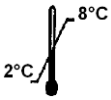
IMMUCOR

Pak Lx™ Assay

REF PLX IVD CE



Caution! Consult Accompanying Documents.

Light Sensitive (Keep away from light)

MATCHIT! Platelet Antibody software v1.0.1 (REF **888622**)

MATCHIT! Platelet Antibody software User Manual (REF **LC1371**)

MATCHIT! Platelet Antibody software Quick Reference Guide (REF **LC1374**)

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Restricted Use Label License:

By opening the packaging containing this Kit (which contains fluorescently labeled microsphere beads authorized by Luminex Corporation) or using this Kit in any manner, you are consenting and agreeing to be bound by the following terms and conditions. You are also agreeing that the following terms and conditions constitute a legally valid and binding contract that is enforceable against you. If you do not agree to all of the terms and conditions set forth below, you must promptly return this Kit for a full refund prior to using it in any manner.

You, the customer, acquire the right under Luminex Corporation's patent rights, if any, to use this Kit or any portion of this Kit, including without limitation the microsphere beads contained herein, only with Luminex Corporation's laser based fluorescent analytical test instrumentation marketed under the name Luminex instrument

INTENDED USE

Pak Lx Assay is a qualitative immunoassay for use on the Luminex instrument. The Pak Lx Assay is designed to detect and differentiate between IgG antibodies to HPA-1, HPA-2, HPA-3, HPA-4, HPA-5, GPIV and Class I HLA in human serum.

SUMMARY AND EXPLANATION

Platelets express a variety of polymorphic proteins. The polymorphic changes in the proteins, while not affecting protein function, might become the targets for antibodies as a result of pregnancy or transfusion. The presence of antibodies that bind to platelet glycoproteins is associated with life-threatening bleeding disorders such as refractoriness to platelet transfusions (PR), post-transfusion purpura (PTP), and fetal and neonatal alloimmune thrombocytopenia (FNAITP). [1-13]

The glycoproteins (GP) targeted by antibodies include GPIIb/IIIa, GPIb/IX and GPIa/IIa, GPIV, and Class I Human Leukocyte Antigens (HLA). The epitopes found on GPIIb/IIIa, GPIb/IX, and GPIa/IIa have been characterized into Human Platelet Antigen (HPA) systems of two alleles each (alleles “a” and “b”). HPA-1, HPA-3, and HPA-4 are all located on GPIIb/IIIa. HPA-2 is located on GPIb/IX and HPA-5 is located on GPIa/IIa.[14-16] The epitopes of GPIV are presented only as isoantigens. In other words, the development of antibodies to GPIV occurs in individuals who do not have or have significantly reduced levels of GPIV expression, but have become exposed to GPIV.[17] Class I HLA is encoded by numerous alleles expressing a large number of diverse epitopes. The vast majority of antibodies produced in response to pregnancy or transfusion will be reactive with the highly polymorphic Class I HLA.[18]

PRINCIPLE OF THE PROCEDURE

The Pak Lx Beads are reconstituted and incubated with serum sample at room temperature. The beads are then washed to remove unbound antibody. An anti-Human IgG antibody conjugated to phycoerythrin (PE) is then added. After further incubation at room temperature, the reaction mixture is diluted and analyzed on the Luminex instrument. The signal intensity from each bead is compared to the signal intensity of three negative control beads included in the bead preparation to determine if the bead is positive or negative for bound antibody.

REAGENTS

Maximum number of tests per kit:

- **PLX: 16 tests per kit**

All reagents should be stored as directed by the label.

