**CHEM.TRIAGE.3.0 TRIAGE BNP TEST FOR EDTA WHOLE BLOOD AND PLASMA**

**PRINCIPLE**

The Triage® BNP Test is a fluorescence immunoassay for the quantitative determination of BNP (B-Type Natriuretic Peptide) in whole blood and plasma specimens in which EDTA is the anticoagulant. After addition of a blood sample to the sample port of the test device, the red blood cells are separated from the plasma via a filter. A predetermined quantity of plasma moves by capillary action into a reaction chamber and is allowed to react with fluorescent antibody conjugates within the reaction chamber to form a reaction mixture. After an incubation period, the reaction mixture flows through the device detection lane. Complexes of the analyte and fluorescent antibody conjugates are captured on discrete zones in the detection lane. Excess plasma sample washes the unbound fluorescent antibody conjugates from the detection lane into a waste reservoir. The concentration of the analyte in the specimen is proportional to the fluorescence bound to the detection lane.

**OWNERS**

Manager, MACL Community Hospital South Laboratory

**SPECIMEN**

A. Patient preparation:

1. No special preparation needed.

B. Specimen type:

1. Whole blood or plasma using EDTA as the anticoagulant. No other anticoagulant is acceptable.

2. Minimum sample requirement of 250 uL.

3. Grossly hemolyzed and / or clotted specimens are unacceptable.

4. Gel separators are unacceptable.

C. Specimen stability:

1. Complete testing as soon as possible after collection. If testing is to be delayed, whole blood or plasma samples may be stored at room temperature or refrigerated at 4° C for up to 7 hours prior to testing. Plasma may be pulled off and refrigerated up to 24 hours. If testing cannot be completed within 24 hours, the plasma should be frozen at -20 C until it can be tested. Specimens that have been refrigerated or frozen must be allowed to warm to room temperature and must be well mixed prior to testing.

**REAGENTS**

A. Triage® BNP Test device.

1. All the reagents necessary for the quantification of BNP in plasma and whole blood are contained in the test device. The BNP test device is made up of the following: Murine monoclonal and polyclonal and stabilizers.

2. The Triage® BNP Test device is stable until the expiration date stamped on the box and foil pouch when stored at 2º - 8ºC.

3. The Triage BNP Test device is stable for 14 days when stored, in the foil pouch, at room temperature (not to exceed the printed expiration date on package).

4. Gently write the date the test device is taken out of the refrigerator and the discard date on the foil pouch and/or the kit box using a soft, felt tip maker if test device is not to be used on the same day.

5. Allow a minimum of 15 minutes for the test device to reach room temperature before use for individual foil pouches. If kit box is removed from refrigeration, allow box to reach room temperature for a minimum of 1 hour.

6. Do not remove the test device from the foil pouch until ready to use.

**EQUIPMENT**

A. Biosite Diagnostics Triage® BNP Test Kit

|  |  |
| --- | --- |
| **Kit contains** | **25 Test Kit** |
| Test Device | 25 each |
| Transfer Pipettes | 25 each |
| Reagent Code Chip | 1 each |
| Printer Paper | 1 roll |

B. Triage® MeterPro

**CALIBRATION/CALIBRATION VERIFICATION**

A. Reagent Code Chip:

1. The Reagent Code Chip contains calibration data that is specific for the test kit lot number. Each box of test kits includes a Reagent Code Chip.

2. Enter the corresponding Reagent Code Chip into the analyzer with each change in kit lot number.

3. Analyze both levels of external QC after a new Reagent Code Chip has been entered and prior to patient testing. See Quality Control Section.

B. Alere® Triage Total Calibration Verification

Total Calibration Verification is used to verify the calibration of the test devices throughout the measureable range.

Alere recommends running Calibration Verification samples every 6 months.

The order number for the Total Calibration Verification set is 88755. The Total Calibration set contain 2 vials of 5 levels X 0.25mls. The Total Calibration Verification Set is stored at -20oC or colder in a non defrosting freezer. Do not store near the door and do not refreeze material.

