- Use the correct size of diameter tube in the block, i.e. one that fits the hole exactly.
- Clean the block regularly as recommended by the manufacturer. Never use caustic cleaning materials. If required, an aluminium block can be autoclaved.
- Disconnect the heat block from the electricity supply by removing the wall plug at the end of each day's work and before cleaning the unit.

4.10 Colorimeter

In district laboratories a colorimeter is used to measure haemoglobin and other substances in body fluids which can alter in concentration in disease and during treatment.

Colorimetry

A colorimeter is used to measure the concentration of a substance in a patient's sample by comparing the amount of light it absorbs with that absorbed by a standard preparation that contains a known amount of the substance being tested. A coloured solution of the substance being measured or a coloured derivative of it is produced. Coloured solutions absorb light at given wavelengths in the visible spectrum.

Visible light spectrum

Wavelengths between 400 nm and 700 nm form the visible light band of the electromagnetic spectrum, referred to as the visible light spectrum. Wavelengths of about 700 nm are seen by the eye as red colours while those of progressively shorter wavelengths give rise in turn to the colours orange, yellow, green, blue and finally violet which is produced by short wavelengths of 400 nm.

Wavelengths greater than 700 nm are known as infrared or heatwaves and cannot be seen by the eye while vibrations with wavelengths of less than 400 nm are known as ultraviolet (UV) light and these also cannot be seen by the eye.

Beer-Lambert law

Most colorimetric analytical tests are based on the Beer-Lambert law which states that under the correct conditions the absorbance of a solution when measured at the appropriate wavelength is directly proportional to its concentration and the length of the lightpath through the solution. Using a standard, this law can be applied to measuring the concentration of a substance in an unknown (test) solution by using the formula:

$$\text{Concentration of test} = \frac{\text{Absorbance (A) of test}}{\text{Absorbance (A) of standard}} \times \text{Concentration of standard}$$

Terminology: Formerly the amount of light absorbed by a coloured solution, i.e. absorbance, was referred to as optical density, or OD.

In colorimetric tests, the lightpath is kept constant by using optically matched cuvettes usually of 10 mm lightpath distance or tubes of known lightpath distance. In selecting the correct band of wavelengths to use, both the maximum absorbance and selectivity of the wavelengths for a particular substance need to be considered.

For the Beer-Lambert law to hold true, both the solution being tested and the instrument used to measure the absorbance must meet certain requirements.

Solution requirements: The solution must be the same throughout (homogeneous) and the molecules of which it is composed must not associate or dissociate at the time absorbance is being measured. The substance being measured in the solution should not react with the solvent.

Reagent blanks must be used to correct for any absorption of light by solvents. A reagent blank solution contains all the reagents and chemicals used in the chemical development of the colour but lacks the substance being assayed. Alternatively, a reagent blank may contain all components except a vital reagent.

Instrument requirements: The instrument used in colorimetric tests must show satisfactory accuracy, sensitivity, reproducibility and linearity at the different wavelengths used.

The cuvettes used in the instrument must be optically matched, free from scratches, clean, and of the correct lightpath distance. A 10 mm lightpath cuvette is recommended for the clinical chemistry tests included in this publication.

Note: To determine whether a test method and the colorimeter used to measure a particular substance obey the Beer-Lambert law, it is essential to prepare what is called a calibration graph (see later text).

INSTRUMENTS USED TO READ ABSORBANCE

Absorbance can be measured using a:

- Colorimeter (filter absorption spectrometer, or filter photometer).
- Spectrophotometer (absorption spectrometer).

The main differences between a colorimeter and a spectrophotometer are as follows:

Colorimeter

When using a colorimeter, absorbance can be measured only within certain wavelength ranges with filters being used to obtain the required wavelength range.

Most colorimeters are supplied with colour filters that correspond to the Ilford Spectrum filters No. 600–609 which cover the wavelength ranges within the visible light spectrum, 400–700 nm. These filters have a wavelength band width of 40 nm and are suitable for most of the colorimetric test methods performed in district laboratories (see clinical chemistry section). This is because as Seaton comments in his paper, *Measuring absorbance on a limited budget*:[13] '....The absorption spectra of many of the chromogens used in routine clinical chemistry assays resemble not so much the steep mountain peaks and valleys but the gently undulating sand-dune.

