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Variables Affecting the Quantitation of CD22 in Neoplastic B Cells

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Abstract

Background: Quantitative flow cytometry (QFCM) is being applied in the clinical flow cytometry laboratory for diagnosis, prognosis, and assessment of patients receiving antibody-based therapy. ABC values and the effect of technical variables on CD22 quantitation in acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), follicular lymphoma (FCL), hairy cell leukemia (HCL) and normal B cells were studied.

Methods: The QuantiBrite System® was used to determine the level of CD22 expression (mean antibody bound per cell, ABC) by malignant and normal B cells. The intra-assay variability, number of cells required for precision, effect of delayed processing as well as shipment of peripheral blood specimens (delayed processing and exposure to noncontrolled environments), and the effect of paraformaldehyde fixation on assay results were studied.

Results: The QuantiBRITE method of measuring CD22 ABC is precise (median CV 1.6%, 95% confidence interval, 1.2–2.3%) but a threshold of 250 malignant cells is required for reliable CD22 ABC values. Delayed processing and overnight shipment of specimens resulted in significantly different ABC values whereas fixation for up to 12 h had no significant effect. ABC measurements determined that CD22 expression is lower than normal in ALL, CLL, FCL, and MCL but higher than normal in HCL.

Conclusions: CD22 expression was atypical in the hematolymphoid malignancies studied and may have diagnostic utility. Technical variables such as cell number analyzed and delayed

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processing or overnight shipment of specimens impact significantly on the measurement of antigen expression by QFCM in the clinical laboratory. Published 2010 Wiley-Liss, Inc.[†]

Keywords

CD22; quantitative flow cytometry; QuantiBRITE; ABC

Flow cytometric evaluation of the levels of expression of various antigens has clinical utility. By measuring cell binding of fluorescently-labeled antibody under saturating conditions, levels of antigen expression can be quantitated. Abnormally dim CD22 expression is characteristic of chronic lymphocytic leukemia (CLL) while abnormally bright CD22 expression is typical in hairy cell leukemia. In several studies, determining the degree of antigen expression is useful in diagnosis, prognostication, and monitoring of therapy in leukemias and lymphomas (1–3). Furthermore, the increased use of antibody-based therapy in hematolymphoid neoplasia has raised interest in correlating levels of expression of the targeted antigen (e.g., CD52 in Campath therapy) with response to therapy. Conventional flow cytometric immunophenotyping provides semiquantitative measures of cell populations (e.g., dim or bright), and mean fluorescence can be determined for the populations of interest and compared between specimens. However, variations in antibody conjugates, changes in instrument performance, variations in instrument settings, and differences in log amplifiers or digital signal processing systems can invalidate comparisons of fluorescent measurement over time, between instruments and between laboratories (4). Quantitative flow cytometry (QFCM) compares the mean fluorescence of cells stained with fluorescent-conjugated monoclonal antibodies to the fluorescent intensity of standard labeled particles or micro beads measured at the same time with the same instrument settings. This provides a standard reference and allows comparison from day to day thus generating reliable data acquired during longitudinal studies.

The measurement of the mean number of fluorescently labeled antibodies bound per cell, or ABC, is one QFCM method. This can be accomplished using standard beads to construct calibrating curves for comparison to a cellular specimen (4). The quantitative indirect immunofluorescence (QIFT) assay is designed for indirect staining methods (4,5). The quantum simply cellular (QSC) and QuantiBRITE assays are designed for studying the binding of antibodies directly conjugated to fluoro-chromes. In the QSC assay, standard beads coated with antimouse immunoglobulin with precalibrated antibody binding capacities are incubated with the antibody being studied (2,4,6). In the QuantiBRITE assay, standard beads with different known numbers of PE molecules are used to generate a calibration curve comparing molecules of PE with mean fluorescent intensity (3,4,7). Using this curve, ABC values for various PE-labeled antibodies can be determined in cell populations of interest. If the PE to antibody ratio is 1:1 (F/P ratio 1:1), then the number of PE molecules per cell (from the calibration curve) equals the number of antibody molecules bound per cell. When the assay is performed under conditions of saturation binding, ABC values give a measure of the overall surface expression of the antigen (8).

