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# Verification of UriSed 3 PRO automated urine microscope in regional laboratory environment

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ABSTRACT

*Background and aims:* Ten UriSed 3 PRO automated microscopes (77 Elektronika, Hungary) were verified for nine HUSLAB laboratories with 160 000 annual urine samples.
*Materials and methods:* Particle counting of the primary UriSed 3 PRO instrument (77 Elektronika, Hungary) was verified against reference visual microscopy with 463 urine specimens, and against urine culture on chromogenic agar plates with parallel 396 specimens. Nine secondary instruments were compared pairwise with the primary instrument.
*Results:* Relative imprecisions compared to Poisson distribution, R(CV), were estimated to be 1.0 for white blood cell (WBC) and 1.5 for red blood cell (RBC) counts, respectively. Spearman's correlations against visual microscopy were $r_S = 0.94$ for WBC, $r_S = 0.87$ for RBC, and $r_S = 0.82$ for squamous epithelial cell (SEC) counts. Agreement with visual microscopy (Cohen's weighted kappa) was 0.94 for WBC, 0.89 for RBC, 0.88 for SEC, 0.59 for combined casts, and 0.49 for non-squamous epithelial cells (NEC). Bacteria were detected with a sensitivity of 90% and specificity of 39 against culture at $10^7$ CFB/L ($10^4$ CFU/mL). Created flagging limits allowed automated reporting for 70–75% of patient results.
*Conclusions:* UriSed 3 PRO instruments were adopted into routine use after acceptance of the verification.

## 1. Introduction

Automation of urine particle counting has become a clinical standard in medium-sized and large laboratories by means of flow cytometry [1,2,3] or several types of image analyzers [4,5,6,7,8]. Reliable implementation of those instruments requires utilization of reference procedures in their verification [9,10].

After a public tender, we have verified the performance of a new model of UriSed (also sediMAX) automated microscope called UriSed 3 PRO (also sediMAX conTRUST PRO), launched in 2018, that takes phase-contrast images on urine particles [11], by using urine samples from our routine of mixed patient populations. The primary ("Gold") UriSed 3 PRO instrument (77 Elektronika, Budapest, Hungary, represented by Mediq Ltd in Finland) was verified against reference visual microscopy at the Department of Clinical Chemistry, and against bacterial culture at the Department of Clinical Microbiology in HUSLAB. After the Gold instrument, additional 9 secondary ("Silver") instruments were verified against the first one, and installed for urinalysis automation in Helsinki and Uusimaa Hospital District, and in Kymenlaakso District in Southern Finland, to produce about 160 000 urine particle counts annually. The performance of UriSed 3 PRO in ruling out negative urine cultures was investigated in a separate study.

*Abbreviations:* BAC, bacteria; CFB, colony-forming bacteria; CFU, colony-forming units; UTI, urinary tract infection; RBC, red blood cells; WBC, white blood cells; NEC, non-squamous epithelial cells; SEC, squamous epithelial cells; HYA, hyaline casts; PAT, pathological casts; CV, coefficient of variation; R(CV), relative coefficient of variation, ratio of observed/theoretical coefficients of variation; U, expanded uncertainty, with a coverage factor = 2; $u_c$, combined uncertainty.

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## 2. Materials and methods

### 2.1. Urine samples

The primary verification was carried out at the Meilahti central laboratory receiving about 200 urine samples for particle counting (Department of Clinical Chemistry), and about 500 samples for urine bacterial culture (Department of Clinical Microbiology) daily, with independent requests for each test. The samples derived from mixed patient populations representing inpatients from Meilahti hospital campus (about 1/3), and outpatients from regional ambulatory units or from homes (2/3 of samples). HUSLAB provided guidance for specimen collection for women, men and children in six languages on paper, and educational videos in three languages. Two parallel sample tubes from simultaneous collection, one for particle counting and the other for bacterial culture were preserved immediately, by using 10 ml C&S boric acid, formate and borate preservative tubes (BD Preanalytical Solutions, Eysins, Switzerland, cat no 364955) for particles and bacterial cultures. The shipment to the central facility was organized at room temperature in temperature-logged boxes together with contracted courier vans. Both mid-stream collections (generally 90% of all specimens) and catheterized specimens were taken into the study, based on particle findings. No bladder punctures or specific urological samples were included based on information on sample label or type of container used. Particle analysis was carried out within 8 h from sample collection [12]. A total of 492 specimens were chosen from routine analysis by UF-1000i instrument (Sysmex, Kobe, Japan) for verification of the primary ("Gold") UriSed 3 PRO instrument against reference visual microscopy during a 6-week period in September - October 2018. After collection of consecutive positive and negative cases for any particle type, an effort was made to enrich samples positive for specific rare particles to maximize comparisons. Out of the 492 original specimens, 463 specimens (94%) provided quantitative results for most particle types both with UriSed 3 PRO and visual microscopy. Samples with a lacking result from either automated or visual microscopy were not compared. Seven samples exceeding the limit of quantitation by UriSed 3 PRO for WBC counts and 6 samples those for RBC counts were then also ignored, resulting in 456 WBC and 457 RBC comparisons.

Verification of the 9 "Silver" or secondary instruments was carried out sequentially at the central Meilahti facility against the "Gold" instrument, analyzing similarly consecutively obtained samples with added samples with rare particles until considered sufficient to verify identical measurement principle with the primary instrument in terms of technical performance, flags, precision and quantities of reported results. Results from these secondary verifications are not presented in this report.

