A Decision-Support System for Flow Cytometry Immunophenotyping

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Key Words: Clinical flow cytometry; Quality assurance; Decision support; Relational databases; Information systems; Quality improvement

Abstract

*We developed a decision-support system, the flow cytometry workstation (FCW), that provides variable panel definitions, age-adjusted reference ranges, and graphic display of immunologic trends. Automated quality assurance functions include validation of flow cytometry data using user-defined monoclonal antibody sums and delta check computations. We evaluated the FCW to determine whether it would reduce CD4+ technical and clerical errors and to discover patterns or trends within flow cytometric data for research purposes. The FCW reduced the number of technical and clerical errors in its first 2 years of use (*P = .003*). User-defined quality assurance summation checks such as CD2+ + CD20+ = 95% ± 5% were applied to a 10-year data set as part of a retrospective analysis. The FCW discovered a relationship between specimen processing and the number of results appearing out of range: 58.11% of reported samples appeared out of range in 1993 compared with 2% in 1996 (*P_1 < .001*). The FCW is a foundation for quality improvement and outcomes-based research for clinical flow cytometry and serves as a platform for state-of-the-art laboratory management.*

One of the most important functions of a diagnostic laboratory is to convey meaningful information that improves the quality of health care. With newer and more sophisticated laboratory instrumentation, medicine has become an information science and requires more complex computational analytic techniques.[1] Specialty laboratories, such as flow cytometry, generate large amounts of data, some of which are readily useful in clinical reporting, while other data may provide the basis for subsequent outcomes-based research.[2]

Flow cytometry immunophenotyping sometimes involves identification of cells by light-scattering properties, and cells are further defined by the binding of fluorochrome-tagged monoclonal antibodies (MAbs). The enumeration of subsets of peripheral blood lymphocytes binding these MAbs is important for the characterization of cellular immunodeficiency diseases.[3] In HIV-infected people, CD4+ lymphocytes are a strong prognostic indicator of AIDS-free survival. Used in conjunction with molecular diagnostic viral load testing, CD4+ lymphocytes are the basis for initiating and monitoring antiretroviral therapy. For this reason, it is critical that each laboratory performing immunophenotyping have methods to evaluate the quality of data.

The need for quality assurance in flow cytometry has been described in the literature.[4] Current quality assurance proficiency testing programs place emphasis on CD4+ T-cell measurements. As part of an intrapanel quality control check, the lymphosum (T + B + natural killer [NK] cell counts) may be used when lymphocyte subset values should equal 100% ± 5% for HIV-seronegative specimens, with a somewhat lower value of 95% ± 5% for HIV-seropositive specimens.[5] Other quality assurance MAb summations have been defined, such as the percentage sum of CD2+ + CD19/CD20+[6] to evaluate

enumeration performance of CD4+ cells, CD8+ cells, B cells, and NK cells. If these limits are not met, a critical reevaluation of gating should be made along with a determination as to whether the data obtained from analyses are valid.

Laboratories may choose to define their own formats for internal quality control checks and clinical reporting; however, relatively few flow cytometry applications permit end users to define clinical and quality assurance panels dynamically. Such applications ideally should permit the linking of demographic and diagnostic data across individual patients or patient populations for data mining. Currently, data-mining techniques applied to clinical flow cytometric data require substantial data processing and collating before analysis.[7] However, a more accessible approach to knowledge discovery is feasible when data are managed and stored within a relational database[8,9] that minimizes the potential problems that arise from using single large spreadsheets to analyze data.[5] Most flow cytometry software offers data output and management only in spreadsheet or word-processing formats.[10]

A software application, the flow cytometry workstation (FCW), was developed to improve the level of decision support within the clinical flow cytometry laboratory. Delta checks[11] and the sum of the percentages (equations: CD2+ + CD20+ = 100% ± 5% and CD2+ + CD20+ = 95% ± 5%[5]) were used as examples of internal quality control measures to detect errors and interesting patterns within a 10-year clinical flow cytometry data set. The quality checks also were implemented as a clinical reporting prerelease check to detect technical and clerical errors.

