

B-Type Natriuretic Peptides

Analytical and Clinical Considerations

BY FRED S. APPLE, PHD

Since 2000 when the first assay for B-type natriuretic peptide (BNP) received clearance from the Food and Drug Administration (FDA), this test has become a "blockbuster" hit for both ruling out and for diagnosing congestive heart failure (CHF). In my 24 years in clinical chemistry, few assays have taken on such a prominent role so quickly in the management of patients.

The rapid acceptance of the BNP test as a diagnostic tool stems in part from the steep rise in CHF over the past decade. At present 5 million Americans have CHF, and nearly 500,000 new cases are diagnosed every year. Not only is the U.S. population aging, but more people are surviving longer with cardiovascular conditions. In addition, the demand for this assay is likely to grow as more studies are published supporting the use of BNP as a risk stratification marker in acute coronary syndrome (ACS) patients.

Echocardiography is considered the gold standard for the detection of left ventricular dysfunction, but it is expensive and does not always reflect acute cardiac conditions. Therefore, when the BNP test became available, physicians welcomed it as a more cost-effective tool for rapidly and accurately differentiating CHF from other causes of dyspnea.

The BNP protein is classified as a cardiac neurohormone, and research has shown that it is secreted from the cardiac ventricles specifically in response to ventricular volume expansion, pressure overload, and the resultant increase in wall tension. Although

guidelines are available, laboratorians need to address how best to implement this test in the lab, as well as decide which assay—BNP or N-terminal-proBNP (NT-proBNP)—to offer clinicians.

Choosing an Assay

The development and commercialization of these assays has been controlled by intellectual property rights related to the use of the BNP antigens and antibodies. Currently, laboratorians have a choice of five BNP and two NT-proBNP assays (Table 1), all of which are used to rule in or rule out CHF.

analyzer, as well as to Response Biomedical, Corp. (Vancouver, B.C.). Response Biomedical plans to develop a rapid, quantitative RAMP test for BNP for the Japanese market.

Roche Diagnostics (Indianapolis, Ind.) maintains patent rights for use of NT-proBNP and has sublicensed their antibodies and antigen to several manufacturers, including Dade Behring (Deer Park, Ill.), Diagnostic Products Corporation (DPC, Los Angeles, Calif.), and others. Dade Behring's assay has received FDA clearance, while DPC is currently developing their test.

In the large majority of laboratories, the choice of BNP assay will likely depend on which manufacturer's instrument the central laboratory uses. The 2004 College of American Pathologists' (CAP) BNP-B Survey shows a summary of participants' assay use for two BNP and two NT-proBNP survey materials (Table 2). Based on the BNP-03 material, of the 2,163 labs that reported BNP results, 73% used the Biosite Triage assay, followed by the Bayer Centaur or ACS 180 assay (16%), the Abbott AxSYM assay (6%), and the Beckman Access assay (5%). For NT-proBNP, the Roche assay had 233 users, which accounts for only 10% of the overall usage. The Dade Behring Dimension assay for NT-proBNP was not available at the time of the survey. Clearly, as more of the licensed manufacturers introduce assays, the distribution of BNP and NT-proBNP usage is likely to change.

Which Assay is Better?

As laboratorians have learned from their experience with the cardiac troponin assays over the past decade, not all assays are created equal. The same is true for BNP assays. Each manufacturer's assay employs different sets of antibodies that detect one of the two forms of BNP, either the N-terminal propeptide (NT-proBNP) or the cleaved peptide (BNP), and therefore the absolute concentration of BNP in a blood sample will vary depending on the assay used. More importantly, classification of the severity of a patient's CHF, as defined by the New York Heart Association's (NYHA) guidelines for mild (I) to severe (IV) disease, depends on the lab's choice of assay. A comparison of the manufacturers' package inserts illustrates the rather substantial impact of this variation on the NYHA classification of CHF severity (Table 3).

the marker has quickly gained acceptance by many cardiologists, the 2001 American College of Cardiology/American Heart Association practice guidelines for the evaluation and management of CHF fell short of recommending BNP testing, stating that its role in the identification of patients with CHF needs to be clarified. Nevertheless, the availability of this blood test is changing the way cardiologists diagnose the severity of heart failure and the response of heart failure patients to therapy. Until more definitive