### 2.2. Automated microscopy with UriSed 3 PRO

The automated UriSed 3 PRO has already been described before [11]. Phase-contrast and bright-field images were obtained with a digital automated camera from 2.2 μL of urine after a short centrifugation to improve focusing and classified then by using neural network-based artificial intelligence. During the initial technical installation and training of the primary = "Gold" instrument, the installation team discussed the detail with representatives of the manufacturer and local vendor to ensure proper performance and to fix some installation problems. Classification of particles included white blood cells (WBC), red blood cells (RBC), bacteria (BAC), squamous epithelial cells (SEC), non-squamous epithelial cells (NEC), casts (CAST, combining hyaline, HYA, and pathological, PAT casts), yeast (YEA), mucus (MUC) and amorphous (AMO) precipitate, and crystals (CRY, and CaOxM, calcium oxalate monohydrate). Out of these, the manufacturer declares quantitative counts for WBC and RBC only. Clinical report included WBC, RBC, BAC, SEC, NEC, and CAST, while the other classes were used for review flags to confirm the reported results only. Selected negative and positive samples from Sysmex UF-1000i analysis were first analyzed with the UriSed 3 PRO, and then counted visually throughout the experiment. During the verification, automated reports were used only, ignoring samples without quantitative report for a given particle. No repeats or visual interpretations by the operator were made in the primary verification to improve that figure.

Precision was estimated both by duplicate counts of 33 patient samples (Dahlberg's procedure) for WBC and RBC, and by commercial control solutions (KOVA®Liqua-Trol ™ with Microscopics; Kova International, Amsterdam, The Netherlands) measured at High Abnormal and Low Abnormal concentrations (mixed from 1 part High Abnormal + 5 parts Normal = negative solution) in 30 consecutive days containing stabilized RBC and WBC particles. Carry-over was assessed as described by the ICSH guideline [13], using the following equation: (B1-B3)/(A3-B3) × 100% , where B1, B2 and B3 represent the 3 sequential low count, and A1, A2 and A3, the following 3 high count samples.

After completing the primary comparison against visual microscopy, the second instrument at the central site (Meilahti) was compared against the Gold instrument for trueness with patient samples, and for precision with KOVA control solutions. Subsequently, the 8 other instruments were compared against the Gold instrument for use in the regional laboratories of the Helsinki and Uusimaa and Kymenlaakso Hospital districts. The eight regional instruments were first tested for trueness at the central site, transferred to their destination, and re-verified mostly for precision with KOVA control solutions, and for trueness with local patient samples during the training of local staff.

Flagging limits for all ten UriSed 3 PRO instruments of the tender were created based on the results from two instruments within the central laboratory by December 2018. Operators are able to review and correct results of particles from 15 high power field-like images on computer screen (each representing about 0.15 μL volume), which was applied in design of the flags. The flagging limits were confirmed with the regional 8 instruments by March 2019.

### 2.3. Visual microscopy

Automated results by UriSed 3 PRO Gold instrument from 456 to 463 urine specimens (variability was related to the number of quantitated samples for each particle) were compared against visual microscopy. The reference phase contrast microscopy was performed blindly to instrumental results. The work was shared by four authors having experience on particle counting, each specimen counted by one person only. Before experimental results, the uniformity of classification between the evaluators was first confirmed with 10–20 patient samples.

We used a modified ISLH reference procedure [10], by counting 1 μL of uncentrifuged, unstained urine samples in disposable Bürker chambers after suspension. The volume of 1 μL was used also in case of low counts, i.e., the statistical uncertainty was accepted for practical reasons despite having impact on uncertainty of classifications.

Comparisons to urine culture results were available for 396 parallel samples from September to October 2018. At the Department of Clinical Microbiology, the samples were cultured by using automated inoculation with 1 μL loop (WASP® automated microbiology instrument, COPAN Wasp, Brescia, Italy) on chromogenic agar plates (CHROMagar™ Orientation medium, RT413-25, CHROMagar, Paris, France) following incubation at 35 °C for 18 h. Results were reported out as CFU/ml (colony forming units/milliliter) and converted to CFB/L (colony forming bacteria/liter) for this study. Significant growth was generally defined as $10^7$ CFB/L (SI unit, corresponding to $10^4$ CFU/mL) or more, with 1–2 identified species of common urinary pathogens [9], independently of the type of collection.

### 2.4. Data analysis

Data were collected from different analyzers and combined on Microsoft Excel spreadsheets. Sample IDs were used to combine the

results from parallel samples and the preanalytical data of the patients as needed. Statistics from Analyze-It® software (Analyse-it Software Ltd., England) was used as a module of Microsoft Excel spreadsheets.

Particle counting follows Poisson statistics with a theoretical standard deviation $s(n) = \sqrt{n}$, where n = total number of counted particles, and a coefficient of variation $CV(n) = \sqrt{n}/n = 1/\sqrt{n}$. Usually, a mean count × in p unit volumes is reported, i.e., particle concentration × = n/p and n = p*x, with a coefficient of variation $CV(x) = \sqrt{x}/x = 1/\sqrt{x}$ [10]. In instrumental counting, the total volume p counted to reach n particles improves the observed imprecision of the mean count, CV(x) with a factor of $(1/\sqrt{p})$. In the UriSed 3 PRO, p = 2.2 μL. The following relation between CV(n) and CV(x) applies:

$$CV(n) = CV(x) / \sqrt{p} \quad (1)$$

Detailed examples and explanatory statements on Poisson statistics were added as **Supplementary material A: Equations (Supplement A1), Consequences (Supplement A2), and a Poisson calculator (Supplement A3).**

For imprecision of particle counts, we used a relative imprecision, i.e., ratio R(CV) of observed/ theoretical imprecision to describe repeatability because of concentration-dependent dispersion of low counts:

$$R(CV) = CV(observed) / CV(x) \text{ from Poisson statistics} \quad (2)$$

In this study, a desirable specification of R(CV) ≤ 2 was adapted from earlier descriptions using a ratio of 2:1 and 3:1 between optimum, desirable and minimum specifications of performance [14,5].

