Automated urinalysis combining physicochemical analysis, on-board centrifugation, and digital imaging in one system: A multicenter performance evaluation of the cobas 6500 urine work area

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A R T I C L E   I N F O

A B S T R A C T

Background: We evaluated the analytical performance of the fully automated cobas® 6500 urine work area and its automated components—cobas u 601 and cobas u 701.

Design and methods: The study was conducted at three European centers using un-centrifuged surplus routine urine samples; all measurements were performed within 2 h of sample collection. Precision, sample carry-over, and method comparisons were evaluated per Clinical and Laboratory Standards Institute guidelines. Method comparisons: cobas u 601 versus Urisys 2400 and cobas u 411 urine test strips; and cobas u 701 versus KOVA® visual microscopy and iQ200 analyzer. Operability and functionality were assessed using questionnaires.

Results: Precision of the entire cobas 6500 system was within predefined acceptance limits and no significant carry-over was observed. Erythrocytes, leukocytes, nitrites, and protein were in good agreement (≥93%) with cobas u 411 reflectometry. High correlation was shown between the cobas u 701 analyzer and KOVA visual microscopy for red blood cells (RBC; slope, 0.89; Pearson's r, 0.95) and white blood cells (WBC; slope, 0.96; Pearson's r, 0.96), demonstrating equivalence of test results. The 97.5% percentile reference values on the cobas u 701 analyzer were 5.3 cells/μL (RBC) and 6.2 cells/μL (WBC). The cobas 6500 system showed good sensitivity for small bacteria (>1 μm) and pathological casts, and the user interface, maintenance wizards, and system design were highly rated by operators.

Conclusions: The fully automated workflow, high precision, and high throughput of the cobas 6500 system have the potential to facilitate standardization of urine screening.

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1. Introduction

Urinalysis is a very common technique used in clinical practice, providing crucial information on the functioning of the kidneys and other organ systems, and aiding clinical decision-making in various diseases, such as diabetes, glomerulonephritis, and suspected urinary tract infections (UTIs) [1–13]. Routine urinalysis involves a two-step approach after initial macroscopic examination, consisting of both physicochemical evaluation and microscopic examination of urine sediment to differentiate and quantify clinically relevant urine particles [14–18]. Physicochemical examination techniques typically involve a refractometry/refractive index method for determination of specific gravity and reflectance spectroscopy using urine test strips for analytes.

Urine test strips are often used as a first diagnostic triage for UTIs, with positive results triggering more specific analyses, such as the identification of bacterial species by urine culture [19,20]. The sensitivity of test strips can be increased by combining the results of individual parameters; for example, a positive result for nitrites and/or leukocytes has a sensitivity of 68–88% to diagnose a UTI, depending on the patient group and clinical setting [21]. One recent study observed a much higher sensitivity of 97% to diagnose a UTI with a positive nitrite and/or leukocyte result in elderly hospitalized patients [19]. However, test strips lack precision and varying predictive values for diagnosing a UTI are reported in the literature [20–22]; thus, automated methods have been proposed to increase precision, accuracy and throughput, especially in an environment with multiple operators [23]. Manual microscopy is still used in ~60% of cases and is considered the reference method for particle analysis in urine sediment [24]. However, manual microscopy requires a high level of operator expertise, and is labor and time intensive, with well-defined pre-analytical steps necessary to avoid potentially detrimental bias [25]. Urine sediment analysis using manual microscopy is also characterized by a high coefficient of variation (CV; >100%) and inter- and intra-observer variability, particularly when performed by non-skilled operators [26–32].

Automated urine analyzers have been developed since the 1970s to integrate the physicochemical and microscopic examination of urine [33,34]. These systems offer practicable and faster urine screening, which may prevent unnecessary culture requests [35]. The increased throughput of these instruments over manual methods enables the reliable and rapid screening of urine samples with reduced labor demands on laboratory staff, thereby improving efficiency and cost-effectiveness [36,37]. Additionally, these instruments can improve reliability and precision by eliminating inter-operator variability, and require a low sample volume [2–12]. Two broad techniques have been developed for automated urine sediment analysis: urinary flow cytometry [33,38–40] and image-based microscopy using a built-in camera [41–43]. It is important that newly developed automated analyzers are rigorously evaluated to ensure they perform similarly to current reference methods (i.e., manual microscopy). Known limitations of some automated systems include limited accuracy for bacteria, casts, and crystals [36,39]. Thus, there is a need to increase accuracy across the whole spectrum of urinalysis in new instruments.

The next-generation cobas® 6500 urine analyzer (Roche Diagnostics GmbH, Mannheim, Germany) integrates physicochemical test strip analysis and image-based microscopic urine sediment analysis in a single platform. Due to its modular design, the individual subsystems can be used as a standalone urine analyzer (cobas u 601) or standalone microscopy analyzer (cobas u 701), or combined as a fully automated urine work area. Although the system analyzes urine particles automatically, microscopic images can also be stored for further manual review. We performed a multicenter study to evaluate the analytical performance of the cobas 6500 urine work area and its modular components, and compared the individual subsystems with harmonized, designated comparison methods. The operability and practicality of the standalone modules and integrated system were also evaluated.

2. Materials and methods

2.1. Study design

The study was carried out from May 2013 to May 2014 at three European centers, which cover different patient populations and a wide range of particle concentrations: two public university hospital laboratories (Leiden University Medical Center, Leiden, The Netherlands; Virgen Macarena University Hospital, Seville, Spain) and one commercial laboratory (CatLab, Viladecavalls, Spain).

All measurements were performed using native human urine samples surplus to requirement (≥5 mL) from routine testing; anonymized samples were used for the precision and correlation experiments, and pseudonymized samples were used for the reference range study (recording of gender only). Samples with any additives or samples collected over a certain time period (e.g. 24-hour urine samples) were excluded. Sample storage and handling were performed according to European urinalysis guidelines [14]. Samples were stored at room temperature, did not undergo any processing or centrifugation, and were analyzed within 2 hours of sample collection. Used samples were stored afterwards for 1 day at 2–8 °C.

