LABScreen™

REF

Catalog ID	Product Name
LS1PRA	LABScreen™ PRA Class I
LS2PRA	LABScreen™ PRA Class II
LS12PRA	LABScreen™ PRA Class I & II
LSM12	LABScreen™ Mixed Class I & II
LS1A04	LABScreen™ Single Antigen HLA Class I - Combi
LS1ASP01	LABScreen™ Single Antigen HLA Class I Supplement - Group 1
LS2A01	LABScreen™ Single Antigen HLA Class II - Group 1
LS2ASP01	LABScreen™ Single Antigen HLA Class II Supplement - Group 1
LS1AEX01	LABScreen™ Single Antigen HLA Class I ExPlex
LS2AEX01	LABScreen™ Single Antigen HLA Class II ExPlex
LSMICA001	LABScreen™ MICA Single Antigen - Group 1

IVD In Vitro Diagnostic Medical Device.

INTENDED USE

LABScreen products are intended for use in detection of HLA antibody using flow cytometric technology

INTENDED PURPOSE

LABScreen™ products are in-vitro diagnostic devices for use by trained healthcare professionals for the qualitative detection of Anti-HLA Class I (HLA-A, B, C, MICA) and Anti-HLA Class II (HLA-DR, DQ, DP) antibodies on Luminex instrument platforms for transplant screening and monitoring. The test is not automated and provides histocompatibility data through the analysis of serum and plasma samples.

SUMMARY AND EXPLANATION

LABScreen products use microbeads coated with purified Class I or Class II HLA antigens and pre-optimized reagents for the detection of Class I or Class II HLA antibodies in human sera. LABScreen products utilize the LABScan™ 100 (Luminex® 100/200) or LABScan3D™ (Luminex® FLEXMAP 3D®) for analysis of up to 100 or 500 bead regions, respectively, in a single test.

The Mixed assay detects the presence of antibody to Class I and/or Class II HLA antigens. The PRA tests can detect antibodies and their specificities against the HLA antigens in each LABScreen panel. The Single Antigen assay allows confirmation of antibody specificity suggested by a previous PRA test, while individual Singles beads are used to focus on reactions against one or a few antigens, e.g. to compare reactivity of different samples from the same individual. A negative control serum is used to establish the background value for each bead in a test batch.

PRINCIPLE(S)

Test sample is incubated with LABScreen beads. Any HLA antibodies present in the test sample bind to the antigens on the beads and then are labeled with R-Phycoerythrin (PE)-conjugated goat anti-human IgG. The LABScan™ 100 or LABScan3D™ flow analyzer(s) simultaneously detects the fluorescent emission of PE and a dye signature from each bead, allowing almost real-time data acquisition. To assign PRA and HLA specificity, the reaction pattern of the test sample is compared to the lot-specific worksheet defining the antigen array.

REAGENTS

A. Identification

See LABScreen Reference Table for product description.

B. Warning or Caution

1. In Vitro Diagnostic Medical Device.

2. **Warning**: LABScreen PRA test reagents contain 0.1% sodium azide (NaN_3) as a preservative. Under acidic conditions, sodium azide yields hydrazoic acid, an extremely toxic compound. Dilute reagents containing sodium azide in running water before discarding, to avoid deposits in plumbing where explosive conditions may develop. (Refer to Material Safety Data Sheet for detail.)

3. **Warning**: All blood products should be treated as potentially infectious. Source material from which this product was derived are controlled/verified to be negative for any potentially infectious agents. No known test methods can offer assurance that products derived from human blood will not transmit infectious agents.

4. **Caution**: For manual flicking of trays, use a quick downward arm motion without wrist movement to prevent repetitive motion effects.
5. Refer to the Material Safety Data Sheet for detailed information.

C. Preparing Reagents for Use

1. See Directions for Use, below.
2. If buffer salts have precipitated out of solution during shipment or storage, re-dissolve by gently warming before preparing working dilution.

D. Storage Instructions

1. LABScreen products are shipped to the end user on dry ice.
2. Store product at -65° C or below until first use, up to the labeled expiration date.
   a. Refer to outer package label for remaining product shelf-life.

3. Once beads are thawed, DO NOT REFREEZE. Store at 2 - 8° C for up to three months or until the expiration date (if earlier).
4. Protect bead mixtures from exposure to light sources.
5. After first use, store wash buffer at 2 - 8° C for up to three months or until the expiration date, if earlier.

E. Purification or Treatment Required for Use

See Directions for Use, below.

