510(k) Summary

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I. Name of Device

Device Name: Platelet Antigen Molecular Typing Assay

Proprietary Name: ThromboType (HPA 1-6, 15) and ThromboType 1 (HPA 1)

Classification Name: DNA Probe, Human Chromosome, Product Code MAO. DNA based assay for the molecular determination of alleles of HPA-1 (PI^A), HPA-2, (Ko), HPA-3 (Bak), HPA-4 (Pen), HPA-5 (Br), HPA-6 (Ca), and HPA-15 (Gov); ThromboType or HPA-1 (PI^A); ThromboType 1.

II. Name of predicate device for claiming equivalence

GTI PAT HPA-1 (PI^A) Genotyping Kit (BK980005)

The GTI PAT HPA-1 (PI^A) Genotyping Kit is a qualitative assay used to identify HPA-1 DNA sequences.

In addition the results from the ThromboType and ThromboType 1 kits were compared to DNA sequencing, which is considered to be the gold standard. This information was also used in claiming equivalence.

III. Description of Device:

Human Platelet Alloantigens (HPAs) are associated with amino acid changes in platelet glycoproteins. While not overtly affecting the function of these proteins, the modifications alter the proteins' local conformation. The resulting antigenic determinants can be targets for alloimmune and autoimmune antibody responses that cause life-threatening bleeding disorders such as refractoriness to platelet transfusions, post-transfusion purpura, and neonatal alloimmune thrombocytopenia. To date, serologic methods for platelet typing have been limited by the lack of well-characterized alloantisera and the scarcity of platelets in thrombocytopenic patients. However, with advances in platelet immunogenetics, it is now possible to genotype individuals for the single nucleotide polymorphisms that characterize the alleles of HPA polymorphisms.

ThromboType is an alternative to platelet serological testing and an adjunct to HLA matching in selecting compatible platelets for alloimmunized recipients. ThromboType contains the reagents necessary to perform allele specific PCR on isolated genomic DNA and to identify an individual's genotype for HPA-1, 2, 3, 4, 5, 6 and 15 using a gel endpoint, (HPA-1 for ThromboType 1).

The ThromboType assay is based on a proprietary modification of a classical allele specific priming polymerase chain reaction (PCR). The assay uses two allele specific PCR amplifications per polymorphism in which a product is produced only if the allele is present. These amplifications determine the presence of alleles for platelet polymorphisms HPA-1 (Pl^A) in the case of ThromboType 1 or HPA-1 (Pl^A), 2 (Ko), 3 (Bak), 4 (Pen), 5 (Br), 6 (Ca), and 15 (Gov) for ThromboType in a DNA sample. Genomic DNA is first isolated from the donor specimen, using one of the many commercial kits or published techniques that can yield high-purity genomic DNA. Sample DNA is amplified using the supplied amplification tubes and reagents. After amplification an aliquot of each reaction is pipetted into a well of a 4% agarose gel. A 15 – 20 minute electrophoresis step separates PCR products by size. The gel is then examined on a UV transilluminator. Presence of a PCR product band of the correct size indicates presence of the allele in the DNA sample. Product bands from internal control primers demonstrate that acceptable PCR conditions were present in each tube. The gel may be photographed as a permanent record of the assay.

Both ThromboType and ThromboType 1 kits consist of two separate boxes (Box A and Box B). Box A provides materials for the PCR (PCR reagents). Box B contains the gels that are used in the electrophoresis step (E-gels). The storage temperature requirements for the PCR reagents and the E-gels are different. Thus the need for two separate boxes with different storage conditions; (Box A is stored at 2 – 8°C and Box B is stored at room temperature). In addition, the components of Box B (the E-gels) have a shorter shelf life than the components of Box A. The E-gels are a replaceable part and additional gels may be provided to customers if the shelf life expires before that of Box A. The E-gels lots are not matched to the PCR reagent lots. Any lot of E-gels may be used with a given lot of PCR reagents.