Three companies manufacture BNP assays: Biosite Diagnostics (San Diego, Calif.), Bayer Healthcare (Tarrytown, N.Y.), and Shionogi & Co., Ltd (Osaka, Japan). In addition, Biosite has partnered with Beckman Coulter (Fullerton, Calif.) to provide an automated version of Biosite's Triage point-of-care (POC) assay. Shionogi, which has exclusive rights to BNP in Japan but does not have an FDA-cleared assay in the U.S., has licensed the assay to Abbott Laboratories (Abbott Park, Ill.) for the AxSYM immuno-



Table 1
**Configuration of Commercial BNP
 And NT-proBNP Assays**

Assay	Antigen	Capture Ab	Detection Ab
Abbott	BNP a.a. 1–32 a.a. 3–32	Scios (ring)	Shionogi (C-terminus)
Bayer	BNP a.a. 1–32 a.a. 3–32	Shionogi (ring)	Shionogi (C-terminus)
Biosite and Beckman	BNP a.a. 1–32	Scios (ring)	Biosite (N-terminus)
Roche and Dade	NT-pro BNP a.a. 1–76	Roche (N-terminus)	Roche (Central)

The table lists the manufacturer of the capture and detection antibodies for the BNP assays sold by each company in the left-hand column. Scios, Inc. (Freemont, Calif.)

BNP is synthesized as a pro-hormone (108 a.a.) that is subsequently cleaved into the physiological active BNP moiety (32 a.a., including a ring in its structure) and the linear 76 a.a. NT-proBNP moiety.

Abbreviations: a.a. (amino acid), Ab (antibody)

A review of the 2004 CAP BNP-B survey results also shows how widely measurements of BNP concentration can vary from lab to lab, where reported BNP values ranged from 213 ng/L to 802 ng/L, approximately a 3.8-fold difference. Furthermore, no primary reference material has been identified to standardize the BNP assays.

Only Biosite currently offers a POC assay for BNP. Therefore labs that use the Beckman Coulter Access analyzer can directly compare results from the central lab to those from POC sites since the two assays use the same antibody, but other central lab and POC results may not be comparable.

Due to the current lack of standardization for BNP assays, laboratorians should refer to the evidence-based literature in order to guide physicians in the interpretation of test results. More importantly, each lab should determine their own BNP age- and gender-specific concentrations, define reference limits, and set medical decision cutoffs for the particular assay system they use. For the measurement of NT-proBNP concentration, these concerns may not be an issue, as the antibodies and antigen materials for all

NT-proBNP assays reported in the literature should be harmonized to those of the Roche Elecsys immunoassay system.

What's the Right Cutoff?

The majority of BNP manufacturers appear to have set their assay's cutoff at 100 ng/L based on Biosite's initial findings for ruling out CHF in dyspnea patients triaged in emergency department settings. However, regression analyses above and below this concentration show substantial variation between assays, and therefore laboratorians need to understand the specific characteristics of the assay they elect to use.

Most importantly, laboratorians should be proactive in educating clinicians about the potential for misinterpreting patients' results. As they read the medical literature on BNP, clinicians may not be aware of the substantial impact the lab's choice of assay manufacturer has on the reported value. Furthermore, clinicians who practice at multiple hospitals also need to be aware of how results from one hospital may not be comparable to those of another hospital. The difference will be quite striking, espe-

Table 2
**Summary of the CAP 2004
 BNP-B Participant Survey***

Assay	Labs (N)	Mean (ng/L)
BNP		
Abbott AxSYM	124	497
Bayer ACS 180	38	575
Bayer ADVIA Centaur	309	476
Beckman Access (Biosite)	118	665
Biosite Triage	1,574	405
NT-proBNP		
Dade Dimension	5	NR
Roche E170	32	286
Roche Elecsys	196	278

NR = not reporting

*Adapted from survey findings.

