

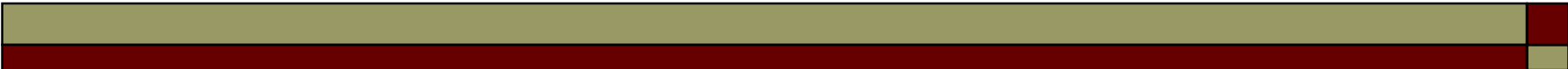
Uriini sademe asemel

Karel Tomberg

SA Põhja-Eesti Regionaalhaigla labor

ELMÜ XXII Suvekool

27. august 2021 Laulasmaa



Millest juttu tuleb

- Uriini sademe mikroskoopia
- Automatiseeritud digitaalne mikroskoopia
- Meetodite võrdlus

Uriinianalüüs

- Ülduuring mitmete haiguste, s.h. kuseteede infektsioonid, neeruhaigus, metaboolsed ja süsteemsed haigused korral

Koosneb 2 komponendist:

- Ribaanalüüs (füsiko-keemiline uuring)
- Uriini sademe analüüs
 - Kamberloendus (Bürker, Fuchs-Rosenthal, KOVA; faaskontrast- või värvinguga valgusmikroskoopia)
 - Vaateväljas loendus (supravitaalvärving, valgusmikroskoopia)

Eelised ja puudused

Uuring	Eelised	Puudused
Uriini ribaanalüüs	Lihtne teha, odav	Riba vale värvumus (niiskus, temp) Interferentsid
Uriini sademe uuring	Info spetsiifiliste rakkude ja partiklite kohta, hõlbustab interpreteerimist	Aeganõudev Töömahukas Suur vaatajate vaheline variatsioon

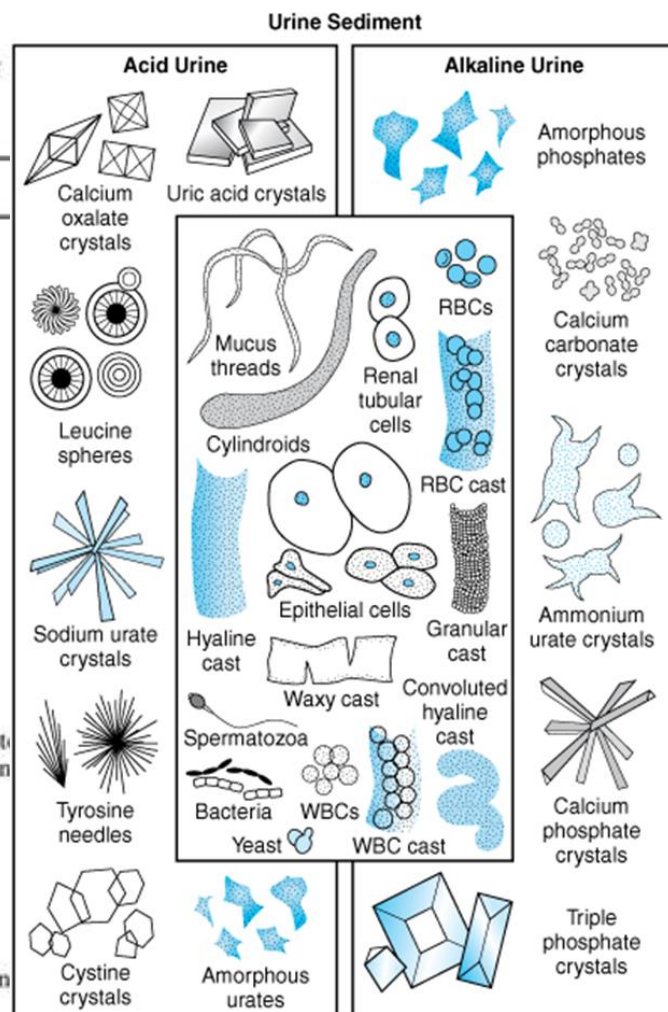
Partiklite/ sademe uuringu tähtsus

Partikkel	Kliinilised seosed
WBC	UTI, glomerulonefriit, interstitsiaalne nefriit, aseptiline tsüstiit
RBC	Hematuuria (düsmorfsed erütrotsüüdid: neeru päritolu)
Lameepiteel	Halva proovivõtu marker, eriti raseduse ajal
Transitoorne epiteel	UTI, uroloogilised haigused: neeruvaagnast põieni
Neeru tuubulusepiteel	Äge tubulaarne nekroos, äge interstitsiaalne nefriit, neeru äratõukereaktsioon, aktiivne proliferatiivne glomerulonefriit, nefrootiline sündroom, Fabry haigus
Silindrid (v.a. hüaliinsilindrid)	Neeruhaigus (spetsiifiline, mitte tundlik)
Kristallid	Enamikel juhtudel pole kliiniliselt oluline; retsidiveeruv neerukivitõbi

European Urinalysis Guidelines

TABLE VI. Levels of microscopy differentiation in clinical urinalysis.

