Results of the 2001 T-Lymphocyte Immunophenotyping Questionnaire Survey Mailed to Laboratories Participating in the Model Performance Evaluation Program

T-Lymphocyte Immunophenotyping by Multi-Platform and Single-Platform Methods

U.S. DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Centers for Disease Control and Prevention

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Information about this report should be addressed to the Model Performance Evaluation Program by calling (770) 488-8091 or (770) 488-8137.
Introductory Comments

The aggregate results from a mailed questionnaire survey conducted by the Model Performance Evaluation Program (MPEP) in May 2001 of laboratories in the United States performing T-lymphocyte immunophenotyping (TLI) are presented in the following figures and tables. Of the 300 laboratories receiving this survey (four enrolled laboratories chose not to receive the survey), 246 (82.0%) 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 may 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 classifications of the responding laboratories are 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 Imagn 2000, or laboratory test, for example, TRAx CD4 or Manual CD4 Count kit. 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 37-38, 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 39. Pages 40 through 43 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.

Responses to Question 45 reflect the external proficiency testing programs in which participant laboratories are enrolled.

Questions 46 and 47 requested information regarding the surrogate-marker tests and the other tests for HIV infection which are performed by participant laboratories.
Number of MPEP TLI Laboratories in the United States and Territories

N = 304
4. In the last year, has your laboratory performed TLI for HIV-infected patients?

- Yes: 232
- No: 11
- Do not know: 1

N=244
Primary Classification of MPEP TLI Testing Laboratories

Total Number of Laboratories in TLI Program

Frequency of Laboratories

<table>
<thead>
<tr>
<th>Primary Classification of Laboratory</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital</td>
<td>190</td>
</tr>
<tr>
<td>Independent</td>
<td>58</td>
</tr>
<tr>
<td>Other</td>
<td>33</td>
</tr>
<tr>
<td>Health Dept</td>
<td>19</td>
</tr>
<tr>
<td>Blood Bank</td>
<td>4</td>
</tr>
</tbody>
</table>

Laboratories Responding to Questionnaire Survey

Frequency of Laboratories Responding

<table>
<thead>
<tr>
<th>Primary Classification of Laboratory</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital</td>
<td>159</td>
</tr>
<tr>
<td>Independent</td>
<td>42</td>
</tr>
<tr>
<td>Other</td>
<td>26</td>
</tr>
<tr>
<td>Health Dept</td>
<td>15</td>
</tr>
<tr>
<td>Blood Bank</td>
<td>4</td>
</tr>
</tbody>
</table>

N=304

N=246
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: 2
- Blood/Plasma center: 2
- Regional: 1
- Non-U.S. Red Cross: 1
- Privately owned: 1
- Hospital blood bank: 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):

- Non-Profit: 74
- University: 48
- Privately owned: 42
- Community: 26
- Regional: 24
- Religious-associated: 23
- City: 20
- County: 15
- Veterans Affairs: 13
- State: 11
- Other: 5
- Military (Federal): 4
- District: 3
- Federal Govt (non-military): 3
- HMO/PPO owned & operated: 2

N=156
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: 9
- County: 5
- City: 3
- National Reference Lab: 1

N=15

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: 26
- Other: 8
- Commercial: 5
- Commercial manufacturer of reagents: 4
- Physician office: 2
- Pharmaceutical: 1

N=39
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: 12
- University clinical: 9
- Other: 4
- Federal Govt research: 4
- Organ procurement: 3
- Privately-funded research: 2
- Military (Federal): 1

N=23
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):

- B.A./B.S. (5 Director, 1 Supervisor)
- Dr.P.H. (1 Director, 0 Supervisor)
- M.A./M.S. (0 Director, 38 Supervisor)
- M.B.A. (9 Director, 1 Supervisor)
- M.D. (167 Director, 10 Supervisor)
- M.P.A. (1 Director, 1 Supervisor)
- M.P.H. (4 Director, 1 Supervisor)
- Ph.D. (68 Director, 24 Supervisor)
- D.V.M. (1 Director, 0 Supervisor)
- J.D. (0 Director, 2 Supervisor)
- A.A./A.S. (0 Director, 2 Supervisor)
- None (0 Director, 2 Supervisor)
- Other (0 Director, 1 Supervisor)

