

N-terminal pro B-type natriuretic peptide (NT-proBNP) (serum, plasma)

1 Name and description of analyte

1.1 Name of analyte

N-terminal pro B-type natriuretic peptide (NT-proBNP)

1.2 Alternative names

None; not to be confused with proBNP or BNP, which are separate analytes

1.3 NLMC code

To follow

1.4. Physiology and function(s) of analyte

Location

B-type natriuretic peptide (BNP) is a 32 amino acid peptide hormone that is part of the family of natriuretic peptides (NPs), which include A-type natriuretic peptide (ANP, previously known as atrial natriuretic factor, ANF) and C-type natriuretic peptide (CNP). BNP is present in the brain, cardiac atria and ventricles although there is a higher concentration expressed in the ventricles than atria.

Expression and release

The gene encoding BNP is located on the short arm of chromosome 1 (1p36.2). Gene transcription is rapid (<1 h) in response to stimuli and the hormone is constitutively synthesized as required; hence it is referred to as a 'cardiac emergency' hormone. Unlike ANP, which is stored in secretory granules in the atria, BNP is not stored. However, there is some evidence that BNP is stored as a 45 amino acid precursor peptide, BNP-45. The main stimulus for BNP (and therefore NT-proBNP) release is cardiac wall stretch or pressure overload of the heart. Other stimuli for release include: ischaemia, hypoxia, endothelin1, angiotensin-II, interleukins, and adrenergic agonists. There are no endogenous inhibitors but pharmacological inhibitors are available e.g. HS-142-1 and anantin.

Processing

The BNP mRNA encodes a 134 amino acid length pre-pro-hormone, which is cleaved by neutral endopeptidase (NEPs) into a 108 amino acid prohormone (pro-BNP) and a small 26 amino acid signal peptide. On secretion into circulation, proBNP is further cleaved by the enzyme corin into a biologically active 32 amino acid C-terminal fragment (BNP) and a 76 amino acid N-terminal fragment (NT-proBNP). BNP and NP-pro-BNP are secreted in equimolar proportions (see Fig. 1).

Clearance and degradation

NEPs expressed in the proximal renal tubules, vascular endothelium, lungs and heart degrade BNP in the circulation. There is also a clearance receptor referred to as the natriuretic peptide receptor C (NPRC) that binds, internalises and then degrades the NPs (receptor-mediated endocytosis). This receptor has higher affinity for CNP and ANP than for BNP, accounting for BNP's comparatively longer half-life in the

circulation. NPRC is usually saturated in disease states in which BNP is increased and NEPs therefore have a more significant role in degradation and clearance. Renal excretion is the main route of clearance for ANP, BNP and NT-proBNP. The half-life of BNP in plasma is about 20 minutes whereas that for NT-proBNP is 60-120 minutes.

