The National Academy of Clinical Biochemistry

Presents

LABORATORY MEDICINE PRACTICE GUIDELINES

LABORATORY GUIDELINES FOR SCREENING, DIAGNOSIS AND MONITORING OF HEPATIC INJURY
LABORATORY GUIDELINES FOR SCREENING, DIAGNOSIS AND MONITORING OF HEPATIC INJURY

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“Laboratory Medicine Practice Guidelines” is the new name for the National Academy of Clinical Biochemistry program formerly known as “Standards of Laboratory Practice”.

The portion of this monograph dealing with laboratory test performance requirements guidelines was jointly developed with the American Association for the Study of Liver Diseases through their Practice Guidelines Committee.

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Introduction

Hepatocyte injury is commonly encountered in the practice of medicine. The incidence of acute viral hepatitis has markedly decreased in the past decade due to the introduction of vaccines for hepatitis A and B and testing of the blood supply for hepatitis C. Other forms of acute hepatic injury have not changed appreciably in incidence, and recognition of chronic hepatic injury has increased. In the United States, an estimated 1 million individuals are chronically infected with hepatitis B and 2.1-2.8 million are chronically infected with hepatitis C (1). Cirrhosis, currently the 9th leading cause of death in the United States (2); deaths from cirrhosis are predicted to increase 223% by 2008 and 360% by 2028 due to cases developing from chronic hepatitis C infection (3). Hepatocellular carcinoma incidence has doubled in the past 20 years (4), and is expected to rise another 68% over the next decade from cases developing in hepatitis C infected individuals (3).

Liver disease is often clinically silent until late in its course. For this reason, laboratory tests are usually needed for recognition and characterization of the type of liver injury present. The most common cause of liver injury worldwide is infection with viruses that primarily infect the liver, often termed hepatitis viruses. Serologic and nucleic acid-based tests are required to document exposure to and presence of these viruses, and are also used to monitor treatment of infected individuals. A number of other diseases may also cause liver injury, particularly autoimmune disorders and congenital or acquired disorders of metabolism. Laboratory tests are critical for recognition of these other diseases, particularly in patients who lack evidence of viral infection. Finally, exposure to ethanol and other drugs can cause hepatic injury; clinical information is the most reliable means to recognize these potential causes of liver damage.

Specific recommendations in this Monograph are based on relevant published information. The strength of scientific data supporting each recommendation is characterized using the scoring criteria adopted by the Practice Guidelines Committee of the American Association for the Study of Liver Diseases (AASLD), as summarized in Table 1.

Table 1. AASLD Categories Reflecting Quality of Evidence on Which Recommendation is Based (Roman Numerals) and Evidence Supporting Guidelines (Letters)

| I | Evidence from multiple well-designed randomized controlled clinical trials each involving a number of patients to be of sufficient statistical power |
| II | Evidence from at least one large well-designed clinical trial with or without randomization, from cohort or case-control analytical studies, or well-designed meta-analysis |
| III | Evidence based on clinical experience, descriptive studies, or reports of expert committees |
| IV | Not rated |

| A | Survival benefit |
| B | Improved diagnosis |
| C | Improvement in quality of life |
| D | Relevant pathophysiologic parameters improved |
| E | Impacts cost of health care |

For each recommendation, the roman numerals I through IV describe the quality of evidence upon which recommendations are based, and the upper case letters A through E describe the significance of the recommendation. Because of the nature of these guidelines, only categories B and E are used in the recommendations.
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Section I. Guidelines for Performance of Laboratory Tests of Liver Function and Injury

Performance Specifications for Laboratory Tests

Laboratory tests are used by clinicians for diagnosis, monitoring, and prognosis in patients with liver disease. A number of factors, primarily preanalytical and analytical, affect the accuracy of test results. The key characteristics of any test are its bias and imprecision. Bias is primarily an analytical characteristic, in which reported results differ from the actual value. Imprecision, or lack of reproducibility, is due to both physiological and analytical factors. In the baseline state, test results fluctuate in an individual due to random and predictable variation, termed intra-individual variation. The degree of variation can be increased under certain conditions, such as food ingestion, time of day, exercise, acute illness, or other forms of stress. In general, for many tests, there are also significant differences from one person to the next, termed inter-individual variation. Intra-individual, inter-individual, and analytical sources of variation must be considered in interpreting the results of laboratory tests as indicating a change in an individual’s health status.

Performance specifications serve as a guide to the laboratory as to the degree of analytical variation that will allow the clinician to accurately determine the physiologic state of an individual. Performance specifications can be established by different methods, including (in decreasing order of importance) medical outcome studies, data on biological variation, opinions of clinicians or professional societies, or data from proficiency testing or government directives. Performance goals should specify acceptable imprecision, bias, and total error (bias + 1.65 * imprecision). When goals are derived from biological data, the target for imprecision is less than half of the intraindividual variation for the test, while the target for bias is less than one-fourth of the average intraindividual (cv_i) and interindividual (cv_g) variation, calculated as \( \frac{1}{4} (cv_i^2 + cv_g^2)^{1/2} \). Table 2 summarizes published data on performance specifications and within-laboratory precision for liver related tests.

Table 2. Performance Specifications and Precision for Liver Tests (Percent)

<table>
<thead>
<tr>
<th>Source</th>
<th>Type</th>
<th>ALT</th>
<th>AST</th>
<th>ALP</th>
<th>GGT</th>
<th>Albumin</th>
<th>Bilirubin</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLIA Mandate</td>
<td></td>
<td>TE 20</td>
<td>TE 20</td>
<td>TE 30</td>
<td>TE 10</td>
<td>TE 20 or 0.4 mg/dL</td>
<td></td>
</tr>
<tr>
<td>European (7)</td>
<td>Biological variation</td>
<td>I 1</td>
<td>B 16</td>
<td>I 3.4</td>
<td>N/S</td>
<td>I 1.4</td>
<td>I 11.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B 16</td>
<td>B 6.2</td>
<td>B 6.4</td>
<td>B 1.1</td>
<td>B 9.8</td>
<td>B 28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TE 18</td>
<td>TE 12</td>
<td>TE 12</td>
<td>TE 3.4</td>
<td>TE 31</td>
<td></td>
</tr>
<tr>
<td>Ricos (8)</td>
<td>Biological variation</td>
<td>I 12.2</td>
<td>B 5.4</td>
<td>I 3.2</td>
<td>I 6.9</td>
<td>I 1.6</td>
<td>I 12.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TE 32</td>
<td>TE 15</td>
<td>TE 12</td>
<td>TE 10.8</td>
<td>B 10</td>
<td>B 31</td>
</tr>
<tr>
<td></td>
<td>Clinician opinion</td>
<td>N/S</td>
<td>TE 26</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
<td>TE 23</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source</th>
<th>Type</th>
<th>ALT</th>
<th>AST</th>
<th>ALP</th>
<th>GGT</th>
<th>Albumin</th>
<th>Bilirubin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lott (10)</td>
<td>Proficiency tests</td>
<td>8</td>
<td>9</td>
<td>5</td>
<td>6</td>
<td>N/S</td>
<td>N/S</td>
</tr>
<tr>
<td>Ross (11)</td>
<td>Proficiency tests</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
<td>4.4</td>
<td>8.9</td>
</tr>
</tbody>
</table>

TE – total error; I – imprecision; B – bias; N/S – not specified
Reference Intervals

In order to determine the likelihood that disease is present, test results are typically compared to values obtained from healthy individuals; the range of such results is termed the reference interval, while the high and low ends of the interval are termed the upper and lower reference limits, respectively. Most laboratories publish a single reference interval for most laboratory tests, defined as the central 95% of results obtained from healthy persons. In many cases, there are recognized factors that can affect the results of tests without indicating the presence of disease, particularly when only a single reference interval is used. For each chemical laboratory test listed, factors that affect results are summarized in tables and figures.

For some tests, reference limits are defined by health outcomes; examples include currently used reference limits for cholesterol and fasting glucose. Use of outcome-based reference limits also requires a high degree of standardization of measurement between laboratories to assure that results from all laboratories have a similar relationship to the upper reference limit. While data from studies on the likelihood of transmission of infection after transfusion suggest that an outcome based upper reference limit may be appropriate for ALT, there is not sufficient standardization of ALT measurements between laboratories to allow use of such an approach at the current time. There are no data on outcome-based reference limits for other tests of hepatic injury and function.

Aminotransferases

Aspartate aminotransferase (AST, also sometimes termed SGOT) and alanine aminotransferase (ALT, also sometimes termed SGPT) are widely distributed in cells throughout the body, with AST primarily in heart, liver, skeletal muscle, and kidney, while ALT is found primarily in liver and kidney, with lesser amounts in heart and skeletal muscle. AST and ALT activity in liver are about 7,000 and 10,000 times serum activities, respectively (12). ALT is exclusively cytoplasmic; both mitochondrial and cytoplasmic forms of AST are found in all cells (13). The half-life of total AST is 17 ± 5 hours, while that of ALT is 47 ± 10 hours (14). The half-life of mitochondrial AST averages 87 hours (15). In adults, AST and ALT activities are significantly higher in males than in females, and reference intervals vary with age (Figures 1 and 2).

![Figure 1](image1.png)

**Figure 1.** Age and Gender Effects on Upper Reference Limits for ALT. The upper reference limit for 25-35 year old males is set at 1.0 relative value units. ALT upper reference limits increase from childhood to about age 40, with greater increases seen in men than in women; upper reference limits are approximately 10% higher in 40 year old men than in those 25 years old. After age 40, ALT upper reference limits again decline, with the decline more pronounced in men than in women. Data from reference 16.

![Figure 2](image2.png)

**Figure 2.** Age and Gender Effects on Upper Reference Limits for AST. The upper reference limit for 25-35 year old males is set at 1.0 relative value units. AST upper reference limits increase from childhood to young adult years, but change relatively little with increasing age in adults until after age 60. At all ages except childhood and old age, AST upper reference limits are approximately 25-30% higher in men than in women. Data from reference 16.
Until about age 15, AST activity is slightly higher than that of ALT, with the pattern reversing by age 15 in males but persisting till age 20 in females (17). In adults, AST activity tends to be lower than that of ALT until approximately age 60, when they become roughly equal. Because upper reference limits vary little between the ages of 25 and 60, age-adjusted reference limits need not be used for this population, which comprises most persons with chronic liver injury. Separate reference limits are needed for children and older adults; these may require national efforts to obtain enough samples from healthy individuals to accurately determine reference limits.

Liver disease is the most important cause of increased ALT activity and a common cause of increased AST activity. A number of factors other than liver disease affect AST and ALT activities; these are summarized in Table 3.

### Table 3. Factors Affecting AST and ALT Activity Besides Liver Injury

<table>
<thead>
<tr>
<th>Factor</th>
<th>AST</th>
<th>ALT</th>
<th>Reference</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of day</td>
<td>45% variation during day; highest in afternoon, lowest at night</td>
<td>45% variation during day; highest in afternoon, lowest at night</td>
<td>18</td>
<td>No significant difference between 9 am and 9 pm; similar in liver disease and health</td>
</tr>
<tr>
<td>Day to day</td>
<td>5-10% variation from one day to next</td>
<td>10-30% variation from one day to next</td>
<td>19</td>
<td>Similar in liver disease and health, and in elderly and young</td>
</tr>
<tr>
<td>Race/gender</td>
<td>15% higher in African-American men</td>
<td>15% higher in African-American men</td>
<td>21</td>
<td>No significant difference between African-American, other women</td>
</tr>
<tr>
<td>Body mass index (BMI)</td>
<td>40-50% higher with high BMI</td>
<td>40-50% higher with high BMI</td>
<td>17, 22, 23</td>
<td>Direct relationship between weight and AST, ALT</td>
</tr>
<tr>
<td>Meals</td>
<td>No effect</td>
<td>No effect</td>
<td>17</td>
<td>Effect of exercise seen predominantly in men; minimal difference in women (&lt; 10%). Enzymes increase more with strength training</td>
</tr>
<tr>
<td>Exercise</td>
<td>3-fold increase with strenuous exercise</td>
<td>3-fold increase with strenuous exercise</td>
<td>24, 25</td>
<td>Stability based on serum separated from cells; stable for 24 h in whole blood, marked increase after 24 h</td>
</tr>
<tr>
<td>Specimen storage</td>
<td>Stable at room temp for 3 d, in refrigerator for 3 wks (10-15% decrease); Marked decrease with freezing/thawing</td>
<td>Stable at room temp for 3 d, in refrigerator for 3 wks (10-15% decrease). Marked decrease with freezing/thawing</td>
<td>26, 27, 28</td>
<td></td>
</tr>
<tr>
<td>Hemolysis/hemolytic anemia</td>
<td>Significant increase</td>
<td>Moderate increase</td>
<td>29</td>
<td>Dependent on degree of hemolysis; usually several-fold lower than LDH elevation</td>
</tr>
<tr>
<td>Muscle injury</td>
<td>Significant increase</td>
<td>Moderate increase</td>
<td>30</td>
<td>Related to degree of elevation in CK</td>
</tr>
<tr>
<td>Other</td>
<td>Maceroenzymes</td>
<td>Maceroenzymes</td>
<td>29, 30</td>
<td>Typically stable elevation, affects only AST or ALT</td>
</tr>
</tbody>
</table>

Unexpectedly abnormal results are often normal on repeat testing. In most types of liver disease, ALT activity is higher than that of AST; an exception is in alcoholic hepatitis. The reasons for the higher AST activity in alcoholic hepatitis appear to be multiple. Alcohol increases mitochondrial AST activity in plasma, while other forms of hepatitis do not (31). Most forms of liver injury decrease hepatocyte activity of both cytosolic and mitochondrial AST, but alcohol leads to decrease only in cytosolic AST activity (32). Pyridoxine deficiency, common in alcoholics, decreases hepatic
ALT activity (33); and alcohol induces release of mitochondrial AST from cells without visible cell damage (34).

AST and ALT are typically measured by catalytic activity (35); both require pyridoxal-5’-phosphate (P-5’-P) for maximum activity, although the effect of deficient P-5’-P on ALT is greater than that on AST (36). In renal failure, AST and ALT are significantly lower than in healthy individuals, perhaps due to serum binders of P-5’-P, as total P-5’-P is elevated (37). Because of marked differences between laboratories, standardization of methods is a priority. In the interim, alternative methods to minimize differences between laboratories, such as expressing results as multiples of the reference limit (38), have been shown to minimize between-laboratory variation (39).