There are of course exceptions but it would be wrong to assume that all assays which instruct, measure the absorbancy at 525 nm, could not equally well be performed by the use of a wide range of wavelengths (such as 450–600 nm) with only marginal if any, loss of accuracy....'

Interference filters

Most filter photometers and some colorimeters are supplied with, or make available as optional accessories, narrow band interference filters (typically 10 nm band width). These are more expensive and sensitive than colour filters and because they are available for reading at wavelengths beyond the visible spectrum (providing the instrument is fitted with a suitable lamp), they can increase the range of tests performed and the number of commercially available test kits that can be used.

Spectrophotometer

When using a spectrophotometer, absorbance can be measured at specific wavelengths. A diffraction grating, prism, or other device is used to disperse white light into a continuous spectrum, enabling wavelengths of monochromatic (one colour) light to be selected.

A spectrophotometer is mainly used when tests require reading at specific wavelengths, e.g. to reduce interference from unwanted chromogens. Compared with a colorimeter, a spectrophotometer is considerably more expensive, usually less rugged, and requires greater technical skill and a stable electricity supply for its reliable performance. Its use is not required in most district laboratories.

Specifications of a colorimeter for district laboratories

- Preferably operating from both AC mains electricity and a DC battery supply.
- Digital or analogue readout of absorbance.
- Wavelength range: 400–700 nm using 40 nm band width colour filters.
- Whenever possible, the colour filters should be glass mounted in a sealed unit inside the colorimeter to avoid them being mislaid or finger marked, fading in bright light, or being damaged from fungal growth.
- Can be used with easily obtainable 10 mm lightpath cuvettes.
- Preferably fitted with a cuvette chamber that holds two cuvettes.
- Capable of reading small sample volumes, to enable semi-micro methods to be used to reduce the cost of tests.
- Easy to use, clean and maintain.

Note: Laboratories carrying out a wide range of clinical chemistry tests using test kits may need to consider using a colorimeter (photometer) fitted with interference filters and capable of providing readouts in both absorbance and concentration units.

WPA C0700D Colorimeter

A colorimeter with the above specifications is the digital readout C0700D Medical Colorimeter shown in Plate 4.19. It is manufactured by Walden Precision Apparatus (WPA). The C0700D colorimeter operates from both mains electricity and a 12 V DC lead-acid battery (leads supplied). All ten Ilford spectrum filters are supplied with the colorimeter (see following text). They are glass sealed and mounted on a special filter wheel housed inside the colorimeter. A filter window shows which filter is in place (see Plate 4.19).

Fluid volumes as low as 1.6 ml can be read in the C0700D colorimeter. It is designed for use with 10 mm lightpath cuvettes. A hand-operated suction system is available as an accessory for the rapid emptying of cuvettes. The cuvette chamber holds two cuvettes, enabling the blank solution to be left in place when reading a batch of tests.

Availability and cost

The WPA C0700D colorimeter is available from Walden Precision Apparatus (see Appendix II) priced £485 (1997). The price includes the colorimeter, ten built in protected filters, twin cuvette chamber, 100 plastic cuvettes, mains adap-

tor, and battery leads. The mains voltage required must be stated when ordering. The colorimeter weighs 1.35 Kg and measures 110 × 215 × 200 mm.

Note: A colorimeter with similar specifications to the WPA C0700D colorimeter but without some of the C0700D advantages, is the Chroma Corning 252 model. This however is more expensive and the price does not include the filters. These need to be ordered separately as also the battery leads.

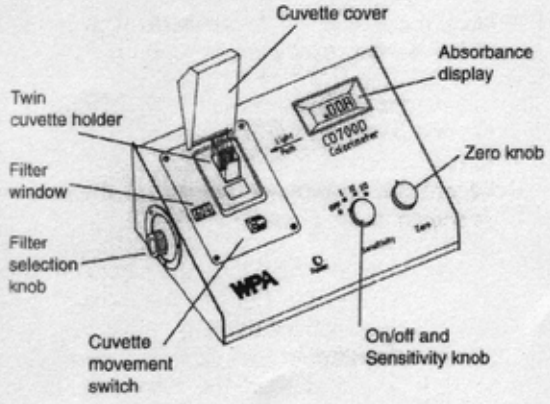

Plate 4.19 WPA C0700D mains and battery operated colorimeter with ten built-in sealed Ilford filters Numbers 600–609. *Courtesy of Walden Precision Apparatus.*

Use and care of a colorimeter

- Read carefully the *User Manual*. Prepare a stock record card and written SOPs covering the use, care, and maintenance of the colorimeter.
- Use the correct type of cuvette or tube in the colorimeter as recommended by the manufacturer.

