CLINICAL MOLECULAR LABORATORY SERVICES
at SPARROW Regional Laboratories

TESTS INSIDE:

HER-2/neu
Grp B streptococcus
Grp A streptococcus
B. pertussis
B. parapertussis
N. gonnorrhoeae
C. trachomatis
MRSA Screen
HIV viral load
Hepatitis C viral load
Hepatitis C Qualitative
HPV
HSV Types I & II
Enterovirus
Factor V Leiden
Prothrombin Mutation

Molecular Center of Excellence
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For Questions: Walid T. Khalife, Ph.D.
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Roche Diagnostics has designated Sparrow Health System as a Molecular Center of Excellence, one of just two clinical laboratory centers in Michigan and 32 Nationwide. Molecular technology is highly sensitive and specific. It identifies pathogens or a mutation by its DNA or RNA sequence. The extreme sensitivity of Molecular PCR testing is used to detect low levels of pathogens and unculturable organisms.

### Molecular Center of Excellence

**Different types of Mutations:**
- **Splice mutations**
  - Intron is not removed from mRNA
  - HbE: missense mutation and splice error
- **Deletions**
  - Complete or partial gene deletions
  - Alpha-thalassemia, cystic fibrosis
- **Insertions**
  - Hemophilia A
- **Unstable trinucleotide repeats**
  - Fragile-X syndrome
- **Chromosomal alterations and translocations**
  - Frequently found in leukemias, lymphomas, other malignancies.

Tests performed at Sparrow Regional Laboratories are validated in our Molecular Laboratory. Sparrow Regional Laboratories is certified under CLIA 88 as qualified to perform high complexity clinical laboratory testing.

Tests that will be added:
- Varicella Zoster Virus
- Mycoplasma pneumonia
- Chlamydia pneumonia
- Respiratory Viruses
- CMV (qualitative & quantitative)
- Epstein-Barr Virus
- HCV genotyping

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There are 9 different molecular technology methodologies utilized by Sparrow Molecular Diagnostics Laboratory.

- Cobas Amplicor PCR
- LightCycler real-time PCR
- Cobas TaqMan real-time PCR
- Smart Cycler real-time PCR
- Third wave (Invader)
- Digene Hybrid Capture
- TMA
- FISH
- GeneXpert real-time PCR

To review a few basic genetic concepts, genomes vary widely in size and the smallest known genome of a free-living organism (a bacterium) contains about 600,000 DNA base pairs, while human and mouse genomes have approximately 3 billion.

Humans have 46 chromosomes:
- 22 pairs of autosomes
- one pair of sex chromosomes; XX or XY.

Genes are the basic unit of heredity. All genes are contributed equally by both parents except the XY chromosome in males. The Human genome is estimated to contain 20,000 to 25,000 genes.
**HER-2/neu**

**Clinical Background**

Her-2/neu is an oncogene on the long arm of chromosome 17 that is amplified in approximately 25-30% of breast cancers. Normally, the Her2-neu gene plays an important role in cell division and growth. When this gene is increased in number, cells appear to respond with uncontrolled growth and become cancerous. Her-2/neu testing is used as a prognostic marker to help determine how aggressive a breast cancer tumor is likely to be and if it may respond to Herceptin therapy.

**Diagnostic Recommendations**

Her-2/neu-positive tumors are susceptible to Herceptin (tratuzumab), a drug therapy that was created to target Her-2/neu protein. Herceptin attaches itself to the excess protein molecules and inhibits the growth of the cancer. Herceptin may be used alone or with some chemotherapy agents but is only useful in those who have Her-2/neu amplification and protein over-expression.

**Test No. & Ordering Info**

6962
Turnaround time is 5 to 10 days
Methodology- Fluorescence in situ Hybridization (FISH)

**Specimen Requirements**

Tissue from breast cancer biopsy procedure and/or tumor Formalin-fixed, paraffin-embedded breast cancer tissue block at room temperature.