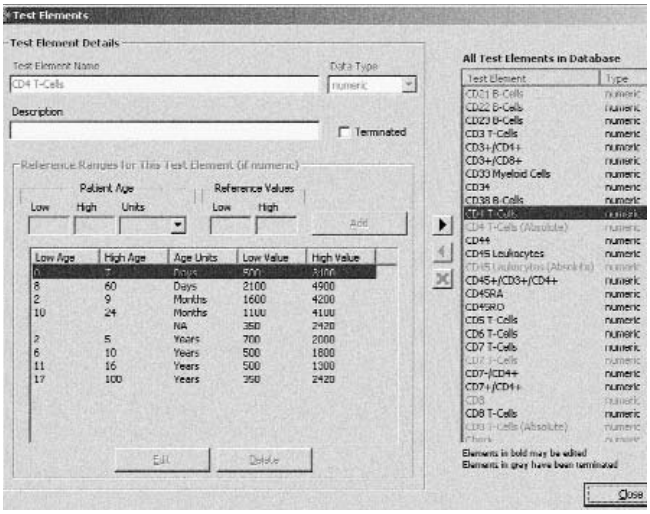

Figure 1 The "Test Elements" form permits end users to create a library of components from which to build panels. The form includes the ability to add age-adjusted reference ranges, expiration dates, lot numbers, and classification headings for inclusion on reports. NA, not applicable.

Materials and Methods

The flow cytometry laboratory at the University of Missouri Health Care, Columbia, uses a flow cytometer (XL-MCL, Beckman Coulter, Miami, FL) and MAbs from various manufacturers with multiple fluorochromes. Before development of the FCW, clinical report generation was performed using a stand-alone data management system developed with Claris FileMaker Pro (Claris, Santa Clara, CA). The FileMaker Pro application, referred to as the legacy system hereafter, permitted generation, storage, and retrieval of laboratory reports; however, it did not address a large number of clerical, quality assurance, and research functions that could improve quality of clinical flow cytometry reporting and management.

FCW Application

The FCW is deployed on an ALR 8300 server (Gateway, Kansas City, MO) using Windows 2000 Server (Microsoft, Redmond, WA) as its operating system. The relational database system is Microsoft SQL Server 2000. The front-end client application is programmed using Microsoft Visual Basic 6.0 operating on 3 workstation terminals. The FCW is 1 component of a larger multilaboratory management application capable of addressing other types of specialty laboratory data for hematology and bone marrow examinations, molecular diagnostics, and cytogenetics.[12,13]

The FCW enables user-defined panel construction and reagent specification. End users construct a "test library" containing elements for clinical reporting and quality assurance purposes **Figure 1**. Absolute numbers, percentages of positive cells, or ± results for each MAb or MAb combination may be defined as a result element. Age-adjusted reference range information[14] may be entered for MAbs for display on final reports in units that range from days to weeks to months to years. Text and image elements also may be defined for interpretive text and histogram images, respectively. Additional elements defined at the user's discretion for quality assurance and reagent tracking reports include expiration dates, lot numbers, and manufacturers. Once test elements have been defined, a panel construction form permits the linking of test elements to a user-defined panel name **Figure 2**. Items defined in the test element form (Figure 1) appear in the "Add/Edit Panels" form in the column "Available Test Elements" (Figure 2). The far-right column shows all panels defined by end users, with a tree-view of the individual test elements. The panel name serves as the parental unit under which test elements are grouped for panel ordering and quality assurance purposes.

