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

A. Legally Marketed Device

Biotest Diagnostics claims substantial equivalence to the DRB-SSO Typing Kit (BK950015) currently in commercial distribution by Biotest Diagnostics.

B. Device Description

The Biotest DRB-SSO Typing Kit is a highly sensitive dot blot assay. In the first step, genomic DNA is purified and the relevant genetic region - for the DRB-SSO Typing Kit this is a DRB fragment - is replicated using PCR*.

Two PCR amplifications with primer pairs DRB-G5/DRB-86G and DRB-G5/DRB-86T, respectively, designed towards the glycine/valine dimorphism at codon 86 of DRB sequences are performed in the high resolution assay. The two primer pairs amplify two non-overlapping groups of DRB sequences; primer pair DRB-G5/DRB-86G amplifies DRB alleles encoding amino acid residue glycine at position -86-, primer pair DRB-G5/DRB-86T amplifies those DRB alleles encoding amino acid residue -86-valine.

The DNA from the two PCR products is attached to a membrane, denatured and, at the same time, immobilized. If the DNA in the sample contains complementary sections, HLA allele-specific oligonucleotide probes can hybridize to the single-stranded DNA (oligotyping, SSO). Unbound oligonucleotide probes are removed by washing. The oligonucleotide probes are labelled with digoxigenin**.

The presence of the DNA-oligonucleotide complex is detected by means of a specific enzyme-labelled antibody (anti-digoxigenin alkaline phosphatase conjugate). The presence of the bound antibody is demonstrated in a subsequent enzyme reaction, in which a colored product is formed. All reactions, including the color reaction, are carried out on the membrane bottom of a microplate well. After completion of the test, the membrane is stripped off, evaluated and archived.

---

*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.

**Licensed from Boehringer Mannheim GmbH

C. Intended Use

The Biotest High Resolution Supplementary Kit is intended for high resolution HLA-DRB typing using the DRB-SSO Typing Kit, an oligonucleotide probe detection system.

D. Comparison with Predicate Device

A summary comparison of the features of the Biotest High Resolution Supplementary Kit and the DRB-SSO Typing Kit is provided in Table 1 below:

Table 1

Feature Comparison of High Resolution Supplementary Kit and DRB-SSO Typing Kit

	High Resolution	DRB-SSO
Intended Use	HLA-DRB Typing	HLA-DRB Typing
Assay Method	Oligonucleotide Probe Detection	Oligonucleotide Probe Detection
Reactive Ingredients	Digoxigenin labeled SSO Probes Primer	Digoxigenin labeled SSO Probes Primer
Specimen: Type	Anticoagulated blood (EDTA, citrate, heparin)	Anticoagulated blood (EDTA, citrate, heparin)
Min. Volume	500 μl x 2	500 μl
Results: Evaluation	Visual color	Visual color
Interpretation	Pattern of positive reactions (-86G-group) (computer or manual scheme)	Pattern of positive reactions (computer or manual scheme)
Kit Size	15 tests	15 tests

E. Performance Data

The Biotest High Resolution DRB-SSO Typing Kit was compared to commercially available anti-HLA serological tests by testing 410 specimens (well characterized, serologically known HLA panel specimens). There was 99.5% concordance (408/410) between the DRB-SSO Typing results and the results obtained using the anti-HLA serological tests.

	Concordant	Disconcordant	Total	%
N	**408**	**2**	**410**	**100**
%	**99.5**	**0.5**		

N = Number of Samples

Upon further testing to resolve discordants using additional methods (standardized in-house DNA methods), there was 100% concordance (410/410).

	Concordant	Disconcordant	Total
N	**410**	**0**	**410**
%	**100**	**0**	**100**

N = Number of Samples