Previous studies indicate that ABC determinations by the QuantiBRITE assay are simple and consistent and that QuantiBRITE PE beads can be employed as a tool for standardized

analysis across instruments and laboratories (2,4,7,9,10). In applying ABC determinations in the clinical flow cytometry laboratory, technical issues such as fixation (required for safety of technical personnel) and specimen age arise. Differences in sample processing (e.g., lysing, fixation, and staining methods), properties of monoclonal antibodies used (antibody clone, valence, and purity of conjugate), and location of the antigen on cell surface produce variability in QFCM values (11–14). We therefore undertook a study to examine the characteristics of our in-house QuantiBRITE assay, and of the technical factors affecting QFCM in our clinical samples. We report on the intra-assay variability and precision of the ABC values generated by our QuantiBRITE assay. We also examine the effect of overnight-shipped specimens, time delay between specimen collection and processing, the effect of fixation, and the number of cells required for reliable ABC determination. We also report on the mean ABC values in normal B cells and in a number of B cell malignancies.

MATERIALS AND METHODS

Case Selection

Specimens from patients with a confirmed diagnosis of acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), follicular lymphoma (FL), or hairy cell leukemia (HCL) were submitted to the Flow Cytometry Unit, Laboratory of Pathology, National Cancer Institute (Bethesda, MD) for QFCM evaluation of cell surface expression of CD22 by malignant and normal B-cells. The specimens included 117 peripheral blood (PB), 3 bone marrow aspirates (BM), and 4 lymph node biopsies (LN). Tumor subclassification was based on the WHO criteria for hematologic malignancies using a combination of morphologic, immunohistochemical (IHC), and flow cytometric (FCM) studies (15). The diagnoses were confirmed by review of the original pathology reports, FCM immunophenotype, IHC immunophenotype, and cytology or histological evaluation by two hematopathologists (C. Yuan, and M. Stetler-Stevenson). All specimens were received fresh within 3 h of collection; the sole exceptions were 30 PB specimens that were shipped to the laboratory by overnight express and were at least 24-h old (but less than 48-h old) upon receipt. The 30 shipped PB specimens were paired with 30 fresh, in-house specimens from the same patient (for determining the effect of shipping on ABC values, see below). In addition, normal B cells and normal T cells were evaluated in 20 specimens each with significant numbers of these cells.

Patients were undergoing eligibility evaluation for IRB-approved research protocols studying the efficacy of anti-CD22 monoclonal antibody therapy in various B cell malignancies (16–18). All patients signed IRB-approved informed consent allowing FCM immunophenotyping. All patients had a confirmed diagnosis of B-cell neoplasia with a distinctively abnormal FCM immunophenotype that could be employed to distinguish malignant from normal cell populations.

Specimen Processing

Cell surface expression of CD22 by malignant and normal B-cells was evaluated. Specimens were stained with a panel of antibodies (fresh specimens within 12 h of collection, shipped specimens within 24–48 h of collection). The complete antibody panels, including antibody

combinations used, were chosen based on the number of cells and previous histological diagnosis and immunophenotypic data. Erythrocytes were lysed by incubating with lysing solution (150 mM NH₄Cl, 10 mM KHCO₃, 0.1 mM EDTA) for 10 min at room temperature (maintained at 21–23°C) at a ratio of 1:9 (volume of sample: volume of lysing solution). Specimens were then washed with phosphate buffered saline (PBS) to remove cytophilic antibodies before determining cell number. Cellularity was manually determined by a hemocytometer and viability by trypan blue uptake. In evaluating levels of CD22 expression, specimens were stained with a cocktail containing: CD22PE (1:1 conjugate of fluorescence:antibody) and other antibodies selected to allow gating on the malignant B cells (antibody concentration for CD22 at the saturation level and the concentrations of other antibodies used according to manufacturer's recommendations), (Table 1). Additional antibody combinations were utilized to confirm diagnosis and immunophenotype of the malignant cells [including antibodies against kappa, lambda, CD19, CD20, CD22, CD5, CD10, CD11c, CD25, CD34, CD45, CD103, and CD123 (BD Biosciences, San Jose, CA)]. Specimens were stained for 30 min at room temperature (maintained at 21–23°C according to Clinical Laboratory Standards Institute (CLSI) document H43-A recommendations (19). After incubation, cells were pelleted by centrifugation (500g for 15 min at room temperature), the media was aspirated, and the cells washed twice in a PBS solution containing 0.1% NaN₃ and 0.5% albumin. Cells were acquired after fixation in 1.0% paraformaldehyde and stored at 4°C for up to 12 h before data acquisition, unless otherwise indicated (see below, in Evaluation of Technical Variables in Quantitative Flow Cytometry). Normal lymphoid cells within specimens served as internal controls. Four-color flow cytometry was performed using a BD Biosciences FACSCalibur. The sensitivity of fluorescent detectors was monitored using CaliBRITE beads (BD Biosciences, San Jose, CA) according to the manufacturer's recommendations. Data (collected in list mode) were analyzed with CellQuest Pro software (BD Bioscience) and/or FCSEXPRESS (De Novo Software). At least 5,000 lymphocytes were acquired per tube. Relevant cell populations were analyzed by examining side scatter (SSC), CD20, CD19, and characteristic markers for each specific disease entity (e.g., bright CD11c and CD20 for HCL, CD5 for CLL and MCL, CD10 for FCL) and the cell population of interest subsequently examined for staining with anti-CD22 (BD Biosciences; see Fig. 1 for examples of analysis gating strategies).