	REF	
PLXB	403620	Lyophilized Bead Blend: A blend of beads to which HPA, GPIV and Class I HLA glycoproteins have been immobilized along with four control beads. The beads are lyophilized in a phosphate-based buffer containing bovine serum albumin and 0.02% methylisothiazolone and 0.02% bromonitrodioxane as preservatives. LIGHT SENSITIVE. Keep out of direct light for extended periods of time (3 hours or less). Store at 2 to 8°C in the dark, in kit box.
PLBD	405160	Bead Diluent: A phosphate-based buffer containing NaCl, Tween-20, ProClin 300, bovine serum albumin and mouse serum. Store at 2 to 8°C.
CCX	405159	Conjugate Concentrate: Goat anti-Human IgG conjugated to phycoerythrin provided in a phosphate-based storage buffer containing NaCl, Tween-20, <0.1 % sodium azide, and bovine serum albumin. LIGHT SENSITIVE. Keep out of direct light for extended periods of time (2 hours or less). Store at 2 to 8°C in the dark.Dilute 1:10 in Conjugate Diluent prior to use.
CDX	403586	Conjugate Diluent: A phosphate-based buffer containing NaCl, Tween-20, 0.1% sodium azide, bovine serum albumin and mouse serum. Store at 2 to 8°C.
LMWB	405314	Wash Buffer: A phosphate-based buffer containing NaCl, Tween-20, 0.1% sodium azide, and bovine serum albumin. Store at 2 to 8°C.
PCX	403595	Positive Control Serum: This serum or sera blend is obtained from individual(s) with known antibodies to HPA-1a with or without antibodies to Class I HLA. Store at 2 to 8°C. Contains 0.1% sodium azide.
NCX	403592	Negative Control Serum: This serum or sera blend is obtained from individual(s) with no known antibodies to HPA, GPIV and HLA. Store at 2 to 8°C. Contains 0.1% sodium azide.

PRECAUTIONS

- For In Vitro Diagnostic Use.
- Do not use reagents that are turbid or contaminated.
- Do not use reagents beyond their expiration date.
- Discard all unused / diluted Positive and Negative Controls and Conjugate after use.
- Reagents contained in the kit are not to be used in conjunction with any other test system.
- Substitution of components other than those provided in this kit may lead to inconsistent or erroneous results.
- When making dilutions, follow pipet manufacturer's instructions for appropriate dispensing and rinsing techniques.
- Care MUST be taken to avoid contamination of Conjugate Diluent or the anti-Human IgG reagent. Inadvertent contamination of these reagents with human serum will result in the neutralization of anti-Human IgG and subsequently result in test failure.
- Care must be taken during pipetting into the filter plate, that beads do not stick to the side of the microplate wells being pipetted, while being careful not to touch the membrane with the tip. Contacting the membrane with the pipet tip can lead to puncture of the membrane and subsequent failure of the assay.
- Care must be taken to ensure, during incubation steps, that the beads are not splashing and sticking to the sides of the wells. The positive and negative controls must be included with each test to help determine if technical error or reagent failures have occurred.
- Care must be taken to control vacuum strength. Strong vacuum pressure can cause beads to stick to the membrane causing bead count failure.

CAUTION

- All human serum used in the Positive and Negative Controls for this product has been tested and found negative for antibody to HIV, HCV and HBsAg by FDA approved methods. No test method, however, can offer complete assurance that HIV, Hepatitis C virus, Hepatitis B virus or other infectious agents are absent. Therefore, these materials should be handled as potentially infectious.
- Some reagents contain sodium azide as a preservative, which may react with lead and copper plumbing to form explosive metal azides. Use large amounts of water when discarding materials down a sink.
- Dispose of all materials after use according to local regulations.

INSTRUMENTATION

The Luminex instrument running the Luminex IS 2.3 or xPONENT 3.1 software is to be used for performing the Pak Lx Assay. The Luminex instrument system is based on the principles of flow cytometry and includes the Luminex analyzer, the Luminex XY plate handling platform, and the Luminex SD sheath fluid delivery system, software and PC. The Luminex System is the combination of three core xMAP Technologies. The first is microspheres, a family of 100 fluorescently dyed 5.6 micron-sized polystyrene microspheres that act as both the identifier and the solid surface to build the assay. The second is a flow cytometry-based instrument, the Luminex analyzer, which integrates key detection components such as lasers, optics, fluidics and high-speed digital signal processors. The third component is the software, which is designed for protocol-based data acquisition.

Information on the features of the software and the instrument installation, operation, maintenance and safety are provided in the Luminex IS Training Manual. The user should contact the Luminex Corporation for specific services regarding the Luminex instrument system.

The assay files required to use the Pak Lx Assay are provided by Immucor on their website for download by the user.
The user should contact Immucor GTI Diagnostics for any product related concerns for Pak Lx Assay and the MATCHIT!® Platelet Antibody Software.

SPECIMEN COLLECTION AND STORAGE

Collect blood without anticoagulant using aseptic technique. Process blood while still fresh to minimize the chance of obtaining false-positive or false-negative reactions due to improper storage or contamination of the specimen.

