## SUMMARY OF SAFETY AND PROBABLE BENEFIT

I. General Information

| | |
|---|---|
| Device generic name: | Ecarin Clotting Time Test |
| Device trade name: | Thrombolytic Assessment System (TAS™) Ecarin Clotting Time (ECT™), hereinafter referred to as TAS ECT |
| Applicant's Name and Address: | Cardiovascular Diagnostics, Inc.<br>5301 Departure Drive<br>Raleigh, NC 27616 |
| HDE number: | H990012 |

Date of Humanitarian Use Device (HUD) Designation: September 23, 1999

Date of Panel Recommendation: Not applicable. (See Section XII for discussion).

Date of Good Manufacturing Practices inspection: January 15, 1998

Date of notice of approval to the applicant: MAY 11 2000

II. Indications for Use

The TAS ECT Test Card is to be used with the TAS Analyzer and is intended to be used to determine the anticoagulant effect of recombinant hirudin (r-hirudin) during cardiopulmonary bypass in patients who have heparin-induced thrombocytopenia (HIT).

III. Device Description

The TAS ECT is to be used with the TAS Analyzer (K990566) to monitor beyond the therapeutic levels (0-3 μg/mL) of r-hirudin to the higher levels (3-5 μg/mL) required during cardiopulmonary bypass (CPB) in citrated whole blood. The test is for *in vitro* diagnostic use, and is specifically intended for professional use during CPB procedures.

A. TAS ECT Test Card

A TAS ECT Test Card is composed of a thin plastic card the size of a standard credit card, upon which is mounted a flat, shallow reaction chamber. The chamber is formed by a spacer, which determines the reaction volume, and an optically transparent cover piece. A sample well is connected to the reaction chamber by a conduit. The reaction chamber contains all the reagents

necessary for a particular test. The reagents are dry, which infers maximum stability. Also contained in the reaction chamber are paramagnetic iron oxide particles (PIOP), which move under the influence of magnetic fields in the instrument. When the sample drop is added to the reaction chamber, the mix of particles and reagents is reconstituted. On the back of the test card is a magnetically encoded stripe containing lot-specific information such as test type, lot number, expiration date, allowed sample types, and mathematical parameters specific for the lot. This information is read by the TAS Analyzer upon initiation of a test.

B. TAS Analyzer

The TAS Analyzer produces an oscillating magnetic field using an electromagnet with an alternating field and a thin permanent magnet mounted at a right angle above it. An inserted test card lies just above this assembly, which also contains a heater strip to maintain a set point temperature (37°C). The reaction chamber is illuminated with a light emitting diode mounted adjacent to the photodiode, and light reflected from the test card surface is measured. The photodetector in the instrument "sees" a light change when the sample is added and begins the test. The electromagnet turns off and on every second. The particles stand up when the magnet is on, causing more light to pass through the detector, and fall down when it is off, causing less light to be detected. This movement of the particles produces the alternating current (AC) signal.

The signal response emerges as a double-sided waveform, with the higher amplitude side detected when the electromagnet is on and the lower amplitude side when the magnet is off. This double-sided waveform is subsequently filtered to yield a single sided waveform, which shows maximum amplitude when the PIOP movement is greatest. Formation of a clot in the sample impedes PIOP movement. The signal produced by the relative movement of the PIOP is interpreted by the analyzer in accordance with algorithms predetermined for this purpose and clotting times are reported in seconds.

C. TAS ECT Test

The TAS ECT Test provides a one stage, two step test which measures the clotting time of a sample after combining it with the prothrombin activator, ecarin. This test consists of a single card that contains calcium chloride and the enzyme, ecarin, which catalyzes the hydrolytic cleavage of the 323Arg-324Ile bond in the human prothrombin molecule, whereby thrombin activity is generated without the release of any zymogen fragment. This form of active prothrombin has been termed "meizothrombin" and is inhibited by r-hirudin, but not efficiently by the heparin-ATIII complex.

