



The One

a publication that highlights new tests and announcements from ARUP

TECHNICAL BULLETINS IN THIS ISSUE

- › Alternative Complement Activation Pathway Functional Assay
- › Bilirubin, Cerebrospinal Fluid
- › Chromatin ELISA
- › *EGFR* Gene Amplification by FISH
- › *EWSR1* Gene Rearrangement by FISH
- › *Francisella tularensis* Antibodies by ELISA, IgG, and IgM
- › Human Papillomavirus (HPV) Genotyping
- › Multiple Endocrine Neoplasia Type 1 (*MEN1*) Sequencing and Deletion/Duplication
- › *SS18 (SYT)* Gene Rearrangement by FISH
- › Testing for Anti-Gangliosides, IgG and IgM Antibodies
- › Thyroid Stimulating Immunoglobulins (TSI) Assay

Table of Contents

Letters and Announcements

Free Webinar: Drug Testing in the Pain Management Setting	1
25th Annual Park City Combined Pathology Update	1

Technical Bulletins

* Please note that test details contained in this publication may change. For the most up-to-date test information, please review ARUP's technical bulletins at <http://www.aruplab.com/Testing-Information/technicalbulletins.jsp>.

Alternative Complement Activation Pathway Functional Assay	2
Bilirubin, Cerebrospinal Fluid	3
Chromatin ELISA	4
<i>EGFR</i> Gene Amplification by FISH	5
<i>EWSR1</i> Gene Rearrangement by FISH	6
<i>Francisella tularensis</i> Antibodies by ELISA, IgG, and IgM	7
Human Papillomavirus (HPV) Genotyping	8
Multiple Endocrine Neoplasia Type 1 (<i>MEN1</i>) Sequencing and Deletion/Duplication	9
<i>SS18 (SYT)</i> Gene Rearrangement by FISH	10
Testing for Anti-Gangliosides, IgG and IgM Antibodies	11
Thyroid Stimulating Immunoglobulins (TSI) Assay	12

Upcoming Trade Shows

SEPTEMBER 12-13

College of American Pathologists (CAP);
Grapevine, TX

SEPTEMBER 21-23

University HealthSystem Consortium (UHC);
Chicago, IL

OCT 4-6

Pathology Informatics; Pittsburgh, PA

OCT 10-11

The Collaborative Groups of the Americas;
Montreal, Canada (CGA)

OCT 12-14

American Society of Human Genetics
(ASHG); Montreal, Canada

OCT 19-21

G-2 Lab Institute; Arlington, VA

OCT 20-21

American Society for Clinical Pathology
(ASCP); Las Vegas, NV

OCT 27-29

National Society of Genetic Counselors
(NSGC); San Diego, CA

Editorial Board:

Edward R. Ashwood, MD
President and CEO

Brian Jackson, MD, MS
Medical Director, Medical Informatics

Dina Hannah, MBA/HCM,
MT(ASCP)H, SBB, CIPP
Vice President; Director, Compliance
and Quality Systems

Mary Suchyta, DO
Medical Content Management

Deanna Lemke
Integrated Marketing Communications

Daria Cassity
Marketing Analytics

Dani Liese, PhD
Integrated Marketing Communications

Donna Cooper, MS, MBA
Marketing Analytics

FREE WEBINAR

Drug Testing in the Pain Management Setting

Join Dr. Gwen McMillin, medical director of the Toxicology and Trace Elements laboratories and co-medical director of Pharmacogenetics at ARUP Laboratories, on September 27 from 11 a.m. to noon MT as she discusses drug testing in the pain management setting.

The presentation will focus on considerations for selecting the most appropriate drug test(s) and interpreting drug testing results. Dr. McMillin will discuss explanations for unexpected positive and negative results, common patterns of urine drug testing results, and evaluation of dose versus drug adherence.

Participants will compare the strengths and limitations of screening and confirmation testing for drugs used in the pain management setting, list scenarios that could explain both positive and negative drug testing result, and understand the limitations of urine drug testing for evaluating dose adherence.

This webinar is provided at no cost to participants and is eligible for P.A.C.E.[®] and Florida credit. For more information, please visit www.aruplab.com.

SAVE THE DATE!

25th Annual Park City Combined Pathology Update Jan 30–Feb 3, 2012, The Canyons Resort, Park City, Utah

Special guest: Brian Rubin, MD, PhD, Cleveland Clinic

For the first time, this conference will combine both anatomic pathology for three days and clinical pathology for two days. This five-day course consists of short lectures and case-oriented discussions led by distinguished faculty from the University of Utah and guest presenters. Faculty consists of clinicians involved in patient care, pathologists, surgical pathologists, and clinical laboratory scientists. Discussion of timely topics by faculty and participants ensures that this course will be informative, interesting, and relevant. Cases are selected to represent common and/or difficult diagnostic problems.

Come enjoy Utah's unparalleled snow and ski terrain while enjoying this educational and informative pathology update.

CME information to come. For more information regarding the update, please contact:

Leita Rogers, (800) 242-2034, leita.rogers@aruplab.com.

For more information, please visit www.arup.utah.edu/parkcityupdate.

Alternative Complement Activation Pathway Functional Assay

Hemolytic radial immunodiffusion assay of the functional activity of alternative complement pathway in serum

Test Highlights

- This test measures the functional ability of the alternative complement pathway to lyse chicken erythrocytes embedded in agarose gel.

Clinical Background

- The alternative complement pathway is a cascade of reactions leading to lysis of cell membranes and cell death. Deficiencies of factors within this pathway contribute to recurrent infections and autoimmune diseases.

