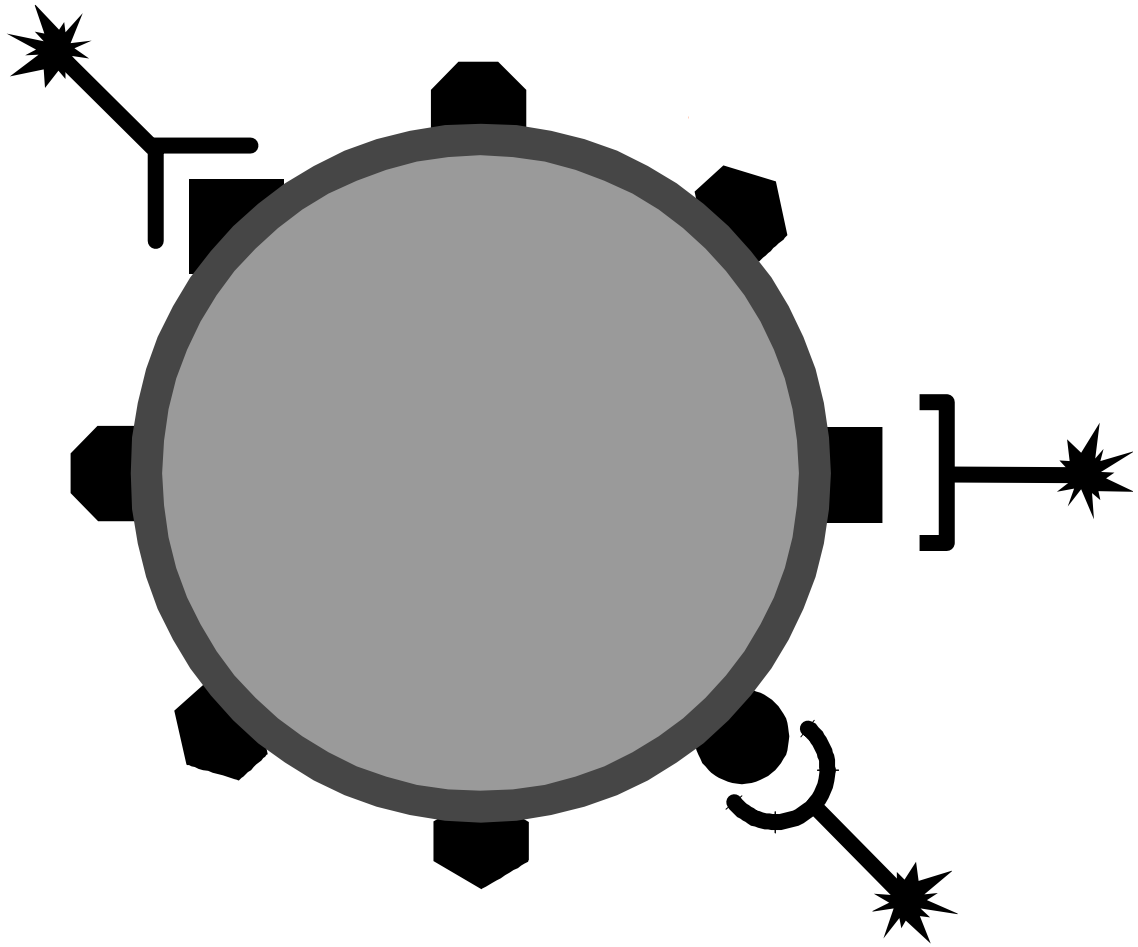
Results of the 1999 T-Lymphocyte Immunophenotyping Questionnaire Survey Mailed to Laboratories Participating in the Model Performance Evaluation Program



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

CENTERS FOR DISEASE CONTROL AND PREVENTION

Report of results for a 1999 T-lymphocyte Immunophenotyping (TLI) laboratory questionnaire survey mailed to laboratories participating in the Model Performance Evaluation Program, Centers for Disease Control and Prevention (CDC).

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Introductory Comments

The aggregate results from a mailed questionnaire survey conducted by the Model Performance Evaluation Program (MPEP) in May 1999 of laboratories in the United States performing T-lymphocyte immunophenotyping (TLI) are presented in the following figures and tables. Of the 321 laboratories receiving this survey, 273 (85.0%) 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 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.

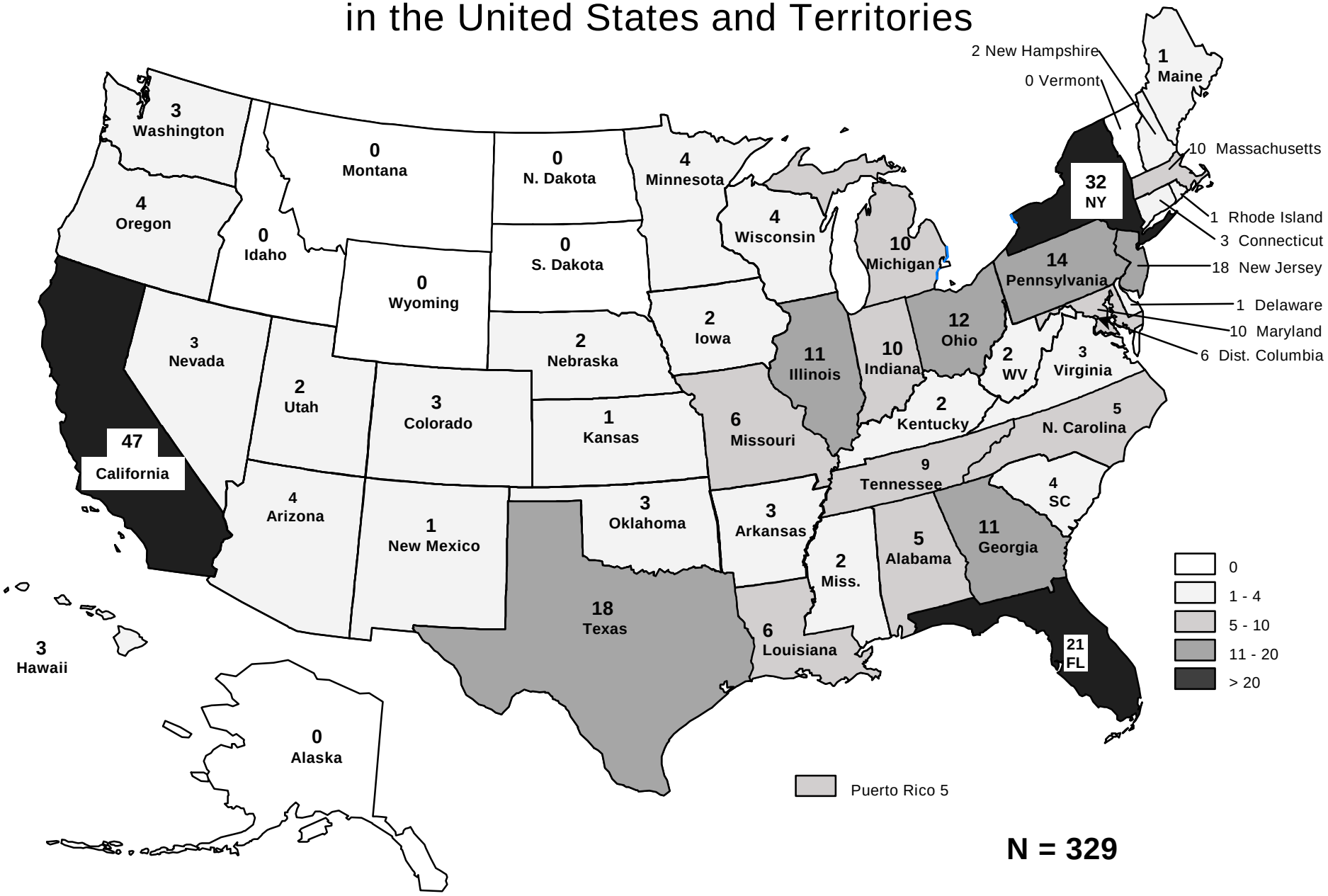
Question 27 requested information regarding the monoclonal antibody manufacturer associated with reagents for each of the cell marker combinations routinely used for performing TLI. The first two pages of results for this question, pages 37-38, show the reagents used for single-color, two-color, three-color, 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.

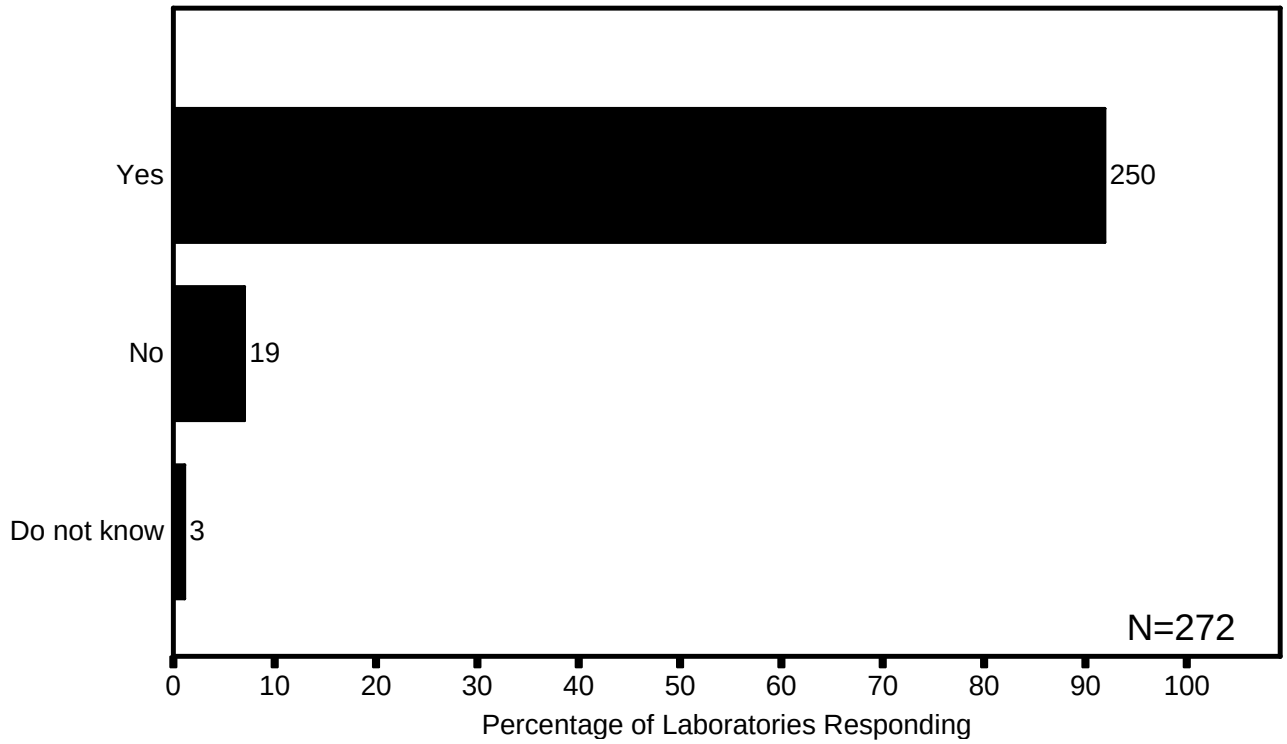
Number of MPEP TLI Laboratories in the United States and Territories



N = 329

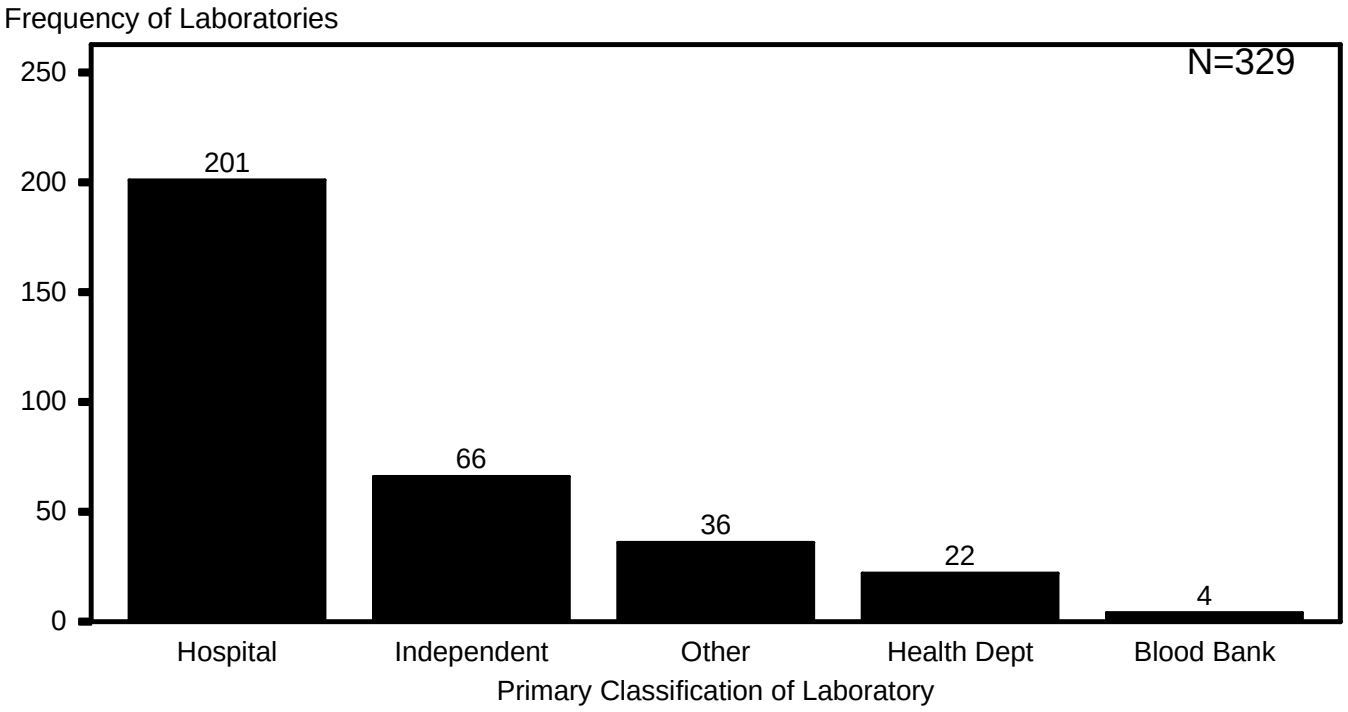
CDC Model Performance Evaluation Program
May 1999 TLI Survey

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

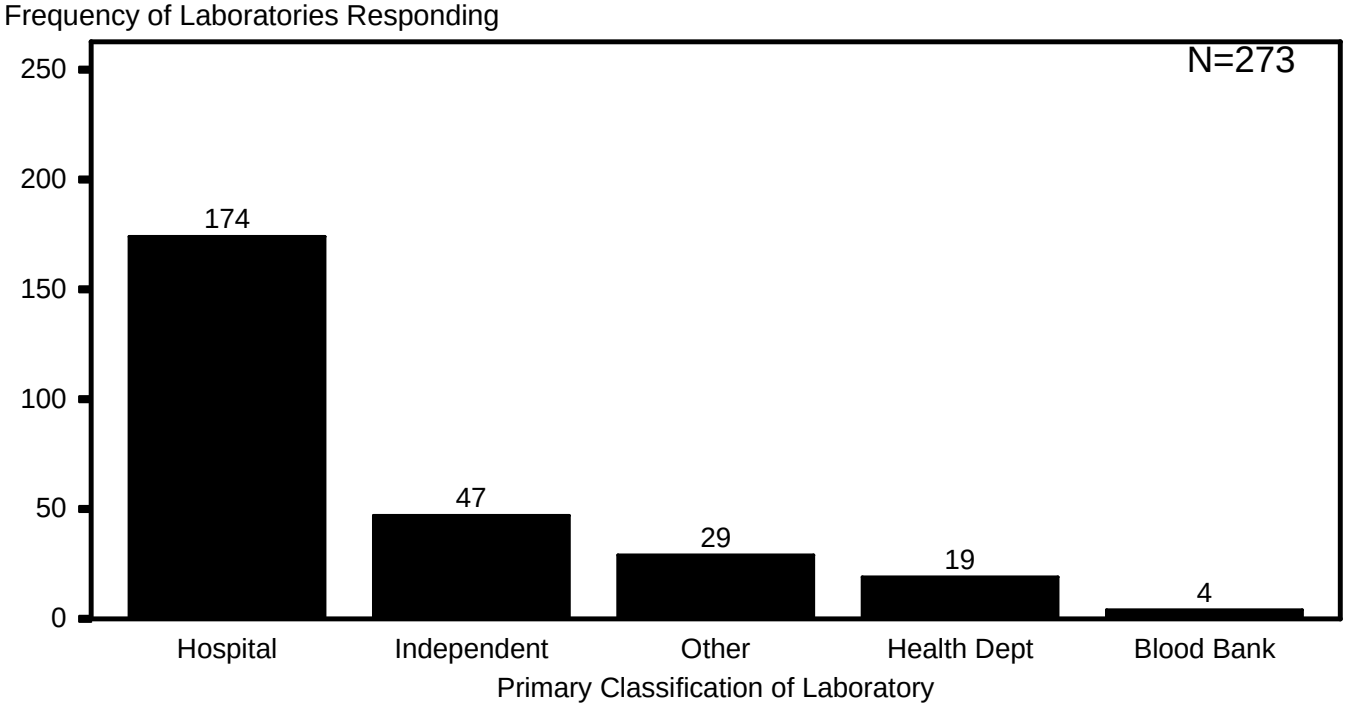


Primary Classification of MPEP Laboratories in Regard to TLI Testing

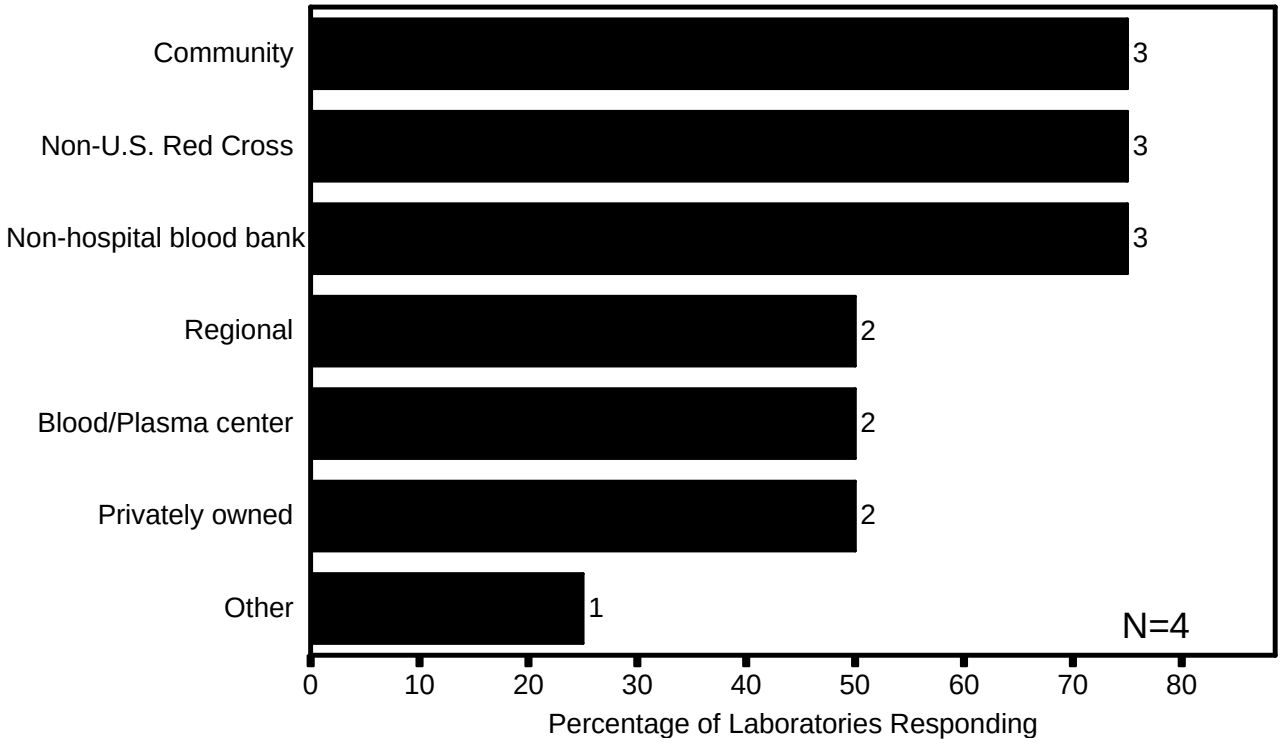
Total Number of Laboratories in TLI Program



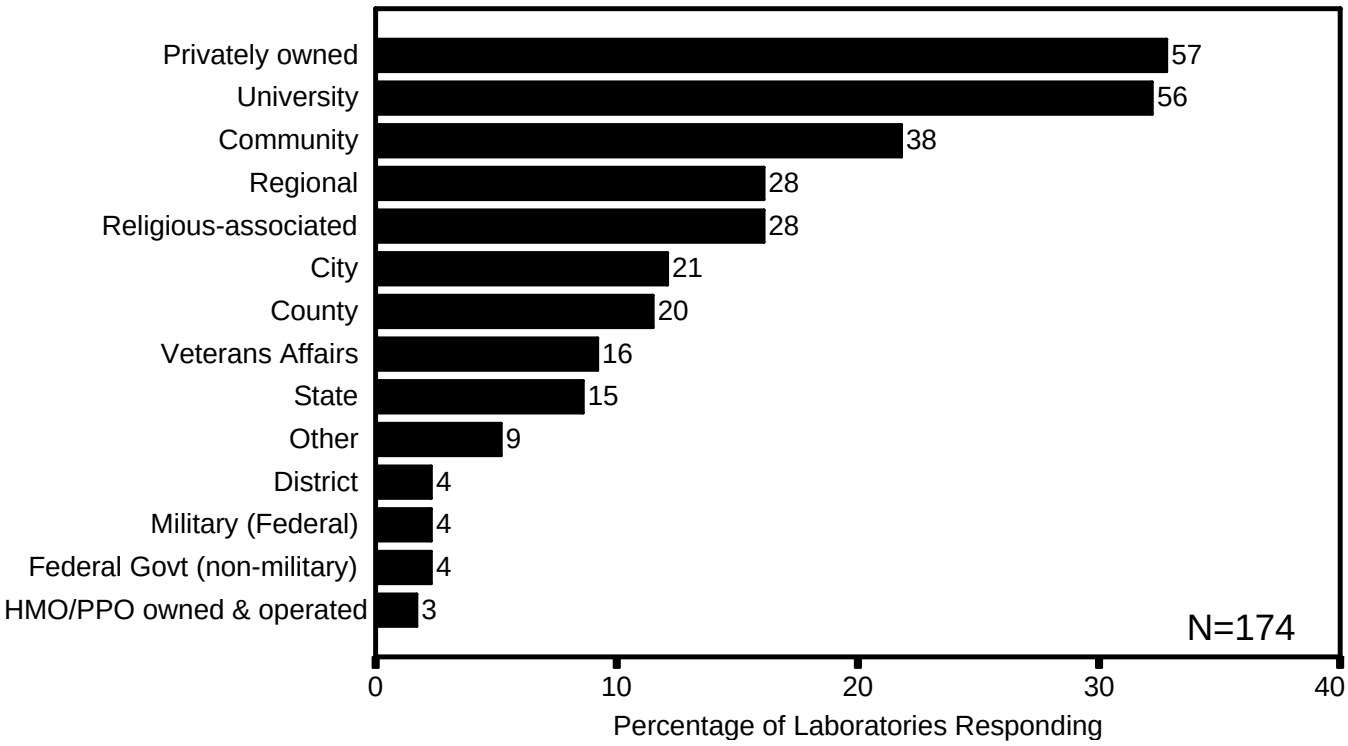
Laboratories Responding to Questionnaire Survey



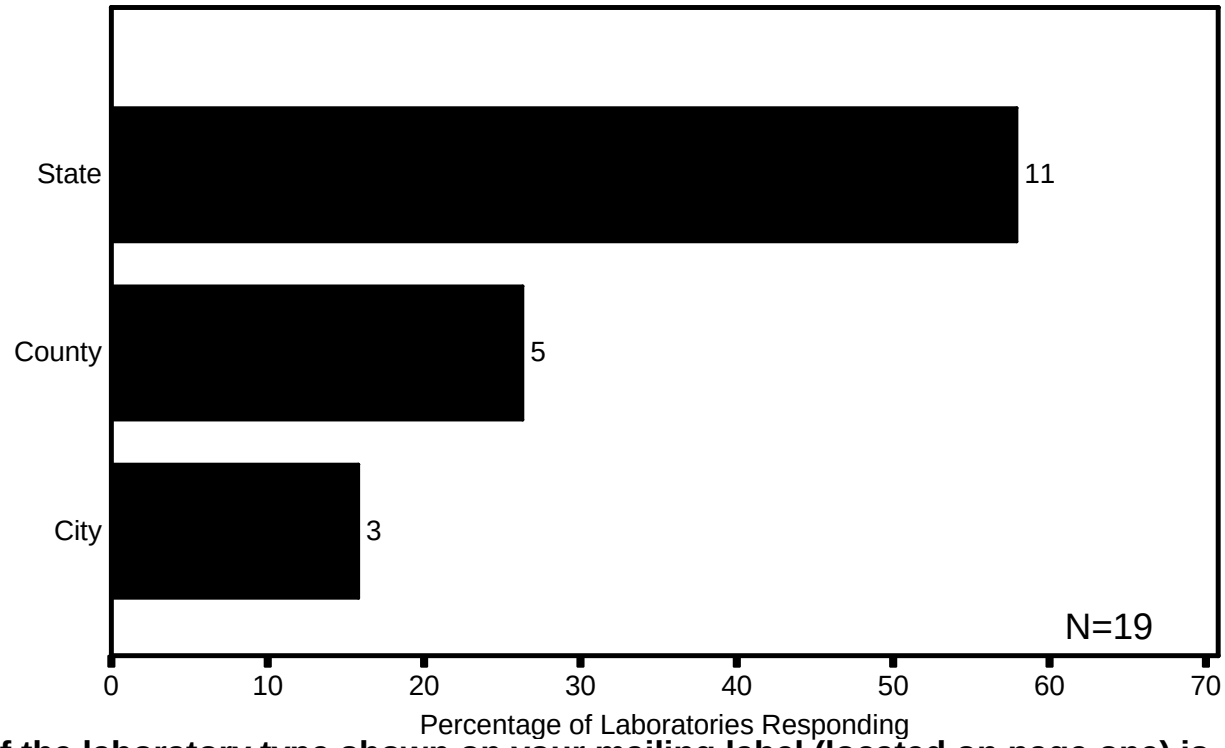
5.(a) If the laboratory type shown on your mailing label (located on page one) is BLOOD BANK, please <u>further describe</u> your TLI testing laboratory (Check <u>all</u> that apply within your Blood Bank laboratory classification.):



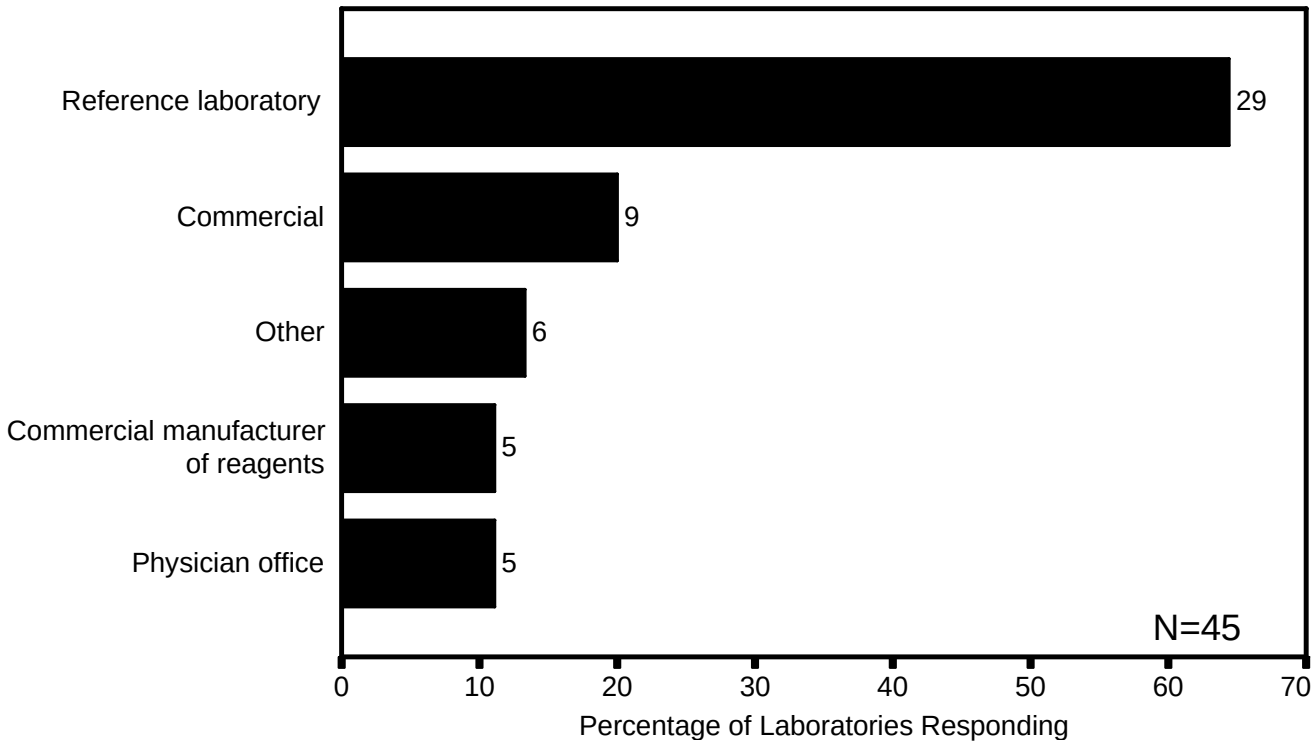
5.(b) If the laboratory type shown on your mailing label (located on page one) is HOSPITAL, please <u>further describe</u> your TLI testing laboratory (Check <u>all</u> that apply within your Hospital laboratory classification.):



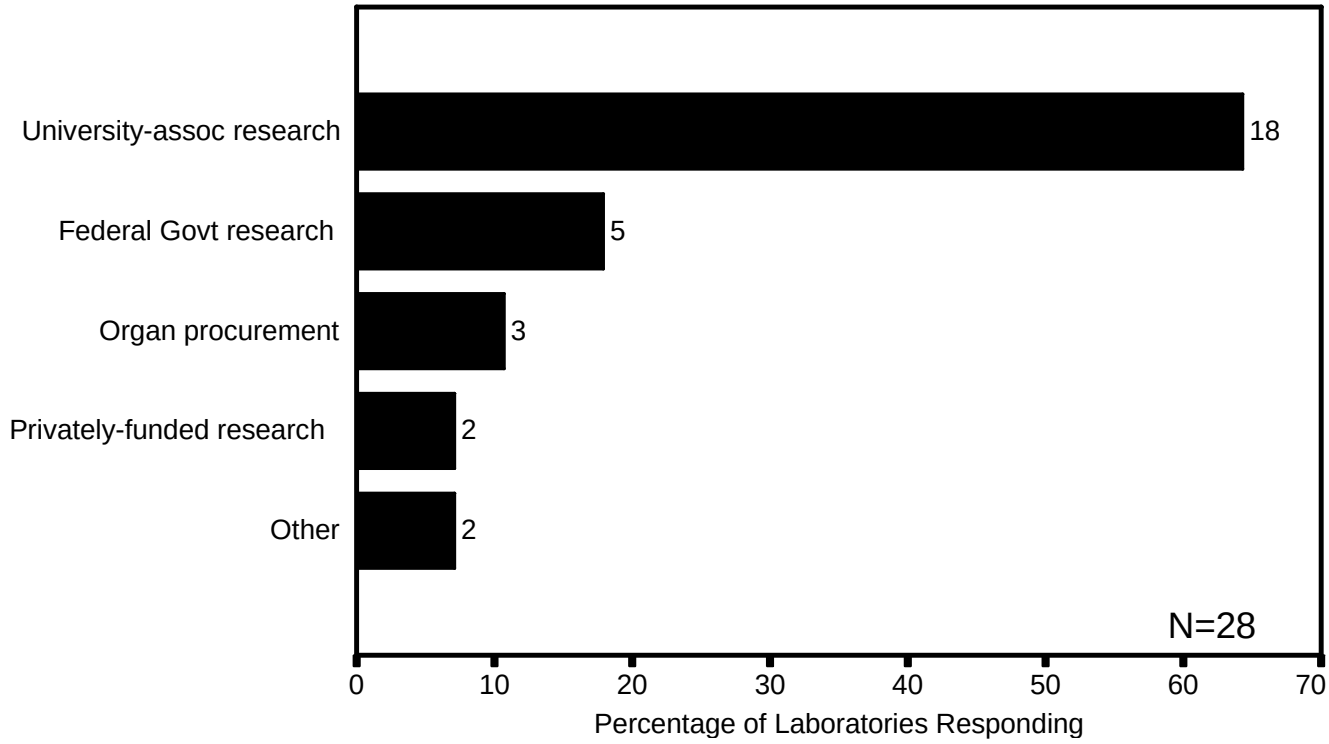
5.(c) If the laboratory type shown on your mailing label (located on page one) is HEALTH DEPARTMENT, please <u>further describe</u> your TLI testing laboratory (Check <u>all</u> that apply within your Health Department laboratory classification.):



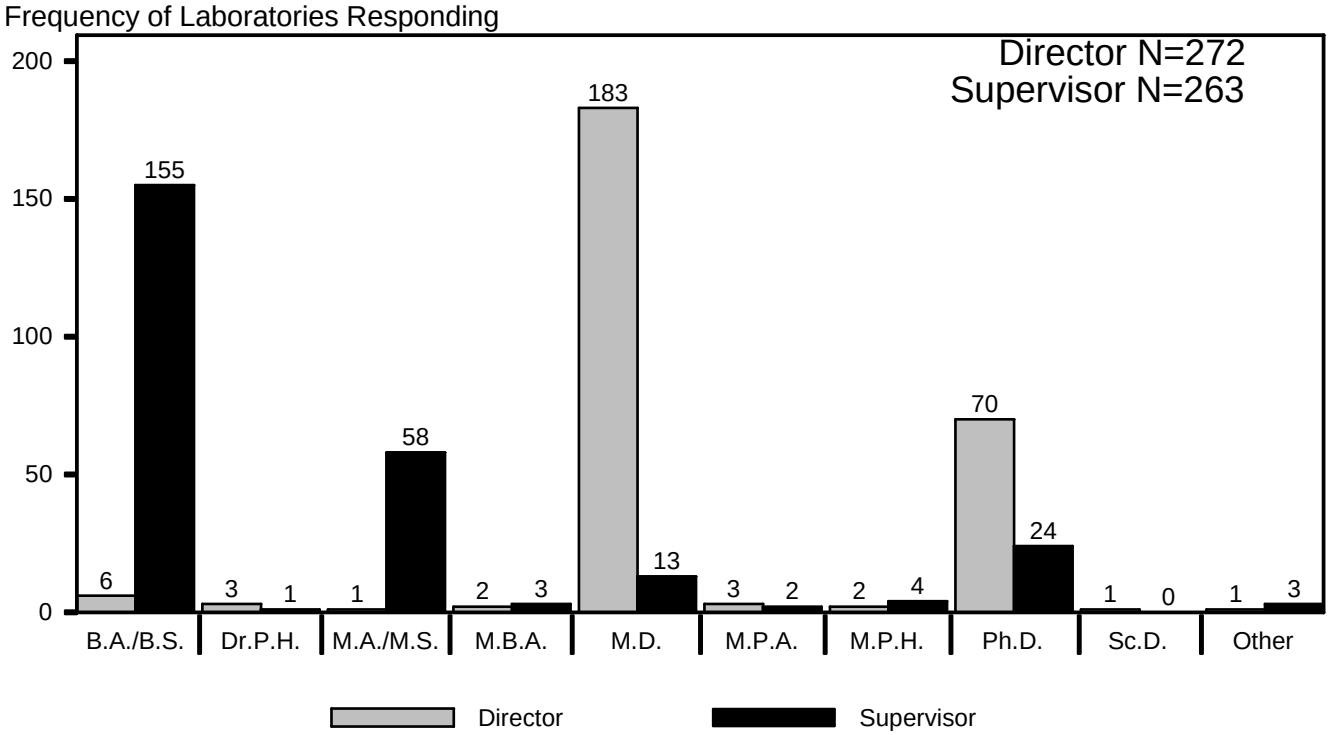
5.(d) If the laboratory type shown on your mailing label (located on page one) is INDEPENDENT, please <u>further describe</u> your TLI testing laboratory (Check <u>all</u> that apply within your Independent laboratory classification.):