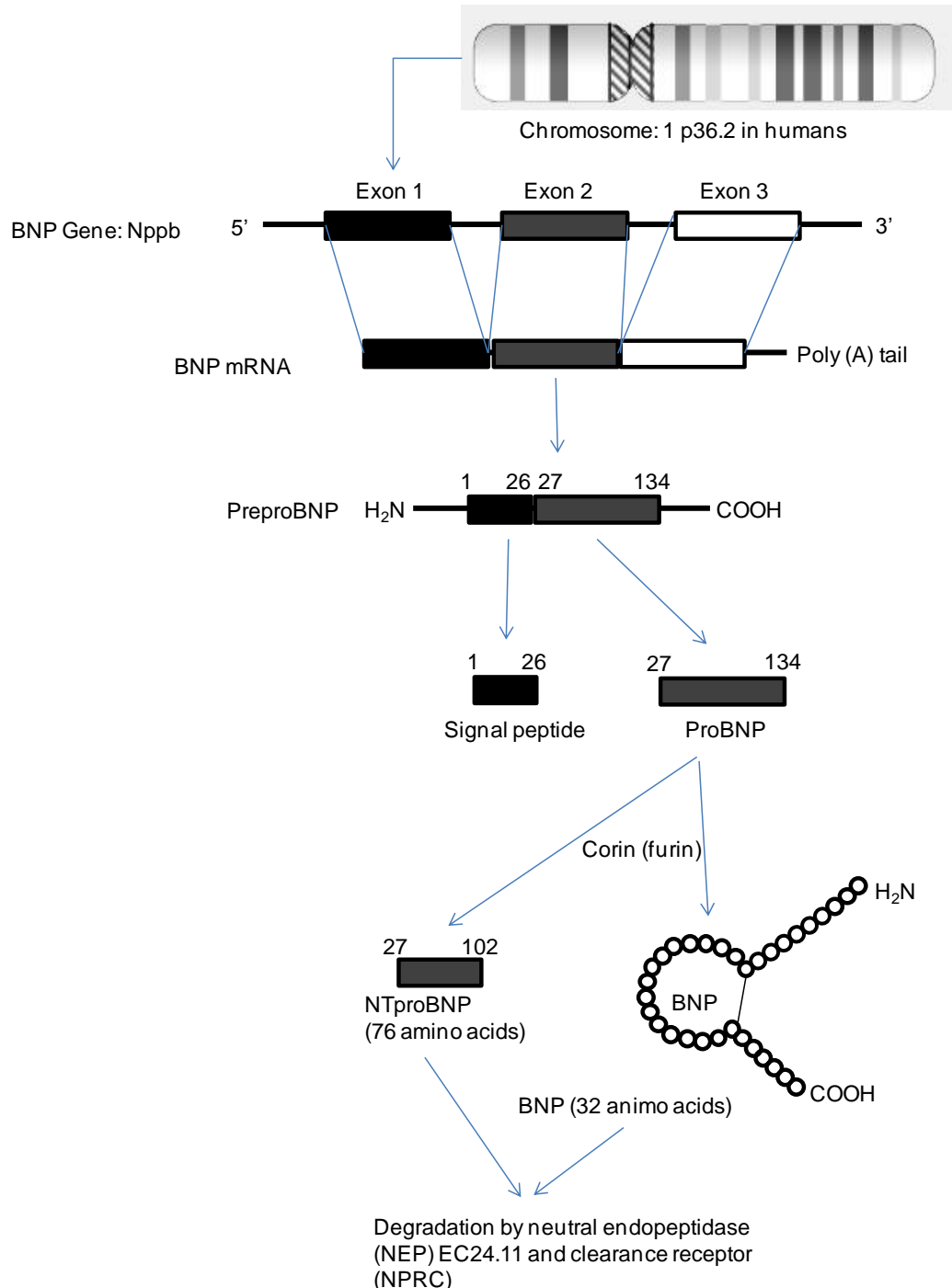


Figure 1. From gene to peptide: processing and clearance of the B-type natriuretic peptide (BNP) and NT-proBNP

Reception and action

BNP (and ANP) mediate their functions on target cells by binding to the natriuretic peptide receptor A (NPRA or guanylyl cyclase receptor A, GC-A), which is linked to the particulate guanylyl cyclase transduction system that catalyse the conversion of guanosine triphosphate (GTP) to cyclic

guanosine monophosphate (cGMP). cGMP is a classic intracellular second messenger that works via cGMP-dependent protein kinases (PKG) and phosphodiesterases (PDEs) to induce biological effects e.g. natriuresis.

Biological functions

BNP is the bioactive peptide and has the following physiological effects:

- antagonism of renin and aldosterone production
- antagonism of the sympathetic nervous system activity
- natriuresis: the natriuretic effect is two staged: short-term increase in glomerular filtration rate coupled with antagonism of sodium reabsorption in proximal tubule and a longer term inhibition of sodium reabsorption in the thick ascending limb of the collecting duct
- diuresis
- vasodilatory and antiproliferative effects on blood vessels.

2 Sample requirements and precautions

2.1 Medium in which measured

NT-proBNP is measured in serum or plasma (heparin or EDTA). Point-of-care (PoC) devices also use whole blood (see Table 1).

2.2 Precautions re sampling, handling etc

There are no specific sampling requirements.

3 Summary of clinical uses and limitations of measurements

3.1 Uses

1. Diagnosis of decompensated heart failure.

The high negative predictive value (NPV) (>97%) enables the rapid exclusion of heart failure (so is a good 'rule-out' test). See section 6.1.

2. Differential diagnosis of dyspnoea/shortness of breath in patients presenting to an Emergency Department or their primary care practice (GPs).

3. Risk stratification and assessment of prognosis in a range of cardiovascular diseases especially acute coronary syndromes (ACS).

