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**Standard Operating Procedure for High sensitivity CRP assay**

**Principle of the method:**

Latex particles coated with monoclonal antibodies to human CRP are mixed with plasma samples. This results in aggregation of latex and protein complexes in the solution. Plane-polarized light passed through the solution is scattered in a manner proportional to the concentration of CRP. The intensity of the light scatter can be measured against a curve from a standard of assigned concentration, and the CRP antigen concentration of the sample is determined.

**Equipment and Reagents**

BN Prospec protein analyser (Dade Behring)

**Buffers and solutions:**

The BN Prospec three different supply tubes supply the analyser with distilled water, reaction buffer, and diluent. Ensure that each of these has an adequate supply, and that the water is fresh. Waste is flushed into a container, which is then emptied into the sewer.

**Cuvettes and Dilution Cups:**

Dilution cups are located under the main hood next to the probe wash station. The BN Prospec uses these to dilute neat plasma using the diluent solution. These are changed at start-up or when the BN Prospec requests it. The cuvettes are also found under the main hood, and have a separate cover for the rotator. Similarly, used strips are changed at start-up or as the BN Prospec requests.

**Sample and Calibrator Segments:**

Sample and calibrator segments are used for inserting multiple samples onto the sample rotator at once. There is one calibrator segment (with 6 spaces) and 3 sample segments (with 15 spaces each). There is room for 3 segments simultaneously on the sample rotator, meaning 45 samples can be run concurrently.

#### Reagents:

The two reagents required for the determination of CRP are hs-CRP reagent and supplementary reagent. These have an evaporation-proof lid put on in line with the barcode on the label. The reagents are then put on the reagent rotator, which is cooled to 10 degrees C, and the barcode of the reagent is be read by the BN Prospec. The BN Prospec is regularly updated with current reagent lots from the manufacturer.

#### Controls:

An in-house control of pooled plasma is used as a control for all hs-CRP assays. The CRP 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.

#### **Operating Procedure:**

##### **Start-up BN Prospec:**

After the main computer terminal is switched on the BN Prospec Software Icon appears on desktop. Prior to accessing this icon, switch on the BN Prospec and allow it to complete a warm-up procedure (end indicated when LEDs in top right-hand corner stop flashing). The software can now be accessed, and the BN Prospec will execute an automatic initiation procedure and check all robotics.

Upon start-up the BN requires a flush through of all system liquids. Access this via the SYSTEMS button and then REPLACE SYSTEM LIQUIDS. Tick all boxes to flush through all liquids and empty the waste container.

The BN requires all dilution cups and cuvettes to be changed at start-up. Change all used strips and cuvettes via the SYSTEM and CUVETTES AND DILUTION CUPS menu. Once replaced, press SELECT ALL and confirm the change.

New CRP reagent lots require a standard curve to be run on the BN Prospec. The CRP calibrator is made up according to the manufacturers instructions and is placed on the BN Prospec in the calibrator segment in the sample loading bay, barcode facing out so the BN Prospec can read it. Go to CALIBRATION in the navigation bar, select the assay for which a reference curve is required, and select the reagent lot. Click on MEASURE. The BN Prospec will perform the curve automatically. The curve can be viewed by the SHOW CURVE option.

### **Running Normal Controls and Samples:**

#### Controls:

The control (plasma pool) is loaded on the sample segment, which is then placed in the sample rotor. Via the LOADING menu, the control ID should be dragged on the graphic of the segment in the appropriate position. The control can then be run using the LAB JOURNAL menu by clicking the REQUEST CONTROL button. The required control measurement field should be selected, the new dilution option ticked, and then click MEASURE. The control will now be run, and the result displayed in the lab journal. The percentage deviation from the mean of the control is displayed with the result. If the control is out -with 10%, it is re-run. If the control is persistently out of range, a new calibration curve is generated before re-running the control.

#### Samples:

To order samples for an assay, sample IDs must be ordered in the NEW menu of the LAB JOURNAL and the appropriate assay (i.e. CRP) must be selected. The list of ordered samples then appears in the LOADING menu from which the samples can be dragged on to the graphic of the segments, in their given position (as for the control.) The segments are then inserted in the sample rotor. The BN Prospec will start sampling automatically and will display the time the run will take. If during a run, the

system liquids run out, the information icon, which is normally green, will turn red and the BN Prospec will stop. They can then be replaced but they need to be flushed through in order for the BN Prospec to restart sampling. This can be done using the same procedure as above.

In a similar manner, when the BN Prospec runs out of cuvettes, dilutions cups or reagents, the appropriate icon turns yellow as a warning and red when the BN Prospec stops. The plastics or reagents can then be replaced as described above.

After a run is completed, the results are listed in the lab journal. If a sample is higher than the normal range, the pre-set dilution may not be sufficient to give an accurate result and the BN Prospec will automatically do a serial dilution on the original dilution. This is then re-run and the result is added to the lab journal. Samples with a CRP value outside the expected range (of the particular experimental subjects) are repeated by highlighting the result and clicking the REPEAT button. The BN Prospec will repeat the procedure, going back to the original sample to make a new dilution.

Once all results and controls are satisfactory, the whole lab journal can be highlighted and printed using the PRINT button.

### **Shutdown and Maintenance:**

#### **Monthly disinfection procedure:**

A 10% bleach solution is made up, into which the distilled water tubing is inserted. In the SYSTEM menu, select DISINFECT SYSTEM and follow the on-screen prompts. The system is rinsed through and this takes approximately 10 minutes. The waste filter is then changed.

#### **Maintenance:**

The weekly maintenance procedure is logged in the maintenance manual. Firstly, in the SYSTEMS menu the option PARK PROBE FOR CLEANING option is selected. The probe is removed from the wash station automatically, and is then cleaned using a

sterile methanol swab. The probe wash station and the BN Prospec work surface are also cleaned at this time.

The CHECK PROBE option is then selected, and the machine runs a stream of water from the tip of the probe so the functional capability of the probe can be visually checked (i.e. no blockages or spraying from the probe tip).

All tubing connections, valves, and syringes are then checked for signs of leakage. Any leakage indicates a fault and may require hardware replacement or an engineer to fix.

#### Shut-Down:

After maintenance the BN Prospec and the computer terminal are shut-down. Click the STATUS option from the main menu and then select EXIT. After confirming this action the BN Prospec will take a few minutes to log results, close all software, and reset hardware. The computer terminal is then switched off.