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PRODUCT INSERT

LAMBDA ANTIGEN TRAY (LAT™) FOR ELISA

IVD **For In Vitro Diagnostic Use.**

REF

			Test Configuration				
			First Incubation			Second Incubation	Third Incubation
Catalog Number	Product Name	Antigens	Antibody Diluent	Serum Control	Test Serum	AP Conjugate	Substrate
LAT1240	Lambda Antigen Tray Class I & II (2 tests/tray)	28 Class I 12 Class II	Row 1 (A-B)* Row 12 (A-B) Row 12 (G-H)*	Row 1 (C-H)	First sample: Row 1 (A-B)* Rows 2-6 Second sample: Rows 7 – 11, Row 12 (G-H)*	Rows 1 - 12	Rows 1 - 12
LAT1288	Lambda Antigen Tray Class I & II (1 test/tray)	56 Class I 32 Class II	Row 1 (A-B)*	Row 1 (C-H)	Row 1 (A-B)* Rows 2 -12	Rows 1 - 12	Rows 1 - 12
LAT140	Lambda Antigen Tray Class I (1 test/tray)	40 Class I	Row 1 (A-B)* Row 12 (A-B)	Row 1 (C-H)	Row 1 (A-B)* Rows 2 – 6	Rows 1 – 6 Row 12 (A-B)	Rows 1 – 6 Row 12 (A-B)
LAT140X2	Lambda Antigen Tray Class I (2 test/tray)	40 Class I	Row 1 (A-B)* Row 12 (A-B) Row 12 (G-H)*	Row 1 (C-H)	First Sample: Row 1 (A-B)* Rows 2-6 Second Sample: Rows 7-11, Row 12 (G-H) *	Rows 1 – 12	Rows 1 – 12
LAT1HD	Lambda Antigen Tray Single Antigen Class I (1 test/tray)	88 Class I	Row 1 (A-B)*	Row 1 (C-H)	Row 1 (A-B)* Rows 2-12	Rows 1-12	Rows 1-12
LAT240	Lambda Antigen Tray Class II (2 Tests/tray)	32 Class II	Row 1 (A-B)* Row 12 (A-B) Row 12 (G-H)*	Row 1 (C-H)	First Sample: Row 1 (A-B)* Rows 2-5 Second Sample: Rows 7-10, Row 12 (G-H) *	Rows 1-12	Rows 1-12
LATM10X5	Lambda Antigen Tray - Mixed Class I & II LATM10X5 (10 tests/tray)	Mixed Class I and Mixed Class II	Row 1 (A-B) Row 2 (C-E)	Row 1 (C-H)	Rows 3 – 12 (A-D) and (E-F) **	Rows 1 - 12	Rows 1 – 12
LATM20X5	Lambda Antigen Tray - Mixed Class I & II LATM20X5 (20 tests/tray)	Mixed Class I and Mixed Class II	Row 1 (A-B) Row 2 (C-E)	Row 1 (C-H)	Rows 3-12 (A-D) or (E-H)	Rows 1 - 12	Rows 1 – 12
LATM120	Lambda Antigen Tray - Mixed Class I	Mixed Class I	Row 1 (A-B) Row 2 (C-E) Rows 3-12 (C-D and G-H)*	Row 1 (C-H)	Rows 3-12 [(A-B) and (C-D) **] or [(E-F) and (G-H) **]	Rows 1 - 12	Rows 1 - 12
LATM220	Lambda Antigen Tray - Mixed Class II	Mixed Class II	Row 1 (A-B) Row 2 (C-E) Rows 3-12 (C-D and G-H)*	Row 1 (C-H)	Rows 3-12 [(A-B) and (C-D) **] or [(E-F) and (G-H)**]	Rows 1 - 12	Rows 1 - 12

Important Notes in Regard to "No Antigen Control" wells (NAC):
**For LAT™ trays, add patient serum to the NAC wells if you need to test for non-specific binding. Otherwise, add Control Diluent to the NAC wells.*
***All LAT™ M assays, except LATM20X5, have NAC reference wells for each serum. For LAT™ M assays, use diluent in "blank" wells (Rows A-B).*
Please consult appropriate LAT™ worksheets for the location of the NAC wells.

INTENDED USE

For the detection of HLA-specific antibody in the serum of pre- and post-transplant organ recipients.

