SECTION 10 EXECUTIVE SUMMARY

The purpose of this 510(k) submission is to get approval for three products used as controls or potentiators in the traditional immunhematology testing (tube technique). In Table 10.a the description of the devices and in Table 10.b. the device comparison is provided.

10.a. Description of the devices

Product	Biotest REF	Intended use	Technology
Seraclone Control ABO+Rh	805171100	Negative control in blood grouping with Biotest Seraclone ABO+Rh Blood Grouping Reagents. Seraclone ABO and Rh reagents react with the corresponding antigens. Samples with autoimmune antibodies, cold antibodies or rouleaux formation may show false positive reactions in testing with monoclonal antibodies. Seraclone ABO+Rh contains all components of Seraclone ABO and Rh reagents, but not the antibodies. Thus it is suited as a negative control in ABO and Rh blood grouping.	Tube
Coombscell-E	816030100	IgG-coated red blood cells for the control of the antiglobulin test with negative test results. Anti-Human Globulin reacts with IgG coated red cells of Coombscell-E. This leads to agglutination of the red cells.	Tube
MLB2	805200100	Modified LISS Biotest 2 is a potentiator (increases the rate of antigen-antibody complex formation) Allowing antigen-antibody reactions to occur in low ionic strength conditions shortens the incubation time for the detection of most of the antibodies. Used in Antibody screening, Antibody identification, cross matches and typing with coombsreactive antisera	Tube

10.b. Device comparison

Product	Indication for use	Technology	510(K), BLA, or CFR Classification	Predicate Linkage
Immucor ImmuAdd™	Used as a potentiator in antibody detection, antibody identification and compatibilty testing	Tube	21 CFR 864.9600	MLB 2 (Modified LISS Solution)

Immucor® Monoclonal Control	To be used as a control for low protein BGRs	Tube	21 CFR 864.9650	Seraclone Control ABO+Rh
Immucor® Checkcell Reagent Red Blood Cells	Used to confirm the validity of negative antiglobulin tests	Tube	21 CFR 864.9650	Coombscell-E

Clinical evaluation of the MLB2, Seraclone Control ABO+Rh and Coombscell-E was conducted as described in the Investigational Plan: "Evaluation of Liquid Antisera for ABO/Rh typing, Red cell phenotyping, Reagent Red Blood Cells for ABO Plasma Testing, Antibody Screening, Antibody Identification and Antiglobulin Reagents for Antibody Screening, Antibody Identification, Compatibility Testing and DAT by Manual Tube Techniques" (Revision Levels: September 2007).

The clinical evaluation was conducted to obtain approval for Biotest Blood Grouping Reagents, Reagent Red Blood Cells and Anti-Human Globulin reagents. Biotest Anti-Human Globulin reagents are Anti-Human Globulin Anti-IgG and Anti-Human Globulin Anti-IgG, -C3d, they are intended for use in detection of unexpected red blood cell antibodies (with Biotestcell 1&2 and Biotestcell 3), identification of unexpected red blood cell antibodies (with Biotestcell-I8 and Biotestcell-I11), cross-matching, direct antiglobulin testing (DAT) and typing with coombs-reactive Blood Grouping Reagents. Except for the DAT, MLB2 is used as a potentiator medium for all intended uses of the Anti-Human Globulin reagents.

Samples were collected in 2005-2006 from both normal blood donors and patients. The Anti-Human Globulin Anti-IgG and Anti-Human Globulin Anti-IgG, -C3d and the Biotest Reagent Red Blood Cells were tested at four investigational sites. The sites included in the study were:

- University of Virginia – Charlottesville, VA
- Heartland Blood Center – Aurora, IL
- Univ. of Colorado Medical Center – Denver, CO
- Wake Forest Baptist Medical Center – Winston-Salem, NC

The purpose of the study was:

- To demonstrate that the Biotest reagents (Anti-Human Globulin reagents, RRBCs and MLB2), for antibody screening, antibody identification, and compatibility testing are equivalent to the reference method used.
- To exhibit sensitivity and specificity comparable to or greater than the reference method used by the testing sites.

The investigational plan called for collecting at least 3,092 ABO/Rh, 3,092 antibody screens using one of three configurations (pooled screen, 2 –cell screen or 3 cell screen) and 500 Rh-K phenotyping samples. In addition to these tests, samples were evaluated for compatibility using the antiglobulin technique, Direct Antiglobulin test (DAT) (100 samples), and antibody identification (ID). The samples included in the study were tested

by the Biotest reagents and by the reference method. The reference method and number of samples tested at each site are indicated in the Table 10.c. to 10.d.