Current target values for performance goals for total error in ALT activity measurements are 10% (CLIA). Clinical data on which to base performance goals is not available for most laboratory tests for liver evaluation, with the exception of ALT. Little data exist on the biological variation of ALT in chronic hepatitis, particularly hepatitis C, although it is commonly stated that ALT results are highly variable. In a study of 275 patients with confirmed HCV infection, the average intraindividual coefficient of variation was 38%, although in a quarter of patients, it was less than 23%. (Dufour, unpublished observations) Several studies have shown that treatment of chronic HCV infection is not indicated if ALT is within the reference range. Thus, accurate determination of ALT at the reference limit is critical for correct treatment of patients with HCV infection. The consensus of the authors and the AASLD Practice Guidelines committee is that performance criteria for ALT should be defined at the upper reference limits, and that current performance goals are inadequate for clinical use. The data from patients with stable ALT suggest that total error of < 10% is required at the upper reference limits for accurate detection of patients who may benefit from treatment for HCV. Current data on within-lab precision (Table 2) suggest that this target cannot be met by current methods. It will likely be necessary to develop a standardization program for ALT measurements, similar to that used for CK-MB. This may require use of other methods, such as immunoassay, to achieve the necessary total error target for management of patients with chronic hepatitis.

Performance goals for total error in AST activity measurements are 15-20%, both by CLIA requirements and based on biological variation. These meet the perceived needs of clinicians for diagnosis and management of liver disease (9). Performance goals are not as critical for AST as for ALT; a lower percentage of AST results are abnormal in chronic HCV compared to ALT (66% vs 71%). AST is seldom (6%) abnormal when ALT is normal, except in cirrhosis or alcohol abuse (Dufour, unpublished observations).

### Recommendation:
- Assays for ALT activity should have total analytical error of ≤ 10% at the upper reference limit (IIB). Current published performance goals for AST, with total error of 15-20%, are adequate for clinical use (IIIB).
- Standardization of ALT values between methods and across laboratories is a priority need for patient care. Until standardization is accomplished, use of normalized results should be considered (IIIB).
- At a minimum, laboratories should have separate upper reference limits for adult males and females; reference limits should also be established for children and adults over age 60 by cooperative efforts (IIB).
- Unexpectedly elevated ALT and/or AST should be evaluated by repeat testing; in individuals engaging in strenuous exercise, repeat should be performed after a period of abstinence from exercise. Research is needed to determine the appropriate time interval required (IIB, E).

### Alkaline Phosphatase

Alkaline phosphatase (ALP), involved in metabolite transport across cell membranes, is found, in decreasing order of
abundance, in placenta, ileal mucosa, kidney, bone, and liver. Bone, liver, and kidney alkaline phosphatase share a common protein structure, coded for by the same gene (40, 41); they differ in carbohydrate content. The half-life of the liver isoenzyme is three days (42). Age and gender related changes in alkaline phosphatase upper reference limits are illustrated in Figure 3.

![Alkaline Phosphatase](image)

**Figure 3. Age and Gender Effects on Upper Reference Limits for Alkaline Phosphatase.** The upper reference limit for 25-35 year old males is set at 1.0 relative value units. Alkaline phosphatase is many fold higher in children and adolescents, reaching adult activities by about age 25. Values are slightly higher in men than in women until late in life. In adult men, upper reference limits do not change with age, while in women upper reference limits increase after menopause. Data from reference 16.

Interpretation of alkaline phosphatase results using appropriate reference populations is particularly important in children; reference limits differ little in adult males and females between the ages of 25 and 60. After age 60, reference limits increase in women, although studies have not consistently evaluated for the presence of osteoporosis, which can increase alkaline phosphatase activity in serum. Separate reference ranges are required for children and pregnant women.

Cholestasis stimulates synthesis of ALP by hepatocytes; bile salts, detergents or other surface-active agents facilitate release of ALP from cell membranes (43, 44). Other factors affecting alkaline phosphatase are summarized in Table 4.
The method for total ALP in widest use is the p-nitrophenylphosphate method of Bowers, McComb and Kelly (50). Complexing agents such as citrate, oxalate or EDTA bind cations such as zinc and magnesium, necessary cofactors for ALP activity measurement, causing falsely decreased values as low as zero. Blood transfusion (containing citrate) causes transient decrease in ALP through a similar mechanism.

Separation of tissue nonspecific ALP forms (bone, liver, and kidney) is difficult owing to structural similarity; high resolution electrophoresis and isoelectric focusing are the most useful techniques. Bone-specific ALP can be measured by heat inactivation (poor method), immunologically and by electrophoretic methods. Immunoassays of bone ALP are now available from several sources (51), and can be used to monitor patients with bone disease. Because there is good agreement between increases in alkaline phosphatase of liver origin and an increase in the activity of other canalicular enzymes such as γ-glutamyl transferase (GGT), elevated GGT is a good indication of a liver source, but does not rule out coexisting bone disease (52).

In contrast to most enzymes, intrapatient variation in ALP is low, averaging slightly over 3% (Table 2). The current average within-laboratory imprecision of 5% is close to recommended performance specifications; a total error of 10-15% should meet health based target values of 12%. The CLIA specified total error range of 30% appears too wide for clinical use and should be narrowed.
**Recommendations:** Assays for alkaline phosphatase activity should have total analytical error of ≤ 10-15% at the upper reference limit (IIIB).

Separate reference limits should be provided for children, based on age and gender, and for pregnant women. A single reference range is adequate for adults over age 25 (IIB).

Specimens for alkaline phosphatase activity should be obtained in the fasting state; if not, mildly elevated patient values should be re-evaluated in the fasting state before further evaluation (IIIB, E).

Assays for alkaline phosphatase isoenzymes or measurement of other associated enzymes (such as GGT) are needed only when the source of an elevated alkaline phosphatase activity is not obvious from clinical and laboratory features (IIIB, E).

**Gamma-Glutamyl Transferase**

Gamma-glutamyltransferase (GGT), a membrane bound enzyme, is present in decreasing order of abundance in proximal renal tubule, liver, pancreas (ductules and acinar cells), and intestine. GGT activity in serum comes primarily from liver. The half-life of GGT in humans is about seven to 10 days; in alcohol-associated liver injury, the half-life increases to as much as 28 days, suggesting impaired clearance. Age- and gender-related differences in GGT are summarized in Figure 4.

![Gamma-Glutamyl Transferase](image)

*Figure 4. Age and Gender Effects on Upper Reference Limits for $\gamma$-Glutamyl Transferase (GGT). The upper reference limit for 25-35 year old males is set at 1.0 relative value units. GGT upper reference limits increase throughout life, with the effect more marked in women than in men. Before age 50, upper reference limits in men are approximately 25-40% higher than those in women, but the differences decrease with increasing age. Data from reference 16.*
In adult men, a single reference range is adequate between the ages of 25 and 80. Although upper reference limits are approximately 2 fold higher in those of African ancestry, information on racial characteristics is not commonly provided to laboratories; it would thus be difficult for laboratories to report values with the appropriate race-based reference interval. In women and children, GGT upper reference limits increase gradually with age, and are considerably lower than those in adult men. Separate reference limits should be established for men and women, and for different age ranges in women and children. In children, this will probably require a cooperative effort of laboratories to obtain adequate numbers of specimens from healthy children.

GGT is slightly more sensitive than ALP in obstructive liver disease. GGT is increased an average of 12 times the upper reference limit in 93-100% of those with cholestasis, while ALP is increased an average of 3 times the upper reference limit in 91% of the same group (52, 53, 54). GGT appears to increase in cholestasis by the same mechanisms as does ALP (54, 55). GGT is increased in 80-95% of patients with any form of acute hepatitis (55, 56). Other factors that affect GGT activity are summarized in Table 5.

| Table 5. Factors Affecting GGT Besides Liver Injury |
|-----------------------------------------------|-----------------------------------------------|
| **Factor** | **Change** | **Reference** | **Comments** |
| Day to day | 10-15% | 19 | Similar in liver disease and health, and in elderly and young |
| Race | Approximately double in African-Americans | 21 | Similar differences in men, women |
| Body mass index (BMI) | 25% higher with mild increase in BMI, 50% higher with BMI > 30 | 22 | Effect similar in men, women |
| Food ingestion | Decreases after meals; increases with increasing time since food ingestion | 57 | |
| Exercise | No significant effect | 57 | |
| Specimen storage | Stable for up to 7 d in refrigerator, for months in freezer | 47 | |
| Pregnancy | 25% lower during early pregnancy | 58, 59 | |
| Drugs | Increased by carbamazepine, cimetidine, diazoxide, heparin, isoniazid, methotrexate, oral contraceptives, phenobarbital, phenytoin, valproic acid | 60 | Values up to 2 times reference limits commonly, may be up to 5 times reference limits, especially with phenytoin |
| Smoking | 10% higher with 1 pack/d; approximately double with heavier smoking | 57 | |
| Alcohol consumption | Direct relation between alcohol intake and GGT | 57, 61 | May remain elevated for weeks after cessation of chronic alcohol intake |

Patients with diabetes, hyperthyroidism, rheumatoid arthritis and obstructive pulmonary disease often have an increased GGT; the reasons for these findings are largely obscure. After acute myocardial infarction, GGT may remain abnormal for weeks (62). These other factors cause a low predictive value of GGT (32%) for liver disease (63).

The International Federation of Clinical Chemistry method described by Shaw (64) is used by most laboratories. Precision with activities less than one-half the upper reference limit is about 10%; at about twice the upper reference limit, it is closer to 5%. Performance goals for GGT are primarily based on biological variation, with total error tolerance limits of approximately 20%. These are adequate for clinical purposes, given the limited clinical utility of GGT measurements.
**Recommendations:** Assays for gamma-glutamyl transferase activity should have total analytical error of ≤ 20% at the upper reference limit (IIIB).

Use of fasting morning specimens is recommended (IIB).

While a single upper reference limit is appropriate for adult men, separate reference limits (based on age) are needed for children and adult women (IIB).

Because of lack of specificity, GGT should be reserved for specific indications such as determining the source of an increased alkaline phosphatase (IIIB, E).

**Bilirubin**

Daily production of unconjugated bilirubin is 250 to 350 mg, mainly from senescent erythrocytes (65). Clearance at normal values is 5 mg/kg/day, or about 400 mg/day in adults; the rate does not increase significantly with hemolysis (66). The half-life of unconjugated bilirubin is <5 minutes (67). UDP-glucuronyltransferase catalyzes rapid conjugation of bilirubin in the liver; conjugated bilirubin is excreted into bile and is essentially absent from blood in normal individuals. Delta bilirubin (δ-bilirubin, also sometimes termed biliprotein) is produced by reaction of conjugated bilirubin with albumin (68); it has a half-life of about 7-20 days (the same as albumin), accounting for prolonged jaundice in patients recovering from hepatitis or obstruction (69). Age and gender related changes in bilirubin reference limits are illustrated in Figure 5.

![Total Bilirubin](image)

**Figure 5. Age and Gender Effects on Reference Limits for Total Bilirubin.** The upper reference limit for 25-35 year old males is set at 1.0 relative value units. Upper reference limits increase throughout childhood and adolescence, reaching peak values at approximately age 20; following this, values gradually decrease with increasing age. At all ages, upper reference limits are higher in men than in women, although the differences are minimal at the extremes of life. Data from reference 16.
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Increases in conjugated bilirubin are highly specific for disease of the liver or bile ducts (70). Increased conjugated bilirubin may also occur with impaired energy-dependent bilirubin excretion in sepsis, total parenteral nutrition, and following surgery (71). With recovery from hepatitis or obstruction, conjugated bilirubin falls quickly, while δ-bilirubin declines more slowly (72). Gilbert’s syndrome, found in about 5% of the population, causes mild unconjugated hyperbilirubinemia due to impaired UDP-glucuronyltransferase activity along with decreased organic ion uptake (73, 74). Total bilirubin rarely exceeds 68-85 μmol/L (4-5 mg/dL), even during prolonged fasting, unless other factors increasing bilirubin are also present (75). Other factors affecting bilirubin are summarized in Table 6.

Table 6. Factors Affecting Bilirubin Besides Liver Injury

<table>
<thead>
<tr>
<th>Factor</th>
<th>Change</th>
<th>Reference</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day to day</td>
<td>15-30%</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Food Ingestion</td>
<td>Bilirubin increases an average of 1-2 fold with fasting up to 48 h</td>
<td>76, 77</td>
<td>Averages 20-25% higher after overnight fast than after meals</td>
</tr>
<tr>
<td>Race</td>
<td>33% lower in African-American men, 15% lower in African-American women</td>
<td>21, 78</td>
<td>Compared to values in other racial/ethnic groups</td>
</tr>
<tr>
<td>Exercise</td>
<td>30% higher in men</td>
<td>72</td>
<td>Significant effect in women</td>
</tr>
<tr>
<td>Light exposure</td>
<td>Up to 50% decrease in one hour</td>
<td>79</td>
<td>Affects unconjugated bilirubin more than direct reacting bilirubin</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>Decreases 33% by second trimester</td>
<td>47</td>
<td>Similar in second, third trimester</td>
</tr>
<tr>
<td>Hemolysis</td>
<td>Cross-reacts in some assays</td>
<td>47</td>
<td>Hemoglobin absorbs light at the same wavelength as bilirubin</td>
</tr>
<tr>
<td>Oral contraceptives</td>
<td>15% lower</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>Hemolytic anemia</td>
<td>Increases in unconjugated bilirubin</td>
<td>47</td>
<td></td>
</tr>
</tbody>
</table>

Bilirubin is typically measured using two assays for total and “direct reacting” or direct bilirubin; subtracting direct from total gives “indirect” or conjugated bilirubin. The direct bilirubin assay measures the majority of δ-bilirubin and conjugated bilirubin, and a variable but small percentage of unconjugated bilirubin (79, 80). High pH or the presence of a wetting agent promotes reaction of unconjugated bilirubin in the “direct” assay; the reagent for “direct” bilirubin should have at least 50 μmol/L HCl to prevent measurement of unconjugated bilirubin (81). Light can convert unconjugated bilirubin to a photoisomer that reacts directly (79); it also causes total bilirubin to decrease by 0.34 μmol/L/hour (0.02 mg/dL/hour). Direct spectrophotometry (dry film methods) measures conjugated and unconjugated bilirubin individually, and can calculate δ-bilirubin as the difference between the sum of these and total bilirubin. Some have suggested conjugated bilirubin better than “direct” bilirubin to measure recovery from liver disease (82).

Performance goals for bilirubin measurement allow 20% (CLIA) to 30% (biological variation) total error. Clinicians felt that a 23% change in bilirubin at the upper reference limits indicates a significant change in condition (9). Thus, CLIA performance goals appear to meet clinical performance needs. At elevated concentrations, a change of 2 mg/dL (5%) was considered clinically significant. Target values for total error should thus specify the concentration of bilirubin.
**Recommendations:** Assays for bilirubin should have total analytical error of $\leq 20\%$ (or $6.8 \mu\text{mol/L}$ [0.4 mg/dL]) at the upper reference limit (IIIB).

Separate upper reference limits should be used for men and women. While bilirubin upper reference limits decline with age in adults, there is little significance to slight elevations in bilirubin and separate adult age-adjusted upper reference limits are not needed. In children, separate reference ranges should be used (IIIB).