SUMMARY AND EXPLANATION

Individuals may be sensitized to Human Leukocyte Antigens (HLA) during pregnancy, by blood transfusions, or by previous organ grafts. The resultant antibodies may be highly cross-reactive or of limited specificity. The existence of pre-formed anti-HLA antibodies is a contraindication for the transplantation of organs that express the specific or crossreactive (CREG) antigens (1-3). Furthermore, the development of antibody to the foreign Class I antigens of an HLA-mismatched graft following transplant may cause hyperacute rejection (4). Thus, the clinical management of these patients has long included screening for Class I HLA-directed antibody (5) with recent interest in detection of Class II antibody, as well (19).

Historically, the established method for determination of anti-HLA antibody has been the lymphocytotoxicity test (6,7). The assay is usually performed with a panel of T lymphocytes with a broad representation of different Class I antigens. Sera that react with a majority, or all, of the antigens on the panel (high percentage of panel reactive antibody, or PRA) are indicative of a high level of presensitization to HLA. If the specificity of the patient's antibody can be determined, it may be possible to predict acceptable crossmatch results in cases where HLA-matched organ donors are not available (8, 9).

Recently, an ELISA technique was introduced as an alternative to the lymphocytotoxicity assay for detection of anti-HLA antibody (10). The Lambda Antigen Tray (LAT™) is based on this principle of presenting purified HLA antigens, instead of cells, as targets for the binding of the patient's antibody. The increased specificity of the assay offers the advantages of eliminating non-HLA false positive reactions and distinguishing Class I and II reactions (11). The Class I and II Mixed (LAT™ M) assay is intended for prescreening of sera to identify positive samples (20). The LAT™ assay presents a panel of HLA antigens, rather than pooled antigen, thus permitting the evaluation of percent PRA and of the specificity of the test sample. LAT high definition provides a panel of purified HLA antigen with single or selected specificities. Such a panel is beneficial for testing high PRA sera to identify specificities that may be obscured by antibodies produced against high frequency common HLA such as A2. Furthermore, like flow cytometry, the LAT™ can detect non-complement binding HLA-specific antibody that is missed by lymphocytotoxicity (12,13). Finally, it is desirable to distinguish IgG from IgM antibody, because the latter appears to be non-detrimental to the transplant (14,15). The lymphocytotoxicity test is often performed on dithiothreitol treated sera in order to eliminate the IgM reactivity (16,17). In contrast, the LAT™ ELISA assay uses a gamma chain-specific second antibody to positively identify IgG antibody in the test sample (10,11). The percent PRA results of the LAT™ assay have been shown to correlate very well (r = 0.88) with cytotoxicity results of DTT-treated sera tested on the Lambda Cell Tray (LCT™) (18). Thus, the LAT™ allows definitive classification of human sera, because it detects only IgG antibody specific for HLA Class I or Class II antigens.

PRINCIPLE OF THE ASSAY

The LAT™ provides pre-calibrated ELISA reagents for the detection of IgG antibody to HLA Class I or Class II antigens in human serum. Defined amounts of affinity-purified HLA antigens are presented in different wells of a Terasaki tray. The specific binding of antibody from the test sample with any of these antigens is detected by a subsequent incubation with alkaline phosphatase-conjugated antibody that recognizes only human IgG. A quantitative measure of the extent of reaction is obtained by spectrophotometric determination following the addition of the appropriate enzyme substrate for the development of color. Qualitative assessment of antibody specificity is performed by analysis of the LAT™ reactivity pattern using the appropriate LAT worksheet.

REAGENTS

A. Identification

Class I and Class II HLA antigens are separately purified from EBV transformed human B cell lines (for phenotypic antigens). Single recombinant HLA antigens are purified from transfected HLA-deficient human lymphoid cell lines. Antigens are directly bound to individual wells of Terasaki microtest trays. The concentration of the antigens is such that their reactivity with monoclonal antibody to either Class I or Class II HLA monomorphic determinant gives a reading of > 1.5 optical density (O.D.) units under standard conditions. These antigens are combined in the Lambda Antigen Tray (LAT™) to allow detection of IgG antibody to a panel of different HLA Class I and Class II HLA specificities (see worksheet). The LATM provides a specific mix of Class I or Class II HLA antigens. The antigen trays (coated with a blocking and stabilizing agent) are presented in a dry format. Reactivity and specificity of these antigens has been confirmed by Quality Control tests with serologically defined reagents from commercially available HLA Class I typing trays.

