Introduction and Purpose

Certification by the American Board of Medical Laboratory Immunology (ABMLI) is the highest credential available to practicing medical laboratory immunologists. This certification is recognized by federal and state governmental agencies as a significant component toward meeting licensure requirements to direct laboratories engaged in immunological diagnosis of human diseases, and it is recognized under the Clinical Laboratories Improvement Amendments of 1988 (CLIA ’88). The successful candidate earns the title of Diplomate of the American Board of Medical Laboratory Immunology.

ABMLI certification reflects the standards of professional expertise and knowledge of microbiology as practiced in the United States. While available worldwide, the examination is not intended to be international in scope or utility.

The ABMLI does not discriminate on the basis of race, religion, national origin, sex, mental or physical disability, or age. The ABMLI complies with the policies set forth by the Americans with Disabilities Act.

EXAMINATION PROCESS

The American Board of Medical Laboratory Immunology certification examination consists of two parts, administered sequentially. Following application approval, candidates have two examination cycles in which to begin the examination process. Part I is a written examination consisting of 200 multiple-choice questions, administered once a year on the second Friday in November. Those who pass the Part I written examination are eligible to sit for the Part II oral examination, which is administered once a year in the spring.

RE-EXAMINATION

Both the Part I and the Part II examinations must be passed within 7 years of the application’s approval date. Candidates who do not pass the Part I written examination the first time have unlimited opportunities to re-examine within that 7-year period. Applications not active (no examinations taken) for a period of 5 years will be withdrawn. Candidates who do not pass the Part II oral examination may be re-examined twice. Re-examination after three failures of the Part II examination requires a new application with full fee.
Study Guide

PART I EXAMINATION FORMAT

A. Objective

The objective is to measure the candidate’s knowledge in subject areas considered to be necessary for effective practice of medical laboratory immunology.

Laboratory immunology is the science concerned with the study of all components of the immune system in healthy and diseased individuals. In particular, it is concerned with performance, development, and interpretation of immunologic tests. Medical laboratory immunology includes the following major areas:

- General principles of immunology
- Basic science relevant to medical laboratory immunology
- Cellular components of the immunologic response
- Humoral components of the immunologic response
- Complement
- Immunogenetics
- Inflammatory substances and mediators
- Granulocytes and monocytes-macrophages
- Autoantibodies
- Microbial immunity
- Immunoproliferative disorders and malignancies of the hematopic system
- Immediate hypersensitivity (e.g., allergy)
- Delayed hypersensitivity
- Major techniques, including cellular, serological, and molecular, in medical laboratory immunology
- Laboratory management and regulatory issues
- Instrumentation

B. Examination Administration

1. Candidates choose a proctor at a convenient location. The ABMLI will assist you in identifying a proctor.
2. The examination will be administered by an approved proctor at that location on the second Friday in November.
3. The proctor will supervise the examination. Candidates are allowed 4 hours to complete the examination, beginning at 9:00 a.m. local time and concluding at 1:10 p.m. local time. This time frame allows for one 10-minute supervised break, if mutually agreed on by the candidate and the proctor. The time allotted is considered to be much greater than required for answering the questions, but the committee does not wish time constraints to be a factor in performance.
4. Bring several #2 lead pencils and a legal document with your photograph and signature to the examination. Acceptable legal documents are a driver’s license, government identification card, passport, or notarized photograph bearing your signature.
5. Reference materials and calculators are not permitted.
6. Results will not be released by telephone.

C. Examination Content and Question Format

The Part I written examination consists of 200 multiple-choice questions. These questions are distributed among three broad domains and 28 specific categories that are described below:

Domain I: Basic Immunologic Mechanisms.
This domain comprises approximately 25% of the Part I examination. Eight categories are included under this domain. Suggested topics to study include, but are not limited to, the following:
1. Antigens and antibodies. The classes of antibodies, structure and function of antibodies, chemistry of antigen-antibody interactions, determination of affinity and avidity.

2. Cells and tissues involved in the immune system. Lymphocytes, monocytes, macrophages, neutrophils, eosinophils, basophils; cell subpopulations; cell markers; functional differentiation and maturation; role in the immune response; lymph node, spleen, thymus, mucosal-associated lymphoid tissue, and bone marrow structure and function.

3. Cell cooperation and immune regulation. Cellular interactions among the various cells involved in immune responsiveness; cellular activation, signal transduction, and apoptosis; immunization and adjuvants; adhesion molecules; major histocompatibility complex (MHC) restriction; mechanisms of action of immunosuppressive and immunomodulatory drugs.

4. Effector mechanisms. Protective and destructive effects of immunologic reactions on the host, microbial and tumor immunity, autoimmunity, transplantation immunity, and immunotherapy.

5. Inflammation. The process of inflammation and the cells involved; phagocytosis; the factors involved (excluding complement).


7. Molecular immunology. Structure of immunoglobulin (Ig) and T-cell receptor genes, other receptor genes, and mediator genes; generation of diversity.


Domain II: Methodology.
This domain comprises approximately 35% of the Part I examination. Eleven categories are included under this domain. Suggested topics to study include, but are not limited to:

1. Assays of soluble and particulate antigen/antibody reactions. Precipitation, agglutination, flocculation, hemolysis, etc.; which methods to use in given circumstances.

2. Complement assays. Various procedures to measure concentration and activity of complement components, calculations, specimen collection.

3. Immunoassays for soluble antigens or antibodies. Radioimmunoassay (RIA), enzyme immunoassay (EIA), and the various configurations of such assays; appropriate use of these assays; advantages and disadvantages of the assays; calculations and interpretation of results.

4. Immunohistology. Configuration, specificity controls, and appropriate use; specimen collection and processing; interpretation of results.

