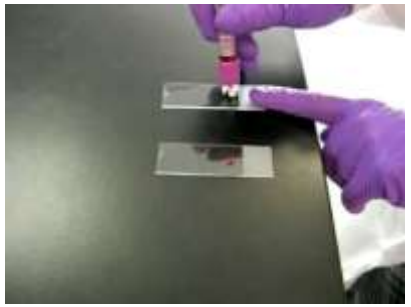
Automated microscopy in laboratory medicine.

Ruggero Buonocore (on behalf of Giuseppe Lippi)

Laboratory of Clinical Chemistry and Hematology, Academic Hospital of Parma, Italy.

Where do we start from?



The Pathologist, in press
Automated microscopy in laboratory medicine
Giuseppe Lippi, MD

The only suitable approach for blood cell enumeration and sizing has been represented for decades by microscopic analysis of peripheral blood smears stained with May-Grünwald Giemsa or other appropriate stains.
Indeed, the procedure is:

- Labour intensive
- Time consuming
- Requires intensive training
- Is plagued by a considerable degree of inter-observer (≈20%) and intra-observer (≈10%) inaccuracy.

The Pathologist, in press
Automated microscopy in laboratory medicine
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Recent technological advances have made it possible to design and introduce automated image analysis systems. They can:

- Be physically connected to other instrumentation (especially with hemocytometers).
- Automatically prepare blood films with customized criteria obtained from CBC
- Scan the slides
- Capture digital images of blood smears at high magnification
- Analysed scans by artificial neural networks according to a preset database of blood elements
- Customize and update original rules by the local users
- The operator can also:
    - Modify the size of the image
    - Magnify single parts
    - Accept actual categorization
    - Shift some elements to other categories
- The scans can else be transmitted to the wards as digital images

ORIGINAL ARTICLE INTERNATIONAL JOURNAL OF LABORATORY HEMATOLOGY

Can automated blood film analysis replace the manual differential? An evaluation of the CellaVision DM96 automated image analysis system

C. BRIGGS*, I. LONGAIR*, M. SLAVIK†, K. THWAITE†, R. MILLS*, V. THAVARAJA*, A. FOSTER†, D. ROMANIN‡, S. J. MACHIN*

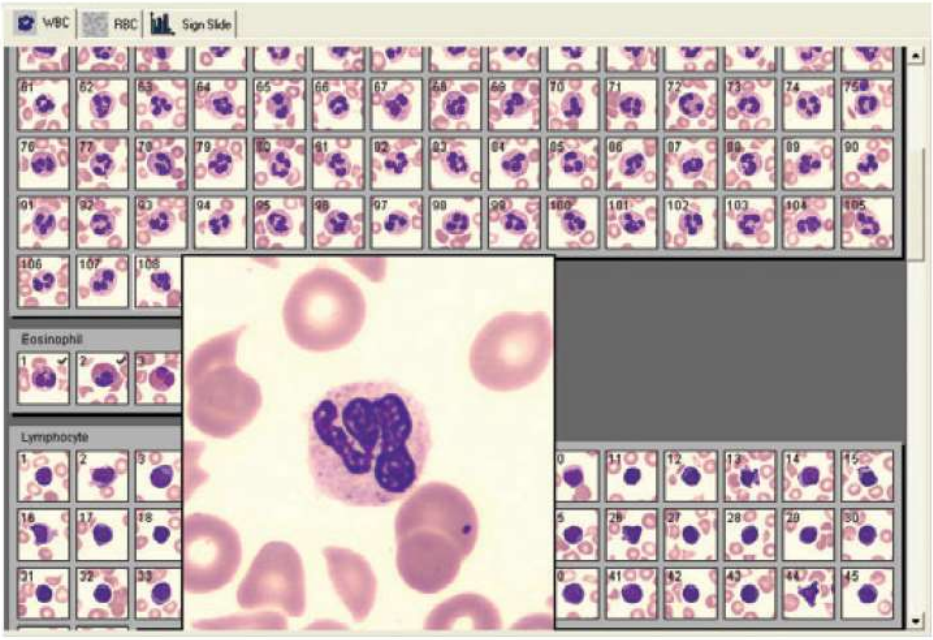
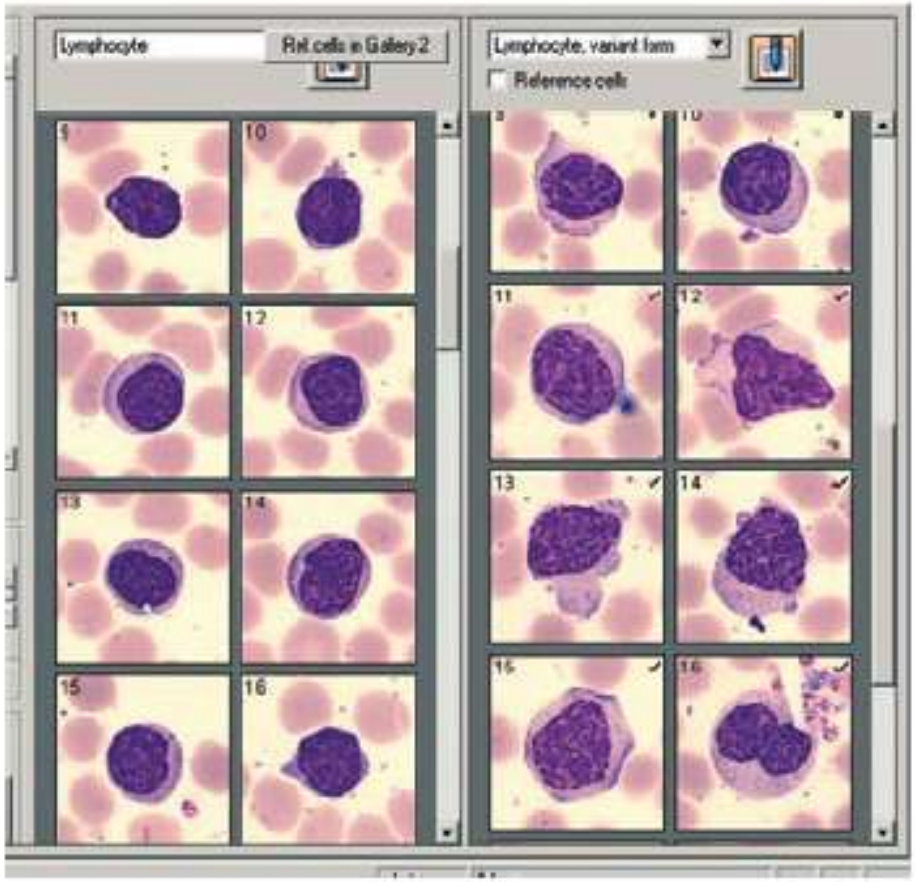