Quantitative Flow Cytometry

The antibody bound per cell (ABC) on the malignant B cells was determined for anti-CD22 clone S-HCL-1 (BD Biosciences, 1:1 antibody to PE conjugate prepared by BD Biosciences) using saturating concentrations of antibody and the BD Biosciences QuantiBRITE system (QuantiBRITE standard beads and QuantiCALC software) for fluorescence quantitation. The antibody combinations used in the QuantiBRITE assay are indicated (Table 1). The ABC value represents the mean value of the maximum capacity of each cell to bind the anti-CD22 antibody. QuantiBRITE PE beads are precalibrated standard beads containing known numbers of PE molecules bound per bead. QuantiBRITE beads were acquired on a FACSCalibur flow cytometer on the same day at the same instrument settings as the individual patient specimens. A standard curve comparing the geometric mean of fluorescence to known PE content of the QuantiBRITE beads was constructed using QuantiCALC software. The regression analysis, slope, intercept, and correlation coefficient

were determined. By gating based upon immunophenotype and cell size, populations were isolated for determination of the geometric mean fluorescence of CD22 staining. The analysis gates for malignant B cell populations were based upon the disease type (Fig. 1). Non-B cell populations were identified in a similar manner. The ABC values were generated from the measured geometric mean fluorescence of the gated malignant cells using the QuantiBRITE standard curve. QuantiBrite beads were analyzed daily and over time.

Two lots of QuantiBRITE beads were evaluated over a 43-day period on a single instrument and with the same settings. Instrument function was assessed with CaliBRITE beads (BD Biosciences, San Jose, CA) according to the manufacturer's recommendations and then QuantiBRITE beads were evaluated. Fluorescence variation was tracked by measuring the relative linear channel number for each of the four levels of PE bound per bead and the daily average residuals [residual—the difference between the assigned and measured PE bead content expressed as a percent; average residual—the mean of the residuals determined for each of the four beads (20)].

Determination of negative CD22 ABC value.—To be able to interpret dim CD22 expression, CD22 ABC values were measured in normal non-B cell lymphocytes (T and NK cells) in 20 specimens.

CD22 expression in B cell lymphoproliferative disorders.—The mean CD22 ABC values in ALL, CLL, MCL, FL, and HCL cases studied were determined and compared with the mean CD22 ABC in normal B cells.

Evaluation of Technical Variables in Quantitative Flow Cytometry

Intra-assay variability.—To assess intra-assay variability inherent in the QuantiBRITE system, CD22 ABC values were measured in triplicate under identical specimen processing and environmental conditions in 31 samples. Specimens included 27 peripheral blood samples (8 HCL, 11 CLL, 7 ALL, 1FL), 3 lymph node biopsies (3FL), and 1 bone marrow aspirate (ALL).

Effects of delayed processing, shipping storage and fixation.—The effects of delayed processing/specimen age on ABC values was evaluated on 22 specimens (11 HCL, 7 CLL, 2 MCL, 2 ALL). Each specimen was split, and then underwent two ABC value determinations: the first within 4 h of receipt, and the second after being held overnight at room temperature (maintained at 21–23°C), for next day processing.

The effects of specimen age and possible exposure to temperature extremes during shipment by overnight express were further evaluated. For comparison, ABC values from 30 FB specimens (25 HCL, 5 CLL) that were shipped to the laboratory by overnight express were paired with ABC values from fresh, in-house specimens processed within 4 h of receipt from the same patient. The shipped specimens were ~24- to 48-h old upon receipt in the laboratory.