B. Warning or Caution

1. For In Vitro Diagnostic Use.
2. Biohazard Warning: The test and control sera should be treated as potentially infectious. The enclosed Control Serum was tested and found negative in tests currently required by the FDA. However, no known test methods can offer complete assurance that products derived from human blood will not transmit infectious agents.

C. Instructions for Use – Reconstitution of Reagents

1. If buffer salts have precipitated out of solution during shipment or storage, re-dissolve them by briefly warming the reagent bottle to 37° C until the precipitate dissolves before preparing working dilution.
2. Lyophilized serum control: reconstitute prior to the first assay. See the **Directions for Use** (next page).
3. Serum control and AP conjugate: dilute the needed amount with antibody diluent.
4. Wash buffer: dilute the needed amount to working concentration with deionized water.
5. Colorimetric substrate components A and B: mix equal volumes just before use.
6. Antigen trays: no pretreatment is necessary. Bring to room temperature before opening package.

D. Storage Instructions

1. Keep reagents at 2 - 5° C until opened. Use the reconstituted 10X serum control within one week or aliquot (8 µl per test), and store the aliquots at -20° C.
2. Do not use reagents if microbial or cross-contamination is suspected. Be sure to use clean pipette tips, or wash channel pipettors thoroughly between dispensing of different reagents. Do not interchange vial caps on reagents.
3. Keep LAT™ trays at 2 - 5° C, and protect them from moisture. Reseal aluminum bag if using only a few trays at a time. Do not remove desiccant from tray package.
4. Do not use past the expiration date on the package.

E. Indications of Product Instability or Degradation:

1. **Micro-ELISA trays**: Moisture in the wells may damage the HLA antigens.
2. **Serum control**: Moisture in the vial may damage the lyophilized antibody. After reconstitution, any cloudiness of the serum could indicate microbial contamination.
3. **AP-conjugated anti-human IgG**: No visible signs of instability. Check QA control values in the LAT assay.
4. **Antibody diluent, wash buffer, sterile water, or stop reagent**: Cloudiness or precipitation may indicate degradation or microbial contamination.

INSTRUMENT REQUIREMENTS

ELISA reader (and printer) with ability to read Terasaki trays.

1. Single wavelength reading at 630 nm for colorimetric (BCIP) assay.
2. Computer software (optional)
3. Follow manufacturer's procedure for instrument calibration.

SPECIMEN COLLECTION AND PREPARATION

A. Serum from aseptically drawn blood specimens can be tested immediately, after brief storage at 2-5°C, or after long-term storage at approximately -20°C.
B. Test samples should be diluted with the antibody diluent provided. Do not use buffers containing EDTA for sample dilution.
C. Do not use heat-inactivated serum, as it will give a high background in this assay.

PROCEDURE

A. Materials Provided

		LAT	LATM	
	Catalog numbers	**96-Well Trays—LAT140, LAT140X2, LAT 240, LAT1240, LAT1288, LAT1HD**	**LATM10X5**	**LATM20X5 LATM120 LATM220**
Reagent Description	**Concentration**	**QUANTITY/VOLUMES PROVIDED**		
Micro-ELISA trays	Not applicable	1 test per tray; 20 trays per package 2 tests per tray; 20 trays per package	10 tests per tray; 5 trays per package	20 tests per tray; 5 trays per package
Serum control; lyophilized anti-HLA alloantiserum (use for positive and negative control wells)	10X	0.2 ml (reconstituted volume)	0.1 ml (reconstituted volume)	
Sterile deionized water (use for reconstitution of serum control)	Not applicable	1 ml	1 ml	
Alkaline phosphatase (AP) conjugated anti-human IgG	100X	0.3 ml	0.1 ml	
Antibody diluent	1X	50 ml	12 ml	
Wash buffer	10X	125 ml	30 ml	
Colorimetric enzyme substrate: BCIP, components A and B (Blue Phos™ from KPL)	1X	Each bottle: 15 ml	Each bottle: 3 ml	
Stop reagent	1X	25 ml	6 ml	

B. Materials Required, But Not Provided

- Microcentrifuge tubes (1.5 ml) for serum dilution
- Plastic bottle for wash buffer
- Deionized water (900 ml)
- Manual, electronic, or 6-channel pipettor
- Disposable pipette tips

C. Step-by-step procedure.

See "Directions For Use."

