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## TECHNICAL NOTES

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# Integration of a Barcode Reader With a Commercial Flow Cytometer<sup>1</sup>

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This report describes the application and installation of a barcode reader on a standard EPICS Elite flow cytometer. The barcode reader system eliminates keyboard entry of sample information on the cytometer. The system automates the transfer of sample information already present in our laboratory database to the cytometer at run time.

The system uses a standard "off-the-shelf" bar code wand with a personal computer keyboard interface and requires no additional software at run time. No typing of sample information is required by the operator at any stage of normal sample operation at the cytome-

ter. All operations are automatically coded into the cytometry software using the macro functions of the software. Tubes are inserted into the tube reader and sample information is transferred automatically into the cytometer. We have found that the system allows rapid and continuous operation of routine clinical and research samples. This automated data entry also reduces the possibility of data input errors.

**Key terms:** Bar code reader, clinical samples, automated analysis, flow cytometry

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Barcode readers have become more evident on biomedical instruments in recent years. Their major advantages include a reduction of data entry errors, increased sample throughput, protection of patient identity, and reduced possibility of computer keyboard contamination from hazardous specimens. The application of barcode readers can be useful to a clinical or research cytometry laboratory. Quality control within flow laboratories is an issue receiving considerable attention (2-4). One of the biggest problems in busy cytometry laboratories is the accidental mix up of patient samples. Many precautions are implemented in clinical and research laboratories to prevent such occurrences. Still, it is almost inevitable that they occur.

The process of analyzing patient samples requires the transfer of patient information or codes from the sample tube label to the cytometer software environment. A data error entered into the cytometer data acquisition software may go undetected. These errors can be avoided by using barcoded sample tube labels generated from a laboratory database. It is more likely

that correct information will be entered for a sample when the label data are being read optically by a barcode reader.

### SYSTEM DESCRIPTION

Effective management of samples and associated data is critical to the efficient operation of our laboratory. A multiuser, relational database (DataEase International, Trumbull, CT) provides the necessary

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framework to control the flow of sample scheduling, sample preparation, data analysis, report generation, and data archiving. A unique, seven digit tube identification number is issued by the database to each flow cytometry sample which is referenced in worksheets and tube labels generated by the database. This number is entered in the sample name field of the flow cytometer acquisition software when the sample is run and recorded as the \$SAMPLE keyword in any FCS data files. The database uses the \$SAMPLE keyword to properly identify these data files for post-acquisition analysis and archive procedures. Typographical errors made when entering this number on the cytometer create subsequent identification and data management problems.

The barcode reader system provides a simple method to transfer accurately the tube identification number from the database to the cytometer. When a sample is run, the number is entered by scanning the tube label or associated worksheet rather than typing the number on a keyboard. This virtually eliminates the problems of data file identification and management previously discussed. The barcode reader system ensures that essential information stored in the cytometer data file will identically match related information in the laboratory database. The barcode reader system is an integral link in our overall laboratory management system.

## FLOW CYTOMETER AND CYTOMETER SOFTWARE

### Flow Cytometer

An Elite cytometer (Coulter Cytometry, Hialeah, FL) was used for application of the bar code system. However, virtually any commercial cytometer could be used with minor adaptations of the system described.

The Elite software runs on an IBM compatible computer interfaced to the instrument through both a serial data connection and a proprietary Coulter communication interface. The data analysis environment is primarily graphical and window-oriented, with movement throughout the environment accomplished by the use of a mouse which is attached to a second serial communication port. In addition, the software allows the creation of "Hotkey" keystroke macros which can be saved and recalled.

Presently available barcode readers allow the connection of the reader in-line with a computer keyboard. The barcode wand can relay information to a software package as though the input has come from direct keyboard entry. An added feature of the barcode reader is the ability to program *preambles* and *postambles*. A preamble is a user-defined series of ASCII codes which are transmitted prior to the transmission of a scanned barcode. Similarly, a scanned barcode is followed by the user-defined postamble. This provides a convenient means of invoking a keystroke macro prior to the

transmission of barcoded information. After the barcoded information is transmitted at the correct prompt within the user environment, the postamble can be used to terminate the entry (i.e., provide a carriage return) and return the user to the interrupted software operation.

The particular macro used in this application consists of left arrow and up arrow keyhits to bring the user to the upper left corner from the lower right corner of the screen. This is followed by right arrow and down arrow keyhits to highlight the sample information menu selection within the pull-down screen menu. Finally, a carriage return is used to go to that screen. This macro was named and saved to disk. After loading, a CTRL-G activates the loaded macro. Many memory-resident macro programs exist (e.g., SuperKey, Borland International, Scotts Valley, CA) which would provide macro capabilities to those cytometer programs which do not support them.

