8-Class Type Tallying Device and Counter

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An electronic device using transistor-transistor-logic integrated circuits as primary building blocks was designed and constructed to accumulate counts for eight class types. Selection of the class types is provided for by three encoders, X, Y, and Z. Using these encoders allows selection of memory counters for accumulations of 0 to 99 counts per class type. Making X, Y, and Z the input equivalents of three genes permitted direct tallying and accumulation of totals of individual phenotypes for recombinant progeny containing three scorable genes. The use of the device as a simple one-of-four colony counter is described. Use of the instrument for obtaining counts of multiply characterized colonies was possible. In all tested instances, tedious and the labor of obtaining differential counts was reduced by more than fourfold with a great increase in accuracy of count accumulations. Complete information is provided for the fabrication of the instrument for use in individual laboratories.

The fundamental treatise of Malmstadt and Enke (1) on digital electronic circuits in use and in design of laboratory instrumentation has been available for several years. With the advent of inexpensive and readily available transistor-transistor-logic (TTL) modules in the form of discrete integrated circuit (IC) packages, relatively straightforward fabrication of specialized microbiological instruments should be on the increase. Generally, however, the microbiologist does not appear to have taken advantage of these technical innovations. Perhaps describing instrumental uses of TTL IC in electronics journals, rather than in journals devoted to microbiology, has accounted for limited use of TTL IC by the microbiologist in design of problem solving instruments.

Frequently the microbiologist is confronted with the necessity of determining how many of each kind are in a total population. With increasing emphasis on investigations of mixed populations of microorganisms such as in microbial ecological investigations, differential counts are becoming more important. Differential counts of cells in microscope fields have been done for many years in the clinical laboratory. The microbial, as well as the classical, geneticist has long recognized the problem of obtaining differential counts of progeny from a single mating. The number of different combinations resulting from a cross is dependent upon the number of heterozygous gene pairs of the parental types. If one gene pair is heterozygous (haploid organisms) two phenotypes may be recovered among the recombinant progeny. If n genes are heterozygous, 2^n phenotypes are theoretically recoverable from an individual mating. The task of tallying the variety of phenotypes and the numbers of each phenotype is often time consuming and tedious. By analogy, the problem of counting small and large colonies among a population of colonies is relatively easy. If one adds characterizations for flat or raised colony type to the determination, the numbers of kinds of colonies rises exponentially. Considering whether each of only three characters are present or absent in a single colony among a population leads to the task of determining if a particular colony should be counted with one of eight possible kinds of colonies.

The difficulties of scoring and tallying a variety of characteristics are illustrated above. The "hand" method of recording all recognized classes and tallying each individually is time consuming and error laden when more than one characteristic is considered. Although it is possible to program computers to tally and accumulate results of these tallies, on-line, benchtop computers for tallying purposes are not generally available at low cost. TTL IC and their present availability provides a means for construction of a device to carry out this task. The present report is a circuit and operational description of an electronic tallying and counting device which was constructed for approximately $125 with TTL IC components. These ICs are readily available and have been described in detail (2). The device helps solve a real microbiological counting problem as well as gives some insight to the opportunities for use in the
microbiological laboratory of digital electronic circuits.

MATERIALS AND METHODS

All TTL IC used in the construction of the clastype tally device, including pin-number designations, have been described in detail (2). The fundamental operations of digital electronic devices were extensively illustrated by Malmstadt and Enke (1).

Sources. All materials and discrete components were obtained from Solid State Systems (Columbia, Mo.), PolyPaks, Inc. (Lynnfield, Mass.), and Allied-Radio Shack electronic supply outlets.

Wiring techniques. To keep construction simple, three Vector circuit boards (4.5 by 6 inch; ca. 11 by 15 cm) were used. In order to contain the circuit of Fig. 3, the crowning circuit of Fig. 4, and the multiplexing circuit of Fig. 4, a ca. 0.1-inch (ca. 0.25 cm) hole spacing was used to mount the indicators of Fig. 5. The size of the circuit board used in the power supply module of Fig. 2 may vary depending upon the size of the transformer used. The pass transistor (Q1 of Fig. 2) was mounted on a heat sink attached to the outer case of the completed instrument.

All wiring was done from point to point. IC were mounted on Molex pins or on standard IC sockets. The former, although somewhat more difficult to use than the latter, were less expensive on a Molex pin-to-socket basis. All interconnections between completed circuit boards were made point to point and were color coded to allow ease in identifying individual leads when necessary.

Components. Complete lists of components for each circuit board are contained, where necessary, in tables and figure legends. Pin numbers of IC and their interconnections are shown in the figures, where possible, to allow easier construction.