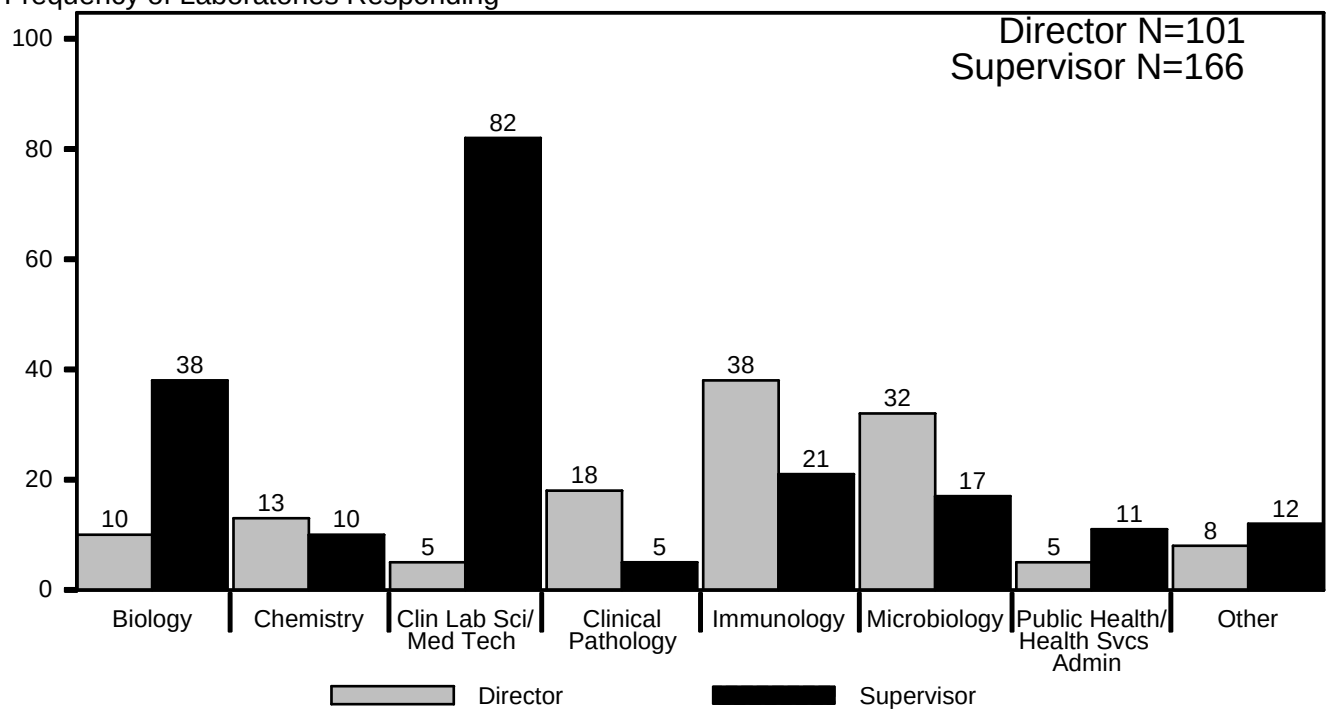
5.(e) If the laboratory type shown on your mailing label (located on page one) is OTHER, please <u>further describe</u> your TLI testing laboratory (Check <u>all</u> that apply within your Other laboratory classification.):



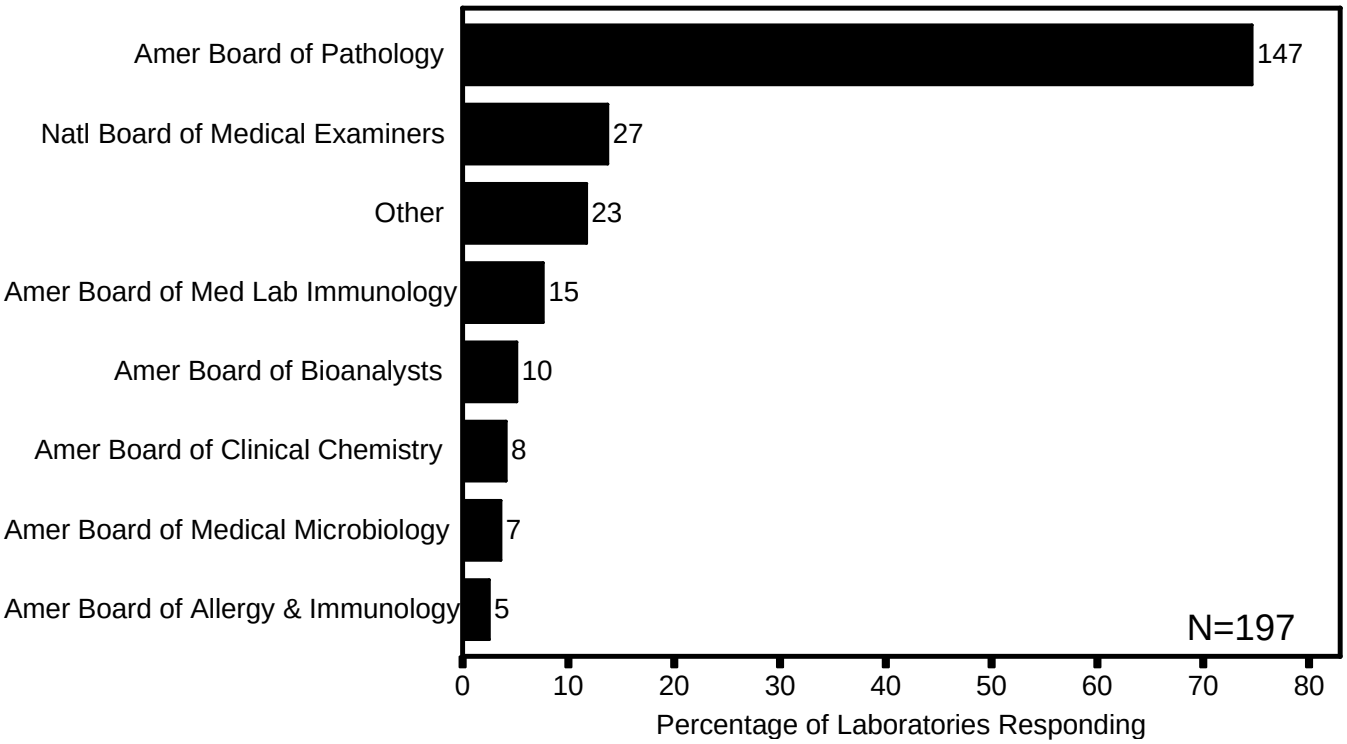
6.(a) Please choose from the list below the *highest academic degree* that has been awarded to your *Laboratory Director* and *Laboratory Supervisor* (Choose only one degree for each person.):



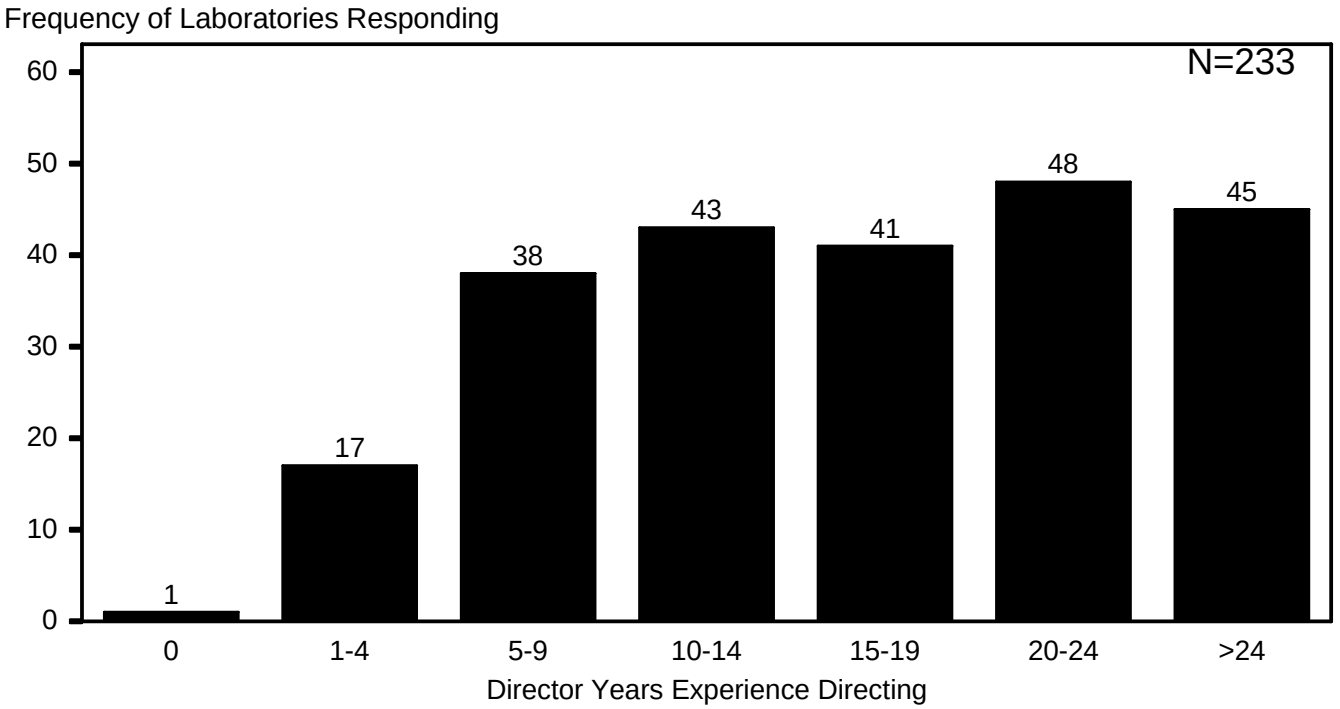
6.(b) If your Laboratory Director or Laboratory Supervisor has a degree other than M.D. or D.O., please indicate the academic discipline in which the degree was awarded (Check all that apply.):



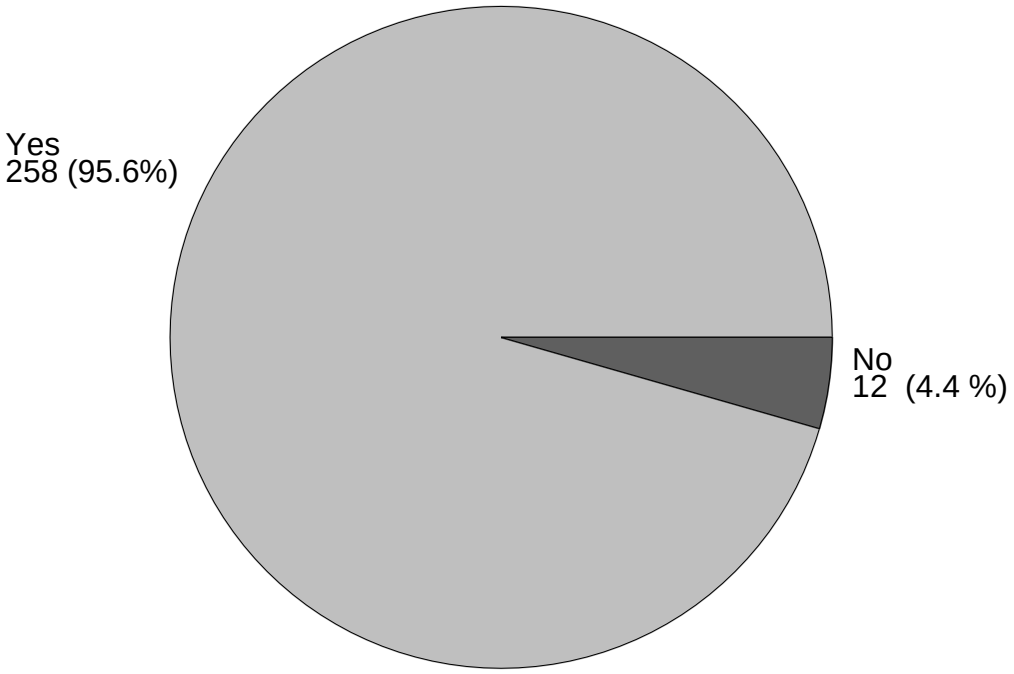
6.(c) What board certifications have been awarded to your Laboratory Director? (Check all that apply.)



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.):

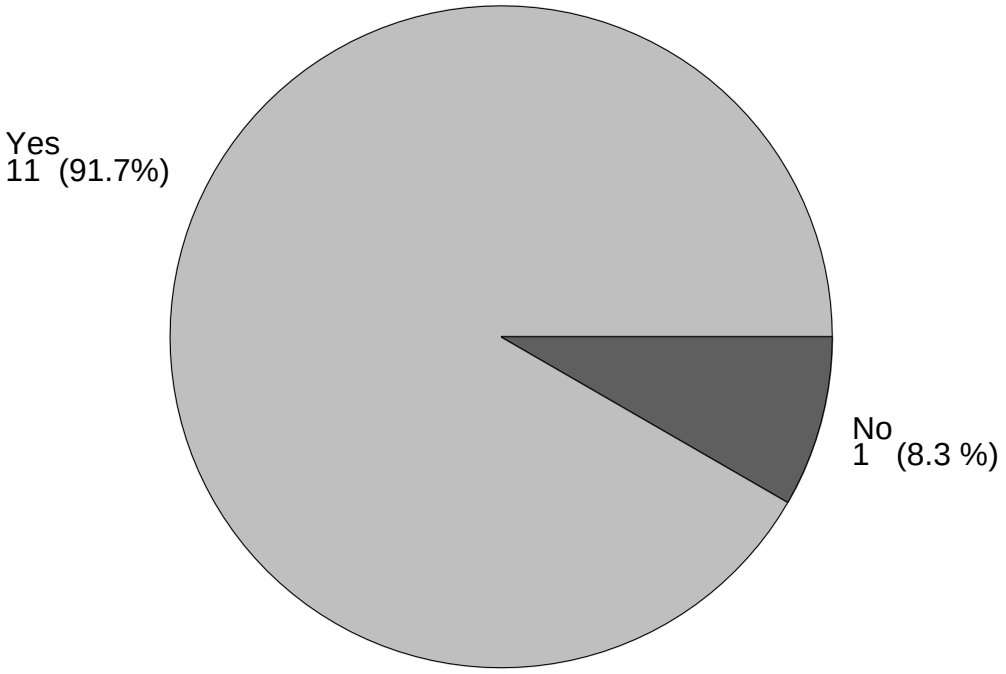


6.(e) Is your <u>Laboratory Supervisor</u> available to provide supervision on-site?



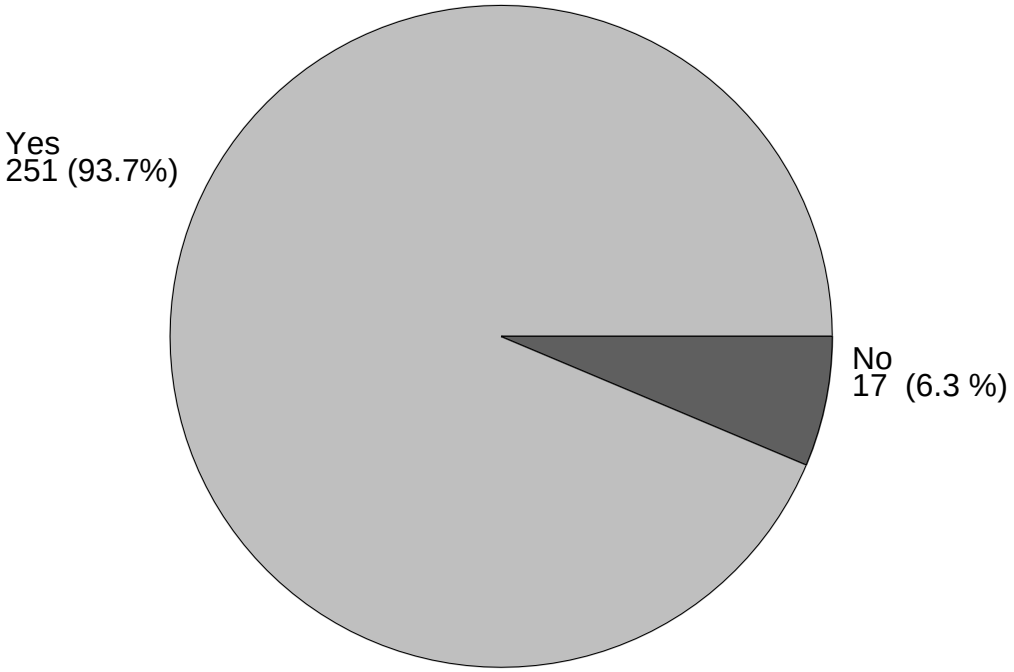
N=270

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



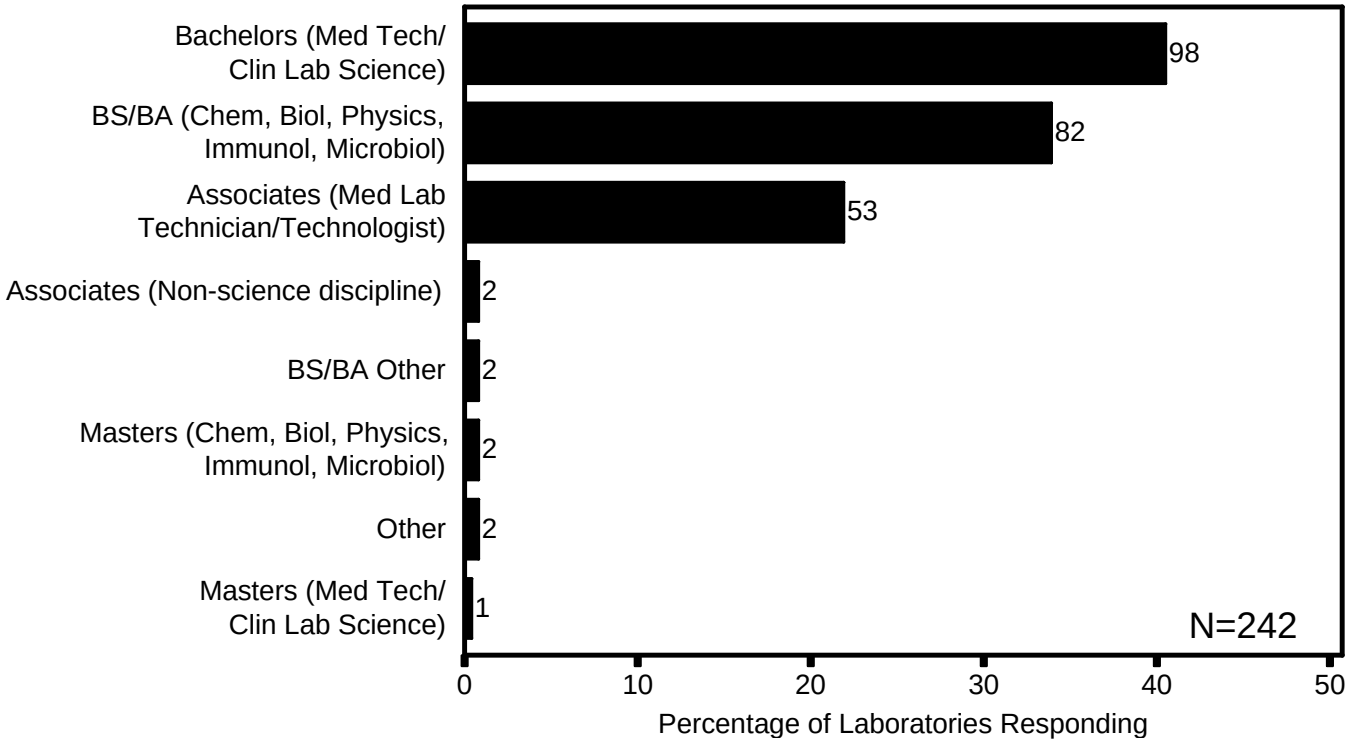
N=12

7.(a) Does your laboratory require that personnel who perform TLI (operate a flow cytometer and analyze resultant data) have a <u>minimum educational degree</u>?

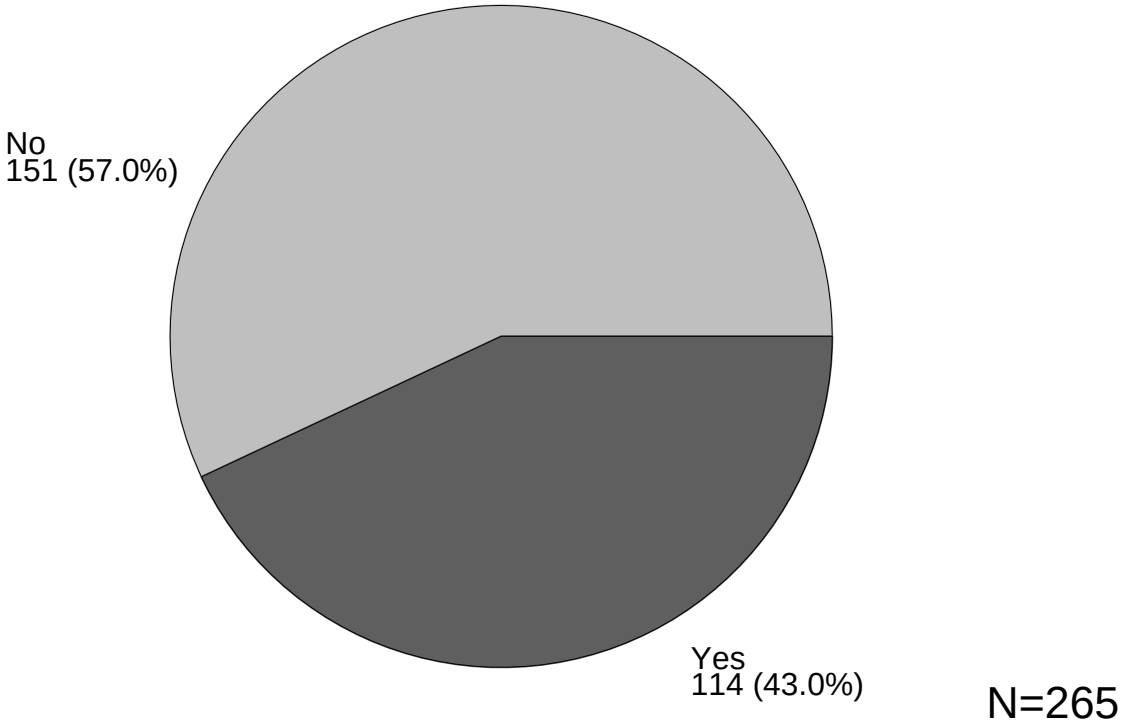


N=268

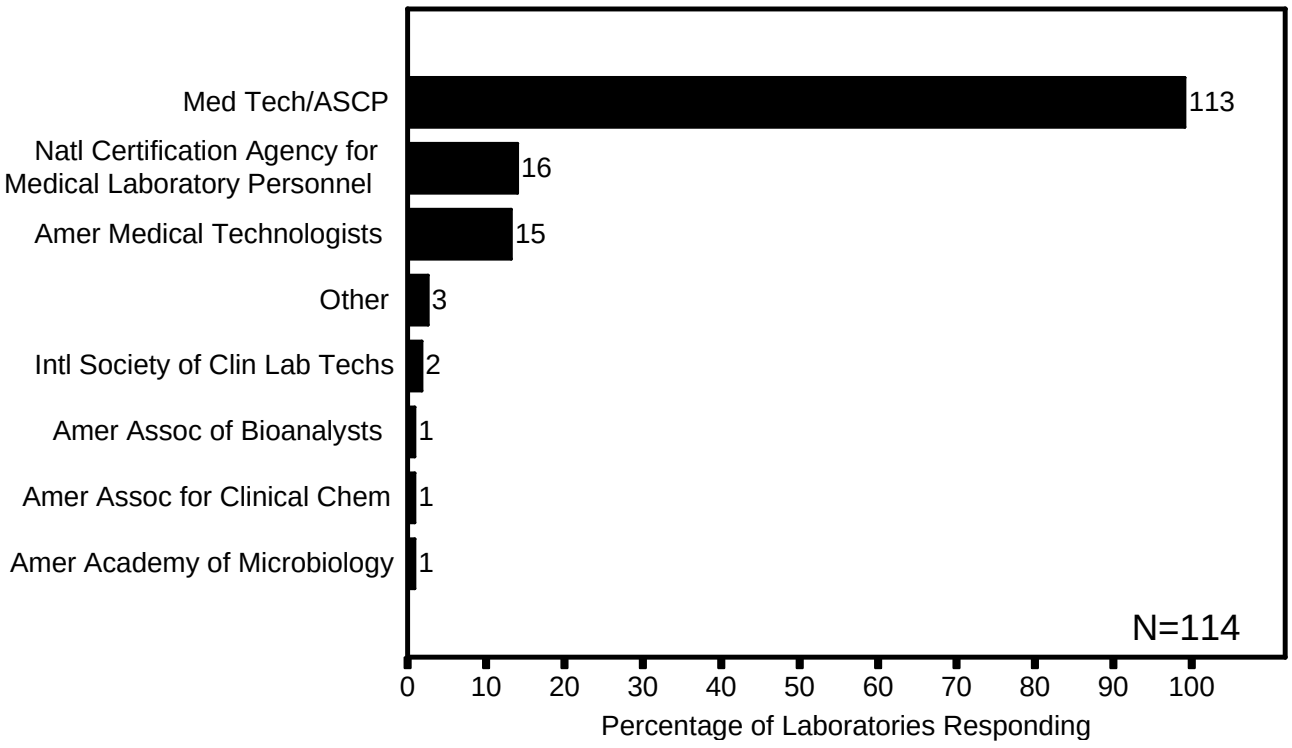
7.(b) What minimum educational degree is required of your TLI testing personnel? (Choose only <u>one</u> degree.)



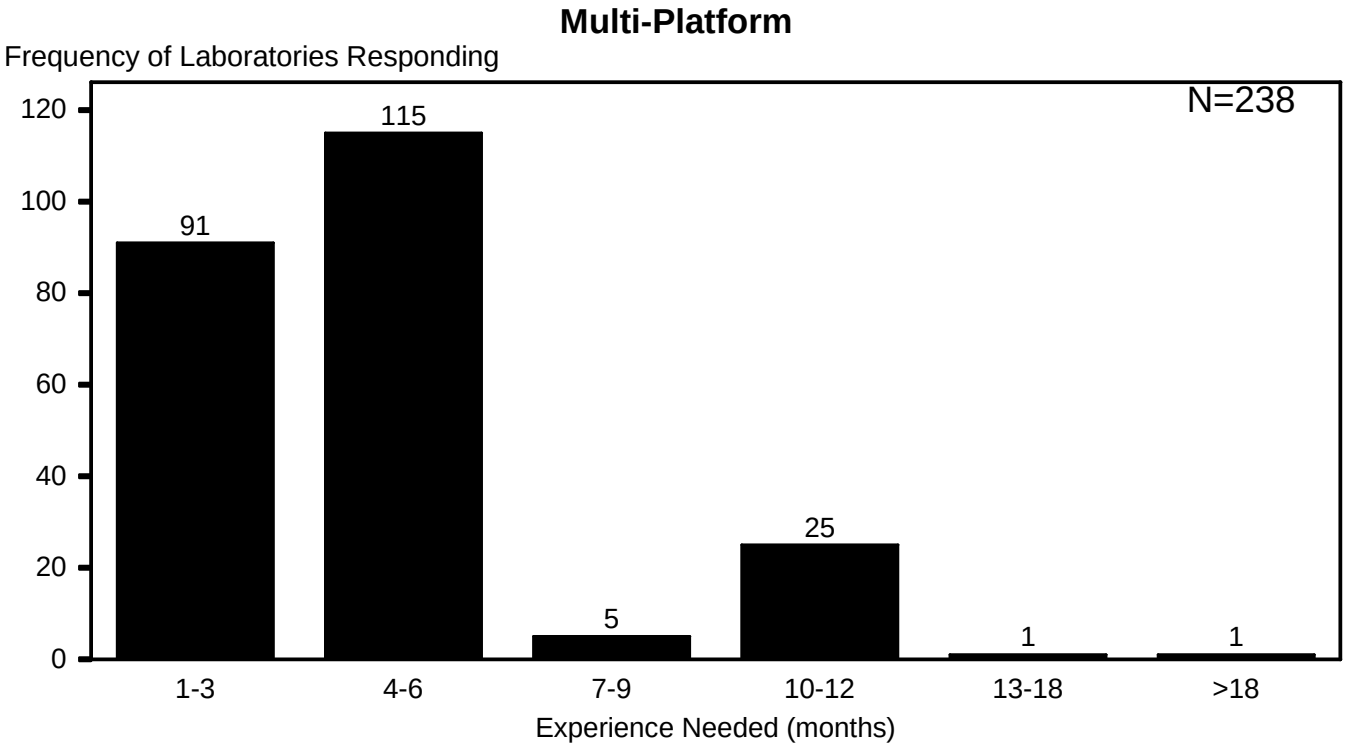
7.(c) Does your laboratory require that your TLI testing personnel have *certification by a professional organization*? (Do not include licensing by city, state, or county.)

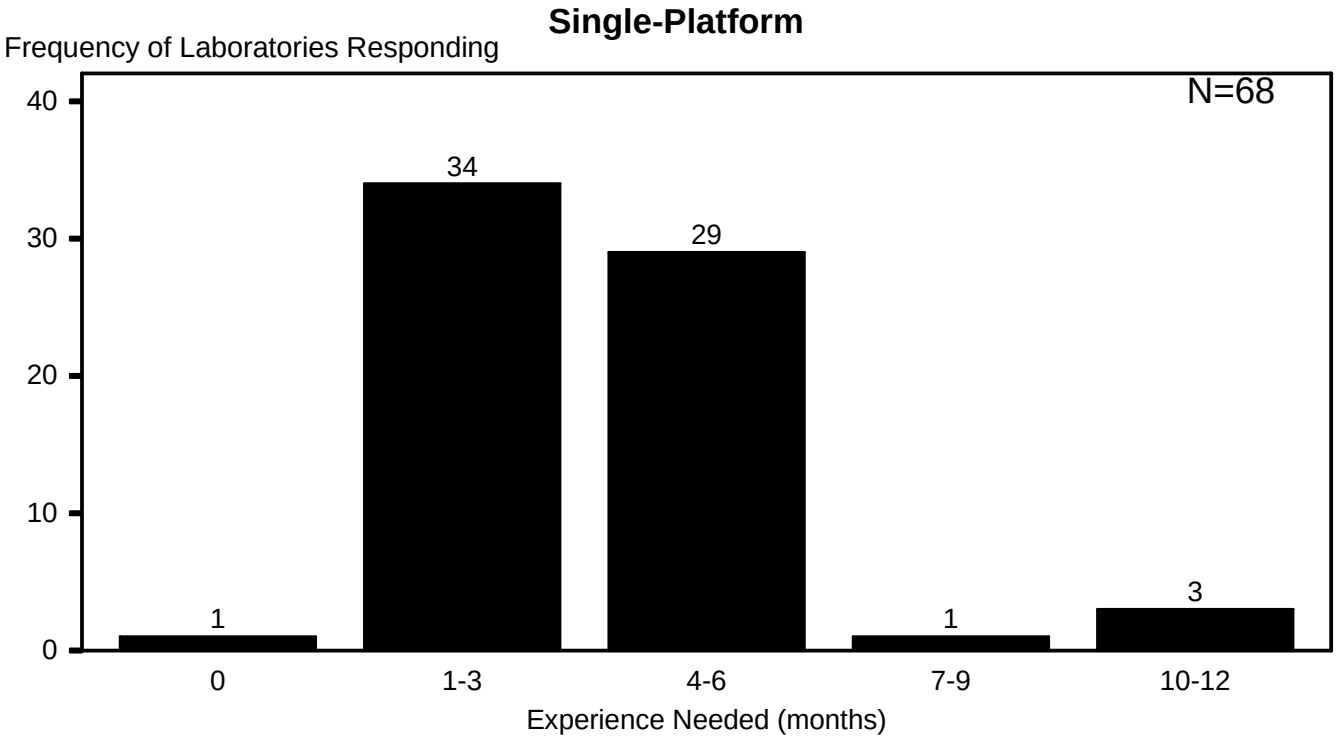


7.(d) Please check the *professional organizations* that have awarded the required *certification* to your *TLI testing personnel* (Check all that apply.):

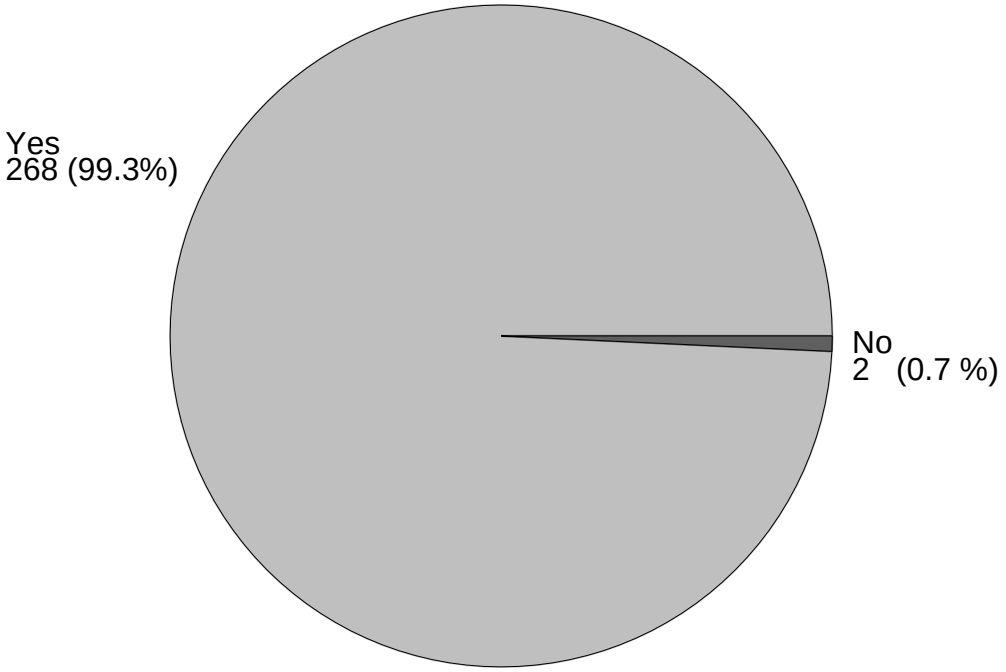


8. On average, how many months of experience do your personnel need to become proficient in performing TLI and analyzing the resultant data? (Indicate number of months of experience needed only for those methods currently in use in your laboratory. Round off to the nearest whole number.)

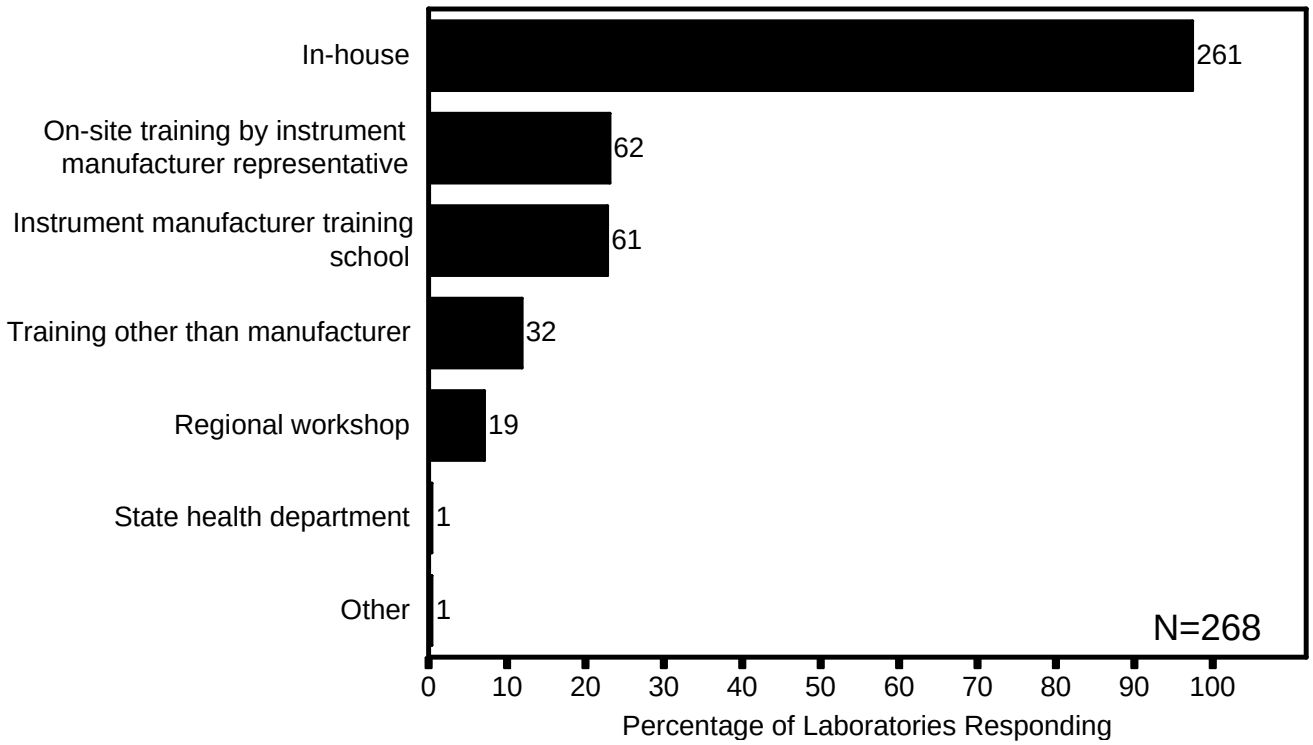




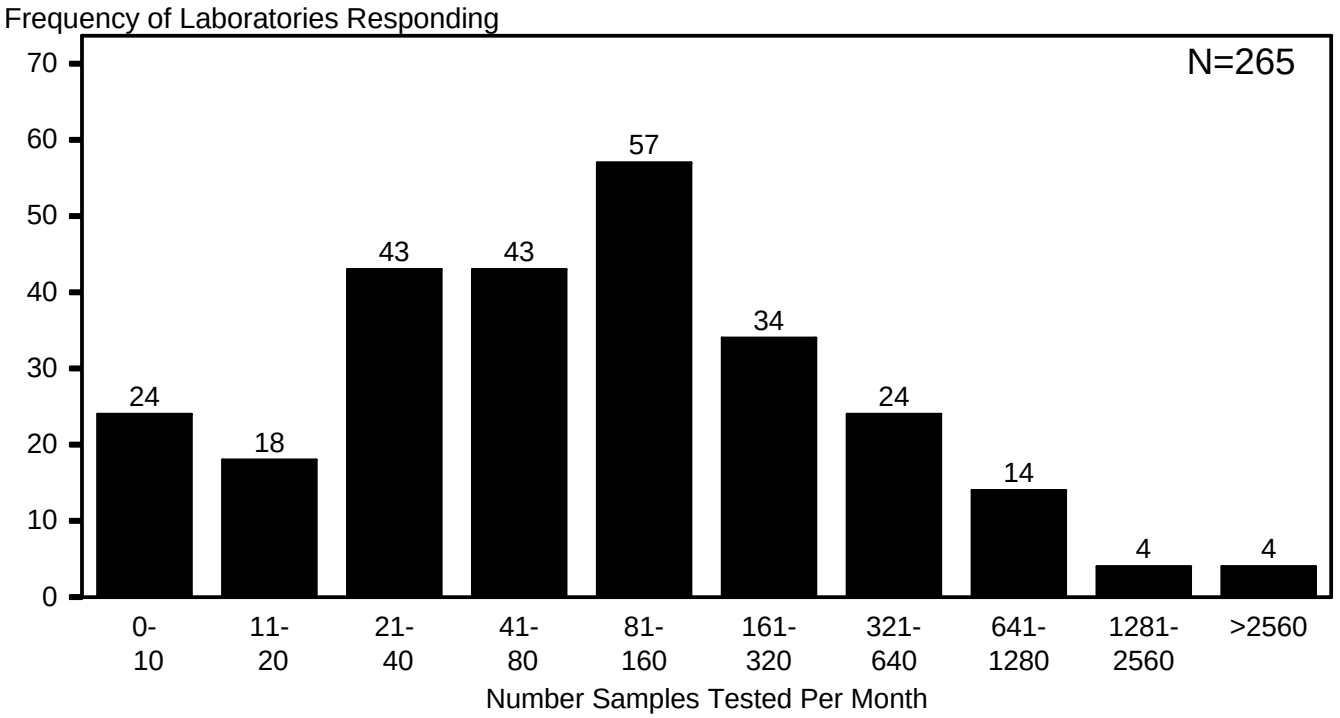
9.(a) Does your laboratory require that personnel who perform TLI have training?