Non-parametric Spearman's correlation coefficients were calculated for quantitative counts (WBC and RBC). Ordinal scale categories of particles were classified as cross-tables and assessed by weighted Cohen's kappa coefficients.

## 3. Results

### 3.1. Precision, imprecision ratios R(CV), linearity and carry-over

Quantitative day-to-day imprecision was assessed by measuring KOVA control solutions containing fixed WBC and RBC after resuspension for 30 consecutive days [Table 1]. Quality specification for a desirable R(CV) ≤ 2 (*Equation (2)*, chapter 2.4.) was fulfilled with the tested WBC and RBC control particles, reaching almost the apparent theoretical Poisson distribution [Table 1].

Imprecision ratios R(CV) were additionally estimated from duplicate measurements of 33 native patient samples with various particles, using Dahlberg's procedure. For WBC counts, all 33 samples fulfilled the desirable requirement of R(CV) ≤ 2, the average R(CV) being about 1 (data not shown). For RBC counts, 4/33 samples exceeded the desirable R(CV) ≤ 2, the average R(CV) being 1.1, but with a standard deviation of 1.1 [Fig. 1]. The causes for less precise results were a very high RBC count (the limit of quantitation for RBC is 1800 × $10^6$/L with UriSed 3 PRO) and WBC > 400 × $10^6$/L in one sample, presence of numerous SEC in another sample, and numerous WBC at infection-related concentrations in two cases. Thus, a safe estimate for the limits of quantitation with UriSed 3 PRO at CV 20% is about 25 × $10^6$/L for WBC with an R(CV) = 1, and about 60 × $10^6$/L for RBC with an R(CV) = 1.5 with patient samples. If calculated at CV 30%, the corresponding figures are about 12 × $10^6$/L for WBC, and about 25 × $10^6$/L for RBC.

**Table 1**
Day-to-day repeatability of RBC and WBC counts obtained with UriSed 3 PRO (Gold instrument) by measuring KOVA®Liqua-Trol TM with Microscopics solutions. High Abnormal = commercial solution directly, Low Abnormal = High Abnormal diluted with Normal (1 + 5 parts) (n = 30). CV = Coefficient of variation. R(CV) = ratio of observed / theoretical Poisson variation based on the mean count.

| Particle type | Quantity | High Abnormal | Low Abnormal |
|---|---|---|---|
| RBC | Mean count × $10^6$/L | 350 | 55.5 |
| | CV %, observed | 8.39 | 15.7 |
| | CV %, theoretical | 5.35 | 13.4 |
| | R(CV) | **1.6** | **1.2** |
| WBC | Mean count × $10^6$/L | 211 | 47.6 |
| | CV %, observed | 8.42 | 15.2 |
| | CV %, theoretical | 6.89 | 14.5 |
| | R(CV) | **1.2** | **1.0** |

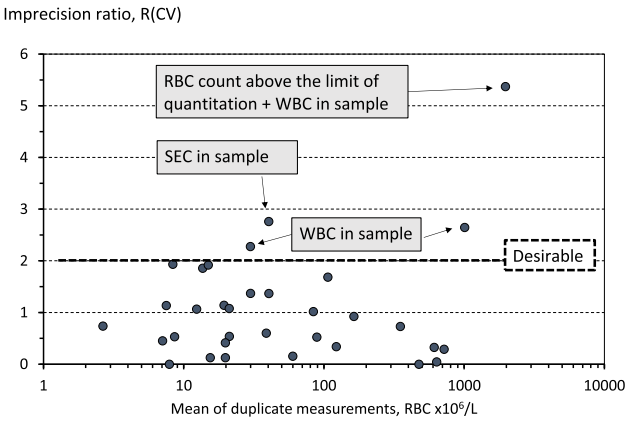


**Fig. 1.** Repeatability of RBC counts from duplicate measurements of 33 samples, expressed as relative imprecision, i.e., ratio R(CV) = (observed CV/theoretical Poisson CV). Quality specification for a desirable performance at R(CV) ≤ 2 is marked with a dashed line. Explanations to the four deviating cases are shown. SEC = squamous epithelial cells, WBC = white blood cells.

Linearity was measured for the two most quantitative particles, RBC and WBC, by diluting a high RBC sample (2000 × $10^6$/L) and a high WBC sample (3600 × $10^6$/L) with phosphate-buffered saline (pH 6). Dilution of RBC succeeded ideally down to 30 × $10^6$/L and that of WBC down to 10 × $10^6$/L with a Pearson's coefficient of correlation $R^2 = 1.00$ (Detail shown in the **Supplement B1**).

Sample carry-over test was started with high counts at 4043 × $10^6$/L for WBC, 290 × $10^6$/L for RBC, and a count of 333 × $10^6$/L for BAC (the limits were at 80 × $10^6$/L for positivity, and 800 × $10^6$/L for abundant bacteria). Calculated carry-over was 0.01%, 1.2% and –1.8%, respectively - a negative value is related to a low background of BAC counts.

### 3.2. Trueness of counts against visual microscopy and uncertainty of measurements

Trueness of UriSed 3 PRO counting was compared with visual phase contrast microscopy by trained persons with a modification of the ISLH reference procedure. Spearman's correlation against visual microscopy was $r_S = 0.94$ (0.93 – 0.95; 95% confidence interval, CI) for WBC counts and $r_S = 0.87$ (0.85 – 0.89; 95% CI) for RBC counts, claimed as quantitative particles by the manufacturer. In addition, squamous epithelial cells (SEC) were quantified with a correlation of $r_S = 0.82$ (0.79 – 0.85; 95% CI). Passing-Bablok regressions of WBC, RBC and SEC are shown in Fig. 2A–2C, respectively. Log-transformed counts are relevant because of exponential changes in disease.