F. Instability Indications

None

INSTRUMENT REQUIREMENTS

A. Required Equipment

- LABScan 100 flow analyzer (Luminex® 100/200) with Luminex® XY platform (for automated 96-sample data acquisition) and sheath fluid delivery system (OLI Cat. # LABSCNXS3) OR LABScan3D flow analyzer (Luminex® FLEXMAP 3D®) with XY platform and sheath fluid delivery system (OLI Cat. # LABSCNXS4)
- Centrifuge
- Rotor for 1.5 ml microcentrifuge tube (9,300 g), or a swinging bucket rotor for 96-well microplate (1,300 g)
- Vortex mixer
- Plate shaker or rotating platform

B. For Filter Plate Option:

- Vacuum manifold, 96-well (Millipore Cat. # MAVM0960R or equivalent)
- Vacuum pump with a pressure less than 100 mm Hg
- Plate shaker or rotating platform

C. Equipment Calibration

Follow manufacturer's instructions for calibration of the LABScan 100 or LABScan3D flow analyzer.

D. Recommended Software

HLA Fusion™ (OLI Cat. # FUSPGR)

SPECIMEN COLLECTION AND PREPARATION

- Unopened blood specimens may be kept at room temperature up to 4 days.
- Separated serum (from clotted samples) or plasma (in ACD or K-EDTA) may be refrigerated up to 7 days, or aliquots may be frozen at -20°C or below and thawed just before the assay. **Caution**: Avoid multiple freeze thaw cycles for test samples.
- Aggregates should be removed from the test serum/plasma by centrifugation (8,000 – 10,000 g for 10 minutes) or filtration (0.2 µm) prior to testing. Any aggregates or contamination of the sample may generate invalid results.
- Samples may be treated to reduce non-specific background or to remove inhibitory factor – see limitation section.

  Note:

  - Test serum or plasma should not be heat inactivated, because it may give a high background in the test.
  - Undiluted serum or plasma is recommended for the test. Diluting serum and plasma may reduce product reactivity (MFI).
  - If a high background sample is diluted for this assay, the negative control sample should be tested at the same dilution.

PROCEDURE

A. Materials Provided

1. See the LABScreen Reference Table in Product Documentation on the One Lambda, Inc. web site for a list of materials provided for each product.
2. The volumes provided exceed the amount required for testing by a small amount to allow for pipetting losses.

B. Materials Required, But Not Provided

1. PE–Conjugated Goat Anti-Human IgG (OLI Cat. # LS-AB2)
2. PBS, filtered [USA Scientific Cat. # 9242 (500 ml 10X) or equivalent]
3. 1.5 ml microcentrifuge tube (USA Scientific Cat. # 1415-2500 or equivalent)
4. Pipette tips
5. Negative Control Serum, containing no HLA antibody when tested by LABScreen method (OLI Cat. # LS-NC or equivalent)

If the test is performed in a 96-well microplate:

1. 96-well microplate, 250 µl, non-treated surface (Whatman Cat. # 7701-3250 or equivalent)
2. Tray seal (OLI Cat. # SSPSEA300 or equivalent)

For the Filter Plate option:

1. Filter plate (Multiscreen-BV, Millipore Cat. No. MABVN1250 or equivalent)

C. Directions for Use

Notes:

- Take special care in the aliquoting process. Failure to follow the steps described below may result in reagent loss.
- Sections A through C indicate the volumes of reagents needed for testing a single bead group. If you are running a combined test, see Section D before proceeding.
- Turn on the LABScan 100 or LABScan3D flow analyzer at least 30 minutes before starting the assay.
- Create a filename and sample code sheet for each test tray.

I. For each test batch, test a negative control serum (e.g. OLI Cat. # LS-NC or equivalent) to establish background values. To complete the test in a 1.5 ml microcentrifuge tube

1. Mix the LABScreen beads well by gently vortexing or pipetting up and down several times prior to use.
2. Incubate 5 µl of LABScreen beads with 20 µl of test sample in a 1.5 ml micro-centrifuge tube for 30 minutes, in the dark at 20 - 25º C with gentle shaking.
3. Dilute 10X wash buffer (OLI Cat. # LSPWABUF) in distilled water to make a 1X solution.
4. Add 1 ml of 1X wash buffer to each bead/sample solution tube and vortex. Centrifuge at 9,300 g for 2 minutes. Aspirate and discard the supernatant.
5. Repeat Step 4 twice.
6. Dilute 1 µl per test of 100X PE-conjugated anti-human IgG (OLI Cat. # LS-AB2) with 99 µl of 1X wash buffer to make a 1X solution.
7. Add 100 µl of 1X PE-conjugated anti-human IgG to each tube. Vortex and then incubate in the dark for 30 minutes at 20 - 25º C with gentle shaking.
8. Repeat Step 4 twice.

9. Add 80 µl 1X PBS to each tube. Proceed to data acquisition and analysis, or store tray at 2 - 8º C in the dark for up to 24 hours before analysis.

II. To complete the test in a 96-well plate

Caution: Seal the 96-well tray carefully and completely to prevent well-to-well sample contamination by pressing the seal against each rim of the 96 wells. Do not re-use tray seals. Use a fresh seal for each step that requires application of a tray seal.

