A simple high-speed recording colorimeter system¹

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The rapid and continuous increase in microbiological assays requested of the routine clinical laboratory has created physical problems by the sheer number of tubes involved. One of the major difficulties is associated with the reading of large numbers of cultures with conventional instruments.

In those assays where it is the rate of growth that is being measured, eg, *Lactobacillus casei* folate assays, standards and tests should theoretically be measured simultaneously. Therefore, in practice, all the cultures to be compared with one set of standards should be read in the shortest possible time. With commercial colorimeters we have found that to read a batch of 500 cultures took two technicians approximately two hours. The method was very prone to copying errors which were not easily determined and the arithmetical processing of the figures was both time consuming and tiring.

In order to alleviate these problems we have devised a simple system which enables large numbers of repetitive readings to be made rapidly with consistent accuracy. Used in conjunction with a vortex mixer over 500 tubes an hour can be read by one person. A foot switch is used to control the flow of samples and the recording of colorimeter readings, leaving both hands free to mix specimens and move the probe. The system (Fig. 1) is made more versatile by using flow cells of different light-path lengths, eg, a 2 mm cell enabled six-day *Euglena gracilis* vitamin B₁₂ assays to be read undiluted. A simple base-board and graticule chart reader enables results to be calculated rapidly.

Apparatus

COLORIMETERS

Bausch and Lomb Spectronic 20 regulated model. EEL Spectra.

FLOW CELLS

Hellma Type 178

RECORDER

Heathbuilt EUW-20A (with Platignum Penline pen).

¹Part of this apparatus is covered by a provisional patent application.

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Fig. 1 General view of the apparatus in use with the Spectronic 20.

SOLENOID VALVE

Black Cat ref. 1115

AIR PUMP

Charles Austin DYMAX Mk I

PERISTALTIC PUMP

Watson Marlow HR flow inducer

FOOT SWITCH

Herga 554/15C

ELECTRICAL COMPONENTS

Radiospares

General Description

Modifications were made to an EEL Spectra and to a Bausch and Lomb Spectronic 20 colorimeter. The system used is essentially the same for both colorimeters although differences in their circuitry necessitate some differences in the control box described below. The basic circuit could be modified for use with many other colorimeter models.

A sample of approximately 1 ml is picked up by the probe (Fig. 2) and sucked through into the flow cell. Operating the foot switch stops the flow and switches the colorimeter output to the recorder. The probe is then moved to the next

Fig. 2 The control box connected to the EEL Spectra illustrating the flow cell in position and the added jack plug connexion to the Spectra. Also shown are a flow cell unit and block for accommodating it in the Spectra.

Fig. 3 Circuit diagram. As illustrated the switch *S1* is set for continuous flow.

- a The EEL Spectra switching circuit
- b The Spectronic 20 switching circuit
- c The modification to the EEL Spectra internal circuit.

sample. On releasing the foot switch the next specimen is sucked into the cell, washing the previous one out into the waste reservoir. While specimens are moving through the flow cell an external reference voltage is applied to the recorder to return the pen to a position equivalent to zero optical density. This prevents the passage of air bubbles through the flow cell between specimens being recorded, and defines each peak relative to the blank solution.

Flow System

An air pump reduces the air pressure in a waste reservoir as shown diagrammatically on the circuit diagram (Fig. 3), and a solenoid valve controls the flow of liquid from a 1 mm diameter steel probe through the flow cell into the waste reservoir. The end of the probe is kept off the bottom of the test tube by a rubber cap liner which rests on the top of the tube. The probe is connected to the optical cell with 1 mm thin-walled silicon rubber pump tubing and thence to the valve with 1/16 in. (id) transmission tubing.

The circuit shown allows the suction line to be normally open or closed, depending upon the position of the flow valve biasing switch (*S1*) with the state reversed whilst the foot switch (*S2*) is operated. In routine use we find it more convenient to have a continuous flow, ie, *S1* switched as in Fig. 3, and to stop temporarily the flow after sucking sufficient material into the cell until the recorder has reached the null point.

