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<th>GROUP</th>
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UNCLASSIFIED
COLLECTION AND ANALYSIS OF COMPLEX ELISA DATA USING A MICROCOMPUTER

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Immunobiology and Transplantation Department
Naval Medical Research Institute, Bethesda, MD 20814-5055, U.S.A.

ABSTRACT

A system for automatic Enzyme-Linked Immunosorbent Assay (ELISA) data collection and processing is described. Hardware for the system consists of a Dynatech microplate reader and Apple II series microcomputer. Software developed by the authors allows automatic configuration of the reader and direct data input from the microplate reader to the microcomputer. After optical density data is input through the interface, it can be printed out as either optical density values, or as scaled values (from 0 to 9) in a color-coded matrix. Data can be stored and later recalled in either format, allowing several sets of data to be merged into one large, color-coded chart. Results from several different microplates can be combined and sorted in a single printout to expedite interpretation of data. (KEY WORDS: ELISA, microcomputer, Apple II, monoclonal antibodies)

INTRODUCTION

The Enzyme-Linked Immunosorbent Assay (ELISA) provides a convenient method for rapid quantitative immunoassay. Several microcomputer programs for microELISA data analysis have been described which allow automated curve fitting of data from competition ELISAs, computer normalization of data, and graphic representation of optical density (1-9). However, such software is not ideal for rapid comparison and analysis of large amounts of data, such as data collected in the process...
of screening a hybridoma fusion for production of appropriate monoclonal antibodies. A program for Apple II computers is described which reads data from a microplate reader, prints data in its original form and as a color-coded scaled matrix, and saves the data to disk. The program allows combining multiple data sets into a single color-coded printout, in order to facilitate analyzing the results from large numbers of assays.

MATERIALS AND METHODS

The program will function identically using either an Apple IIe or an Apple IIe (Apple Computer, Cupertino CA) with two disk drives. A standard parallel printer interface is required. An Apple super serial card interfaces the computer to the RS-232 port of the MR600 microplate reader (Dynatech, Alexandria VA) with the serial card switches set as shown (Table 1). A clock card is optional but useful for automatic file dating using David-DOS II (David Data, Los Angeles CA). For printing assay results, the Epson JX-80 color printer was used (Epson America, Torrance CA). If color-coded output is not desired, any Epson-compatible dot matrix printer (with 12 cpi printing capability) will work with no software modification.

The main program is written in Applesoft BASIC. A short input/output (I/O) subroutine in machine language was provided by Dynatech, along with accompanying BASIC subroutines which allow placing all functions of the ELISA reader under software control. The Dynatech BASIC routines facilitate setting the MR600 filters, blanking on a specified well, transferring the optical density (OD) for all 96 wells in the microplate to the computer's memory, and calculation of a 0-9 scaled matrix from the OD data. The scaled values in the matrix are calculated...
TABLE 1

DIP Switch Settings for Serial Interface Card

<table>
<thead>
<tr>
<th>BANK #</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
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<td>1</td>
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<td>OFF</td>
<td>ON</td>
<td>OFF</td>
<td>OFF</td>
<td>OFF</td>
</tr>
</tbody>
</table>

using the following formula for each OD value, where \( L \) = lower OD limit, \( U \) = upper OD limit, \( INT \) = the integer function (in BASIC), and \( OD \) = optical density:

\[
\text{Scaled value} = \text{INT}(10 \times (\text{OD}-L)/(U-L))
\]

RESULTS

The software developed by the authors provides the functions listed in Table 2. After booting the ELISA program disk, the user may select to run a configuration program. The hardware configuration (slot numbers of each interface card) and the microplate reader default settings are stored in a disk file and may be viewed or changed using this program. As the configuration rarely needs to be changed, the main ELISA program is usually entered directly from the boot-up menu. The user is instructed to insert a data disk in drive 2 and to turn on the microplate reader. Then, the program verifies proper clock-card installation and establishes communication with the microplate reader.
TABLE 2
Functions Provided by the ELISA Data Processing Program