Basic level	Advanced level in addition
Red blood cells (RBC)	<i>Detailed subclasses erythrocytes:</i> dysmorphic erythrocytes
White blood cells (WBC)/Granulocytes	<i>Differentiation of leukocytes:</i> Granulocytes, lymphocytes, macrophages (monocytes and eosinophils)
<i>Epithelial cells:</i>	<i>From non-squamous epithelial cells:</i>
Squamous epithelial cells	Squamous epithelial cells
Non-squamous = small epithelial cells	Renal tubular epithelial cells
	Transitional epithelial cells (superficial and deep)
	Intestinal epithelial cells (occurring after bladder surgery)
	Atypical cells (experienced cytopathologist)
<i>Casts:</i>	<i>From non-hyaline casts:</i>
Hyaline casts	Erythrocyte, granulocyte casts
Non-hyaline casts	Renal tubular cell casts
	Hyaline, granular, waxy, fatty casts
	Bacteria and yeast-containing casts
	Haemoglobin and myoglobin casts
	Bilirubin casts
Bacteria	Gram-staining characteristics of bacteria (microbiology laboratory)
Yeasts, Trichomonas	<i>Schistosoma haematobium</i> (in appropriate geographical location)
Spermatozoa	Spermatozoa
Artefacts (hair, paper and textile fibres, starch, glass) and mucus	Artefacts and mucus as on the left
<i>Lipids:</i>	<i>Lipids, in addition to droplets:</i>
Droplets (isolated and aggregated)	Oval fat bodies (lipid-laden tubular cells), cholesterol crystals
<i>Crystals:</i>	<i>Additional rare crystals:</i>
Urate, oxalate (mono- and dihydrated), phosphate and cystine	Drugs, cystine, leucine, tyrosine, 2,8-dihydroxyadenine, xanthine



Uriini sademe mikroskoopia metoodika

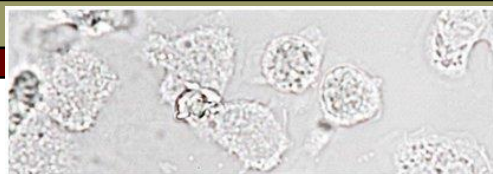
- ❑ Tsentrifuugi 10 ml segatud uriini 5 min (1600 p/min, kesktõmbejõud 400 g) ümarapõhjalises gradueeritud katsutis
- ❑ Aspireeri 9,5 ml supernatanti (sademeks 0,5 ml).
- ❑ Sademele lisa 50-60 µl Sternheimeri supravitaalvärvi (Alcian Blue, Pyron B).
- ❑ 5 minuti pärast võta hästi segatud värvitud sademest 20 µL (13 µL) suurune tilk alusklaasile ja kata 22x22 mm (18x18 mm) suuruse katteklaasiga. Väldi õhumullide teket või uriini laialivalgumist.

Koivula T, et al. Basic urinalysis and urine culture. Finnish recommendations from the working group on clean midstream specimens. Scan J Clin Lab Invest 1990;50(Suppl.200):26-33.

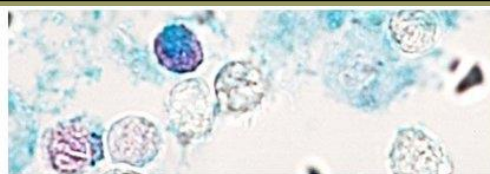
Uriini sademe mikroskoopia metoodika (2)

- Alusta mikroskopeerimist 10x suurendusega – keskendu silindritele, atüüpilistele rakkudele või teistele haruldastele leidudele.
- Täpsem hindamine toimub 40x suurendusel. Loe 10 vaatevälja, tulemused esita ühe keskmise arvuna vaateväljade kohta.
- Referentsväärtused: Leukotsüüdid 3-4 rakku/vv, Erütrotsüüdid 1-2 rakku/vv, Lameepiteel – üksikud, Hüaliinsilindrid - üksikud

Koivula T, et al. Basic urinalysis and urine culture. Finnish recommendations from the working group on clean midstream specimens. Scan J Clin Lab Invest 1990;50(Suppl.200):26-33.



