9. 510 (k) Summary of Information Respecting Safety and Effectiveness

A. Legally Marketed Device

Biotest Diagnostics claims substantial equivalence to the LYMPHOTYPE Microtrays with pre-dropped anti-HLA sera (BK830027) currently in commercial distribution by Biotest Diagnostics.

B. Device Description

The Biotest DQB-ELPHA (Enzyme Linked Probe Hybridization Assay) is a DNA-hybridization test for the determination of HLA-DQB alleles. Sequence-specific oligonucleotide (SSO-) probes are used as diagnostic tools to identify polymorphic sequence motifs. The hybridization between probe and target DNA is detected by a method adapted from the protein-ELISA technique. The microtest plate format allows the use of standardized apparatuses for automation of the test procedure and data evaluation. The test, however, also can be easily performed by hand.

Starting from genomic DNA which can be readily obtained from peripheral blood, the polymorphic region in the 2nd exon of the DQB gene is amplified by means of the polymerase chain reaction (PCR[1]). For targeting and starting the PCR Biotin-labeled primers are used. After amplification and denaturation of the PCR products the resulting biotinylated single strand molecules are bound to Streptavidin-coated wells of a microtest plate. The wells contain FITC-labeled SSO-probes in a dried form which redissolve when the diluted solution of the PCR-products is transferred to the wells. Fixation of the PCR products to the wells and hybridization with the SSO-probes thus occur in one step. The specificity of the assay is dependent on the subsequent stringency washing step in which probes of insufficient sequence homology to the PCR-products are removed. Hybridization probes are visualized in a color reaction involving FITC-specific antibody fragments coupled to Horeseradish peroxidase (POD). The results are quantitated photometrically in an ELISA reader. The combination of DQB alleles in the test material is determined from the pattern of positive reactions either by using the Biotest HLA-DQB-typing program on a personal computer or by means of the DQB-SSO reaction scheme.

C. Intended Use

The Biotest DQB-ELPHA is an enzyme linked probe hybridization assay intended for the determination of HLA-DQB alleles.

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[1]The PCR process is covered by US patents owned by Hoffmann-La Roche Inc. The use of the PCR process requires a license. Nothing in this publication should be construed as an authorization or an implicit license to practice PCR under any patents held by H-LR Inc.

D. Comparison with Predicate Device

A summary comparison of the features of the Biotest DQB-ELPHA and the LYMPHOTYPE Microtrays is provided in Table 1 below:

Table 1
Feature Comparison of DQB-ELPHA and LYMPHOCYTE Microtrays

	DQB-ELPHA	LYMPHOCYTE Microtrays
Intended Use	HLA Typing (Detection of HLA Antigens)	HLA Typing (Detection of HLA Antigens)
Assay Method	Enzyme Linked Probe Hybridization	Lymphocytotoxicity
Reactive Ingredients	Biotin-labeled primers FITC-labeled SSO-probes	Anti-HLA antisera
Specimen: Type	Defibrinated or heparinized blood	Anticoagulated blood (heparin, sodium citrate, EDTA)
Min. Volume	500 μl	5 ml
Storage	2 hours (defibrinated)/ RT 24 hours (heparinized)/ RT	20 - 25°C/3 days -20°C/several months
Controls	Positive Control (Probes E2 and H2) Inhouse panels	HLA Positive & Negative Controls
Results:		
Evaluation	ELISA reader @ 450 nm	Phase contrast microscope
Interpretation	Pattern of positive reactions (computer or manual reaction scheme)	Pattern of positive reactions (manual work sheet)
Kit Size	12 analyses	60, 72 or 120 tests

E. Performance Data

The Biotest DQB-ELPHA was compared to commercially available anti-HLA serological tests by testing 312 specimens (random transplant candidates and potential donors) at two geographically distinct locations. There was 95.8% concordance (299/312) between the DQB-ELPHA results and the results obtained using the anti-HLA serological tests.

Upon further testing to resolve discordants using additional methods (standardized in-house DNA methods), there was 100% concordance (312/312).

Lot-to-lot reproducibility has been demonstrated in tests of a panel of 7 serologically known HLA types with at least two different lots.