510(k) Summary

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I. Name of Device:

Device Name: Granulocyte Antigen DNA Typing Assay

Proprietary Name: GranType™

Classification Name: DNA Probe, Human Chromosome, Product Code MAO. DNA based assay for the molecular determination of alleles of polymorphism HNA – 1: HNA – 1a (NA1), HNA – 1b (NA2), and HNA – 1c (SH).

II. Name of predicate device for claiming equivalence

One Lambda Micro SSP™ HLA Class II DNA Typing Kit (BK960062)

The One Lambda Micro SSP™ HLA Class II DNA Typing Kit is used for DNA typing of HLA Class II HLA alleles.

III. Description of Device:

Antibodies to neutrophil antigens are involved in neonatal alloimmune neutropenia, febrile transfusion reactions, transfusion-related neutropenia, and transfusion-related acute lung injury (TRALI). Antigens HNA-1a (NA1), HNA-1b (NA2), and HNA-1c (SH) are located on the granulocyte Fc receptor FcγRIIIb. These antigens are coded by multiple nucleotide polymorphisms on the FcγRIIIB gene. However, an adenosine at nucleotide 227A of the FcγRIIIB gene accurately predicts the HNA-1a (NA1) phenotype, while a thymidine at nucleotide 147 is predictive of the HNA-1b (NA2) phenotype. A C→A substitution at nucleotide 266 results in HNA-1c (SH), and nucleotide 266A predicts that antigen.

Both serological and DNA-based methods are used for typing the HNA-1 polymorphism. However, serologic methods can be limited by the availability of specific antisera, the requirement of specialized training in the complex techniques used, and the need to obtain large numbers of type-specific neutrophils. Current DNA-based methods can require extensive manipulation of PCR-amplified products, complex gel electrophoresis techniques, and use of solutions containing mutagenic DNA stains for visualization of product bands on

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gels. By contrast, GranType provides the reagents necessary to perform PCR on isolated genomic DNA. Visualization of PCR product bands on precast agarose gels is quick and easy and requires no stain-contaminated solutions. Presence or absence of allele-specific PCR product bands for each sample determines assignment of alleles.

The GranType™ assay uses three allele-specific polymerase chain reaction (PCR) amplifications in which a product is produced only if the allele is present. These amplifications determine the presence of alleles of neutrophil polymorphism HNA-1: HNA-1a (NA1), HNA-1b (NA2), and HNA-1c (SH). Genomic DNA is first isolated from the 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 PCR reagent. After amplification, an aliquot of each reaction is pipetted to a well of a 4% agarose gel. A 15 - 30 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.

A Grantype™ kit consists 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). Since the storage temperature requirements for the PCR reagents and the E-gels are different, these components are provided in separate boxes which are to be stored at two different conditions; Box A is shipped and stored at 2 – 8°C and Box B is shipped and 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

GranType™ is a DNA – based assay for the molecular determination of alleles of neutrophil polymorphism HNA – 1: HNA -1a (NA1), HNA – 1b (NA2), and HNA – 1c (SH).

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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 GranType™ kit and the predicate device: One Lambda Micro SSP™ HLA Class II DNA Typing Kit

Feature/Characteristics	One Lambda Micro SSP™ HLA Class II DNA Typing Kit	GranType™
Type of Test	Qualitative	Qualitative
Intended Use	DNA typing of Class II HLA alleles	DNA based typing of HNA -1a (NA1), HNA – 1b (NA2), and HNA – 1c (SH)
Technology Used in Assay	SSP PCR (sequence specific priming PCR) Separation of PCR products by agarose electrophoresis Detection of separated product bands by staining with ethidium bromide	Allele specific PCR using sequence specific priming (SSP) Separation of PCR products by agarose electrophoresis Detection of separated product bands by staining with ethidium bromide
Detection Method	Visual detection of product bands under UV light	Visual detection of product bands under UV light

The following further summarizes the differences and similarities between the GranType™ assay and the predicate device One Lambda Micro SSP™ HLA DNA Typing Trays.

The GranType™ assay is similar to the One Lambda Micro SSP™ HLA Class DNA Typing Kit in the following:

1. Both GranType™ and the One Lambda Micro SSP™ HLA Class II DNA Typing Kit are PCR based molecular assays which use sequence specific priming.
2. Both GranType™ and the One Lambda Micro SSP™ HLA Class II DNA Typing Kit are PCR based molecular assays using gel electrophoresis and staining of the product bands using ethidium bromide as the detection system.
3. Both GranType™ and the One Lambda Micro SSP™ HLA Class II DNA Typing Kit are used to type for polymorphisms on cell surface proteins.