Figure 1. Preclassified white blood cells presented on the CellaVision DM96 computer screen.

ORIGINAL ARTICLE INTERNATIONAL JOURNAL OF LABORATORY HEMATOLOGY

Performance evaluation and relevance of the CellaVision™ DM96 system in routine analysis and in patients with malignant hematological diseases

E. CORNET*, J.-P. PEROL†, X. TROUSSARD*



How did it work, in Parma?

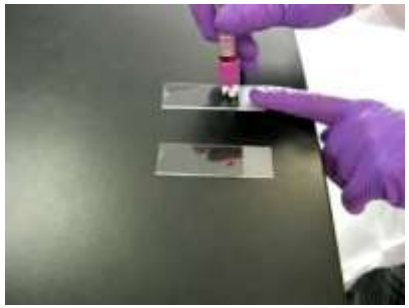
Sample collection

Sample analysis

Lab report

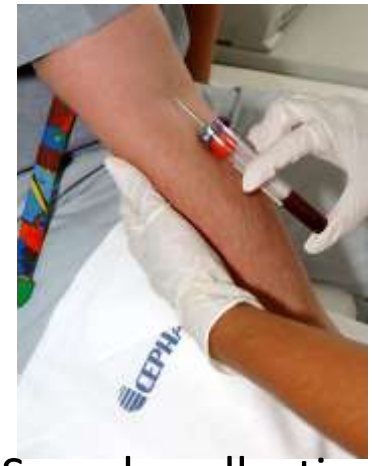
Smear analysis

Smear staining



Smear preparation

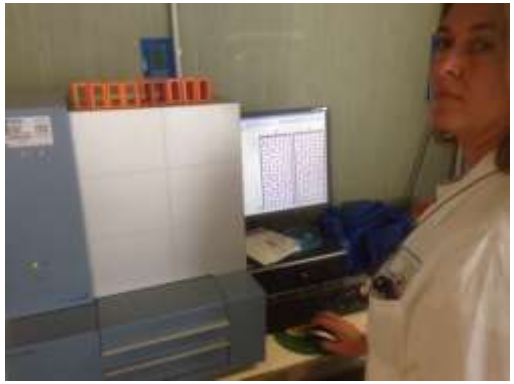
How does it work now, in Parma?



Sample collection

Sample analysis

Web transmission



Digital analysis & reclassification

Auto-preparation & staining

How does it perform?

VS.

ORIGINAL ARTICLE INTERNATIONAL JOURNAL OF LABORATORY HEMATOLOGY

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Table 1. Percentage of cells from 286 blood films correctly preclassified by the CellaVision DM96

Cell class	Preclassifying agreement (%)
Neutrophil (Neut)	99.5
Lymphocyte (Lymph)	94.9
Monocyte (Mono)	87.6
Eosinophil (Eos)	79.9
Basophil (Baso)	54.1
Metamyelocyte	32.6
Myelocyte	37.7
Promyelocyte	77.6
Blast	76.6
Nucleated red blood cell	89.6
Neut, Lymph and Mono	97.3
Neut, Lymphs, Mono, Eos and Baso	87.2
All cell classes	89.2
Abnormal cells called normal	0.9
Normal cells misclassified as other normal cells	9.1
Normal cells called abnormal cells	1.8