Mounting. Switches SW1, SW2, SW3, SW4 and SW5, controlling the clastype selection circuit, the count pulse circuit, and the error correction, were mounted in a small aluminum box (2 by 2.5 by 3 inch; ca. 5.1 by 7.3 by 7.6 cm). This box and its switches provide a “keyboard” for operation. The scanning rate selector switch (SW6), scanning enable switch (SW7), power supply switch (see Fig. 2), and count reset switch (see Fig. 4) were mounted on the front panel of the completed instrument. For ease in reading, the indicators and their attendant circuitry were also mounted on the front panel. The power supply, control board, memory counter board, and multiplexing board were mounted in an aluminum box 6.5 inches high by 5.5 inches wide by 12 inches long (ca. 17 by 14 by 30 cm). Vertical mounting of the modular circuit boards permitted ease in locating these circuits in the case. The power supply was mounted horizontally at the rear to allow the pass transistor, Q1, to be mounted externally on an appropriate heat sink.

RESULTS AND DISCUSSION

Basic principles. The tallying device was designed to accommodate accumulation of clastypes represented by the presence or absence of three characteristics, labeled X, Y, and Z. When the presence of X, Y, or Z is expressed as the condition “I” and the absence of these characteristics is expressed as the condition “0,” a table can be constructed, presenting all possible states of X, Y, and Z (Table 1). With three characteristics, decimal equivalents of each specific condition of the clastype of these three characteristics can be obtained. Table 1 is an example of binary to decimal coding, and in the reverse direction, decimal to binary coding. If “1” and “0” are considered as switch conditions where “1” represents the closing of a switch and “0” represents the opening of a switch, three switches (X, Y, and Z) can be used specifically to encode for eight clastypes, decimals 0 through 7. An additional switch allows encoding of 16 clastypes, and n switches permits encoding of 2^n clastypes. The direct analogy of switch conditions (1 = +; 0 = −) with gene expression or other characteristics is obvious. Consequently it is possible to obtain a binary coded decimal number for each clastype of interest. Digital circuits, in the form of a large variety of TTL IC, have been devised to manipulate such binary coded decimal numbers (1). In general, such devices are used to code decimal numbers into binary equivalents and to code binary numbers into decimal equivalents.

The tallying device described was designed by appropriate selection of available IC to carry out the necessary manipulations.

Table 1. Binary and decimal equivalents for the presence or absence of the characteristics X, Y, and Z

| Binary equivalent* | Decimal equivalent |
|-------------------|--------------------|
| X     | Y | Z |            |
| 0     | 0 | 0 | 0          |
| 1     | 0 | 0 | 1          |
| 0     | 1 | 0 | 2          |
| 1     | 1 | 0 | 3          |
| 0     | 0 | 1 | 4          |
| 1     | 0 | 1 | 5          |
| 0     | 1 | 1 | 6          |
| 1     | 1 | 1 | 7          |

* 0, Absence of characteristic; 1, presence of characteristic.
through the classtype control, enables activation of the multiplexing system and the indicators. There are three indicators, or readout devices. The leftmost, separated physically from the remaining two, serves to identify the classtype selected by X, Y, and Z positions. The remaining indicators serve to permit determination of the number of counts (0 to 99) which have been accumulated in the classtype memory counter specified. Although it is possible to have direct readouts for each classtype, the cost of the large number of required indicators would be prohibitive. Therefore, one set of indicators was used. This was accomplished by taking the contents of the eight classtype counter memories and multiplexing them such that one set of output lines from the multiplex system goes directly to one set of indicators. The identification of and the contents of each classtype memory counter then are read sequentially on one readout device consisting of only three indicators.

**Power supply.** Figure 2 is a schematic diagram of the power supply used in the device and the individual components and their values are listed in the legend. The power supply makes use of a standard design. A center-tapped transformer, T1, is used in conjunction with rectifying diodes, CR1 and CR2, to produce a pulsating DC voltage from the transformed voltage from an AC power line. This pulsating DC current is filtered with C1 and further AC components of the supply are eliminated with voltage regulator LM335 and pass transistor Q1. Although an LM335 voltage regulator IC can supply more than 500 mA of well-regulated DC current, this current is not sufficient to supply all the power needed by the remaining circuits. Therefore this IC is used to control the current through a pass transistor, Q1. This in turn supplies the higher current requirements of the remaining stages. Q1 is mounted on a heat sink (see Fig. 6) on the case of the completed instrument to ensure that the transistor will not be damaged by excessive heating while supplying approximately 8 W of power to the circuits. Layout for the power supply is not critical. Room should be left to allow air circulation around the components to help dissipate excess heat. A V+ bus and a ground bus should be provided for all other circuit boards. Pin connection from the IC to V+ and ground are presented in the tables and figure legends.

