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Premarket Notification Summary

TpP™ EIA

The ABS, Inc. TpP™ EIA is an enzyme linked immunoassay for the quantitative determination of soluble fibrin polymers in human plasma. It is indicated as an aid in assessing the risk of intravascular thrombosis and monitoring the efficacy of anticoagulant (heparin) therapy. The TpP™ EIA employs a murine monoclonal antibody (MAb), specific for soluble fibrin polymer, as a capture antibody immobilized on a microtiter plate (MTP). This MAb recognizes a conformational epitope present only on the TpP™ entities but which is absent from fibrinogen and degradation products of fibrin and fibrinogen. During the first incubation phase TpP™ in human plasma specimens bind to the capture antibody. Afterwards the plate is rinsed, and a conjugate, another murine monoclonal antibody, labeled with horseradish peroxidase (HRP) is added to the well. This peroxidase conjugated MAb binds to a separate site on the TpP™ molecule during a second incubation period. Excess enzyme conjugated MAb is washed out and a subsequent application and incubation with tetramethylbenzidine (TMB) substrate follows. The reaction after TMB incubation is terminated with dilute sulfuric acid. The level of the TpP™ present in the specimen sample is determined colorimetrically from the enzymatic activity of detection MAb conjugate. The intensity of the color is proportional to the concentration of TpP™. Calibrator standard is provided with the kit.

The data and information in this submission demonstrate that American Biogenetic Sciences' TpP™ EIA is substantially equivalent to Organon Teknika Corporation's immunochemical assay Thrombonostika F1.2®, ~~K9911434~~. K911434

These devices are similar in their intended use and methodology. Both utilize enzyme conjugated antibodies that bind to the analyte and react with the substrate TMB to directly measure species in blood which reflect activation of the coagulation system. These devices are dissimilar in that F1.2 tests measure a molecular entity which is generated in the penultimate stage of fibrin formation, i.e. during the conversion of prothrombin to thrombin. Although prothrombin is converted to thrombin, the latter is not necessarily coincident with fibrin (clot) formation. This is due to multiple inhibitors and substrates of thrombin. TpP™ consists of polymeric soluble fibrin entities which ultimately form fibrin in a clot. TpP™ is, therefore, coincident with fibrin formation as well as an indicator of thrombin activity.

These differences are not considered significant and have no effect on the safety and effectiveness of the product.

In order to determine the expected normal range for the TpP™ EIA, as well as to determine an effective cutoff point, several control populations were tested at several sites.

There were a total of 140 subjects used (115 healthy volunteers from Site #1, 8 healthy volunteers and 17 outpatients from site #2). The best estimate of an effective cutoff value was determined to be 6.65 µg/mL by employing a percentile evaluation.

A clinical study was conducted at Johns Hopkins Medical School, Baltimore, MD. Ninety (90) patients were recruited from a population of adults of both sexes, age 17 and older who were undergoing procedures to repair aortic aneurysm. There was a positive correlation (0.58, p=0.008) for the increase in the two analytes following surgery. The mean value for all F1.2 patients started to decrease at 6 hours which is further confirmed by a lower correlation coefficient of 0.3 (p=0.19) as calculated for the increase in TpP™ and F1.2 after 6 hours. As F1.2 approached normal levels at t=12 hours post surgery TpP™ levels remained elevated and consequently the correlation between the ratios at 12 hours post-surgery decreased to 0.23 (p=0.31). From these results it can be concluded that elevations of TpP™ and F1.2 are correlated immediately following surgery and TpP™ levels persist at an elevated level for longer time periods. This physiological response suggests that TpP™ could serve as an alternative but equivalent marker to F1.2 for evaluating patients in a hypercoagulable state and as an aid to assess patients at risk of intravascular thrombosis.

The utility of the TpP™ EIA to distinguish patients with increased risk of thrombosis during a surgical procedure (PTCA) was evaluated at the Philadelphia Heart Institute. In the study, 25 PTCA patients were administered systemic heparin to achieve an activated clotting time of >300 seconds. Four (4) of the 25 patients proceeded to serious thrombotic complications. The TpP™ values for these four patients never returned to normal values indicating active thrombosis. High TpP™ levels post heparinization suggest that the anticoagulant therapy was not adequate to maintain normal hemostatic function. From this data it may be concluded that the TpP™ EIA could serve as an aid in monitoring the efficacy of anticoagulant (heparin) therapy.

The performance of the TpP™ EIA was confirmed by linearity, precision, and interference testing. Results are reported below.

Linear Reportable Range : 0 to 40 µg/ml

Minimum Detectable Level: 0.177 µg/mL

Interference: The TpP™ EIA has been evaluated with potential interfering substances. This interference study utilized a dose response method utilizing Hemoglobin (5, 2.5, 1, 0.5, 0.1 mg/mL), Bilirubin (0.2, 0.1, 0.05, 0.025, 0.01 mg/mL), Triglycerides (400, 250, 200, 125, 50 mg/dL), and Urokinase (1000, 500, 400, 200, 100 NIHU/ml). No significant interference was observed between normal plasma samples and normal plasma samples spiked with the noted level of analyte (interferent).

Precision

Variability was determined by testing two samples over 10 days, one run per day, with replicates of 2 in each run. A single manufactured lot of kits, one kit utilized for each run, was employed. The concentrations were calculated from a calibration curve, with sample A, mean=23.64 µg/mL, selected well above the pathological cutoff and sample B, mean=7.91 µg/mL, selected near the pathological cutoff. Assay variances, standard deviation and coefficient of variation, were determined according to the NCCLS guideline EP5-T2.

Sample	Mean TpP™ value (µg/mL)	Within-Run Standard Deviation	Within-Run Coefficient of Variation (%)	Total Precision Standard Deviation	Total Precision Coefficient of Variation (%)
A	23.64	0.36	1.50	2.40	10.15
B	7.91	0.29	3.70	0.85	10.80