Perazella, M.A. (2014) Light chain crystalline kidney disease: diagnostic urine microscopy as the "liquid kidney biopsy" (2014) Clin Nephrol. 2014 Dec;82(6):387-91. doi: 10.5414/CN108424. PMID: 25295579.

[29] Luciano, R.L and Perazella, M.A. (20160 Crystalline-induced kidney disease: a case for urine microscopy. Clin Kidney J. 2015 Apr;8(2):131-6. doi: 10.1093/ckj/sfu105. Epub 2014 Oct 20. PMID: 25815167; PMCID: PMC4370296.

[30] Perazella, M.A.& Herlitz, L.C. (2015) The crystalline nephropathies. Kidney International Reports, 2021.

[31] Daudon. M. & Frochot, V. (2015) Crystalluria. Clin Chem Lab Med. 2015 Nov;53 Suppl 2:s1479-87. doi: 10.1515/cclm-2015-0860. PMID: 26509782.

[32] Daudon, M.; Letavernier, E.; Frochot, V.; Haymann, J-P.; Bazin, D. & Jungers, P. (2016) Respective influence of calcium and oxalate urine concentration on the formation of calcium oxalate monohydrate or dihydrate crystals. Comptes Rendus Chimie. 19(11–12), 1504-1513. ISSN 1631-0748. Available from: https://doi.org/10.1016/j.crci.2016.08.009 [Accessed 8th February 2022].

[33] Fogazzi, G.B.; Baronim S.; Garigali, G. et al. (2004) An unusual type of crystalluria (appearing only one every 130 years?). Nephrol Dial Transplant 2004;19:1907-9.

[34] Edvardsson, V.O.; Sahota, A. & Palsson, R. (2012) Adenine phosphoribosyltransferase deficiency. 2012 Aug 30 [updated 2019 Sep 26]. In: Adam, M.P. , Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Mirzaa G, Amemiya A, et al. (eds). GeneReviews® [Internet]. Seattle, WA, University of Washington, Seattle; 1993–2021. PMID: 22934314.As.

[35] de Liso, F.; Garigali, G.; Ferraris Fusarini, C.; Daudon, M. & Fogazzi, G.B. (2016) How to identify sulfamethoxazole crystals in the urine. Clin Chim Acta. 2016 Jan 15(452). 106-108. doi: 10.1016/j.cca.2015.11.006. Epub 2015 Nov 7. PMID: 26554520.

[36] Poloni, J.A.; Beltrão Lda, F.; Keitel, E.; Tasca, T. & Rotta, L.N. (2016) Trichomonas vaginalis erythrophagocytosis in the urine sediment. Int J STD AIDS. 2016 Feb; 27(2):157-158.

[37] Pasqualotto, A.C.; Zborowski, M.F.; dos Anjos, M.; Poloni, J.A.; dos Santos, A.P. & Torelly, A.P. (2009) Strongyloides stercoralis in the urine. Trans R Soc Trop Med Hyg. 2009 Jan;103(1):106-7. doi: 10.1016/j.trstmh.2008.08.011. Epub 2008 Sep 21. PMID: 18809190.

[38] Maino, A.; Garigali, G.; Grande, R.; Messa, P. & Fogazzi, G.B. (2010} Urinary balantidiasis: diagnosis at a glance by urine sediment examination. J Nephrol. 2010 Nov-Dec;23(6):732-7. PMID: 20349417.

[39] Pasqualotto, A.C.; Oliveira, F.M. & Severo, L.C. (2009) Histoplasma capsulatum recovery from the urine and a short review of genitourinary histoplasmosis. Mycopathologia. 2009 Jun;167(6):315-23.

[40] Khetan, S.; Khetan ,P.; Katkar, V. & Kusulkar, M. (2018) Urinary tract infection due to Fusarium oxysporum in an immunocompetent patient with chronic kidney disease. J Biomed Res. 2018 Mar 26;32(2):157-160.

[41] Su, C.C; Hsu, H.J.; Wu, J.J. & Chou, C.W. (2007) Diagnosis of fusariosis in urine cytology. J Clin Pathol. 2007 Apr;60(4):422-4.

[42] Tesser Poloni, J.A.; Perazella, M.A. & Neild, G.H. (2013) Macrophages at work: phagocytosis of urinary fungi. Clin Kidney J. 2013 Apr;6(2):233-4. doi: 10.1093/ckj/sfs184. Epub 2013 Feb 3. PMID: 26019856; PMCID: PMC4432439.

[43] Poloni, J.A.T.; Garcia, C.D.; Rotta, L.N. & Urban, C.F. (2021) Neutrophils phagocytosing fungal hyphae in urinary sediment. J Bras Nefrol. 2021 Jul-Sep;43(3):431-433. doi: 10.1590/2175-8239-JBN-2019-0245. PMID: 33350430; PMCID: PMC8428652.

[44] Poloni, J.A.T. & Rotta, L.N. (2020) Urine sediment findings and the immune response to pathologies in fungal urinary tract infections caused by candida spp. J Fungi (Basel). 2020 Oct 23;6(4):245. doi: 10.3390/jof6040245. PMID: 33114117; PMCID: PMC7711825.

[45] Flores-Mireles, A.L.: Walker, J.N.; Caparon, M. & Hultgren, S.J. (2015) Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol. 2015 May; 13(5): 269–284.

[46] Suwantarat, N. & Jacobs, M.R. (2013) Photo quiz: positive blood culture in a patient with sickle cell crisis. Answer: Klebsiella pneumoniae bacteremia showing filamentous forms and spheroplasts due to the presence of subinhibitory concentrations of beta-lactams. J Clin Microbiol 2013;51(8):2475;2807.

[47] Poloni, J.A; Meinerz, G.; Monteiro Ade, A.; Keitel, E. & Rotta, L.N. (2016) Klebsiella pneumoniae ESBL forming spheroplasts in the fresh and unstained urine sediment. J Bras Nefrol. 2016 Jun;38(2):269-270.

[48] Falbo, R.; Fogazzi, G.B.; Sala, M.R.; Garigali, G.; Sulejmani, A.; Brambilla, P. & Leoni, V. (2021) Spheroplasts, poorly known but clinically relevant particles of urinary sediment. Clin Chim Acta. 2021 Apr;515:13-15. doi: 10.1016/j. cca.2020.12.026. Epub 2020 Dec 24. PMID: 33359495.

[49] Nikler, A.; Radišić Biljak, V.; Čičak, H.; Marić, N.; Bejuk, D.; Poloni, J.A.T. & Simundic, A.M. (2019) Escherichia coli spheroplasts in a Croatian patient misclassified by two urine sediment analysers as erythrocytes: case report. Biochem Med (Zagreb). 2019 Oct 15;29(3):030801.

[50] Becker, G.J.; & Nicholls, K. (2015) Lipiduria—with special relevance to Fabry disease. Clin Chem Lab Med. 2015 Nov;53 Suppl 2:s1465-70. doi: 10.1515/cclm-2015-0499. PMID: 26124059.

[51] Koene, R.A.P. & Bogman, M.J.J.T. (1993) Sperm bodies in the urinary sediment. Nephrol Dial Transplant 1993;8:1296.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5. Microscope Equipment

At the risk of stating the obvious, urine microscopy requires proper equipment. In general, brightfield microscopes are the instrument of choice and they are indeed used extensively in the work. However, urinalysis guidelines also recommend the use of microscopes equipped with phase contrast and polarized light filters [1, 2]. These filters are recommended because they contribute to the identification of urine particles like dysmorphic erythrocytes and casts (phase contrast) as well as lipids and crystals (polarized light).

ZEISS Axiolab 5 with ZEISS Axiocam 208 color

ZEISS Primostar 3 with ZEISS Axiocam 208 color

LED illumination systems increase the quality of sample visualization, and they are also a useful resource for any kind of microscopic technique.

The following examples show uses in which phase contrast and polarized light filters are relevant:

Phase Contrast

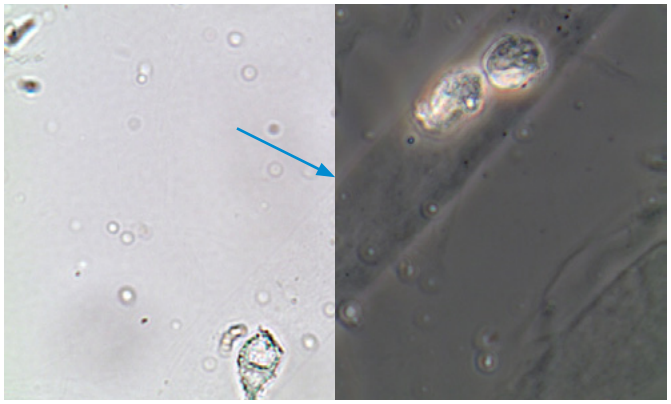

Figure 5.1: Epithelial cast. Fresh and unstained urine sediment. Bright field microscopy (left); Phase contrast microscopy (right). Original magnification 400×. Courtesy: Controllab.

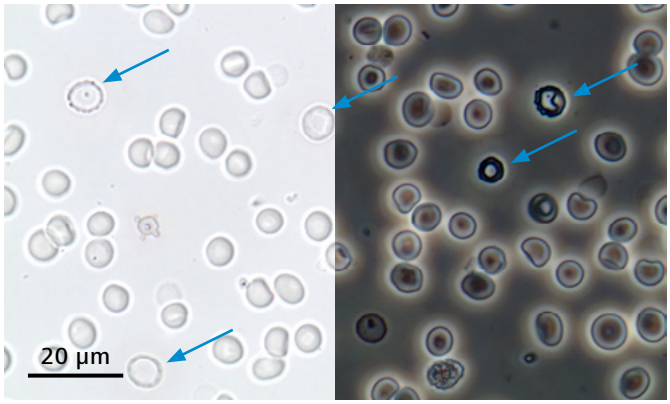

Figure 5.2: Dysmorphic erythrocytes (some pointed by arrows). Fresh and unstained urine sediment. Bright field microscopy (left); Phase contrast microscopy (right). Original magnification 400×. Courtesy: Controllab.