The use of patient samples complied with all relevant national regulations and institutional policies. Prior to study start, the responsible ethics committees for the three study sites (Commissie Medische Ethiek [No: C13/146] for the Leiden University Medical Center; Ethics Committee of the Virgin Macarena University Hospital [internal code: 2014]; Assessment of the Quality Management for CATLAB) confirmed that no formal ethical approval was required, as only surplus samples from routine testing were used in the study.

2.2. cobas 6500, cobas u 601, and cobas u 701 analyzers

The cobas 6500 urine work area provides fully automated urinalysis and consists of cobas u 601 and cobas u 701 modular subsystems, with a throughput of up to 116 complete urine results (test strip + sediment) per hour; cobas 6500 test parameters and their abbreviations are shown in Fig. 1.

Fig. 1. cobas 6500 parameters and their abbreviations.

The cobas u 601 analyzer is a reflectance photometry urine test strip analyzer, which also provides physical cell measurements for determination of clarity and specific gravity. It has a throughput of up to 240 samples per hour. Test strips are provided in cobas u packs with a quantity of 400 strips per pack.

The cobas u 701 analyzer uses digital microscopic imaging technology and is intended for quantitative, semi-quantitative, and qualitative examination of urine sediment particles. The measurements are performed using unspun urine. Sample measurement is performed with 170 μL of urine in disposable plastic cuvettes using an efficient pipetting mode (three times aspirating and rinsing) to ensure optimal sample mixing. The automated workflow of the cobas u 701 after pipetting and an example result image are shown in Fig. 2.

2.3. Comparison methods

The cobas u 411 and Urisys 2400 analyzers (Roche Diagnostics GmbH, Mannheim, Germany) are urine test strip analyzers that use reflectance photometry. Urisys 2400 test strip cassettes were used with the Urisys 2400 analyzer and Combur [10] Test® M test strips were used with the cobas u 411 analyzer.

Visual microscopy using KOVA® chamber technology (Cat. No. 3518345001, Kova International Inc., Garden Grove, CA), a standardized methodology according to European urinalysis guidelines [14], was used as a harmonized comparison method across the three evaluation centers. Two KOVA slides of each sample were prepared and counted using bright field microscopy, each by two experienced operators, to reduce counting error; the mean value was then calculated. Further details on the KOVA procedure are provided in Supplementary Material S2.

The iQ200 analyzer (Iris Diagnostics, Chatsworth, CA) is a second-generation automated microscopy analyzer that captures images from planar flow of urine particles. A neural network (Auto-Particle Recognition™) is used to classify and quantify particles in the sample; 500 photographs are taken from each urine sample and compared with standard images.

2.4. Study assessments

Testing was performed in three phases by first assessing the cobas u 601 analyzer, then the cobas u 701 analyzer, and finally the cobas 6500 urine work area. Precision (repeatability and intermediate precision), sample carry-over (according to Broughton et al. [44]), and method comparison were evaluated according to Clinical and Laboratory Standards Institute guidelines. Negative and positive quality control samples (qUAntify® Control and Liquichek™ Urinalysis Control; Bio-Rad Laboratories Ltd, Watford, UK) and human urine samples were used for repeatability testing. Human samples were selected to cover relevant concentration ranges for each test; 21 measurements were performed per sample. Control samples alone were used for intermediate precision testing, due to the limited stability of human samples.

Method comparisons were performed for the cobas u 601 analyzer versus Urisys 2400 and cobas u 411 urine test strip systems, and for the cobas u 701 analyzer versus KOVA visual microscopy and the iQ200 analyzer. For the bacteria parameter, a comparison was only performed between the cobas u 701 analyzer and KOVA visual microscopy, due to the reported limitation of the iQ200 system in detecting small bacterial cocci [45,46].

Recovery of predefined semi-quantitative concentration ranges for non-squamous epithelial cells, hyaline casts, squamous epithelial cells, and bacteria on the cobas u 701 analyzer was assessed by diluting strongly positive samples. To avoid problems associated with
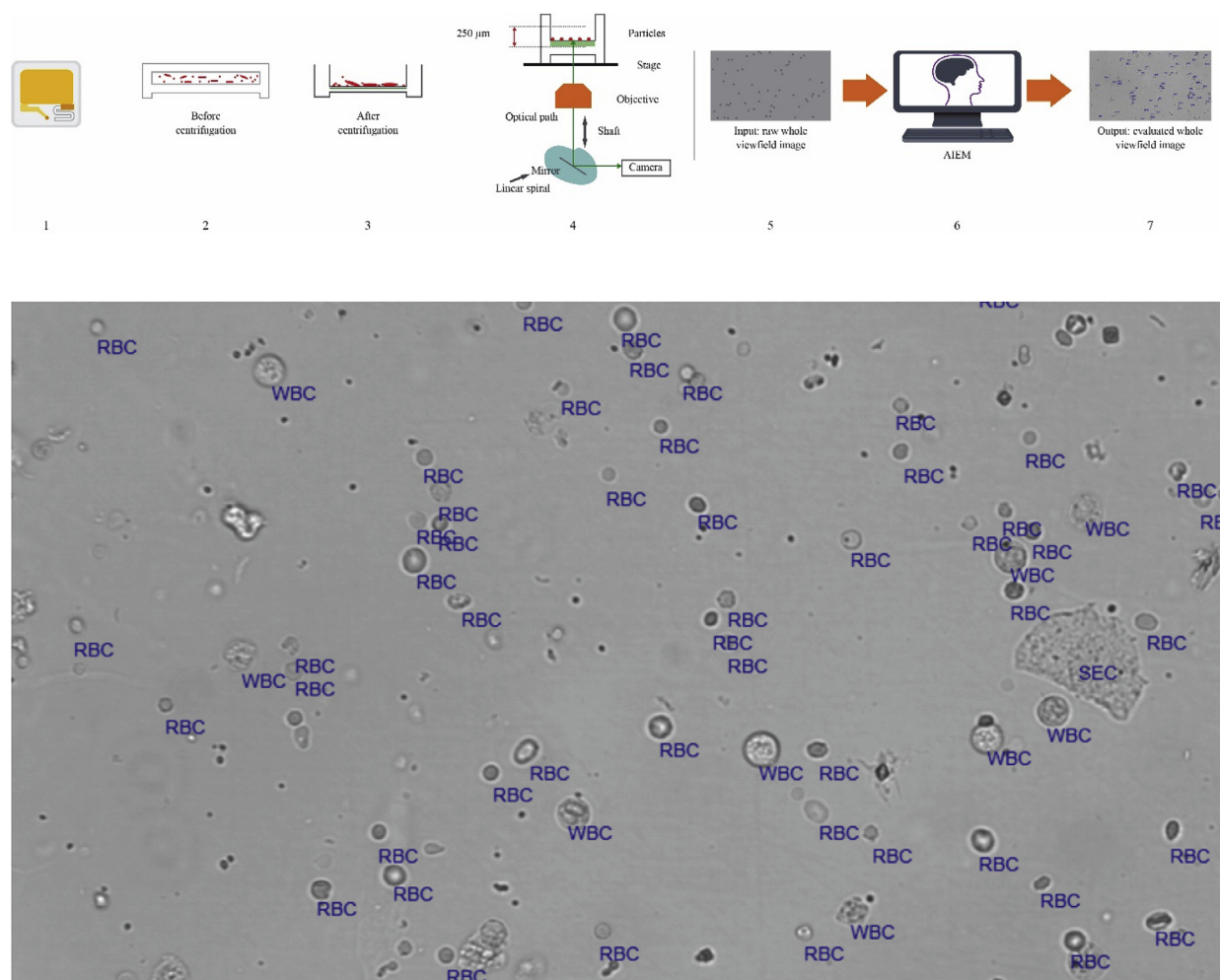
Fig. 2. Workflow diagram for sample processing on the cobas u 701 analyzer (upper panel). The filled cuvettes (images 1 and 2) are briefly centrifuged (10 s at 260×g) to obtain a single layer of particles (image 3). The microscope then automatically records high-resolution images (image 4); 15 images (image 5) are used in the analysis by the Automated Image Evaluation Module (AIEM), which utilizes a neural-network-based algorithm (image 6). The AIEM comprises different stages for generation of the final results, using probability plots followed by comparison with a characteristic image database (image 7). The evaluation takes approximately 3–4 s per image. Example of result image from the cobas u 701 analyzer (lower panel). Additional images are shown in Supplementary Material S1. RBC, red blood cells; SEC, squamous epithelial cells; WBC, white blood cells.