Pathophysiology

- The complement system consists of plasma enzymes, regulatory proteins, and proteins activated in a cascading fashion.
- The alternative pathway is activated by complex polysaccharides, including endotoxin.
- Bacterial lipopolysaccharides, virus components, and other pathogens have the ability to activate the alternative pathway.
- Deficiency of C3 component is seen in systemic lupus erythematosus (SLE), pyogenic infections, and glomerulonephritis.
- Deficiencies of factor D, properdin, and membrane attack complex are associated with increased susceptibility to infection, particularly due to *Neisseria spp.* species.
- Alternative pathway activation begins with the breakdown of C3 by C3 convertase (C3b and factor B), which is stabilized by properdin.
- C3 cleavage results in the subsequent activation of C5, C6, C7, C8, and C9 and formation of the membrane attack complex that binds to the surface of the target, leading to lysis and local inflammation.
- Intermediate components of complement activation C3a and C5a function as anaphylotoxins. C5a is also a major chemo-attractant for neutrophils and macrophages to the site of activation.

Indication for Ordering

- Screening for functional ability of the alternative pathway of the complement system.

Additional Ordering Notes

- If the test result is abnormal, order specific tests for evaluation of individual components of the alternative pathway.

Authors: Julio Delgado, MD, MS, and Igor Pavlov, PhD

Interpretation

- A test result below reference interval can occur due to hereditary absence or acquired functional activity of any of the individual components of the alternative pathway.

Limitations

- Test does not evaluate individual components of the alternative pathway.
- Rare complement activation can occur during blood draw.

Methodology

- Radial immunodiffusion method measuring functional ability of the alternative complement pathway to lyse chicken erythrocytes embedded in agarose gel.

Related Tests

- Complement Activity Enzyme Immunoassay, Total (0050198)
- Complement Factor Bb (2003042)
- Complement Component 2 (0050148)
- Complement Component 3 (0050150)
- Complement Component 4 (0050155)
- Complement Component 4A (2003180)
- Complement Component 5 (0050156)
- Complement Component 6 (0099072)
- Mannose Binding Lectin (0051692)
- Complement Factor B (0051720)

References

1. Walport MJ. Complement. First of two parts. *N Eng J Med* 2001;344:1058–66.
2. Walport MJ. Complement. Second of two parts. *N Eng J Med* 2001;344:1140–4.
3. Thurman JM, Holers VM. The central role of the alternative complement pathway in human disease. *J Immunol* 2006;176:1305.
4. Wen L, Atkinson JP, Giclas PC. Clinical and laboratory evaluation of complement deficiency. *J Allergy Clin Immunol* 2004;113:585.
5. Glovsky MM, Ward PA, Johnson KJ. Complement determinations in human disease. *Ann Allergy Asthma Immunol* 2004;93(6):513–22.

For specific collection, transport, and testing information, refer to Complement Activity, Alternative Pathway (AH50) (2005373) on the ARUP website at www.aruplab.com. For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.

Bilirubin, Cerebrospinal Fluid

For use in the evaluation of suspected subarachnoid hemorrhage

Test Highlights

- The concentration of bilirubin in cerebrospinal fluid (CSF) is too low to be measured using standard chemical techniques, and the visual inspection of CSF for the presence of xanthochromia is an unreliable method of detecting bilirubin.
- Spectrophotometric detection of bilirubin in CSF can help detect subarachnoid hemorrhage (SAH) in patients in whom hemorrhage is suspected but who have a negative computed tomography scan. This method can also eliminate the possibility of SAH in the remainder without the need for angiography.

Clinical Background

- Most SAH cases occur due to the rupture of intracranial aneurysms that release blood into CSF, which rapidly increases intracranial pressure.
- The most frequent symptom of SAH is a sudden, severe headache often described by patients as the worst headache they have ever experienced. This symptom may or may not be accompanied by loss of consciousness, vomiting, and nuchal rigidity. Patients who present with these classic symptoms usually do not pose a diagnostic challenge, but up to half of all patients with SAH experience only minor bleeding and may not present with classic symptoms.
- Approximately 30 percent of SAH is not correctly diagnosed. In these cases, outcome is generally poor.
- A computed tomography (CT) scan of the head is the mainstay of an SAH diagnosis and is most sensitive when performed within the first 12 hours after the hemorrhage but is often negative in patients with a minor SAH. The sensitivity of a head CT scan decreases rapidly over time.
- Spectrophotometric detection of bilirubin in CSF can be useful in identifying SAH in those patients for whom a CT scan is unrevealing.

Pathophysiology

- Following an SAH, red blood cells rapidly disseminate through the subarachnoid space, where they are gradually lysed and release intracellular oxyhemoglobin. The released oxyhemoglobin is enzymatically metabolized to bilirubin in a time-dependent process that imparts a yellow tint to the CSF. This yellowish tint is commonly referred to as xanthochromia.
- The conversion of oxyhemoglobin to bilirubin only occurs in vivo. Thus, the presence of blood in the CSF from a traumatic lumbar puncture will not result in an increase in CSF bilirubin.

Indications for Ordering

- To investigate the possibility of SAH in patients for whom a CT scan of the head is unrevealing.

Additional Ordering Notes:

- A blood sample must be obtained at the time of CSF collection. This sample is used to aid in interpretation of the test result.

Interpretation

- The detection of bilirubin in the CSF supports a diagnosis of SAH in patients for whom it is suspected.
- The absence of bilirubin in the CSF in conjunction with a negative CT scan of the head is sufficient to rule out SAH.

Limitations

- CSF samples should be collected 12 hours after the suspected hemorrhage, as the formation of bilirubin from oxyhemoglobin is time-dependent, and bilirubin will not be detectable within 12 hours of an SAH.
- A traumatic lumbar puncture can increase the amount of oxyhemoglobin in the sample, which may interfere with the detection of bilirubin.
- Samples should be protected from light because bilirubin is degraded when exposed to light.

Methodology

- Scanning spectrophotometry is used to determine the absorbance of a CSF sample at each wavelength between 350 and 550 nm. The number of absorbance units (AU) that the curve deviates from a base line at 476 and ~414 nm is recorded as the net bilirubin absorbance (NBA) and the net oxyhemoglobin absorbance (NOA), respectively. Reference limits for the NBA and NOA are ≤ 0.007 and ≤ 0.020 AU, respectively.