Polarized Light

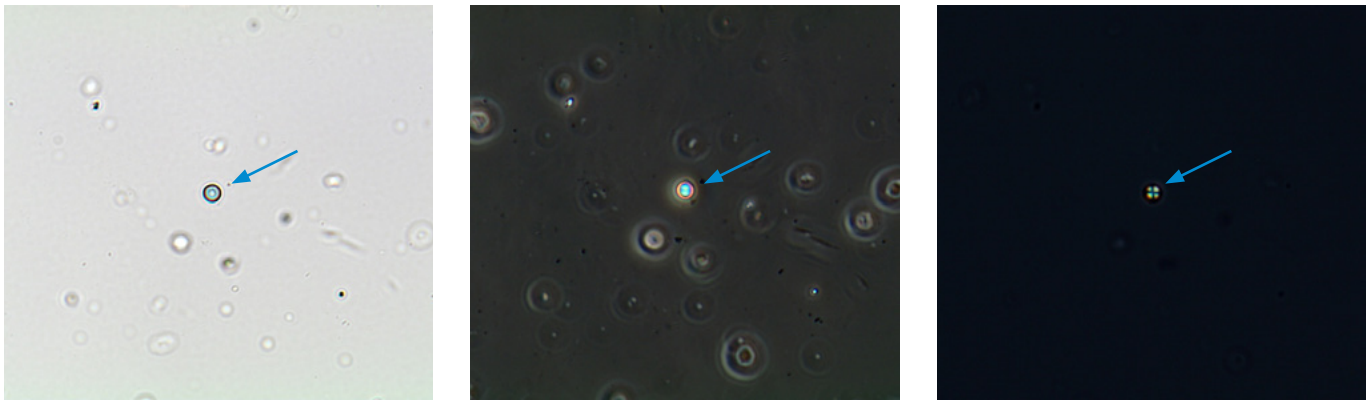

Figure 5.3: Lipid droplet. Fresh and unstained urine sediment. Bright field microscopy (left); Phase contrast microscopy (center); Polarized light microscopy (right). Original magnification 400×. Courtesy: Controllab.

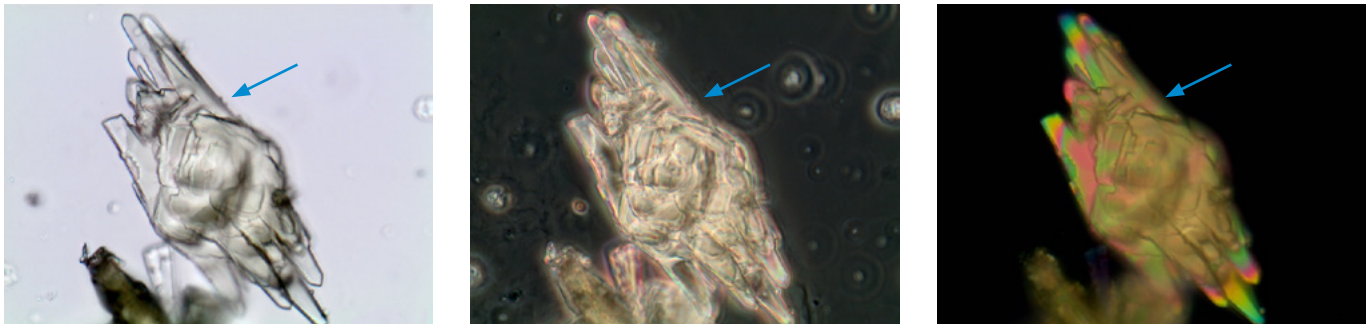

Figure 5.4: Uric acid crystals (polychromatic pattern). Fresh and unstained urine sediment. Original magnification 400×. Courtesy of Controllab.

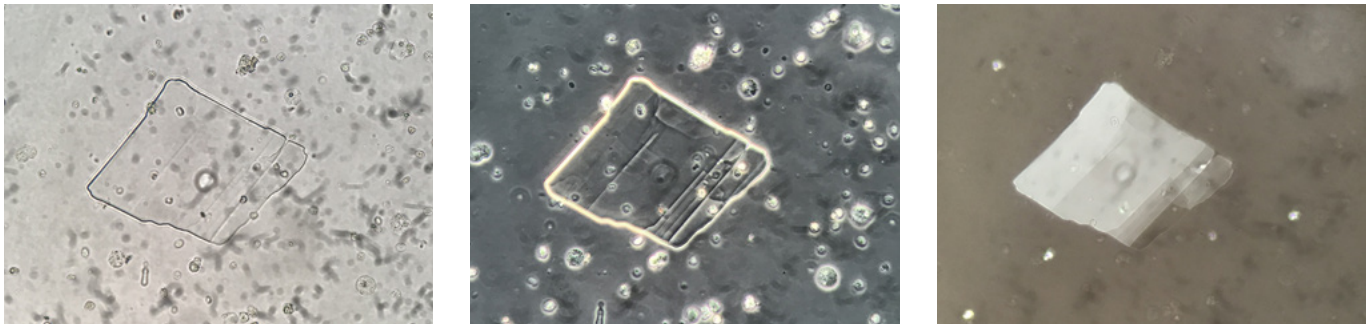

Figure 5.5: Cholesterol crystals (monochromatic pattern). Fresh and unstained urine sediment. Original magnification 400×. Courtesy of J. A. T. Poloni.

Phase contrast microscopy is much better than brightfield microscopy for identifying casts with a hyaline matrix, erythrocytes with low hemoglobin content [3, 4] and cell details. For this last reason, it is the best instrument for differentiating normal from dysmorphic erythrocytes, and also renal tubular epithelial cells from deep transitional epithelial cells [3].

Polarized light filters are useful—often indispensable—for identifying lipids containing cholesterol esters and free cholesterol (observed forming Maltese cross patterns), crystals (observed with positive birefringence—monochromatic in some, polychromatic in others), and some contaminants such as starch and synthetic fibers [3].

Köhler Illumination

Two condenser settings – centering and Köhler illumination – provide an optimal view of the light field. They must be set every time an objective is changed. In fact, these procedures must be adopted as part of daily maintenance and be performed every day at the beginning of the routine. To centralize the condenser and achieve Köhler illumination, the following steps must be carried out:

- Place the condenser in the upper position (Figure 5.6).
- Place the 5× objective in the working position (Figure 5.6). Make a movement to close the lower diaphragm and look through the eyepiece, checking the formation of a geometric figure. It can be a hexagon or octagon, depending on the microscope manufacturer (Figure 5.7). Check that the edges of the geometric Figure touch the edge of the black circle that corresponds to the microscopic field (Figure 5.8). If the geometric Figure is misaligned (Figure 5.9), use the screws on the microscope condenser base (Figure 5.10) to align it in the central position of the field (Figure 5.8).

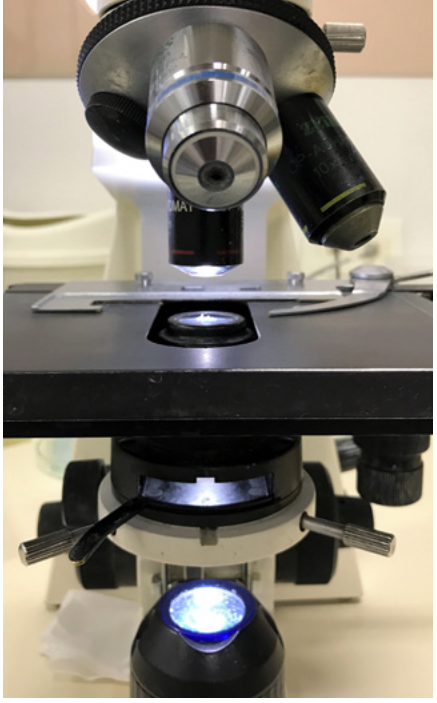

Figure 5.6

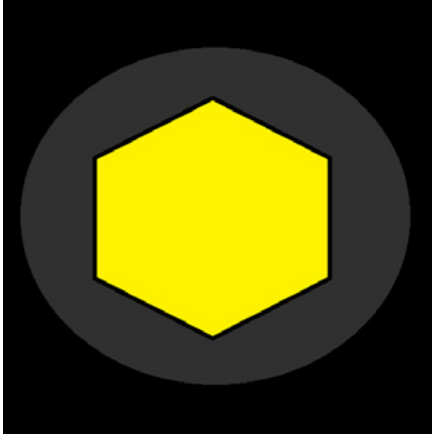

Figure 5.7

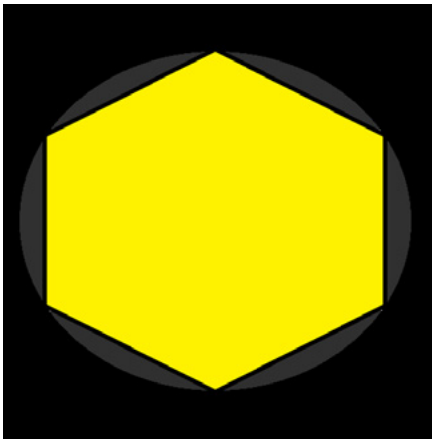

Figure 5.8

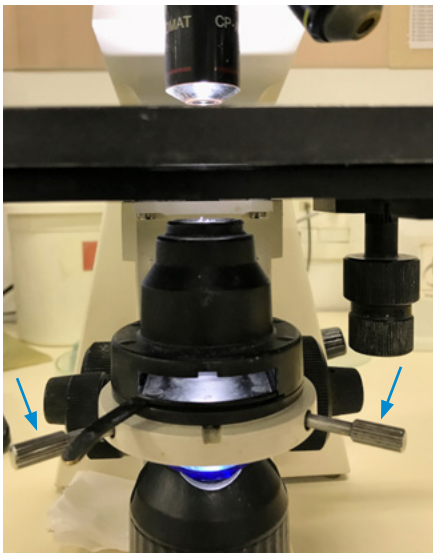

Figure 5.10

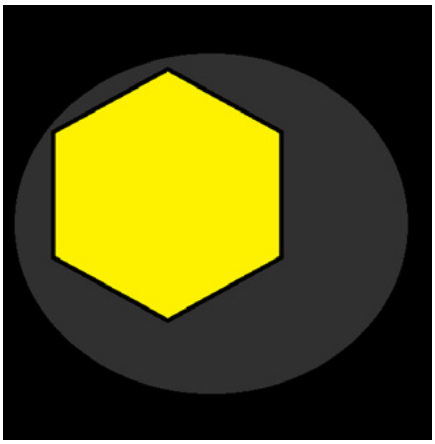

Figure 5.9

Performing preventive maintenance procedures on the microscope ensures good optical performance. The microscope should always be covered when not in use to protect it from dust. If any optical surface becomes covered with dust, remove the dust carefully using a camel hair brush. Optical surfaces should be cleaned with lens paper or cotton. Clean any contaminated lens immediately with a commercial lens cleaning solution. Alternatively, use a 90 % ether solution (ethyl or petroleum) and 10 % alcohol–the purer the better [5].