Only serum is suitable for this assay.

Serum should be separated from red cells when stored or shipped.

Samples containing particulate matter should be clarified by centrifugation prior to testing.

Serum samples that cannot be tested immediately should be stored at 2 to 8°C for no longer than 48 hours or frozen. Samples frozen at or below –20°C remain in good condition for up to 2 years. Avoid frost-free freezers. Avoid repeated freezing and thawing of the serum samples.

PROCEDURE

Materials Provided:
(See REAGENTS section for more specific information)

Vials may contain more reagent than stated on the labels. Be sure to measure the reagent with an appropriate device when making dilutions.

1. 3 x Lyophilized Bead Blend
2. 1 x 850 µL Bead Diluent
3. 1 x 120 µL Conjugate Concentrate
4. 1 x 1.2 mL Conjugate Diluent
5. 1 x 50 mL Wash Buffer
6. 1 x 80 µL Positive Control Serum
7. 1 x 80 µL Negative Control Serum

Additional Materials Required:
(as listed or equivalent)

1. 5 µL to 50 µL adjustable pipets with appropriate pipet tips
2. 250 µL multichannel pipet with matching tips and buffer trough
3. 1.5 mL microcentrifuge tubes for conjugate dilutions
4. Test tubes
5. Timer
6. Marking pen
7. Plate sealers
8. Luminex Sheath Fluid
   REF 628005 (1X); **REF** 628025 (20X)
9. Luminex Calibration and control beads
   REF 628006 (CAL1); **REF** 628007 (CAL2); **REF** 628008 (CON1); **REF** 628009 (CON2)
10. Distilled water
11. Rotator or vortex with plate adapter
12. Millipore Multiscreen filter plates
    REF 888633 Millipore MSBVN1210/MSBVN1250/MABVN1210/MABVN1250
13. Multiscreen vacuum manifold
    REF 888315 Millipore MAVM0960R; Qiagen 19504; Pall 5017
14. Microcentrifuge
15. Lot specific Luminex Acquisition Template file; available on website
    - IDT file for use with IS 2.3
    - LXT file for use with xPONENT 3.1
16. Lot specific Electronic Data Sheet (EDS) file; available on website
17. Luminex instrument with IS 2.3 or xPONENT 3.1 software
18. **PFS** Plate Format Sheet
19. **PACD** Platelet Antibody Software Installation CD
    MATCHIT! Platelet Antibody Software v1.0.1
    MATCHIT! Platelet Antibody software User Manual; also available on website
    MATCHIT! Platelet Antibody software Quick Reference Guide; also available on website

Test Procedure

1. Download the lot specific Luminex acquisition template (IDT/LXT file) from the website (www.immucor.com) and save it on the computer connected to the Luminex instrument. Note the location of the saved file. This file will be imported to the Luminex instrument in a later step.

2. Bring all reagents except the Conjugate Concentrate and the Conjugate Diluent to room temperature (21 to 24°C) prior to use.

3. Reconstitute the Lyophilized Bead Blend as noted in Table 1 below.

   The number of vials provided in Table 1 includes enough volume to run one positive and one negative control along with the number of samples listed in column 1.

Table 1

Number of samples	Vial(s) of Lyophilized Bead Blend
1-4	1
5-10	2
11-16	3

a. Add 275 µL of Bead Diluent to each vial of lyophilized beads. Allow the reconstituted vial to sit at room-temperature for at least 5 minutes.

b. **Do not** mix or vortex the Lyophilized Beads with the Bead Diluent at this time.

- Reconstituted vials may be stored at 2-8°C, in the dark for up to 15 days.

CAUTION: DO NOT POOL VIALS RECONSTITUTED ON DIFFERENT DAYS INTO ONE.
CAUTION: DO NOT VORTEX THE BEADS AT ANY TIME DURING THE ASSAY TO AVOID FROTHING.

4. Prepare samples by vortexing. Centrifuge at ≥ 10,000 x g for at least 60 seconds to settle any insoluble matter before use.

5. Record sample information on the Plate Format Sheet. The plate format sheet is available for download from the website (www.immucor.com).