The TAS ECT Test Card is to be used with the TAS Analyzer and is intended to determine the anticoagulant effect of recombinant hirudin in plasma diluted, citrated whole blood. Samples are obtained by drawing whole blood into sodium citrate (3.2 or 3.8%), in a ratio of nine parts blood to one part anticoagulant, and by mixing briefly with gentle inversion. The sodium citrate chelates the calcium in the blood. This allows the operator to control the start of the clotting reaction. The test card has a magnetic stripe on the back, which encodes lot specific information such as lot number, expiration date, and mathematical parameters specific to that lot. A room temperature test card is removed from the pouch and the card is passed through the TAS instrument's magnetic reader to initiate the instrument to run a TAS ECT test. The instrument instructs the operator to insert a TAS ECT test card and then requests patient and sample information. The card is warmed quickly and the operator is prompted to add a drop of sample to the card well. The sample is drawn into the card and rehydrates the TAS ECT reagent, which begins the clotting reaction. As the reaction proceeds and clotting begins, the movement of the particles decreases, and the instrument signals the clotting time.

The greater the amount of an antithrombin drug in the patient, the more meizothrombin (generated by the action of ecarin on prothrombin in the sample) is inhibited and the longer the clotting time reported by the TAS system.

IV. Contraindications

The use of the TAS ECT test is contraindicated in the following individuals:

- Patients on coumadin therapy with an INR > 4.5,
- Patients with > 25 percent hemolysis,
- Patients with > 30 percent hemodilution,
- Patients with > 0.5 U/mL, concentration of unfractionated heparin,
- Patients with < 30 percent prothrombin activity or < 15 mg/dL fibrinogen concentration, and
- Patients on thrombolytic therapy.

The use of acid citrate blood collection tubes is also contraindicated with the TAS ECT.

V. Warnings and Precautions

See "Warnings and Precautions" in the labeling.

VI. Alternative Practices and Procedures

The process of cardiopulmonary bypass (CPB) causes significant hemodilution and the potential activation of the coagulation cascade with unpredictable and occasionally progressive consumption of several coagulation factors. Usually

high dose heparin is used to anticoagulate during CPB, but it is contraindicated and life-threatening in patients experiencing heparin-induced thrombocytopenia (HIT). Refludan (r-hirudin) has been found to be a reasonable substitute for heparin in HIT.

While there exists alternative in vitro diagnostic procedures (IVDs), including global coagulation tests, such as the activated clotting time (ACT), activated partial thromboplastin time (aPTT), or prothrombin time (PT), performance has not been established for monitoring high dose r-hirudin (3-5 μg/mL) in blood during CPB. Non-controlled studies using global coagulation tests, such as the aPTT and the ACT, for r-hirudin monitoring during CPB have yielded variable results because the coagulation factors necessary for the aPTT and ACT may be depleted or absent. Additionally, potential matrix effects (hemodilution, consumption of coagulation factors), lack of standardization, and an apparent non-linear, dose-response of aPTT to higher concentrations (>1.5 μg/mL) of r-hirudin (Tripodi et. al., 1993) also contribute to variable results.

VII. Marketing History

The TAS ECT test has been provided to two consultants in Germany for Investigational Use Only. A limited number of clinical uses (10), performed under Compassionate Use, have been recently done in the United States.

VIII. Potential Adverse Effects of the Device on Health

Inaccurate information on the coagulation status of the patient poses a significant risk to the patient during CPB, since both over-treatment and under-treatment can have fatal consequences (bleeding in the patient and clots in the CPB system, respectively). Possible risks of using the TAS ECT Test Card could include under-estimation or over-estimation of the level of hirudin in the patient. Currently, an antidote for hirudin is unavailable, and the clinical data available are insufficient to establish the probabilities or incidence of erroneous medical judgements and their clinical outcomes.

IX. Summary of Preclinical Studies

A. Precision Studies.

Precision studies were performed on the TAS Analyzer with TAS ECT cards and the indicated sample types. Test cards and samples were allowed to reach room temperature before testing. Unless otherwise stated, thirty replicates of each sample type were tested, to allow coefficients of variation to be determined at a 95% confidence level.

1. Within day

In this study, one operator performed all of the tests on a single day, using a single lot of TAS ECT cards. The test was done with two sample types: citrated whole blood (CWB) and CWB with 4.0 ug/mL r-Hirudin, individually diluted at 30% with phosphate-buffered saline (PBS). All samples were diluted 1:1 with normal human plasma before addition to the TAS ECT test. Testing was done on 30 TAS Analyzers simultaneously, to minimize changes in the samples that might occur during the course of the experiment, therefore the results of this test also include analyzer variation.