References

1. Cruickshank A, et al. Revised national guidelines for analysis of cerebrospinal fluid for bilirubin in suspected subarachnoid haemorrhage. *Ann Clin Biochem* 2008;45:238–44.
2. Edlow JA and Caplan LR. Avoiding pitfalls in the diagnosis of subarachnoid hemorrhage. *New Eng J Med* 2000;342:29–36.
3. Perry JJ, et al. Is the combination of negative computed tomography result and negative lumbar puncture result sufficient to rule out subarachnoid hemorrhage? *Ann Emerg Med* 2008;51:707–13.

Author: David Grenache, PhD

For specific collection, transport, and testing information, refer to Bilirubin, CSF (2005248) on the ARUP website at www.aruplab.com. For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.

Chromatin ELISA

Aids in the diagnosis of drug-induced lupus (DIL) and systemic lupus erythematosus (SLE)

Test Highlights

- Chromatin ELISA detects the presence of chromatin antibodies in human serum, found predominantly in people with systemic lupus erythematosus (SLE) and drug-induced lupus (DIL).

Clinical Background

- SLE is an autoimmune disorder that can affect multiple organs. The development of SLE may be associated with numerous drugs.
- One of the main uses of this test is to aid in the diagnosis of drug-induced Lupus (DIL). Anti-chromatin antibodies have been found in patients with lupus induced by drugs such as procainamide, quinidine, penicillamine, acebutalol, and methyldopa.
- Anti-chromatin antibodies were found in people taking procainamide shortly before the development of lupus-like symptoms.
- About 50 to 90 percent of SLE patients have been reported to have anti-chromatin antibodies.
- Anti-chromatin antibodies are predominantly found in patients with SLE or DIL and occasionally in people with other diseases, such as primary Sjögren syndrome and primary antiphospholipid syndrome.
- In general, more SLE patients are positive for anti-chromatin antibodies than for anti-histone or anti-DNA antibodies.
- The presence of anti-chromatin antibodies has also been linked to proteinuria, glomerulonephritis, and disease activity in SLE patients.

Pathophysiology

- Chromatin antibodies are also called anti-nucleosome, anti-(H2A-H2B) DNA, anti-DNP, and LE-cell factor.
- Naturally found in the nucleus of cells, chromatin is comprised of native DNA wrapped around the (H2A-H2B-H3-H4)₂ histone octamer associated with histone H1 and some non-histone proteins.
- Chromatin antibodies are directed to epitopes consisting of the native histone-DNA complex, native DNA, and the exposed histone regions of chromatin. Consequently, anti-nuclear DNA (anti-nDNA) is a subset of anti-chromatin.

Indications for Ordering

- Patient with systemic symptoms suggesting DIL or SLE, including:
 - Arthritis
 - Arthralgias
 - Skin rashes

- Anemia
- Renal dysfunction
- Pleuritis
- Pericarditis

Interpretation

- A positive result signifies the presence of chromatin antibodies and is suggestive of a possible diagnosis of DIL or SLE.
- A negative result indicates the absence of chromatin antibodies or levels below the negative cutoff of the assay.
- Results should be used in combination with clinical findings and other serological tests to determine the presence of disease.

Limitations

- Presence of immune complexes or other immunoglobulin aggregates in patient serum may result in an increased level of nonspecific binding, producing false positives in this assay.
- The absence of chromatin antibodies does not rule out DIL or SLE.
- Results should be used in conjunction with clinical findings and other serological tests.
- Serum is the only matrix for which assay performance characteristics have been established.

Methodology

- The presence of anti-chromatin antibodies in serum is detected by ELISA using microwell plates coated with purified chromatin antigen.

Related Tests

- Anti-chromatin antibodies can also be measured by LE cell test, immunoprecipitation, and immunofluorescence of histone-reconstituted, acid-extracted cells.

References

1. Gómez-Puerta JA, Burlingame RW, Cervera R. Anti-chromatin (anti-nucleosome) antibodies: diagnostic and clinical value. *Autoimmun Rev* 2008;7(8):606–11.
2. Cervera R, et al. Anti-chromatin antibodies in systemic lupus erythematosus: a useful marker for lupus nephropathy. *Ann Rheum Dis* 2003;62(5):431–4.
3. Souza A, et al. Anti-nucleosome and anti-chromatin antibodies are present in active systemic lupus erythematosus but not in the cutaneous form of the disease. *Lupus* 2009;18(3):223–9.
4. Gómez-Puerta JA, Burlingame RW, Cervera R. Anti-chromatin (anti-nucleosome) antibodies. *Lupus* 2006;15(7):408–11.
5. Burlingame RW, Cervera R. Anti-chromatin (anti-nucleosome) autoantibodies. *Autoimmun Rev* 2002;1(6):321–8.

Authors: Brenda Suh-Lailam, PhD, and Anne Tebo, PhD

For specific collection, transport, and testing information, refer to Chromatin Antibody, IgG (2005287) on the ARUP website at www.aruplab.com. For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.

EGFR Gene Amplification by FISH

For the detection of *EGFR* amplification in carcinomas

Test Highlights

- *EGFR* FISH analysis is a sensitive and specific method used to detect *EGFR* gene amplification, which is useful as a prognostic and therapeutic indicator in several carcinomas.

Disease Overview

- *EGFR* amplification has been shown to occur in a variety of solid tumors, including glioblastoma, non-small cell lung carcinoma, head and neck carcinomas, and carcinomas of the colon, breast, prostate, stomach, and ovary.
- Studies have shown that amplification of the *EGFR* gene is correlated with a poor prognosis in some types of tumors, including glioblastomas.

Genetics

- *EGFR* is located on the short arm of chromosome 7 (7p12). Amplification of this gene may result in overexpression of *EGFR*, leading to progression of malignancies through increased angiogenesis, metastasis, and inhibition of apoptosis.

Indication for Ordering

- Patients diagnosed with a neoplasm in which *EGFR* amplification has been shown to be a prognostic or therapeutic indicator.

Contraindication

- This test is not recommended for detection of minimal residual disease.

Additional Ordering Note

- The biopsy site and fixative used should be provided. The submitted sample should contain sufficient viable tumor.

Interpretation

- Presence of *EGFR* gene amplification is predictive of a poor prognosis.