During entry of a test requisition, the panel order form displays panels currently available that have been defined by the laboratory. The result entry form displays fields that

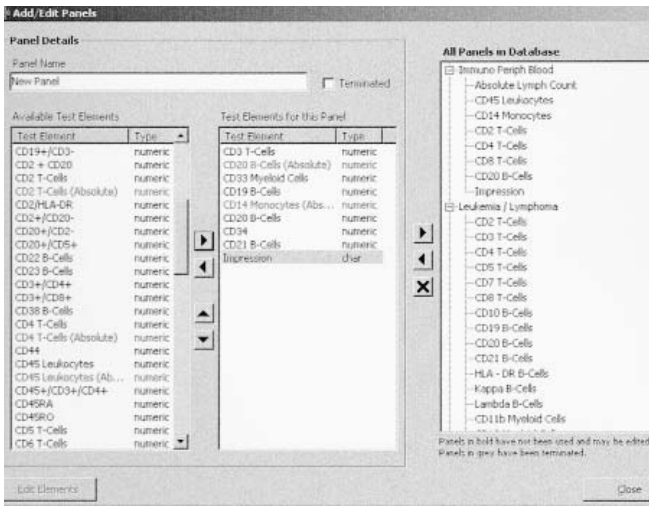

Figure 2 The "Add/Edit Panels" form permits construction of monoclonal antibody panels. The end user selects and moves the test elements from the "Available Test Elements" box to the "Test Elements for this Panel" box. The order in which the elements are to appear on the report can be defined using the up and down arrows. The box, "All Panels in Database," shows all panels currently defined and their associated data elements (reagents). Those in gray have been terminated. Clicking the tree-view "+" expands a panel to show its test elements.

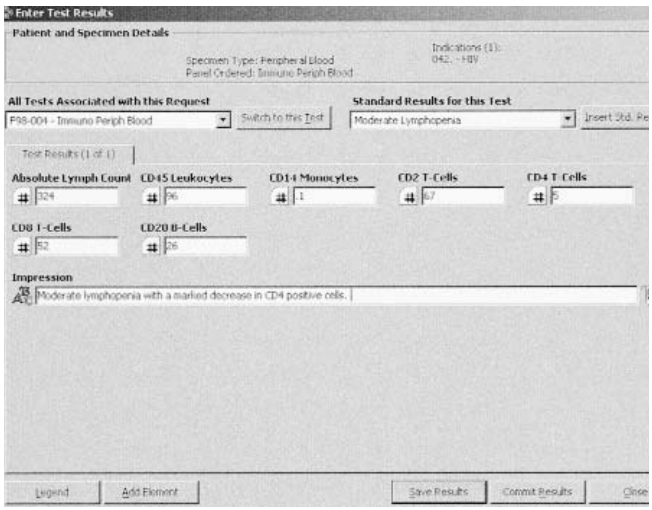

Figure 3 The "Enter Test Results" form permits manual entry or capture of data from the flow cytometer. Only the items selected during panel construction (Figure 2) appear on the Enter Test Results form. Selections from the "Standard Results for this Test" list box automate retrieval of standard comments from the standard report library. Patient and physician's names displayed are fictitious. Lymph, lymphocyte.

are dynamically "painted" or adjusted to show only the test elements associated with the ordered panel(s) **Figure 3**. The data entry fields in Figure 3 match the elements shown in Figure 2 for an "Immuno Periph Blood" panel. A selection from a "standard result" menu leads to the insertion of user-defined interpretive comments previously defined by end users using the "standard comments" library. Standard comments are inserted primarily for clinical report interpretations. The final report generated by the application includes user-defined longitudinal plots for patient results along with age-adjusted reference ranges **Figure 4** that had been defined in the Test Elements form (Figure 1). Report formats automatically adjust to the number and type of elements defined for the panel. While this article addresses mainly CD4+ testing, the FCW also is used for all other testing, such as leukemia and lymphoma immunophenotyping.

