Lymphoid neoplasms are known to be complex, and the prognosis and clinical course of patients with lymphoma is highly variable. Genetic abnormalities have emerged as one of the most reliable criteria for categorizing lymphomas. Several chromosome anomalies and variants of these anomalies have been associated with various kinds of lymphoma (see table below). Unfortunately, conventional chromosome studies cannot be employed on paraffin-embedded tissue and molecular genetic analyses are often problematic in the study of lymphomas. New methods using chromosome specific fluorescent-labeled DNA probes and fluorescence in situ hybridization (FISH) now permit detection of abnormal genes associated with various chromosome anomalies in lymphoma (see table below).

### Common chromosome anomalies in lymphomas

<table>
<thead>
<tr>
<th>Lymphoma</th>
<th>Chromosome anomaly</th>
<th>FISH for Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burkitt</td>
<td>t(8;14)(q24;q32)</td>
<td>c-MYC, IgH</td>
</tr>
<tr>
<td>Mantle Cell</td>
<td>t(11;14)(q13;q32)</td>
<td>CCND1, IgH</td>
</tr>
<tr>
<td>Anaplastic large cell</td>
<td>t(2;var)(p23;var)</td>
<td>ALK</td>
</tr>
<tr>
<td>Follicular</td>
<td>t(14;18)(q32;q21)</td>
<td>IgH, BCL2</td>
</tr>
<tr>
<td>Diffuse large B-cell</td>
<td>t(14;18)(q27;var)</td>
<td>BCL6</td>
</tr>
<tr>
<td></td>
<td>t(8;14)(q24;q32)</td>
<td>c-MYC, IgH</td>
</tr>
</tbody>
</table>

### Useful For

- Detecting chromosome anomalies in paraffin-embedded lymphoid tissue
- Providing diagnostic and prognostic genetic information for the following lymphomas: mantle cell, follicular, Burkitt, anaplastic large cell, and diffuse large B-cell lymphoma
- We recommend this test in cases where the diagnosis is questionable.

### Interpretation

A neoplastic clone is detected when the percent of cells with an abnormality exceeds the normal reference range for any given probe.

### Cautions

- It is possible to order 1 or any combination of these probes, but this must be done in consultation with 1 of our hematopathologists.
- This test has not been approved by the FDA and is best used as an adjunct to existing clinical and pathological information.

### Supportive Data

We tested the efficacy of FISH to study paraffin-embedded tissue in 6 normal lymph nodes or tonsils, and 32 malignant lymphomas including 5 mantle cell, 5 follicular, 5 Burkitt, 5 anaplastic large cell, 7 diffuse large B-cell, and 5 extranodal marginal zone lymphomas of mucosa-associated lymphoid tissue. FISH studies were successful for each of the 38 specimens. The expected chromosome anomalies were detected in each malignant specimen, but not in the normal lymphoid tissue. The correct chromosome anomaly was detected in 22 of 22 specimens with genetic abnormalities that were established by other genetic techniques.

### References

Locus and Centromere Anomalies for Lymphoma, Fluorescence In Situ Hybridization (FISH)  
#80027

References

Method
Hematopathologists at Mayo will examine the paraffin-embedded tissue blocks and select the region that contains malignant cells based on comparison with hematoxylin and eosin sections. Nuclei are extracted from paraffin-embedded tissue using a technique in which tissue cores are processed with xylene, proteinase K, citric acid and pepsin. FISH is used to study individual extracted nuclei. Fusion of CCND1 and IgH, BCL2 and IgH, and c-MYC and IgH are detected using probes with a dual fusion FISH strategy. Anomalies involving ALK and BCL6 are detected using break-apart FISH probes. At least 200 interphase nuclei are screened for each probe and results for each abnormal probe(s) are expressed as percent abnormal nuclei.

Specimen Required:
SUBMIT ONLY 1 OF THE FOLLOWING SPECIMENS:

Lymph Node
A paraffin block with 1 corresponding hematoxylin and eosin slide containing lymph node tissue. Although formalin is the preferred fixative, tissue blocks may be fixed with B5 fixative. Specimens that are processed with Prefer fixative are not acceptable. Unstained slides are not acceptable.