5. Phagocyte assays. Types of procedures, appropriate use, specimen collection, controls, calculations, and interpretation of results.

6. Cell-mediated immunity assays. In vitro and in vivo assays, including skin tests, proliferation, cytotoxicity, and mediator release assays; advantages and disadvantages of procedures; controls, calculations, and interpretation of results.

7. Protein analysis and preparation. Chemicals and techniques used, purification, electrophoretic techniques, measurement.

8. Quality assurance and laboratory management. Regulatory and legal issues; proficiency testing; laboratory safety; personnel requirements and testing qualifications; Federal
laws and agencies (e.g., Clinical Laboratories Improvement Amendments [CLIA], Occupational Safety and Health Administration [OSHA], Centers for Disease Control and Prevention [CDC], Food and Drug Administration [FDA]; other agencies (e.g., College of American Pathologists [CAP], Joint Commission on Accreditation of Healthcare Organizations [JCAHO], American Society for Histocompatibility and Immunogenetics [ASHI], NCCLS); quality control measurement and statistical analysis; critical pathways; cost of testing.

9. Molecular biology-based techniques. Principles and performance of Southern, Northern, and Western blots, PCR, restriction fragment length polymorphism; various DNA- and RNA-based analyses; fluorescent in situ hybridization (FISH); microarray technology; sequencing; advantages and disadvantages.

10. Instrumentation. Microscopy, image analysis, automated immunoassay systems, and other instruments and equipment used in a clinical immunology laboratory; the use of these instruments, basic understanding of the principles of operation, controls, calibration, and quality assurance related to the procedures.

11. Flow cytometry. Types of procedures, specimen collection, controls, interpretation of histograms, cluster of differentiation (CD) nomenclature, selection of reagents, gating.

**DOMAIN III: Immunodiagnosis and Clinical Laboratory Correlation.**

This domain comprises approximately 40% of the part I examination. Nine categories are included under this domain. Suggested topics to study include, but are not limited to:

1. Critical evaluation of laboratory tests. Decision-making strategies for test selection and implementation; preanalytical variables, including proper and appropriate specimen collection and transport; pretest clinical consultation; critical interpretation of test results; test algorithms and result reporting; predictive value of results.

2. Infectious diseases. Diagnostic strategies based on disease processes, appropriate selection of tests, timing and analysis of appropriate specimen for disease staging based on immunologic analysis.

3. Autoimmune diseases. Various systemic autoimmune diseases, including hemolytic and collagen-vascular diseases; diagnostic tests available and advantages or disadvantages of each; interpretation of test results.

4. Organ-specific autoimmune diseases. Diseases of various organs and associated immunologic causes or parameters; tests and interpretation of results.

5. Immunodeficiency disorders. Tests for differential diagnosis of immunodeficiencies, acquired (including HIV infection) and congenital immunodeficiencies, monitoring and prognostic tests, interpretation of results.


8. Transplantation. HLA system and MHC antigens, HLA matching and detection of humoral sensitization, ABO-Rh compatibility, analysis of rejection or tolerance, stem cell collection and enumeration, posttransplant complications and monitoring.

Question Format

1. Each question is multiple choice, with one correct answer.

2. Questions have a stem and four or five possible responses.

3. In some cases, questions may require calculations. Examples of such questions include assessment of sensitivity, specificity, dilution factors, and cost-accounting results.

4. Two types of questions are incorporated in the examination:
   a. Questions designed to test basic recall knowledge, direct interpretation of data, or simple synthesis of information.
   b. Questions that require a higher level of thought process, reasoning skills, or interpretation to arrive at the correct answer.

5. Questions are updated and re-evaluated every year by the examination committee, which consists of five or six ABMLI Diplomates. Candidates should expect to see questions on technical advances or immunologic issues that occurred during the past year.

6. There is no penalty for guessing.

7. Laboratory experience is the single most important way to prepare for the examination. It is important to become familiar with all areas of the laboratory, including administrative functions, serology, flow cytometry, molecular diagnostics, and laboratory instrumentation. In addition, examinees have identified the following activities as beneficial for examination preparation:
   a. Studying clinical and basic immunology textbooks and reference manuals such as Manual of Clinical Immunology and Manual of Clinical Microbiology (both published by ASM Press).
   b. Reviewing recent articles in clinical immunology- and laboratory-oriented journals, CAP inspection checklists, manufacturer’s technical manuals and procedures, and laboratory procedural manuals.
   c. Working in or visiting those areas of the laboratory (e.g., flow cytometry, autoimmunity, infectious disease serology, electrophoresis, administration) with which the candidate is less familiar.
   d. Attending rounds in immunology, tumor, transplantation, transfusion, infectious disease, etc.

D. Scoring

1. The examination answer sheets are scored electronically. Scores falling within five points of the breakpoint are verified by hand.

2. The ABMLI uses a criterion-referenced scoring system. This method sets a standard of performance in absolute, not relative, terms. As a result, candidates are not graded on a curve and do not compete against each other. Each question is rated individually by its relative difficulty and scored according to a predetermined standard of performance determined by a consensus of at least five examination committee members. Thus, if more difficult questions are chosen for a particular examination, the passing score will be lower than that of another examination of equal length but consisting of easier questions, as determined by the examination committee. Each candidate’s score is based only on the number of correct answers; there is no comparison among candidates.
3. After the examination has been scored, the examination committee evaluates the responses. Occasionally, questions fail to perform as expected and are dropped from the scoring; the examinations are then rescored.

4. Examination results are mailed to candidates within 10 to 12 weeks. Results are not released over the telephone.

E. Sample Questions

The sample questions included are actual questions from previous examinations. They have been removed from the question pool. Do not judge the content as indicative of content in current questions, but use these sample questions as templates for the format and design of questions and answers.

