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**Standard Operating Procedure for Determination of clottable (Claus) fibrinogen**

**Principle of method**

This method used was initially described by von Clauss (1957).

When thrombin is added to diluted citrated plasma samples, it converts fibrinogen to fibrin, which in turn, polymerises and forms a fibrin network. Over time, this produces a visible clot, which is detected by a light absorption method. The elapsed time from thrombin addition to the generation of a visible clot is inversely proportional to the fibrinogen concentration. A standard of known concentration is used to generate a standard curve, from which concentrations of fibrinogen can be deduced.

**Equipment**

**MDA 180 coagulometer (Biomerieux)**

**Buffers and solutions:**

Two on-board containers supply distilled water and imidazole buffer (which is supplied by the manufacturer) to the instrument. These are topped up as required and changed on a weekly basis. Care must be taken when changing the distilled water container to rinse it with 20% methanol and then dry before re-use. This prevents the growth of algae etc. The imidazole buffer contains a preservative and this container can be washed in the usual way. The waste from the instrument is flushed into another container, which is then emptied into the sewer.

Two different sizes of rectangular containers are used in the MDA wash station; a 130 ml container for MDA probe cleaner (supplied by the manufacturer) and an 80 ml container for bleach solution. A 30% sodium hypochlorite solution is used in the bleach station. This is made up from a concentrated solution from Sigma Aldrich (product code 53,200-2). 3.4mls of this stock solution is made up to 100mls with distilled water. Gloves MUST be worn when making up and handling this solution,

which is corrosive. This solution must be made fresh daily. The probe cleaner is stable on the machine for one week.

#### Cuvettes:

Cuvette cassettes contain 500 cuvettes. When the number of available clean cuvettes gets to 60, the MDA 180 will flag a warning to replace the cassette. This can be done via the Reagent Service and Load Cuvette Cassette menus.

#### Sample carrier:

Each sample carrier can hold up to 10 samples and has a unique barcode label to identify it to the MDA system.

#### Safety shield:

The plastic safety shield protects the MDA operator from possible injury by the moving probes, and can only be raised when the MDA is not processing specimens. It can be raised or closed via the Reagent Service menu.

#### Reagent bay:

The reagent bay can hold up to 30 reagents in four colour-coded rows. The rows are labelled A, B, C, and D. These correspond to the pump and probe designations. In addition, each reagent position has a bar code. The Fibriquik reagent for the fibrinogen assay is loaded in row D. The control plasma is loaded in row A.

#### MDA Start up

System must be in the standby state.

Select Start Menu on the touch screen.

Select Start Up System with water reference. This will initiate bleaching (cleaning) of the probes. Pumps are primed for eight cycles. The optics lamp will be switched on and will be ready for use after heating for 15 minutes. Water reference will run automatically. This evaluates the functioning of the optics and prints a report.

#### Reagents, Controls and Standards

The thrombin reagent (Fibriquik) is made up with 2.5 mls distilled water and is ready for use when the lyophilised material has completely dissolved. This requires gentle mixing for about 5 minutes.

The standard curve for fibrinogen is constructed using the 9<sup>th</sup> British Standard for Coagulation Factors from NIBSC (South Mimms, UK).

This curve is stored and can be used for a month provided the control plasma is within the accepted range. A new curve has to be run for each batch change in the Fibriquik reagent. The software will prompt the operator to do this when the new bar code for the reagent is scanned in.

An in-house control of pooled plasma is used as a control for all fibrinogen assays. The fibrinogen mean concentration of this plasma is assigned after at least 20 separate runs. When this value is keyed into the computer software, a Levey-Jennings curve is generated, and QC results are displayed graphically. The QC is loaded onto the reagent bay as described above, using the QC serial code instead of a barcode.

#### Loading reagents into the reagent bay.

Select Stop Menu from the touch screens, then Suspend Operation. When screen has greyed out, select Enter And Review Reagents and Open Safety Shield. The bar code for the appropriate position for the reagent and control are scanned using the bar code reader. The barcodes of the reagent, standard and control plasma are then scanned into the MDA. The MDA software contains information on current lots for each the reagent type and will confirm these reagents.

The bleach and probe cleaning containers are loaded at the same time.

#### Running the standard curve.

Go to Order Assay Menu. Select Order Control.

Select Fibrinogen II (Clauss) from assay list.

Select Save request.

Select Force Generation Of New Curve.

Select Cancel and exit to return to the main menu.

The MDA will then make serial dilutions of the standard and prepare the calibration curve. The control plasma will be run automatically and read from this curve. If the curve has an  $r^2$  of less than 0.998, or if the control plasma value is outwith 2SDs on the Levey Jennings plot, the curve should be re-run.

Select the Results menu, then select the Levey Jennings option. The Levey Jennings plot will be displayed graphically.

### Print Bar Code Labels

Select Order Assay Menu

Select Patient Bar Code Print Menu

Select Print Sequential Bar Codes

Enter number of labels desired from 1-100

Press enter on computer keyboard.

Select Start Printing

Working from an Excel spreadsheet with patient details and assigned bar codes as sample ID (SID), attach each bar codes to relevant sample.

Place samples in sample carrier ensuring that the bar code is in the correct position to be read by the bar code reader.

### Ordering assays

Select Order Assay Menu.

Select Order Patient Assays.

Type study ID

Press enter on computer keyboard.

Select Fibrinogen II

Select Save Request.

Samples will be moved along the track. Plasma is diluted with buffer at probe A and incubated in a micro-cuvette at 37° C. Fibrinogen is added at probe D, and the optical system monitors the formation of the clot. The time (in seconds) taken for the clot to form is the value used to read the fibrinogen concentration from the standard curve.

Results are printed out as each sample assay is finished.

Results are also stored on the MDA computer for 72 hours.

Select Results Menu

Select Patient results

Select bar code or other subject ID

Select Search.

Select results to be reviewed. Selected results change from grey to blue.

Touch summary report.

Samples with values below 2.0 g/L or above 5.0 g/L should be repeated.

Results are then keyed into the relevant data base.

### Daily Shut Down

Select Reagent Menu.

Select Clean Probes Menu

Select Clean all Probes.

Select Stop Menu.

Select Shut Down for Maintenance.

Reference

Clauss, A. Gerinnungsphysiologische Snellmethode zur Bestimmung des Fibrinogens.

Acta Haematol 1957; 17: 237-46