SPECIMEN CANNOT BE FROZEN. Advise Express Mail or equivalent if not on courier service.

NOTE: Please complete either a “Hematopathology/Molecular Oncology Request Form” (Supply T241) or a “MayoConnect Additional Test Information Form” (Supply T357) and forward it with the specimen.

Solid Tumor
A paraffin block with 1 corresponding hematoxylin and eosin slide containing solid tumor tissue. Although formalin is the preferred fixative, tissue blocks may be fixed with B5 fixative. Specimens that are processed with Prefer fixative are not acceptable. Unstained slides are not acceptable.

SPECIMEN CANNOT BE FROZEN. Advise Express Mail or equivalent if not on courier service.

NOTE: Please complete either a “Hematopathology/Molecular Oncology Request Form” (Supply T241) or a “MayoConnect Additional Test Information Form” (Supply T357) and forward it with the specimen.

Reference Values:
An interpretive report will be provided.

Analytic Time:
1-3 days

Days Set Up:
Sunday through Friday

Fee:
$62.70 for #8874 “Path Consult, Limited w/o review of patients medical record”
$393.50 for #80027 “Locus Anomalies, Lymphoma, FISH”
An additional fee will be charged if 1 or more of the following are ordered:
$207.30 for #19169 “One Additional FISH Probe” (if appropriate)
$257.40 for #19170 “Two Additional FISH Probes” (if appropriate)
$359.50 for #19171 “Four Additional FISH Probes” (if appropriate)

Test Classification:
This test was developed and its performance characteristics determined by Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN. It has not been cleared or approved by the US Food and Drug Administration.
#8874 (Billable code) Path Consult, Limited w/o review of patients medical record
80500
#80027 (Billable code) Locus Anomalies, Lymphoma, FISH
88275/Interphase in situ hybridization, 100-300 cells
88291/Interpretation and report
88271x2/DNA probe, each
#19169 (80024 - Billabale code) One Additional FISH Probe
88271/DNA probe, each
#19170 (80025 - Billabale code) Two Additional FISH Probes
88271x2/DNA probe, each
#19171 (80026 - Billabale code) Four Additional FISH Probes
88271x4/DNA probe, each
Morphine, Unconjugated, Serum
#83132

Clinical
Morphine is an opioid receptor agonist used for major pain analgesia. It can be administered by intravenous, intramuscular, subcutaneous, intrathecal, or oral routes to provide analgesia for approximately 6 hours. The major elimination pathway for morphine is via glucuronidation. Morphine is metabolized to morphine-3-glucuronide (M-3-G) and morphine-6-glucuronide (M-6-G). Both metabolites are active. M-6-G produces stronger analgesic effect than morphine. M-3-G also is active, but is less potent than M-6-G. UDP-glucuronyl transferase is the major enzyme catalyzing conjugation. Morphine also is metabolized by demethylation [cytochrome P(450) 2D6] to normorphine, an inactive minor metabolite; this is considered a minor metabolic pathway. At steady state, 20% of morphine circulating in blood is the unconjugated form.1,2

Useful For
• Monitoring morphine therapy
• Assessing toxicity
• Routine drug monitoring is not indicated in all patients. Compliance monitoring is indicated in patients who are being treated for acute pain requiring excessive dose.

Interpretation
• The minimal effective peak serum concentration of unconjugated morphine for analgesia is 70 ng/mL. Peak therapeutic serum concentrations of 70-450 ng/mL occur 30 minutes after intravenous dose, 1 hour after intramuscular or subcutaneous dose, or 2 hours after oral dose.
• Patients continuously administered morphine develop tolerance; they can tolerate serum concentrations up to 1,500 ng/mL.
• Death may be associated with serum total morphine >700 ng/mL in the nontolerant subject.3,4

Cautions
• This test quantifies unconjugated morphine. This test does not quantify M-3-G and M-6-G.
• This test cannot distinguish the source of morphine from heroin, codeine, or prescription morphine.

