Prostate Specific Antigen (PSA) predominantly exists in human serum as a complex with the serum protease inhibitor α₁-antichymotrypsin (PSA-ACT). It is also present in significant quantities as unbound, free PSA (fPSA), and in very small quantities as a complex with other serum protease inhibitors, such as α₂-macroglobulin (PSA-A2M). Elevated serum levels of total PSA (tPSA = fPSA + PSA-ACT) are detectable in individuals with prostate cancer or benign prostatic hyperplasia (BPH), but the relative amount of fPSA is greater in BPH. Thus, measuring the relative amounts of fPSA and PSA-ACT is useful in distinguishing prostate cancer from BPH. In 1993 Leinonen et al. simultaneously measured fPSA and PSA-ACT in a dual-label immuno-fluorometric assay and reduced the frequency of false-positive prostate cancer diagnoses by 38%. Further evidence was presented at the AACC 1995 Annual Meeting and Clinical Laboratory Exposition held in Anaheim, CA.

Using an assay specific for fPSA and one for PSA-ACT, Chan et al. determined the fPSA/tPSA ratios of 81 subjects. Using a cut-off of 0.12, fPSA:tPSA ratios displayed 82% sensitivity and 63% specificity in distinguishing prostate cancer from BPH. In a larger study, Ward et al. measured the fPSA:tPSA ratios in 243 subjects (cut-off = 0.15) and demonstrated 78% sensitivity and 69% specificity. In contrast, tPSA measurements alone yielded 76% sensitivity and 33% specificity for diagnosing prostate cancer.

What is an Equimolar PSA Assay?

In the Summer '94 issue of Scripps News (Vol. 8, No. 1) we recognized the importance of calibrating PSA assays to account for varying fPSA and PSA-ACT concentrations. A year later, research has shown that PSA test kits must measure fPSA and PSA-ACT on an equimolar basis. That is, the tPSA measurement of a serum sample should be unaffected by changes in the relative concentrations of fPSA and PSA-ACT. To test this, several sets of calibrators (range: 0 - 200 ng/ml) should be prepared, each with a unique percentage of fPSA and PSA-ACT: For instance, calibrator set 1 would be prepared with 100% fPSA and 0% PSA-ACT; Set 2, 80%:20%; Set 3, 60%:40%; Set 4, 40%:60%; Set 5, 20%:80%; and Set 6, 0%:100%

Patient samples would then be run in six different assays calibrated with one of the preceding calibrator sets. If the assay is equimolar, tPSA values will not vary significantly from assay to assay, regardless of the fPSA:PSA-ACT ratio present in the sample. If the assay does not equally detect fPSA and PSA-ACT, the standard curve generated by each calibrator set will vary significantly from assay to assay. As a result, a single patient sample will yield different tPSA measurements between the assays. This is supported by several posters that reported a discrepancy between tPSA values measured by various commercially-available PSA tests. Blankenstein et al. measured tPSA in a sample from a patient with advanced prostate cancer, using nine different PSA assays; they
found that there were equimolar and five showed a preference for fPSA. In another investigation, Strobel et al. compared three PSA assays and found that differences in calibration, antibody specificity, and assay conditions largely contributed to the discrepant results. Arai and Tsukada compared eight PSA assays and found large variances in the kits’ reactions to fPSA: Selecting one kit's reactions to fPSA: 80.7% cross-reactivity with PSA-ACT.

Wang et al. developed a sandwich immunoassay with fPSA:PSA-ACT ratios in the diagnosis and management of prostate cancer. After heat treatment, all PSA reactivity was blocked or conformationally changed. After heat treatment, all PSA reactivity was blocked or conformationally changed. Immunoassays continued to evaluate many commercial PSA kits. The new PSA assays allowed detection was simple, rapid, and gave reproducible results with several different kits. These results suggest that heat inactivation may be useful in the determination of the relative concentrations of fPSA, PSA, and PSA-ACT.

We mentioned above that PSA binds to A2M in human serum. In addition to prostate tissue, PSA is reportedly produced in ovarian tissue, breast carcinoma, amniotic fluid and maternal sera, and in the milk of lactating women. In another report, the PSA gene cloned into an insect cell line and the PSA expressed was virtually identical to native PSA.

In summary, the data presented at the 1995 AACC Annual Meeting confirmed the usefulness of determining fPSA:PSA-ACT ratios in the diagnosis and management of prostate cancer.

Several posters described the importance of equimolar assays for fPSA, ones that will measure fPSA and PSA-ACT equally. Furthermore, many reports reflected the need for an international PSA:PSA-ACT ratio.