- Assign labels to rows and columns of a plate
- Choose plate reader filter combinations (default provided)
- Choose well for blanking plate reader (default provided)
- Create a color-coded 0-9 matrix of scaled OD values for each plate
- Merge data from up to 3 plates into one color-coded 8X12 matrix
- Merge data from up to 25 plates into a color coded chart organized by well label
- Extended file directory system
- Preset sequences of commonly used functions are provided as defaults

Next, the main menu is displayed. The user can choose to read optical density (OD) data from the microplate reader, or to use previously stored OD data from a disk file. After input of OD data (from the microplate reader or from a file), the program calculates a 0-9 scaled range matrix, prints both the original OD data and the scaled data, and saves the data to disk for archival storage.

The program allows the user to label rows and columns of a 96 well plate, including the option of using those labels entered for the last plate analyzed. Microplate reader default settings are also displayed and the user is given the opportunity to change reference or sample filters and/or the well that the reader will use as the blank. When the user responds that the settings are correct, the computer signals the reader to begin reading, and receives the OD data through the serial interface.
The OD data is automatically printed out in an 8 by 12 matrix that matches the plate (Figure 1). Then, the operator has the option of scaling the data by entering lower and upper OD limits, which are used by the computer to calculate a value between 0 and 9 for each OD value. A "*" or "-" is assigned if the original OD is outside the upper and lower limits. The 0-9 scaled matrix can then be printed (Figure 2). Colors are used in the printout to group values into four ranges (Table 3). This facilitates identification of wells in a certain optical density range during visual inspection of the data. Finally, the original data with the labels and the 0-9 matrix may be saved to a data disk. Saving the 0-9 matrices allows later recall and merging of up to 3 matrices in one 8 by 12 printout (Figure 3), or merging of up to 25 matrices in a color-coded chart sorted by well labels (Figure 4). This charting capability is effective for quickly locating an antibody with the desired positive and negative binding pattern to several antigens. Color coding of the results produces a more compact chart for visual inspection than does graphing of such data.

A second main menu option executes the same functions described above, except that previously stored data is used instead of reading new data from the microplate reader. The remaining menu options allow the user to execute specific functions separately, such as viewing the extended directory, or saving plate data already in memory. The extended directory is a file written by the program to each data disk which contains the filenames and descriptions of all ELISA data files stored on that disk. Each new data disk is automatically assigned a unique number, which can be seen as part of the catalog on the disk. These two features allow easy location of previously stored data, even when using multiple data disks.
### Scaled Values (0-9 Matrix)

**08/10/86 17:17**  
**TYPE: IMM 1 STRIPS**  
**PLATE # 2**

**TREATMENT**  
GAM 1/40

**COMMENT**  
EXAMPLE OUTPUT OF ELISA PROGRAM

**RANGE : LOWER = 0  UPPER = .2**

<table>
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<tr>
<th></th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>LOTO</th>
<th>DESCRIPTION</th>
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<td>-</td>
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<td>-</td>
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<td>-</td>
<td>8</td>
<td>-</td>
<td>1</td>
<td>4</td>
<td>12</td>
<td>KGIA</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>5</td>
<td>7</td>
<td>-</td>
<td>2</td>
<td>5</td>
<td>1</td>
<td>344</td>
<td>HPBALL</td>
</tr>
<tr>
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<td>6</td>
<td>-</td>
<td>0</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>-</td>
<td>7</td>
<td>5</td>
<td>1</td>
<td>979</td>
<td>MOLT 3</td>
</tr>
<tr>
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<td>-</td>
<td>3</td>
<td>6</td>
<td>2</td>
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<td>5</td>
<td>-</td>
<td>9</td>
<td>1</td>
<td>974</td>
<td>U937</td>
<td></td>
</tr>
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<td>-</td>
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<td>0</td>
<td>7</td>
<td>9</td>
<td>-</td>
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<td>+</td>
<td>8</td>
<td>-</td>
<td>7</td>
<td>-</td>
<td>52</td>
<td>DAUDI</td>
<td></td>
</tr>
<tr>
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<td>3</td>
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<td>340</td>
<td>K562</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>7</td>
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<td>3</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>3</td>
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<td>J.M.</td>
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**9876343210**