References

Method
Morphine is extracted from serum by solid phase chromatography, derivatized, and then analyzed by gas chromatography-mass spectrometry using selected ion monitoring (GC-MS/SIM). (Burt M, Kloss J, and Apple F: Postmortem blood free and total morphine concentration in medical examiner cases. J Forensic Sci 2001;46:1138-1142)
Test Title: Morphine, Unconjugated, Serum #83132

Specimen Required: Draw blood in a plain red-top tube(s) or a serum gel tube(s). Spin down and send 2.0 mL of serum refrigerated.

Reference Values: Therapeutic: 70-450 ng/mL
Tolerant Patients: <1500 ng/mL
Toxicity: >700 ng/mL

Analytic Time: 2 days
Days Set Up: Monday through Friday
Fee: $147.30
CPT Code: 83925
Hepatitis A Virus (HAV) is endemic throughout the world, occurring most commonly, however, in areas of poor hygiene and low socioeconomic conditions. The virus, which is transmitted primarily by the fecal-oral route, is spread by close person-to-person contact and by food- and water-borne epidemics. Outbreaks frequently occur in overcrowded situations and in high-density institutions and centers, such as prisons and health care or day care centers. Viral spread by parenteral contact (with blood or oropharyngeal secretions) is possible but rare because infected individuals are viremic for a short period of time (usually less than 3 weeks). There is little or no evidence of transplacental transmission from mother to fetus or of newborns contracting HAV infection during delivery.

In most cases, antibody against hepatitis A antigen is detectable by the onset of symptoms (usually 15-45 days after exposure). Initial antibody consists almost entirely of the IgM subclass. Anti-HAV IgM usually falls to undetectable levels 3-6 months after hepatitis A infection. Anti-HAV IgG levels rise quickly once the virus is cleared and may persist for many years.

Useful For
- Demonstration of previous exposure and immunity to HAV
- Differentiation between acute and convalescent states of HAV infection

Interpretation
This assay tests for both the IgG and the IgM (combined) subclasses of antibody. A positive anti-HAV (IgG and IgM) indicates that the patient has had either a recent or a past HAV infection.

Testing for hepatitis A antibody IgM only, (#8342 Hepatitis A Antibody, IgM only [Anti-HAV, IgM], Serum) is necessary to confirm an acute or recent infection, if clinically indicated. A positive anti-HAV (IgG and IgM) and negative anti-HAV (IgM) may indicate immunity to HAV.

Cautions
- Passively acquired antibody (i.e., recent immune globulin administration, transfusion, etc) may result in transient-positive test results.
- When used alone, this test may not be useful for detection of acute hepatitis A infection following exposure or prior to symptoms.

References
**Method**

In the assay procedure, patient specimens and controls are incubated in antibody-coated microwells. IgM antibodies present in a specimen or control bind to the antibody. Excess specimen is removed by a wash step, and a solution of hepatitis A virus (HAV) and the enzyme tracer are then added to the microwells and allowed to incubate. The presence of IgM anti-HAV enables the HAV and the enzyme tracer to bind to the solid phase: enzyme activity is therefore related to the IgM anti-HAV concentration present in the specimen or control. Excess HAV and enzyme tracer are removed by a wash step, and a chromogen/substrate solution is added to the microwells and allowed to incubate. If a specimen contains IgM anti-HAV, the bound enzyme (horseradish peroxidase) chemically reduces the substrate peroxide, which concurrently oxidizes the chromogen tetramethylbenzidine (TMB) to a blue color. The blue color turns to yellow (450 nm) after addition of the stop solution. If a specimen does not contain IgM anti-HAV, the microwell will be colorless after the chromogen/substrate solution is added and will remain colorless after the stop solution is added. Color intensity, which is measured spectrophotometrically, is directly related to the concentration of IgM anti-HAV. Absorbance value readings for patient specimens are compared to a cutoff value determined from the mean of the calibrators. (Package insert: ETI-HA-IGMK PLUS, DiaSorin, Inc, Stillwater, MN)

**Specimen Required:**

Draw blood in a plain, red-top tube(s) or a serum gel tube(s). Spin down, remove serum from clot within 24 hours, and send 2.0 mL (pediatric: 0.5 mL) of serum frozen in a screw-capped, round-bottom, plastic vial.