The effect of fixation and storage on the assay was studied. In a series of 13 samples (6 HCL FB, 3 CLL PB, 3 ALL FB, 1 ALL BM) cells were processed and stained (if red cells were

present, lysis was performed) Three ABC values were then determined on each sample as follows: (1) on an unfixed preparation, (2) fixed in 1.0% paraformaldehyde and acquired within 2 h of fixation, and 0) fixed in 1.0% and acquired after being stored at 4°C overnight.

Accuracy determination.—The number of events required for accuracy in ABC determinations was determined in 28 specimens (12 CLL FB, 9 HCL FB, 4 ALL FB, 1 ALL BM, 1 FL LN, 1 MCL PB) stained with anti-CD22 1:1 antibody. Each specimen was acquired 11 times, so that the number of malignant cells collected varied. The acquisition was performed so that 10, 20, 30, 40, 50, 100, 250, 500, 1,000, 2,500, and 5,000 malignant cells were collected for ABC determinations.

RESULTS

A variety of specimens were received for evaluation of cell surface expression of CD22, and were utilized in this study. These included peripheral blood, bone marrow aspirates, and lymph node biopsies. The diagnoses included acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), follicular lymphoma (FCL), mantle cell lymphoma (MCL), and hairy cell leukemia (HCL), (Table 2). In addition, CD22 ABCs were measured in normal B cells and in normal T plus NK cells. The mean and standard deviation (S.D.) for ABC values were determined. Since T and NK cells are negative for CD22, the mean CD22 ABC for this population of lymphocytes (mean ABC = 63) plus 2 S.D., i.e., an ABC of 75 can be utilized as the cut off for positivity. All CD22 ABC values measured were considerably above this value (lowest CD22 ABC value 505). When comparing mean CD22 ABC values, CD22 expression was lower in ALL, CLL, FCL, and MCL than in normal B cells but it was markedly increased in HCL.

Intra assay variability in ABC values was determined by performing the QuantiBRITE assay in triplicate in 30 specimens (Supporting Information Table Is). The standard deviation of each specimen's values was divided by the mean of the values to obtain the coefficient of variation (CV). The median CV was 1.6% (95% confidence interval, 1.2–2.3%), indicating a good precision for the test.

The effect of delayed processing on ABC values was evaluated. A total of 22 peripheral blood specimens (11 HCL, 7 CLL, 2 MCL, 2 ALL) were split so that ABC values were determined (1) within the first 4 h of receipt and (2) after being held overnight at room temperature (maintained at 21–23°C) for next day processing (Table 3). As expected, CD22 ABC correlated with diagnosis (i.e., lower CD22 ABC in CLL and ALL and higher CD22 ABC in HCL). Delayed processing resulted in a statistically significant decrease in ABC values. A decrease in ABC values was noted in 20/22 samples (range –54% to –3%) whereas 2/22 showed an increase (+3% and +16%). The median change was –19% (95% Q, –24% to –6%, $P < 0.0001$).

The effect of delayed processing and specimen shipment (delayed processing and exposure to noncontrolled environments) was further studied in 30 peripheral blood samples (25 HCL, 5 CLL). Paired. ABC values from overnight-shipped specimens compared to ABC values of in-house specimens from the same patients showed a statistically significant change in the

ABC values (Table 4). of the shipped specimens, 23/30 had decreased malignant cell CD22 ABC values (range -64% to -13%), while 7/30 had increased malignant cell CD22 ABC values (+1% to +73%). The average difference among the shipped specimens was -21% (95% CI, -26% to -18%, $P = 0.0012$ by the Wilcoxon signed rank test). More variation was noted between shipped and in-house specimens (Table 4), than in in-house specimens in which processing was simply delayed (Table 3).

The effect of fixation was evaluated in 13 specimens (12 peripheral blood and 1 bone marrow aspirate). For each sample, ABC values were determined on (1) an unfixed preparation, (2) fixation within 2 h, and (3) fixation with overnight storage at 4°C and acquisition the next day. No statistically significant change in ABC values above the normal assay variability (determined above) were noted between unfixed cells, fixed cells acquired within 2 h of fixation and cells held overnight in fixative and acquired the next day (Table 5).