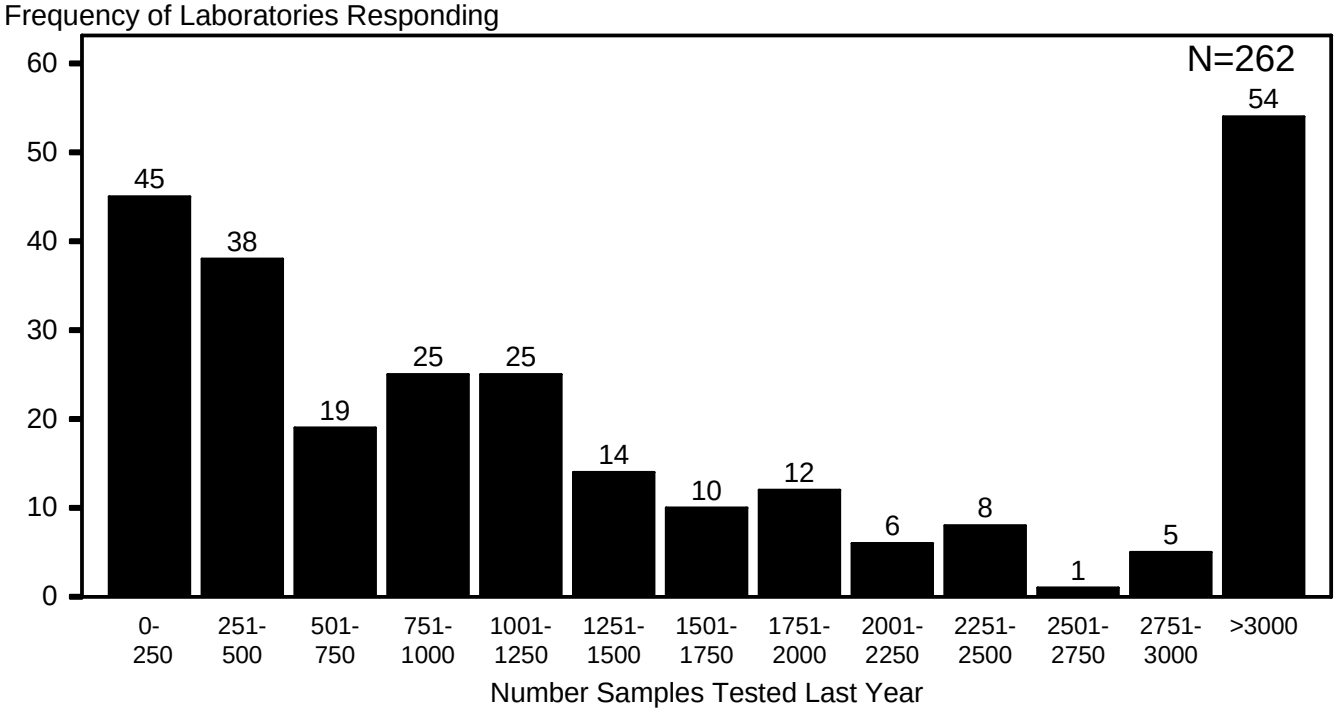
9. (b) What training must your laboratory personnel complete before they are considered qualified to perform TLI? (Check <u>all</u> that apply.)



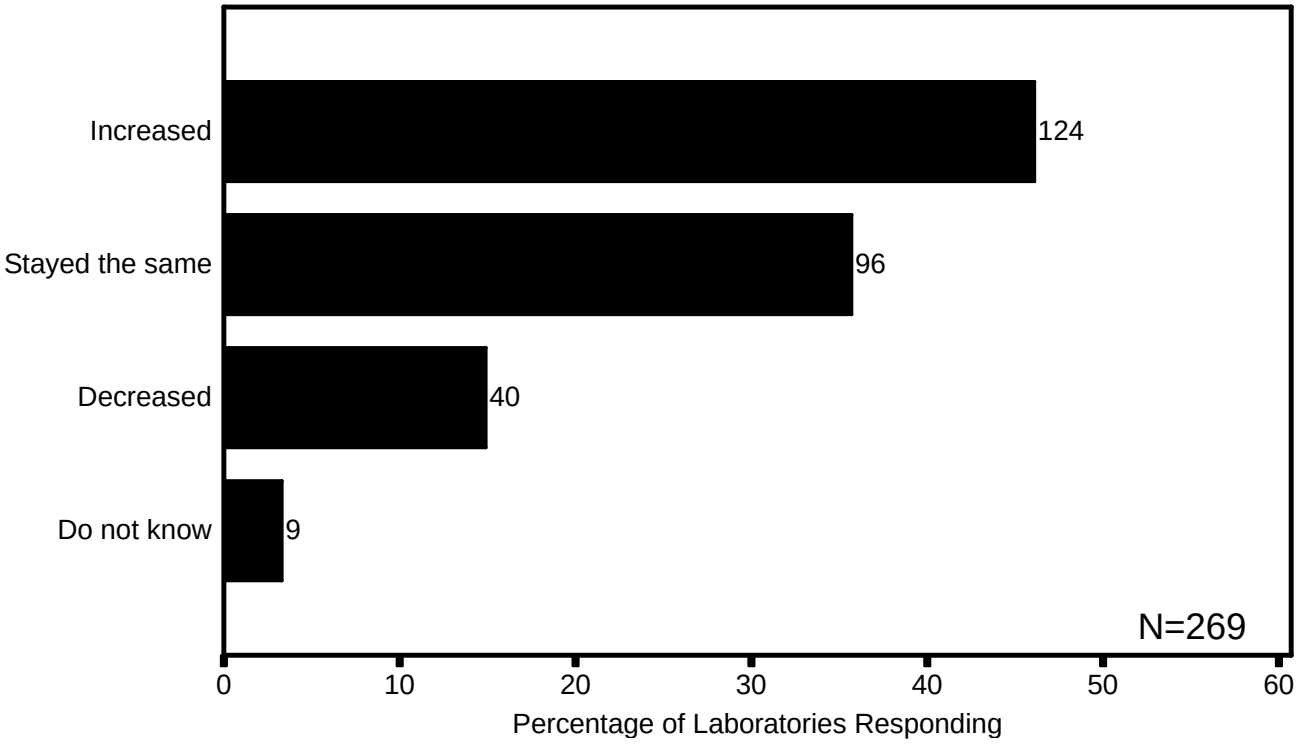
10.(a) On average, how many TLI specimens are tested in your laboratory in a <u>month</u>? (Number of single patient and/or blood donor specimens, <u>not</u> tests. Round off to the nearest whole number.)



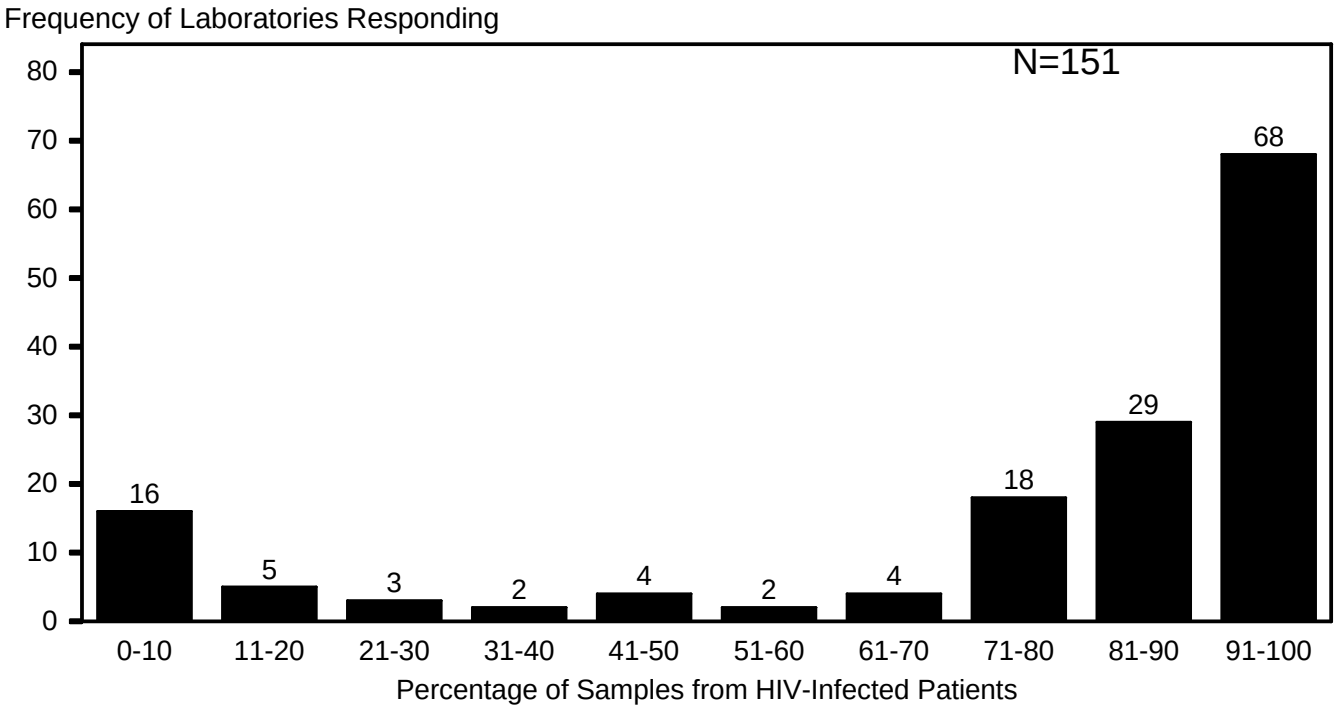
10.(b) How many TLI specimens were tested in your laboratory in the last <u>year</u>? (Number of single patient and/or blood donor specimens, <u>not</u> tests. Round off to the nearest whole number.)



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.)



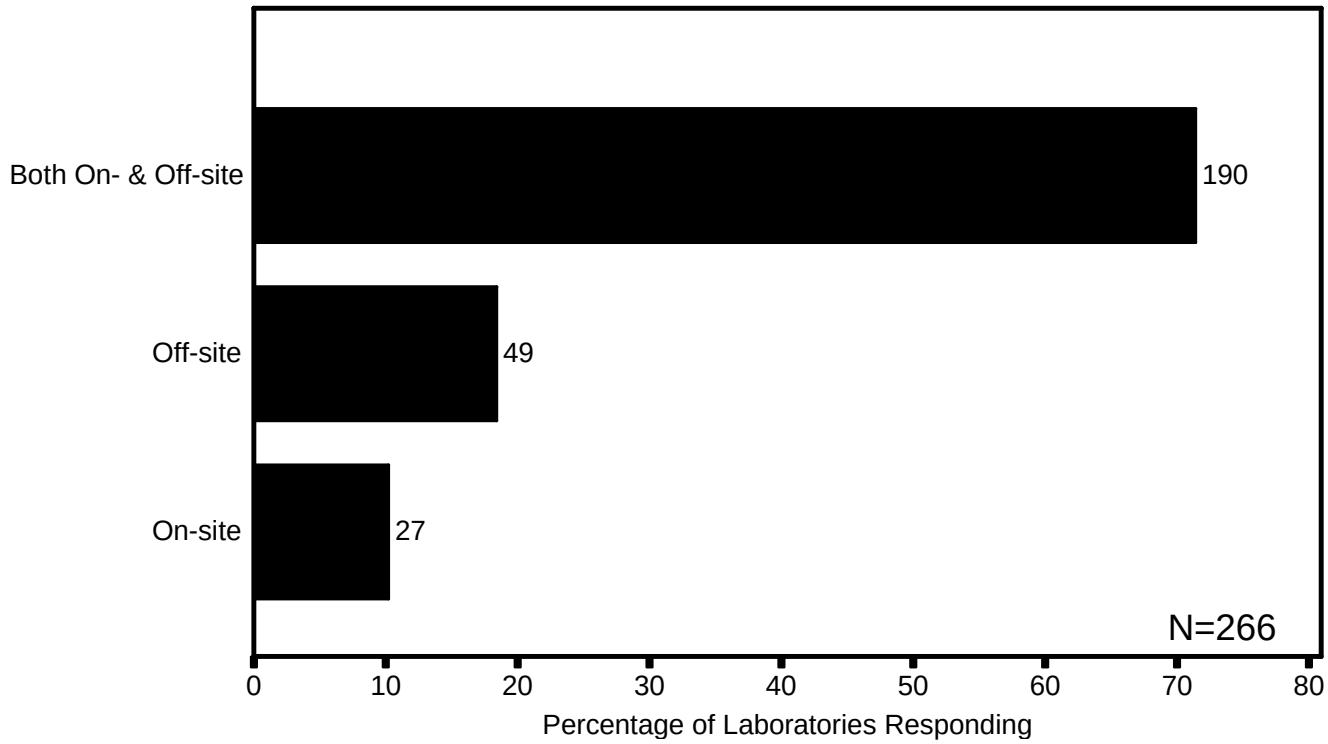
11. In the last year, what percentage of your TLI specimens have come from patients known by your laboratory to be HIV-infected? (Round off to the nearest whole number.)



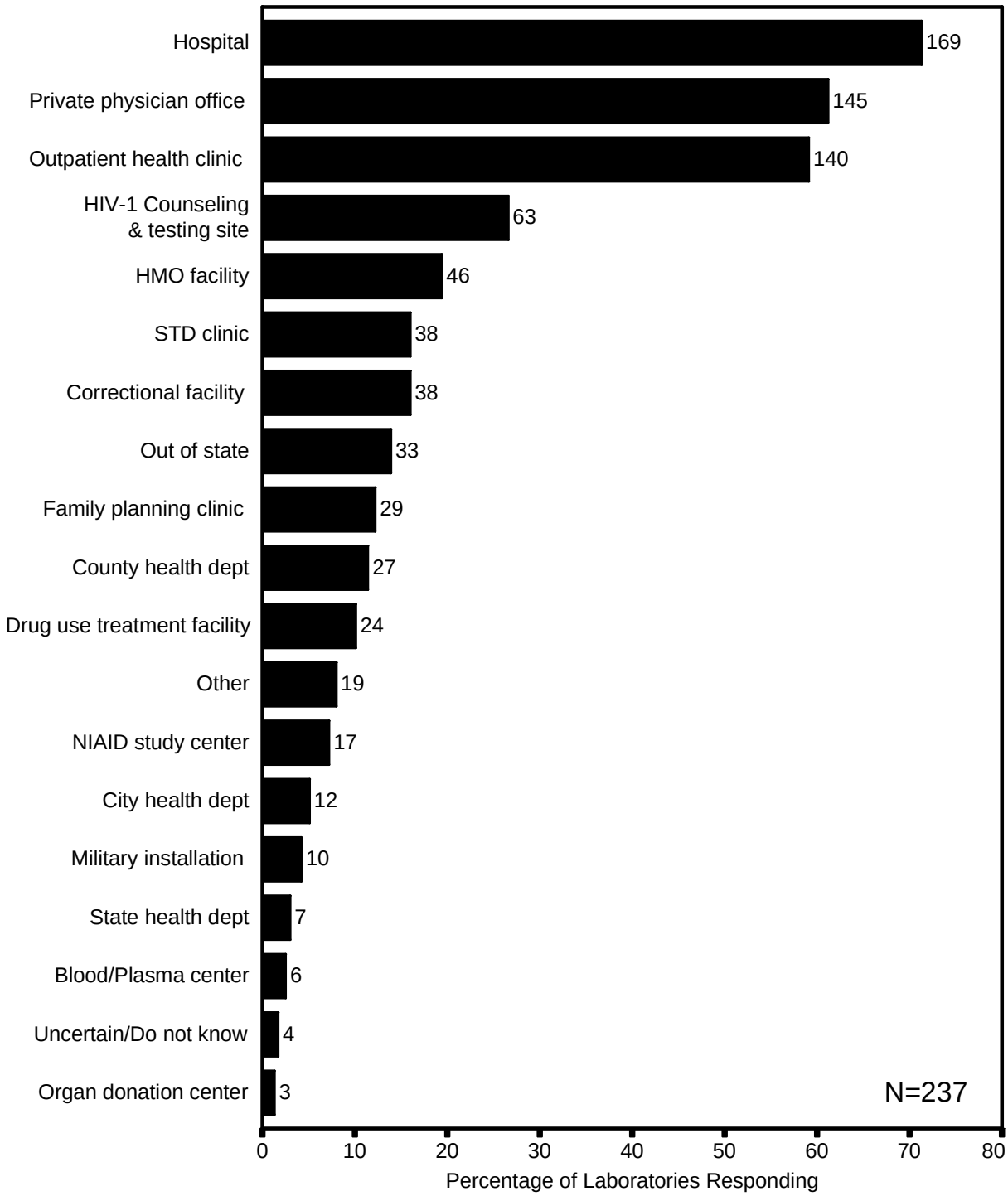
12.(a) If written instructions are provided to collection site personnel for collecting, labeling, and transporting TLI specimens, who provides these instructions? (Check <u>all</u> that apply.)

Type of Instruction	Instructions NOT Provided	Testing Laboratory	Associated Institution	Person Ordering Test	Other	N =
Collecting	9	228	51	20	2	259
Labeling	10	223	56	17	2	259
Transporting	9	228	49	14	2	258

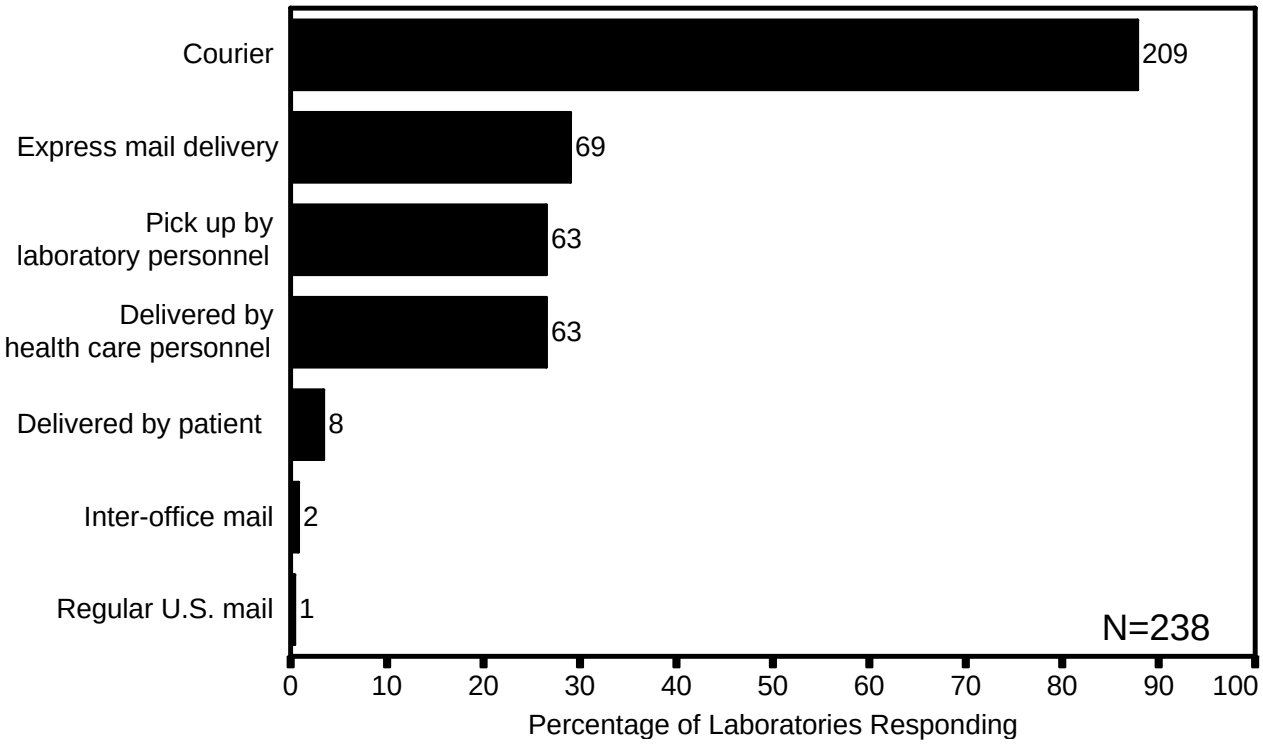
12.(b) Where are your specimens collected for TLI? (Choose only <u>one</u>.)



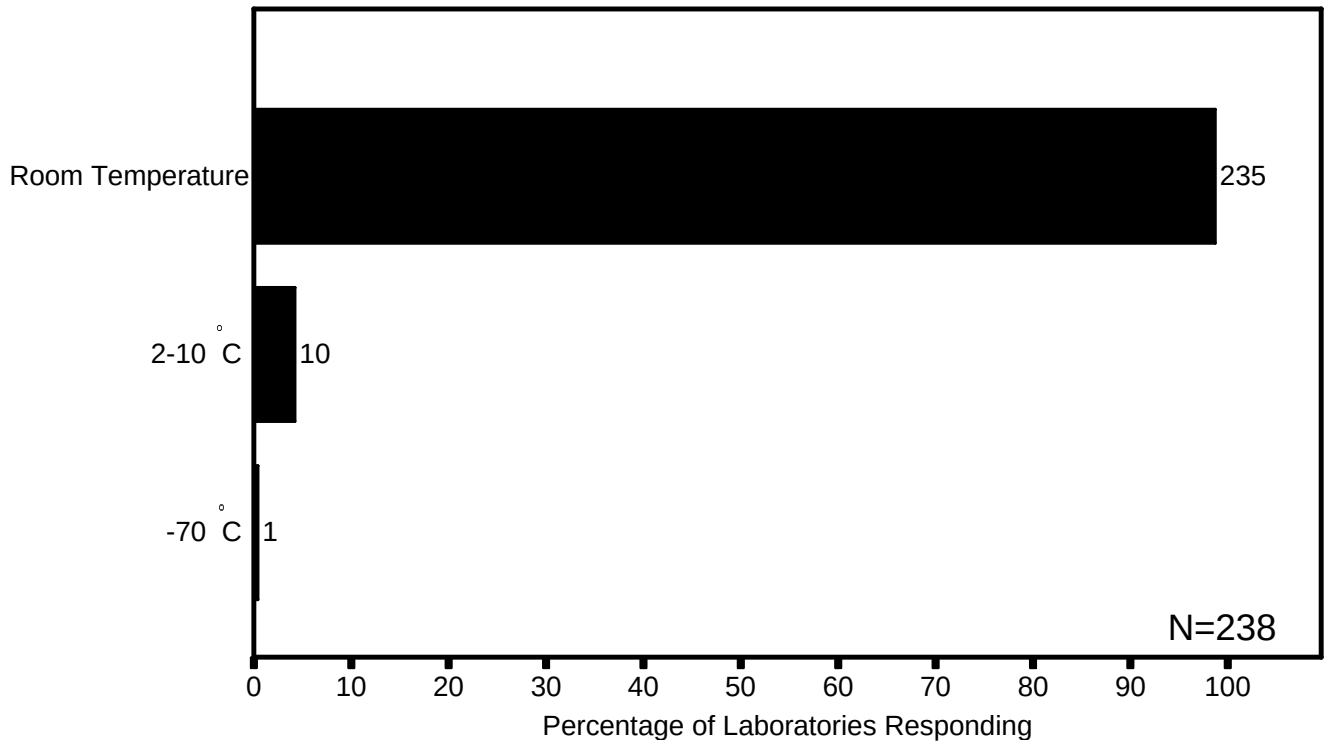
12.(c) When your laboratory tests TLI specimens that are collected off-site, where are the specimens collected? (Check <u>all</u> that apply.)



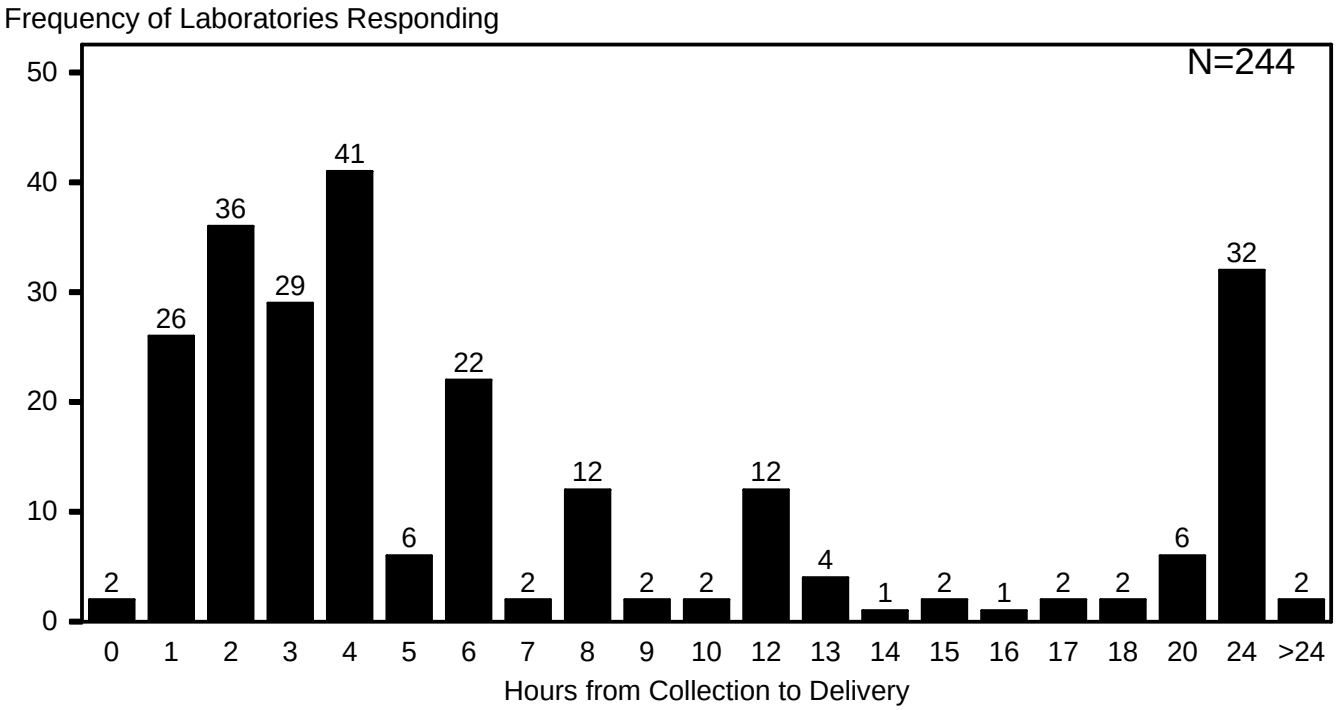
12.(d) How are the off-site TLI specimens delivered to your laboratory? (Check <u>all</u> that apply.)



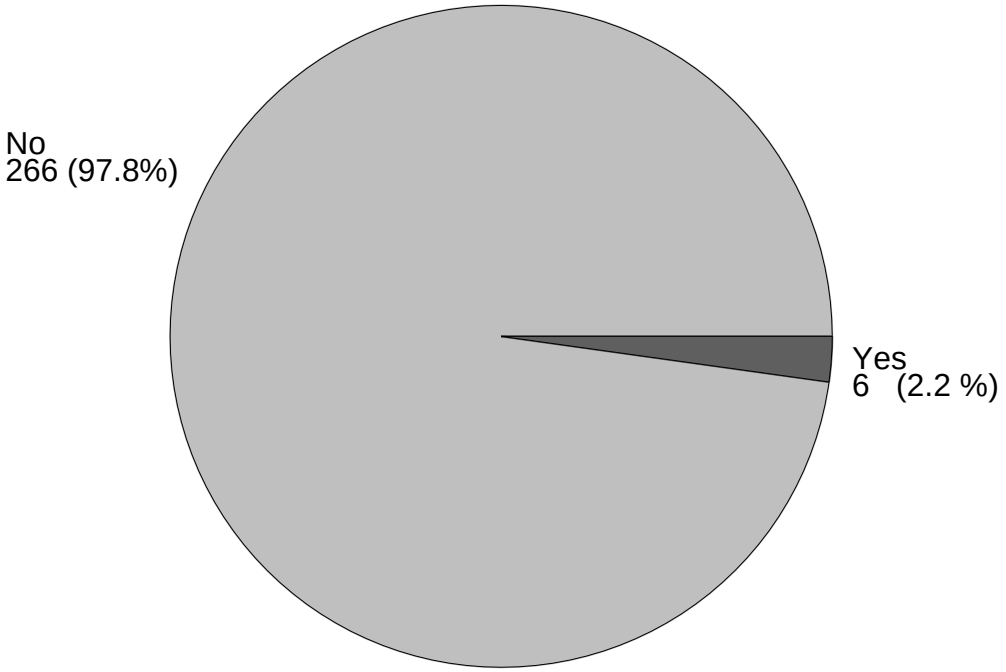
12.(e) At what temperature are TLI specimens transported to your laboratory? (Check <u>all</u> that apply.)



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.)

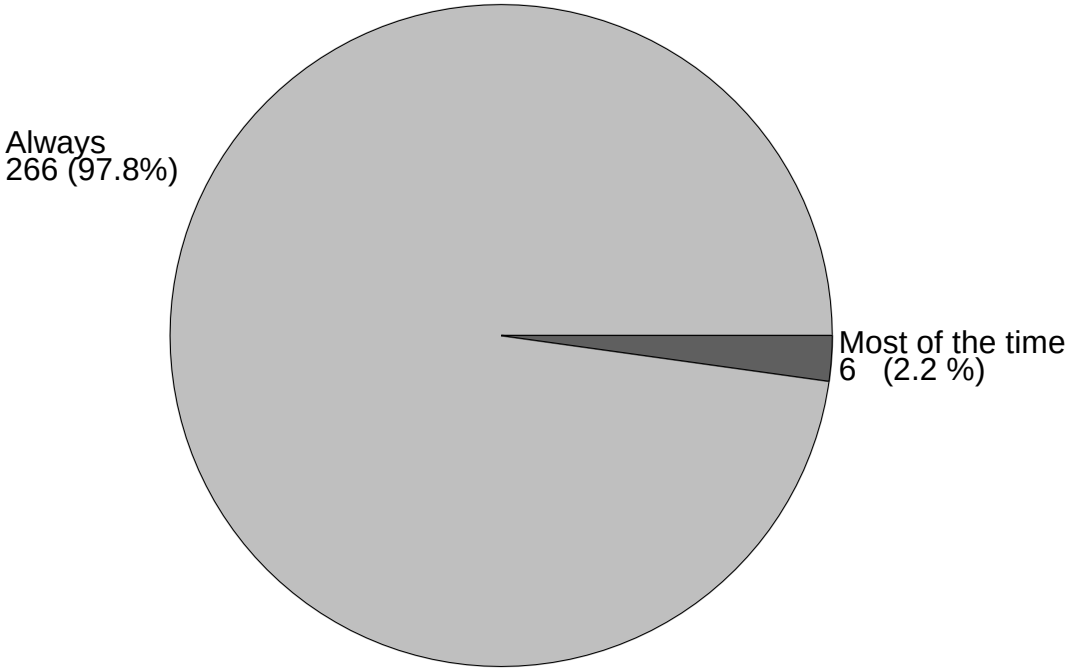


14.(a) Are the procedures your laboratory uses for labeling a suspected HIV-1-positive specimen different from the procedures you use for other specimens?



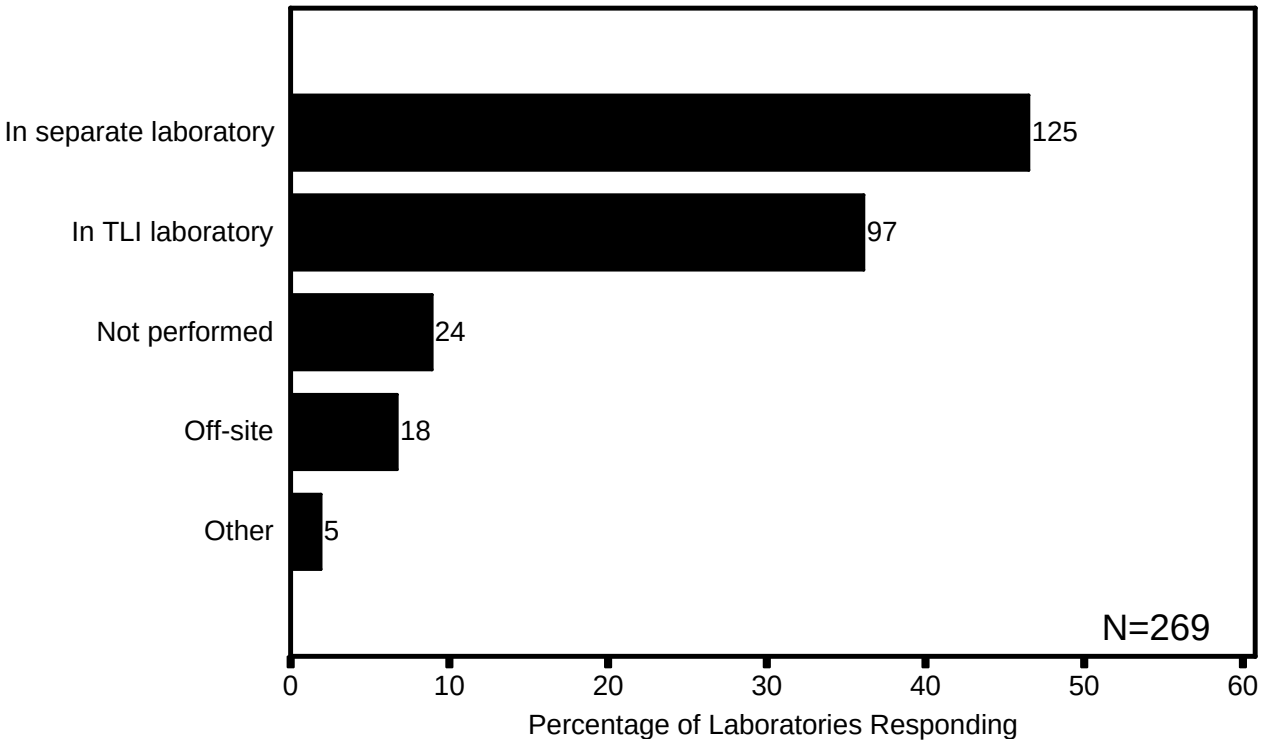
N=272

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

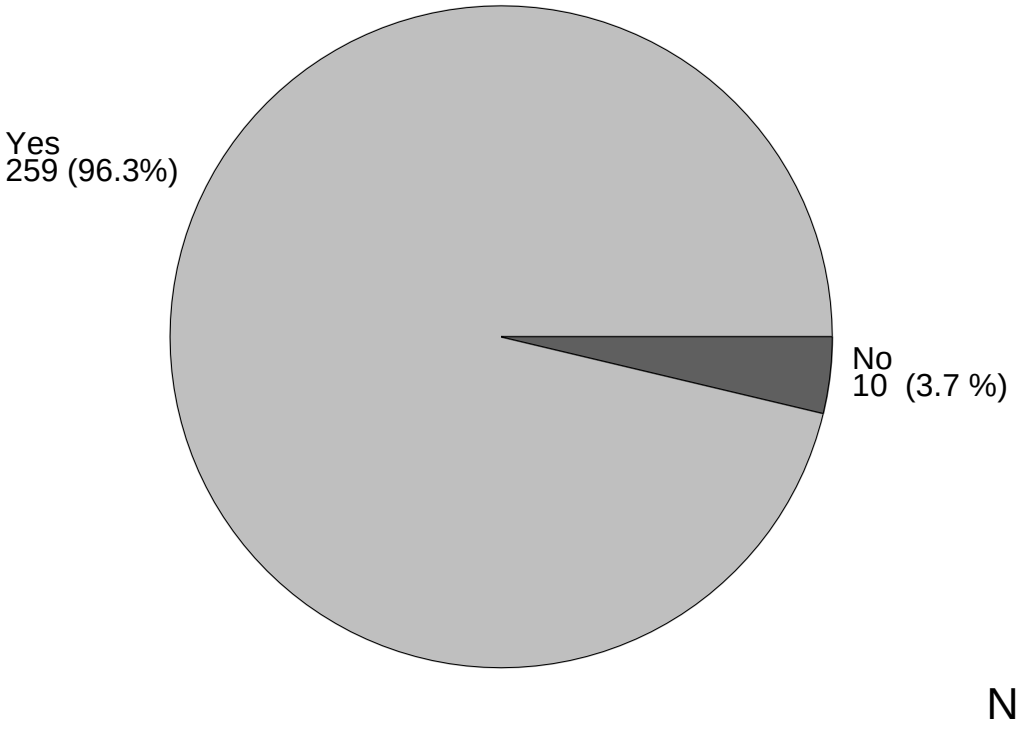


N=272

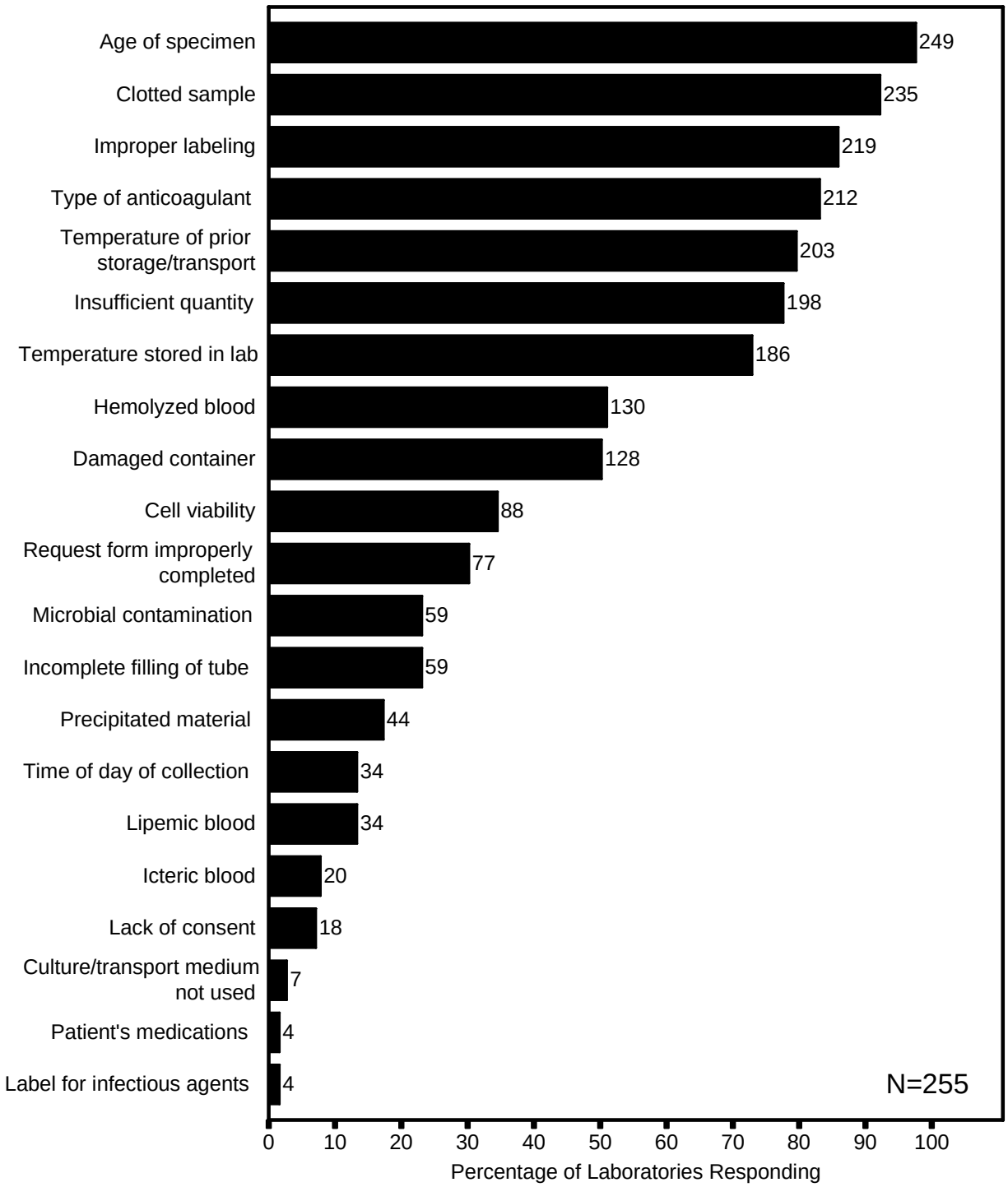
15. Where is the hematology testing (e.g., complete blood count [CBC]) for your laboratory's TLI specimens usually performed? (Choose only <u>one</u>.)