1. Mix the LABScreen beads well by gently vortexing or pipetting up and down several times prior to use.
2. Add 5 µl of LABScreen beads with 20 µl of test sample in each well of a 96-well plate, seal plate, and incubate for 30 minutes in the dark at 20 - 25º C with gentle shaking.
3. Dilute 10X wash buffer (Cat. # LSPWABUF) in distilled water to make a 1X wash solution.
4. After incubation, remove seal and add 150 µl of 1X wash buffer to each well of the plate. Cover with tray seal (OLI Cat. # SSPSEA300 or equivalent) and vortex. Centrifuge at 1,300 g for 5 minutes.
5. Remove wash buffer from wells of plate by flicking or with vacuum aspiration.
6. Add 200 µl of 1X wash buffer to each well of the plate. Cover with a new tray seal and vortex. Centrifuge at 1,300 g for 5 minutes.
7. Remove supernatant from wells of plate by flicking or with vacuum aspiration.
8. Repeat Steps 6 and 7.
9. Dilute 1 µl per test of 100X PE-conjugated anti-human IgG (OLI Cat. # LS-AB2) with 99 µl of 1X wash buffer to make a 1X solution.
10. Add 100 µl of 1X PE-conjugated anti-human IgG to each well. Cover with tray seal and vortex. Incubate in the dark for 30 minutes at 20 - 25º C with gentle shaking.
11. Centrifuge at 1,300 g for 5 minutes.
12. Remove supernatant from wells of plate by flicking or with vacuum aspiration.
13. Repeat Steps 6 and 7 twice.
14. Add 80 µl 1X PBS to each well. Cover with a new tray seal and vortex. Proceed to data acquisition and analysis, or store tray at 2 - 8º C in the dark for up to 24 hours before analysis.

III. To complete the test in a 96-well filter plate

1. Mix the LABScreen beads well by gently vortexing or pipetting up and down several times prior to use.
2. Dilute 10X wash buffer (OLI Cat. # LSPWABUF) in distilled water to make a 1X solution (approximately 3.2 ml/tray/wash).
3. Cover any wells of the plate that will remain unused during the test with a tray seal to assure that the unused wells remain dry. Pre-wet filters in the filter plate by dispensing 300 µl wash buffer into only those wells that will be used for the assay.
4. Incubate the plate for 10 minutes on a platform plate shaker at low speed.
5. Aspirate all wash buffers from the wells using a Millipore vacuum manifold. Do not exceed 100 mm Hg vacuum pressure!
6. Add 5 µl of LABScreen beads and 20 µl of test sample per test well.

   Note: During bead and sample dispensing steps, press pipette tip gently against filter plate well to avoid filter rupture.
7. Incubate the plate in the dark for 30 minutes at 20 - 25º C with gentle shaking.
8. Add 175 µl wash buffer per well.
9. Turn on vacuum pump. Press the plate firmly on the vacuum manifold. Make sure liquid drains out slowly. Make sure all liquid has drained from the wells before proceeding.

Caution: Do not exceed 100 mm Hg vacuum pressure. A rapid vacuum will cause loss of beads due to be entrapment in the pores of the filter paper.

10. Repeat Steps 8 and 9, above, four times.
11. Add 100 µl of 1X PE-conjugated anti-human IgG to each well.
12. Incubate in the dark for 30 minutes at 20 - 25º C with gentle shaking.
13. Repeat Steps 8 and 9 five times.
14. Add 80 µl of 1X PBS to each well.
15. Read sample on the LABScan 100 or LABScan3D flow analyzer, adjusting probe height if necessary.

    Note: LABScreen Single Antigen HLA Class I and Class II ExPlex products can be used on the LABScan3D flow analyzer only.

IV. Combined tests

Any of the above protocols can be used for a combined test of certain LABScreen products.

- For acceptable lot combinations of LS12PRA see www.onelambda.com (Antibody Detection>LABScreen>LABScreen PRA/ Product Documentation: LABScreen Bead Combo – Multiple IDs DataSheet).
- Do not combine LABScreen Single Antigen Class I Combi and Class II panels (bead IDs would overlap).

1. Mix equal volumes of beads. Then dispense the appropriate aggregate amount (10 or 15 µl) of bead mixture per test.
2. Bead combinations and amounts to dispense are listed in the table below.

Catalog ID	Bead Volume per Test	Control (NC/PC) Beads	Test Sample per Test	Instrument
LS12PRA (CI and CII beads)	5 µl + 5 µl	Included	40 µl	LABScan 100 or LABScan3D
LS1A04 + LS1AEX01	5 µl + 5 µl	Included	40 µl	LABScan3D
LS2A01 + LS2AEX01	5 µl + 5 µl	Included	40 µl	LABScan3D
LS1A04	5 µl	Included	20 µl	LABScan 100 or LABScan3D

