HLA Typing Tray
Class I (ABC)
(72, 72BL, 72L, 72OR, 72AB1/72AB2, 72C
72ABC1/72ABC2, 96, 96C)

INTENDED USE

For use in the qualitative determination of HLA cell surface antigens using a complement-dependent microlymphocytotoxic technique.

For In Vitro Diagnostic Use.

SUMMARY AND EXPLANATION OF THE TEST

GTI HLA Typing Trays contain specific human antisera plus one positive and one negative control. The positive control, well 1A, is a rabbit anti-human lymphocyte antibody. The negative control serum, well 1B, is from a healthy non-transfused group AB male and has no cytotoxic reactivity. All antisera are characterized by serological testing.

The differing styles of trays available provide various abbreviated or extensive choices for testing. The 72 trays contain sera to test for A,B loci; A,B,C loci; or ethnically associated loci as seen in the oriental (72OR) or black (72BL) trays. The 96 well tray contains a more extensive set of sera to test for A,B or A,B,C loci. The 72C tray contains sera to test for Cw1-Cw4 and the 72ABC2 and 96C tray provides sera to test for Cw1-Cw8.

HLA characterization may be used for patient and/or donor assessment as applicable in Organ Transplant, Platelet Transfusion, Bone Marrow Transplant and Disease Association Studies.

PRINCIPLE OF THE PROCEDURE

Viable lymphocytes are incubated with specific antisera and rabbit complement. If antigens present on the cell surface correspond to the antibodies in the sera, cell death will occur. Dead cells can be observed using phase microscopy after differential uptake of eosin dye. Interpretation of the results is aided by the enclosed Sera Analysis sheet.

REAGENTS

- HLA Typing Trays: 72 or 96 typing sera with controls.
- Complement: Rabbit Complement, non-toxic to normal lymphocytes.
  - COMP02: 0.5 mL aliquot,
  - COMP03: 0.75 mL aliquot, or
  - COMP07: 5.0 mL aliquot
- Mineral Oil: All antisera in the tray are covered with mineral oil to prevent evaporation.

PRECAUTIONS

- All materials provided are to be used without dilution.
- Store trays at or below -65ºC.
- Use trays before expiration date.
- Thaw trays at room temperature for 15 minutes and use within 30 minutes of thawing. Do not refreeze.
- Do not store trays in dry ice after sealed bag is opened.

CAUTION

- All human serum used in the preparation of the HLA Typing Trays have 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, HLA Typing Trays should be handled in the same manner as potentially infectious material.
- Some of the sera may contain sodium azide as preservative. **WARNING**: Sodium azide reacts with lead and copper plumbing forming highly explosive metal azides. When discarded in a sink, the sink should be flushed with a large volume of water to prevent azide build up. Sodium azide is a poison and is toxic if ingested.
- Discard all components of this kit when completed according to local regulations.

SPECIMEN COLLECTION

Peripheral blood samples collected in Sodium Heparin, ACD or cells that have been cryopreserved using acceptable technique may be used for testing.

> Sodium Heparin collection: 10cc of blood in 143 USP units of Sodium Heparin. Heparinized blood should be kept at room temperature at all times prior to lymphocyte isolation and should be processed within 24 - 48 hours of collection.
> ACD collection: ACD blood should be kept at room temperature at all times prior to lymphocyte isolation and should be processed within 24 - 48 hours of collection.

- Samples must be collected prior to patient myeloablative therapy.
- Collect samples prior to blood transfusion or at least 48 hours post blood transfusion.
- Excessive platelet contamination may mask the lymphocyte reaction or cause false negative reactions.

Lymph Node or Spleen tissue from organ donation may also be used. Process tissue and perform testing as soon as possible after tissue harvest.

PROCEDURE

The test procedure listed is our recommended protocol. The user may establish protocols with variations on the test procedure (e.g. fluorescence, incubation times, cell concentration); however, the user is responsible for determining protocols that are acceptable for use with the GTI HLA Typing Trays.