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<th>COL7</th>
<th>1-J-2 M-10</th>
</tr>
</thead>
<tbody>
<tr>
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<td>PBS W/O NO 1</td>
<td>COL8</td>
<td>1-J-2 D-3</td>
</tr>
<tr>
<td>COL3</td>
<td>PBS W/O NO 2</td>
<td>COL9</td>
<td>1-J-2 H-9</td>
</tr>
<tr>
<td>COL4</td>
<td>MY10</td>
<td>COL10</td>
<td>1-J-2 H-10 2</td>
</tr>
<tr>
<td>COL5</td>
<td>L243</td>
<td>COL11</td>
<td>2-J-4 G-23</td>
</tr>
<tr>
<td>COL6</td>
<td>2-J-4 D-2</td>
<td>COL12</td>
<td>1-J-2 D-3</td>
</tr>
</tbody>
</table>

**FIGURE 2.** Example printout of scaled values calculated from the data printed in Figure 1. Printout was made with a non-color printer, but would have color grouping of values if printed with the JX-80 printer. Values less than the lower range value print as "-", while values greater than the upper range value print as "*".
### O.D. DATA

**08/18/86 17:17**

**TREATMENT**

**GAM 1/40**

**COMMENT**

**EXAMPLE OUTPUT OF ELISA PROGRAM**

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>LOT#</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.000 UNDER 0.113</td>
<td>-0.07</td>
<td>0.040 UNDER 0.006 UNDER 0.171</td>
<td>UNDER 0.022</td>
<td>0.098</td>
<td>12</td>
<td>K61A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.036 0.084 0.059</td>
<td>-0.035</td>
<td>-0.047</td>
<td>-0.030 0.105 0.106 0.153</td>
<td>-0.028 0.059</td>
<td>0.104</td>
<td>344</td>
<td>HPBALL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>-0.004 0.121</td>
<td>-0.009</td>
<td>0.017 0.138 0.091 0.051</td>
<td>0.071 0.162</td>
<td>-0.086 0.142</td>
<td>0.106</td>
<td>979</td>
<td>MOLT 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>-0.002 0.087 UNDER</td>
<td>-0.052</td>
<td>0.075 0.122 0.059</td>
<td>0.063 0.110 UNDER</td>
<td>0.194 0.210</td>
<td>874</td>
<td>U937</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>0.095 0.127 OVER</td>
<td>0.210 0.092 0.006</td>
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<td>-0.081 0.152</td>
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<td></td>
</tr>
<tr>
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<td>OVER 0.163 0.209 0.171</td>
<td>-0.058 0.141</td>
<td>-0.010</td>
<td>52</td>
<td>DAUDI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
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<td>0.143 0.072</td>
<td>0.077 0.006</td>
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<td>-0.009 UNDER</td>
<td>348</td>
<td>K562</td>
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</tr>
<tr>
<td>H</td>
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<td>0.020 0.067 0.021</td>
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<td>-0.100 0.078 UNDER</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

**COL1 PBS W/O**

**COL2 PBS W/O NO 1**

**COL3 PBS W/O NO 2**

**COL4 MY10**

**COL5 L243**

**COL6 2-J-4 G-2**

**CDI**

**CDL1 1-J-2 H-10**

**CDL2 1-J-2 D-3**

**CDL3 1-J-2 H-9**

**CDL4 1-J-2 H-10 2**

**CDL5 2-J-4 G-23**

**CDL6 1-J-2 D-3**

---

**FIGURE 1.** Example printout of OD values from an ELISA assay, showing labels of rows and columns of the 96 well plate. OD data that is less than that of the "blank" well prints as *under*. 