IV. Intended Use

ThromboType is an assay for the molecular determination of HPA-1 (Pl^A), HPA-2, (Ko), HPA-3 (Bak), HPA-4 (Pen), HPA-5 (Br), HPA-6 (Ca), and HPA-15 (Gov), using PCR amplification of human genomic DNA.

ThromboType 1 is an assay for the molecular determination of HPA-1 (Pl^A) using PCR amplification of human genomic DNA.

V. Support of substantial equivalence based on comparison of features, characteristics and components to the predicate device

The following table provides a comparison between the ThromboType and ThromboType 1 kits and the predicate device GTI-PAT HPA-1 (Pl^A) Genotyping Kit

Feature/Characteristics	GTI-PAT	ThromboType 1	ThromboType
Type of Test	Qualitative	Qualitative	Qualitative
Intended Use	Qualitative assay used to identify HPA-1 DNA sequences	Qualitative DNA-based assay for the molecular determination of alleles of Human Platelet Alloantigen HPA-1 (Pl^A)	Qualitative DNA-based assay for the molecular determination of alleles of Human Platelet Alloantigen HPA-1 (Pl^A), HPA-2 (Ko), HPA-3 (Bak), HPA-4 (Pen), HPA-5 (Br), HPA-6 (Ca), and HPA-15 (Gov)
Technology Used in Assay	PCR based molecular diagnostic assay; allele specific capture/hybridization Detection of bound PCR product using sheep anti-fluorescein IgG (Fab) – alkaline phosphatase enzyme conjugate along with PNPP substrate	Allele specific PCR Separation of PCR products by agarose electrophoresis Detection of separated product bands by staining with ethidium bromide	Allele specific PCR Separation of PCR products by agarose electrophoresis Detection of separated product bands by staining with ethidium bromide
Detection Method	Colorimetric measurement	Visual detection under UV light	Visual detection under UV light

The following further summarizes the differences and similarities between the ThromboType and ThromboType 1 kits and the predicate device GTI-PAT HPA-1 (PI^A) Genotyping Kit

The ThromoboType and ThromboType 1 assays are similar to the GTI-PAT assay in the following:

1. Both ThromboType 1/ThromboType assays and the GTI-PAT assays are PCR based molecular assays.

2. Both ThromboType 1 and the GTI-PAT assay are used for the detection of HPA-1 DNA sequences.

The ThromboType and ThromboType 1 assays are different from the GTI-PAT assay in the following:

1. Difference in technologies

The PAT assay uses PCR to amplify the segment of DNA that includes the site for the polymorphism HPA-1a/b. The amplified product results in the incorporation of a fluorescein molecule. Once PCR product is obtained, it is denatured. Subsequently the denatured product is added to a microwell which contains captured DNA allele specific sequences (these sequences contain a biotin molecule and are captured in the microwell which contains immobilized streptavidin). The denatured PCR product is allowed to hybridize to the captured allele specific sequences and unbound material is removed by washing. A sheep anti-fluorescein antibody fragment (Fab) – alkaline phosphatase enzyme conjugate is added. After a wash step, the bound conjugate is detected colorimetrically at 405 nm by the addition of pNPP substrate.

In contrast, the ThromboType and ThromboType 1 assays use two allele specific PCR amplifications per polymorphism. A product is only produced if the allele is present. The products are detected visually after separation by agarose gel electrophoresis and staining with ethidium bromide.

2. Difference in polymorphisms detected

ThromTybope detects additional polymorphisms that are not detected by the GTI-PAT assay: HPA – 2a/b, HPA – 3a/b, HPA – 4a/b, HPA – 5 a/b, HPA – 6a/b, HPA - 15a/b.