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The GranType™ assay is different from the One Lambda Micro SSP™ HLA Class II DNA Typing Kit in the following:

GranType™ detects polymorphisms found in the HNA antigen located on the Fc receptor, FcγRIIIb a cell surface protein found on granulocytes. The One Lambda Micro SSP™ HLA Class II DNA Typing Kit detects polymorphisms found on HLA Class II proteins present on the surface of B lymphocytes, macrophages, and dendritic cells.

VI. Support of substantial equivalence with performance data

While there are similarities in the technologies used between GranType™ and the predicate device, the performance of the GranType™ assay cannot be compared to that of the predicate device, since each assay types for polymorphisms on different cell surface proteins. Therefore we are relying on additional data which demonstrates the performance of the GranType™ assay. Detailed information on these studies can be found in Section 8 (Performance Data) of this 510(k) submission. A brief summary of this data is provided below (VII).

VII. Summary of additional performance data to demonstrate safety and effectiveness of the GranType™ assay

A. Agreement by Comparison of Methods:

1. Comparison of GranType™ assay to typing results from an external evaluation at North Central Blood Services Neutrophil Laboratory, St. Paul, MN

An independent external evaluation of the GranType™ assay was performed at the North Central Blood Services Neutrophil Laboratory in St. Paul, MN (NCBS). A comparison of methods study was conducted in which the GranType™ assay was compared to an in house developed serologic typing assay termed "Granulocyte Immuno – Fluorescence Typing" or GIFT. Briefly, 50 individual samples were tested in both the GranType™ and the GIFT methods. The results showed 100% agreement (95% Confidence: 94.3% - 100%) between the two methods for typing of HNA – 1a, HNA – 1b, and HNA – 1c.

2. Comparison of GranType™ assay to typing results from an external evaluation at the Blood Center of Southeastern Wisconsin, Milwaukee, WI

An independent external evaluation of the GranType™ assay was performed at the Blood Center of Southeastern Wisconsin, Milwaukee, WI (BCSEW). A comparison of methods study was conducted in which the GranType™ assay was compared to an in house developed DNA based typing assay which uses an allele specific PCR based DNA typing methodology. This assay is based on the same technology as the GranType™ assay. Thirty of the samples which were previously typed in the GranType™ and GIFT assays, were then typed for HNA – 1a, HNA – 1b, and HNA – 1c by the Blood Center's in house assay and again by the GranType™ assay. The results showed 100%

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agreement (95% Confidence: 90.5% - 100%) between the two methods. Furthermore, the results from the GranType™ assay were reproducible between the NCBS and BCSEW laboratories.

B. Assay Precision

Three samples having the neutrophil antigens as shown in the table below were used to test the precision of the GranType™ assay.

Sample #	Sample ID	HNA - 1a	HNA - 1b	HNA - 1c
1	NAP	neg	pos	pos
2	JBA817	pos	pos	neg
3	AT032	neg	pos	neg

To determine within run precision, 20 replicates of each sample were tested in a single assay (n = 20). The results demonstrated 100% agreement between the replicates for each of the samples.

To determine between run precision, each sample was tested in duplicate over 5 days (n = 10 for each sample). In addition, the kit control which is positive for all three HNA alleles was run on each day. The results for each sample and the kit control showed 100% agreement between the duplicates and between days.

C. GranType™ Kit Stability

An accelerated stability study was conducted at elevated temperatures (25°C, 32°C, and 40°C) over a period of 8 weeks. The data from this accelerated stability study predicted a 12 month shelf life for Box A at 2 – 8°C storage.

A real time stability study was conducted in which Box A was stored at 2-8°C and tested periodically over a period of 13 months (0, 2, 4, 6, 8, 10, 12, 13 months). The samples used in the study supplied the presence and absence of all 3 HNA – 1 alleles. The data showed 100% agreement in results at each time point tested to date, confirming the predicted stability. Another real time stability study has been initiated on two additional GranType™ lots; Box A. This stability study is being carried out to 37 months and includes the following time points: 0, 3, 6, 12, 18, 24, 30, and 37 months. At this point there is limited data as this stability study is in the 4th month.

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

VIII. Conclusions

Based on the similarities in technology between GranType™ and the predicate device, as well as the performance data summarized here, the safety and effectiveness of the GranType™ is equivalent to that of the predicate device.

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