**FOR EACH DEVICE TYPE:**

1. Remove 1 set of 5 levels of Calibration Verification samples from freezer.
2. Thaw at room temperature (19oC to 25oC) for 30 minutes. Use within one hour of removal from frozen storage.
3. Mix the control thoroughly by vortexing or inversion prior to testing.
4. Open a device and label device with the vial lot number (can be obtained from the expected values card or the vial).
5. Hold tube with tip facing upward. Make sure all the liquid is at bottom of the vial.
6. Open vial by twisting the tab at the top. Turn the tube over and dispense entire contents into sample port of the test device. Discard the empty tube.
7. Allow the fluid to absorb into the filter.
8. To run in meter: Highlight **Run Test** on the display panel. Press **Enter** to select.
9. Enter your ID number and press **Enter** (if applicable).
10. Use the arrow key to highlight **Patient Sample**. Press **Enter**.
11. Type in the lot number of the calibration verification vial. Enter 1 for the first sample. Press **ENTER** until you see the message ***Measurement in Progress*** on the meter screen.
12. You may apply the second Calibration Verification sample to a test device a few minutes after the first test device is put into the meter.
13. Review the results printed out by the meter to make sure all the test results are within + 2 SD of the mean.
14. Run the rest of the Calibration Verification samples following the same instructions.
15. **METER-TO-METER VERIFICATION:** If multiple meters are used, take the device from the first meter and run it on the second meter and so on. The first reading should take approximately 15 minutes. Subsequent reading should take approximately 2 minutes each. Once inoculated, each device may be read for up to 30 minutes.
16. If the Cal Ver results are > + 2 SD, remove a 2nd vial, thaw 30 minutes and rerun. If it fails again, call Alere technical service. 1-877-308-8287.
17. Enter all data and information, including all meter serial numbers, device, control and cal ver lot numbers, into the form and email to: William.sbai@alere.com.

**QUALITY CONTROL**

A. QC Simulator

1. The QC Simulator mimics a test device and checks the following instrument functions:

a. Optics / calibration

b. Laser stability

c. Carrier alignment

2. The QC Simulator provided with the Triage® Meter must be tested daily to verify instrument performance.

B. External Quality Controls:

Triage® Total Control 5 Level 1 (5 x .25ml) with control chip.

Triage® Total Control 5 Level 2 (5 x .25ml) with control chip.

**WARNING:** The human source material used to produce this product has been tested for hepatitis B antigen, antibodies to hepatitis C, HIV – 1 and –2 and found to be non-reactive when using licensed reagents. Inasmuch as no known test method can offer complete assurance that infectious agents are not present, the reagents and patient samples should be handled as though they are capable of transmitting disease.

1. External Quality Controls are to be analyzed with each new shipment or lot of product, and at least every thirty days. Controls may be run to verify test performance as needed.

2. Controls are stable until the expiration date on the box when stored at < -20ºC in a non-defrosting freezer where possible.

3. Thaw controls until room temperature and mix well by inversion prior to use. Controls should be used within one hour of thawing.

4. Do not refreeze control material. Each vial should be used once and then discarded.

C. Internal Quality Controls

1. Each Triage® BNP test device contains two internal positive controls that satisfy routine quality control requirements. These controls indicate that sufficient sample was applied to the panel, that the unbound fluorescent label washed sufficiently from the detection zone, and that the panel was inserted and read properly by the Triage® Meter.

2. An unacceptable result from either control causes a warning message on the Triage® Meter indicating that the test should be repeated. Lack of sample results will be indicated by dashes.

a. If a whole blood sample results in an error message, “Warning: Internal QC Out of Range,” spin the sample down and test the EDTA plasma. If the plasma does not result in an error message, the results are reportable.

b. If the error message “Measurement Failure” is displayed, insufficient sample was added to the device. This could be due to:

i. Improper use of the Biosite provided pipette.

ii. Clot or fibrin strands present in sample.

**PROCEDURE**

A. QC Simulator

1. Upon initial receipt of a QC Simulator, the Simulator data must be loaded into the meter by installing the QC Simulator Code Chip.

a. Switch the Triage Meter on.

b. Select “Install Code Chip” Using up/down keys, from the Main Menu.

c. Press <Enter>.

d. Install selected code chip in code chip port reader.

e. Press <Enter>

f. Code chip information is automatically installed into meter memory.

g. Remove code chip from port.

h. If installation is unsuccessful call Biosite Technical Service.