Limitations

- Tissues fixed in alcohol-based or non-formalin fixatives have not been tested using this method.

Methodology

- This test uses a commercially available DNA FISH probe.
- This test is conducted by counting the number of probe signals within 40 cells and calculating the average probe number per cell. Neoplasms containing an *EGFR*/CEP7 ratio greater than or equal to two are considered amplified.

References

1. Vysis® LSI *EGFR* SpectrumOrange/ CEP 7 SpectrumGreen Probe (package insert). Des Plaines, IL: Abbott Molecular, Inc.; 2001.
2. Layfield LJ, et al. Epidermal growth factor receptor gene amplification and protein expression in glioblastoma multiforme: prognostic significance and relationship to other prognostic factors. *Appl Immunohistochem Mol Morphol* 2006;14(1):91–6.
3. Dacic S. *EGFR* assays in lung cancer. *Adv Anat Pathol* 2008;15(4):241–7.
4. Rosell R, et al. Screening for epidermal growth factor receptor mutations in lung cancer. *N Engl J Med* 2009; 361(10):958–67.
5. Chua W, et al. Predictive biomarkers of clinical response to targeted antibodies in colorectal cancer. *Curr Opin Mol Ther* 2009; 11(6):611–22.
6. Markman B, Rodriguez-Freixinos V, Tabernero J. Biomarkers in colorectal cancer. *Clin Transl Oncol* 2010;12(4):261–70.
7. Jansen M, Yip S, Louis DN. Molecular pathology in adult gliomas: diagnostic, prognostic, and predictive markers. *Lancet Neurol* 2010; 9(7):717–26.
8. Chang SS, Califano J. Current status of biomarkers in head and neck cancer. *J Surg Oncol* 2008; 97(8):640–643.

Authors: Lester Layfield, MD, and Carlynn Willmore-Payne, MT(ASCP)

For specific collection, transport, and testing information, refer to *EGFR* Gene Amplification by FISH (0049234) on the ARUP website at www.aruplab.com. For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.

EWSR1 Gene Rearrangement by FISH

For the diagnosis of Ewing sarcoma

Test Highlights

- *EWSR1* FISH is a sensitive and specific method used to detect *EWSR1* gene rearrangements and aid in the diagnosis of Ewing sarcoma.

Disease Overview

- Ewing sarcoma is a round-cell tumor occurring in children and young adults.
- The tumor most often presents in the long bones of the body, such as the arms, legs, pelvis, or chest, and frequently metastasizes to the lungs or other bones.

Epidemiology

- Ewing sarcoma is more common in males, with a peak age for occurrence between 10 and 20 years.
- About four cases of Ewing sarcoma per 1,000,000 children are diagnosed each year in the United States.

Genetics

- Ewing sarcoma is strongly associated with t(11;22) and, in some cases, t(7;22) or t(21;22) involving the *EWSR1* gene on chromosome 22.

Pathophysiology

- The exact cause of Ewing sarcoma is not known. Translocations within the *EWSR1* gene on chromosome 22 can increase the rate of cell division and growth.
- The distinction of Ewing sarcoma from other sarcomas provides prognostic and therapeutically relevant information.

Indication for Ordering

- Patients diagnosed with or suspected of having Ewing sarcoma based on morphology or immunophenotypic studies.

Contraindication

- This test is not recommended for detection of minimal residual disease.

Additional Ordering Note

- The biopsy site and fixative used should be provided. The submitted sample should contain sufficient viable tumor.

Interpretation

- Presence of an *EWSR1* gene rearrangement is strongly supportive of a diagnosis of Ewing sarcoma.

Limitations

- Tissues fixed in alcohol-based or non-formalin fixatives have not been tested using this method.
- This test will not identify the specific *EWSR1* translocation partner.

Methodology

- The detection of *EWSR1* gene rearrangements in formalin-fixed, paraffin-embedded tissue uses a commercially available DNA FISH probe. The presence of two fusion signals per nucleus indicates an intact *EWSR1* gene. The presence of a single orange and single green signal indicates a rearranged *EWSR1* gene.
- This test is conducted by noting the probe-signal configuration within the nuclei and reporting the result as rearranged or normal.

References

1. Vysis® LSI *EWSR1* Dual Color Breakapart Probe (package insert). Des Plaines, IL: Abbott Molecular, Inc.; 2002.
2. Sandberg AA, Bridge JA. Updates on cytogenetics and molecular genetics of bone and soft tissue tumors; Ewing sarcoma and peripheral primitive neuroectodermal tumors. *Cancer genet cytogenet* 2002;123(1):1–26.
3. Balamuth NJ, Womer RB. Ewing's sarcoma. *Lancet Oncol* 2010; 11(2) :184–92.
4. Osuna D and de Alava E. Molecular pathology of sarcomas. *Rev Recent Clin Trials* 2009; 4(1):12–26.
5. Toomey EC, Schiffman JD, Lessnick SL. Recent advances in the molecular pathogenesis of Ewing's sarcoma. *Oncogene* 2010;29(32):4504–16.
6. Khoury JD. Ewing sarcoma family of tumors. *Adv Anat Pathol* 2005; 12(4):212–20.

Authors: Lester Layfield, MD, and Carlynn Willmore-Payne, MT(ASCP)

For specific collection, transport, and testing information, refer to *EWSR1* (22q12) Gene Rearrangement by FISH (0049335) on the ARUP website at www.aruplab.com. For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.

Francisella tularensis Antibodies by ELISA, IgG, and IgM

Test Highlights

- This assay is useful in the detection of IgG and IgM antibodies against *Francisella tularensis*.

