Abstract

Introduction: The cobas m 511 integrated hematology analyzer conducts a complete blood count (CBC), white blood cell (WBC) differential, reticulocyte count, and nucleated red blood cell count using automated digital microscopy. This multicenter study validated the analytical performance of the cobas m 511 system.

Methods: Repeatability, reproducibility, carryover, mode-to-mode comparison, cytomorphology, WBC clinical sensitivity, and method comparison were analyzed at four clinical sites using residual whole blood clinical samples (n = 2546) and fresh whole blood from healthy volunteers (n = 480). For WBC clinical sensitivity, the cobas m 511 system automated CBC and WBC differential, system flags, cobas m 511 images, and stained cobas m 511 slides were compared with manual microscopy. Sysmex® XN analyzers were used for interinstrument method comparison.

Results: Repeatability and reproducibility results showed low variability. There was no significant sample carryover and no difference between open/closed modes. The overall percentage agreement of morphology assessments with manual microscopy (n = 163 samples) was 95.6% for cobas m 511 images and 95.7% for cobas m 511 slides. The sensitivity and specificity for detecting distributional and/or morphological abnormalities were 94.4% and 74.6% for cobas m 511 automated differential, and 95.9% and 73.3% for cobas m 511 image assessment, compared with a manual 400-cell reference differential (n = 439 samples). Some discordance was seen for monocytes and basophils. Correlations between cobas m 511 and Sysmex XN system data were very good (Pearson's R ≥ 0.95 for most CBC parameters).

Conclusion: The cobas m 511 system performs robustly in the clinical laboratory and is suitable for routine clinical use.

KEYWORDS
cobas m 511 system, digital morphology, integrated hematology analyzer, microscopy, multicenter evaluation
1 | INTRODUCTION

Current automated hematology analyzers for the quantitative analysis of complete blood counts (CBC), white blood cell (WBC) differentials, reticulocytes, and nucleated red blood cell (NRBC) counts are methodically based on electrical impedance and optical or fluorescence flow cytometry.\(^1\) Abnormal cases are flagged for review, necessitating preparation of a blood smear and a manual microscopic morphological differential count to identify the abnormalities.\(^2\) As manual microscopy is time consuming, requires experienced medical staff, and is subject to significant variance, automated digital imaging systems were developed to address these issues for routine hematology diagnostics.\(^3\)\(^4\)\(^6\)

The cobas m 511 integrated hematology analyzer (Roche Diagnostics Operations Inc., Boston, MA, USA) combines a slide maker, slide stainer, digital image-based cell locator, cell counter, and cell classifier in one system. Unlike existing impedance- or flow cytometry-based automated hematology analyzers, all analyses with the cobas m 511 system are performed using microscope slides. Slides are prepared using a precision printing method from ethylenediaminetetraacetic acid (EDTA)-anticoagulated whole blood and stained automatically.\(^7\) The CBC, automated differential, reticulocyte, and NRBC counts are performed using digital morphologic analysis. Stained cells are classified, enumerated, and analyzed using computer image analysis, providing an image-based assessment of cell type and morphology with flags for abnormalities. A viewing station enables manual verification and reclassification of the results, and the classification of unclassified abnormal WBCs.

This study validated the analytical performance of the cobas m 511 integrated hematology analyzer in a multicenter clinical laboratory setting.

2 | MATERIALS AND METHODS

The study was conducted from June 2016 to January 2017. Sites started at different timepoints and the study duration at each site averaged 14 weeks including the reviews of the slides and images.

2.1 | Instrumentation and instrument setting

A nominal 1 μL of EDTA-anticoagulated whole blood was automatically printed on DigiMAC3\(^8\) slides (Roche) by the cobas m 511 system using Bloodhound\(^9\) technology. Slides were stained automatically with a modified Romanowsky stain and with a supravital stain on a separate slide for analysis of reticulocytes. These stains were modified for high-speed application and provide consistency across all cobas m 511 systems. The system then digitally identified and counted the red blood cells (RBCs), WBCs, platelets (PLTs), and NRBCs on the microscope slide using low-magnification (×10) multispectral imaging. Randomized locations of between 600 and 700 WBCs were recorded, imaged using high-magnification (×50) multispectral imaging, and an automated WBC differential performed using computer algorithms. Unclassified cells that did not fall into the five normal WBC types were flagged and presented in the viewing station for review. RBC and PLT indices were also measured during the high-magnification analysis.

The cobas m 511 system was evaluated at two European (Department of Clinical Chemistry, Erasmus MC, University Medical Center, Rotterdam, the Netherlands; Institute of Laboratory Medicine, University Hospital, LMU Munich, Germany) and two US sites (TriCore Reference Laboratories, Albuquerque, NM, USA; Virtua Hospital, Voorhees, NJ, USA) sites. Sysmex XN analyzers (XN-10, XN-20; Sysmex, Kobe, Japan) were used for interinstrument method comparison. Default settings for test and flagging algorithms were used for all cobas m 511 systems.

