Discussion

The peroxidatic activity of haemoglobin in the presence of hydrogen peroxide catalyses the oxidation of TMB to a coloured product. Haemoglobin peroxidase has been termed a "pseudoperoxidase" because, unlike true peroxidases which exhibit specificity for phenols, erythrocytes contain a peroxidase (erythrocyte glutathione peroxidase) specific for oxidation of reduced glutathione. This causes the in vitro detoxification of hydrogen peroxide. Glutathione peroxidase activity has been found to be associated with a relatively stable, non-dialysable, heat-labile, intracellular component which can be separated from haemoglobin by gel filtration and ammonium sulphate precipitation. The pH optimum of glutathione peroxidase has been shown to be pH 8.0 with negligible activities below pH 6.0.2 Pretreatment of the fixed slides with resorcinol before staining with TMB and hydrogen peroxide inhibits myeloperoxidase staining. This increases specificity of the stain and reduces error in enumeration of erythroid colonies. By omitting counter staining only erythroid colonies and precursors are stained.

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References


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An automated method for recording the Westergren erythrocyte sedimentation rate

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An automated system for recording the Westergren erythrocyte sedimentation rate (ESR) is described. The method was developed to cater for the frequently occurring but small numbers of late specimens which otherwise delay staff after normal working hours.

Principle

The method involves photography of the ESR tests exactly 1 hour after being set up, in such a way that the photographs are easily and rapidly obtainable when required and the results can be easily read.

Equipment

A Polaroid MP-4 camera with Tominon f4-5, 135 mm lens and Copal shutter is used. This camera employs bellows focusing so that close-up work is easily possible.

A base was constructed to hold the camera and ESR rack at a fixed distance apart, which gives optimum framing of the rack on the film. This base is made mainly of perspex and wood. The circular camera base is held in place by and can rotate in a perspex ring. Using a focusing screen, the camera was aligned as required; then a line was engraved on the camera base and perspex ring. The focus was also marked on the bellows guides, and in this way the camera can be set up rapidly without having to re-align or refocus each time (Fig. 1). The film used is Polaroid Land Type 107C. This is a black-and-white film rated at 3000 ASA (36 Din).

The timing device consists of a shutter-activating system, which uses a simple mains-driven timer (ORMON—Type STPNH, 72 min) which switches on an electric motor after a pre-set time. The motor has a large reduction gear box which gives a shaft speed of 1 revolution per minute. This gives adequate torque from a low-power motor to drive a cam which activates the camera shutter.

The electrical control circuit is simple and as foolproof as possible. There are two parts to it: one is the setting up procedure, and the second is the timer and motor operation.

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With the mains switched on the indicator neon is illuminated. The reset button is pressed by the operator, and the motor starts and drives the cam around until a microswitch is operated. This action closes the contact on the microswitch, and the timer is activated. The timer neon is now on and the reset button can be released.

After the pre-set time (1 hour in our case) the timer contacts close, the motor is switched on, and the cam rotates and operates the camera shutter. As the cam rotates further the microswitch is operated and switches off the timer. The circuit is now disabled with only the indicator neon operative until the operator resets the cycle. It is imperative to keep the friction on the cam as small as possible to reduce the risk of malfunction. A circuit diagram is shown in Figure 2.

The ESR equipment used is as supplied by Accu-Tech Ltd. Modifications were made to the rack in order to record, on film, the time at which the tests are set up and the time at which the photograph is taken (this interval of time being 1 hour). Inset into the lower part of the ESR rack are four 0-9 thumbwheel switches which can be manually set to represent the time at which the tests were set up. Above the thumbwheel switches is inset a Citizen quartz analogue watch which will indicate the time at which the photograph was taken. This watch was
selected for the clarity of the figures and hands. It will operate continuously for approximately one year before battery replacement is required (Fig. 3).

LIGHTING FOR PHOTOGRAPHY
The very sensitive emulsion of the film makes photography possible in artificial light. However, whether natural or artificial light is used, it is essential that even and constant illumination falls on the rack. We have found that the standard fluorescent lighting in the laboratory is perfectly adequate for a satisfactory photograph with the camera set at f5·6 and 1/60th second exposure.