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



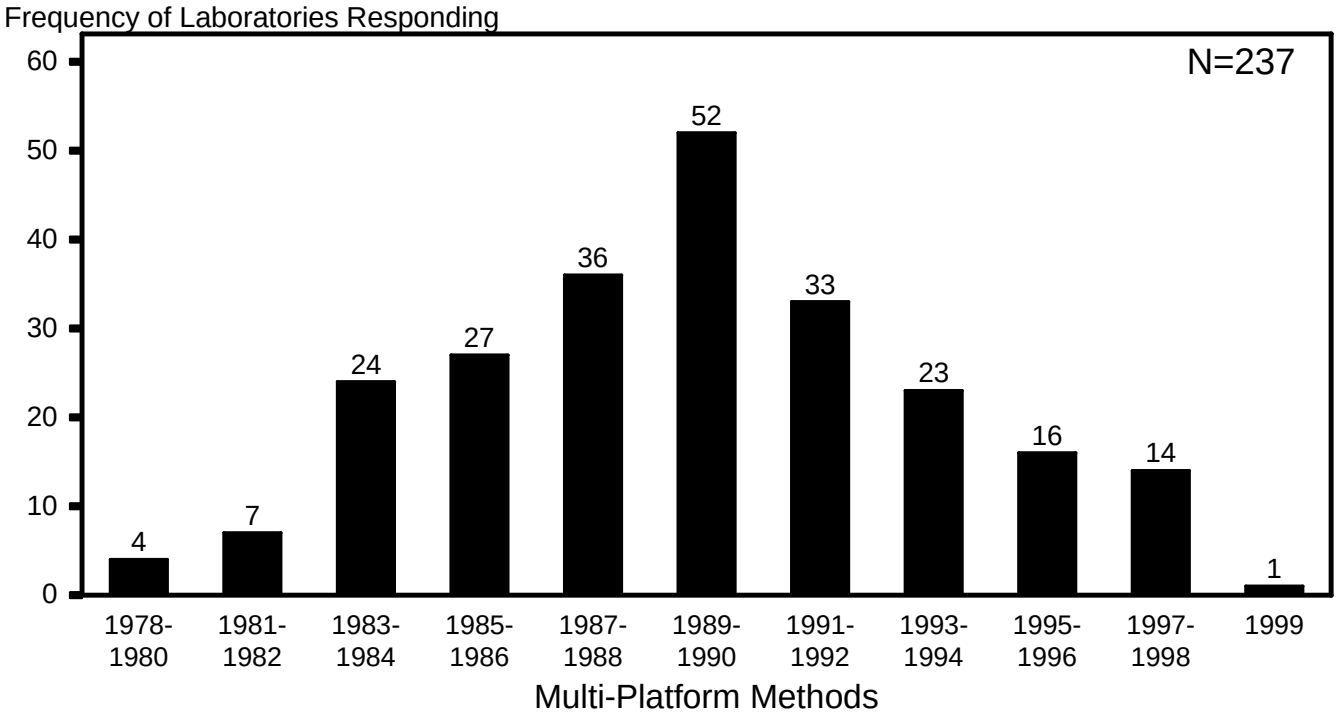
16.(b) What specimen collection criteria are used at your laboratory to determine whether or not a specimen is acceptable for TLI? (Check all that apply.)

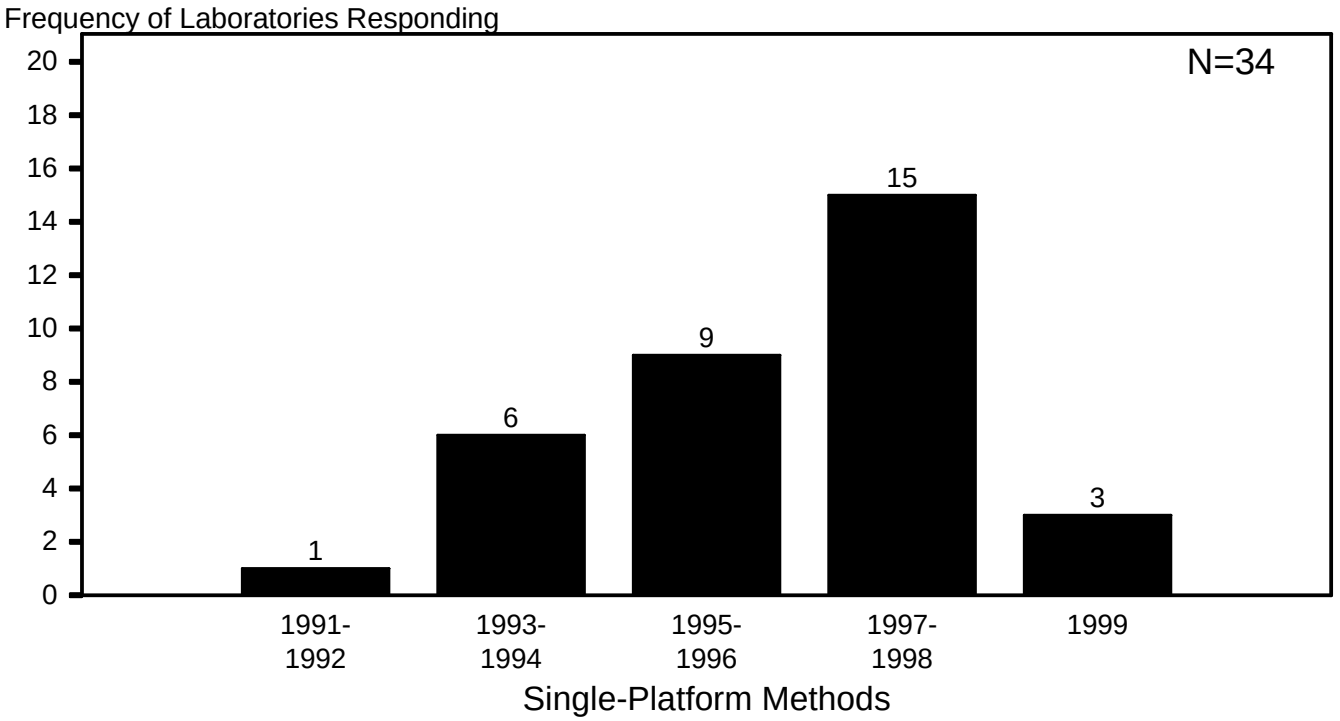


17. At what temperatures does your laboratory store TLI specimens until they are processed? (Check all that apply.)

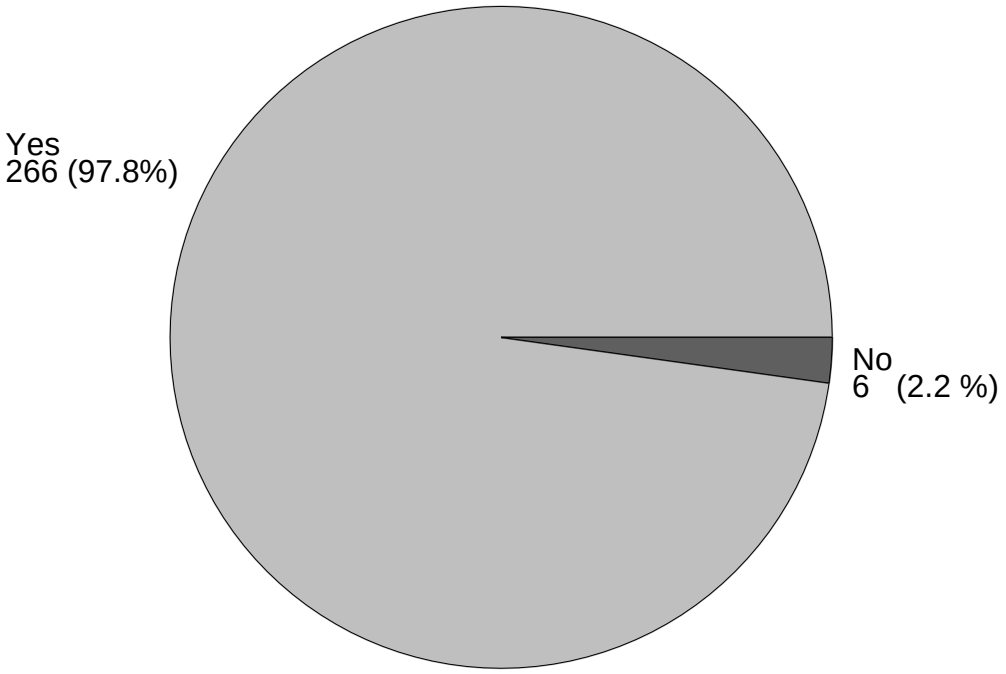
Type of Specimen	Room Temperature	2 - 10 °C	Other Temperature	N =
Whole Blood	267	3	1	268
Separated Cells	25	47	1	72
Other	19	24	1	42

18. When did your laboratory begin performing TLI? (Please indicate month and year only for those methods currently in use in your laboratory.)

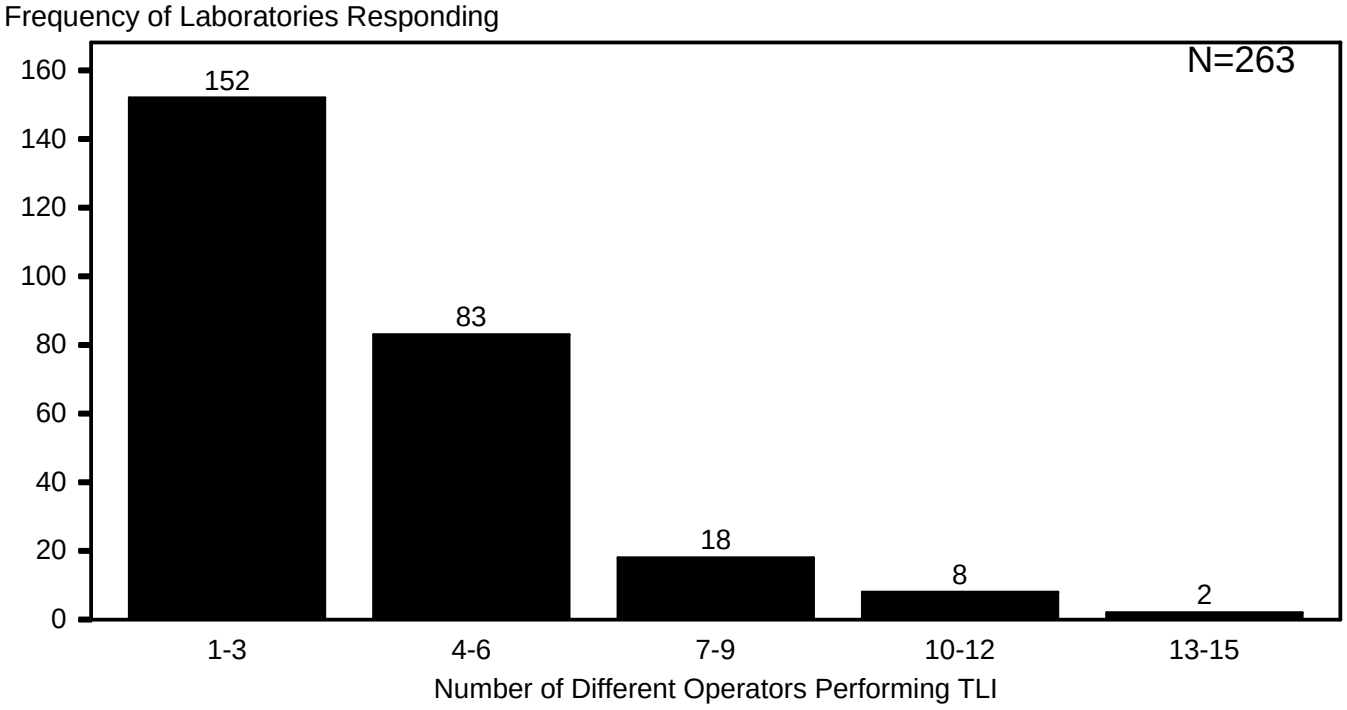




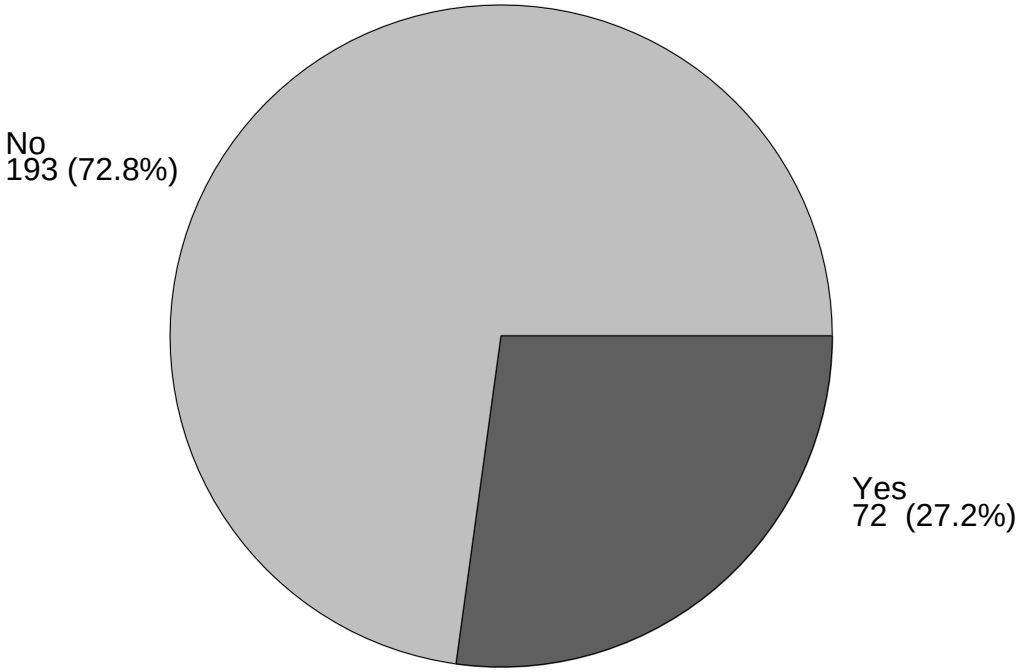
19. Does your laboratory perform TLI using a flow cytometry instrument?



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

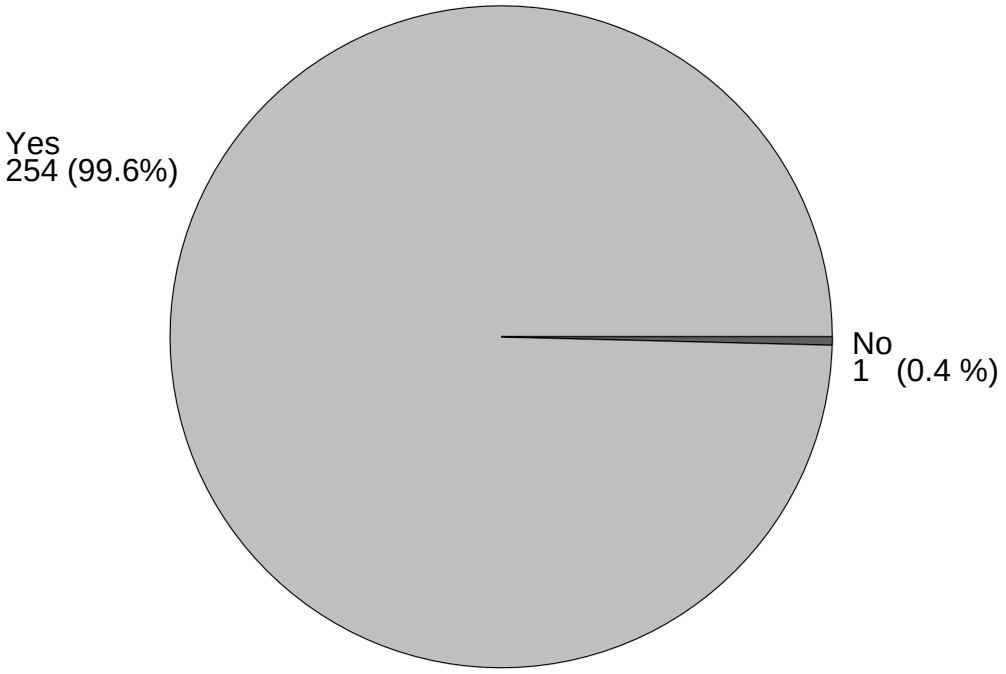


20.(b) Do any of your laboratory's flow cytometer operators routinely perform TLI on more than one flow cytometer?



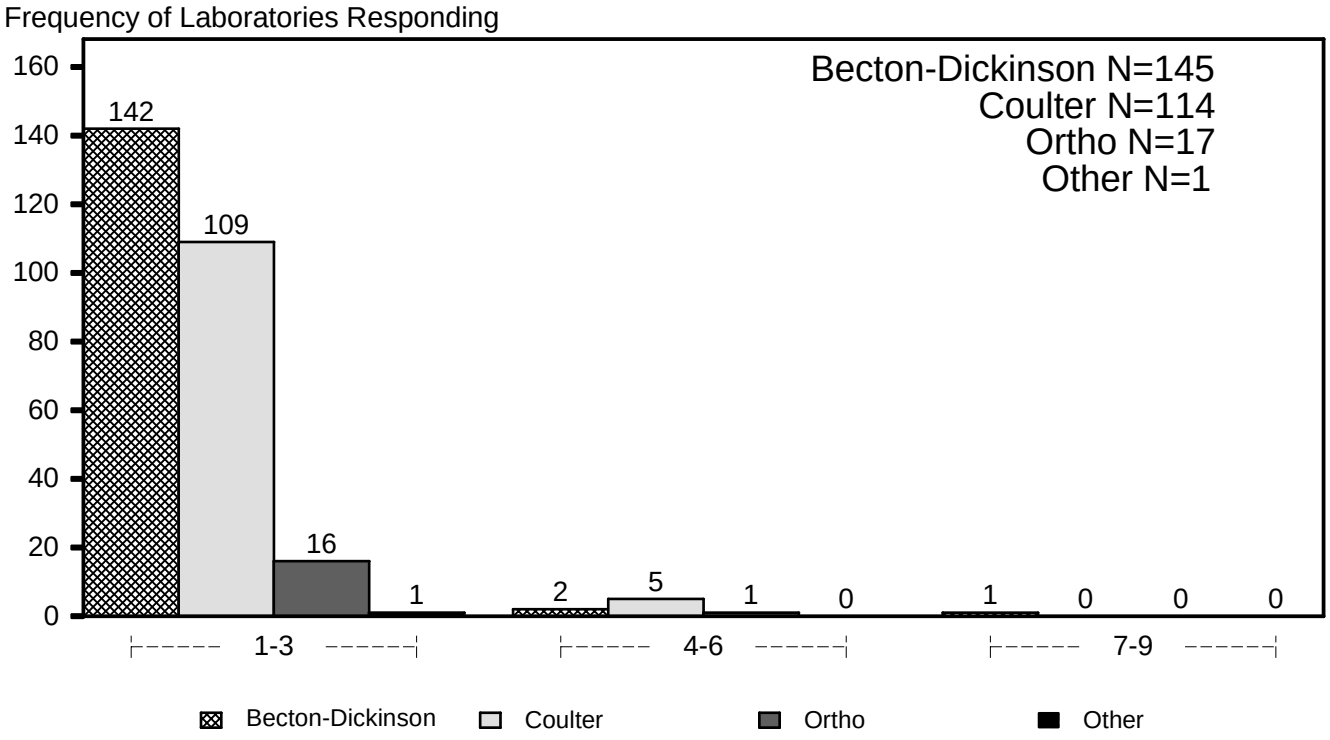
N=265

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

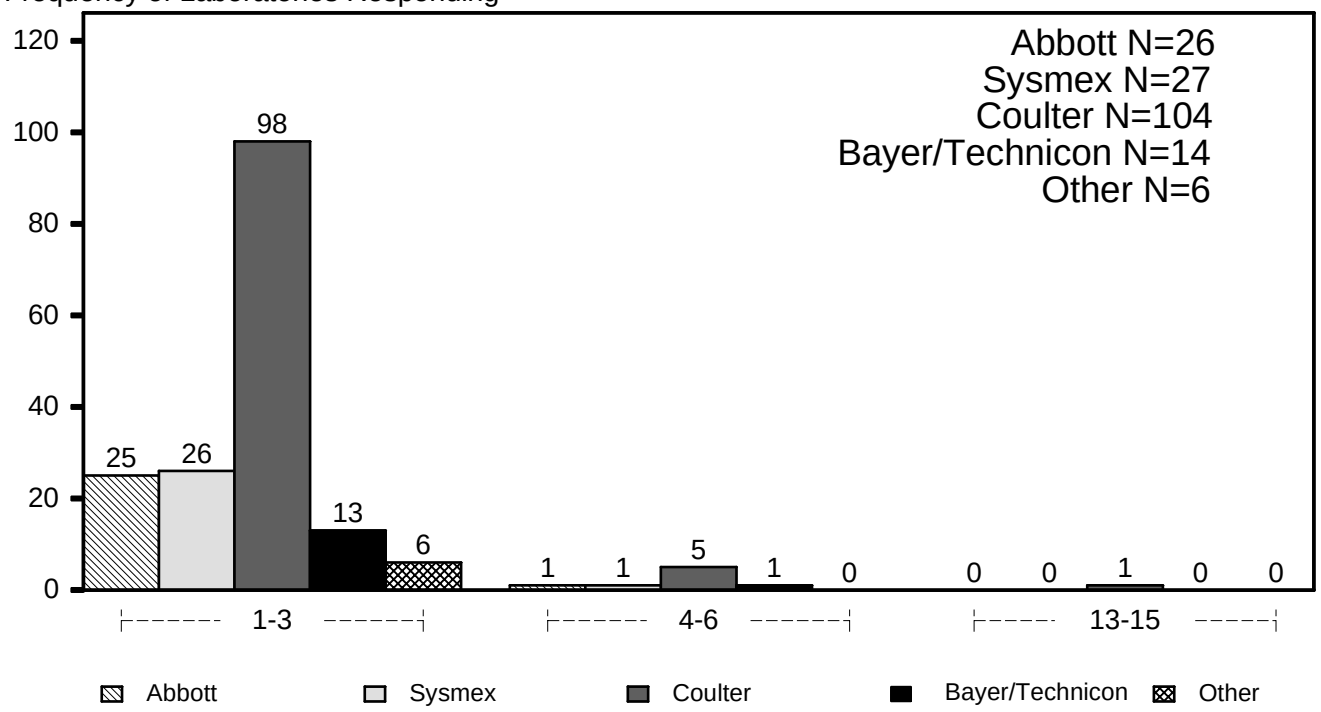


N=255

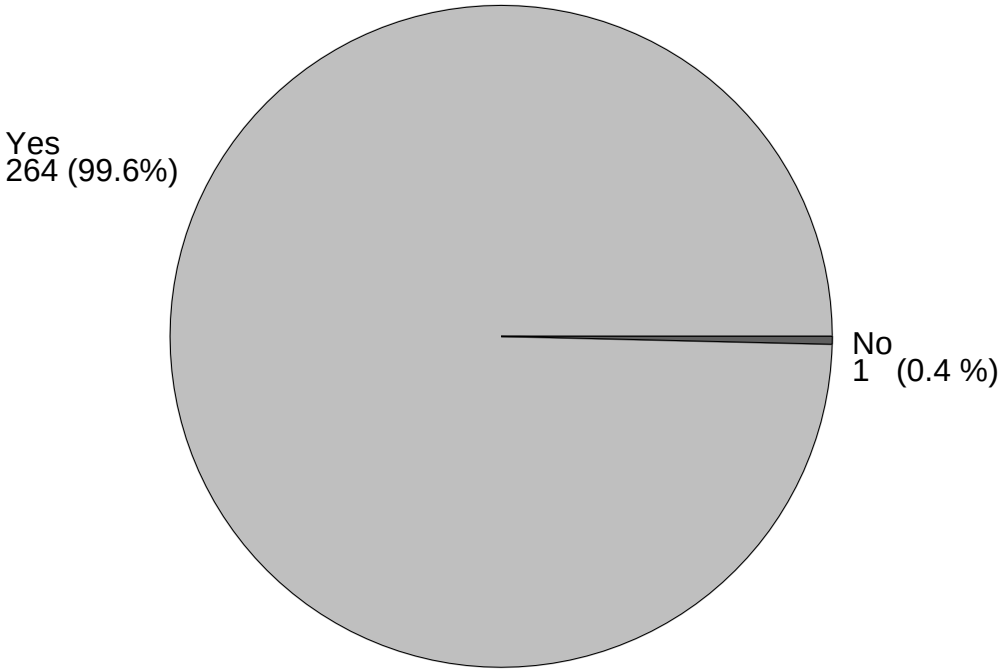
21. What are the quantities and manufacturers of your laboratory's flow cytometers that are used for TLI?



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

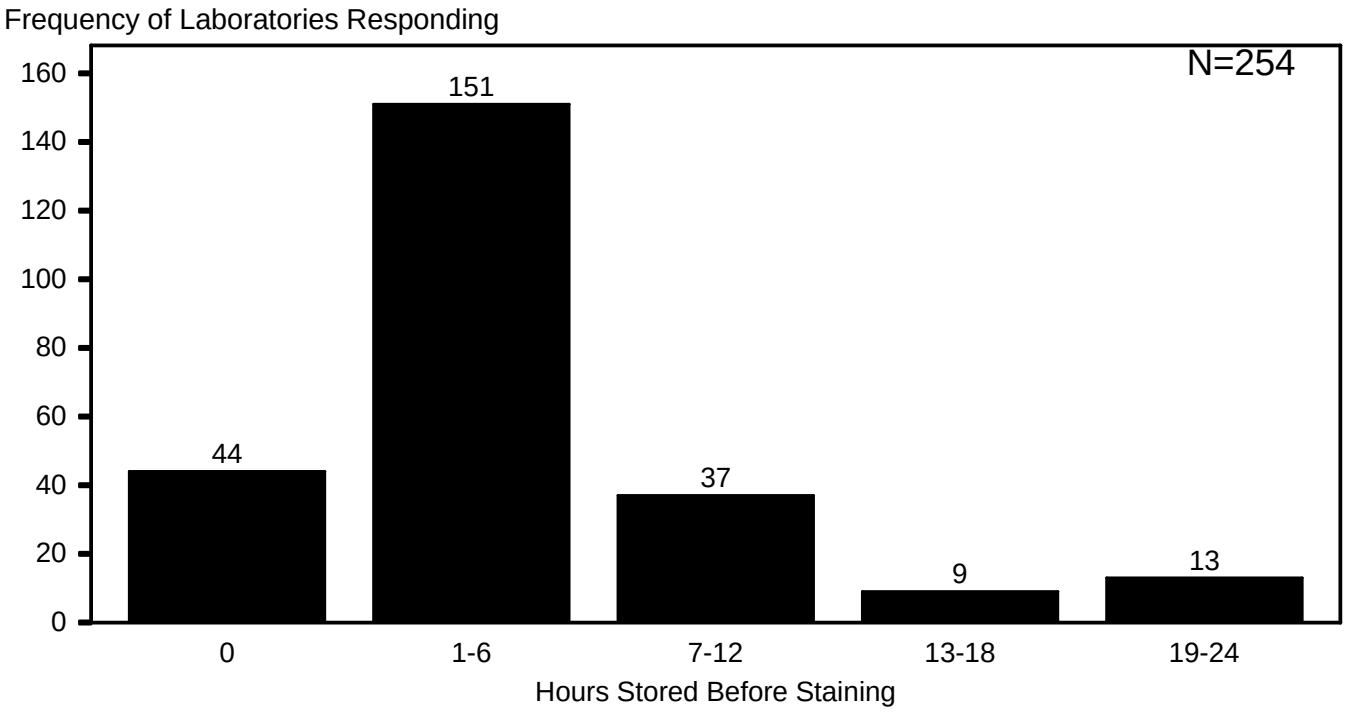


22. Can multicolor analysis techniques (two or more markers tagged with different color fluorochromes as a single test) be used to perform TLI on any of the flow cytometry instruments you indicated?

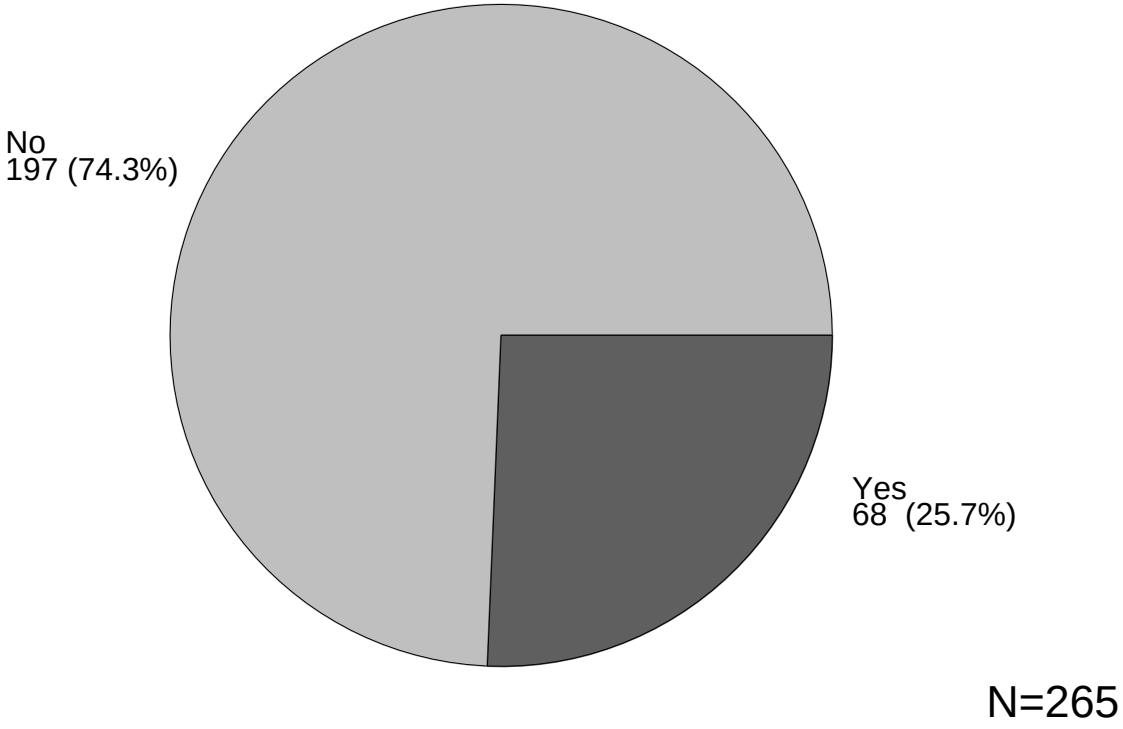


N=265

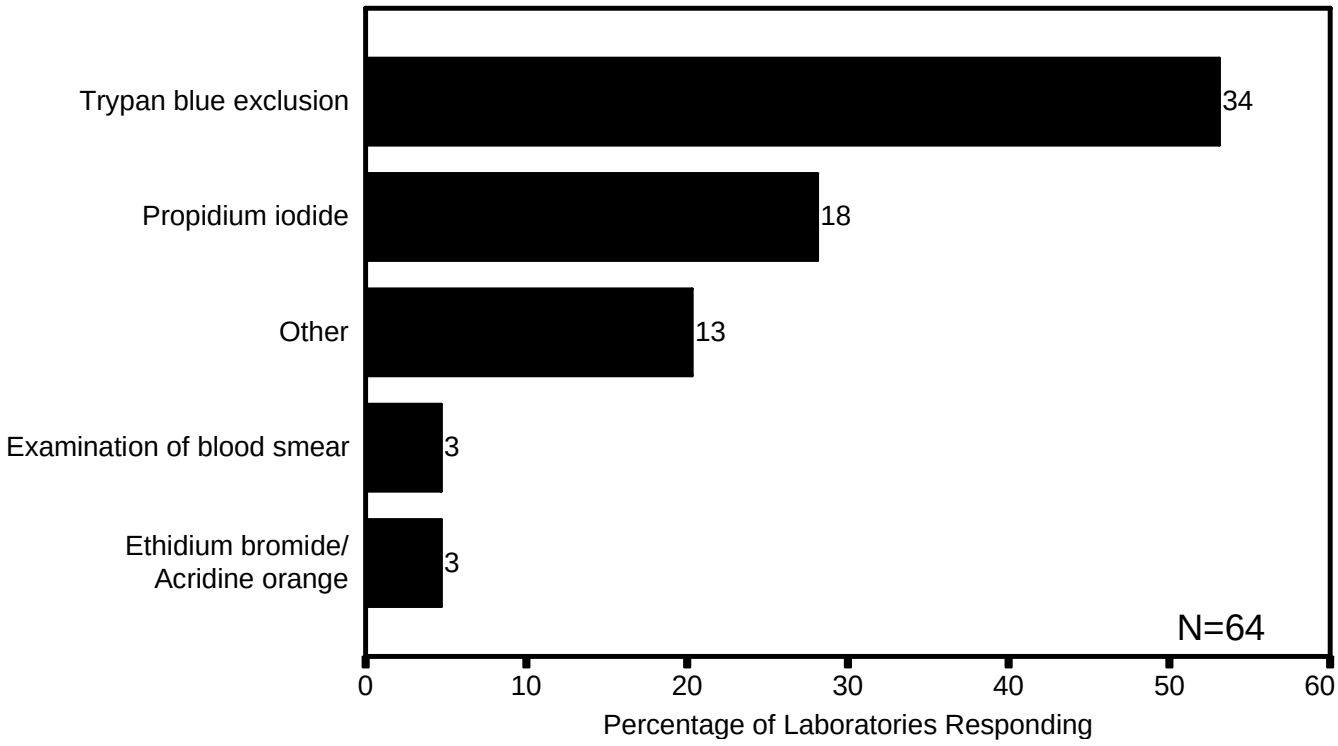
23. On average, how many hours is a specimen stored at your laboratory before it is <u>stained</u> for TLI? (If the specimen is processed immediately upon receipt, please indicate 0 hours, otherwise, round off to the nearest whole number.)