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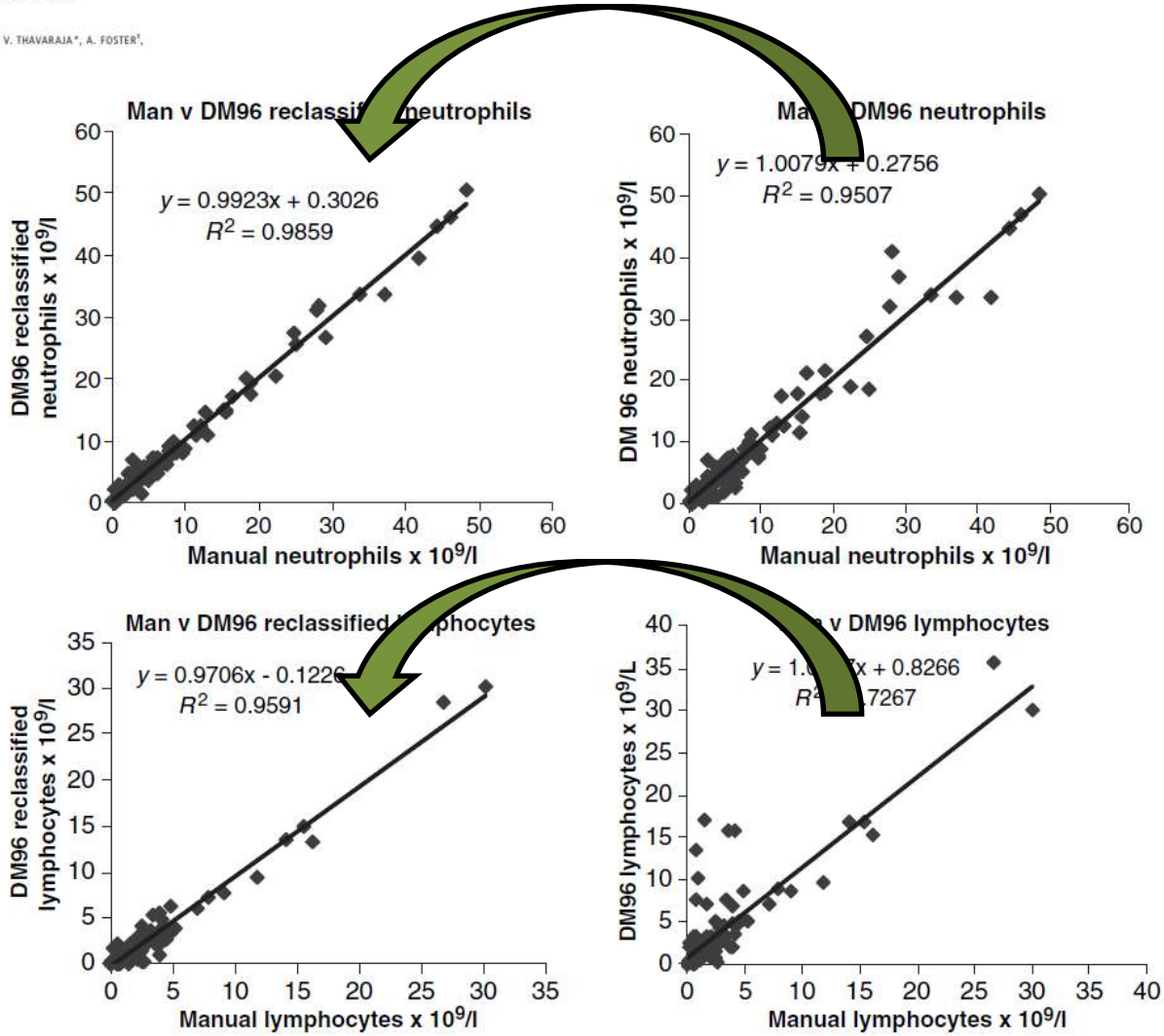

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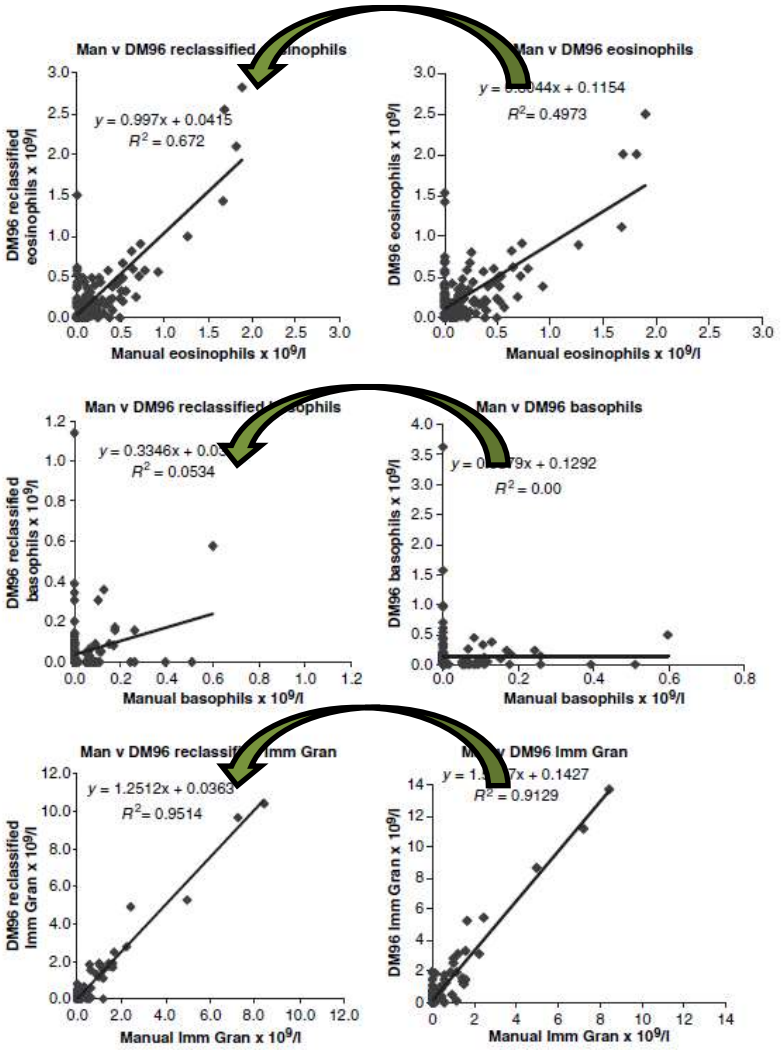