6. Cover the unassigned wells of the filter plate with a plate sealer.

7. Add 250 µL of wash buffer to the assigned wells.

   a. Allow the wash buffer to sit in the wells for at least 3 minutes.

   b. Remove the wash buffer by gentle aspiration using the vacuum manifold. (See manufacturer's recommendations for proper use.)

8. Mix the reconstituted beads by pipetting up and down about 15 to 20 times taking care to **avoid any frothing**.

9. Add 40 µL of Reconstituted Beads to each test well of the filter plate. Ensure that the Reconstituted Beads are mixed during bead addition to the filter plate. Pipet up and down 2-3 times after delivery to every 2-3 wells.

   CAUTION: It is important to keep the beads resuspended to ensure uniform bead addition to the wells. Failure to mix beads intermittently will cause beads to settle towards the bottom of the vial. This will result in differential amount of beads being dispensed into wells which may adversely affect run-times and analysis of results.

10. Add 10 µL of patient serum or control serum referring to the plate layout sheet.

11. Return unused portions of control sera and beads to storage at 2 to 8°C in the dark for future use.

12. Cover the plate with a plate sealer.

13. Incubate for 60 minutes at room temperature (21 to 24°C) in the dark on a rotating platform (approximately 200 rotations per minute) or a vortex shaker with a plate adaptor set at a speed that will allow proper mixing without splashing of the samples.

    a. During this incubation, bring the Conjugate Concentrate and Conjugate Diluent to room temperature (21 to 24°C).

    b. During this Incubation, warm up and prepare the Luminex instrument.

    c. Set up the assay on the Luminex instrument by using the lot specific IDT/LXT file (acquisition template) downloaded from the web site (www.immucor.com). Refer to the Luminex software manual on how to add/import a template file into the software.

       NOTE: When using the Automated Batch Setup feature, refer to the MATCHIT! Platelet Antibody software User Manual which is available on the Platelet Antibody Software Installation CD PACD and on the website (www.immucor.com).

       CAUTION: Failure to use the correct IDT/LXT file will result in the collection of data that is out of sequence and will incorrectly or incompletely capture the assay data from the Luminex instrument.

14. Prepare diluted Conjugate Concentrate by mixing the volumes of Conjugate Concentrate (CCX) with Conjugate Diluent (CDX) as listed in Table 2 below. The volumes shown in Table 2 below include enough volume to run one positive and one negative control along with the number of samples listed in column 1.

    a. Cover the diluted conjugate with foil or store in the dark at room temperature until used.

    b. Return the unused portion of Conjugate Concentrate and Conjugate Diluent to storage at 2 to 8°C in the dark for future use.

    Table 2

Number of Samples	Volume of CCX (µL)	Volume of CDX (µL)
1	20.0	180.0
2	25.0	225.0
3	30.0	270.0
4	35.0	315.0
5	40.0	360.0
6	45.0	405.0
7	50.0	450.0
8	55.0	495.0
9	60.0	540.0
10	65.0	585.0
11	70.0	630.0
12	75.0	675.0
13	80.0	720.0
14	85.0	765.0
15	90.0	810.0
16	95.0	855.0

15. After the 60 minute incubation, remove the plate sealer and add 200 µL of Wash Buffer to each well. Remove the wash buffer by gentle aspiration using the vacuum manifold.

    CAUTION: Use of excessive vacuum strength will cause beads to stick to the membrane and can result in an assay failure. Apply **the minimum** vacuum pressure required to aspirate samples. Refer to the Manufacturer's instructions for specific use of the filter plates and vacuum manifold.

16. Add 250 µL of Wash Buffer to each well, aspirate. Repeat two more times.

    CAUTION: Failure to wash completely may reduce the ability of the conjugate to detect IgG bound to sensitized beads.

17. Add 50 µL of diluted conjugate to each well. Cover with plate sealer. Incubate for 30 minutes at room temperature (21 to 24°C), in the dark on a rotating platform (approximately 200 rotations per minute) or a vortex shaker with a plate adaptor set at a speed that will allow proper mixing without splashing of the samples. Do not save the diluted conjugate for future use.

18. Remove the plate sealer. Add 150 µL of Wash Buffer to each well containing the sample. Return the unused portion of Wash Buffer to storage at 2 to 8°C for future use.

19. Immediately collect data with Luminex instrument using the manufacturer's instructions.

    NOTE: A minimum of 60 events must be collected per bead region.

