UZ LEUVEN | LABORATORIUMGENEESKUNDE

CAT
Critically Appraised Topic

Title: Critical evaluation of the usefulness of microscopic leucocyte differential in the follow-up of oncological patients

Exploration of the possibilities to reduce turn-around-time (TAT) of leucocyte differential and optimalisation of workflow: ‘Flag’ settings automatic analyser, automatic digital microscopy, smear scanning, frequency reduction of microscopic leucocyte differential

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CLINICAL BOTTOM LINE

Clinicians insist on microscopic review of blood smears to identify possible erythrocyte or leucocyte anomalies, and even when results are negative, they feel reassured by the simple fact that an expert checked the smear and did not find anomalies [1]. This attitude contrasts with the need to reduce microscopic checks to a minimum, limiting them to specimens that did not pass medical review [1]. With this critically appraised topic, we aimed at finding ways to reduce the TAT of the white cell differential in oncological units and more in general. With the introduction of a new version of the data management system Remisol, Advance 2000, reconsideration of the flagging rules, and introduction of smear scan, the TAT of a white cell differential (automatic and/or microscopic) was reduced from 119 to 63.3 min on weekdays at the oncological day clinic. These changes also lead to a reduction in review rate from 32.5% (manual white cell differentials) to 13.9% manual white cell differentials and 13.9% smear scans. Blasts and myeloid precursors can be reported in a second time. Oncologists phone the lab to inquire the absolute neutrophil count before chemotherapy is started. Accordingly all oncology samples have priority for microscopic review. Haematologists are still interested in a complete microscopic review, white cell differential and red cell morphology included. A study of peripheral blood smears with routine manual and automated microscopy (Cellavision™ DM96) showed that automated microscopy did not reduce the TAT. This finding could be influenced by a learning phase with this new way of interpreting a blood smear. The instrument indeed leads to a reduction in TAT in case of neutropenic patients. On the other hand however, many cells had to be reclassified into another cell class in our short experience with Cellavision™ DM96. Smear scan was able to reduce the TAT in some cases of red and white cell abnormalities. A smear scan takes only about one third of the time of a manual white cell differential. It is worth considering counting band and segmented neutrophils as one group since there are other signs of toxicity. Moreover automated microscopy cannot distinct well between these two cell subgroups. For newborns with bacteraemia, the distinction band-segment is however still used by neonatologists. Finally, although there is little consensus in literature about the minimum time interval between two successive manual white cell differentials, oncologists of the Imelda Hospital seem to agree with the introduction of a blocking period. For hospitalised oncology patients, a time interval of 48 hrs could be discussed. Oncology patients visiting the day clinic still should receive a manual white cell differential when needed.

CLINICAL/DIAGNOSTIC SCENARIO

The Imelda Hospital is a tertiary care 502-bed centre serving a growing number of haemato-oncological patients. These patients have to wait upon the white cell differential, and in particular on the neutrophil count, before chemotherapy can be given in the oncological day clinic. Therefore, it is of utmost importance to have a white cell differential available as soon as possible.

By means of literature study, we want to evaluate in how far the patient’s physician is getting useful information with the white cell differential in band forms, metamyelocytes, myelocytes and promyelocytes. Does the presence of blasts influences the therapy on that moment?

Before the result reaches the treating physician, there are several steps to be performed. The whole process can be divided in a pre-analytical phase (prescription, sample, sample transport, reception and registration at the lab, internal transport to the workbench), an analytical phase (measurement, reflex testing, microscopic review) and a post-analytical phase (technical and medical validation, reporting and interpretation).
The aim of the laboratory is to report the results of more than 90% of the prescriptions within 90 min. A sample on which a white cell differential is requested undergoes several steps, each with their particular influence on the total turn-around-time (TAT).
An extended process analysis has already lead to adaptations of the pre-analytical phase. As such, specific mark signs have been introduced on the transport boxes and request forms in case it concerns samples of oncological patients requiring chemotherapy. Likewise, transport boxes from the oncological day clinic have got a higher priority. An extra centrifuge is foreseen for the analysis of urgent samples. In the electronic medical records of the laboratory information system (LIS) (GLIMS), one has added an electronic ‘Onco’ label to which different actions can be coupled.
In the analytical phase, samples are run on the Beckmann Coulter LH755/LH750 haematology analysers.
Result management of the haematology parameters takes place with Remisol software. The results are transferred to the LIS automatically in case they fall within the validation- and reference limits. All samples with particular ‘flags’ (f. eg. leucopenia, morphologic aberrations) are transferred for manual microscopy. In that case, the white cell differential of the analyser (Coulter LH755) is withheld from the LIS. It is up to the laboratory technician to validate the white cell differential manually. We will analyse the existing ‘flags’ in Remisol and in the Coulter, with particular attention on further reduction of the TAT. Furthermore, we have the intention to evaluate more methods which could possibly reduce the TAT of the white cell differential. The staining of the slides and the microscopic review extend the TAT enormously.
A rather new technique is the automated digital microscopy of peripheral blood. Some years ago, Sysmex has introduced Cellavision™ DM96. Analis® and/or Siemens will bring the HemaCAM® computed assisted microscopy on the market in the year 2009. These instruments determine automatically the location of white blood cells on a peripheral blood smear and preclassify them. Cellavision™ DM96 and possibly also HemaCAM® will be evaluated for their potential to reduce the TAT of the white cell differential.
Smear scanning, i.e. the microscopic screen of the leucocytes on a peripheral blood smear without counting them, is a different technique meriting evaluation.
In conclusion, we can ask ourselves whether the daily repetition of a white cell differential is useful. Is it clinically possible to prolong the time interval between two microscopic white cell differentials? Can we use delta checks for this latter?

QUESTIONS

1. Has the introduction of a pre-sorting of slides for manual white cell differential, based on the ‘Onco’ label, significant influence on the TAT of samples from oncological patients?
2. Is it possible to adapt/change the ‘flag’ setting in the automated haematology analyser (Coulter LH755) in such a way that TAT is reduced?
3. Is it possible to report blasts and myeloid precursors in a second time?
4. Can automated digital microscopy (Cellavision™ DM96 Sysmex / HemaCAM® Computer Assisted Microscopy) reduce the TAT of a white cell differential?
5. Is there place for smear/slide scanning instead of a complete manual white cell differential, and does this reduce TAT?
6. Is it possible to discontinue the differentiation into band forms and segments as separate classes, and does this reduce TAT?
7. Is deltacheck an acceptable way to increase the time interval between two successive microscopic white cell differentials?

SEARCH TERMS

1) *MeSH Database (PubMed): MeSH term: “white cell differential”, peripheral blood smear”, peripheral blood review”, “smear scan”, “segments”, “band forms” ,”delta check”, “automated digital microscopy”*

2) *PubMed Clinical Queries (from 1966; http://www.ncbi.nlm.nih.gov/entrez/query.fcgi): Systematic Reviews; Clinical Queries using Research Methodology Filters (diagnosis + specific, diagnosis + sensitive, prognosis + specific): white cell differential and flagging, white cell differential and automated microscopy*
3) *Pubmed (Medline; from 1966), SUMSearch (http://sumsearch.uthscsa.edu/), National Guideline Clearinghouse (http://www.ngc.org/), Institute for Clinical Systems Improvement (http://www.icsi.org), The National Institute for Clinical Excellence (http://www.nice.org.uk/), Cochrane (http://www.update-software.com/cochrane, Health Technology Assessment Database (http://www.york.ac.uk/inst/crd/htahp.htm)*
4) *National Committee for Clinical Laboratory Standards (NCCLS; http://www.nccls.org/), International Federation of Clinical Chemistry (IFCC; http://www.ifcc.org/ifcc.asp), Westgard QC (http://www.westgard.com), Clinical Laboratory Improvement Amendments (CLIA; http://www.cms.hhs.gov/clia/)*
5) *UpToDate Online version 17.1 (2009)*

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1 Has the introduction of a pre-sorting of slides for manual white cell differential, based on the ‘Onco’ label, significant influence on the TAT of samples from oncological patients?

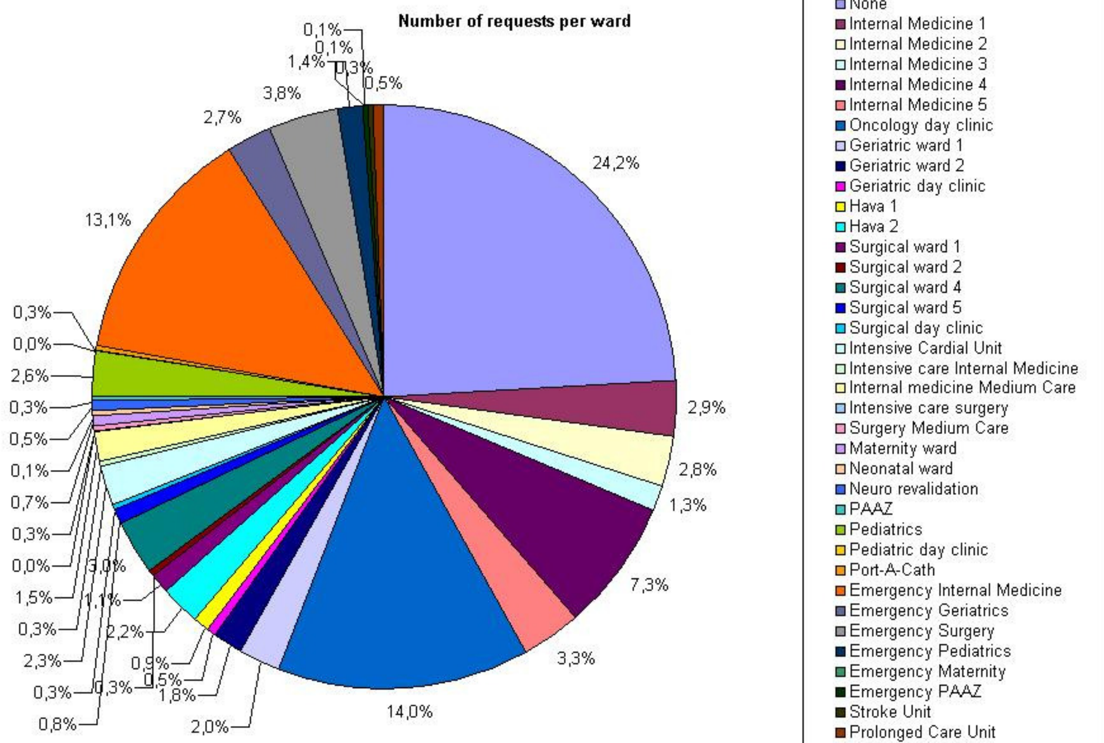
The ‘Onco’ label was introduced for the registration of the samples from the oncological day clinic on the lab [2]. Thanks to this fluo green label, the laboratory staff and technicians know that these samples have to get priority for analyzing. It should be possible to sort the samples for white cell differential in such a way that samples from the oncological day clinic get priority. We will not discuss this pre-sorting of slides based on this Onco label further, because a new update of the Remisol software, Advance 2000, has been installed which should make it possible to couple a pathology label to each of the patients of the oncological day clinic (f. e.g. Chronic lymphatic leukaemia, acute myeloid leukaemia, M. Kahler). We would like to focus on the rest of the topics in more detail.

2 Is it possible to adapt/change the ‘flag’ setting in the automated haematology analyser (Coulter LH755) in such a way that TAT is reduced ?

2.1 Introduction

The Imelda hospital is a 502-bed care center. Each day, on average 150 complete blood counts with white cell differential are performed with a Beckman Coulter LH755/LH750. Samples of the oncological day clinic represent 14%, those of the emergency department internal medicine 13.1%, those of the intensive care units 0.0-2.3%, those of the surgical units 0.3-3.0% and those of the medical units 1.3-7.3% of the demands (cfr Figure 1).

Figure 1. Percentage of requests per hospital ward in pie chart (over a period of 3 months).



In the Imelda Hospital, Beckman Coulter LH 755/LH750 haematology analyzers are used. The instruments use the combination of impedance, conductivity and laser light scatter [3]. They provide a 5-part leucocyte differential count (lymphocytes, monocytes, neutrophils, eosinophils and basophils) by analysing events in a flow cell with three different technologies: volumetric impedance using direct current (related to cell size or volume), conductivity using high frequency electromagnetic energy (signal related to internal complexity), and laser light scatter (related to both cell size and structure)[3]. The system uses a series of flags that indicate the need for manual review of automated results. In determining which of abnormal findings need be followed-up with a microscopic review, one must first decide what is clinically significant and then choose those among the clinically significant abnormal findings, which will yield the lowest (hopefully negligible) false negative rate even at the expense of a reasonable false positive rate [4]. This fact must be kept in mind when deciding which flags of the haematology analyser will be used for further microscopic review. The percentage of flagged test results and accordingly the rate of samples with a request for a white cell differential which are reviewed microscopically is 32.5% in the Imelda hospital. Because this rate is rather high compared with tertiary care centers (12-50%[5]) and literature data (12-30% for tertiary care hospitals with haematology-oncology units [6-12]), we were urged to take steps to reduce this high rate of flagged samples. The TAT of a white cell differential for all wards considered together was 171 min on average (N = 7534, over a period of 3 months, TAT of neutrophils on weekdays counting from the electronic request of the lab test until reporting the result. For the absolute white cell count, we reached a TAT of 45.6 min when considering all hospital wards (N = 6676, January 1st, 2009 – February 1st, 2009), and a TAT of 25.9 min when considering the oncological day clinic (N = 402, January 1st, 2009 – February 1st, 2009)(cfr. Figure 2). These data were compared with those of other laboratories in Antwerp. There seems to be a high difference in the TAT for absolute white cell count and white cell differential, review rates and work method on the oncological day clinic in the different laboratories (Table 1). The way of reporting results is also highly variable between centers, some laboratories report results in the same way as other routine samples, others have a separate haematology analyser on the oncological day clinic with the aim of obtaining a very quick result. For the oncology day clinic of Imelda hospital, the mean TAT of a white cell differential was 119 min on weekdays. On Mondays and Tuesdays, the TATs were longest with 103 and 170 min respectively.

Figure 2: Mean Turn-around time of white cell count and white cell differential over three months

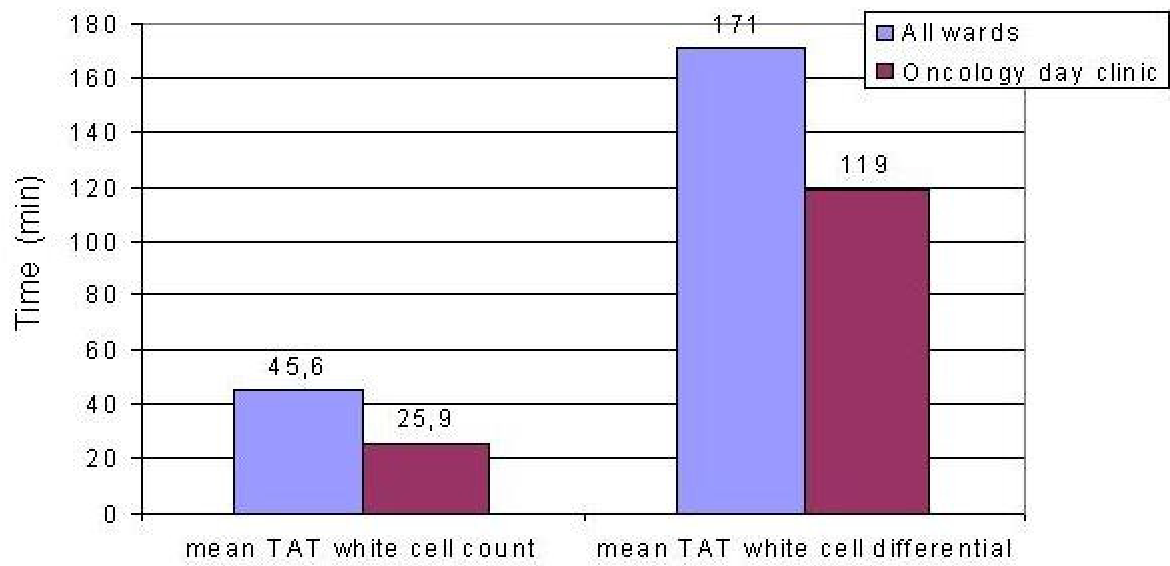
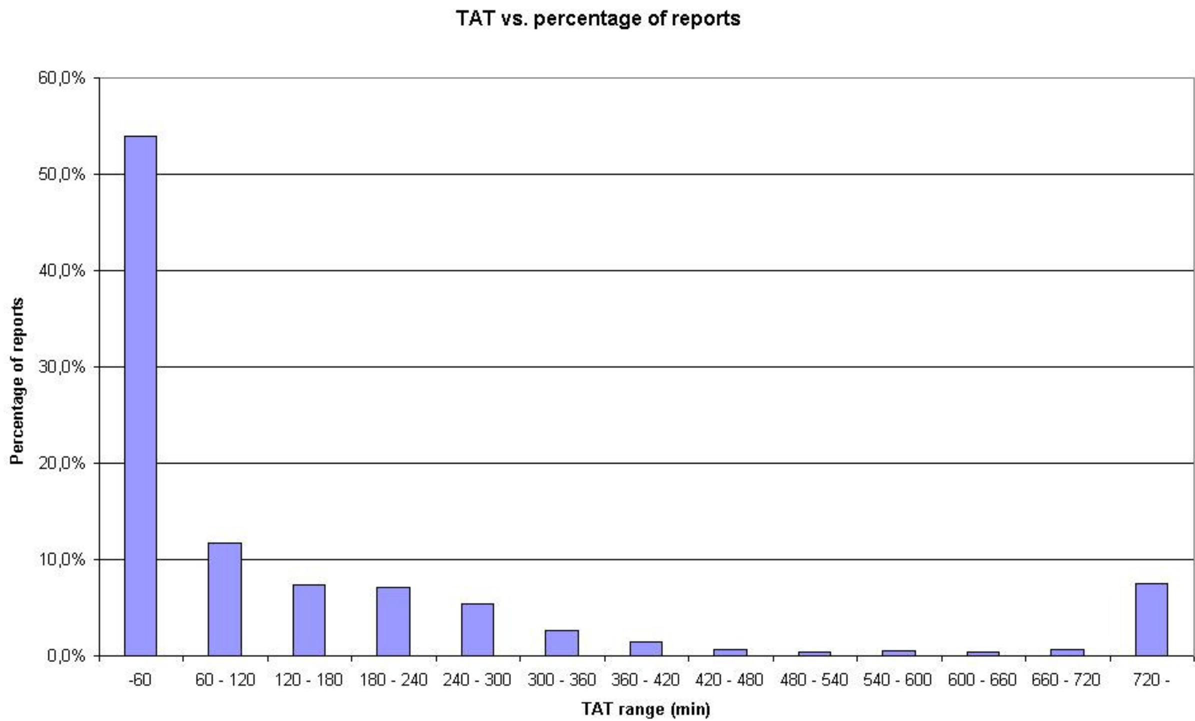


Table 1. TAT, review rate and method of different laboratories in the region of Antwerp.