Disease Overview

- *Francisella tularensis* is a small, gram-negative pathogenic pleomorphic coccobacilli that can cause infection in humans and small mammals.¹
- *F. tularensis* is the causative agent of tularemia, a disease with variable symptoms depending on the site of infection. Five typical forms include ulceroglandular, glandular, oculoglandular, pharyngeal, and pneumonic.
- Ulceroglandular tularemia is characterized by a skin ulcer at the site of a tick or fly bite. This is the most common form of tularemia.²
- Glandular tularemia will present with tender regional lymphadenopathy without a skin ulcer.
- Oculoglandular tularemia occurs when infection occurs through the conjunctiva and presents with photophobia and excessive lacrimation followed by painful conjunctivitis, chemosis, and small yellowish conjunctival ulcers or papules in some patients.
- Oropharyngeal tularemia is similar to ulceroglandular tularemia; however, the primary site of infection is the oropharynx. Pharyngeal tularemia presents with fever and tonsillitis; one or more ulcers may be present.
- Pneumonic tularemia symptoms include cough, chest pain, and difficulty breathing. Pneumonic tularemia is the most serious form of tularemia.

Epidemiology

- Tularemia is found primarily in the northern hemisphere.
- The number of cases of tularemia has steadily declined from 0.15 cases per 100,000 in the 1950s to around 0.05 cases per 100,000 in 2001.
- The number of tularemia cases, which are typically associated with tick bites, peaks during the summer months. Fewer cases occur late in the fall and are often associated with hunting.

Indications for Ordering

- These ELISA assays should be ordered if patients have any of the following symptoms: skin ulcer at the site of a tick or fly bite; tender regional lymphadenopathy near the site of a tick or fly bite; photophobia and excessive lacrimation followed by painful conjunctivitis, chemosis, and small yellowish conjunctival ulcers or papules; ulcers of the oropharynx if surface water of hunted game has recently been consumed; cough, chest pain, and difficulty breathing.

Methodology

- Enzyme immunoassay.

Interpretation

- A positive result of greater than 15 U/mL is indicative of a detectable and significant level of IgG or IgM antibodies to *F. tularensis*.
- A result of 10–15 u/mL is considered to be equivocal, and retesting is suggested in 10–14 days.
- A result of less than 10 u/ml is considered negative for antibodies to *F. tularensis*.

Limitations

- This test has been validated for serum and plasma samples only. No other sample types may be used.
- Antibody testing has limited utility in patients who are HIV-positive or otherwise immunocompromised.

References

1. Mariathasan S, et al., Innate immunity against *Francisella tularensis* is dependent on the ASC/caspase-1 axis. *J Exp Med* 2005;202(8):1043–9.
2. Mendell GL, Bennett JE, Dolin R. 2010. *Mendell, Douglas, and Bennett's principles and practice of infectious diseases*, 7th ed. Philadelphia, PA: Churchill Livingstone Elsevier.

Authors: Julio Delgado, MD, MS, Stephen Merrigan, and Ryan Welch

For specific collection, transport, and testing information, refer to *Francisella tularensis* Antibodies, IgG and IgM (2005350) on the ARUP website at www.aruplab.com. For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.

Human Papillomavirus (HPV) Genotyping

For detection of HPV type 16 and 18 in clinical specimens

Test Highlights

- In contrast to the high-risk HPV assay that tests for all high-risk HPV types, this test is designed to detect HPV types 16 and 18 only.
- HPV types 16 and 18 are the types most commonly found in cervical cancer.
- This test is performed on the same specimen types used for high-risk HPV testing and can aid in triage of women over age of 30 with negative Pap cytology but a positive high-risk HPV test.^{1,2}

Clinical Background

- HPV is the most common sexually transmitted infection in the United States.
- There are more than 100 different HPV types. Approximately 40 HPV types infect the anogenital mucosa. These types can be classified as high-risk, intermediate risk, and low-risk types, depending on propensity of causing cervical cancer.
- Infections with high-risk HPV types are also associated with cancers of the vulva, vagina, anus, penis, and oropharynx.
- Most infections are asymptomatic and resolve spontaneously.
- Among high-risk HPV types, infections with types 16 and 18 confer the highest risk for persistence and the subsequent development of cervical cancer.
- Screening with cervical cytology (Pap smear) has decreased the incidence of cervical cancer deaths in developed countries.
- Detection of high-risk HPV types in cervical specimens has also been used for screening and aiding in risk stratification and patient management.

Epidemiology

- Cervical cancer is the second leading cause of cancer-related deaths worldwide.
- About 11,000 women are diagnosed with cervical cancer in the United States every year.

Indication for Ordering

- Current ASCCP guidelines recommend HPV genotyping for types 16 and 18 in women over the age of 30 who have negative Pap cytology but a positive high-risk HPV screening test. Presence or absence of HPV 16 or 18 can assist in further patient-management decisions (e.g., immediate colposcopy versus repeat testing at a later time).^{1,3}

Interpretation

- The HPV genotyping test detects HPV high-risk types 16 and 18, which are strongly associated with cervical cancer and its precursor lesions.
- Results of this test should be correlated with the clinical context, including cytologic and histologic findings.
- Sensitivity may be affected by specimen integrity and/or cellularity.
- A negative test result does not exclude the possibility of HPV 16 and/or HPV 18 infection, as very low levels of infection or sampling error may cause false-negative results.

Authors: Robert Schlaberg, MD, MPH, and Rosemary She, MD

For specific collection, transport, and testing information, refer to Human Papillomavirus (HPV), Genotypes 16 and 18 (2005277) on the ARUP website at www.aruplab.com. For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.

- A false-negative result may also be obtained in specimens that are contaminated with high levels of contraceptive jelly and/or antifungal creams.

Limitations

- This test only detects DNA of HPV types 16 and 18. It does not detect DNA of the other high-risk HPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) which are also associated with cervical cancer and its precursor lesions. It is NOT intended to test for high-risk HPV types and should be used only in patients who have tested positive with a test for high-risk HPV types.
- Cross-reactivity to high levels of HPV high-risk type 31 can be seen.⁴
- High levels of contraceptive jelly and/or anti-fungal creams may cause false-negative results.⁴
- HPV genotyping is not suitable for women under age 30.¹
- HPV genotyping is not intended for women with abnormal cervical cytology, since results do not affect management.¹
- This test is not recommended for evaluation of suspected sexual abuse.⁴

Methodology

- Invader method.