2.2 | Samples and evaluation protocol

Samples were comprised of residual whole blood clinical samples (n = 2546 in total) and fresh whole blood samples from healthy volunteers (n = 480 in total). Sample totals were obtained from collecting approximate equal proportions from the four clinical sites. Residual whole blood samples were collected randomly or selected based on specific laboratory results. These samples were provided with birth year, sex, sample draw time, and laboratory results from the comparative instrument, when required. Fresh whole blood samples were collected from healthy volunteers after they had signed an informed consent and completed a questionnaire to verify health status. These volunteers were males and females aged ≥18 years, and sample collection was in accordance with the Clinical Laboratory Standards Institute (CLSI) EP28-A3c guideline.\(^8\) All samples were collected in standard K2-EDTA collection tubes (Sarstedt, Nümbrecht, Germany; Becton Dickinson, Franklin Lakes, NJ, USA) and processed within 8 hours of venipuncture. Standard blood smears were prepared according to site-specific methods within 2 hours of running on automated analyzers. Dedicated study coordinators at each site performed all instrument-related analytical evaluations and managed the image and slide reviews by study technologists.

The study protocol was approved by each site’s Ethics Committee or Institutional Review Board.

The analytical performance assessments of the cobas m 511 system comprised: whole blood repeatability, reproducibility using DigiMAC3\(^8\) controls (Roche), carryover, mode-to-mode comparison, morphology, WBC clinical sensitivity, and method comparison (Table 1).

2.3 | Whole blood repeatability

Repeatability evaluated within-run precision using whole blood samples according to the CLSI EP05-A3 guideline\(^2\) and CLSI H26-A2 standard.\(^10\) The repeatability of reticulocyte (RET) related parameters (%RET, #RET, RET-HGB) was not evaluated.

Overall, 144 residual whole blood samples were selected for WBC, RBC, hemoglobin (HGB), and PLT parameters at targeted low, middle, and high ranges generally encountered in the laboratory. Forty-eight samples at medical decision levels for anemia (n = 12),
thrombocytopenia (n = 12), severe leukopenia (n = 12), and elevated NRBCs (n = 12) were also evaluated. Samples were processed 31 consecutive times over 35 minutes on the cobas m 511 system. The mean, standard deviation (SD), and coefficient of variance (%CV) were calculated for each sample separately, and the range and mean of the samples means, and repeatability derived by a mixed model analysis, were determined for each parameter.

2.4 Reproducibility

Reproducibility of results was determined using three levels of DigiMAC3® quality control materials in a multiday assessment according to CLSI EP05-A3 guideline. The same lot was used at each site to minimize lot-to-lot bias.

The assessment used a 4 × 5 × 2 × 3 design, that is four clinical sites, 5 days, two runs per day, and three replicates per run. Within-run precision (repeatability), between-run precision (same day), between-day precision, between-laboratory precision, and reproducibility (total precision) were calculated. For each parameter and control level, the mean, SD, and %CV of the components of precision were determined (with 95% CI of the SD and %CV for repeatability and reproducibility). Low SD and %CV results are indicative of good reproducibility.

2.5 Carryover

Carryover was assessed at each site for high target value (HTV) samples containing high numbers of WBCs, RBCs, and PLTs according to CLSI H26-A2 standard. Samples with high numbers of blasts were also assessed. The HTV samples were characterized by WBC counts ≥90 × 10⁹/L, RBC counts ≥6.20 × 10¹²/L, and PLT counts ≥900 × 10⁹/L. The blast samples had WBC counts ≥20 × 10⁹/L and ≥30% blast percentage. One of these four sample types was assessed on each test day: Three HTV replicates were run on the cobas m 511 system followed immediately by three low target value (LTV) replicates (filtered serum). There was a minimum of 1-day separation between the test days for each different sample type. The assessment resulted in 12 samples per site (including three blast samples per site) and 48 samples combined from all sites. Individual site results were averaged by sample type. By design, the cobas m 511 system rejects a LTV sample at the low-magnification imaging stage due to insufficient cells (less than approximately 0.4 × 10⁹/L). Rejected cobas m 511 slides from all LTV samples from all sites were retrieved and assessed microscopically by a single hematology expert for evidence of carryover, determined for each cell type:

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\text{Carryover\%} = \frac{\text{total # cells of interest on serum slides (cobas m 511 slide results)}}{\text{# cells per µL in high taget sample (Sysmex XN result)}} \times 100\%
\]
2.6 Mode-to-mode comparison

Mode-to-mode evaluations assessed whether consistent results were obtained when using the closed (automated) and open (manual) modes on the cobas m 511 system.