Table 3
**Comparison of Manufacturers' Values
 for CHF Severity**

Manufacturer	New York Heart Association Classification			
	I	II	III	IV
Abbott BNP	133	266	335	1531
Biosite BNP	95	221	459	1006
Bayer BNP	211	365	536	940
Roche NT-proBNP	1,015	1,666	3,029	3,465

Median concentrations (ng/L) are for males and females combined. Values are taken from the manufacturer's package insert.

cially if the clinician compares a BNP result to a NT-proBNP result.

Two laboratory medicine groups—the National Academy of Clinical Biochemistry (NACB) and the International Federation of Clinical Chemistry (IFCC) Committee for Standardization of Markers of Cardiac

Damage (C-SMCD)—are preparing manuscripts for publication that will address analytical specifications and clinical interpretation guidelines for BNP and NT-proBNP assays. These documents will be essential for clinicians' understanding of the role of BNP in managing CHF patients. The guidelines

Quality Specifications for BNP and NT-proBNP Assays

Analytical

1. Calibration characterization
2. Assay specification, including antibody cross reactivity characteristics to structurally related, metabolized, and degraded forms
3. Imprecision characteristics
4. Lower detection limits
5. International standardization of immunoassays

Pre-analytical

1. Specimen collection tubes
2. Temperature dependant stabilities of specimens post collection

will also help laboratorians better understand the subtleties of the various BNP and NT-proBNP assays, as well as foster closer collaboration among manufacturers, clinicians, and laboratory scientists.

Over the next year, the IFCC C-SMCD will work on two initiatives: developing primary and secondary materials for BNP and NT-proBNP, and determining a better understanding of what forms of BNP (i.e. proBNP) cross react with the current assays in the marketplace. From the laboratory's perspective, it will also be important for manufacturers to address quality specifications for both markers, including the analytical and pre-analytical issues (see box above).

Growing Clinical Utility

Numerous clinical studies have shown BNP and NT-proBNP to be powerful diagnostic and prognostic markers of heart failure. In addition, evidence is growing for the use of these markers in the diagnosis and management of ACS, and recent findings continue

to confirm the important pathophysiological relationships between the severity of an acute ischemic insult and the increase in levels of circulating BNP. Overall, many experts believe these findings suggest that BNP should be incorporated into a multi-marker strategy for risk stratification to improve the management of myocardial dysfunction associated with myocardial ischemia.

Undoubtedly, there is a long road ahead to optimize clinical utilization of BNP or NT-proBNP. Although there has been some debate about which marker—BNP or NT-proBNP—is better, based on studies reported in the literature, I don't believe either form of the marker is clearly superior. Each marker has subtle differences both clinically and analytically, and presently, both laboratory medicine and cardiology groups are endorsing use of either. **CLN**

SUGGESTED READING

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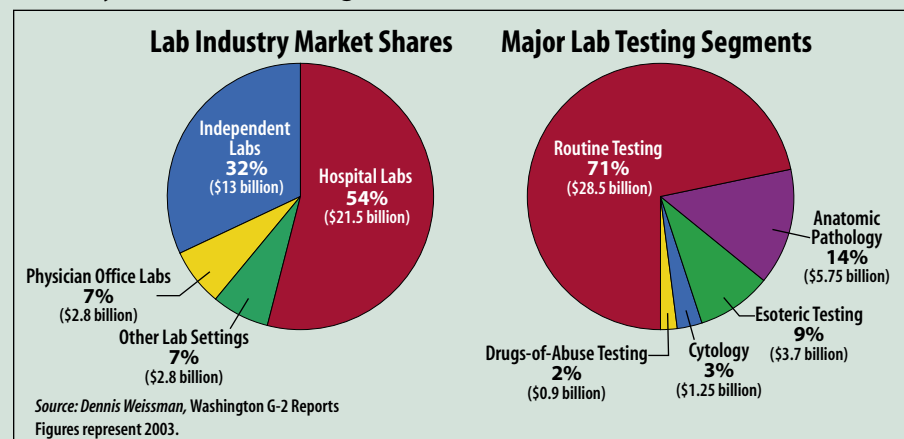
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Sizing Up the Lab Testing Market Study Puts Growth at Annual Rate of 6.8%

BY NANCY SASAVAGE, PHD

Often clinical laboratorians may feel like they are fighting a losing battle. Budgets are constantly being squeezed as the health care system attempts to control expenses. But a look at recent growth in the U.S. lab testing market could give the lab community a reason to smile.