---

ROSS AND REYNOLDS
TABLE 3
Color Coding for Scaled OD Values

<table>
<thead>
<tr>
<th>Scaled OD Value</th>
<th>Assigned Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>purple</td>
</tr>
<tr>
<td>0-3</td>
<td>purple</td>
</tr>
<tr>
<td>4-5</td>
<td>green</td>
</tr>
<tr>
<td>6-7</td>
<td>blue</td>
</tr>
<tr>
<td>8-9</td>
<td>red</td>
</tr>
<tr>
<td>+</td>
<td>red</td>
</tr>
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</table>

DISCUSSION

The software presented in this paper provides a flexible and cost-effective system for the collection and processing of large amounts of ELISA optical density data from a microplate reader, using an inexpensive, general-purpose microcomputer. Although designed principally for analyzing assays of hybridoma supernatants in the process of producing monoclonal antibodies, the program will be useful for other types of Enzyme-linked Immunosorbent Assay (ELISA). The system automates collection and comparison of large amounts of data to a higher degree than previously described methods (1-9). Using an Apple II computer interfaced to the Dynatech MR600, filter setting, blanking and reading are all under software control, and default settings are stored on disk. Although the software was developed for a Dynatech MR600 reader, it should be adaptable to other ELISA readers. However, complete software control of reader functions may not be possible with some ELISA readers.
**SCALED VALUES (0-9 MATRIX)**

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<th>LOWER RANGE</th>
<th>UPPER RANGE</th>
</tr>
</thead>
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<td>.05</td>
<td>.15</td>
</tr>
<tr>
<td>2. NEINB</td>
<td>.01</td>
<td>.1</td>
</tr>
<tr>
<td>3. NEINC</td>
<td>.05</td>
<td>.15</td>
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</table>

<table>
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</tbody>
</table>

**FIGURE 3.** Combination of scaled values from 3 "replica" assay plates into a single matrix. As in Figure 2, values would show color grouping if printed with the JX-80 printer.
### FIGURE 4

Chart assembled by the program for 7 different microplate assays. Only a portion of the total chart is shown. The program sorts the well labels so that duplicates are displayed together, regardless of their position in the assay plate. If printed with the JX-80 printer, scaled values would be color-coded. Each column of data shown is from a single 96 well plate. Upper and lower limits used to calculate scaled values for each plate are printed at the bottom of the data column.

The ELISA Data Processing program achieves a balance between user friendliness and flexibility (10) by allowing execution of either sequences of functions in a preset order, or of each function separately from the main menu. The data merging features of the software facilitates compiling large amounts of ELISA data into a single printout for analysis. We have found this approach useful for analyzing antibody binding on many different cell lines when screening hybridoma fusions for desired...
COMPLEX ELISA DATA

monoclonal antibodies. The charting program allows quick analysis of binding patterns for multiple antibodies to large panels of cell lines or other antigens. Also, the ELISA program facilitates determination of the stability of hybridoma antibody production by collecting the results of multiple assays of the same clone (or subclone) into a color-coded chart. The charting programs are especially useful for any type of experiment where multiple plates are used in a "replica plate" configuration, allowing comparison between the same positions of each plate.

Interfacing a microplate reader to a microcomputer offers the following advantages: 1) the computer can be used for analysis or storage of a wide variety of data types and 2) the microcomputer stores the data in machine-readable form, allowing recall and further analysis or output. Also, calculations employing large quantities of data can be performed rapidly. By interfacing the computer directly to the instrument, data can be collected and analyzed by the computer without time-consuming (and error-prone) keyboard entry. Use of a general-purpose microcomputer for such instrument interfacing offers a significant advantage over dedicated instrument monitor/recorders, as the microcomputer can be used for other tasks in the laboratory. The Apple II computer is useful in word processing (11), statistical analysis (12), database management (13,14) and several types of laboratory instruments have been successfully interfaced to Apple II computers (15-17). As the software described here was developed in BASIC, it can be adapted to other laboratory microcomputers, such as the IBM-PC or compatibles.

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Note: A listing of the source code for the software described here will be provided on request. The software is available on disk by sending 2 blank disks and a self-addressed, stamped envelope. Address requests for reprints and source code to:

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REFERENCES


