KAISER PERMANENTE®
COLORADO LABORATORY

BNP TEST FOR BECKMAN / COULTER ACCESS II

PRINCIPLE

B-type natriuretic peptide (BNP) is a member of a class of hormones that regulate blood pressure. The heart is the main source of circulating BNP in humans. The molecule is released into the blood in response to increased heart pressure. Various studies have demonstrated that increased levels of circulating BNP are found in early stages of CHF. The level of BNP in the blood continues to increase as the CHF disease advances. The Triage BNP test offers an objective, noninvasive measurement for assessing patients for CHF and risk stratification in patients with acute coronary syndromes (ACS).

The Triage BNP test is a two-site immunoenzymatic ("sandwich") assay. A sample is added to a reaction vessel with mouse monoclonal anti-human BNP antibody-alkaline phosphatase conjugate and paramagnetic particles coated with mouse omniclonal anti-human BNP antibody. BNP in human plasma binds to the immobilized anti-BNP on the solid phase, while the mouse anti-BNP conjugate reacts specifically with bound BNP. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. A chemiluminescent substrate, Lumi-Phos* 530, is added to the reaction vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of BNP in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

SCOPE

All Medical Technologists and Medical Laboratory Technicians working at the Franklin Laboratory.

SPECIMEN REQUIREMENTS

1. Plasma (EDTA) is the required sample. Other specimen types have not been evaluated.
2. Collect all blood samples observing routine precautions for venipuncture.
3. Mix the blood specimen by gently inverting the tube several times.
4. Keep tubes stoppered at all times.
5. Samples should be tested as soon as possible after collection. However, if it is not possible to test the samples immediately, the following is recommended:
    - Plasma samples may be stored for 8 hours at room temperature or up to 24 hours refrigerated prior to testing.
    - For longer storage, transfer at least 500 µL of cell-free sample to a storage tube. Tightly stopper the tube immediately and freeze at -20 °C or colder in a non-defrosting freezer. When thawing, allow samples to warm to room temperature for a minimum of 30 minutes prior to testing.
    - Thaw samples only once.
6. Transport specimens at room temperature or chilled and avoid extreme temperatures.
7. Avoid using severely hemolyzed specimens whenever possible. If a specimen appears to be severely hemolyzed, another specimen should be obtained and tested.

8. Ensure residual fibrin and cellular matter has been removed prior to analysis.

- **SPECIMEN REJECTION**

1. Non-EDTA plasma
2. Severely hemolyzed plasma must be redrawn.

- **ORDER CODES**

Cerner Code	Cerner Description	Health Connect Code	Health Connect Description
BNP	BNPeptide	83880B	B-Type natriuretic peptic

EQUIPMENT AND MATERIALS

1. Beckman Coulter Access II Analyzer(Franklin Facility ID# 982972/SN #504760)
2. Beckman Coulter Access reaction Vessels Ref#81901
3. Beckman Coulter Access Waste Bags Ref#81904
4. Transfer Pipettes Item # 9990 5580
5. Fiber-free polyester swabs
6. 2.0 mL sample cups(3 for maintenance)

REAGENTS

1. BNP reagent packs. Cat. No. 982002: 100 determinations, 2 packs, 50 tests/pack
    a. Store upright and refrigerate at 2 to 10°C.
    b. Refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument.
    c. Stable until the expiration date stated on the label when stored at 2 to 10°C.
    d. Stable at 2 to 10°C for 28 days after initial use.
    e. Signs of possible deterioration are a broken elastomeric layer on the pack or control values out of range.
    f. If the reagent pack is damaged (i.e., broken elastomer), discard the pack.
    g. All antisera are polyclonal unless otherwise indicated.

R1a:	Paramagnetic particles coated with mouse omniclonal anti-human BNP antibody suspended in TRIS buffered saline, with bovine serum albumin (BSA), 0.1% ProClin** 300, and < 0.1% sodium azide.
R1b:	Purified mouse and goat IgG in TRIS buffered saline with 0.1% ProClin 300 and < 0.1% sodium azide.
R1c:	Mouse monoclonal anti-human BNP antibody-alkaline phosphatase bovine conjugate in PBS buffered saline with BSA, 0.1% ProClin 300, and < 0.1% sodium azide.