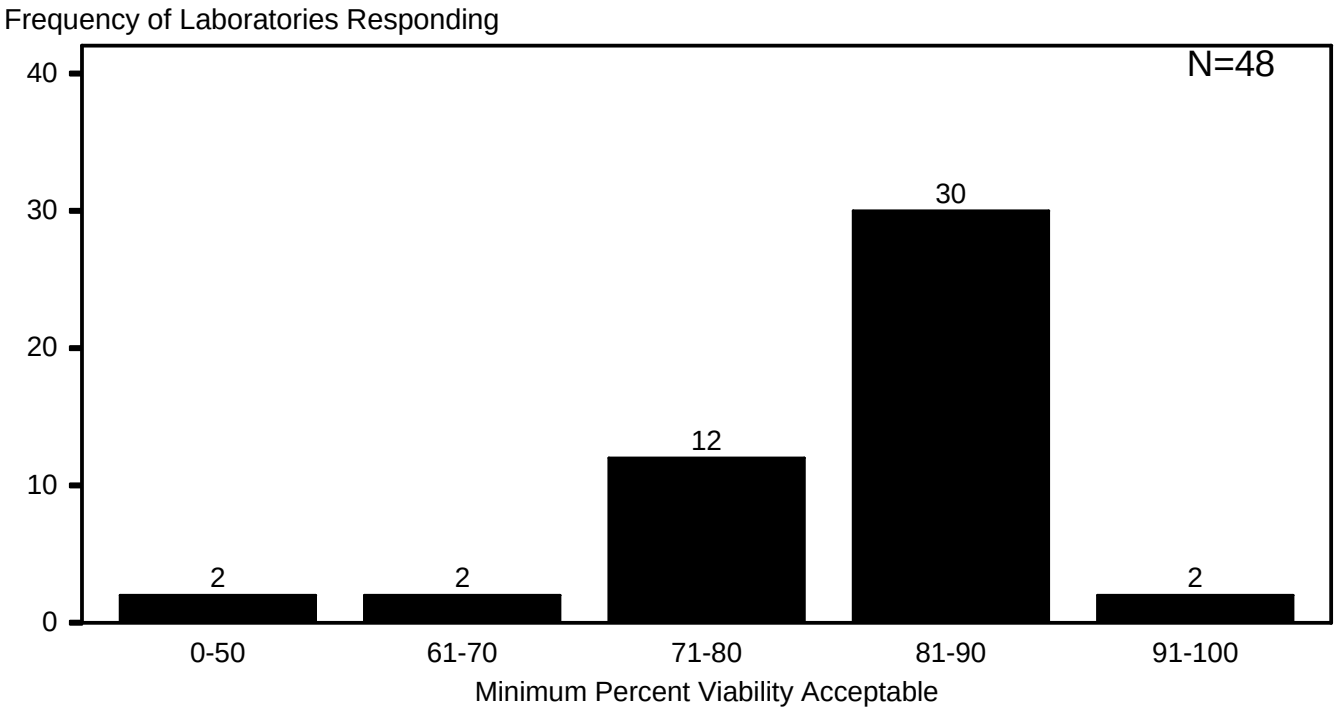
24.(a) Is testing for viability of TLI specimens performed by your laboratory?



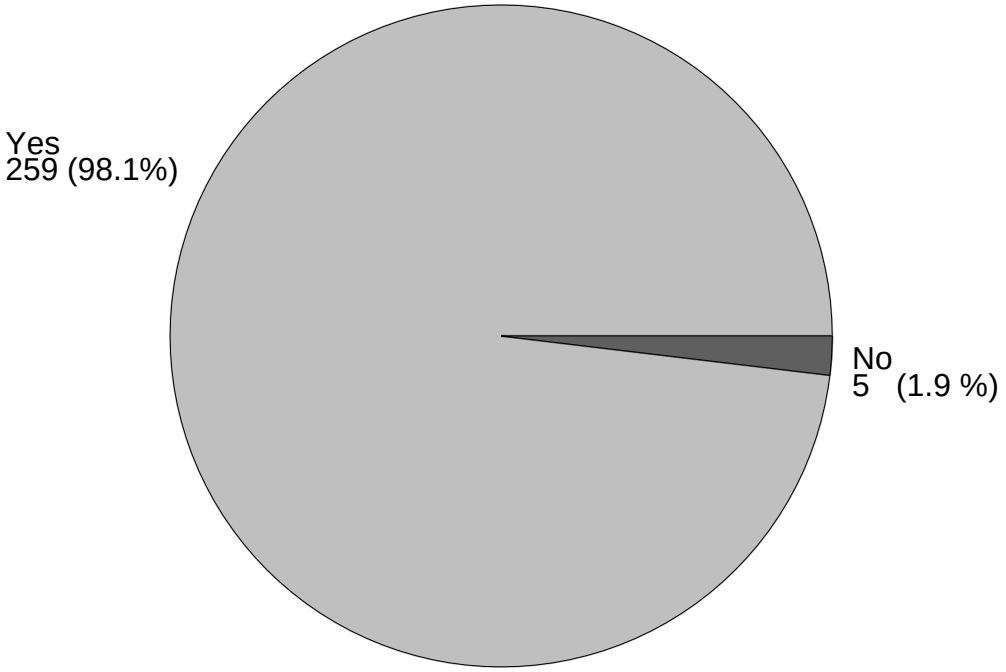
24.(b) How is testing for viability performed? (Check <u>all</u> that apply.)



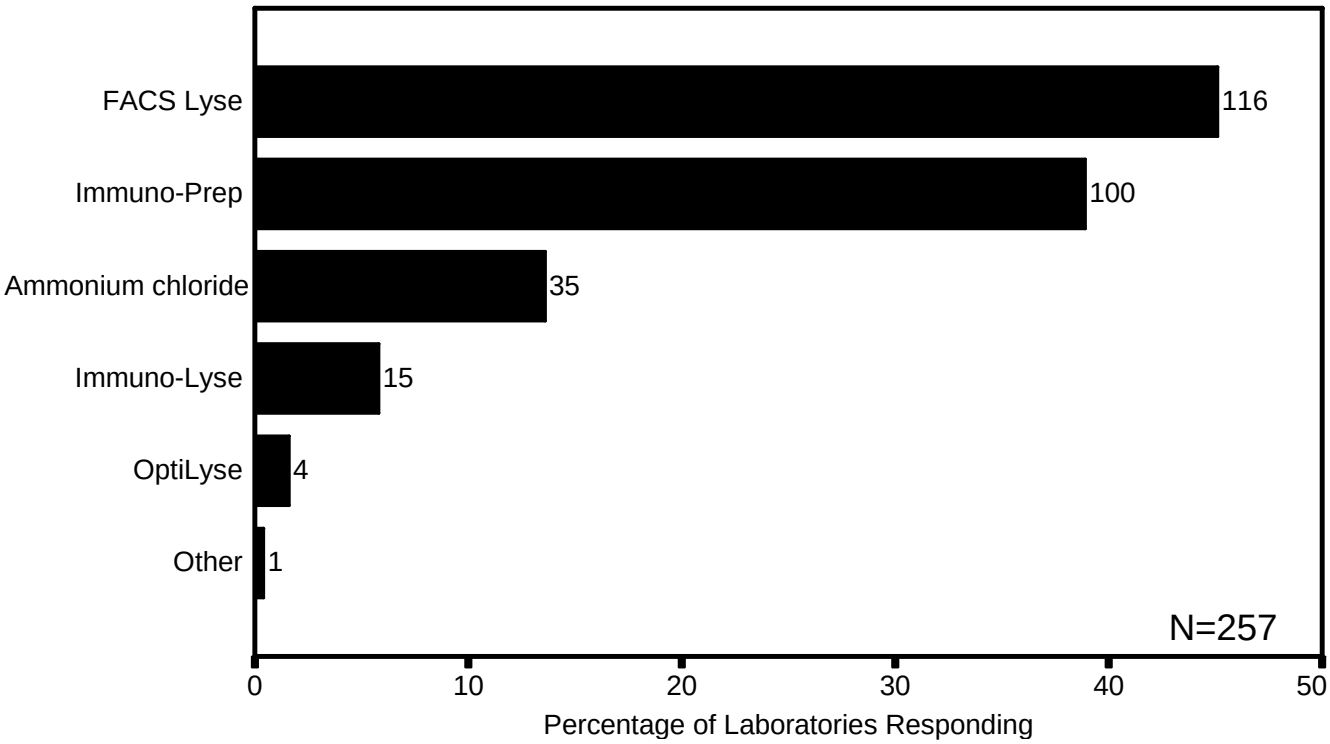
24.(c) What is the minimum percent viability acceptable by your laboratory for TLI? (Round off to the nearest whole number.)



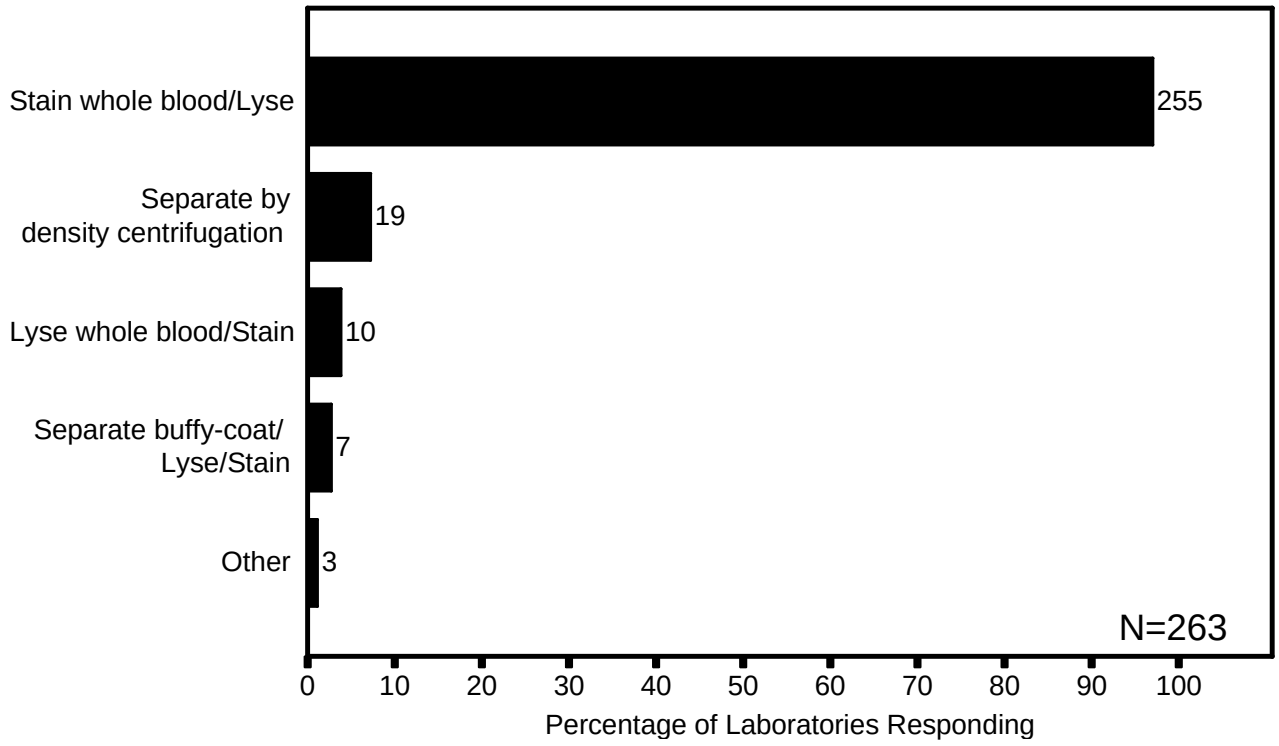
25.(a) Does your laboratory use a whole blood lysis method for staining TLI specimens?



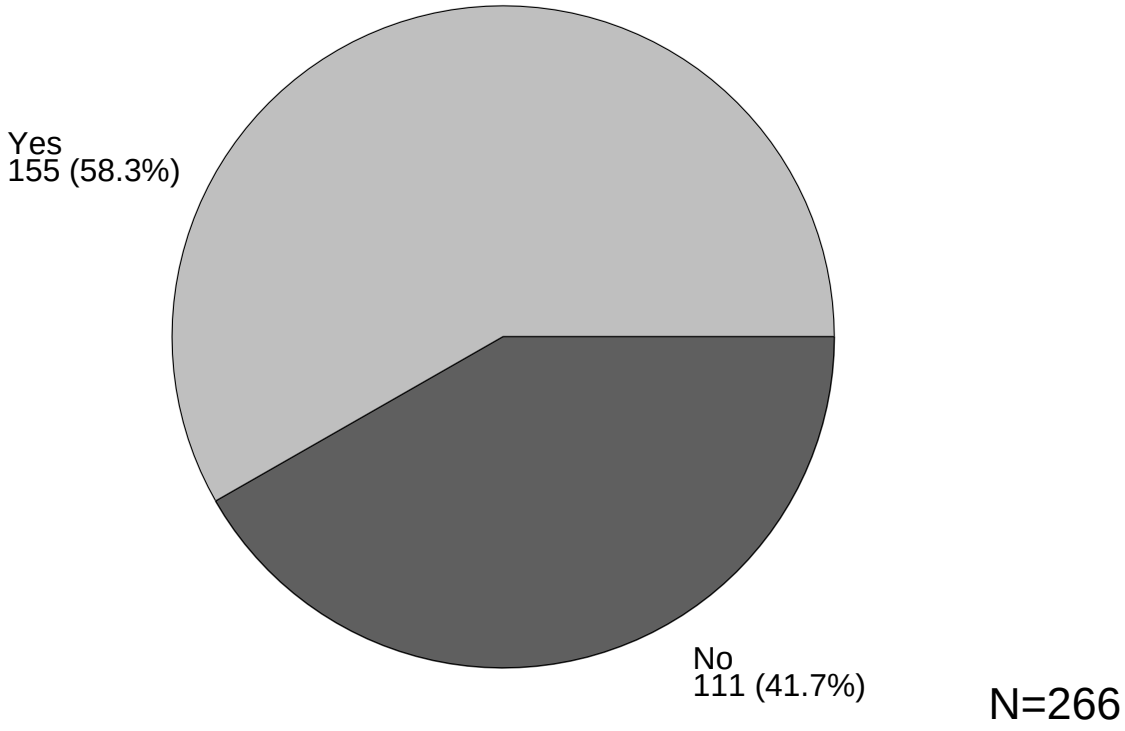
25.(b) Which lysis method does your laboratory use? (Check <u>all</u> that apply.)



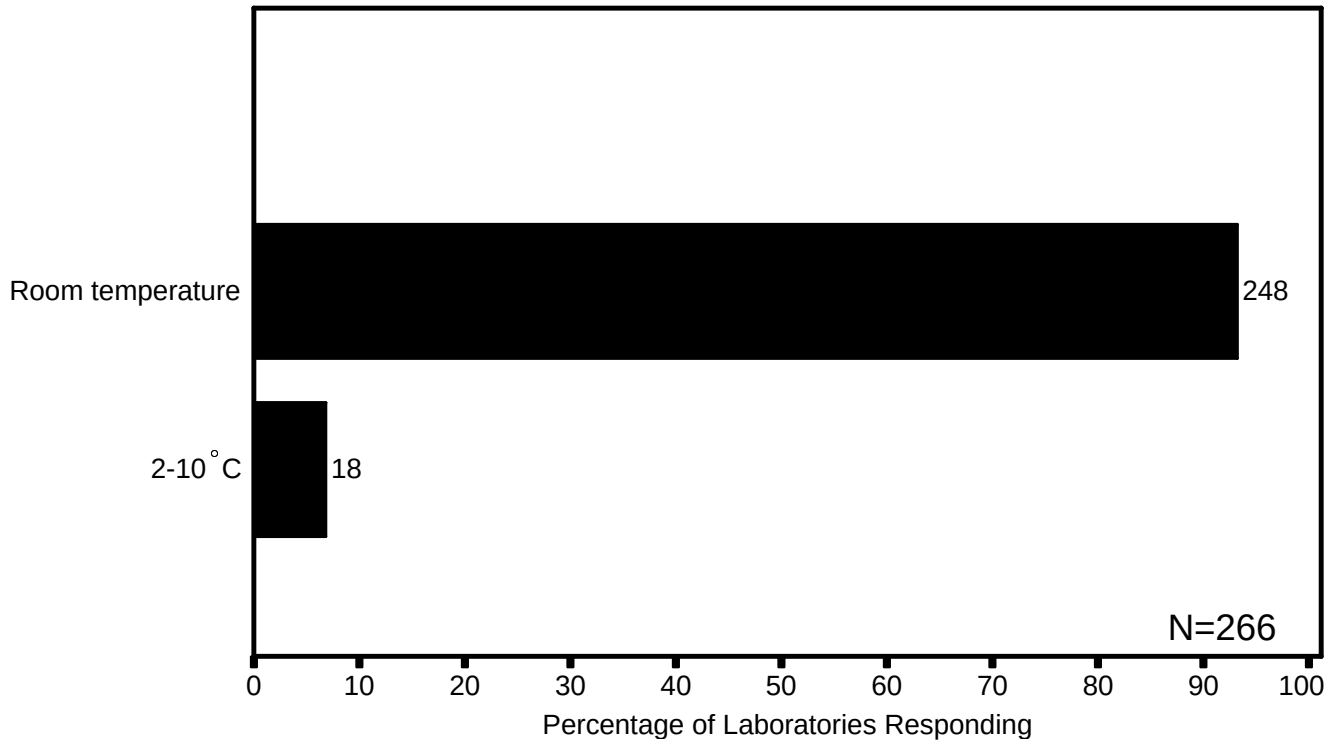
25.(c) What sample staining procedure(s) does your laboratory use for TLI? (Check <u>all</u> that apply.)



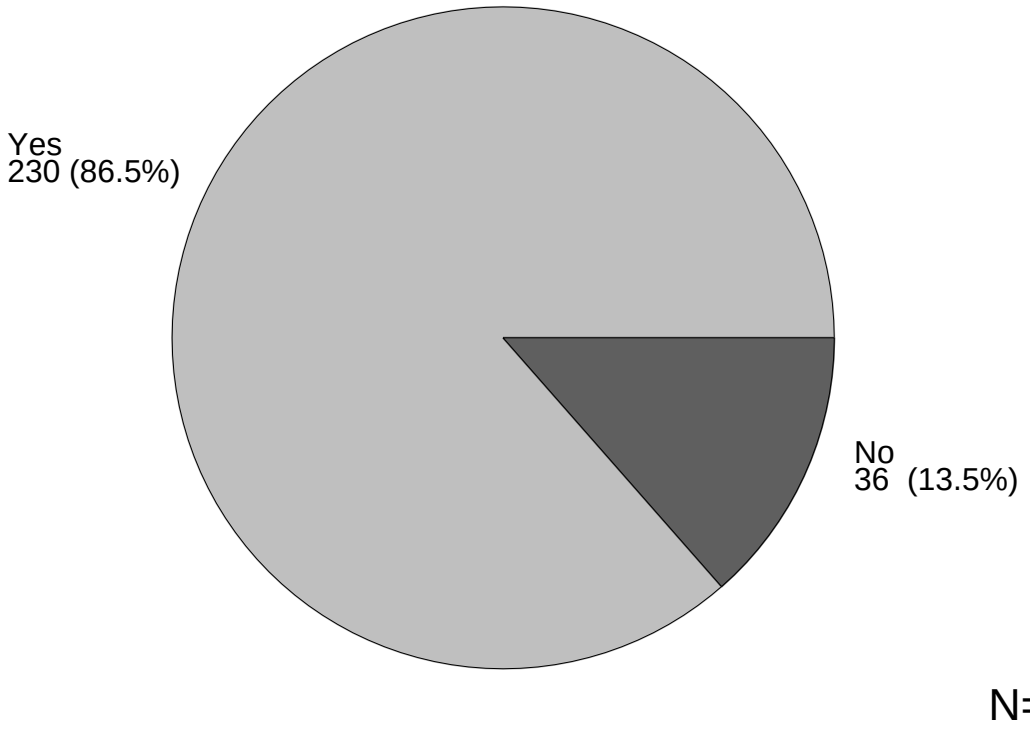
25.(d) Does your laboratory routinely use isotype controls as part of the staining procedure?



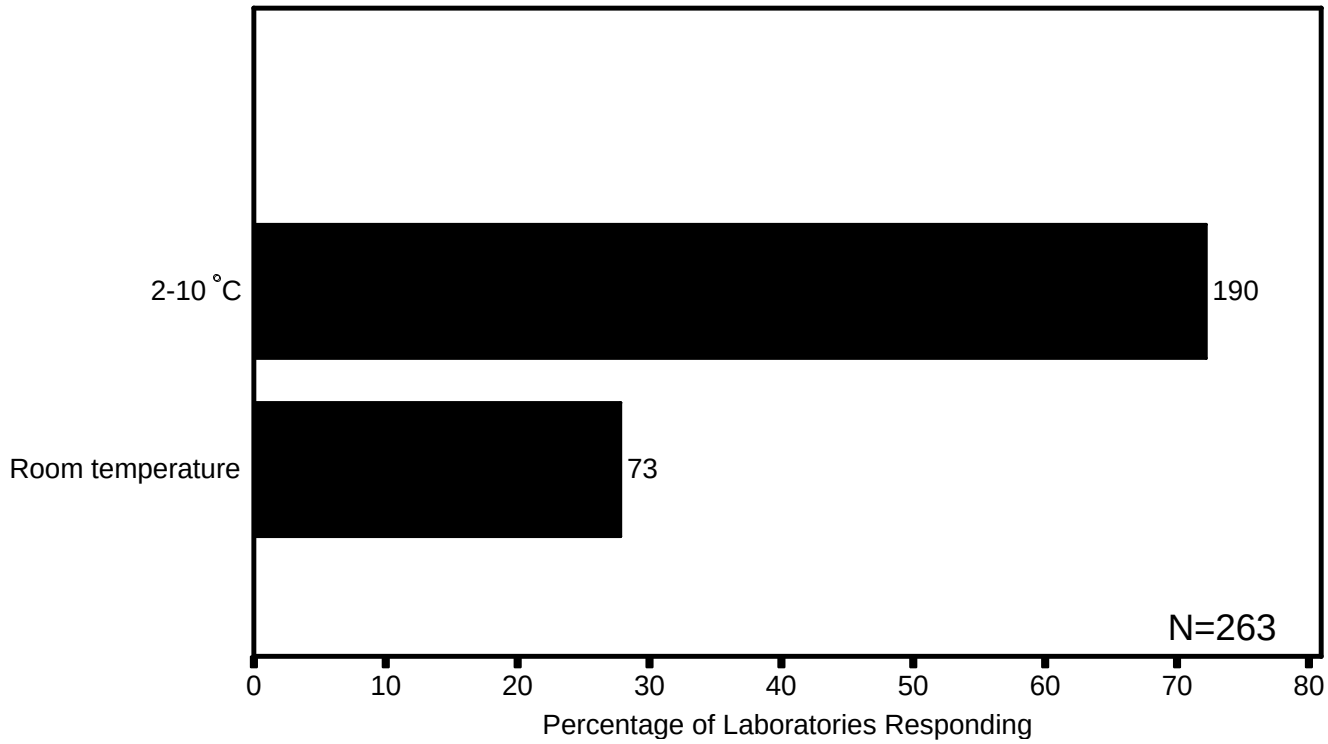
25.(e) At what temperature does your laboratory routinely perform the staining procedure? (Choose only <u>one</u>.)



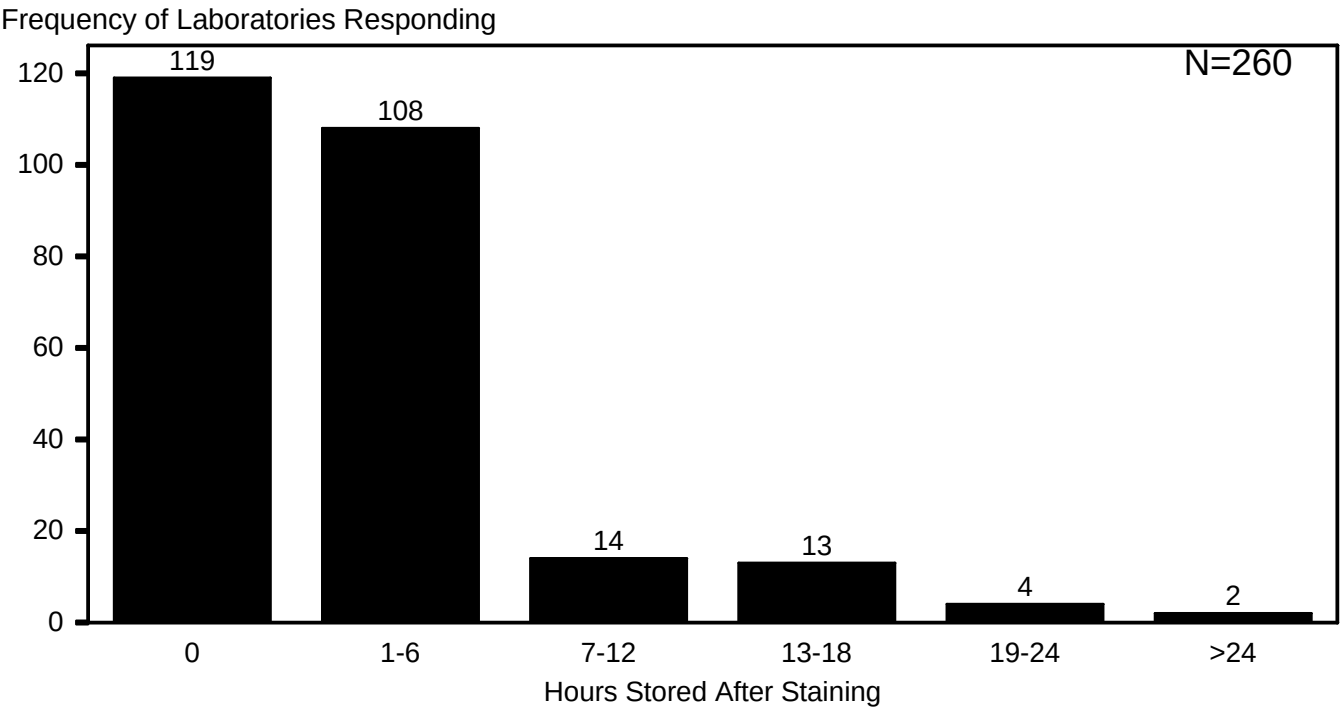
25.(f) Does your laboratory fix cells before TLI flow cytometry is performed?



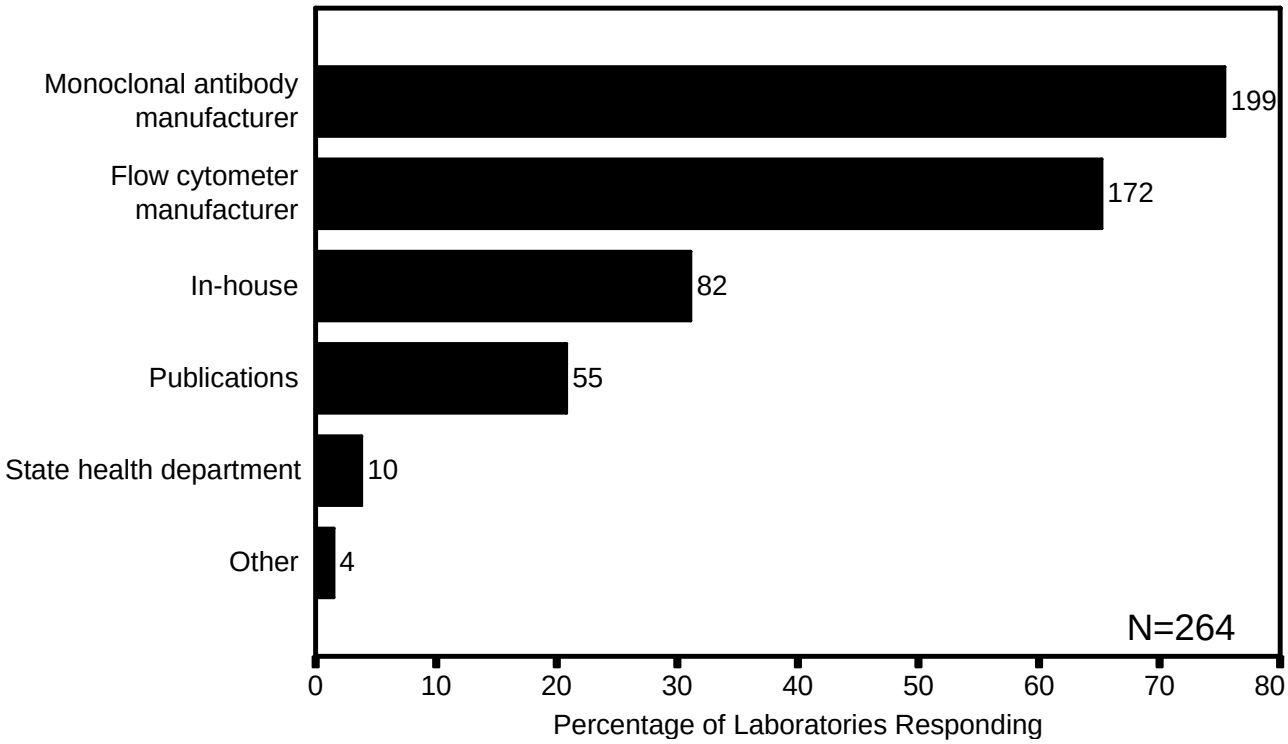
25.(g) At what temperature does your laboratory routinely store cells after staining? (Choose only <u>one</u>.)



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



26. From what source did your laboratory obtain its TLI staining procedure? (Check <u>all</u> that apply.)



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 performingTLI.

Single-color Tests
Monoclonal Antibody Manufacturers Used

Cell Marker	A	B	C	D	E	F	G	H	I	J	K
CD3	1	17	11	1					1		
CD4	2	14	6	1					1		
CD8	1	14	6	1					1		1
CD19	2	19	9	4							
CD16		14	3					1			
CD56		15	10	2							
CD56 & 16	2	16	3								

Two-color Tests
Combinations of Monoclonal Antibody Manufacturers Used

Cell Marker	A/A	A/C	B/A	B/B	B/C	B/H	C/A	C/C	C/D	D/D	H/C	H/H	I/B	I/I	K/K
CD45/CD14	7			68				55		5		3	1		1
CD3/CD4	1			66				53		4		3		1	1
CD3/CD8	1			65				52		4		3		1	1
CD3/CD19	3			59	1			30		4		2	1		1
CD3/CD16		1	1	16		1	1	3				1		1	1
CD3/CD56	5	1		18				13	1	1	1		1		1
CD3/CD56 & 16	2		1	72	1		1	3							

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
Combinations of Monoclonal Antibody Manufacturers Used

Cell Marker	A/A/A	A/A/C	A/B/B	A/C/A	A/C/C	B/A/B	B/B/B	B/C/B	B/D/B	C/C/A	C/C/C	D/D/D	H/H/H	K/B/B	K/B/K	K/D/D	K/K/K
C45/CD3/CD4	2				1		59				11	1		2			2
C45/CD3/CD8	1				1		55				10	1		1			2
C45/CD3/CD19	1			1	2		45				3	1		1			1
C45/CD3/CD56 & 16			1	1			40							1			1
CD3/CD4/CD8	8				1		10			1	10		5	2			1
CD3/CD19/CD16	3	1					4						5		1		
CD3/CD19/CD56	3						5			1	4						
CD3/CD19/CD56 & 16						1	7	1	1							1	

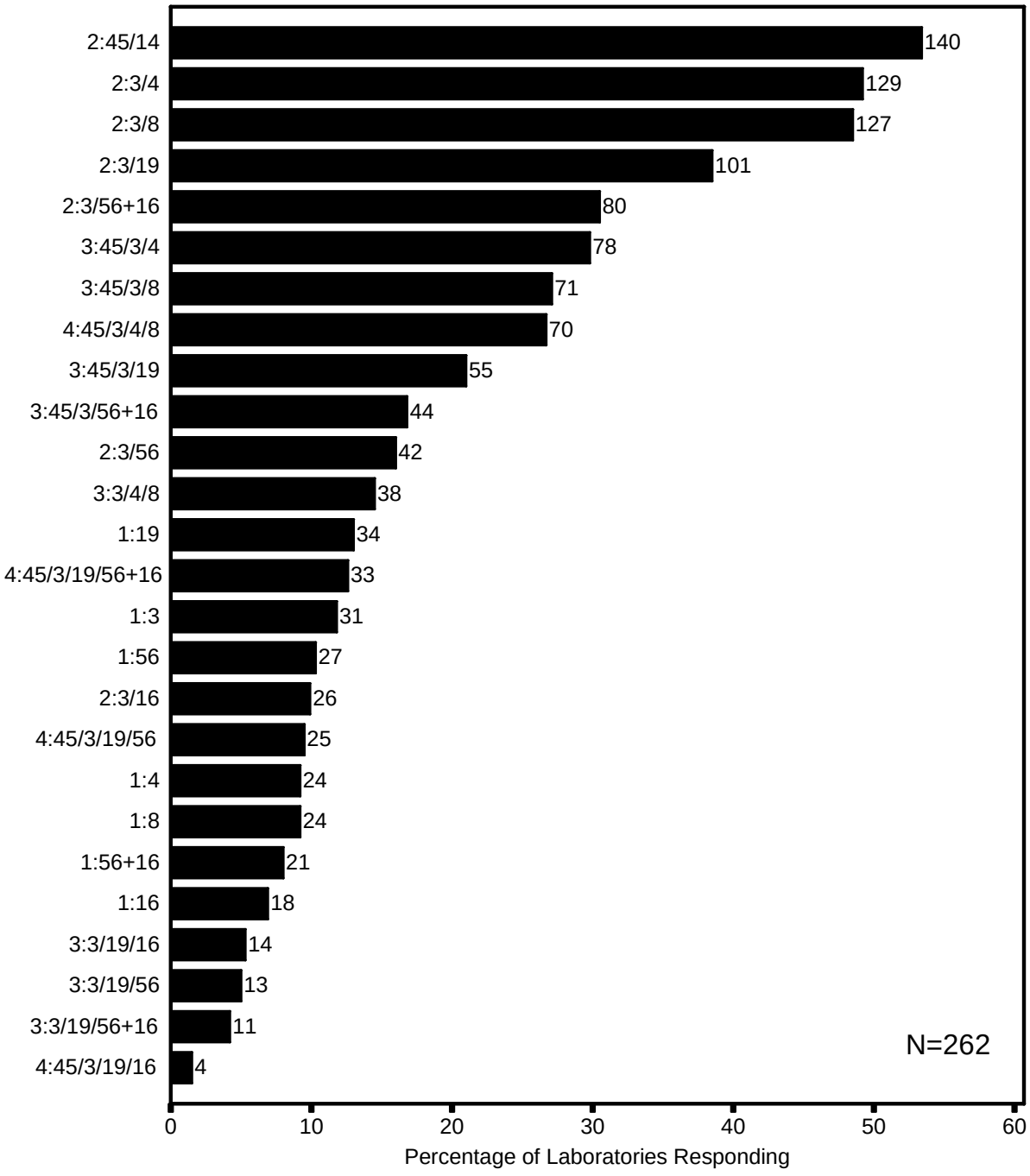
Four-color Tests
Combinations of Monoclonal Antibody Manufacturers Used

Cell Marker	B/B/A/C	B/B/B/B	B/B/B/C	B/B/C/B	B/B/K/B	C/B/C/B	C/C/C/C	K/B/C/B	K/C/C/C
CD45/CD3/CD4/CD8		31	1		1		36		1
CD45/CD3/CD19/CD16		2					2		
CD45/CD3/CD19/CD56	1	2					22		
CD45/CD3/CD19/CD56 & 16		26		1		1	4	1	

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. continued:

Summary of monoclonal antibody reagents used by participant laboratories

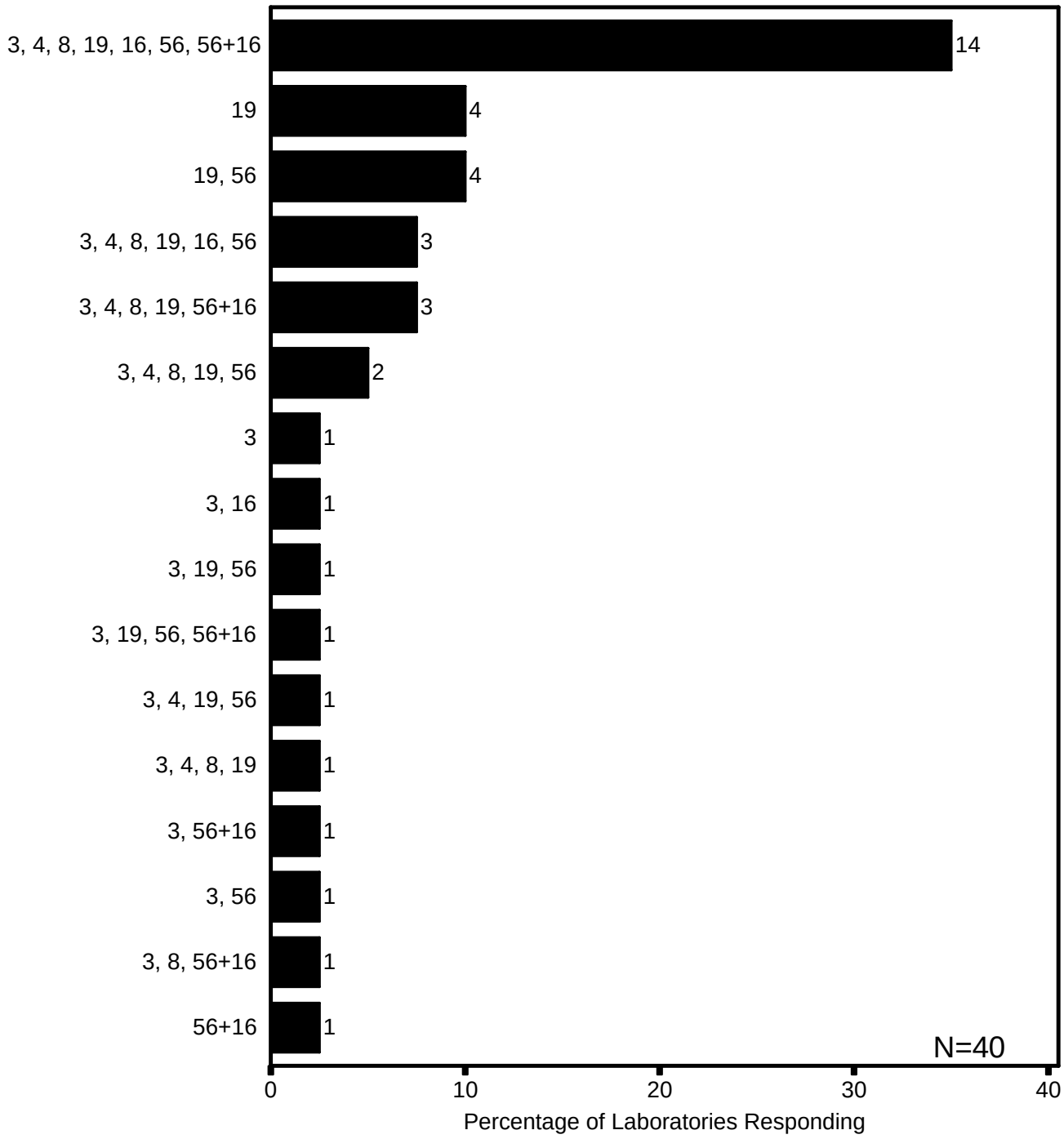


Key for interpretation

1: one-color 2: two-color 3: three-color 4: four-color

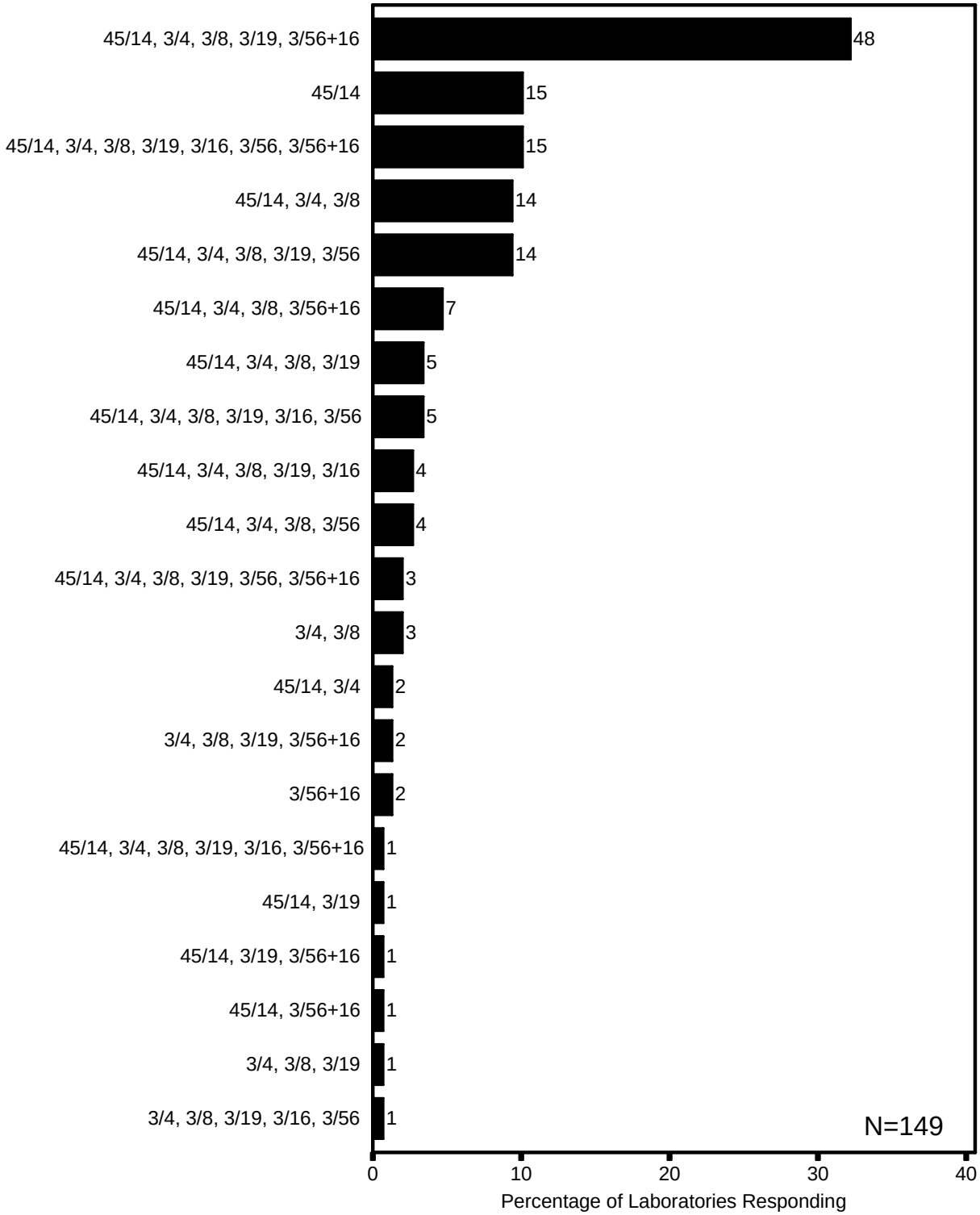
27. continued:

One-color monoclonal antibody reagent panels used by participant laboratories



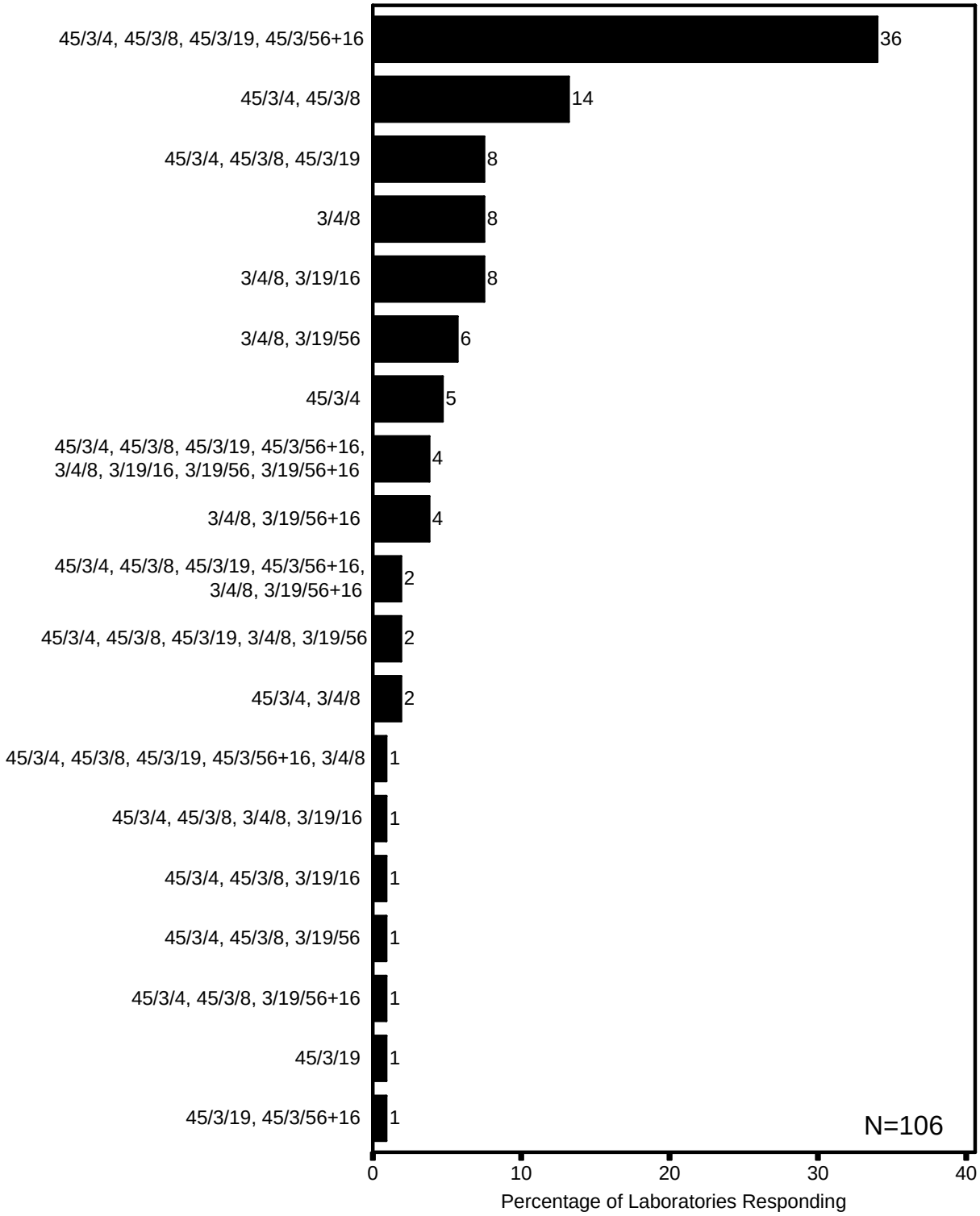
27. continued:

Two-color monoclonal antibody reagent panels used by participant laboratories



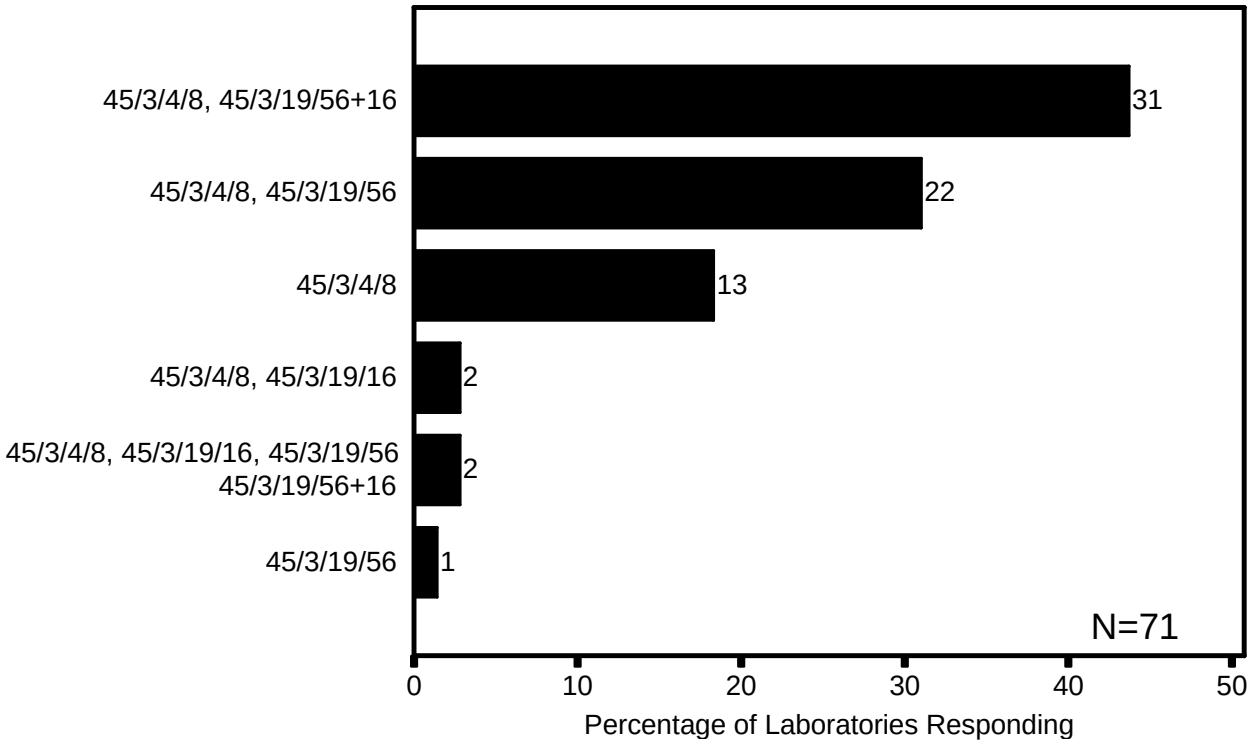
27. continued:

Three-color monoclonal antibody reagent panels used by participant laboratories

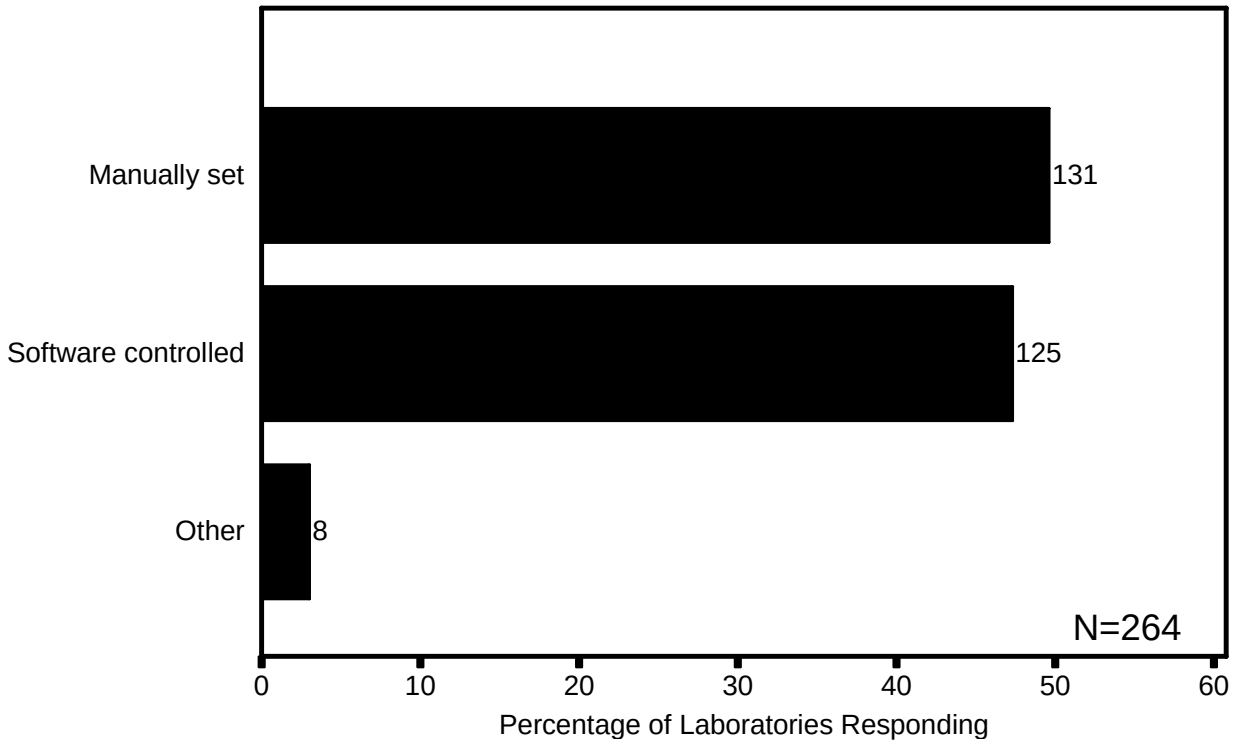


27. continued:

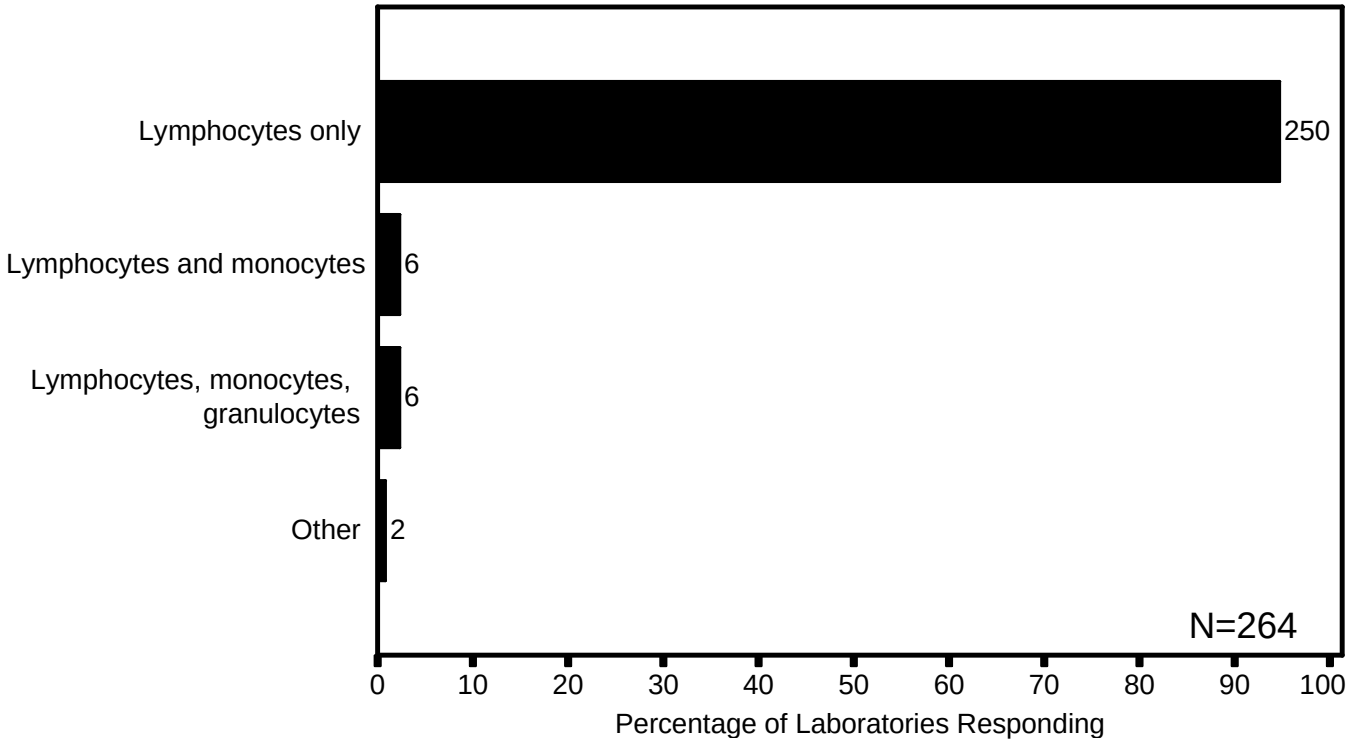
Four-color monoclonal antibody reagent panels used by participant laboratories



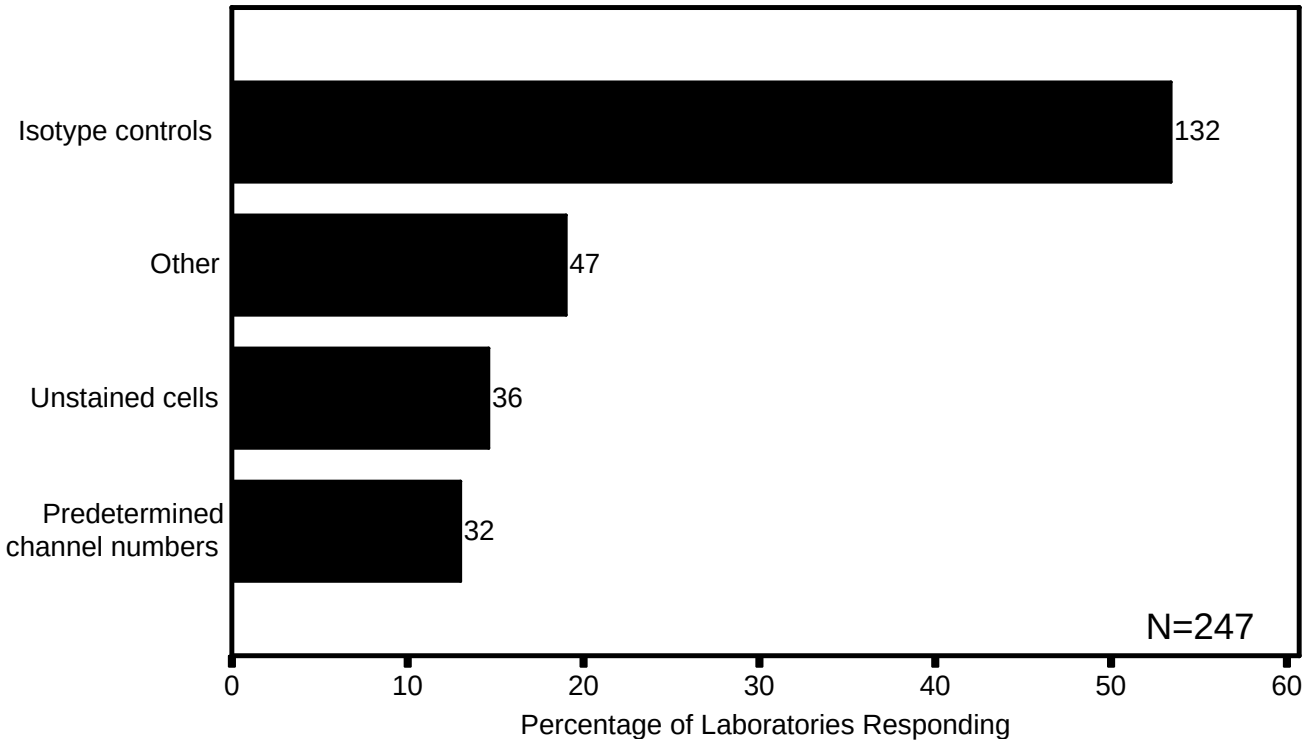
28.(a) How is the gating set when performing TLI on your flow cytometers? (Choose only <u>one</u>.)



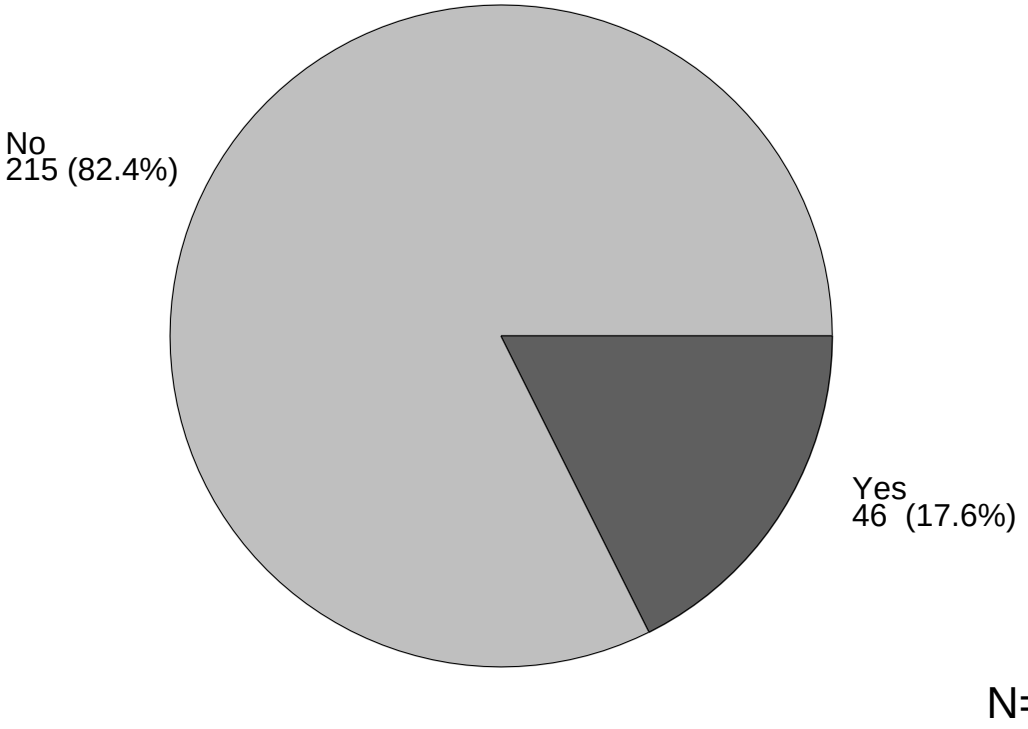
28.(b) Which cell populations are included in the gates for analyses of lymphocyte phenotypes? (Choose only <u>one</u>.)



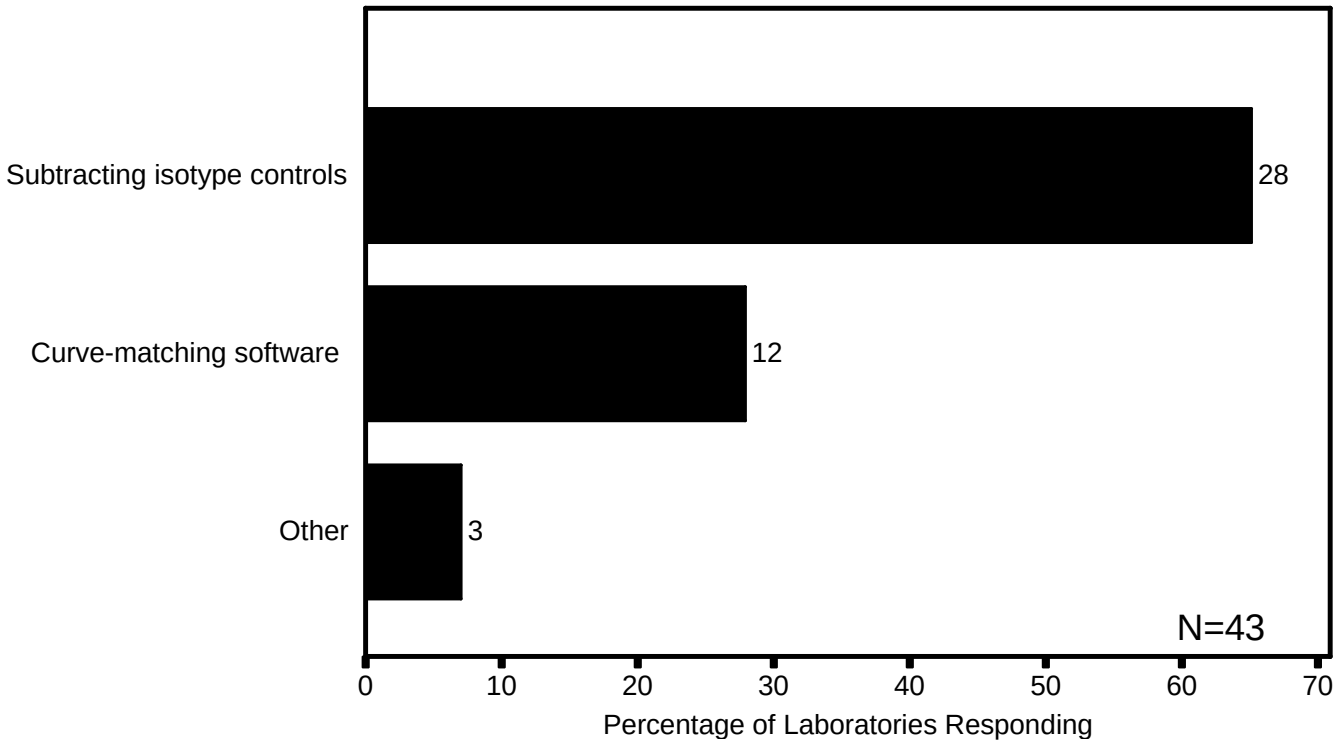
29. How are the integration windows set? (Choose only one.)



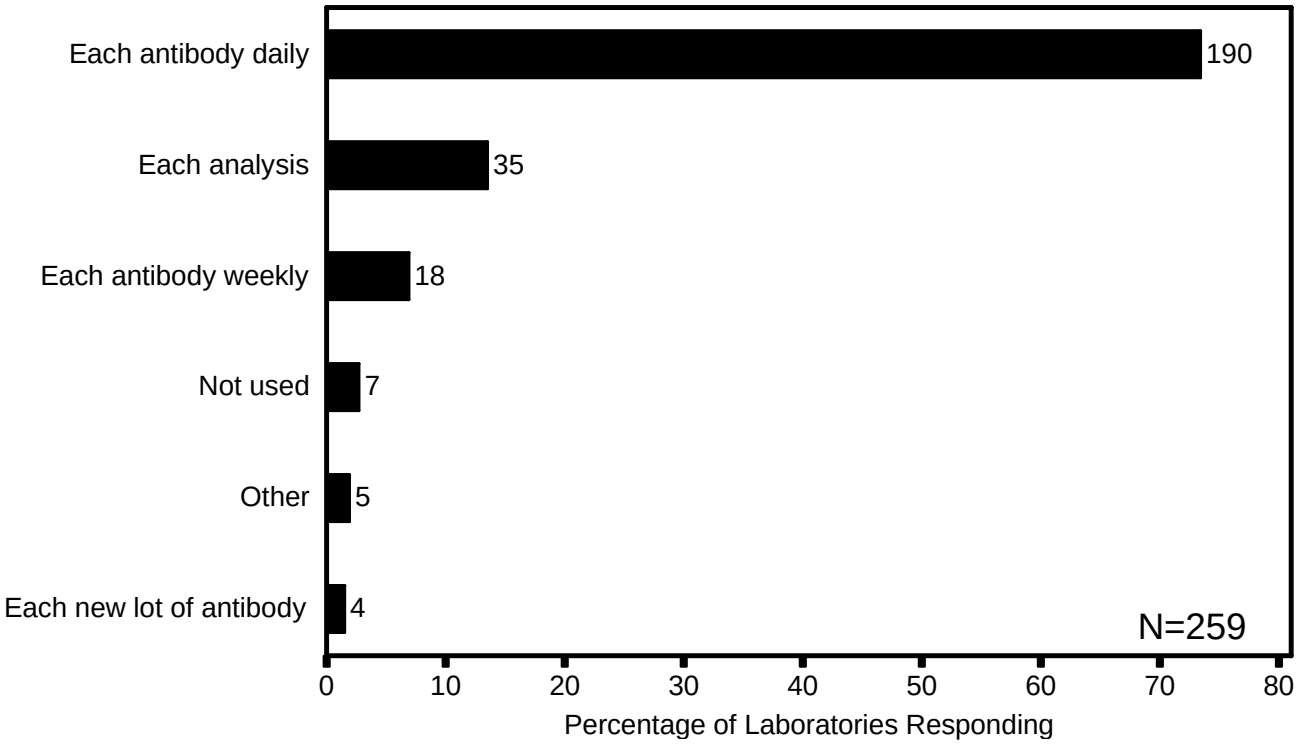
30.(a) Do you mathematically adjust your phenotype values for isotype control values?



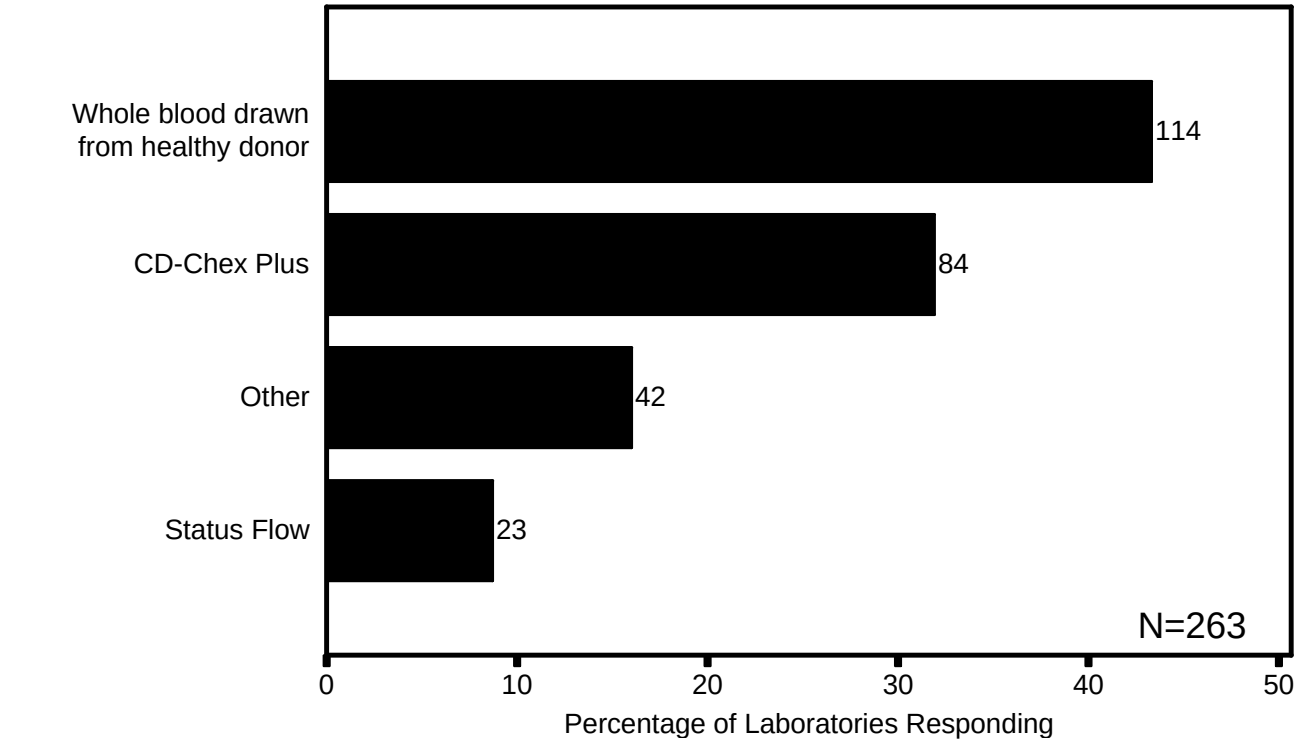
30.(b) How do you adjust your phenotype values? (Choose only <u>one</u>.)



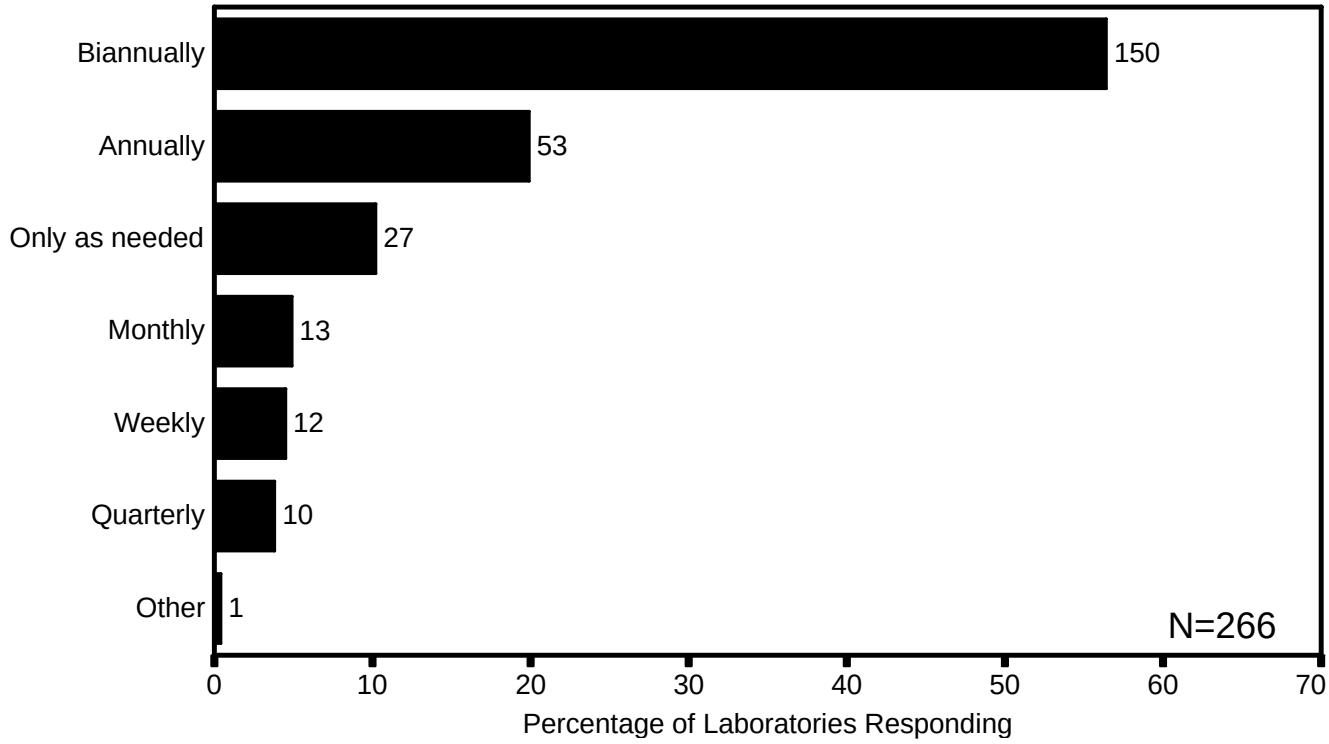
31.(a) How often are normal cell controls used? (Choose only one.)