For accurate longitudinal plots and quality assurance measures, it is critical that patients be identified accurately during order entry. The FCW determines whether the patient listed on the test order has previous data within the system by matching the patient's last name, first name, medical record number, sex, and date of birth with other records. Laboratory personnel make the final decision as to whether the current patient should be associated with previous entries returned from the database query's result set. This ensures

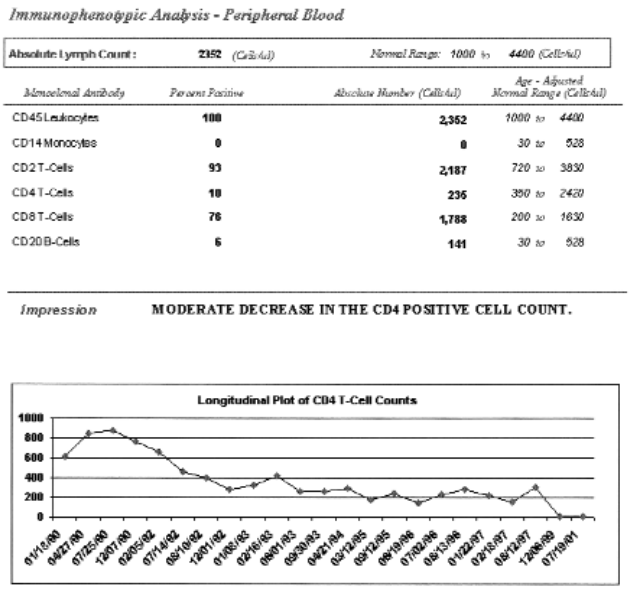

Figure 4 An immunosuppression panel report with a longitudinal plot of CD4+ T-cell counts for an individual patient. Age-adjusted reference ranges, defined by the user during panel construction, appear along with absolute number computations. Lymph, lymphocyte.

that patient values are not missed and that erroneous data are not associated with the current patient's data when performing analyses. Within the University of Missouri Health Care, patients may have multiple medical numbers

based on the ordering institution; therefore, medical record numbers cannot be used as the sole identifier for patients.

Currently, the University of Missouri Health Care Information Technology group is developing software tools for integrating output from the FCW into the main hospital information system. The FCW accepts output from Beckman Coulter's EXPO 32 flow cytometer software using middleware tools that upload flow cytometer files exported in Microsoft Excel format.

Evaluating Clerical Error Reduction

By using delta checks and the CD2+ + CD20+ equation, we compared the number of clerical errors during the preintervention period, the period during which the laboratory used the legacy system, with those during the intervention period. The delta check, defined as Absolute = Current Value – Previous Value,[11] was applied specifically to CD4+ and CD45+ values. End users have other options for quality assurance measures **Figure 5**. Data are also validated through longitudinal graphing of MAbs to compare current results with the patient's previous data **Figure 6**. The preintervention period includes 3,297 records processed from July 1989 to August 1999. The intervention period includes 867 records processed from August 1999 to December 2001.

Evaluating Laboratory Technical Proficiency

Several algorithmic rules may be applied to assess the quality of the data reported by the clinical flow cytometry laboratory. In a multicenter quality assurance program study, the use of the lymphosum approached 100% in specimens from HIV-seronegative healthy donors, suggesting that in healthy donors, nearly all peripheral blood lymphocytes can be accounted for in the T-, B-, or NK-cell populations. In the same study, HIV-seropositive patient specimen values were lower (85% of the values were 95% ± 5%). The study was unclear as to whether this was due to a technical artifact or a reflection of "null" lymphocytes in these patients. The FCW applied the following summation equations to determine laboratory technical proficiency during a 10-year period: CD2+ + CD20+ = 100% ± 5% and CD2+ + CD20+ = 95% ± 5%. The 100% ± 5% range was deemed acceptable for HIV-seronegative samples and the 95% ± 5% range, for HIV-seropositive samples. To determine whether out-of-range values were due to laboratory technical error or to immunologic processes within particular patients, we checked whether out-of-range values occurred repeatedly within certain patients.

