

Low level leucocyte counting: a critical variable in the validation of leucodepleted blood transfusion components as highlighted by an external quality assessment study

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Summary Leucocyte counts of $< 5 \times 10^6$ per blood transfusion product are currently recommended in the UK in order to reduce transfusion-related infections and febrile reactions. Routine leucocyte depletion, however, requires the development of reliable internal and external quality assurance (EQA) programmes. We report preliminary findings from the UK NEQAS for Low-Level Leucocyte Counting from 18 UK Transfusion Centres over a four month period. Data analysis showed that the IMAGN 2000 had the lowest CVs (range 7.5–36%, mean 16.7) for samples with counts of 5–30 cells/ μl when compared to the flow cytometric (range 13.8–88%, mean 29.5) and Nageotte methods (range 20.6–117%, mean 61.8). In addition, laboratories using commercial nuclear stains (LeucoCOUNT™) had consistently lower CVs than those using 'in-house' propidium iodide staining methods. Important differences in flow cytometric gating strategies were also identified. This study highlights the current variability in low level leucocyte counting, especially within the critical range of 5–30 cells/ μl (equating to $< 5 \times 10^6/\text{l}$). The acceptance of consensus protocols, including gating strategies and nuclear staining techniques, is required to reduce the observed interlaboratory variation. Finally, we demonstrate that stabilized blood preparations can be successfully used to provide a national/international low-level leucocyte EQA scheme.

Keywords Flow cytometry, quality control, standardization, absolute counting, leucodepletion

Introduction

The use of leucocyte-depleted blood products has been shown to reduce, or prevent, adverse transfusion reactions, transfusion-related bacterial sepsis, febrile reactions, HLA antigen alloimmunization and transmission of viruses such as cytomegalovirus (CMV) (Wenz *et al.*, 1980; Sniecinski *et al.*, 1988; De Graan-Hentzen *et al.*, 1989; Orlin & Ellis, 1997; Raife, 1997; Wagner, 1997). Furthermore, the emergence of variant Creutzfeldt–Jakob Disease

(vCJD) in the United Kingdom (Will *et al.*, 1996), coupled with increasing evidence that the distribution of this disease in human tissue may be different to that of the classical form of CJD (Hill *et al.*, 1999), has raised questions regarding the safety of UK plasma products (Ludlam, 1997).

Recent guidelines suggest that blood product units should have less than 5×10^8 leucocytes in order to prevent febrile transfusion reactions (Venglen-Tyler, 1996) and fewer than 5×10^6 to prevent alloimmunization and the transmission of cytomegalovirus (Venglen-Tyler, 1996; British Committee for Standards in Haematology 1998). The latter level has recently been adopted by the UK Transfusion Services and all issued

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blood components are required to have leucocyte counts of $< 5 \times 10^6$ in 99% of components, with 95% statistical confidence. The expansion in production of leucocyte-depleted blood products has therefore increased the requirement for reliable and robust validation processes and quality control measures both at internal and external level.

The enumeration of leucocytes in peripheral blood samples is routinely achieved using automated leucocyte counters. However, the detection level required for monitoring leucocyte depletion, i.e. $< 0.1 \times 10^9/l$, is below the sensitivity of routine haematology analysers. Alternative methods are required and currently include the Nageotte counting chamber, volumetric capillary cytometry and flow cytometry (Conte *et al.*, 1997; BEST working Party of the International Society of Blood Transfusion 1996). Flow cytometry has been the method of choice, particularly as large numbers of samples can be processed routinely. Absolute cell subset counting by single platform flow cytometry has been shown to reduce the interlaboratory variation for CD4⁺ T lymphocyte and CD34⁺ stem cell counting (Strauss *et al.*, 1996; Barnett *et al.*, 1999) and the approach has been recommended for low level leucocyte counting (Conte *et al.*, 1997).

The decision by the government health departments to leucocyte deplete all blood components, prompted UK NEQAS for Leucocyte Immunophenotyping, in conjunction with the UK Transfusion Services, to initiate an external quality assessment (EQA) program for low level leucocyte counting, using blood products stabilized using a previously described whole blood process (Barnett & Granger, 1998). In this report, we present data demonstrating that such an approach can be used to provide a valuable national/international low-level leucocyte counting EQA scheme.