DIRECTIONS FOR USE

A. Reagent Preparation

1. Reconstitute lyophilized serum control with **0.1 or 0.2** ml of sterile deionized water (as specified on the vial) at least 20 minutes prior to the first assay. Keep the reagent at room temperature and tap the vial occasionally until the antibody is solubilized.
2. Just prior to assay, dilute each test sample with antibody diluent (1:3 for LAT and 1:2 for LATM; however, if prescreen indicated a strong antibody, then you may wish to dilute further to assign specificity on LAT).
3. Take 8 µl (per tray) of reconstituted 10X serum control and dilute with antibody diluent.
4. Take 10 µl (per tray) of 100X AP-conjugated anti-human IgG and dilute with antibody diluent just before first wash step.
5. Take 5 ml (per tray) of 10X wash buffer and dilute with 45 ml deionized water (enough for 4 washes/tray).
6. Combine equal volumes of enzyme substrate reagents A and B (500 µl of each per tray) just before second wash step. Take care to change pipette tips to avoid cross-contamination of stock solutions.

DIRECTIONS FOR USE

B. Test Procedure

Refer to table on page 1 to identify rows to which reagents should be added. See Data Record and Worksheet for additional information.

1. Add 10 µl of (diluted) test sample or quality control reagent to the appropriate wells of the LAT™.
   Note: *To avoid cross-contamination of reagents, change pipette tips regularly.*
2. Replace tray lid, and incubate for **1 hour** at **20 - 25°C** (Optional: Incubate on rotary platform at low speed).
3. Remove diluent and test or control serum from wells by hand flicking of trays. Strike inverted tray into paper toweling to blot. Approximately one lambda (1µl) of liquid should remain in the wells. Keep trays inverted or covered until addition of the next reagent. Do not let trays dry out.
4. Add 15-20 µl of 1X wash buffer with a six-channel pipettor or bottle reservoir/dispenser, or pour buffer into trays to fill all wells. Swirl gently, and pour off excess buffer. Then, remove wash buffer from wells as above. Repeat wash and buffer removal.
5. Dispense 10 µl of diluted AP-conjugate to each well. Discard leftover diluted reagent.
6. Replace tray lid, and incubate for **40 minutes** at **20 - 25°C**. (Optional: Incubate on rotary platform at low speed).
7. Remove AP-conjugate from wells, as in step 3. Do not let trays dry out.
8. Add and remove wash buffer twice as in step 4. Approximately one lambda (1 µl) of liquid should remain in the wells.
9. Dispense 10 µl of enzyme substrate to each well of the LAT™. Discard leftover reagent.
10. Replace tray lid, and incubate at 37º C for 10-15 minutes protected from light. (Optional: Incubate on rotary platform at low speed.)
    Note: Do not let the reaction proceed longer than 15 minutes unless it is carefully monitored. The positive control wells should be dark blue and the negative wells should be clear. It is important to stop the color development before non-specific background arises.
11. Stop the reaction by adding 5 µl of stop reagent to each well of the LAT™ tray. Replace tray lid. Wait 15 minutes for reagents to equilibrate.
12. Read the results within 1 hour with an ELISA reader adapted for the Terasaki tray format. Remove lid for tray reading. Trays (with lids) can be stored at 2 - 5°C for up to 3 days after stopping the reactions and can be read again, if needed to confirm reaction pattern. However, O.D. values will decrease slightly during storage, and weak reactions may be lost.

Note: *If pNPP is substituted for BCIP as the colorimetric substrate, incubation time (step 10 of* ***Test Procedure****) should be for 30 minutes at 37°C.*

RESULTS

A. Validation of the assay:

1. Correcting for background (wells 1A and 1B), divide the Pos. HLA control value by the Neg. HLA control value to get a ratio, as indicated by the formula:

$$\text{POS/NEG Ratio} = \frac{\text{Av. Pos. HLA – Av. NAC (or Blank)}}{\text{Av. Neg. HLA –Av. NAC (or Blank)}}$$

2. If the denominator is less than 50, substitute a default value of 50

Note*: This value for (Av. Neg.* ***-*** *HLA-NAC) was determined by testing 200 negative sera from non-transfused male donors in the LAT assay.*

3. The POS/NEG ratio must be >5. To call "2" reactions positive, a higher POS/NEG ratio may be needed for statistical confidence, according to each laboratory's results and their sensitivity requirements.
4. QA wells coated with human IgG are sometimes included to check the performance of the secondary antibody-enzyme conjugate. The average reading for these wells should always be >1,000 (generally >2,000).