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2 green signals indicate the presence of 2 copies of chrom. 17.  
2 orange signals indicate the presence of 2 copies of HER-2 genes in the same nucleus. The ratio of HER-2 to CEP 17 is 1.0. **Non-amplified**

3 green signals indicate the presence of 3 copies of chrom. 17.  
13 orange signals indicate the presence of 13 copies of HER-2 genes in the same nucleus. The ratio of HER-2 to CEP 17 is approximately 4. **Amplified**

4 green signals indicate the presence of 4 copies of chrom. 17.  
10 orange signals indicate the presence of 10 copies of HER-2 genes in the same nucleus. The ratio of HER-2 to CEP 17 is 2.5, **Amplified**

3 green signals indicate the presence of 3 copies of chrom. 17.  
The orange signals are showing in clusters. When focusing up and down, there are actually approximately 20 signals indicating approximately 20 copies of the HER-2 gene. The ratio of HER-2 to CEP 17 is approximately 7. **Amplified**  

Source: Abbott laboratories/Vysis
Group B Strep (GBS) is the leading cause of neonatal sepsis, morbidity and mortality. Infected infants may display symptoms within six hours of birth or as late as two months of age. If untreated, the baby may become septic, develop pneumonia, suffer hearing or vision loss, or develop varying degrees of physical and learning disabilities.

The use of intrapartum antibiotics based on screening results has decreased the incidence of early-onset GBS disease by approximately 70%. Current literature indicates that recovery of GBS from properly collected samples is significantly improved by PCR. In clinical trials, Davies et al showed that the PCR test was superior to antenatal cultures (sensitivity, 94% vs. 42%; P<.0001) without significant differences in specificity.

Source: Davies et al CID2005

Diagnostic Recommendations

The guidelines for prevention of prenatal GBS disease revised in 2002 by the US Centers for Disease Control and Prevention recommend universal prenatal screening for GBS colonization in all pregnant women at 35-37 weeks gestation as well as intrapartum antibiotic prophylaxis for those identified as positive for GBS.

Test No. & Ordering Info

1412

Turnaround time is 1 to 2 days
Susceptibility testing of isolates from penicillin allergic patients will be performed on request from samples submitted for PCR.

Specimen Requirements

Use the culturette II swab and collect from Vaginal and rectal areas. Samples must be collected as vaginal/rectal to increase the sensitivity of the test.

Test Result Interpretation

A positive amplification for GBS is reported as PCR Result: ****Detected**** on the result report and designates the woman as a GBS carrier.
Human Papillomavirus is the primary cause of cervical cancer. Over 100 types of HPV have been identified and approximately 30 HPV types primarily infect the squamous epithelium of the lower anogenital tracts of men and women. HPV can cause cervical cancer if not detected and treated properly. HPV infection can be latent for many years but most women will clear an HPV infection within 9-15 months. HPV testing is performed using the Digene Hybrid Capture 2 for High risk HPV types 16, 18, 31,33, 39,45,51,52, 56, 58, 59, 68.

According to the ACOG guidelines, HPV DNA test may be used with the PAP test for primary adjunctive screening for women age 30 and older to detect the presence or absence of High Risk HPV. The ACOG guidelines also state that reflex testing for HPV testing on women of any age with ASC-US PAP results or inconclusive PAP results should be performed.

A woman with a negative HPV DNA with PAP can be reassured that she is unlikely to develop cervical cancer before her next routine Ob-gyn visit.

The Negative predictive value of HPV is 99%. Results are reported out as High Risk Group: Positive or Negative.

Algorithm for the management of women using a combination of cervical cytology and HPV DNA testing for primary cervical cancer screening:

ASCUS = atypical squamous cells of undetermined significance.
ACOG, ASCCP & ACS, 2004
Herpes Simplex Virus Types I & II

Clinical Background

Herpes simplex virus causes several clinical manifestations in both normal and immunocompromised hosts. The infected anatomical sites include lips, oral cavity, eyes, genital tract, skin and central nervous system. Disseminated HSV infection may occur in patients compromised by organ transplantation, neoplasia, AIDS and through neonatal infection acquired by transmission of the virus through an infected birth canal. Most disseminated disease is fatal.

HSV is one of the most commonly detected viruses in diagnostic labs and has the ability to establish latency with subsequent reactivation manifested usually by recurrent local disease. HSV infection of the genital tract, particularly with recurrent episodes, can be asymptomatic and is associated with ongoing risk of sexual and perinatal transmission. Thus the importance of a sensitive, accurate, and rapid diagnostic test that can detect symptomatic or asymptomatic viral shedding that leads to early treatment in order to reduce the transmission of infection. Real time PCR has been shown in several studies to be more sensitive and rapid than the cell culture. At Sparrow Regional Laboratories, we use real time PCR for the routine diagnosis and genotype determination of dermal, genital, respiratory and CSF HSV infections.