From all sites combined, 145 random samples and sixteen leukopenic samples were collected (n = 161 in total). Bias between the closed and open mode results was assessed per the CLSI EP09-A3 guideline. Bias was calculated at high and low critical bias limits defined either at generally recognized medical decision points or at the extremes of the reference interval. If a parameter had both an absolute and a proportional bias limit, the bias was calculated at the crossover point where they were equal. A Passing-Bablok regression model was used for most parameters, except those with many valid results of zero (i.e., %EO, #EO, %BASO and #BASO) in which case a Deming regression model was used. The #NRBC parameter had a limited number of valid, nonzero results, so bias was calculated as the mean difference between open and closed modes, and no regression lines were estimated with CI derived using a paired t test. %NRBCs were excluded from this analysis due to very low prevalence.

2.7 Morphology

Cellular morphology was assessed to ensure that morphological characteristics that were present on blood smears could be identified by a trained technologist using the matched cobas m 511 images and slides.

From all sites combined, 163 residual blood samples were collected and processed on the cobas m 511 system. The cobas m 511 images, cobas m 511 slides, and the corresponding blood smear from each sample were randomized and split between two technologists at each site. Each technologist performed a 100-cell differential with morphology review on the set of cobas m 511 images, cobas m 511 slides, and blood smears from each sample to minimize inter-reviewer bias. Overall agreement for each morphological characteristic with the 100-cell reference differential was calculated for each test method (cobas m 511 images or slides) using the total number of results that agreed (A) or disagreed (D) within ± one grade of the blood smear result:

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\text{Agreement (\%)} = \frac{A_{\text{total}}}{A_{\text{total}} + D_{\text{total}}} \times 100\%
\]

2.8 WBC clinical sensitivity

Consistent with the CLSI H20-A2 standard, WBC clinical sensitivity was assessed in two parts. Part one determined method-specific reference ranges for the cobas m 511 automated differential, images, and slides using 480 samples from normal healthy donors. Part two investigated the sensitivity, specificity, and efficiency of the cobas m 511 system for detecting distributional and morphological abnormalities in 439 mostly abnormal residual samples (granulocytosis with left shift, monocytosis, eosinophilia, lymphocytosis, atypical/reactive/variant lymphocyte forms, lymphopenia, immature granulocytes, blasts, and NRBCs) compared with a manual 400-cell reference differential (combined from two technologists). The goal was to determine whether the cobas m 511 automated WBC differential, system messages, cobas m 511 images, and cobas m 511 slides generated results that were consistent with the manual microscopy. The primary review mechanism for the cobas m 511 system is the images; however, the cobas m 511 slides were also studied to ensure they could be used, if needed, for additional review by the laboratory.

Two qualified technologists at each site each reviewed one-half of the cobas m 511 images and cobas m 511 slides for WBC differentials. Three corresponding blood smears per sample were also produced for the reference differential. Both technologists performed a 200-cell WBC differential on one of these smears and combined their results to create the 400-cell reference differential. A third technologist or laboratory physician with special knowledge in hematology microscopy reviewed the third blood smear if the difference in results of the two technologists exceeded the 99% Fisher exact limits. In these cases, the two results which were closest in agreement were used to derive the 400-cell reference differential. Technologists were blinded to the matching of the cobas m 511 images, cobas m 511 slides, and blood smears during the WBC clinical sensitivity analyses.

For the distributional abnormalities, the five normal WBC types from the cobas m 511 automated differential, images, and slides were evaluated vs method-specific reference ranges established in this study in normal healthy donors consistent with the CLSI H20-A2 standard. Results exceeding the upper and lower limits of the reference range were considered abnormal (positive). The cobas m 511 system messages, images, and slides were also reviewed against the 400-cell reference differential for morphological abnormalities, comprising immature and/or abnormal WBCs. Results exceeding a predefined threshold were considered abnormal (positive). Thresholds for the reference method were blasts >1%, variant lymphocytes >10% (containing lymphocytes suspected to be reactive and to be malignant), immature granulocytes >2%, and left shift (band neutrophils) >5%.

It was expected that distributional and morphologic abnormalities seen on the cobas m 511 system should also be present on the corresponding reference 400-cell reference differential. The sensitivity, specificity, and efficiency of the different cobas m 511 system modalities compared with the 400-cell reference differential were calculated.