dilution, such as cell lysis, the samples were split into two portions; the second portion was centrifuged and its supernatant was used as a diluent for the first portion. All samples (including original samples, prepared dilutions, and used diluents) were measured in triplicate.

The cobas u 701 analyzer includes software for image reclassification by the operator. Reclassification was performed by all operators to confirm software functionality and to assess the impact on statistical result calculations. All sites performed ≥10 reclassifications.

Reference values for the quantitative parameters (red blood cells [RBC] and white blood cells [WBC]) on the cobas u 701 analyzer were estimated using urine samples from apparently healthy donors. Healthy urine status was determined by measuring samples using three different methods: test strip reading (cobas u 601 analyzer), iQ200 analyzer, and visual microscopy using KOVA counting.

Concordance testing between the cobas u 601 and cobas u 701 analyzers was conducted using 626 routine samples with positive test strip results for RBC, WBC, nitrites, and proteins, on both systems.

System functionality and practicality of the cobas 6500 urine work area was continually assessed throughout the study. Routine simulation and workflow experiments were performed with up to 1000 samples per day to evaluate whether the system is fit for high-volume clinical laboratory demands. Operators answered questionnaires using a ranking scale from 1 (very poor) to 5 (excellent) to assess usability and functionality.

2.5. Statistical analysis

All data were recorded and analyzed using the Windows-based Computer-Aided Evaluation (WinCAEv) tool (Roche Diagnostics International Ltd, Rotkreuz, Switzerland). For precision testing of the cobas u 601 analyzer and quantitative parameters of the cobas u 701 analyzer, the mean, standard deviation (SD), and CV were calculated; for semi-quantitative and qualitative parameters of the cobas u 701 analyzer, agreement rates of the tested concentration ranges were calculated. Statistical analysis of carry-over testing was performed by comparing affected samples versus results from the reference part. Method comparison results for the cobas u 601 analyzer were assessed by calculation of agreement rates or Deming regression (specific gravity, cobas u 601 versus Urisys 2400). Method comparison results for the cobas u 701 analyzer were assessed using Bland-Altman difference plots and Passing–Bablok regression analysis (including Pearson's *r* and Kendall's Tau correlation coefficients) for quantitative parameters (RBC and WBC); calculation of agreement rates (best fit; ±1 adjacent range; overall) for semi-quantitative parameters; and calculation of sensitivity and specificity for semi-quantitative and qualitative parameters. For reference value estimation in samples from apparently healthy individuals, the mean and 97.5th and 99th percentile values were calculated for RBC and WBC using the cobas u 701 analyzer and KOVA visual microscopy.

Predefined acceptance criteria for validation of results are presented in Supplementary Material S3.

3. Results

3.1. cobas u 601

3.1.1. Precision

Repeatability and intermediate precision results for all parameters were within two adjacent concentration ranges, and 90% of negative samples were correctly identified, which is within the predefined acceptance limits (Table 1; Supplementary Material S4).

3.1.2. Carry-over

A total of 33 experiments were performed by assessing the sequence of high-positive and low-positive sample concentrations, of which 32 provided results within the acceptance criteria. For erythrocytes, carry-over was observed in the analysis of the raw data, but was not observed in the final results. Further statistical analysis of the estimated effect on a patient result showed a maximum possible deviation from negative to trace concentration range; thus, there is no relevant risk for patients.

3.1.3. Method comparison

In the correlation study, 1430 samples were measured at the three sites. Results were compiled to cover the concentration range with ≥60 measurements in each bin; 84 samples were spiked to cover the measurement range for ketones (n = 16; concentration range, 4+), bilirubin (n = 19; concentration range, 2+ [3 mg/dL] and 3+ [6 mg/dL]), urobilinogen (n = 17; concentration range, 3+ [8 mg/dL] and 4+ [12 mg/dL]), and urobilinogen/bilirubin (n = 32), as native samples did not cover the full measurement range. Samples were spiked

Table 1

21-day intermediate precision results for all parameters on the cobas u 601 module using quality control samples (Bio-Rad Liquichek Urinalysis Control).