Materials Provided:
- HLA Typing Tray
- Recording Sheet
- Sera Analysis Sheet
- ABC Complement

Additional Materials Required (User Provided):

- Lymphocyte suspension in 5% Fetal Calf Serum in RPMI-1640.
- 0.05 mL syringe with needle in repeating dispenser (single or multiple) set to deliver 1 µL.
- 0.25 mL syringe with needle in repeating dispenser (single or multiple) set to deliver 5 µL.
- 1 mL syringe with a 6 nozzle head capable of delivering 3.3 µL per nozzle in repeating dispenser.
- Cover slides: 75 X 50 mm.
- Eosin Y: 5.0 g per 100 ml HBSS. Filter through #1 Whatman filter paper.
- Formaldehyde: Add 2 mL of 0.5% phenol red to 500 mL formaldehyde (10%). Adjust to pH 7.2 with concentrated HCl.
- Inverted phase microscope.

Test Procedure:

1. Prepare cells according to user laboratory procedures for lymphocytotoxicity testing.
2. Determine if cell viability is acceptable for test. Lymphocyte viability must be at least 85%.
3. Adjust cells to a desired concentration of (2×10^6 cells/mL) in 5% Fetal Calf Serum in RPMI-1640.
4. Remove tray from freezer and let thaw for at least 15 minutes at room temperature but not longer than 30 minutes.
5. Using a 0.05 mL syringe with needle attached, carefully add 1 µL of a 2.0×10^6 cells/mL suspension of lymphocytes just under the oil. Ensure that cells and sera are adequately mixed.
6. Incubate for 30 minutes at 20-25ºC.
7. Using a 0.25 mL syringe with needle attached to a repeating dispenser add 5 µL of rabbit complement.
8. Incubate for 1 hour at 20-25ºC.
9. Add 3.3 µL of 5% Eosin to each well using a microdispenser.
10. Allow eosin to penetrate the dead cells for 5 minutes.
11. Add 6.6 µL of Buffered Formalin to each well to fix the reaction.
12. Allow cells to settle for 5-10 minutes before covering wells with a 75 x 50 mm coverslip.
13. Store trays, if not being read immediately, in the refrigerator prior to reading to reduce bubble formation.
14. Place the tray on an inverted phase microscope and examine each well at 100X magnification. Read the tray in the following serpentine pattern which corresponds to the Recording Sheet:
    - 1A through 1F; 2F through 2A, etc. (72 well trays)
    - 1A through 1H; 2H through 2A, etc. (96 well trays)
15. Record reactions as observed on the Recording Sheet provided.

Quality Control:

Specific Controls

a) The positive control serum is in well 1A. Valid tests should have at least 51% greater cell death than the negative control.

b) The negative control serum is in well 1B. There should be no cell death in this well. The "baseline" viability of the lymphocyte preparation can be determined by the reaction. Other reactions in the tray are scored by comparing the viability to the viability of the negative control which should be between 0-20% dead cells.

INTERPRETATION OF RESULTS

Cell death should occur in any test well for which the cell surface antigen and the serum antibody are matched. Viewed using phase microscopy, live cells will appear bright and refractile, whereas dead cells will appear somewhat larger and stained dark with eosin dye.

Eosin dye in the concentrations recommended in this procedure is effective only with phase-contrast microscopy. Without phase-contrast the dead cells do not appear sufficiently dark to allow proper discrimination. Because swelling of the dead cells is increased by the addition of eosin, it is important to allow the dye to penetrate the dead cells for exactly 5 minutes prior to the addition of formalin.

Results are recorded using a grading system which corresponds to the percentage of dead cells (seen as large, dark and nonrefractile), within each well.

8 = 81-100% dead cells
6 = 51-80% dead cells
4 = 21-50% dead cells
2 = 11-20% dead cells
1 = 0-10% dead cells
0 = Not readable

Following recording of the results, compare the positive reactions to the specificities contained in each well. Positive reactions occur where antigens on the lymphocytes correspond to antibody present in the antisera. Identify the antigens which are present on the lymphocyte preparation being tested.