**Albumin**

Albumin is the most abundant plasma protein produced by hepatocytes. Rate of production is dependent on several factors, including supply of amino acids, plasma oncotic pressure, levels of inhibitory cytokines (particularly IL-6), and number of functioning hepatocytes (83). The half-life of plasma albumin is normally about 19-27 days. Plasma albumin concentrations are low in neonates, typically 28 to 44 g/L (2.8-4.4 g/dL). By the first week of life, adult values of 37 to 50 g/L (3.7-5.0 g/dL) are reached, rising to 45-54 g/L (4.5-5.4 g/dL) at age 6 and remaining at these concentrations through young adulthood before declining to typical adult values. There is no significant difference in reference limits between males and females (84). Increased albumin is typically due to hemoconcentration, caused either by dehydration, prolonged tourniquet use during collection or specimen evaporation. The main causes for decreased albumin include protein loss (nephrotic syndrome, burns, protein losing enteropathy), increased albumin turnover (catabolic states, glucocorticoids), decreased protein intake (malnutrition, very low protein diets), and liver disease. Plasma albumin is seldom decreased in acute hepatitis due to its long half-life, but in chronic hepatitis albumin gradually falls with progression to cirrhosis. Albumin concentrations are a marker of decompensation and prognosis in cirrhosis.

Albumin is most commonly measured by dye binding methods, particularly bromcresol green and bromcresol purple; currently, about 50% of laboratories use each method. Bromcresol green methods may overestimate albumin (85), although differences between the two methods are small (83). Bromcresol purple underestimates albumin in renal failure (86) and in patients with increased $\delta$-bilirubin (87) making this method unsuitable for patients with jaundice. Estimation of albumin from protein electrophoresis is not recommended due to significant overestimation of albumin based on higher dye binding (83). Immunoassays for albumin are available but not widely used in plasma (88).

Performance goals for albumin measurement based on biological variation are typically around 4%, while CLIA allows an error of 10%. The clinical use of albumin measurements for liver disease is primarily in recognition of cirrhosis, and in determining its severity; these require significant changes from reference limits. Data from CAP surveys indicate that only 2% of laboratories can meet the error limits based on biological variation. The opinion of the committee is that the CLIA performance goals are adequate for clinical purposes.

**Recommendations:** Total error of $< 10\%$ at the lower reference limit is adequate for clinical purposes; performance goals based on biological variation, while an ideal goal for measurement, cannot be met by most laboratories (IIIB).

Assays for albumin in patients with liver disease should use bromcresol green. Bromcresol purple and electrophoresis determinations of albumin may be inaccurate in patients with liver disease (IIIB).

**Prothrombin Time**

Prothrombin time (PT) measures the time required for plasma to clot after addition of Tissue Factor and phospholipid;
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it is affected by changes in the activity of factors X, VII, V, II (prothrombin) and I (fibrinogen). All of these factors are synthesized in the liver, and three (II, VII, and X) are activated by a vitamin K-dependent enzyme through addition of a second, \( \gamma \)-carboxyl group on glutamic acid residues. Warfarin, a vitamin K antagonist, causes anticoagulation by inhibition of \( \gamma \)-carboxylation, rendering the factors unable to bind calcium and reducing their activity. Individuals on warfarin or with vitamin K deficiency synthesize normal amounts of the clotting factor precursors, but in an inactive form termed “proteins induced by vitamin K antagonists (PIVKA)”. Immunoassays are available to measure the most abundant PIVKA, des-\( \gamma \)-carboxy prothrombin. PT is relatively insensitive to deficiency of any single clotting factor; there is no significant increase until concentrations fall below 10% of normal (89).

PT is commonly reported in seconds and compared to patient reference values. The time required for a specimen to clot is inversely related to the amount of Tissue Factor present in the reagents. To minimize variation among reagents with different amounts of Tissue Factor, each is assigned an International Sensitivity Index (ISI); the lower the amount of Tissue Factor, the lower the ISI value and the longer the prothrombin time. To adjust for differences in the ISI of reagents, the international normalized ratio (INR) is used; the value is calculated as:

\[
INR = \left( \frac{PT_{\text{patient}}}{PT_{\text{control mean}}} \right)^{\frac{1}{\text{ISI}}}
\]

Use of reagents with low ISI improves the reproducibility of INR measurement, making use of low ISI reagents ideal for monitoring anticoagulant therapy (90).

The effect of ISI is much greater on PT in warfarin use than in liver disease, so that INR does not accurately reflect inhibition of coagulation in liver disease (89, 91, 92). A sample from a patient receiving warfarin has a PT of 20 s with high ISI reagents and a PT of 40 s when tested with low ISI reagents, but INR is essentially identical with both reagents (89). INR thus normalizes results in a patient on warfarin, despite differences in the ISI of reagents used. In liver disease, decreasing the ISI of reagents used causes only a slight increase in PT. For example, a sample from a patient with liver disease has a PT of 20 s with high ISI reagents but a PT of 23.6 s with low ISI reagents. In contrast to the patient on warfarin, where INR is virtually identical when differing ISI reagents are used, INR was 2.90 with high ISI reagents and 1.86 with low ISI reagents (89). If reagents with low ISI are used, INR thus markedly underestimates degree of coagulation impairment in liver disease. A possible cause for the discrepancy in INR utility between warfarin use and liver disease is the marked difference in the relative amounts of native prothrombin versus des-\( \gamma \)-carboxy prothrombin present in the two conditions. Patients on warfarin or with vitamin K deficiency have marked elevation of des-\( \gamma \)-carboxy prothrombin and decrease in native prothrombin, while patients with acute hepatitis or cirrhosis have decreased native prothrombin but only slight elevation of des-\( \gamma \)-carboxy prothrombin (93). Some preparations of Tissue Factor are inhibited by des-\( \gamma \)-carboxy prothrombin (93).

PT is reproducibly increased, usually at least 3 sec beyond the population mean, in acute ischemic (94, 95) and toxic (96) hepatitis, but is only elevated more than three seconds in viral (97) or alcoholic (98, 99) hepatitis. PT is often elevated in obstructive jaundice, and may respond to parenteral vitamin K administration. In chronic hepatitis, PT is typically within reference limits, but increases as progression to cirrhosis occurs, and is elevated in cirrhotic patients (100). Other factors affecting prothrombin time are summarized in Table 7.
Table 7. Factors Affecting Prothrombin Time

<table>
<thead>
<tr>
<th>Factor</th>
<th>Change</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen storage</td>
<td>No change at room temperature up to 3 d; refrigeration falsely shortens PT</td>
<td>101</td>
</tr>
<tr>
<td>Citrate concentration</td>
<td>3.2% citrate minimizes problems compared to other concentrations</td>
<td>102</td>
</tr>
<tr>
<td>Inadequate tube filling</td>
<td>Falsely increases PT</td>
<td>102</td>
</tr>
<tr>
<td>High hematocrit</td>
<td>Falsely increases PT</td>
<td>102</td>
</tr>
<tr>
<td>Other factors</td>
<td>Warfarin, malabsorption, vitamin K deficiency, drugs that decrease vitamin K production (especially antibiotics, fibr acid derivatives), consumptive coagulopathy increase PT</td>
<td>102</td>
</tr>
</tbody>
</table>

Reagents with the same ISI typically give different results on different instruments even of the same model (103). In addition, when using different manufacturer’s reagents with the same ISI, the same specimen can give different INRs (104). The reproducibility of PT results in laboratories using the same instrument and reagents is from 3-8% when prothrombin times are prolonged; variation is greater for INR than it is for the prothrombin time itself. Within a single laboratory, average variation in INR is estimated to be ±10% (104). The difference in PT between laboratories using different reagents may be marked; in one study, the average difference was 20% (104). Recently, use of calibrant plasmas to determine ISI in each laboratory for its own reagents and instrument has been shown to significantly improve reproducibility of INR (104, 106, 107).

**Recommendations:** PT (in seconds) rather than INR should be used to express results of prothrombin time in patients with liver disease; however, this does not standardize results between laboratories (IIB). Additional research into standardization of reagents and use of derived indices (percent activity, INR) in liver disease is needed (IVB).

Ammonia (NH₃)

Ammonia is a product of amino acid metabolism; it is cleared primarily by urea synthesis in the liver. *Helicobacter pylori* in the stomach appears to be an important source of ammonia in patients with cirrhosis (108). In liver disease, increased NH₃ is typically a sign of hepatic failure. High concentrations are seen with deficiency of urea cycle enzymes (Reye's syndrome (111), and with acute or chronic hepatic encephalopathy (112, 113). Mild increases in plasma NH₃ are seen in patients with chronic hepatitis, in proportion to the extent of disease (114). Use of NH₃ for monitoring of patients with encephalopathy is controversial; some studies have shown good correlation of NH₃ concentrations with degree of encephalopathy (111, 113), while others have not (115). NH₃ appears to enhance the effects of γ-aminobutyric acid (GABA) (116) and increases benzodiazepine receptors (117); both GABA and benzodiazepines have been implicated in the pathogenesis of hepatic encephalopathy. On the other hand, clinical features seen in persons with isolated hyperammonemia are not identical to those of hepatic encephalopathy (118). Other factors affecting NH₃ are summarized in Table 8.
### Table 8. Factors Affecting Ammonia Besides Liver Injury

<table>
<thead>
<tr>
<th>Factor</th>
<th>Change</th>
<th>Reference</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>4-8 fold higher in neonates; 2-3 fold higher in children &lt; 3 yr; reach adult concentrations by adolescence</td>
<td>119</td>
<td>Only arterial ammonia correlates with change in liver function. Tourniquet use, clenched fist increase venous ammonia.</td>
</tr>
<tr>
<td>Specimen source</td>
<td>Arterial higher than venous; difference greater in renal, hepatic disease. Capillary blood falsely increased due to NH₃ in sweat if skin inadequately cleaned.</td>
<td>112, 120</td>
<td>Increase greater in males than in females.</td>
</tr>
<tr>
<td>Exercise</td>
<td>Increases up to 3 fold after exercise</td>
<td>121</td>
<td>Increase greater in males than in females.</td>
</tr>
<tr>
<td>Smoking</td>
<td>Increases 10 µmol/L after 1 cigarette</td>
<td>120</td>
<td>Increase greater in males than in females.</td>
</tr>
<tr>
<td>Delay in analysis</td>
<td>Ammonia increases due to cellular metabolism: 20% in 1 hour and 100% by 2 hours.</td>
<td>122</td>
<td>Use of ice water, rapid centrifugation and separation of plasma minimize increases.</td>
</tr>
<tr>
<td>Other factors</td>
<td>Increased in acute leukemia, blood transfusion, bone marrow transplantation, portal-systemic shunts, GI bleeding or high protein intake</td>
<td>123, 124</td>
<td>Increase higher in liver disease due to high GGT activity in specimens.</td>
</tr>
<tr>
<td>Medications</td>
<td>Valproic acid, glycine (in irrigation fluids used in prostate, endometrial resection) increase ammonia production</td>
<td>125, 126</td>
<td>Increase higher in liver disease due to high GGT activity in specimens.</td>
</tr>
</tbody>
</table>

Specimens should have plasma separated from cells within one hour of collection; in patients with liver disease, separation within 15 minutes is ideal (120, 122).

Several methods have been used to measure ammonia (120), with enzymatic assays currently the most widely employed. One manufacturer uses slide technology with alkaline pH to convert ammonium to ammonia and measurement of ammonia with bromphenol blue. Reproducibility within laboratories using the same method averages 10 to 20%, with mean values using different methods differing by less than 10% on average (127).

**Recommendations:** Measurement of plasma ammonia for diagnosis or monitoring of hepatic encephalopathy is not routinely recommended in patients with acute or chronic liver disease; it may be useful in patients with encephalopathy of uncertain etiology (IIIB).

For most accurate measurement, arterial, rather than venous, specimens should be used (IIB).

Plasma should be separated from cells within 15 minutes of collection to prevent artifactual increases in ammonia (IIB).
Section II.
Hepatitis Serologic Markers and Nucleic Acid Testing

Hepatitis A virus (HAV) – Hepatitis A virus is an RNA virus of the picornavirus family. HAV is spread by the fecal-oral route, and causes hepatic injury after an incubation period of only a few weeks. HAV RNA is present in stool and plasma for most of the period before onset of clinical symptoms, but disappears soon after onset of clinical illness. Immunoglobulin M (IgM) antibodies to HAV (anti-HAV IgM) are typically present at onset of symptoms, and remain detectable for an average of 3 to 6 months after infection (range <30-420 days, with 95% positive longer than 4 months) (128). Total anti-HAV persists for long periods after infection, perhaps for life (129). Seroprevalence increases with increasing age, ranging from 11% in children < 5 years to 74% in those > 50 years (130). HAV vaccine induces detectable anti-HAV within 2 to 4 weeks of the initial dose of vaccine (131) and antibody remains detectable at 5 years in 99% of individuals completing vaccination (132). There are no commercially available antigen or nucleic acid detection tests for HAV. Immune electron microscopy and immunoassay methods have been used to detect HAV antigen in stool filtrates and other specimens in research settings, and HAV RNA assays have been employed to document sources of epidemics and in research studies.

Recommendations: IgM anti-HAV should be used to diagnose acute HAV infection (IB).
Total antibody should be used for determining immune status for HAV (IB).

Hepatitis B virus (HBV) – Hepatitis B is a DNA virus of the hepadnavirus group. These viruses replicate by forming an RNA intermediate, which is copied using the enzyme reverse transcriptase to regenerate DNA strands. HBV is transmitted by exchange of body fluids; major methods of transmission include serum, sexual intercourse, and transmission from mother to infant (usually occurring after birth). While HBV infection is typically acute with complete recovery in adolescents and immunocompetent adults, chronic infection can also occur. Approximately 1-3% of healthy adults, 5-10% of immunocompromised adults, and 90% of neonates exposed to HBV develop chronic infection.

HBV produces several protein antigens that can induce an antibody response. The most abundant, HBV surface antigen (HBsAg) is produced in excess along with viral particles, but can also be present when HBV DNA is integrated into cellular DNA and no longer produces infectious virions. HBV core antigen and e antigens (HbcAg and HBeAg) are produced in the same genetic region in the virus and are found in infectious particles. A typical serological and clinical course of acute HBV infection is shown in Figure 6 (133).
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Figure 6. Time Course of Serologic Markers in Acute Hepatitis B Infection with Resolution. Following infection, the first marker of infection to appear is the surface antigen (HBsAg), from 1 to 3 months after exposure. Approximately 1-2 months later, the first antibody response is IgM antibody to the hepatitis B core antigen (IgM anti-HBc), generally around the time of increase in AST and ALT activities in plasma. At the time of onset of jaundice, most patients have both HBsAg and IgM anti-HBc. With clearance of virus, anti-HBs will become detectable. In a small percentage of patients, there may be a transient period when neither HBsAg nor anti-HBs can be measured; the only commonly measured marker present at this time will be IgM anti-HBc, a pattern termed the “core window”. Although not illustrated in this diagram, usually such patients will be positive for anti-HBe if a second test is needed to confirm the anti-HBc result. With recovery from HBV infection, anti-HBc and anti-HBs persist for life in most individuals.