PART I WRITTEN EXAMINATION SAMPLE QUESTIONS

1. Which of the following cell surface markers are normally associated with both T and B cells?
   a. CD19 antigens
   b. Receptors for tumor necrosis factor (TNF)
   c. CD3 antigens
   d. MHC class I (MHC-I) gene products
   e. CD2 antigens

2. The skin biopsy of a patient having a delayed hypersensitivity reaction is characterized by:
   a. the deposition of Ig and complement in the arterial wall.
   b. neutrophil infiltrates around arteries.
   c. necrosis of the epidermis.
   d. mononuclear cell infiltrates surrounding small vessels.
   e. edema.

3. Serum samples from patients on heparin therapy, particularly those receiving renal dialysis, may contain fragments of fibrin. In solid-phase RIA for HBsAg with polystyrene beads, these fragments:
   a. produce false-negative results by trapping radiolabeled antibody.
   b. trap radiolabeled antibody on the bead with resulting false positives.
   c. interfere with binding of the radiolabeled antibody on the bead.
   d. do not affect the specificity of the test result.

4. In a patient with a positive antinuclear antibody (ANA) and a history compatible with systemic lupus erythematosus (SLE), the MOST specific test is:
   a. anti-single-stranded DNA (anti-ssDNA).
   b. anti-double-stranded DNA (anti-dsDNA).
   c. anti-Ro.
   d. positive immunofluorescence of uninvolved skin with an intracellular pattern.
   e. anti-RNP.

5. Interleukin-3 (IL-3) stimulates:
   a. hematopoiesis of lymphoid and myeloid stems.
   b. development of lymphokine-activated killer (LAK) cells.
   c. generation of NK cells.
   d. proliferation of helper T cells.
   e. differentiation of NK cells.
6. A cell with phenotype CD2\(^-\), terminal deoxynucleotidyltransferase negative (TdT\(^-\)), HLA-DR\(^+\), slg\(^-\), clg\(^+\) is MOST likely to be a:
   a. monoblast.
   b. pre-B cell.
   c. mature B cell.
   d. plasma cell.
   e. myeloblast.

7. Optimal efficiency of PCR is obtained when primers:
   a. are random hexamers.
   b. are complementary to sequences which are over 5,000 bp apart.
   c. are complementary to positive DNA strands.
   d. are complementary to negative DNA strands.
   e. complement both positive and negative DNA strands.

8. Antibodies to polysaccharides in humans are MOST likely to be of which one of the following isotypes?
   a. IgG1
   b. IgG2
   c. IgG3
   d. IgG4
   e. IgA2

9. Which of the following best correlates with active SLE?
   a. Deposition of Ig and complement along the glomerular basement membrane
   b. High-titer ANAs and anti-centromere antibodies
   c. Circulating cryoglobulin complexes formed by IgM-IgG aggregates
   d. Antibodies to dsDNA and depressed levels of serum complement
   e. marked increase of polyclonal light chains.

10. The marginal zone of a secondary follicle contains high numbers of:
   a. activated T cells.
   b. nonactivated B cells.
   c. dendritic macrophages.
   d. large cleaved lymphocytes.
   e. equal mixtures of T and B cells.

11. Laboratory diagnosis of Goodpasture’s syndrome is largely dependent on the demonstration of:
   a. anti-glomerular basement membrane antibodies in serum by complement fixation.
   b. lumpy staining of glomerular basement membrane by electron microscopy.
   c. linear staining of tubular basement membrane by indirect immunofluorescence.
   d. linear staining of glomerular basement membrane by indirect immunofluorescence.
   e. antibody in kidneys cross-reactive with cardiac sarcolemma.

12. A 62-year-old female with progressive rheumatoid arthritis and a tubular-type proteinuria by urine electrophoresis has a positive heat test for Bence Jones proteins in concentrated urine. The MOST probable cause is:
   a. marked increase of polyclonal light chains.
   b. gamma heavy-chain disease.
   c. light-chain disease.
   d. plasma cell myeloma.
   e. non-Hodgkin’s lymphoma.
13. The following results are obtained by nephelometry from a patient suspected of having hereditary angioedema.

<table>
<thead>
<tr>
<th>Test</th>
<th>Result (mg/dl)</th>
<th>Reference range (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>1,158</td>
<td>723–1,685</td>
</tr>
<tr>
<td>IgA</td>
<td>221</td>
<td>69–312</td>
</tr>
<tr>
<td>IgM</td>
<td>144</td>
<td>56–353</td>
</tr>
<tr>
<td>C3</td>
<td>127</td>
<td>83–177</td>
</tr>
<tr>
<td>C4</td>
<td>29</td>
<td>12–43</td>
</tr>
<tr>
<td>C1 esterase inhibitor</td>
<td>17.3</td>
<td>11.5–19.5</td>
</tr>
</tbody>
</table>

The physician calls and explains that the patient appears to have a classic case of hereditary angioedema, but the laboratory results do not confirm this. What additional tests would you recommend?

a. C3b inactivator, functional  
b. C3 activator  
c. CH50  
d. C1 esterase inhibitor, functional  
e. Total C1

14. A 35-year-old man presents with a history of episodic subcutaneous swelling and incapacitating, colicky, abdominal pain lasting 1 to 3 days that is sometimes associated with nausea and vomiting. The abdominal pain occurs independently of the swelling. He has also had episodes of swelling in the throat. He has a positive family history: his mother had similar episodes when she was younger; one son has similar attacks; another son is completely normal. Several distant cousins have the same symptoms.

Physical examination shows a male in no acute distress. Height, 5' 11"; weight, 177 lb; blood pressure, 125/80 mm/Hg, pulse, 82 beats/min. The rest of the examination is unremarkable.