To determine the optimal cell number needed for reliable CD22 ABC values, 28 samples (26 PB, 1 LN, 1 BM) were acquired multiple times, increasing the number of the cellular events collected each time such that 10, 20, 30, 40, 50, 100, 250, 500, 1,000, 2,500, and 5,000 malignant cells were collected for ABC determinations (Fig. 2, Supporting Information Table 2s). The standard deviation (SD) of the relative deviations from the sample average showed a steady decrease as the number of tumor cell events acquired and analyzed increased from 10 to 250. There was no significant variability in results for 250, 500, 1,000, 2,000, or 5,000 cellular events. This showed that at least 250 cellular events were required for reliable CD22 ABC values.

Two lots of QuantiBRITE beads were studied on the same instrument with the same settings (43 consecutive days for each lot). Instrument function was validated with CaliBRTTE beads. There was little variation in fluorescence of the four standard beads over time, as measured by the relative linear channel number. The daily average residuals for the two lots were compared and for both lots is less than 1%. The linear regression curves of the highest and lowest slopes were compared and little to no difference was noted.

DISCUSSION

Quantitative Flow cytometry (QFCM) is being investigated as a diagnostic tool in the clinical laboratory and as a prognostic indicator in patients receiving antibody-based therapy (21–23). In the current study, CD22 expression was quantitated by measuring the mean number of fluorescently-labeled antibodies bound per cell, or ABC, utilizing the QuantiBRITE assay and a custom conjugated anti-CD22 antibody with 1:1 PE to antibody ratio (F/P ratio 1:1). Although the current study is mainly technically oriented, it also provides reference ABC values for the levels of CD22 encountered in B cell neoplasms. Based upon CD22 ABC values, CD22 expression is lower in CLL, ALL, MCL, and FCL (in order from lowest to highest) than in normal B cells but is markedly increased in HCL. These results further support a diagnostic role for QFCM in hematolymphoid neoplasia. The data is in line with previous observations made on the basis of semi-quantitative measurements (arbitrary units of fluorescence intensity). Huang et al found aberrant CD22 expression in CLL, MCL, marginal zone lymphoma, HCL and FCL (24) using a non-

quantitative assessment of levels of CD22 expression in malignant and normal B cells. Later studies demonstrated aberrant CD22 expression in CLL, ALL, prolymphocytic leukemia, HCL, splenic marginal lymphoma, and MCL using multiple quantitative methods (3,5,25). In a study by D'Arena et al. mean $\pm$ standard deviation ABC values were determined for normal B cells ($26,283 \pm 5,091$), CLL ($7,726 \pm 6,315$), HCL ($108,199 \pm 53,348$), FCL ($22,094 \pm 16,954$), and MCL ($7,042 \pm 8,440$). The ABC values determined in the current study are consistently lower than those of D'Arena et al., namely normal B cells $21,642 \pm 1,329$, CLL $2,757 \pm 1,901$, HCL $50,558 \pm 21,642$, FCL $11,951 \pm 1,278$, and MCL $5,687 \pm 661$. In the current study, however, the standard deviation is significantly lower and we used a 1:1 antibody to PE conjugate (not reported used in D'Arena et al. study). In addition, the previous study does not mention daily running of QuantiBRITE beads for calibration so drift of instrument function may have lead to inaccuracies. Because of the ability of QFCM to make absolute measurements (ABC), these ABC values may be compared to future studies using the QuantiBRTTE approach, thus generating a rich database of CD22 levels.

Before QFCM is incorporated into the clinical setting, technical factors affecting this assay must be evaluated. We studied a variety of factors impacting QFCM of CD22 expression in malignant B cells using the QuantiBRITE method for measuring ABC. The QuantiBRTTE beads and our instrument function were stable over time, as evidenced by the minimal variance in the relative linear channel number for each of the four levels of PE beads, linear regression curves of the highest and lowest slopes, and the daily average residuals for two lots of beads studied. The QuantiBRITE method of measuring CD22 ABC is quite precise (median CV 1.6%, 95% confidence interval, 1.2–2.3%). However, several technical variables impacted the quality of data measured by QFCM. We determined that acquisition 250 cells of interest (malignant B cells, in this study) is needed for reliable CD22 ABC values. Delaying processing of peripheral blood specimens for 12 h by holding them at room temperature overnight resulted in a different ABC value from that obtained from fresh cells. In the vast majority of specimens, (91%), the CD22 ABC value was lower than that obtained in fresh cells when the specimen was held overnight. Shipping peripheral blood specimens by overnight express resulted in further significant ABC value changes; again, the majority of shipped peripheral blood specimens (77%) had decreased tumor cell CD22 ABC values compared to fresh peripheral blood specimens on the same patient. The degree of variation in shipped specimens was greater than in specimens that were simply delayed in processing, and may be due to exposure to temperature extremes during shipping. No significant effect of paraformaldehyde fixation for up to 18 h was noted on CD22 ABC values. Importantly, although delayed processing impacted the accuracy of ABC quantification, it did not effect qualitative determination of the presence or absence of CD22 expression by malignant cells.