Frequency of Laboratories Responding

Director N=245
Supervisor N=238

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):

- Chem (10 Director, 13 Supervisor)
- Biol (40 Director, 2 Supervisor)
- Molecular Biology (5 Director, 6 Supervisor)
- Epi (1 Director, 1 Supervisor)
- Microbiol (32 Director, 23 Supervisor)
- Immuno (37 Director, 20 Supervisor)
- Path (15 Director, 15 Supervisor)
- Clin Lab Sci (61 Director, 8 Supervisor)
- Admin (3 Director, 8 Supervisor)
- Pharma (4 Director, 2 Supervisor)
- Other (3 Director, 3 Supervisor)

Frequency of Laboratories Responding

Director N=92
Supervisor N=142
6.(c) What board certifications have been awarded to your Laboratory Director? (Check all that apply.)

- Amer Board of Pathology: 133
- Natl Board of Medical Examiners: 21
- Amer Board of Med Lab Immunology: 13
- Other: 11
- Amer Board of Medical Microbiology: 9
- Amer Board of Internal Medicine: 8
- Amer Board of Allergy & Immunology: 6
- Amer Board of Bioanalysts: 6
- Amer Board of Clinical Chemistry: 4

N=180

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

N=214

Director Years Experience Directing:
- 1-4: 17
- 5-9: 31
- 10-14: 38
- 15-19: 38
- 20-24: 41
- >24: 49
6.(e) Is your Laboratory Supervisor available to provide supervision on-site?

Yes 239 (98.0%)

No 5 (2.0%)

N=244

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

Yes 4 (80.0%)

No 1 (20.0%)

N=5
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 230 (94.3%)

No 14 (5.7%)

N=244

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

- BS/BA (Chem, Bio, Phys, Immunol, Microbiol) 80
- Bachelors (Med Tech/Clin Lab Science) 78
- Associates (Med Lab Technician/Technologist) 54
- Associates (Non-science discipline) 2
- BS/BA Other 1
- Other 2
- Doctorate (Chem, Bio, Phys, Immunol, Microbiol) 1
- Masters (Chem, Bio, Phys, Immunol, Microbiol) 1
- Masters (Med Tech/Clin Lab Science) 1

N=220
7.(c) Does your laboratory require that your TLI testing personnel have certification by a professional organization? (Do not include licensing by city, state, or county.)

- No 130 (54.2%)
- Yes 110 (45.8%)

N=240

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: 100
- Natl Certification Agency for Medical Laboratory Personnel: 28
- Amer Medical Technologists: 10
- Amer Assoc for Clinical Chem: 3
- Other: 3
- Intl Society of Clin Lab Techs: 2
- Amer Assoc of Bioanalysts: 1
- Amer Academy of Microbiology: 1

N=103
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**

Frequency of Laboratories Responding

<table>
<thead>
<tr>
<th>Experience Needed (months)</th>
<th>N=222</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3</td>
<td>90</td>
</tr>
<tr>
<td>4-6</td>
<td>105</td>
</tr>
<tr>
<td>7-9</td>
<td>9</td>
</tr>
<tr>
<td>10-12</td>
<td>17</td>
</tr>
<tr>
<td>13-18</td>
<td>1</td>
</tr>
</tbody>
</table>

**Single-Platform**

Frequency of Laboratories Responding

<table>
<thead>
<tr>
<th>Experience Needed (months)</th>
<th>N=63</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3</td>
<td>33</td>
</tr>
<tr>
<td>4-6</td>
<td>25</td>
</tr>
<tr>
<td>7-9</td>
<td>2</td>
</tr>
<tr>
<td>10-12</td>
<td>3</td>
</tr>
</tbody>
</table>
9.(a) Does your laboratory require that personnel who perform TLI have training?