Parameter	Result	Target range	SD [% remission]	Exact agreement [%]	Agreement within two adjacent ranges [%]
Bio-Rad Liquichek Urinalysis Control, Level 1					
BIL	Negative	Negative	0.6–0.7	100	100
ERY	Negative	Negative	0.5–1.4	100	100
KET	Negative	Negative	0.5–1.0	100	100
GLU	Normal	Normal	0.6–1.2	100	100
LEU	Negative	Negative	0.7–2.7	100	100
NIT	Negative	Negative	0.5–2.0	100	100
PRO	Negative	Negative	0.6–0.8	100	100
UBG	Normal	Normal	0.6–0.7	100	100
pH	6.5	5–6.5	0.5–0.6	76–100	100
Bio-Rad Liquichek Urinalysis Control, Level 2					
BIL	6 mg/dL	3–6 mg/dL	0.7	100	100
ERY	250 ERY/µL	150–250 ERY/µL	1.3–1.4	100	100
KET	150 mg/dL	50–150 mg/dL	0.6–2.2	100	100
GLU	1000 mg/dL	300–1000 mg/dL	0.4–0.7	100	100
LEU	500 LEU/µL	100–500 LEU/µL	1.4–1.9	100	100
NIT	Positive	Positive	0.7–2.3	100	100
PRO	150 mg/dL	150–500 mg/dL	0.5	75–100	100
UBG	12 mg/dL	8–12 mg/dL	0.8	100	100
pH	7	7–8	0.5–0.7	100	100

21-day precision experiments were performed at each site, with four measurements per day, for a total of 84 measurements per parameter per control per site; a total of 252 measurements per parameter were performed. The overall range of values from the three sites is shown.

BIL, bilirubin; ERY, erythrocytes and hemoglobin; GLU, glucose; KET, ketones; LEU, leukocytes; NIT, nitrite; PRO, protein; SD, standard deviation; UBG, urobilinogen.

according to internal standard procedures using the following materials: lithium acetate (A8609-5G; Sigma-Aldrich) for ketones; bilirubin (B4126-1G; Sigma-Aldrich); and urobilinogen (A1536; AppliChem). All results were within the defined acceptance limits (either for slope, intercept, and correlation coefficient, or for agreement rates).

The best-fit agreement for the compiled test strip results from all sites was ≥85% and overall agreement rates were ≥90%. Fig. 3 provides an overview of the four most important test strip parameters (according to European urinalysis guidelines [14]): erythrocytes, leukocytes, nitrites, and proteins. A summary of all other parameters is available in Supplementary Material S5. Deming regression analysis of results for specific gravity (cobas u 601 versus Urisys 2400) yielded a regression equation of $y = 1.04x - 0.0417$ ($n = 1334$; Pearson's $r = 0.995$).

3.2. cobas u 701

3.2.1. Precision

Repeatability was assessed in 141 experiments for all parameters using quality-control and human samples with different concentrations; all results met predefined acceptance criteria (Supplementary Material S6). Intermediate precision results for quantitative parameters (RBC and WBC) were within acceptance limits; SDs and CVs are presented in Table 2. Modified intermediate precision data for semi-quantitative parameters are shown in Table 3.

3.2.2. Recovery of semi-quantitative parameters

A total of 12 runs were performed, of which 11 provided good recovery of the different concentration ranges; representative data are shown for squamous epithelial cells in Fig. 4. There were two borderline results in the low-positive measurement for hyaline casts with values of 1.76 p/μL, which was just below the transition point of 2 p/μL (Fig. 4).

3.2.3. Carry-over

Carry-over was assessed in 36 experiments for quantitative and semi-quantitative parameters. There was no evidence of a potential carry-over effect and all results for quantitative parameters were within the acceptance criteria. A representative example of the carry-over testing for WBC and a summary of all the quantitative carry-over results are shown in Supplementary Material S7.

3.2.4. Method comparison

Bland-Altman difference plots for RBC and WBC counts measured using the cobas u 701 analyzer versus KOVA visual microscopy are shown in Fig. 5. Passing-Bablok regression analysis yielded the following correlation coefficients (Pearson's r) for measuring RBC counts: 0.95 ($n = 378$) between the cobas u 701 analyzer and KOVA visual microscopy (Fig. 5); 0.86 ($n = 383$) between the cobas u 701 analyzer and iQ200 analyzer; and 0.84 ($n = 444$) between the iQ200 analyzer and KOVA visual microscopy (Table 4). The following

ERY	cobas u 411				LEU	cobas u 411		
		Negative	Positive	Σ			Negative	Positive	Σ

ERY — cobas u 411 (cobas u 601)

	Negative	Positive	Σ
Negative	678	25	703
Positive	38	638	676
Σ	716	663	1379

Specificity	95%
Sensitivity	96%
Overall agreement rate	95%

LEU — cobas u 411 (cobas u 601)

	Negative	Positive	Σ
Negative	716	20	736
Positive	72	571	643
Σ	788	591	1379

Specificity	91%
Sensitivity	97%
Overall agreement rate	93%

NIT — cobas u 411 (cobas u 601)

	Negative	Positive	Σ
Negative	1027	18	1045
Positive	55	279	334
Σ	1082	297	1379

Specificity	95%
Sensitivity	94%
Overall agreement rate	95%

PRO — cobas u 411 (cobas u 601)

	Negative	Positive	Σ
Negative	744	41	785
Positive	35	559	594
Σ	779	600	1379

Specificity	96%
Sensitivity	93%
Overall agreement rate	94%

Fig. 3. Method comparison results between cobas u 601 and cobas u 411 analyzers for selected parameters. ERY, erythrocytes and hemoglobin; LEU, leukocytes; NIT, nitrite; PRO, protein.

Table 2

21-day intermediate precision results for determination of quantitative parameters (RBC and WBC) with the cobas u 701 module using quality control samples (Bio-Rad qUAntify Control).

Parameter	n	Target [p/µL]	Mean	Precision
Bio-Rad qUAntify Control, Level 1				
RBC	252	0–25	0.3–2.2	SD 0.6–2.5
WBC	252	0–25	0.0–0.0	SD 0.0–0.1
Bio-Rad qUAntify Control, Level 2				
RBC	252	220–659	435–479	CV 8.2–8.6%
WBC	252	139–418	250–268	CV 7.2–9.3%

21-day precision experiments were performed at each site, with four measurements per day, for a total of 84 measurements per parameter per control per site; a total of 252 measurements per parameter were performed. The overall range of values from the three sites is shown.
CV, coefficient of variation; RBC, red blood cells; SD, standard deviation; WBC, white blood cells.