	AZ Sint-Jozef Malle	H Hart Leuven	AZ St jozef Turnhout	St Augustinus (St Vincentius)	AZ Herentals	CMA	Middelheim (ZNA)	Stuyvenberg - ZNA	Imelda
instrument	Beckman Coulter LH750	Beckman Coulter LH750	Sysmex	Sysmex XE-2100	Siemens ADVIA 120	Sysmex XE-2100	Sysmex Poch100i Sysmex XE-2100 Sysmex XS- 1000	Sysmex Cellavision™ DM96	Beckman Coulter LH755
request form		colored request form	fluo mark on sample tube and request form	paper requests	the previous day, the request forms are registered and labelled tubes are brought to day clinic tubes are labbeled with orange label and have priority				green fluo onco label on request form and sample tubes
TAT white cell differential automatically	on week days (24/24) all hospital units: p50 = 32 min p90 = 163 min	for 2 days: p5 = 5 min p50 = 15 min p95 = 172 min for 5 days, oncology day clinic: mean = 14.5 min	- weekdays, TAT all white cell differentials automatically or manually: mean = 265 min - remark: sample appears in red when TAT > 1 hr	mean = 18 min p95 = 50 min p99 = 90 min		p90 = 100 min p95 = 130 min	80% of the results of the Sysmex Poch100i are accurate (on oncolgy day clinic)	60 min	-weekdays oncology day clinic, automatic and microscopic white cell differentials: mean = 119 min p50 = 38 min p90 = 452 min -all wards: mean = 171 min
TAT white cell differential microscopically		mean = 2 u 28.5 min p5 = 61 min p95 = 232 min						24-48u	
TAT white cell count automatically		mean = 13 min 57 sec p5 = 5.0 min p95 = 38.85 min	2 weekdays: mean = 45 min	p95 = 50 min p99 = 90 min					mean = 25.9 min
rate of white cell differentials / white cell counts	82.58%	100%			99.2%	90-95%			58%
review rate (smear scan and manual white cell differential)	20.7%	18 - 20%		?	9.7%	+/- 10%			32.5%
reporting to oncology day clinic	oncologist satisfied with reporting time	- result TAT < 60 min -technically validated results are transferred for emergency department, day and week clinic -clinically validated results are marked with [V]	preliminary hematology protocol is transferred to LIS	STAT results are immediately printed by clinical biologist on a printer on the day clinic	in case manual microscopy is necessary, laboratory technician/ clinical biologist checks for neutropenia		hematology analyser Sysmex Poch100i on oncology unit tube and results are transferred to the lab and repeated in case of discrepancy, clinical biologist / oncology unit is alerted	TAT oncology ward: < 60 min, not medically validated results ? In futur: small hematology analyser 3-5 diff on day clinic	oncologist telephones the lab for urgent counts

Among all requests (N = 7534) from all hospital wards, 54% of the requested white cell differentials were reported within 60 min (Figure 3).

Figure 3. Range in TAT of white cell differentials versus reporting percentage for all blood samples during 3 months



The Beckman Coulter LH 755 haematology analyzer flags have different origins. There are "suspect" and "definite" flags from the Coulter LH755 on the one hand, and flags from the Remisol, a data management system on the other hand. "Suspect" flags are built into the instrument technology and are based on aberrations in cell distribution (exceeding the 95th percentile values) or potential interference with these distributions (e.g. blasts, variant ly, imm NE1, imm NE2…) "Definite" flags are user-defined and based on quantitative thresholds defined by the laboratory (e.g. hemoglobin, lymphocytosis, macrocytosis…) [13, 14]. Flags from the Remisol dictionary are all user-defined.

An overview of the existing and new rules (see infra) in the LH and Remisol software is given in Table 2.

Table 2. Remisol rules: old and new.

old LH criteria	slide	smear scan	differential count	rerun	delta flag	comment	new LH criteria	slide	smear scan	differential count	telephone	rerun	comment
PLATELETS (PLT)													
PLT <= 40.0	1					stolsel? Indien pt niet gekend, verwittig KB, bel!	PLT <= 40.0	1			1	1	als pt niet gekend, verwittig KB
PLT <= 150.0	0					stolsel, controleer buisje!	PLT <= 50.0	1					
PLT >= 800.0	1	1				plt > 1000, patient gekend?	PLT <= 150.0						stolsel? Controleer buisje
PLT ---- or PLT R or PLT :::: or PLT ?????	1			1		plt error, rerun plt	PLT >= 800.0	1					
suspect: platelet clumps or suspect: giant platelets	1					controle PLT op citraat	PLT R	1	1				histogrammen? Fitted curve?
suspect: cellular interferene or suspect: high PLT interference or suspect: red cell agglutination	1					PLT clumps? Erythroblasten? Cryoglobulines?	PLT ---/::::/????					1	plt error, rerun plt
							PLT CLUMPS/GIANT PLT	1	1				controle PLT op citraat/heparine
							HIGH PLT INTERFERENCE	1	1				PLT clumps? NRBC? Cryoglobulines? RBC fragments? Microcytic RNC?
							PLT +++++					1	verdun, breng dilutiefactor in , rerun?
RED BLOOD CELLS (RBC)													
RDW >= 20.00	1					anisocytose? RBC morf	RDW >= 19.00	1					
HGB >= 18.00	1						HGB >= 18.00	1					
HGB <= 8.00 and ne% >= 0.0*	1			1		stolsel? Rerun en bel!!! ; verwittig KB	HGB <= 7.00	1	1			1	stolsel? Rerun op LH2
							HGB <= 7.00				1		
							HGB <= 6.00						verwittig KB
definitive: anemia and ne% >= 0.0	1		1			diff manueel							
definitive: hypochromia and ne% >= 0.0	1	1				verifieer microscopisch							
HGB --- or HGB ::: or HGB ???	0			1		HGB error, rerun	HGB ---/:::/???					1	HGB error, rerun
HGB delta check (run1-run2)# >= 1.00	0				1	HGB verschil te groot: delta flag							
MCHC >= 37.00	1			1		KA? Rerun na 60 min op 37°	MCHC >= 37.00	1	1				KA? Rerun na 60 min op 37°C
MCV >= 110.00 and ne% >= 0.0	1	1				macro 3+							
MCV >= 105.00 and ne% >= 0.0	1	1				macro 2+	MCV >= 105.00	1					
MCV <= 75.00 and ne% >= 0.0	1	1				micro 2+	MCV <= 75.00	1					
MCV <= 70.00 and ne% <= 0.0	1	1				micro 3+							
							MCV# >= 5.00						MCV verschil te groot: staalverwisseling
WBC +++ or RBC +++ or PDW +++	1			1		verdun, breng dilutiefactor in, rerun	RBC +++++	1				1	verdun, breng dilutiefactor in, rerun
WBC >= 400.00 or RBC >= 8.000 or PLT >= 3000.0	1			1		verdun, breng dilutiefactor in, rerun							
							HGB <= 12.50 and RDW >= 15.00 and MCHC >= 35.20	1	1			1	rerun retics --> MCV >= MSCV --> sferocyten?
H&H check failed	0			1		rerun, verh HGB & HCT onjuist	H&H check failed	1				1	1) rerun na 60 min op 37°C en 2) verdun 1/2
suspect: dimorphic reds and ne% >= 0.0	1		1			diff manueel	dimorphic reds and ne% >= 0.0	1	1				
NRBC % >= 2.1 or suspect: NRBC and ne% >= 0.0	1		1			NRBC?	NRBC % >= 2.1 or suspect: NRBC	1		1			NRBC?
suspect: cellular interferene or suspect: high PLT interference or suspect: red cell agglutination	1					PLT clumps? Erythroblasten? Cryoglobulines?		1					PLT aggr, NRBC? Cryoglobulines?
							high WBC count	1					RBC parameters: mogelijks interferentie van WBC
suspect: abnormal retic pattern	0			1		rerun retics	suspect: abnormal retic pattern	1	1				
							Retic R	1	1				
							Retic ::::					1	rerun retics
HGB <= 10.0 and RDW >= 18.00 and MCV <= 70.00	1	1?				ijzer te kort???							
HGB >= 10.00 and MCV <= 65.00 or Suspect: Thalassemia	1	1?				thalassemie?							
WHITE BLOOD CELLS (WBC)													
WBC >= 20.00 and WBC <= 40.00	1					controleer scatterplot	WBC >= 20.00	1					
WBC >= 40.00	1		1			als pt niet gekend, verwittig KB, bel!!!	WBC >= 40.00			1	1		als pt niet gekend, verwittig KB
WBC <= 2.00 and ne% >= 0.0	1		1			als pt niet gekend, verwittig KB, bel!!!	WBC <= 2.00 and existne%	1	1				als pt niet gekend, verwittig KB
definitive: leukopenia and ne% >= 0.0	1		1			diff manueel	WBC <= 2.00						bel
suspect: cellular interference or suspect: high PLT interference or suspect: red cell agglutination	1		1			PLT clumps? Erythroblasten? Cryoglobulines?	suspect: cellular interference	1		1			PLT CLUMPS, NRBC? Cryoglobulines?
WBC --- or WBC .... or WBC :::: or WBC ?????	0			1		WBC error! Rerun	WBC ---/..../::::					1	WBC error, rerun
WBC +++ or RBC +++ or PDW +++	1			1		verdun, breng dilutiefactor in, rerun	WBC +++++	1				1	verdun, breng dilutiefactor in, rerun
WBC >= 400.00 or RBC >= 8.000 or PLT >= 3000.0	1			1		verdun, breng dilutiefactor in, rerun	high WBC count					1	verdun staal , rerun
							WBC R	1	1				
WHITE CELL DIFFERENTIAL													
ba % >= 3.0 or ba# >= 0.3	1		1			baso verhoogd	ba % >= 3.0 or ba# >= 0.3	1		1			baso's verhoogd
eo % >= 20.0 or eo# >= 0.6	1		1			eo verhoogd	eo % >= 10.0 or eo# >= 1.0	1	1				eo's verhoogd
suspect: Mo blasts or suspect: NE blasts or suspect: LY blasts	1		1			verwittig KB	blast	1		1			
MO# >= 1.5 or MO % >= 20.0	1		1			mono verhoogd	Mo# >= 1.5	1		1			mono's verhoogd
MO# <= 0.2	1		1			mono < 0.2, pt gekend?							
NE# <= 1.0	1		1			NE# <= 1.0: bel, verwittig KB!	NE# <= 1.0	1			1		als pt niet gekend, verwittig KB
NE# >= 11.0	1		1			NE>11.0: NE verhoogd	NE# >= 11.0	1	1				
NE% :::: or LY% :::::	1			1		LYSE RESIST RBC? Verdun 1/2 en rerun	NE% ::::	1					Lyse resistent RBC? Verdun 1/2 en rerun. Flowcell verstopt?
Suspect: Variant Ly	1		1			ATYPISCHE LYMFOCYTEN, als pt niet gekend, verwit	Variant LY	1		1			atypische lymfocyten?
LY# >= 4.0	1		1			Lymfo >4.0, pt gekend?	Ly#>= 4.00 and > 16yrs	1		1			
							Ly#>= 6.00 and >1 yrs and < 16 yrs	1		1			
							Ly# >= 10.00 and < 1 yr	1		1			lymfo's verhoogd
Definitive: Lymphocytosis%	1		1			ATYPISCHE LYMFOCYTEN							
Suspect: Imm NE2	1		1			JONGE VORMEN??	Suspect: Imm NE2	1		1			jonge vormen?
Suspect: verify diff	1		1			Diff manueel	verify diff	1		1			
GENERAL													
Suspect: Low event # or Part asp. or Sys. Alarm	0			1		MOGELIJK TE WEINIG STAAL, RERUN	Suspect: Low event						differentiatie op minder dan 800 cellen
Suspect: Low event # or Part asp. or Sys. Alarm	0			1		MOGELIJK TE WEINIG STAAL, RERUN	Part. Asp.					1	mogelijk te weinig staal, rerun
Suspect: Aging sample	0					OUD STAAL? VRAAG CONTROLE STAAL							
WBC <= 3.00 and HGB <= 10. And PLT <= 100.0	1					retic?							
WBC +++ or RBC +++ or PDW +++	1			1		verdun, breng dilutiefactor in, rerun							
WBC >= 400.00 or RBC >= 8.000 or PLT >= 3000.0	1			1		verdun, breng dilutiefactor in, rerun							
	43	6	19	14	1			39	13	12	4	13	
*NE% >= 0.0 : a white cell differential is performed													

As an example, we will discuss the restricted literature considering the flagging criteria of haematology analysers.

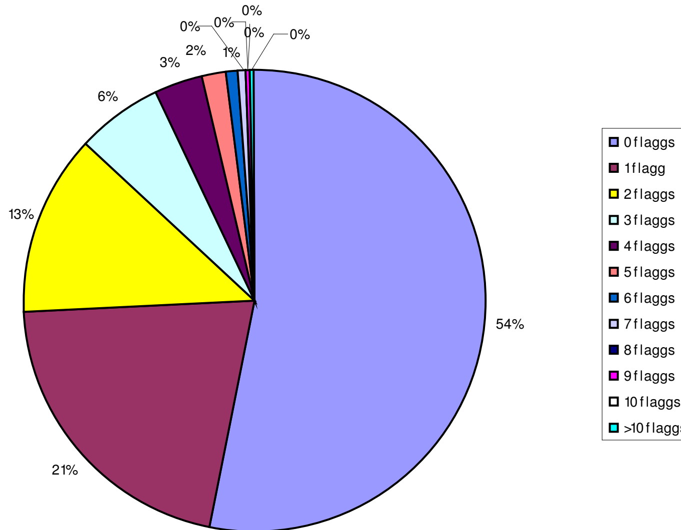
Lantis et al have studied the effect of revising instrument-driven reflex manual differential leucocyte counts on a Beckman-Coulter GenS analyzer (Hialeah, FL) [13]. They eliminated all these instrument-driven reflex rules for manual differential except when the instrument was unable to issue any differential result owing to marked interference (dot-out results) [13]. Since there are no evidence based guidelines regarding the level at which immature granulocytes (metamyelocytes and myelocytes) become clinically significant, the arbitrary limit of greater than 2 immature granulocytes discovered on scanning was chosen for the performance of a manual differential count [13]. If 1 or 2 immature granulocytes or more than 2 nucleated red blood cells (NRBC) were seen on scanning of the peripheral blood smear, the technologist was instructed to release the automated leucocyte differential with the following comments, as applicable: ‘Rare metamyelocytes/myelocytes seen on scan’. The finding of even a single promyelocyte or blast on scanning triggered the ordering of a manual leucocyte differential [13]. An overview of other reported criteria for slide review are given in Table 3.