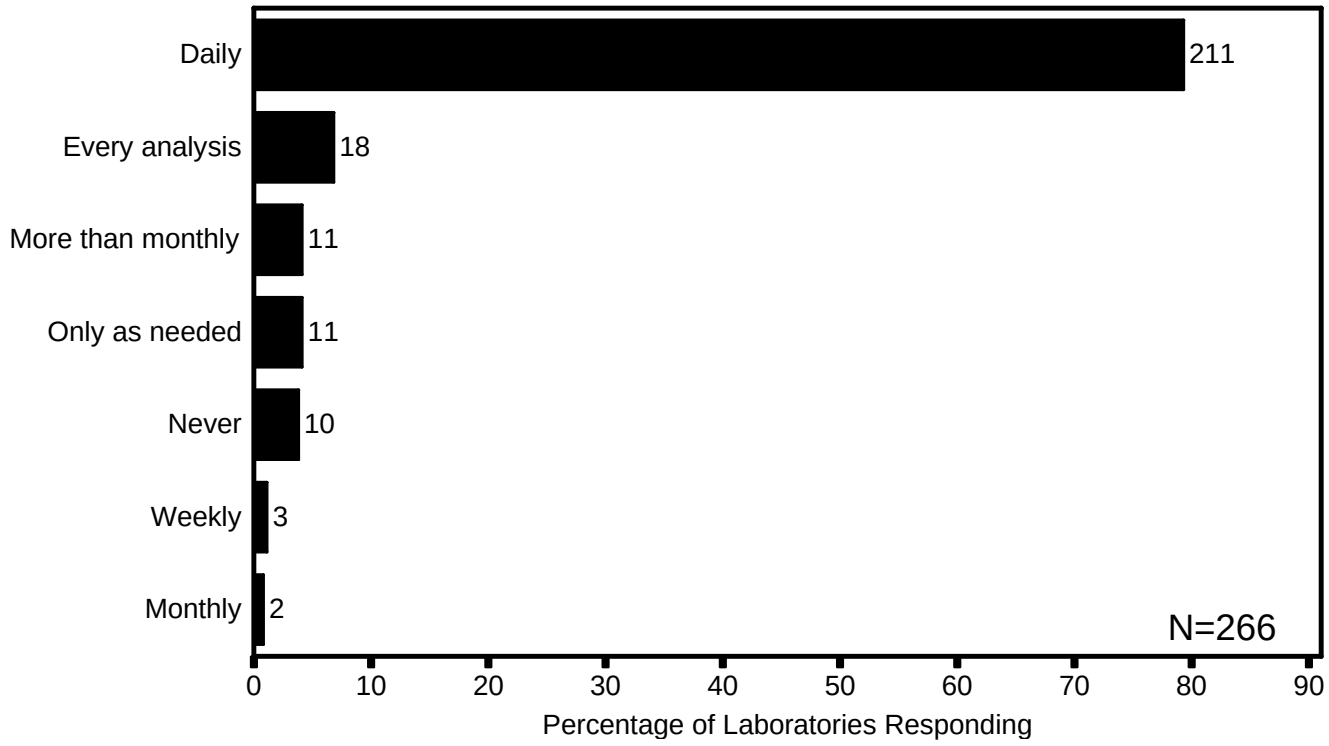
31.(b) Which normal cell control is primarily used in your laboratory? (Choose only one.)



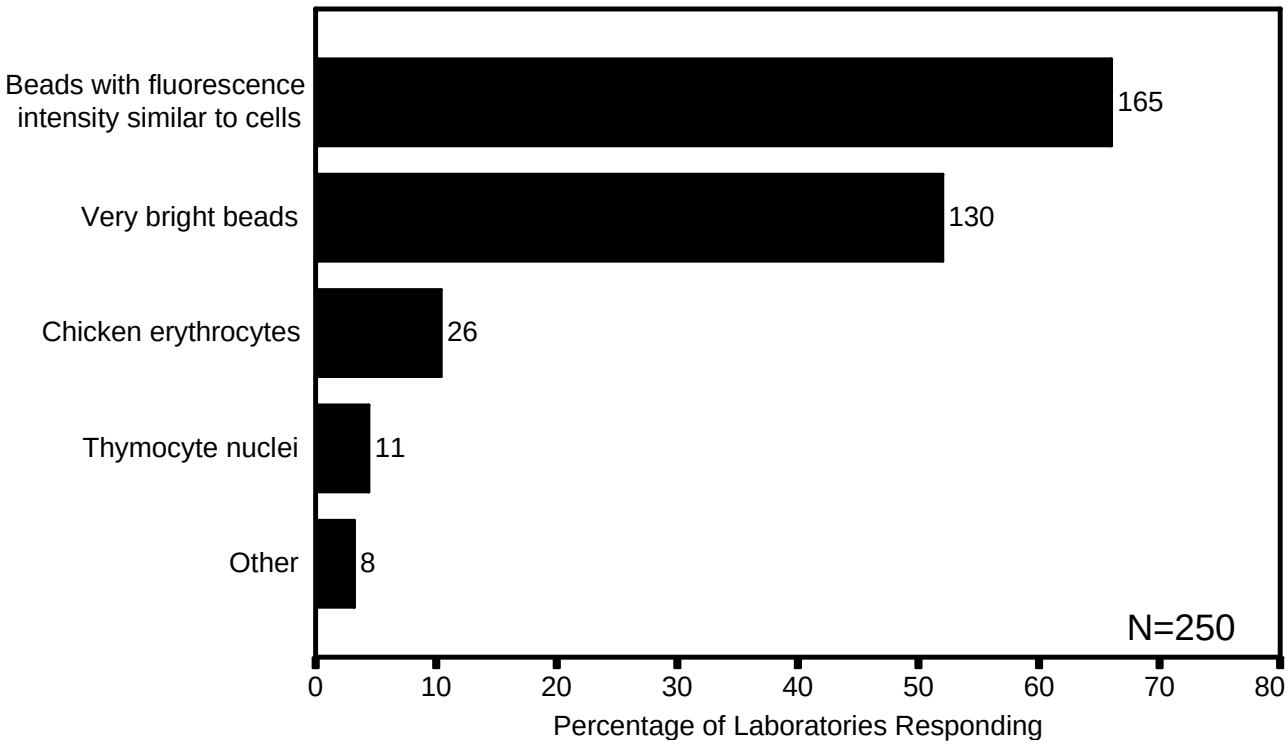
32. How often do your flow cytometer(s) receive preventive maintenance (e.g., cleaning of optical filters and lenses, and fluidics check)? (Choose only <u>one</u>.)



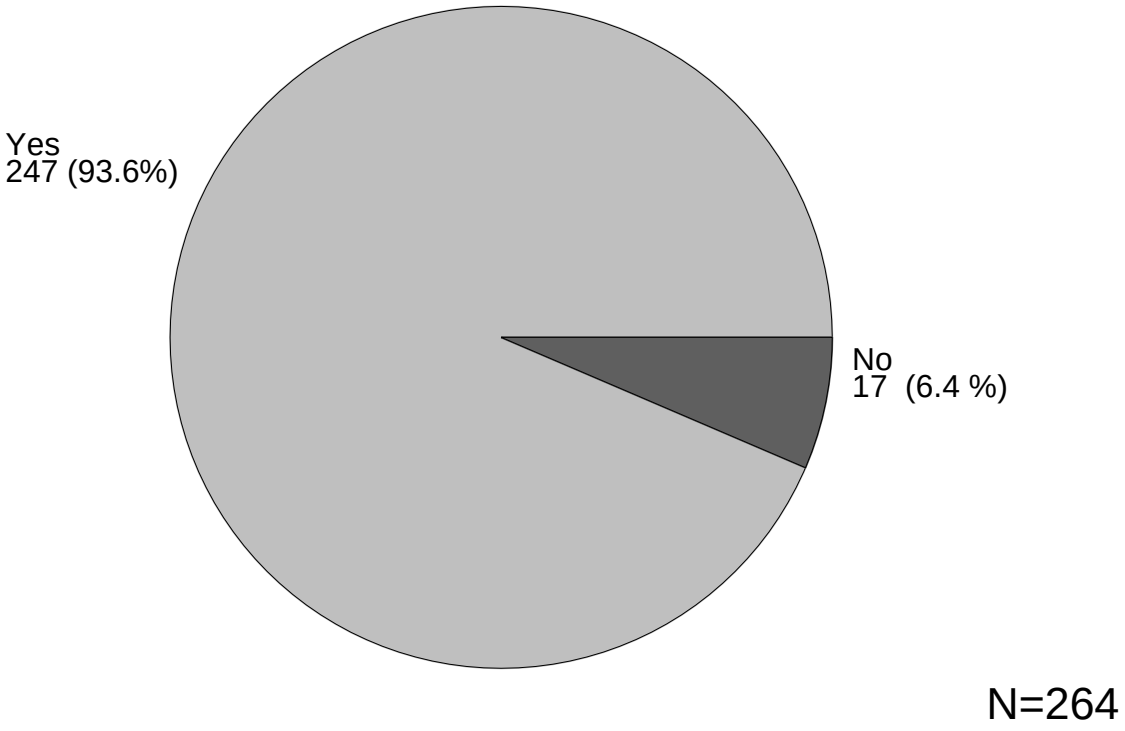
33.(a) How often does your laboratory check the optical alignment of your flow cytometer(s)? (Choose only one.)



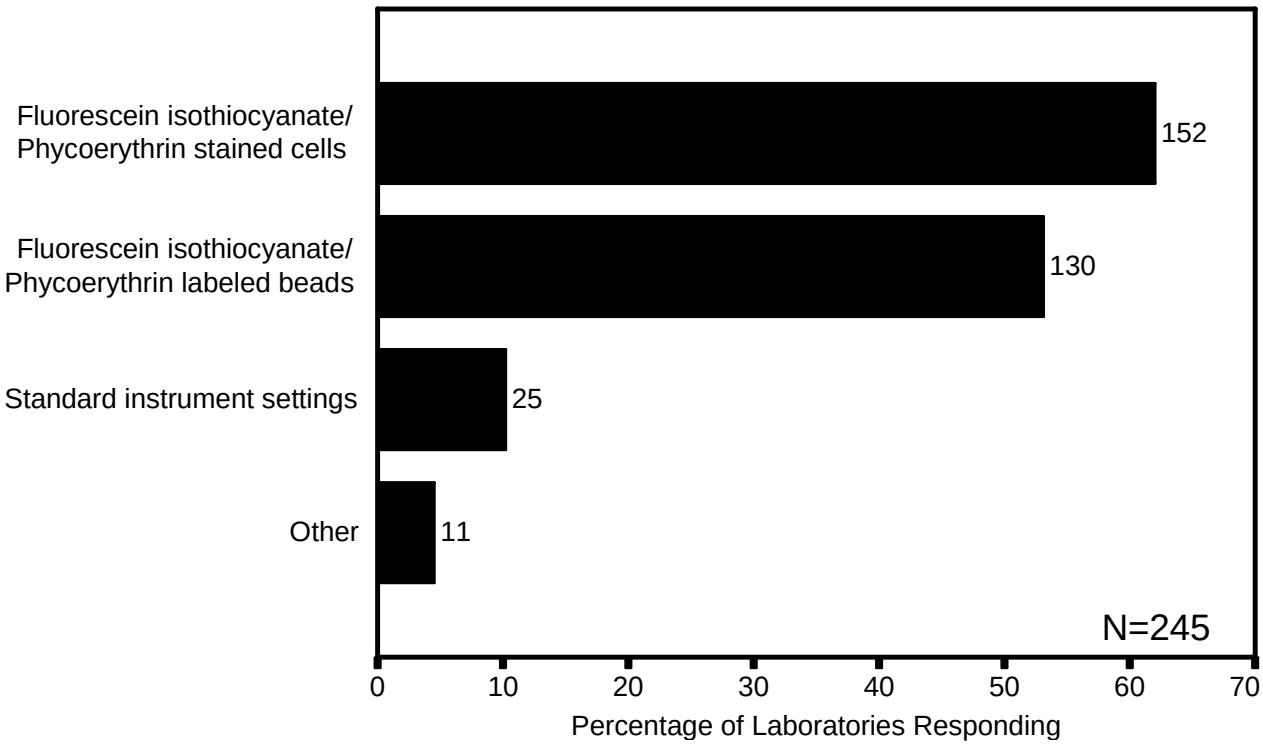
33.(b) What types of particles are used by your laboratory to align your flow cytometers? (Check all that apply.)



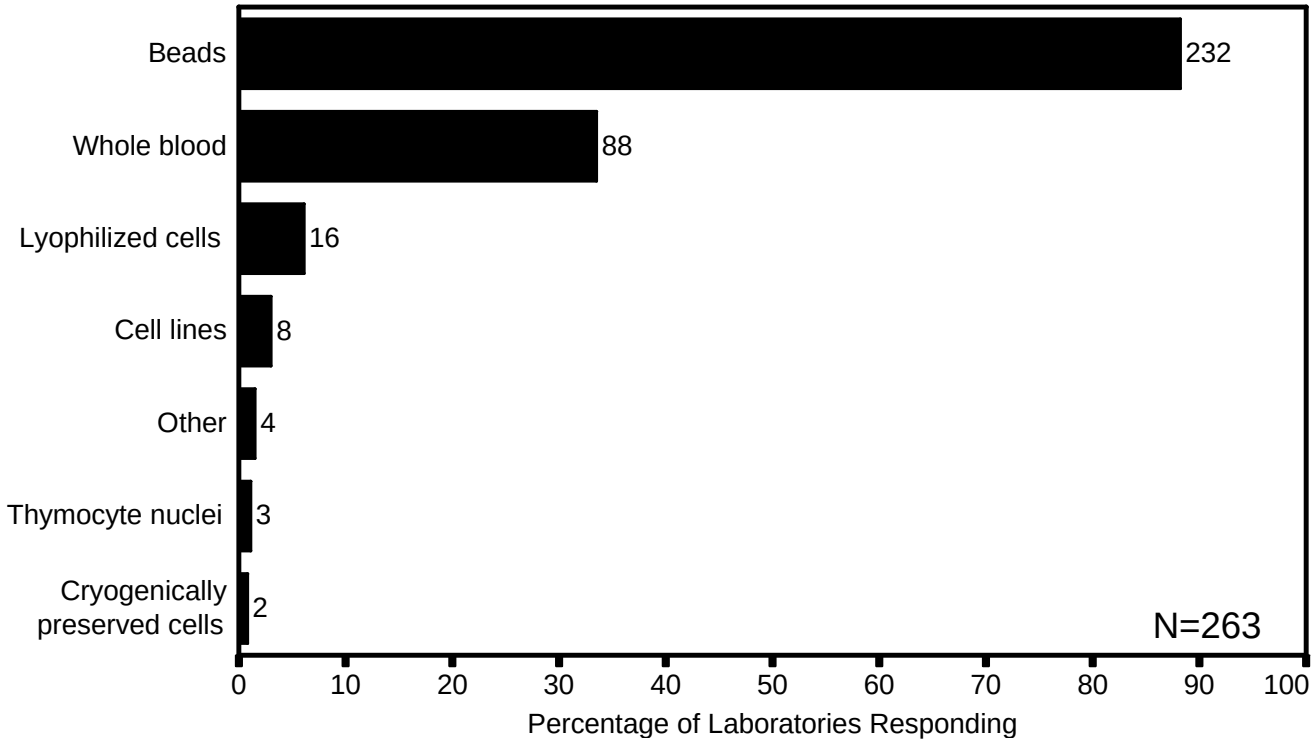
34.(a) Does your laboratory set fluorescence overlap compensation?



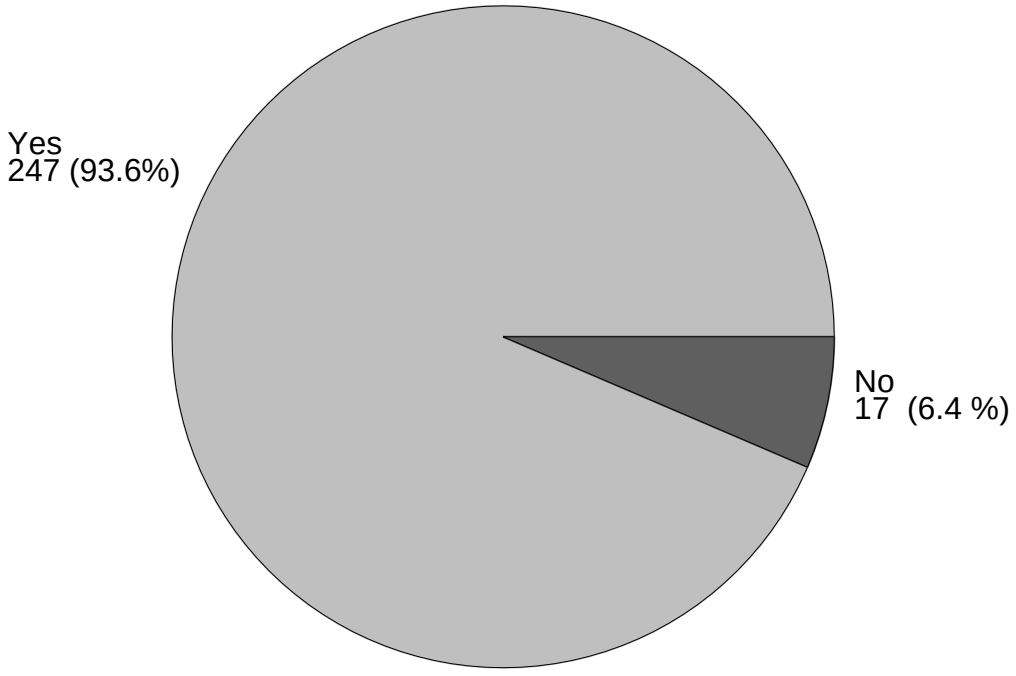
34.(b) What reference materials are used to set fluorescence overlap compensation? (Check <u>all</u> that apply.)



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 <u>all</u> that apply.)

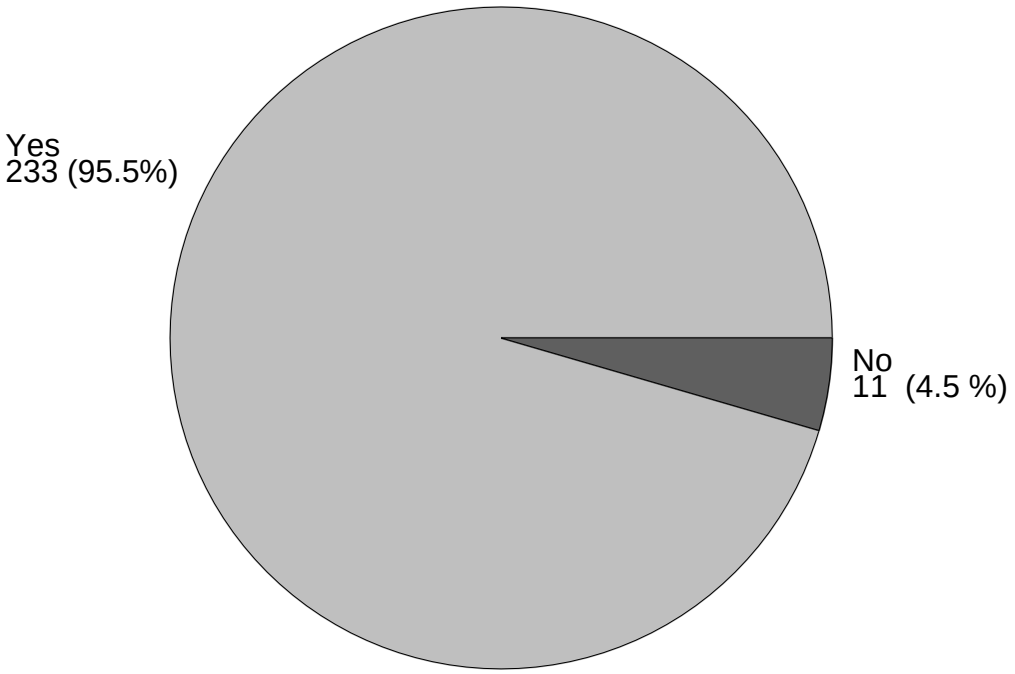


35.(b) Does your laboratory routinely record the instrument settings used to reach the target conditions for FALS?



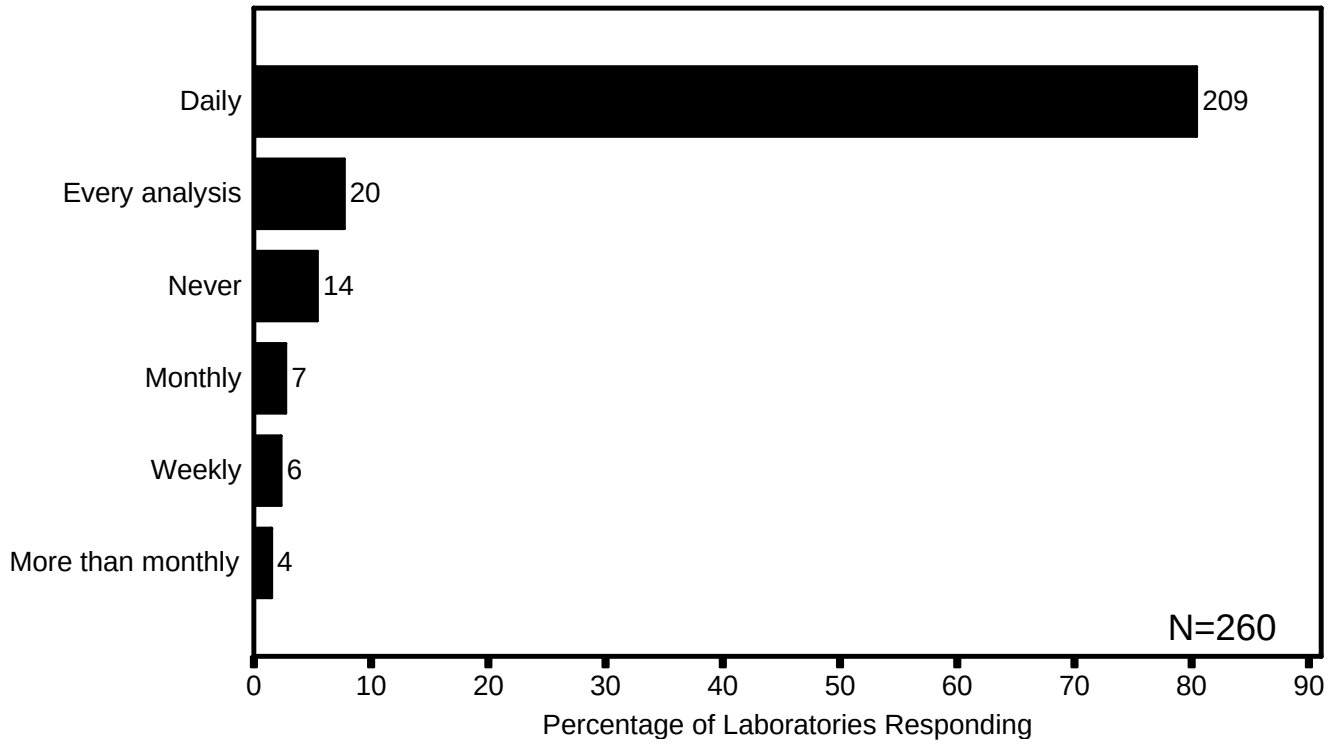
N=264

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

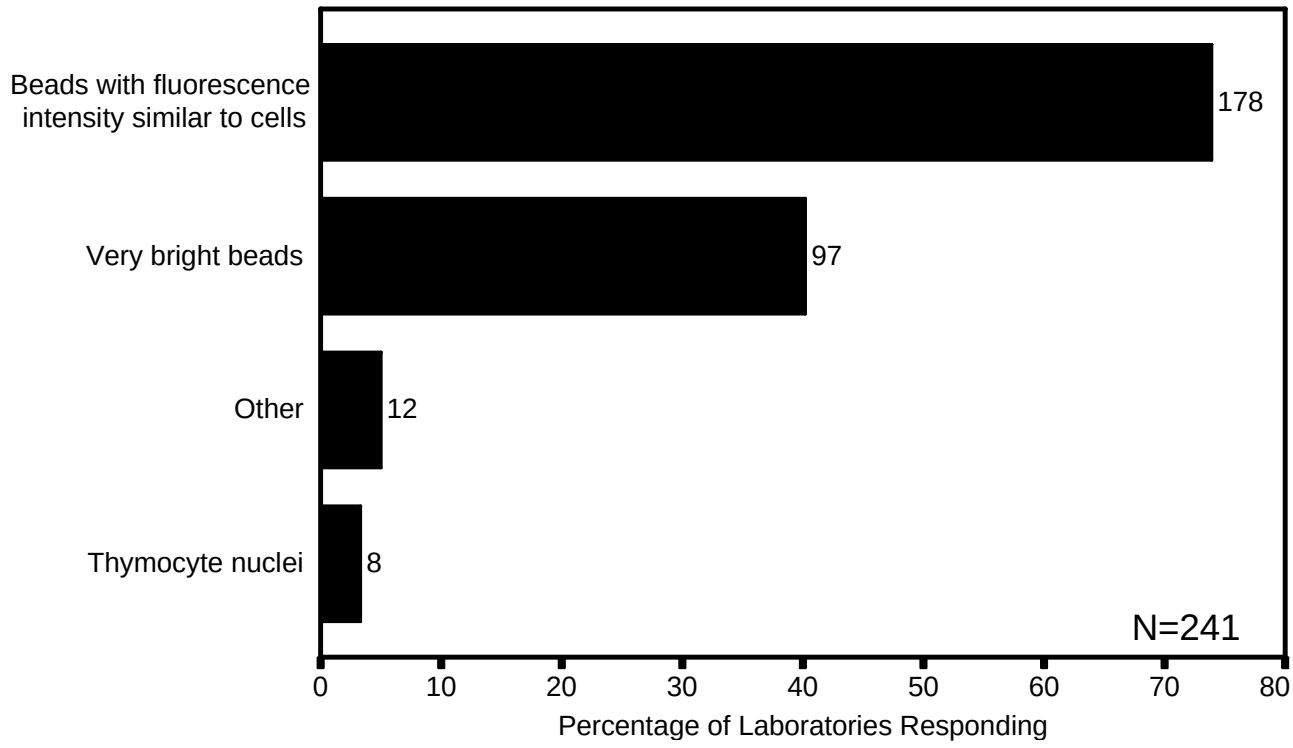


N=244

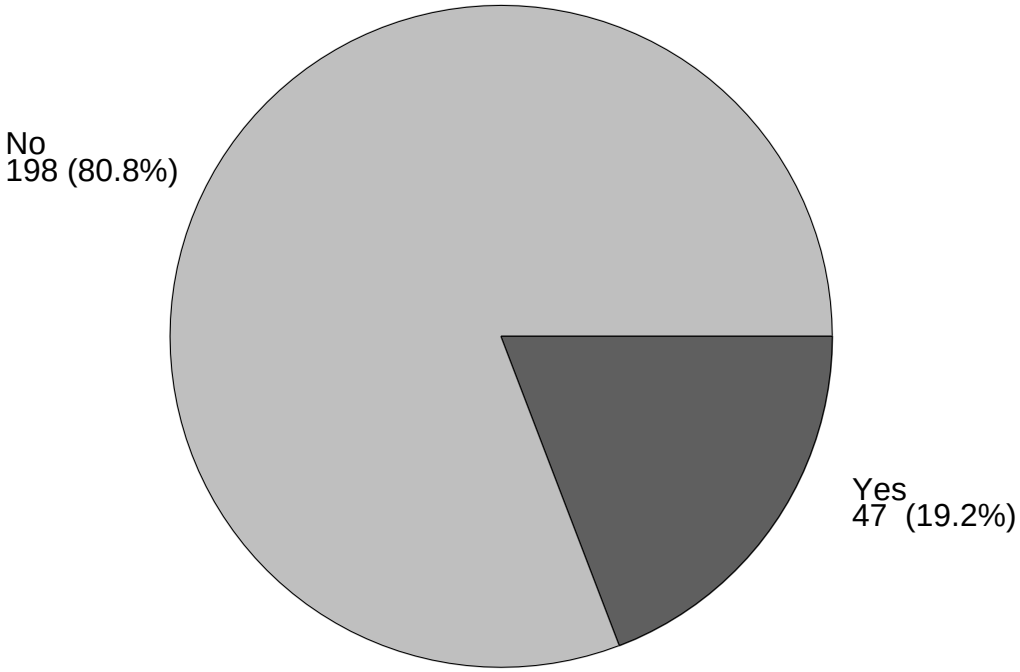
36.(a) How often does your laboratory standardize your flow cytometer(s)? (Choose only <u>one</u>.)



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

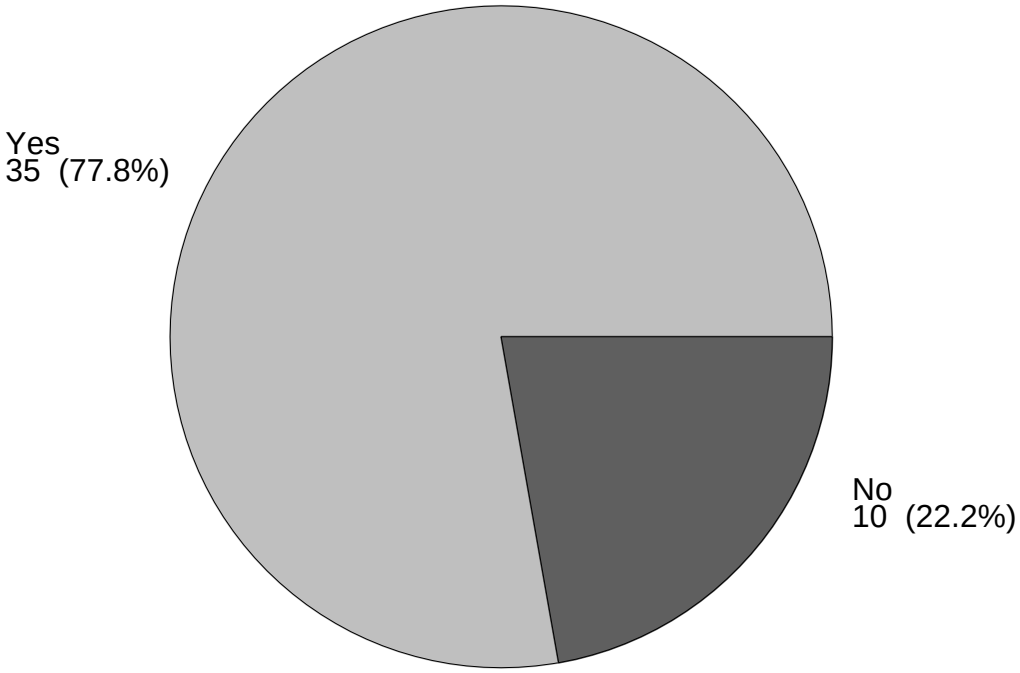


36.(c) Does your laboratory use reference standards to plot standard curves of mean channel fluorescence vs. molecules of equivalent soluble fluorochrome?



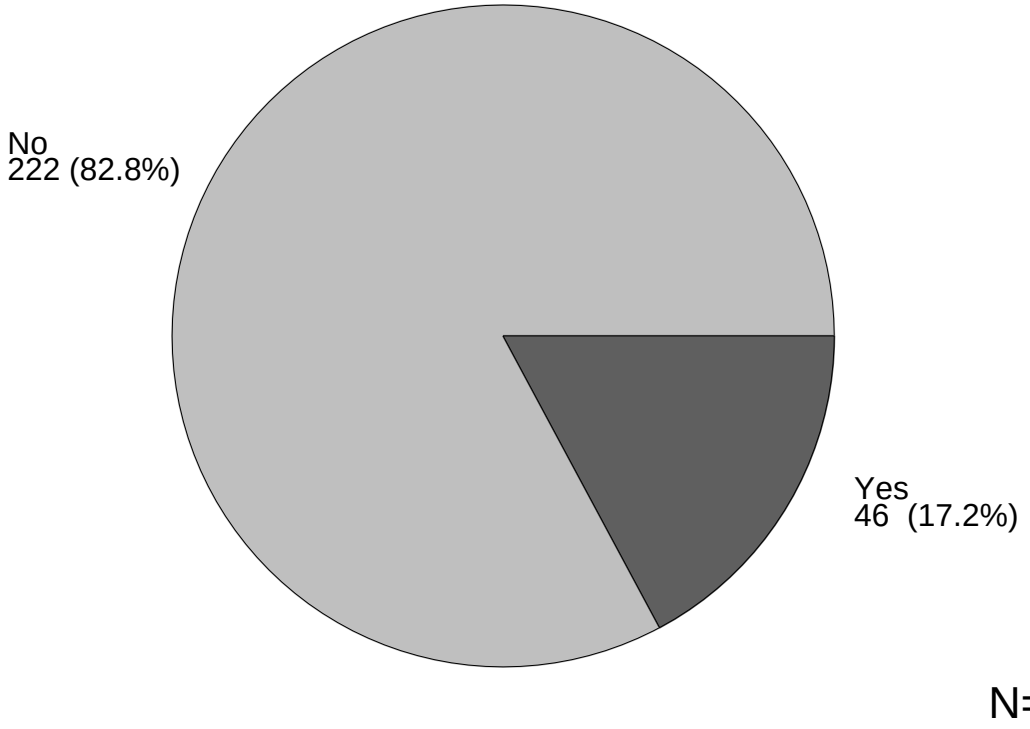
N=245

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

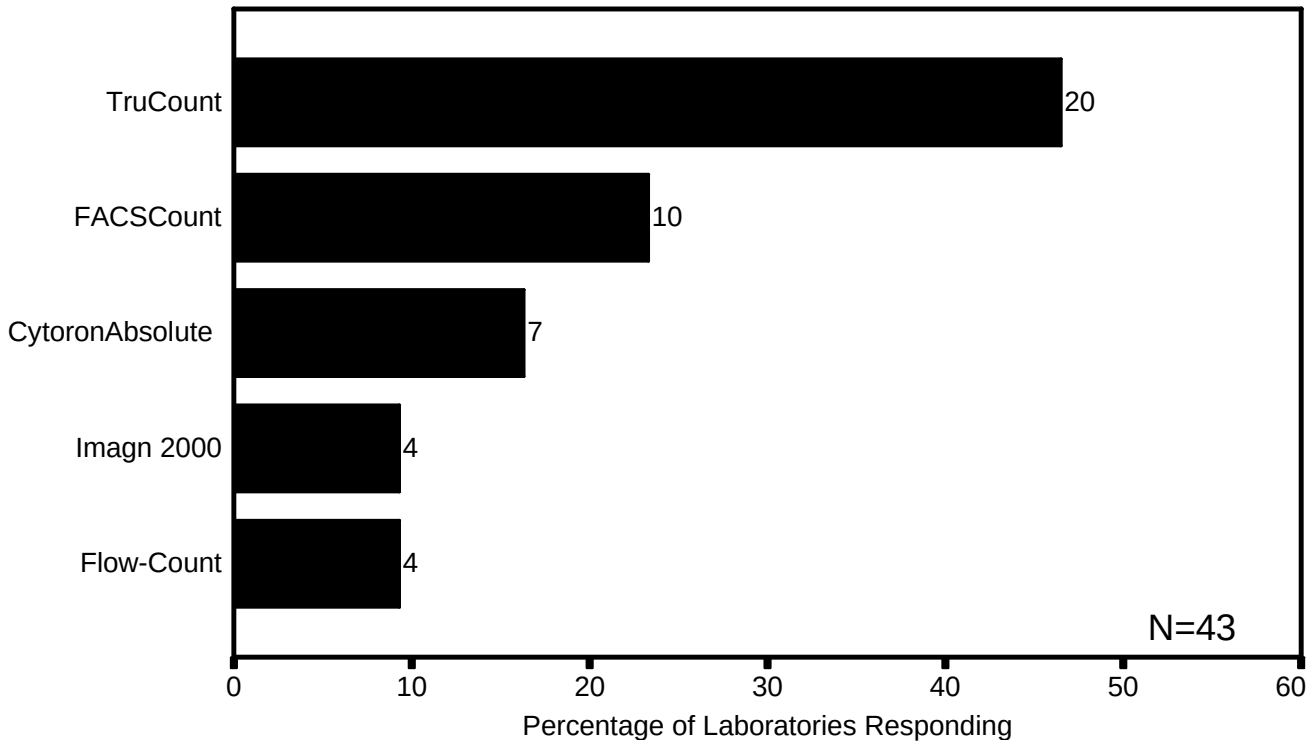


N=45

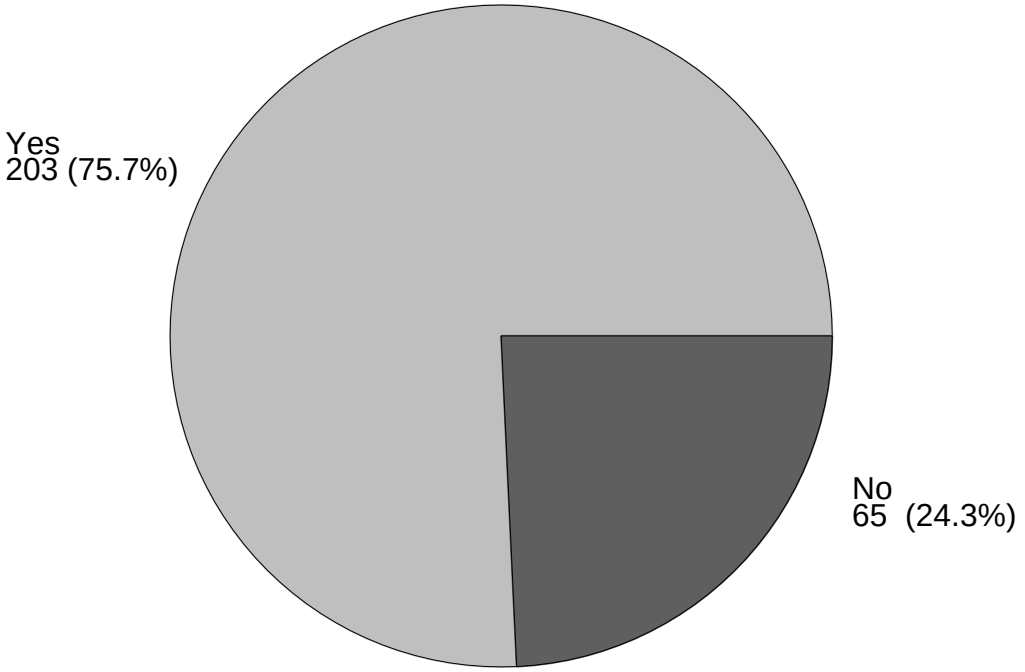
37. Does your laboratory obtain marker-specific absolute counts (e.g., absolute CD4 count) using a single-platform method?