Materials and methods

Leucocytes obtained following leucopheresis of a leukaemia patient were stabilized and then added by limiting dilution to stabilized leucocyte-depleted whole blood, or pooled platelet products, to achieve a final concentration range of 0–70 cells/ μ l. These materials are stable for up to two years at 4 °C (Barnett *et al.*, 1996, 1998; Barnett & Granger, 1998). The leucocyte count of each sample was checked using LeucoCOUNT™ (Becton Dickinson BioSciences, San Jose, USA), a single-platform flow cytometric method that employs a proprietary nuclear staining solution and a known number of lyophilized reference beads. Essentially, 100 μ l of sample and 400 μ l of

LeucoCOUNT™ kit solution were added to TruCount™ tubes, mixed and incubated for a minimum of 5 min (maximum 60 min) in the dark at room temperature. The samples were then analysed using the LeucoCOUNT™ protocol within CellQuest when 10,000 bead events were acquired, equivalent to 20 μ l of sample. Analysis was undertaken using FL1 vs. FL2, with two analysis regions being set around the beads (R1) and leucocytes (R2). The observed ratio between the known bead count and the number of leucocyte events allows the calculation of the absolute leucocyte count:

$$\frac{(R2) \text{ WBC events}}{(R1) \text{ bead events}} \times \frac{\text{number of beads/tube}}{\text{sample volume } (\mu\text{l})} = \text{WBC } (\mu\text{l})$$

Eighteen UK National Blood Service Centres were issued with six 2 ml samples of stabilized blood each month (three peripheral blood and three pooled platelet). Each centre was required to determine the absolute leucocyte count for each sample using their routine analyser (e.g. flow cytometry, volumetric scanning flow cytometry (IMAGN 2000) and/or the Nageotte chamber). A nominal volume was provided with each specimen (reflecting the donor volume postleucocyte depletion) to enable the total number of leucocytes in the original blood product to be calculated. Participating centres were required to state if the unit met the criteria of $< 5 \times 10^6$ leucocytes per unit blood product (BCSH 1998) and whether therefore it was suitable for release. Two staining techniques were used, the LeucoCOUNT™ staining system (Becton Dickinson BioSciences, San Jose, USA) and a nonstandardized propidium iodide (PI) method, prepared according to local protocols.

All data was returned to UK NEQAS within two weeks of specimen issue and detailed statistical analysis undertaken using in-house software (Visual dBASE, Borland International Inc., CA, USA).

Results

A total of 600 complete data sets for the blood and platelet from 24 samples were returned from the 18 participating centres (Tables 1 and 2). Inter-laboratory coefficients of variation (CV) were dependent on sample type (Tables 3 and 4) and leucocyte count. For example, samples with leucocyte counts < 1 cell/ μ l gave interlaboratory CVs $> 100\%$. However, within the approximate critical range of 5–30 cells/ μ l (equating to blood products with leucocyte counts $< 5 \times 10^6/l$ depending on blood volume) the mean CVs were lower, ranging from 4.8 to 35.7%. The manual Nageotte method resulted in consistently higher interlaboratory CVs for all samples, except for P10 that

Table 1. Overall summary of the low-level leucocyte counting results obtained for the 12 whole blood specimens issued

Sample	Number in group	25th centile (cells/ μ l)	Median (cells/ μ l)	75th centile (cells/ μ l)	Inter-laboratory CV (%)
R1	18	36.9	39.1	44	18.2
R2	18	16.8	21	22.2	25.7
R3	18	0.4	0.9	2.1	189
R4	25	9	10.09	11.03	20.1
R5	25	25	27.4	28.5	12.8
R6	25	15.2	18.1	19.9	26
R7	27	7.4	9	10	28.9
R8	27	1	1.3	1.7	53.8
R9	27	2.4	3	3.7	43.3
R10	30	1	1.54	2	64.9
R11	30	9.5	11.75	13	29.8
R12	30	5.5	7	8	35.7

All values expressed as cells/ μ l.