Brightfield Microscopy

Brightfield microscopy has been used traditionally for the analysis of urinary sediment and is still widely used today [3, 6]. However, with this type of microscopy the elements of the urinary sediment are hard to differentiate from the bottom of the slide, with some exceptions such as lipids, crystals and waxy casts. Therefore, it is easy to overlook particles with a low refractive index such as hyaline casts and erythrocytes with a low hemoglobin content. Furthermore, cellular details are hard to distinguish [3].

Some improvement in viewing the elements can be achieved by using the microscope's condenser in its lowest position and slightly closing the condenser diaphragm. However, even with these adjustments the results are not as good or consistent as those using phase contrast microscopy [3].

Phase Contrast Microscopy

In this type of microscopy, the condenser contains an annular diaphragm (Figure 5.11) that transforms the incident light into a hollow cone of light.

Figure 5.11

The objective, in its posterior focal plane, contains an engraved circular ring, also known as a phase ring, which is covered by a translucent layer of silver. After penetrating the object under study, the light beam is composed of both direct light and diffracted light, whose photons have interacted with the object. While direct light passes through the phase ring, diffracted light passes only around thicker areas. This difference in light path lengths results in a phase difference between the two light beams of a quarter of the wavelength. In this way, an evident contrast between the bottom of the slide and the elements under investigation is obtained and that, precisely,

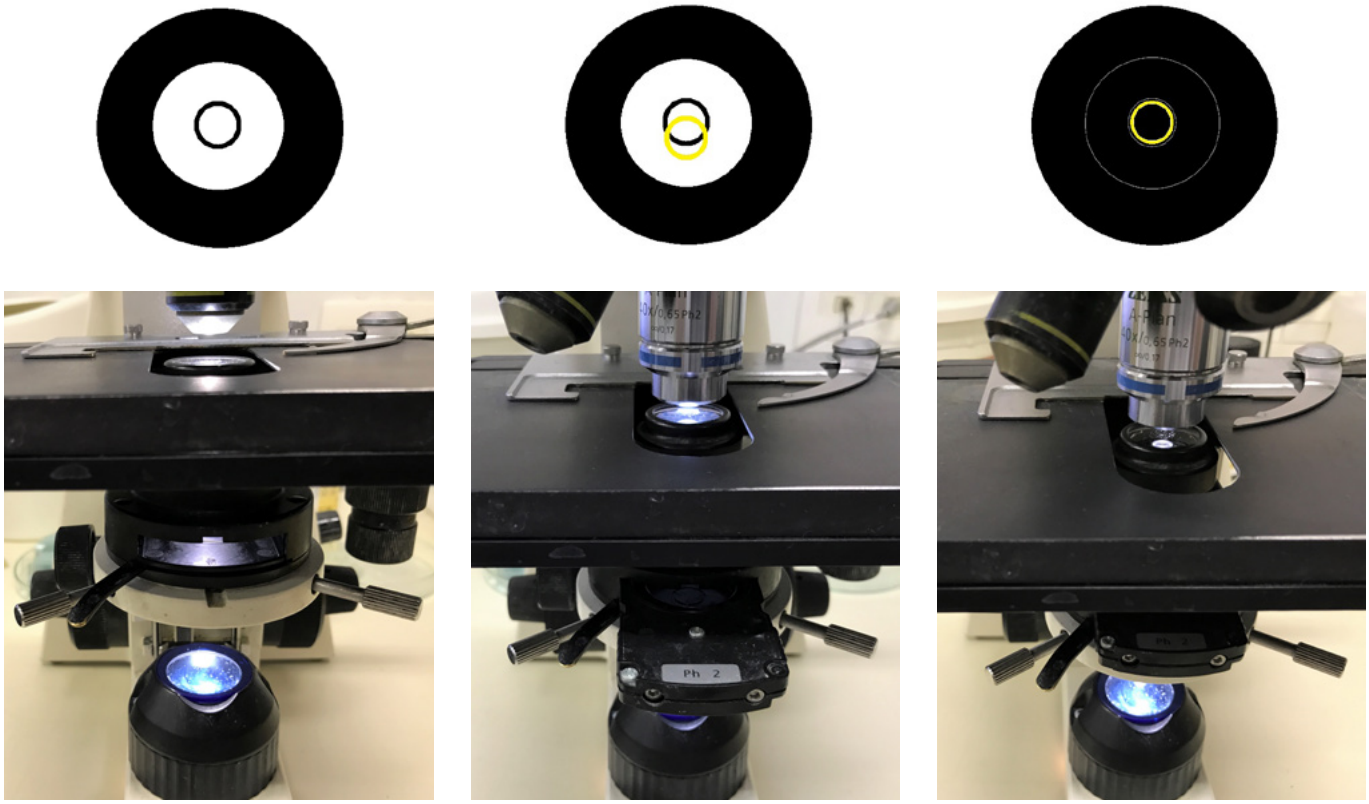

Figure 5.12: Using a phase contrast microscope involves centering the condenser ring with the objective phase ring.

is the main characteristic of phase contrast microscopy. Another feature peculiar to phase contrast microscopy is the presence of a halo (that is, a light zone around dark details and a dark zone around light details. Phase contrast microscopy makes the use of dyes unnecessary [3].

In newer types of microscopes this is easily done by adjusting knobs placed on the condenser. For less recent models this is done by using the phase telescope or auxiliary microscope, removing an eyepiece. The centering maneuver has to be done frequently, but it only takes a few seconds. When changing the objective to a different magnification objective, the annular condenser must be exchanged in parallel since the annular diaphragm ring and objective rings need to be compatible. For this reason, the best microscopes have a "universal condenser" with several different annular diaphragms that are compatible with different objectives. Analysis with polarized light filters is also possible in phase contrast microscopy, but the results are not as good as those obtained when polarization is performed with brightfield optics. However, as phase contrast objectives can also be used with brightfield lenses, all you have to do is change the condenser from the phase contrast to the brightfield position to obtain perfect polarization [3].

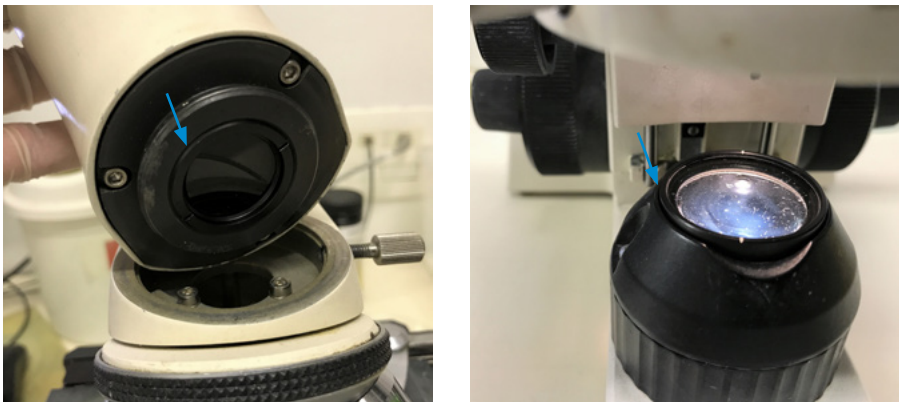

Figure 5.13

Polarized Light Microscopy

Your microscope must also be equipped with filters for polarized light (Figure 5.13). The 90° rotation of one of the filters placed below the condenser causes the microscope field to be completely darkened. When an anisotropic object is placed between the two filters, the plane of oscillation of the light beam changes by interacting with the anisotropic particle under study and a fraction of the light reaches the eyes of the microscopist. Elements that polarize light are seen as glowing particles against a dark background. This phenomenon is known as birefringence [3].

References

[1] European Confederation of Laboratory Medicine (2000) European urinalysis guidelines. Scand J Clin Lab Invest Suppl. 2000;231:1-86. PMID: 126477

[2] Clinical and Laboratory Standards Institute (2009) Urinalysis; Approved Guideline, 3rd edition. CLSI document GP16-A3. Wayne, PA.

[3] Fogazzi, G.B. (2010) The urinary sediment: an integrated view, 3rd edition. San Francisco, Elsevier.

[4] Brody, L.; Webster, M.C. & Kark, R.M. (1968) Identification of elements of urinary sediment with phase contrast microscopy. J Am Med Assoc 1968; 206: 1777-81.

[5] Strasinger, S.K. & DiLorenzo, M.S. (2008) Urinalysis and body fluids, 5Th edition. Philadelphia, PA, F.A. Davis.

[6] Fogazzi, G.B.; Garigali, G.; Pirovano, B. et al. (2007) How to improve the teaching of urine microscopy. Clin Chem Lab Med 2007; 45: 407-12.


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6. Clinical Profiles

Like any other type of microscopic analysis, urine sediment analysis depends on a number of important factors, among them the equipment, the experience of the professional performing the test and the quality of the sample collection. The way a sample is evaluated and interpreted under the microscope plays a crucial role in the quality of the information the microscopist will be able to obtain during the work. That's why understanding the clinical profile of the sample is extremely relevant to the final information the microscopic analysis will furnish. This chapter will show some examples of clinical profiles using text, schematic drawings and pictures to create a flow of "mind work" during routine analysis.

The Urinary Tract Infection Profile

A urinary tract infection (UTI) caused by bacteria is one of the world's most common medical conditions, especially cystitis. Laboratory personnel are usually familiar with this condition, given the large number of patients undergoing tests to diagnose it so it's no mystery to anyone.