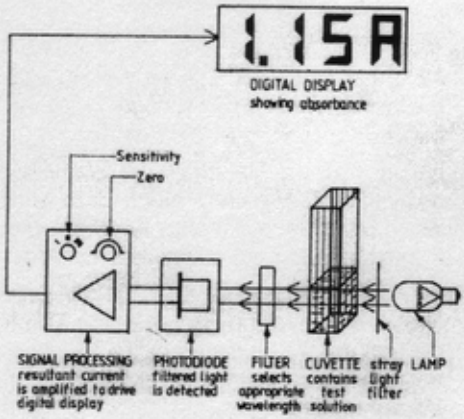

Fig. 4.20 Internal components of a colorimeter based on the WPA C0700D colorimeter.

Make sure the cuvette (or tube) is clean and its optical surfaces are dry and free from finger marks and scratches.

- Bring the filter into place before switching on the colorimeter. Before use, allow sufficient time for the colorimeter to stabilize as instructed in the *User Manual*.
- Before reading the absorbance of a solution, check that it is clear, there are no air bubbles in it, and that it is at room temperature to avoid condensation forming on the outside of the cuvette or tube.
- Calibrate the colorimeter for each test method (see later text).
- If a spillage occurs, immediately clean the colorimeter.
- To prolong the life of the lamp, switch off the colorimeter after use. At the end of the day's work disconnect it from the battery or mains socket and cover the instrument with its protective dust cover.
- When needing to replace the lamp, usually indicated by difficulty in zeroing the instrument, use a lamp of the correct specifications as detailed in the *User Manual*. Always keep spare lamps in stock. Replace the lamp as instructed by the manufacturer. Take care not to finger mark the glass bulb.

Use of a colorimeter, based on the WPA C0700D instrument

The essential components of a typical digital colorimeter such as the WPA C0700D are shown in Fig. 4.20 and Plate 4.19 (lower).

Method of use

1 Place the colorimeter on a solid surface that is free from mechanical vibration. Do not place the instrument in direct sunlight or on the same bench as a centrifuge.

2 Ensure that the colorimeter is connected correctly to its 12 V battery supply or to its adaptor which plugs into the mains electricity socket, as instructed in the manufacturer's *User Manual*.

3 Turn the *Filter Selector Knob* until the required filter is shown in the *Filter Window*. The filter to use will usually be stated in the test method. If only the wavelength is given refer to the following table:

Wavelength required	*Ilford filter to use* *pt = peak transmission*
400–419 nm	No. 600 Deep violet, pt = 408 nm
420–449 nm	No. 601 Violet, pt = 430 nm
450–479 nm	No. 602 Blue, pt = 470 nm
480–504 nm	No. 603 Blue-green, pt = 490 nm
505–534 nm	No. 604 Green, pt = 520 nm
535–564 nm	No. 605 Yellow-green, pt = 550 nm
565–589 nm	No. 606 Yellow, pt = 580 nm
590–639 nm	No. 607 Orange, pt = 600 nm
640–689 nm	No. 608 Red, pt = 680 nm
690–700 nm	No. 609 Deep red, pt = 700 nm

4 Turn on the colorimeter by moving the *Sensitivity Knob* from the OFF position to *Setting 1*. The green *Power Indicator* lamp will come on. Allow at least 2 minutes for the instrument to stabilize.

5 Transfer at least 1.6 ml of the reagent blank solution or distilled water (as specified in the test method) to a plastic or glass cuvette of 10 mm lightpath distance.

Important: Do not finger mark the clear optical sides of the cuvette and avoid air bubbles forming when transferring the solution to the cuvette see Fig. 4.21. If reusing a plastic cuvette, make sure it is completely clean and its optical sides are scratch free.

6 Insert the blank solution in the rear cuvette holder, making sure the clear optical sides of the cuvette are dry and facing sideways for the light to pass through.

7 Transfer at least 1.6 ml of the sample solution (standard, control, or patient's) to another cuvette. Insert the sample solution in the front cuvette holder.

8 Move the *Cuvette Movement Switch* to the white position to bring the blank solution into the arrowed *Light Path* position. Close the *Cuvette Cover*.