Table 2. Overall summary of the low-level leucocyte counting results obtained for the 12 platelet specimens issued

Sample	Number in group	25th centile (cells/ μ l)	Median (cells/ μ l)	75th centile (cells/ μ l)	Inter-laboratory CV (%)
P1	18	0	0.19	0.6	316
P2	18	8	9.3	10	21.5
P3	18	22.3	23	23.4	4.8
P4	25	9.4	10.65	12	24.4
P5	25	24.4	29	30.9	22.4
P6	25	15	20.9	21.5	31.2
P7	27	1	1.4	1.9	64.3
P8	27	0	0.35	0.5	143
P9	27	1.69	1.95	2.1	21
P10	30	65	69.8	72.2	10.3
P11	30	0.2	0.5	0.8	120
P12	30	17.6	18.9	20.5	15.3

All values expressed as cells/ μ l.

Table 3. Comparative analysis of counting technologies used for low level leucocyte determination in the 12 whole blood samples issued

Sample	Flow cytometry					IMAGN 2000					Nageotte				
	<i>n</i>	LQ (cells/ μ l)	Median (cells/ μ l)	UQ (cells/ μ l)	ICV (%)	<i>n</i>	LQ (cells/ μ l)	Median (cells/ μ l)	UQ (cells/ μ l)	ICV (%)	<i>n</i>	LQ (cells/ μ l)	Median (cells/ μ l)	UQ (cells/ μ l)	ICV (%)
R1	10	36.8	40.2	44.3	18.7	5	36.9	42	42.1	12.4	3	n/a	33.2	n/a	n/a
R2	10	16.6	21	21.5	23.6	5	20	22.4	22.5	11.2	3	n/a	11.6	n/a	n/a
R3	10	0.5	0.9	1	59	5	0	2.1	2.1	100	3	n/a	0.4	n/a	n/a
R4	12	9.3	9.9	11	17.2	9	9.3	10.8	11.4	19.4	4	4	8.3	9	60.2
R5	12	25.2	27.3	29	13.8	9	25.9	27.6	28	7.6	4	10.8	19.6	25	72.5
R6	12	17	18.1	19.6	14.2	9	13.5	18.9	20.3	36	4	6.8	11.3	15.2	74.3
R7	13	8	9	9.3	14.4	10	7.4	9.6	10.3	30.2	4	4	6.7	7.2	47.8
R8	13	1	1	1.3	30	10	0.8	1.7	1.7	52.9	4	1.1	2.8	4.2	111
R9	13	2.5	3	3	16.8	10	1.7	3.7	4.1	66	4	0.5	2.8	3.2	96
R10	13	1	1	1.6	58	13	1.4	1.7	2.1	41	4	0.5	2	3.2	135
R11	13	9	11.3	13	35.4	13	10.6	12.9	13.4	21.7	4	4.8	8.8	9.5	53.7
R12	13	5	6.6	7	30.4	13	6.9	8	8.5	20	4	5.5	7.3	8	34.3

All values expressed as cells/ μ l. UQ, 75th centile; LQ, 25th centile; n/a, not applicable (statistically invalid); ICV, inter-laboratory coefficient of variation.

Table 4. Comparative analysis of counting technologies used for low level leucocyte determination in the 12 stabilized platelet samples issued

Sample	Flow cytometry				IMAGN 2000				Nageotte						
	<i>n</i>	LQ (cells/ μ l)	Median (cells/ μ l)	UQ (cells/ μ l)	ICV (%)	<i>n</i>	LQ (cells/ μ l)	Median (cells/ μ l)	UQ (cells/ μ l)	ICV (%)	<i>n</i>	LQ (cells/ μ l)	Median (cells/ μ l)	UQ (cells/ μ l)	ICV (%)
P1	10	0	0.7	0.19	271	5	0.5	0.6	0.6	16.8	3	n/a	3.8	n/a	n/a
P2	10	8	9.9	11.1	31.1	5	7.7	9.3	9.3	17.2	3	n/a	6.2	n/a	n/a
P3	10	21	23.2	25.8	20.7	5	20.7	22.9	23	10	3	n/a	22.8	n/a	n/a
P4	11	3	9.6	11.4	88	9	9.9	10.6	10.9	9.4	4	14.2	23.6	31.1	72
P5	12	21.8	28.9	30.5	30	9	27.9	29.6	31	10.5	4	24.4	31.4	36.8	39.4
P6	12	12	20	21	44.9	9	19.9	21.3	21.5	7.5	4	11.4	19.7	24.4	66
P7	13	1	1.1	1.5	44.6	10	1.4	1.7	1.9	30.3	4	0.5	2.5	4.2	150
P8	13	0	0.1	0.4	400	10	0.2	0.4	0.6	107	4	0.45	3.9	6	142
P9	13	1.5	1.7	2	30	10	1.7	2.1	2.1	19.5	4	1.8	5.8	8.6	117
P10	13	64	68.6	72	11.7	13	63	70.1	72.2	13.1	4	64.4	69.9	70.6	8.9
P11	13	0	0.4	0.5	125	13	0.2	0.6	1	133	4	0.1	0.7	1	139
P12	13	17	19	20.8	20.1	13	16.2	18.4	19.3	16.6	4	16.8	19.6	20.9	20.6