A peristaltic pump running at 100 rpm with 3/16 in. pump tubing can be used as an alternative means of generating the flow. This is connected between the solenoid valve and the waste reservoir. By wiring the pump to a socket on the control box it is switched on and off by the foot switch. Without the solenoid valve, flow control is imprecise because of overrun by the pump.

Main advantages with this more expensive pump are much less noise and a higher flow rate.

Optical Cell

We use flow cells of various light path lengths (10 mm and 2 mm for routine microbiological assay work), but all have standard 12.5 mm cross section. A simple cell carrier was formed from thin steel strip so that it fitted the standard Spectronic 20 sample holder with spring clips removed. The position of the light path was determined (so that cells with a masked aperture could be used) by wrapping bromide paper around

a cell mounted in the cell carrier. The paper was exposed by switching on the instrument for five seconds. In the Spectronic 20 with our cell carriers the light path was 17 mm above the bottom of the cell.

A block (Fig. 2) to take the same cell carriers was made for the EEL Spectra and replaced the existing one. We have made these by casting Tetrabond filler resin (blackened by adding a small amount of activated charcoal) and also by laminating black perspex. A suitable slot accommodated the cell carrier which was retained by a metal plate. The blocks were designed so that the apertures in the cells we had obtained for the Spectronic 20 came into the centre of the Spectra light path. The cells can be changed through the hole in the instrument cover where the original flow cell had been. These cells have worked entirely satisfactorily in both colorimeters.

Circuit Notes

The circuit shown is of a control box designed to operate with either of the colorimeters discussed, the appropriate circuit being selected by the five-pole, three-way switch (S3-S7). In practice the control box would be used with only one colorimeter and a suitable two-way switch would suffice. For convenience the two circuits are shown separately although many parts are common to both.

Mains Switching

It is essential that adequate screening is incorporated to prevent induction in the recorder input from mains equipment. The circuit was therefore built into an aluminium box with the mains side contained in a smaller aluminium box inside. Simultaneous switching of the mains-operated solenoid valve (and the 2 amp socket) and of the colorimeter output or reference voltage to the recorder is achieved by using two single-pole changeover relays. Initial work with a two-pole, two-way switch proved inadequate due to a leakage from the mains side causing deflection of the recorder pen. Further problems associated with induction in long leads to a foot switch, despite adequate screening and insulation of the micro-switches, led to the adoption of the present system. By using two relays the recorder input circuit was physically separated from the mains equipment and the intermittent current being carried by the leads to the foot switch was minimized.

The neon indicator is illuminated when the flow is held and the colorimeter output is being recorded. This has proved invaluable to people learning to use the system.

Reference Voltage

Both of the instruments modified in this work have electrical outputs which are proportional to 'percent transmission' and are therefore maximal with a blank solution. As increased optical density results in a lower voltage output, a reference voltage equal to the colorimeter output at 100% T is switched to the recorder between specimens so that the peak height increases with increased optical density. This voltage is obtained from a potential divider circuit powered by a 1½ volt U2 battery and is adjustable from 5 to 30 mV.

Back-off Voltage

The output from the EEL Spectra is derived from a selenium barrier layer cell and is therefore electrically zero in the dark requiring no back-off voltage. The Bausch and Lomb Spectronic 20 output is amplified from a phototube which passes a small current when not illuminated. In order to present a zero voltage to the recorder when no light is falling onto the phototube a back-off voltage is produced by a Wheatstone bridge circuit (as produced by Bausch and Lomb in their Spectro chart 25) and powered by a 1½ volt U2 battery shown in Fig. 3b (zero match).

Selector Switch

In the 'meter' position the colorimeter meters are switched into use for calibrating and checking the system, or to allow use of the colorimeters without the recorder. In this position also the recorder input is shorted for zeroing the recorder pen and the internal batteries in the control box are disconnected. When the appropriate colorimeter position is selected the output or reference voltage is applied to the recorder depending on the position of relay 2.