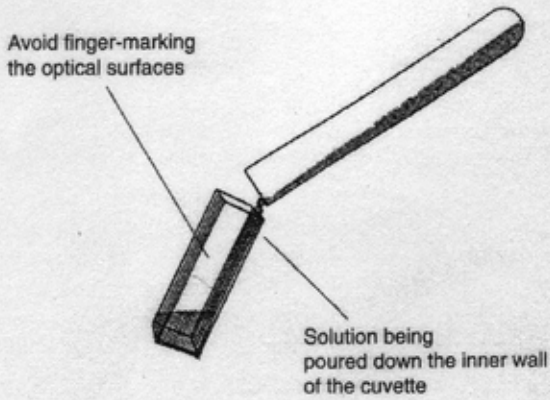

Fig. 4.21 How to transfer a solution from a tube to a cuvette, avoiding air bubbles.

9 Adjust the *Zero Knob* to give R reading on the display (R indicates Absorbance units). If it is not possible to zero on setting 1, move the *Sensitivity Knob* to setting 11 and if necessary to setting 111. The lowest setting should always be used to zero the colorimeter. Difficulty in zeroing the colorimeter is usually an indication that the lamp needs changing.

Note: If the display does not show R at the end of the reading, turn the zero knob to the right.

10 When the display reads .00R, move the *Cuvette Switch* to the red position to bring the sample solution into the *Light Path* position. Record the absorbance reading of the sample as shown in the display.

11 Return the blank solution to the *Light Path* position by moving the *Cuvette Switch* back to its white position. Check that the display still reads .00R. If the display does not read zero, adjust to .00R and read again the absorbance of the sample solution. The blank solution should continue to read zero each time it is returned to the *Light Path*.

12 Calculate the concentration of the control and test samples as instructed in the test method.

Calculation factor given in test kit methods

Where a test kit method provides a factor to calculate test results, this is based on reading the test at the wavelength specified. The factor will be different when using a colorimeter because of the 40 nm band width of the filter. To obtain the correct factor, when using a colorimeter with a colour filter, calibrate the test method (see following text).

Calibrating a colorimeter

As previously mentioned, when introducing a new test method it is necessary to establish whether the absorbance of the substance being measured increases in a linear way with its concentration. From the calibration graph, test results can be obtained.

Calibration of a test method involves:

- Preparing and testing a series of dilutions of the substance being assayed, i.e. standards, as described in the test methods in the clinical chemistry section (see *Calibration* text). For the calibration of most tests, five standards are used.
- From the absorbance readings obtained for the standards, prepare a calibration graph by plotting the absorbance of each standard against its concentration and examining whether a straight line (linear) calibration is produced. The preparation and use of a calibration graph is described as follows:

Method of preparing a calibration graph

1 Take a sheet of graph paper and rule the vertical axis and the horizontal axis. Mark the vertical axis *Absorbance*. Mark the horizontal axis *Concentration* and give the units of measurement (see Fig. 4.22).

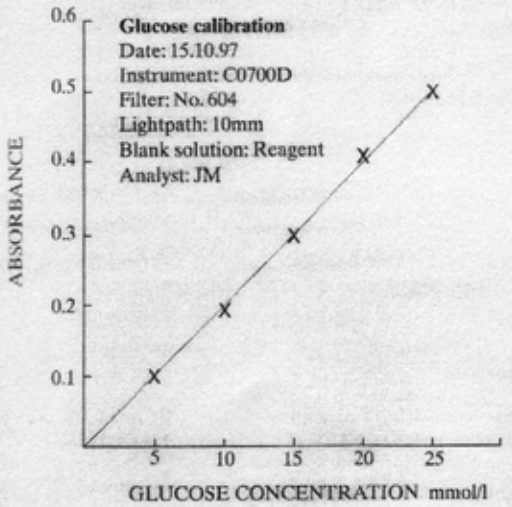

Fig. 4.22 Example of a calibration graph used to calibrate a glucose test method.

2 Depending on the range of concentrations and absorbance readings, divide each axis into the most suitable units for easiest reading. Use as much of the graph paper as possible.

3 Test each standard following the specified method. Plot the absorbance of each standard solution against its concentration, marking each point with a small neat cross (see Fig. 4.22).

4 Join each cross, making sure that the line passes through the point of origin in the bottom left hand corner (equivalent to the reagent blank).