All values expressed as cells/ μ l. UQ, 75th centile; LQ, 25th centile; n/a, not applicable (statistically invalid); ICV, inter-laboratory coefficient of variation.

had the highest count (Table 4). If the 75th and 25th centiles for each sample (see Tables 3 and 4) are taken to indicate the upper and lower limits of acceptability for the median value for a given technique, then the flow cytometric and IMAGN 2000 systems had fewer outlying results (2) when compared to the Nageotte method (15). The IMAGN 2000 had the lowest CVs for samples with counts of 5–30 cells/ μ l (range 7.5–36%, mean 16.7) when compared to the flow cytometric (range 13.8–88%, mean 29.5) and Nageotte methods (range 20.6–117%, mean 61.8). However, in the four whole blood specimens where the count was < 5 cells/ μ l the interlaboratory CV for the IMAGN 2000 system was higher than flow cytometry in three specimens (Table 3). Conversely, in the 4/5 platelet samples issued with leucocyte counts of < 5 cells/ μ l the interlaboratory CVs for the IMAGN 2000 system were lower than flow cytometry.

During the study, the number of centres using a nonstandard PI staining approach fell such that only two centres continued to use 'in-house' methods after the sixth issue. The first five issues revealed an interlaboratory variation of between 6.7 and 30.4% for the LeucoCOUNT™ method compared to 3.3–63.2% for the PI method. Using the 75th and 25th centiles to define the limits of acceptability for the median value for a given method (Tables 5 and 6), it was noted that all the LeucoCOUNT™ results were satisfactory, whilst the PI method gave unsatisfactory results for five platelet (P3, P9–12) and two whole blood samples (R3–4).

A number of gating strategies were noted (Figures 1–3). Site A, for example, employed the simplest approach, utilizing only two histograms, whilst site C used seven histograms, incorporating a time vs. fluorescence parameter, in order to monitor laser performance during acquisition. The placement of regional gates was also noted to vary. Site B used a large analysis region, encompassing leucocytes and possible debris, whilst site C used a restricted gate placement, thereby excluding a number of leucocyte events (Figure 3, region f). All centres differed in their definition of the bead population, with the result that doublet and quadruplet populations were either included or excluded in the final analysis.

For each issue, the number of centres reporting for a given sample as 'out of consensus' and which would theoretically have led to an inappropriately released unit was: sample R1 (one centre), sample R2 (five centres), sample R5 (two centres) and sample R6 (three centres), and for platelet preparations: sample P3 (six centres), sample P4 (two centres), sample P5 (four centres), sample P6 (four centres) and sample P12 (one centre). There was one laboratory (sample P8) that failed the unit when the consensus was that the unit met the BCSH criteria. Further detailed analysis identified that one centre consistently passed all the whole blood samples and one platelet sample while another passed all the platelet samples when the consensus was a fail. The centre that failed the platelet unit when the consensus was a pass used the Nageotte counting method.