Note: *QA wells are not included in the LAT1288 trays. Refer to the Pos. HLA wells. The latter wells check antigen integrity and secondary antibody simultaneously.*

5. The average value of the Pos. HLA wells should always be >800 (optimum >1500).

6. The average value of the NAC (Blank) wells should be <250 if antibody diluent is used in these wells, and <500 if test serum is used.

Note: *Certain test sera may exhibit high non-specific binding to the blocking reagent (or to the plastic) in the No Antigen Control (NAC wells), so correction for this background is desirable.*

B. Determination of assay cut-off values:

1. Cut-offs are calculated as a percentage of the range of the reactivity of the provided Serum Control (SC) tested in the Positive HLA wells minus the non-specific background of the Test Serum (or antibody diluent) tested in the NAC wells.
2. The data printout shows the original raw data. Therefore, to be able to scan the printout for values above or below a given cut-off, an adjusted cut-off can be calculated. For example, for the 20% cut-off:

   Adj. Cut-off = [(Av. Pos. HLA - Av. NAC) x 0.2] + [Av. NAC]

3. For the LAT140, LAT240, LAT1240, and LAT1288 assays, readings are separated into ranges and assigned a score (similar to the lymphocytotoxicity assay). The suggested scoring system is shown below:

   0-10% = 1 (negative)
   11-20% = 2 (weak positive or negative reaction)
   21-50% = 4 (positive reaction)
   51-80% = 6 (strong positive reaction)
   81 to >100% = 8 (very strong positive reaction)

4. For LATHD, we recommend considering only "6" or "8" reactions as positive. Since this assay has a higher degree of signal amplification, lower scores may be due to non-specific binding.
5. For the LATM assays, a single cut-off is calculated, The average test serum value for duplicate wells for either Class I or Class II mixed antigen is generally considered positive if it exceeds the 20% cut-off. Samples within +/- 5% of the cut-off (grey area) may give inconsistent results.

C. Calculation of % PRA (not applicable to LATM or LATHD):

1. For calculation of % PRA, divide the number of wells with values over the cut-off by the total number of antigen preparations on the test panel, and convert to a percent as in the equation below:

   $$\%\ PRA = \frac{\#\ of\ positive\ HLA\ wells}{n} \times 100$$

2. Percentages are rounded off to whole numbers. The lower limit of detection depends on the panel size, e.g., 2.5% PRA (n = 40) or 3.6% (n=28) for Class I and 3% (n=32) or 6% (n = 18) for Class II. For determination of % PRA, we recommend a standard cut-off value of 4 for a 1:3 dilution of test serum.

D. Determination of antibody specificity (not applicable to LATM):

1. Determine the HLA antibody specificity by entering the reaction score for each well (determined in section B3, above) into the tray worksheet (or available software) and analyzing the reaction pattern. As in lymphocytotoxicity, each serum will have a different titer. Flexibility in analyzing either the "**8**", "**6, 8**" or "**4, 6, 8**" reactions allows one to tailor the analysis of specificity for the strength of each serum.
2. For some "weak" sera, specificity can be assigned even with "2" reactions. Such samples can be re-tested at a lesser dilution if desired.
3. For high PRA sera, specificity might better be analyzed by testing a dilution of the serum (perhaps 1:20 or 1:40). For strong sera, the % PRA usually drops from 100% to 0% between a dilution of 1:40 and 1:160.

E. Determination of Strength Index (not applicable to LATM or LATHD):

An individual **Strength Index** (S.I.) for specific antibodies identified in a given test sample can be calculated as the % of "8" reactions out of the total number of antigen-specific positive ("4, 6, or 8") reactions, as in the lymphocytotoxicity assay.

F. Determination of the Average Positive Score (not applicable to LATM or LATHD)

For calculation of the **Average Positive** of a reactive (non-specific or multi-specific) serum, determine the Mean of positive ("4, 6, or 8") reactions for the test sample.