QUALITY CONTROL

The Positive and Negative Control Sera serve as external assay controls and must be included with each test run. The Positive Control Serum is prepared from available serum donors and may change with each lot of Pak Lx. The Positive Control Serum should have a positive reported result for those antibodies reported on the Certificate of Quality Control. The Negative Control Serum should have a negative reported result for any HPA, GPIV or HLA Class I bead. If these requirements are not met, the affected samples are to be considered invalid.

Quality control of the Pak Lx assay is built into the test system by the inclusion of four control beads, (one positive control bead and three negative control beads). The Positive Control Bead is coated with human IgG and should yield MFI values $\geq$10,000 with the Negative Control Sera. If values of <10,000 MFI are obtained with the Negative Control serum, the assay may be insufficiently washed or the conjugate may be compromised. Samples may show a wide range of reactivity with the Positive Control Bead, but should produce a signal of $\geq$3500 MFI. If these requirements are not met, the affected samples are to be considered invalid.

The interpretation of sample data is based on having a minimum number of each bead collected during an assay. When too few bead events are acquired for a sample, a "Bead Failure" error is noted and all antibody results for the affected sample are to be considered invalid.

Please see the troubleshooting section of these instructions for further details.

INTERPRETATION OF RESULTS

Interpretation by software: The MATCHIT! Platelet Antibody software v1.0.1 is required for the interpretation of the results. For the analysis, a lot-specific EDS file is required and is available for download from the website (www.immucor.com).

Determination of individual bead reactivity: To determine if an antigen-specific bead is positive or negative, the MFI of the antigen-specific bead is divided by the MFI for each Negative Control Bead (CON1, CON2, CON3), producing three ratios for each antigen-specific bead. Note: when the MFI of any CON bead is less than the Minimum Cutoff (MC), then the MC is used in the calculation. From the three calculated ratios, a predetermined ratio, the Background Adjustment Factor (BAF) is subtracted from each antigen-specific bead/con combination to obtain three Adjusted Ratios.

- A positive value for two or more of the three Adjusted Ratios indicates a positive bead reaction.
- A negative value for two or more of the three Adjusted Ratios indicates a negative bead reaction.

Interpretation of results: Refer to the MATCHIT! Software report for the results. The table below lists the possible results that could be obtained when testing any given sample.

Antigen	**GPIV**	**HLA Class I**	**HPA-1, -3, -4 (GPIIb-IIIa)**	**HPA-2 (GPIb-IX)**	**HPA-5 (GPIa-IIa)**
Possible result	Pos Neg	Pos Neg	Reactive Neg	Pos Neg Indeterminate	Pos Neg Indeterminate

- Results for HLA Class I, GPIV, HPA-2 and HPA-5 are directly reported by the MATCHIT! software
- Any indeterminate results for HPA-2 and HPA-5 should be repeated using the Pak Lx Assay, or using an alternate method.
- To determine HPA-1, -3 and -4 reactivity, use the following table:

HPA coated beads	**Pattern of Bead Reactivity****					
HPA – 1a-3a-4a	Pos	Neg	Pos	Neg	Pos	Neg
HPA – 1a-3b-4a	Pos	Neg	Neg	Pos	Pos	Neg
HPA – 1b-3a-4a	Neg	Pos	Pos	Neg	Pos	Neg
HPA – 1b-3b-4a	Neg	Pos	Neg	Pos	Pos	Neg
HPA – 1ab-3ab-4a	Pos*	Pos*	Pos*	Pos*	Pos*	Neg
HPA - 1a-3ab-4b	Pos	Neg	Pos*	Pos*	Neg*	Pos
Interpretation of results	Antibodies to HPA-1 detected		Antibodies to HPA-3 detected		Antibodies to HPA-4 detected	
This pattern may mask the presence of an antibody reactive with:	HPA-4b	HPA-4b not masked	HPA-4b	HPA-4b	HPA-1a, -1b, -3a, -3b	HPA-1a, -1b, -3a, -3b not masked

* Heterozygous beads may be positive or negative depending on the titer of the antibody in the sample or the level of background on the negative control beads.

**Any pattern not represented in the table above should be considered an indeterminate result and may be due to the presence of auto-antibodies or a combination of auto- and allo- antibodies. This may also include reactivity to polymorphic variants not identified in the assay.