Table 3. Overview of literature criteria for slide review [4, 8, 13, 15].

author	College of American Pathologists	Hyun et al (1991)		Lantis et al (Hematopathol 2003)		ISCH Consensus guidelines 2007	
complete blood count	p90 triggering manual review	criteria	action	criteria	action	rules	action
WBC ($*10^3/\mu l$)	< 3.0	< 4.0	slide review			< 4.0 + first time/ delta failed and < 3 d	slide review
WBC ($*10^3/\mu l$)	> 40.0	> 50.0	slide review	> 50	smear scan	> 30.0 + first time / delta failed and < 3 d	slide review
Retics						exceeds linearity	dilute sample and rerun
WBC, PLT						lower than lab verified instrument linearity	flow lab SOP
WBC, RBC, Hb, PLT						vote out	check sample for clot, rerun, if persists, perform alternate counting method
BP ($*10^3/\mu l$)	< 100.0	< 100.0	slide review	< 50		< 100.0 + first time	slide review
BP ($*10^3/\mu l$)	> 1000.0	> 1000.0	slide review			> 1000.0 + first time	slide review
BP ($*10^3/\mu l$)						any value + delta check failed	slide review
Hb (g/dl)	< 10.0	< 8.0	slide review	< 8.0	smear scan	< 7.0 + first time	slide review and verify sample integrity if indicated
Hb (g/dl)	> 21.0	> 18.0	slide review			> 2.0 above upper reference range for age and sex + first time	slide review and verify sample integrity if indicated
RBC ($*10^6/\mu l$)	< 3.0						
RBC ($*10^6/\mu l$)	> 8.0						
MCV (fl)	< 79.0	< 75	slide review	< 77	smear scan	< 75.0 + first time + specimen <24 hrs old	slide review
MCV (fl)	> 120.0	> 104	slide review			> 105.0 (adult) + first time + specimen < 24hrs old	slide review
MCV (fl)				> 115	smear scan	> 105.0 + adult + specimen > 24hrs old	slide review for macrocytic associated changes, request sample if no macrocytic associated changes seen, report with comment if fresh sample is not available
MCV (fl)						any value and delta fails and specimen < 24 hrs old	verify sample integrity/identity
MCH (pg)				< 21	smear scan		
MCHC (g/dl)		31	slide review			>= 2 units above upper limit of reference range	check for lipemia, hemolysis, RBC agglutination, spherocytes
MCHC (g/dl)						< 30 + normal/high MCV	investigate possible IV contamination or other sample specific cause
RDW (%)		> 20	slide review	> 17	smear scan	> 22 + first time	slide review
neonate						first sample	slide review
differential							
no diff or incomplete diff							manual diff and slide review
neutrophils # ($*10^3/\mu l$)		> 7.5	slide review			< 1.0 + first time	slide review
neutrophils # ($*10^3/\mu l$)						> 20.0 + first time	slide review
lymfocytes # ($*10^3/\mu l$)				> 8		> 5.0 (adult) + first time	slide review
lymfocytes # ($*10^3/\mu l$)		> 6.0	slide review			> 7.0 (<12 yrs) + first time	slide review
monocytes # ($*10^3/\mu l$)				> 2		> 1.5 (adult) + first time	slide review
monocytes # ($*10^3/\mu l$)		> 1.0	slide review			> 3.0 (< 12 yrs) + first time	slide review
eosinophils # ($*10^3/\mu l$)		> 1.0	slide review	> 1.5	smear scan	> 2.0 + first time	slide review
basophils # ($*10^3/\mu l$)		> 0.3	slide review	basophilia	smear scan	> 0.5 + first time	slide review
NRBC #				any	smear scan	any value + first time	slide review
retic absolute #						> 0.100 + first time	slide review
::::					differential		
R flag					smear scan		
suspect flags							
suspect flag (except ImmG/band)				ImmNE2	smear scan	positive flag + first time + adult/child	slide review
WBC unreliability flag						positive flag +any	check sample integrity and rerun, if persists, review instrument output, slide review with manual diff if indicated
RBC fragment					smear scan	positive flag + any	slide review
dimorphic RBC					smear scan	positive flag + first time	slide review
lyse resistant RBC						positive flag + any	review WBC histogram/cytogram, validate by lab SOP eg. Review smear for abnormal RBC morphology
RBC agglutination					smear scan		
PLT clump flag						any count	check sample for clots, slide review (PLT estimate), if clumps persist, follow lab SOP
platelet flags						PLT & MPV flags except PLT clumps	slide review
giant platelets					smear scan		
flag						positive flag + first time	slide review
flag						positive flag + previous confirmed result + positive delta fail for WBC	slide review
left shift flag						positive flag	follow lab SOP
atypical/variant lymphs		> 0.5 * 10^9/L	slide review		smear scan	positive flag + first time	slide review
atypical/variant lymphs						positive flag + previous confirmed result + positive delta fail for WBC	slide review
blast flag		any	slide review		smear scan	positive flag + first time	slide review
blast flag						flag + previous confirmed result + delta pass or negative delta for WBC + within 3-7 d	follow SOP lab
blast flag						positive flag + previous confirmed result + positive delta fail for WBC	slide review
verify diff					smear scan		
NRBC flag		2/100 WBC	slide review			positive flag	slide review, if positive, enumerate NRBC count, correct WBC if appropriate
retics						anormal pattern	look at instrument output, repeat if aspiration problem, if persists, review slide
pancytopenia					smear scan		
::::							

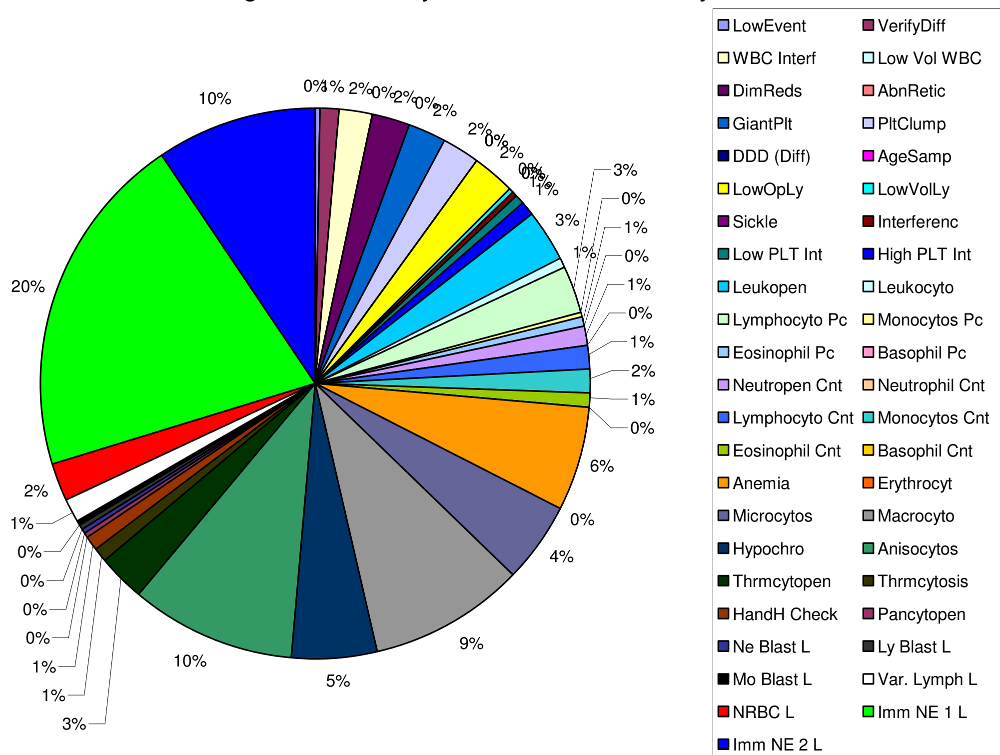
We evaluated the flagged samples from the Coulter LH from the First of January 2009 until the First of February 2009. In this period, 3641 (47%) of the 7769 samples had flags. 73% of the 3641 flagged samples had one or two flags (Figure 4).

Figure 4. The rate of flags per sample, from January 1st until February 1st 2009 (N = 3641 samples).



The Coulter's flags reported most frequently were: immNE1 (band forms) (20% of all flags), imm NE2 (promyelocytes, myelocytes and metamyelocytes) (10%) and RBC anisocytosis (10%) (Figure 5).
Figure 5. Distribution of the different flags from January 1st, 2009 until February 1st, 2009.

(p75- p25



We evaluated the flagged blood samples of the oncology department from 12 January until 17 March 2009 (2 months), which had a TAT of more than 2 hrs. We chose this cutoff since the key performance indicator for the TAT of the white cell differential for oncological day clinic is less than 2 hrs for more than 90% of the samples [2]. We presume that these samples have been reviewed microscopically. As such, we collected 307 samples. The mean TAT of these samples was 5.53 hrs. Table 4 shows the flags occurring in these samples.
In summary, of all flags occurring, the most frequent flags (N > 40) appeared on red cell morphology (anisocytosis, macrocytosis), leukopenia, neutropenia, lymphocytosis, monocytosis, immNE1 and platelet clumps. The flags supposed to be the most important ones for the 307 samples are atypical lymphocytes, immNE2 and verify diff. These are flags which inevitably will require a microscopic review. They are remarkably frequent. It should be possible though to reduce the review rate for these samples with 26% when red cell flags are not taken into consideration for oncological patients. In this 26%, a red cell flag was the main rule played when analysing the peripheral blood sample on the Beckman Coulter.

Table 4: Main flags of 307 samples of oncology day clinic														
Red blood cells														
flag type	definitive						suspect	user						
description of flag	anisocytosis	hypochromia	microcytosis	macrocytosis	anemia	H&H check failed	dimorphic RBC	MCHC > 37: cold agglutinins, rerun after 60 min at 37°C	sferocytes (Hb < 12.5, RDW > 15, MCHC > 35.2)	retics? (WBC < 3, Hb < 10 and BP < 100)	NRBC			
all flags (N)	29	4	1	18	27	2	34	4	32	18	34			
most important flags per sample (N)	29	4	1	18	1	0	33	0	0	0	0			
White blood cells														
flag type	definitive							suspect						user
description of flag	lymphocytosis (lymphocytes > 4.0)	leucocytosis	leukopenia	neutropenia	eosinophilia	basophilia	monocytosis (user flag added: mono > 20% / > 1,5)	atypical/variant lymphocytes	immNE1	immNE2	NE blasts	MO blasts	lymphoblasts	monocytopenia (mono < 0.2)
all flags (N)	55	2	98	50	5	4	54	43	72	59	1	2	1	73
most important flags per sample (N)	11	0	16	3	0	2	0	40	1	58	1	2	1	0
Blood platelets							**Other**							
flag type	definitive		suspect		user		definitive	suspect						
description of flag	thrombopenia	thrombocytosis	platelet clumps	giant platelets	clot? BP < 150		pancytopenia	cellular interference	verify diff	low event				
all flags (N)	45	1	59	6	34		11	21	57	1				
most important flags per sample (N)	6	0	18	0	0		3	21	57	1				

Because microscopic review leads to a prolonged TAT, we reevaluated all flagging criteria with the associated comments/actions in Remisol (software) and LH (Beckman Coulter). The ultimate aim was to reduce the review rate of peripheral blood samples. The reconsideration of the existing criteria was performed in conjunction with an update of the Remisol software (Remisol Advance 2000). New rules were established after a number of meetings. Assisted by Lobke Tremmerie (representative of Analis®), a new dictionary of flagging criteria was established both in the LH computer and in Remisol software (Table 2). After some meetings, a virtual setting was set up in order to test the new flagging criteria. In a second test phase, experienced laboratory technicians were asked to test the new flagging criteria in 'life view'. The introduction of the new Remisol Advance2000 software lead to some practical changes for the laboratory technicians. Instead of the usual single diffpad sheet, separate sheets are now present on the Remisol screen for the blood smears requiring only smear scan and those requiring a full white cell count. Because we have two Beckman Coulter haematology analysers, two separate sheets were made for the samples run on LH755 and on LH750. An extra sheet is available for the samples needing a rerun. Because in this new setting, we have separate hosts to which the samples should be assigned when a rule is in action, a priority order had to be established for the different hosts. As such, the priority order will be: I) rerun, ii) manual white cell differential, iii) smear scan.
When a blood smear is scanned, a comment 'scanned' is added and when a white cell differential is performed, the comment 'counted' is added to the sample. This enables later statistics in the host (GLIMS).
Lastly, it would be interesting when oncological patients have a demographic label in the attributes of GLIMS that differentiates them from other patients. In that way, Remisol software could use this label for incorporation into a 'clinical' comment. In case the patient has a chronic lymphatic leukemia for instance, it would be helpful for the laboratory technicians knowing this in advance. Other reasons for coupling this clinical information to the Remisol software are: the lymphocytosis % and neutropenia flags, which are frequently present in oncological patients, should not be a reason for a manual white cell differential in these patients, in case no other flags have been played.

An overview of review guidelines published by some organizations is given in Table 3. It is clear that there still is little uniformity among different laboratories on criteria for action. There are no generally accepted guidelines [16]. According to Lantis et al (2003), it is sufficient to perform a manual white cell differential in case the automated white cell count percentages seem inaccurate by smear scan on the one hand and when 'suspect' cells are observed on the other hand [13, 17].
In what follows, we discuss the most important changes in our dictionary of rules and some ideas about the clinical usefulness of certain flagging criteria.

- White blood cells ≤ 2000/µL and white cell differential is asked by the clinician
- Neutrophils ≤1000/µL and white cell differential is asked by the clinician
    - This condition lead to a manual white cell differential previously. We omitted these rules in the revised dictionary of rules since it is clear from Rümke's tables [5] that we cannot count and differentiate white blood cells better than the haematology analyser performs this.
- Verify diff (suspect message)
    - This flag is generated by an internal algorithm when WBC > $1.5 * 10^3$ /µL and monocytes >= 20%. An 'R' flag appears next to DIFF%, DIFF#, nucleated red blood cells (NRBC)% and NRBC# when such an unexpected data pattern is encountered. A manual white cell differential is always needed.
- White blood cells: "cellular interference"
    - There is interference at the 35 fl region of the histogram. The WBC count can be artificially high because of NRBC, platelet clumps, unlysed red cells, or cryoglobulins. On the other hand, falsely decreased WBC counts can be the result of clotting [3, 11]. It is of importance to perform a manual white cell differential because of the possibility of NRBC interfering with the automated white cell differential.
- Lymphocytes >= 4000/µL
    - In case there is an absolute lymphocytosis, the possibility of atypical lymphocytes should be checked in the context of a leukaemic non-Hodgkin's lymphoma. Neonates and children have higher lymphocyte counts. Therefore, the rule was adapted. For children up to 1 year, a slide is performed without smear or manual white cell differential when the absolute lymphocyte count exceeds 10000/µL. For children between 1 and 16 years old, a slide is performed when the absolute lymphocyte count exceeds 6000/µL. For adults of more than 16 years old with an absolute lymphocyte count of more than 4000/µL, a manual white cell differential is required.
- Atypical/variant lymphocytes

- According to Hoffmann and Hoedemakers, this flag has no diagnostic value for the Abbott® Cell-Dyn 4000. Its performance is inadequate, as it fails to flag clinically relevant abnormal lymphocytes (e.g. hairy cells) with a rather high rate of false positives [18]. Furthermore, the flag appeared to be based solely on the level of lymphocytosis and the blast flag. And a blast flag is, independently, an indication for microscopic review [18].
- For the Beckman Coulter LH755 the variant lymphocyte flag is a reason to review a peripheral blood smear microscopically. In our opinion, this flag has diagnostic value.

- ImmNE1 = band forms
  - The clinical importance of left shift remains a subject of debate. Still, it represents an inexpensive, fast, screening method, especially in pediatrics [19]. In Imelda, this flag is not taken into consideration and accordingly does not result in a manual white cell differential. The automatic white cell differential is automatically transferred to the host.
- ImmNE2 = immature neutrophils : metamyelocytes, myelocytes, promyelocytes
  - There are three levels of sensitivity at the flagging preferences. We have adapted the level of sensitivity from 3 to 2. This action could help in reducing the rate of flagging.
- Eosinophils > 20% or # > 600/µL
  - In stead of performing a manual white cell differential, we have changed this rule into a smear scan in case the eosinophil count exceeds 600/µL. The relative rate of 20% is left out the rule, because this most often represents an overlap with the absolute count. It is of importance to check the morphology of the eosinophils in case of a high eosinophil count, since it can reveal a chronic eosinophilic leukemia with eosinophils and eosinophil myelocytes containing some granules with basophilic staining characteristics [20, 21]. In this condition, mature eosinophils often show hypogranularity, vacuolisation, and hypolobulation [20, 21]. The commonest causes of eosinophilia are allergic conditions (asthma, hay fever, eczema), parasitic infections, and drug allergy. When the eosinophil count is higher than 10.000/µL though, the likely causes are far fewer. In case of such marked eosinophilia, the main causes are parasitic infections, drug hypersensitivity, Churg-Strauss variant of polyarteritis nodosa, Hodgkin's disease, idiopathic hypereosinophilic syndrome and eosinophilic leukemia [21]. This means, that we probably may set the limit for smear scan at a higher level of 1-2000/µL.
- Platelets
  - Increased results can be caused by WBC fragmentation, severe microcytosis or cryoglobulinemia, decreased results can be caused by satellitism or clumping [3, 11]. A slide scan can reveal clumping, retesting the patient's blood platelets on citrate or heparin can reveal a pseudothrombopenia (as advised in Imelda).
- Hb ≤ 8 g/dL → 'clot?, rerun on LH2'
  - Spuriously decreased results can be obtained in case of clotting [11]. In Imelda, it is advised to rerun the sample on the condition that the patient did not have a previous Hb level ≤ 10 g/dL. In case the same result is found after rerun, the blood tube is inspected macroscopically for large blood clots. If there are no visible clots, there is probably no blood clot and the result is accepted.
- MCHC >= 37 g/dL
  - Increased results can be the result of hyperlipidemia or cold agglutinins, decreased levels can result from WBC > 50000/µL [3, 11]. In Imelda, it is recommended to rerun the sample after incubation at 37°C for 60 min, this action will neutralize the cold agglutinins.
- MCV >= 105 or <= 75 fl
  - The diagnostic possibilities are rather limited in these cases [11]. In early anaemias, either iron deficiency anemia and in > 50% of anaemias of chronic disease, MCVs are normal and morphologic discrimination between the two is difficult [11]. The combination of Hb, RDW and MCV serve better to this purpose [11].
  - Spurious high results can be caused by cold agglutinins, hyperglycemia, WBC count > 50000/µL, while spurious low results can be caused by cryoglobulins [11].
  - In Imelda, only a slide is made, no smear scan or manual white cell differential is needed. The slide is useful in case the patient would need a bone marrow examination afterwards.
  - We also use a delta check rule on the MCV value. In case a patient has a difference of more than 5 compared with the previous result (no matter the time interval between the two samples), it is possible that the blood sample is from a different patient (except for transfusion). The possibility of sample confusion should be checked.