Bacteria and leukocytes in urine (usually in large amounts) are major pointers for UTIs and are common findings for both cystitis and pyelonephritis.

Dipstick results normally present a positive reaction to leukocyte esterase. Depending on the type of bacteria that is causing the UTI, an alkaline pH reaction is possible—for example, if the UTI is caused by urea split bacteria—and a nitrite positive test is also possible if the UTI is caused by bacteria that can convert nitrate into nitrite. It is also common to observe a positive result to hemoglobin because usually there is injury to the urothelium.

In the microscopic analysis, bacteria and leukocytes are expected and erythrocytes are also common.

The dipstick and microscopic findings are basically the same in cystitis and pyelonephritis except for one particular structure: the cast containing leukocytes (white blood cells). WBC casts are exclusive to pyelonephritis cases within the context of urinary tract infection. This favors pyelonephritis when observed in the differential diagnosis.

Another important point about WBC casts: they can be observed not only in pyelonephritis but in any kind of clinical condition that leads to intratubular inflammation—acute interstitial nephritis, for example. The words "exclusive to pyelonephritis" are used here only because our subject is UTIs [1 – 5].

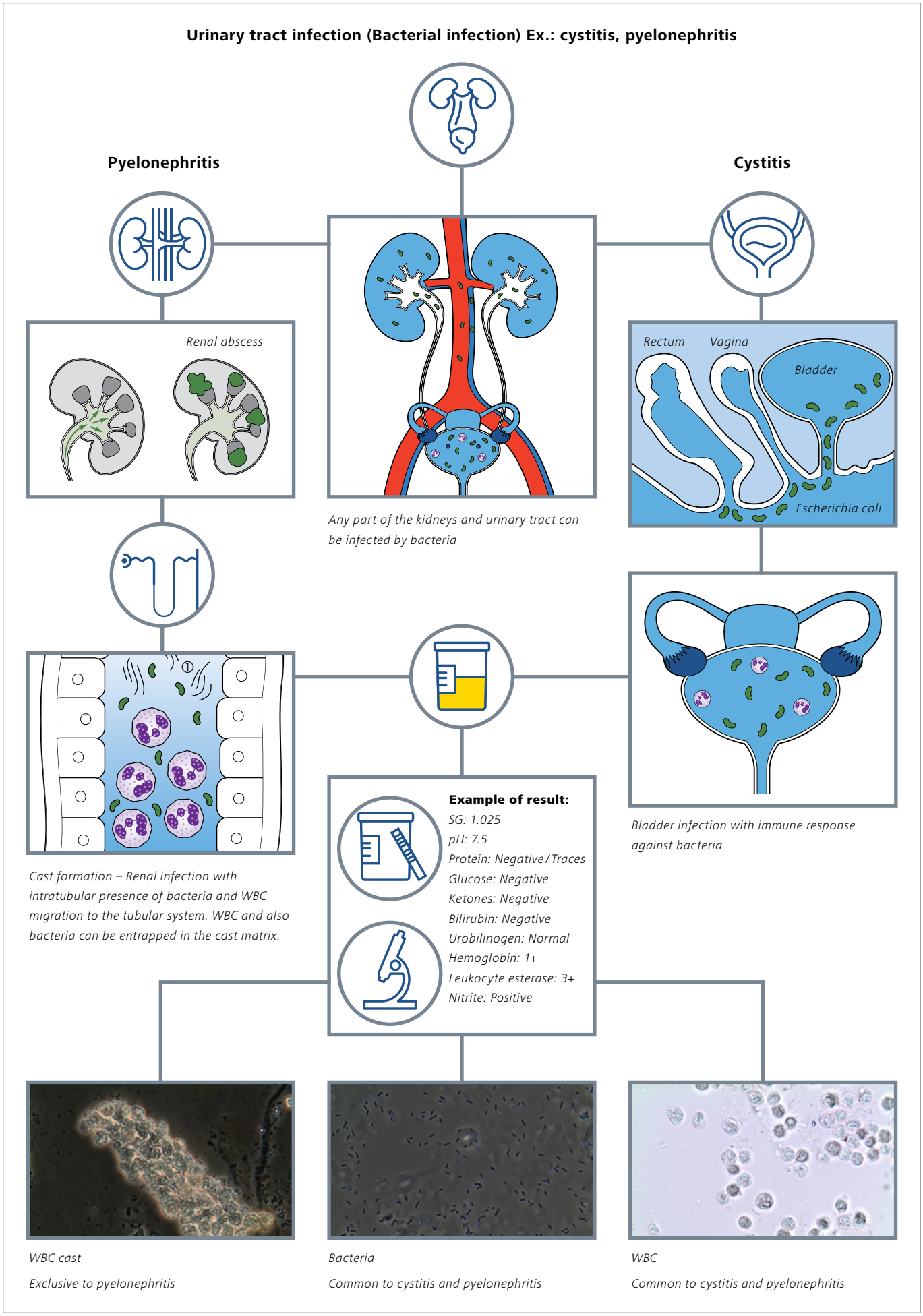


Figure 6.1: The Urinary Tract Infection Profile

① *Tamm-Horsfall protein polimerization and cast formation*

The Tubular Injury Profile (Figures 6.2 to 6.6)
A tubular injury profile (TIP) can start with several different conditions, five of which will be used as examples in this chapter. What is common to all types of TIPs is the observation in urine microscopy of renal tubular epithelial cells (RTECs), casts containing RTECs (RTEC casts) and granular casts.

Tubular injury (Ischemic/nephrotoxic) Ex.: Drug toxicity
Nephrotoxic drugs can promote metabolism disruption leading to the death of renal tubular epithelial cells (RTECs). The dead cells detach from the tubules and enter the tubular lumens, where they promote obstruction. The obstruction process is marked by an intraluminal increase in sodium and polymerization of the Tamm-Horsfall glycoprotein (THG), leading to cast formation. The detached RTECs and the remnants of dead cells (cellular debris) can become entrapped in the cast matrix where the RTEC casts and the granular casts originated. These structures are eliminated in the urine and are the hallmark of tubular injury when found during microscopic examination of the sediment. Their diagnostic utility is for their potential link to loss of kidney function.

The dipstick information related to this TIP can be unremarkable, requiring total attention from the professional evaluating the sample on the microscope since sometimes the chemical test will offer no clue as to tubular injury.

Tubular injury (associated to crystals)
Ex.: Primary hyperoxaluria
This urinary profile presents the same features as the previous example, but with additional findings: crystals, both free, in urinary casts, and also (more rarely) in RTECs. The most common type of crystal observed here is the calcium oxalate monohydrate (CaOxm). However, other types of crystal can also be observed—for example, uric acid crystals.

Intratubular precipitation of crystals is a sign of an imbalance between the promoters of crystal formation and the inhibitors. Crystals in urinary casts are a clear clue as to the presence of this type of structure in the tubular system. This is very useful in the identification of important clinical conditions—primary hyperoxaluria, for example.

The dipstick test, as with the previous TIP, can be unremarkable, again reinforcing the relevance of the microscopic resources available for the sample analysis and the knowledge of the professional performing the test.

Tubular injury (associated to direct bilirubin)
Ex.: alcoholic cirrhosis and hepatitis C infection
Patients with high levels of direct bilirubin in the blood, usually caused by severe liver diseases, can present with intense bilirubinuria. Direct bilirubin can enter the urinary system after being filtered by the glomerulus (it will usually originate a yellow/brown discoloration in the patient's urine). At tubular level, direct bilirubin can cause necrosis and apoptosis, leading to RTECs and debris being shed in the tubular lumens. The presence of these structures will contribute to tubular obstruction and cast formation, originating as the same types of microscopic findings observed in the first example of TIP (RTECs, RTEC casts and granular casts) – all intensely pigmented in yellow. In addition to these findings, the microscopic analysis can also present bilirubin crystals and, less often, leucine and tyrosine crystals. Bilirubin crystals can be seen free, in RTECs and in leukocytes (WBCs). Leucine crystals can also be observed entrapped in urinary casts.

The dipstick analysis will present a strong reaction to bilirubin and usually to urobilinogen. Protein is also commonly positive in these patients.

However, not all patients with severe liver diseases will present with this urinary profile. Also, findings related to this profile can be linked to loss of kidney function, but this is not necessarily so. In any case, it is important to note that the urine test is only part of the diagnostic toolkit required to understand the patient's clinical condition.

Tubular injury (associated to hemoglobin)
Ex.: hemolytic anemia
Patients with intravascular hemolysis (e.g., hemolytic anemia) will present with large amounts of free hemoglobin in the circulation. This excess hemoglobin will arrive in the kidneys and be filtered by the glomerulus (usually originating as a brown/red discoloration in the urine). At tubular level, the heme group (present in the hemoglobin molecule) will break down products and labile iron will be deposited in the tubular cells, stimulating the process of cell death. Dead RTECs detach from the tubules, entering the tubular lumens together with cell debris. This process will contribute to tubular obstruction and cast formation, originating [in] the same microscopic findings that are characteristic of the TIPs (RTECs, RTEC casts and granular casts) – frequently presenting as a brown/red color.

