Results of the 1997 T-Lymphocyte Immunophenotyping Questionnaire Survey Mailed to Laboratories Participating in the Model Performance Evaluation Program

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Information about this report should be addressed to the Model Performance Evaluation Program by calling (770) 488-8091 or (770) 488-8098.
Introductory Comments

The aggregate results from a mailed questionnaire survey conducted by the Model Performance Evaluation Program (MPEP) in October 1997 of laboratories in the United States performing T-lymphocyte immunophenotyping (TLI) are presented in the following figures and tables. Of the 310 laboratories receiving this survey, 261 (84.2%) reported results. The "N" numbers appearing in each figure or table reflect the total number of laboratories responding to the specific question. For multiple response questions, the total number of responses may exceed the actual number of laboratories responding to that specific question.

The map located on page 2 reflects the enrollment in the MPEP TLI program at the time this survey was mailed, and does not reflect the current enrollment in this program.

The primary classification of all the laboratories in the MPEP TLI program at the time the survey was mailed is shown in the top figure on page 4. The primary classification of only those laboratories responding to the survey is shown in the bottom figure on the same page. The further classification of the responding laboratories is shown in the responses for questions 5(a)-5(e).

Please note that wording for questions 6 and 7, regarding the education and certification requirements of the laboratory director and supervisor, reflect current regulatory requirements related to the Clinical Laboratory Improvement Amendments of 1988 (CLIA ’88), as published in CFR 42, Part 493.

The term "single-platform method" was defined to be those methods for obtaining absolute CD4+ T-cell counts using a single-instrument, for example, FACSCount or Imag 2000, or laboratory test, for example, TRAx CD4 or Zymmune assay. The term "multi-platform method" was defined to be those methods that derive absolute CD4+ T-cell counts by using the percent CD4+ T-cells obtained from a flow cytometer in combination with the absolute lymphocyte count obtained from a hematology instrument.

Responses to question 8 reflect the amount of experience necessary to perform either single-platform or multi-platform methods.

Question 27 requested information regarding the monoclonal antibody manufacturer associated with reagents for each of the cell marker combinations routinely used for performing TLI. The first two pages of results for this question, pages 38-39, show the reagents used for single-color, two-color, three-color, and four-color tests. A summary of monoclonal antibody reagents used by participant laboratories is shown on page 40. Pages 41 through 44 show the monoclonal antibody reagent panels used by the participant laboratories.

Question 44 requested information regarding the price charged for TLI performed by single-platform or multi-platform methods.
Number of MPEP TLI Laboratories in the United States and Territories

CDC Model Performance Evaluation Program
October 1997 TLI Survey

N = 323
4. In the last year, has your laboratory performed TLI for HIV-infected patients?

Laboratories Performing TLI for HIV-Infected Patients

- Yes: 251
- No: 9
- Do not know: 1

N=261
Primary Classification of MPEP laboratories in regard to TLI testing

Total Number of Laboratories in TLI Program

<table>
<thead>
<tr>
<th>Primary Classification of Laboratory</th>
<th>Frequency of Laboratories</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital</td>
<td>208</td>
</tr>
<tr>
<td>Independent</td>
<td>54</td>
</tr>
<tr>
<td>Other</td>
<td>38</td>
</tr>
<tr>
<td>Health Dept</td>
<td>20</td>
</tr>
<tr>
<td>Blood Bank</td>
<td>3</td>
</tr>
</tbody>
</table>

N=323

Laboratories Responding to Questionnaire Survey

<table>
<thead>
<tr>
<th>Primary Classification of Laboratory</th>
<th>Frequency of Laboratories Responding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital</td>
<td>168</td>
</tr>
<tr>
<td>Independent</td>
<td>45</td>
</tr>
<tr>
<td>Other</td>
<td>28</td>
</tr>
<tr>
<td>Health Dept</td>
<td>17</td>
</tr>
<tr>
<td>Blood Bank</td>
<td>3</td>
</tr>
</tbody>
</table>

N=261
5.(a) If the laboratory type shown on your mailing label (located on page one) is BLOOD BANK, please further describe your TLI testing laboratory (Check all that apply within your Blood Bank laboratory classification.):