### Barcode Reader

The barcode reader used in this implementation was a model P10 Keyboard Barcode Reader with a medium resolution wand (Worthington Data Solutions, Santa Cruz, CA). This model uses an interface which couples directly to the synchronously serial personal computer keyboard lines (1) as shown in Figure 1. This interface, called a *keyboard wedge*, allows the direct input of barcoded information into the keyboard input of a computer with no interruption in normal keyboard operation and no additional hardware or software modifications. This reader also allows user programming of preambles and postambles which can include the complete alphanumeric ASCII set, F1 through F10, all ALT and CTRL keys, as well as ESC, CR/LF, and a variety of punctuation symbols.

Barcode reader units are available to interface with many types of computers. The particular reader we used is able to be interfaced with either an IBM compatible or an Apple Macintosh, via a cable change. The barcode wand and keyboard interface are powered by the 5VDC line within the standard keyboard cable. No additional power connections are necessary.

A variety of computer configuration settings are allowed by the programmable barcode reader. These include computer type, keyboard transmission speed, and barcode format (see Table 1). The preamble used consisted of a CTRL-ON, "G", CTRL-OFF, eight NULLs, and a carriage return. The CTRL-ON/G/CTRL-OFF serve to activate the preprogrammed Elite macro to bring the user to the sample information screen. The eight NULLs provide a delay while the macro is executing. If this delay were not present, the sample information would be transmitted before the software requested the data. The carriage return is optional depending upon whether the macro brings the user directly into the sample information input field or just to the field location.

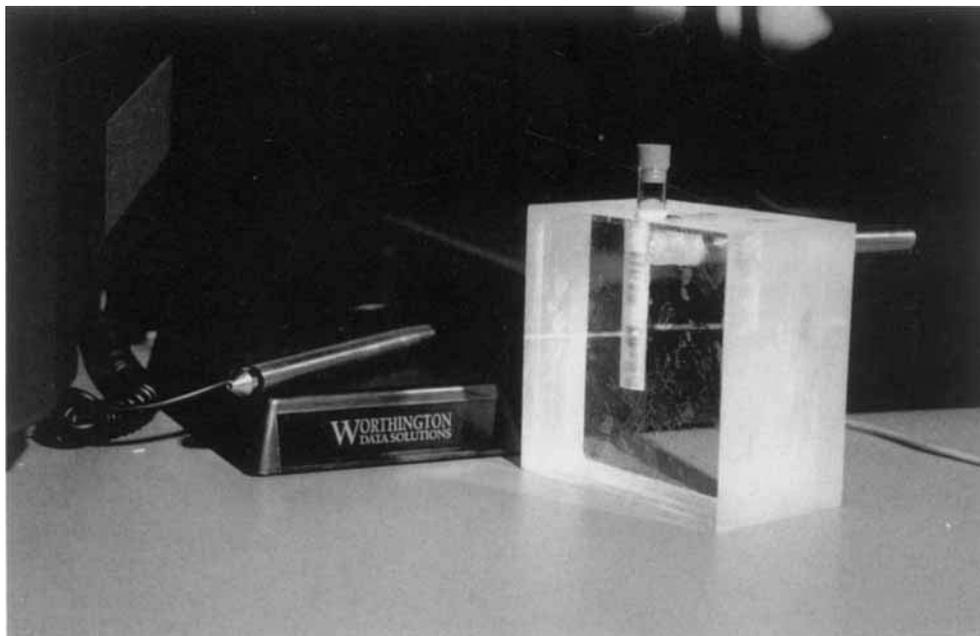


FIG. 1. Shown here are the keyboard wedge and sample tube reader with a labelled tube being inserted into the reader. On the left is the keyboard interface or wedge. The clear plastic cube on the right is the sample tube reader built in our laboratory. Tubes are placed into the center hole of the holder and pushed past the wand which is located at 90° to the tube face. A handheld barcode reader wand is also shown on top of the keyboard interface.

Table 1  
Computer Configuration Settings<sup>a</sup>

Computer interface	Type 7 (clone)
Data transmission timing	Long
Code 3 of 9	Full ASCII mode Accumulate off

<sup>a</sup>The configuration settings used in the programmable keyboard wedge for communication with the Elite cytometer. These configuration settings define the choice of barcode format and computer hardware interfacing specifics.

### SOFTWARE Barcoded Labels

The barcode labels can be generated from almost any wordprocessor or database program using available memory-resident print utilities. These software utilities monitor all data transmissions to the printer and upon detecting defined starting and ending strings of characters, the utilities convert any enclosed data into a barcoded data sequence.

For our implementation, the BarRes utility (Worthington Data Solutions, Santa Cruz, CA) was used. This utility allows barcodes of types Code 39, Interleaved 2 to 5, UPC-A, UPC-E, and Full-ASCII Code 39 to be printed on Epson-compatible dot matrix printers. Additional barcode formats are allowed on the HP LaserJet, which is also supported. The format chosen for our laboratory was Code 39 due to its popularity and compressed representation of ASCII data.