4. Monitoring treatment for heart failure.

There is evidence that using natriuretic peptides to guide therapy gives better outcomes than clinical judgement. For example, the BATTLESCARRED trial (Lainchbury JG *et al.* J Am Coll Cardiol 2010; 55:53-60) showed improved outcomes up to 2 years with NT-proBNP-guided treatment strategy in patients with heart failure aged <75 years of age. However, in those >75 years, this may not be better than symptom-guided therapy (Maisel AJ. Am Coll Cardiol 2010; 55:61-4).

5. To establish the severity of heart failure.

BNP correlates with the New York Heart Association (NYHA) classification for heart failure.

3.2 Limitations

See section 6.2.

4 Analytical considerations

4.1 Analytical methods

The methods available for NT-proBNP and the principles of method used and other pertinent variables are listed in Table 1.

Table 1. N-Terminal pro-B-type Natriuretic Peptide (NT-proBNP) assays (aa: amino acid length)

| Assay: analyser | Principle of method | Capture antibody | Detection antibody | Standard material | Sample type required |
|---|---|--|---|-----------------------------|--|
| Roche NT-proBNP I: Elecsys E170, Cobas e601, e602 | Sandwich electro- chemiluminescence immunoassay (ECLIA) | N-terminal polyclonal sheep antibody, aa 14–21 | Central molecule, polyclonal sheep, aa 39–50 | Synthetic NT-proBNP 1–76 | Serum or plasma (heparin or EDTA) |
| Roche NT-proBNP II: Elecsys E170, Cobas e601, e602 | Sandwich electro- chemiluminescence immunoassay (ECLIA) | Murine monoclonal antibody, aa 27–31 | Sheep monoclonal antibody, aa 42–46 | Synthetic NT-proBNP 1–76 | Serum or plasma (heparin or EDTA) |
| Siemens (Dade Behring): Dimension RxL, Stratus CS, Dimension VISTA | Chemiluminescence immunoassay | N-terminal monoclonal sheep antibody, aa 22–28 | Central molecule, sheep monoclonal antibody, aa 42–46 | Synthetic NT-proBNP 1–76 | Serum or plasma (heparin or EDTA) |
| Siemens (DPC): Immulite 1000, 2000, 2500 | Chemiluminescence immuno-assay | N-terminal polyclonal sheep antibody, aa 1- 21 | Central molecule, polyclonal sheep antibody, aa 39-50 | Synthetic NT-proBNP 1-76 | Serum or plasma (heparin or EDTA) |
| Ortho-Clinical Diagnostic: Vitros ECI | Chemiluminescence immunoassay | N-terminal polyclonal sheep antibody, aa 1- 21 | Central molecule, polyclonal sheep antibody, aa 39–50 | Synthetic NT-proBNP 1–76 | Serum or plasma (heparin or EDTA) |
| Point-of-Care (POC) assays | | | | | |
| bio-Merieux: VIDAS | ELISA with fluorescent detection (ELFA principle) | N-terminal polyclonal sheep antibody, aa 1- 21 | Central molecule, polyclonal sheep antibody, aa 39–50 | Synthetic NT-proBNP 1–76 | Plasma (heparin or EDTA) or whole blood |
| Mitsubishi Chemical: PATHFAST | Chemiluminescence immuneassay with | N-terminal polyclonal sheep antibody, aa 1- | Central molecule, polyclonal sheep | Synthetic NT-proBNP 1–76 | Plasma (heparin or EDTA) or whole blood |

| | | | | | |
|----------------------------------|---|---|--|--------------------------|---|
| | patented Magstration technology (separation of magnetic particles coated with antibody) | 21 | antibody, aa 39–50 | | |
| Response Biomedical RAMP | Quantitative immunochromatographic test with fluorescent detection | Murine monoclonal antibody, aa 27–31 | Central molecule, polyclonal sheep antibody, aa 39–50 | Synthetic NT-proBNP 1–76 | Whole blood |
| Nanogen: LifeSign DXpress Reader | Immunochromatographic test with biotin coupled anti-NT-proBNP antibody and streptavidin solid-phase | Monoclonal mouse and polyclonal goat antibodies | Polyclonal sheep antibody | Synthetic NT-proBNP 1–76 | Plasma (heparin or EDTA) or whole blood |
| Radio-meter: AQT90 FLEX | Time-resolved fluorescence immunoassay | Similar to Roche above | Europium chelate-labelled fluorescent tracer antibody (similar to Roche above) | | Plasma (heparin or EDTA) or whole blood |

For latest details, refer to the manufacturer's websites.