Diagnostic Recommendations

- Women presenting with both classical and atypical symptoms
- Symptomatic pregnant women or those with infected partners
- As part of a complete STD screen for women with one or more risk factors

Specimen Requirements

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF or Body Fluid</td>
<td>0.5 ml of body fluid or spinal fluid in a screw-capped, sterile vial. Maintain sterility and forward promptly.</td>
</tr>
<tr>
<td>Dermal Specimens (vesicles):</td>
<td>Collect lesion and dermal specimens using a culture transport swab. If a culture transport swab is not available, M4 or M5 media is acceptable.</td>
</tr>
<tr>
<td>Genital Specimens:</td>
<td>Collect a specimen of the cervix, rectum, urethra, vagina, or other genital sites using a culture transport swab. If a culture transport swab is not available, M4 or M5 media is acceptable.</td>
</tr>
<tr>
<td>Respiratory Specimens:</td>
<td>1.5 ml of bronchial washing, bronchoalveolar lavage, naso-pharyngeal aspirate or washing, sputum, or tracheal aspirate.</td>
</tr>
<tr>
<td>Throat Swabs:</td>
<td>Swab the area with a culture transport swab.</td>
</tr>
<tr>
<td>Transport and storage:</td>
<td>Transport at ambient temperatures Store refrigerated/frozen</td>
</tr>
<tr>
<td>Special Notes:</td>
<td>Write specimen source on request form for specimen laboratory processing. Specimens grossly contaminated with blood may inhibit the PCR and produce false-negative results. Calcium alginate tipped swabs or transport swab containing gel is not acceptable for PCR testing. Turn around time is 1 to 3 days.</td>
</tr>
</tbody>
</table>

Test Number

8069

Test Result Interpretation

The detection of Herpes Simplex Virus Type I and Type II is based upon real-time PCR amplification and detection of specific HSV DNA sequences by PCR from total DNA extracted from the specimen. Probes specific for HSV I and HSV II are used to identify and differentiate the products of the PCR amplification.
B. pertussis and parapertussis

Clinical Background

Pertussis, also known as whooping cough, is caused by Bordetella pertussis or B. parapertussis. Incidence of Pertussis continues to rise in the US according to the CDC. From the onset of symptoms, the disease can take 6-8 weeks to resolve.

Pertussis is highly contagious, infecting 80-90% of susceptible individuals. Incubation period is 4 to 21 days post exposure. Mortality from this disease is low among healthy adults. However, they are a potential reservoir for pediatric infections. Pertussis can be very severe in young infants and adults age 60 years and older. Early diagnosis is essential to limit complications and minimize transmission of the disease.

Diagnostic Recommendations

PCR offers a fast tool with high sensitivity and specificity and better turnaround time for the diagnosis of B. pertussis and parapertussis infections suitable for implementation in a routine diagnostic laboratory.

At Sparrow Hospital, pertussis testing by PCR detected 100% of the B. pertussis positive specimens (93/93), while only 76.3% (71/93) were detected by DFA and culture. Sloan et al from Mayo Medical Laboratories reported that culture detected only 22.5% (9/40) of the pertussis positive specimens.

Test Number

8099

Turn around time is 1 to 4 days

Specimen Requirements

Collect one nasopharyngeal (not throat) specimen on a rayon swab with an aluminum or plastic shaft. Place in transport medium such as Stuart’s or Amies with charcoal. Alternatively, a nasopharyngeal aspirate can be collected and transported in a screw-capped, sterile container within 24 hours of collection. Send specimen refrigerated.
Clinical Background

Group A Streptococcus DNA testing can detect and identify Streptococcus pyogenes from throat swabs. Group A Strep can range from a mild skin infection or sore throat to severe, life threatening conditions such as toxic shock syndrome and necrotizing fasciitis, commonly known as flesh eating disease. Most people are familiar with strep throat which along with minor skin infection, is the most common form of the disease. Health experts estimate that more than 10 million mild infections (throat and skin) like these occur every year.

Untreated group A strep infection can result in rheumatic fever and post-streptococcal glomerulonephritis (PSGN). Rheumatic fever develops about 18 days after a bout of strep throat and causes joint pain and heart disease. PSGN is an inflammation of the kidneys that may follow an untreated strep throat but more often comes after a strep skin infection.

Diagnostic Recommendations

Strep throat symptoms usually include red and painful sore throat. Patients may have white patches on the tonsils, swollen lymph nodes in your neck, run a fever and have a headache.