| **Table 1: Within Day Precision of ECT Test Cards** | | | |
|---|---|---|---|
| | **Mean (seconds)** | **Standard Deviation (seconds)** | **%CV** |
| **r-Hirudin Concentration** | | | |
| **0.0 ug/mL** | **55.1** | **2.8** | **5.0** |
| | | | |
| **4.0 ug/mL** | **443.4** | **27.4** | **6.2** |

2. Day to day

Day to day precision studies of 10 TAS ECT cards were done each day, for 20 consecutive days. Single lots of TAS ECT normal and abnormal control plasma and of TAS ECT cards were used in the study. A single operator did the tests on multiple TAS Analyzers. These data were analyzed according to Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline, NCCLS publication EP5-A, Volume 19 Number 2.

| **Table 2: Day to Day Variation of ECT Test Cards** | | | | | | |
|---|---|---|---|---|---|---|
| | **Normal Control (seconds)** | | | **Abnormal Control (seconds)** | | |
| | | **Range** | | | **Range** | |
| | | **Min.** | **Max.** | | **Min.** | **Max.** |
| **Mean** | **52.9** | **50.8** | **54.7** | **154.0** | **146.1** | **159.9** |
| **Total Precision (SD)** | **2.2** | | | **9.2** | | |
| **Total CV** | **4.1** | | | **6.0** | | |

Day to day precision studies were also done on CWB samples, neat and CWB containing 4.0 ug/mL r-hirudin. Results are in Table 2a.

| Table 2a: Day to Day Variation of ECT Test Card (CWB) | | | | | | |
|---|---|---|---|---|---|---|
| Day to Day Variation of ECT Test Card: Citrated Whole Blood (CWB) (N=5 samples per day for 20 days) | | | | | | |
| | CWB with 0.0 μg/ml r-hirudin | | | CWB with 4.0 μg/ml r-hirudin | | |
| | | Range | | | Range | |
| | | Min | Max | | Min | Max |
| Mean (seconds) | 55.9 | 49.0 | 60.6 | 439.4 | 393.0 | 529 |
| Total Precision (S.D.) | 4.1 | | | 35.5 | | |
| Total CV (%) | 7.0 | | | 6.7 | | |

3. Operator to operator variability

Three different operators performed all of the tests on a single day, on 45 TAS Analyzers, with a single lot of TAS ECT cards. The test was done with two sample types, diluted at 30% with PBS: citrated whole blood and citrated whole blood with 4.0 ug/mL r-hirudin. To minimize variation that might occur due to sample changes with time, the tests were performed with 15 analyzers/operator, by 3 operators simultaneously.

| Table 3: Operator to Operator Precision Results Individually Diluted Samples | | | | | | |
|---|---|---|---|---|---|---|
| | Mean (seconds) | Standard Deviation (seconds) | % CV | Grand Mean (seconds) | Grand SD | Grand CV |
| **Results for 0.0 ug/mL r-Hirudin** | | | | | | |
| Operator I | 49.8 | 3.1 | 6.2 | | | |
| Operator II | 50.7 | 2.7 | 5.3 | 50.9 | 2.9 | 5.8 |
| Operator III | 52.2 | 2.6 | 5.0 | | | |
| **Results for 4.0 ug/mL r-Hirudin** | | | | | | |
| Operator I | 405.3 | 30.1 | 7.4 | | | |
| Operator II | 396.0 | 22.8 | 5.8 | 406.8 | 27.0 | 6.6 |
| Operator III | 419.2 | 23.1 | 5.5 | | | |

4. Lot to lot

Lot to lot precision studies were performed with TAS ECT tests using r-hirudin at 0.0 and 2.0 ug/mL, in citrated whole blood, with 40 replicate values. A single operator did the tests on multiple TAS Analyzers. This experiment was performed without the 1:1 pooled normal plasma dilution, to test the relative response of the different lots of TAS ECT tests and lot variation that would occur from the 1:1 plasma dilution.

| Table 4: Lot to Lot Precision Results | | | | |
|---|---|---|---|---|
| Lot # | r-hirudin | Mean (seconds) | SD (seconds) | % CV |
| 1 | 0.0 | 48.6 | 2.4 | 5.0 |
| | 2.0 | 428.6 | 19.9 | 4.7 |
| 2 | 0.0 | 49.3 | 2.0 | 4.1 |
| | 2.0 | 397.6 | 27.8 | 7.0 |
| 3 | 0.0 | 48.6 | 2.0 | 4.0 |
| | 2.0 | 434.1 | 25.2 | 5.8 |