Yes
243 (99.6)

No
1 (0.4 %)

N=244

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

- In-house: 237
- On-site training by instrument manufacturer representative: 54
- Instrument manufacturer training school: 53
- Training other than manufacturer: 21
- Regional workshop: 8
- Other: 8
- State health department: 1

N=240
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

Number Samples Tested Per Month

N=238

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

Number Samples Tested Last Year

N=235
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: 104
- Stayed the same: 102
- Decreased: 30
- Do not know: 2

N=238

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.)
12.(a) 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>
</tr>
</thead>
<tbody>
<tr>
<td>Collecting</td>
<td>3</td>
<td>208</td>
<td>42</td>
<td>12</td>
<td>4</td>
<td>233</td>
</tr>
<tr>
<td>Labeling</td>
<td>5</td>
<td>200</td>
<td>46</td>
<td>12</td>
<td>3</td>
<td>230</td>
</tr>
<tr>
<td>Transporting</td>
<td>4</td>
<td>205</td>
<td>41</td>
<td>11</td>
<td>2</td>
<td>231</td>
</tr>
</tbody>
</table>

12.(b) Where are your specimens collected for TLI? (Choose only one.)

- Both On- & Off-site: 174
- Off-site Only: 40
- On-site Only: 26

N=240
12.(c) When your laboratory tests TLI specimens that are collected off-site, where are the specimens collected? (Check all that apply.)

- Hospital: 160
- Private physician office: 129
- Outpatient health clinic: 118
- HIV-1 Counseling & testing site: 51
- Correctional facility: 41
- STD clinic: 35
- Home health care agency: 32
- Out of state: 29
- HMO facility: 27
- Family planning clinic: 26
- Nursing home: 21
- County health dept: 19
- Other: 18
- City health dept: 12
- Drug use treatment facility: 12
- Military installation: 12
- State health dept: 8
- NIAID study center: 7
- Uncertain/Do not know: 7
- Tuberculosis clinic: 5
- Blood/Plasma center: 4
- Organ donation center: 3

N=214
12.(d) How are the off-site TLI specimens delivered to your laboratory? (Check all that apply.)

- Courier: 188
- Express mail delivery: 62
- Pick up by laboratory personnel: 50
- Delivered by health care personnel: 45
- Inter-office mail: 5
- Delivered by patient: 3
- Regular U.S. mail: 2

N=214

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

- Room Temperature: 212
- 2-10 °C: 10
- Do not know: 1

N=214
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.)
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** 241 (99.2%)
- **Yes** 2 (0.8%)

**N=243**

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** 239 (97.6%)
- **Most of the time** 6 (2.4%)

**N=245**
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: 115
- In TLI laboratory: 83
- Not performed: 28
- Off-site: 12
- Other: 7

N=245

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

Yes: 238 (97.9%)

No: 5 (2.1%)

N=243
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
- Clotted sample
- Improper labeling
- Type of anticoagulant
- Insufficient quantity
- Temperature of prior storage/transport
- Temperature stored in lab
- Hemolyzed blood
- Damaged container
- Cell viability
- Request form improperly completed
- Incomplete filling of tube
- Precipitated material
- Microbial contamination
- Time of day of collection
- Lipemic blood
- Icteric blood
- Lack of consent
- Patient's medications
- Culture/transport medium not used
- Label for infectious agents
- Other

N=236
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>240</td>
<td>2</td>
<td>1</td>
<td>243</td>
</tr>
<tr>
<td>Separated Cells</td>
<td>20</td>
<td>30</td>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>Other</td>
<td>8</td>
<td>8</td>
<td>1</td>
<td>15</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

<table>
<thead>
<tr>
<th>Multi-Platform Methods</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1979-1980</td>
<td>2</td>
</tr>
<tr>
<td>1981-1982</td>
<td>5</td>
</tr>
<tr>
<td>1983-1984</td>
<td>19</td>
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<tr>
<td>1985-1986</td>
<td>18</td>
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<tr>
<td>1987-1988</td>
<td>31</td>
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<tr>
<td>1989-1990</td>
<td>38</td>
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<td>1991-1992</td>
<td>30</td>
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<td>1993-1994</td>
<td>16</td>
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<td>1995-1996</td>
<td>16</td>
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<td>1997-1998</td>
<td>19</td>
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<tr>
<td>1999-2000</td>
<td>14</td>
</tr>
<tr>
<td>2001</td>
<td>1</td>
</tr>
</tbody>
</table>