In conclusion, the QuantiBRTTE system of QFCM is stable over time, making it an appropriate test for longitudinal studies. The CD22 ABC determinations using the QuantiBRTTE system are very precise if a sufficient number of cells of interest are acquired and analyzed. Paraformaldehyde fixation (recommended for routine clinical specimens) does not significantly impact the ABC results for CD22. Reporting of CD22 ABC values should be avoided in specimens shipped overnight or in which processing is delayed. As the effect of specimen age, fixation, and other factors on QFCM of different antigens may vary, the possible impact of technical variables upon QFCM should be considered and evaluated when

developing new assays for antigen quantitation. CD22 expression is frequently abnormal in B cell neoplasms and further studies to determine its utility in diagnosis are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Analysis gating strategies. **A.** Chronic lymphocytic leukemia (CLL): (1) Ungated data—Gate drawn on CD19 positive B cells. (2) CD19 positive B cells—Gate drawn on CD19 and CD5 positive CLL cells. (3) CD 19 and CD5 positive CLL cells: CD22 expression. FITC channel is blank. **B.** Hairy cell leukemia (HCL): (1) Jngated data—Gate drawn on CD20 positive B cells. (2) CD20 positive B cells—Gate drawn on CD20 and CD11c bright positive HCL cells. (3) CD20 and CD11c positive HCL cells: CD22 expression. FITC channel is blank. **C.** Acute lymphoblastic leukemia (ALL): (1) Ungated data—Gate drawn on CD19 positive cells. (2) CD19 positive cells—Gate drawn on CD19 and CD10 positive ALL cells. (3) CD19 and CD10 positive ALL cells: CD22 expression.

Fig. 2. Optimal cell number for reliable CD22 ABC values. Twenty eight samples were analyzed multiple times collecting 10, 20, 30, 40, 50, 100, 250, 500, 1,000, 2,500, and 5,000 malignant/aberrant cells. The standard deviation (SD) of the relative deviations of CD22 ABC from the sample average (y axis) was plotted versus number of aberrant cells the CD22 ABC was determined from.

Table 1

Antibody Cocktails for CD22 ABC Determinations

Diagnosis	FITC	PE	PerCP 5.5	APC
ALL	CD19	CD22 1:1	CD45	CD10
CLL and MCL	–	CD22 1:1	CD19	CD5
FCL	–	CD22 1:1	CD19	CD10
HCL	–	CD22 1:1	CD20	CD11c

ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; MCL, mantle cell lymphoma; FCL, follicular lymphoma; HCL, hairy cell leukemia.

Table 2

Specimens Received for Evaluation of Cell Surface Expression of CD22

Diagnosis	Specimens	ABC mean (S.D.)
ALL	14 (11 PB, 3 BM)	4,695 (2,665)
CLL	31 (31 PB)	2,757 (1,901)
FCL	4 (1 PB, 3 LN)	11,951 (1,278)
MCL	3 (3 PB)	5,687 (661)
HCL	72 (72 PB)	50,558 (21,454)
Normal B cells	20 (20 PB)	21,642 (1,329)
T cells + NK cells	20 (20 PB)	63 (6)
Total	164	

ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; MCL, mantle cell lymphoma; FL, follicular lymphoma; HCL, hairy cell leukemia; PB, peripheral blood; BM, bone marrow; LN, lymph node.