B. Normal Range and Expected Values.

Samples from 120 normal individuals (72 females and 48 males ranging in age from 20 to 63 years, median 38 years) were tested using the TAS Analyzer and TAS ECT Test Cards. Citrated whole blood was diluted 1:1 with pooled normal human plasma. The results are presented in Tables 5-7.

| Table 5: Summary of Normal Range Data - All Values Used | | | |
|---|---|---|---|
| | | | |
| | REP 1 | REP 2 | Mean |
| | Seconds | | |
| Geomean | 48.5 | 48.3 | 48.5 |
| Mean | 48.7 | 48.5 | 48.6 |
| STDEV | 3.8 | 4.0 | 3.4 |
| %CV | 7.7 | 8.3 | 7.1 |
| Minimum | 32.5 | 29.3 | 30.9 |
| Maximum | 58.8 | 57.1 | 54.9 |
| Number | 120 | 120 | 120 |

| Table 6: Summary of Normal Range Data – Sorted by Age | | | | | |
|---|---|---|---|---|---|
| | | | REP 1 | REP 2 | Mean |
| | Age | N= | Seconds | | |
| Median Age = 38 | ≤ 38 | 63 | 48.9 | 48.5 | 48.7 |
| | > 38 | 57 | 48.4 | 48.5 | 48.5 |
| T-test | | | 0.526 | 0.955 | 0.706 |

| Table 7: TAS-ECT Values for Normal Population | |
|---|---|
| | Normal TAS-ECT (seconds) |
| Mean | 48.5 |
| STDEV | 3.4 |
| % CV | 7.1 |
| Minimum | 30.9 |
| Maximum | 54.9 |



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C. Interfering Factors.

Several studies were performed to determine whether certain factors could interfere with the TAS-ECT test. The results are summarized in Table 8.

Table 8: Interfering Factors

| **Factor Sensitivity** |
|---|
| Factor VII tested 0 to 100 % normal - No effect on TAS ECT Test performance |
| Factor IX tested 0 to 100 % normal - No effect on TAS ECT Test performance |
| Factor X tested 0 to 100 % normal - No effect on TAS ECT Test performance |
| Factor II tested 0 to100 % normal – No effect on TAS ECT Test performance > 30%normal |
| Fibrinogen tested at 15 to 1000 mg/dl – No effect on TAS ECT Test performance |
| **Interference Studies** |
| Acid Citrate blood collection tubes – contraindicated |
| Hematocrit tested at HCT of 8 to 64% - no effect on TAS ECT Test performance |
| Heparin tested at 0.0, 0.5, and 5.0 U/mL – No effect up to 0.5 U/mL unfractionated heparin on TAS ECT Test performance |
| Lipemia tested at 0.0 to 15.0 g/l – No effect on TAS ECT Test performance |
| Nitroglycerin tested at 0 to 1000 ug/mL – No significant effect on TAS ECT Test performance |
| Dextran tested at 0 to 5 mg/mL – No significant effect on TAS ECT Test performance |
| Plasminogen tested at 0 to 100 % normal – No effect on TAS ECT Test performance >20% normal |
| Protamine tested at 0 to 100 ug/mL – No effect on TAS ECT Test performance |
| Hemodilution tested at 0 to 100% No significant effect on TAS ECT Test performance at <30% hemodilution |
| Aprotinin test at 0.0 to 1000 KIU/mL – No effect on TAS ECT Test performance |
| Citrate at 3.2% vs. 3.8% has no effect |
| Hemolysis of Sample tested at 0 to 100 % hemolysis; up to 25% hemolysis no effect |

In addition, studies demonstrated that there was no interference on the assay when:

- Sample temperature was 4°C, 24°C, or 37°C;
- Test cards were 4°C or ambient temperature;
- Samples were stored in glass or polypropylene tubes.