- Community: 3
- Non-U.S. Red Cross: 2
- Regional: 1
- Blood/Plasma center: 1
- Privately owned: 1
- Non-hospital blood bank: 1
- Other: 1

N = 3

5.(b) If the laboratory type shown on your mailing label (located on page one) is HOSPITAL, please further describe your TLI testing laboratory (Check all that apply within your Hospital laboratory classification.):

- Privately owned: 56
- University: 53
- Community: 34
- Regional: 28
- City: 22
- Religious-associated: 22
- County: 19
- State: 18
- Veterans Affairs: 15
- Other: 11
- District: 7
- HMO/PPO owned & operated: 7
- Federal Govt (non-military): 4
- Military (Federal): 3

N = 167
5.(c) If the laboratory type shown on your mailing label (located on page one) is HEALTH DEPARTMENT, please further describe your TLI testing laboratory (Check all that apply within your Health Department laboratory classification.):

- State: 10
- County: 5
- City: 3
- Regional: 1

Total (N=17)

5. (d) If the laboratory type shown on your mailing label (located on page one) is INDEPENDENT, please further describe your TLI testing laboratory (Check all that apply within your Independent laboratory classification.):

- Reference laboratory: 37
- Commercial: 11
- Other: 4
- Physician office: 3
- Commercial manufacturer of reagents: 2
- HMO satellite clinic: 2

Total (N=45)
5.(e) If the laboratory type shown on your mailing label (located on page one) is OTHER, please further describe your TLI testing laboratory (Check all that apply within your Other laboratory classification):

- University-assoc research: 16
- Other: 9
- Federal Govt research: 5
- Organ procurement: 3

N=26

Percentage of Laboratories Responding
6.(a) Please choose from the list below the highest academic degree that has been awarded to your Laboratory Director and Laboratory Supervisor (Choose only one degree for each person.):

Frequency of Laboratories Responding

Director N=257
Supervisor N=253

6.(b) If your Laboratory Director or Laboratory Supervisor has a degree other than M.D. or D.O., please indicate the academic discipline in which the degree was awarded (Check all that apply.):

Frequency of Laboratories Responding

Director N=99
Supervisor N=165
6.(c) What board certifications have been awarded to your Laboratory Director? (Check all that apply.)

- Amer Board of Pathology: 142
- Amer Board of Med Lab Immunology: 18
- Natl Board of Medical Examiners: 15
- Amer Board of Allergy & Immunology: 10
- Amer Board of Bioanalysts: 9
- Amer Board of Medical Microbiology: 9
- Amer Board of Clinical Chemistry: 3

6.(d) Please indicate the years of experience your Laboratory Director has in directing or supervising laboratory testing (Round off to the nearest whole number.):

Frequency of Laboratories Responding

- <5: 23
- 5-9: 32
- 10-14: 51
- 15-19: 39
- 20-24: 36
- >24: 41
6.(e) Is your Laboratory Supervisor available to provide supervision on-site?

Yes 250 (96.9%)
No 8 (3.1%)

N=258

6.(f) If no, is there another person on-site that has been assigned to provide supervision?

Yes 8 (100%)

N=8
7.(a) Does your laboratory require that personnel who perform TLI (operate a flow cytometer and analyze resultant data) have a minimum educational degree?

- **Yes**: 239 (92.3%)
- **No**: 20 (7.7%)

N=259

7.(b) What minimum educational degree is required of your TLI testing personnel? (Check only one.)

<table>
<thead>
<tr>
<th>Degree Type</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>BS/BA (Chem, Biol, Physics, Immunol, Microbiol)</td>
<td>87</td>
</tr>
<tr>
<td>Bachelors (Med Tech/ Clin Lab Science)</td>
<td>87</td>
</tr>
<tr>
<td>Associates (Med Lab Technician/Technologist)</td>
<td>51</td>
</tr>
<tr>
<td>BS/BA Other</td>
<td>4</td>
</tr>
<tr>
<td>Masters (Med Tech/ Clin Lab Science)</td>
<td>3</td>
</tr>
<tr>
<td>Masters (Chem, Biol, Physics, Immunol, Microbiol)</td>
<td>1</td>
</tr>
<tr>
<td>Associates (Non-science discipline)</td>
<td>1</td>
</tr>
</tbody>
</table>

N=234
7.(c) Does your laboratory require that your TLI testing personnel have certification by a professional organization? (Do not include licensing by state or county.)