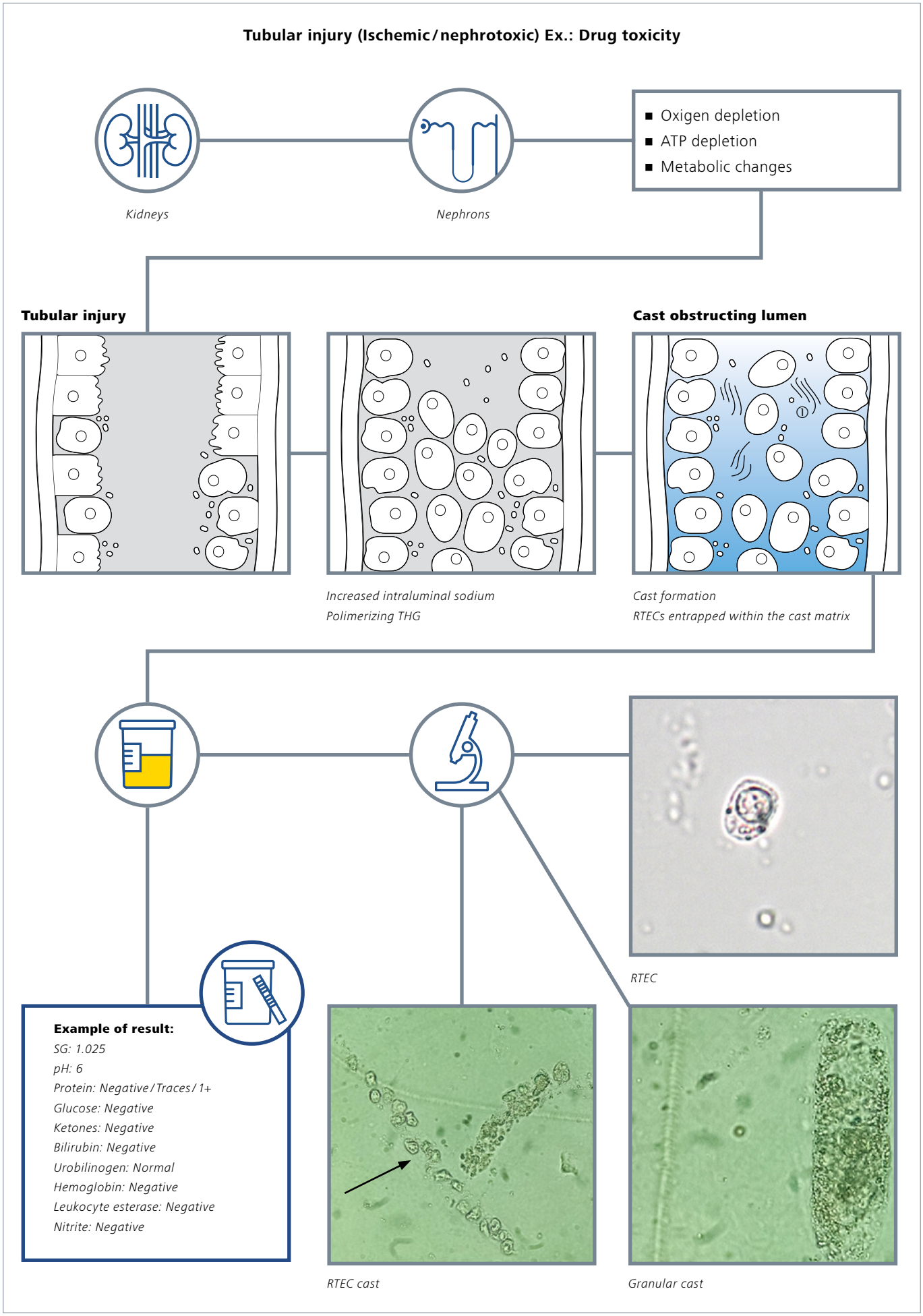


Figure 6.2: TIP Example 1: Drug Toxicity

① Tamm-Horsfall protein polimerization and cast formation

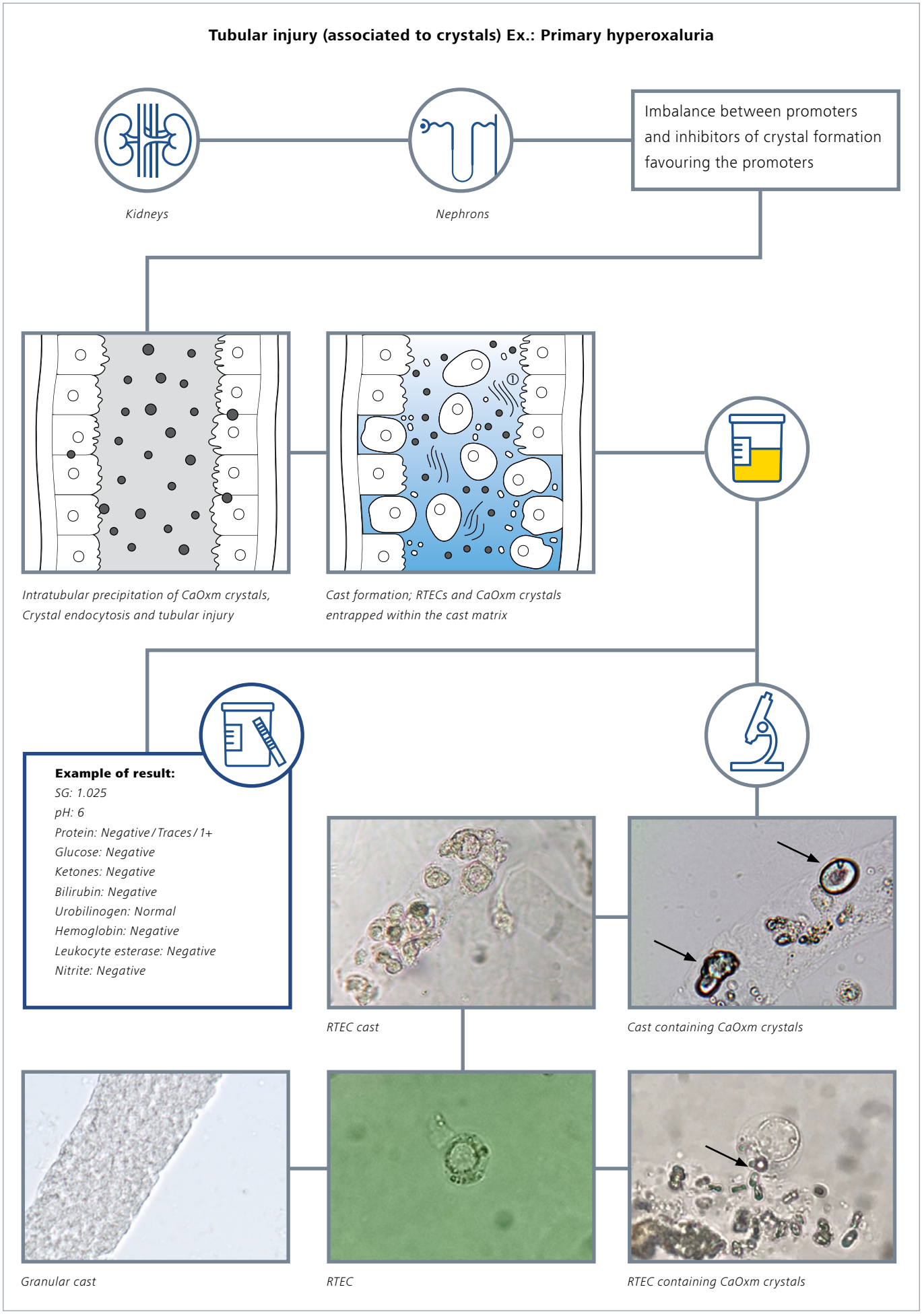


Figure 6.3: TIP Example 2: Intratubular Crystal Precipitation

① *Tamm-Horsfall protein polimerization and cast formation*

Tubular injury (associated to direct bilirubin) Ex.: alcoholic cirrhosis and hepatitis C infection

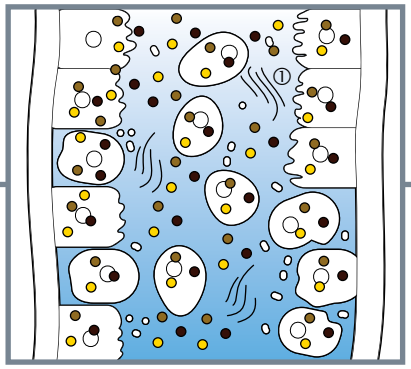
Liver damage

High levels of direct bilirubin enters the bloodstream

Direct bilirubin arrives in the kidneys

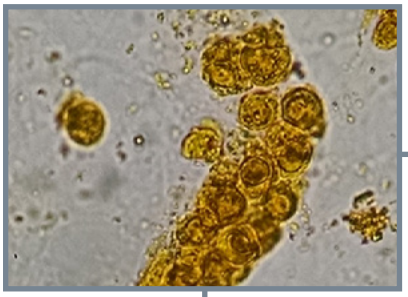
Direct bilirubin is filtered by the glomerulus

- Direct bilirubin affects mitochondrial function, causes phosphaturia, uricosuria, glucosuria.
- Changes tubular architecture with necrosis and apoptosis.

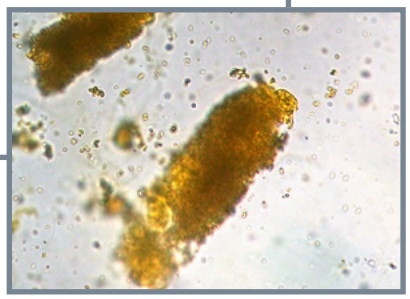


Cast formation; RTECs and cell debris (all bile stained) entrapped within the cast matrix

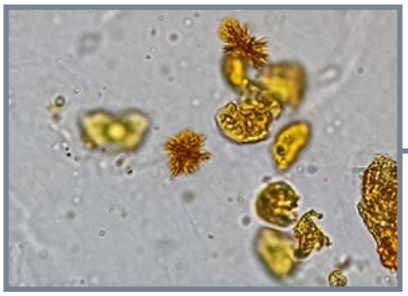
Example of result:
SG: 1.030
pH: 5,5
Protein: 1+/2+
Glucose: Negative
Ketones: Negative
Bilirubin: 3+
Urobilinogen: Excess
Hemoglobin: Negative
Leukocyte esterase: Negative
Nitrite: Negative