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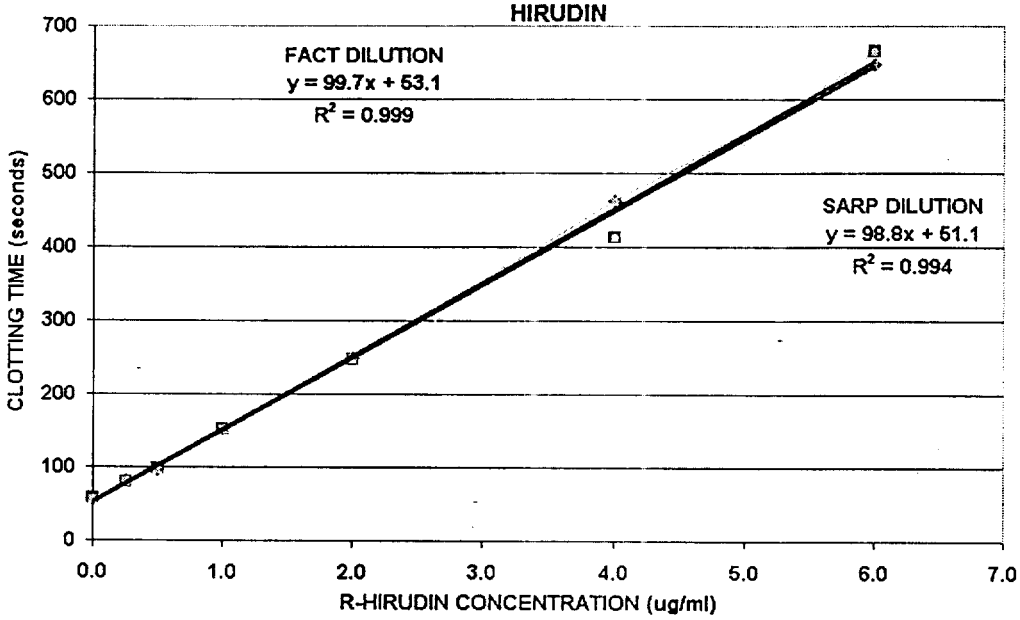
D. Dilution with normal human plasma.

Fresh citrated whole blood was diluted at 30% with phosphate buffered saline (PBS). The PBS/blood mixture was supplemented with various concentrations of r-hirudin and the aliquots were diluted 1:1 with either FACT or SARP plasma, and 5 replicate measurements were performed on TAS ECT tests (30µl/test). Results are shown in the graph below. Both FACT and SARP normal pooled plasmas yielded equivalent sensitivity to r-hirudin.

**FIGURE 1: COMPARISON OF TWO DIFFERENT POOLED NORMAL PLASMAS ON THE RESPONSE OF TAS-ECT (1:1 DILUTION WITH PLASMA) TO R-HIRUDIN**




FACT – (F)actor (A)ssay (C)on(T)rol
SARP – (S)pecialty (A)ssayed (R)eference (P)lasma

E. Analytical Sensitivity of TAS ECT Test.

The lowest level of hirudin that could be detected in plasma was 0.1 µg/mL.

F. Stability Studies.

Studies conducted support a stability of 24 months for the unopened test cards when stored at 2-8°C.

X. Summary of Clinical Studies

The TAS ECT Test was used, when requested, on a compassionate use basis, in



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10 patients at 9 geographically diverse clinical sites. Six males and three females were identified and ranged in ages from 14-82 years. Age was not provided for one male patient, and no patient information was provided for another. In cases where r-hirudin dosing information was provided, the 1:1 plasma diluted ECT test responded to increases in dosing. The diluted ECT, during the cardiac procedures, was generally maintained between 200-450 seconds.