Frequency of Laboratories Responding

<table>
<thead>
<tr>
<th>Single-Platform Methods</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1987-1988</td>
<td>1</td>
</tr>
<tr>
<td>1989-1990</td>
<td>1</td>
</tr>
<tr>
<td>1991-1992</td>
<td>5</td>
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<tr>
<td>1993-1994</td>
<td>3</td>
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<tr>
<td>1995-1996</td>
<td>6</td>
</tr>
<tr>
<td>1997-1998</td>
<td>8</td>
</tr>
<tr>
<td>1999-2000</td>
<td>20</td>
</tr>
<tr>
<td>2001</td>
<td>2</td>
</tr>
</tbody>
</table>
19. **Does your laboratory perform TLI using a flow cytometry instrument?**

Yes 243 (99.2%)

No 2 (0.8%)

N=245

20.(a) **How many flow cytometer operators actually performed TLI in your laboratory over the last 12 months?**

Frequency of Laboratories Responding

<table>
<thead>
<tr>
<th>Number of Different Operators Performing TLI</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3</td>
<td>133</td>
</tr>
<tr>
<td>4-6</td>
<td>85</td>
</tr>
<tr>
<td>7-9</td>
<td>16</td>
</tr>
<tr>
<td>10-12</td>
<td>2</td>
</tr>
<tr>
<td>13-15</td>
<td>4</td>
</tr>
<tr>
<td>&gt;15</td>
<td>1</td>
</tr>
</tbody>
</table>

N=241
20.(b) Do any of your laboratory's flow cytometer operators routinely perform TLI on more than one flow cytometer?

No 176 (73.0%)

Yes 65 (27.0%)

N=241

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

Yes 235 (100 %)

N=235
21. **What are the quantities and manufacturers of your laboratory's flow cytometers that are used for TLI?**

![Flow Cytometers Frequency Chart]

- Becton-Dickinson N=138
- Coulter N=105
- Ortho N=2
- Other N=1

21. **What are the quantities and manufacturers of your laboratory’s hematology analyzer that are used for TLI?**

![Hematology Analyzer Frequency Chart]

- Abbott N=36
- Baker/Biochem N=3
- Bayer/Technicon N=23
- Coulter N=83
- Roche/Sysmex N=21
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 240 (99.2%)
No 2 (0.8%)

N=242

23. On average, how many hours is a specimen stored at your laboratory before it is stained for TLI? (If the specimen is processed immediately upon receipt, please indicate 0 hours, otherwise, 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</td>
<td>47</td>
</tr>
<tr>
<td>1-6</td>
<td>121</td>
</tr>
<tr>
<td>7-12</td>
<td>40</td>
</tr>
<tr>
<td>13-18</td>
<td>11</td>
</tr>
<tr>
<td>19-24</td>
<td>13</td>
</tr>
</tbody>
</table>

N=232
24.(a) Is testing for viability of TLI specimens performed by your laboratory?

No 207 (85.2%)
Yes 36 (14.8%)

N=243

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

- Trypan blue exclusion: 13
- Propidium iodide: 9
- Other: 7
- 7AAD: 6
- Examination of blood smear: 3
- Ethidium bromide/Acridine orange: 1

N=35
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

<table>
<thead>
<tr>
<th>Minimum Percent Viability Acceptable</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-50</td>
<td>3</td>
</tr>
<tr>
<td>51-60</td>
<td>0</td>
</tr>
<tr>
<td>61-70</td>
<td>4</td>
</tr>
<tr>
<td>71-80</td>
<td>5</td>
</tr>
<tr>
<td>81-90</td>
<td>17</td>
</tr>
</tbody>
</table>

N=29
25.(a) Does your laboratory use a whole blood lysis method for staining TLI specimens?

Yes 237 (97.9%)
No 5 (2.1 %)

N=242

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

FACS Lyse 118
Immuno-Prep 91
Ammonium chloride 23
Immuno-Lyse 12
OptiLyse 7
Other 4

N=236
25.(c) What sample staining procedure(s) does your laboratory use for TLI? (Check all that apply.)