RESULTS

A. Data Acquisition

1. Set up the LABScan 100 or LABScan3D flow analyzer for sample acquisition and calibration according to the Luminex User's Manual.[1]
2. Choose a template according to product kit catalog ID and lot number.
   a. Acquisition templates are available from OLI by CD or via our download website.
   b. To create your own acquisition template, follow instructions in the Acquisition chapter of the Luminex User's Manual.
   c. Luminex software versions - LABScan 100 (xPONENT 3.1 or higher); LABScan 3D (xPONENT 4.2 or higher) must be used.
3. Create a file name for the samples to be run.
4. Make sure all the template settings are correct. Template specifications are:
   a. Set sample volume to 50 µl.
   b. Set sample time-out to 80 seconds.
   c. Set doublet discriminator gate to 8,000 (low limit) and 16,000 (high limit).
   d. Set number and ID of beads selected according to the product-specific worksheet provided with the product.
   e. Set minimum events collected to 100 per bead.
5. Enter the sample IDs (if the same sample is tested more than once, assign a different ID).
6. Load the plate onto the XY platform and fill the reservoir with sheath fluid.
7. Click the START button to initiate the session. After the samples have finished running, save the data output in a .csv file.
8. Wash the machine twice with sheath fluid at the end of the session.

B. Data Analysis

1. The reactivity of a test sample is calculated from the "raw" fluorescence values recorded by the LABScan device (.csv file) for each HLA coated bead.
2. Calculate anti-HLA sample reactivity by correcting for non-specific binding to the negative control bead and background values (obtained by testing with a negative control serum (OLI Cat. # LS-NC) to determine the normalized background ratio (NBG ratio). See Calculations, below.
3. For LABScreen PRA or LABScreen Single Antigen, the normalized fluorescent value for each HLA coated bead equals the value of that bead divided by the value of the NC bead. (For LABScreen Mixed, the normalized fluorescent signal equals the value of the Class I or Class II coated bead minus the value of the NC bead.)

   Note: The fluorescent signal (value) can be either the trimmed mean or median value.

C. Calculations

1. The abbreviations used in this section are defined below:

NBG ratio	Normalized Background ratio used to assign strength of each anti-HLA reaction
S#N	Sample-specific fluorescent value for bead #N
SNC bead	Sample-specific fluorescent value for Negative Control bead
BG#N	Background NC Serum fluorescent value for bead #N
BGNC bead	Background NC Serum fluorescent value for Negative Control bead

NC Serum Negative Control Serum (OLI Cat. # LS-NC) validated for a given lot of LABScreen beads

2. For LABScreen PRA or LABScreen Single Antigen:

$$\text{NBG ratio} = \frac{\text{S\#N / SNC bead}}{\text{BG\#N / BGNC bead}}$$

For LABScreen Mixed:

$$\text{NBG ratio} = \frac{\text{S\#N - SNC bead}}{\text{BG\#N - BGNC bead}}$$

Note: If (BG#N-BGNC bead) <50 then use 50 as a default threshold value.

D. Determination of Positive/Negative Cut-Off

1. For LABScreen PRA and LABScreen Mixed:
   a. Select the NBG ratio that gives a significant shift over background fluorescent value when the background value is obtained using the negative control serum in 3 - 5 replicate tests. If you prefer, test 5 - 10 samples from non-transfused, non-transplanted male donors to obtain an average background value.
   b. Validate the cut-off using 5 - 10 reference samples with defined HLA antibody specificity. The NGB ratio values for expected positive antigen reactions should be above the cut-off. Reference samples with defined HLA specificities can be obtained from commercial sources such as Vitalant, UK NEQAS, or Bloodworks Northwest.
   c. Additional positive/negative reactions may be noted. If necessary, adjust the LABScreen assay cut-off to match the sensitivity of a previously accepted antibody detection assay.
   d. For high PRA samples, the patient's own antigen(s) may show weak positive reactions. For such cases, the fluorescence value for the patient's own antigen may be used as the cut-off.
2. For LABScreen Single Antigen:
   a. Test negative control sample or several negative samples (see 1a, above).
   b. Define working range:

      Working Range = NBG ratio maximum - NBG ratio minimum

   c. Define cut-off points within the working range:

      Relative NBG ratio cut-off = X% (working range) + NBG ratio minimum, where X% = user-defined percent cut-off point within the working range for negative (1), gray area(2), weak positive (4) and strong positive (8).

   d. Set criteria to define positive vs. negative reactions, for example:
      (1) If [NBG ratio max/NBG ratio min] >8, apply the calculation in 2c.
      (2) If [NBG ratio max/NBG ratio min] <8 AND
         (a). NBG ratio max >5, then NBG ratio min should be adjusted to one half of the NBG ratio max and the relative NBG ratio cut-off should be re-computed (as in 2c) based on the adjusted NBG ratio min. The reaction is then scored as above.
         (b). NBG ratio max <5, then the reaction of the test sample with that bead is negative. Assign a score of "1".
   e. Test several reference samples as in 1b above, using the relative NBG ratio to validate the cut-off.
      (1) Establish a strong and weak reactivity cut-off based on the performance of the reference samples, relative to an established assay.