2. Perform Simulator test.

a. At MAIN MENU, select RUN TEST and <ENTER>. Select QC Simulator and <ENTER>.

b. At the prompt “Insert QC Simulator into meter the QC Simulator into the meter until it clicks and <ENTER>.

c. The device will be drawn into the meter.

d. PASS or FAIL results will appear on the screen and the meter will release the QC Simulator upon test completion. Results will automatically print.

e. Remove the QC Simulator from the meter and return it to the black storage box.

**NOTE:** The QC Simulator is light sensitive and must be stored in the black provided.

f. Record the Simulator results on the BNP Daily Maintenance log.

g. If any of the tested functions fail, clean the QC Simulator with canned air or a lint free cloth and repeat the test. If the failure continues, contact Biosite Technical Service. If any of the QC Simulator checks fail, no patient of QC samples can be tested.

B. QC SAMPLE

1. Install Reagent Code Chip, if necessary.

2. The chip does not need to remain in the port.

3. Install QC Code Chip.

4. Remove QC Code Chip from meter port, and store with remaining frozen controls.

5. Log the QC sample onto the appropriate log sheet, if applicable.

6. Open the pouch, remove test device and label with an identifier.

7. Add well-mixed quality control material into the sample chamber using the transfer pipette provided. To pipette, depress the larger (top) bulb completely and insert the tip into the specimen. Release the bulb slowly. The tube should fill completely with some fluid overflowing into the smaller overflow bulb.

8. Place the tip of the pipette into the sample port of the device and depress the larger bulb completely. The entire amount of sample in the pipette tube must be dispensed. The sample in the smaller overflow bulb will not be expelled.

9. Discard the transfer pipette into the biohazard trash.

10. At MAIN MENU, select RUN TEST and <ENTER>.

11. Select QC SAMPLE and <ENTER>.

a. Enter QC lot number from the label on the side of the vial, not from the side of the box, using numbers only. To correct if necessary, press DELETE and retype correct numbers. Press <ENTER>.

**NOTES:**

1. External QC samples must be run in pairs. Both Level 1 and Level 2 are to be run consecutively. The meter will not recognize that QC is valid if only one level is run and the NUMBER OF CONTROLS setting is set to 2.

2) Do not re-insert the reagent code chip after QC has been run. Inserting the reagent code chip resets the QC Sample timer and QC samples will need to be repeated.

3) If the NUMBER OF CONTROLS setting is 2 and the 2nd level is rerun, the meter resets the counter and both levels must be run again. Ex. Level 1 passes, Level 2passes but technician decides to run Level 2 again, the meter will reset the counter with the 2nd run and require Level 1 to be run again.

12. Insert the test device gently into the Triage® Meter until a click is heard.

**NOTE:** Once sample has been pipetted into a device, the device must be inserted into the meter within 30 minutes. If for any reason there is a delay, the test must be set up again using a new test device.

13. The results will be available in approximately 15 minutes.

14. The test device will be released from the analyzer upon test completion. Discard the test device appropriately. Up to four additional devices may be set up within the first 15 minutes of the initial device’s insertion.

15. Results may be read on the display screen or from meter printout. Document appropriately.

16. If results is backlit on screen or printout then QC sample is greater than +/-2 SD and must be repeated. If External QC continues to fail, contact BIOSITE Technical Service.

17. Reagent Lots – QC a list of Reagent Code Chips in memory, along with corresponding expiration dates for QC sample can be recalled from memory. The list will contain the Lot number, Panel type and expiration date, (if the reagent lot expires prior to the QC sample, the reagent lot expiration date will be displayed).

a. If the QC expiration date has passed, the date will be in reverse video.

b. If the QC for the lot has failed, “FAILED” will appear in reverse video.

c. If a Code Chip has been installed for a new lot, but QC has yet to be established, the words “NOT RUN” will appear in place of this expiration date.

d. To access the information at MAIN MENU, select recall results, QC results, Reagent Lots – QC.