IgM antibody to HBcAg (anti-HBc) is usually considered the gold standard for diagnosis of acute hepatitis B (134). It may also be present at fluctuating, low titers in patients with chronic hepatitis B, particularly when patients also have positive plasma HBeAg, HBV DNA, or episodes of rising ALT indicating reactivation of disease (135). Total anti-HBc typically persists for life (136). HBsAg is characteristically present and anti-HBs absent at presentation in patients with acute HBV infection, but both are occasionally absent (134), leaving IgM anti-HBc the only marker of infection (“core window”). Isolated positive anti-HBc also may represent low level viremia, loss of anti-HBs many years after recovery, or a false positive result (136, 137, 138). Two factors are associated with likelihood of false positive results: low level of anti-HBc reactivity and absence of anti-HBs using sensitive immunoassays. In several studies, virtually none of those with low levels of anti-HBc and negative anti-HBs showed an anamnestic response to a single injection of HBsAg vaccine, whereas 35-40% of those with weakly positive anti-HBs and 50-80% of those with high level of anti-HBc responded (137, 139, 140). Convalescence from infection is indicated by loss of HBsAg and development of anti-HBs. Concomitant HBsAg and anti-HBs may be seen in a small number of patients with chronic HBV infection. This phenomenon appears to be particularly common in patients on maintenance hemodialysis (7%) compared to other HBsAg positive patients (2%) (141). The presence of anti-HBs in these settings does not appear to have clinical importance. Patterns of serological markers in various forms and phases of HBV infection are shown in Table 9 (142).
### Table 9. Serological diagnosis of hepatitis B virus infections (modified from reference 142)

<table>
<thead>
<tr>
<th>Marker</th>
<th>Incubation</th>
<th>Acute Infection</th>
<th>Past Infection</th>
<th>Chronic Infection</th>
<th>Vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAg</td>
<td>+(^a)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>HBeAg</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+/-</td>
<td>-</td>
</tr>
<tr>
<td>HBV DNA</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+/-(^b)</td>
<td>-</td>
</tr>
<tr>
<td>Anti-HBc IgM</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+/-(^c)</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Anti-HBe</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
<td>+/-(^d)</td>
<td>-</td>
</tr>
<tr>
<td>Anti-HBs</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^a\) detectable; - not detectable; +/- may be detectable.
\(^b\) Non-PCR method
\(^c\) May be positive in 10-15% patients with reactivation of infection
\(^d\) Patients with chronic HBV infection usually have detectable HBeAg or anti-HBe.

Examples of discordant or unusual hepatitis profiles are given in Table 10.

### Table 10. Discordant or unusual hepatitis B serologic profiles requiring further evaluation.

- HBsAg positive/Anti-HBc negative
- HBsAg, anti-HBs, and anti-HBc positive
- Anti-HBc positive only
- Anti-HBs positive only in a non-immunized patient
- HBsAg negative/HBeAg positive
- Positive for HBeAg and anti-HBe
- Total anti-HBc negative/IgM anti-HBc positive

Tests with discordant results should be repeated and testing for additional serological markers may be indicated to establish the correct diagnosis (143).

**Recommendations:** Tests for HBsAg, anti-HBs, and anti-HBc should be performed for diagnosis of current or past HBV infection. In suspected acute HBV infection, tests for IgM anti-HBc should be utilized (II).

HBeAg and anti-HBe are not required for diagnosis of acute hepatitis B or for routine evaluation of HBV status (IIIB, E).

In patients with discordant results, tests should be repeated; persistently discordant results should be evaluated by a hepatologist or gastroenterologist (IIIB).

In patients with chronic presence of HBsAg, HBeAg and anti-HBe are useful tests for determining the status of infection. HBV DNA can be present in hepatocytes in two forms: as replicating virus, leading to production of infectious particles, or integrated into the host DNA, a non-replicative form. HBeAg is only produced as part of replicating virus, and thus can be used to indirectly determine the state of HBV DNA production in the hepatocyte. In the HBeAg positive patient, loss of HBeAg and seroconversion to anti-HBe positivity is typically associated with loss of circulating HBV DNA by methods other than polymerase chain reaction (PCR), normalization of aminotransferases and histo-
logic improvement, implying a low replication state and significant clinical improvement (144). HBV DNA measurements are more useful in following chronic hepatitis B patients receiving antiviral therapy. Loss of detectable HBV DNA by a solution phase hybridization assay is an earlier indicator of response to antiviral therapy than loss of HBeAg (143). Several assays for detection of serum HBV DNA are commercially available; sensitivity limits are given in Table 11. There is currently no standardization of HBV DNA assays between laboratories.

**Table 11. Lower Detection Limits of HBV DNA Assays**

<table>
<thead>
<tr>
<th>Method</th>
<th>Detection Limit (copies/mL)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hybrid Capture</td>
<td>(3.0 \times 10^6)</td>
</tr>
<tr>
<td>Branched DNA</td>
<td>(0.7 \times 10^6)</td>
</tr>
<tr>
<td>Liquid Hybridization</td>
<td>(4.0 \times 10^4)</td>
</tr>
<tr>
<td>Polymerase Chain Reaction</td>
<td>(10^2 - 10^3)</td>
</tr>
</tbody>
</table>

\(^a\)Results can also be expressed as pg/mL of HBV DNA by dividing by 2.85 \(\times 10^5\)

Circulating HBV DNA can be found by sensitive PCR methods in a high percentage of patients with negative HBsAg and positive anti-HBs, anti-HBe, and anti-HBc months to years after clinical recovery from acute hepatitis (145) or chronic hepatitis (146). The significance is not clear, as most viral DNA is found in immune complexes (145), and may not represent the entire genome. A recent study of 67 potential liver donors who were HBsAg negative and anti-HBs and anti-HBc positive found replicating forms of the virus within the hepatocyte in 6 of the 7 individuals (147). Similarly, in patients with chronic hepatitis C infection, HBV viral DNA is commonly found (using sensitive PCR methods) in both liver and serum, particularly in patients with anti-HBc as an isolated HBV marker (137, 138). These studies suggest that many patients who were formerly thought to have recovered from HBV actually have low, but controlled, levels of viral replication persisting for many years after clinical recovery. It is not clear what level of HBV DNA viremia should be used to consider a patient “cured” of HBV infection for clinical purposes.

**Recommendations:**

- HBeAg and anti-HBe are useful in monitoring patients with chronic HBsAg positivity (IIB).
- Quantitative HBV DNA should be used for monitoring response to antiviral therapy (IIB).
- An international standard for HBV DNA tests should be established and manufacturers should calibrate kits against it. (IIIB).
- Tests for HBV DNA should be quantitative and the clinically useful dynamic range for HBV DNA tests should be defined (IIIB).

**Hepatitis C virus (HCV)** - Hepatitis C virus (HCV) is an RNA virus of the flaviviridae family. To date, HCV has not been cultured; it was recognized by detecting viral sequences through recombinant technology, and the entire genome has now been sequenced. Currently, no commercial assays are available to detect HCV antigens, although a highly sensitive assay for HCV core protein has been developed (148).

Most HCV tests measure antibodies to HCV. Screening tests for HCV infection detect antibodies to HCV proteins,
usually apparent by an average 80 days (range 33-129 days) after infection using second generation anti-HCV enzyme immunoassays (EIA-2) (149). Immunocompromised patients and those on dialysis may rarely lack detectable antibodies by EIA-2 despite other evidence of active viral infection (150). A third generation EIA (EIA-3) for anti-HCV has been approved by the FDA for screening blood products; it contains reconfigured core and NS3 antigens and an additional antigen (NS5) not found in EIA-2. EIA-3 provides a slight increase in sensitivity but lower specificity than EIA-2, and shortens the time to detection of antibody to an average of 7-8 weeks after infection (151). In patients who have cleared HCV from the circulation, titers of anti-HCV gradually fall (152), and eventually become negative in 6-10% of infected individuals (153, 154). In evaluating possible perinatal transmission of HCV, maternal antibody clears by 12 months in 90% of non-infected infants and by 18 months in 100% (155). Approximately 90% of infected infants have detectable plasma HCV RNA by 3 months of age (156).

Supplemental tests for anti-HCV help resolve suspected false-positive EIA test results. Recombinant immunoblot assays (RIBA) contain the same HCV antigens as do the EIA tests, along with superoxide dismutase (SOD) to detect non-specific antibodies to yeast proteins (recombinant HCV antigens are typically derived using yeast as the vector). A positive RIBA is defined as reactivity against two or more HCV antigens from different regions of the genome, without reactivity to SOD. Reactivity to a single HCV antigen or multiband reactivity with reactivity to SOD are considered indeterminate. In populations at high risk for HCV infection less than 1% of EIA-2 positive specimens will be false-positives. Additionally, in recently infected individuals, RIBA is positive in only 85% of cases (157). Therefore, RIBA testing in high-risk populations is not necessary for the diagnosis of hepatitis C (158).

Active HCV infection is defined by presence of HCV RNA in plasma. HCV RNA can be detected within 1-2 weeks after acute infection, weeks before ALT becomes abnormal and prior to appearance of anti-HCV (152). The time course of markers in typical HCV infection is illustrated in Figure 7.

**Figure 7 – Time Course of Serologic Markers in Acute Hepatitis C Infection.** Following infection, the first marker to appear is HCV RNA, usually detectable by 1-2 weeks following exposure to the virus. HCV RNA concentration gradually increases, but begins to fall with development of antibody response; it may be transiently negative in about 15% of cases. Anti-HCV appears at an average of 8-10 weeks after exposure; the time is shorter with third generation than with second generation anti-HCV assays. After the acute episode, which is clinically silent in most individuals, 75-85% will develop chronic infection with HCV. During the transition from acute to chronic infection, both ALT and HCV RNA may be intermittently positive; they are more likely to be persistently positive many years after infection, although 15-25% of chronically infected individuals may have persistently normal ALT.
Although not FDA-approved, reverse transcription (RT) PCR assays for HCV RNA are used commonly in clinical practice; the most sensitive can detect > 100 HCV RNA copies/mL. HCV RNA assays are not standardized, and quantitative assay results may vary significantly between different laboratories using different assays (158, 159). HCV RNA is very susceptible to degradation by the high activities of RNase present in blood; therefore, serum specimens for HCV RNA should be centrifuged as soon as possible after clot formation. EDTA or sodium citrate plasma are preferred specimens for HCV RNA tests. Heparinized plasma is inhibitory for many nucleic acid amplification assays, and serum specimens provide suboptimal stability unless serum is frozen soon after specimen collection. If centrifugation is performed immediately, less than 10% of HCV RNA is lost even if the plasma or serum is not separated from the formed elements for up to 6 hours (160). If a serum separator tube is used, specimens are stable after centrifugation for up to 24 hours (160). Short term (< 7 days) storage of serum or plasma at 4°C is acceptable. Once frozen, samples are stable through at least three freeze-thaw cycles (160). Quantitative HCV RNA assays are often less sensitive than qualitative RNA assays using the same technology, but this is not universal. The current version of the branched DNA assay is the least sensitive, with a lower limit of detection of 200,000 copies/ml; however, branched DNA assays have better linearity and reproducibility than do PCR assays. A more sensitive branched DNA assay is being introduced commercially in the United States. In patients with chronic HCV who are untreated, it is unusual to encounter specimens with undetectable HCV RNA by branched DNA but positive by PCR. Results from different methods cannot be directly compared because different standards are used. A World Health Organization international standard for HCV RNA for nucleic acid amplification assays is now available (161), and is being introduced to use by kit manufacturers.

**Recommendations:**
- EIA screening tests for HCV antibody are adequate for diagnosis of past or current HCV infection in a patient population with a high prevalence of disease; supplemental testing is not needed in such patients. If confirmation of active infection is required, HCV RNA should be used (IIB, E).
- Supplemental anti-HCV tests (RIBA) should be used in populations with low prevalence of disease, or to confirm prior infection by HCV in a patient whose HCV RNA negative (IIIB, E).
- Improved inter-method agreement and precision are needed for HCV RNA tests; methods should use a standard such as that developed by the World Health Organization (IIB).
- Specimens for HCV RNA should either be collected as EDTA or citrated plasma, or be promptly centrifuged to prevent falsely low results (IIB).

There are six major genotypes and >90 subtypes of HCV that vary in their world-wide distribution. In addition, HCV has a high rate of spontaneous mutation, producing discrete “quasispecies” that vary from one individual to the next (162). Genotypes 1a and 1b account for about 2/3 of infections in the United States; genotype 1 represents 90-95% of infections in African-Americans compared to about 60% in white patients (163). Genomic amplification and sequencing, followed by sequence comparison and phylogenetic tree construction is the reference method for genotype determination (164). A variety of genotype screening assays have been described, including PCR using genotype-specific primers (165), restriction fragment length polymorphism of amplified sequences (166), and a commercially available line probe assay (167). These methods compare favorably with the reference method for determining HCV genotype (168).

**Hepatitis D virus** - HDV is a defective RNA virus that replicates only in the presence of HBsAg. Testing for evidence of HDV infection should be considered in HBsAg-positive patients with symptoms of acute or chronic hepatitis, particularly in those with fulminant hepatitis or where there is a high risk for HDV infection. The only HDV serological test widely available commercially detects total anti-HDV. In patients in whom virus is cleared, antibody typically disappears between 1 and 5 years (169). In most clinical situations, HBsAg, IgM anti-HBc, and total anti-
HDV are adequate to diagnose HDV infection. Patients with acute HDV co-infection are usually positive for IgM anti-HBc, while patients with HDV superinfection are usually negative for IgM anti-HBc.

**Hepatitis E virus** - HEV is an enterically transmitted RNA virus that causes sporadic and epidemic acute hepatitis in developing countries; it does not cause chronic hepatitis. In the United States, HEV infection has been seen rarely as a cause of hepatitis, predominantly among those who have traveled to endemic areas, although at least one case has occurred without history of travel (170). Immunoassays for anti-HEV have been developed for diagnostic use (171). An evaluation of multiple anti-HEV methods showed significant variation in titers reported, and discordance between methods, although tests detecting antibodies to ORF2 were most accurate (172). Antibody reactive with HEV antigens was found in 15-25% of homosexual men, intravenous drug users, and blood donors in Baltimore, suggesting lack of specificity of assays (173).
Section III.
Acute Hepatic Injury

Hepatic injury is defined by damage to hepatocytes. Traditionally, two main patterns of hepatic injury are recognized, acute and chronic. These are often termed “hepatitis”, indicating the presence of inflammation in the liver. With some causes of hepatic injury, however, inflammation is minimal or absent; the more specific term hepatic injury will thus be used in this document. Acute hepatic injury refers to hepatocyte damage that occurs abruptly and over a short period of time. The most consistent feature of acute hepatic injury is significant elevation of aminotransferases (usually more than eight times the upper reference limit), often accompanied by increased bilirubin. Protein synthesis is affected in some cases, particularly those due to direct injury to hepatocytes by ischemia or toxin ingestion. Chronic hepatic injury refers to continuing hepatocyte damage over long periods of time, usually defined as a period greater than 6 months. Chronic hepatocyte injury is usually recognized by slight elevation of aminotransferases (usually less than 4 times the upper reference limit), although activities may be intermittently elevated and, in a small percentage of cases, persistently within reference limits. Bilirubin excretion and protein synthesis are generally normal. Alkaline phosphatase is generally within reference limits in most cases of acute and chronic hepatic injury; measurement is generally used to recognize hepatic disorders with obstruction of biliary drainage, which may otherwise resemble acute or chronic hepatic injury. Total protein, often included in hepatic panels, is generally not useful in evaluating hepatic function, since it is affected by changes in immunoglobulin levels as well as by changes in liver synthesis. An increase in globulins is helpful in patients with acute or chronic hepatic injury in suggesting the possibility of autoimmune disease as a cause of injury.