Transmission can occur by direct contact with saliva or nasal discharge from an infected person. A person can get sick within 3 days after being exposed to the bacteria. Once infected, the infection may be passed on to others for up to 2 to 3 weeks even if there is not any symptoms. After 24 hrs of antibiotic treatment, the spread of the bacteria to others will stop.

Rapid Group A strep kits have good specificity (greater than 90%) but poor sensitivity (from 45 to 80%). We recommend Group A Strep DNA test for screening or as a confirmatory test for negative rapid antigen tests.

Specimen Requirements

Collect throat specimen by asking the patient to say ah, use a tongue depressor to and guide the swab over the tongue into the posterior pharynx. The mucosa behind the uvula and between the tonsillar pillars is swabbed with a gentle back and forth sweeping motion. Transport swabs to the laboratory at ambient temperatures for up to 48 hours after specimen collection.

Test Number

2258

Turnaround time is 3 hours to 1 day
Neisseria gonorrhoeae

Clinical Background

Gonorrhea is a sexually transmitted disease caused by Neisseria gonorrhoeae, a bacterium that can grow and multiply easily in the warm, moist areas of the reproductive tract, including the cervix, uterus and fallopian tubes in women, and in the urethra in women and men. The bacterium can also grow in the mouth, throat, eyes, and anus. Gonorrhea is the second most commonly reported infectious disease in the United States, with 339,593 cases reported in 2005. Following a 74 percent decline in the rate of reported gonorrhea from 1975 through 1997, overall gonorrhea rates appear to have plateaued in recent years. Like chlamydia, gonorrhea is substantially under-diagnosed and under-reported, and approximately twice as many new infections are estimated to occur each year as are reported.

In the United States, the highest reported rates of infection are among sexually active teenagers, young adults, and African Americans. Gonorrhea is spread through contact with the penis, vagina, mouth, or anus. Ejaculation does not have to occur for gonorrhea to be transmitted or acquired. Gonorrhea can also be spread from mother to baby during delivery.

Although many men with gonorrhea may have no symptoms at all, some have some signs or symptoms that appear two to five days after infection; symptoms can take as long as 30 days to appear. Symptoms and signs include a burning sensation when urinating, or a white, yellow, or green discharge from the penis. Sometimes men with gonorrhea get painful or swollen testicles. In women, the symptoms of gonorrhea are often mild, but most women who are infected have no symptoms. Even when a woman has symptoms, they can be so non-specific as to be mistaken for a bladder or vaginal infection. The initial symptoms and signs include a painful or burning sensation when urinating, increased vaginal discharge, or vaginal bleeding between periods. Women with gonorrhea are at risk of developing serious complications from the infection, regardless of the presence or severity of symptoms. Symptoms of rectal infection may include discharge, anal itching, soreness, bleeding, or painful bowel movements.

Untreated gonorrhea can cause serious and permanent health problems in both women and men. Gonorrhea can spread to the blood or joints. This condition can be life threatening. Among women, gonorrhea is a major cause of PID, which can lead to chronic pelvic pain, ectopic pregnancy, and infertility. In men, untreated gonorrhea can cause epididymitis, a painful condition of the testicles that can result in infertility.

In addition, studies suggest that the presence of gonorrhea infection makes an individual three to five times more likely to acquire HIV, if exposed.

Test Numbers - GC and CT

6970 & 6971

Test performed daily, Monday thru Friday
Chlamydia trachomatis remains the most commonly reported infectious disease in the US. This sexually transmitted disease (STD) is caused by the bacterium, Chlamydia trachomatis.

In 2005, 976,445 chlamydia diagnoses were reported, up from 929,462 in 2004. Even so, most chlamydia cases go undiagnosed. It is estimated that there are approximately 2.8 million new cases of chlamydia in the US each year. Chlamydia is known as a “silent” disease because about 3/4 of infected women and about 1/2 of infected men have no symptoms. If symptoms do occur, they usually appear within 1 to 3 weeks after exposure.

In women, the bacteria initially infect the cervix and the urethra. Women who have symptoms might have an abnormal vaginal discharge or a burning sensation when urinating. When the infection spreads from the cervix to the fallopian tubes, some women still have no signs or symptoms; others have lower abdominal pain, low back pain, nausea, fever, pain during intercourse, or bleeding between periods.

Complications among men are rare. Men with signs or symptoms might have penile discharge or a burning sensation when urinating or a burning and itching at the opening of the penis.