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Percentage of Laboratories Responding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stain whole blood/Lyse</td>
<td>233</td>
</tr>
<tr>
<td>Separate by density centrifugation</td>
<td>10</td>
</tr>
<tr>
<td>Lyse whole blood/Stain</td>
<td>8</td>
</tr>
<tr>
<td>Other</td>
<td>6</td>
</tr>
<tr>
<td>Separate buffy-coat/Lyse/Stain</td>
<td>3</td>
</tr>
<tr>
<td>Separate using BD LeucoPREP/Stain</td>
<td>2</td>
</tr>
</tbody>
</table>

N=241

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

- Yes 109 (45.4%)
- No 131 (54.6%)

N=240
25.(e) At what temperature does your laboratory routinely perform the staining procedure? (Choose only one.)

- Room temperature: 234
- 2-10 °C: 8

N=242

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

- Yes: 201 (83.4%)
- No: 40 (16.6%

N=241
25.(g) At what temperature does your laboratory routinely store cells after staining? (Choose only one.)

![Temperature Storage Distribution](chart1.png)

- 2-10 °C: 151 responses
- Room temperature: 91 responses

Total responses: 242

25.(h) On average, how many hours is a specimen stored at your laboratory after staining before being analyzed? (If the specimen is analyzed immediately after staining, please indicate 0 hours, otherwise, round off to the nearest whole number.)

![Hours Stored After Staining Distribution](chart2.png)

- 0 hours: 124 responses
- 1-6 hours: 82 responses
- 7-12 hours: 15 responses
- 13-18 hours: 10 responses
- 19-24 hours: 6 responses

Total responses: 237
26. From what source did your laboratory obtain its TLI staining procedure? (Check all that apply.)

- Monoclonal antibody manufacturer: 193
- Flow cytometer manufacturer: 168
- In-house: 69
- Publications: 42
- State health department: 12
- Other: 2

N=241
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 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>
<th>J</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>4</td>
<td>18</td>
<td>1</td>
<td></td>
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<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>CD4</td>
<td>4</td>
<td>17</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>CD8</td>
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<td></td>
</tr>
<tr>
<td>CD19</td>
<td>5</td>
<td>19</td>
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<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>CD16</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD56</td>
<td>3</td>
<td>15</td>
<td>2</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD56+16</td>
<td>2</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
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### Two-color Tests

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<th>B/B</th>
<th>C/A</th>
<th>C/C</th>
<th>D/D</th>
<th>I/B</th>
<th>I/I</th>
<th>C/B</th>
<th>A/D</th>
<th>A/B</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD45/CD14</td>
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</tr>
<tr>
<td>CD3/CD4</td>
<td>21</td>
<td>50</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>CD3/CD8</td>
<td>21</td>
<td>51</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>CD3/CD19</td>
<td>12</td>
<td>46</td>
<td>2</td>
<td>1</td>
<td></td>
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<td></td>
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<tr>
<td>CD3/CD16</td>
<td>4</td>
<td>14</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<td></td>
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<td></td>
</tr>
<tr>
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<td>1</td>
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<td></td>
</tr>
<tr>
<td>CD3/CD56+16</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Manufacturer Key:
A=Coulter/Immunotech  B=Becton Dickinson/PharMingen  C=Caltag  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

#### Combinations of Monoclonal Antibody Manufacturers Used

<table>
<thead>
<tr>
<th>Cell Marker</th>
<th>A/A/A</th>
<th>B/A/A</th>
<th>B/B/B</th>
<th>C/A/A</th>
<th>C/B/B</th>
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<tbody>
<tr>
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<tr>
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<td>C45/CD3/CD19</td>
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<td>36</td>
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<td>1</td>
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<td>C45/CD3/CD56+16</td>
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<td></td>
<td>31</td>
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</tr>
<tr>
<td>CD3/CD19/CD56</td>
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<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD3/CD19/CD56+16</td>
<td></td>
<td></td>
<td>5</td>
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</table>