The laboratory data include the following results:

- Hemoglobin: 14 g/dl  
- Hematocrit: 48%  
- White blood cells (WBC): 8,000/mm³  
- Differential: 26% lymphocytes, 68% segmented neutrophils, 3% monocytes, 2% eosinophils, 1% basophils

Electrocardiogram, SMA-12, chest X-ray, and urinalysis results were within normal limits. Which of the following tests is more likely to be abnormal in this patient?

a. CH50  
b. C3  
c. C4  
d. C5  
e. IgE

15. Graves’ autoimmune thyroiditis is associated with all of the following immunologic observations EXCEPT:

a. an antibody directed against the thyroid-stimulating hormone (TSH) receptor.  
b. an antibody directed against the thyroid cell receptor.  
c. infiltration of macrophages into the thyroid.  
d. activation of HLA-DR antigens on thyroid epithelial cells.  
e. a frequent association with HLA-DR3 expression.
16. Which of the following characteristics is shared by radioimmunoprecipitation (RIPA) and Western blot assays for specific antibodies recognizing viral antigens?

a. An electrophoretic step is required to separate the viral antigens on the basis of molecular weight.
b. The antigen-antibody reaction takes place before the electrophoretic step.
c. The viral antigens are radiolabeled.
d. An enzyme-conjugated goat anti-mouse Ig reagent is used.

17. Which of the following approaches would be best to deal with the problem of an increasing number of celebrations (birthdays, etc.) by laboratory personnel during normal working hours?

a. Prohibit all celebrations except on break time and lunch periods.
b. Predesignate a reasonable time for all celebrations each month, chosen to least interfere with laboratory performance.
c. Allow each section supervisor to deal with the problem.
d. Act only if you have gathered data showing that celebrations are interfering with laboratory performance and productivity.

18. Isoelectric focusing is a technique that can be used to analyze proteins. Which of the following is true of isoelectric focusing?

a. It is commonly used to quantitate IgA.
b. It separates proteins in an aqueous environment where one is able to maintain a net charge of +1 on the proteins.
c. It is restricted to the analysis only in an acidic environment (pH < 6).
d. It is restricted to the analysis only in an alkaline environment (pH > 6).
e. It separates proteins based on the pH at which the net charge on a protein is zero.

19. The skin biopsy of a patient having a Jones-Mote reaction is characterized by:

a. a deposit of IgG and complement in the arterial wall.
b. neutrophils around the arteries.
c. basophil-rich infiltrates in the dermis.
d. mononuclear cell infiltrates surrounding small vessels.
e. subcutaneous edema.

20. The finding by nephelometry of low IgG, IgA, and IgM levels in a patient with a monoclonal protein of gamma mobility (8 g/dl) and no Bence Jones protein should first be followed by:

a. repeating the assay of IgG, IgA, and IgM with higher dilutions of serum.
b. immunofixation using anti-IgD and anti-IgE.
c. repeating the assay with different antisera.
d. reevaluating the quality control in your laboratory.
e. reassignment of the technician in charge of the nephelometer.

21. In the direct antiglobulin test (DAT), it is essential that the antiglobulin reagent contain anti-IgG antibodies and:

a. anti-IgM antibodies.
b. anti-C1r antibodies.
c. anti-C3d antibodies.
d. anti-P antibodies.
e. anti-MN antibodies.

22. Which of the following is NOT observed when complement is activated by the alternative pathway?

a. Breakdown of C3 into C3a and C3b
b. Breakdown of C4 into C4a and C4b
c. Breakdown of C5 into C5a and C5b
d. Activation of the membrane attack complex
e. Activation of C1 complex
23. Prekallikrein can be activated by:
   a. components of the extrinsic pathway of coagulation.
   b. tissue thromboplastin and coagulation factor IX.
   c. plasmin and coagulation factor Xa.
   d. thrombin and kininogen.
   e. activated factor XII.

24. Chronic inflammation in response to foreign bodies is characterized by the accumulation of:
   a. polymorphonuclear leukocytes.
   b. sensitized T lymphocytes.
   c. platelets.
   d. macrophages.
   e. basophils.

25. Which of the following factors attracts neutrophils?
   a. C-reactive protein
   b. Soluble antibody-antigen complexes
   c. C5a
   d. IL-2
   e. Amyloid A protein

26. Which of the following cell types are positive for TdT analysis by immunofluorescence?
   a. Germinal center cells
   b. Resting cells in diffuse cortex
   c. Plasma cells
   d. Peripheral T cells
   e. Mantle cells

27. The finding of a positive result with the serum control in a complement fixation test is MOST likely explained by the presence of:
   a. anti-Forssman antibodies.
   b. anti-human red blood cell antibodies.
   c. free antigen in circulation.
   d. high levels of heat-labile IgE.
   e. soluble immune complexes.

28. An activated CD4-positive lymphocyte will:
   a. recognize antigens associated with MHC-I.
   b. release large amounts of IL-2.
   c. interact with CD2+ lymphocytes.
   d. interact with antigen-presenting cells through the MHC-I molecule.
   e. express MHC-I molecules but NOT MHC-II molecules on its membrane.

29. A laboratory bills Medicare for the individual component tests of a panel or profile with individual Current Procedural Terminology (CPT) codes (totaling $25.00) rather than one code that reflects the composite testing (totaling $20.00). What is the most important consequence of this action?
   a. Increased revenue
   b. Decreased fixed cost per test
   c. Improved billing efficiency
   d. Fraud and abuse charges
   e. Faster turnaround time
30. The radioallergosorbent test (RAST) is used instead of a skin test under which of the following circumstances?

a. The suspected allergen is not present.
b. The patient is very young.
c. C1q binds to an IgE-allergen complex in patient serum.
d. IgG antibody to suspected allergen interferes with the skin test.
e. The patient has hypogammaglobulinemia.