2.9 Method comparison

Method comparison assessed correlation and bias of results obtained on the cobas m 511 system vs the Sysmex XN-10 or XN-20 automated hematology analyzer. Sample processing was randomized and occurred over 2 weeks to minimize sampling bias and to capture the routine populations of each laboratory. From all sites combined, 1591 residual whole blood samples were analyzed. Correlation and bias between results were determined according to CLSI EP09-A3 guideline using either a Passing-Bablok or Deming regression model as described for the mode-to-mode comparison.
2.10 | Data analysis and statistics

Microsoft Excel 2013 (Microsoft Corp., Redmond WA, USA) and r statistical software version 3.3.1 (The R Foundation for Statistical computing Vienna, Austria) were used for the statistical analysis of anonymized data.

The cobas m 511 system reports 26 parameters. For the method comparison and mode-to-mode assessments, only results that were valid from both assessments (instruments or modes) were used in the statistical analysis. If one or more individual parameters were considered invalid, the remaining valid parameters were reported and included in the dataset. All results and conclusions are from combined data from all four clinical sites unless stated otherwise.

3 | RESULTS

3.1 | Whole blood repeatability

The cobas m 511 system demonstrated high repeatability for most of the testing parameters (Table 2), with relatively lower repeatability seen for basophil and NRBC counts.

### TABLE 2 Whole blood repeatability results

| Parameter (units) | Sample range | Samples/observations (N/N) | Range of sample means | Repeatability %CV (95% CI) |
|-------------------|--------------|-----------------------------|-----------------------|---------------------------|
| WBC (10⁹/L)       | All          | 144/4436                    | (1.98, 130.75)        | 1.93 (1.89, 1.97)         |
|                   | <4.0 × 10⁹/L | 17/520                      | (1.98, 3.95)          | 3.01 (2.84, 3.21)         |
|                   | ≥4.0 × 10⁹/L | 127/3916                    | (4.00, 130.75)        | 1.85 (1.81, 1.90)         |
| RBC (10¹²/L)      | All          | 144/4436                    | (1.92, 6.40)          | 0.84 (0.82, 0.86)         |
| HGB (g/dL)        | All          | 144/4436                    | (5.98, 20.26)         | 1.09 (1.06, 1.11)         |
| HCT (%)           | All          | 144/4436                    | (18.39, 65.15)        | 0.99 (0.97, 1.01)         |
| MCV (fL)          | All          | 144/4436                    | (66.36, 109.80)       | 0.65 (0.63, 0.66)         |
| MCH (pg)          | All          | 144/4436                    | (20.16, 36.19)        | 0.62 (0.61, 0.64)         |
| MCHC (g/dL)       | All          | 144/4436                    | (30.22, 34.47)        | 0.43 (0.42, 0.44)         |
| RDW (%)           | All          | 144/4436                    | (12.23, 27.18)        | 1.82 (1.78, 1.86)         |
| RDW-SD (fL)       | All          | 144/4436                    | (40.26, 83.41)        | 1.72 (1.68, 1.76)         |
| PLT (10⁹/L)       | All          | 143/4405                    | (14.74, 936.84)       | 2.73 (2.67, 2.79)         |
|                   | <150 × 10⁹/L | 31/950                      | (14.74, 149.61)       | 3.24 (3.10, 3.40)         |
|                   | ≥150 × 10⁹/L | 112/3455                    | (152.71, 936.84)      | 2.59 (2.53, 2.65)         |
| MPV (fL)          | All          | 141/4347                    | (8.76, 14.91)         | 1.58 (1.55, 1.62)         |
| #NRBC (10¹²/L)    | All          | 144/4436                    | (0.00, 0.132)         | SD: 0.009 (0.009, 0.009)  |
|                   | <0.25 × 10⁹/L| 142/4374                    | (0.00, 0.20)          | SD: 0.007 (0.007, 0.007)  |
|                   | ≥0.25 × 10⁹/L| 2/62                        | (0.28, 1.32)          | SD: 0.048 (0.041, 0.059)  |
| #NEUT (10⁹/L)     | All          | 142/4365                    | (0.87, 49.70)         | 3.08 (3.02, 3.15)         |
| #LYMPH (10⁹/L)    | All          | 142/4365                    | (0.21, 116.87)        | 7.52 (7.36, 7.68)         |
| #MONO (10⁹/L)     | All          | 142/4365                    | (0.03, 7.32)          | SD: 0.132 (0.129, 0.135)  |
| #EO (10⁹/L)       | All          | 142/4365                    | (0.00, 0.87)          | SD: 0.050 (0.049, 0.051)  |
| #BASO (10⁹/L)     | All          | 142/4365                    | (0.00, 0.25)          | SD: 0.028 (0.028, 0.029)  |
| WBC (10⁹/L)       | All (leukopenic samples) | 12/371 | (0.21, 2.03) | 4.51 (4.21, 4.87) |
| RBC (10¹²/L)      | All (anemia samples) | 12/371 | (2.33, 3.78) | 0.81 (0.76, 0.87) |
| HGB (g/dL)        | All          | 12/371                      | (7.58, 9.96)          | 1.14 (1.06, 1.23)         |
| HCT (%)           | All          | 12/371                      | (22.95, 31.24)        | 1.06 (0.98, 1.14)         |
| PLT (10⁹/L)       | All (thrombocytopenia samples) | 12/372 | (3.48, 51.55) | 6.96 (6.48, 7.50) |
| #NRBC (10¹²/L)    | All (NRBC samples) | 12/366 | (0.40, 39.19) | 1.90 (1.77, 2.05) |