### Four-color Tests

#### Combinations of Monoclonal Antibody Manufacturers Used

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<tr>
<th>Cell Marker</th>
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<th>A/A/B/A</th>
<th>A/B/A/B</th>
<th>A/C/A/C</th>
<th>B/A/A/A</th>
<th>B/B/A/B</th>
<th>B/B/A/B</th>
<th>B/B/B/B</th>
<th>C/C/C/C</th>
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<tbody>
<tr>
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<td>59</td>
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<td>CD45/CD3/CD19/CD16</td>
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<td></td>
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</tr>
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</tr>
<tr>
<td>CD45/CD3/CD19/CD56+16</td>
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<td>49</td>
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<tr>
<td>CD3/CD4/CD8/CD56+16</td>
<td>1</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

Manufacturer Key:
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- C=Caltag
- 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

![Histogram showing the distribution of one-color monoclonal antibody reagent panels used by participant laboratories. The panel shows that 14 laboratories used the panel 3, 4, 8, 19, 16, 56, 56+16, 2 laboratories used the panel 4, 8, 19, 16, 56, 1 laboratory used the panel 19, 56+16, 19, 56, 3, 19, 3, 4, 56, 3, 4, 8, 3, 4, 8, 19, 3, 4, 8, 19, 16, 56+16, 3, 4, 8, 19, 56+16, 3, 4, 8, 19, 56, 3, 56, and 4, 8. The total number of laboratories responding is N=29.]
Two-color monoclonal antibody reagent panels used by participant laboratories

<table>
<thead>
<tr>
<th>Panel Description</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>45/14, 3/4, 3/8, 3/19, 3/56+16</td>
<td>24</td>
</tr>
<tr>
<td>45/14, 3/4, 3/8, 3/19, 3/56</td>
<td>11</td>
</tr>
<tr>
<td>45/14, 3/4, 3/19</td>
<td>10</td>
</tr>
<tr>
<td>45/14, 3/4, 3/8, 3/19, 3/56+16</td>
<td>8</td>
</tr>
<tr>
<td>45/14, 3/4, 3/8, 3/19, 3/56</td>
<td>6</td>
</tr>
<tr>
<td>45/14, 3/4, 3/8</td>
<td>5</td>
</tr>
<tr>
<td>45/14, 3/4, 3/8, 3/56+16</td>
<td>5</td>
</tr>
<tr>
<td>45/14, 3/4, 3/8, 3/19, 3/16, 3/56</td>
<td>4</td>
</tr>
<tr>
<td>3/4, 3/8</td>
<td>4</td>
</tr>
<tr>
<td>45/14, 3/4, 3/8, 3/19, 3/16</td>
<td>3</td>
</tr>
<tr>
<td>45/14, 3/56+16</td>
<td>2</td>
</tr>
<tr>
<td>45/14, 3/4, 3/8, 3/19, 3/16, 3/56+16</td>
<td>1</td>
</tr>
<tr>
<td>45/14, 3/4, 3/8, 3/16</td>
<td>1</td>
</tr>
<tr>
<td>45/14, 3/4, 3/8, 3/56</td>
<td>1</td>
</tr>
<tr>
<td>45/14, 3/8</td>
<td>1</td>
</tr>
<tr>
<td>45/14, 3/19</td>
<td>1</td>
</tr>
<tr>
<td>45/14, 3/19, 3/56+16</td>
<td>1</td>
</tr>
<tr>
<td>3/4, 3/8, 3/19, 3/56+16</td>
<td>1</td>
</tr>
<tr>
<td>3/4, 3/8, 3/56+16</td>
<td>1</td>
</tr>
<tr>
<td>3/19, 3/16</td>
<td>1</td>
</tr>
</tbody>
</table>

N=91
continued:

Three-color monoclonal antibody reagent panels used by participant laboratories

- 45/3/4, 45/3/8, 45/3/19, 45/3/56+16: 25
- 45/3/4, 45/3/8: 15
- 45/3/4: 7
- 45/3/4, 45/3/8, 45/3/19: 6
- 45/3/4, 45/3/8, 45/3/19, 45/3/56+16: 5
- 3/4/8, 3/19/56, 3/19/56+16: 5
- 3/4/8, 3/19/56: 5
- 45/3/19: 3
- 45/3/4, 45/3/8, 3/4/8: 2
- 45/3/4, 45/3/8, 45/3/56+16, 3/4/8: 1
- 45/3/4, 45/3/8, 45/3/56+16: 1
- 45/3/4, 45/3/8, 3/4/8, 3/19/56: 1
- 45/3/4, 45/3/19: 1
- 3/4/8, 3/19/16: 1