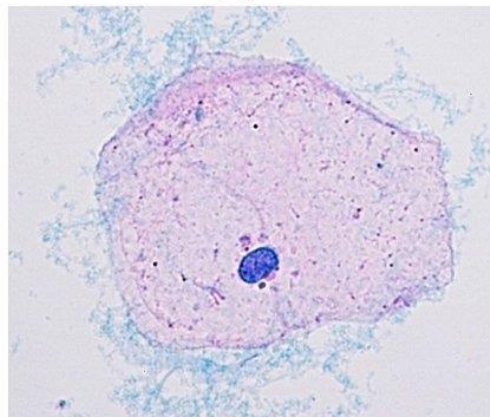
1-U



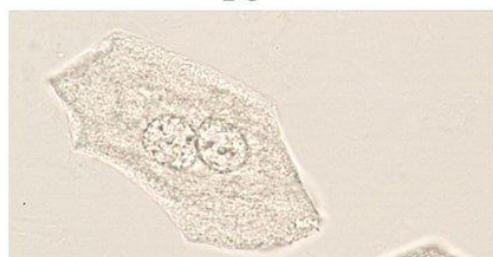
1-S



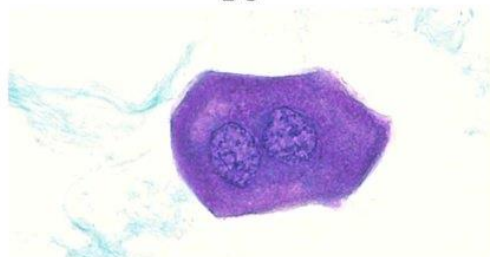
2-U



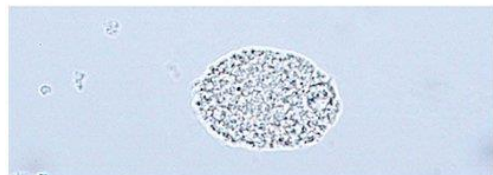
2-S



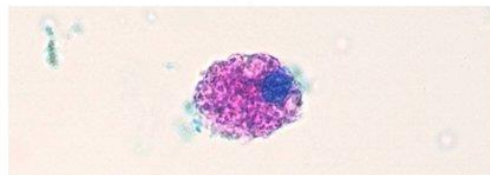
3-U



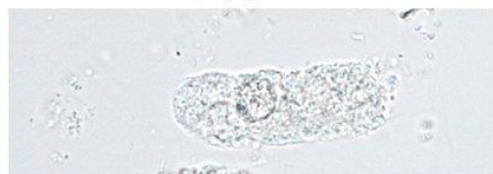
3-S



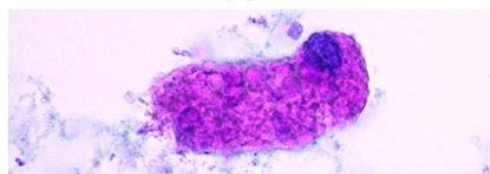
4-U



4-S

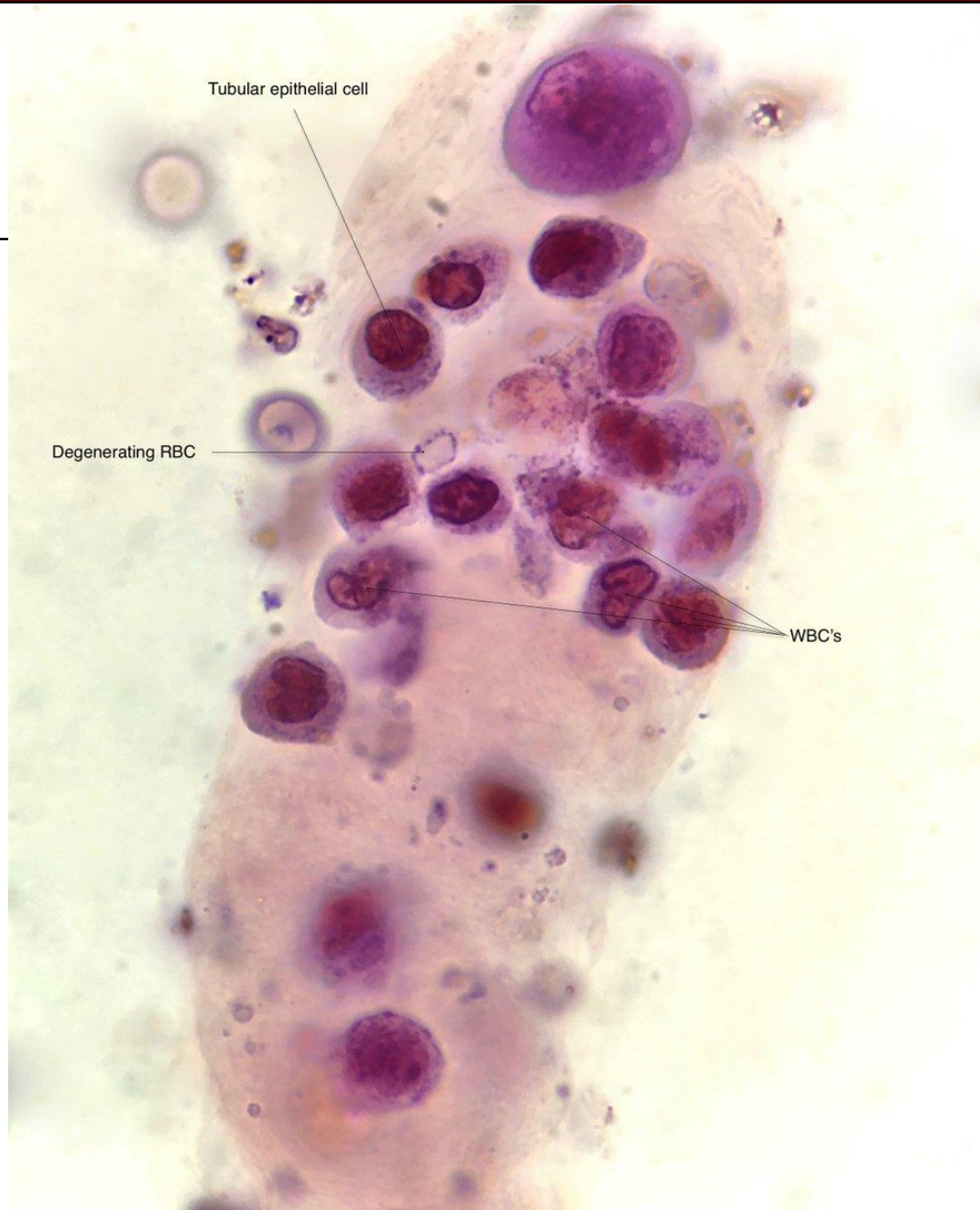


5-U



5-S

50 μ m



Tubular epithelial cell

Degenerating RBC

WBC's

Digitaalmikroskoopia süsteemid

**Cobas u701 (cobas 6500,
Roche Diagnostics)**

**UAS800 (Atellica 1500,
Siemens Healthineers)**



Digitaalmikroskoopia süsteemid (2)