# count; BASO, basophils; CI, confidence interval; CV, coefficient of variance; EO, eosinophils; HCT, hematocrit; HGB, hemoglobin; LYMHPH, lymphocytes; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MONO, monocytes; MPV, mean platelet volume; NEUT, neutrophils; NRBC, nucleated red blood cells; PLT, platelets; RBC, red blood cells; RDW, red cell distribution width; RDW-SD, red cell distribution width standard deviation; SD, standard deviation; WBC, white blood cells.

*Only samples with ≥20 × 10⁹/L PLT are used for calculation of repeatability of MPV.

*Only samples with ≥2.0 × 10⁹/L WBC are used for calculation of repeatability of differential parameters.

Data for percentage parameters are not shown. Data are from all sites combined.
### TABLE 3  Clinical sensitivity analysis of white blood cells

| Sample size (N) | TP  | FP  | FN  | TN  | Sensitivity (%) | Specificity (%) | Efficiency (%) |
|----------------|-----|-----|-----|-----|----------------|-----------------|---------------|
| **cobas m 511 automated differential** |     |     |     |     |                |                 |               |
| Any distributional abnormality or combination of distributional abnormalities | 355\(^a\) | 272 | 17  | 16  | 50             | 94.4            | 74.6          | 90.7          |
| **cobas m 511 images** |     |     |     |     |                |                 |               |
| Any morphological or distributional abnormality, or combination thereof | 426 | 351 | 16  | 15  | 44             | 95.9            | 73.3          | 92.7          |
| **cobas m 511 slides** |     |     |     |     |                |                 |               |
| Any morphological or distributional abnormality, or combination thereof | 438 | 332 | 17  | 45  | 44             | 88.1            | 72.1          | 85.8          |
| **cobas m 511 automated flags** |     |     |     |     |                |                 |               |
| Any message or combination\(^b\) | 439 | 118 | 10  | 9   | 302            | 92.9            | 96.8          | 95.7          |
| “Suspect blasts?” | 439 | 76  | 5   | 0   | 358            | 100.0           | 98.6          | 98.9          |
| “Suspect immature granulocytes?” | 439 | 53  | 10  | 8   | 368            | 86.9            | 97.4          | 95.9          |
| “Suspect variant lymphocytes?” | 439 | 13  | 8   | 1   | 417            | 92.9            | 98.1          | 97.9          |
| “Suspect left shift?” | 439 | 27  | 4   | 46  | 362            | 37.0            | 98.9          | 88.6          |

FN, false negative; FP, false positive; TN, true negative; TP, true positive.

\(^a\)Sample size for automated differential is lower because if the cobas m 511 system flagged the results with differential not measurable the system did not report results.

\(^b\)Excluding left shift samples based on high variability seen in technologist identification of this occurrence during manual review and the limited clinical value of the left shift flagging.

The reference method was a 400-cell manual differential derived from combined results of two technologists at each site.

Data are from all sites combined.
TABLE 4  Method comparison of the cobas m 511 system vs Sysmex XN