N=78
27. continued:

Four-color monoclonal antibody reagent panels used by participant laboratories

- 45/3/4/8, 45/3/19/56+16: 51
- 45/3/4/8, 45/3/19/56: 37
- 45/3/4/8: 20
- 45/3/4/8, 45/3/19/16: 7
- 45/3/4/8, 45/3/19/16, 45/3/19/56, 45/3/19/56+16, 45/3/14/19: 4
- 45/3/4/8, 45/3/19/16, 45/3/19/56: 2
- 45/3/4/8, 45/3/19/56, 45/3/19/56+16: 1
- 45/3/14/19: 1

N=123

Percentage of Laboratories Responding
28.(a) How is the gating set when performing TLI on your flow cytometers? (Choose only one.)

- Software controlled: 142
- Manually set: 94
- Other: 3

N=239

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

- Lymphocytes only: 226
- Lymphocytes and monocytes: 7
- Lymphocytes, monocytes, granulocytes: 6

N=239
29. How are the integration windows set? (Choose only one.)

- Isotype controls: 87
- Predetermined channel numbers: 44
- Unstained cells: 42
- Software controlled: 26
- Other: 19

N=218
30.(a) Do you mathematically adjust your phenotype values for isotype control values?

- No: 203 (86.0%)
- Yes: 33 (14.0%)

N=236

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

- Subtracting isotype controls: 18
- Curve-matching software: 12
- Other: 1

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

- Each antibody daily: 174
- Each analysis: 22
- Each antibody weekly: 13
- Other: 10
- Each new lot of antibody: 7
- Each antibody monthly: 4
- Not used: 2

N=232

31.(b) Which normal cell control is primarily used in your laboratory? (Choose only one.)

- CD-Chex Plus: 87
- Whole blood drawn from healthy donor: 79
- Cytotrol/Immunotrol: 63
- Status Flow: 8
- Other: 2
- FluoTrol-CD4: 1

N=238
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.)

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Number of Respondents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biannually</td>
<td>124</td>
</tr>
<tr>
<td>Annually</td>
<td>42</td>
</tr>
<tr>
<td>Only as needed</td>
<td>28</td>
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<tr>
<td>Weekly</td>
<td>15</td>
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<tr>
<td>Monthly</td>
<td>14</td>
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<tr>
<td>Quarterly</td>
<td>12</td>
</tr>
<tr>
<td>Other</td>
<td>4</td>
</tr>
<tr>
<td>Never</td>
<td>1</td>
</tr>
</tbody>
</table>

N=240
33.(a) How often does your laboratory check the optical alignment of your flow cytometer(s)? (Choose only one.)

- Daily: 196
- Every analysis: 14
- Only as needed: 12
- Never: 6
- More than monthly: 5
- Weekly: 4
- Monthly: 3
- Do not know: 1

N=241

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

- Beads with fluorescence intensity similar to cells: 156
- Very bright beads: 116
- Chicken erythrocytes: 15
- Thymocyte nuclei: 7
- Other: 7

N=228
34.(a) Does your laboratory set fluorescence overlap compensation?

Yes
224 (94.1%)

No
14 (5.9 %)

N=238

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

- Fluorescein isothiocyanate/Phycoerythrin labeled beads: 130
- Fluorescein isothiocyanate/Phycoerythrin stained cells: 128
- Standard instrument settings: 20
- Other: 5

N=217
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: 208
- Whole blood: 67
- Lyophilized cells: 14
- Cryogenically preserved cells: 5
- Cell lines: 4
- Other: 3
- Thymocyte nuclei: 1

N=228

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

- Yes: 214 (91.5%)
- No: 20 (8.5%)

N=234
35.(c) Are these data analyzed to monitor trends or changes in instrument performance?

Yes
203 (96.7%)

No
7 (3.3%)

N=210
36.(a) How often does your laboratory standardize your flow cytometer(s)? (Choose only one.)

