510(k) Summary

I. Submitter:

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II. Name of Device:

Device Name: Micro Antibody Monitoring System
Proprietary Name: MICROAMS®
Classification Name: Test, Qualitative, for HLA, Non-Diagnostic
Product Code: MZI

III. Name of predicate device for claiming equivalence

GTI Antibody Monitoring System (AMS®)

IV. Description of Device

HLA glycoproteins are prepared from donor lymphocytes by solubilizing the cells with a non-ionic detergent. Once solubilized, the lymphocyte lysates are added to microwells to which monoclonal antibodies specific for HLA class I or class II have been immobilized. HLA glycoproteins are allowed to bind to the monoclonal antibodies, and unbound glycoproteins are washed away. The microwells containing the bound glycoproteins are tested with human serum to detect antibodies against HLA molecules. Unbound antibodies are then washed away. An alkaline phophatase labeled anti-human globulin reagent (Anti-IgG) is added to the wells and incubated. The unbound Anti-IgG conjugate is washed away and the substrate PNPP (p-nitrophenyl phosphate) is added. After a 30-minute incubation period, the reaction is stopped by a sodium hydroxide solution. The optical density of the color that develops is measured in a spectrophotometer.

V. Intended Use

MICROAMS® is a qualitative solid phase enzyme linked immunosorbent assay (ELISA) designed to detect IgG antibodies to donor-specific HLA class I and class II glycoproteins.

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

The characteristics of the two devices can be summarized as follows:

Features	GTI AMS®	GTI MICROAMS®
Type of Test	Qualitative	Qualitative
Intended Use	Antibody Monitoring System: HLA Class I and Class II (AMS 1+2) is a qualitative solid phase enzyme linked immunosorbent assay (ELISA) designed to detect IgG antibodies to donor-specific HLA class I and class II antigens.	MICROAMS® is a qualitative solid phase enzyme linked immunosorbent assay (ELISA) designed to detect IgG antibodies to donor-specific HLA class I and class II glycoproteins.
Technology Used in Assay	ELISA	ELISA
Detection Method	Optical Density	Optical Density
Target Antigen Immobilization	Antigen is captured in the microwells by an HLA class I-specific monoclonal antibody and an HLA class II-specific monoclonal antibody.	Antigen is captured in the microwells by an HLA class I-specific monoclonal antibody and the HLA class II-specific monoclonal antibody. The same monoclonal antibodies are used in both the MICROAMS® and AMS® kits.
Detection of captured antigen	Captured HLA class I antigens are detected by an alkaline phosphatase labeled pool of 5 monoclonal antibodies reactive with class I HLA glycoproteins.	Captured HLA class I antigens are detected by a single alkaline phosphatase labeled monoclonal antibody reactive with class I HLA glycoproteins. The

	Captured HLA class II antigens are detected by an alkaline phosphatase labeled monoclonal antibody reactive with class II HLA glycoproteins.	single monoclonal antibody used in MICROAMS® is one of the five pooled monoclonal antibodies used in the AMS® kit. Captured HLA class II antigens are detected by an alkaline phosphatase labeled monoclonal antibody reactive with class II HLA glycoproteins. The same monoclonal antibody is used in both the MICROAMS® and AMS® kits.
Detection of antibodies to HLA antigens	The presence of antibodies to HLA class I and class II antigens is detected by an alkaline phosphatase labeled anti-human IgG antibody.	The presence of antibodies to HLA class I and class II antigens is detected by an alkaline phosphatase labeled anti-human IgG antibody. The same reagent is used in both the MICROAMS® and the AMS® kit.

In addition, MICROAMS® and AMS® have the same performance characteristics and scientific technology.

The differences between the two products are related to changes of materials.

The first material change was to replace full-size microwell strips with low-volume microwell strips. The identity of the monoclonal antibodies immobilized on the microwells as well as the process used for strip manufacturing is the same for both kits. The use of the low-volume microwell strips requires the use of different volumes of reagents throughout the manufacture of the kit as well as throughout the performance of the assay.

The second change was to replace the Class I Lysate Control Reagent (LCR1), which is a pool of 5

monoclonal antibodies conjugated to alkaline phosphatase specific for HLA class I antigens with one that consists of a single monoclonal antibody(LCRI). The AMS® LCR1 contains a blend of the following monoclonal antibodies: 198.2, 198.3, 198.4, 198.9 and 198.10. The MICROAMS® LCRI consists of the single monoclonal antibody 198.10.

The third change was the addition of a stabilizer to the storage buffer for the alkaline phosphatase conjugated reagents. GTI's experience using alkaline phosphatase/PNPP based detection systems in other ELISAs demonstrated that in general, alkaline phosphatase antibodies are more stable when a metal ion is incorporated into their storage buffer. The Anti-IgG conjugate, the Class I Lysate Control Reagent (LCRI) and the Class II Lysate Control Reagent (LCRII) are now provided in the new stabilized storage buffer. The effect of the new storage buffer on the stability of the MICROAMS® conjugates is being addressed with on-going stability studies discussed in the Performance Data Section of this submission.