The BarRes utility is loaded as a memory resident TSR before entering the laboratory database. When a new sample identification number is entered, the appropriate sample identification information is sent to a dedicated barcode label printer. With the BarRes software, sending the character string "@@BC 123456@@BE" to the printer produces a barcoded label of the number 123456, when @@BC and @@BE are the respective beginning and ending barcode delimiters.

The standard label at our facility displays the sample identification number in both alphanumeric and barcode formats as shown in Figure 2. Additional patient information could be included in either format. In cases where confidentiality is important, the barcode system provides a simple way of encrypting patient identification information.

### Tube Reader

The tube reader was constructed from Plexiglass by laminating several layers of half inch plate together. Holes were drilled to accommodate the 12 × 75 mm tubes routinely used in this laboratory. Any tube size could be accommodated by using the appropriate diameter hole. A second hole was drilled at 90° for the barcode probe. The barcode probe used was a conventional hand-held barcode wand. More compact barcode probes are available, although they are much more costly. Continued laboratory use has proven this arrangement to be both functional and reliable.



FIG. 2. This figure shows a representative bar code label consisting of the tube number, laboratory database accession number, and the three monoclonal antibodies used for a multiparametric analysis.

### Operation

The barcode labeling system can be used in two distinct manners, both of which have been tested in our laboratory. The first method of automatically entering sample information is by generating the barcoded information labels and placing them directly onto the sample tube. The tube is then placed into the tube reader and immediately removed. When the tube reader detects and reads the barcoded sample information, the preprogrammed preamble places the user in the sample information screen. The barcoded sample information is then transmitted by the barcode system. Finally, the postamble adds a carriage return to enter the data and an ESC to return the user to their previous screen.

A second method of automating the sample information entry procedure is to print the barcoded sample information numbers directly onto the flow cytometer sample run sheet. The identical preambles and postambles perform as before with the only difference being the location of the barcoded information and the need for the operator to handle the barcode wand.

### DISCUSSION

Several important factors must be considered when implementing a barcode system, including the type of barcode reader to be used and the placement of the barcode labels. Barcode readers are available as keyboard input, serial transmission, or PC bus installed devices. Often the serial and bus interfaces require additional address space and reserved processor interrupts which may not be available on the cytometer computer. It is important to ensure that the type of interface selected is compatible with the configuration of the cytometer computer system. We chose the keyboard interface to avoid these problems.

The two barcode labelling methods previously outlined were the placement of the label directly on the tube or on the sample set run sheet. These two methods provide distinct advantages and disadvantages. The label-on-tube method provides a means of entering sample data at run time with the identifier attached directly to the sample tube. It does require the construction of a sample tube reader as described. The tube reader allows single-handed label scanning as the

tube is being placed on the instrument. A disadvantage of this approach is that the small size of the test tube limits the amount of information which can be encoded on the label. Label placement is important when working with assays involving the continual immersion of the samples in water. When assays involve incubating tubes in water baths, no label degradation occurs if the label is placed a little higher on the tube. There are also commercially available printer inks and labels which are water resistant.

Using the second method, the instrument operator scans tube information printed on the run sheet. A large amount of sample information can be encoded due to the space available on the run sheet. This method does, unfortunately, separate the tube from the barcode label, which still allows for a mix up of tubes.

A problem encountered with our present integration involved the manner in which the Elite data analysis software handles keyboard events. The barcode reader system with keyboard interface was designed to provide barcode input through the keyboard port as if the data had been typed at the keyboard. The fact that the data originated from the barcode reader rather than the keyboard should not be detectable by any software package. Our experience has shown that the Elite software can "detect" the difference when the amount of barcoded data exceeds ten characters. The result is the reception of a random portion of the barcode label on the first scan and a completely accurate reception on the second scan. As a solution, a barcoded sequence was created that invoked the macro separate from the barcode label with the tube number. Included in this barcode were DEL characters which deleted the spurious input. The scan of the sample number then proceeded without error. These two barcoded sequences are placed on the run sheet. Tests with a number of other software packages showed normal operation with the barcode system. Our method operates well with the Elite software.

### CONCLUSIONS

The application of a barcode reader to routine flow cytometry provides a particularly useful option. There are several direct benefits to the user. It reduces errors for sample input into the cytometer. It increases the speed of running a series of tubes because no typing operations are performed during sample run. It creates a more uniform sample analysis procedure because tubes are moved from the rack to the barcode reader to the cytometer in a smooth operation. It also requires exactly the same period of time to perform this operation regardless of the amount of information encoded about the sample, creating a uniform pace. Because of the versatility of the system as implemented in our laboratory, several different functions can be performed with the barcode system. We believe that implementation of this simple system has provided a most effective and inexpensive addition to a commercial instrument. Addition to image cytometers or other re-

lated instrumentation would be possible using the methods presented. Barcodes have provided a major reduction in probability of errors and increased sample throughput at our facility.

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