- Daily: 184
- Never: 17
- Every analysis: 16
- Monthly: 5
- More than monthly: 5
- Weekly: 5

N=232

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: 153
- Very bright beads: 83
- Other: 10
- Thymocyte nuclei: 3

N=212
36.(c) Does your laboratory use reference standards to plot standard curves of mean channel fluorescence vs. molecules of equivalent soluble fluorochrome?

No
160 (75.5%)

Yes
52 (24.5%)

N=212

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

Yes
40 (80.0%)

No
10 (20.0%)

N=50
37. Does your laboratory obtain marker-specific absolute counts (e.g., absolute CD4 count) using a single-platform method?

- Yes: 56 (23.3%)
- No: 184 (76.7%)

N=240

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

- TruCount: 31
- Flow-Count: 11
- FACSCount: 9
- GEN-S: 2
- Other (flow): 2
- Other (not flow): 1

N=54
39. Before TLI results are reported, are they routinely reviewed by someone other than the person(s) who performed the tests?

Yes
184 (76.7%)

No
56 (23.3%)

N=240
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: 224
- CD4/CD8 ratios: 215
- Cell marker counts: 202
- Absolute lymphocyte counts: 186
- Range of normal results: 162
- Percent lymphocytes: 159
- Total WBC count: 148
- Interpretation of results: 112
- Leukocyte differential: 60
- Sample condition: 24
- Normal control results: 23
- Suggested follow-up testing: 15
- Fluorescence histograms/plots: 12
- Light scatter histograms/plots: 8
- Cell viability: 8
- Other: 5

N=240
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? (If results are returned on the same day the specimen is received, please indicate 0 days, otherwise, 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 (92.9%)

No

17 (7.1 %)

N=239
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

<table>
<thead>
<tr>
<th>TLI Results Inquiries Per Month</th>
<th>Frequency</th>
</tr>
</thead>
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<tr>
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</tr>
<tr>
<td>&gt;20</td>
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</tbody>
</table>

N=199
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>
</tr>
</thead>
<tbody>
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</tr>
<tr>
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<td>401-450</td>
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</tr>
<tr>
<td>&gt;450</td>
<td>3</td>
</tr>
</tbody>
</table>

**Single-Platform**

<table>
<thead>
<tr>
<th>Price Charged (dollars)</th>
<th>Frequency of Laboratories Responding</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-50</td>
<td>8</td>
</tr>
<tr>
<td>51-100</td>
<td>10</td>
</tr>
<tr>
<td>101-150</td>
<td>3</td>
</tr>
<tr>
<td>151-200</td>
<td>3</td>
</tr>
<tr>
<td>201-250</td>
<td>1</td>
</tr>
<tr>
<td>251-300</td>
<td>0</td>
</tr>
<tr>
<td>301-350</td>
<td>0</td>
</tr>
<tr>
<td>351-400</td>
<td>1</td>
</tr>
<tr>
<td>&gt;450</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: 91
- Do not know: 76
- Increased: 47
- Decreased: 9

N=223

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: 94
- Do not know: 78
- Increase: 49
- Decrease: 2

N=223
45.(a) Does your laboratory participate in an external TLI proficiency testing program?

- Yes: 233 (95.5%)
- No: 11 (4.5%)

N=244

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: 215
- Other: 38
- NIAID DAIDS: 31
- FAST Systems, Inc.: 15
- Instrument mnfr program: 12
- U.S. Army: 3

N=229
46.(a) In the last year, has your laboratory performed surrogate-marker tests for TLI in HIV-infected patients?

No
228 (94.6%)

Yes
13 (5.4 %)

N=241

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

- Interleukin-2 receptor: 10
- Beta-2-microglobulin: 6
- Neopterin: 4
- Other: 2

N=13
47.(a) In the last year, has your laboratory performed other tests for HIV-1 infection?

No 125 (51.9%)
Yes 116 (48.1%)
N=241

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

- EIA: 92
- HIV-1 RNA: 61
- Western blot: 59
- HIV-1 p24 antigen: 33
- HIV-1 DNA: 17
- Antiretroviral drug resistance testing: 13
- Other: 13
- Viral culture: 8
- IIF: 6
- Particle agglutination: 1
N=116