(2) It may be helpful to plot the NBG ratio values in a histogram for visualization of the HLA reactivity pattern of each sample.

3. Higher or lower sensitivities can be obtained by adjusting the cut-off.
4. Optional analysis – HLA Fusion™ software.

LIMITATIONS OF THE PROCEDURE

- Sera or plasma samples that contain contaminants or aggregates may clog the LABScan flow analyzer and generate inaccurate data. Aggregates in the test specimen should be removed by centrifugation or filtering the sample prior to testing.
- The presence of IgG-IgM immune complex may cause inhibition in some patient samples. Samples should be treated to reduce this presence according to the protocols determined by the laboratory, however, samples should not be heat treated as they may cause non-specific background – please reference bibliography section for more information.7,8,9,10, 11
- Ambient temperature may affect LABScan 100 and LABScan3D performance. If the ambient temperature changes, the machine may need to be re-calibrated. Consult the manufacturer's manual for more information.
- The LABScan 100 and LABScan3D flow analyzer must be properly calibrated and maintained. If insufficiently flushed, aggregates of the sample may cause the machine to clog and generate invalid data.
- Assignment of antibody specificity is limited to the HLA antigens presented in each bead panel (see lot-specific worksheet).
- The bead region used for each antigen and the antigen composition of the panel may change from lot-to-lot of product (see the lot specific worksheet).
- Because of the complexity of the HLA allelic definitions, a certified HLA technician or specialist should review and interpret the data, and assign the HLA typing.
- LABScreen is suitable for use in accredited HLA laboratories (i.e. ASHI (American Society for Histocompatibility and Immunogenetics), EFI (European Federation for Immunogenetics), and/or CLIA (Clinical Laboratory Improvement Amendments) regulations). LABScreen is designed to be used by trained HLA technologists and personnel.
- This test must not be used as the sole basis for making a clinical decision.
- The impact of sample filtration prior to use with LABScreen has not been evaluated.

EXPECTED VALUES

A. LABScreen PRA Class I or Class II

- The reactivity strength of a test sample to each bead can be compared to distinguish the strong positive, weak positive and negative reactions. Reactivity ratios can be ranked within different ranges, if a scoring system is desired.
- Our data show NBG ratios > 1.5 in the LABScreen PRA test (using the LABScan 100) correlate well with positive reactions in the FlowPRA test.
- For calculation of percent PRA (Panel Reactive Antibody), divide the number of positive reactions by the number of valid reactions for that test sample.
- To determine the specificity of HLA antibody, enter the reaction score into the lot specific Worksheet to analyze the reaction pattern.

B. LABScreen Mixed

- Score HLA Class I and Class II reactions separately, according to reactivity strength of the sample for each bead set.
- If anyone bead in the mixed assay is positive, then the result should be assigned as positive.

- Our data show that NBG ratios >2.2 in the LABScreen Mixed test (using the LABScan 100) correlate well with positive reactions in LAT™ Mixed.

C. LABScreen Single Antigen

- Test samples may produce signal/background ratios that are much higher than those obtained in the PRA assay. Establishing the assay cut-off(s) using the relative NBG ratio is one way of normalizing the data (see Results, Section D-2c).
- Our data show that a positive/negative cut-off or relative NBG ratio >15% of the working range NBG ratio calculated for each test sample (using the LABScan 100) will give results comparable to the LABScreen PRA assay.

D. General Guidelines

- Each bead count should be over 50. A lower bead count may be due to sample loss during the wash steps. It could also be due to improper calibration or clogging of the LABScan 100 or LABScan3D flow analyzer, or by photo-bleached beads that dropped out from the mapped region.
- Signal values are the fluorescence intensity of each bead set vs. the test sample. A negative control should be tested with the same batch of samples to establish the background value(s) for that test run.
- Negative Control Serum (OLI Cat. # LS-NC or equivalent) is recommended. Using any other negative control sample may require adjustment of cut-off values.
- Negative Control Beads (Ag ID = NC) are not coated with HLA antigen. The fluorescence value may vary among different samples due to non-specific binding or to insufficient washing. The NC value is usually less than 500 except for samples with a high background. It should always be lower than 1500 and less than or equal to half of the PC value.
- Positive Control Beads are coated with purified human IgG, which should bind to the secondary antibody to produce a positive signal. The PC value should be over 500 and at least twice the NC value.

E. Validation of the Assay

- The cut-off value of signal to background should be validated if a new negative control sample is used.
- For a given sample, the value for PC/NC should be greater than 2. A lower value may be due to an extremely high NC bead background value for the test sample, a high HLA bead signal for the NS control, or a low signal from the secondary antibody or the LABScan 100 and LABScan3D flow analyzer. In this case, the data may have to be confirmed.
- Each user should evaluate the performance of the assay in their laboratory to validate the cut-off value(s) selected.
- Plasma samples may give lower FI or higher background values than serum. The user may wish to normalize the data if comparing results between sera and plasma samples (see Reference 5) for the same or different test subjects.