Iris iQ®200SPRINT
(Beckman Coulter)



URiSCAN® PlusScope (YD;
Korea Vabariik)



Digitaalmikroskoopia süsteemid (3)

UriSed 3 PRO (77

Elektronica, Hungary)



Cobas u 701 süsteem (Roche Diagnostics)

- Analüüsib ja loendab uriini rakke sademest, mis on tsentrifuugitud spetsiaalselt disainitud cobas u küvettides
- 200 µl uriini kantakse küvetti
- Tsentrifuugitakse kesktõmbejõuga 260 g 10 sek
- Sisseehitatud kaamera teeb sademest 15 digitaalset pilti erinevatest küveti osadest (≈ 400 x suurendus)
- (kõik osakesed 15-l pildil on ca 2,3 µl-s uriinis)
- Pilte hindab Auto Image Evaluation Module (AIEM) tarkvara (Roche Diagnostics)

cobas 6500 parametrid

cobas 6500 test parameters

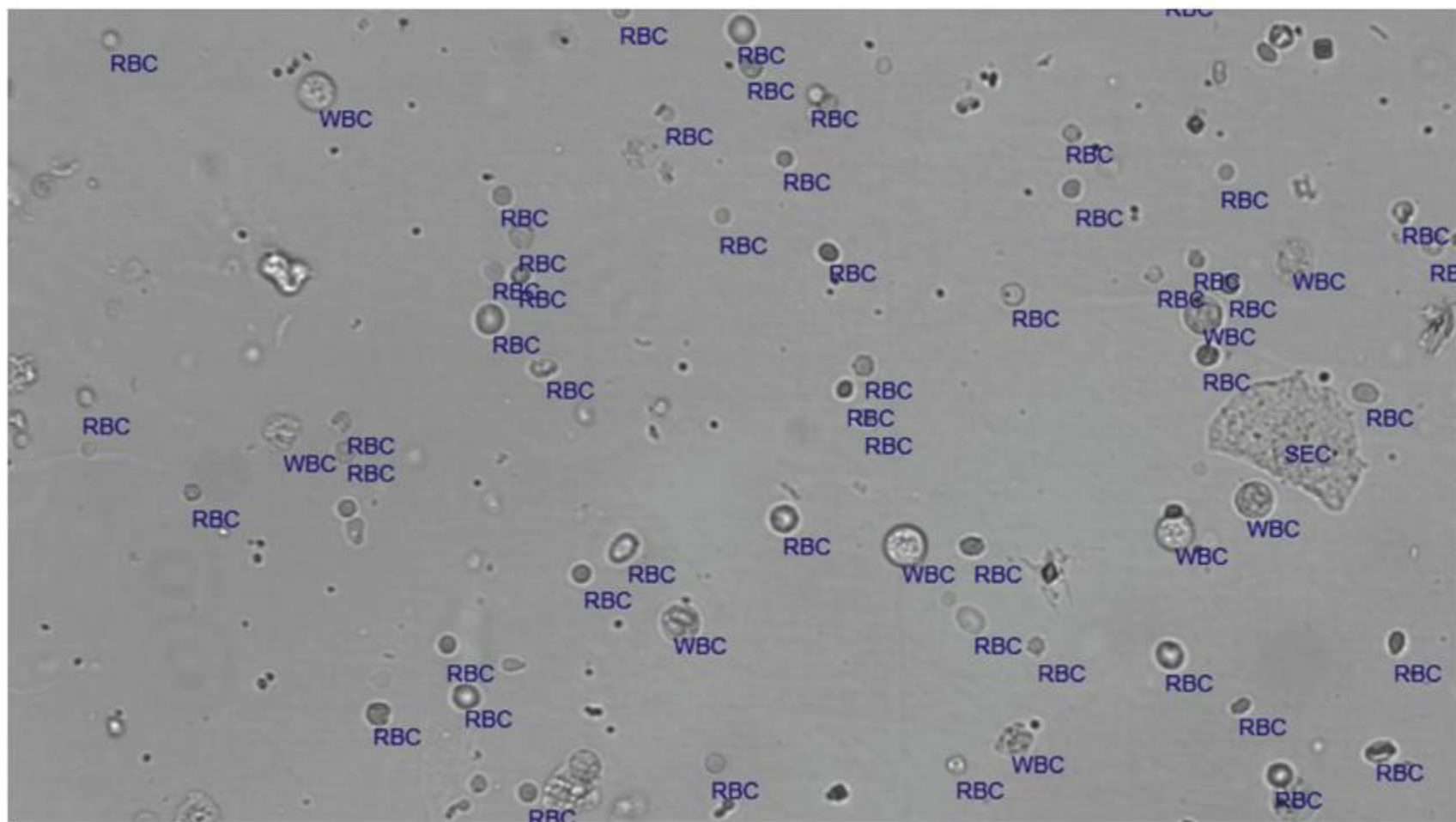
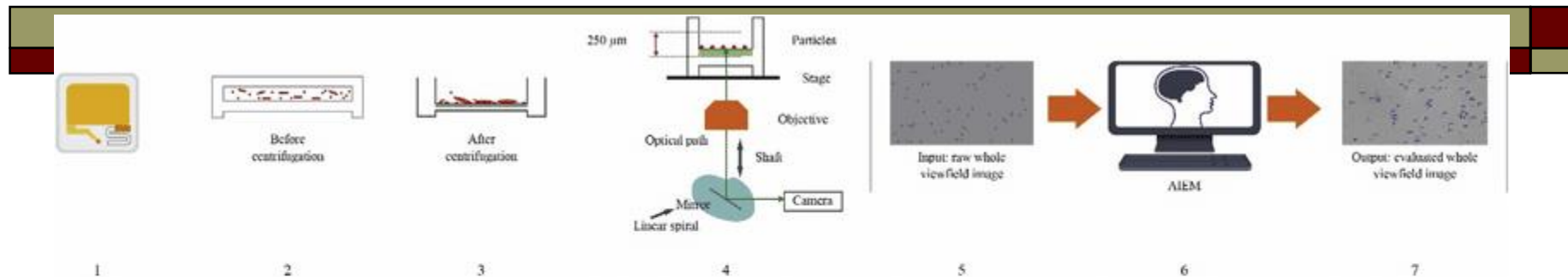
cobas u 601