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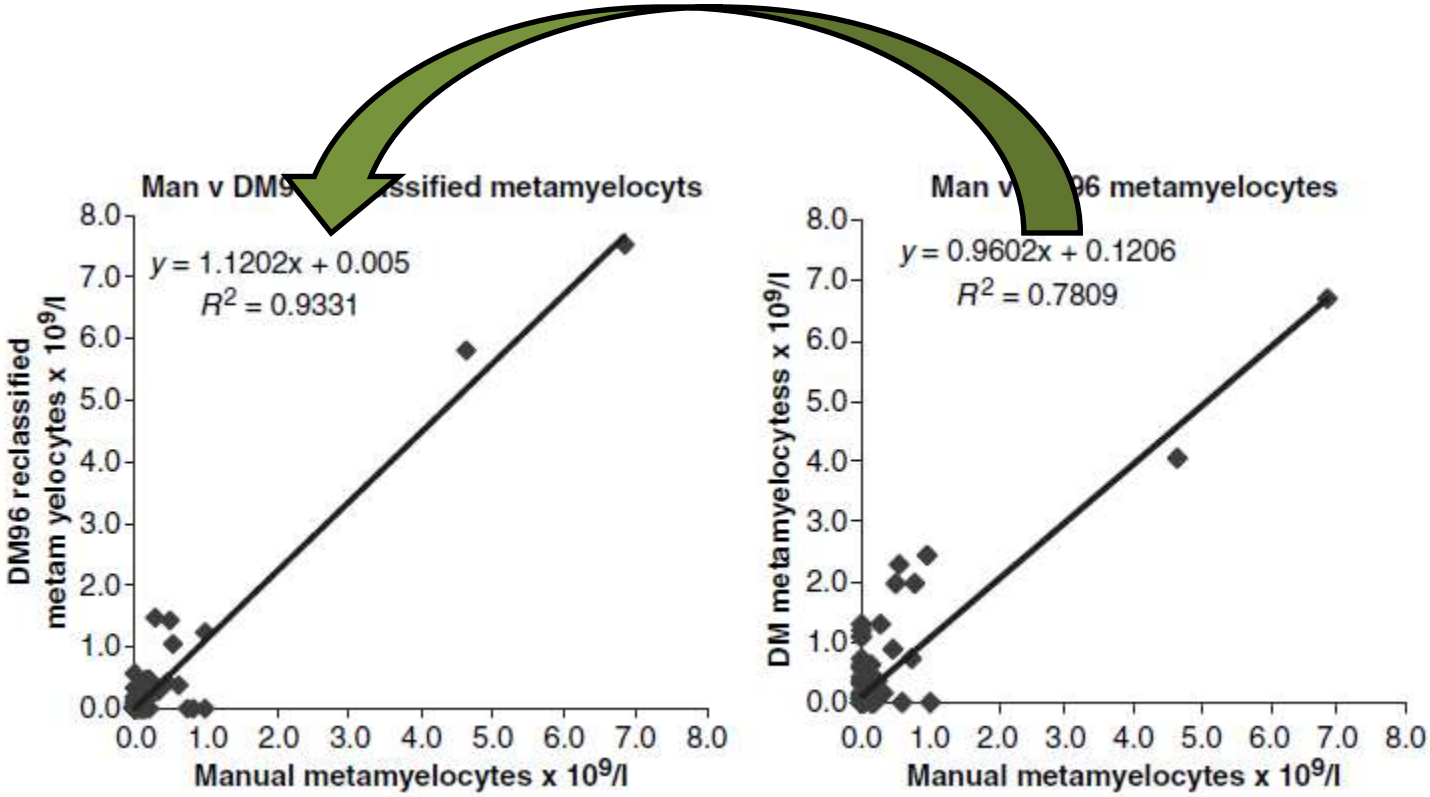

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Table 3. Correlation coefficients (r^2 values) for comparison of five different operators' differentials to the 400-cell reference differential

Operator	Ref *vs.* man	Ref *vs.* reclass	Ref *vs.* preclass	Ref *vs.* Man	Ref *vs.* reclass	Ref *vs.* pre-class	Ref *vs.* man	Ref v Re-class	Ref v Pre-class
	Neutrophils			Basophils			Metamyelocytes		
1	0.994	0.993	0.995	0.295	0.001	0.056	0.274	0.330	0.822
2	0.993	0.991	0.974	0.060	0.012	0.031	0.537	0.701	0.723
3	0.968	0.986	0.988	−0.053	−0.049	−0.152	0.845	0.238	0.758
4	0.843	0.994	0.989	0.023	0.009	0.063	0.789	0.928	0.914
5	0.990	0.990	0.990	0.089	−0.197	−0.284	0.863	0.728	0.956
	Lymphocytes			Blasts			Myelocytes		
1	0.897	0.752	0.218	0.998	0.989	0.804	0.804	0.670	0.707
2	0.788	0.841	0.240	0.999	0.998	0.999	0.763	0.712	0.963
3	0.752	0.764	0.324	0.996	0.998	0.982	0.264	0.927	0.831
4	0.442	0.077*	0.179	0.965	0.975	0.952	0.688	0.333	0.329
5	0.686	0.782	0.193	0.998	0.999	0.986	0.712	0.503	0.712
	Monocytes			Nucleated red blood cell			Promyelocytes		
1	0.624	0.663	0.677	0.804	0.978	0.973	0.309	0.000	0.690
2	0.674	0.768	0.707	0.995	0.991	0.973	0.948	0.702	0.936
3	0.752	0.540	0.823	0.861	0.960	0.956	0.227	0.423	0.643
4	0.848	0.822	0.805	0.921	0.953	0.915	0.018	0.600	0.706
5	0.521	0.805	0.724	0.992	0.962	0.956	0.551	0.540	0.600
	Eosinophils			Immature granulocytes					
1	0.802	0.461	0.323	0.831	0.987	0.910			
2	0.451	0.528	0.155	0.909	0.971	0.917			
3	0.624	0.554	0.301	0.750	0.748	0.950			
4	0.338	0.395	0.465	0.777	0.670	0.851			
5	0.694	0.394	0.112	0.898	0.887	0.956			

ORIGINAL ARTICLE INTERNATIONAL JOURNAL OF LABORATORY HEMATOLOGY

Performance evaluation and relevance of the CellaVision™ DM96 system in routine analysis and in patients with malignant hematological diseases

E. CORNET*, J.-P. PEROL†, X. TROUSSARD*

Table 2. Comparaison between DM96™ before and after classification of unidentified cells and manual differential counts

Cell per cell analysis with DM 96: pool of 62 904 cells

DM96™\user	Neutrophils	Eosinophils	Basophils	Lymphocytes	Monocytes	IG	NRBC	total
Before unidentified cells classification								
Unidentified	796	36	9	56	214	353	157	1621
Accuracy (%)	95.6	96	80	99	92	58	56	95
After unidentified cells classification (%)								
Accuracy	98	98	83	99	98	86	82	98
False negative	2.3	1.5	17.3	0.5	2.4	14.4	17.9	
False positive	0.0	12.3	39.3	2.2	4.9	46.2	6.3	