SPECIFIC PERFORMANCE CHARACTERISTICS

A. Using the assay cut-offs referenced under Expected Values, above, LABScreen assays have given results comparable to the results of the One Lambda FlowPRA® and LAT™ assays. However, HLA antibody patterns may be quite complex. A given test sample may contain several HLA Class I and Class II antibody specificities, each with a different avidity; however, not all specificities will be recognized in assays with lesser sensitivity. Therefore, each laboratory should establish and validate the assay cut-offs for their own use based on their expertise in recognizing HLA CREG patterns and an evaluation of the assay performance using HLA allosera with defined specificities.

B. Comparison of serum vs. plasma for 1,000 blood donors in the NIHLBI REDS-II study (6) showed good correlation within the working range of the assay. For anti-HLA CI and CII antibodies the R2 values were 0.88 and 0.91, respectively. However, the NBG ratio was generally 1.3-fold higher for serum samples.

C. If high background is seen, this may indicate improper washing during the test protocol. High negative control background may cause inaccurate normalized MFI values.

D. Analytical Performance Characteristics

1. Accuracy (Trueness)

   Concordance percentages for accuracy studies were obtained by comparing the data output of LABScreen devices to well characterized reference samples and/or predicate reference devices. Table 1 below provides a summary of study data collected during accuracy studies.

Table 1: Accuracy

SKU	Number of Users	Instrument	Total Data points	Positive Percent Agreement (PPA)	Negative Percent Agreement (NPA)	Overall Concordance	95% Lower-Bound Confidence Interval (Clopper-Pearson)
LS1A04	1	LS100	6,000	92.9%	96.3%	95.7%	95.2%
LS2A01	1	LS100	2,964	99.4%	96.4%	96.9%	96.4%
LS1A04	1	LS3D	6,640	94.4%	97.1%	96.6%	96.2%
LS2A01	1	LS3D	2,925	98.1%	98.2%	98.2%	97.7%
LS1ASP01	1	LS100	432	100.0%	100.0%	100.0%	99.3%
LS2ASP01	1	LS100	240	100.0%	100.0%	100.0%	98.8%
LS1ASP01	1	LS3D	432	100.0%	100.0%	100.0%	99.3%
LS2ASP01	1	LS3D	240	100.0%	100.0%	100.0%	98.8%
LS1AEX01	1	LS3D	3,348	95.1%	97.7%	96.2%	95.6%
LS2AEX01	1	LS3D	1,488	99.8%	95.2%	96.9%	96.1%
LS1PRA	1	LS100	2,210	93.0%	90.2%	91.2%	90.1%
LS2PRA	1	LS100	1,406	99.8%	90.4%	94.2%	93.1%
LS1PRA	1	LS3D	2,210	90.5%	93.6%	92.6%	91.6%
LS2PRA	1	LS3D	1,444	99.1%	92.3%	95.0%	94.0%
LSMICA0001	1	LS100	315	97.5%	99.2%	98.7%	97.1%
LSMICA0001	1	LS3D	315	91.1%	100.0%	97.8%	95.9%
LSM12	1	LS100	515	93.9%	93.2%	93.4%	90.9%
LSM12	1	LS3D	515	92.6%	92.3%	92.4%	90.2%

2. Analytical Sensitivity (Limit of Detection)

   Limit of detection studies were performed on LABScreen devices by comparing neat to diluted serum samples (1:2, 1:4, 1:8, 1:16, and 1:32). Increasing dilution caused a drop in reactivity (MFI) and concordance rates when compared to the undiluted serum. Therefore, diluting of test serum may cause a drop in performance and is not recommended.

3. Analytical Specificity (Interfering Substances)

   Interfering Substances that may be present in blood specimens or isolated serum samples and could potentially interfere with LABScreen devices were evaluated. For these potential interfering substances, the impact on device performance was evaluated. All devices maintained optimal performance when in the presence of the tested interferents. See Table 2 for information about tested substances.

   Table 2: Tested Concentration of Interfering Substances

Substance	Test Concentration (mg/mL)
Albumin	15
D-glucose	9.9
Bilirubin, unconjugated	0.2
Cholesterol	5.031
Creatine	0.05
Hemoglobin	2.0
Ascorbic Acid	0.0299
Caffeine	0.0598
Nicotine	0.001
Acetaminophen	0.2
Ibuprofen	0.5
Rituximab	10.0
Salicylic Acid	0.000694

4. Precision (Repeatability and Reproducibility)

   Concordance percentages for precision studies were obtained by comparing the data output of LABScreen devices to well characterized reference samples and/or predicate reference devices. Results were generated through comparing data between different users, days, and runs. Table 3 below provides a summary of study data collected during precision studies.