5 Write in the top right-hand corner of the graph:
   - date
   - instrument used
   - filter or wavelength at which the absorbances were measured
   - lightpath distance of the cuvette (usually 10 mm)
   - blank solution used to zero the instrument, i.e. water or a reagent blank solution
   - initials of analyst (person who performed the calibration).

Linear calibration: If the line obviously looks as though it should be a straight line but one or two points are slightly off the line, this usually indicates inaccurate pipetting. A line of 'best fit' can be drawn if only one or two points are off the line. If a line of 'best fit' cannot be drawn, the calibration must be repeated.

If only one point is off the line this should be disregarded and a straight line drawn through the origin and all the other points. Ideally the line passing through zero should be at an angle of 45°. If this is not so and the angle is too steep or too flat, repeat the readings using the filter or wavelength near to maximum absorption.

If a straight line can be drawn through the points plotted as shown in Fig. 4.23, then absorbance is directly proportional to concentration. The Beer-Lambert law applies and its formula can be used (see following text).

Non-linear calibration: When the calibration is non-linear, the points will follow a curve, not a straight line. The curved line should be drawn smoothly and pass through the point of origin as shown in Fig. 4.24.

When the graph is non-linear, the Beer-Lambert law and its formula cannot be applied (see following text).

Note: An analytical method where the Beer-Lambert law applies will have better performance characteristics than a method where the law cannot be applied.

Using a calibration graph to obtain test results

The procedures for determining the concentration of test and control samples depend on whether the calibration is linear or non-linear.

Using a linear calibration graph

When the calibration graph is linear, the following formula can be used to calculate the concentration of unknown samples:

Concentration of test (CT) =

$$\frac{\text{Absorbance of test (AT)}}{\text{Absorbance of standard (AS)}} \times \text{Concentration of standard (CS)}$$

or in the abbreviated form:

$$CT = \frac{AT}{AS} \times CS$$

Alternatively, a table covering the appropriate range of values can be prepared from the calibration graph. Results can be read from the table providing the reading of the standard (put through with each batch of tests) agrees with the table and the control result is correct.

Note: If the reading of the test solution is beyond the limits of the graph or the range of the instrument, the final coloured solution can be diluted with an appropriate diluent and the absorbance of the diluted fluid read. The result is multiplied by the dilution factor. It is however better practice to dilute the specimen and repeat the test.

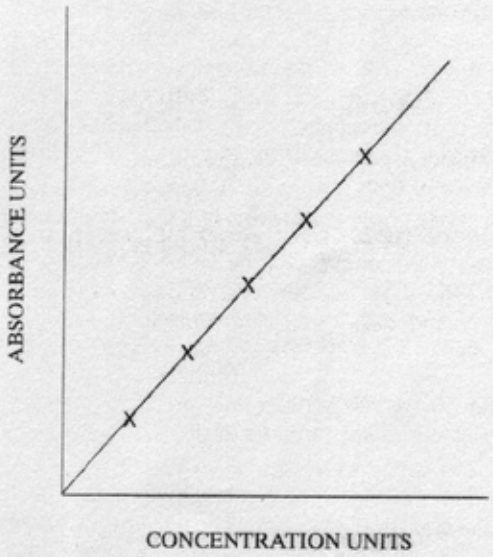

Fig. 4.23 Linear calibration showing a straight line joining the points plotted.

Using a non-linear calibration graph

When a test method produces a non-linear calibration, the values of the test and control samples must be read from the calibration graph. The formula based on the Beer-Lambert law *cannot* be used. A table covering the appropriate range of values can be prepared from the calibration graph.

The reading of the standard must be checked to make sure it agrees with the calibration graph and the control result must be correct.

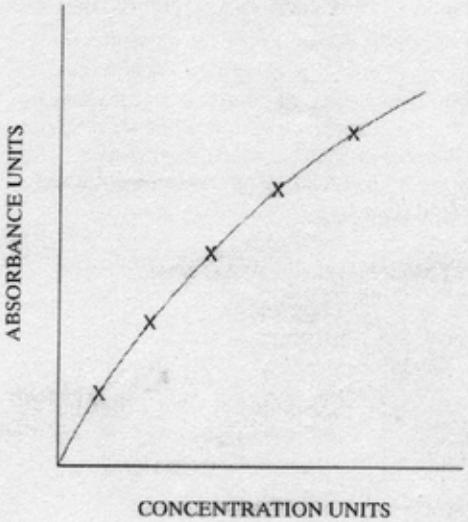

Fig. 4.24 Non-linear calibration showing a curved line joining the points plotted.