No 141 (56.0%)
Yes 111 (44.0%)

N=252

7.(d) Please check the professional organizations that have awarded the required certification to your TLI testing personnel (Check all that apply.):

- Med Tech/ASCP: 101
- Natl Certification Agency for Medical Laboratory Personnel: 20
- Amer Medical Technologists: 12
- Other: 4
- Amer Assoc for Clinical Chem: 1
- Intl Society of Clin Lab Tech: 1

N=107
8. On average, how many months of experience do your personnel need to become proficient in performing TLI and analyzing the resultant data? (Indicate number of months of experience needed only for those methods currently in use in your laboratory. Round off to the nearest whole number.)

**Multi-Platform**

<table>
<thead>
<tr>
<th>Experience Needed (months)</th>
<th>Frequency of Laboratories Responding</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3</td>
<td>93</td>
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<tr>
<td>4-6</td>
<td>117</td>
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<td>7-9</td>
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<td>&gt;18</td>
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N=240

**Single-Platform**

<table>
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<th>Experience Needed (months)</th>
<th>Frequency of Laboratories Responding</th>
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<td>39</td>
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</tr>
<tr>
<td>10-12</td>
<td>2</td>
</tr>
<tr>
<td>13-18</td>
<td>1</td>
</tr>
</tbody>
</table>

N=61
9.(a) Does your laboratory require that personnel who perform TLI have training?

Yes
258 (99.2%)

No
2 (0.8 %)

N=260

9.(b) What training must your laboratory personnel complete before they are considered qualified to perform TLI? (Check all that apply.)

- In-house: 251
- Instrument mnfr training school: 64
- On-site training by mnfr rep: 59
- Training other than manufacturer: 35
- Regional workshop: 17
- State health department: 4
- Other: 4
- Natl Laboratory Training Network: 2

N=254
10.(a) On average, how many TLI specimens are tested in your laboratory in a month? (Number of single patient and/or blood donor specimens, not tests. Round off to the nearest whole number.)

Frequency of Laboratories Responding

10.(b) How many TLI specimens were tested in your laboratory in the last year? (Number of single patient and/or blood donor specimens, not tests. Round off to the nearest whole number.)

Frequency of Laboratories Responding
10.(c) Has the number of requests per month for TLI to be performed by your laboratory increased, decreased, or stayed the same compared to twelve months ago? (Choose only one.)

Increased: 113
Stayed the same: 98
Decreased: 43
Do not know: 4

N=258

11. In the last year, what percentage of your TLI specimens have come from patients known by your laboratory to be HIV-infected? (Round off to the nearest whole number.)

N=147
12.(a) Where are your specimens collected for TLI? (Choose only one.)

- **Both On- & Off-site**: 175
- **Off-site**: 50
- **On-site**: 34

N = 259

12.(b) If written instructions are provided to collection site personnel for collecting, labeling, and transporting TLI specimens, who provides these instructions? (Check all that apply.)

<table>
<thead>
<tr>
<th>Type of Instruction</th>
<th>Instructions NOT Provided</th>
<th>Testing Laboratory</th>
<th>Associated Institution</th>
<th>Person Ordering Test</th>
<th>Other</th>
<th>N =</th>
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<tbody>
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<td>Collecting</td>
<td>6</td>
<td>227</td>
<td>47</td>
<td>17</td>
<td>6</td>
<td>250</td>
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<td>217</td>
<td>53</td>
<td>16</td>
<td>6</td>
<td>250</td>
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<tr>
<td>Transporting</td>
<td>7</td>
<td>220</td>
<td>49</td>
<td>13</td>
<td>5</td>
<td>248</td>
</tr>
</tbody>
</table>
12.(c) Does your laboratory test TLI specimens collected off-site?

Yes 226 (86.9%)

No 34 (13.1%)

N=260
12.(d) When your laboratory tests TLI specimens that are collected off-site, where are the specimens collected? (Check all that apply.)

- Hospital
- Private physician office
- Outpatient health clinic
- HIV-1 counseling & testing site
- HMO facility
- Correctional facility
- STD clinic
- Family planning clinic
- Out of state
- County health dept
- Drug use treatment facility
- City health dept
- Military installation
- NIAID study center
- State health dept
- Other
- Organ donation center
- Blood/Plasma center
- Uncertain/Do not know

N=224
12.(e) How are the off-site TLI specimens delivered to your laboratory? (Check all that apply.)