- Dimorphic red cells and a manual white cell differential is asked by the clinician
  - In the former dictionary of rules, a manual white cell differential would be performed when this flag was present. It is more important to scan the slide for the presence of a double red cell population and possible dysplastic changes in the red cells, white cells and thrombocytes. A dimorphic red cell population can be an indication of a myelodysplastic syndrome when no blood transfusion has been given. The automatic white cell differential can be reported without delay though.
- RDW (red cell distribution width) >= 20
  - In the former rules, an increased RDW lead to smear scan of the red cell morphology. We know that this smear scan will reveal anisocytosis. The RDW appears on the report to the clinician, he is expected to be able to interpret this parameter correctly. In case the patient has an iron deficiency anaemia, we can deduce that from the decreased Hb, the decreased MCV, the decreased MCH, and the increased RDW. Therefore, smear scan in case of a high RDW has been left out the rules. In the consensus guidelines of the International Consensus Group for Haematology though, it is advised to perform a slide review in case the RDW exceeds 22 for the first time [15]. In case we would perform a slide review for a RDW > 22 in Imelda, this would mean 4.2% slides to review (slides originating from all hospital wards).

2.2 Analytical performance characteristics (analytical validation report)

2.2.1 Analytical considerations (reproducibility, accuracy, correlation, linearity, reference range)

Accuracy (bias)

Since the haematology analyser counts and differentiates some 8.000 events or cells, this instrument is much more accurate or precise than our manual microscopy [3, 22]. Rümke has demonstrated the statistical imprecision of a manual microscopic white cell differential [5, 9]. This statistical sampling error is the greatest source of error in the microscopic white cell differential [9]. This is of particular importance in peripheral blood of oncology patients with often a low total white cell count.

Reproducibility

There is not only the statistical imprecision, but also the inter- and intra-individual variability when differentiating white cells microscopically (more specifically the band forms) [23]. Therefore, it is important that all laboratory technicians performing manual microscopy have been educated in standardized morphologic criteria of the different blood cell series. Automated microscopy can, in part, reduce this variability. These systems guide the laboratory technician in recognizing particular cell class.

Correlation with current method

The reference method is according to CLSI guidelines [24] a manual count of 400 WBC, in particular: the counting of 200 WBC by two different technicians on two peripheral blood smears. The technicians have neither clinical information, nor information of flaggings of the analyser [5].

2.2.2 Turnaround time (TAT)

The time required to perform and report a 100-cell white cell differential has been reported to lie between 1.9 and 6 minutes in literature [14]. We were however interested in the TAT defined as the time from registration of the lab request at the reception until reporting the white cell differentiation in the laboratory information system. These figures were kindly reported by some other hospitals and private laboratories. A comparison of the TATs is given in Table 1. Since we are interested in the TAT of the white cell differential of the oncological patients in particular, we performed queries in GLIMS of TAT of the segmented neutrophils on weekdays. From these queries, the mean TAT of the oncological day clinic was 119 min. This was intermediate to what other laboratories guarantee their oncologists. We aim at performing better though.
After revision of the Remisol and LH rules, we reanalyzed the TAT of the automatic/microscopic white cell differential on weekdays at the oncological day clinic. From April 6th until April 30th 2009, the mean TAT for the white cell differential was 63.3 min at the oncological day clinic and 119 min for all hospital units. This is clearly better than the results before revision.
The rate of microscopic reviews has been reduced by i) the introduction of the new rules together with ii) the different hosts ‘smear scan’ and ‘manual white cell differential’. Instead of 32.5%, the microscopic review rate of white cell differentials is now 27.9%, with 13.9% for manual white cell

differential and 13.9% for smear scan. As such, our review rate comes closer to that of other peripheral centres/hospitals (cfr Table 1) and tertiary care centres (5).

2.3 Diagnostic performance: sensitivity, specificity

Detailed information concerning the sensitivity and specificity of the individual flags generated by the automated analysers, though probably available in the data files of the manufacturers, is scarce in the published literature and thereby not readily available to the laboratory community [4].

2.4 Cost impact: in and outside the laboratory

Actual cost
Novis et al showed that laboratory productivity was inversely related to manual differential count review rates: the higher the manual differential count rate, the lower the productivity [8]. Therefore we aim at limiting our review rate to 20% of the peripheral blood samples.

3 Is it possible to report blasts and myeloid precursors in a second time?

The delay for patients to receive their chemotherapy is a concern. Most, if not all, standard regimens to treat cancer patients include dose-exclusion criteria based on a minimum absolute neutrophil count (ANC), usually in the range of 0.5 – 1.0 * 10^9/L [25]. At the Imelda Hospital, one uses a cut-off of 1.5 * 10^9/L to initiate chemotherapeutic treatment. When a sample is flagged for microscopic review, the white cell differential is not transferred to the patient's electronic medical record. The clinician has to wait for the microscopic review to know the number of neutrophils. Clinicians regularly phone the lab in order to receive the absolute neutrophil count reported by the haematology analyzer. This reduces the TAT, patients can receive their chemotherapy faster in this way. Parham et al studied the difference between automated and manual neutrophils counts [25]. They concluded that the automated absolute neutrophil count would be a clinically valid replacement for manual ANCs, for the purpose of decision making regarding initiation of chemotherapy (comparing automated versus manual ANC, Pearson correlation coefficient was 0.97 for all haematology/oncology specimens tested) [25]. We have adapted our dictionary of rules in such a way that no manual white cell differential follows in case the WBC are less than 2000/µL and/or the neutrophils are less than 1000/µL. We cannot count better than the haematology analyser. The automatic white cell differential is transferred to the host provided no other rules have been played requiring slide review (eg. lymphoblasts, verify diff).
It is clear that newly admitted patients require a complete evaluation of the peripheral blood [6]. For inpatients under treatment though, the ANC often provides sufficient information about the function of the bone marrow to guide further treatment or monitor already initiated treatment [6].
In this setting, Friis-Hansen et al have established a simple algorithm allowing extraction of valid ANC from flagged Sysmex XE-2100 test results even though the five part differential count cannot be given because of flaggings [6]. Only samples flagged with the 'WBC abnormal scatter gram' indicating that the Sysmex XE-2100 cannot separate different cell types from each other have to be reviewed microscopically [6]. This algorithm provided a means for reliable ANC with which it is possible to rapidly evaluate bone marrow function which in many situations will be the information required for treatment of the patient [6]. If needed, the complete blood count can be performed at a later time [6].
Sysmex analysers have the advantage of reporting the rate of immature granulocytes (6-diff in stead of 5-diff such as Beckman Coulter analyser). Immature granulocytes have a higher DNA and RNA content than mature granulocytes and have therefore a higher side fluorescence intensity. In this way, they can be separated as a different population from the mature granulocytes [26]. With this parameter, immature granulocytes < 1.0 (oncology/haematology ward, UH Leuven) - 2% (not-oncology wards UH Leuven / Sint-Augustinus) are reported as such on the haematology protocol without further slide review. At UH Leuven, this change would reduce the manual white cell differential rate with 16% [26].
Lantis et al have studied the effect of revising instrument-driven reflex manual differential leucocyte counts on a Beckman-Coulter GenS analyzer (Hialeah, FL) [13]. Since there are no evidence based guidelines regarding the level at which immature granulocytes (metamyelocytes and myelocytes) become clinically significant, the arbitrary limit of greater than 2 immature granulocytes discovered on scanning was chosen for the performance of a manual differential count [13]. If 1 or 2 immature granulocytes or more than 2 nucleated red blood cells (NRBC) were seen on peripheral blood smear scan, the technologist was instructed to release the automated leucocyte differential with the following comments, as applicable: 'Rare metamyelocytes/myelocytes seen on scan'. The finding of even a single promyelocyte or blast on scanning triggered the ordering of a manual leucocyte differential [13]. At Imelda, we choose for the continuation of a manual white cell differential when immature flags f. eg. immNE2, lymphoblasts, monoblasts, myeloblasts have been played.

We were interested in the specific needs from the clinicians when they order a white cell differential. Therefore, we performed a query amongst oncologists (N = 6) of the Imelda hospital. The raised questions were the following:

1. To what extent is there a need for the complete leucocyte differential in blasts, promyelocytes, myelocytes, metamyelocytes, band forms, segments in the oncology day clinic?
2. Does the presence of blasts (and from which % onwards?) influences the therapy on that moment?
3. Does the ANC suffice to start up chemotherapy?
4. Is a leucocyte differential sufficient or is there a specific need for a systematic review of the red cell morphology?
5. Is it useful to repeat the leucocyte differential every 24 hrs? Is there a possibility to introduce a minimum time interval in which a second leucocyte differential will not be performed?

It seemed that clinicians dealing with gastro-enterological and pulmonary oncology mainly were interested in the absolute leucocyte count and the rate of neutrophils.
In hematological oncology though, it is wishful to perform a complete leucocyte differential (1.), the presence or increase in the rate of blasts in leukemia patients will have therapeutic implications (2.). The ANC is sufficient for most patients coming to the oncology day clinic for chemotherapy, but the thrombocyte count is also an important parameter for chemotherapy. Hemoglobin is an important parameter to evaluate the need for transfusion (3.) Although red cell morphology is not critical, its evolution is followed and checked for possible confusion of samples (4.). For hospitalized patients (e.g. neutropenic fever, post-chemotherapy to follow nadir, probability of infection,…), a leucocyte count repeated every 24 hrs is useful to follow the patient’s evolution.

Conclusion:
As already practiced, clinicians can phone the lab to inform after the ANC of oncology patients. All oncology samples accordingly have priority for microscopic review.
For haematology, it seems appropriate to perform a complete microscopic review, white cell differential and red cell morphology included.

4 Can automated digital microscopy (Cellavision™ DM96 Sysmex / HemaCAM® Computer Assisted Microscopy) reduce the TAT of a white cell differential?

Some authors believe that image recognition systems clearly improve quality by standardization of results, but that they nevertheless only reduce TAT marginally [6].

We aimed at evaluating two systems for automated digital microscopy: Cellavision™ DM96 (Sysmex®) and HemaCAM (Analis®).
In particular, we wanted to evaluate:
1) the performance of two types of automated microscopy systems: Cellavision™ DM96 and HemCAM®. Manual microscopy will be used as a reference method.
2) the possibility to reduce the turn-around-time with an automated digital microscope.

A demo instrument of Cellavision™ DM96 was available at Imelda from November 2008 until February 2009. A total number of 583 samples were analysed both with Cellavision™ DM96 technology and with manual microscopy. Although we had the intention to evaluate HemaCAM®, the demo instrument was not available and eventually could not be included in this study.

4.1 Materials and methods

Cooperating personnel
In our laboratory, there are 7 laboratory technicians experienced in performing manual white cell differentials. Every employee working with Cellavision™ DM96 was instructed in the use of the instrument by a representative of the firm or by the supervisor of the laboratory technicians (Isa Vierendeels).

Sample collection and preparation
Routine patient blood samples having been flagged for microscopic review were used in this study. The Coulter LH755 automated cell counter is used for analysis of patient EDTA blood samples. When particular decision rules and flagging criteria are met, coming either from the Coulter LH755 or the result management programme Remisol, an automatic slide is performed by the Coulter LH slide maker and stained with the May-Grünwald Giemsa stain. In the standard workflow, microscopic white cell counts can also be ordered by a physician. In that case, it occurs that manual slides have

to be performed. Both automatic and manual slides requiring microscopic review were used in the study.

Each of the 7 laboratory technicians was asked to analyse 50 randomly selected slides requiring review both with manual microscopy and Cellavision™ DM96. The laboratory technician can see the automated cell counter's results when analysing a sample with manual microscopy and with Cellavision™ DM96.

Special cases (acute leukaemia, chronic lymphatic leukaemia, toxic samples, myelodysplastic syndromes, abnormal red cell morphology…) had been collected and were analysed with manual microscopy and Cellavision™ DM96 by dr. ass. V. Saegeman.
We aim at analysing 500 slides for review both with manual microscopy and Cellavision™ DM96.

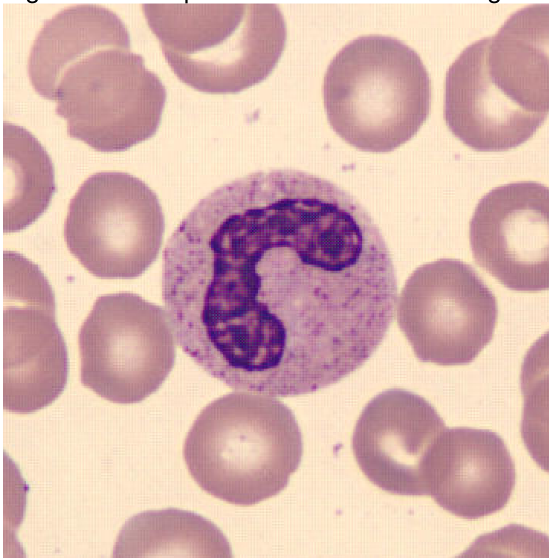
Manual white cell differential
For each of the 50 slides, each laboratory technician performed a microscopic white cell differential. This includes the counting and categorisation of 100 white cells based on pre-established morphologic criteria, results are expressed as a percentage of each cell type identified [4]. Although the CLSI recommends to count 400 cells, this method is time consuming and laborious [24]. Moreover, also in daily practice, only 100 white blood cells are counted. Therefore, we believe that counting 100 white blood cells is sufficient to the purpose of method comparison [4].
Morphology of the red cells and white cells was evaluated.

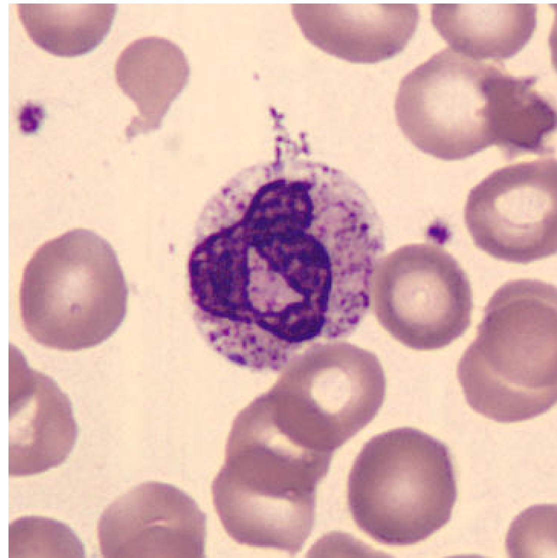
Turn-around-time study
Twelve slides prepared for microscopic review inclusive white cell differential were analysed consecutively with the light microscope while the time from evaluating the first slide to confirmation of the twelfth slide was registered. Each of the seven technicians was asked to record this turn-around-time.