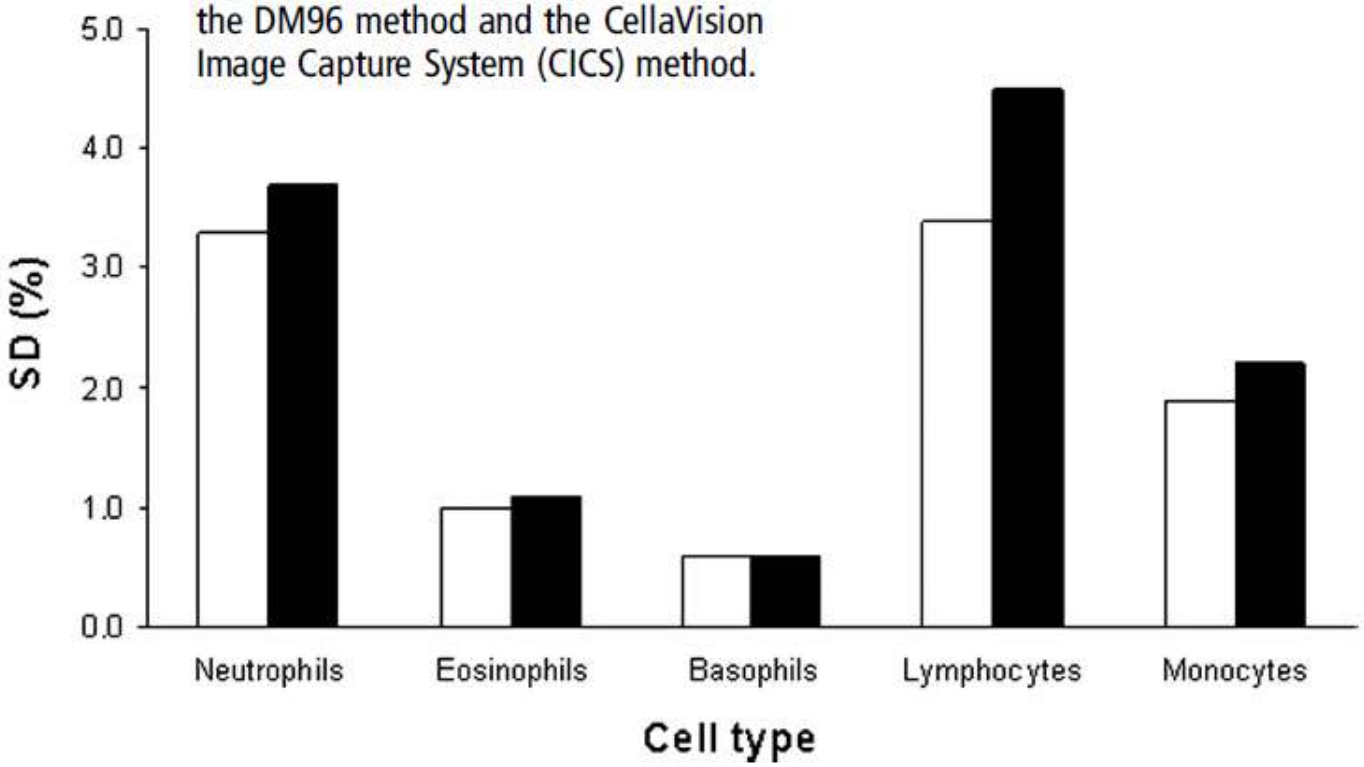
IG, Immature Granulocytes; NRBC, nucleated red blood cell.

Clinical performance evaluation of the CellaVision Image Capture System in the white blood cell differential on peripheral blood smears

Simone M Smits, Anja Leyte

Optical microscopy : ≈10%

Figure 2 Short-term imprecision for the DM96 method and the CellaVision Image Capture System (CICS) method.



Clinical performance evaluation of the CellaVision Image Capture System in the white blood cell differential on peripheral blood smears

Simone M Smits, Anja Leyte

Table 1 Regression coefficients and regression lines with their 95% CI for neutrophils, eosinophils, lymphocytes, monocytes and blast cells

Cell class	Intercept	95% CI intercept	slope	95% CI slope	R^2
Neutrophils	0.11	−1.01 to 1.52	0.99	0.97 to 1.01	0.98
Eosinophils	0.00	0.00 to 0.01	1.05	0.99 to 1.11	0.92
Lymphocytes	0.19	−0.56 to 0.57	1.01	0.98 to 1.04	0.96
Monocytes	0.13	−0.37 to 0.68	0.97	0.87 to 1.06	0.71
Blasts	0.25	−0.29 to 0.25	1.09	0.86 to 1.17	0.96

Research Article

Performance of CellaVision DM96 in leukocyte classification

Lik Hang Lee[1], Adnan Mansoor[1,2], Brenda Wood[2], Heather Nelson[2], Diane Higa[2], Christopher Naugler[1,2]

Table 2: Correlation coefficients between DM96 and manual microscopy in the classification of leukocytes. Correlation for the nextslide digital review network and correlation between technologists and an expert reference are included for comparison