   Table 3: Precision

Test Category	SKU	Instrument	Total Lots Evaluated	Variables Evaluated (Users x Days x Runs/Day)	Overall Concordance*
Precision: Reproducibility	LS1A04	LS100	1	3 x 5 x 2	90.4%
	LS1A04	LS3D	1	3 x 5 x 2	95.9%
	LS1AEX01	LS3D	1	3 x 5 x 2	95.9%
	LS2A01	LS100	1	3 x 5 x 2	94.1%
	LS2A01	LS3D	1	3 x 5 x 2	97.4%
	LS2AEX01	LS3D	1	3 x 5 x 2	97.7%
Precision: Repeatability	LS1A04	LS100	3	1 x 1 x 1	95.1%
	LS1A04	LS3D	3	1 x 1 x 1	95.1%
	LS1AEX01	LS3D	3	1 x 1 x 1	95.9%

Test Category	SKU	Instrument	Total Lots Evaluated	Variables Evaluated (Users x Days x Runs/Day)	Overall Concordance*
	LS2A01	LS100	3	1 x 1 x 1	97.6%
	LS2A01	LS3D	3	1 x 1 x 1	97.4%
	LS2AEX01	LS3D	3	1 x 1 x 1	94.1%

*Minimum concordance values observed during study is reported in Table 3.

E. Clinical Performance Characteristics

Clinical studies using LABScreen products have been conducted to establish clinical performance characteristics for the device. Both Clinical Accuracy and Clinical Reproducibility were executed at clinical laboratories. Summaries of the studies performed are provided in the following sections.

1. Clinical Accuracy

Clinical Accuracy studies were performed using LABScreen products. LABScreen kits were compared to equivalent on market devices, well characterized reference samples and/or different instrument systems at external clinical laboratories to establish clinical accuracy performance characteristics. A summary of the resultant data for each study conducted has been provided below.

a. Clinical Accuracy Study 1

Correlation coefficients for clinical accuracy study 1 were obtained by comparing the data output of LABScreen devices to a predicate device. Table 4 below provides a summary of study data collected.

Table 4: Results of Clinical Accuracy Study 1

SKU Evaluated	Predicate Device	Number of External Sites	Total Samples	Correlation Coefficient
LS1PRA	FlowPRA Class I Specific Beads (FL1SP)	1	95	0.95
LS2PRA	FlowPRA Class II Specific Beads (FL2SP)	1	95	0.94

b. Clinical Accuracy Study 2

Overall percent agreements for clinical accuracy study 2 were obtained by comparing the data output of LABScreen devices on the LABScan3D to a predicate instrument, the LABScan 100. Bead counts for all tests evaluated were greater than 100 beads. Table 5 below provides a summary of study data collected.

Table 5: Results of Clinical Accuracy Study 2

SKU Evaluated	Test Instrument	Predicate Instrument	Number of External Sites	Total Samples	OA[1]	95% LB CI[2]
LS1PRA	LABScan3D	LABScan 100	3	83	96%	96%
LS2PRA	LABScan3D	LABScan 100	3	83	97%	97%
LSM12	LABScan3D	LABScan 100	3	83	98%	97%
LS1A04	LABScan3D	LABScan 100	3	82	95%	94%

[1]Overall Agreement
[2]Lower-Bound Confidence Interval (Clopper-Pearson)

c. Clinical Accuracy Study 3

Overall percent agreements for Clinical Accuracy Study 3 were obtained by comparing the data outputs from LABScreen Single Antigen devices to an on-market predicate device (LABScreen PRA) and an on-market reference method (Lambda Antigen Tray – Single Antigen). This study was conducted at four separate clinical study sites. Table 6 below provides a summary of study data collected.

Table 6: Results of Clinical Accuracy Study 3

SKU Evaluated	Number of External Sites	Total Samples	OA[1] (%)
LS1A04	4	1163	96.7%
LS2A01	4	1150	95.7%

[1]Overall Agreement

d. Clinical Accuracy Study 4

Overall percent agreements for Clinical Accuracy Study 4 were obtained by comparing the data outputs from LABScreen Single Antigen Explex devices to reference devices. Table 7 below provides a summary of study data collected.

Table 7: Results of Clinical Accuracy Study 4

Test Device	Reference Device	Site Number	Lot Number	OA[1] (%)	95% LB CI[2] (%)
LS1AEX01	LS1ASP01	1	1	99.40%	99.10%
		1	2	99.40%	99.10%
		2	1	98.30%	97.80%
		2	2	98.30%	97.80%
		3	1	97.90%	97.30%
		3	2	97.80%	97.30%
LS2AEX01	LS2ASP01	1	1	99.50%	99.00%
		1	2	99.30%	98.70%
		2	1	98.40%	97.70%
		2	2	98.60%	97.90%
		3	1	97.80%	96.90%
		3	2	98.10%	97.20%
LS1A04 + LS1AEX01	LS1AEX01	1	1	99.70%	99.40%
		1	2	99.80%	99.60%
		2	1	99.50%	99.20%
		2	2	99.70%	99.40%
		3	1	98.70%	98.20%
		3	2	98.90%	98.50%
LS2A01 + LS2AEX01	LS2AEX01	1	1	99.50%	99.00%
		1	2	99.70%	99.30%
		2	1	99.80%	99.40%
		2	2	99.90%	99.50%
		3	1	98.60%	97.90%
		3	2	98.60%	97.90%