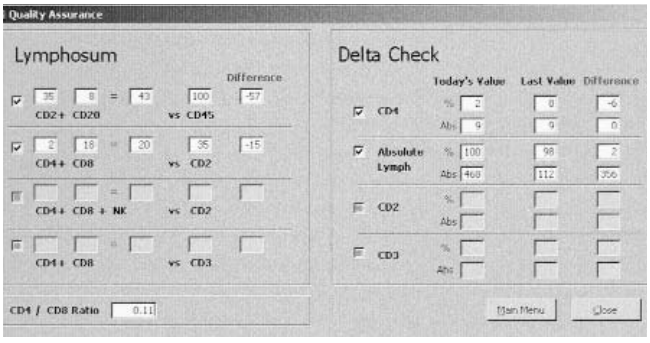

Figure 5 The use of lymphosum and delta checks for validating quality before release of the final report. End users select their preferred lymphosum equation(s). Delta checks include monitoring of both percentage and absolute number changes for CD4, absolute lymphocytes, CD2, or CD3. Lymph, lymphocyte; NK, natural killer.

Statistical Analysis

The Fisher exact test[15] was used to show significance in clerical error reduction of CD2+, CD4+, and CD45+ values by comparing errors in the preintervention period with those in the intervention period. A chi-square test[15] was used to show significance of out-of-range values during specific time intervals using the CD2+ + CD20+ = 95% ± 5% equation.

Results

Clerical Error Detection

The delta check found 7 patients during the preintervention period with values increasing or decreasing more than 30% from a previous result, and the result subsequently returned to its relative baseline level within a short period. The use of CD4+ T-cell delta checks marginally reduced the preintervention period clerical error rate for CD4+ T cells from 3 of 3,297 to 0 of 867 during the intervention period (P = .05) **Table 1**. An example of this type of error is shown in Figure 6, where the value is plotted with previous and subsequent values for the patient. The CD45+ delta check reduced the CD45+ clerical error rate of 4 of 3,297 during the preintervention period to 0 of 867 during the intervention period (P = .58). While the reduction in the number of CD4+ and CD45+ errors using the FCW was not statistically significant, any error warrants attention since clinical decisions could be based on a single erroneous result.

The CD2+ + CD20+ = 100% ± 5% and CD2+ + CD20+ = 95% ± 5% equations applied iteratively to the preintervention period data set showed 34 clerical errors (Table 1). Incorrect data entry led to 27 CD2+ errors (Table 1). No CD2+ errors occurred during the intervention period. The reduction in the number of reported CD2+ errors using the FCW is statistically significant (P = .003).

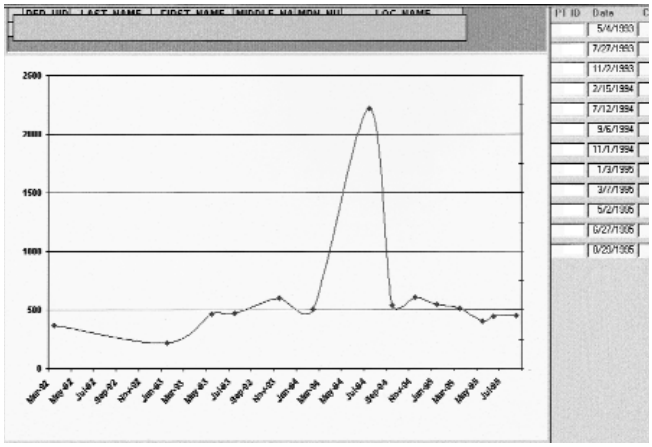

Figure 6 Longitudinal plotting of monoclonal antibodies is an additional quality-check mechanism used to validate data before release of the report. One of the 3 incorrect CD4+ T-cell values found while using the flow cytometry workstation during the preintervention period is plotted with previous and subsequent values demonstrating the usefulness of longitudinal plots and the storage of data using a relational database system. PT ID, patient identification number.