- Courier: 190
- Express mail delivery: 70
- Pick up by laboratory personnel: 68
- Delivered by health care personnel: 66
- Delivered by patient: 4
- Other: 4
- Inter-office mail: 2
- Regular U.S. mail: 2

N=224

12.(f) At what temperature are TLI specimens transported to your laboratory? (Check all that apply.)

- Room Temperature: 222
- 2 - 10 °C: 6
- -70 °C: 1
- Other: 1

N=224
13. On average, how many hours does it take from the time a TLI specimen is collected until the time it is delivered to your laboratory? (Round off to the nearest whole number.)

Frequency of Laboratories Responding

<table>
<thead>
<tr>
<th>Hours from Collection to Delivery</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
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<tr>
<td>1</td>
<td>24</td>
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<td>4</td>
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<td>19</td>
<td>2</td>
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<tr>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>&gt;24</td>
<td>1</td>
</tr>
</tbody>
</table>

N=236
14.(a) Are the procedures your laboratory uses for labeling a suspected HIV-1-positive specimen different from the procedures you use for other specimens?

- **No**: 251 (96.5%)
- **Yes**: 9 (3.5%)

N=260

14.(b) Universal precautions assume that all blood and body fluids are potentially infectious for blood borne pathogens. How often do your laboratory employees follow universal precautions when handling specimens for TLI? (Choose only one.)

- **Always**: 255 (97.7%)
- **Most of the time**: 6 (2.3%)

N=261
15. Where is the hematology testing (e.g., complete blood count [CBC]) for your laboratory’s TLI specimens usually performed? (Choose only one.)

- In separate laboratory: 130
- In TLI laboratory: 88
- Off-site: 21
- Not performed: 17
- Other: 4

N=260

16.(a) Does your laboratory use TLI specimen collection criteria to determine whether or not a specimen is acceptable for TLI?

- Yes: 255 (97.7%)
- No: 6 (2.3%)

N=261
16.(b) What specimen collection criteria are used at your laboratory to determine whether or not a specimen is acceptable for TLI? (Check all that apply.)

- Age of specimen: 249
- Clotted sample: 223
- Improper labeling: 219
- Type of anticoagulant: 218
- Insufficient quantity: 200
- Temperature of prior storage/transport: 195
- Temperature stored in lab: 194
- Hemolyzed blood: 142
- Damaged container: 131
- Cell viability: 100
- Request form improperly completed: 71
- Incomplete filling of tube: 63
- Precipitated material: 62
- Microbial contamination: 60
- Time of day of collection: 29
- Lipemic blood: 23
- Icteric blood: 16
- Lack of consent: 15
- Culture/transport medium not used: 4
- Patient medication: 2
- Label for infectious agents: 1

N=254
17. At what temperatures does your laboratory store TLI specimens until they are processed? (Check all that apply.)

<table>
<thead>
<tr>
<th>Type of Specimen</th>
<th>Room Temperature</th>
<th>2 - 10°C</th>
<th>Other Temperature</th>
<th>N =</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Blood</td>
<td>259</td>
<td>4</td>
<td>1</td>
<td>260</td>
</tr>
<tr>
<td>Separated Cells</td>
<td>37</td>
<td>55</td>
<td>0</td>
<td>91</td>
</tr>
<tr>
<td>Other</td>
<td>24</td>
<td>13</td>
<td>0</td>
<td>36</td>
</tr>
</tbody>
</table>
18. When did your laboratory begin performing TLI? (Please indicate month and year only for those methods currently in use in your laboratory.)

Frequency of Laboratories Responding

N=229

Frequencies of Laboratories Responding

N=41
19. Does your laboratory perform TLI using a flow cytometry instrument?

- Yes: 259 (99.2%)
- No: 2 (0.8%)

N=261

20.(a) How many different flow cytometer operators actually performed TLI in your laboratory over the last year?

Frequency of Laboratories Responding:

- 1-3: 144
- 4-6: 88
- 7-9: 16
- 10-12: 7
- 13-15: 1

N=256
20.(b) Do any of your laboratory’s flow cytometer operators routinely perform TLI on more than one flow cytometer?