Cell type	This study	Briggs *et al.**[9]	Kratz *et al.*[10]	Cornet *et al.*[11]	Ceelie *et al.*[12]	Yu *et al.***[13]	Koepke *et al.****[4]
Neutrophils (total)		0.9859	0.9536			0.9134	
Lymphocytes	0.9547	0.9591	0.9393		0.9405	0.901	0.73
Monocytes	0.8316	0.805	0.6658		0.7004	0.8176	0.41
Eosinophils	0.8821	0.672	0.73		0.846	0.7671	0.83
Basophils	0.7637	0.0534				0.5592	0.32
Segmented neutrophils	0.9611		0.8771		0.9528		0.87
Bands	0.874		0.6852		0.7961	0.8868	
Metamyelocytes	0.717	0.9331					
Myelocytes	0.8806	0.3709					
Promyelocytes	0.7357	0.4175					
Blasts	0.9861	0.9953		0.9	0.984	0.9769	
Immature granulocytes (meta-, myelo-, and promyelocytes)	0.9064	0.9514				0.9285	
Atypical lymphocytes						0.9326	

*Cells per liter used for correlation coefficient calculation rather than percentage of cell type, **Correlation between nextslide digital review network and manual microscopy, ***Correlation between 73 technologists and expert reference

ORIGINAL ARTICLE INTERNATIONAL JOURNAL OF LABORATORY HEMATOLOGY

Automated digital cell morphology identification system (CellaVision DM96) is very useful for leukocyte differentials in specimens with qualitative or quantitative abnormalities

S. H. PARK, C.-J. PARK, M.-O. CHOI, M.-J. KIM, Y.-U. CHO, S. JANG, H.-S. CHI

Table 2. Clinical performance of the CellaVision DM96 system compared with manual counts on the microscopy as the reference method

	Clinical performance, overall (low leukocyte count/abnormal leukocyte/failure for differential count by automatic hematology analyzer)				
Abnormal findings	Agreement rates (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Atypical lymphocytes	74.5 (76.5/78.0/69.0)	78.4 (83.6/71.9/76.6)	71.3 (67.4/80.9/62.3)	69.1 (76.7/63.9/64.3)	80.1 (76.3/85.9/75.0)
Blasts	99.0 (98.0/100.0/99.0)	98.2 (90.0/100.0/100.0)	99.2 (98.9/100.0/98.3)	96.6 (90.0/100.0/97.7)	99.6 (98.9/100.0/100.0)
Promyelocytes $\geq 3\%$	99.0 (98.0/100.0/99.0)	100.0 (NC/NC/100.0)	99.0 (98.0/100.0/99.0)	25.0 (0.0/NC/50.0)	100.0 (100.0/100.0/100.0)
Myelocytes $\geq 3\%$	95.3 (96.9/94.0/95.0)	88.8 (66.7/87.0/92.2)	97.7 (98.9/96.1/98.0)	93.4 (80.0/87.0/97.9)	95.9 (97.8/96.1/92.3)
Metamyelocytes $\geq 3\%$	95.0 (98.0/97.0/90.0)	93.2 (85.7/89.5/95.7)	95.6 (98.9/98.8/84.9)	87.2 (85.7/94.4/84.9)	95.6 (98.9/97.6/95.7)
Nucleated RBCs	80.2 (82.7/86.0/72.0)	76.1 (72.7/60.0/82.9)	81.4 (83.9/90.6/64.4)	54.3 (36.4/52.9/61.8)	92.2 (96.1/92.8/84.4)

PPV, positive predictive value; NPV, negative predictive value; RBC, red blood cells; NC, not calculated.

Original Article

Experience with CellaVision DM96 for peripheral blood differentials in a large multi-center academic hospital system

Marian A. Rollins-Raval, Jay S. Raval, Lydia Contis

Table 4: Adult cancer center calculations and analysis

Cell type	Proportion of total events* (%)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Unidentified	1.11	N/A	N/A	N/A	N/A
Band neutrophil	4.29	74.57	97.83	60.60	98.85
Segmented neutrophil	52.69	94.82	98.08	98.21	94.44
Eosinophil	1.42	94.24	99.71	82.64	99.92
Basophil	0.36	80.26	99.69	48.85	99.93
Lymphocyte	10.55	97.76	99.76	97.94	99.74
Monocyte	5.36	93.02	99.87	97.56	99.61
Promyelocyte	0.02	87.76	99.86	13.07	100.00
Myelocyte	0.41	66.78	99.81	59.21	99.86
Metamyelocyte	0.82	48.67	99.83	70.52	99.58
Blast	0.31	64.74	99.85	57.72	99.89
Variant lymphocyte	0.64	67.11	99.73	61.60	99.79
Plasma cell	0.01	100.00	99.82	6.31	100.00
Large granular lymphocyte	0.00	0.00	100.00	N/A	100.00
Other	0.01	0.00	100.00	N/A	99.99
Erythroblast	1.28	98.38	99.63	77.57	99.98
Giant platelet	3.90	97.68	99.81	95.38	99.91
Platelet aggregation	0.17	81.02	99.86	50.00	99.97
Megakaryocyte	0.00	0.00	100.00	N/A	100.00
Smudge cell	9.11	90.85	99.90	98.91	99.09
Artefact	8.63	94.14	99.93	99.18	99.45

Original Article

Experience with CellaVision DM96 for peripheral blood differentials in a large multi-center academic hospital system

Marian A. Rollins-Raval, Jay S. Raval, Lydia Contis

Table 6: Children's hospital calculations and analysis

Cell type	Proportion of total events (%)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Unidentified	0.91	N/A	N/A	N/A	N/A
Band neutrophil	3.06	68.16	99.72	88.36	99.00
Segmented neutrophil	33.50	97.17	98.61	97.24	98.57
Eosinophil	1.47	89.83	99.66	79.70	99.85
Basophil	0.39	80.65	99.49	37.88	99.92
Lymphocyte	19.18	97.98	99.88	99.47	99.52
Monocyte	5.84	95.73	99.92	98.68	99.74
Promyelocyte	0.07	100.00	99.95	60.00	100.00
Myelocyte	0.14	81.82	99.97	81.82	99.97
Metamyelocyte	0.22	77.78	99.95	77.78	99.95
Blast	1.71	90.51	99.91	94.66	99.83
Variant lymphocyte	1.21	88.66	99.97	97.73	99.86
Plasma cell	0.01	100.00	99.83	6.67	100.00
Erythroblast	1.40	100.00	99.67	81.16	100.00
Giant platelet	4.51	99.17	99.79	95.72	99.96
Platelet aggregation	0.05	100.00	99.69	13.79	100.00
Smudge cell	14.28	93.53	99.93	99.53	98.93
Artefact	12.95	96.43	99.84	98.91	99.47