Table 3

Modified intermediate precision results for determination of semi-quantitative parameters on the cobas u 701 module.

Parameter	Exact agreement [%]	Agreement within two adjacent ranges [%]
Negative samples		
BAC	100	100
HYA	100	100
NEC	95–100	100
SEC	100	100
Low-positive samples		
BAC	60–100	100
HYA	55–95	100
NEC	55–100	100
SEC	60–100	100
High-positive samples		
BAC	100	100
HYA	85–100	100
NEC	90–100	100
SEC	50–95	100

Due to low stability of analytes, a multi-day intermediate precision experiment was not possible. Instead, 20 measurements were made over five runs on 1 day on each sample. The overall range of values from the three sites is shown.
BAC, bacteria; HYA, hyaline casts; NEC, non-squamous epithelial cells; SEC, squamous epithelial cells.

correlation coefficients (Pearson's r) were demonstrated for measuring WBC counts: 0.96 ($n = 501$) between the cobas u 701 analyzer and KOVA visual microscopy (Fig. 5); 0.94 ($n = 515$) between the cobas u 701 analyzer and iQ200 analyzer; and 0.90 ($n = 507$) between the iQ200 analyzer and KOVA visual microscopy (Table 4). The equivalence shown between the cobas u 701 analyzer and KOVA visual microscopy for absolute number of RBC (slope, 0.89) and WBC (slope, 0.96) particles suggests metrological traceability of the cobas u 701 analyzer test results for these two parameters to the designated comparison method KOVA visual microscopy (Table 4; the pre-defined acceptance criterion was a slope between 0.8 and 1.2). In contrast, the iQ200 analyzer showed approximately 40% under-recovery of RBC counts versus KOVA visual microscopy (slope, 0.57; Table 4).

Correlation testing between the cobas u 701 analyzer and KOVA visual microscopy for semi-quantitative and qualitative parameters demonstrated sensitivities of 69.5–91.9% and specificities of 78.0–96.6% across the three sites ($n = 564$; Table 5). Good sensitivity rates for bacteria and pathological casts compared with KOVA visual microscopy were observed. The higher number of false-positive casts ($n = 46$) may lead to additional operator intervention; however, there were only 9 out of 55 false-negative results. Correlation between cobas u 701 and iQ200 analyzers for semi-quantitative and qualitative parameters was considerably lower, with sensitivities ranging from 43.8–93.1% and specificities from 73.8–94.5% across the three sites ($n = 584$; Table 5). In particular, the agreement between the two systems for detection of pathological casts (sensitivity 43.8%) and yeast (sensitivity 50.7%) was different. Comparison with manual microscopy highlights the advantage of the new fully automated system and limitations of the iQ200 analyzer for detecting these parameters.

3.2.5. Reclassification

A total of 115 reclassifications were performed across the three sites. The results obtained following reclassification were similar to the original results, confirming the quality of the automated measurements (Table 5). It should be noted that reclassification at one site improved the cobas u 701 analyzer's sensitivity for spermatozoa, due to the fact that some positive samples were not detected in the original measurement (images of these measurements were provided to Roche Diagnostics for further investigation).

3.2.6. Reference values

Of the 400 healthy urine samples included in the study, 395 were used for reference value estimation (five samples were incorrectly selected and were excluded). The 97.5% percentile upper reference limits (URLs) obtained on the cobas u 701 analyzer were 5.3 cells/µL
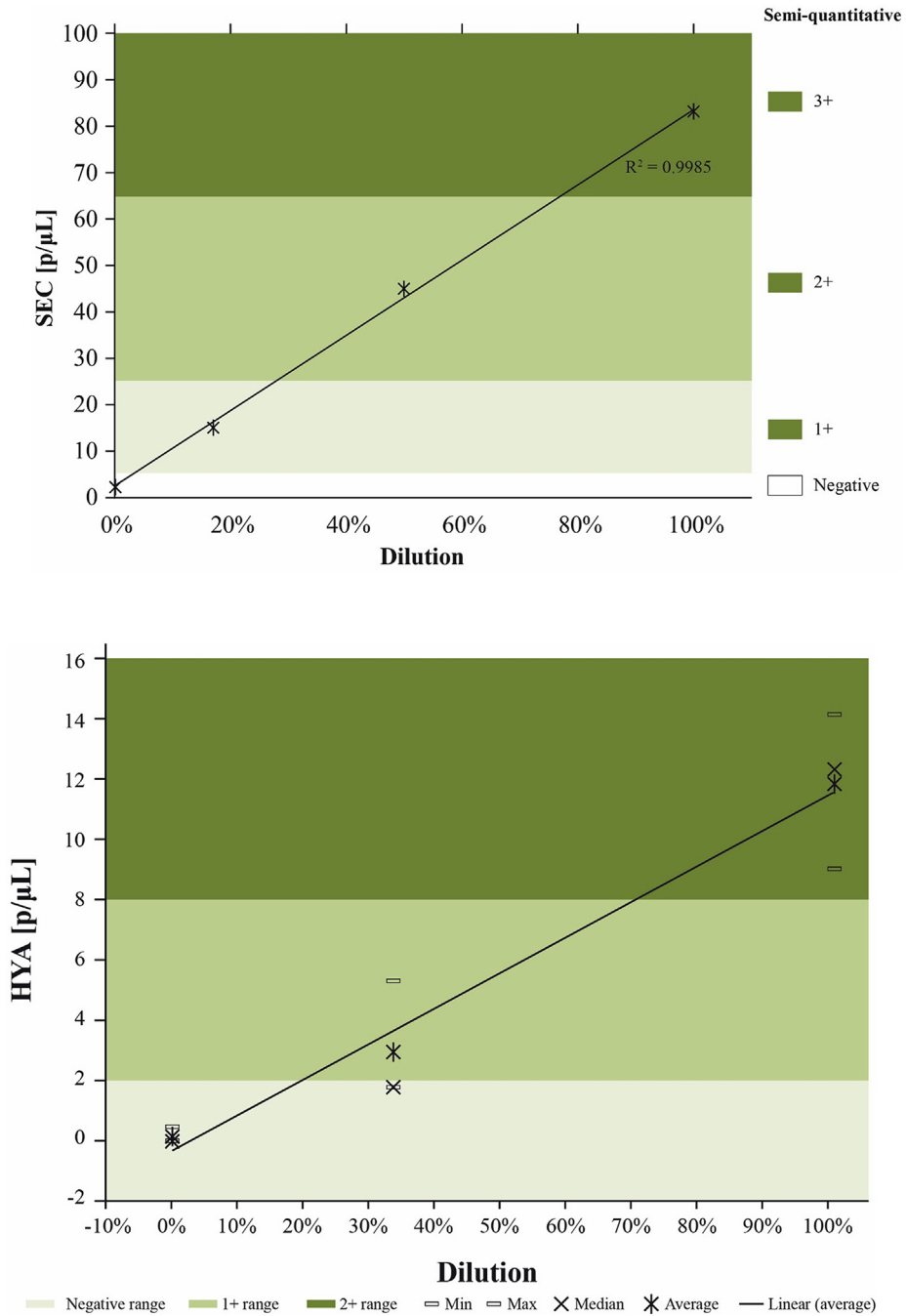
Fig. 4. Data from a dilution series linearity experiment for squamous epithelial cells; the average of three values is shown for each dilution (upper panel). The green shading demarcates the range borders from the semi-quantitative results. Hyaline recovery experiment data from site 1 (lower panel). For each dilution (100%, 33%, and 0%), the three obtained results are shown (minimum, median, and maximum) and the average. HYA, hyaline casts; SEC, squamous epithelial cells.