No 192 (74.1%)

Yes 67 (25.9%)

N=259

20.(c) Have your flow cytometer operators received training on each of the instruments that they are required to operate?

Yes 246 (98.8%)

No 3 (1.2%)

N=249
21. What are the quantities and manufacturers of your laboratory's flow cytometers that are used for TLI?

Frequency of Laboratories Responding

Becton-Dickinson N=129
Coulter N=130
Ortho N=23

21. What are the quantities and manufacturers of your laboratory's hematology analyzers that are used for TLI?

Frequency of Laboratories Responding

Abbott N=19
Sysmex N=20
Coulter N=113
Technicon N=15
Other N=3
22. Can multicolor analysis techniques (two or more markers tagged with different color fluorochromes as a single test) be used to perform TLI on any of the flow cytometry instruments you indicated?

Yes 258 (99.6%)

No 1 (0.4 %)

N=259

23. On average, how many hours is a specimen stored at your laboratory before it is stained for TLI? (Round off to the nearest whole number.)

Frequency of Laboratories Responding

<table>
<thead>
<tr>
<th>Hours Stored Before Staining</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-6</td>
<td>184</td>
</tr>
<tr>
<td>7-12</td>
<td>44</td>
</tr>
<tr>
<td>13-18</td>
<td>8</td>
</tr>
<tr>
<td>19-24</td>
<td>13</td>
</tr>
<tr>
<td>&gt;24</td>
<td>1</td>
</tr>
</tbody>
</table>

N=250
24.(a) Is testing for viability of TLI specimens performed by your laboratory?

No
192 (74.4%)

Yes
66 (25.6%)

N=258

24.(b) How is testing for viability performed? (Check all that apply.)

Trypan blue exclusion
30

Propidium iodide
19

Other
15

Examine blood smear
5

Ethidium bromide/ Acridine orange
1

N=65
24.(c) What is the minimum percent viability acceptable by your laboratory for TLI? (Round off to the nearest whole number.)

Frequency of Laboratories Responding

Minimum Percent Viability Acceptable

N=52

Frequency: 5, 1, 1, 16, 26, 3

Range: 0-50, 51-60, 61-70, 71-80, 81-90, 91-100
25.(a) Does your laboratory use a whole blood lysis method for staining TLI specimens?

Yes
251 (97.3%)

No
7 (2.7%)

N=258

25.(b) Which lysis method does your laboratory use? (Check all that apply.)

Immuno-Prep
105

FACS Lyse
98

Ammonium chloride
38

Immuno-Lyse
18

OptiLyse
8

Other
4

N=249
25.(c) What sample staining procedure(s) does your laboratory use for TLI? (Check all that apply.)

- Stain whole blood/Lyse: 246 (75.6%)
- Separate by density centrifugation: 12
- Lyse whole blood/Stain: 10
- Separate buffy-coat/Lyse/Stain: 4
- Other: 4

N=255

25.(d) Does your laboratory routinely use isotype controls as part of the staining procedure?

- Yes: 195 (75.6%)
- No: 63 (24.4%)

N=258
25.(e) At what temperature does your laboratory routinely perform the staining procedure? (Choose only one.)

- Room temperature: 240 (86.4%)
- 2-10 °C: 18 (6.6%)

N=258

25.(f) Does your laboratory fix cells before TLI flow cytometry is performed?

- Yes: 228 (88.4%)
- No: 30 (11.6%)

N=258
25.(g) At what temperature does your laboratory routinely store cells after staining? (Choose only one.)

- 2-10 °C: 200
- Room temperature: 58

N=258

25.(h) On average, how many hours is a specimen stored at your laboratory after staining before being analyzed? (Round off to the nearest whole number.)

- 0-6: 209
- 7-12: 19
- 13-18: 18
- 19-24: 7
- >24: 1

N=254
26. From what source did your laboratory obtain its TLI staining procedure? (Check all that apply.)

- Monoclonal antibody mnfr: 214
- Flow cytometer mnfr: 167
- In-house: 82
- Publications: 59
- State health department: 17
- Other: 5

N=257
27. Please complete the table below and the tables on the following pages, by choosing from the list below, the monoclonal antibody manufacturer associated with reagents for each of the cell marker combinations you routinely use for performing TLI.