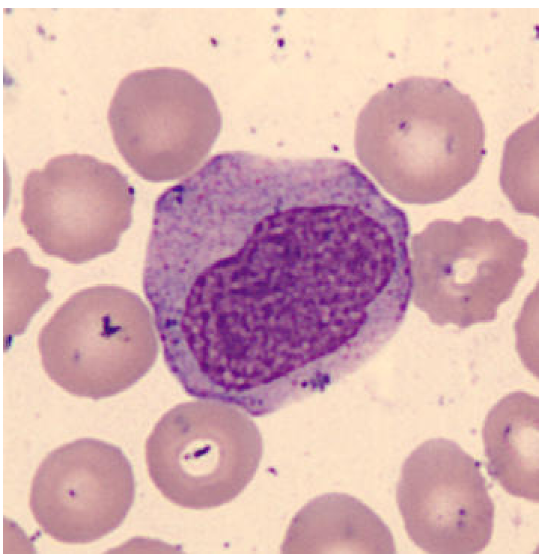
Analysis with the Cellavision™ DM96
The Cellavision™ DM96 consists of a slide scanning unit and a computer with the Cellavision Blood (Cellavison™, AB, Lund, Sweden) differential software (version 2.0). The scanning unit consists of a motorized microscope (x 10, x 50, and x 100 objective), a digital CCD camera, an automatic immersion oil unit, slide feeder unit with barcode reader, rack feeder unit and a control unit which controls motors, sensors, oil applying and illumination [27].
All slides were labeled with a barcode and loaded onto Cellavision. Slides are processed immediately and continuously [27]. Cellavision scans the slide, identifies potential white blood cells, takes digital images and uses artificial neural network-based software to analyse the cells. Each cell is separated using a segmentation algorithm based on several techniques, including tresholding and watershed transforms [12]. Several hundred calculations are made in order to extract specific features such as color, size and shape from the cell [12]. The networks were trained using feature sets (vectors of values) from about 37000 cells preclassified by experts [12]. The system counts on average 120 cells [28]. This setting is meant to allow for the possible inclusion of artifacts in the WBC count. Digital images of preclassified cells are presented to the laboratory technician on a user definable computer display (Figure 6) [27]. The laboratory technician can enlarge single cells for a more detailed view. The laboratory technician accepts the preclassification or reclassifies cells (by clicking on the cell and using the drag and drop function) when necessary [27]. Results are not released until all preclassified cell categories are reviewed [27]. Band neutrophils are added to the segmented neutrophils, the laboratory technician has to reclassify the band forms in the class of band neutrophils. For the evaluation of the red cell morphology, the DM96 provides an image which corresponds to eight areas of the smear viewed at x 100 objective. Red blood cell morphology is partially classified by the DM96; polychromasia, hypochromia, microcytosis, macrocytosis, anisocytosis and poikilocytosis are reported using a scale 0-3. Abnormalities in red cell shape such as fragmented red cells, sickle cells, target cells, are not automatically classified by the DM96 but can be added by the user after examining the image [27]. An estimate of platelet count can be made by the DM96 [27]. The image is subdivided into sub-images and the user is then required to count the number of platelets in each field. A calculated estimate of the platelet concentration is then made based on the counts per field and a platelet estimate factor [27]. This characteristic was not evaluated in our study since the haematology analyser gives us the platelet count. The laboratory technicians were asked to perform the analysis on Cellavision at least one day later then the analysis with manual microscopy. In this way, the memory of certain characteristics on a particular slide is minimal.

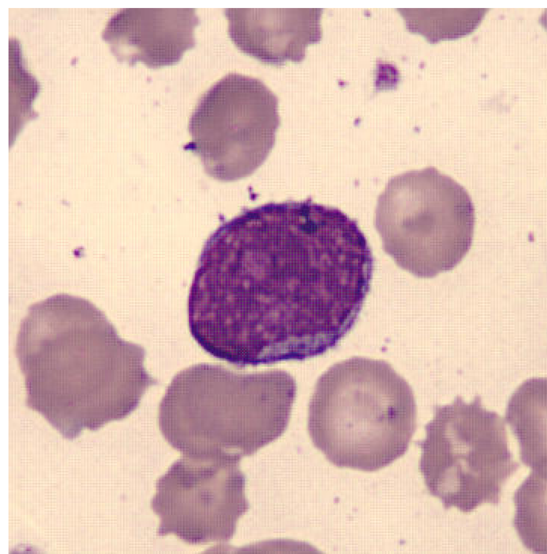
Turn-around-time study
The same 12 slides as used for manual differential are placed in a cassette and loaded in Cellavision™ DM96. The time needed to analyse these twelve slides, starting from the moment the cassette was loaded in Cellavision™ DM96 to reclassification and autorisation of the twelfth slide was registered. Each of the seven technicians was asked to record this turn-around-time.

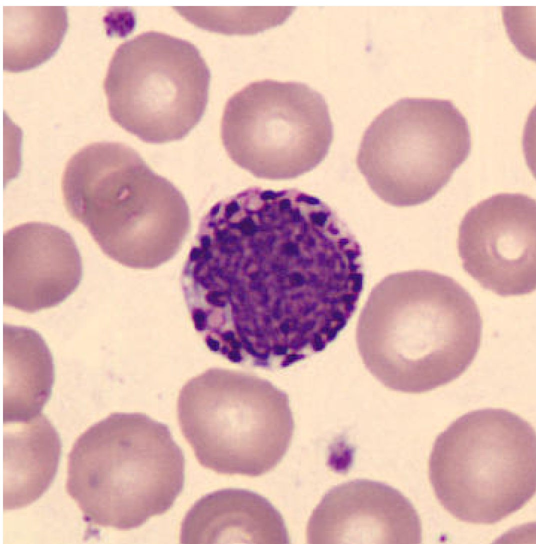
Figure 6. Example of white blood cell images from Cellavision™ DM96.

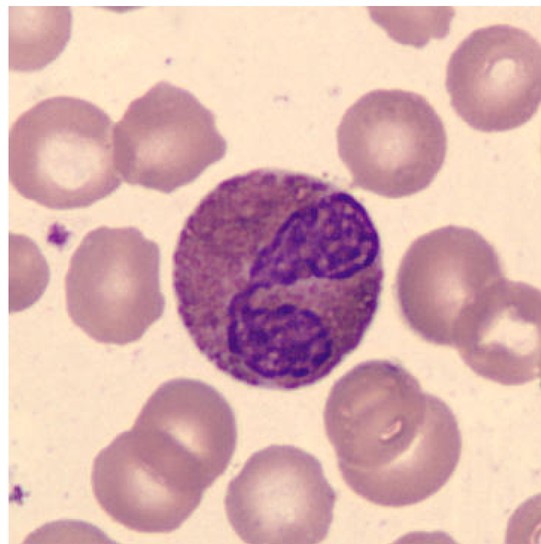

(a) band neutrophil


(b) segmented neutrophil


(c) monocyte


(d) lymphocyte


(e) basophil


(f) eosinophil

Analysis of data

Statistical analysis was performed with Microsoft Excel software. Pre-classification agreement and agreement with final results was calculated. Remark that for the calculations, the class of total lymphocytes consisted out of lymphocytes, variant lymphocytes, plasmocytoid cells, virocytes, reactional lymphocytes, atypical lymphocytes, large granular lymphocytes, Sézary cells, hairy cells, and prolymphocytes. We made a distinction between band forms and segmented neutrophils since we also count them separately in the routine manual white cell differential. Bland-Altman plots, for comparison of measurements, were constructed for the following cell classes: neutrophils (band forms and segmented neutrophils), total lymphocytes, eosinophils, basophils and monocytes. With this plot, we can more easily evaluate whether problems exist at certain ranges caused by nonlinearity of one of the methods [29].

Correlation analysis was used to compare the reclassified data of Cellavision™ DM96 with manual microscopy.

Paired t-tests were used to compare the time needed to analyse slides with the two different methods. A p-value < 0.05 was considered significant.

4.2 Results and Discussion Cellavision DM96

In total, 583 blood smears with a total count of 80185 white blood cells were analysed. Only blood smears for which both manual microscopy and analysis on Cellavision™ DM96 was possible were included in the study.

There were 8 blood smears on which there was a difference of more than 50 cells between the total cells in the preclassification made by Cellavision™ DM96 and the total cell number in the postclassification results. This was due to smudge cells in the preclassification result, transferred to the segmented neutrophils in the postclassification results. These 8 samples were excluded from the calculations. For 3 other samples, there were less than 20 identifiable cells on the Cellavision™ DM96 system. Because of the limited reproducibility of differential results based on such low counts, these samples were excluded too. This leaves 572 blood smears analysed both with Cellavision™ DM96 and manual microscopy.

4.2.1 Analytical performance characteristics

4.2.1.1 Analytical considerations (reproducibility, accuracy, correlation, linearity, reference range)

System Reliability

During the time the Cellavision™ DM96 demo was used, a ‘critical oil error’ was regularly noticed. There was, however, no problem with the oil dispenser.

We also noticed that the system counted the same cell several times in the same cell class. This phenomenon was not exclusively present among cells reaching each other with their cell margins. According to the technicians of Cellavision™ DM96, this phenomenon would be theoretically impossible (personal communication). They believe it could be that a cell is partly lysed in such way that the two different parts of the cells are counted separately. However, we noticed this problem also with intact cells. We believe this problem might be related to our staining protocol, which is, although being a very good staining according to the firm, not specifically adjusted for use on Cellavision™ DM96. The cells counted several times were reclassified in the artifact class.

Thirdly, the red cell morphology was very difficult to interpret. The only image of the red cells was regularly flawed by an unclear line in the image. This problem could not be solved by a technician who cleaned the objectives. The majority of the laboratory technicians thought the red cell view was inadequate to evaluate the red cell morphology. The limited number of red cells displayed on Cellavision™ DM96 did not allow to give a general idea of red cell morphological characteristics. This was also noticed in the study of Kratz et al, in 23% of the cases, technologists were not satisfied with the red cell view of Cellavision™ DM96 [28]. Moreover, Ceelie et al reported that there was variable agreement (35-78%) between pre- and post-characterisation for moderate and severe changes in red cell morphology [30]. Therefore, we did not take the interpretation of the red cell morphology into account in the further calculations.

Reproducibility

To evaluate the reproducibility, we analysed 3 slides 10 times with the Cellavision™ DM96 (pre-classification data). For the relevant cell categories, we found a moderate reproducibility (Table 5). Segmented neutrophils though had a SD of more than 5. On the contrary, Ceelie et al found a reproducibility of less than 2.5 SD for all relevant cell classes [30].

Table 5. reproducibility of Cellavision™ DM96 (pre-classification data).

	slide 1		slide 2		slide 3	
	mean (%) N = 10	SD	Mean (%) N = 10	SD	mean (%) N = 10	SD
segmented neutrophils	72,7	5,70	48,3	3,77	59,8	3,10
band neutrophils	11,3	2,79	0,2	0,42	2,4	0,73
eosinophils	0,9	0,57	0,4	0,52	14,3	1,45
basophils	2,1	1,29	0,3	0,67	0	0,00
lymphocytes	5,6	3,63	63	4,16	25,5	1,79
monocytes	11,1	2,42	6,5	0,85	17,2	1,50
blasts	0,7	0,95	0	0,00	0	0,00

Accuracy (bias)

The overall pre-classification accuracy in our study population was 82%. When segmented and band neutrophils were combined in one group (neutrophils), the pre-classification accuracy increased to 86%. Table 6 shows the pre-classification agreement of the different cell classes. In a European study of Cellavision™ DM96, the agreement of pre-classification for all cell classes was 84%, and 86% when segmented and band neutrophils were considered as one group [31]. Kratz et al also found an identical pre-classification agreement of 82% of all cells and of 86% if band forms were combined with segmented neutrophils. Ceelie et al found an overall pre-classification agreement of 92% [30] and Briggs reported a pre-classification agreement of 89.2% [27]. The agreement levels were lowest for abnormal cells (variant lymphocytes, plasma cells) and precursor white blood cells, corresponding to our findings and those of Briggs et al [27]. In the study of Briggs et al, blasts were underestimated with Cellavision™ DM96, the preclassification agreement was 76.6% compared with 68.3% in our study [32]. In our study too, blasts were counted with Cellavision™ DM96 with a sensitivity of 67.0% and a specificity of 99.8%. Cornet et al though found that for all patients with blasts on their smears, the blasts were detected by DM96 [12]. DM96 only underestimates the number of blast cells, especially in ALL where a huge number of them is misclassified as lymphocytes [12].

Table 6. Rate of cells correctly pre-classified by Cellavision DM96.

Cell-class	Pre-classifying agreement[a]	In agreement with final result[b]
Segmented neutrophils (N = 42461)	98,9%	86,6%
Band neutrophils (N = 830)	8,8%	37,7%
Eosinophils (N = 1196)	74,6%	74,3%
Basophils (N = 421)	84,4%	80,8%
Lymphocytes (N = 14743)	90,8%	82,7%
Variant Lymphocytes (N = 447)	17,3%	11,6%
Monocytes (N = 5077)	68,9%	93,7%
Plasma cells (N = 7)	4,3%	42,9%
Blast cells (N = 698)	68,3%	50,7%
Promyelocytes (N = 89)	26,1%	83,1%
Myelocyte (N = 357)	15,6%	48,7%
Metamyelocytes (N = 346)	31,4%	29,8%
Erythroblast (NRBC) (N = 320)	52,2%	44,4%

[a] Fraction of the cells correctly classified in that group by Cellavision, the laboratory technician agreed with Cellavision's preclassification.

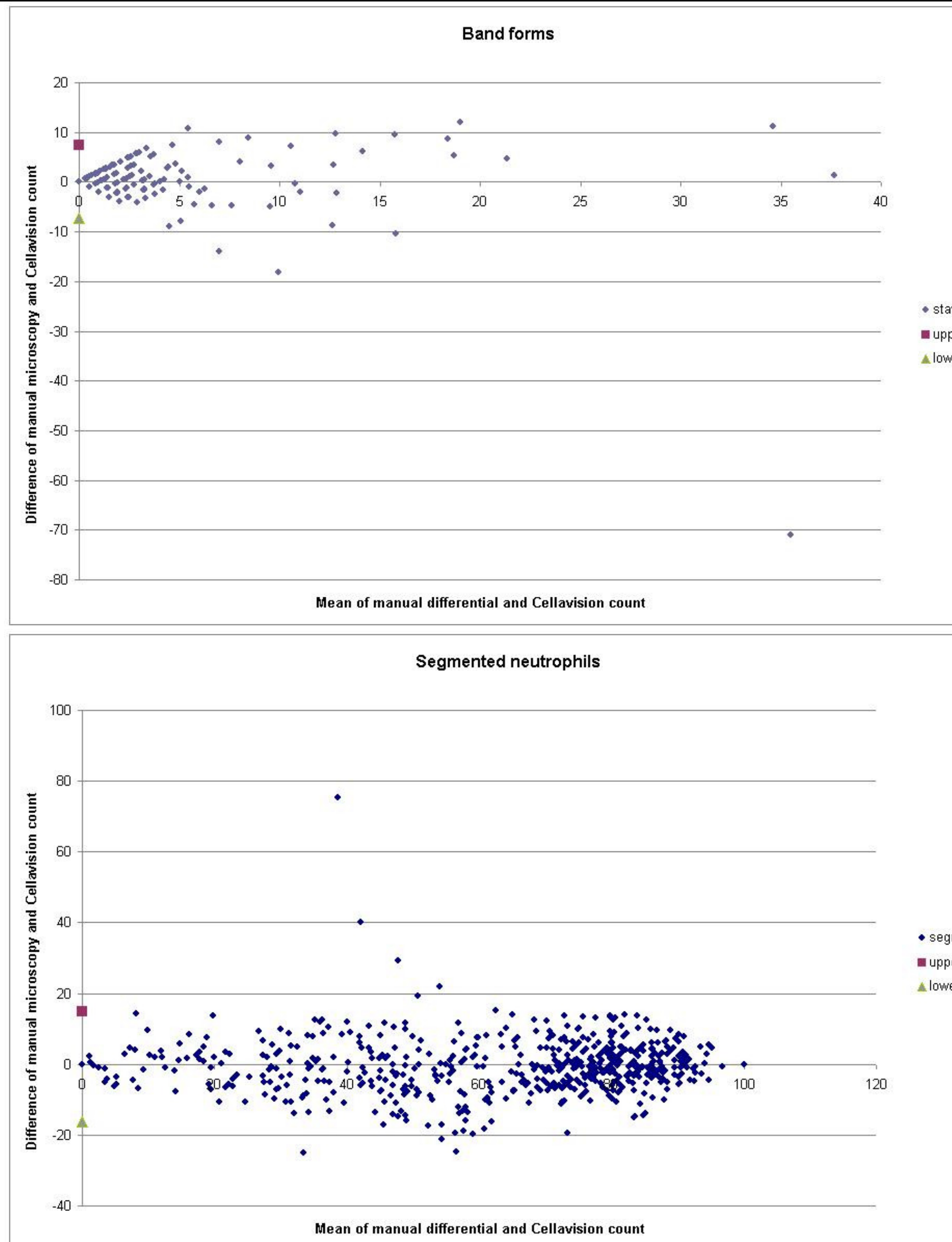
[b] The fraction of correct pre-classified cells of the total number of verified cells for the different cell classes.