for RBC and 6.2 cells/μL for WBC; respective 99% percentile URLs were 6.2 cells/μL and 8.8 cells/μL. These results were in good agreement with 97.5% (RBC, 6.3 cells/μL; WBC, 6.3 cells/μL) and 99% (RBC, 7.5 cells/μL; WBC, 8.8 cells/μL) percentile URLs obtained from KOVA visual microscopy, and confirmed results previously reported in the literature [47,48].

3.3. cobas 6500

Equivalency testing (precision, carry-over, and concordance experiments) was performed after assembling the two standalone cobas
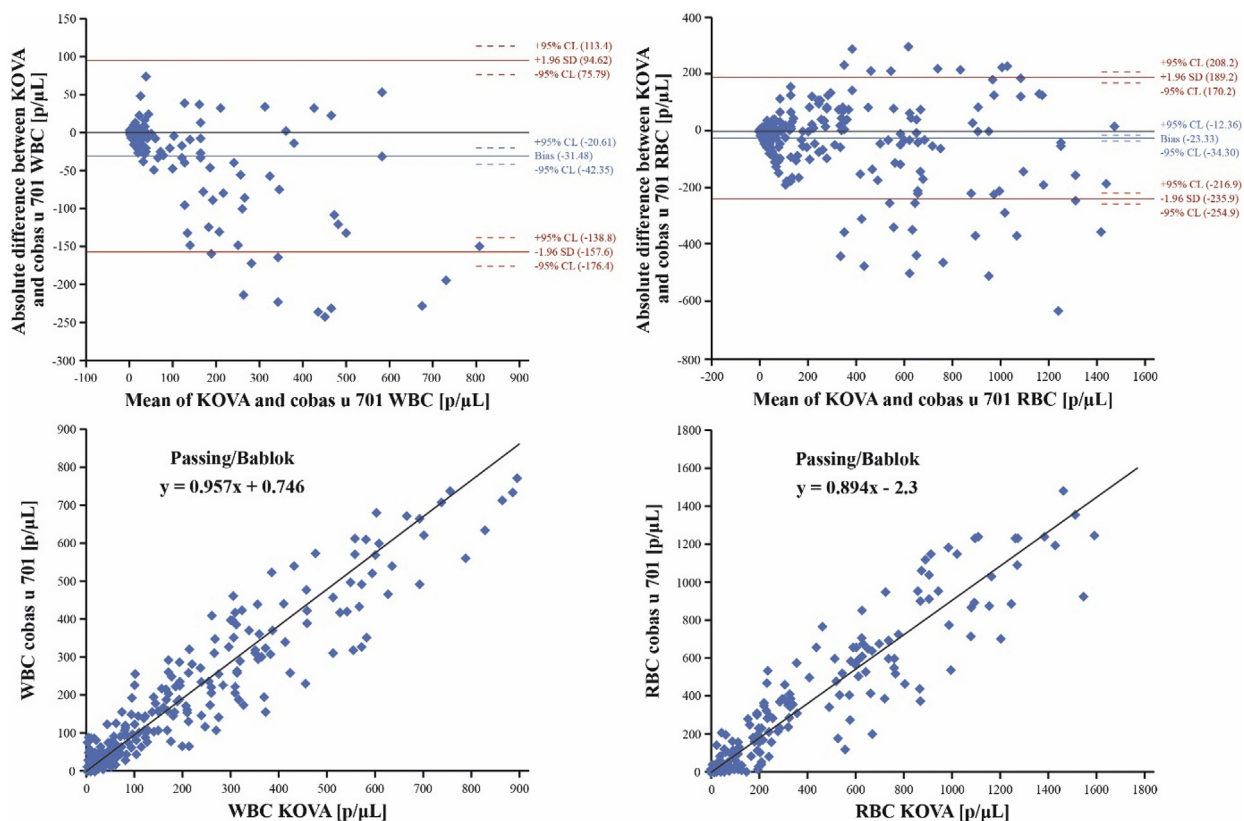
Fig. 5. Bland-Altman difference plots (upper panels) and Passing-Bablok regression plots (lower panels) for RBC and WBC counts measured using the cobas u 701 analyzer versus KOVA visual microscopy. RBC, red blood cells; WBC, white blood cells.

Table 4
Passing-Bablok regression analysis of RBC and WBC counts measured using the iQ200 analyzer versus KOVA visual microscopy, the cobas u 701 analyzer versus KOVA visual microscopy, and the cobas u 701 analyzer versus the iQ200 analyzer.