Table 1
Reduction of Clerical Errors During the Use of the Flow Cytometry Workstation (Intervention)

Quality Assurance Checks	Monoclonal Antibodies	No. of Clerical Errors: Preintervention	No. of Clerical Errors: Intervention
Delta checks	CD4	3	0
	CD45	4	0
CD2+ + CD20+ = 95% ± 5%	CD2	27	0

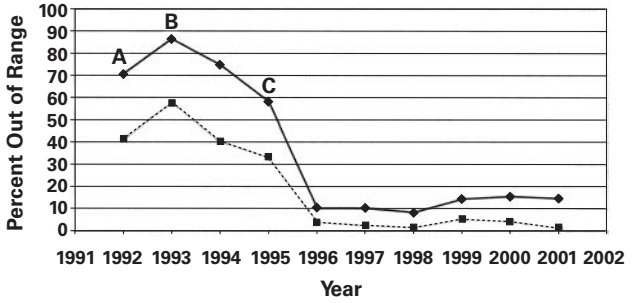

Figure 7 Frequency in out-of-range values using the CD2+ + CD20+ = 100% ± 5% (diamonds) and CD2+ + CD20+ = 95% ± 5% (squares) monoclonal antibody quality assurance equations. Point A shows the time point at which laboratory protocol was changed from cell separation by density gradient to manual whole blood lysis (WBL). Point B shows the time at which the automated WBL preps were used, and point C shows continued use of automated WBL preparations along with the introduction of protease inhibitors as treatment options and viral load testing.

Evaluating Technical Proficiency

After removing errors due to incorrect data entry, preintervention and intervention period data were combined to determine the frequency of out-of-range values using the CD2+ + CD20+ = 100% ± 5% and the CD2+ + CD20+ = 95% ± 5% MAb summation equations. Of all patient samples, 62.43% were in the 100% ± 5% range, while 80.09% of all patient samples were in the 95% ± 5% range. The range of all values was from 33% to 124% with no discrimination made between HIV-seropositive and HIV-seronegative samples. To determine whether the frequency of out-of-range values was time-dependent, out-of-range values were grouped by year **Figure 7**. (Out-of-range values from 1989 to 1991 were excluded from the analysis owing to small samples.) The highest out-of-range frequency was in 1993 (86.81% at 100% ± 5%; 58.11% at 95% ± 5%); a gradual decline occurred in 1995 (58.43% at 100% ± 5%; 33.81% at 95% ± 5%), with a precipitous drop occurring in 1996 (10.14% at 100% ± 5%; 3.89% at 95% ± 5%). From 1996 to 2001, the frequency of out-of-range values remained stable (<15% at 100% ± 5%; <5% at 95% ± 5%).

Similar to values derived using the lymphosum (T + B + NK cell counts),[5] HIV-seronegative specimens should have CD2+ + CD20+ = 100% ± 5%, and HIV-seropositive specimens should have values of 95% ± 5%. The values not within range should be reexamined closely to determine whether a technical or transcribing error occurred.[5] Since the majority of cases received for repeated immunophenotyping are most likely HIV-seropositive specimens, focus was placed on values outside the 95% ± 5% level, where samples less than 90% may have been subject to technical error. The 100% ± 5% range was included as a reference point for comparison.

Similar to the lymphosum, applying the CD2+ + CD20+ equation to the laboratory's combined 8-year caseload showed that 80.21% of samples were in the 95% ± 5% range. Having more than 80% of our laboratory's values at 95% ± 5% is near the cited 85% found in the multicenter quality assurance program for HIV-seropositive patient samples.[5] However, when viewing the data by year, there is great variability in terms of the percentage of cases meeting the expected range (Figure 7).