31. The lectin pathway leads to activation of the classical complement pathway through:

a. antibody binding.
b. production of C1 complex.
c. mannose-binding lectin (MBL) and activation of MBL-associated serum proteases (MASP).
d. C5 convertase.
e. decay-accelerating factor (DAF).

32. Which one of the following statements demonstrates the linkage disequilibrium of HLA-A1 and HLA-B8 alleles in the Caucasian population?

a. The observed A1-B8 haplotype frequency is usually higher than the expected frequency.
b. The observed A1-B8 haplotype frequency is usually lower than the expected frequency.
c. The observed A1-B8 haplotype frequency is the same as the expected frequency.
d. The observed A1-B8 haplotype frequency is the sum of the A1 and B8 gene frequencies.
e. The observed A1-B8 haplotype frequency is the product of the A1 and B8 gene frequencies.

33. Which one of the following is NOT the cause of a female adult’s serum lymphocytotoxic antibodies?

a. Her pregnancy history
b. Her blood transfusion history
c. Her kidney transplantation history
d. Her active SLE status
e. Her hepatitis B antigen carrier status

34. The function of MHC-I molecules is to present:

a. endogenous antigen peptides to T helper cells.
b. exogenous antigen peptides to T cells.
c. processed antigen peptides to CD4+ T cells.
d. processed antigen peptides to CD8+ T cells.
e. processed antigen peptides to B cells.

35. The function of MHC-II molecules is to present:

a. exogenous antigen peptides to T killer cells.
b. endogenous antigen peptides to T cells.
c. processed antigen peptides to CD4+ T cells.
d. processed antigen peptides to CD8+ T cells.
e. intact antigen molecules to B cells.

**Answers**

1. d  8. b  15. c  22. b  29. e
2. d  9. d  16. b  23. e  30. b
3. b  10. b  17. b  24. d  31. c
4. b  11. d  18. e  25. c  32. a
5. a  12. a  19. c  26. a  33. e
6. b  13. d  20. a  27. e  34. d
7. e  14. c  21. c  28. b  35. c
PART II EXAMINATION FORMAT

A. Objective

To measure the candidates’ cognitive skills, including depth of knowledge, problem-solving, and clinical judgment, related to directing a medical immunology laboratory.

B. Examination Administration

1. The examination is administered annually in early spring at ASM headquarters in Washington, DC. Examinations are scheduled at 90-minute intervals over a 1- or 2-day period, depending on the number of candidates. Several examinations may be conducted simultaneously by different panels. The examinees may be asked to show identification before the examination.

2. All candidates in a given year are presented with the same set of issues and questions. The topic issues vary from year to year.

3. The examination is administered by a panel of three ABMLI Diplomates, none of whom are well acquainted with the candidate or have participated in his/her education and/or training. One member of the panel functions as Chair and is responsible for the administrative aspects of the examination.

4. Each examiner is responsible for asking one or more of a set of predetermined questions pertaining to a topic issue. During the examination, the examiners keep a written record of the appropriateness of the candidate’s responses. The examination will last approximately 1 hour.

C. Examination Content

The examination consists of a series of questions based on major issues involved in medical laboratory immunology. The questions are designed to assess the candidate’s knowledge in the following content areas:

3. Laboratory practice. Specimen handling and distribution, quality control, standards and reference centers, data handling and reporting, personnel and training.

D. Question Format

1. Each of the major issues will be introduced by a case history, clinical situation, or other problem similar to one that may be faced by a medical immunology laboratory director. The candidate will be provided with a written copy of the history, and the history will be read aloud by one of the examiners. The candidate may take a few moments to collect his/her thoughts and make any written notes desired.

2. The candidate is asked a series of predetermined questions pertaining to that topic by one examiner. The candidate is not given a copy of these questions. The questions are designed to determine the depth of the candidate’s knowledge and cognitive processes and to rate his/her verbal and interpersonal skills. Immediate correct deduction of the answer is less important than the demonstration of problem-solving ability and judgment. Each topic may test one or more of the three content areas. After the predetermined questions have been asked, the other examiners may ask additional questions to clarify the candidate’s responses.

E. Scoring

1. At the end of each candidate’s oral examination, each examiner assigns a pass or fail grade to each question for content areas addressed by that question. Each candidate will be tested in each content area by a combination of five questions. The scoring grid (Table 1) will be used to determine whether the candidate has passed or failed the examination. If a majority of examiners pass a candidate in a content area, the candidate will pass that area. The candidate must pass all three content areas in order to pass the examination.
Table 1. Sample scoring grid.

<table>
<thead>
<tr>
<th>Content area</th>
<th>Question and score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Clinical implications</td>
<td>XXXX</td>
</tr>
<tr>
<td>Technical aspects</td>
<td>XXXX</td>
</tr>
<tr>
<td>Laboratory practice</td>
<td>XXXX</td>
</tr>
</tbody>
</table>

2. The candidate’s answers to each question are evaluated based on “model” answers. Panel members decide individually on the candidate’s success or failure to address all significant aspects of the problem and assign a pass/fail score based on their evaluation. Each examiner’s vote carries equal weight when the final results are tallied. Two examples follow in which the candidate passes (Table 2) or fails (Table 3) the examination.

Table 2. Candidate passes the oral examination.

<table>
<thead>
<tr>
<th>Content area</th>
<th>Question and score</th>
<th>Examination score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Clinical implications</td>
<td>Pass</td>
<td>Pass</td>
</tr>
<tr>
<td>Technical aspects</td>
<td>XXXX</td>
<td>Pass</td>
</tr>
<tr>
<td>Laboratory practice</td>
<td>Pass</td>
<td>XXXX</td>
</tr>
</tbody>
</table>

Table 3. Candidate fails the oral examination.