Table 5. Comparative analysis of the flow cytometric determination of low level leucocyte counts using LeucoCOUNT and 'in-house propidium iodide' staining methods for whole blood samples

Sample	LeucoCOUNT					Propidium iodide				
	<i>n</i>	LQ (cells/ μ l)	Median (cells/ μ l)	UQ (cells/ μ l)	ICV (%)	<i>n</i>	LQ (cells/ μ l)	Median (cells/ μ l)	UQ (cells/ μ l)	ICV (%)
R1	5	37	38	44.3	19.3	5	36	42.3	44	18.9
R2	5	16.6	21	21.5	23.5	5	14	20.9	21	33.5
R3	5	0.6	0.84	0.86	30.4	5	0.4	0.95	1	63.2
R4	8	9.3	9.7	10.1	8.2	4	9	11.4	12.2	28
R5	8	25	26.3	27.2	8.3	4	29	29.9	30	3.3
R6	8	16.9	18	18.1	6.7	4	18.2	19.9	20	9
R7	11	7.8	9	10	25	2	n/a	8.5	n/a	n/a
R8	11	0.8	1.1	1.7	80.9	2	n/a	1	n/a	n/a
R9	11	2	3	3.1	36.7	2	n/a	3	n/a	n/a
R10	11	1	1	1.6	58.4	2	n/a	1.5	n/a	n/a
R11	11	7	10.5	13	56.9	2	n/a	12.5	n/a	n/a
R12	11	4	6	7.6	60	2	n/a	7	n/a	n/a

All absolute counts expressed as cells/ μ l. n/a, not applicable (statistically invalid); ICV, inter-laboratory coefficient of variation.

Table 6. Comparative analysis of the flow cytometric determination of low level leucocyte counts using LeucoCOUNT and 'in-house propidium iodide' staining methods for platelet samples

Sample	LeucoCOUNT					Propidium iodide				
	<i>n</i>	LQ (cells/ μ l)	Median (cells/ μ l)	UQ (cells/ μ l)	ICV (%)	<i>n</i>	LQ (cells/ μ l)	Median (cells/ μ l)	UQ (cells/ μ l)	ICV (%)
P1	5	0	0.05	0.15	300	5	0	0.09	0.5	556
P2	5	8	10	10.7	26.7	5	8	9.8	13	51
P3	5	22.5	23	23.4	4.1	5	21	25	28	28.2
P4	7	1.3	8.3	11	117	4	3	11	11.4	76.5
P5	8	20.2	26.7	29	32.9	4	22	30.7	30.9	29
P6	8	12	18	21	50	4	11	21.1	21.5	49.8
P7	11	1	1.2	1.6	53.3	2	n/a	1	n/a	n/a
P8	11	0	0.15	0.4	267	2	n/a	0	n/a	n/a
P9	11	1.5	1.7	2	29.4	2	n/a	1	n/a	n/a
P10	11	62	65.9	70	12.1	2	n/a	76.5	n/a	n/a
P11	11	0	0.4	0.5	125	2	n/a	1.5	n/a	n/a
P12	11	17	19	20.5	18.4	2	n/a	23.5	n/a	n/a

All absolute counts expressed as cells/ μ l. n/a, not applicable (statistically invalid); ICV, inter-laboratory coefficient of variation.

Discussion

The decision by the UK Government to implement a leucocyte depletion policy for blood components prompted UK NEQAS for Leucocyte Immunophenotyping, in collaboration with the UK Transfusion Services, to introduce an EQA scheme for low-level leucocyte counting in 1999. Stabilized whole blood and platelet samples, containing different leucocyte counts, were issued to Blood Transfusion Centres to facilitate their validation processes for leucocyte depletion. Such an approach has been previously documented to eliminate variability due to specimen

degradation and to provide sample integrity for over 600 days (Barnett *et al.*, 1996, 1998, 1999).

It is now well recognized that low level absolute leucocyte counting, particularly for CD4⁺ T lymphocytes and CD34⁺ peripheral blood stem cells, is best performed using the 'single-platform' flow cytometric method (Connelly *et al.*, 1995; Strauss *et al.*, 1996; Barnett *et al.*, 1999; Reimann *et al.*, 2000; Schnizlein-Bick *et al.*, 2000). This is because the 'dual-platform' approach has to rely upon the white cell count (WCC) generated by haematology analysers, a factor that contributes to a high CV (Robinson *et al.*, 1992; Barnett *et al.*, 1999). Furthermore,