**Single-color Tests**

<table>
<thead>
<tr>
<th>Cell Marker</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
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<td>CD3</td>
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<td>9</td>
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**Two-color Tests**

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<td>85</td>
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</tbody>
</table>

Manufacturer Key:
A=Immunotech  B=Becton Dickinson  C=Coulter  D=Dako  E=GenTrak  F=Tago  G=Olympus  H=Ortho  I=In-House  J=Non Commercial  K=Other
27. Please complete the table below and the tables on the following pages, by choosing from the list below, the monoclonal antibody manufacturer associated with reagents for each of the cell marker combinations you routinely use for performing TLI.

### Three-color Tests

<table>
<thead>
<tr>
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<td>43</td>
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<tr>
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### Four-color Tests

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<th>B/A/C</th>
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<th>B/B/B/B</th>
<th>B/B/B/K</th>
<th>B/B/C/B</th>
<th>C/B/C/B</th>
<th>C/C/C/C</th>
<th>K/B/C/B</th>
<th>K/C/C/C</th>
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</thead>
<tbody>
<tr>
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<td>1</td>
<td>6</td>
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<tr>
<td>CD45/CD3/CD19/CD16</td>
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<td></td>
<td>1</td>
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<tr>
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</tbody>
</table>

Manufacturer Key:
A=Immunotech B=Becton Dickinson C=Coulter D=Dako E=GenTrak F=Tago G=Olympus H=Ortho I=In-House J=Non Commercial K=Other
Summary of monoclonal antibody reagents used by participant laboratories

Key for interpretation
1: one-color  2: two-color  3: three-color  4: four-color
27. continued:

One-color monoclonal antibody reagent panels used by participant laboratories

<table>
<thead>
<tr>
<th>Panel</th>
<th>Percentage of Laboratories Responding</th>
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<tbody>
<tr>
<td>1:3, 1:4, 1:8, 1:19, 1:16, 1:56, 1:56/16</td>
<td>7</td>
</tr>
<tr>
<td>1:19</td>
<td>4</td>
</tr>
<tr>
<td>1:3, 1:4, 1:8, 1:19, 1:16, 1:56</td>
<td>4</td>
</tr>
<tr>
<td>1:19, 1:56</td>
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<td>1:3, 1:19</td>
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<tr>
<td>1:3, 1:4, 1:8, 1:19, 1:56</td>
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</tr>
<tr>
<td>1:16, 1:56</td>
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<td>1:3, 1:16</td>
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<tr>
<td>1:3, 1:4, 1:8</td>
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<tr>
<td>1:3, 1:4, 1:8, 1:19</td>
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</tr>
<tr>
<td>1:3, 1:4, 1:8, 1:19, 1:56/16</td>
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</tr>
<tr>
<td>1:56</td>
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<tr>
<td>1:3, 1:4, 1:56/16</td>
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<tr>
<td>1:3, 1:4, 1:8, 1:19, 1:56/16</td>
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</tr>
<tr>
<td>1:3, 1:56</td>
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</tr>
<tr>
<td>1:4, 1:8</td>
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<tr>
<td>1:4, 1:8, 1:19</td>
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</tbody>
</table>

N=41

Key for interpretation
1: one-color, marker (e.g., 1:3, one-color CD3)
27. continued:

Two-color monoclonal antibody reagent panels used by participant laboratories

Key for interpretation

2: two-color, marker (e.g., 2:45/14, two-color CD45/CD14)
27. continued:

Three-color monoclonal antibody reagent panels used by participant laboratories

Key for interpretation
3: three-color, marker (e.g., 3:45/3/4, three-color)
27. continued:

Four-color monoclonal antibody reagent panels used by participant laboratories

Key for interpretation
4: four-color, marker (e.g., 4:45/3/4/8, four-color)
28.(a) How is the gating set when performing TLI on your flow cytometers? (Choose only one.)

- Manually set: 152
- Software controlled: 92
- Other: 13

N=257

28.(b) Which cell populations are included in the gates for analyses of lymphocyte phenotypes? (Choose only one.)

- Lymphocytes only: 240
- Lymphocytes and monocytes: 9
- Lymphocytes, monocytes, granulocytes: 7
- Other: 1

N=257
29. How are the integration windows set? (Choose only one.)