According to a recently completed study by *Washington G-2 Reports*, labs experienced healthy growth in the first part of this decade. "Spending on lab services, including anatomic pathology, grew at an annual rate of 6.8% from 2001–2003, to \$40.1 billion dollars," said Dennis Weissman, President of Dennis Weissman & Associates, LCC (Washington, D.C.), an independent news and information company serving the health care industry. "This represents a big increase from the estimated 1% growth during the previous three years." Weissman founded G-2 Reports, an independent news and information company serving the health care industry, and he spoke about the lab market and trends at a December conference sponsored by Ortho-Clinical Diagnostics (Raritan, N.J.).



On top of this recent market growth, the forecast for the future also looks positive. "The lab [market] is projected to grow by 7.1% over the next ten years, following the overall health [care] spending trend," Weissman predicted. While lab expenditures comprise only 2.9% of total health care spending, Weissman contends that lab testing is the cornerstone for most treatment decisions. And most laboratorians likely would agree.

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FDA Clears First Gene Chip For Pharmacogenomics

BY BILL MALONE

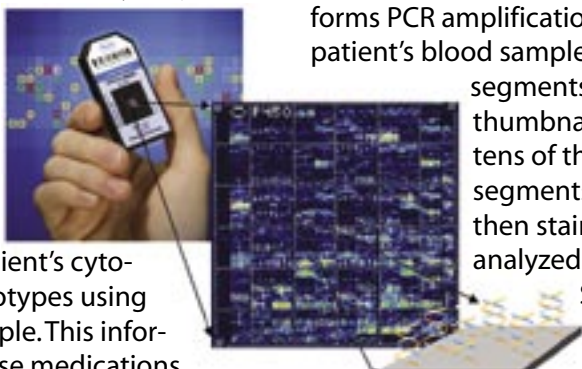
In a decision marking the first time a microarray chip has been cleared as a diagnostic device, the FDA recently granted 510(k) clearance to Roche Molecular Systems' (Pleasanton, Calif.) AmpliChip CP450 Genotyping test for use on Affymetrix's (Santa Clara, Calif.) GeneChip Microarray Instrumentation System. Many researchers and others have hailed gene chips as the beginning of a new era of personalized medicine. The AmpliChip will allow labs to determine a patient's cytochrome P450 2D6 and 2C19 genotypes using DNA extracted from a blood sample. This information can help physicians choose medications and optimize drug doses based on an individual's genotype, thereby avoiding possible adverse side effects.

The CYP450 2D6 and 2C19 genes encode liver enzymes that make certain classes of drugs more water soluble and easier for the body to eliminate. Variations in the CYP450 genes play a key role in determining how individuals metabolize commonly prescribed drugs, including treatments for depression, cardiovascular disease, and cancer. Knowing which genetic variation an individual may have is important because poor metabolizers risk having excessive or prolonged levels of a drug in their blood, while those who metabolize a drug

too quickly may require higher doses. Roughly 10% of Caucasians and 20% of Asians metabolize drugs poorly, while a smaller proportion of both groups quickly metabolize the same drugs.

To perform the AmpliChip test, a technician performs PCR amplification on purified DNA from a patient's blood sample and then applies labeled DNA segments to the AmpliChip microarray, a thumbnail-sized glass chip arrayed with tens of thousands of DNA fragments. DNA segments that bind to the microarray are then stained with fluorescent dye and analyzed by the Affymetrix laser scanner. Special software analyzes the scan and produces a report of which CYP2D6 and CYP2C19 variations have been detected.

Roche launched the new test in Europe last fall. In the U.S., the company introduced the AmpliChip test in June 2003 as an analyte specific reagent (ASR), but the FDA ruled soon after that the device required 510(k) clearance. The ensuing disagreement between Roche and the FDA regarding the chip's regulatory status stirred a controversy about how IVD companies could interpret the FDA's definition of ASRs and how emerging pharmacogenomic testing technologies would be regulated (*CLN* September 2003 and December 2004).



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