In urine samples with low particle concentrations in general, statistical imprecision affects evaluation of trueness, as seen with the otherwise easily identified WBC. The Bland-Altman plot of log-transformed WBC counts against visual microscopy included uncertainties of both measurements (Fig. 3A). The maximum differences between the two procedures describe limits of maximum uncertainty at each concentration, including bias and imprecision. A difference of +/- 30% in $^{10}$Log counts represents 2-fold or +/- 100% difference between the actual counts of the instruments, since $^{10}$Log 2 = 0.30 = 30%. The maximum uncertainty of $^{10}$Log(WBC) was +/- 30% at 1.5 $^{10}$Log(Visual WBC), corresponding to 30 Visual WBC × $10^6$/L (Fig. 3A). No bias was observed after removing six samples (out of 425) with falsely high WBC

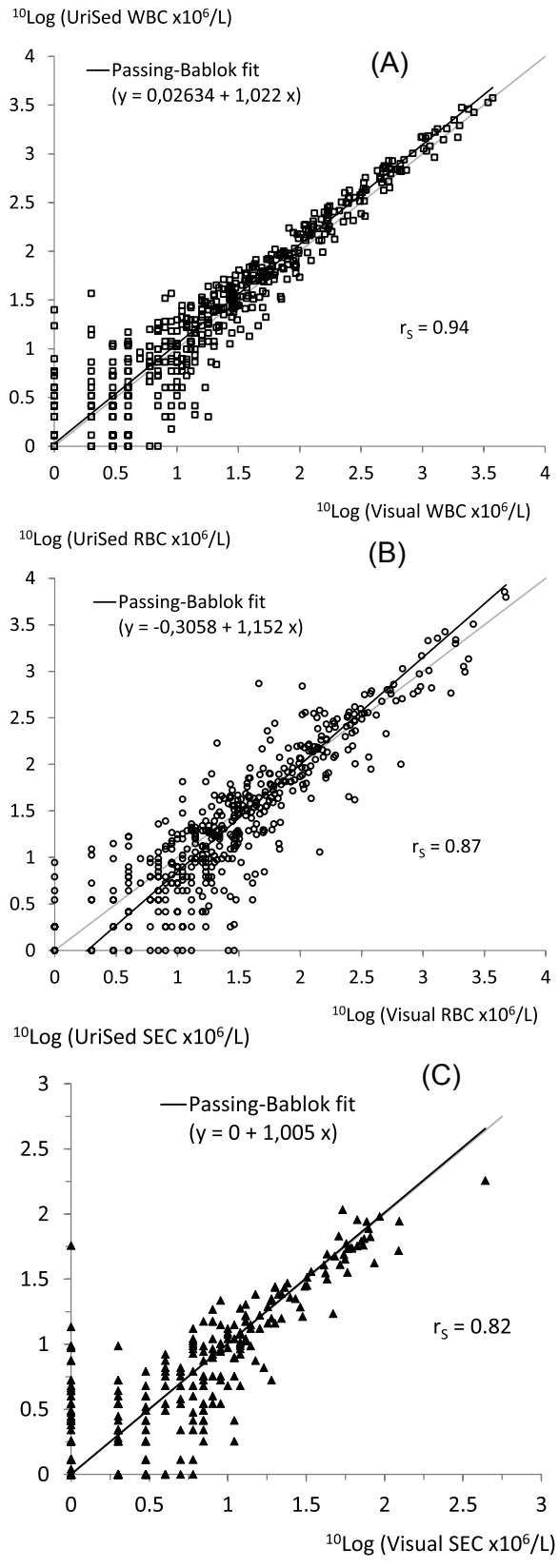


**Fig. 2.** Passing-Bablok regressions of UriSed 3 PRO counting against visual phase contrast microscopy in Bürker chamber. The graphs represent comparisons of WBC (Fig. 2A), RBC (Fig. 2B) and SEC (Fig. 2C) counts. Specimens with zero counts are excluded from the figures due to logarithmic transformation of counts: $^{10}$Log 1 = 0.