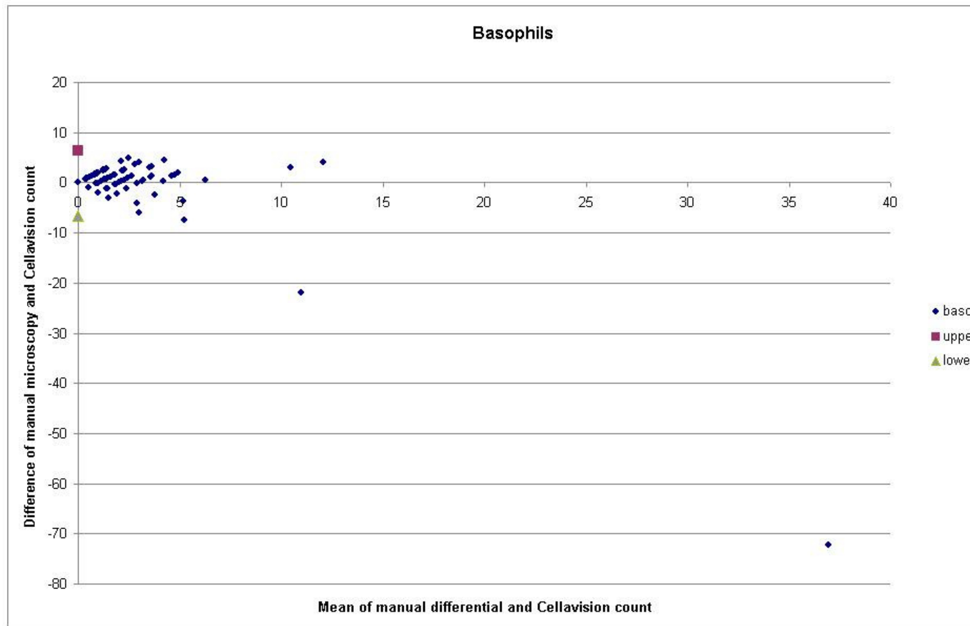
In our study, Cellavision™ DM96 preclassified 2.2% of the abnormal cells as normal cells, 7.1% of the normal cells as other normal cells and 3.7% of the normal cells were preclassified as abnormal cells. These figures were only 1.6%, 3.0% and 0.6% for Cellavision™ DM96 respectively in a study of Ceelie et al [30]. Briggs et al found only 0.9% of the abnormal cells misclassified as normal with Cellavision™ DM96 [27]. This type of misclassification is a major error. Although this major error represented only 2.2% in our study, it should be possible to get it lowered. Possibly, this high rate of major errors is due to the use of only blood smears flagged by the haematology analyser for microscopic review.

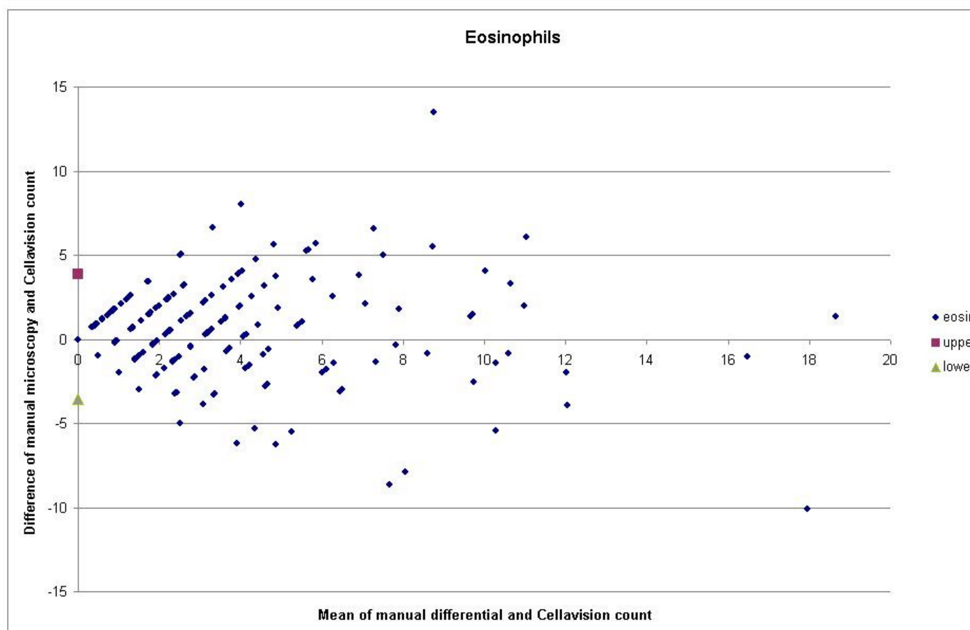
For the preclassification data, Cellavision™ DM96 had higher counts for monocytes (bias = 2.66) and band forms (bias = 4.00). Cellavision™ DM96 counted less lymphocytes (bias = -3.29) and

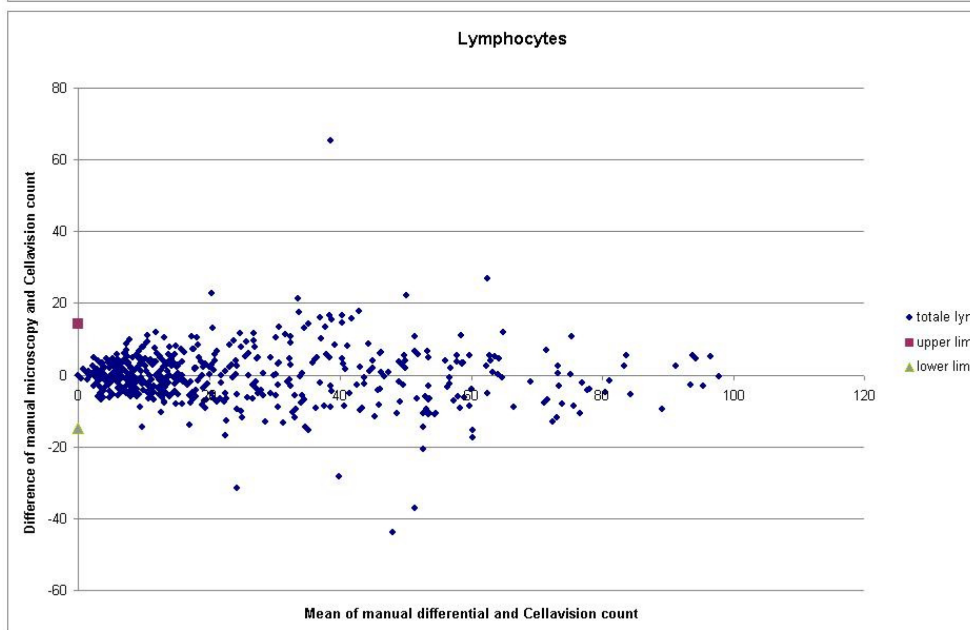
segmented neutrophils (bias = -7.52) than was counted with manual microscopy. For the eosinophils, basophils and blast cells, the bias was smaller than +/- 0.50. Postclassification all biases were smaller than +/- 0.62, indicating a good agreement (little discrepancy) between Cellavision^{TM} DM96 and manual microscopy.
The Bland-Altman (Figure 7) plots show that there is a high variation for the eosinophils and the blast cells. The individual points have a large bias at the higher mean levels. For the band forms, basophils, eosinophils, monocytes and blast cells there was a proportionally higher difference for the higher rates of band forms, basophils, eosinophils, monocytes and blast cells.

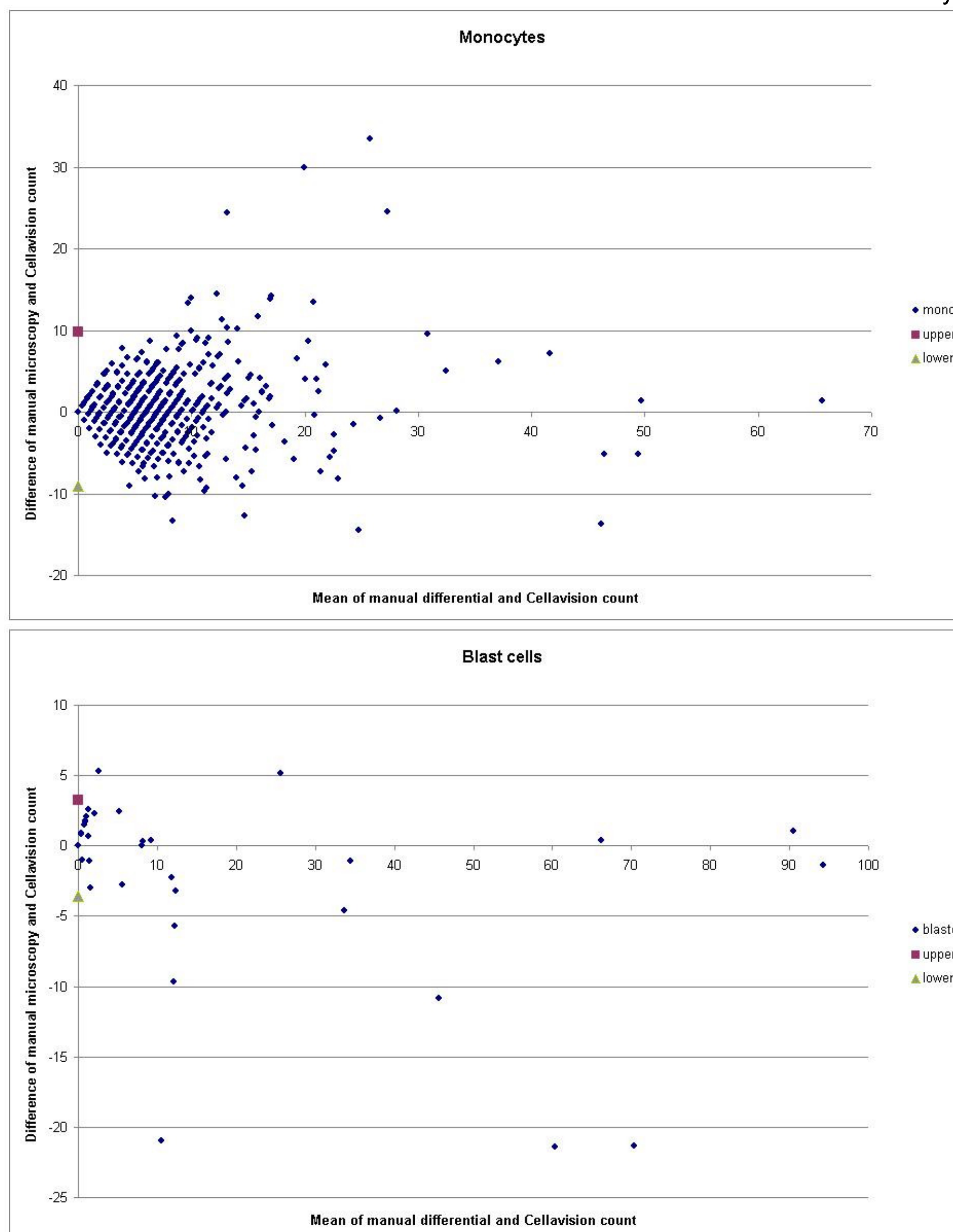
Figure 7. Bland-Altman plots of manual microscopy versus Cellavision^{TM} DM96 (postclassification) for band forms, segments, basophils, eosinophils, lymphocytes, monocytes and blast cells.




Basophils
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
0
5
10
15
20
25
30
35
40
Difference of manual microscopy and Cellavision count
Mean of manual differential and Cellavision count
basol
uppei
lower


Eosinophils
15
10
5
0
-5
-10
-15
0
2
4
6
8
10
12
14
16
18
20
Difference of manual microscopy and Cellavision count
Mean of manual differential and Cellavision count
eosin
uppei
lower


Lymphocytes
80
60
40
20
0
-20
-40
-60
0
40
60
80
100
120
Difference of manual microscopy and Cellavision count
Mean of manual differential and Cellavision count
totale lym
upper limi
lower limi



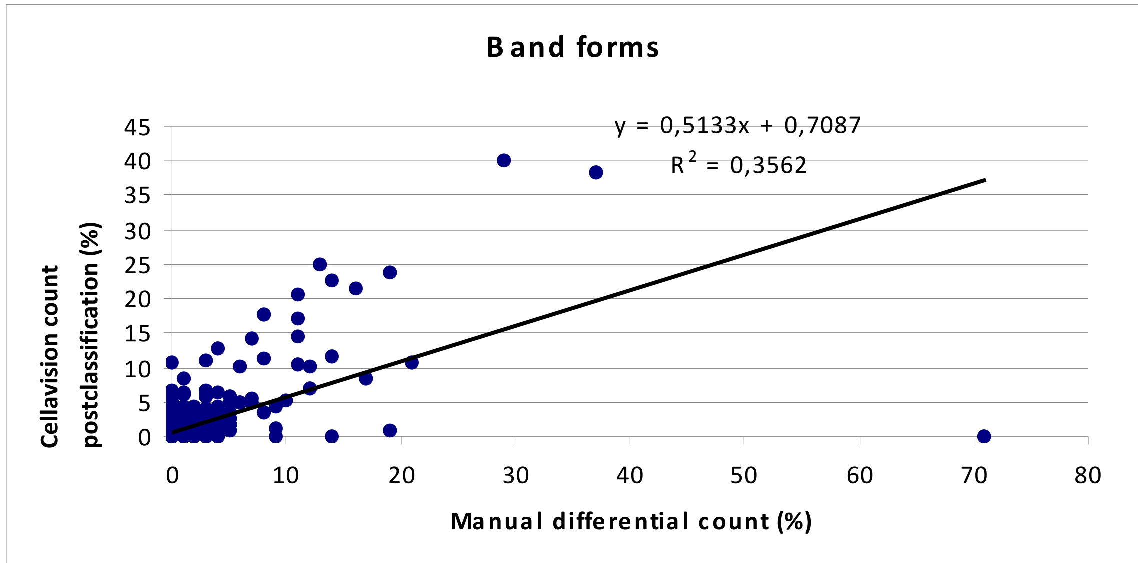
Correlation with current method

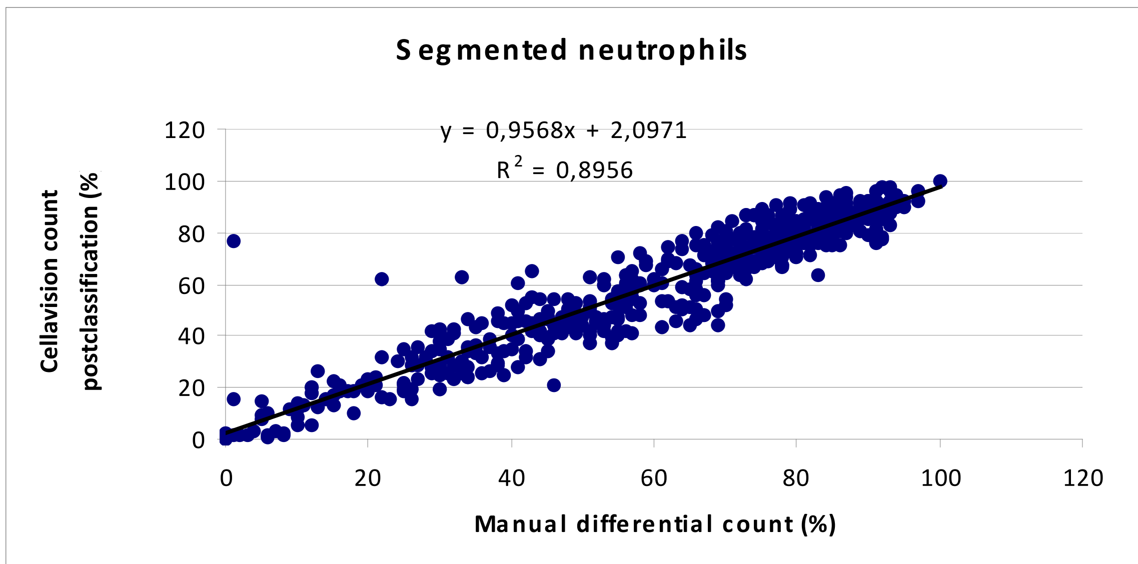
The correlation coefficients in our study were higher after the cells had been reclassified (Table 7, Figure 8). Correlation of the Cellavision™ DM96 postclassification results with manual microscopy was performed for the normal cell classes: neutrophils (band forms and segmented neutrophils), lymphocytes, eosinophils, basophils, monocytes, and for blast cells. Bland-Altman plots were obtained with Analyse-it®. Pearson correlation coefficients were highest for blast cells (R^2 = 0.96), segmented neutrophils (R^2 = 0.90) and lymphocytes (R^2 = 0.89). For eosinophils, basophils, monocytes and band forms, the correlation coefficient was between 0.04 (band forms) and 0.60 (monocytes). Ceelie et al found correlation coefficients between Cellavision™ DM96 postclassification and manual microscopy for blasts (R^2 = 0.98), lymphocytes (R^2 = 0.94), and segmented neutrophils (R^2 = 0.95) comparable with our results [30]. The study of Kratz et al showed the highest correlation postclassification for lymphocytes (R^2 = 0.94), followed by segmented neutrophils (R^2 = 0.88), eosinophils (R^2 = 0.73), band neutrophils (R^2 = 0. 69), monocytes (R^2 = 0.67) [10, 28]. Briggs had the best results with correlation coefficients for neutrophils (R^2 = 0.99), followed by lymphocytes (R^2 = 0.96), monocytes (R^2 = 0.81) and eosinophils (R^2 = 0.67) [27].