LIMITATIONS

- This product has not been designed to detect antibodies of the IgA or IgM class of immunoglobulin.
- The results of this assay should not be used as the sole basis for a clinical decision. A positive test only indicates the presence of antibodies specific for HLA Class I, GPIV, or HPA-1, HPA-2, HPA-3, HPA-4 and HPA-5, and is not intended to diagnose a clinical condition.[19-21]
- The HPA systems occur in different frequencies in different populations. The higher frequency HPA systems are deemed to be "public" while the lower frequency HPA systems are deemed to be "private". The Pak Lx assay uses glycoproteins captured from donors that have only been typed for the public HPA systems: HPA-1, -2, -3, -4, and -5. Three of these public HPA systems (HPA-1, -3, -4) are displayed on the same glycoprotein, GPIIb/IIIa.[1,5,11,14] Serum samples may have antibodies to one or more of these public HPA systems, and the presence of antibodies to one HPA system may mask the presence of antibodies to other

systems. For example, the presence of a high titer antibody reactive with one HPA-4 epitope may mask the presence of lower titer antibodies reactive with one of the HPA-1 epitopes. Please see the section of these instructions labeled Interpretation of Results for further detail.

- The Pak Lx assay uses glycoproteins captured from donors that have not been typed for private HPA systems. Serum samples may have antibodies to one or more of these private HPA systems. The presence of these antibodies to private epitopes may simulate reactivity patterns that would indicate the presence of an antibody reactive with a public HPA epitope and/or cause indeterminate results.

- Some low titer, low avidity antibodies, including antibodies to low incidence HLA Class I antigens may not be detected by this product.

- This assay has not been validated for use in detecting autoantibodies.

TROUBLESHOOTING

PROBLEM	POSSIBLE CAUSE	SOLUTION
Positive control Serum shows additional Antibody reactivity not indicated on the QC certificate	Positive Control contaminated with another sample	User to employ good laboratory technique including proper aliquoting methods to eliminate carryover of samples to adjacent wells; Repeat the assay
	Poor washing	Repeat assay following wash instructions
Negative Control Serum shows Antibody reactivity	Contaminated reagents	User to employ good laboratory technique including proper aliquoting methods to eliminate carryover of samples to adjacent wells; Repeat the assay
	Poor washing	Repeat the assay following wash instructions
For the Negative Control Serum, the MFI value on the Positive Control Bead (Probe 77) is <10,000	Photobleached conjugate	Use new kit; Repeat the assay
	Poor washing	Repeat the assay following wash instructions
For test samples, the MFI value on the Positive Control Bead (Probe 77) is <3,500	Photobleached conjugate	Use new kit; Repeat the assay
	Poor washing	Retest the sample following wash instructions
MFI values on antigen beads are approximately 30 MFI while Positive Control bead MFI is > 10,000	Sample not added	Retest the sample
Bead Failure (insufficient bead events counted) (<60 events collected)	Beads not mixed well or resuspended	Mix well with pipet to completely resuspend the beads; Retest the sample
	Instrument failures - out of calibration	See Instrument Manual; Repeat the assay
	Instrument failures - sample flow blocked	See Instrument Manual; Repeat the assay
	Photobleached beads	Use new kit; Repeat the assay
	Vacuum pressure too strong/beads stuck to membrane	Reduce vacuum strength; Retest the sample
Uninterpretable results	Use of incorrect acquisition template (IDT/LXT) file.	Check to see if the lot number in the IDT/LXT file used for the acquisition of data matches the Pak Lx kit used for testing. If incorrect, repeat the assay and acquire data using the correct IDT/LXT file.
	Use of incorrect EDS (BAF) file for performing the analysis by MATCHIT! Platelet Antibody software	Check to see if the lot number in the EDS file used for the analysis of data matches the Pak Lx kit used for testing. If incorrect, repeat the data analysis using the correct EDS file.

SPECIFIC PERFORMANCE CHARACTERISTICS

The following tables list the co-positivity (sensitivity), co-negativity (specificity), and agreement for the numbers of samples tested for antibodies reactive with each HPA or GPIV when compared to results obtained using monoclonal antibody immobilization of platelet antigens (MAIPA); or antibodies reactive with Class I HLA when compared to results obtained with LifeScreen Deluxe Assay (LMX).