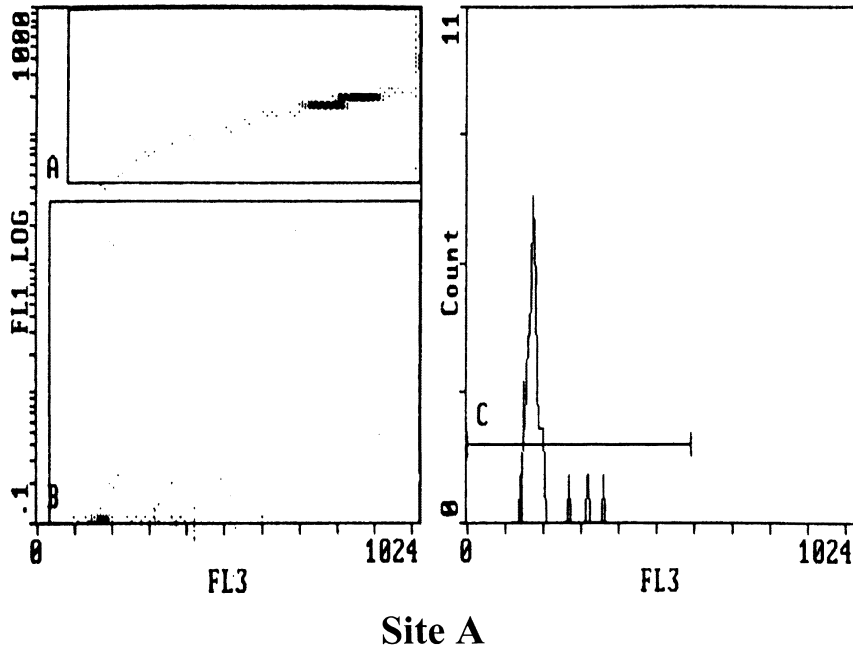


Figure 1. Gating strategy employed by site A to enumerate leucocytes.