**NOTE:** Collection date is required on request form for processing.

**Reference Values:**

Negative

**Analytic Time:**

1 day

**Days Set Up:**

Monday through Sunday

**Fee:**

$68.70

**CPT Code:**

86708
**Mycoplasma pneumoniae** Antibodies, IgM, Qualitative, Serum  
#84117

### Clinical

*Mycoplasma pneumoniae* is estimated to cause 20-30% of all cases of pneumonia but asymptomatic infection with this organism is common. Infection with *Mycoplasma pneumoniae* is usually limited to the respiratory tract from the nasopharynx through the bronchioles, producing symptoms more consistent with bronchitis than pneumonia. Symptoms from extrapulmonary involvement (e.g., skin and mucous membranes, central nervous system, heart) also have been reported.

The disease occurs in 4-7 year cycles. Infections are most common in young children (3-14 years of age) and young adults (14-19 years of age); it is less frequent in infants and adults over 50 years of age.

Direct detection of *Mycoplasma pneumoniae* infection is difficult because of the slow growth in culture (4-20 days) and the fastidious growth requirements of the organism. For this reason, serology is generally the best laboratory method available for diagnosis of this infection.

### Useful For

Laboratory diagnosis of acute phase infection with *Mycoplasma pneumoniae*

### Interpretation

Presence of IgM class antibodies to *Mycoplasma pneumoniae* is consistent with acute phase infection with this organism.

Specific IgM antibodies to *Mycoplasma pneumoniae* are usually detected in patients with a recent primary infection. However, they may be found in patients with reactivated or secondary infections and are sometimes found in patients with no other detectable evidence of recent infection. In addition, IgM to *Mycoplasma pneumoniae* has been shown to persist for extended periods (2-12 months) in some patients.

Absence of IgM class antibodies to *Mycoplasma pneumoniae* is consistent with lack of acute phase infection with this organism.

Specimens obtained too early during infection may not contain detectable levels of IgM antibody. If a *Mycoplasma pneumoniae* infection is suspected, a second specimen should be obtained in 7-14 days and tested.

### Cautions

- Test results should be used in conjunction with information available from the patient's clinical evaluation and other available diagnostic procedures.
- Significance of negative test results in immunosuppressed patients is uncertain.
- Positive test results may not be valid in persons who have received blood transfusions or other blood products within the past several months.
- False-negative results due to competition by high levels of IgG, while theoretically possible, have not been observed.
- Rare cross reactions with sera from patients with autoimmune diseases (e.g., with herpes erythematous) have been noted, but not with sera from patients with the following infections: parainfluenza virus, influenza viruses A & B, adenovirus, cytomegalovirus, and Epstein-Barr virus.
Test Title: Mycoplasma pneumoniae Antibodies, IgM, Qualitative, Serum
#84117

References

Method
The ImmunoCard Mycoplasma EIA detects the presence of IgM to Mycoplasma pneumoniae in serum. Patient serum is added to each of the 2 sample ports. After allowing the sample to enter the device and migrate along the membrane and through the reaction ports, 3 drops of antihuman IgM alkaline phosphatase conjugate are added to the sample ports and allowed to enter the device. Three drops of wash and 2 drops of substrate are then added to each of the reaction ports. Reaction ports are observed for the development of any blue color after 5 minutes. The control port serves as a procedural control, containing immobilized human IgM in the reaction port. The test port contains Mycoplasma pneumoniae antigens and serves as the patient test port. The development of the blue color in the test port indicates a reactive test result for IgM to Mycoplasma pneumoniae. No blue color in the test port indicates a nonreactive result. (Package insert: ImmunoCard Mycoplasma EIA. Meridian Bioscience, Cincinnati, OH)

Specimen Required: Draw blood in a plain, red-top tube(s) or a serum gel tube(s). Spin down and send 0.5 mL of serum refrigerated.

Reference Values: Negative
Analytic Time: Same day
Days Set Up: Monday through Sunday
Fee: $122.70
CPT Code: 86738