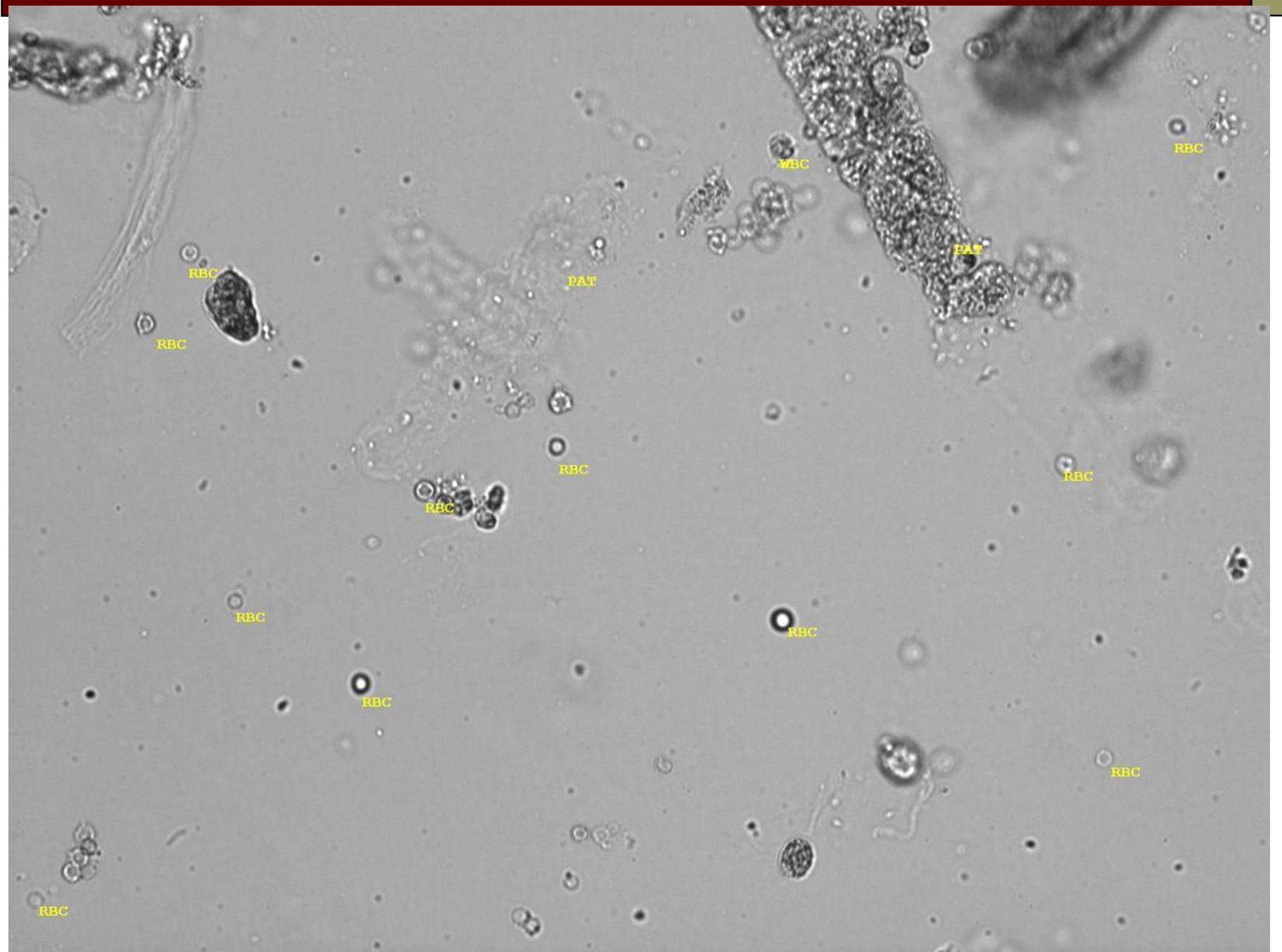
ERY	Erythrocytes and hemoglobin
LEU	Leukocytes
NIT	Nitrite
PRO	Protein
GLU	Glucose
KET	Ketones
UBG	Urobilinogen
BIL	Bilirubin
pH	pH
SG	Specific gravity
COL	Color
CLA	Clarity