Recommendation: A liver panel that contains the following tests should be used to evaluate patients with known or suspected liver disease: aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, direct bilirubin, total protein, and albumin. Such a panel is currently approved by the Health Care Financing Administration for Medicare reimbursement (IIB, E).

Acute Hepatic Injury

Acute hepatic injury can be recognized by the presence of jaundice or non-specific symptoms of acute illness accompanied by elevation of AST and/or ALT activities. An estimated 80% of individuals with acute viral hepatitis are never diagnosed clinically, although some may be detected by elevated aminotransferases in the face of non-specific or absent clinical symptoms. AST and ALT activities are seldom greater than 10x the upper reference limit in liver diseases other than acute hepatic injury. ALP is over 3x the upper reference limit in less than 10% of cases of acute hepatic injury (175). Over the past decade, there has been a significant decline in the incidence of acute viral hepatitis; in the Centers for Disease Control and Prevention Sentinel Counties Study, hepatitis B declined by 55% and non-A, non-B hepatitis (most of which are hepatitis C) by 80% (175a). Other liver diseases are now more commonly encountered as causes of increased AST or ALT activities; in a recent study, 25% of those with AST more than ten times the upper reference limit had obstruction as a cause (176). Overall, about 1-2% of patients with bile duct obstruction have transient increases in AST and/or ALT activities of greater than 2000 U/L (177, 178); aminotransferase activities usually fall to within reference limits by 10 days even if obstruction persists (174, 176, 177).

The best discriminant values for recognizing acute hepatic injury appear to be 200 U/L for AST (sensitivity 91%, specificity 95%) and 300 U/L for ALT (sensitivity 96%, specificity 94%) (175). AST is over ten times the upper reference limit in slightly over half of patients at the time of presentation (175). In uncomplicated alcoholic hepatitis, AST and ALT values are almost never over 10x the upper reference limit, AST/ALT ratio is over 2 in 80%, and elevated
alkaline phosphatase is present in 20% of cases (98, 179, 180). Jaundice occurs in 60-70% of cases of alcoholic hepatitis (179, 180). The frequency of jaundice in patients with acute viral hepatitis differs both by age and etiologic agent. Jaundice is rare in children with viral hepatitis, and when present less severe than in adults. In one study, only 1% of children with acute hepatitis had peak bilirubin over 171 \( \mu \text{mol/L} \) (10 mg/dL), while 27% of adults did (181). In adults, jaundice develops in 70% of cases of acute hepatitis A (182), 33-50% of cases of acute hepatitis B (183, 184) and 20-33% of cases of acute hepatitis C (185, 186). There is a direct correlation between age and peak serum bilirubin in children; an increase of 10 years in age was associated with an average increase of 85 \( \mu \text{mol/L} \) (5 mg/dL) in bilirubin. In adults, there is no relationship between age and peak bilirubin (187). The distribution of direct bilirubin as a percentage of total bilirubin is similar in acute hepatic injury and obstructive jaundice: only 16% of those with acute hepatic injury have direct bilirubin < 50% of total bilirubin. Lower percentages of direct bilirubin suggest another cause for jaundice such as hemolysis (187).

**Recommendations:** Acute hepatic injury can be diagnosed by ALT more than 10x upper reference limits and alkaline phosphatase less than 3x the appropriate upper reference limit (IIB).

Direct bilirubin is needed to rule out other causes of increased total bilirubin such as hemolysis, but does not differentiate hepatic injury from obstructive jaundice (IIB).

### Markers of Severity

Acute viral hepatitis A or B is usually a self-limited illness, and most patients recover completely. In those with acute hepatitis C infection, approximately 80-85% develop chronic hepatitis, although the percentage appears to be lower in children or in young women receiving Rh immune globulin (188, 189). Rarely, acute hepatic injury causes severe liver damage and acute liver failure. Testing should identify patients at highest risk for liver failure.

Aminotransferase activities are more related to the cause of hepatic injury, rather than to severity. There is weak correlation between aminotransferase activities and bilirubin in viral hepatitis (175) and none in ischemic or toxic hepatic injury (190). Peak aminotransferase activities bear no relationship to prognosis, and may fall with worsening of the patient’s condition; in all causes of hepatic injury, aminotransferase activities begin to fall before peak bilirubin occurs regardless of whether recovery or deterioration occurs (175, 191). Prothrombin time (PT) is the most important predictor of prognosis; cutoff times > 4 seconds beyond control, > 20 seconds, or INR > 6.5 have been used to identify patients at high risk of death (99, 191). In ischemic or toxic hepatic injury, prolongation of PT is common early after injury, with peak abnormality occurring by 24-36 hours and then rapidly returning to normal. In acetaminophen injury, marked prolongation of PT does not by itself indicate likelihood of liver failure (94, 96), but persistent elevation or rising PT more than 4 days after acetaminophen ingestion does (192). Other tests may be prognostically helpful with specific causes of hepatic injury (191). In viral hepatitis, total bilirubin > 257 \( \mu \text{mol/L} \) (15 mg/dL) indicates severe liver injury and mandates close monitoring for encephalopathy (193). In alcoholic hepatitis, bilirubin > 428 \( \mu \text{mol/L} \) (> 25 mg/dL), or albumin < 25 g/L (2.5 g/dL) predicts a high likelihood of death (91, 180).

**Recommendations**

Total bilirubin > 257 \( \mu \text{mol/L} \) (15 mg/dL) or PT > 4 seconds above the upper reference limit in an individual with viral hepatitis, in the absence of other factors affecting results, indicates severe liver injury (IIB).

With acetaminophen toxicity, persistent elevation or rising prothrombin time more than 4 days after ingestion indicates severe liver injury (IIB).
**Table 12. Patterns of Laboratory Tests in Types of Acute Hepatic Injury**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Peak ALT (x URL)</th>
<th>AST/ALT Ratio</th>
<th>Peak Bilirubin (mg/dL)</th>
<th>Prothrombin Time Prolongation (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viral Hepatitis</td>
<td>10-40</td>
<td>&lt; 1</td>
<td>&lt; 15</td>
<td>&lt; 3</td>
</tr>
<tr>
<td>Alcoholic Hepatitis</td>
<td>2-8</td>
<td>&gt; 2</td>
<td>&lt; 15</td>
<td>1-3</td>
</tr>
<tr>
<td>Toxic injury</td>
<td>&gt; 40</td>
<td>&gt; 1 early</td>
<td>&lt; 5</td>
<td>&gt; 5 (transient)</td>
</tr>
<tr>
<td>Ischemic injury</td>
<td>&gt; 40</td>
<td>&gt; 1 early</td>
<td>&lt; 5</td>
<td>&gt; 5 (transient)</td>
</tr>
</tbody>
</table>

x- times; URL- upper reference limit

It is often possible to suspect the type of agent causing hepatic injury from the pattern seen. Initial evaluation of patients with the most common (immunologic) pattern of acute hepatic injury should include a drug history and testing for antibodies to hepatitis A, B, and C viruses (HAV, HBV, and HCV). Most hepatic drug reactions occur within 3 to 4 months of initiating treatment. However, in some instances, hepatic injury may become manifest as late as 12 months after beginning treatment and in a few instances injury may become evident days to weeks after stopping the responsible drug (198). Hence, it is important to ask about all drugs the patient may have received or has continued to receive during the past year or so. Evaluation for viral hepatitis should use the Health Care Financing Administration approved acute hepatitis panel (IgM anti-HAV, IgM anti-HBC, BsAg, and anti-HCV) (Figure 8).

Figure 8 – Approach to acute hepatic injury – In a patient with signs or symptoms of acute hepatic injury (fever, loss of appetite, dark urine, light stools jaundice), initial evaluation includes measurement of AST and ALT activities. Slight elevations in AST (< 10x the upper reference limit), particularly with AST > ALT, suggest acute alcoholic hepatitis, while marked elevations (> 100x the upper reference limit) strongly suggest ischemic or toxic liver injury. In those with intermediate values, an acute hepatitis panel (see text) is the initial test for evaluation. Presence of IgM anti-HAV or IgM anti-HBC are considered diagnostic for acute hepatitis A and B respectively. Acute hepatitis C cannot be definitively diagnosed by serologic tests, but can be suspected by appearance of anti-HCV in a patient with jaundice (when previously negative), or positive HCV RNA in a patient lacking anti-HCV. In patients with negative results for viral markers, another cause of acute hepatic injury such as obstruction, other infectious agents, Wilson's disease, or autoimmune hepatitis should be considered (see Table 13).
IgM anti-HAV, the diagnostic test of choice for acute HAV infection, disappears by 4-6 months (194), while total HAV antibodies persist for life (129) and are found in a high percentage of the population (130). Because of its brief period of transmissibility, diagnosis of acute HAV infection should be made as soon as possible after presentation, ideally within 48 hours, to allow immune globulin treatment of exposed individuals. IgM anti-HBc and HBsAg are the most reliable tests for acute HBV infection (134, 193); IgG (and thus total) anti-HBc persists for years, and is not helpful in diagnosis of acute hepatitis B infection (136). Other HBV viral markers and antibodies are not of use in the diagnosis of acute HBV infection. There is currently no test to definitively diagnose acute hepatitis C, since anti-HCV and HCV RNA can be present in both acute and chronic HCV infection. Anti-HCV is detectable with EIA-2 in only 77% of acute HCV cases at the time of initial enzyme elevation, while HCV RNA is positive in essentially all cases (195), although it is intermittently present in 15% (157, 196). By the time of clinical presentation, about 90% are detectable as anti-HCV (196). Patterns that would support a diagnosis of acute hepatitis C are negative anti-HCV but positive HCV RNA, or (if HCV RNA was not tested) anti-HCV results that convert from negative to positive within a short time period. Use of anti-HDV to detect delta (HDV) infection should be limited to patients with positive HBsAg, particularly if accompanied by severe acute hepatitis, high risk factors (IV drug abuse, hemophilia) or a biphasic pattern of illness (197). If a patient with chronic hepatitis B becomes superinfected with HDV, a clinical picture resembling severe acute hepatic injury and hepatic failure may evolve (197).

**Recommendations:** Initial evaluation of acute hepatic injury should include a detailed drug history and viral markers (IgM anti-HAV, IgM anti-HBc, HBsAg, and anti-HCV) (IIB).

Because of the need for post-exposure prophylaxis, turnaround time of IgM anti-HAV should be < 48 hours (IIIC, E).

If cost-effective (based on prevalence), laboratories may use total antibody to HAV and anti-HBc initially, performing IgM antibodies only if one or both is positive, if a turnaround time needs can be met (IIIE).

Diagnosis of acute HCV infection (in a patient with a clinical picture of acute hepatic injury) can be presumptively made by negative HAV and HBV markers, recent exposure, and either negative anti-HCV and positive HCV RNA or negative anti-HCV at initial presentation with development of positive anti-HCV within 3 months (IIIB).

Testing for HDV should be limited to patients with positive HBsAg, atypical clinical course, and high risk for HDV infection (IIB, E).

**Workup of Patients without Obvious Cause for Acute Hepatic Injury (Table 13)**

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Key Feature</th>
<th>Laboratory Tests</th>
<th>Associated findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wilson’s disease</td>
<td>Young individuals, low alkaline phosphatase, high bilirubin.</td>
<td>Low ceruloplasmin in only 40%. Abnormal gene on chromosome 13</td>
<td>Urine, serum copper not reliable in patients with acute Wilson’s. Often associated with hemolysis, renal insufficiency</td>
</tr>
<tr>
<td>Autoimmune hepatitis</td>
<td>Young individuals; increased gamma globulins; low albumin, ascites often present</td>
<td>Positive ANA and/or ASMA</td>
<td>Other autoimmune disorders in some cases</td>
</tr>
<tr>
<td>Hepatitis E</td>
<td>Travel to endemic area</td>
<td>Anti-HEV</td>
<td>Similar to hepatitis A</td>
</tr>
<tr>
<td>Other viruses</td>
<td>Clinical features of mononucleosis often present</td>
<td>Anti-CMV, anti-EBV</td>
<td>Elevated alkaline phosphatase</td>
</tr>
</tbody>
</table>

ANA – antinuclear antibody; ASMA – anti-smooth muscle antibody; HEV – hepatitis E virus; CMV – cytomegalovirus; EBV – Epstein-Barr virus
Ischemic and Toxic Hepatic injury - Values of AST or ALT over 100x normal are rare in viral hepatitis (174, 175), but common in both toxin ingestion, especially acetaminophen (96, 199, 200), and ischemic hepatic injury (94, 95, 190). In acetaminophen-induced hepatic injury, peak AST is over 3,000 U/L in 90% of cases (200). Toxic or ischemic hepatic injury cause over 90% of cases of acute hepatic injury with AST activity > 3,000 U/L (200a). In both ischemic and toxic hepatic injury, AST and ALT activity typically peak early (often in the first 24 hours after admission) with AST activity initially higher than that of ALT. After peaking, activities of both fall rapidly; AST may fall by 50% or more in the first 24 hours (199, 200), and declines more rapidly than ALT due to its shorter half-life (175). AST activity reaches near normal values an average of 7 days after injury (174). Prothrombin time is more than 4 seconds above the reference limits in 90% of cases (94, 96), and rapidly falls after peak AST is reached (94). Bilirubin is less than 34 μmol/L (2 mg/dL) in 80% of cases of toxic or ischemic injury (94, 96, 99). Lactate dehydrogenase (LDH) activity is often higher than that of AST at presentation in toxic or ischemic hepatic injury (94, 199, 200), while it is increased on initial determination in only 55% of cases of viral hepatitis with average values being only slightly above the upper reference limit (175).