RTEC cast

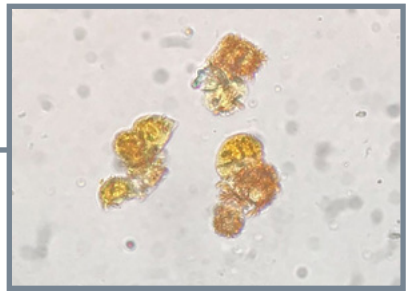


Granular cast



RTECs and bilirubin crystals

Leucine crystal



RTECs with engulfed bilirubin crystals

Figure 6.4: TIP Example 3: Hyperbilirubinemia / Hyperbilirubinuria

① Tamm-Horsfall protein polimerization and cast formation

Tubular injury (associated to hemoglobin) Ex.: hemolytic anemia

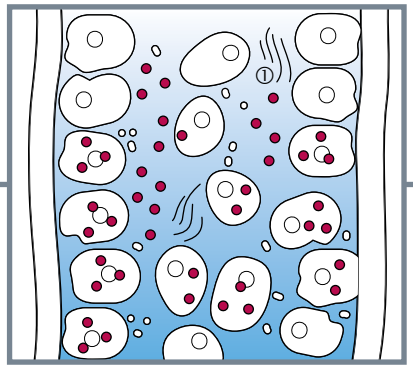
Intravascular hemolisis

High amount of hemoglobin in the bloodstream arrives in the kidneys

Hemoglobin is filtered by the glomerulus and enters the tubular system

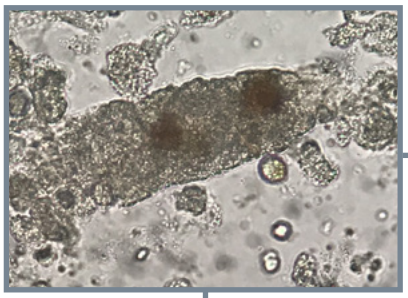
In the tubular system the hemoglobin promotes:

- Mitochondrial damage and tubular injury due to the action of heme group breakdown products and labile iron that depositates in the kidney tubules

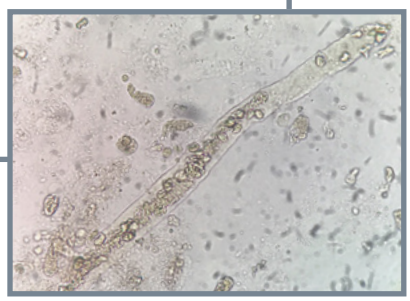


Cast formation; RTECs and cell debris entrapped within the cast matrix

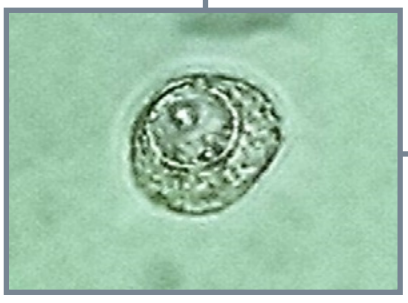
Example of result:
SG: 1.020
pH: 6
Protein: 1+/2+
Glucose: Negative
Ketones: Negative
Bilirubin: Negative
Urobilinogen: Excess
Hemoglobin: 3+
Leukocyte esterase: Negative
Nitrite: Negative



Granular cast



RTEC cast



RTEC

No Erythrocytes

Figure 6.5: TIP Example 4: Hemoglobin

① Tamm-Horsfall protein polimerization and cast formation

Tubular injury (associated to myoglobin) Ex.: rhabdomyolysis

Muscle damage

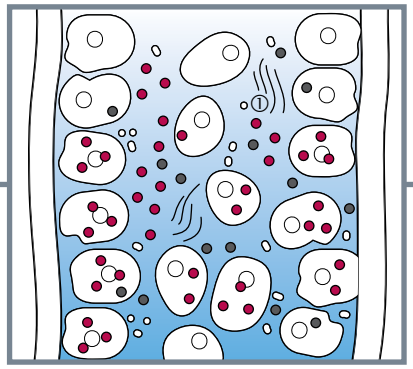
High levels of myoglobin enters the bloodstream

Myoglobin arrives in the kidneys

Myoglobin is filtered by the glomerulus and enters the tubular system

In the tubular system the myoglobin promotes:

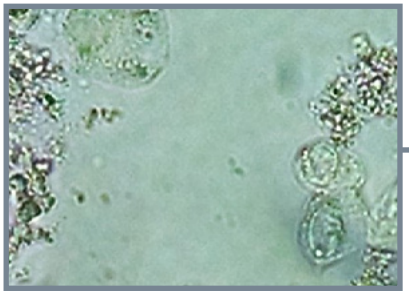
- Ischemia due to renal vasoconstriction
- Direct tubular toxicity
- Tubular damage due to ischemia
- Distal tubular obstruction due to precipitation of THG-myoglobin complex
- Celular cast formation



Cast formation; RTECs and cell debris entrapped within the cast matrix

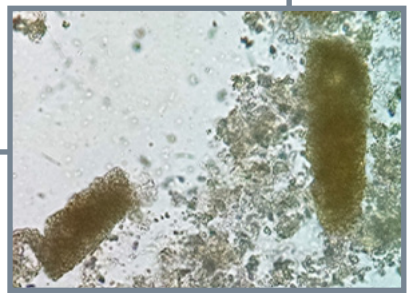
Example of result:

SG: 1.015
pH: 5
Protein: 1+/2+
Glucose: Negative
Ketones: Negative
Bilirubin: Negative
Urobilinogen: Normal
Hemoglobin (Myoglobin): 3+
Leukocyte esterase: Negative
Nitrite: Negative



RTECs

RTEC cast



Granular cast

Figure 6.6: TIP Example 5: Myoglobin

① Tamm-Horsfall protein polimerization and cast formation

Dipstick analysis presents a strong reaction to hemoglobin and urobilinogen, and protein is also usually positive. Despite the strong reaction to hemoglobin, there will be no or very few erythrocytes (red blood cells/RBCs) observed because hemolytic anemia is promoting the destruction of RBCs at vascular level and only the hemoglobin is passing on to the urine.

As with any example of a tubular injury profile, the urinary findings can be linked to loss of kidney function so the information from this test is of clinical interest.

Tubular injury (associated to myoglobin)
Ex.: rhabdomyolysis
Patients with rhabdomyolysis will present large amounts of myoglobin in the blood, caused by the breakdown of the muscle fibers. Myoglobin molecules will be filtered by the glomerulus (usually originating as a dark brown discoloration in the urine) and enter the tubular system. At tubular level, myoglobin promotes cell death, tubular obstruction and cast formation. The microscopic findings of this TIP will be the same as mentioned in the other examples of TIP (RTECs, RTEC casts and granular casts – usually with a brown color).

Dipstick analysis is usually marked by a strong reaction in the hemoglobin area that is also reactive to myoglobin so it will be positive in this case—and by a negative result to urobilinogen. Usually, protein will also be positive. It is important to mention that despite the strong reaction to hemoglobin (in reality myoglobin), no RBCs will be observed because there is no hemoglobinuria or hematuria, but there is myoglobinuria. Also, this patient usually presents with acidic pH and uric acid crystals/urates can be observed, sometimes also in the tubular system forming casts and contributing to tubular obstruction [1 – 9].

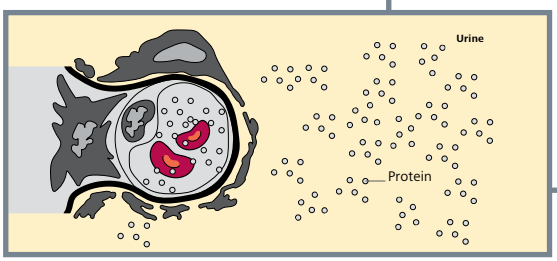
Nephrotic profile (Nephrotic syndrome)
Ex.: Diabetic nephropathy, amyloidosis.
Nephrotic syndrome is the clinical condition that originates in the nephrotic profile. It is characterized by intense loss of protein (albumin) in urine, which causes hypoalbuminemia along with elevated serum cholesterol levels. This particular profile represents cases where the glomerular disease is not caused by an inflammatory injury in the glomerulus (e.g., diabetic nephropathy, amyloidosis) so red and white blood cells are not expected in this example. One of the key findings in the nephrotic profile is lipiduria, due to elevated serum cholesterol levels coupled with intense proteinuria. Proteinuria and lipiduria play an important role in the tubular injury that usually is observed in patients with nephrotic syndrome, including the formation of oval fat bodies, detachment of RTECs and cast formation. The urine sediment of patients with the nephrotic profile is characterized by the presence of lipid droplets (both free, and in oval fat bodies and urinary casts), RTECs and RTEC casts. Less common is the observation of cholesterol crystals, which can also be observed free or in urinary casts. In fact, several types of casts (hyaline, hyaline-granular, granular, waxy and the previously-mentioned RTEC casts) can be observed in the nephrotic profile and cylindruria is a usual observation in this case.

Dipstick analysis provides key information for the identification of nephrotic syndrome: an intense reaction the reactive area to protein, usually 3+ or 4+. The professional performing the microscopic analysis needs to pay close attention to this kind of information and, since the dipstick test is always performed before the microscopic evaluation, the results observed in the dipstick test will help guide the microscopic analysis [1 – 5].

Nephritic profile (Nephritic sediment)
Ex.: acute post-infectious glomerulonephritis, Schönlein-Henoch purpura nephritis, cryoglobulinemic glomerulonephritis
The nephritic profile is observed in patients with acute nephritic syndrome (e.g., acute post-infectious glomerulonephritis). Usually the nephritic profile originates with diseases that are characterized by immune-mediated injury in the glomerular capillaries. WBCs find immune complex deposited in the spaces between the podocytes and the capillary wall, then release their lytic enzymes in an attempt to destroy the immune complex. However, this also destroys the capillary membrane and causes the formation of "holes" in the glomerual capillaries. Protein and RBCs pass through these spaces, entering the urinary system. Depending on the size of the holes RBCs need to "squeeze" past, this causes mechanical deformation to the cell body. When the RBCs enter the tubular system it is exposed to severe osmotic variations. The mechanical deformation associated with the osmotic variations and also the lytic enzymes released by WBCs are the main factors associated with the formation of the dysmorphic RBCs, one of the key findings in the nephritic profile. Usually,

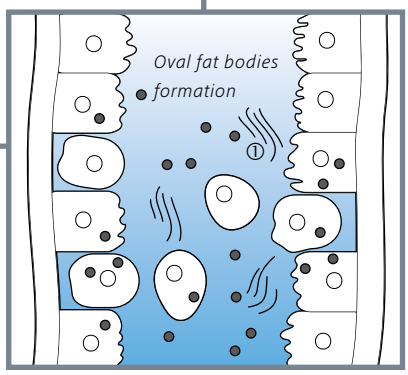
Nephrotic profile (Nephrotic syndrome) Ex.: Diabetic nephropathy, amyloidosis.