Risk analysis did not identify any new issues of safety or effectiveness.

VII. Support of substantial equivalence with performance data:

The performance data used to validate that the reportable results obtained from MICROAMS® and AMS® were equivalent is detailed in Section 8.1 of this submission. Briefly, 101 unique lysate/serum pairs were tested for class I and class II reactivity using both kits. For both class I and class II, 100% agreement in reportable results were observed. The 2x2 tables as well as the calculated 95% confidence intervals for class I testing are included below.

		AMS® Class I	
		Positive	Negative
MICROAMS® Class I	Positive	52	0
	Negative	0	49

Class I	Co-positivity/ Sensitivity	Co-negativity/Specificity
Value	100%	100%
95% confidence level (lower)	93.1%	92.7%
95% confidence level (upper)	100%	100%

The 2x2 tables as well as the calculated 95% confidence intervals for class II testing are included below.

		AMS® Class II	
		Positive	Negative
MICROAMS® Class II	Positive	49	0
	Negative	0	52

Class II	Co-positivity/ Sensitivity	Co-negativity/Specificity
Value	100%	100%
95% confidence level (lower)	92.7%	93.1%
95% confidence level (upper)	100%	100%

Studies were conducted to determine the within-run precision of MICROAMS®. Testing was conducted using 2 different lysates with 2 known positive and 2 known negative sera samples; each sample run in 20 replicates. The data in section 8.4 demonstrated that the %CV between replicates was less than 20% and there was 100% agreement between the reportable results for all replicates. This level of within-run precision is equivalent to that demonstrated by the predicate device, AMS®.

The between-run precision for MICROAMS® was determined as outlined in section 8.5 of this submission. Testing was conducted using 2 different lysates with 2 known positive and 2 known negative sera samples in 10 different assay runs. MICROAMS® demonstrated 100% agreement of the reportable results between all 10 separate assay

runs. This between-run precision was equivalent to that demonstrated by the predicate device, AMS®.

Real-time stability studies are on-going as described in Sections 8.7 and 8.8 of this submission. These studies will confirm the expected shelf life of the MICROAMS® product. The shelf-life currently given to MICROAMS® is based on the stability data originally obtained for the unmodified AMS® kit. All components of the AMS® and MICROAMS® kits except for the microwell strips, Lysate Control Reagents, and the anti-IgG conjugate are the same. It is expected that the stability of the low-volume microwell strips used in the MICROAMS® kit would be the same as the strips in the AMS® kit. Both microwell strips are made of the same plastic, with the same hydrazide coating, and the same monoclonals are covalently attached to both kinds of strips with the same chemistry and process.
It is expected that the use of a single monoclonal antibody in the MICROAMS® Class I Lysate Control Reagent instead of a pool of 5 monoclonal antibodies used in AMS® will not affect the stability of the kit. It is also expected that with the addition of a stabilizer to the storage buffer used in the Anti-human IgG and the Class I and Class II Lysate Control Reagents, that these reagents will have equal or greater stability when compared to the AMS® reagents. At this point in time only the data from t=0 and t=3 months is available. Based on this data, all three validation lots of MICROAMS® tested still give the expected reportable results and the values for the control wells still meet the requirements for a valid test as stated in the MICROAMS® direction insert.

VIII. Support of Safety and Effectiveness:

Additional studies included in this Special 510(k) submission assess the choice of and reactivity of the class I Lysate Control Reagent. The studies described in Section 8.2 show that the monoclonal antibody 198.10 is able to bind to a broad variety of

class I HLA antigens. In addition, 198.10 can bind to class I HLA in the presence of the monoclonal antibody used for antigen capture (138.5). This data supports the decision to change from a pool of 5 monoclonal antibodies to a single monoclonal antibody in our Class I Lysate Control Reagent

In the AMS® kit, the direction insert required that the average of the Lysate Control wells be over 0.900 OD. When extensive testing was conducted on MICROAMS® it was determined that the lysate control values depended on which lysate was tested and that the Lysate Control wells were indicators that HLA antigen was bound, but could not be used to determine the quality of the lysates used. More specifically correct reportable results could be obtained for lysates with lysate control values below the 0.900 cutoff. Experiments detailed in Section 8.3 show the lysate control values obtained on class I and class II testing using 35 different donor lysates. All of the lysates were determined to be quality lysates due to the ability of MICROAMS® to identify the expected reactivities. The MICROAMS® direction insert indicates that a valid lysate reagent control must have an OD value greater than twice the average of the negative control wells. This positive reactivity indicates that HLA antigen can be captured from the prepared lysates. The MICROAMS® direction insert also contains the average OD and ranges of the OD values obtained from these 35 lysates. This data is included in the direction insert as a guide for our product users.

IX. Conclusion:

Based on the performance data summarized here and detailed in Sections 8.1-8.8 of this Special 510(k) submission, the MICROAMS® kit performs equivalently to the predicate device, AMS®.