Other Causes - Rarely, Wilson’s disease and autoimmune hepatitis (discussed in more detail under chronic hepatic injury) can present as acute hepatic injury (Table 13). Hepatitis E is endemic in parts of the world; individuals with acute hepatic injury who have traveled to or reside in endemic areas should be tested for anti-HEV. Several viruses other than the classical agents (HAV, HBV, HCV, HEV) have been associated with hepatitis, including herpesvirus, cytomegalovirus (CMV), enterovirus, coronavirus, reovirus, adenovirus, parvovirus B6 (in pediatric populations), varicella-zoster virus, and Epstein-Barr virus. Syphilis, leptospirosis, and toxoplasmosis may also cause hepatic injury, as may other less common infectious agents. Rarely, non-infectious disorders including lymphoma, Budd-Chiari syndrome, and venoocclusive disease may present with a picture of acute hepatic injury. In general, hepatic injury associated with these etiologies is either unusual, or is associated with a specific syndrome (chicken pox with varicella-zoster virus, mononucleosis with Epstein-Barr virus or cytomegalovirus). Most patients with other infectious causes of hepatic injury have signs and symptoms that suggest a particular agent as the cause. Specific diagnosis of infection by other agents should be pursued when the etiology remains unknown after more common causes are excluded, and when establishment of a specific diagnosis appears clinically indicated. Superinfection with other hepatitis viruses may occur in a patient with other forms of hepatic injury. For example, patients with chronic HCV or alcoholic hepatitis may become infected with either HAV or HBV and develop an acute hepatitis due to the superimposed infection. In chronic hepatitis, an acute rise in aminotransferases mimicking acute hepatic injury can occur with clearance of HBeAg (208) or with emergence of quasispecies of HCV (209).

Recommendations: In patients with negative viral markers and initial AST > 100x upper reference limit, toxic exposure or ischemia should be suspected (IIB).

In patients with negative viral markers and enzyme levels 8-100 times the upper reference limit, testing must exclude the possibility of Wilson’s disease and autoimmune hepatitis (IIB).

Testing for antibody to hepatitis E is recommended in those with negative serologies for other viruses and history of recent travel to or residence in an endemic area (IIIE).

Tests for other infectious agents (Epstein-Barr and cytomegalovirus, syphilis, toxoplasmosis) may be used if no other causes are evident (IIB).
Monitoring

Aminotransferases - Aminotransferase activities tend to rise before and peak near onset of jaundice in viral hepatitis, falling gradually from that point on (191). Activities tend to fall slowly in viral hepatitis and alcoholic hepatitis; AST and ALT decrease, on average, 11.7% and 10.5% per day, respectively, and remain elevated 22 ± 16 and 27 ± 16 days, respectively (175). In hepatitis A, a secondary rise in enzymes occurs in 5-10% of cases before activities return to baseline, associated with circulating HAV RNA and viral particles in stool, indicating potential for transmission of infection (210). As discussed above, AST and ALT fall rapidly after reaching peak activities in ischemic and toxic hepatic injury. Once aminotransferases have shown a consistent pattern of decrease, they need not be checked again until the patient has clinically recovered. Return of aminotransferases to normal is not a reliable sign of recovery in hepatitis B or C. In patients with chronic HCV infection, 49% with normal ALT on initial visit after seroconversion developed elevated ALT on subsequent follow-up (211). In hepatitis B, AST and ALT may return to normal despite persistence of infection (212).

Bilirubin - Bilirubin peaks later than aminotransferases, often by a week or so, and then gradually decreases. Peak bilirubin over 257-342 μmol/L (15-20 mg/dL) is unusual in viral hepatitis. Only 10-12% of patients with viral hepatitis have peak values over 257 μmol/L (15 mg/dL) and only 4% have peak values over 342 μmol/L (20 mg/dL); higher bilirubin is more common in HBV infection (175, 181). As total bilirubin declines, the proportion of δ-bilirubin increases, often reaching 70-80% of total bilirubin (213, 214). In adults with viral hepatitis, bilirubin remains elevated 30.3 ± 19.7 days after peak levels are reached (175), but clears more quickly in children (181); jaundice remains more than 6 weeks in 34% of adult HBV cases but in only 15% of other forms of viral hepatitis (181). Prolonged elevation of conjugated bilirubin occasionally occurs with viral hepatitis, particularly with HAV, but does not signify a poor prognosis if synthetic function remains intact (215). Significant elevation of bilirubin is uncommon in toxic and ischemic hepatic injury. Once serum bilirubin has begun to decline, there is no reason to measure it again unless jaundice worsens clinically.

Coagulation Tests – Elevated prothrombin time is a common finding in ischemic and toxic hepatic injury, often with results > 15 seconds or 4 seconds above the reference limit before rapidly returning to normal. There are no data on the degree of elevation affecting prognosis in ischemic hepatic injury. Elevation of prothrombin time > 15 seconds or more than 4 seconds above reference limits in viral or alcoholic hepatitis is a marker of more severe disease (98, 99, 180).

Serologic Markers – In individuals with acute hepatitis B, HBsAg is the best indicator of viral clearance. Patients who lose HBsAg and develop anti-HBs virtually never develop recurrence of liver injury, and can be considered to have recovered from HBV infection. In acute HCV infection, most individuals never develop a clinical picture of acute hepatic injury (154, 211). The only reliable marker of clearance of HCV is repeatedly (on at least two occasions) negative HCV RNA, using sensitive qualitative tests.

Recommendations: Prothrombin time > 4 seconds above reference limits, bilirubin > 257 μmol/L (15 mg/dL) or development of encephalopathy identify high risk patients that require close monitoring and consideration of referral to a gastroenterologist or hepatologist (IIB).

In patients with acute hepatitis B, repeat HBsAg should be performed within 6-12 months; if negative and anti-HBs is positive, no further follow-up is needed (IIE).

In patients with acute hepatitis C, ALT should be repeated periodically over the next 1-2 years to assure continued normal results. Clearance of virus should be confirmed with qualitative HCV RNA measurement (IIB).
Section IV.

Chronic Hepatic Injury

Chronic hepatic injury is a relatively common disorder with minimal symptoms, yet with long term risk of significant morbidity and mortality. It is defined pathologically by ongoing hepatic necrosis and inflammation in the liver, often accompanied by fibrosis. It may progress to cirrhosis (15-20% in the case of chronic HCV) and predispose to hepatocellular carcinoma. Most commonly, it is due to chronic viral infection. In the United States alone, there are estimated 2.1-2.7 million people chronically infected with HCV (1). There are also approximately 1-1.25 million chronic carriers of HBV in the United States. While prevalence rates for HCV infection generally are between 0.5 and 5% in other parts of the world, prevalence rates for HBV vary markedly, and in many areas HBV is an endemic infection. The prevalence of endemic HBV in children is declining in many parts of the world due to use of HBV vaccine. Clinical findings and laboratory investigation are often adequate to establish the most likely diagnosis, with a predictive value of 88% for alcoholic hepatitis and 81% for chronic viral hepatitis (before availability of HCV tests) compared to biopsy (216).

**Recommendation:** In the absence of liver biopsy showing chronic hepatitis, one of the following clinical definitions should be used to diagnose chronic hepatitis:

- Persistence of increased ALT for more than 6 months after an episode of acute hepatitis
- OR
- Elevation of ALT (without another explanation) on more than one occasion over a period of 6 months. A shorter time may be appropriate in patients with risk factors for chronic viral hepatitis, genetic causes of hepatic injury, or autoimmune liver injury; or in the presence of clinical signs or symptoms of liver disease (IIB).

Although the definition of chronic hepatic injury by elevated ALT is widely accepted, 15-50% of individuals with chronic hepatitis C infection have persistently normal ALT (211). The likelihood of continuously normal ALT decreases with increasing number of measurements; even after three normal ALT values, 11% of those with chronic HCV viremia subsequently developed persistently elevated ALT (211). ALT often fluctuates between normal and abnormal, particularly in chronic hepatitis C; 60% of patients with multiple ALT measurements have at least occasional normal ALT values (Dufour DR, unpublished observations). The majority of patients with persistently normal ALT have histologic evidence of chronic hepatitis on biopsy, but, in general, milder inflammation, less fibrosis, and lower rates of progression to cirrhosis than do HCV patients with elevated ALT (185, 217). Centers for Disease Control and Prevention guidelines do not recommend treatment of patients with HCV and persistently normal ALT (218). While long term studies are needed, it appears that the clinical definition proposed will not miss a significant group of patients who require and benefit from treatment.

It is not always possible to distinguish acute from chronic hepatic injury. Most patients with chronic hepatitis C (the most common form of chronic hepatic injury) have ALT values between 1-4 times the upper reference limit, and 90% have maximum ALT less than 7 times the upper reference limits, values lower than typically seen in acute hepatitis. In about 5% of cases, however, peak ALT may be over 10 times the upper reference limit, often associated with jaundice, in a pattern similar to that seen in acute hepatic injury (Dufour DR, unpublished observations). In such cases, it is often necessary to do additional testing to rule out another cause of acute hepatic injury.

**Screening**
General screening of the population for chronic hepatic injury is not cost effective; testing should be limited to high risk individuals (218, 219). These include those with a family history of genetic diseases known to affect the liver, as discussed below, or risk factors for chronic viral infection (Table 14).

### Table 14. Risk Factors for Chronic Viral Hepatitis (Reference 218)

<table>
<thead>
<tr>
<th>Established Risk Factors</th>
<th>Possible Risk Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Injection drug use</td>
<td>• Body piercing or tattooing</td>
</tr>
<tr>
<td>• Chronic hemodialysis</td>
<td>• Multiple sexual partners or sexually transmitted diseases</td>
</tr>
<tr>
<td>• Blood transfusion or transplantation prior to 1992 (HCV)</td>
<td>• Health care workers (HCV)</td>
</tr>
<tr>
<td>• Receipt of blood (including needlestick) from a donor subsequently testing positive for HCV</td>
<td>• Contacts of HBV or HCV positive persons</td>
</tr>
<tr>
<td>• Receipt of clotting factor concentrates produced before 1987</td>
<td></td>
</tr>
<tr>
<td>• Asian ancestry (HBV)</td>
<td></td>
</tr>
<tr>
<td>• Unvaccinated health care workers (HBV)</td>
<td></td>
</tr>
<tr>
<td>• Birth to mother with chronic HBV or HCV</td>
<td></td>
</tr>
<tr>
<td>• Males having sex with males (HBV)</td>
<td></td>
</tr>
</tbody>
</table>

ALT is consistently higher than AST with all causes of chronic hepatic injury except alcohol; AST is normal in a significant number of cases. ALT may be normal in patients with cirrhosis, while AST remains elevated (100, 220). Total and direct bilirubin and alkaline phosphatase are normal in essentially all patients, and not useful in screening (216, 221, 222). If an elevated ALT is found on routine testing, this should be confirmed by repeat testing before further evaluation. A minority of individuals with only one elevated ALT is found to have liver disease (221, 223). Patients with slightly elevated ALT (1-2 times the upper reference limit) are more likely to have transient elevation not due to disease (216, 222, 223); however, about 30% of those with chronic HCV infection have peak ALT less than 2 times the upper reference limit (Dufour DR, unpublished observations). Since ALT is also found in skeletal muscle, it is advisable to consider history of exercise and, if positive, consider measurement of creatine kinase to rule out skeletal muscle origin for ALT (221, 223).

In patients with risk factors for chronic HBV or HCV infection (Table 14), HBsAg and anti-HCV should be measured to screen for chronic infection. Chronic “carriers” of HBV typically have normal ALT (224), and 15-30% of patients with chronic HCV infection have persistently or intermittently normal ALT; however, the likelihood of only normal values falls with frequency of testing (225). Because occasional individuals with anti-HCV have no detectable viremia, persons with positive anti-HCV and normal ALT should have qualitative HCV RNA performed to identify those with persistent infection. HCV RNA may be transiently present in the early stages of infection (157). If a patient has persistently elevated ALT, positive anti-HCV, but negative HCV RNA, the test should be repeated.

**Recommendations:** Screening for chronic hepatitis is recommended in asymptomatic high risk individuals (IIB, E).

ALT is the most cost-effective screening test for metabolic or drug-induced liver injury; AST should also be measured with history of alcohol abuse (IIB, E).

Specific viral serologies (HBsAg, anti-HCV), in addition to ALT, should be used in individuals at high risk for viral hepatitis (IB).

If necessary, confirmation of chronic HCV infection in an anti-HCV positive individual should be made by HCV RNA tests; if negative and ALT elevated, HCV RNA should be repeated (IIB).
Differential Diagnosis

If the clinical history suggests alcohol abuse and/or AST is greater than ALT (especially if > 2x ALT), the most likely diagnosis is alcoholic hepatitis. Virtually no other form of chronic hepatic injury causes AST to be higher than ALT unless cirrhosis develops (221, 222). While the majority of cases of chronic hepatic injury is caused by viruses, drugs, or ethanol, a number of other disorders may produce chronic hepatic injury. Additional tests are not needed if initial evaluation is consistent with hepatitis B or C or alcoholic hepatitis (222, 226). Prescription drugs may cause persistently increased ALT, most commonly with drugs such as sulfonamides, cholesterol lowering agents, and isoniazid (198). In one study from an area with low prevalence of viral hepatitis, history of prescription drug use was common in those with chronic hepatic injury and no recognizable etiology despite extensive laboratory testing (227). In patients with elevated ALT, negative viral markers, and negative history for drug or alcohol ingestion, workup should consider less common causes of chronic hepatic injury (Table 15).

Table 15. Other Causes of Chronic Elevation of ALT and/or AST

<table>
<thead>
<tr>
<th>Cause</th>
<th>Key Feature</th>
<th>Screening Tests</th>
<th>Confirmatory Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-alcoholic steatohepatitis</td>
<td>Most common cause other than viral, alcoholic</td>
<td>None</td>
<td>Biopsy</td>
</tr>
<tr>
<td>Hemochromatosis</td>
<td>Autosomal recessive trait; 1:200 among Northern European ancestry</td>
<td>Transferrin saturation &gt; 45%; unsaturated iron binding capacity &lt;28 μg/dL (155 μg/dL)</td>
<td>HFE gene analysis for C282Y mutation</td>
</tr>
<tr>
<td>Wilson’s Disease</td>
<td>Autosomal recessive trait; 1:30,000 individuals; hemolytic anemia, renal injury</td>
<td>ceruloplasmin in 65-95% of homozygous, 15% of heterozygous</td>
<td>Genetic analysis, low serum copper, high urine copper</td>
</tr>
<tr>
<td>Autoimmune hepatitis</td>
<td>Up to 18% of non-viral hepatitis, mainly in young women; increased γ-globulins</td>
<td>ANA and ASMA; false positive anti-HCV common</td>
<td>Biopsy</td>
</tr>
<tr>
<td>Primary biliary cirrhosis</td>
<td>Middle aged women; usually mainly elevation of alkaline phosphatase; often associated with Sjogren’s syndrome</td>
<td>Anti-mitochondrial antibody</td>
<td>Biopsy</td>
</tr>
<tr>
<td>Sclerosing cholangitis</td>
<td>Young to middle aged men; usually mainly elevation of alkaline phosphatase; often associated with inflammatory bowel disease</td>
<td>Anti-neutrophil cytoplasmic antibodies; ASMA, ANA may also be positive</td>
<td>Bile duct imaging</td>
</tr>
<tr>
<td>Alpha-1-antitrypsin deficiency</td>
<td>Autosomal recessive trait; 1:1000 to 1:2000. Controversial whether it causes chronic liver disease in adults</td>
<td>Alpha-1-antitrypsin phenotyping</td>
<td></td>
</tr>
</tbody>
</table>
**Recommendations:** Initial evaluation should include a detailed drug history along with measurement of HBsAg and anti-HCV. If anti-HCV is positive, chronic infection should be confirmed by qualitative HCV RNA measurement (IIB, E).