SAMPLE CALCULATIONS

Calculations will be shown based on the following tray layout and sample values for LAT140. Similar steps would be followed for each LAT product by reference to the specific Worksheet and data output.

	A	B	C	D	E	F	G	H
Row 1	NAC 290	NAC 310	Neg. HLA 400	Neg. HLA 420	Neg. HLA 440	Pos. HLA 1650	Pos. HLA 1700	Pos. HLA 1750
Row 2	HLA A1 1200	HLA A1 1800	HLA A1 980	HLA A1 1600	HLA A1 1750	HLA A2 400	HLA A2 420	HLA A2 400
Rows 3-6	Assume all other test serum readings vs. HLA antigens are negative							
Rows 7-11	Not used in the assay							
Row 12	QA 2000	QA 2002	Not used in the assay					

A. For assay validation:

1. The POS/NEG ratio =

$$\frac{\text{Av. Wells 1(FGH)} - \text{Av. Wells 1(AB)}}{\text{Av. Wells 1(CDE)} - \text{Av. Wells 1 (AB)}} = \frac{1700 - 300}{420 - 300} = \frac{1400}{120} = 11.7 \quad (>5 \text{ is OK})$$

2. The average Pos. HLA value = 1700 **(>800 is OK)**
3. The average QA value = 2001 **(>1000 is OK)**
4. The average NAC value = 300 **(< 500 is OK)**

B. For calculation of the (adjusted) cut-off values:

[Av. Wells 1(FGH) – Av. Wells 1(AB)] x (X%) + [Av. Wells 1(AB)] =

1. For **2,4,6,8** reactions +, the cut-off = **(1700-300)(0.1) + (300) = (1400)(0.1) +300 = 140 +300 = 440**
2. For **4,6,8** reactions +, the cut-off = **(1700-300)(0.2) + (300) = (1400)(0.2) +300 = 280 +300 = 580**
3. For **6**,**8** reactions +, the cut-off = **(1700-300)(0.5) + (300) = (1400)(0.5) +300 = 700 +300 = 1000**
4. For **8** reactions +, the cut-off = **(1700-300)(0.8) + (300) = (1400)(0.8) +300 = 1120+300 = 1420**

C. For calculation of % PRA (% positive):

$$\frac{\text{\# of positive HLA wells}}{n} \times 100 = \frac{5}{40} \times 100 = 12.5\% \text{ PRA}$$

D. For assignment of antibody specificity:

1. Five of five HLA A1 wells are positive (wells 2A –2E)
2. Therefore, the antibody specificity of this test serum is assigned as **A1**.

E. For calculation of the Strength Index:

1. Three of five readings for wells 2A –2E are over the **8** cut-off (1420).
2. Therefore, 3/5 x 100 = **60% S.I.**

F. For calculation of the Average Positive Score:

1. The five positive wells (2A –2E) have scores of **6,8,4,8** and **8**.
2. The average positive is the total of these scores/ # wells positive = (34/5) = 6.8

LIMITATIONS OF THE PROCEDURE

A. This product detects only IgG antibody and not IgM, which may be present in the early phase of an anti-HLA response. The range of detectable positives is limited to the specific HLA antigens of the cell lines used to purify HLA molecules (see product worksheet for HLA antigen panel composition and mixed antigen frequency information).

B. The percent PRA is not an absolute value. Results will differ depending on the antigen composition of the test panel. Determination of percent PRA is considered a first screen diagnostic test, but not the sole basis for a clinical decision affecting the patient's treatment. A final crossmatch test is routinely required prior to transplant.

C. With some rabbit anti-globulin (ATG), a drug to reduce rejection risk, a high non-specific background may occur in Class I or Class II antigen wells if the patient's serum contains over 100 µg/ml of ATG. In this case, diluting the AP-conjugated anti-human IgG with the antibody diluent containing 10% rabbit serum may reduce the background. For sera with extremely high background, results may be inconclusive.

EXPECTED VALUES

A. The percent PRA (range from 0 to 100%) values obtained are an indication of the degree of sensitization to HLA antigens. Patients who have had multiple blood or platelet transfusions can be expected to have a high percent PRA. The antibody titer may drop over time (from > 1: 160) and this will finally be reflected by a drop in percent PRA in the LAT™ assay with the sera diluted 1:10. For sensitivity comparable to an AHG-enhanced cytotoxicity, use a 1:3 dilution of sera; for 100% PRA sera, dilute further to call major specificity.