Need for control and standard with each batch of tests

A quality control sample must be carried through at the time of calibration and with each batch of tests. Only if the control sample gives the correct result can a calibration graph be used to determine patients' samples.

Control sample

A control sample contains a known amount of the substance being assayed. It is carried through every stage of the procedure in exactly the same way as patients' samples. It is the best indication that a method, reagents, and instrument are functioning satisfactorily.

When determining the concentration of unknown samples, the instrument, the method, and the reagents must be the same as those used for the standard solutions.

At least one of the standards used to prepare the calibration graph should be included in each batch of tests carried through. The amount of analyte in

this standard solution should be carefully chosen to give the best performance possible. Best performance is required at concentrations where clinical decisions are made.

Note: The reagent blank must be treated in exactly the same way as the standards.

4.11 Mixers

Mixers that may be required in district laboratories, depending on the type of work performed and workload, include:

- Roller mixer, particularly for mixing blood specimens and control samples.
- Rotator (orbital mixer) for the controlled mixing of agglutination tests such as the RPR test.
- Vortex mixer for the safe mixing and emulsifying of samples particularly in microbiology and clinical chemistry laboratories and for dissolving substances in the preparation of reagents.
- Combined magnetic stirrer and hot plate, mainly used in the preparation of culture media and reagents, and when needing to heat liquids.

Roller mixer

A roller mixer, rather than a revolving disc type of mixer (rotary mixer), is preferred for mixing blood and other specimens because it is easier to use, provides gentle but thorough mixing of specimens, can

Specifications of a roller mixer for district laboratory work

- Five roller capacity.
- Provides rotary and tilt mixing of specimens.
- Fixed speed (33 rpm or 40 rpm).
- Easy to decontaminate and clean.

mix specimens in a variety of containers including Universals, and can be easily decontaminated and cleaned.

Availability and cost of a roller mixer

An example of a 5-roller electric mixer with the above specifications is the mix and tilt *Movil-Rod* mixer shown in Plate 4.20. It is manufactured by J. P. Selecta (see Appendix II) and is available from Developing Health Technology, priced £314 (1997). The rollers and base tray are easy to clean. The *Movil-Rod* roller mixer weighs 3.5 Kg and measures 440 mm long × 190 mm wide × 100 mm high.

Stuart Scientific Ltd (see Appendix II) also manufacture a 5 roller mixer similar to the *Movil-Rod* mixer. It costs about £340 (1997).

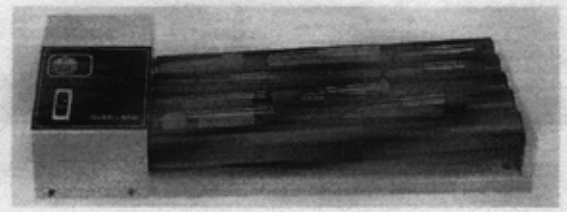

Plate 4.20 Selecta *Movil-Rod* 5-roller mixer that rotates and tilts specimens. *Courtesy of Jencons Scientific.*

Rotator (Orbital mixer)

A rotator with the following specifications is required for mixing RPR agglutination tests used in the investigation of syphilis. Such a rotator is also useful for other slide or tile agglutination tests performed in microbiology, haematology, and blood transfusion laboratories.

Specifications of a rotator for RPR and other slide agglutination tests

- Operating at a speed of 100 rpm.
- Rotating in a 15–20 mm diameter orbit.
- Preferably with timer (RPR tests require 8 minutes rotation).
- Supplied with a lid to prevent drying of samples.

Becton Dickinson rotator, model 54

A rotator with the above specifications is the electric Becton Dickinson *Macro-Vue Card Test Rotator, Model 54*, shown in Plate 4.21. It operates at a fixed speed of 100 rpm in 20 mm diameter rotating orbit.

The size of the rotating platform is 127 × 203 mm (holds two agglutination plates). It has a 0–15 minute timer and a humidifying lid (dampened piece of sponge can be fitted in the lid). The Becton Dickinson rotator weighs 1.9 Kg and measures 190 mm long × 127 mm deep × 114 mm high.

Availability and cost

The *Macro-Vue Card Test Rotator, Model 54* is available from Becton Dickinson (see Appendix II), priced US $386, about £244 (1997).