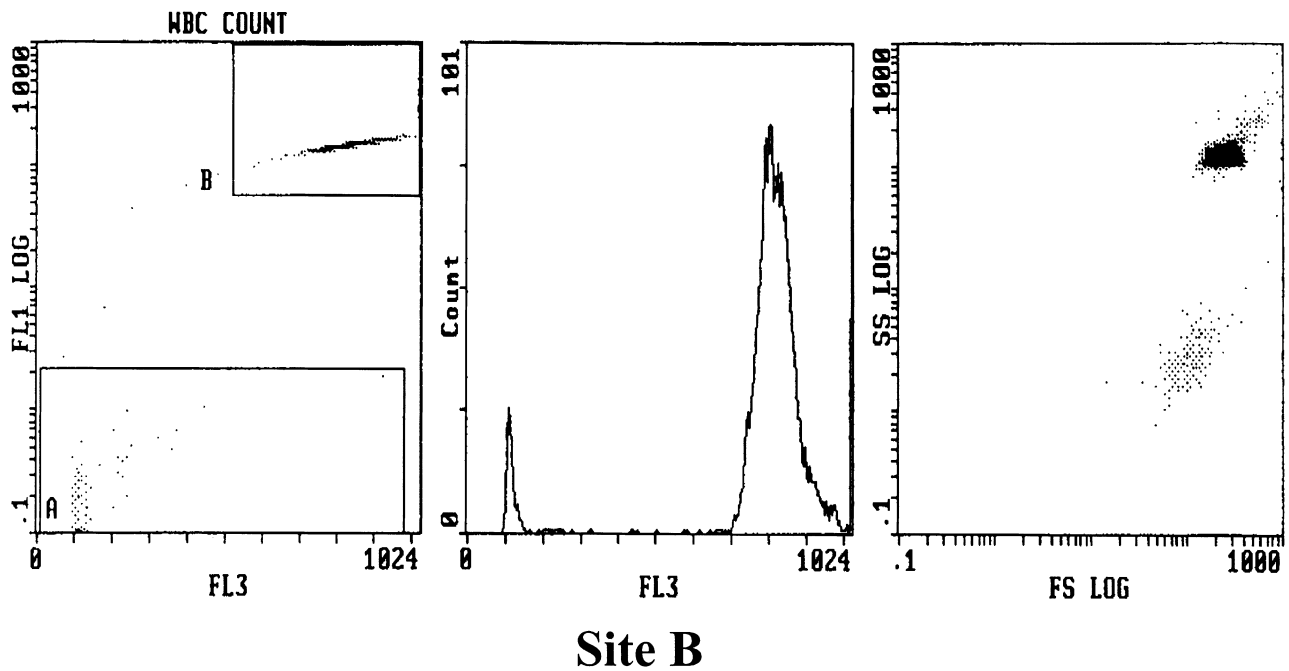
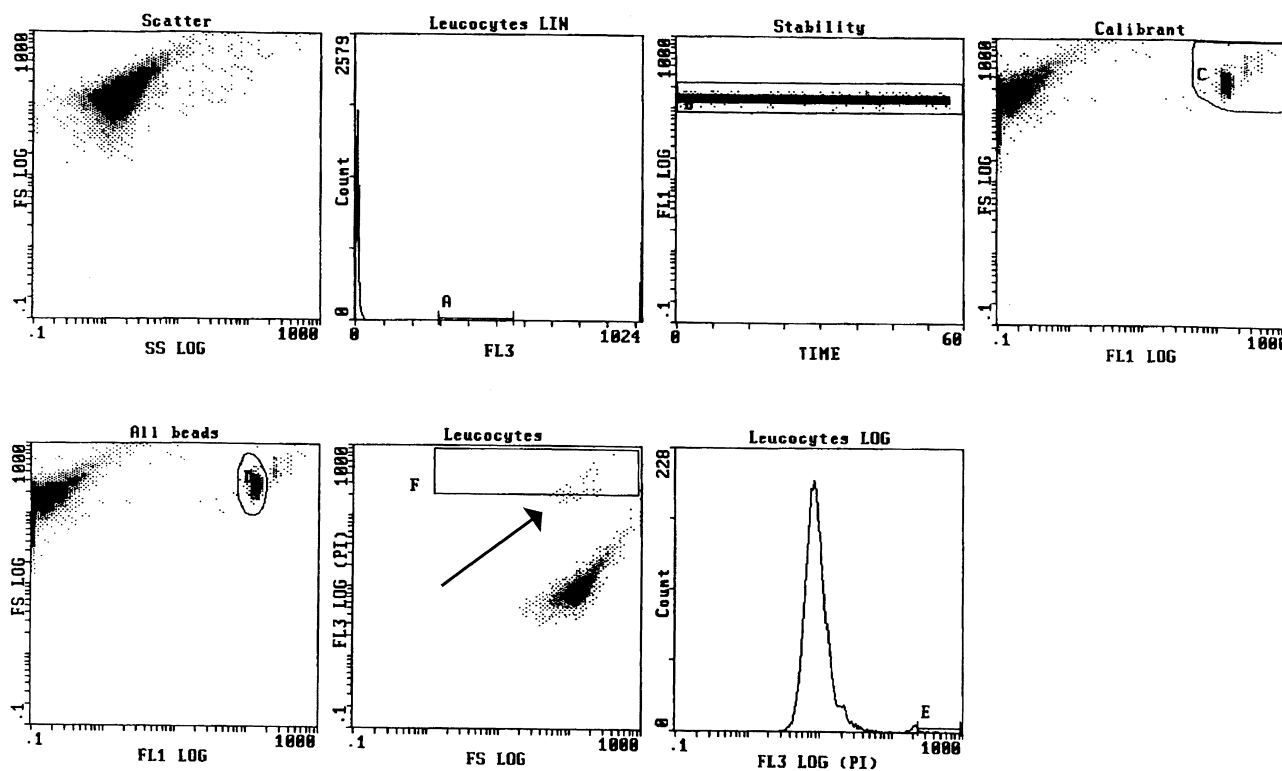


Figure 2. Gating strategy employed by site B to enumerate leucocytes.

haematology analysers cannot be reliably used for the enumeration of leucocytes in leucocyte-depleted blood components, as the levels are below their detection limit. The Nageotte counting method has been routinely employed to circumvent these problems although it is laborious and time-consuming (Szufiad & Dzik 1997). As a result of these problems, single platform flow cytometry

has become the method of choice. However, Conte *et al.*, (1997) suggested that the use of a method that can count to extremely low levels of leucocytes also increases the likelihood of underestimating the leucocyte content in postfiltration blood components. Furthermore, Rebullia & Dzik (1994) reported, using blood that was less than 24-h-old, that flow cytometry and the Nageotte method were



Site C

Figure 3. Gating strategy employed by site C to enumerate leucocytes. Arrow on plot highlights gate placement and the excluded leucocyte events.

equal on performance at levels above 1 cell/ μ l but flow cytometry showed less variability at levels below this value.