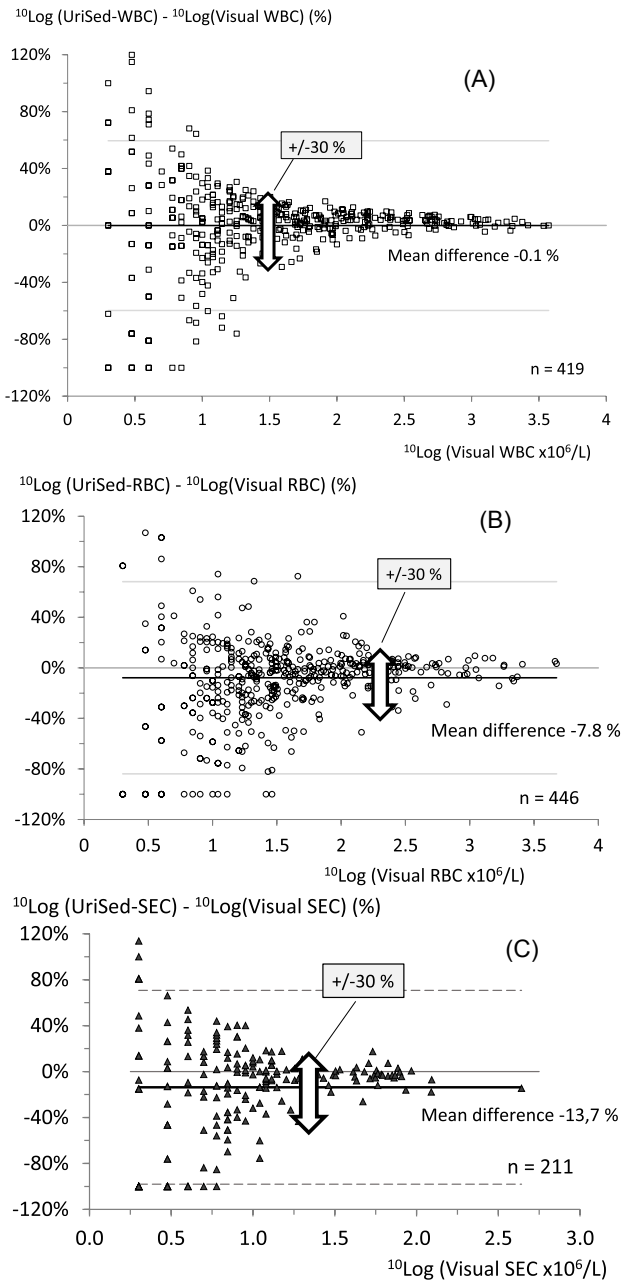


**Fig. 3.** Bland-Altman plot of relative differences between UriSed 3 PRO and visual microscopy counts, using visual counts as reference. Logarithmic transformation clearly shows the effect of low concentrations on imprecision, due to Poisson statistics. The broad arrow depicts +/- 30% uncertainty limits of $^{10}$Log counts, or +/- 100% differences of original counts between the two procedures. Differences of WBC, RBC and SEC counts are shown in Fig. 3A, Fig. 3B, and Fig. 3C, respectively.

counts against 2 WBC × $10^6$/L in visual microscopy.

Counting of RBC with UriSed 3 PRO resulted in an average bias of −7.8% (Fig. 3B). The lower RBC counts against visual microscopy were mostly seen at $^{10}$Log (Visual RBC × $10^6$/L) less than 2, or below 100 RBC × $10^6$/L, seen also in Fig. 2B. The maximum uncertainty between the procedures was reduced to +/- 30% $^{10}$Log(RBC) at about 2.3 $^{10}$Log(Visual RBC), corresponding to 200 Visual RBC × $10^6$/L (Fig. 3B). At 50 RBC × $10^6$/L in visual counting, the maximum uncertainty was still about +/- 50% $^{10}$Log(RBC) and at 30 RBC × $10^6$/L it was about +/- 70% $^{10}$Log(RBC). Quantitative counts of SEC were also comparable with UriSed 3 PRO against visual microscopy, despite not being claimed as

quantitative by the manufacturer (Fig. 3C). The average bias was −13.7% with the maximum uncertainty of +/- 30% at 1.3 $^{10}$Log(Visual SEC), or +/- 100% at 20 × $10^6$/L Visual SEC.

For other particles, uncertainties were larger than those of WBC or RBC, partially due to low counts, suggesting ordinal scale comparisons. We used ordinal scale categories with limits of 3, 10, 30, 100, 300 and 1000 × $10^6$/L, depending on detected particle concentrations (see **Supplementary material B2** for full detail of the cross-tables). The casts were combined from HYA and PAT casts because of their low prevalence at concentrations > 10 × $10^6$/L in our patient samples, despite pre-selection attempts (Table 2). The low frequency of clearly positive cases affected somewhat the weighted kappa coefficient of 0.59 for CAST. When using a limit of 10 × $10^6$/L for a positive result of casts, a sensitivity of 31% with a specificity of 99% was reached. Using a less precise level of 3 × $10^6$/L in the comparison, the sensitivity was increased to 49% with the specificity remaining at 98% (Table 2).

The occurrence of samples positive at concentrations > 10 × $10^6$/L was low for NEC like that for CAST samples (Table 3). The weighted kappa coefficient of 0.49 was obtained for NEC. Using the cut-off of 3 × $10^6$/L for positivity, a sensitivity of 42% with a corresponding specificity of 93% was obtained (Table 3).

For bacteria (BAC) detection, the performance of UriSed 3 PRO was compared against bacterial culture at CFB $10^7$/L (CFU $10^4$/mL). UriSed 3 PRO counts at a lower limit of 80 × $10^6$/L reached a sensitivity of 90% with a specificity of 39% against culture. When using a higher limit of 800 × $10^6$/L in UriSed 3 PRO, a specificity of 96% against culture was achieved with a reduced sensitivity to 24%. Detailed data with different cut-off limits by UriSed 3 PRO against urine culture as compared to those of reference visual microscopy is given as the **Supplementary material B3**. Combined detection of all classified particles with the UriSed 3 PRO Gold instrument against visual microscopy is shown in Table 4. A double presentation of selected cut-offs of positive results is shown, understanding both health-related reference limits and uncertainties of counts. Using a less precise reference counting with 1 μL volume of Bürker chamber as compared to 3,2 μL Fuchs-Rosenthal chamber caused a major part of this ambiguity.

In sequential testing, all other 9 UriSed 3 PRO instruments fulfilled the requirement of agreement against the Gold instrument both in RBC and WBC counting quantitatively (Spearman's $r_S$ at least 0.95), and in cross-tabulated categories for BAC and SEC particles (Cohen's weighted kappa at least 0.85). Positive cases for NEC and CAST were too rare in our patient samples to be compared individually for regional instruments (detail not shown).