Colorimeter Connexions

The Spectronic 20 was connected to the control box with a Bausch and Lomb accessory cord with a plug fitting the socket underneath the instrument. The line socket was removed and a suitable five-pin plug substituted.

The Spectra was connected with screened twin lead. A closed three-pole jack socket was fitted to the colorimeter as shown on the circuit diagram (Fig. 3C). By means of the white/orange connexion the meter on the colorimeter could be switched into use or damped by earthing at switch S7 to prevent damage.

Recorder Connexions

It was found essential to connect the control box to the recorder with braided co-axial lead. Lapped screening proved inadequate.

Calibration

SPECTRA

The meter on the Spectra is adjusted to infinity by turning the 'adjust zero' control fully clockwise. The selector switch is set to Spectra, the flow held, and the recorder pen adjusted to zero with the recorder zeroing control. A blank solution is sucked into the flow cell and the Spectra meter set to zero with the 'adjust zero' control. After switching the output to the recorder, the pen is adjusted to give fullscale deflection with the recorder range and calibration controls

(Spectra fsd is approximately 15 mV). The 'reference voltage' control is used to adjust the pen to full-scale deflection with the flow sucking to provide a simple check with the blank solution for drift by the colorimeter or recorder during use.

SPECTRONIC 20

The recorder is first zeroed with the input shorted by setting the selector switch to the 'meter' position. The flow cell is removed from the colorimeter and the meter adjusted to infinity. The pen is adjusted to zero with the coarse and fine 'zero match' controls. After replacing the flow cell, zero and the reference voltage are adjusted in the same way as described for the Spectra. The Spectronic 20 meter gives full-scale deflection with approximately 25 mV.

We wish to thank Mr P. M. Langham for the illustrations.

Obituary

honorary consultant pathologist to the Royal Free Group of Hospitals.

After qualifying in 1936 at the London School of Medicine for Women and holding several house appointments at the Royal Free Hospital, she became A. M. Bird scholar in pathology and joined the Emergency Public Health Laboratory Service at the beginning of the war. In 1943 she was appointed lecturer in clinical pathology at the Royal Free Hospital, and senior lecturer in 1947. In 1952 she spent a year at New York University as the William Marsden travelling professor; she became a reader of the University of London in 1964 and the title of professor was conferred on her early this year.

Since the beginning of her professional life, Nuala's primary interest was in streptococci, and her work with Griffiths, Elliott, and Maxted, and C. M. MacLeod in America fostered this interest. Most of her publications have been on the enzymic activity of B haemolytic streptococci.

She liked to teach and was good at it. Many generations of undergraduates, and those who were lucky enough to work in her department, will remember her sometimes caustic wit, and the wide experience which she brought to her teaching. The 'Manual of practical bacteriology' which she recently published with her colleagues at the Royal Free Hospital will serve as an additional reminder.

Above all, Nuala had a great capacity for enjoyment stemming from deeply held religious convictions. She had wide interests in reading and politics and an appreciation of beautiful things. She had many friends both in and outside her profession and indeed in her last illness she derived great pleasure and comfort from the friends who met each other round her bed.

M.P.J.

Letter to the Editor

Elastic Changes and Carcinoid

Those interested in the recent paper by Anthony and Drury (1970) might care to refer to an earlier study (Kirkland *et al.* 1959) of the classic changes adjacent to invasive carcinoids. This curious reaction in the material studied in Dundee seemed in some places to be the formation of new elastica within stromal tissue without relationship to vessels, almost as if 'some product of the tumour has a directly stimulating effect on elastic tissue'. On the other hand, there is an intense accumulation and thickening of vascular elastica and not least at the internal elastic lamina, a site that would seem unlikely to be affected by diffusion from neoplasia situated alongside the arteries (see Figs 6 and 7 of the 1959 paper). These phenomena defeated our attempts at interpretation, but clearly they merit further study as Anthony and Drury have shown so excellently.

References

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Professor Nuala Crowley

Professor Nuala Crowley died on 27 May after years of debilitating illness. She was Professor of Microbiology at the Royal Free Hospital School of Medicine and