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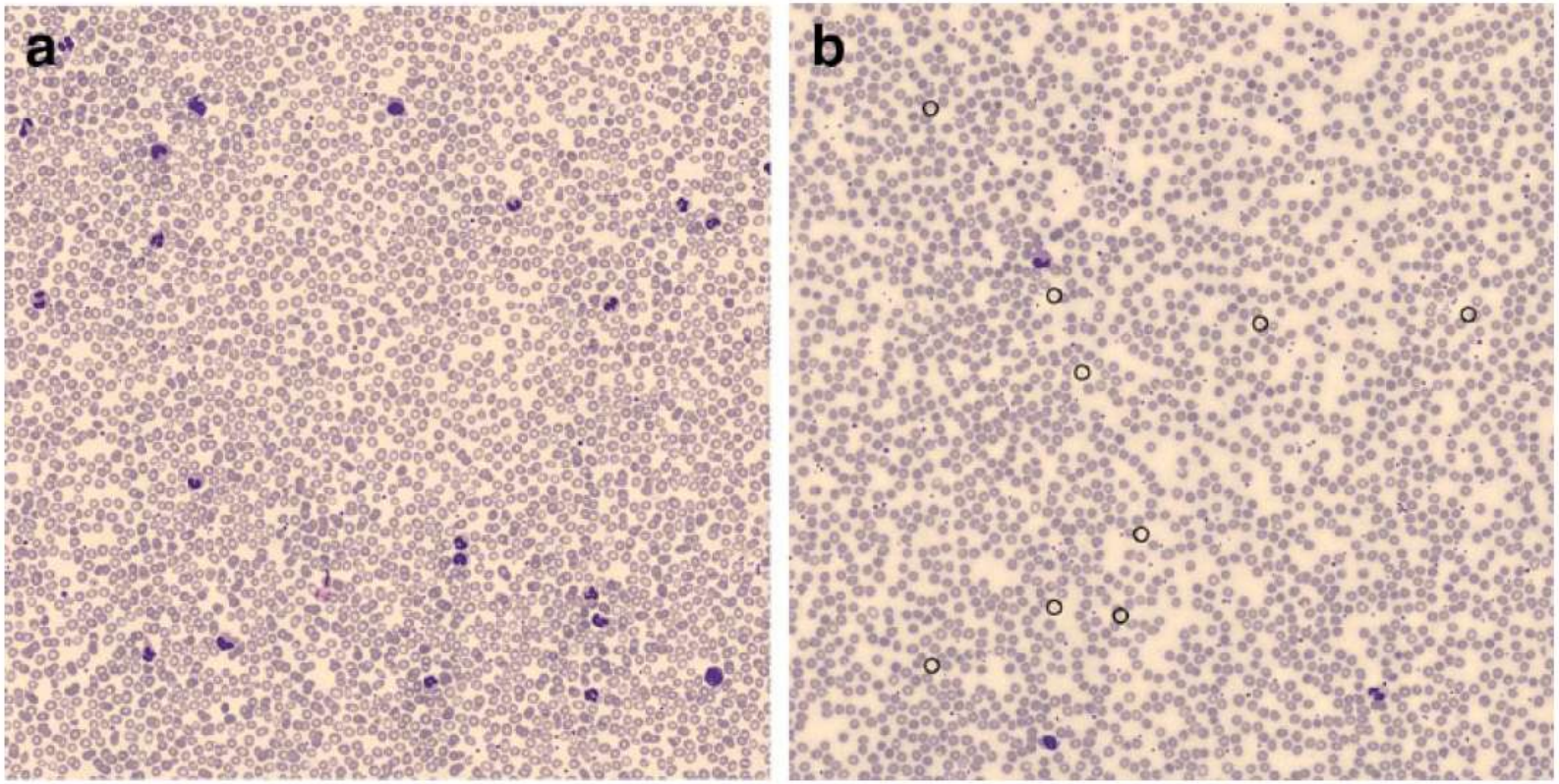
Table 2. Percentage agreement for red cell morphology on the CellaVision DM96

Red cell abnormality	Preclassification agreement (%)	Reclassification agreement (%)
Polychromasia	76	76
Hypochromia	87	84
Microcytosis	85	84
Macrocytosis	42	85
Anisocytosis	51	74
Poikilocytosis	59	74

Preclassification is the number of correct suggestions by the DM96 and Reclassification is agreement with the manual method after the operator has altered the results originally presented by the instrument. Two hundred and eighty-six blood films were evaluated.

What Do Hemolyzed Whole-Blood Specimens Look Like? Analysis with a CellaVision DM96 Automated Image Analysis System

Giuseppe Lippi[1], Fernanda Pavesi[1], Anna Benegiamo[1], and Silvia Pipitone[1]



What Do Hemolyzed Whole-Blood Specimens Look Like? Analysis with a CellaVision DM96 Automated Image Analysis System

Giuseppe Lippi[1], Fernanda Pavesi[1], Anna Benegiamo[1], and Silvia Pipitone[1]

Table 1. Complete Blood Cell Count and CellaVision DM96 Data of a Normal Blood Sample and a Paired Specimen after Spurious Hemolysis.