### 3.3. Flagging limits, data transfer and process adaptation

Manufacturer provided the primary software to detect and verify or correct doubtful findings, based on flagging for crowded samples, mucous samples or amorphous material that prevent detection of separate particles. Additional technical flags were available for a non-representative sample, an empty cuvette, and small sample volume. Unexpectedly, false identification of particles occurred occasionally, which was intensively discussed with the manufacturer to understand the automated optics. The users at HUSLAB utilized the possibility to adapt local review rules based on selected particle concentrations when installing the automated workflow. They were created based on technical flags and observed performance in detecting the reportable particles during the verification. Quantitative flagging limits were first designed and then reviewed iteratively, using stored results that were compared against stored particle images on instrument screen. Further detail is explained in the **Supplementary material C**.

The following flagging limits were adapted at the mentioned particle concentrations, based on experience gained from instrument performance and local prevalence of findings: RBC 2000, WBC 5000, CRY (crystals) 40, CaOxM (calcium oxalate monohydrate crystals) 10, HYA 10, PAT 10, NEC 10, EPI 30, YEA 30, or BAC 800 × $10^6$/L. The aim was to confirm the detection of particles related to urinary tract infection (WBC and BAC), hematuria (RBC), and renal damage (NEC and CAST) to the levels that were considered unreliable or prone to matrix errors based on verification experience. E.g., WBC counts were reliable enough to set a flag at the limit of quantitation (5000 $10^6$/L) only. BAC results at 800 × $10^6$/L or higher were reviewed to confirm the presence of the abundant bacteriuria that may be significant in emergency cases, thus avoiding false positive reporting mostly due to AMO. Additional flags of non-reportable particles (CRY, CaOxM, YEA, and AMO) were used to avoid false positive RBC reports. Still other particles (CRY, MUC, and sperms) were flagged to reveal a possible false focus or false negative findings in various types of reported particles.

In addition to counts, 16 different statements were created to supplement or replace the quantitative report with a written statement (e.g., due to crowded sample) or increased uncertainty of results (e.g., due to degeneration of cells, dilution of sample or presence of mucus). About 100 technologists in 9 hospital laboratories were trained to use the 10 instruments. They were trained to compare automated counts suggested by the instrument with the amounts seen on the 15 images of each sample, and to use visual microscopy as needed. After adaptation of the flagging limits, 70–75% of the samples could be automatically reported, while 25–30% needed visual review on instrument display. Typically, reviews could be handled by editing the report on screen only, but 5–10% of all samples needed a dilution of sample at our empirical ratio 1:4 with phosphate-buffered saline, pH 6, to dissolve most amorphous precipitate, after which a rerun was performed. Two to three per cent of flagged cases consisted of mucous samples. Finally, only 1–5% of cases needed a genuine visual microscopy, less than routinely needed with the earlier flow cytometric instrument. A workload of 150–200 samples was achieved by a trained technologist in a working shift.

During the period of installation, software of the UriSed 3 PRO did not support a full bi-directional data transfer as usual in large automated

**Table 2**
Detection of casts (CAST, combined HYA and PAT casts) by UriSed 3 PRO against visual microscopy. Statistical uncertainty zone is marked with **bold face**, particle concentrations expressed as × $10^6$/L.

| | VISUAL MICROSCOPY | | | | |
|---|---|---|---|---|---|
| UriSed 3 PRO (Gold) | *Categories* | | | | |
| *Categories* | *0–3* | ***4–10*** | ***11–30*** | *31 or more* | *Total* |
| *0–3* | 348 | **42** | **11** | 2 | 403 |
| ***4–10*** | **7** | **20** | **15** | 1 | 43 |
| ***11–30*** | **1** | **3** | **7** | 4 | 15 |
| *31 or more* | 0 | 0 | 0 | 2 | 2 |
| Grand Total | 356 | 65 | 33 | 9 | 463 |
| Agreement (diagonal) | | | 81.4% | | |
| Agreement (diagonal + neighbor) | | | 96.8% | | |
| Cohen's weighted kappa | | | 0.591 | | |

**Table 3**
Detection of non-squamous epithelial cells (NEC) by UriSed 3 PRO against visual microscopy. Statistical uncertainty zone is marked with **bold face**, particle concentrations expressed as × $10^6$/L.

| | VISUAL MICROSCOPY | | | | |
|---|---|---|---|---|---|
| UriSed 3 PRO (Gold) | *Categories* | | | | |
| *Categories* | *0–3* | ***4–10*** | ***11–30*** | *31 or more* | *Total* |
| *0–3* | 309 | **65** | **11** | 0 | 385 |
| ***4–10*** | **24** | **26** | **14** | 1 | 65 |
| ***11–30*** | **0** | **5** | **5** | 1 | 11 |
| *31 or more* | 0 | 0 | 1 | 1 | 2 |
| Grand Total | 333 | 96 | 31 | 3 | 463 |
| Agreement (diagonal) | | | 73.7% | | |
| Agreement (diagonal + neighbor) | | | 97.4% | | |
| Cohen's weighted kappa | | | 0.485 | | |

**Table 4**
Performance of UriSed 3 PRO (Gold) against visual reference microscopy in detection of urine particles (number of specimens given separately for each type of particle). Cases classified as positive exceeded the shown limits. Bacteria (*BAC*) were also assessed against chromogenic cultures at two cut-off limits of UriSed 3 PRO.

| Particle type | Limit of positivity(x $10^6$/L) | Number of positive cases | Total number of samples | Sensitivity (%) | Specificity (%) | Agreement (Cohen's kappa, weighted) |
|---|---|---|---|---|---|---|
| WBC | 30 | 187 | 456 | 95 | 90 | 0.94 |
| | 10 | 300 | | 92 | 77 | |
| RBC | 3 | 206 | 457 | 85 | 92 | 0.89 |
| | 10 | 350 | | 82 | 84 | |
| SEC | 10 | 83 | 463 | 81 | 96 | 0.88 |
| | 3 | 161 | | 88 | 90 | |
| NEC | 10 | 34 | 463 | 24 | 99 | 0.49 |
| | 3 | 130 | | 42 | 93 | |
| CAST[a] | 10 | 42 | 463 | 31 | 99 | 0.59 |
| | 3 | 107 | | 49 | 98 | |
| *BAC*[b] | *80*[b] | 146 | 396 | 90 | 39 | |
| | *800*[b] | | | 24 | 96 | |