We present data, resulting from the issue of 24 samples to 18 laboratories, that reveals a wide range of inter-laboratory CVs. However, within the critical range of approximately 5–30 cells/ μ l depending on blood volume, which equates approximately to blood components with leucocyte counts of $< 5 \times 10^6$ (BCSH, 1998), the CVs improved, although still ranged from 4.8 to 35.7%. Analysis by technique showed the IMAGN 2000 approach to have the lowest overall CV, whilst the Nageotte had the greatest variation in this critical range. However, the interlaboratory CV of the IMAGN system was higher than flow cytometry in the majority of whole blood specimens when the leucocyte count was < 5 cells/ μ l. This finding may reflect the way in which the IMAGN 2000 system differentiates positive and negative fluorescence peaks and the fact that red cells in the whole blood samples results in higher 'background' noise which may produce increased variability for the flow cytometric methods. The wide range of CVs with the Nageotte supports the findings of the

Biomedical Excellence for Safer Transfusion International Working Party (BEST, 1996) that studied the variation between seven sites using a modified Nageotte method. In an attempt to improve the reproducibility of the technique, this study required a 20-fold concentration of leucocytes prior to analysis. Nevertheless, CVs of $< 50\%$ were still reported when cells counts were equivalent to 10 cells/ μ l. Furthermore, Conte *et al.*, (1997) compared flow cytometry with the modified Nageotte method and reported that the Nageotte yielded a one log higher value. They also found a difference in the postfiltration leucocyte content, and suggested that the cell loss related to the method employed and the various technical manipulations involved. Interestingly, we have previously shown that interlaboratory CVs are improved if minimal sample manipulation is undertaken, particularly in rare event analysis, such as CD34⁺ stem cell enumeration (Barnett *et al.*, 1998, 1999). We also found that the IMAGN 2000 system gave lower mean interlaboratory CVs than the flow cytometric methods. This probably reflects the fact that the IMAGN 2000 is a closed system, using preprogrammed software to identify rare cell events, whereas

the flow cytometric approach is more operator dependent. However, this variation increases for the IMAGN 2000 at cell concentrations of < 5 cells/ μl particularly when red cells are present.

This study has highlighted a number of additional factors that may lead to variation of results. Firstly, there was no consensus regarding the use of histograms or gating strategies. One laboratory, for example, used a two-histogram approach whereas another employed seven histograms for leucocyte enumeration. Secondly, significant variability existed with regard to analysis region placement, with one centre excluding some leucocyte events as the result of inappropriate gate placement. Recently, the National Blood Authority for England has introduced a standardized national protocol for those laboratories using flow cytometric methods. Studies are underway to evaluate if the use of such an approach will result in an improvement in interlaboratory CVs. Finally, laboratories using commercial nuclear stains (LeucoCOUNT™) had consistently lower CVs than those using 'in-house' propidium iodide staining methods. The former is a standardized staining kit that incorporates specifically designed software analysis, using a single platform bead-based method and a standardized nucleic acid staining reagent, whereas the propidium method is not only nonstandardized but also relies on a user-defined gating strategy. Such variability clearly influenced the decision of individual sites on whether the blood components met UK recommendations for the number of leucocytes permitted in blood transfusion products (BCSH, 1998). However, we noted that upon introduction of a standardized protocol the number of laboratories out of consensus was significantly reduced.

In conclusion, this study highlights the current variability in low level leucocyte counting, especially within the critical range of 5–30 cells/ μl . The Nageotte approach, for example, gave the highest interlaboratory CVs, whereas the IMAGN 2000 produced the lowest CVs. Furthermore, the use of nonstandardized nuclear staining reagents resulted in higher CVs than the LeucoCOUNT™ method. As a result, we would recommend that a standardized protocol (incorporating targeted training) and nuclear staining reagent be employed for the routine validation of leucocyte-depleted blood components. Such an approach has been successfully used on an international basis to obtain interlaboratory CVs of $< 10\%$ for the enumeration of CD34⁺ stem cells (Barnett *et al.*, 2000). Finally, we demonstrate that stabilized blood preparations can be successfully used to provide a national/international low-level leucocyte counting scheme.

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