Related Tests

- Human Papillomavirus (HPV) High-Risk DNA by Invader Method with Reflex to Human Papillomavirus (HPV), Genotypes 16 and 18 (2005283)
- Human Papillomavirus (HPV) DNA Probe, High Risk, Cervical Brush (Digene) (0065999)
- Human Papillomavirus (HPV) DNA Probe, High Risk, Surepath® (AutoCyte) (0060744)
- Human Papillomavirus (HPV) DNA Probe, High Risk, ThinPrep® (0060750)

References

1. American Society for Colposcopy and Cervical Pathology (ASCCP). Consensus guidelines. <http://www.asccp.org/ConsensusGuidelines/HPVGenotypingClinicalUpdate/tabid/5963/Default.aspx> (accessed on April 25, 2011).
2. American Society for Colposcopy and Cervical Pathology. Use of HPV genotyping to manage HPV HR positive/cytology negative. Women 30 years and older. http://www.asccp.org/Portals/9/docs/pdfs/Consensus%20Guidelines/hpv_genotyping_20090320.pdf (accessed on July 6, 2011).
3. Wright TC, et al. 2006 consensus guidelines for the management of women with abnormal cervical cancer screening tests. *Am J Obstet Gynecol* 2007;197:346–55.
4. Cervista™ HPV 16/18 (package insert). Madison, WI: Third Wave Technologies, Inc.

Multiple Endocrine Neoplasia Type 1 (*MEN1*) Sequencing and Deletion/Duplication

For diagnostic and presymptomatic identification of multiple endocrine neoplasia type 1, *MEN1* syndrome, multiple endocrine adenomatosis, and Wermer syndrome

Disease Overview

- Multiple endocrine neoplasia type 1 (*MEN1*) syndrome can include the development of multiple endocrine and non-endocrine tumors.
- Common *MEN1*-related endocrine tumors include parathyroid (90–95 percent), pancreatic islet cell (30–80 percent), and pituitary (15–90 percent).
- Non-endocrine tumors include facial angiofibromas, collagenomas, lipomas, meningiomas, ependymomas, and leiomyomas.
- Primary hyperparathyroidism is the most common and often the initial manifestation of *MEN1*.
- Gastrinoma and carcinoid tumors are the most frequent causes of mortality.
- Treatment of *MEN1* is dependent on tumor type and may include surgery and/or drug therapy.
- Recommendations for screening of asymptomatic individuals with a known *MEN1* mutation include routine biochemical testing (i.e., prolactin, calcium, gastrin, and parathyroid hormone) and imaging (i.e., head MRI and abdominal CT or MRI) to identify potential tumors in early stages.

Epidemiology

- Prevalence of *MEN1* is approximately one in 30,000.

Genetics

- Autosomal dominant inheritance.
- *MEN1* syndrome is caused by inactivating mutations of the *MEN1* tumor suppressor gene.
- De novo mutation rate is approximately 10 percent.
- Variable expressivity.
- Penetrance is approximately 50 percent by age 20 and greater than 95 percent by age 40.

Indication for Ordering

- Diagnostic testing for individuals with clinical and/or biochemical evidence of *MEN1*.

Contraindication for Ordering

- If the specific familial mutation has already been identified in a relative, testing can be performed on at-risk family members by ordering Familial Mutation, Targeted Sequencing (ARUP test code 2001961). A copy of the relative's genetic test result is required.

Additional Ordering Note

- Please complete the patient history form for *MEN1* and submit with the sample for optimal interpretation of test results.

Interpretation

- Identification of a known pathogenic *MEN1* mutation predicts the presence of *MEN1* syndrome.
- Absence of a pathogenic mutation reduces but does not exclude the possibility that the individual is affected with *MEN1*. Medical management should rely on clinical findings and family history.
- *MEN1* mutations of unknown clinical significance may be detected by this assay.

Methodology

- PCR followed by bidirectional sequencing of the entire coding region and intron-exon boundaries of the *MEN1* gene.
- Multiplex ligation-dependent probe amplification (MLPA) to identify large exonic deletions/duplications in the *MEN1* gene.
- Combined clinical sensitivity for sequencing and deletion/duplication analysis is up to 94 percent. Approximately 90 percent of detectable mutations are sequence variants, while up to 4 percent of causative mutations are large deletions.
- Analytical sensitivity and specificity are 98 percent.

Limitations

- Rare diagnostic errors may occur due to primer- or probe-site mutations.
- Regulatory region and deep intronic mutations will not be detected, and breakpoints of large deletions/duplications will not be determined.
- Genes other than *MEN1* will not be evaluated by this assay.

Related Test

- Familial Mutation, Targeted Sequencing (2001961)

References

1. Falchetti A. Genetic screening for multiple endocrine neoplasia syndrome type 1 (*MEN1*): when and how. *F1000 Med Rep* 2010;24:2:Pii 14.
2. Falchetti A, et al. Multiple endocrine neoplasia type 1 (*MEN1*): not only inherited endocrine tumors. *Genet Med* 2009;11(12):825–35.
3. Marini F, et al. Multiple endocrine neoplasia type 1. *Orphanet J Rare Dis* 2006;1:38.
4. Brandi ML, et al. Guidelines for diagnosis and therapy of *MEN1* type 1 and type 2. *J Clin Endocrinol Metab* 2001;86(12):5658–71.

Author: Kimberly Hart, MS, LCGC

For specific collection, transport, and testing information, refer to Multiple Endocrine Neoplasia Type 1 (*MEN1*) Sequencing and Deletion/Duplication (2005360), Multiple Endocrine Neoplasia Type 1 (*MEN1*) Sequencing (2005359), and Multiple Endocrine Neoplasia Type 1 (*MEN1*) Deletion/Duplication (2005346) on the ARUP website at www.aruplab.com. For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.

SS18 (SYT) Gene Rearrangement by FISH

For the diagnosis of synovial sarcoma

Test Highlights

- SS18 (SYT) FISH is a sensitive and specific method used to detect SYT gene rearrangements aiding in the diagnosis of synovial sarcoma.

