I. SUMMARY
GenTrak, Inc. Controls for Lymphocyte Typing are intended for use as a positive and negative serum controls in lymphocyte microcytotoxicity testing procedures.

II. REAGENTS
A. Positive Control for Lymphocyte Typing - 1 mL frozen anti-human lymphocyte serum produced in goats.
B. Negative Control for Lymphocyte Typing - 1 mL Frozen Human AB sera that does not demonstrate cytotoxicity to human lymphocytes.

Controls available separately from GenTrak, Inc.
Store at -65°C or colder.
Controls may be dispensed as small aliquots immediately after thawing and stored frozen at -65°C or colder. Frozen aliquots may be stored until the expiration date of the product. Repeated freezing and thawing is not recommended.
Human source material from which this product was derived was found non-reactive for HBsAg, Anti-HCV and HIV 1/2 when tested with licensed reagents.
Not for In-Vitro Diagnostic Use. For Research Use Only.

III. ADDITIONAL MATERIALS REQUIRED
1. Lymphocyte suspending medium
2. HLA typing trays
3. Pipettes capable of delivering up to 10 µl.
4. Microliter syringes
5. Inverted phase contrast microscope, 125x magnification
6. Nontoxic light or heavy mineral oil
8. Eosin Y, 5% (w/v). Prepare by completely dissolving 5 g. eosin Y in distilled water. Bring the volume to 100 mL with distilled water. Filter and store between 2°C and 8°C.
10. Microscope slides, 50 x 75 mm.
11. Temperature controlled room or incubator maintained at 22°C +/- 2°C.
12. Freezer capable of maintaining temperature of -65°C or colder.

IV. PROCEDURE
A. Preparation of Typing Trays
1. Add 2 to 5 µl of nontoxic light or heavy mineral oil to each well.
2. Using a microliter syringe, carefully deliver 1 µl of Positive and Negative controls, and Leukocyte Typing Serum under the mineral oil to the bottom of the test well. The serum must adhere to the bottom of the well.
3. These trays may be stored at -65°C or colder for a maximum of 1 year but not beyond the expiration date of the product. Avoid open exposure to carbon dioxide (dry ice). To use, thaw at room temperature immediately prior to performing the test.

B. Performance of Test
The following procedure is a standard two-step lymphocyte microcytotoxic technique. It is strongly recommended that more than

V. RESULTS

<table>
<thead>
<tr>
<th>Non-viable Lymphocytes</th>
<th>Grade Interpretation</th>
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<tbody>
<tr>
<td>0-19%</td>
<td>1  Negative</td>
</tr>
<tr>
<td>20 - 29%</td>
<td>2  Weak Negative</td>
</tr>
<tr>
<td>30 - 49%</td>
<td>4  Weak Positive</td>
</tr>
<tr>
<td>50 - 79%</td>
<td>6  Positive</td>
</tr>
<tr>
<td>80 - 100%</td>
<td>8  Strong Positive</td>
</tr>
<tr>
<td>Unreadable</td>
<td>0  Invalid</td>
</tr>
</tbody>
</table>

NOTE: If the Negative Control contains less than 80% viable lymphocytes (more than 19% stained cells) the test is unacceptable and must be repeated.

If duplicate determinations are made, readings should differ by no more than 20% with both results being either positive or negative.

CAUTION: Extreme care should be taken in the interpretation of the results, and the assignment of antigen specificities. Use more than one antiserum for each specificity.

VI. PERFORMANCE CHARACTERISTICS
The controls have been tested with human lymphocytes (both T and B) in the lymphocyte microcytotoxicity assay. The Negative Control was determined to produce negative reactions (19% or less "killed" cells). The Positive Control was determined to produce positive reactions (80% or more "killed" cells).

VII. LIMITATION
Cell viability prior to testing should be at least 90% to assure accurate negative control test result.
The Positive Control is not to be used as a specificity control in lymphocyte cytotoxicity procedures. It contains nonspecific antibodies to human lymphocytes.

VII. BIBLIOGRAPHY

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one serum representing each specificity be used. When only one serum per specificity is available, the test should be performed in duplicate to detect discrepancies. A positive and negative control should be included for each cell suspension being tested.