The steady increase of out-of-range values during the 1992-1993 period at the 95% ± 5% level likely was related to a change in the cell-separation method from density gradient centrifugation (Pharmacia, Piscataway, NJ) to a manual whole blood lysis (WBL) method (AMAC, Marseilles, France) in August 1992 (Figure 7). In 1992, 41.12% of

values were out of range, while in 1993, 58.11% were out of range ($P_1 < .001$). The wide range in values may have been caused by variable mixing and adjusting of solutions at variable time intervals while using the manual WBL method. In 1993, the laboratory switched to an automated WBL method (Q prep, Beckman Coulter) that used set reagent times and automated solution dispensing. The automated WBL method led to greater consistency in sample preparation and reduced the number of out-of-range values to 40.08% in 1994 and 33.81% in 1995. The change in out-of-range values from the 1992-1993 period using the manual WBL protocol compared with those from the 1994-1995 period using the automated WBL method was statistically significant ($P_1 < .001$), as was the change in the number of out-of-range values in 1994 compared with the number in 1995 ($P_1 < .001$). Out-of-range values were lowest (2%) in 1998. The continued use of the automated WBL method helps explain this decrease. However, the introduction of protease inhibitors as a treatment option in August 1995 may have had an influence on reducing the level of result variability.[16] The change in out-of-range values in the 1992-1995 period compared with values in the 1996-2001 period was statistically significant ($P_1 < .001$). After 1996, all values were within acceptable limits, even at the 100% ± 5% level. Changes in the numbers of out-of-range values by individual year from 1996 to 2001 were not statistically significant ($P_5 = .245$; Figure 7).

Discussion

In general, the manner in which data are presented can have a substantial impact on the action taken by clinicians. It is common for laboratory results to be overlooked or their significance underestimated.[17] To improve the level of decision support to both clinicians and laboratory staff, flow cytometry software applications should make more use of graphic longitudinal result plotting for more rapid interpretation of quality assurance and clinical result data. Tabular result formats are the current norm.[17] Graphic displays improve the interpretability of data such as CD4+ counts in which trends often are of more clinical importance than single-point values. Since CD4+ values usually trend up or down over time, unexpected sharp deviations from a developed trend should result in an alert to reexamine values. Before implementation of the FCW, physicians manually paged through medical records to identify previous results and visualize trends, or they asked the laboratory to have a technologist manually find previous values for a patient.[18] Inclusion of this graphic display capability now provides a current historic record of all previous values in a way that helps clinicians to observe trends.

A laboratory information system that produces graphic plots of drug concentrations over time and detects results violating preprogrammed rules has been developed.[19] The FCW also is capable of detecting preprogrammed violations by generating alerts to encourage review of the results. The ability of the FCW to include user-defined delta checks and MAb quality assurance summation equations serves as an untapped resource for improving result quality. Delta checks typically are used to detect specimen labeling and contamination problems; however, they are also useful for detecting transcriptional errors or changes in a patient's condition.

The FCW maintains relationships within the data using a relational database system. Such relationships are not maintainable using the flat-file or spreadsheet formats currently used by the majority of flow cytometric software. In addition to the obvious clinical reporting functions that are improved through the use of a relational database, other potential advantages include computational intelligence approaches to data mining, development of teaching cases, "expert" assistance using anonymous patient data, and development of innovative methods of quality control and quality assurance. Based on the inherent flexibility of the FCW in defining any type of data element, the incorporation of additional parameters and programmatic rules could assist in the evaluation of malignant neoplasms. These include rules that can use fluorescence intensity and antigen density such as bright CD20+ in hairy cell leukemia, HLA-DR in prolymphocytic leukemia, dim CD5+ and CD45+ in B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma, CD19+/CD20+ in B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma, and multiple CD19+/CD45+ peaks in resurgent bone marrow hyperplasia.[20,21] We currently are programming the FCW to cross-check previous immunophenotypic results for comparison of leukemia and lymphoma samples. For example, a switch in immunoglobulin light chain expression on a recurrent lymphoma is improbable and should result in reexamination. Similarly, a change in expression of selected MAbs in acute or chronic leukemias elicits a warning message. We also are exploring ways to link the FCW report parameters and results to public databases such as PROW (Protein Reviews on the Web available at http://www.ncbi.nlm.nih.gov/prow) and PubMed (available at http://www4.ncbi.nlm.nih.gov/PubMed).

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Funded in part by grant LM07089-07 from the National Library of Medicine, Bethesda, MD.

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