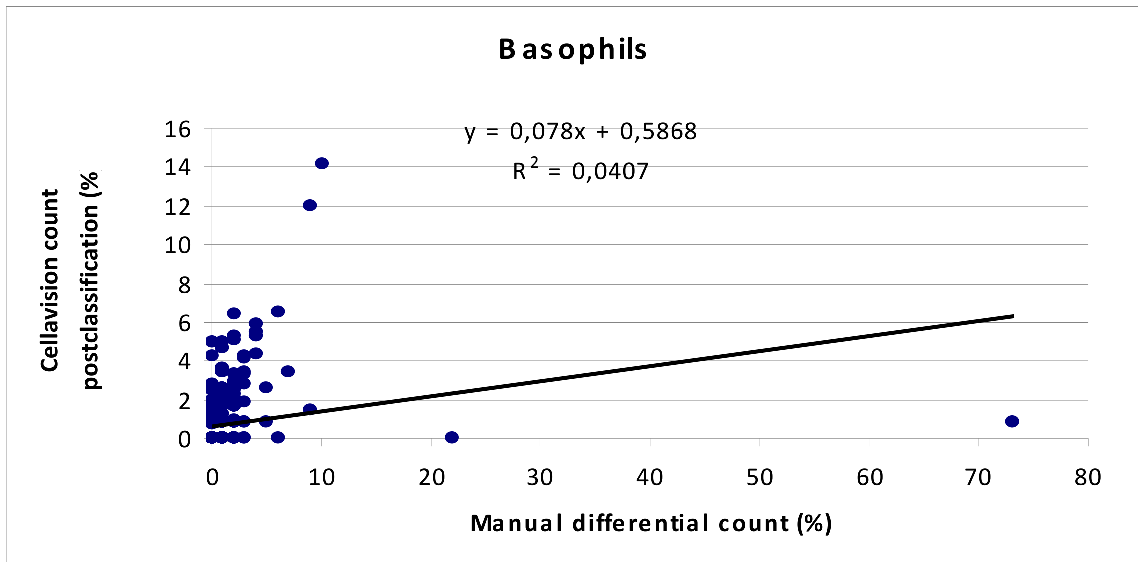
Table 7. Pearson correlation coefficient (R^2) between Cellavision™ DM96 and manual white cell differential, pre- and post- classification.

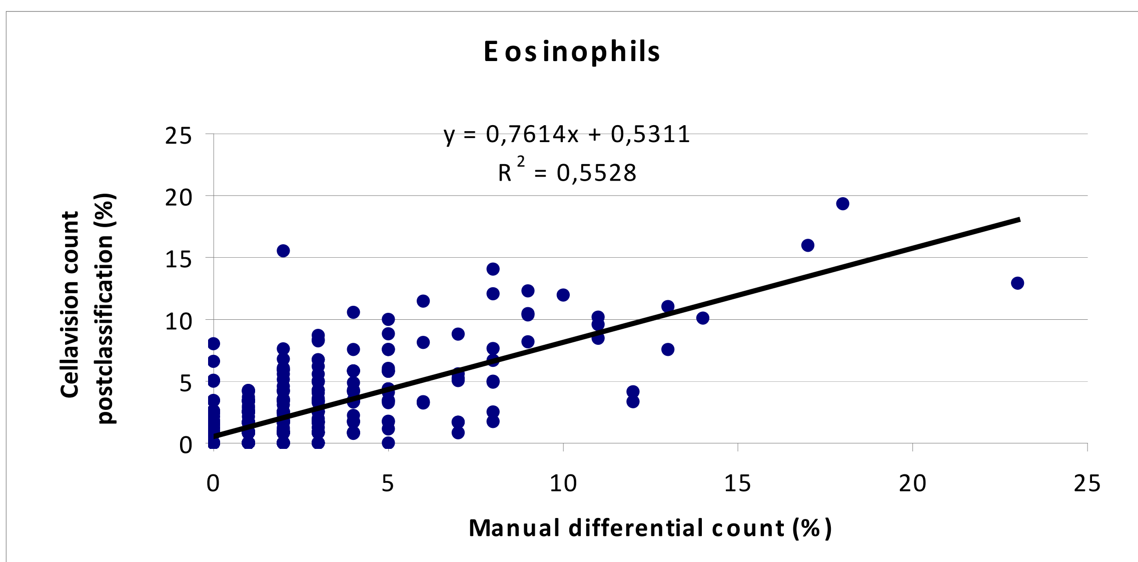
	Pre-classification	Post-classification
Band neutrophils	0.07	0.36
Segmented neutrophils	0.82	0.90
Basophils	0.04	0.04
Eosinophils	0.35	0.55
Total lymphocytes	0.76	0.89
Monocytes	0.34	0.60
Blasts	0.69	0.96

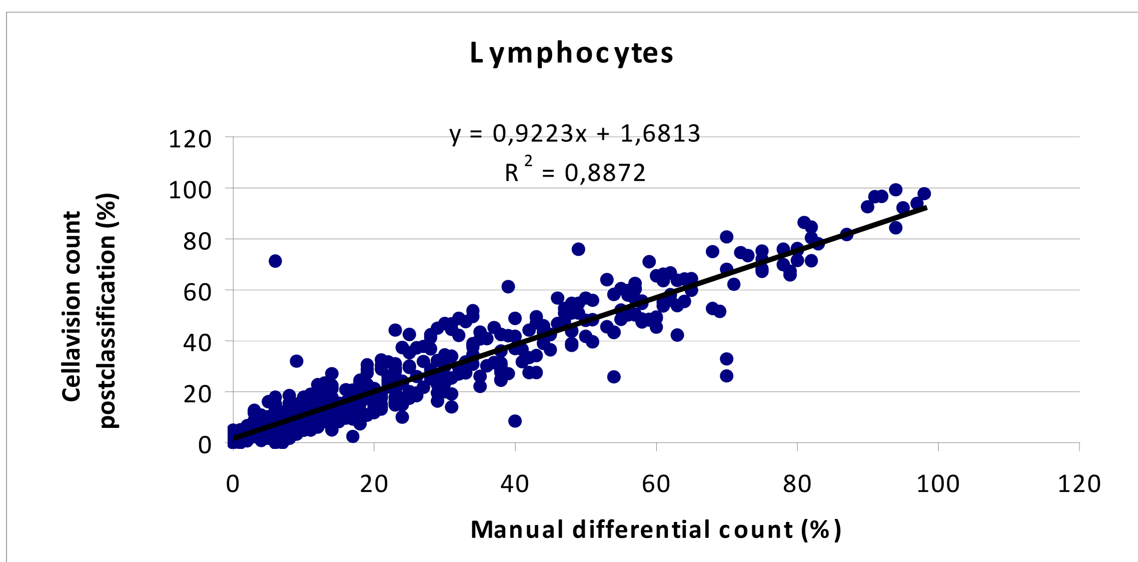
Figure 8. Correlation of manual white cell differential with Cellavision™ DM96 post classification for band forms, segments, basophils, eosinophils, lymphocytes, monocytes an blast cells.

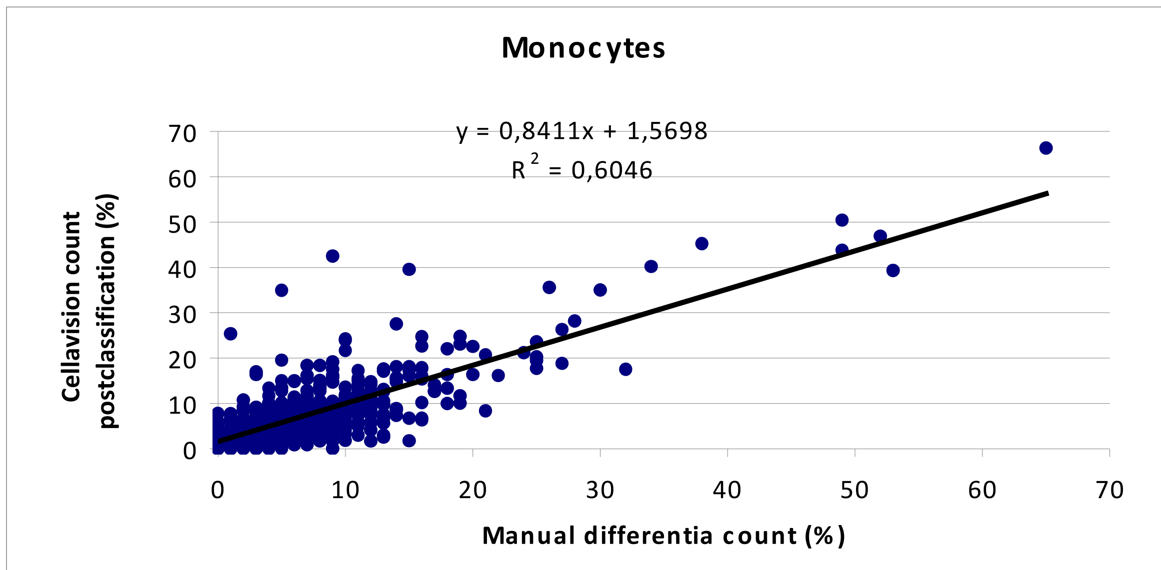


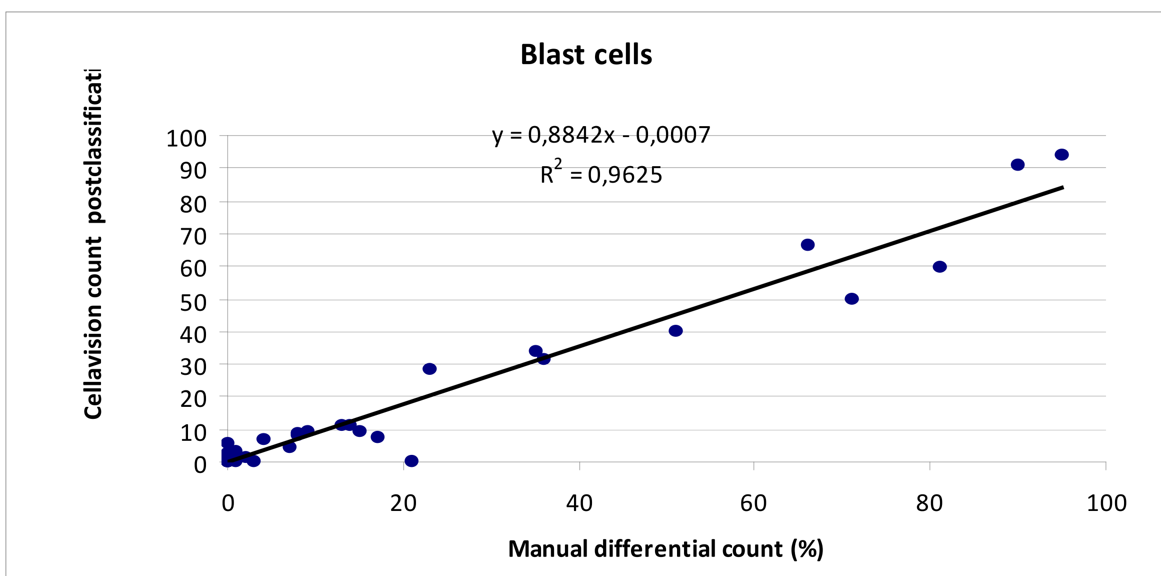



Basophils
y = 0,078x + 0,5868
R 2 = 0,0407
Cellavision count postclassification (%
Manual differential count (%)
16
14
12
10
8
6
4
2
0
0
10
20
30
40
50
60
70
80


Eosinophils
y = 0,7614x + 0,5311
R 2 = 0,5528
Cellavision count postclassification (%)
Manual differential count (%)
25
20
15
10
5
0
0
5
10
15
20
25


Lymphocytes
y = 0,9223x + 1,6813
R 2 = 0,8872
Cellavision count postclassification (%)
Manual differential count (%)
120
100
80
60
40
20
0
0
20
40
60
80
100
120





Briggs et al evaluated the Cellavision™ DM96 and the 100-cell manual white cell differential with the reference method (200-cells, counted by two scientists, according to CLSI) [27]. The authors found that correlation with the reference method is similar whether the differential is preformed manually or by Cellavision™ DM96 with reclassification of cells. Only for eosinophils, correlation of Cellavision™ DM96 (postclassification) with the reference method is not as good as for the manual method [27]. This is understandable since the same statistical errors are made when performing a manual white cell differential on 100 instead of 400 cells as when performing a differential with Cellavision™ DM96 (postclassification) on 120 cells instead of 400 cells with the reference method. In other words, correlation with the reference method is similar either when the differential is performed manually or with the Cellavision™ DM96 with reclassification of cells [27]. Taking this finding into account, our correlation coefficients between Cellavision™ DM96 postclassification and a manual white cell differential are fairly well.

We wondered whether the correlation between manual microscopy and the automatic white cell differential of Beckman Coulter LH 755 haematology analyzer on the one hand and Cellavision™ DM96 (post-classification) and the automatic white cell differential on the other hand is comparable. In case this would be the case, we could confirm once more that the differences between manual microscopy and Cellavision™ DM96 are mainly related to the statistical error made by counting just 100-120 cells for the white cell differential. As is evident from Table 8, correlation is as good between the Beckman Coulter haematology analyser and manual microscopy as between Beckman Coulter haematology analyser and Cellavision™ DM96 (postclassification). Manual microscopy performs slightly better, but with Cellavision™ DM96, we have to take into account a learning phase.

Table 8. Pearson correlation coefficients (R^2) between haematology analyser (Beckman Coulter) and manual microscopy or haematology analyser and Cellavision™ DM96 postclassification.

	R^2 haematology analyser Beckman Coulter vs.	
	Manual microscopy	Cellavision™ DM96 postclassification
Neutrophils (Segmented + band forms)	0.80	0.80
Basophils	0.02	0.08
Eosinophils	0.73	0.69
Lymphocytes	0.86	0.85
Monocytes	0.42	0.37

4.2.1.2 Turn around time (TAT)

The objective of the automatic image processing differential devices was to complete a 100- or 200-cel differential count in approximately 1 minute, and to classify the differential as normal or abnormal with an accuracy comparable with that of the microscopic white cell differential [14]. As such, Cellavision™ DM96 claims to be able to perform a white cell differential in 1.7 min, i.e. 35 slides per hour [9, 31].

We were interested in a possible reduction of the TAT when using automated microscopy. Therefore, the 7 laboratory technicians experienced in performing white cell differentials performed a white cell differential on 12 consecutive slides, both with Cellavision™ DM96 and with the microscope. As can be derived from Table 9, there was no statistical difference ($p > 0.05$) in the time needed to perform a white cell differential with Cellavision™ DM96 or with the microscope. The mean time needed with Cellavision™ DM96 was 2 min 8 sec, while with manual microscopy one needed on average 2 min 5 sec. Some laboratory technicians worked faster with Cellavision®, while others spent less time counting on the microscope.

Table 9. Time needed to perform a white cell differential with Cellavision™ DM96 and manual microscopy.

Laboratory technician	Cellavision™ DM996, time per slide (mm:ss)	Manual microscopy, time per slide (mm:ss)	Difference in time Cellavision - manual microscopy (mm:ss)
1	02:30	02:18	00:12
2	03:16	03:20	-00:04
3	01:18	01:18	00:00
4	02:00	02:45	-00:45
5	01:34	01:54	-00:20
6	02:05	01:05	00:59
7	02:13	01:52	00:22
mean time	02:08	02:05	
paired t-test, p =	0.8		

Kratz et al showed that laboratory technicians needed on average 1.3 min longer with Cellavision than with manual microscopy ($p < 0.01$) [10, 28]. With manual microscopy, 5.1 min were needed to perform a differential, with Cellavision™ DM96 the time required was 6.4 min [28].

Van Gelder et al reported that Cellavision™ DM96 lead to a reduced microscopic review rate, < 5% of the samples analysed with Cellavision™ DM96 required a manual microscopic review. The turn-around-time for a white cell differential by using Cellavision™ DM96 was reduced with 50% [10]. Ceelie et al found that 3.2 min were needed to evaluate one blood smear with Cellavision™ DM96 and 4.2 min with manual microscopy [33]. In the study of Briggs et al, 2.7 min were needed for analyzing a slide with Cellavision™ DM96 and 5.8 min were needed for a manual white cell differential [27]. In this latter study, introduction of Cellavision would lead to a reduction in the TAT of half its current length. According to the flyer of the Cellavision™ DM96, 35 slides can be completely differentiated and evaluated on RBC and platelet morphology within 1 hr, i.e. 1.7 min per slide [34]. For most of the laboratory technicians evaluating blood smears on Cellavision™ DM96, this time was slightly longer (2 min 8 sec).

We have to take into account that more time might be saved when familiarity with the use of Cellavision™ DM96 increases.

4.2.2 Diagnostic performance: sensitivity, specificity

For normal cells, we found a sensitivity of 98% and a specificity of 93% (comparing preclassified with postclassified cells on Cellavision™ DM96). For the blast cells, the sensitivity was 66%, while the specificity was 100%.
When comparing Cellavision™ DM96 with manual microscopy, Kratz et al found for blast cells a sensitivity of 86% and a specificity of 91% [28]. Overall, the sensitivity of Cellavision™ DM96 versus manual microscopy was 25 to 91% and the specificity was 82 to 93% in the study of Kratz et al [28]. These figures are not so good.