Disease Overview

- Synovial sarcoma is a soft-tissue tumor that usually occurs near the joints in the legs and arms but has been documented in almost all tissue types.
- The separation of synovial sarcoma from other spindle-cell sarcomas and benign spindle-cell lesions is best achieved by identification of chromosome 18 (SYT) rearrangements.
- Since synovial sarcomas are high-grade neoplasms, identification of a spindle-cell sarcoma as a synovial sarcoma allows for the timely initiation of aggressive adjunct therapy.

Epidemiology

- Synovial sarcoma occurs primarily in the young, with a peak incidence before 30 years of age.
- Synovial sarcoma accounts for 5 to 10 percent of soft-tissue sarcomas, with approximately 800 new cases diagnosed each year.

Genetics

- Synovial sarcoma is strongly associated with t(X;18). This translocation involves the SYT gene on chromosome 18 and one of the three SSX genes on the X chromosome (SSX1, SSX2, and SSX4).

Pathophysiology

- The exact cause of synovial sarcoma is not known. Most patients with synovial sarcoma have an acquired t(X;18). These translocations can increase the rate of cell division and growth.
- The distinction of synovial sarcoma from other sarcomas is important and provides prognostic and therapeutically relevant information.

Indications for Ordering

- Patients diagnosed with or suspected of having synovial sarcoma based on morphology or immunophenotypic studies.

Contraindications

- This test is not recommended for detection of minimal residual disease.

Additional Ordering Notes

- The biopsy site and fixative used should be provided. The submitted sample should contain sufficient viable tumor.

Interpretation

- Presence of a SYT gene rearrangement is strongly supportive of a diagnosis of synovial sarcoma.

Limitations

- Tissues fixed in alcohol-based or non-formalin fixatives have not been tested using this method.
- This test will not identify the specific SS18 (SYT) translocation partner.

Methodology

- The detection of SYT gene rearrangements in formalin-fixed, paraffin-embedded tissue uses a commercially available DNA FISH probe.
- The presence of two fusion signals per nucleus indicates an intact SYT gene. The presence of a single orange and single green signal indicates a rearranged SYT gene.
- This test is conducted by noting the probe-signal configuration within the nuclei and reporting the result as rearranged or normal.

References

1. Bovee JV and Hogendoorn PC. Molecular pathology of sarcomas: concepts and clinical implications. *Virchows Arch* 2010; 456 (2) :193–99.
2. Jain S, et al. Molecular classification of soft tissue sarcomas and its clinical applications. *Int J Clin Exp Pathol* 2010; 3(4):416–28.
3. de Alava E. Molecular pathology in sarcomas. *Clin Transl Oncol* 2007; 9(3) :130–144.
4. Sandberg AA, Bridge JA. Updates on the cytogenetics and molecular genetics of bone and soft tissue tumors synovial sarcoma. *Cancer Genet Cytogenet* 2002;133:1–23.

Authors: Lester Layfield, MD, and Carlynn Willmore-Payne, MT(ASCP)

For specific collection, transport, and testing information, refer to SS18 (SYT) (18q11) Gene Rearrangement by FISH (0049380) on the ARUP website at www.aruplab.com. For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.

Testing for Anti-Gangliosides, IgG and IgM Antibodies

Two new panels for the detection of autoantibodies against GM1, GD1b, and GQ1b

Clinical Background

- Elevated antibody levels to ganglioside-monosialic acid (GM1) have been associated with motor neuropathy.
- Anti-GM1 may occur as IgM (polyclonal or monoclonal) or IgG antibodies.
- Anti-GM1 antibodies may also be found in patients with diverse connective-tissue diseases, as well as normal or healthy individuals.
- Anti-GD1b is predominantly found in sensory-motor neuropathy and may also be associated with cranial nerve deficit.
- Anti-GQ1b antibodies are seen in >80 percent of patients with Miller-Fisher syndrome and may be elevated in Guillain-Barre syndrome (GBS) patients with ophthalmoplegia. They have also been associated with sensory-motor neuropathies that involve the brainstem or cranial nerve.

Indications for Ordering

- These assays may be clinically useful in the evaluation of patients suspected of having an autoimmune-mediated neuropathy.
- With the elimination of the Asialo GM1 IgM and IgG assays, the new GM1 antibody panel (ARUP test code 0050591) will be more cost-effective and efficient in the evaluation of multifocal motor neuropathy (MMN) and acute motor axonal neuropathy (AMAN).
- The new GM1, GD1b & GQ1b IgG and IgM panel (ARUP test code 2004998) utilizes a more targeted approach for screening anti-ganglioside antibodies. Unlike the Ganglioside (Asialo-GM1, GM1, GM2, GD1a, GD1b, & GQ1b) Antibody, IgG/IgM panel (ARUP test code 0051033), this panel is capable of distinguishing between IgM and IgG antibodies directed against GM1, GD1b, and GQ1b markers.

Interpretation

- Positive results in association with clinical findings may help support the diagnosis of specific autoimmune-mediated neuropathy.

Limitations

- Test results alone are not diagnostic and should be used in conjunction with other clinical parameters to confirm disease.

Methodology

- Enzyme immunoassay (EIA).

Related Tests

- *Campylobacter jejuni* Antibody, IgG (0098841)
- Myelin Associated Glycoprotein (MAG) Antibodies, IgM & Sulfate-3-Glucuronyl Paragloboside (SGPG) Antibodies, IgM (2004412)
- Ganglioside (Asialo-GM1, GM1, GM2, GD1a, GD1b, & GQ1b) Antibody, IgG/IgM (0051033)
- GM1 Antibody Panel (0050591)
- Motor & Sensory Neuropathy Evaluation with Immunofixation Electrophoresis & Reflex to ANNA Titer & ANNA Immunoblot (0051223)
- Motor & Sensory Neuropathy Evaluation with Reflex to ANNA Titer & ANNA Immunoblot (0051224)
- Acetylcholine Receptor Blocking Antibody (0099580)

References

1. Ariga T, Yu RK. Antiglycolipid antibodies in Guillain-Barre syndrome and related diseases: review of clinical features and antibody specificities. *J Neurosci Res* 2005;80(1):1-17.
2. Bromberg MB. Acute neuropathies. *Front Neurol Neurosci* 2009;26:1-11.
3. Kaida K, et al. Ganglioside complexes as new target antigens in Guillain-Barre syndrome. *Ann Neurol* 2004;56(4):567-71.
4. Nobile-Orazio E, et al. How useful are anti-neural IgM antibodies in the diagnosis of chronic immune-mediated neuropathies? *J Neurol Sci* 2008;266(1-2):156-63.