Disorders of the filtration barrier in the glomerular capillaries results in proteinuria – if severe: nephrotic syndrome



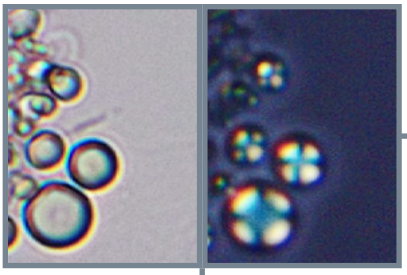
Large amounts of protein passes through the glomerular capillaries

- Severe urinary protein loss leads to hipoalbuminemia and hypercholesterolemia.
- Lipids in excess in blood passes through the urine.
- Formation of oval fat bodies and casts.

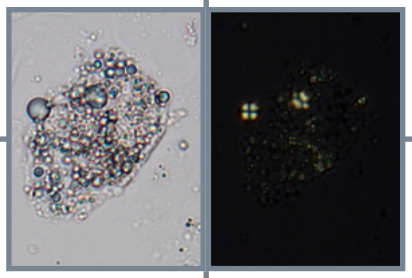


Cast and oval fat bodies formation RTECs with engulfed lipid droplets and lipid droplets entrapped within the cast matrix

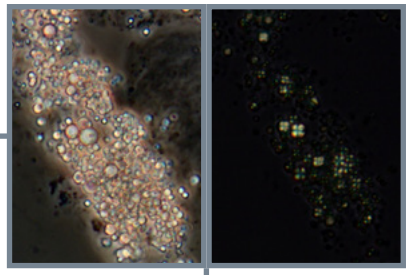
Example of result:
SG: 1.020
pH: 6
Protein: 3+/4+
Glucose: Negative
Ketones: Negative
Bilirubin: Negative
Urobilinogen: Normal
Hemoglobin: Negative
Leukocyte esterase: Negative
Nitrite: Negative



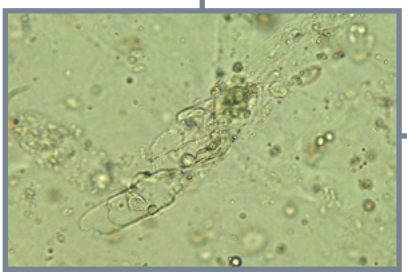
Free Lipid droplets



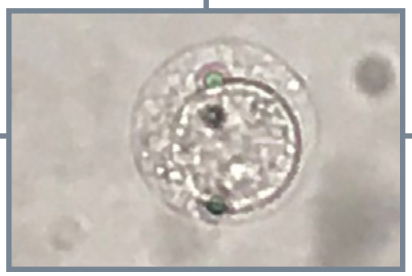
Oval fat body



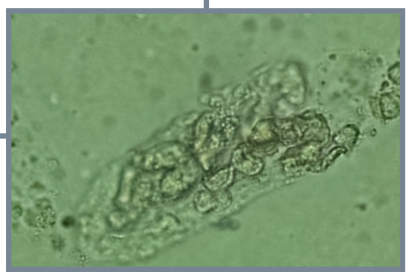
Fatty cast



Cholesterol crystals



RTEC



RTEC cast

Figure 6.7: The Nephrotic Profile

① Tamm-Horsfall protein polimerization and cast formation

Nephritic profile (Nephritic sediment) Ex.: acute post-infectious glomerulonephritis, Schönlein-Henoch purpura nephritis, cryoglobulinemic glomerulonephritis

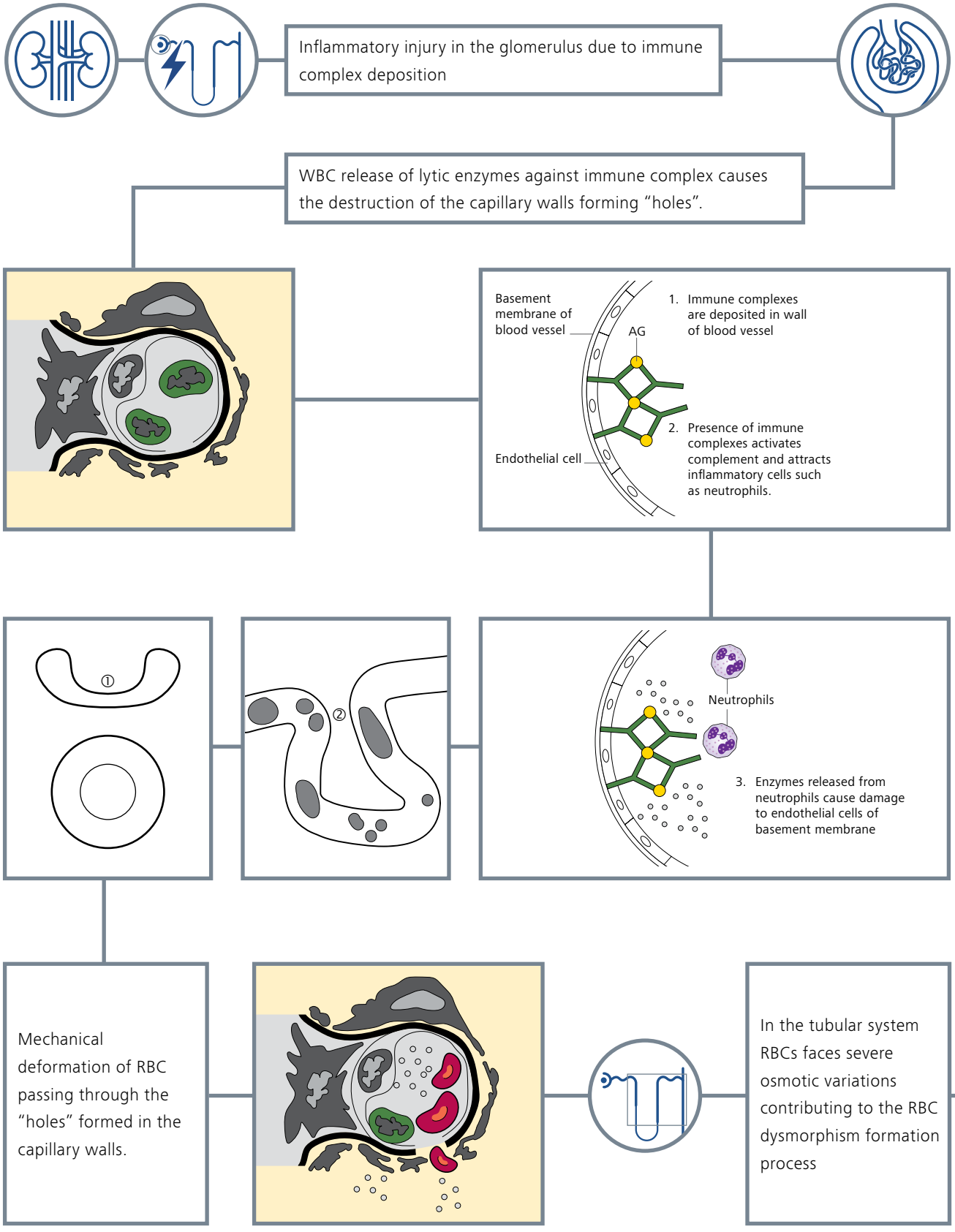


Figure 6.8: The Nephritic Profile (Part 1)