	Normal Blood	Hemolyzed Blood
Complete blood cell count (XE-2100)		
White blood cells (× 10^9/L)	8.65	8.54
Neutrophils (× 10^9/L)	6.68	6.82
Lymphocytes (× 10^9/L)	1.11	1.09
Monocytes (× 10^9/L)	0.84	0.58
Eosinophils (× 10^9/L)	0.01	0.03
Basophils (× 10^9/L)	0.01	0.02
Red blood cells (× 10^{12}/L)	4.20	3.29
Reticulocytes (× 10^{12}/L)	0.035	0.028
Red blood cell ghosts (× 10^{12}/L)	0.02	0.02
Hemoglobin (g/L)	139	139
Hematocrit (%)	40.8	31.5
Mean corpuscular volume (fL)	97.1	95.7
Mean corpuscular hemoglobin (pg)	33.1	42.2
Mean corpuscular hemoglobin concentration (g/dL)	34.1	44.1
Red blood cell distribution width (%)	13.8	14.3
Platelets (× 10^9/L)	278	275
Mean platelet volume (fL)	9.3	11.0
Plateletcrit (%)	0.26	1.38
Platelet distribution width (%)	10.2	13.1
CellaVision DM96		
Polychromasia (%)	0	0
Hypochromia (%)	0.6	0.1
Anisocytosis (%)	2.8	4.8
Microcytosis (%)	0.3	0.6
Macrocytosis (%)	2.5	2.2
Poikilocytosis (%)	1.2	1.1
Band neutrophils (%)	0.0	2.6
Segmented neutrophils (%)	85.6	76.8
Lymphocytes (%)	12.4	2.6
Monocytes (%)	7.7	9.2
Other cells (%)	0.0	3.0
Large platelets (%)	1.0	9.0
Smudge cells (%)	3.1	9.2
Artifacts (%)	2.1	7.7

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Table 4. Comparison of time taken to complete the 30 differentials on the CellaVision DM96 including reclassification of cells with time taken to perform the same differentials manually

Operator	Time for analysis on DM96	Time for manual differential analysis
1	1 h 5 min	1 h 45 min
2	1 h 10 min	1 h 40 min
3	1 h 30 min	3 h 45 min
4	1 h 40 min	4 h 10 min
5	1 h 14 min	3 h 10 min

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Automated digital cell morphology identification system (CellaVision DM96) is very useful for leukocyte differentials in specimens with qualitative or quantitative abnormalities

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Table 4. Comparison of the average process time and total cell count per slide in the samples with low leukocyte count (<1000/μL) between the Cellavision DM96 system and manual microscopic examination when the instrument was ordered to count 300 or 500 cells from the operator

		CellaVision DM96 system							
		Total processing time, seconds†, mean ± SD				Total cell count‡, mean±SD			
Groups*	Leukocyte count, cells/μL	100 cells§	300 cells§	500 cells§	Manual count	100 cells§	300 cells§	500 cells§	Manual count
1	<100	110.0 ± 21.7	104.3 ± 19.3	105.0 ± 15.3	102.0 ± 21.6	9.3 ± 6.6	11.6 ± 5.5	10.6 ± 5.6	8.0 ± 6.0
2	100–200	176.0 ± 6.1	134.0 ± 21.7	123.0 ± 16.5	113.6 ± 5.5	58.6 ± 22.7	69.6 ± 28.9	68.6 ± 29.1	44.3 ± 17.0
3	200–300	150.0 ± 14.5	154.6 ± 18.1	147.6 ± 22.5	114.3 ± 5.0	83.6 ± 18.7	108.0 ± 31.4	108.6 ± 36.2	68.0 ± 8.5
4	300–400	202.6 ± 29.7	209.0 ± 38.1	199.0 ± 21.9	133.3 ± 5.7	90.3 ± 7.0	128.6 ± 36.5	122.6 ± 39.1	86.6 ± 23.0
5	400–500	163.3 ± 23.2	208.6 ± 33.3	208.3 ± 46.6	116.6 ± 4.9	95.0 ± 1.0	197.3 ± 36.1	199.6 ± 34.2	100.0 ± 0.0
6	500–600	158.3 ± 10.6	200.0 ± 47.1	212.0 ± 58.2	116.6 ± 5.2	98.0 ± 1.0	218.3 ± 7.2	220.0 ± 5.1	100.0 ± 0.0
7	600–700	169.3 ± 28.5	258.3 ± 6.6	236.0 ± 17.0	108.6 ± 10.9	97.3 ± 1.1	234.6 ± 34.0	237.0 ± 37.3	100.0 ± 0.0
8	700–800	178.3 ± 31.2	276.6 ± 21.9	248.6 ± 43.3	122.6 ± 15.1	95.3 ± 4.1	241.3 ± 19.1	238.0 ± 22.5	100.0 ± 0.0
9	800–900	136.0 ± 7.5	264.3 ± 36.1	242.3 ± 21.8	109.3 ± 8.6	97.3 ± 0.5	279.3 ± 26.2	300.0 ± 37.5	100.0 ± 0.0
10	900–1000	158.6 ± 6.8	249.6 ± 21.2	256.0 ± 29.5	117.6 ± 4.0	92.6 ± 3.0	242.6 ± 49.1	252.3 ± 62.7	100.0 ± 0.0
Total		160.2 ± 29.5	205.9 ± 61.6	197.8 ± 58.8	115.4 ± 11.8	81.7 ± 28.3	173.1 ± 88.8	175.7 ± 94.1	80.7 ± 31.6

The Pathologist, in press
Automated microscopy in laboratory medicine
Giuseppe Lippi, MD

Table 1.

Advantages of automated microscopy in laboratory medicine

- Standardized approach to cell classification
- Transmission of digital images to skilled hematologists in various locations
- Storage of a large number of digital images
- Training tool for students and laboratory professionals
- Fully automated selection, preparation, staining and capturing of blood film images
- Screening of potentially unsuitable specimens

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Despite remaining the gold standard in white blood cell differentials, microscopic analysis of blood smear carries a number of technical and practical drawbacks that can be at least in part overcome by automated microscopy.

As for our local experience, **the high NPV has allowed to reduce the blood smear review from 7% to 2%.**