[a] CAST represents a sum of hyaline (HYA) and pathological (PAT) casts.
[b] BAC counts of UriSed 3 PRO allowed classification of results in categories of "negative", "positive" at 80 × $10^6$/L, or "abundant" at 800 × $10^6$/L bacteria or more. Bacterial cultures were defined as positive at $10^7$ CFB/L (equal to $10^4$ CFU/mL) or more, including mixed growth.

laboratories. After several changes by the manufacturer, a reasonable routine workflow was established, enabling a throughput of 500 samples analyzed daily in the HUSLAB laboratories on 24/7 emergency basis.

## 4. Discussion

In the verification, the ten UriSed 3 PRO instruments fulfilled the quality specifications of trueness and precision for routine counting of urine RBC, WBC, BAC and SEC. Screening of CAST and NEC representing renal particles was achieved at a sensitivity of 40–50%, which was accepted by the nephrology unit of the hospital. With local flagging limits and operating procedures, the 10 instruments were successfully adapted into the nine HUSLAB laboratories (a duplicate was installed in the central site).

### 4.1. Precision

Analytical performance of UriSed 3 PRO was assessed by paying a special attention to the reference procedure [10] and uncertainty of measurements. In particle counting, WBC and RBC counts of quality control solutions obtained by UriSed 3 PRO easily fulfilled the quality requirement of R(CV) ≤ 2 (Table 1), reaching an apparent Poisson CV(x) of the reported counts, due to a total volume of 2.2 μL that reduces the observed CV with a factor of $1/\sqrt{2.2} = 0.675$ (*Equation (1)*, chapter 2.4.). In addition, the preserved particles do not adhere to each other in the prepared suspension. Similar relative imprecisions R(CV) for control particles could be calculated with the *Equation (2)* from earlier results on iQ200 [4], and sediMAX [11].

In counting of 33 patient samples, the biological nature of particles and matrix effects created an additional challenge. The R(CV) of WBC counts by Dahlberg procedure was better than the desirable R(CV) < 2 in all cases. Four out of 33 RBC samples exhibited difficulty of quantitation due to other particles. These examples remind of matrix interferences (e.g., mucus, lots of bacteria or precipitates, and crystallization) related to clinical urine samples. On the other hand, pipetting and disposable chamber seemed to tolerate high bacteria counts without noticeable carry-over during the evaluation. In addition to the variable background, the RBC particles themselves show variation in their morphology and size, creating inaccuracy in counting. A safe estimate of the average imprecision ratio of natural RBC or WBC in UriSed 3 PRO counting was a R(CV) of 1.5 or 1, respectively. Within-day repeatabilities of natural RBC and WBC with a few selected samples were both about R(CV) of 0.4 with the Sysmex UF-5000, due to a larger volume of 7.8 μL counted by UF-5000 [3].

A starting point defining the clinical need for the limit of quantitation in urine particle counting could be a CV of 30% that allows detection of 100% differences between two measurements ($2 \times \sqrt{2} \times$ CV). These 30% limits of quantitation at CV 30% were estimated to be about 12 × $10^6$/L for WBC with an R(CV) = 1, and about 25 × $10^6$/L for RBC with an R(CV) = 1.5, as opposed to the conventional 20% limits used for solutes that were reached at 25 × $10^6$/L for WBC and 60 × $10^6$/L for RBC, respectively.

In patient samples, variation of urine concentration may be even tenfold (100 – 1000 mOsm/kg $H_20$) or a difference of 1000% during a day [15]. If converted to a maximum imprecision with +/- 2 s limits, an imprecision of +/- 250% describes preanalytical uncertainty related to diuresis. A similar frame comes from diagnostically important classifications of pyuria and hematuria, where typical particle counts may differ up to 1000% from each other (such as WBC 10 vs 100 × $10^6$/L), allowing an uncertainty of about 3-fold differences = 300%, by dividing 1000% with ($2 \times \sqrt{2}$), out of which the analytical component might be up to 50–100%.

### 4.2. Trueness

In the assessment of trueness, direct chamber counting by phase contrast optics and trained professionals are needed [10]. Our exception from the reference procedure was the use of 1 μL volume only, rather than 3,2 μL of Fuchs-Rosenthal chamber for practical reasons: Bürker chamber had been in the continuous routine use, and the smaller volume speeded up the assessment. We obtained Spearman's correlation coefficients of 0.94, 0.87 and 0.82 in counting of WBC, RBC and SEC, respectively, as seen in Passing-Bablok regressions of log-transformed counts (Fig. 2A to 2C). Previously, Spearman's correlation of 0.91 for WBC counts and that of 0.87 for RBC counts against visual microscopy have been reported [11]. These figures agree also with the information provided by the manufacturer. Erythrocytes were more difficult to detect by UriSed 3 PRO than leukocytes even at diagnostic concentrations < 100 × $10^6$/L (Fig. 2B). This is due to the variability of RBC shapes in urine, and difficulty in detecting ghost cells among other particles with higher microscopic contrasts. Also, a difficulty of instrumental counting is exaggerated if compared with specially trained visual microscopists [5]. SEC were counted surprisingly well (Fig. 2C). The most important factor in our RBC counting was, however, the presence of samples with numerous inflammatory cells and squamous epithelial cells obscuring the background optics. This is seen in counts of Fig. 2A-2C that derive from samples from the same patients (excluding only cases with 0 or 1 particles × $10^6$/L in logarithmic transformations, and some non-quantitative results).