| Parameter (units) | N   | Pearson’s R | Sample range | Intercept (95% CI) | Slope (95% CI) |
|------------------|-----|-------------|--------------|------------------|----------------|
| WBC (10^9/L)     | 1583| 0.999       | (0.04, 247.04)| 0.02 (-0.01, 0.04)| 1.005 (1.000, 1.009) |
| RBC (10^12/L)    | 1581| 0.993       | (1.15, 7.21) | 0.00 (-0.03, 0.00) | 1.000 (1.000, 1.007) |
| HGB (g/dL)       | 1574| 0.991       | (4.20, 21.20) | -0.38 (-0.46, -0.30) | 1.055 (1.048, 1.063) |
| HCT (%)          | 1581| 0.981       | (13.50, 66.00) | -0.73 (-1.07, -0.41) | 1.051 (1.041, 1.060) |
| MCV (fl)         | 1581| 0.858       | (58.20, 119.20)| 2.80 (-0.50, 5.34) | 1.000 (0.972, 1.037) |
| MCH (pg)         | 1566| 0.951       | (17.58, 40.75) | 1.72 (1.17, 2.22)  | 0.964 (0.947, 0.983) |
| MCHC (g/dL)      | 1566| 0.562       | (26.59, 36.80) | 17.16 (16.27, 18.13) | 0.474 (0.444, 0.500) |
| RDW (%)          | 1581| 0.910       | (10.70, 29.40) | 2.72 (2.41, 3.02)  | 0.852 (0.830, 0.875) |
| RDW-SD (fl)      | 1581| 0.900       | (32.00, 97.10) | 7.89 (6.75, 8.93)  | 0.893 (0.868, 0.918) |
| PLT (10^9/L)     | 1533| 0.982       | (1.00, 1061.00)| -5.94 (-7.75, -4.08) | 0.982 (0.973, 0.990) |
| MPV (fl)         | 1415| 0.818       | (8.00, 13.00)  | -0.10 (-0.20, 0.52) | 1.000 (0.937, 1.000) |
| #NRBC (10^9/L)   | 1586| 0.995       | (0.00, 9.59)  | N/A              | N/A               |
| #NEUT (10^9/L)   | 1335| 0.997       | (0.37, 37.66) | 0.05 (0.03, 0.08)  | 1.031 (1.025, 1.038) |
| #LYMPH (10^9/L)  | 1398| 0.979       | (0.02, 5.99)  | -0.03 (-0.04, -0.02) | 1.000 (0.993, 1.012) |
| #MONO (10^9/L)   | 1398| 0.947       | (0.01, 6.14)  | -0.03 (-0.04, -0.01) | 0.979 (0.957, 1.000) |
| #EO (10^9/L)     | 1427| 0.986       | (0.00, 7.17)  | -0.00 (-0.01, -0.00) | 1.042 (1.033, 1.051) |
| #BASO (10^9/L)   | 1513| 0.679       | (0.00, 0.46)  | -0.02 (-0.03, -0.02) | 1.646 (1.556, 1.736) |
| %RET (%)         | 1567| 0.968       | (0.05, 12.93) | -0.23 (-0.26, -0.20) | 1.048 (1.029, 1.066) |
| #RET (10^12/L)   | 1559| 0.951       | (0.00, 0.42)  | -0.01 (-0.01, -0.01) | 1.027 (1.010, 1.047) |
| HGB-RET (pg)     | 1465| 0.789       | (16.23, 45.00) | -1.66 (-3.19, -0.26) | 1.107 (1.063, 1.153) |

#, count; BASO, basophils; CI, confidence interval; EO, eosinophils; HCT, hematocrit; HGB, hemoglobin; HGB-RET, reticulocyte hemoglobin; LYMPH, lymphocytes; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MONO, monocytes; MPV, mean platelet volume; NEUT, neutrophils; NRBC, nucleated red blood cells; PLT, platelets; RBC, red blood cells; RDW, red cell distribution width; RDW-SD, red cell distribution width standard deviation; RET, reticulocytes; WBC, white blood cells.

Data for percentage parameters are not shown. Data are from all sites combined.

3.2 | Reproducibility

Table S1 shows the results of various components of precision calculated using three levels of DigiMAC3® controls. The SD or %CV results for all parameters indicate that the cobas m 511 system produces reproducible results.

3.3 | Carryover

The mean percent carryover was below 0.01% for WBCs, RBCs, and PLTs and below 0.001% for blasts indicating virtually no carryover between samples, including blast cases.

3.4 | Mode-to-mode comparison

The results of the mode-to-mode comparison demonstrated satisfactory bias and correlation with Pearson’s R values ranging from 0.674 to 0.997 for CBC and differential parameters (Table S2; %NRBCs were excluded from this analysis).

3.5 | Morphology

The overall percent agreement between the results obtained with the viewing station and the gold standard manual microscopy 100-cell reference differential was 95.6% for WBC, RBC, and PLT characteristics. For cobas m 511 slide microscopy, the overall percent agreement was 95.7%. In general, this demonstrates that the results from the cobas m 511 images and slides compare well with results obtained by technologists in routine practice using a blood smear.

3.6 | WBC clinical sensitivity

The cobas m 511 system assessments correlated well with the manual 400-cell reference differential method for detecting abnormal distribution of WBCs and the presence of morphological abnormalities. The sensitivity, specificity, and efficiency results vs the gold standard manual differential method were 94.4%, 74.6%, and 90.7%, respectively, for the automated differential and 95.9%, 73.3%, and 92.7%, respectively, for the manual image review on the viewing station (Table 3). Slightly lower values were seen for cobas m 511 slides vs the reference method. A lower efficiency was observed for monocytes with all cobas m 511 system modalities (Table S3).

