trained employees to follow the standard operating procedures manual.

Linden and Kaplan\(^4\) emphasized that rapid identification of transfusion errors (especially AHTR) as well as properly identifying the cause of such errors and implementation of corrective actions are critical steps in the reduction of transfusion-associated fatalities. They cited previous work\(^2\) and recommended a number of strategies that may decrease the risk of AHTR. A modified version of these recommendations, including suggestions put forth by Sazama,\(^1\) is provided in [T2].

**Conclusion**

Erroneous transfusion of ABO-incompatible blood is the most prevalent transfusion error and almost always reflects a preventable breakdown in transfusion protocol and standard operating procedure. These errors can have disastrous outcomes, accounting for significant iatrogenic morbidity and mortality. While most transfusion errors occur at the patient’s bedside and thus are remote from the blood bank and the immediate oversight of the transfusion service director, the hospital-wide implementation and enforcement of transfusion policies can help minimize the risk and occurrence of transfusion errors.

striking the cells, the light from either source passes through focusing and shaping optics to form an elliptical beam with a broad, maximal intensity in the center. The beam then passes through the center of a flow chamber, which is a quartz cuvette with an approximately 200-µm inner diameter. The specimen is also injected through the middle of this flow chamber, where the laser beam strikes the cell [F2].

When the laser strikes the cell, light is diffracted around the edges of the cell, producing a diffraction pattern along the path of the laser beam. This scattered light is approximately equivalent to the cell circumference and is the same wavelength as the exciting laser light (usually blue). Termed forward scatter (FSc) or forward angle light scatter (FALS), it is collected along the same axis as the laser beam. Because this is a very strong signal, a simple photodiode can convert the forward-scattered light into an electrical pulse. However, the direct laser beam could enter this photodiode, giving an overwhelming signal. A physical barrier eliminates the direct laser beam by blocking about a 2-degree angle of light along the path of the laser, allowing only the light diffracted at greater angles to pass.

In addition to the forward-scattered light, blue laser light is also reflected off the cell and off internal structures in the cell. This is termed side scatter (SSc), right-angle light scatter (RALS), or 90-degree light scatter (90LS) because it is detected by a collection objective set at 90 degrees to the incident laser light. It is a measure of granularity of the cell. Granulocytes have a much larger side-scattered light signal than do lymphocytes ([F1]; see also [F5]). After being focused by the objective lenses, the blue light is reflected by a dichroic mirror. These mirrors direct specific wavelengths of light into the correct detector. The detector is a photomultiplier tube, which converts the light signal into an electrical signal.

Fluorescent probes can also be added to the cell for detecting specific molecules in the cell. These probes are typically antibodies to cellular antigens with a covalently attached fluorochrome. The fluorochrome, excited by the laser light, will fluoresce at a longer wavelength. Fluorescein gives a green fluorescence, phycoerythrin (an algal protein) gives orange, and peridinin chlorophyll protein or phycoerythrin coupled to cyanin 5 produces red fluorescence. Some flow cytometers have a second laser that can excite other fluorochromes. Like the side-scattered blue light, all of these fluorescent signals pass through the objective set at 90 degrees to the incident laser light. Additional dichroic mirrors reflect specific wavelengths of light, allowing separation of the signals from each fluorochrome with minimal overlap. In front of each photomultiplier, a final bandpass filter precisely defines the wavelength of light entering that detector.
Fluidics

The flow cytometer has 2 sources of fluid passing through it: a sheath fluid that is usually a buffered saline, and the specimen containing the cells to be analyzed. The specimen forms a core, which is injected into the middle of the sheath fluid, and both pass through the flow chamber \([F3]\). The outer sheath fluid causes hydrodynamic focusing, which compresses the specimen core into a small area. Cells are forced to pass singly through the focal point of the laser, which is crucial for collecting accurate information about each cell in the specimen.

Electronics

The photodiode detectors or photomultiplier tubes first collect the light signal from the cells. In both cases, the photons produce a pulse of electrical current. Photomultiplier tubes have a voltage applied across a series of plates. This increases their sensitivity so that fewer photons are required to produce an electrical current. The initial small current requires amplification. The amplified current ranges from 0 to 10 V depending on the strength of the signal. The voltage signal is an analog signal and cannot be recognized by the computer. It is converted by an analog-to-digital converter into a digital signal, called a channel, which can range from 0 to 1,023 channels. The channel is a counter, and all signals of a specific voltage are counted in a channel corresponding to the strength of the signal.

The amplification of the initial signal can be either linear or logarithmic. Linear amplification provides a direct visual relationship and is useful for scatter signals and fluorescent measurements of DNA. Linear amplification allows a dynamic range of approximately 200- to 1,000-fold. Logarithmic amplification is used for most other biologic signals, including immunofluorescence, owing to the extreme dynamic range of these signals. Logarithmic amplification gives a dynamic range of 2,000- to 10,000-fold.