With persistently elevated ALT and negative viral markers, workup should include antinuclear antibodies and iron and iron binding capacity (or unsaturated iron binding capacity) (IIIB).

In patients under age 40, ceruloplasmin should also be measured (IIIB).

In patients negative for these markers, α1-antitrypsin phenotype may be of use (IIIB).

If these tests are negative or inconclusive, diagnostic liver biopsy should be performed (IIB).

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**Workup of Patients Without Obvious Cause for Chronic Hepatic Injury**

**Nonalcoholic Steatohepatitis (NASH)** - Occurrence of chronic liver disease histologically resembling alcoholic hepatitis (fatty change or steatosis, neutrophilic inflammatory response and Mallory bodies) in patients without alcohol abuse has been termed NASH. Many individuals with elevated ALT have steatosis without the complete histologic picture of NASH (227). NASH is the most common cause of chronic liver injury other than viruses and alcohol and the most common cause of cryptogenic cirrhosis (216, 228). Although it occurs most commonly in middle aged women with obesity and/or diabetes, it also occurs in men and persons without these risk factors (228). Patients with NASH commonly have abnormal lipid profiles, although normal results do not rule out this disease. It differs from alcoholic hepatitis in having ALT higher than AST (except in those with cirrhosis) (229). Weight loss may cause significant improvement in enzyme results; in one study, a 1% reduction in weight caused an average fall of 8.1% in ALT (230). There are no clinical features or laboratory tests that definitively establish a diagnosis of NASH; biopsy is the only diagnostic procedure with adequate specificity.

**Recommendations:** Biopsy is necessary to establish the diagnosis of NASH (IIB).

**Hemochromatosis** - An Autosomal recessive trait, hemochromatosis is the most common inherited genetic defect in persons of northern European ancestry (approximately 1:200-1:300 in the United States) (231). The vast majority of cases are due to one of two point mutations of the HFE gene on chromosome 6. The majority (60-90%) of affected individuals is homozygous for the C282Y (845A) mutation, while a minority has compound heterozygosity for this mutation and the H63D (187G) mutation (232). Screening involves detection of increased transferrin saturation (saturation = serum iron (Fe) / total iron binding capacity (TIBC)) (233) or low unsaturated iron binding capacity (234). A transferrin saturation cutoff of ≥ 45% or unsaturated iron binding capacity cutoff ≤ 28 μmol/L (155 μg/dL) has a sensitivity of 90-100% for homozygosity for the C282Y mutation; if fasting specimens are used, specificity is 43% (235, 236). A recent consensus conference recommends that definitive diagnosis be made by genetic analysis (237). While several recent publications have shown the feasibility of hemochromatosis screening using transferrin saturation, most organizations and researchers do not currently recommend screening because of unresolved issues regarding ability to convince young adults to be tested, specificity and reproducibility of screening tests, and questions about natural history of untreated disease (237). Screening has been advocated by the College of American Pathologists (238), and has been estimated to save $3.19 per blood donor screened (239).
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**Recommendations:** Initial evaluation for hemochromatosis should be by fasting serum transferrin saturation or unsaturated iron binding capacity (IIB).

Transferrin saturation ≥ 45% or unsaturated iron binding capacity ≤ 28 μmol/L (155 mg/dL) should be followed by analysis for HFE gene mutations (IIB).

Screening of the population may be beneficial but is not currently recommended pending clarification of screening benefits (IIB, E).

**Wilson’s Disease** - An autosomal recessive disorder, Wilson’s disease occurs in about 1 in 30,000 individuals in Europe and North America. It is caused by a mutation of a gene on chromosome 13 coding for an ATPase needed for copper transport (240). Wilson’s disease may present as liver disease, neurologic problems, or with psychiatric symptoms, almost always before age 40. Most patients who present with liver disease do not have neurologic manifestations (201). The most common diagnostic finding is low plasma ceruloplasmin. Low levels also occur with malnutrition, protein loss, and advanced liver disease, and falsely normal values can occur with pregnancy, estrogen administration, and acute inflammation (241). Most references report low ceruloplasmin in 95% of homozygotes and 20% of heterozygotes (241). One study found normal ceruloplasmin in 25% of patients with chronic liver disease due to Wilson’s disease (confirmed by genetic studies in 80%), but in only 15% of patients with Wilson’s without overt liver involvement (201). Other expected findings in Wilson’s disease include increased serum free copper, decreased total serum copper, increased urine copper excretion, and increased liver copper content. These tests may also provide misleading results in Wilson’s disease patients (201, 242). Multiple tests are frequently needed to establish the diagnosis.

**Recommendations:** Testing for Wilson’s disease with ceruloplasmin is indicated in patients under age 40 with chronic hepatic injury or fatty liver, and negative workup for viral hepatitis, drug-induced liver injury, and hemochromatosis (IIB).

Screening for Wilson’s disease in all patients with chronic hepatic injury is not indicated (IIB, E).

Genetic marker testing may be useful in equivocal cases, but testing must be able to detect multiple mutations in the Wilson’s disease gene (IIIB).

**Autoimmune hepatitis** - Autoimmune hepatitis (AIH) is responsible for up to 18% of chronic hepatitis not due to viruses or alcohol (243). Several variants of AIH have been described (244). Type 1, found primarily in young and middle-aged women, is the most common form; it is associated with high titers of anti-nuclear antibody (ANA) and/or anti-smooth muscle antibody (ASMA). Type 2, found primarily in children, is common in western Europe but rare in the United States; it is associated with antibodies to liver-kidney microsomal antigen (anti-LKM1), but rarely with positive ANA or ASMA. Many patients with type 2 also have HCV infection. Type 3, found primarily in young women, is associated with systemic autoimmune disease in many cases. Most affected individuals lack ANA, ASMA, or anti-liver-kidney microsomal antibodies, but are positive for antibodies for soluble liver antigen (anti-SLA). Standardized diagnostic criteria and a scoring system have been defined by an international panel (206). The classic features of the most common type 1 include elevated aminotransferases; minimal or no elevation of alkaline phosphatase; polyclonal hypergammaglobulinemia (at least 1.5 times the upper reference limit); no evidence of viral infection, risk factors for viral infection, or exposure to drugs or alcohol; and positive ANA or ASMA (at least 1:80) (206). Approximately 40% of patients with chronic HCV infection have a positive ANA or ASMA, usually in low titers (244). False positive anti-HCV has been reported in 60% of patients with AIH using second generation tests and in 20% using third generation
assays (245); anti-HCV typically disappears with successful treatment (246). In equivocal cases, HCV RNA (or recombinant immunoblot assay) can be used to establish the diagnosis (245).

**Recommendations:** Autoimmune hepatitis should be suspected in patients with chronic hepatic injury and increased immunoglobulins and absence of viral markers or risk factors for viral hepatitis (IIIB).

The diagnosis of type 1 AIH can be clinically supported by positivity for either anti-nuclear antibodies (ANA) or anti-smooth muscle antibodies (ASMA) in high titers (IIIB).

**Primary Biliary Cirrhosis and Primary Sclerosing Cholangitis** – Primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) are autoimmune diseases causing destruction of bile ducts. Although characteristically causing elevation in ALP and GGT, patients with PBC and PSC may have elevations of AST and ALT and be considered to have chronic hepatitis. Primary biliary cirrhosis is associated with destruction of intrahepatic bile ducts; it is often associated with other autoimmune disorders, particularly Sjogren’s syndrome (up to 80% of cases) (247). An autoimmune marker, antimitochondrial antibody (AMA), is found in almost all patients with PBC. Although other diseases may be associated with positive AMA, in PBC the antibody is directed against the 2-oxalate dehydrogenase complex (so-called M2 type of AMA), particularly to dihydrolipoamide acetyltransferase (E2) and E3-binding protein (248). About 5-10% of patients have features of both PBC and AIH (249). PSC is often detected in asymptomatic individuals by finding an elevated ALP. AST and ALT are elevated in about half of cases, although values are above 2x the reference limit in only 20% (250). Primary sclerosing cholangitis is associated with damage to both intra- and extrahepatic bile ducts; 70% of cases are associated with inflammatory bowel disease (Crohn’s disease or ulcerative colitis) (251). Perinuclear anti-neutrophil cytoplasmic antibodies are found in about 2/3 of cases (252). In PSC, antibodies are commonly directed against bactericidal/permeability-increasing protein, chymase G, and/or lactoferrin. There appears to be no prognostic significance to the different antibody specificities, although patients with cirrhosis more commonly have antibody to multiple antigens and to antigen other than lactoferrin (253). Anti-smooth muscle and anti-nuclear antibodies are also present in up to 70% of cases (254).

**Recommendations:** Primary biliary cirrhosis or primary sclerosing cholangitis should be suspected in patients with chronic elevation of alkaline phosphatase (IIIB).

The diagnosis can be clinically supported by positivity for anti-mitochondrial antibodies (PBC) or anti-neutrophil cytoplasmic antibodies (PSC) in high titers (IIIB).

**Alpha-1-antitrypsin (A1AT) Deficiency** – Alpha-1-antitrypsin is the most important protease inhibitor; congenital deficiency occurs in approximately 1 in 1000 to 1 in 2000 persons of European ancestry. The gene for A1AT is located on chromosome 14 (255); deficiency is usually due to a single amino acid substitution that alters carbohydrate binding and impairs release from hepatocytes (256). The most important deficiency involves homozygosity for the Z variant, termed Pi (for protease inhibitor) ZZ. Deficiency is associated with emphysema and neonatal hepatitis (257); chronic hepatic injury with cirrhosis and hepatocellular carcinoma have also been reported (256). Almost all Pi ZZ neonates have evidence of liver injury at birth; this usually resolves by age 12 years (257). In adults, 50% of Pi Z positive individuals (either homozygotes or heterozygotes) develop cirrhosis and 31% develop hepatocellular carcinoma (256). There is also an excess of Pi Z heterozygotes among patients referred for liver transplant, particularly among patients with cryptogenic cirrhosis where approximately 25% of patients are Pi Z positive (258). There is evidence, however, that A1AT deficiency or heterozygosity for PiZ phenotype may not directly cause liver disease, but increase susceptibility to liver damage by other agents, especially viruses. Two controlled studies found the same frequency of
Pi Z (either homozygous or heterozygous) in patients with liver disease and controls (259). In a study of 164 patients with Pi Z, 40% had chronic liver disease; 87% were also positive for HCV antibodies or HBV markers, and only 11% had no other liver disease risk factors (260). Because A1AT is an acute phase reactant, quantitative levels may be falsely normal with infection or inflammation, and falsely low levels may occur with malnutrition, protein losing states, or end stage liver disease. In one study, quantitative levels were normal in 42% of heterozygous Pi Z patients with liver disease (261). Testing for A1AT deficiency should use phenotype analysis rather than quantitative plasma concentration (256).

**Recommendations:** Testing for alpha-1-antitrypsin deficiency may be of benefit in patients with chronic hepatic injury and no other apparent cause, although the role of A1AT deficiency in liver disease in adults is not clearly defined (IIB).

Testing is especially important in neonates with evidence of hepatic injury (IIB).

Testing for A1AT variants should be performed by determination of phenotype (IIB).

Screening patients with chronic hepatic injury for alpha-1-antitrypsin deficiency is not recommended (IIB, E).

**Other Viruses**

Two other viruses have been suggested as possibly involved in the pathogenesis of chronic hepatitis: hepatitis G (HGV) and TT virus (TTV). Both viruses can be transmitted by transfusion, and chronic viremia is present with both. To date, evidence suggests that infection with these viruses is common, but there is no clear proof that they play a role in liver injury. HGV (and the related GBV-C) are members of the flavivirus family, as is HCV. HGV was first isolated from patients following transfusion, although most showed no evidence of liver injury (262). HGV can also be found commonly in chronic hepatitis (263), but does not appear to be a common cause of cryptogenic chronic liver disease (264). This may be because HGV RNA is rarely found in the liver in chronically viremic patients (265). TTV was first identified in patients with post-transfusion hepatitis (266). TTV DNA is found in 1-7% of blood donors in the United States (267, 268). Presence of TTV DNA is no more common in persons with acute non-A-E hepatitis than in other causes of acute hepatitis or in control patients (268, 269).

**Recommendations:** Testing for HGV or TTV, in other than a research setting, is not recommended (IIIE).

**Monitoring**

While ALT is the most clinically used laboratory test for monitoring liver injury, there is often considerable fluctuation in enzyme activities over time (particularly in chronic HCV infection) (217, 270). It is important to measure ALT repeatedly in chronic HCV before concluding that ALT is normal (225); 43% of chronically infected individuals have ALT values fluctuating between normal and abnormal, and 16% of those with normal ALT on their first two visits and 11% of those with normal ALT on their first three visits subsequently developed increased ALT (211). In patients with chronic HBV infection without elevated ALT (“chronic carriers”), approximately 10% will develop increased ALT on follow-up (224); ALT should therefore be measured periodically even if initially normal.

With both chronic HBV and HCV, clearance of viral markers is the most reliable method for detecting resolution of
infection. In untreated hepatitis B, a small percentage of patients spontaneously clear viral antigens; in long term studies, loss of HBeAg occurs in 1/3 to 1/2 of patients (208, 270). In those that lose HBeAg, 5-10% will subsequently clear HBsAg over 10 years of follow-up (224, 271). HBeAg should be rechecked periodically if initially positive. If HBeAg is negative and anti-HBe is positive, this may indicate either the beginning of viral clearance from the body, or integration of HBV DNA into host DNA and loss of ability to form replicating virus. HBsAg and anti-HBs should be measured periodically to look for viral clearance, as HBsAg will remain positive in those with integration of HBV DNA. In treatment of HBV, likelihood of viral clearance is related to baseline ALT levels; those with elevated ALT are more likely to respond than those with initially normal ALT activity (272). Successful treatment is associated with loss of HBV DNA, HBsAg, and HBeAg. While there is evidence that quantitative HBeAg correlates well with HBV DNA (273), quantitative HBeAg assays are not commercially available. HBeAg may disappear even if patients fail to show no response to therapy (274). Moreover, there is an increasing frequency of “pre-core” mutants that do not produce HBeAg, particularly in endemic areas in Asia and the Mediterranean region (275). Patients infected with such mutants have anti-HBe, but continue to have circulating HBV DNA. In infection with normal strains, HBV DNA remains detectable longer than does HBsAg in recovery (276). When viral DNA integrates into the host genome, HBsAg is still produced, although HBeAg and HBV DNA are commonly negative in plasma (277). With lamivudine treatment, however, production of viral nucleic acid through reverse transcriptase is inhibited (277), although viral DNA levels in the hepatocytes are not changed (278). For these reasons, use of HBV DNA, HBeAg, and HBsAg may all be useful in monitoring patients with chronic HBV, as no single test provides unequivocal evidence of viral clearance.