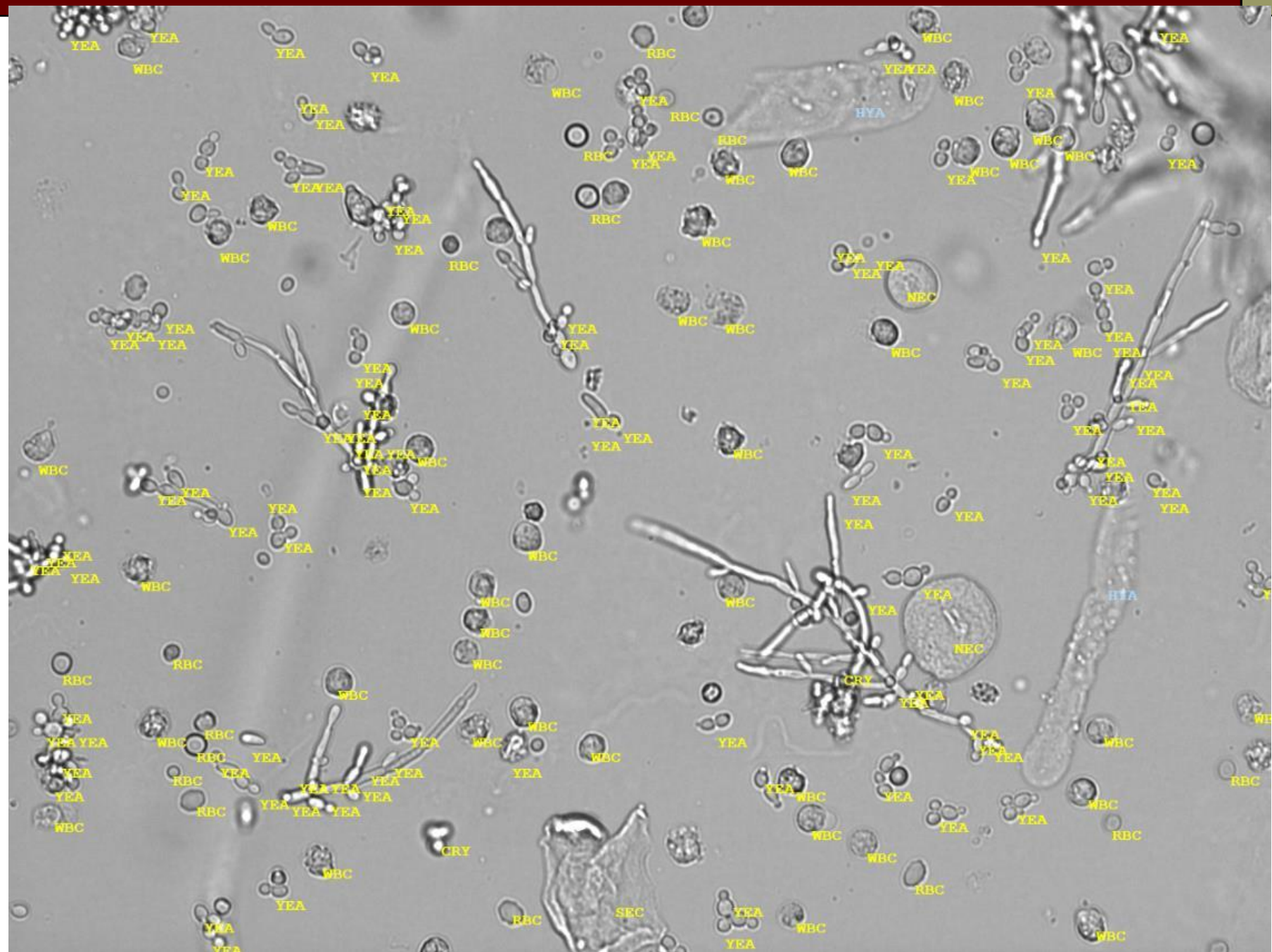
cobas u 701

RBC	Red blood cells
WBC	White blood cells
BAC	Bacteria
SEC	Squamous epithelial cells
HYA	Hyaline casts
NEC	Non-squamous epithelial cells
PAT	Pathological casts
CRY	Crystals
YEA	Yeast
MUC	Mucus
SPRM	Sperm

Quantitative	Semi-quantitative	Qualitative
--------------	-------------------	-------------







Manuaalse ja digimikroskoopia metoodika võrdlus

	Manuaalne mikroskoopia	Cobas u701
Uriini algkogus	10 ml	200 µl küvetis
Tsentrifugimise kiirus	400 g	260 g
Tsentrifugimise aeg	5 min	10 sek
Lõppkogus	500 µl (kontsentratsioonifaktor 20)	
Uuritav kogus	20 µl (13 µl) olenevalt katteklaasist	Ca 2,3 µl
Suurendus	400 x	400 x
Uuritavate vaateväljade arv	10 (5, kui WBC, RBC >10/VV)	15
Hinnang	Visuaalne	Tarkvara (AIEM)

Referentsväärtused

Parameeter	Manuaalne mikroskoopia (rakku/HPF) Koivula T, et al, 1990	Cobas u701 (rakku/HPF) 97,5%
WBC (naised, mehed)	3-4	<1,4 (n = 199/ 196)
RBC (naised)	1-2	<1,2 (n = 199)
RBC (mehed)	1-2	<0,8 (n = 196)

$$\begin{aligned} \text{p/HPF} \times 4,4 &= \text{p}/\mu\text{L} \\ \text{p}/\mu\text{L} \times 0,227 &= \text{p/HPF} \end{aligned}$$