The manual differential count identified 76 samples with blasts, 14 samples with variant lymphocytes, and 61 samples with immature granulocytes (IGs) as the most clinically relevant abnormal cell types. Performance testing of the cobas m 511 automated flags for the respective cell types in comparison with the manual 400-cell
reference differential count as reference revealed an overall sensitivity and specificity of 92.9% and 96.8%, respectively (Table 3). In particular, all 76 blast-positive samples identified by manual differential count were correctly flagged by the cobas m 511 system. Five other samples were flagged for the presence of blast cells by the cobas m 511 system that were not detected by manual differential count. The system also correctly flagged 13 of 14 samples with variant lymphocytes detected with manual differential count and flagged a further eight samples that were not verified by microscopy.

### 3.7 | Method comparison

Intercomparison between the cobas m 511 and Sysmex XN systems revealed excellent correlations with Pearson's R values ≥0.95 for most CBC parameters (WBC, RBC, and PLT counts, HGB, hematocrit [HCT], and MCH; Table 4 and Figure 1), reticulocyte counts (R = 0.95), and NRBC counts (R = 1.00). The cobas m 511 system consistently revealed slightly higher values for HGB and HCT (slope 1.055 and 1.051, respectively). Pearson's R values for automated neutrophil, lymphocyte monocyte, and eosinophil counts (absolute numbers) were all ≥0.94, whereas the R value for absolute basophil counts was lower (0.68). Ten outlier samples from one site were excluded from the analysis after the study was completed because it had been discovered they had been rerun on the Sysmex XN system in open mode after sitting for an extended period. Improper hand mixing is thought to be the reason for the outlier values.

### 4 | DISCUSSION

The cobas m 511 system is the first fully automated slide-based hematology analyzer that uses digital microscopy to provide numerical and cell morphology results simultaneously. This multicenter evaluation validated the performance characteristics of the cobas m 511 system. Comparison of cobas m 511 automated blood counts, automated system flags, images, slides, and morphology with standard manual microscopy—and interinstrument comparison with the Sysmex XN hematology analyzer—revealed a high R value representing excellent agreement. These results validate the performance of the cobas m 511 system and indicate it is suitable for routine clinical use.

The basic performance characteristics of the cobas m 511 system such as repeatability, reproducibility, carryover, and comparison between closed and open modes all demonstrated acceptable results. This demonstrates the robustness of the cobas m 511 system to provide consistent and precise results in routine diagnostic settings over time regardless of location, system, and operator.

All cobas m 511 system modalities, automated counts, automated system flags, review on viewing station, and review using cobas m 511 slides, were validated in WBC clinical sensitivity testing as compared to manual microscopy as standard method. The overall agreement between cobas m 511 automated analysis of neutrophil, lymphocyte, and eosinophil counts with microscopy was high. This indicates that the cobas m 511 system delivers reliable results for these cell types when compared to the 400-cell reference differential, thereby automating much of the blood analysis process. Comparable results were also observed in previous studies comparing differential blood counts from flow cytometry-based hematology analyzers with microscopy.\(^3\)\(^{13}\)\(^{14}\) The lower correlation of basophil counts between automated and manual counts is largely due to statistical uncertainty, due to low basophil counts in normal samples and the lack of samples with higher basophil counts. Poor correlations between basophil counts are seen in most comparative studies of impedance and flow-based analyzers.\(^3\)\(^{13}\)\(^{14}\)

The main function of routine hematology analyzers is to correctly identify samples containing pathological cell types with a need for further evaluation. A key requirement is that the rate of false-positive flags is low to reduce unnecessary reviews. The automated flagging of samples for the presence of blast cells and variant lymphocytes with the cobas m 511 system was very good, especially considering the cobas m 511 system is a first-of-a-kind instrument. The system identified samples with blast cells detected by microscopy with a sensitivity of 100% and a specificity of 99%. Recent publications show significant differences for blast cell flagging quality between different hematology analyzers with sensitivities ranging from 97% to 100% for the Sysmex XN system to 72% for the Beckman ClDXH 800 system and specificities of 93%-98%.\(^3\)\(^{15}\) The cobas m 511 system automatically flagged samples for the presence of variant lymphocytes with a sensitivity of 93% and a specificity of 98% compared with microscopy, although numbers of positive samples were low (n = 14). For comparison, existing hematology analyzers demonstrated a sensitivity for variant lymphocyte flagging in the 80% range (for the Sysmex XN system), and specificities of 95%.\(^3\)\(^{15}\) The cobas m 511 system correctly flagged IG-containing samples with a sensitivity of 87% and a specificity of 97%, compared with standard microscopy. For current instruments, a sensitivity in the range of 90% was demonstrated for flagging IG-containing samples (Sysmex XN system), while specificities in the range of 86% have been shown.\(^3\)\(^{15}\) The sensitivity for “suspect left shift” was low.