These data were compiled from two clinical studies conducted at Sanquin Diagnostic Services (Amsterdam, The Netherlands) and The BloodCenter of Wisconsin (Milwaukee, Wisconsin).

MAIPA (HPA-1)

Pak Lx assay (HPA-1)	Positive	Negative	Total
Positive	52	2	54
Negative	0	292	292
Total	52	294	346

Agreement:	99.4%	95% Confidence Interval =	97.9 – 99.8%
Co-positivity:	100.0%	95% Confidence Interval =	93.1 – 100.0%
Co-negativity:	99.3%	95% Confidence Interval =	97.6 - 99.8%

MAIPA (HPA-2)

Pak Lx assay (HPA-2)	Positive	Negative	Total
Positive	8	0	8
Negative	1	336	337
Total	9	336	345

Agreement:	99.7%	95% Confidence Interval =	98.4 – 99.1%
Co-positivity:	88.9%	95% Confidence Interval =	56.5 – 98.0%
Co-negativity:	100.0%	95% Confidence Interval =	98.9 – 100.0%

MAIPA (HPA-3)

Pak Lx assay (HPA-3)	Positive	Negative	Total
Positive	4	0	4
Negative	2	341	343
Total	6	341	347

Agreement:	99.4%	95% Confidence Interval =	97.9 – 99.8%
Co-positivity:	66.7%	95% Confidence Interval =	30.0 - 90.3%
Co-negativity:	100.0%	95% Confidence Interval =	98.9 – 100.0%

MAIPA (HPA-4)

Pak Lx assay (HPA-4)	Positive	Negative	Total
Positive	4	0	4
Negative	0	160	160
Total	4	160	164

Agreement:	100.0%	95% Confidence Interval =	97.7 – 100.0%
Co-positivity:	100.0%	95% Confidence Interval =	51.0 – 100.0%
Co-negativity:	100.0%	95% Confidence Interval =	97.7 – 100.0%

MAIPA (HPA-5)

Pak Lx assay (HPA-5)	Positive	Negative	Total
Positive	29	1	30
Negative	2	297	299
Total	31	298	329

Agreement:	99.1%	95% Confidence Interval =	97.4 – 99.7%
Co-positivity:	93.5%	95% Confidence Interval =	79.3 - 98.2%
Co-negativity:	99.7%	95% Confidence Interval =	98.1 - 99.9%

MAIPA (GPIV)

Pak Lx assay (GPIV)	Positive	Negative	Total
Positive	5	0	5
Negative	1	162	163
Total	6	162	168

Agreement:	99.4%	95% Confidence Interval =	96.7 – 99.9%
Co-positivity:	83.3%	95% Confidence Interval =	43.6 - 97.0%
Co-negativity:	100.0%	95% Confidence Interval =	97.7 - 100.0%

LMX (HLA CI-I)

Pak Lx assay (HLA CI-I)	Positive	Negative	Total
Positive	67	1	68
Negative	3	104	107
Total	70	105	175

Agreement:	97.7%	95% Confidence Interval =	94.3 – 99.1%
Co-positivity:	95.7%	95% Confidence Interval =	88.1 - 98.5%
Co-negativity:	99.0%	95% Confidence Interval =	94.8 - 99.8%

Precision

The Pak Lx assay showed 100% agreement in reported results for eight samples when tested with one lot in duplicate over 20 separate testing events by two operators. The selected samples included samples with antibody reactivity to HPA-1a, -1b, -3a, -4a, -5b, GPIV, and Class I HLA.

Interfering substances

Interfering substances studies were conducted using CLSI EP07-A2 Interference testing in clinical Chemistry; Approved Guideline. The following substances showed no interference in the Pak Lx assay at the concentrations indicated:

Hemoglobin	≤ 500 mg/dl
Triglycerides	≤ 500 mg/dl
Bilirubin	≤ 20 mg/dl

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303285.IFUEN Rev H
2015-05-29

Warning	Warning
H317	May cause an allergic skin reaction
P261	Avoid breathing dust/fume/gas/mist/vapours/spray
P272	Contaminated work clothing should not be allowed out of the workplace
P280	Wear protective gloves/protective clothing/eye protection/face protection
P302 + P352	IF ON SKIN: Wash with plenty of soap and water.
P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P501	Dispose of contents/container to an approved waste disposal plant.