Table 10.c Samples Collected and Analyzed per Investigational Site (2006)

Investigational Site	ABO/Rh Testing	Antibody screen	Pheno-typing	DAT	Cross-match	Antibody ID	Rare Antisera Testing
Univ. of Virginia	795	795	70	39	55	31	30
Heartland Blood Center	1600	1608	150	0	0		0
Univ. of Colorado	490	483	34	26	25	10	0
Wake Forest Baptist Med. Center	550	550	95	17	0	0	0
TOTAL	**3435**	**3436**	**349**	**82**	**80**	**41**	**30**

Note: only the highlighted tests will be presented in this section in order to prove the performance of the MLB2 reagent

Each site used a currently approved method or licensed reagent for the test of record. Table 10.d lists the test methods used and the resolution testing at each investigational site.

Table 10.d. Reference Test Methods used at each Investigational Site 2006

Site	ABO/Rh	ABO Resolution	Antibody Screen	Antibody Screen Resolution	Pheno-typing	Pheno-typing Resolution
University of Virginia	Manual Tube Test	Manual Tube Test	LISS Manual Tube Test	PEG, Ortho MTS Anti-IgG card	Manual Tube Test	Manual Tube Test
Heartland Blood Center	Olympus PK7200 Automated Blood Grouping Analyzer	Manual Tube Test	Ortho Diagnostics MTS Anti-IgG card	LISS Manual Tube Test	Manual Tube Test	Manual Tube Test
Univ. of Colorado	Manual Tube Test	Manual Tube Test	Immucor Capture R Ready Screen or LISS Manual Tube	LISS Manual Tube, PEG, Ortho MTS IgG card	Manual Tube Test	Manual Tube Test
Wake Forest Baptist Med Center	Manual Tube Test	Manual Tube Test	Ortho Diagnostic MTS Anti-IgG card	LISS Manual Tube or PEG manual tube	Manual Tube Test	Manual Tube Test

Additional data to support use of different specimen, sample age and sample storage In-House Data at Biotest – 2007):

The 2006 trial protocol did not include a study to support use of Biotest Anti-Human

Globulin Anti-IgG and Anti-Human Globulin Anti-IgG ,-C3d, Biotest Reagent Red Blood Cells for the detection and identification of red cell antibodies as well as MLB2 with different sample specimen and aged samples.

In the former field trial (2006), control results (Seraclone control ABO+Rh and Coombscell-E) were not reported. Both control reagents were carried along with the additional testing.

In addition testing was performed on the following samples:

- 200 EDTA (100 fresh[1] samples and 100 samples which were at least 10 days old)
- 200 clotted samples (100 fresh samples and 100 samples which were at least 10 days old)
- 200 donor segments (citrated samples-100 fresh samples and 100 samples which were at least 36 days old) for representative products of the monoclonal Blood Grouping Reagents and Seraclone control ABO+Rh).
- Reagent Red Blood Cells for detection of unexpected antibodies were tested with 200 EDTA (100 fresh samples and 100 samples which were at least 10 days old); and 200 clotted samples (100 fresh samples and 100 samples which were at least 10 days old). All negative antiglobulin test results were verified with Coombscell-E.
- 38 samples of elderly people (>80 years old) and 101 samples with known red blood cell antibodies were tested with the Reagent Red Blood Cells for antibody screening and Anti-Human Globulin. All negative antiglobulin test results were verified with Coombscell-E.

Interference testing:
The Biotest Reagent Red Blood Cells for the detection of unexpected antibodies, MLB2 and the Anti-Human Globulin reagents were also tested with at least 10 blood samples per interfering substance (hemolyzed, icteric, lipaemic).

Additional antibody screen testing:
In addition, 101 samples with known red cell antibodies were tested with Biotest Anti-Human Globulin reagents, MLB2 and Biotest RRBCs to increase the number of positive samples (tested in 2006). The positive agreement with the reference method was calculated with the higher number of positive samples.

Reference Test Methods
Currently FDA approved reagents were used as a reference method. The test method was the manual tube test.

Results
This section summarizes the data for each of the protocol endpoints. Refer to Tables 10.e. to 10.g.

[1] up to 3 days old

Table 10.e. Positive agreement testing for Antibody Screen Results (combined data 2006 and 2007)

Trial Reagent Name	Correct Result Positive	Incorrect Negative Results	Positive agreement [95% lower confidence interval]	
Biotest AHG Anti-IgG	155	5	155/160	96.88% [93.54%]
Reference AHG Anti-IgG	153	7	153/160	95.63% [91.94%]
Biotest AHG Anti-IgG, -C3d; Polyspecific	140	8	140/148	94.60% [90.46%]
Reference AHG Anti-IgG, C3d	143	5	143/148	96.62% [93.03%]

The evaluation of the negative agreement was not part of the 2007 trial. For the general view, table 10.f. shows the calculated negative agreement based on the data of the multi-center field trial (2006).