4.2.3 Clinical impact: health outcome

Cellavision™ DM96 makes training and monitoring of staff in blood cell morphology skills easier and more efficient [12, 27]. There is also a possibility of remote viewing of blood cells. One can verify certain cell types from a different location. A small laboratory without morphology expertise can send images to the central laboratory for classification and diagnosis [12, 27].

4.2.4 Cost impact: in and outside the laboratory

Actual cost
Ceelie et al are convinced that the introduction of automated microscopy such as Cellavision™ DM96 can reduce the workload by efficiently processing non-pathological smears, giving more time for analysis of pathological slides [30]
Microscopic examination requires trained personnel. There is an error associated with the number of cells analyzed, a cell distribution error and error associated with examiner variability.
Preclassification by DM96 is not examiner dependent. This could lead to more uniformity in evaluating cell classes.

Profit elsewhere in the hospital
The introduction of SIS (Sysmex Information system) software in combination with Cellavision™ DM96 lead to a reduced personal cost of 50% in a study of van Gelder et al [10]. Also in UH Leuven, the introduction of Cellavision™ DM96 has reduced the workload for laboratory technicians; 1.2 in stead of 3 technicians perform the same work as before introduction of Cellavision™ DM96 [35]. We do not know though whether this introduction also included an adaptation of the SIS rules.

5 Is there place for smear/slide scanning instead of a complete manual white cell differential, and does this reduce TAT?

Smear scan is a method in which one performs a microscopic screen, but in which one does not replace reflexively the automatic white cell differential by a manual white cell differential for the presence of analyser flags. Only in case there are aberrant cells at microscopy, one reports the manual white cell differential [26].

5.1 Analytical considerations

It has been shown several times that a haematology analyzer can produce a much more precise and more reproducible count than one can ever become with manually microscopy [13, 17] This is explained by the high number of cells counted by the analyser, reducing the statistical variation. In comparison, the coefficient of variation of a white cell count with a haematology analyser is +/- 1.5% compared with +/- 16% for a manual white cell differential performed with a hemocytometer [36] . In case we would use smear scan in conjunction with the automatic white cell differential, this would improve the accuracy of the reported result. Above this, TAT could be reduced, certainly when we have to look for 100 cells to differentiate in cases with leucopenia.

5.2 General Considerations

The topic of smear scan has been studied in the Critically Appraised Topic of Apr. M. Van Gijsel. She found that there is no straightforward answer on this question [17]. It depends on the hospital population and the size of the hospital. In laboratories with a high ratio of review (haematological centers), one more often decides to introduce smear scan instead of a complete manual white cell

differential [17]. Remark that there are different ways to perform such a smear scan. There are no criteria about the number of cells to screen [17].
Lantis et al introduced a revised manual review policy in the University of Michigan Medical Center haematology laboratory [13]. Specific instrument criteria lead to the performance of microscopical smear scan before determining the appropriateness of a manual white cell differential [13]. These criteria are illustrated in Table 10.

Table 10. Criteria for manual scan review of peripheral blood smears based on instrument flags [13]

Criteria
MCV ≥ 115 fL
‘Suspect’ and ‘Definitive’ flags as follows:
Anaemia (Hb < 8.0 g/dL)
Anisocytosis > 1+ (RDW > 17)
Eosinophilia (> 1500/µL)
Hypochromia > 1+ (MCH < 21 pg)
ImmNE2 and WBC count ≤ 15.000/µL
Leucocytosis (WBC count > 50.000/µL)
Microcytosis > 1+ (MCV < 77 fL)
NRBCs
Thrombocytopenia (BP < 50.000/µL)

The extent of the manual microscopic scan performed by the laboratory technician is left to the professional discretion of the individual technician based on the leucocyte count and distribution on the slide being scanned [13]. Technicians are encouraged to evaluate at least as many cells as they would evaluate for a manual white cell differential. The scan is not limited to an evaluation of the flagged result [13].
If 1 or 2 immature granulocytes or more than 2 nucleated red blood cells (NRBC) were seen on scan, the technologist was instructed to release the automated leucocyte differential with the following comments, as applicable: ‘Rare metamyelocytes/myelocytes seen on scan’. The finding of even a single promyelocyte or blast on scanning triggered the ordering of a manual leucocyte differential [13].
This revision lead to a reduced rate of manual white cell differentials (microscopic reviews) (from 125/ day to 35/ day), and so a reduced TAT [13].
In our oncological patient population, the main flags concerning red blood cell abnormalities counted for 26% of the main flags (N = 86/327). It should therefore be possible to reduce the rate of complete microscopic slide reviews, when we limit extreme red cell abnormalities to a smear scan.
In the establishment of the new dictionary of rules for the Remisol Advance 2000, we also tried to incorporate more rules leading to smear scan than to complete manual white cell differentials (see Table 2). As such, we have changed the rule for a manual white cell differential in case of eosinophils exceeding 600/µL or 20% into a smear scan (when eosinophils exceeds 600/µL).
The introduction of the different hosts ‘smear scan’ and ‘manual white cell differential’ has lead to a reduction of the TAT for the scanned blood smears to one third of the TAT for a manual white cell differential.

6 Is it possible to discontinue the differentiation into band forms and segments as separate classes, and does this reduce TAT?

6.1 Pathophysiology

Band cells are often believed to be indicators of possible bacterial or viral infection when their frequency or number in blood samples exceeds the generally accepted upper normal limit of 5% [37]. Detection of a granulocytic left shift, often used as an indicator of infection or sepsis, has been defined as an elevated neutrophil band count [23]. But neither neutrophilia, nor an elevation of band neutrophils is specific for infection. A variety of other stimuli cause band form elevations, including inflammatory processes, tissue damage or necrosis, seizures, neoplasia, intoxication, poisoning, metabolic abnormalities, acute hemorrhage or hemolysis, myeloprolipherative disorders, and drugs [7]. The band count has limited utility in the diagnosis of infection in sick, hospitalised patients, who are likely to have bandemia associated with an underlying illness or medication [7].

6.2 Definition

Band neutrophils are defined in at least three different ways in literature. The Clinical and Laboratory Standards Institute (formerly NCCLS), the College of American Pathologists (CAP) and the Committee on Clarification of the Nomenclature of Cells and Diseases of the Blood and Blood

Forming Organs (1948)' differentiate a band from a segmented neutrophil by requiring a segmented neutrophil to have complete separation of the lobes, with a clearly visible strand that appears as a solid threat-like dark line, containing no visible chromatin between the margins [7]. Any neutrophil with less complete restriction is classified as a band. Novak defines a band as a cell, 10 to 15 µm in diameter, with a nuclear to cytoplasm ratio of 1:1.5 to 1:2. Its nucleus is indented to more than one half the width of the hypothetic round nucleus, yielding a nucleus with S, C, or U shapes [38]. The morphologic criteria of the Netherlands Society for Laboratory Haematology defines a band cell as a neutrophil with a nuclear constriction width of one-half to one third of the maximum nuclear width [7, 37]. Laboratories using this liberal definition classify more neutrophils as segmented and fewer neutrophils as bands, and so have a lower upper cutoff for the band count reference range [7]. This upper limit for band neutrophils varies from 1 to 21.5% [7, 38]. Recommendations for standardising the morphological criteria for band neutrophils have largely failed [37].

6.3 Analytical considerations

6.3.1 Reproducibility

There are major limitations in the reproducibility of the band count. The first is inaccuracy introduced by sampling alone. Repeated counts might lead to a wide range, depending on the number of cells counted for the white cell differential (i.e. statistical variability, cfr. Tables of Rümke et al) [38].
Secondly, there is a heterogeneous distribution of white blood cells on a blood smear. Lymphocytes are mainly located in the center of the smear, while neutrophils and monocytes are found at the edges [39]. A third problem is the intra- and inter-observer variability in cell identification [37, 38]. Van der Meer et al showed there is a low intra-observer reproducibility (weighted kappa κ for category agreement < 0.8 in 4/5 samples) concerning the band count with or without previous knowledge of the left shift or immature granulocyte flag of the analyser [37]. Supplementary flagging information had no influence on the observer bias, pointing at observer inconsistency. The inter-observer reproducibility was low too, i.e. there was a wide range in observed values of band neutrophils for all blood samples. The authors believe this variability is due to the less well-defined leucocyte fractions (e.g. band neutrophils). The inter-observer variability was much lower for the immature granulocyte fraction [37]. In case band neutrophils would be reported qualitative instead of quantitative, this would result in a reduced SD for neutrophils of 2 % (in stead of 11%) [23]. Van der Meer et al therefore suggested not to report band cell counts unless they are present in increased numbers (eg. comment appended: 'normal' or 'increased') [23, 37].
Cornbleet came to the same conclusion. Even when using the CAP definition to identify band neutrophils, there was an enormous inter-laboratory variability in the distinction of band neutrophils and segments (10-90 percentile of band forms: 25 to 55.6%) [7]. This greatly limits the value of the band count as laboratory test [7].
Bands are counted in the group immature granulocytes to determine the leucocyte index (total immature / total mature count) [40]. This index cannot be used properly as an indicator of sepsis because of poor inter-observer correlation (inter-observer variability) and bad reproducibility [7, 40].

6.3.2 Reference values

As mentioned above, the establishment of a clear reference value of the band neutrophils is problematic. This is dependent upon the used definition of band neutrophils, individual interpretation, sex, age, and race [38, 39].

6.3.3 Diagnostic performance and clinical impact

Cornbleet assessed the clinical utility of the band count in various clinical scenarios using the positive and negative likelihood ratios as an indicator of the quality of the laboratory test [7]. This review provides little support for the clinical utility of the band count in patients greater than three months [7, 38]. The absolute white cell count and the automated neutrophil count are better diagnostic tests for adults and most children. Absolute numbers of bands are required for the Rochester criteria. This is a diagnostic algorithm using clinical and biological parameters, for acutely ill, febrile children less than three months of age (28 to 90 days)[39]. No studies, however, assess the independent contribution of bands to the performance of the algorithm, or the use of the automated neutrophil count as a replacement of the band count. Band counts also are required to calculate an immature to total neutrophil ratio (I:T ratio), an index widely used to aid in the diagnosis of neonatal sepsis. Studies, however, show a wide range of sensitivity and specificity for the I:T ratio, indicating variable performance [7]. The white cell differential is not very specific for serious infection (sepsis) among neonates, because meconium aspiration, hypoglycemia, pneumothorax, and prolonged labor can all cause a manifest significant bandemia [38].

Yilmaz et al reported that the percentage of neutrophils, absolute neutrophil count, percentage of band neutrophils, absolute band count, and the band/neutrophil ratio in peripheral blood provide diagnostic information about bacteraemia in children (aged 3 to 36 months) with fever [41].

Seebach et al compared the sensitivity and specificity of several left-shift parameters (manual band counts, I:T ratio, WBC count, neutrophil count). The level of C-reactive protein was used as the gold standard [42]. When using a cut-off of ≥ 20%, the manual band count had the highest specificity (79%) [42]. The sensitivity, however, was only 53% at this cut-off [42]. Accordingly, the band count is of low diagnostic value in the screening of infectious diseases (high number of false negatives). Likewise, the area under the curve was highest for the manual band count (A = 0.72), but not that different from the other left-shift parameters (A > 60). Several authors concluded that the microscopic band count is of limited diagnostic value, and that similar results can be obtained with left-shift parameters of automated haematology analysers [42, 43].
Badgett et al showed that physicians over rely on the band count for taking clinical decisions [38].

At UH Leuven, band forms are only reported for neonates. Neonatologists use the band count in the Rochester criteria (cfr above).

Conclusion
There are alternatives for the band count. One can use characteristic signs of toxicity such as Döhle bodies, toxic granulation, neutrophil vacuolisation, which can indicate bacterial infection [39]. These characteristics neither are specific for sepsis.
The WIV recommends not to report the manual band counts, not even for newborns [39]. The search for toxic characteristics is the best alternative [39]. It is worth considering reporting the segmented and band neutrophils as one group. Moreover, automated digital microscopes have difficulties in counting band neutrophils and count them default in one group.
On the other hand, automated haematology analysers produce a left shift flag for bandemia (immNE1) which is not specific. Several studies have shown that this flag is unreliable and should not be used [39].

7 Is deltacheck an acceptable way to increase the time interval between two successive microscopic white cell differentials?

The delta check measures differences between results obtained in two consecutive analyses of the same patient. This difference depends on many variables both in the specimen analysed and in the method used, and the limits that are proposed are a compromise between physiological variations due to biologic intra-individual variability and modifications taking place with the changes in health profile of the patient or analytical or pre-analytical errors [1]. There is no consensus on delta check limits, which depend on the type of the patient and the time limit used between consecutive analyses [1]. Buttarello et al used an experimental delta check with a time-limit of 72 hrs for inpatients and last appointment for outpatients (Clinical Pathology Laboratory, Geriatric Hospital, Padua) [1]. For the neutrophils for instance, the checking limit included an absolute value of 8 initially and if not exceeded, a second criterion was used based on the percentage variation of 50% [1]. Unfortunately, the authors do not state the results of this experimental setup. In the following table 11, some examples of delta check limits are given for the different cell classes leading to action (slide review).

Table 11. Some suggestions for delta check [1].

	Groner/Simson (absolute variations)	Buttarello (absolute variations / percent)	Klee/Schryver (absolute variations)
Leucocytes (/µL)	5000	10000 (50)	12800
Neutrophils (/µL)	100	8000 (50)	10900
Lymphocytes (/µL)	100	1500 (30)	2030
Monocytes (/µL)	100	1000 (30)	900
Eosinophils (/µL)	100	800 (50)	540
Basophils (/µL)	100	200 (-)	360

In the Los Angeles Country/University of Southern California Medical Center, a consensus was reached between clinicians and the laboratory that no differential counts were reported at intervals of less than 1 week. There were few situations where the actual differential count was required for clinical management, if the erythroid values, white cell count, and platelet count were available

whenever needed [14]. In case the clinician informed the laboratory of a specific indication for the slide review, the microscopic white cell differential was performed. No adverse effects on patient management have been reported over a three-year period in this institution [14]. The author recommends institutions/clinicians to review the medical necessity of a daily white cell differential on patients undergoing drug testing or chemotherapy [14].
In the University College London Hospital (Department of Haematology), the number of times a blood film of haematology patients is looked at has been restricted to twice a week [27]. There still are daily full blood counts however [27]. This action lead to a review rate of less than 10% [27].
At UH Leuven, one performs only one manual white cell differential per 48 hours for the same hospitalised oncological patient (on Mondays, Wednesdays and Fridays), except for clinical emergency cases [35]. Up to this moment, only two remarks have been arisen, coming from surgeons. One will continue with this way of working. Complete blood counts and automatic differentials can be requested at any time.
Brecher et al believe it is worthless to repeat manual white cell differentials daily for the monitoring of patients under immunosuppressive therapy only for knowing the number of neutrophils [17, 44]. They recommend to repeat the manual white cell differential once in 24-48 hrs after an initial white cell differential [44]. Afterwards, only absolute white cell count is analysed every 48 hrs. Only in case of a significant change in the leucocytosis, the manual white cell differential is repeated [17, 44]. This strategy would result in loss of information in about 1% of instances, at a considerable saving in differential counts [44].
An interesting suggestion was made by Chapman et al. In case results are concordant within +/- 10% with the previous results, a slide review could be postponed for 72 hrs for hospitalised patients and for 7 days for outpatients [17]. This is rather difficult though in patients under chemotherapy. These patients visit the oncology day clinic after and for a new course of chemotherapy. Bone marrow toxicity of the previous course or a decreasing leucocytosis should be evaluated each time before the patient can have his following course of chemotherapy. In these situations, the clinical decision can be influenced by the manual white cell differential [17]. In non-leukopenic or mild leukopenic patients with a stable leucocytosis, one manual white cell differential per week is sufficient [17]. In conclusion, as was already reviewed in the Critically Appraised Topic of Apr M. Van Gijsel, there is little consensus in the minimal time interval between two successive manual white cell differentials [17].
At Imelda, oncologists seem to agree with the introduction of such a minimum time interval between two consecutive manual white cell differentials. For hospitalised oncology patients, a time interval of 48 hrs could be discussed with the oncologists. It is obvious that oncology patients visiting the day clinic should receive a manual white cell differential when needed. The introduction of this type of blocking period shall be discussed with clinicians if the number of microscopic reviews would increase again.

At the Imelda hospital, the query among the oncologists made clear that they need the white cell differential (or at least the total neutrophil count) before starting chemotherapy at the oncology day clinic. The automatic white cell differential is transferred to the host under the condition no Remisol rules requiring a manual white cell differential have been played. For instance in case the white cell count is lower than 2000/µL or when the neutrophil count is lower than 1000/µL a manual white cell differential is not longer required leading to a decrease in the TAT.
Delta check rules have been implemented to adapt the blocking period in case of minor differences in cell counts; f. eg. in case Hb is ≤ 8 g/dL and the previous Hb value was also ≤ 10 g/dL, no smear scan is necessary. A same type of rule is established for low platelets.

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TO DO/ACTIONS

1) Evaluation HemaCAM, cfr study protocol ?