① RBC mechanical deformation ② Holes formed in the capillary walls

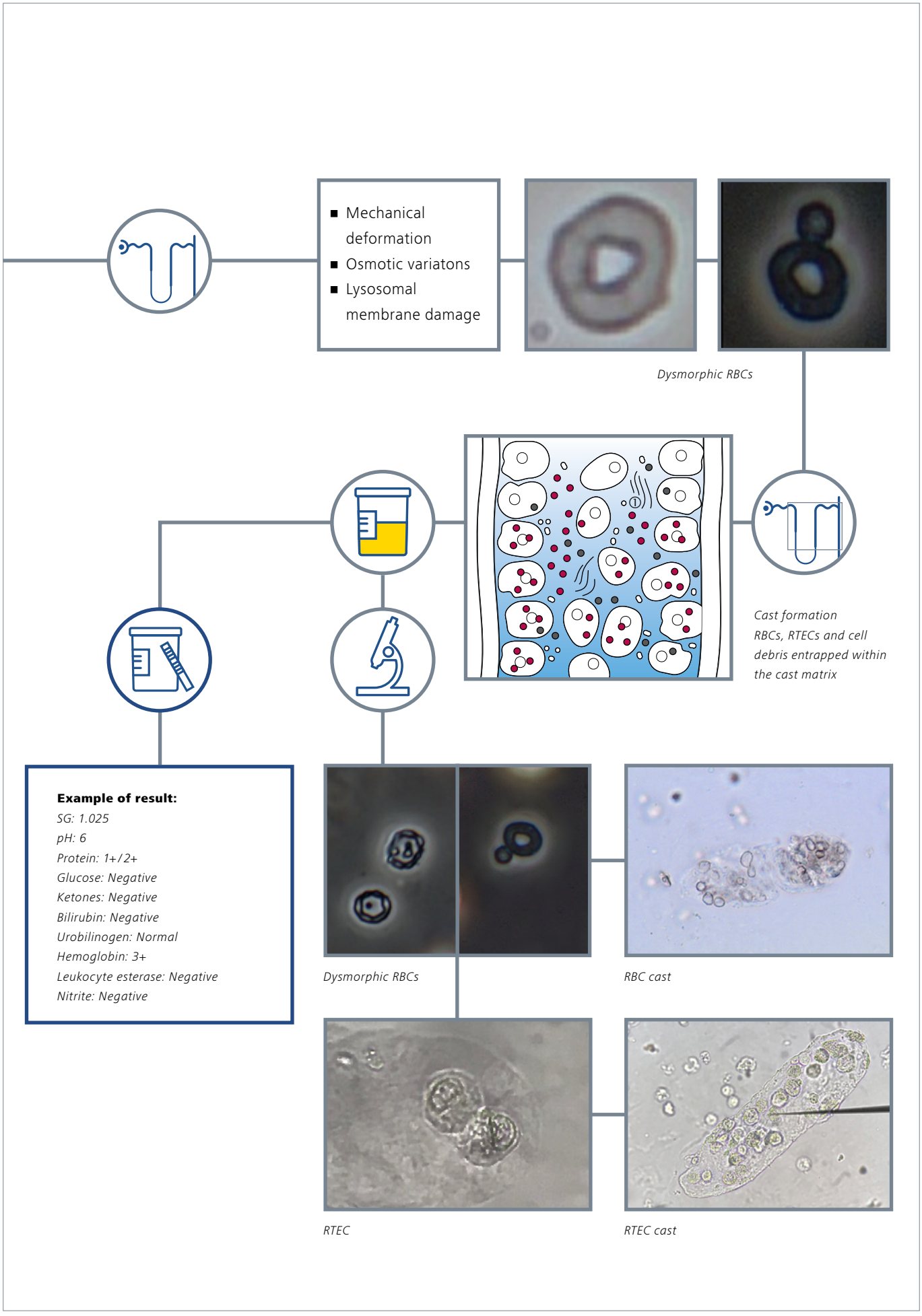


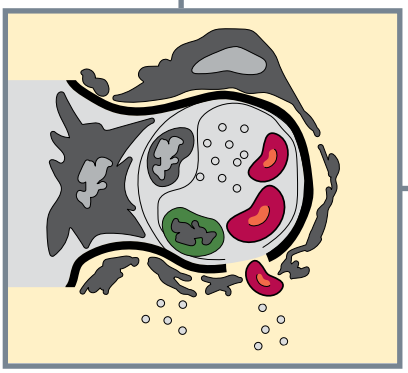
Figure 6.8: The Nephritic Profile (Part 2)

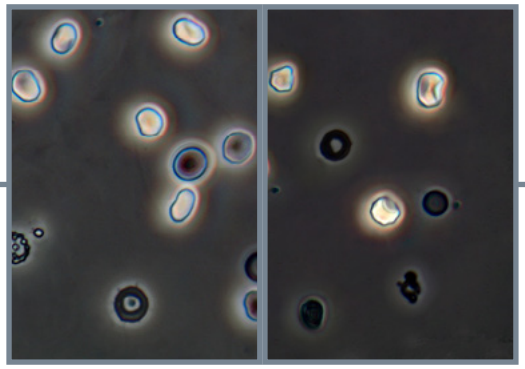
① Tamm-Horsfall protein polimerization and cast formation

Nephrotic and nephritic profile (Proliferative glomerulonephritis)
Ex.: IgA nephropathy, class IV lupus nephritis

For example, IgA deposits activated the immune system and damaged the capillary wall leading RBCs and protein to pass to the urine.

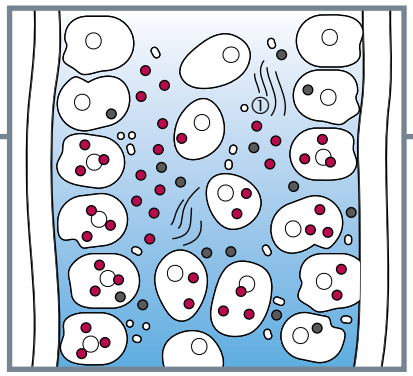
As explained in the nephritic profile RBCs suffers deformation when passing through the "holes" formed in the capillaries and when passing in the tubular system originating dysmorphic RBCs.



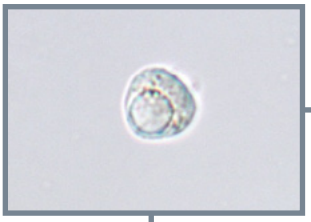


Due to severe protein loss, the same process explained in the nephrotic profile happens leading to hipercholesterolemia and lipiduria

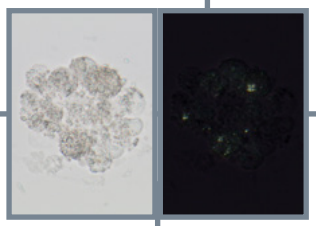
Example of result:
SG: 1.020
pH: 6
Protein: 3+/4+
Glucose: Negative
Ketones: Negative
Bilirubin: Negative
Urobilinogen: Normal
Hemoglobin: 3+
Leukocyte esterase: Traces/1+
Nitrite: Negative



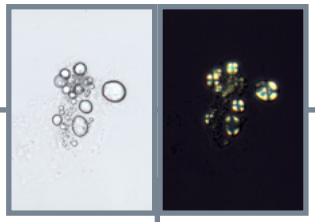
Cast formation; RBCs, lipid droplets, RTECs and cell debris entrapped within the cast matrix



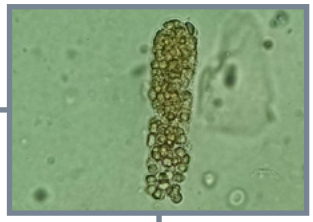
RTEC



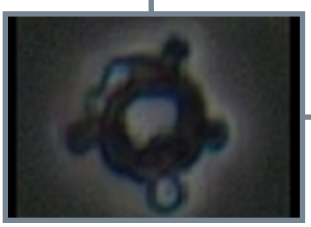
Oval fat body



Free lipid droplets

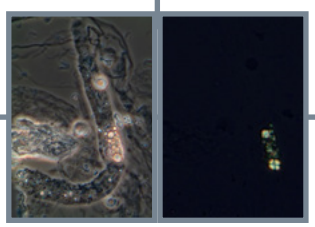


RBC cast

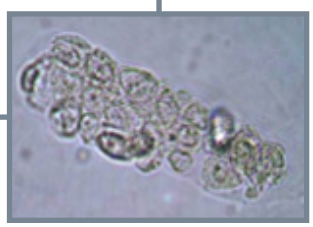


Dysmorpic RBC (Acanthocyte)

Cholesterol crystals



Fatty cast



RTEC cast

Figure 6.9: The Nephrotic and Nephritic Profile

① *Tamm-Horsfall protein polimerization and cast formation*

associated with the glomerular damage, the tubular cells are also injured, detaching from the tubules and contributing to tubular obstruction together with cast formation. The casts that originated in the tubular system can contain the RTECs and also, in this case, the RBCs, originating [from] the RBC cast—another key finding in the nephritic profile. The urine microscopy findings from this profile are hematuria (with dysmorphic RBCs), RBC casts, RTECs and RTEC casts. Other types of casts can also be observed (e.g., hyaline, hyaline-granular, granular and waxy).

The dipstick findings on the nephritic profile usually present a strong reaction to hemoglobin (3+) and a positive reaction to protein (1+/2+). The observation of an intense reaction to hemoglobin and a positive reaction to protein during dipstick analysis guides the professional to search for the findings that indicate the nephritic profile [1–5].

Nephrotic and nephritic profile (Proliferative glomerulonephritis)
Ex.: IgA nephropathy, class IV lupus nephritis

The nephrotic and nephritic profile—basically a mix of the two—is observed in patients with proliferative glomerulonephritis (e.g., IgA nephropathy). The urinary findings are the same as mentioned earlier on the nephrotic and nephritic profiles (hematuria with dysmorphic RBCs, RBC casts, free lipid droplets, oval fat bodies, fatty casts, RTECs, RTEC casts and, less often, cholesterol crystals). Other types of casts are also common (e.g., hyaline, hyaline-granular, granular and waxy).

At dipstick evaluation, these patients usually present with strong reactions both to protein (3+/4+) and hemoglobin (3+) which, as mentioned in the other profiles, is a useful guide in the microscopic analysis.

Important points:

- On both the nephritic profile and the nephrotic/nephritic profile, WBCs can be observed as both free and in urinary casts because they are the immune agents that promote the injury in the glomerular capillaries that lead to the findings of these two profiles. The dipstick test will present a positive reaction to leukocyte esterase when WBCs are observed. Mixed casts (casts containing RTECs, and/or RBCs, and/or WBCs) can also be found in these two profiles.
- Mixed casts tell us two or more pieces of information—for example, a cast containing RBCs and WBCs informs the analyst that the patient has intratubular presence of RBCs and WBCs (hematuria and inflammation) [1–5].

References

[1] Fogazzi, G.B. (2010) The urinary sediment: an integrated view, 3rd edition. San Francisco, Elsevier.

[2] Reilly Jr, R.F. & Perazella, M.A. (2014) Nephrology in 30 days, 2nd edition. New York, McGraw Hill.

[3] Strasinger, S.K. & Schaub DiLorenzo, M. (2008) Urinalysis and body fluids, 5th edition, photography by B. Wang et. al., illustration by S. Bonomelli. Philadelphia, PA, F.A. Davis.

[4] Mundt, L.A. & Shanahan, K. (2011) Graff's textbook of routine urinalysis and body fluids, 2nd edition. Philadelphia, PA, Lippincott Williams & Wilkins.

[5] Brunzel, N.A. (2013) Fundamentals of urine & body fluid analysis, 3rd edition. USA, Saunders.

[6] Poloni, J.A.; Garcia, C.D.; Rotta, L.N. & Perazella, M.A. (2016) Calcium oxalate crystalluria points to primary hyperoxaluria type 1. Kidney Int. 2016 Jan;89(1):250. doi: 10.1016/j.kint.2015.11.001. PMID: 26759051.

[7] Poloni, J.A.T.; Perazella, M.A.; Keitel, E.; Marroni, C.A.; Leite, S.B. & Rotta, L.N. (2019) Utility of a urine sediment score in hyperbilirubinemia/hyperbilirubinuria .. 2019 Clin Nephrol Sep;92(3):141-150. doi: 10.5414/CN109673. PMID: 31198169.

[8] Poloni, J.A.T & Hickmann, F.H. (2020) AKI in a patient with hemolytic anemia. Kidney 360 Jan 2020, 1 (1) 72-73; doi: 10.34067/KID.0000082019.

[9] Tesser Poloni, J.A. & Perazella, M.A. (2017) A rarely recognized cause of acute kidney injury in rhabdomyolysis (2018) Am J Med Sci. 2018 Sep;356(3):e27. doi: 10.1016/j.amjms.2017.03.028. Epub 2017 Mar 22. PMID: 30049408.

Notes

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