Mis läks paremaks

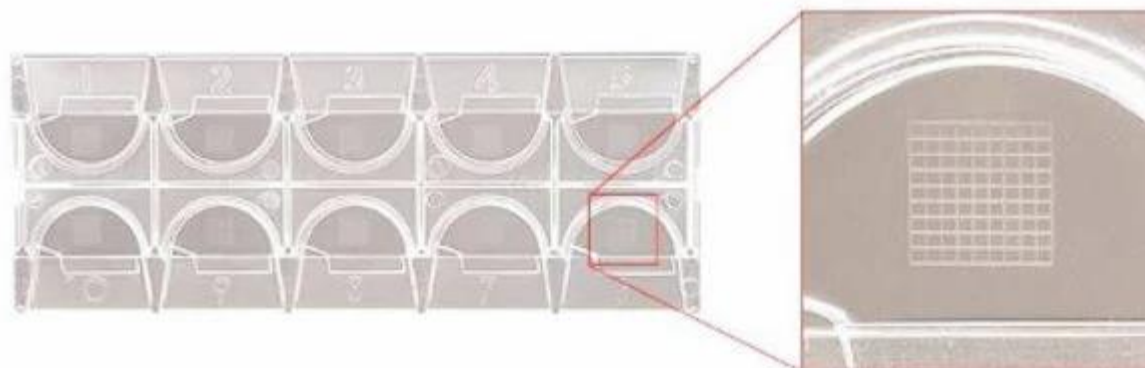
- ❑ Ei ole uriinide kogumist, manuaalset tsentrifuugimist, supernatandi eraldamist, värvimist
- ❑ Ribatesti tulemuste automaatne kontroll, proovid liiguvad reeglite alusel mikroskoopiale
- ❑ Täpsem – 15 pildi keskmine
- ❑ Sademel eraldi kalibratsioon ja kontrollid kahel tasemel
- ❑ Ekraanipilt on hea õppematerjal praktikantidele
- ❑ Väga mugav, kui tööd palju

Probleemsed kohad

- Kui uriin on punane või mädane – ei loe rakke, ei saa vastust väljastada (vastusele – „hemorraagiline uriin“, „mädane uriin“)
- Ei toimu neeru tuubulusepiteeli eristamist kuseteede transitoorsest epiteelist (parameeter: *non-squamous epithelial cells*, NEC)

Uriini sademe analüsaatorite võrdlus

- ❑ Cho J, et al võrdlesid 5 sademe analüsaatorit manuaalse mikroskoopiaga (KOVA kamer)
- ❑ UF-5000 (Sysmex), Cobas u 701 (Roche), UAS800 (Siemens), Iris iQ200SPRINT (Beckman Coulter), URiSCAN PlusScope (YD)
- ❑ 2 kogenud laboranti (CV RBC 3,7%, CV WBC 3,6%)
- ❑ N = 1016

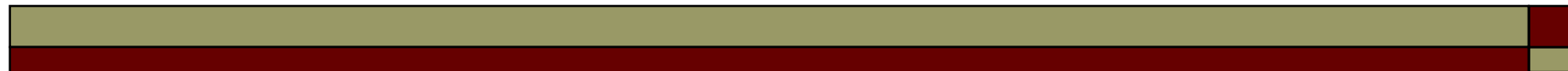


Võrdlusuuringu tulemused

- Proportsionaalne nihe RBC, WBC ja SEC osas oli $<\pm 20\%$ manuaalse mikroskoopia tulemustest, v.a. URiSCAN PlusScope (RBC) ja Iris iQ200SPRINT (WBC)
- Roche Cobas u701 ja Siemens UAS800 tundlikkus patoloogiliste silindrite suhtes oli hea (73,6% ja 81,1%), kristallide suhtes 62,2% ja 49,5%
- Roche Cobas u701 ja Siemens UAS800 andsid valepositiivseid tulemusi bakterite suhtes, Sysmex UF-500 selles suhtes parim
- *Sysmex UF-500-l* saab kristallide ja silindrite otsusepiire muuta, koos sellega ka ülevaatamise osakaalu