Table 3

Effect of Delayed Processing of Peripheral Blood Specimens on CD22 ABC Determinations

Patient	Diagnosis	CD22 ABC-fresh in-house specimen	CD22 ABC-delayed processing of specimen	Percent variation
1	CLL	922	708	23%
2	CLL	1,701	1,411	17%
3	MCL	4,706	4,544	3%
4	CLL	5,047	3,849	24%
5	CLL	5,224	3,028	42%
6	CLL	5,630	5,268	6%
7	CLL	5,784	5,235	10%
8	CLL	6,059	4,921	19%
9	ALL	6,830	4,797	30%
10	ALL	9,380	6,464	31%
11	FCL	13,121	15,168	16%
12	HCL	21,323	20,320	5%
13	HCL	24,402	11,206	54%
14	HCL	25,292	19,935	21%
15	HCL	26,514	12,894	51%
16	HCL	28,922	29,773	3%
17	HCL	40,007	25,536	36%
18	HCL	44,637	43,388	3%
19	HCL	54,242	44,585	18%
20	HCL	61,773	48,205	22%
21	HCL	75,745	60,711	20%
22	HCL	79,911	71,309	11%

Comparison of tumor cell CD22 ABC values determined in specimens split and one portion processed within 4 h of collection (CD22 ABC-fresh in-house specimen) and another portion stored overnight at room temperature (CD22 ABC-delayed processing of specimen). Delayed processing had a significant impact on CD22 ABC ($P < 0.0001$). CLL = chronic lymphocytic leukemia, MCL = mantle cell lymphoma, ALL = acute lymphoblastic leukemia, FCL = follicular lymphoma, HCL = hairy cell leukemia.

Table 4

Effect of Shipping Peripheral Blood Specimens on CD22 ABC Determinations

Patient	Diagnosis	CD22 ABC-fresh in-house specimen	CD22 ABC-shipped overnight specimen	Percent variation
1	CLL	1,082	385	64%
2	CLL	1,160	950	18%
3	CLL	1,401	1,063	24%
4	CLL	1,463	1,877	28%
5	CLL	2,222	1,093	51%
6	HCL	14,058	16,332	16%
7	HCL	25,292	20,552	19%
8	HCL	31,542	23,526	25%
9	HCL	35,473	22,572	36%
10	HCL	35,615	61,470	73%
11	HCL	36,494	36,983	1%
12	HCL	37,226	45,718	23%
13	HCL	38,939	28,771	26%
14	HCL	40,007	33,565	16%
15	HCL	41,824	29,338	30%
16	HCL	42,566	26,117	39%
17	HCL	49,509	61,106	23%
18	HCL	52,929	33,152	37%
19	HCL	54,242	36,657	32%
20	HCL	60,123	46,244	23%
21	HCL	60,454	61,558	2%
22	HCL	61,450	49,453	20%
23	HCL	61,773	50,565	18%
24	HCL	64,139	48,144	25%
25	HCL	79,726	47,046	41%
26	HCL	80,679	64,681	20%
27	HCL	81,125	48,787	40%
28	HCL	82,870	66,788	19%
29	HCL	87,448	67,299	23%
30	HCL	112,120	97,171	13%

Comparison of tumor cell CD22 ABC values determined In fresh In house specimens (processed within 4 h of collection) and specimens shipped by overnight express. Overnight shipping had a significant impact on CD22 ABC ($P = 0.0012$). CLL = chronic lymphocytic leukemia, HCL = hairy cell leukemia.

Table 5

Effect of Fixation with 1% Paraformaldehyde on CD22 ABC Determinations

Specimen	Diagnosis	CD22 ABC; no fixation	CD22 ABC; 2-h fixation	%Variation <2-h fixation from no fixation	CD22 ABC; overnight fixation	%Variation overnight fixation from no fixation
1	CLL	1,584	1,593	<1%	1,544	3%
2	ALL	1,639	1,702	4%	1,621	1%
3	CLL	1,996	1,926	4%	1,890	5%
4	ALL	3,077	3,353	9%	3,388	10%
5	ALL	6,442	6,447	<1%	6,986	8%
6	ALL	6,544	6,605	1%	7,098	8%
7	CLL	6,665	6,649	<1%	7,001	5%
8	HCL	36,181	33,080	9%	30,457	16%
9	HCL	43,887	40,170	9%	39,356	10%
10	HCL	49,132	47,176	4%	46,279	6%
11	HCL	50,089	48,996	2%	48,560	3%
12	HCL	55,017	52,396	5%	50,300	9%
13	HCL	67,554	60,454	11%	58,992	13%

Comparison of malignant cell CD22 ABC values for unfixed and cells fixed in 1% paraformaldehyde. Each specimen was split three ways and processed (1) unfixed (2) fixed 2 h, and (3) fixed and stored overnight at 4°C. No significant difference above assay variability was noted in ABC values between these three methods ($P=0.15$). CLL = chronic lymphocytic leukemia, ALL = acute lymphoblastic leukemia, HCL = hairy cell leukemia.