<table>
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<tr>
<td>Technical aspects</td>
<td>XXXX</td>
<td>Fail</td>
</tr>
<tr>
<td>Laboratory practice</td>
<td>Pass</td>
<td>XXXX</td>
</tr>
</tbody>
</table>

3. Diplomate status is granted to candidates who pass the Part II examination. Results are mailed to the candidate within 5 business days of the examination.

Feedback from Former Examinees

The following comments have been chosen from feedback received from examinees as advice to future examinees:

“Be prepared for difficult questions that require extensive knowledge of basic principles. Facts alone will not do.”

“Pay more attention to the technical and clinical aspect of the subject.”

“Relax. Remember, you will be asked questions concerning major areas. Do not drive yourself crazy studying minutiae—the examiners are not there to trick you with the minor areas of clinical laboratory immunology.”
PART II EXAMINATION SAMPLE QUESTIONS

The questions presented here were used in previous examinations and will not be repeated. Model answers are used as guides for the panel, but candidates may pass the oral examination without responding to every issue cited in the model answer.

QUESTION I

A female baby, 4½ months old, was admitted to the hospital for further evaluation and treatment for cough, tachypnea, and hypoxia associated with severe thrush and failure to thrive. Her mother received no prenatal care during pregnancy. The infant had been born at 33 weeks of gestation, weighing 4 lb, 6 oz. She had had diarrhea since birth; for 1 month, she also had oral thrush and a cough without congestion or fever. At the time of admission, the baby had a severe cough and gasping respiration and required the use of a ventilator.

What should be done to work up this infant from the immunology laboratory standpoint?

EMPHASIS: CLINICAL IMPLICATIONS

Suggested follow-up questions:

1. What immunologic diseases should be included in your list of differential diagnoses?

2. How would you evaluate this infant for human immunodeficiency virus (HIV) infection?

3. How would you evaluate an infant for non-HIV-associated immunodeficiency disorder?

MODEL ANSWER

CLINICAL IMPLICATIONS

The clinical history suggests a possible immunodeficiency disorder. Possible diseases to be considered are primary immune deficiencies including T-cell immunodeficiency, such as thymic aplasia (Di George syndrome) or chronic mucocutaneous candidiasis, or a combined immunodeficiency disorder, such as Nezelof syndrome or severe combined immunodeficiency (SCID). However, the lack of prenatal care suggests a secondary immune deficiency, such as AIDS.

Laboratory data to be requested include a complete blood count (CBC) with differential, quantitative immunoglobulin (Ig) levels, and lymphocyte subset analysis. The immunophenotyping results reveal decreased CD4+ T-cell levels and elevated CD8+ T-cell levels. These results are suspicious for HIV infection. The infant also had a bronchial alveolar lavage, which was positive for cytomegalovirus (CMV) and Pneumocystis carinii. The patient’s urine was positive for CMV, and she was diagnosed with CMV colitis. There was evidence of esophagitis due to Candida albicans.

Ig levels.

<table>
<thead>
<tr>
<th>Test</th>
<th>Result (mg/dl)</th>
<th>Reference range (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>427</td>
<td>206–1,026</td>
</tr>
<tr>
<td>IgA</td>
<td>28</td>
<td>8–93</td>
</tr>
<tr>
<td>IgM</td>
<td>42</td>
<td>10–83</td>
</tr>
</tbody>
</table>

Immunophenotyping results.

<table>
<thead>
<tr>
<th>Test</th>
<th>Result (%)</th>
<th>Reference range (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>74</td>
<td>55–79</td>
</tr>
<tr>
<td>CD4</td>
<td>11</td>
<td>29–53</td>
</tr>
<tr>
<td>CD8</td>
<td>57</td>
<td>14–35</td>
</tr>
<tr>
<td>CD20</td>
<td>20</td>
<td>5–20</td>
</tr>
</tbody>
</table>
TECHNICAL ASPECTS

With the laboratory results, testing for HIV should be ordered. An HIV antibody test may be performed at 4½ months; however, some maternal antibody may remain. The best test for a neonatal HIV diagnosis is an HIV DNA PCR assay or an ultrasensitive HIV RNA quantitative assay. If the infant’s PCR assay is positive, then testing of both parents is also indicated, if not already performed.

HIV screening was repeatedly reactive for both parents of this infant, and the parents’ respective Western blots contained all significant antibodies for a positive result. According to Association of State and Territorial Public Health Laboratory Directors (ASTPHLD) criteria, a test must have two out of three antibodies to gp120/160, gp41, or p24 to be considered positive.

The infant’s antibody results may be influenced by the continued presence of maternal IgG that has crossed the placenta. Normally, maternal antibody reaches its lowest level when the infant is about 6 months old. However, in some cases, maternal HIV-specific antibody has been detected in an infant for 12 to 18 months.

LABORATORY PRACTICE

Proper attention to good laboratory practice is essential to ensure the safety of laboratory workers and to obtain valid and reproducible results. Universal blood and body fluid precautions should be followed at all times for all laboratory samples. A manual detailing these procedures should be readily available at the workbench. Any laboratory staff performing HIV nucleic acid assays must do their work in a biosafety level 2 (BSL 2) hood. Technologists must wear gowns, gloves, masks, etc., while performing the test and must have extensive safety training. All materials, including reagents and cultures, must be terminally sterilized before disposal and/or removal from the facility.