C. PATIENT SAMPLE

1. Log patient information onto the appropriate log sheet, it applicable.

2. Open pouch, remove test device and label with a patient identifier.

3. Add well mixed EDTA whole blood or plasma into the sample chamber using the transfer pipette provided. To pipette, depress the larger (top) bulb completely and insert the tip into the specimen. Release the bulb slowly. The tube should fill completely with some fluid overflowing into the smaller overflow bulb.

4. Place the tip of the pipette into the smaller port of the device and depress the larger bulb completely. The entire amount of sample in the pipette tube must be dispensed. The sample in the smaller overflow bulb will not be expelled.

5. Discard the transfer pipette into the biohazard trash.

6. At MAIN MENU, select RUN TEST and <ENTER>.

7. Select PATIENT SAMPLE and <ENTER>. If correct, press <ENTER>.

8. Confirmation of the correct entry is required. If correct, press <ENTER>. If incorrect, select correct patient ID, and press (ENTER). Then press DELETE and retype the numbers. Press <ENTER>.

9. Insert the test device gently into the Triage® Meter until a click is heard.

**NOTE:** Once sample has been pipetted into a device, the device must be inserted into the meter within 30 minutes. If for any reason there is a delay, the test must be set up again using a new test device.

10. The results will be available in approximately 15 minutes.

11. The test device will be released from the analyzer upon test completion. Discard the test device appropriately. Up to four additional devices may be set up within the first 15 minutes of the initial device’s insertion.

12. Results may be read on the display screen or from the meter printout. Document appropriately.

13. If the device’s Internal QC results are unacceptable, the patient result will not be displayed. A warning will appear on the display and printout. To troubleshoot, see QUALITY CONTROL, Section C.

**REPORTING RESULTS**

A. Normal Range: 5 – 100 pg/mL

B. Critical Range: Not applicable

C. Computer Functions

|  |  |  |
| --- | --- | --- |
|  | **Misys** | **QLS** |
| **Function** | MEM | 3,3,1 |
| **Worksheet** | Specific to Site | Specific to Site |
| **Method** | Specific to Site | N/A |
| **Test Code** | BNP | 51054 |

D. Online entry:

Function: OEM

Device: (method/device specific for site)

E. Report patient results in whole numbers.

F. Report BNP results less than 5 pg/mL as <5.

G. Report BNP results of greater than 5000pg/mL as >5000.

H. See Laboratory Computer Downtime Policy (QA.LIS.10.0) if system is unavailable.

**PROCEDURE NOTES**

A. When testing cannot be performed follow appropriate back up policy.

B. Notification of instrument downtime should be made to MACL Client Response, the nursing unit and the backup laboratory receiving specimens.

**LIMITATIONS**

A. AMR: 5 – 5,000 pg/mL

B. The results of Triage® BNP Test should be evaluated in the context of all the clinical and laboratory data available. If Triage® BNP Test results do not agree with the clinical evaluation, additional tests should be performed.

C. Some patients may have circulating BNP concentrations that are higher than the measurable range of the Triage® BNP Test (> 5000 pg/mL).

D. This test has been evaluated with whole blood and plasma using EDTA as the anticoagulant. Serum and blood or plasma specimens obtained using other anticoagulants (e.g. heparin or citrate) have not been evaluated and should not be used.

E. There is the possibility that factors such as technical or procedural errors, as well as additional substances in blood specimens that are not listed in the BNP product insert, may interfere with the test and cause erroneous results.

F. Interfering substances: The compounds listed below when added to human plasma did not interfere with the recovery of BNP.

|  |  |
| --- | --- |
| **Compound** | **Concentration Tested** |
| HGB | 10,000 mg/dL |
| CHOL | 1000 mg/dL |
| TRIG | 1000 mg/dL |
| BILI | 20 mg/dL |

G. Severely hemolyzed specimens should be avoided

**REFERENCES**

1. Triage® BNP Product Insert; 22342 Rev G, Biosite Incorporated, 2008/04/14.
2. Triage® MeterPro Users Guide, 22719 Rev C, 2007/02/09.
3. Triage® Total Controls 5 Product Insert, Rev E, 2009.