Detailed differences between UriSed 3 PRO and visual microscopy were assessed by Bland-Altman plots of WBC, RBC and SEC (Fig. 3A to 3C). The maximum uncertainty of WBC counts between the two methods

reached +/- 100% at 30 × $10^6$/L (Fig. 3A). No bias was observed. If the observed maximum uncertainty is modelled to represent an expanded uncertainty (U) with a coverage factor of two standard deviations (+/-2s, or 95% confidence limits), a single combined uncertainty ($u_c$) between the two measurement principles was +/- 50% at 30 × $10^6$/L, despite the use of 1 μL volume in visual microscopy. The observed inaccuracy would not affect classification of healthy and inflammatory states. This was also a result from comparisons of two measurements. With UriSed 3 PRO counting alone, the imprecision at 20 WBC × $10^6$/L was 20 +/- 9 WBC × $10^6$/L (+/- 2 s limits) or a 95% confidence interval of 11–29 × $10^6$/L that can be interpreted as a diagnostic grey zone from 10 to 30 WBC × $10^6$/L, above which WBC counts differ significantly from the arbitrary upper health-related reference limit of 10 WBC × $10^6$/L, until pyuria is detected.

For RBC, the maximum uncertainty between UriSed 3 PRO and visual procedures was larger than that of WBC, since a +/- 100% maximum uncertainty of RBC counts was reached at about 200 RBC × $10^6$/L (Fig. 3B). Difficulty in identifying RBC in some samples resulted in a bias of −7.8 RBC × $10^6$/L in addition to increased uncertainty as discussed with Fig. 2B. Hematuria should be detected at 50 × $10^6$/L or higher RBC concentrations, after which the clinical hematuria may reach concentrations up to 5000 RBC × $10^6$/L or more. We could see an expanded uncertainty U of about +/-50% $^{10}$Log(RBC) or +/- 300% RBC × $10^6$/L at 50 RBCx$10^6$/L that gradually improved at counts above 100 RBC × $10^6$/L (Fig. 3B). Clinical suspicion of hematuria is important after treating the infection, after which counting of RBC succeeds better. Without other particles and using UriSed 3 PRO alone, a satisfactory limit of quantitation of 25 RBC × $10^6$/L with a CV of 30% was estimated, as described above. Squamous epithelial cells demonstrated a good quantitative agreement against visual microscopy with a minor bias of −13.7% and a expanded uncertainty of +/- 100% at 20 × × $10^6$/L SEC between the two measurements (Fig. 3C). Suspicions of unsuccessful mid-stream collections are associated with higher than this count.

Detection of renal damage is a traditional indication of urine particle examination [16]. Sensitivity of UriSed 3 PRO to detect casts (Table 2) or NEC (Table 3) was from 49% to 42%, respectively, at a cut-off of 3 × $10^6$/L, as compared to visual microscopy, but with a high specificity of 98–93%, respectively. In the **Supplementary Tables B2-B3**, categorized data of all tested particles are shown. Results from classifications depend on chosen cut-off limits, different patient populations and prevalence of diagnostic findings, in addition to performance of instruments. A high specificity is typically targeted in the artificial intelligence to reach correct classifications, reducing sensitivity of detection against visual microscopy when particles exhibit variable features. For sediMAX, a sensitivity of 62–71% with a specificity of 97% has been shown earlier for casts [11]. For flow cytometers, the following figures in detecting casts have been reported: a sensitivity of 59% with a specificity of 80% for Sysmex UF-1000i, and a sensitivity of 72% with a specificity of 83% for the newer UF-5000 [3]. The reduced sensitivities or specificities reveal a difficulty by current automated instruments in differentiation of various renal particles from other particles in urine. Thus, a sensitive screening of renal particles still requires visual microscopy in skilled hands, in addition to other diagnostic tests [16]. The verified performance of UriSed 3 PRO was, however, sufficient for our local nephrology department to eliminate traditional detailed visual microscopy from their laboratory routine since they use also other tests, including specific proteinuria measurements, in detection and follow-up of renal disease.

The detection of bacteria by UriSed 3 PRO was more sensitive than routine visual microscopy even by phase contrast optics in most laboratories. The compiled Table 4 summarizes our verification assessment on UriSed 3 PRO with two optional cut-off limits for positive results. The performance was judged to reach an acceptable analytical accuracy for routine use in our laboratories.

### 4.3. Review flags

Flagging limits by manufacturer were tested, applied partially, and further developed locally. The UriSed 3 PRO had not been installed in many laboratories of our size at the time of the verification. That is why the created flagging limits by the user may be considered preliminary and might be improved after gaining more experience in routine. We reached the capacity to analyze and report 200–300 samples in 24 h in the central laboratory, and a total of about 500 samples in 24 h altogether in 9 laboratories. Software allowed us to adjust the flagging rules and to create new rules to select efficiently samples for human review, while 70–75% of samples were released automatically into the laboratory information system, including most samples with disease-related counts. Computerized data interface raised several suggestions for improvements from the HUSLAB laboratories, some of them remaining for future developmental phases.

## 5. Conclusions

We were able to install 10 UriSed 3 PRO instruments in a regional laboratory environment at HUSLAB, providing about 160′000 automated particle counts annually at 24/7 basis, thus satisfying both routine testing and emergency analysis of clinical samples. Analytical performance fulfilled the analytical performance specifications and expectations obtained from preliminary testing before the public tender.

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2021.01.005.

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