Letter to the Editor

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Could the UKNEQAS program "Manual Differential Blood Count" be performed by the use of an automated digital morphology analyzer (Sysmex DI-60)? A feasibility study

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To the Editor,

Automated white blood count (WBC) differential counters relieve the clinical laboratory of a labor-intensive activity. Since predetermined criteria are substituted for visual perception of laboratory personnel with varying skill and training, automation improves reproducibility of the results both within a single institution and within the professional and scientific communities [1]. Automated morphologic analysis of blood cells shows good correlation with the reference manual microscopy and has been proposed and employed in addition to regular microscopy in high volume haematology laboratories [2]. The Sysmex DI-60 system (DI-60, Sysmex, Kobe, Japan) is a fully-integrated cell image analyzer that can be connected with the XN haematology analyzer (Sysmex) and slide making/staining device SP-10 (Sysmex). When a single sample is placed on the automation line Complete Blood Count (CBC), smear preparation and digital cell location are carried out. DI-60 enhances the function of a microscope and, using Artificial Intelligence algorithms, it pre-classifies WBC, pre-characterizes Red Blood Cells (RBC) and estimates the number of platelets (PLT) on peripheral blood smears [3]. DI-60 does not simply magnify an image but scans a portion of a slide and independently identifies an appropriate analysis area in which it captures and pre-classifies images of the cells using its image analysis software.

These images are not produced by direct microscope examination, so the setup of Internal Quality Control (IQC) and External Quality Assurance (EQA) procedures are highly valuable since haematology requirements in terms of high resolution of finite details such as granules, nucleoli, and vacuoles are the most demanding. Digital slides have been used by clinical laboratories in programs for proficiency testing in the field of haematology by authoritative organizations such as The College of American Pathology (CAP) and United Kingdom National EQA Scheme (UK NEQAS) [4]. In these proficiency programs the participants could examine, compare and comment their findings on the same cells to those of the other participants, to consensus data from glass slides, and to expert opinion. These programs start from an already digitalized image: by this way it is possible to correctly evaluate the staff competence but not to simulate the real laboratory practice activity.

Currently, to the best of our knowledge, no sample specific for DI-60 is distributed by organizations that deal with quality assurance. Therefore, we investigated the use of an EQA sample previously distributed for microscope evaluation to assess the DI-60 performance.

UKNEQAS, a leading organization in EQA field, distributes three programs relative to microscope evaluation: differential counting, morphology comments and parasite identification.

In this letter we describe our experience in assessing the DI-60 capacity to select an appropriate area of the smear, to acquire high-quality images and to correctly recognize different cell types in five UKNEQAS surveys for differential counting in samples of different pathologies (acute leukemia, lymphocytosis, leukemoid reaction, myeloproliferative disorder, and eosinophilia).

Since EQA surveys should mimic routine specimens [5], every sample was processed by a single analyzer

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and the final images data were randomly evaluated by one of two biologist's expert in haematology assisted by a laboratory physician haematologist as consultant. The DI-60 was set to acquire 200 WBC, to classify them into 13 subtypes (neutrophils, lymphocytes, monocytes, eosinophils, basophils, promyelocytes, myelocites, metamyelocytes, promonocytes, prolymphocytes, blasts, plasma cells, hairy cells), and to place the unrecognized cells in the "unidentified cell" area. A clinical validation of a differential count will be done only if all the cells present in the "unidentified cells" area will be shifted from the user to their specific subtype (WBC or not).

To evaluate the overall performance of DI-60, we assessed the following four aspects:

- the quantification of unrecognized cells
- the quantification of the elements misrecognized as cell
- the reliability of the differential count carried out by the analyzer
- the reliability of the differential count carried out by the analyzer and modified by the user

The percentage of "unidentified cells" of the EQA samples ranged from 1.5 to 9.5%, and can be considered satisfactory since the percentage of patients' "unidentified cells" in our daily routine laboratory practice usually ranges from 0.5 to 10%.

The wrong recognition of an element as a cell was rare (less than 1%) and it mostly involved smear cells or giant platelet, that were identified as basophils, myelocytes or unidentified cells (Figure 1).