4.2 Reference method

There is no reference method for NT-proBNP.

4.3 Reference materials

All assays are traceable to purified, synthetic NT-proBNP material in human plasma but as of February 2013 there was no listed certified standard reference material (SRM).

4.4 Interfering substances

In general, the assay is unaffected by icterus (bilirubin <428 µmol/L), haemolysis (haemoglobin <0.621 mmol/L or <1.0 g/dL) and lipaemia (triglycerides <17.1 mmol/L). If the patient is on high-dose biotin therapy, sampling should be undertaken after at least 8 h of the last dose. For the AQT90 FLEX POC assay, notable interference was observed by some drugs, e.g. acetylcysteine, ciclosporin and furosemide. There is no high-dose hook effect up to 33,400 pmol/L (300,000 ng/L) for the Cobas assay.

4.5 Sources of error

See section 6.2

5 Reference intervals and variance

5.1.1 Reference interval (adults)

Decision thresholds for NT-proBNP:

<75 years: >125 ng/L

≥75 years: >450 ng/L

NT-proBNP values <125 ng/L (pg/mL) exclude cardiac dysfunction in patients suspected of having heart failure. Values >125 ng/L may indicate cardiac dysfunction and are associated with an increased risk of cardiac complications (MI, heart failure or death). Data using Roche proBNP II assay from the manufacturer's kit insert (2012) states that in comparing patients with stable heart failure (n=721) to the reference group (n=2264), ROC-plot analysis at the cut-off value of 125 ng/L showed a sensitivity of 88%, specificity of 92%, negative predictive value of 96.7% and positive predictive value of 80.6%.

5.1.2 Reference intervals (others)

The NT-proBNP concentration had been ascertained in children (1–18 years) using the Roche proBNP and Biomedica NT-proBNP assays (Albers *S et al. Clin Chem Lab Med* 2006; 44:80-85). There was no significant age-dependency with the Biomedica assay although values were generally higher ranging from a median concentration 1954 (1–3 years) to 1247 ng/L (18 years). The Roche NT-proBNP age-dependent reference range is shown in Table 2 below.

5.1.3 Extent of variation

5.1.3.1 Interindividual CV: not known

5.1.3.2 Intraindividual CV: 33–50% (Wu AH *et al. Am J Cardiol* 2003; 92:628-631)

5.1.3.3 Index of individuality: not known

5.1.3.4 CV of method: using human plasma pools, within-run CV of 1.3–2.4% and total CV of 2.9–6.1% are achievable with a wide measuring range (30 – 35,000 ng/L) (Wu AH *et al.* Clin Chim Acta 2003; 338:107-115)

5.1.3.5 Critical difference: it has been established that a change of at least 90% for NT-proBNP was required for serially collected data to be considered significantly different (Wu AH *et al.*, Am J Cardiol 2003; 92:628-631)

Table 2. Age-dependent reference range for the Roche NT-proBNP assay.

| Age (years) | Roche NT-proBNP (ng/L) | |
|-------------|--------------------------|----------------------------|
| | 75 th centile | 97.5 th centile |
| 1–3 | 231.2 | 319.9 |
| 4–6 | 112.6 | 189.7 |
| 7–9 | 94.4 | 144.7 |
| 10 | 72.5 | 112.4 |
| 11 | 93.4 | 317.1 |
| 12 | 95.0 | 186.4 |
| 13 | 113.6 | 369.9 |
| 14 | 68.2 | 362.8 |
| 15 | 73.6 | 216.7 |
| 16 | 84.9 | 206.0 |
| 17 | 71.0 | 134.9 |
| 18 | 53.3 | 114.9 |

5.1.4 Sources of variation
See section 6.2.

6 Clinical uses of measurement and interpretation of results

6.1 Indications and interpretation

NT-proBNP is an important adjunct to clinical assessment, ECG, chest X-ray and echocardiography in patients suspected of having heart failure. BNP concentrations have been shown to be raised in patients with left ventricular dysfunction and correlate with the New York Heart Association (NYHA) classification for heart failure (Maisel AS *et al.* N Engl J Med 2002; 347:161-167). The clinical indications for BNP/NT-proBNP are listed in section 3.1.