Table 10.f. Negative agreement testing for Antibody Screen Results (data 2006)

Trial Reagent Name	Correct Result Negative	Incorrect Positive Results	Negative agreement [95% lower confidence interval]	
Biotest Anti-Human Globulin Anti-IgG	1286	0	1286/1286	100.0% [99.77%]
Biotest Anti-Human Globulin Anti-IgG, -C3d	2067	0	2067/2067	100.0% [99.86%]
Reference Method	3353	0	3353	100.0%[99.86%]

Table 10.g. Rate of Agreement for Biotest RRBCs; MLB2 and Anti-Human Globulin Anti-IgG and Anti-Human Globulin Anti-IgG, -C3d; Polyspecific (combined data - 2006 field trial and 2007 testing of additional samples)

Trial Reagent	Number in Agreement	Number of Tests	Rate (%)	Lower 95% confidence bound
Biotest AHG Anti-IgG	1441	1446	99.65%	99.27%
Biotest AHG Anti-IgG, -C3d;	2207	2215	99.64%	99.35%
Reference Method	3636	3649	99.66%	99.43%

Summary: The antibody detection with Biotest reagents (Anti-Human Globulin Anti-IgG or Anti-Human Globulin Anti-IgG, -C3d, Biotest RRBCs and MLB2) met the acceptance criteria that the rate of agreement between the Biotest reagents and the reference method is at least 99% (95% lower confidence interval).
The Anti-Human Globulin Anti-IgG, -C3d did not meet the requirement that the sensitivity is equal or greater than the reference reagent. The antibody specificity of the antibodies not detected by the Biotest Anti-Human Globulin Anti-IgG, -C3d were: 1x Anti-Lea (not be considered clinically significant), 1x Anti-c (w+ reaction with the reference method), 3x passive Anti-D (not be considered clinically significant), 1x Anti-E, 1xAnti-K, 1x Anti-Lua (Biotest screen cell was negative for the Lu (a+) antigen).Due to the fact that 4/8 antibodies were not clinically significant and 1/8 was not detectable by the Biotest RRBCs (due to the antigen make up of the used Biotest Reagent Red Blood Cells), the number of non-detected red blood cell antibodies was acceptable. Please note, that the sensitivity was not recalculated after the exclusion of these 5 samples.

The 2007 data demonstrated that Biotest reagents (Anti-Human Globulin, MLB2, RRBCs and Coombscell-E) can be used with different sample specimen (EDTA anti-coagulated and clotted samples). The data also support the use of aged samples. Hemolysis, jaundice or lipemia do not interfere with the outcome of the antibody detection and antibody identification results.
All negative test results among those sample testing were tested with Coombscell-E. The reactions were all clear strong positive.

Cross-matching results
Twenty-one (21) patient samples were tested with 80 donor units for a total of 80 compatibility tests. The study contained both antibody positive samples (anti-D, anti-K anti-E,c) and antibody negative samples. The samples were cross-matched against both ABO compatible and ABO incompatible donor units. For samples that contained unexpected alloantibodies, antigen negative and antigen positive donor units were selected for compatibility testing.
There was 100% concordance between the reference method and the trial reagents. All ABO incompatible units were correctly incompatible with the trial reagents. All cross-

matches that were incompatible due to other red cell alloantibodies were incompatible with the trial reagents. All ABO compatible units were compatible with the trial reagents.

Results with Seraclone Control ABO+Rh:
In the former multi-center field trial (2006), control results were not reported. In the 2007 additional trials, Seraclone Control AB0+Rh was tested with 486 samples. Seraclone Control AB0+Rh is intended to be used as a control for the Seraclone Blood Grouping Reagents with patient samples that exhibit interference with testing due to positive DATs, cold agglutinins, etc. For the trial, random samples were chosen to be tested. Details are provided in Table 10.h.

10.h. Number of samples with Seraclone Control ABO+Rh

Sample Type	Number in Agreement	Number of Tests	Rate (%)
EDTA 0-3 days old	50	50	100%
EDTA 0-7 days old	80	80	100%
EDTA at least 11 days old	90	90	100%
Citrated 0-3 days old	99	99	100%
Citrated at least 36 days old	127	127	100%
Clotted samples at least 11 days old	40	40	100%

This data shows the Seraclone Control AB0+Rh reacts correctly negative with 489 of 489 samples of varying age and anticoagulant type.

Final Conclusions
The analysis of the data demonstrated that the Biotest Blood Grouping Reagents, Anti-Human Globulin Reagents and Reagent Red Blood Cells including MLB2 as a potentiator and Coombscell-E as a control for antiglobulin test with negative results and Seraclone Control ABO+Rh are safe and effective in performing ABO/Rh grouping, typing of rare antigens, antibody screening, antibody identification, cross-matches for patients and donors. All test reagents performed satisfactorily.