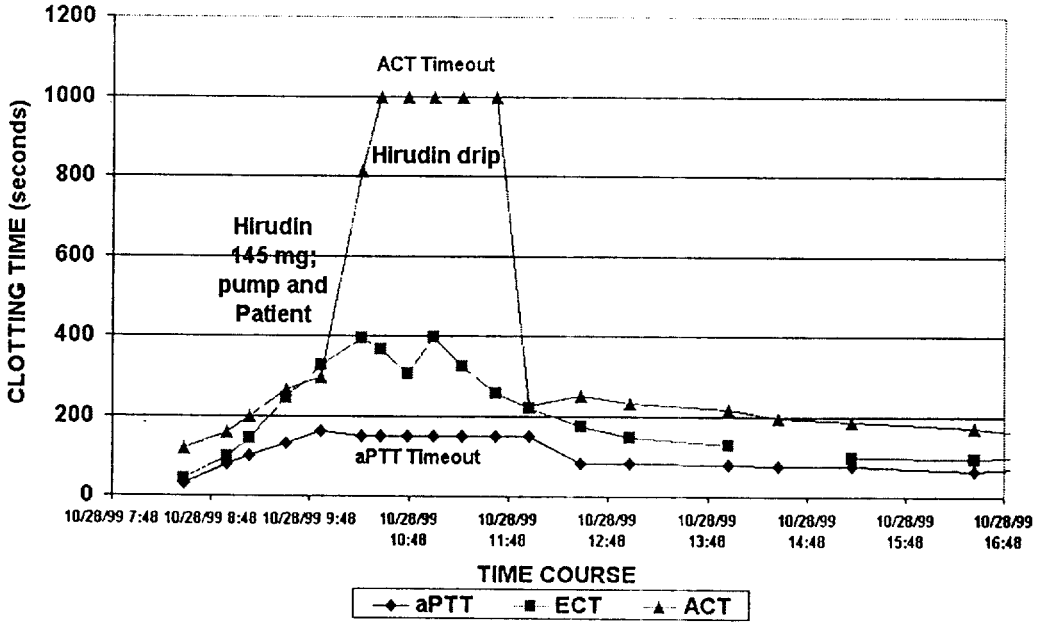
**Summary of patient data**

The patient population included persons identified with and/or confirmed as a high risk for HIT, and requiring high dose anticoagulation with recombinant hirudin for a scheduled or emergency CPB procedure.

The patients were treated and monitored for their responses to the drug, r-hirudin. The device was used according to procedures outlined in the package insert, with sites taking readings at baseline, during bolus and/or pump dosing and/or titers. Response to the drug was evident in all 10 patients.

In one subset of four patients, a comparative analysis with the aPTT and ACT provided results which indicated that neither test was sufficiently sensitive nor linear to monitor r-hirudin at the higher doses used during CPB (See the example in Figure 2).

**FIGURE 2: COMPARISON OF TYPICAL TAS ECT, APTT, AND ACT RESPONSE TO r-HIRUDIN BOLUS AND INFUSION DURING CPB SURGERY**




In another subset, three patients demonstrated that an undiluted ECT exceeds the linear range for monitoring the drug at those higher levels (See the example in Figure 3).

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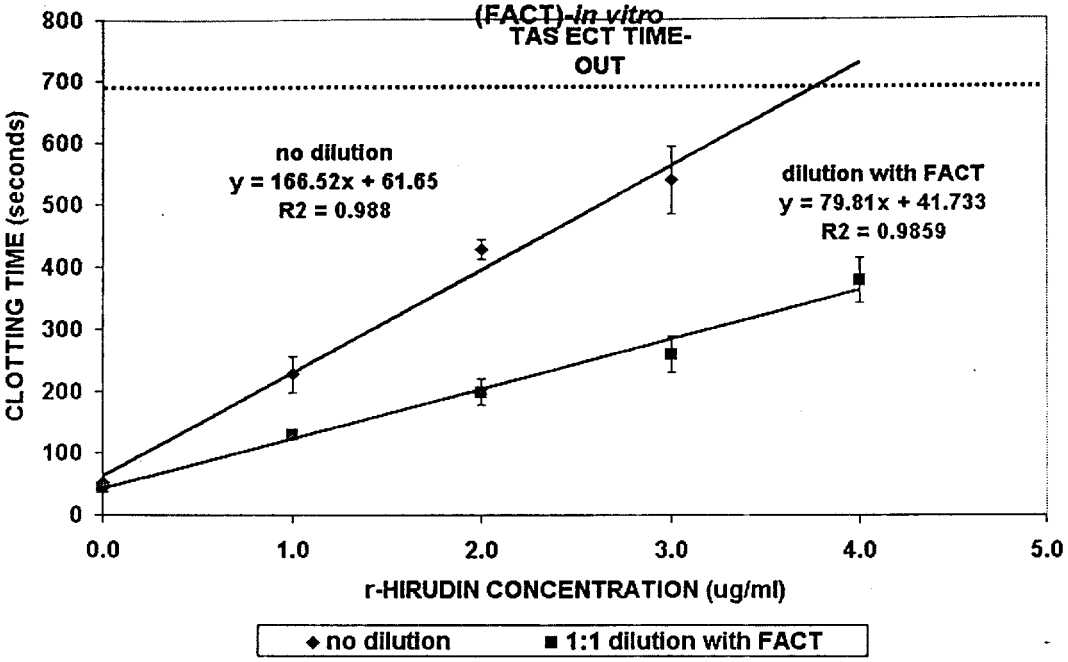
FIGURE 3: TAS ECT RESPONSE TO r-HIRUDIN IN WHOLE CITRATED BLOOD AND DILUTED 1:1 WITH POOLED NORMAL HUMAN PLASMA (FACT)-*In vitro*




Although instructions were provided to the sites, and data were requested, each site provided varying levels and complexities of data. During cardiac procedures, the collective ranges of the diluted ECT were between 200 - 450 seconds. Values not exceeding 600 seconds during the testing period, ranged from 141.8 - 485.1 seconds.