QUESTION 2

The patient is a 73-year-old male who is referred to your institution because of an increased white blood cell count (153,000/mm), decreased platelets (22,000/mm), and abdominal pain. He has a history of chronic myelogenous leukemia (CML) since 1983, which was treated with chemotherapy (busulfan and hydroxyurea). Studies done at that time showed the patient to be positive for the Philadelphia chromosome. A blast crisis occurred in January 1990. The blast cells were large and primitive looking and were shown by flow cytometry to be of myeloid origin (see the table “Flow cytometric results for peripheral blood,” column 1). In the current admission (September 1993), the blast cells are small to medium sized, with round nuclei and inconspicuous nucleoli (see the table “Flow cytometric results for peripheral blood,” column 2).

What is your interpretation of these results?

<table>
<thead>
<tr>
<th>Test</th>
<th>Date of blast crisis and result</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>January 1990</td>
<td>September 1993</td>
</tr>
<tr>
<td>WBC/mm</td>
<td>96,000</td>
<td>153,000</td>
</tr>
<tr>
<td>% PMNs</td>
<td>40</td>
<td>36</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Blastocysts, LUCs</td>
<td>22</td>
<td>41</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Basophiles</td>
<td>19</td>
<td>2</td>
</tr>
</tbody>
</table>

Abbreviations: WBC, white blood cells; PMNs, polymorphonuclear leukocytes; LUCs, large unstained cells.
EMPHASIS: TECHNICAL ASPECTS AND LABORATORY PRACTICE

Suggested follow-up questions:

1. How common is a lymphoid relapse in CML?

2. What is the genetic rearrangement that leads to the Philadelphia chromosome?

3. Discuss appropriate quality control in flow cytometry, including instrument, reagents, sample preparation, and operator training.

4. How might clonality of these B cells be determined?

MODEL ANSWER

CLINICAL IMPLICATIONS

The initial diagnosis of CML is supported by the presence of a positive Philadelphia chromosome. More than 95% of patients with CML are positive for the Philadelphia chromosome, which is a translocation involving chromosomes 22 and 9 (c-abl on chromosome 9 is translocated to chromosome 22 at the bcr gene). Blast crisis is common in CML and usually occurs 3 to 4 years after the initial diagnosis. The blast crisis is myeloid in two-thirds of cases and lymphoid in one-third of cases.

The first blast crisis in this patient involved primitive myeloid cells, expressing CD13, CD33, CD34, and CD45. The second blast crisis involved immature B cells, expressing CD10, CD19, CD34, CD45, and HLA-DR. No light-chain expression was detected, so the clonal nature of the B cells could not be established by immunophenotyping. Gene rearrangement studies would have to be done. This case is very unusual in that it shows both types of blast crisis.

TECHNICAL ASPECTS

The determination of the myeloid or lymphoid nature of the blast cells may be of use in the choice of treatment. Flow cytometry is the appropriate procedure for this determination. The accuracy of flow cytometric results depends on many factors, including the window or bitmap chosen for analysis. The number of cells analyzed in this bitmap is important, i.e., the larger the number of events, the more accurate the results. The percentage of events in the bitmap should correspond to the percentage of lymphocytes and large unstained cells seen on the differential.

Flow cytometry should be used to establish the lymphoid or nonlymphoid nature of the blast cells. It is usually not necessary to run a large panel of monoclonal antibodies. In differentiating a malignant population from a nonmalignant one, several characteristics can be of importance. First, the forward versus side scatter pattern may detect a population in an unusual position. Second, a population of cells may fail to express an antigen that is normally present, i.e., CD3 may be absent while other mature T-cell surface markers are seen. Third, a population of cells may express a surface antigen that is usually not expressed, such as a myeloid antigen in the presence of primarily T-cell antigens. Fourth, the fluorescence intensity of a surface marker may be either brighter or dimmer than is usually seen.

LABORATORY PRACTICE

Proper attention to good laboratory practice is essential to ensure the safety of the laboratory workers and to ensure valid and reproducible results. Universal blood and body fluid precautions should be followed at all times for all laboratory samples. A manual detailing these procedures should be readily available at the workbench.

Appropriate normal controls must be used for all monoclonal antibodies. The results of these normal controls should be within preset limits. Isotypic controls should be used to detect nonspecific binding of the antibodies to cells. Instrument setup and alignment should be within preset limits. The window or bitmap should contain
an adequate number of events. The scattergram parameters should be reasonable, to guard against problems with the cell fixative.

Data analyses should be performed by appropriately trained individuals. All results must be checked for errors before being reported by the person in charge of the laboratory. Their qualifications should be monitored periodically. The laboratory itself should be quiet and conducive to careful work.

**Flow cytometric results for peripheral blood.**

<table>
<thead>
<tr>
<th>CD marker (%)</th>
<th>L + LUCs</th>
<th>Reference range (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>January 1990</td>
<td>September 1993</td>
</tr>
<tr>
<td>CD 19</td>
<td>1</td>
<td>69</td>
</tr>
<tr>
<td>Kappa</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>Lambda</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>CD 10</td>
<td>0</td>
<td>74</td>
</tr>
<tr>
<td>CD 7</td>
<td>2</td>
<td>ND</td>
</tr>
<tr>
<td>CD 5</td>
<td>ND</td>
<td>5</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>ND</td>
<td>90</td>
</tr>
<tr>
<td>CD 13</td>
<td>69</td>
<td>19</td>
</tr>
<tr>
<td>CD 33</td>
<td>83</td>
<td>21</td>
</tr>
<tr>
<td>CD 34</td>
<td>50</td>
<td>69</td>
</tr>
<tr>
<td>CD 14</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>CD 45</td>
<td>98</td>
<td>99</td>
</tr>
</tbody>
</table>

Abbreviations: ND, not done; L + LUCs, lymphocytes plus large unstained cells.