Although BNP has potent natriuretic and diuretic effects, paradoxically in heart failure there is congestion, sodium retention and oedema. Recent studies have proposed that this is due to insufficient post-translational maturation of the biosynthetic precursors (Goetze JP *et al.* Eur Heart J 2003; 24:1471-1472) affecting the bioactive potency of the hormone.

The National Institute for Health and Care Excellence (NICE) clinical guideline 187 (2014) on diagnosing and managing acute heart failure in adults recommends a single measurement of NT-proBNP (or BNP) at presentation to rule out heart failure. The suggested thresholds for

excluding acute heart failure are: NT-proBNP less than 300 ng/L or BNP less than 100 ng/L. If these levels are exceeded, echocardiography is recommended within 48 hours to guide early specialist management.

The NICE clinical guideline CG108 (2010) on the management of chronic heart failure in adults in primary and secondary care recommends:

- In patients presenting with symptoms such as breathlessness in whom heart failure is suspected, refer directly for specialist assessment and echocardiography within 2 weeks if the patient has a history of myocardial infarction. A systematic review by Mant et al that informed this clinical guideline (Health Technol Assess 2009; 13: 1-207) previously also recommended basal crepitations or male with ankle oedema as triggers for referral within 2 weeks.
- Otherwise, measure BNP or NT-proBNP and refer for specialist assessment and echocardiography depending on the results:
 - High concentrations (BNP > 400 ng/L or 116 pmol/L; NT-proBNP > 2000 ng/L or 236 pmol/L) refer within 2 weeks.
 - Raised concentrations (BNP > 100-400 ng/L or 29-116 pmol/L; NT-proBNP > 400-2000 ng/L or 47-236 pmol/L) refer within 6 weeks.

6.2 Confounding factors

NT-proBNP/BNP concentrations are affected by the following factors:

- dietary sodium intake
increases NT-proBNP/BNP release
- age
increases with age (see section 5 above) probably reflecting diminishing kidney function
- gender
higher in women than men but the reason remains unknown.
- renal failure
there is an inverse, moderate but significant correlation between eGFR and NT-proBNP or BNP concentrations. Moreover, there is increased cardiovascular disease in patients with chronic kidney disease (CKD). Vickery et al (Am J Kidney Dis, 2005; 46:610-20) concluded that eGFR and left ventricular mass index have independent effects on NTproBNP or BNP concentrations in patients with CKD. Taken together, elevated NTproBNP and BNP are due to impaired renal excretion (NTproBNP to a greater extent than BNP) and the presence of concomitant heart disease
- exercise
increases circulating NT-proBNP and BNP concentrations. The mechanisms remain unknown
- heart rate
inverse relation with an increment of 10 beats/min resulting in a 9% reduction in BNP and 15% reduction in NT-proBNP
- pulsatility and intra-individual variability
BNP release has been shown to be pulsatile and there is significant intra-individual variability in both healthy and stable heart failure subjects

- drugs
decreased by diuretics, ACE inhibitors, aldosterone antagonists, angiotensin-II receptor inhibitors and nitrates
- obesity
decreases both NTproBNP and BNP but the mechanism is unknown
- other cardiovascular disease
disorders such as myocardial infarction, pulmonary embolism, valvular disease, hypertension, tachyarrhythmias are stimuli for NT-proBNP/BNP release
- diabetes mellitus
increases NT-proBNP/BNP and may be related to underlying heart or renal disease
- endocrine disorders
hyperaldosteronism and hyperthyroidism have been reported to increase NT-proBNP and BNP concentrations.

7 Causes of abnormal results

7.1 High values

7.1.1 Causes

- heart failure
- other conditions (see 6.2)

7.1.2 Investigation

NT-proBNP should only be measured when heart failure is suspected as discussed in sections 3.1 and 6.1.

7.2 Low values

7.2.1 Causes

- obesity
- drugs (see 6.2)

7.2.2 Investigation

No specific investigations. Medication (see 6.2) may need to be discontinued (with close monitoring) before repeat NT-proBNP measurement.