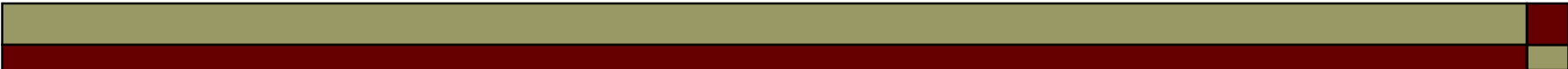
	Parameter	Agreement	Sensitivity	Specificity	PPV	NPV	Kappa (95% CI)		
	Crystal (n = 111)								
	UF-5000 ^a								
	Overall	91.7%	29.7%	99.8%	94.4%	91.6%	0.42 (0.37–0.47)		
	With the exception of amorphous crystal	96.0%	46.8%	99.8%	93.8%	96.0%	0.59 (0.53–0.65)		
	Cobas® u 701	91.2%	62.2%	95.1%	61.7%	95.0%	0.57 (0.52–0.61)		
	UAS800	92.3%	49.5%	97.9%	75.0%	93.7%	0.55 (0.51–0.60)		
	Iris iQ®200SPRINT	87.9%	68.5%	90.4%	47.9%	95.7%	0.49 (0.46–0.53)		
	URiSCAN® PlusScope	87.7%	18.0%	96.7%	41.2%	90.2%	0.20 (0.15–0.24)		
	Hyaline cast (n = 26)								
	UF-5000 ^b	95.3%	23.1%	97.2%	17.6%	98.0%	0.18 (0.11–0.25)		
	Cobas® u 701	92.3%	19.2%	94.2%	8.1%	97.8%	0.08 (0.03–0.13)		
	UAS800	86.0%	65.4%	86.6%	11.3%	99.0%	0.16 (0.12–0.19)		
	Iris iQ®200SPRINT	96.4%	3.8%	98.8%	7.7%	97.5%	0.04 (–0.02 to 0.09)		
	URiSCAN® PlusScope	96.4%	3.8%	98.8%	7.7%	97.5%	0.04 (–0.02 to 0.09)		
	Pathologic cast (n = 53)								
	UF-5000	92.0%	23.6%	95.9%	25.0%	95.6%	0.20 (0.14–0.26)		
	Cobas® u 701	89.4%	73.6%	90.2%	30.4%	98.4%	0.38 (0.34–0.43)		
	UAS800	84.6%	81.1%	84.8%	23.6%	98.8%	0.31 (0.27–0.35)		
	Iris iQ®200SPRINT	94.1%	0.0%	99.5%	0.0%	94.6%	–		
	URiSCAN® PlusScope	94.6%	0.0%	–	0.0%	–	–		
	Non-squamous epithelial cell (n = 211)								
	UF-5000	86.6%	44.4%	88.2%	12.1%	97.7%	0.14 (0.10–0.18)		
	Cobas® u 701	80.1%	44.4%	81.4%	8.1%	97.6%	0.08 (0.05–0.11)		
	UAS800	94.1%	19.4%	96.8%	18.4%	97.0%	0.16 (0.10–0.22)		
	Iris iQ®200SPRINT	96.0%	8.3%	99.2%	27.3%	96.7%	0.11 (0.05–0.18)		
	URiSCAN® PlusScope ^c	–	–	–	–	–	–		
	Yeast (n = 36)								
	UF-5000	95.3%	44.4%	97.1%	36.4%	97.9%	0.38 (0.31–0.45)		
	Cobas® u 701	94.4%	58.3%	95.7%	33.3%	98.4%	0.39 (0.33–0.46)		
	UAS800	94.9%	50.0%	96.5%	34.6%	98.1%	0.38 (0.32–0.45)		
	Iris iQ®200SPRINT	91.8%	33.3%	94.0%	16.9%	97.5%	0.19 (0.13–0.24)		
	URiSCAN® PlusScope	96.7%	25.0%	99.3%	56.3%	97.3%	0.33 (0.25–0.42)		
	Sperm (n = 5)								
	UF-5000	99.6%	20.0%	100.0%	100.0%	99.6%	0.33 (0.09–0.58)		
	Cobas® u 701	96.3%	100.0%	96.2%	11.6%	100.0%	–		
	UAS800	97.9%	0.0%	98.4%	0.0%	99.5%	–		
	Iris iQ®200SPRINT	99.5%	0.0%	–	0.0%	–	–		
	URiSCAN® PlusScope	99.5%	0.0%	–	0.0%	–	–		
	Mucous thread (n = 145)								
	UF-5000	88.8%	39.3%	97.0%	68.7%	90.6%	0.44 (0.40–0.49)		
	Cobas® u 701	89.6%	89.7%	89.6%	58.8%	98.1%	0.65 (0.62–0.68)		
	UAS800	91.7%	81.4%	93.5%	67.4%	96.8%	0.69 (0.66–0.72)		
	Iris iQ®200SPRINT	88.3%	75.2%	90.5%	56.8%	95.6%	0.58 (0.54–0.61)		
	URiSCAN® PlusScope	92.5%	70.3%	96.2%	75.6%	95.1%	0.69 (0.65–0.72)		

PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval. ^aThe UF-5000 dissolves amorphous crystals using a special reagent, so amorphous urate and amorphous phosphate could not be detected. ^bThe UF-5000 reports total casts instead of hyaline casts. ^cThe URiSCAN® PlusScope reports the number of casts instead of the percentage of casts.



	Pathologic cast		
	Cellular (n=8)	Granular (n=45)	Total (n=53)
UF-5000	12.5%	26.7%	24.5%
Cobas [®] u 701	50.0%	77.8%	73.6%
UAS800	87.5%	80.0%	81.1%
Iris iQ [®] 200SPRINT	0.0%	0.0%	0.0%
URiSCAN [®] PlusScope	0.0%	0.0%	0.0%

Cho, Jooyoung, Oh, Kyeong Jin, Jeon, Beom Chan, Lee, Sang-Guk and Kim, Jeong-Ho. "Comparison of five automated urine sediment analyzers with manual microscopy for accurate identification of urine sediment" Clinical Chemistry and Laboratory Medicine (CCLM), vol. 57, no. 11, 2019, pp. 1744-1753. <https://doi.org/10.1515/cclm-2019-0211>



Uuringu järelused

- Uriini sademeanalüsaatoritelt loodetakse käsitöö vähendamist koos usaldusväärsete tulemustega
- Igal analüsaatoril on oma erijooned
- Neid tuleb arvesse võtta kliiniliste algoritmide ja labori tööprotsesside juures