Most studies have shown that HCV RNA fluctuates over time, but rarely varies by more than 1 log, and in most cases variation is less than 0.5 log (279). In untreated individuals tested repeatedly over several years, HCV RNA rises by an average of 0.25 log/year (281). In some series, however, up to a 3 log difference is seen in patients with elevated ALT when HCV RNA is measured monthly (282); in about 1/3 of chronically infected patients, HCV RNA can fluctuate between a mean of 10^6 copies/mL and undetectable (283).

Currently, antiviral treatment is recommended for patients with chronic HCV infection who have elevated ALT and more than mild inflammatory changes on biopsy. The most effective therapy currently available is combined ribavirin and interferon. Laboratory tests have been found helpful in predicting response to varying lengths of therapy and in detecting those who do not respond to treatment, and in whom therapy should probably be discontinued. In those treated with combination therapy, both viral load and genotype have been found to identify patients who may respond to 24, rather than 48, weeks of therapy (284, 285). In a combined analysis of these two studies, five factors were found useful in predicting response (Table 16).

### Table 16. Favorable and Unfavorable Risk Factors in HCV Treatment with Interferon and Ribavirin (Reference 286)

<table>
<thead>
<tr>
<th>Favorable Factors</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genotype</strong></td>
<td>2, 3</td>
</tr>
<tr>
<td><strong>Viral load</strong></td>
<td>&lt; median (3.5 x 10^6 copies/mL)</td>
</tr>
<tr>
<td><strong>Female gender</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>&lt; 40 years</td>
</tr>
<tr>
<td><strong>None or only portal fibrosis</strong></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Unfavorable Factors</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genotype</strong></td>
<td>1, 4, 5, 6</td>
</tr>
<tr>
<td><strong>Viral load</strong></td>
<td>&gt; median</td>
</tr>
<tr>
<td><strong>Male gender</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>40 or above</td>
</tr>
<tr>
<td><strong>Septal or more severe fibrosis</strong></td>
<td></td>
</tr>
</tbody>
</table>

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HISTORICAL
Persons with genotype 2 or 3, along with 3 or 4 other favorable risk factors, can be treated effectively with only 24 weeks of therapy; all other patients do better with 48 weeks of therapy (286). The best indicator of viral clearance is persistent absence of HCV RNA (determined by qualitative HCV RNA assays). Absent HCV RNA 6 months after completion of treatment is associated with less than 10% likelihood of recurrent HCV viremia (287). Decrease in viral load in the absence of clearance is not reliable evidence of treatment success; however, failure of HCV RNA to decline to less than 400,000 copies/mL by 12 weeks of therapy is associated with 100% likelihood of persistent HCV RNA at end of treatment (286). An approach to monitoring treatment of patients with HCV by combination therapy is outlined in Figure 9.

**MONITORING COMBINED THERAPY**

<table>
<thead>
<tr>
<th>HCV RNA, ALT at 24 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive or Abnormal ALT</td>
</tr>
</tbody>
</table>

- Stop Therapy
- Evaluate Risk Factors

<table>
<thead>
<tr>
<th>Negative and Normal ALT</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 or 5 favorable</td>
</tr>
</tbody>
</table>

- Genotype 2
- Genotype 3
- Genotype 4, 5, 6

- Treat for 1 year

- Stop Therapy, Return at 48 weeks

**Figure 9 – Approach to Treatment of Hepatitis C** — At baseline, HCV RNA (using a quantitative assay linear to at least $4 \times 10^6$ copies/mL) and genotype should be collected; if facilities do not allow storage at $-70^\circ\text{C}$ for at least 6 months, testing should be performed prior to therapy. After six months of treatment, ALT and a sensitive HCV RNA measurement (lower detection limit < 1,000 copies/mL) should be performed. If ALT remains elevated and HCV RNA remains detectable, treatment is stopped. In patients showing a response, risk factors (Table 16) are used to decide whether another 6 months of treatment is needed. In patients who are genotype 2 or 3 and who have at least 3 other favorable risk factors, treatment can be stopped.

Some patients cannot take ribavirin; the only current treatment option for such patients is interferon monotherapy. With this form of treatment, failure of HCV RNA to fall to undetectable or failure of ALT to return to normal at 12 weeks after initiation of therapy is associated with over 95% likelihood of treatment failure, and is considered a reason to discontinue therapy (288).

The optimal frequency of measurement of laboratory tests in patients with chronic hepatitis C has not been determined. The European Association for the Study of the Liver Consensus Conference on Hepatitis C recommends that complete blood count and liver enzymes be performed every 6 months in untreated patients (289). The major complications of treatment with interferon are depression, thrombocytopenia and hypothyroidism, while hemolytic anemia is the major complication of ribavirin therapy. The European Association for the Study of the Liver recommends complete blood count weekly during the first four weeks of treatment, and regular determinations after the first four weeks. They also recommend measurement of TSH every 6 months during therapy.
**Recommendations:** In viral hepatitis, viral markers are the most reliable indicators of resolution of hepatitis (IIB).

HCV RNA quantitation and genotype are important determinants of duration of combination therapy. To reduce expenses of testing, if feasible, specimens should be obtained before treatment and stored at –70°C pending results of treatment. If this is not possible, testing should be performed before treatment is begun (IIB, E).

In patients with HCV treated with interferon and ribavirin, qualitative HCV RNA should be measured after 24 weeks of treatment to determine potential responders. If genotype and quantitative HCV RNA were not performed but specimens were frozen for their analysis before treatment, those with negative HCV RNA and favorable risk factors should have those tests performed (IB, E).

In patients with HCV treated with interferon monotherapy, qualitative HCV RNA and ALT should be measured after 12 weeks of treatment to determine non-responders (IIB).

Following treatment in those with negative HCV RNA at 24 weeks, sensitive HCV RNA measurements (currently qualitative assays) should be performed 6 months after the end of treatment to document sustained virologic remission (IIB).

In untreated patients with HBV, HBeAg should be monitored periodically; once negative and anti-HBe is positive, HBsAg should be monitored periodically to determine viral clearance. With anti-viral therapy, HBV DNA should also be used to document viral clearance (IIB).

In treated patients, CBC with platelet count should be measured every week for the first four weeks, then monthly thereafter. TSH should be measured every 3 to 6 months, or sooner if symptoms of thyroid dysfunction develop. Measurement of ALT should be performed at least monthly (IIIB).

ALT is the best marker of inflammatory activity available, but is of limited utility in predicting degree of inflammation and of no use in estimating severity of fibrosis (IIB).
Section V.

Cirrhosis

The major significance of chronic hepatitis is its possible progression to cirrhosis, an end stage of the process of scarring and regeneration of the liver in response to chronic damage. The scarring causes increased resistance to blood flow through the portal vein (carrying blood from the intestine to the liver), leading to ascites, esophageal varices, and increased risk of infection. Eventually, cirrhosis can cause liver failure, and is the major cause of liver transplantation. Currently, the “gold standard” for evaluation of patients with chronic hepatitis is liver biopsy, which allows determination of the severity of damage.

Chronic hepatitis has two major components: inflammatory damage and fibrosis. While the extent of inflammation reflects the degree of damage at that point in time, the extent of fibrosis more closely relates to likelihood of developing cirrhosis. Plasma activities of aminotransferases are not related to degree of fibrosis, and there is at best a weak correlation between plasma ALT activity (290, 291) or (in chronic HCV) HCV RNA levels (291) and histological activity. At best, ALT explains only 30-50% of variation in histologic activity, and there is considerable overlap in values in patients with mild, moderate, or severe activity (290, 291). The activity of inflammation has weak correlation with rate of progression of fibrosis (292).

Liver fibrosis is associated with deposition of a number of proteins in the liver. Among the proteins produced as part of fibrosis are collagen, laminin, elastin, and fibronectin; and enzymes produced in collagen synthesis such as lysyl- and proline hydroxylase. Various proteoglycans such as hyaluronate, are also produced in the process of fibrosis. Fibrosis is removed by a variety of related enzymes, termed matrix metalloproteinases; these enzymes and their inhibitors are also produced in chronic hepatitis. Numerous studies of plasma levels of proteoglycans, proteins of fibrosis, and their precursors (293, 294) have shown at best a weak correlation between marker levels and extent of fibrosis. Levels reflect degree of fibrogenesis at the time of sampling, and there is considerable overlap in values with varying degrees of fibrosis.

In the process of progression from chronic hepatitis to cirrhosis, a number of changes occur in basic laboratory test results. Several studies have shown that the ratio of AST to ALT is typically < 1 in patients with chronic hepatitis (except that due to alcohol), but with progression to cirrhosis the ratio often increases to > 1: the specificity of a ratio > 1 is 75-100%, with sensitivity 32-83% (100, 220). In one study (220), the ratio also increased with increasing fibrosis score. This appears to result from a reduction of ALT production in damaged liver (295). Other routine tests that predict likelihood of cirrhosis are thrombocytopenia and prolonged prothrombin time; an index using these two variables with AST/ALT ratio has a sensitivity of 46% and specificity of 98% for cirrhosis (100). Albumin is commonly measured in patients suspected of progressing to cirrhosis. While it is not as sensitive as other markers, it is used as a marker of severity as part of the Child-Pugh classification of cirrhosis. AFP is more likely to be elevated as degree of hepatic fibrosis increases (296), especially in cirrhosis; AFP greater than 17.8 ng/mL has a sensitivity of 35%, specificity of 83.6%, and positive predictive value of 97.7% for cirrhosis (297).

Recommendations:

- Biopsy is the only definitive marker of progression from chronic hepatitis to cirrhosis (IIB).
- Laboratory markers of fibrosis should not be used except in research studies (IIIB, E).
- Markers of hepatic function that may indicate progression to cirrhosis (AST/ALT ratio, albumin, prothrombin time, platelet count) should be measured every 3-6 months in patients with chronic hepatitis (IIIB).
Section VI.
Hepatocellular Carcinoma

Primary liver cancer (hepatocellular carcinoma, HCC) is a serious late complication of chronic hepatic injury, particularly in cirrhosis due to HBV, HCV, and hemochromatosis. Infrequently, HCC is seen in patients with chronic HCV and in asymptomatic HBV carriers without cirrhosis. It is the fifth most common malignancy world-wide and is particularly common in Eastern Asia and Africa (298). The incidence of HCC has increased by 70% in the United States over the past 20 years, particularly among younger patients (299), and is increasing in other parts of the world as well (298). The risk of developing HCC in cirrhosis due to chronic HBV or HCV infection is 1.5% per year (300, 301). In a study of 448 cases of HCC, 75% occurred in patients with cirrhosis; however, in only 30% was cirrhosis recognized clinically before hepatocellular carcinoma was diagnosed (302). These data support the screening programs, if instituted, must include patients with chronic hepatic injury as well as patients with diagnosed cirrhosis. In one study, however, hepatocellular carcinoma developed only in 325 patients with severe chronic hepatitis or cirrhosis, and not in any of 800 patients with mild or moderate chronic hepatitis (303). Since patients with normal ALT generally have mild inflammation on biopsy (185, 217, 218), it is reasonable to exclude from screening those persons without cirrhosis and with normal ALT or less than severe hepatitis on biopsy. Other risk factors include male gender and age > 55 years.

The prognosis of patients with HCC detected by development of symptoms is grim, with few patients surviving over 6 months. Detection of small tumors offers the potential for operative resection and forms the rationale for considering screening. Current practice suggests measurement of a-fetoprotein (AFP) and ultrasound of the liver every 6 months (304). Unfortunately, AFP interpretation is complicated by intermittent elevations of AFP in 12-13% of patients with chronic HBV or HCV (305), often (but not always) associated with transient increases in ALT (306). A Consensus Development workshop recommended screening chronic HBsAg carriers at least once, and preferably twice, yearly with AFP only, while patients with other risk factors (known cirrhosis, family history) should have both AFP and ultrasound (307). In chronic hepatic injury, high risk of HCC is present in patients with hemochromatosis or with cirrhosis due to HBV, HCV, or alcohol abuse. Other causes of chronic hepatic injury and cirrhosis have lower risk of HCC (308).

In Western countries, the predictive value of AFP is low, often in the range of 10-30%, with sensitivity of AFP between 40-80% (309, 310). In 147 patients with cirrhosis, none of the 30 patients with HCC had AFP > 105 ng/mL at the time of diagnosis and 60% had AFP < 10 ng/mL; however the frequency of HCC in patients with AFP < 50 ng/mL was 17%, compared with 42% in those with higher AFP (310). In another study of 260 patients with cirrhosis, HCC developed in 66% of patients with initial AFP < 20 ng/mL, but 46% in those with higher levels. Moreover, those with even transient increases above 100 ng/mL had a significantly higher risk of HCC than those whose AFP was consistently < 20 ng/mL (311). A decision analysis on published papers of screening for HCC in Western patients with compensated cirrhosis concluded that, for patients with a likelihood of survival of 85% at 5 years, screening would likely add 4.9 months to average life expectancy at a cost of $26,000 - $55,000 per year of life gained, figures that compare favorably to those of colon cancer and breast cancer screening (312). In patients with lower likelihood of survival, screening provided minimal or no gain in life expectancy and does not appear indicated. A systematic analysis of all published studies concluded that there is inadequate data to determine the benefit of screening for HCC among patients with chronic liver disease (313). If screening is used, frequency of testing of every 6 months appears to be optimal based on observed doubling times of HCC, reported to average around 3-5 months (314).

Des-γ-carboxy prothrombin has also been suggested as a screening test. Levels are elevated occasionally in chronic liver disease, but there is less overlap with values seen in HCC than for AFP (315, 316). Occasional high levels are encountered in metastatic carcinoma to the liver, but they are usually minimally increased. While des-γ-carboxy prothrombin appears less sensitive (50-70%) than AFP, it is more specific. There is poor correlation between AFP and
des-γ-carboxy prothrombin, and some tumors are only detected by des-γ-carboxy prothrombin (315, 316). Vitamin K deficiency can also cause significant elevation; repeating testing after administration of vitamin K improves specificity (315, 316). Recently, a more sensitive immunoassay has shown promise in detection of small HCC, with positivity in 27% of cases compared to 3% with older assays (317). Assays for des-γ-carboxy prothrombin are not widely available, in contrast to AFP assays. Other laboratory tests, including AFP variants (318) and lectin affinity chromatography of alkaline phosphatase (319) have been evaluated in too few patients to make definitive recommendations. A recent study identified high levels of abnormal forms of GGT in 78 of 91 patients with HCC, but in only 2.5% of 116 patients with other liver diseases (320).

**Recommendations:** Screening for hepatocellular carcinoma is of questionable benefit in Western populations (IIB, E).

Screening should be confined to high risk patients (those with severe chronic hepatitis or cirrhosis due to alcohol, HBV, HCV, or hemochromatosis) who are candidates for treatment of hepatocellular carcinoma, if detected (IIIB, E).

If screening is used, measurement of α-fetoprotein and ultrasound at intervals no more frequently than every 6 months is recommended (IIB).

There is currently little data to support the use of other tests (IIIB).
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Appendix

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