7.3 Notes

None

8 Performance

8.1 Sensitivity, specificity etc. for individual conditions

A systematic review by Mant *J et al*, (Health Technol Assess 2009; 13(32): 1-207) reported that:

- dyspnoea was the only clinical symptom or sign with high sensitivity (89%), but it had low specificity (51%) for heart failure
- the sensitivities for ECG, BNP and NT-proBNP were all high at 89, 93 and 93% respectively

- BNP was more accurate than ECG
- there was no difference between the diagnostic accuracy of BNP and NT-proBNP
- a model was derived, which has subsequently been incorporated into the NICE guideline 108 (see section 6.1)
- this decision rule was found to be cost-effective to the NHS in terms of cost per additional case detected and resulted in improved patient life expectancy.

The ProBNP Investigation of Dyspnoea in the Emergency Department (PRIDE) study (Januzzi J *et al.* Am J Cardiol 2005; 95: 948-954) enrolled 600 patients presenting with dyspnoea in the Emergency Department. The clinical diagnosis was adjudicated by physicians blinded to NT-proBNP results. The sensitivity and specificity of NT-proBNP at various cut-offs is shown in Table 3 below. The study concluded that whereas NT-proBNP was superior to clinical judgment alone for diagnosing acute heart failure, NT-proBNP together with clinical judgement was superior to NT-proBNP or clinical judgement alone i.e. NT-proBNP was an important adjunct to clinical assessment in the identification and exclusion of acute heart failure in the Emergency Department.

Table 3. Receiver-operating characteristics (ROC) analysis for NT-proBNP in the diagnosis of acute heart failure.

| Cut-off (ng/L) | Sensitivity (%) | Specificity (%) | Positive predictive value (%) | Negative predictive value (%) | Accuracy (%) |
|----------------|-----------------|-----------------|-------------------------------|-------------------------------|--------------|
| 300 | 99 | 68 | 62 | 99 | 79 |
| 450 | 98 | 76 | 68 | 99 | 83 |
| 600 | 96 | 81 | 73 | 97 | 86 |
| 900 | 90 | 85 | 76 | 94 | 87 |
| 1000 | 87 | 86 | 78 | 91 | 87 |

9 Systematic reviews and guidelines

9.1 Systematic reviews

Mant J, Doust J, Roalfe A *et al.* Health Technol Assess 2009; 13(32): 1-207. Systematic review and individual patient data meta-analysis of diagnosis of heart failure, with modelling of implications of different diagnostic strategies in primary care.

9.2 Guidelines

1. The National Institute of Health and Care Excellence (NICE). Chronic heart failure. Management of chronic heart failure in adults in primary and secondary care. Clinical Guideline 108 (2010).

<http://www.nice.org.uk/guidance/cg108>

2. The National Institute of Health and Care Excellence (NICE). Chronic heart failure. Acute heart failure. Diagnosing and managing acute heart failure in adults. Clinical Guideline 187 (2014).

<http://www.nice.org.uk/guidance/cg187>

- 9.3 Recommendations
See sections 6.1

10 Links

- 10.1 Related analytes
1. B-type (brain) natriuretic peptide (BNP)

2. proBNP – the precursor of BNP and NT-proBNP

There is emerging data that proBNP may be a better biomarker for heart failure than either BNP or NT-proBNP (Emdin M, Passino C. J Am Coll Cardiol, 2011; 57: 1396-1398) but this is beyond the scope of this article. It is also important to note that all current immunoassays for NT-proBNP and BNP cross-react with proBNP.

BNP versus NT-proBNP

In the foregoing sections, the biomarkers may have been described interchangeably. As described in Section 1, BNP and NT-proBNP are produced in equimolar proportions with the former being the bioactive.

It is generally accepted that both biomarkers provide essentially the same information although in one study, NT-proBNP had been shown to be far superior to BNP in predicting mortality, morbidity and hospitalisation for heart failure (Masson *S et al*, Clin Chem 2006; 52; 1528-1538). The NICE guidelines listed above do not give preference to either biomarker.

NT-proBNP has better in vitro stability than BNP. In addition, BNP is degraded in glass bottles as a result of enzyme activation by glass.

Renal insufficiency has a greater effect on NT-proBNP than BNP as the former is mainly cleared by the kidney. The half-life of BNP is about 20 minutes whereas that for NT-proBNP is 60-120 minutes. See section 1.4 and 6.2.

- 10.2 Related tests
None

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