Comparison		n	Passing-Bablok regression			
x axis	y axis		Slope	Intercept	Pearson's r	Kendall's Tau
RBC						
KOVA visual microscopy	iQ200 analyzer	444	0.57	1.55	0.84	0.60
KOVA visual microscopy	cobas u 701 analyzer	378	0.89	−2.30	0.95	0.75
iQ200 analyzer	cobas u 701 analyzer	383	1.57	−5.88	0.86	0.65
WBC						
KOVA visual microscopy	iQ200 analyzer	507	0.87	1.82	0.90	0.67
KOVA visual microscopy	cobas u 701 analyzer	501	0.96	0.75	0.96	0.76
iQ200 analyzer	cobas u 701 analyzer	515	1.16	−1.65	0.94	0.74

RBC, red blood cells; WBC, white blood cells.

u 601 and cobas u 701 systems to the cobas 6500 urine work area. Results for repeatability and carry-over on the combined cobas 6500 platform were in good agreement with those obtained for the standalone analyzers.

Concordance between the cobas u 601 and cobas u 701 analyzers was 82% for WBC and 76% for RBC. The positive predictive value for nitrite/bacteria was 94.4% (95% confidence interval [CI] 89.9–97.3) and negative predictive value for protein/casts was 97.2% (95% CI 95.1–98.6). It should be noted that these numbers were obtained during the technical assessment of the cobas 6500 system functionality, but did not include a detailed clinical assessment of the samples.

3.3.1. Usability and practicality

Claimed throughputs of 240 samples per hour on the cobas u 601 analyzer and 116 samples per hour on the cobas u 701 analyzer/ cobas 6500 urine work area were confirmed.

Questionnaires on usability and functionality were completed by the 10 operators involved in the study (ranking scale: 1 [very poor] to 5 [excellent]). The average rating was 4.0 for the cobas u 601 analyzer and 4.1 for the cobas u 701 analyzer; the average rating for routine urinalysis systems currently in use was 3.3. Automated workflow showing high result quality, ease-of-use of the interface,

availability of maintenance wizards, and system design were rated particularly highly by operators. Regarding the cobas u 701 analyzer, operators liked that the imaging display allowed online, retrospective visual inspection and further differentiation of complex urine specimens. Other functionalities—such as sieve criteria, result interpretation rules, and throughput—fulfilled users' expectations of a modern urinalysis system. Operators also highlighted the possibility of using different working modes, and the ability for defining cross-check rules, on the cobas 6500 urine work area. In future software updates, operators commented that they would like to see further subclassification functionality for the recognition of dysmorphic RBC and amorphous salts, as well as the provision of a results library.

4. Discussion

The present multicenter study demonstrates that automated urinalysis can be performed at high throughput in a precise and accurate manner across multiple sites and for all test parameters. The new cobas 6500 system enables reliable detection of bacteria, yeast, and pathological casts, and consistently shows higher sensitivity (with similar specificity) versus that of the iQ200 analyzer, as demonstrated in the present study and in previous reports [11,37]. Thus, the cobas 6500 system meets the sensitivity criteria for these parameters, which have been postulated to be at least 80% [37]. The high sensitivity for pathological casts is particularly important for the early detection of acute kidney injury [11]. The cobas 6500 system also demonstrated excellent precision across laboratories, with within-individual CVs (CVi) for 21-day intermediate precision consistently below 10%. This is better than the between-laboratory CVs shown for manual microscopy in a College of American Pathologists survey, in which the inter-operator CVi was found to be 21–42% and 15–58% for RBC and WBC counts, respectively [49,50], and is similar to standard-of-care physicochemical analysis [51]. Clinically acceptable performance criteria for urinalysis thus need to reflect current standard-of-care performance estimates [52]. Further improvements in the performance of automated urine analyzers are likely to occur with the development of new techniques and subsequent updates to College of American Pathologists criteria, as observed in other disease areas (e.g., performance criteria for hemoglobin A_{1c}) [53]. For example, current precision goals for urinary protein content have been postulated to be CVi 20% (optimal) and CVi 25% (desirable) [54].

The cobas 6500 system provides a precise and reliable tool for complete urinalysis with a throughput of 116 samples per hour on the cobas u 701 analyzer, which is faster than other automated techniques and manual microscopy [43]. The throughput for the standalone cobas u 601 analyzer is 240 samples per hour. Our findings complement previous studies, which have shown that the cobas 6500 system can reduce turnaround time and workload [43], and that the cobas u 701 analyzer can be used for rapid screening for UTI [55].

When evaluating new medical tests, it is important to first define the unmet clinical need that the test is aiming to address, as well as the test purpose and role in the clinical care pathway [56,57]. Urinalysis is an integral part of clinical practice, providing important information on the functioning of renal/urogenital and other organ systems, and is a vital part of the diagnostic test menu in clinical laboratories [1–13]. Urinalysis is generally used as a screening test; however, manual microscopy of urine sediment requires a high level of expertise, and is time and labor intensive to perform [25]. In addition, many clinical laboratories are experiencing ever-increasing demands on their workloads, often in the context of limited resources and tighter financial constraints [58–60]. As such, there is a need to improve the efficiency and cost-effectiveness of urinalysis tests, with a focus on increasing sample throughput, ease-of-use, and turnaround time, while maintaining the accuracy and reproducibility of results. These factors may also improve patient care by decreasing the time between testing and result availability, thereby reducing the delay in treatment initiation or hospital discharge.

The cobas 6500 urine work area aims to address these clinical needs by integrating the physicochemical and microscopic analysis of urine, and eliminating inter-operator variability, increasing sample throughput, and reducing resource demands on laboratory staff. Furthermore, the modular nature of the cobas 6500 system provides alternative options for users in different laboratory settings with differing needs. For example, the combined platform may be particularly suitable for use in laboratories with a pre-analytical phase that does not allow urine samples to be analyzed within 2 h (where the ability to cross-check with the urine test strip analyzer may be essential), while the cobas u 701 analyzer could be used as a standalone screening analyzer in hospital settings that have full track-and-trace on biospecimens and require rapid availability of results within guaranteed turnaround times. Importantly, the cobas 6500 platform enables the rapid and reliable screening of four key urine parameters (RBC, WBC, bacteria, and protein) in both routine primary care and academic settings. According to European urinalysis guidelines [14], a high analytical sensitivity is required for urine screening of these four parameters to avoid false-negative results, while also aiming to limit false positives. It should be noted that there were some discrepancies between the cobas u 601 and cobas u 701 analyzers, which could be due to the different methodologies used. For example, lysed cells can still be detected by test strip analysis while no longer being visible in the microscopy images [61]. Also, detection of nitrite-negative bacteria can only be achieved by microscopic analysis [61]. However, the combination of both methodologies in the cobas 6500 urine work area ensures optimal identification and information for the operator.