**Control circuit.** Figure 3 presents the circuit and components list for the control circuit of the system. This circuit is mounted on one Vector board (4.5 by 6 inch). IC pin numbers to aid in interconnection of the IC are given in Fig. 3. Three momentary contact, single-pole, single-throw switches, SW2, SW3, and SW4, corresponding to characteristics X, Y, and Z, respectively (see Fig. 1), determine the output state (either "0" or "1") of the dual flip-flop (FF) IC U1 and U2. The closing of any of these switches causes a "1" to appear at Q of the associated FF. If a switch is not depressed, Q of the associated FF is at "0." The Q outputs of these lead to the binary to decimal encoder, U3. An appropriate memory address line, MA0...MA7,
Fig. 3. Circuit diagram of control board. Components are as follows. R1, R2: Resistor, 1.8 kΩ. C1, C2, C3, C4: Capacitor, 0.002 μF. Ceramic disk. C7: Capacitor, 2.2 μF, 10 V. C5, C6: Capacitor, 50 μF, 10 V, electrolytic. SW1: Switch, s.p.d.t., push button. SW2, SW3, SW4, SW5: Switch, s.p.s.t., normally open, momentary contact, push button. SW6: Switch, three pole, rotary wafer. SW7: Switch, s.p.s.t., slide. U1, U2: IC, 7476, dual J-K Master-slave flip-flop. U3: IC, 7422, binary-coded decimal-to-decimal decoder. U5, U9: IC, 7400, quad 2-input NAND gate. U6, U7: IC, 7493, 4-bit binary counter. U8, U10: IC, 7402, quad 2-input NOR gate. U11: IC, 7492, 4-line-to-1-of-10 line decoder.

is selected by the action of SW2, SW3, or SW4. Lines MA0...MA7 are directed to eight pairs of cascaded binary-coded decimal counters serving as memories. The remaining FF of U1, connected to SW1, acts to enable a count pulse to the individually selected line MA0...MA7. Its Q output (pin 15) controls U5 and becomes the fourth line of the 4-line-to-1-of-10 line decoder, U3. When this FF controlled by SW1 is not actuated, none of lines MA0...MA7 can pass a count pulse. When SW1 is actuated it produces a single pulse to the individual line MA0...MA7 selected and controlled by the outputs of FF attached to SW2, 3, and 4. The Q output of U1 serves as a reset mechanism. This output is fed through a time-delay monostable multivibrator, U4, back to the reset inputs of U1 and U2 after the single-count pulse has been delivered. Keying switches 2, 3, or 4 and then keying SW1 automatically keys a single pulse into line MA0...MA7 leading to individual counter memories and resets all Q outputs of U1 and U2 to the "0" state, classtype 0. To prevent errors, an error correction is provided by the switch SW5. Actuating this switch before pressing SW1 automatically clears U1 and U2, without passing a count pulse through the selected line.

Interconnect lines X, Y, and Z (Fig. 3) control the classtype indicator (I1 of Fig. 5). They also control the multiplexing system of Fig. 4. U8 and U9 in combination provide "OR" gates for these lines. A classtype scanning system (U5, U6, and U7; Fig. 3) is disabled when SW7 is in the open position. Therefore, the outputs of U1 and U2 directly control the multiplexing system and I1 through the OR gate. When SW7 is in the closed position, U7 provides a binary output code to control I1 and the multiplexing system. This binary output code is generated by a multivibrator, U5, which pulses at a frequency of ca. 0.5 Hz. The pulses are fed to a binary counter, U6, and three switch-selected positions are offered by SW6 to divide this frequency. U7 converts decimals 0 through 7 into their specific binary coded equivalent. In turn these binary-coded decimals control the multiplex system and I1 at a constant rate. Consequently, while accumulating counts each classtype can be sequentially displayed on the readout devices at a rate determined by the position of SW6. This sequential display allows a readout of the contents of each classtype during counting. Therefore, either automatic scanning or manual control of scanning is accomplished through SW7.