Authors: Troy Jaskowski, PhD, and Brenda Suh-Lailam, PhD

For specific collection, transport, and testing information, refer to Ganglioside (GM1) Antibodies, IgG & IgM (0050591) and Ganglioside (GM1, GD1b & GQ1b) Antibodies, IgG & IgM (2004998) on the ARUP website at www.aruplab.com. For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.

Thyroid Stimulating Immunoglobulins (TSI) Assay

To confirm a diagnosis of Graves disease

Disease Overview

- Hyperthyroidism is a medical condition that is defined by overproduction of thyroid hormone. Hyperthyroidism is most commonly caused by autoantibodies to the thyroid stimulating hormone receptor (TSHR), a condition known as Graves disease, but can also be caused by thyroid nodules, thyroid tumors, or excessive administration of thyroid hormone.

Pathophysiology

- TSHR is a G-protein coupled receptor that has structural homology with other glycoprotein hormone receptors.¹
- Binding of TSH to the TSHR activates both the adenylate cyclase/cyclic adenosine monophosphate (cAMP) and the phospholipase C signaling pathways.¹
- Adenylate cyclase and cAMP regulate I⁻ uptake and transcription of thyroglobulin (Tg), thyroid peroxidase (TPO), and sodium/iodide symporter (NIS), while the phospholipase C pathway regulates I⁻ efflux, H₂O₂ production, and Tg iodination.¹
- Increased cAMP levels have been used as a biomarker for physiological conditions that cause stimulation of the TSHR, such as thyroid stimulating immunoglobulins (TSI).
- Autoantibodies to the TSHR may be stimulating, blocking, or neutral.² Stimulating antibodies mimic the action of TSH and cause hyperthyroidism (Graves disease), whereas blocking antibodies block the binding of TSH and cause hypothyroidism.² Both stimulating and blocking antibodies usually occur together in any given patient and may change over time.³

Epidemiology

- Prevalence of hyperthyroidism is approximately 2–3 percent of the general population.
- The majority of hyperthyroidism, 60–80 percent, occurs as a consequence of Graves disease.

Indications for Ordering

- Subsequent to a diagnosis of hyperthyroidism (below normal TSH levels or elevated free T4 levels), this assay would be used to confirm TSI involvement.

Methodology

- ARUP Laboratories has recently developed an ectopically expressing TSHR cell line for use in this assay. These cells also fuse the alpha subunit of the appropriate G protein (GNAS) to the TSHR to yield greater sensitivity. In addition, the method using the improved cells demonstrates better precision compared to the previously offered TSI assay.
- Ectopically expressing TSHR-GNAS cells are seeded into microplates, grown for two days, then incubated with a dilution of patient serum and subsequently lysed.

Authors: Gregory Gillespie, MLS(ASCP)^{CM}, A. Wayne Meikle, MD, and Michael Pierce, PhD

For specific collection, transport, and testing information, refer to Thyroid Stimulating Immunoglobulin (0099430) on the ARUP website at www.aruplab.com. For information on test selection, ordering, and interpretation, refer to ARUP Consult[®] at www.arupconsult.com.

- Levels of cAMP are measured using a commercially available kit (DiscoverX[®]).
- Results are reported as a percent of normal control.

Interpretation

- Using a cutoff of 114 percent of normal yields an 82.1 percent sensitivity and a 98.4 percent specificity for serum samples considered to be autoimmune hyperthyroid vs. normal serum.
- Results between 114 percent and 127 percent of normal are considered indeterminate, as serum samples from both autoimmune and nonautoimmune hyperthyroid patients can yield results in this range.
- Results that are 128 percent of normal and above are considered positive for the presence of TSI.

Limitations

- The TSHR expressed in the assay cells is the native form and will bind to all anti-TSHR antibodies (i.e., stimulating, blocking, and neutral). Consequently, even though a serum sample contains stimulating antibodies, the presence of blocking antibodies may obscure the results from the stimulating antibodies.^{2,4–5} However, this net response most closely represents the physiological one.
- Serum levels of TSH at or above 76mU/L are known to stimulate cAMP levels in the assay and accentuate stimulation as a result of TSI.

Related Tests

- Thyroid Stimulating Hormone Receptor Antibody (TRAb) (2002734)
- Thyroid Peroxidase (TPO) Antibody (0050075)
- Thyroglobulin Antibody (0050105)

References

1. De La Vieja A, et al. Molecular analysis of the sodium/iodide symporter: impact on thyroid and extrathyroid pathophysiology. *Physiol Rev* 2000;80:1083–105.
2. Michalek K, et al. TSH receptor autoantibodies. *Autoimmun Rev* 2009;9:113–6.
3. Demers LM and Spencer CA. Laboratory medicine practice guidelines: laboratory support for the diagnosis and monitoring of thyroid disease. *Clin Endocrinol* 2003;58:138–40.
4. Evans M, et al. Monoclonal autoantibodies to the TSH receptor, one with stimulating activity and one with blocking activity, obtained from the same blood sample. *Clin Endocrinol* 2010;73:404–12.
5. Kohn LD and Harii N. Thyrotropin receptor autoantibodies (TSHRABs): epitopes, origins, and clinical significance. *Autoimmunity* 2003;36:331–7.



AN ENTERPRISE OF THE UNIVERSITY OF UTAH
AND ITS DEPARTMENT OF PATHOLOGY

QUESTIONS OR SUGGESTIONS? Contact ARUP Marketing at (800) 242-2787, extension 3635.

ARUP LABORATORIES
500 Chipeta Way
Salt Lake City, UT 84108-1221
Phone: (800) 522-2787
Fax: (801) 583-2712
www.aruplab.com
www.arupconsult.com

©Copyright 2011.