The cobas u 701 analyzer is based on a method principle similar to that of manual microscopy and uses two-dimensional images to recognize urine sediment particles, but may miss some particles in high-density samples compared with manual microscopy. In our study, the cobas u 701 analyzer demonstrated acceptable repeatability and intermediate precision. The analyzer's pipetting functionality, with three aspirating and rinsing cycles to ensure optimal sample mixing, likely contributed to the good precision observed. Although there were two borderline recovery results in the low-positive measurement for hyaline casts on the cobas u 701 analyzer, any comparison from continuous to discrete values may provide false allocations, due to typical imprecision of primary data. From a clinical perspective, hyaline casts are not thought to be indicative for any disease process, as increased numbers can be seen in healthy, concentrated urine specimens (0–5 particles per low-power field is commonly defined as the reference value of hyaline casts and can be considered physiological) [62].

The ability of the cobas u 701 analyzer to screen for certain urine parameters, such as casts, crystals, and dysmorphic RBC, remains limited and further analysis by manual microscopy is required for accurate identification of clinically relevant urine particles in complex

Table 5

Sensitivity and specificity of all cobas u 701 parameters when using the iQ200 analyzer or KOVA visual microscopy as a predicate device, and comparison of the iQ200 analyzer versus KOVA visual microscopy.

Parameter	cobas u 701 vs KOVA		cobas u 701 vs iQ200		iQ200 vs KOVA		cobas u 701 reclassification vs KOVA	
	Specificity [%]	Sensitivity [%]	Specificity [%]	Sensitivity [%]	Specificity [%]	Sensitivity [%]	Specificity [%]	Sensitivity [%]
BAC	78.0	89.3	*	*	*	*	78.0	89.7 ↑
NEC	82.4	73.8	73.8	82.1	95.9	39.5	82.7 ↑	74.3 ↑
SEC	91.2	91.9	87.5	93.1	92.4	80.2	90.1 ↓	91.9
HYA	96.6	82.0	90.8	88.2	98.8	18.0	97.2 ↑	82.0
PAT	91.0	83.6	84.7	43.8	98.0	10.9	94.5 ↑	78.2 ↓
CRY	93.0	83.8	91.0	88.7	98.3	67.5	96.3 ↑	87.5 ↑
YEA	94.5	86.5	94.1	50.7	91.3	73.0	95.6 ↑	89.2 ↑
MUC	86.8	69.5	82.5	84.4	96.0	36.4	86.8	69.2 ↓
SPRM	96.3	73.3	94.5	76.5	98.9	32.4	97.7 ↑	76.5 ↑
n = 564								

Arrows indicate whether reclassification based on the cobas u 701 results changed the sensitivity or specificity. *Bacteria were not assessed on the iQ200 analyzer.

BAC, bacteria; CRY, crystals; HYA, hyaline casts; MUC, mucus; NEC, non-squamous epithelial cells; PAT, pathological casts; SEC, squamous epithelial cells; SPRM, sperm; YEA, yeast.

samples [37,63–70]. Importantly, the cobas system facilitates the re-evaluation of complex samples by enabling visual review of the automated images. In unclear cases, the images are flagged and the operator can evaluate the stored images manually. Images in which bacteria of 1 μm size (e.g. Gram-positive cocci) have been automatically identified are also stored for later manual inspection (Supplementary Material S8), as these bacteria can be difficult to identify with other automated solutions [71]. This has been shown to reduce the requirement for conventional manual microscopy, compared with other automated urine analyzers, and thereby reduce operator workload and turnaround time [43]. This feature may be of particular relevance in academic centers and teaching hospitals, where higher proportions of complex urine samples with abnormal particles may be encountered compared with non-academic centers. Rapid response times for urinalysis are also essential for efficient collaboration with microbiology departments in order to guide decisions on patient management; up to 60% of urine cultures can be avoided with early recognition (within 10–15 min) of a negative urine screen in patients with suspected UTI [72]. Finally, there was good agreement for the quantitative determination of sample WBC content between the cobas u 701 module, iQ200 system and KOVA visual microcopy. However, our results suggest that the iQ200 system underestimated RBC content of samples, compared with the cobas u 701 analyzer and KOVA visual microscopy.

Our study was conducted at three European centers to ensure a good representation of normal and pathological urine samples. However, the performance of urine analyzers may differ depending on the patient cohort under investigation and so should be evaluated in the intended-use population. For example, the complexity of urine samples may be different in a primary care setting versus a specialist care setting.

In conclusion, we have shown that the fully automated cobas 6500 urine work area meets the analytical quality specifications recommended by European urinalysis guidelines [14], produces results comparable to those obtained by manual microscopy, and is suitable for routine clinical laboratory use. Furthermore, we confirmed the excellent performance of the individual modular subsystems (cobas u 601 and cobas u 701 analyzers), which provide alternative test options for users with different clinical needs. The claimed throughputs for the combined cobas 6500 platform and each subsystem were confirmed, and overall usability was rated higher than for routine systems in current use.

Author contributions

All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Conflict of interest

Christa M. Cobbaert and Antonio Carmona-Fernández declare support for research and travel expenses and speaker honoraria from Roche Diagnostics, but no economic compensation for the study. Figen Arslan declares support for research and travel expenses from Roche Diagnostics, but no economic compensation for the study. Imma Caballé Martín, Antoni Alsius Serra, and Ester Picó-Plana declare support for research from Roche Diagnostics, but no economic compensation for the study. Víctor Sánchez-Margalet declares support for research from Roche Diagnostics, but no compensatory payment nor relationship with the company. John Burden, André Ziegler, and Walter Bechel are employees of Roche Diagnostics and hold shares in F. Hoffmann-La Roche Ltd.

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Appendix A. Supplementary data

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Transparency document

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