2. Triage BNP Calibrators. Cat. No. 98202
   Provided at zero, and approximately 25, 100, 500, 2500, and 5000 pg/mL.
3. Triage BNP QC Controls or other commercially available control material. Cat. No. 98201
   Provided at approximately 80, 400, and 2200 pg/ml
4. Access Substrate. Cat. No. 81906
5. Access Wash Buffer II. Cat. No. A16792
6. Beckman Coulter Access 2 analyzer.

CALIBRATION-See separate procedure

QUALITY CONTROL-See separate procedure

PROCEDURE

- **Loading patient samples – Step by step instructions**

1. Place small LIS label on the 0.5 ml cup and place aliquot of plasma in cup (fill it to the top)
2. Use rack series 2500 for the 0.5 ml cups
3. Go to "sample manager" and barcode the rack.
4. Select "test request"
5. Use the barcode scanner and read the barcode on the plasma cup.
6. Select the test "Triage BNP" and press ENTER.
7. Turn on batch mode to program the rest of the samples on the same rack for the same test.
8. Use the barcode scanner and read the barcode of all the samples you have on the rack.
9. Load the rack on the instrument – remember to tell, wait, do, done – then press RUN.
10. Check QC on log to make sure it is OK before reporting patient results.
11. The completed report will automatically print
12. Enter results in CERNER – see below
13. Cap and save samples in the back refrigerator and discard after 24 hours.

REPORTING RESULTS

1. Patient results are entered in the "ARE" application of CERNER – accession or instrument queue mode.
2. Enter the accession # (by typing or scanning the barcode) in the accession # field.
3. PERFORM then VERIFY.

The Access Immunoassay System calculates the test results automatically. A number in pg/mL represents the amount of BNP present in the sample.

- BNP results less than or equal to 100 pg/mL are representative of normal values in patients without CHF.
- BNP results greater than 100 pg/mL are considered abnormal and suggestive of patients with CHF.
- BNP results of > 5000 pg/mL are considered very high values for BNP and exceed the upper limits of the BNP test. We report >2200 per established AMR.
- Higher BNP concentrations measured in the first 72 hours after an acute coronary syndrome are associated with an increased risk of death, myocardial infarction, and CHF.
- Higher BNP concentrations or the lack of a decrease in the BNP concentration from hospital admission to discharge indicate an increased risk of hospitalization or death in patients with heart failure

REFERENCE RANGES

- 0 – 100 pg/mL

- **TABLE 6 ANALYTICAL RANGE-FRANKLIN**

SAMPLE TYPE	ANALYTICAL MEASUREMENT RANGE	CLINICAL REPORTABLE RANGE
EDTA Plasma	20 – 2200pg/mL	20 – 2200 pg/mL

LIMITATIONS OF THE PROCEDURE

1. This test has been evaluated with plasma using EDTA as the anticoagulant. Serum and blood or plasma specimens obtained using other anticoagulants (e.g., heparin or citrate) has not been evaluated and should not be used.
2. Other factors may interfere with the Triage BNP test and may cause erroneous results. These include technical or procedural errors, as well as additional substances in blood specimens that are not listed or exceed the concentrations listed in the Interfering Substances and the Analytical Specificity sections.
3. Samples can be accurately measured within the analytic range of the lower limit of detection and the highest calibrator value [approximately 1–5000 pg/mL].
    a. If a sample contains more than the stated value of the highest Triage BNP Calibrator (S5), report the result as greater than that value [i.e. > 5000 pg/mL]. Alternatively, dilute one volume of sample with one volume of Access Wash Buffer. Refer to the appropriate system manuals and/ or Help system for instructions on entering a sample dilution in a test request. The system reports the results adjusted for the dilution.
    b. See AMR range.
2. Human anti-mouse antibodies (HAMA) may be present in samples from patients who have received immunotherapy utilizing monoclonal antibodies. Additionally, other heterophile antibodies such as human anti-goat antibodies, may be present in patient samples. This test has been specifically formulated to minimize the effects of these antibodies on the assay. However, carefully evaluate results from patients suspected of having such antibodies.
3. The Triage BNP test does not demonstrate any “hook” effect up to BNP concentrations greater than 150,000 pg/mL.
4. The Triage BNP test results should be interpreted in light of the total clinical presentation of the patient, including: clinical history, data from additional tests and other appropriate information.

.ASSISTANCE

If you have any questions regarding the use of this product, please call Biosite’s Technical Services number at 1-888-BIOSITE/1-888-246-7483 (toll-free in the U.S.) or 858-455-4808, 7 days per week, 24 hours per day.

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