[1]Overall Agreement
[2]Lower-Bound Confidence Interval (Clopper-Pearson)

2. Clinical Reproducibility

Clinical Reproducibility studies were performed using LABScreen products. LABScreen kits were evaluated against reproducibility conditions at external clinical sites. This examination included comparing different users, instruments, times of day, and other relevant conditions. A summary of the results from each study can be found below.

a. Clinical Reproducibility Study 1

Overall percent agreements for Clinical Reproducibility Study 1 were obtained by comparing the data output of LABScreen devices on LABScan3D instruments across three different testing sites. Results were compared between different instruments, lots, technicians, test days and test runs. Table 8 below provides a summary of study data collected during precision studies.

Table 8: Results of Clinical Reproducibility Study 1

SKU Evaluated	Number of External Sites	Number of Lots Evaluated	Number of Users per External Site	OA[1] (%)	95% LB CI[2] (%)
LS1PRA	3	3	3	99%	99%
LS2PRA	3	3	3	99%	99%
LSM12	3	3	3	98%	97%
LS1A04	3	3	3	98%	98%

[1]Overall Agreement
[2]Lower-Bound Confidence Interval (Clopper-Pearson)

b. Clinical Reproducibility Study 2

Repeatability, within site precision, and reproducibility was evaluated in Clinical Reproducibility Study 2 by comparing the data output of LABScreen devices across three different testing sites. Results were compared between different product lots, technicians, test days and test runs. Table 9 below provides a summary of study data collected during precision studies.

Table 9: Results of Clinical Reproducibility Study 1

SKU Evaluated: LS1A04+LS1AEX01			**Repeatability**		**Within Site Precision**		**Reproducibility**	
Number of Sites	MOA[1] (%)	p-value	SD[2]	%CV[3]	SD	%CV	SD	%CV
3	96.1	0.69	3.9	4.00%	4.3	4.50%	4.4	4.60%
SKU Evaluated: LS2A01+LS2AEX01			**Repeatability**		**Within Site Precision**		**Reproducibility**	
Number of Sites	MOA (%)	p-value	SD	%CV	SD	%CV	SD	%CV
3	97.6	0.06	4.2	4.40%	5.6	5.80%	6.3	6.40%

[1] Mean Overall Agreement
[2] Standard Deviation
[3] Percent Covariance

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TRADEMARKS

DISCLAIMERS

All One Lambda products are designed to assist personnel experienced in HLA analysis by suggesting typing results or antibody assignments. All test results must be carefully reviewed by such qualified personnel to assure correctness.

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NOTIFICATIONS OF SERIOUS INCIDENT

Should the user observe any serious incident has occurred in relation to this in vitro medical device, the user shall report the serious incent to the manufacturer, any local regulatory agency and the competent authority of the Member State in which the user is established.

EXPLANATION OF SYMBOLS

(Reference EN ISO 15223-1: medical devices – symbols to be used with medical device labels, labeling and information to be supplied.)

Symbol	Description
REF *ISO 7000 Reg No. 2493*	Catalog number
IVD	In vitro diagnostic medical device
 *ISO 7000 Reg No. 1641*	Consult instructions for use
 *ISO 7000 Reg No. 0434A*	Caution
 *ISO 7000 Reg No. 0659*	Biological risks
 *ISO 7000 Reg No. 0633*	Upper limit of Temperature
 *ISO 7000 Reg No. 3082*	Manufacturer
EC REP	Authorized representative in the European Community
 *ISO 7000 Reg No 2497*	Date of Manufacture
 *ISO 7000 Reg No 2607*	Use By Date
 *ISO 7000 Reg No 0624*	Protect from Light Sources
LOT	Batch Code

Batch field on the label is for traceability of manufacturing event

For Summary of Safety and Performance, contact TDX Customer Service.

REVISION HISTORY

Revision	Date	Revision Description
06	30 Mar 2021	Update IVD verbiage on page 1 to align with symbols table, Update Performance characteristics (B) to reference correct bibliography designation.
07	20 May 2021	Added additional information to sections for ExPlex catalog IDs: (1) Serum dilution statement to Specimen Collection Prep Section; and (2) added tables to Table A - Clinical Performance & Table B - Clinical Reproducibility. Removed reference to Rainin brand pipette tips from the *Materials Required, But Not Provided* section. Pipette tips do not need to be brand specific. Alignment to standard for caution symbol in Symbols table.
08	Current	Update to template, Clarification to storage instructions in REAGENTS Section D, Added intended purpose section, Clarified limitations and disclaimers sections, Re-formatted and added relevant performance characteristics to SPECIFIC PERFORMANCE CHARACTERISTICS section, Clarified details in specimen handling sections.

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