The reliability of the differential counts (DI-60 pre-classification and the user's modified one) was assessed considering as reference the value reported in the UKNEQAS program: the mean value and the standard deviation (SD) of about 500 laboratory haematology professionals that participated to the specific EQA survey by microscope evaluation of the smear. Table 1 shows all performances in differential count: there are only five cases outside the two SDs and a single clinically significant inadequate performance: the underestimation of Blasts in the DI-60 pre-classification in case 1701. Really, acute leukemia cannot be diagnosed when the percentage of Blasts in peripheral blood is lower than 20%. The right adjustment of the percentage made by the user means that DI-60 selected an appropriate area of the smear and acquired anyway high-quality images. Figure 1 shows this wrong cell classification executed by DI-60: Blasts were placed in "lymphocyte area" or "unidentified area".

Our study demonstrates the feasibility of the extension of the UKNEQAS differential count EQA program to digital images devices, as DI-60. In conclusion, our results give even more strength to the applicability of automated digital imaging analyzers in laboratory practice. They have

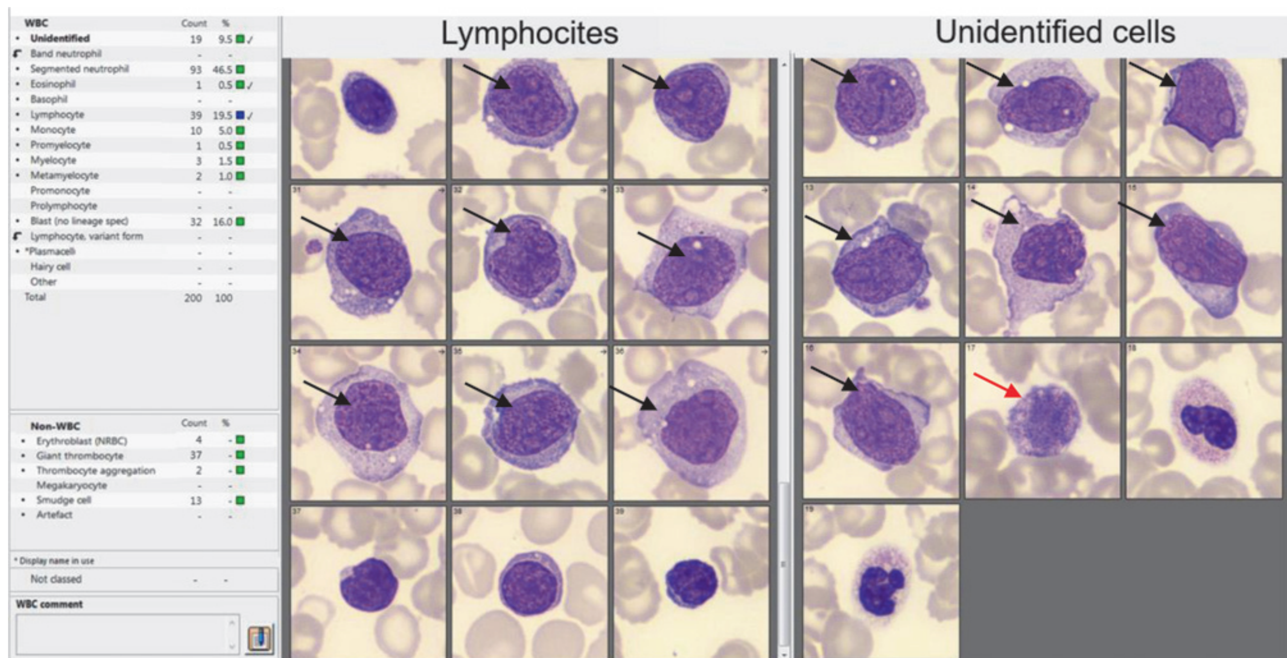

Figure 1: DI-60 validation screen of the sample 1701. Black arrows show blasts wrongly identified as lymphocytes or unidentified cells; red arrow shows a giant platelet wrongly identified as a cell (unidentified cell).

Table 1: Difference in WBC count between UKNEQAS results and DI-60 value (pre-classified and modified by the user) in the analyzed surveys. In bold are reported the results outside the two standard deviations (2SDs).