VI. Support of substantial equivalence with performance data

A. Accuracy by Comparison of Methods

To support substantial equivalence between the ThromboType/ThromboType 1 assay and the predicate device GTI – PAT assay a comparison of methods study was performed. Briefly, the HPA 1a/b genotype was determined for a population consisting

of eight HPA – 1a/a, three HPA – 1b/b, and four HPA – 1a/b samples using the ThromboType 1 assay and the GTI – PAT assay (n = 30 results). The results showed 100% agreement between the two methods.

B. Accuracy by Comparison to a "Gold Standard"

To support the accuracy of the ThromboType assay, DNA sequencing was performed to determine the HPA – 1a/b, HPA – 2a/b, HPA – 3a/b, HPA – 4 a/b, HPA – 5a/b, HPA – 6a/b, and HPA – 15a/b genotype of 10 samples (n = 140 results). The number of samples representing each allele used in this study is shown in the table below. The samples were then tested in the ThromboType assay and the results were compared to those obtained by DNA sequencing. The results of the ThromboType assay showed 100% agreement with the results from DNA sequencing.

	a/a	a/b	b/b
HPA - 1	6	2	2
HPA - 2	7	2	1
HPA - 3	3	5	2
HPA - 4	9	0	1
HPA - 5	7	2	1
HPA - 6	10	0	0
HPA -15	2	5	3

VII. Additional performance data to demonstrate safety and effectiveness of the ThromboType/ThromboType 1 assays.

A. Comparison of ThromboType and DNA based molecular typing results from an external evaluation

An external evaluation of the ThromboType assay was performed at the Blood Center of Southeastern Wisconsin (Milwaukee, WI). The Blood Center of Southeastern Wisconsin (BCSEW) is a recognized reference laboratory for platelet alloantigen typing (P. Metcalfe, et al. 2003. Vox Sanguinis 85: 240 – 245). A comparison of methods study was conducted in which the ThromboType assay was compared to a molecular typing assay developed by the BCSEW. The BCSEW assay is a multiplex genotyping assay for the molecular determination of Human Platelet alloantigen alleles for HPA – 1, HPA – 2, HPA – 3, HPA – 4, HPA – 5, HPA – 6, and HPA – 15. The assay uses proprietary "multi-code" technology developed by EraGen Biosciences (Madison, WI) for the Luminex^{100} instrument. The "in house" HPA genotyping assay was co-developed by the Blood Center and EraGen (refer to Appendix 3 for a description of the assay).

Thirty six samples representing a wide range of genotypes were used for the study, (the number of samples representing each allele used in this study is shown in the table below).

	a/a	a/b	b/b
HPA - 1	22	7	7
HPA - 2	27	7	2
HPA - 3	11	20	5
HPA - 4	35	1	0
HPA - 5	29	6	1
HPA - 6	36	1	0
HPA -15	7	22	7

The samples were genotyped in both the EraGen and the GTI ThromboType assay for HPA – 1a/b, HPA – 2a/b, HPA – 3a/b, HPA – 4a/b, HPA – 5a/b, HPA – 6a/b, and HPA 15a/b. Fourteen results for each sample (504 total assay results) were obtained. A single rare sample was typed in the HPA – 6 a/b system only. The analysis of the 506 assay results showed 99.8% agreement between the EraGen method and the ThromboType method.

B. Assay Precision

The reproducibility of the ThromboType assay was determined. Briefly, three samples representing a range of alleles were tested in duplicate in the ThromboType assay on nine separate days. The results demonstrated 100% agreement between the duplicates tests and the day to day to results.

C. ThromboType Kit Stability

Accelerated stability test data predicts a 12 month shelf life for Box A at 2 – 8°C storage. Real time stability studies are being conducted with the intent to confirm and extend a 12 month expiration dating. To date the real time studies have been carried out to 6 months with no indication of instability.

The shelf life of Box B was determined to be 10 months at room temperature storage.

VIII. Conclusions

Based on the performance data summarized here, the ThromboType and ThromboType 1 kits are safe and effective and perform as well as the predicate device and "gold standard."