**Figure 1** Correlation plots from method comparison analysis. Figures show Passing-Bablok regression fits except for those parameters with a large number of valid results of zero (%NRBC, %NBRC, %EO, %EO, %BASO and %BASO) which are represented by Deming regression fits. Data for percentage parameters, MPV, HGB-RET, RDW-CV, and RDW-SD are not shown. Dashed lines indicate line of identify (y = x); gray-shaded regions indicate 95% confidence bounds (calculated with the bootstrap [quantile] method for Passing-Bablok regression fits or the analytical method for Deming regression fits). Data are from all sites combined. Corresponding bias plots are shown in Figure S1. # count; BASO, basophils; EO, eosinophils; HCT, hematocrit; HGB, hemoglobin; LYMHPH, lymphocytes; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MONO, monocytes; MPV, mean platelet volume; NEUT, neutrophils; NRBC, nucleated red blood cells; PLT, platelets; RBC, red blood cells; RET, reticulocytes; WBC, white blood cells.
(37.0%, Table 2), likely reflecting how the technologists defined left shift using manual microscopy (based on band counts), and the typically poor inter- and intraobserver reproducibility.\textsuperscript{16}

The widely used Sysmex XN hematology analyzer was selected for interinstrument comparison for automatically generated numerical and differential results.\textsuperscript{3,13-15} This comparison revealed excellent agreement between the cobas m 511 and Sysmex XN systems for most CBC parameters, WBC differential, reticulocytes, and NRBC analyses, despite the two systems using widely different technologies. A lower correlation was seen for mean corpuscular volume (MCV; $R = 0.86$) and mean corpuscular hemoglobin concentration (MCHC; $R = 0.56$). The low correlation for MCHC is not uncommon as the typical data range for this parameter is very limited which causes low $R$ values. A recent study comparing the performance of hematology analyzers that use somewhat similar technologies demonstrated good correlations for CBC, but poorer or low agreement for reticulocyte and NRBC counts.\textsuperscript{3}

An overall adequate agreement to standard manual microscopy was demonstrated for results achieved by review using the cobas m 511 viewing station or microscopy using cobas m 511 slides. Results of WBC, RBC, and platelet morphology assessment indicate a general agreement between the evaluation of morphology on the cobas m 511 images and the manual blood smear. Monocyte counts identified using the viewing station and cobas m 511 slide microscopy were lower than those seen with standard blood smears, and a lower agreement between eosinophil counts generated by cobas m 511 slides and standard microscopy was observed. This may be caused by the low numbers of these types of cells found in most samples. Further experience will help to clarify these results.

Several potential areas where the cobas m 511 system would be useful can be envisaged. First, automated analysis combined with digital morphology would be valuable for mid-size laboratories or for laboratories with high volumes of pathological samples with a need for microscopic review. The high analytical and flagging quality of the cobas m 511 system in relation to standard microscopy and to the Sysmex XN system presented here need to be verified in subsequent studies; however, the results suggest that the cobas m 511 system would be a valuable additional system in larger laboratories to augment flow cytometry-based systems for combined high throughput analysis. The small blood sample volume (30 μL) required by the cobas m 511 system may be useful for analyzing pediatric samples, although these were not evaluated in this study. A key strength of the cobas m 511 system compared with flow cytometry-based systems is the ability to instantly (and remotely) view and sort all the stored cell images on a cobas m 511 viewing station. The clinical value of this viewing station for digital morphology compared with existing digital imaging systems must be further validated. A potential limitation of the cobas m 511 system compared with flow cytometry-based instruments is the relatively low sample throughput, 60 samples per hour, due to the need to print and stain the slides. This may be mitigated, however, by the system’s ability to enable review of potentially abnormal cases on a real-time basis.

In conclusion, the novel cobas m 511 slide-based automated hematology analyzer demonstrated very good agreement with the Sysmex XN analyzer for most parameters, despite the different technologies used. Agreement with standard manual microscopy was also very good, and basic quality performance characteristics of the cobas m 511 system were all well within acceptable limits. These results indicate that the slide-based cobas m 511 system is suitable for clinical use and may reduce laborious manual microscopy in routine hematology diagnostics.

**CONFLICT OF INTEREST**

Tracy I. George and Henk Russcher have received consultancy fees from Roche. Timothy R. Allen, Dan Bracco, and David J. Zahniser are employees of Roche Diagnostics and received stock options. David J. Zahniser is a coinventor of the cobas m 511 system; he holds several patents related to the technology and receives royalty payments through his partnership in Cell Imaging Systems, LLC, the company at which the technology was developed. All other authors have no conflict of interests to declare.

**AUTHOR CONTRIBUTION**

Mathias Bruegel, Tracy I. George, Bo Feng, and Henk Russcher performed the research. David J. Zahniser, Dan Bracco, and Timothy R. Allen designed the research study and analyzed the data. Dan Bracco coordinated the running of the study. All authors drafted and critically revised the paper and approved the final version.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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