Cell type	UKNEQAS mean value	UKNEQAS range of acceptability (±2SDs)	DI-60 not modified	DI-60 modified by the user
Survey 1701DF acute leukemia				
Unidentified cells	/	/	9.5%	/
Neutrophils	50.4%	40–60.8%	46.5%	50%
Lymphocytes	8%	0–17.8%	**19.5%**	7.8%
Monocytes	4%	0–11.4%	5%	4.8%
Eosinophils	1%	0.4–1.6%	0.5%	**0%**
Basophils	0%	0–0%	0%	0%
Metamyelocytes	0%	0–2.2%	1%	0%
Myelocytes	0.5%	0–3.5%	1.5%	1%
Promyelocytes	0%	0–0%	**0.5%**	0%
Blasts	33%	15.2–50.8%	16%	36.4%
Nucleated RBC	2%	0.6–3.4%	2%	2%
Survey 1703DF lymphocytosis				
Unidentified cells	/	/	1.5%	/
Neutrophils	8%	2–14%	11%	10.6%
Lymphocytes	88%	60–100%	84%	86.4%
Monocytes	1%	0–2.4%	2%	2%
Eosinophils	0.5%	0–1.9%	0.5%	1%
Basophils	0%	0–0%	**0.5%**	0%
Metamyelocytes	0%	0–0%	**0.5%**	0%
Myelocytes	0%	0–0%	0%	0%
Promyelocytes	0%	0–0%	0%	0%
Blasts	0%	0–0%	0%	0%
Nucleated RBC	0%	0–0%	0%	0%
Survey 1801DF leukemoid reaction				
Unidentified cells	/	/	5.5%	/
Neutrophils	78%	91.4–64.6%	74.5%	77.7%
Lymphocytes	5%	0.6–9.4%	4%	5.1%
Monocytes	5%	0.6–9.4%	4%	4.6%
Eosinophils	0%	0–1.4%	0%	0%
Basophils	0%	0%	0%	0%
Metamyelocytes	3%	0–8.4%	2.5%	4.6%
Myelocytes	5.2%	0.8–9.6%	4%	4.6%
Promyelocytes	2%	0–5.6%	5%	3.6%
Blasts	0%	0–0%	0%	0%
Nucleated RBC	25%	7.2–42.8%	18%	19.3%
Survey 1901DF myeloproliferative disorder				
Unidentified cells	/	/	2%	/
Neutrophils	63.3%	52.9–73.7%	67.5%	66.8%
Lymphocytes	9%	3.8–14.2%	7.5%	8.9%
Monocytes	14%	5.2–22.8%	15%	14.8%
Eosinophils	0.5%	0–1.9%	0%	0%
Basophils	1%	0–3.2%	0.5%	0.5%
Metamyelocytes	2%	0–5%	3.5%	2%
Myelocytes	5%	0.2–9.8%	2.5%	3.5%
Promyelocytes	1%	0–4%	0.5%	0.5%
Blasts	2.9%	0–5.9%	0.5%	3%
Nucleated RBC	2%	0–5%	1.5%	0%
Survey 1903DF eosinophilia				
Unidentified cells	/	/	2%	/
Neutrophils	37%	28.2–45.8%	37%	37.4%
Lymphocytes	12%	4.6–19.4%	6.5%	6.6%
Monocytes	7%	1–13%	9.5%	9.1%
Eosinophils	37%	28.2–45.8%	36.5%	38.9%
Basophils	1%	0–3.2%	1%	1%

Table 1: (continued)

Cell type	UKNEQAS mean value	UKNEQAS range of acceptability (±2SDs)	DI-60 not modified	DI-60 modified by the user
Metamyelocytes	1.5%	0–4.1%	2%	2.5%
Myelocytes	3%	0–6%	3.5%	3%
Promyelocytes	1%	0–3.2%	2%	1.5%
Blasts	0.5%	0–1.9%	0%	0%
Nucleated RBC	1%	0–3.2%	0%	0%

SDs, standard deviations.

shown a good performance in agreement with the assessment of a group of about 500 laboratory haematology professionals (UKNEQAS users group). Finally, it should be emphasized that since an automated digital morphology analyzer is an instrument more sophisticated than a microscope, the monitoring of its performance with an EQA programme is desirable when it is used in a clinical laboratory.

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