B. Sera from healthy non-transfused males give 0% PRA values in the LAT™ assay. Sera from multiparous females may have higher percent PRA and antibody specificity for their children's paternal HLA.

C. The LAT™ is about 10 times more sensitive than the lymphocytotoxicity test in regard to the titer of the sera. The overall correlation of percent PRA between the two assays is high (see Figure 1). The percent PRA value of a given sample may vary from cytotoxicity results due to differences in antigen frequency on the panel or possibly are due to the complement, AHG, or incubation time of the cell-based assay in each individual laboratory.

D. Since the LAT™ assay measures different parameters than the lymphocytoxicity assay, i.e., IgG HLA-specific antibody only, and direct binding versus complement activation and cytotoxicity, matching results between the two assays are not expected for certain sera. In these cases the LAT™ data may have greater clinical relevance.

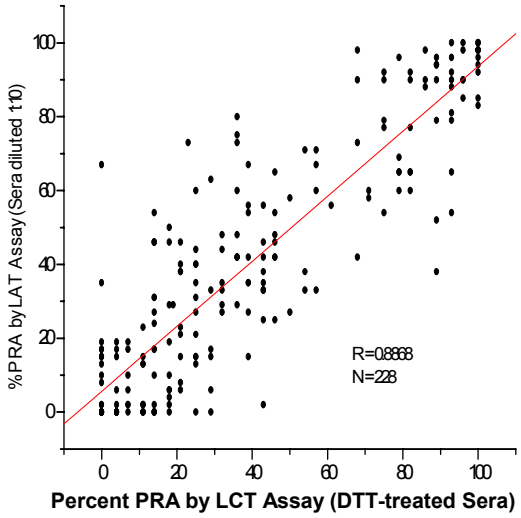
E. This product is designed to detect only the IgG-class of antibody for diagnostic use. For research purposes, the assay could be conducted with AP-conjugated secondary antibody (not provided) specific for IgA or IgM to determine if these are present in the test sample.

F. For LATM, sensitivity of the mixed antigen may be slightly less than for the individual antigens. Therefore, the sera are tested at a lesser dilution, and any "2" reactions could be positive. These sera can then be tested on the LAT antigen panel to confirm reactivity and to assign specificity.

SPECIFIC PERFORMANCE CHARACTERISTICS

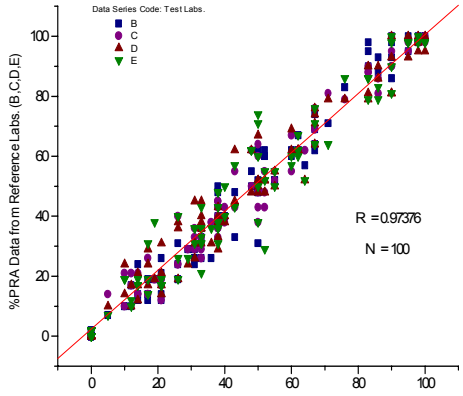
The LAT™ assay compares favorably with the Lambda Cell Tray (LCT™) lymphocytotoxicity assay, as shown in Figure 1 for detection of HLA Class I antibody. A linear regression analysis of the data showed a significant correlation when the LAT™ was conducted with sera diluted 1:10 and the LCT™ was conducted with undiluted DTT-treated sera.

Figure 1. Concordance between LAT and LCT assays. Detection of IgG anti-HLA Panel Reactive Antibody (PRA) in Human Sera.



The LAT™ assay demonstrates excellent stability and reproducibility. The linear regression analysis in Figure 2 shows minimal inter-laboratory variation in the percent PRA data on replicate samples.

Figure 2. Reproducibility of LAT between Replicate Testing of a Panel of PRA Sera



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REVISION HISTORY

Revision	Date	Revision Description
17	*2004/11*	*Add "Results" and "Sample Calculations" Sections. Revision to "Reagents" Sections A., D.1 and "Limitations of the Procedure" Sections B, C.*
18	*2005/04*	*Clarify fact that LATM20X5 does not have reference wells for serum background; LATM10X5 trays do have designated well for controls.*