Test procedure
Blood samples for ESR tests which arrive at the laboratory during the last working hour of the day are set up as a batch just before the staff leave. Using the Accu-Tech suction pump, stand, and tubes, it will take no more than 30 seconds to set up all the tests, and all other aspects of the test are as recommended in the recent ACP Broadsheet.\(^1\) Immediately the tests have been set up, the timer is activated. The thumbwheel switches are set, and the camera settings are checked. These should include camera alignment, focus, and exposure settings. The photograph is taken after 1 hour and the exposed film remains in the camera until it is convenient to develop it, usually the next morning. At this time the exposure is removed from the camera and developed according to instructions supplied with the film. This will generally take less than 1 minute. After confirming from the photograph that the exposure has taken place at 1 hour, the tests are read. It is useful to arrange the framing of the photograph in such a way that the specimen numbers appear within the photograph (Fig. 3).

The use of a camera and watch in the laboratory, especially after working hours, may present a security problem. In the equipment described, this has been minimised by locking the camera to the base. The watch is attached to the rack by means of a metal block screwed securely in place.

The cost of film is £2·48, and with eight exposures each photograph costs 31p. The rack may contain from one to 10 tests so that the individual cost of the tests is between 31p and 3·1p. It can be seen that running costs are considerably less than the payment of staff who might be required to stay after hours.

The capital cost of the equipment to this department, including camera, watch, rack, base, and timer was about £260.

The above equipment has been in use in this laboratory for some months and has proved reliable as well as providing a convenient way of dealing with these late and time-consuming tests. It may also be of use at times throughout the normal working day.
We thank the staff of the Regional Physics Department for their skilled assistance and co-operation, Dr HB Goodall for his help with the script, and Mr RS Fawkes for Figure 1.

Reference
1 Lewis SM. Erythrocyte sedimentation rate and plasma viscosity. ACP Broadsheet 94, 1980.

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Letter to the Editor

UCH microbiology computer system

After the meeting of the Association of Clinical Pathologists at Reading in 1979, at which Dr Ridgway described the data processing system he and his colleagues subsequently described in the Journal, negotiations were started between Selly Oak Hospital and University College Hospital to transfer the software to the microbiology department at Selly Oak.

The transfer has now been completed and the system is being operated at Selly Oak for about three months. Most of the delay was in getting the necessary finance and in having extra wiring put in, so that the interval implied between spring 1979 and autumn 1980 could have been much less.

The UCH microbiology software had been implemented at SOH on a DEC 11/34 processor with 64K words of memory and dual RLO1 (5 Mbyte) disks. Five visual display units, a label printer, and a character printer complete the hardware necessary for routine operation. As part of the agreement with the West Midlands Regional Health Authority, who funded the scheme, an additional RLO1 disk drive and two modems and GPO lines were also installed to permit shared access by up to five other microbiology laboratories within the Region. User laboratories do not share the UCH laboratory system in a routine sense but make use of subsets of it and other purpose-written programs for limited routine and ad hoc data processing functions.

About the only modification to the UCH software that was required for the Selly Oak users was to alter the hospitals, wards, and staff codes, and we changed the specimen code from a numeric to an alphanumeric one. These modifications to the MUMPS program were completed literally within a few hours. We were given the salary of a programmer for one year and had a haematology technician with an interest in computers seconded to us from another local hospital for this. He made several journeys to London to discuss the project with Mr Batchelor of UCH and then tackled the problems of writing programs based on the UCH system for the 'bureau' users, each of whom has his own particular requirements.

As far as the staff at SOH is concerned, two members of the technical staff took a particular interest in the scheme before it was installed and were able to help the others to 'get to know the ropes'. We had a fortuitous after the hardware and software had been installed to get us into the system by putting together the work of the whole department on the system on 1 July 1980.

Although it would be easy to revert to our old system of processing reports should a major breakdown occur, we decided not to run the two systems in parallel, and the 'into the deep end' approach has worked.

Ultimately, the SOH users will require new programs to be written to cover the statistics that we wish to recover, which are different from those extracted by UCH, but the workings of the two departments are so similar that we have not yet found any major problems.

We were pleasantly surprised at the smoothness of the transfer and are very conscious of the effort made on our behalf by Mr Batchelor of UCH and members of the staff of the Wolfson Research Laboratories at the Queen Elizabeth Hospital, Birmingham, who have acted as advisers and are still giving us support.

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Reference