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

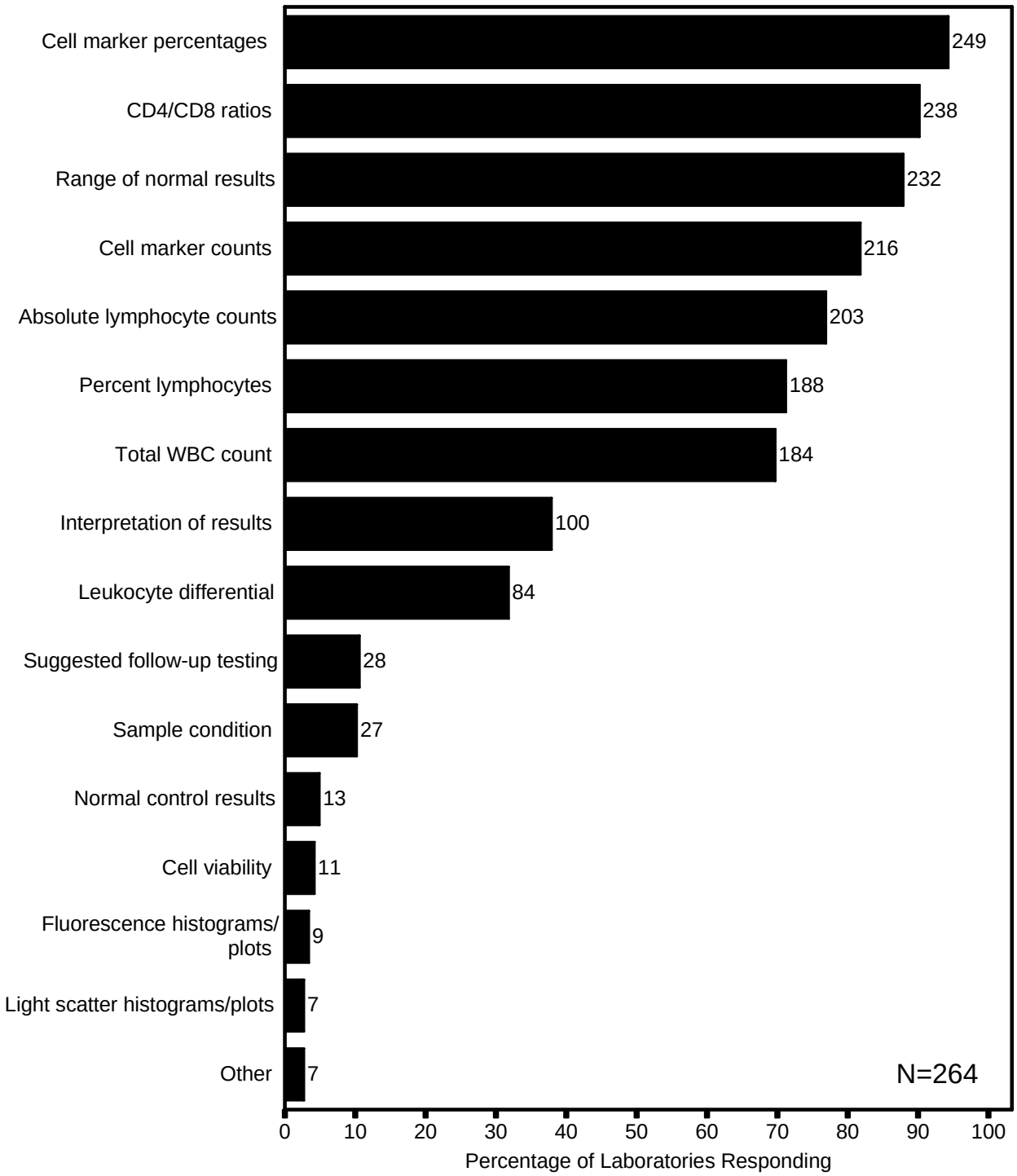


39. Before TLI results are reported, are they routinely reviewed by someone other than the person(s) who performed the tests?

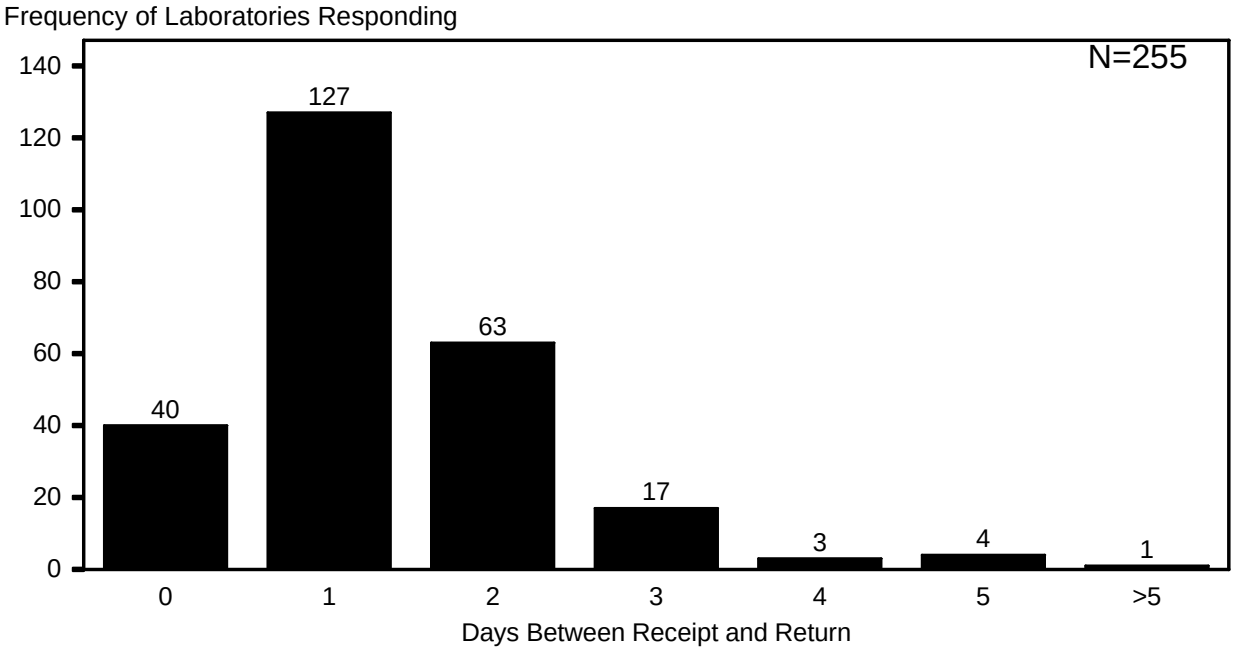


N=268

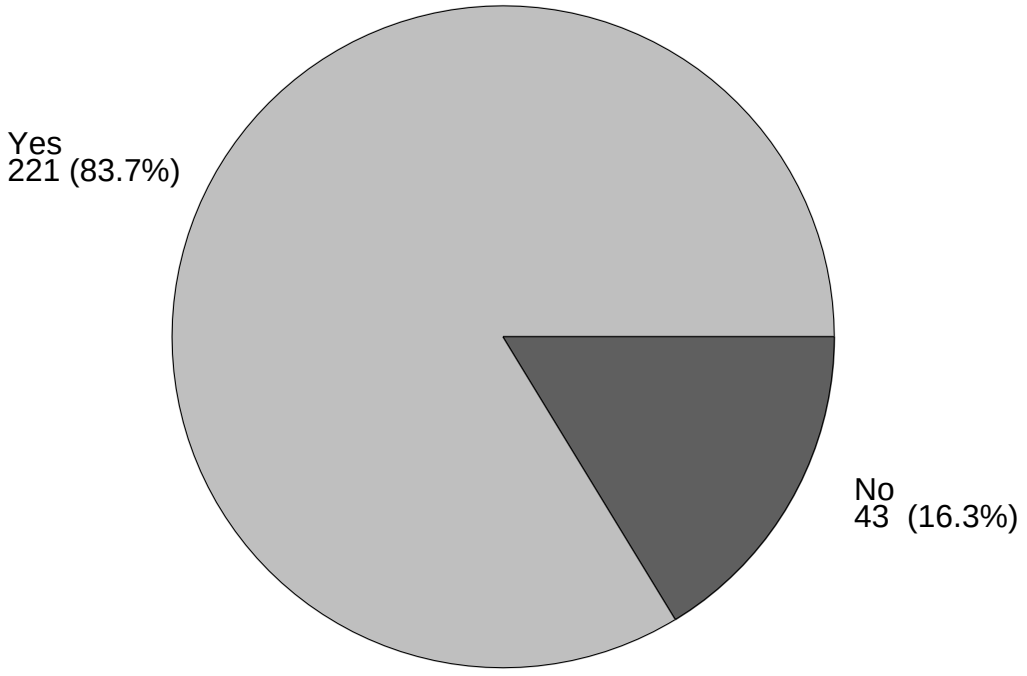
40. What information is included in the report returned to the person or institution initiating the request for TLI? (Check <u>all</u> that apply.)



41. On average, how many days elapse between <u>receipt</u> of the specimen in your laboratory and the time the results of the test are <u>returned</u> 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.)

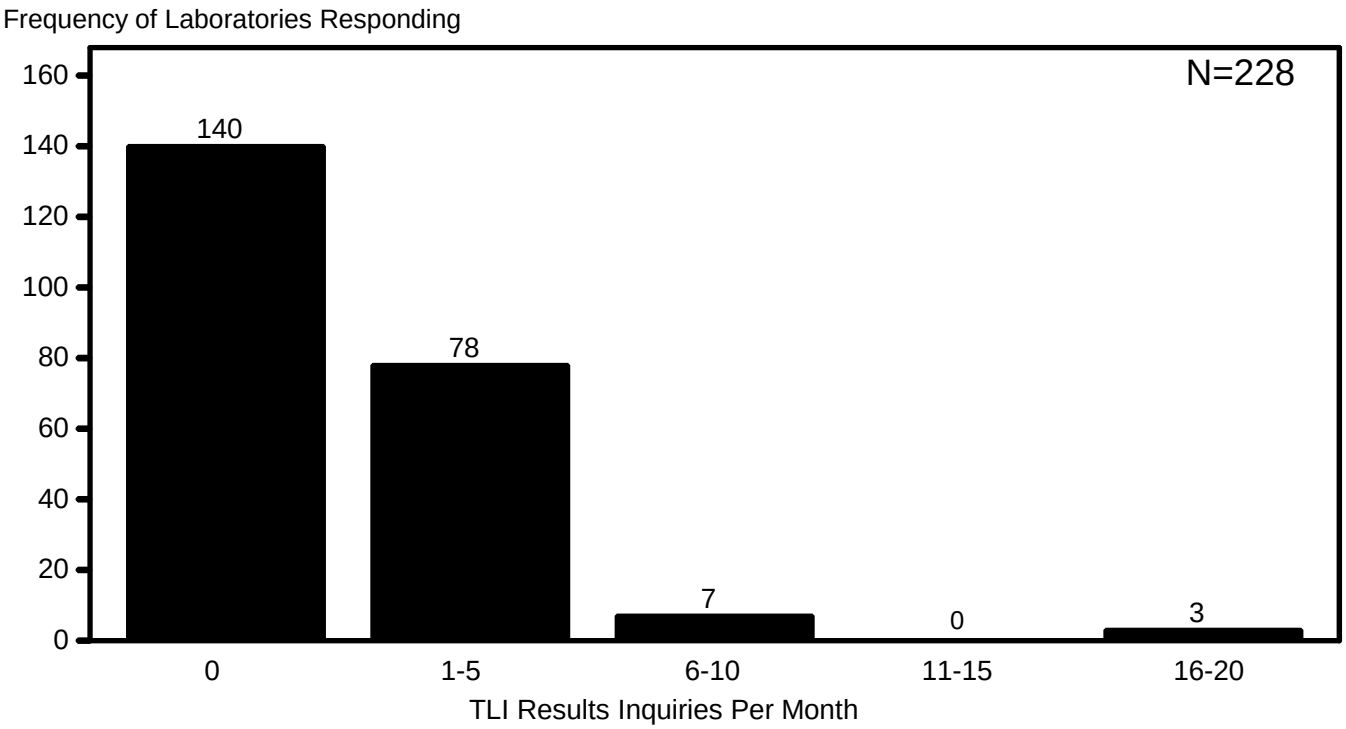


42. Does your laboratory have procedures for protecting the confidentiality of TLI results?

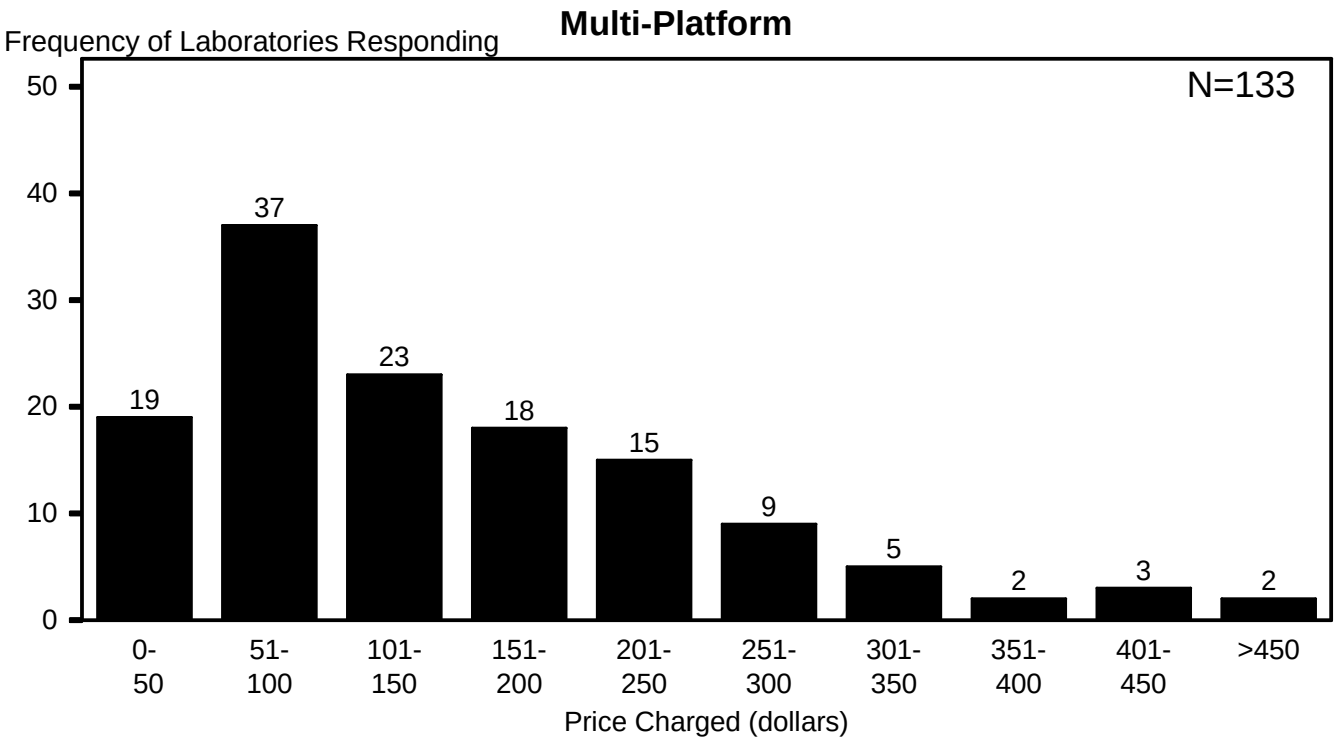


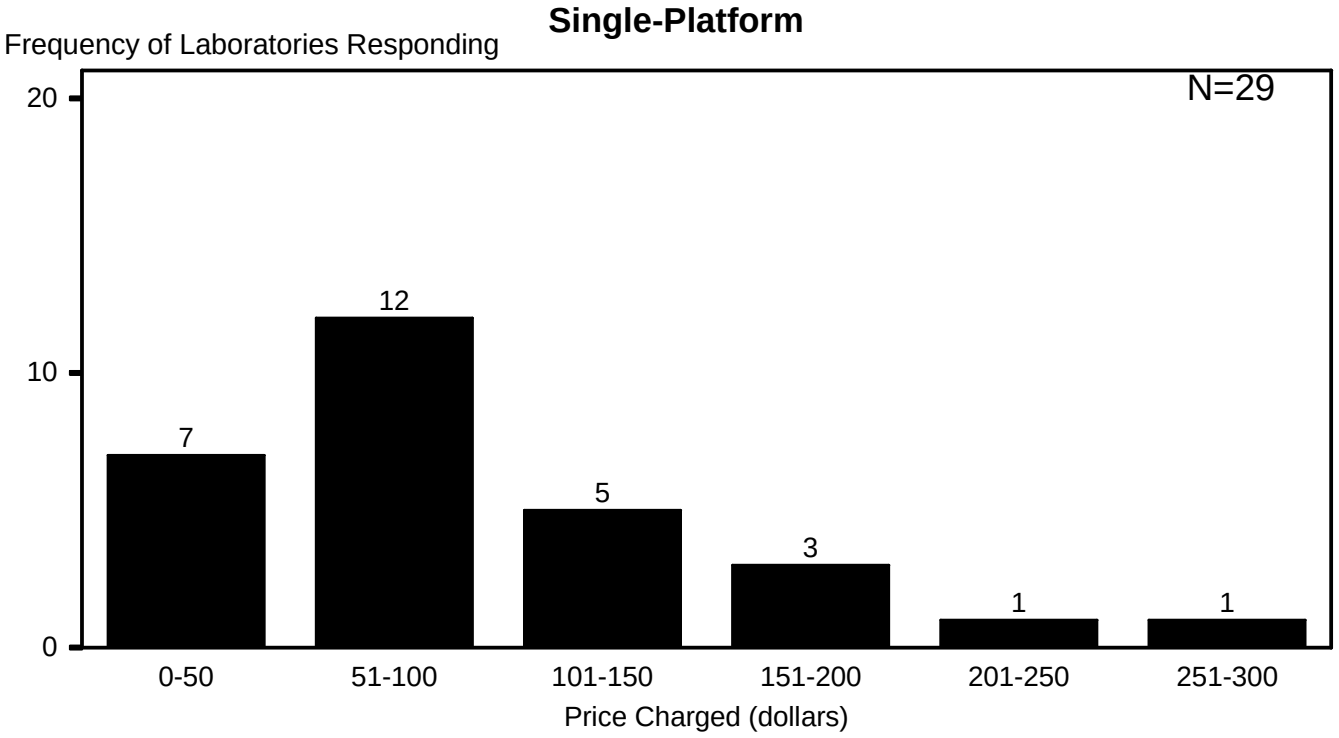
N=264

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.)

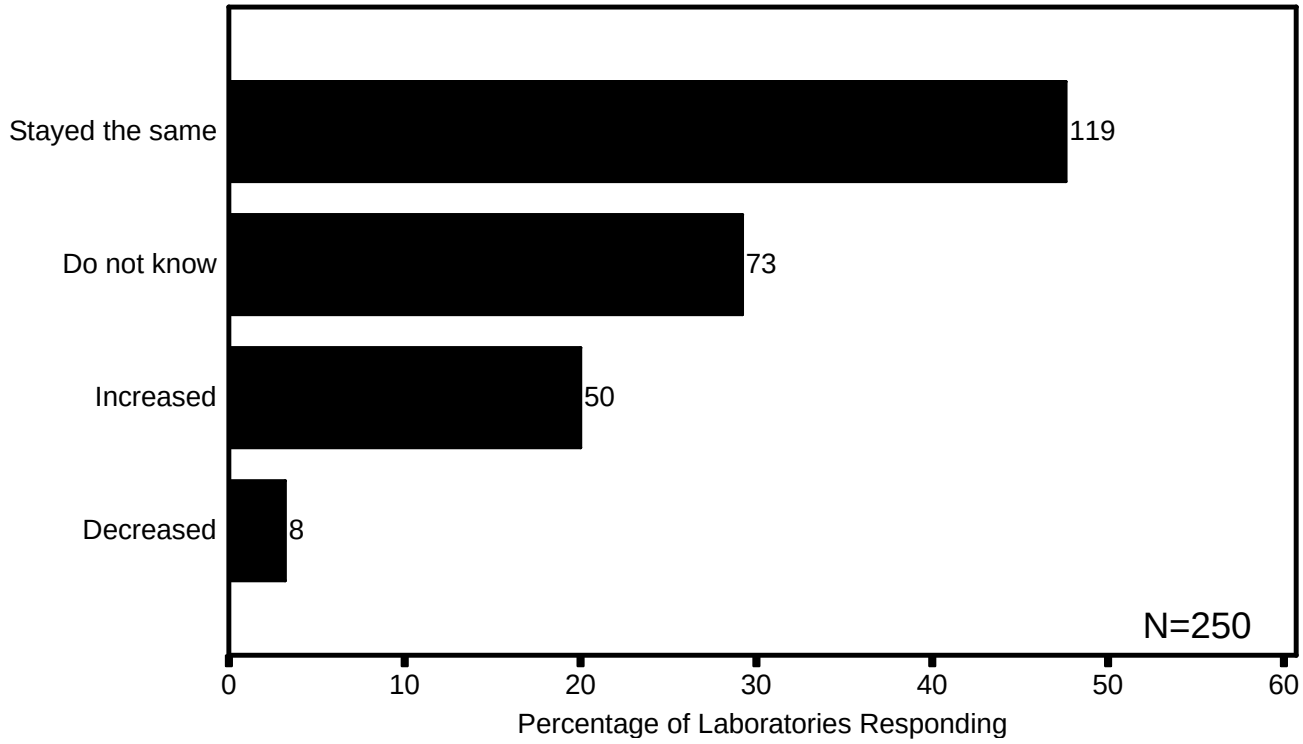


44.(a) What is the average full price currently charged by your laboratory for TLI for each patient/blood donor sample? (Please indicate the price charged only for those methods currently in use in your laboratory. Round off to the nearest whole dollar.)

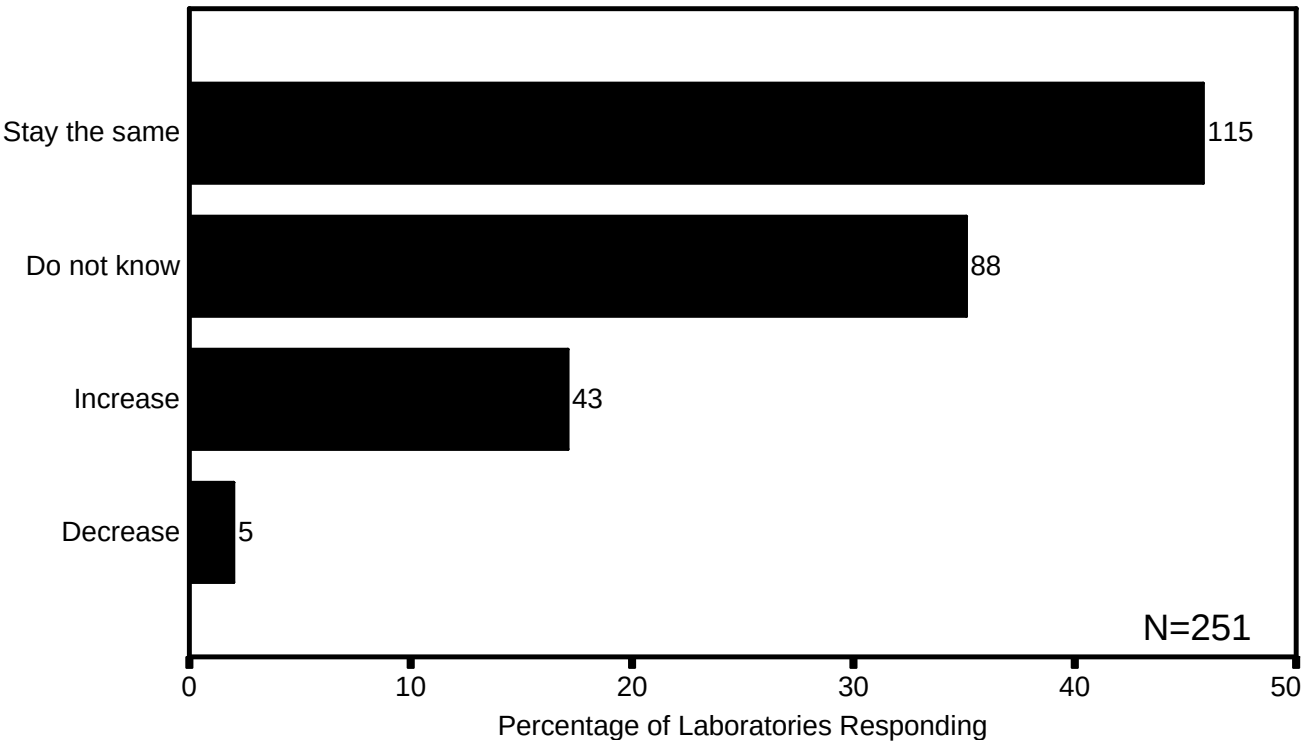




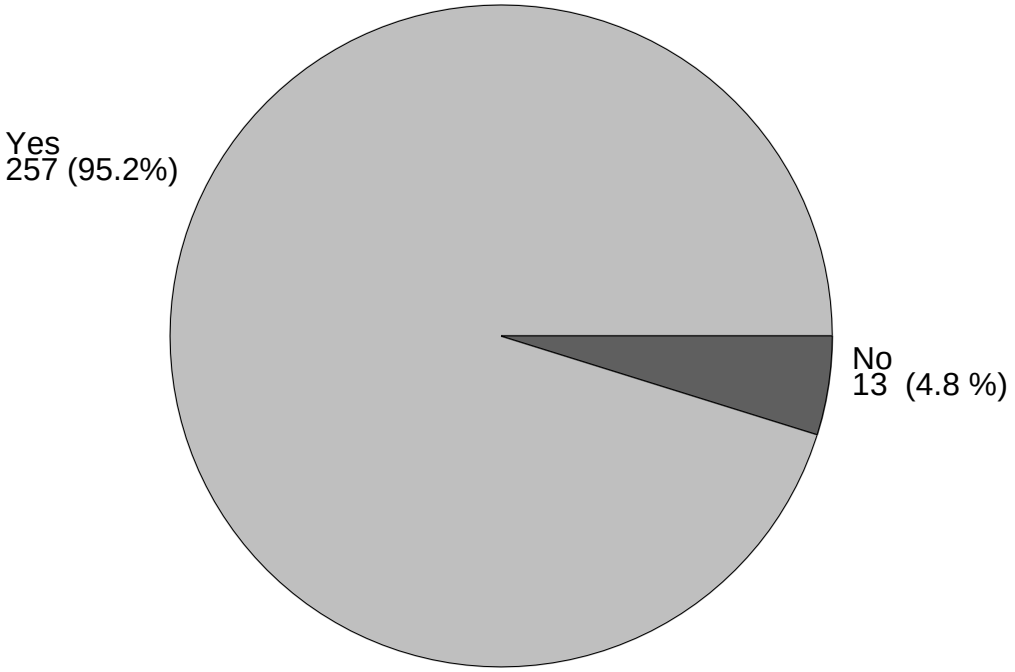
44.(b) Has the average full price that your laboratory charges for TLI for each patient/blood donor sample increased, decreased, or stayed the same compared to twelve months ago? (Choose only <u>one</u>.)



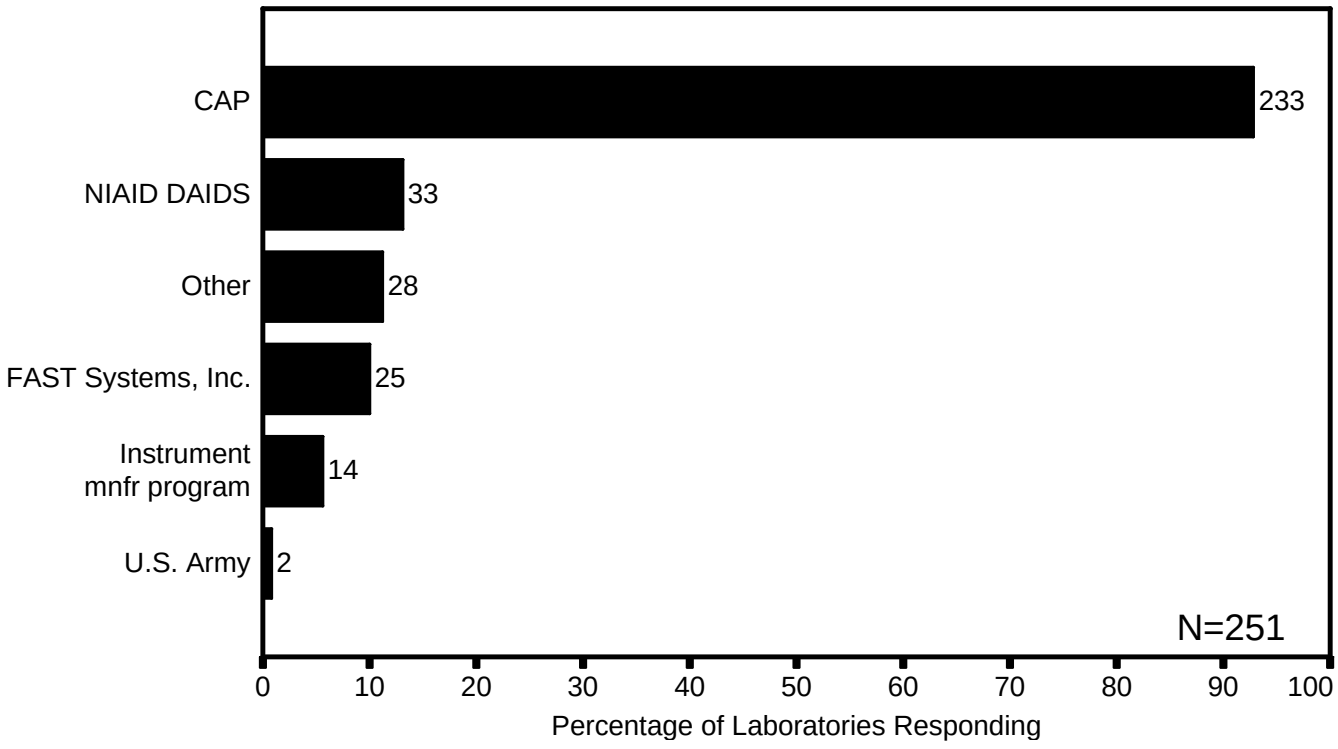
44.(c) Do you anticipate the price charged for TLI by your laboratory will increase, decrease, or stay the same in the next twelve months? (Choose only <u>one</u>.)



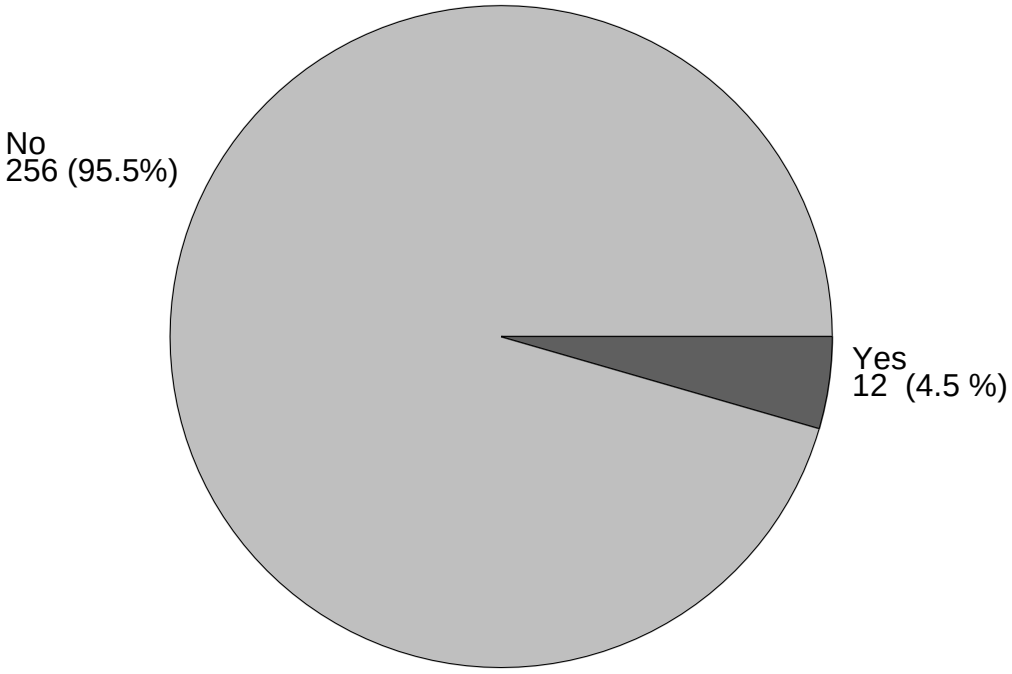
45.(a) Does your laboratory participate in an external TLI proficiency testing program?



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

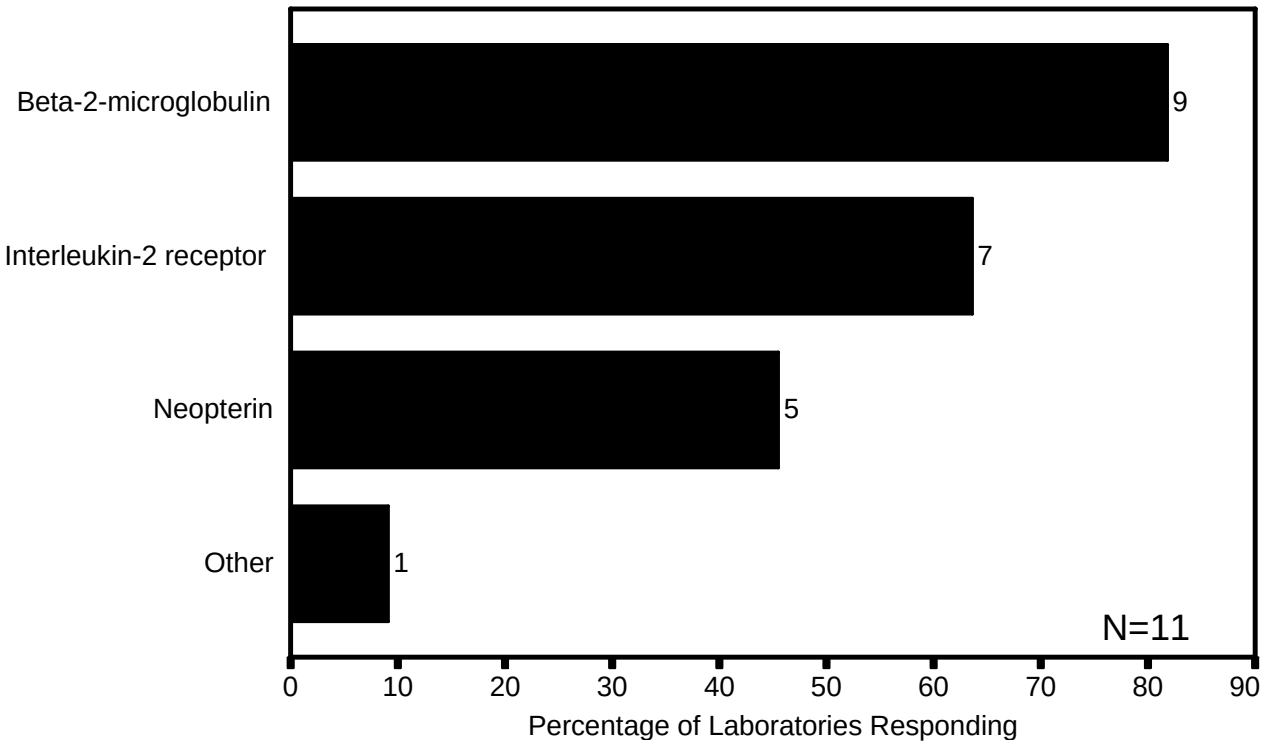


46.(a) In the last year, has your laboratory performed surrogate-marker tests for TLI in HIV-infected patients?

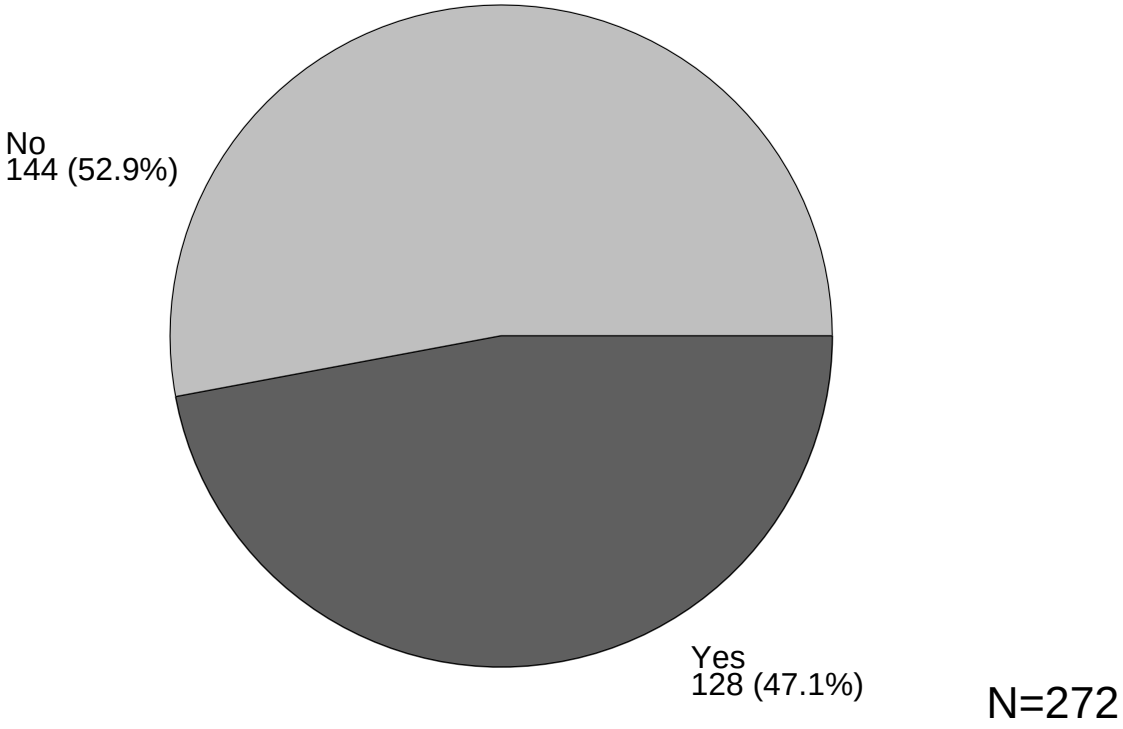


N=268

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



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



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