Memory counting circuit. Figure 4 shows the wiring diagram of the memory counters U10 and U11 which accumulate the count pulses from the single line MA8. Pulses into pin 14 of U10 are accumulated and stored in the form of a binary-coded decimal number. For each 10 pulses received by U10, U11 stores one pulse in a binary coded decimal form. Thus U10 accumulates units and U11 accumulates decades. The maximal accumulation of pulses for this cascaded pair of counters is 99 pulses or counts. The outputs of U10 and U11 are wired to the inputs of the multiplexers, U26 to U33. The count reset switch is wired to pin 2 of both U10 and U11. Pin 2 of all other memory counters is also connected to this switch. The switch allows resetting of all memory counters to zero and permits new counts to be accumulated.

Fig. 4. Circuit diagram of complete wiring for memory counter pair U10-U11 and multiplexers U26 through U33. See Tables 2, 3, and 4 for other interconnections.
Except for input and output connections, the remaining counter memory pairs, U14 through U25, are wired like pair U10-U11. Table 2 lists the interconnections from input lines MA0...MA7 from the control board to the respective counter memory pairs of the counter memory board. All 16 decade counters, U10 through U25, are placed on one board. Tables 3 and 4 present the interconnections between the memory counters and the multiplexers contained on another board.

**Multiplexing circuit.** All information in the memory counters is stored in the form of binary-coded decimals. To obtain the stored information, eight lines from each memory counter pair must ultimately reach readout decoders U35 and U36. All output "A" lines (e.g., pin 12 U10 = output A line) from each of the eight units counters must be connected to U26. In a similar manner, the output "B" lines of the units counters must be connected to U27. Figure 4 illustrates the complete wiring of memory pair U10-U11 to the respective multiplexing IC U26 through U33. Table 3 lists interconnections from all of the units counters to the units multiplexers. Table 4 lists interconnections between all of the decades counters to the decades multiplexers. The multiplexers, U26 through U33, are controlled in parallel by the X, Y, and Z lines from the control board. Interconnections between the X, Y, and Z lines of the control board (Fig. 3) and the X, Y, and Z lines of the multiplexers (Fig. 4) must be made. Consequently, the information from the control board X, Y, and Z lines appearing at the multiplexers determines which of the memory counter pairs is selected to pass information through the outputs of multiplexers, U26 through U33. Appropriate color coding of the wires leading from the memory counter board to the multiplex board is mandatory. Should wiring mistakes occur during construction, color coding will help considerably in determining the location of a wiring error. The interconnections between the memory counter board and the multiplexing board is the most complex, albeit symmetrical, wiring necessary. A total of 64 interconnections must be made between these two circuit boards.

**Indicator circuit.** The indicator circuit of Fig. 5 contains the readout devices necessary to obtain the information stored in the memory counters and passed through the multiplexing system and the legend specifies the components. The output lines of U26 through U29, containing the units information, are connected directly to the input lines of U35. This IC "translates" the binary coded decimal into a "seven-segment" readout code. This translation results in the production of arabic numerals by the readout, I3, attached to U35. In a similar manner, the input information from the decades multiplexers, U30 through U33, is translated by U36 for I2. Consequently, I2 and I3 allow readout of the accumulated contents of the memory counters corresponding to class-types 0 through 7. The class-type indicator, I1,
Table 4. Interconnections from decades counters on memory counter board to decades multiplexers on multiplexer board

| From pin no. | To pin no. | U30 | U31 | U32 | U33 |
|--------------|------------|-----|-----|-----|-----|
| U11          | 12         | 4   | 4   | 8   | 11  |
| U13          | 12         | 3   | 3   | 8   | 11  |
| U15          | 12         | 2   | 9   | 8   | 11  |
| U17          | 12         | 1   | 9   | 1   | 11  |
| U19          | 12         | 15  | 9   | 15  | 11  |
| U21          | 14         | 14  | 14  | 14  | 11  |
| U23          | 12         | 13  | 9   | 13  | 11  |
| U25          | 12         | 9   | 12  | 8   | 12  |

* U30-U33. IC no. 74151 8-line-to-1-line data selector/multiplexer. V+ = pin 16; ground = pin 8.

decodes the information presented from lines X, Y, and Z. The X, Y, and Z lines contain the information determining the classtype memory pair selected by the multiplexers for information transfer to readouts I2 and I3. The complete readout, from left to right, is: classtype (0 to 7); number of counts in the classtype (0 to 99). The decoding system for I2 and I3 was devised so that if there are no counts in a classtype, these readouts are blank. This "zero blanking" allows an easier correspondence between the classtype indicator and the count contents when only a few classtypes contain counts. The zero indicating classtype 0 is not blanked.