- Isotype controls: 176
- Other: 30
- Predetermined channel numbers: 20
- Unstained cells: 20

N=246
30.(a) Do you mathematically adjust your phenotype values for isotype control values?

- No: 196 (77.8%)
- Yes: 56 (22.2%)

N=252

30.(b) How do you adjust your phenotype values? (Choose only one.)

- Subtracting isotype controls: 39
- Curve-matching software: 12
- Other: 3

N=54
31. How often are normal cell controls used? (Choose only one.)

- Each antibody daily: 181
- Each analysis: 34
- Each antibody weekly: 19
- Other: 8
- Not used: 6
- Each new lot of antibody: 4
- Each antibody monthly: 3

N=255

32. How often do your flow cytometer(s) receive preventive maintenance (e.g., cleaning of optical filters and lenses, and fluidics check)? (Choose only one.)

- Biannually: 142
- Annually: 61
- Quarterly: 16
- Weekly: 13
- Only as needed: 11
- Monthly: 10
- Other: 5

N=258
33.(a) How often does your laboratory check the optical alignment of your flow cytometer(s)? (Choose only one.)

- Daily: 194
- Every analysis: 17
- Only as needed: 15
- Never: 13
- More than monthly: 9
- Weekly: 6
- Do not know: 4

N=258

33.(b) What types of particles are used by your laboratory to align your flow cytometers? (Check all that apply.)

- Very bright beads: 150
- Beads with fluorescence intensity similar to cells: 123
- Chicken erythrocytes: 28
- Thymocyte nuclei: 10
- Other: 5

N=231
34.(a) Does your laboratory set fluorescence overlap compensation?

- Yes: 249 (96.5%)
- No: 9 (3.5%)

N=258

34.(b) What reference materials are used to set fluorescence overlap compensation? (Check all that apply.)

- Fluorescein isothiocyanate/Phycoerythrin stained cells: 161
- Fluorescein isothiocyanate/Phycoerythrin labeled beads: 132
- Standard instrument settings: 34
- Other: 5

N=239
35.(a) What reference materials does your laboratory use to achieve the target conditions for forward angle light scatter (FALS) and fluorescence intensity (FI)? (Check all that apply.)

- Beads: 222 (86.9%)
- Whole blood: 98 (38.1%)
- Lyophilized cells: 19 (7.5%)
- Other: 6 (2.4%)
- Thymocyte nuclei: 4 (1.6%)
- Cryogenically preserved cells: 3 (1.2%)
- Cell lines: 2 (0.8%)

35.(b) Does your laboratory routinely record the instrument settings used to reach the target conditions for FALS?

- Yes: 240 (93.8%)
- No: 16 (6.2%)

N=256
35.(c) Are these data analyzed to monitor trends or changes in instrument performance?

Yes  235 (98.3%)

No    4  (1.7 %)

N=239
36.(a) How often does your laboratory standardize your flow cytometer(s)? (Choose only one.)

- Daily: 196
- Every analysis: 28
- Never: 15
- Monthly: 7
- More than monthly: 7
- Weekly: 4

N=257

36.(b) What material(s) does your laboratory use to standardize your flow cytometer(s)? (Check all that apply.)

- Beads with fluorescence intensity similar to cells: 168
- Very bright beads: 103
- Other: 11
- Thymocyte nuclei: 2

N=237
36.(c) Does your laboratory use reference standards to plot standard curves of mean channel fluorescence vs. molecules of equivalent soluble fluorochrome?

- Yes: 46 (19.3%)
- No: 192 (80.7%)

N=238

36.(d) Does your laboratory maintain written records of the slope, intercept and correlation coefficients of the standard curves?

- Yes: 39 (86.7%)
- No: 6 (13.3%)

N=45
37. Does your laboratory obtain marker-specific absolute counts (e.g., absolute CD4 count) using a single-platform method?

- No: 215 (84.0%)
- Yes: 41 (16.0%)

N=256

38. What single-platform method(s) does your laboratory use? (Check all that apply.)

- CytoronAbsolute: 12
- FACSCount: 11
- TruCount: 6
- STKS: 6
- Imagn 2000: 3
- Flow-Count: 2
- Other (not flow): 1

N=36
39. Before TLI results are reported, are they routinely reviewed by someone other than the person(s) who performed the tests?