LIMITATIONS

Erroneous results can occur at several stages. These are grouped below according to the steps in the procedure.

a. Cell identification

When testing several samples simultaneously, the following switching errors may occur while isolating or testing:

1. Interchanging cells
2. Testing one cell twice while omitting another
3. Mixing two samples during isolation

b. Cell isolation

The lymphocyte preparation must be as pure as possible. Cell contamination can result in the following problems:

1. Erythrocyte contamination can make microscopic evaluation difficult because of visual confusion with lymphocytes. Also, erythrocytes can deplete complement necessary for lymphocytotoxic reaction.
2. Platelet contamination can deplete antibody and complement, thereby causing false negative reactions.
3. Granulocyte contamination can cause false positives due to phagocytosis of eosin.
4. Cell concentration is also important since the test is standardized using a certain antigen-antibody ratio. Cell suspensions of $<2x10^6$ lymphocytes may result in false positive reactions. Cell suspensions of $>2x10^6$ lymphocytes may result in false negative reactions.

c. Adding the lymphocytes

Because the addition of lymphocytes to the wells can be done rapidly, several potential errors can occur:

1. Failure to mix lymphocytes with antisera (this is a common cause of negative reactions).
2. Skipping a well or row of wells.
3. Carryover of sera from one well to the next by the dispensing tips.

d. Microscopic Evaluation ("reading")
This phase is susceptible primarily to errors of carelessness such as reading a tray in reverse order or recording errors. It is essential to have the phase-contrast microscope properly adjusted to view both dead and live cells.

e. Temperature
The microlymphocytotoxicity test is temperature dependent. A temperature of 20-25ºC is required to perform the test.

f. Complement
Rabbit complement must be nontoxic to normal lymphocytes. Careful handling of the complement is required. It must be completely thawed, mixed (gently), and kept cool continuously before using. DO NOT refreeze rabbit complement.

Bacterial contamination of the reagents or lymphocyte preparations may cause false positive reactions.

PERFORMANCE CHARACTERISTICS

Reproducibility studies have shown less than 2% errant reactions. Studies comparing HLA Typing Trays to DNA typing results showed 97.4% agreement. Sensitivity and specificity cannot be determined for the product as a whole. However the sensitivity and specificity of each typing serum included on the tray is provided in the Sera Analysis Sheet. Positive reactivity is defined as 50% or greater cell death caused by the antiserum. Parentheses indicate specificities which have been known to react with (~50%) of cells possessing these antigens.

The antiserum specificity has been confirmed with a 50 cell frozen panel by an unaffiliated clinical histocompatibility laboratory accredited by the American Society for Histocompatibility and Immunogenetics (ASHI) in combination with 75 fresh cell samples representing various ethnic groups or by a panel of approximately 125 fresh cell samples representing various ethnic groups. The Sera Analysis sheet is a summary of these results.

BIBLIOGRAPHY

1. Histocompatibility Testing 1980: Report of an International Workshop and Conference, Los Angeles 1980, Ed. Terasaki, P.I., Los Angeles, CA. UCLA Tissue Typing Laboratory, UCLA.
2. Histocompatibility Testing 1984: Report of the Ninth International Histocompatibility Workshop, Munich and Vienna 1984, Eds. Albert, E.D.; Baur, M.P.; Mayr, W.R.
3. Topics in Clinical Histocompatibility Testing: Vol. 1: Schacter, B., Ed. In Chief, 1979 American Association for Clinical Histocompatibility Testing.
4. Manual of Tissue Typing Techniques: (1979) Ed. Ray, J.G., NIAID, National Institutes of Health, Bethesda, MD 20205. Publ. No. 80-545.
5. Miller, W.V., and Rodey, G: 1981. HLA Without Tears. Chicago: Educational Products Division ASCP.
6. Mittal, K.K.: Standardization of the HLA Typing Methods and Reagents. Vox Sang 34:58-63, 1978.
7. Microdroplet Testing for HLA-A, -B, -C, and –D Antigens. American Journal of Clinical Pathology. Volume 69, Number 2, February 1978, pages 103-120.
8. Rodey, G.E.: 2000. HLA Beyond Tears, 2nd edition. Colorado: De Novo, Inc.
9. ASHI: 2000. ASHI Laboratory Manual, 4th edition.

GTi DIAGNOSTiCS®
Good science starts with people.®

HLA Typing Tray
Class I (ABC)
- **For In Vitro Diagnostic Use**
- **Store at -65ºC or colder**

20925 Crossroads Circle, Suite 200
Waukesha, WI 53186-4054 USA
(262) 754-1000 OR 1-800-233-1843

0459

Cat. NO. 72, 72BL, 72L, 72OR, 72AB1/72AB2, 72C, 72ABC1/72ABC2, 96, 96C

Rev. 04 November 2008

Qarad b.v.b.a.
Volmolenheide 13
B-2400 Mol
Belgium