Instrument operation. Turn on the power supply switch. It should display a number, 0 to 7. Place the reset count switch in the reset position and then return to the count position. Close SW7 and set SW6 to the desired scan rate for automatic scanning. To disable the automatic scan, open switch SW7; in this case the position of SW6 is immaterial. Using the keyboard, press X, Y or, Z (SW2, SW3 or, SW4) for the desired characteristics. If an error is made, press SW5, and reenter X, Y, and Z. Press the count switch, SW1. Continue entering conditions for X, Y and, Z, and after each full entry press the count switch until all classtypes have been tallied. To record the contents of each classtype, allow the indicators to automatically scan through one complete classtype cycle. To obtain new counts, place the reset count switch in the reset position and return it to the count position.

The device can be used as a single counting unit by pressing the count switch. Each count will automatically accumulate in classtype 0. By holding down X, Y or, Z (or any combination) and continually pressing the count switch, counts can be placed in a specific classtype without resetting X, Y, and Z positions.

To read out the contents of the classtypes manually, open SW7. Key in the combination of X, Y, and Z appropriate for the classtype and readout the contents on the indicators. To select a new classtype, press the error switch (automatically returning the display to classtype 0) and key in the address for a new classtype.

General considerations. Using the classtype tally described above results in considerable time savings. Scoring nocardial recombinants for three characteristics (e.g., resistance-sensitivity to three antibiotics) was found to be consistent in replicate trials with the device. It greatly relieves the tedium of phenotype scoring and tallying. Several hundred recombinants may be accurately tallied in approximately one-fourth the time required for conventional

![Circuit diagram for indicating readouts.](http://aem.asm.org/Downloaded from http://aem.asm.org/on March 22, 2020 by guest)
hand tallying methods. When used to count two colony types on one plate, the device was relatively simple to operate and consistently limited error to small amounts in replicate counts. Because of the nature of the coding, four clastypes, "0," "1," "2," and "4" can be counted without considering more complex encoding requiring two or more switch positions. For these clastypes, no switch (except the count switch) or the position of only X or Y or Z is necessary to encode for a total of four clastypes.

Expense and circuit wiring complexity limited design of an instrument to accumulate more than 99 counts per individual clastype or more than three characteristics for encoding. However, the device could be expanded. The addition of one decade counter to each memory counter pair permits tallying of 999 counts. Of course, concomitant with the addition of one decade counter there is the necessity of expanding the readouts by one indicator and expansion of the multiplex system by four IC to achieve the additional decade of counts. To expand the device for an additional clastype encoder switch requires twice the number of memory counters! For reasons such as these, the device was limited in scope. Using other electronic circuitry techniques embodying large scale integrated circuits would allow performing counts on larger numbers of clastypes and permit greater than 99 counts per clastype to be accumulated. However, large scale integrated discrete circuits require multiple power supplies and much more care in assembling modules than the TTL IC circuitry employed here, and costs are also higher.

With some care in layout of the components and patience on the part of the fabricator, this device could be made by most individuals who have had some "kit-building" experience. Certainly, members of electronic engineering departments in industry or academe in concert with the microbiologist could easily fabricate the described instrument. Construction times for each of the circuit modules were not recorded since some design changes were made during the prototype fabrication. The wiring symmetry of the memory counter board and of the multiplexing board reduce wiring time for these circuits. Someone skilled in the art of constructing IC-containing modules could probably build such an instrument in 6 to 8 h. This must be considered as a very rough estimate since construction skills and availability of appropriate tools can be expected to vary considerably from laboratory to laboratory.

Two features of the device are not readily apparent. It is possible to obtain the needed binary-coded decimal output and the triggering pulses necessary to drive a digital printer directly from the instrument. The multiplex outputs of U26 through U33 are appropriately coded for binary-coded decimal input digital printers. The automatic scanning circuit is readily modified to trigger such a printing device. With such modifications, clastype identification and contents can be automatically printed if desired. A second feature of the device allowing further automation is inherent in the inputs to U1 and U2. Not only can these inputs be set by switches (Fig. 3), but optoelectronic devices or other transducers can be used in place of these switches. Consequently, modifying the inputs and outputs of the device would permit completely automatic gathering and print out of data.

Digital instrumentation is going to become more and more a part of the future of microbiology laboratories. Whereas this report describes an instrument offering a specific solution to some counting problems peculiar to the microbiologist and geneticist, I hope it also serves to kindle the imagination of the microbiologist. With relatively little knowledge of electronics, common sense, and the use of TTL IC digital circuits like those employed in the described device, the microbiologist should have little difficulty in designing and fabricating instruments needed to solve own one specific laboratory problem at fractions of the costs of extant, commercially available instruments. If, indeed, the needed instrument actually exists!

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