In one patient, an episode of bleeding occurred due to an inability to reverse the effects of r-hirudin. Another patient, who was hypercoagulable and presented with compromised renal function, developed significant postoperative coagulopathy; and required multiple units of platelets, packed red cells, cryoprecipitate and fresh frozen plasma. Although postoperative bleeding events occurred in these two patients, they were soon corrected with no further problems.

**Safety evaluation**: Two patient deaths were reported, the first of which was a 63-year old male. The cause of death was reported as due to "severe myocardial depression and the severity of his disease," and was not attributed to the use of the device for monitoring anticoagulation.

The second patient died post-operatively. This patient was noted to have a severe coagulopathy possibly related to the r-hirudin therapy, possibly attributable to other causes.

The precision and accuracy of the TAS ECT under conditions of actual clinical use are not known. The available data do suggest, however, a general response to the ECT to increasing or decreasing doses of r-hirudin drug.

Two of ten patients whose r-hirudin therapies were monitored with this device experienced profound coagulopathies. The TAS ECT appeared to not be sensitive to the abnormalities of hemostatis observed in these patients.

XI. Conclusions Drawn from the Studies

The TAS ECT test is to be used only for patients who have been identified with and/or confirmed as a high risk for heparin induced thrombocytopenia (HIT) and require high dose anticoagulation with recombinant hirudin for a scheduled or emergency cardiopulmonary bypass (CPB) procedure.

Preclinical studies show that the TAS ECT test provides a method for monitoring high dose (3 – 5 ug/mL) r-hirudin. In the required monitoring range, the test is not significantly affected by most interferences that would be commonly found during CPB. The TAS ECT test results are affected by very low concentrations of prothrombin (< 30% normal), hemolysis > 25% and hemodilution > 30%. These conditions are not likely to be encountered, even during CPB procedures.

The limited clinical data are not adequate to establish the safety and effectiveness of this device in the indicated patient population. The limited clinical reports, of 10 patients, show that the TAS ECT test responds to increasing levels of r-hirudin in the high dose range used for CPB and provides a tool for monitoring r-hirudin during CPB. Limited clinical studies have also shown that neither aPTT nor ACT tests provide a reliable means for monitoring high dose r-hirudin, whereas, during the same procedures, the TAS ECT responded as predicted from the preclinical studies. Clinical data are limited, but indicate satisfactory monitoring of r-hirudin drug levels.

In conclusion, the pre-clinical safety and performance studies provide reasonable assurance that the device materials and design are appropriate for this intended use. The limited clinical data suggest that the device will not expose patients to an unreasonable or significant risk of illness or injury. Considering the risks and benefits of currently available devices or alternative forms of monitoring high-dose r-hirudin in blood during CPB, it appears that the probable benefit to health from using the device outweighs the risk of injury or illness.

XII. Panel Recommendations

The HDE was not reviewed by an FDA Advisory Panel. A similar device, the activated whole blood clotting time test (ACT), for a different indication, was reviewed by the Hematology and Pathology Devices Panel on September 12, 1980. Since that meeting, many similar devices have been cleared by the FDA.

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Therefore, it was determined that this application substantially duplicates information previously reviewed by the Advisory Panel.

XIII. CDRH Decision

CDRH has determined that, based on the data submitted in this HDE application, the TAS ECT Test will not expose patients to an unreasonable or significant risk of illness or injury; and that the probable benefit to health from using the device outweighs the risk of illness or injury, and issued an approval order on MAY 11 2000.

XIV. Approval Specifications

Directions for Use. See professional labeling (attached).
Contraindications, Precautions and Warnings. See professional labeling.

XV. References (Bibliography)

1. Tripodi A, Chantarangkul V, Arbini AA, Moia M, Mannucci PM. Effects of Hirudin on Activated Partial Thromboplastin Time Determined with Ten Different Reagents. Thrombosis and Haemostasis, 70 (2) pp. 286-288, 1993.