The fluorescent signals have been discussed above. Although these signals appear mainly as a single color under the microscope, actually the emission from any fluorochrome is broad. Although fluorescein isothiocyanate (FITC) appears apple-green, it also has lower-level emissions in the orange area of the spectrum. Hence, any green FITC-stained cell will also generate a signal in the orange channel, making the cell appear to be labeled by 2 fluorochromes. To allow multicolor fluorescence, this apparent overlap must be eliminated. This is done by fluorescence compensation. A percentage of any bright signal, such as FITC, is subtracted from any other associated signal. The instrument is set up at the beginning of a run with pure signals of each fluorochrome, and any associated fluorescence at the other wavelengths is compensated \([F4]\).

Specimen Analysis

Specimen Preparation

Specimens are often whole blood but may be other tissues. For analysis, the specimen must contain greater than 95% single cells for accurate interpretation of the data. Whole blood is a single cell suspension, but tissues must be disaggregated into single cells before analy-
Data can be displayed on several plots and electronically gated to analyze pure populations. a, Dot plot of lysed whole blood showing forward scatter vs side scatter with a gate surrounding lymphocytes. b, Overlaid histograms of fluorescein fluorescence showing the difference with total and gated population analysis. The positive cells within the markers are 1% of the total particles, including debris, but 89% of the gated lymphocytes. c, Dot plot of fluorescein vs phycoerythrin fluorescence showing quadrant statistics. FL1-H, x-axis, green, fluorescein; FL2-H, y-axis, orange, phycoerythrin.

sis. Often antibodies are used to stain cells and select subpopulations or evaluate protein expression. In most cases, these antibodies are commercial products with robust staining properties. However, the staining patterns should be confirmed in the laboratory on each new lot of antibody using appropriate control materials. Unknown antibodies must be carefully evaluated for specificity and efficacy of staining. This is best done under a microscope initially because nonspecific staining or unusual patterns can be better distinguished with a microscope than with a flow cytometer. Once approximate staining characteristics are determined, the procedure can be fine-tuned for the flow cytometer.

Data Processing

The initial light signal is first converted by the detectors into an electric current, which is amplified and measured as an analog quantity, such as 5 V of a maximum of 10 V. Because this signal is analog, it must be converted into a digital signal for computer processing. The signal then becomes an event with a specific digital channel value such 512 on a 1,024-channel scale. The digital data can be displayed as 1-parameter histograms [F5b] or as 2-parameter plots [F4, F5a, F5c]. The information on a 1-parameter histogram is described either as the percentage of cells within a set of markers or as the mean fluorescence intensity of a population. For logarithmically amplified signals, the geometric mean fluorescence is used, since it is less influenced by the extreme ends of the distribution. A 2-parameter plot is usually divided into 4 quadrants, each containing a percentage of the total population [F5c]. This division is used to distinguish between fluorescent and nonfluorescent cells. It also defines expression and nonexpression of a cell molecule marked by a fluorescent antibody or other fluorochrome.

Because the specimen that is analyzed may be complex with several populations plus debris, such as lysed whole blood, analysis of the total specimen would give variable and confusing results. Therefore, it is often necessary to analyze a single population, such as lymphocytes. Electronic gating allows separation of the total population into parts, which can be individually analyzed [F5a].

Quality Control

There are several kinds of quality controls. First the flow cytometer itself must be evaluated for proper function. This is usually accomplished with standardized fluorescent beads. These give very precise, reproducible patterns, which quickly assess instrument function. In addition, the beads are fairly stable and can be used daily with little change in values. The bead values for instrument settings and signal intensity are used to generate Levy-Jennings plots and determine instrument errors.

A second quality control material is used to set up the appropriate instrument settings for the type of staining used. These can be beads or antibody-stained cells. One of the primary functions of this second control is to establish the compensation settings for the instrument; therefore, either beads or cells should mimic the staining that will be used on actual specimens.

The third level of quality control is a control substance that mimics actual specimens. These controls are available commercially and usually consist of stabilized blood, sometimes with added tissue-culture cells that mimic a specific cancer cell. These controls can be used to assess the sensitivity of the instrument. They will have published ranges for many of the antibodies used in the laboratory. Because they usually contain normal, stabilized blood cells, some proteins characteristic of specific cancer cells will not be present. For this reason, some tissue-culture cells may be included because they express specific proteins that can be used to evaluate other antibodies.
Conclusion

Flow cytometry is used in the clinical laboratory for a variety of analyses including diagnostic immunophenotyping of lymphomas, leukemias, and congenital immunodeficiencies; detection of minimal residual disease in leukemias; monitoring CD4+ T-lymphocyte counts in HIV patients; DNA content analysis for prognosis of malignancies; screening of hematologic disorders such as paroxysmal nocturnal hemoglobinuria and idiopathic thrombocytopenic purpura; evaluation of peripheral stem cell products for transplantation; monitoring monoclonal antibody therapy; and determination of the extent of maternal/fetal bleeding by detection of hemoglobin F.

Flow cytometry remains a labor-intensive area of laboratory medicine requiring extensive training. Learning the fundamental principles of flow cytometry enhances both patient care and work satisfaction. In addition, these principles apply to other areas using cytometry techniques, such as hematology. For more details and theoretical discussion, the reader is referred to several books and Internet sites.