Yes 207 (79.3%)

No 54 (20.7%)

N=261
40. What information is included in the report returned to the person or institution initiating the request for TLI? (Check all that apply.)

- Cell marker percentages: 239
- CD4/CD8 ratios: 234
- Range of normal results: 227
- Cell marker counts: 209
- Absolute lymphocyte counts: 208
- Percent lymphocytes: 187
- Total WBC count: 184
- Interpretation of results: 95
- Leukocyte differential: 82
- Suggested follow-up testing: 33
- Sample condition: 24
- Normal control results: 9
- Fluorescence histograms/plots: 7
- Other: 7
- Cell viability: 6
- Light scatter histograms/plots: 5

N=258
41. On average, how many days elapse between receipt of the specimen in your laboratory and the time the results of the test are returned to the person or institution initiating the request for TLI? (Round off to the nearest whole number.)

Frequency of Laboratories Responding

Days Between Receipt and Return

42. Does your laboratory have procedures for protecting the confidentiality of TLI results?

Yes 222 (86.0%)

No 36 (14.0%)
43. On average, how many times in a month does your laboratory receive inquiries from clinicians requesting interpretation of TLI results? (Round off to the nearest whole number.)

Frequency of Laboratories Responding

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<th>TLI Results Inquiries Per Month</th>
<th>Count</th>
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<td>114</td>
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<tr>
<td>1-5</td>
<td>91</td>
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<tr>
<td>6-10</td>
<td>8</td>
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<td>11-15</td>
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<tr>
<td>&gt;30</td>
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</table>

N=217
44.(a) What is the average full price currently charged by your laboratory for TLI for each patient/blood donor sample? (Please indicate the price charged only for those methods currently in use in your laboratory. Round off to the nearest whole dollar.)

**Multi-Platform**

<table>
<thead>
<tr>
<th>Price Charged (dollars)</th>
<th>Frequency of Laboratories Responding</th>
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<tbody>
<tr>
<td>0-50</td>
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<td>51-100</td>
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**Single-Platform**

<table>
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<th>Price Charged (dollars)</th>
<th>Frequency of Laboratories Responding</th>
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<td>0-50</td>
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<td>201-250</td>
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<tr>
<td>251-300</td>
<td>1</td>
</tr>
</tbody>
</table>
44.(b) Has the average full price that your laboratory charges for TLI for each patient/blood donor sample increased, decreased, or stayed the same compared to twelve months ago? (Choose only one.)

- Stayed the same: 133
- Do not know: 67
- Increased: 35
- Decreased: 10

N=245

44.(c) Do you anticipate the price charged for TLI by your laboratory will increase, decrease, or stay the same in the next twelve months? (Choose only one.)

- Stay the same: 118
- Do not know: 80
- Increase: 39
- Decrease: 11

N=248
45.(a) Does your laboratory participate in an external TLI proficiency testing program?

Yes 250 (96.2%)
No 10 (3.8 %)

N=260

45.(b) In which program(s) does your laboratory participate? Please exclude the CDC Model Performance Evaluation Program, since it is not designed for proficiency testing. (Check all that apply).

- CAP: 230
- FAST Systems, Inc.: 43
- Other: 28
- NIAID DAIDS: 27
- Instrument mnfr program: 15
- U.S. Army: 2

N=247
46.(a) In the last year, has your laboratory performed surrogate-marker tests for TLI in HIV-infected patients?

No 224 (86.2%)
Yes 36 (13.8%)

N=260

46.(b) Which surrogate-marker tests did your laboratory perform? (Check all that apply).

- p24 antigen: 24
- Beta-2-microglobulin: 15
- Interleukin-2 receptor: 7
- Neopterin: 3
- Other: 3

N=32
47.(a) In the last year, has your laboratory performed other tests for HIV-1 infection?

Yes 134 (51.9%)

No 124 (48.1%)

N=258

47.(b) Which HIV-1 tests did your laboratory perform? (Check all that apply.)

- EIA 112
- Western blot 68
- RNA-PCR 49
- HIV-1 p24 antigen detection 46
- DNA-PCR 23
- Branch deoxyribonucleic acid (bDNA) 21
- Viral culture 11
- IIF 6
- Other 6

N=131