The poster sessions also revealed that PSA-A2M levels may be significant in prostate cancer and that PSA is not a prostate tissue-specific antigen as once believed. In the last several years, PSA has received much attention in the diagnosis and management of prostate cancer, and given the results presented here, may be the subject of other cancer research in the future.

**PSA References**


**Table 1. New Assays presented at the 1995 AACC Annual Meeting**

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<th>Specificity</th>
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Recently, the FDA approved the use of two new diagnostic tests to aid the detection of myocardial infarction (MI): human cardiac Troponin T (cTnT) and human cardiac Troponin I (cTnI). cTnI and cTnT are two of the three subunits of the troponin complex, a contraction-regulating protein found in both striated and cardiac muscle. Several posters presented at the 1995 AACC Annual Meeting indicated that these markers are equivalent to, or better than, creatine kinase MB (CK-MB) in confirming MI. However, in agreement with the Summer 1993 issue of Scripps News (Vol. 7, No. 2), additional evidence suggested that cTnI is the only marker specific for cardiac injury.

In a clinical study involving 241 patients, Green et al. measured levels of cTnI, CK-MB, and total CK. Their results revealed 64 subjects with discordant cTnT and CK-MB values. Of these, 84% had elevated cTnT, while CK-MB levels were normal. This led the authors to conclude that cTnT is more sensitive than CK-MB in MI diagnosis. In another study, Collinson et al. monitored cTnT levels in 460 patients at risk for unstable angina (UA). Of the 183 with confirmed UA, 33.9% had cTnT levels above the threshold of 0.2 µg/L. Their findings indicated that cTnT levels may help predict cardiac events in patients presenting with UA.

Likewise, cTnI was studied and found clinically useful in MI diagnosis. As part of a multi-center study, Alonsozana et al. measured cTnI, CK-MB, and total CK in 105 subjects. Their study proved cTnI to be equivalent to CK-MB in distinguishing patients with MI from healthy controls. Both cTnI and cTnT were found to remain elevated in the bloodstream longer than CK-MB. In fact, cTnI or cTnT may be equivalent to the late-stage cardiac markers, lactate dehydrogenase isoenzymes 1 and 2 (LDH-1, LDH-2). Landt et al. found cTnT to be more sensitive than the ratio of LDH-1:LDH-2 in diagnosing MI up to seven days after admission. In a similar study, Murthy & Karmen determined that cTnT remains elevated well above threshold levels for at least one week after CK-MB levels return to normal. They proposed that cTnT is not continually released after MI, but persists in the serum for a longer period of time than other cardiac markers; in addition, they suggested that cTnT may be a better late-marker for MI than LDH-1.

Another poster presented a study suggesting that cTnT and CK-MB may be complementary. Pickeral et al. measured cTnT and CK-MB levels in 50 patients with MI symptoms. The sensitivities for the two markers were identical in diagnosing MI (100%), but the specificities varied slightly: cTnT, 76%; CK-MB, 71%. The authors suggested that in certain cases, combining cTnT and CK-MB assay results may provide more useful data than the results of either test alone.

Two posters reported that cTnT may be useful as an indicator of MI during cardiac surgery. Elevated cTnT levels were indicative of perioperative MI in patients receiving coronary artery bypass grafting, while CK-MB levels were not.12,13

**cTnT and CK-MB are not Cardiac-Specific**

Regarding specificity, cTnI is apparently the only marker specific for cardiac events. CK-MB is known to elevate in skeletal muscle trauma and in renal failure.21,23 Similarly, several posters reported elevated levels of cTnT in patients with renal insufficiency or renal failure.3,14,15,16,17

In addition, Bodor et al. presented data indicating that cTnI is expressed in both healthy and regenerating skeletal muscle. Thus, these reports question the cardiac specificity of cTnT. In contrast, significant levels of cTnT were not found in any of these studies.

In closing, over 30 posters were presented that discuss cTnI, cTnT, or both (Table 1 lists the new troponin assays that were presented at the AACC). In many of these studies, cTnI or cTnT was compared to CK-MB and found to be more sensitive in detecting MI. In addition, they remained elevated in the bloodstream much longer than CK-MB, suggesting that either cTnI or cTnT may be useful when diagnosis is made several days after the onset of symptoms. Additional studies may determine whether either cTnI or cTnT can replace the late-stage markers LDH-1 and LDH-2. One of the most significant findings was that cTnT is elevated in renal disease, while cTnI is not. This suggests that elevated cTnT is not always indicative of cardiac injury, and could contribute to diagnostic confusion in distuinguishing MI from non-MI patients. Thus, it appears that cTnI is the lone biochemical marker specific for cardiac injury.

**Troponin References**