**QUESTION 3**

A 47-year-old Hispanic male presented to his primary physician reporting that he had recently donated blood at a local blood bank. The blood bank informed him that he had positive serology for hepatitis C. Upon examination, the patient appeared well except for slight physical evidence in the hands and feet of rheumatoid arthritis, which the patient had for 7 years. He had no evidence of skin or eye color changes consistent with jaundice. He received several blood transfusions in 1979, following a serious car accident. The physician ordered liver enzymes and a hepatitis C enzyme-linked immunosorbent assay (ELISA). The results are listed below.

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>22 IU/liter</td>
<td>&lt;30 IU/liter</td>
</tr>
<tr>
<td>ALT</td>
<td>19 IU/liter</td>
<td>1–40 IU/liter</td>
</tr>
<tr>
<td>HCV ELISA</td>
<td>Positive</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; HCV, hepatitis C virus.

**EMPHASIS: Clinical Implications and Laboratory Practice**

**Does the patient have hepatitis?**

The normal liver enzyme results do not indicate evidence of active damage of liver hepatocytes. However, the patient may have chronic hepatitis C infection that has not yet led to significant liver damage.

**FOLLOW-UP QUESTIONS**

**CLINICAL IMPLICATIONS**

1. The patient is referred to a hepatologist, who orders additional laboratory tests. What additional tests should be ordered?
The positive hepatitis C ELISA tests may be followed by a hepatitis C recombinant immunoblot assay (RIBA), especially in the absence of clinical disease. The RIBA is not indicated in the presence of a strong positive ELISA and elevated liver enzymes. In that case, a hepatitis C viral load test would be preferable to the RIBA.

In this case, the physician ordered the RIBA, a viral load test, and hepatitis C genotype. Because of the lack of documentation, a hepatitis C ELISA should be redone, along with a follow-up confirmatory RIBA. The confirmatory assay may be important because of the patient’s history of rheumatoid arthritis and the high rate of false-positive results in the screening ELISA assay for patients with autoimmune diseases. A hepatitis C viral load (quantitative RNA) assay may be used as a confirmatory assay in place of, or following, the RIBA.

The following test results were obtained.

<table>
<thead>
<tr>
<th>HCV test</th>
<th>Result</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis C genotype</td>
<td>4</td>
<td>None detected</td>
</tr>
<tr>
<td>RIBA</td>
<td>C100–C103; NS4 A/B C33c; NS3 C22-3; core C200; NS3/NS4</td>
<td>Negative Negative Positive Negative</td>
</tr>
<tr>
<td>RNA virus</td>
<td>&lt;2,000 IU/ml</td>
<td>&lt;2,000 IU/ml</td>
</tr>
</tbody>
</table>

2. Are these results consistent with chronic HCV infection?

No. Although the screening test is positive, the RIBA test requires antibody against at least two regions of the virus. A positive result against the core region alone is not sufficient to confirm HCV infection. Core antibody alone is frequently seen in patients with autoimmune diseases. Also, the test for HCV RNA is negative.

3. Are these results consistent? What is the most likely explanation for these laboratory results?

The negative result for HCV RNA virus is inconsistent with the HCV genotype result. Genotyping presupposes the presence of the virus in the blood. The genotyping assays are usually less sensitive than the HCV RNA assays and it would be unlikely that low levels (<2,000 IU/ml) of virus would give a positive genotype with a negative HCV RNA assay. The most likely explanation is a false-positive HCV genotype result. The genotype 4 result is very uncommon (seen almost exclusively in samples taken from patients in Africa and the Middle East). However, it is possible that the confirmatory assay and the HCV tests are not positive because of insensitivity of the RIBA to the genotype 4 antibodies and lack of adequate priming for the genotype 4 virus by the HCV RNA assay (the PCR-based commercial assay is relatively insensitive to genotypes that are uncommon in patients in the United States).

On follow-up, repeat testing for this patient of both the HCV RNA and genotyping assays was negative for the virus, indicating an initial false-positive genotyping assay.

FOLLOW-UP QUESTIONS

LABORATORY PRACTICE

1. How should HCV RNA quantitative and HCV genotyping assays be used to monitor therapy?

HCV genotyping assays should be done prior to initiation of therapy and are used by physicians to determine the length of therapy. Genotypes 1a and 1b are less amenable to interferon treatment than types 2 and 3. Genotype 1 predominates in the United States, however. HCV quantitative assays should be done
prior to the initiation of therapy and at 6 and 12 months to monitor the effects of therapy. However, quantitative assays used at 1 or 3 months may discriminate between patients who respond to therapy from those who do not respond.

2. **Describe the strategies used in molecular laboratories to prevent false-positive PCR amplification.**

There should be a separation of physical space between pre- and postamplification materials. Procedures to minimize all aerosols (special positive displacement pipettes, plugs in the disposable tips), frequent glove changes, decontamination of surfaces with DNA- and RNA-destroying substances like Clorox or UV radiation, control of air circulation, use of biocontainment hoods, and the performance of multiple replicates of the PCR amplification, should be performed.

3. **The College of American Pathologists (CAP) molecular diagnostics inspection checklist requires the recording of failed nucleic acid isolations and documentation of corrective actions taken. What procedures can be used to satisfy this requirement?**

For DNA extraction of cells, a careful monitoring of the number of cells extracted and a measurement of DNA yield can be done. A coamplification of a constitutive DNA sequence of similar length and primer amplification conditions can be carried out in the same amplification vial or a separate vial.

Multiple replicates of the DNA or RNA extraction can be performed.

For RNA extraction, it is most common to spike the sample prior to the extraction with known quantities of RNA that can be coamplified during the subsequent amplification reaction. This can be a sequence that uses the same primers with different internal sequence, or it can be the viral amplicon sequence containing a small deletion or additional RNA made in such a way as to be distinguished by size or hybridization characteristics from the wild-type RNA virus.

Extraction failure rates can be easily monitored as a quality control parameter by determining failure rates for each run.