In women, untreated infection can spread into the uterus or fallopian tubes and cause pelvic inflammatory disease (PID). This happens in up to 40% of women with untreated chlamydia. PID can cause permanent damage to the fallopian tubes, uterus, and surrounding tissues. The damage can lead to chronic pelvic pain, infertility, and potentially ectopic pregnancy.

Clinical Background

GC and CT Diagnostic Recommendations

CDC recommends that primary care clinicians routinely screen all women, pregnant or not, if they:
- Are sexually active and age 25 or younger.
- Have more than 1 sexual partner at any age.
- Have had an STD in the past, regardless of age.
- Do not use condoms consistently & correctly, regardless of age.
- Pregnant women with risk factors & those < 25

Specimen Requirements

Collection for both GC & CT testing:

Endocervical, vaginal & urethral swabs in Aptima transport tube:

Endocervical: Remove excess mucus from cervical os using the white swab. Discard this swab. Next, Insert the blue specimen collection swab into endocervical canal. Rotate swab clockwise for 15 sec. for adequate sampling. Withdraw swab carefully to avoid any contact with vaginal mucosa.

Male Urethra specimens
Patient should not have urinated for at least 1 hour prior to collection. Insert collection swab 2-4 cm into urethra. Rotate swab clockwise for 3 sec. for adequate sampling. Withdraw swab carefully. Immediately place specimen collection swab into the aptima transport tube. Break swab at scoreline and recap aptima transport tube tightly.

Urine Aptima transport tube:
Patient should not have urinated for at least 1 hour prior to collection. Instruct patient to collect the first 10-20 ml of voided urine in a sterile, leakproof container. Urine samples must be transferred into the APTIMA urine transport tube within 24 hrs of collection. Fill sample between the black lines on the sample tube. Take care not to overfill or underfill.

Alternate specimens: Liquid PAP vials from ThinPrep or SurePath vials a culturette swab, M4 transport media swab, Genprobe PACE swab or BD (Viper) NAT.
**MRSA SCREENING**

**Clinical Background**

The PCR MRSA test is a qualitative in vitro diagnostic test for the direct detection of nasal colonization by methicillin-(oxacillin) resistant Staphylococcus aureus (MRSA) to aid in the prevention and control of MRSA infections in healthcare settings.

Staphylococcus aureus (S. aureus) has long been recognized as one of the most important bacteria that cause disease. Although most staph infections are minor, serious infections such as bloodstream, surgical wound, bone, joint infections or pneumonia can occur. It is the leading cause of skin and soft tissue infections. S. aureus is a part of the normal flora and humans are most often colonized with S. aureus in their noses, on the skin and other body sites. 30% of the population is colonized in the nose with S. aureus (MRSA and MSSA present, without infection). Of these 30% S. aureus colonization in the nose, 50% are MRSA.

S. aureus is most often spread to others by contaminated hands. Other factors, that are associated with the spread of MRSA skin infections, include skin-to-skin contact, openings in the skin such as cuts, contaminated items and surfaces, crowded living conditions, and poor hygiene. In the past, most serious S. aureus infections were treated with the beta-lactam antibiotics. Treatment of these infections has become more difficult because of the MRSA strains that are now resistant to commonly prescribed β-lactam agents, including cephalosporins and carbapenems.

The MRSA DNA tests have the same sensitivity, same turnaround time and 88% specificity when compared to culture. Currently, we do recommend ordering nasal culture for MRSA instead of this test.

This test is not intended to diagnose MRSA infections nor to guide or monitor treatment for MRSA infections. MRSA outbreak can occur when one strain is transmitted to other patients or close contacts of the infected persons in the community. Handwashing and screening patients for MRSA should be performed to decrease transmission and reduce the number of patients infected with MRSA.

**Specimen Requirements**

**Nasal swab collection:** Carefully insert the swab into the nostril, with swab tip about 2.5 cm from the edge of the nares. Roll the swab 5 times. Insert the same swab into the 2nd nostril and repeat steps 2 and 3. Place the swab in its container.

Label with patient name, site and source.

**Test Result Interpretation**

Staphylococcus aureus resistance to methicillin occurs when an isolate carries an altered penicillin-binding protein, PBP2a, which is encoded by the mecA gene. This test does not detect the mecA gene. It detects the Staphylococcal Cassette Chromosome mec(SCCmec). This leads to 12% false positive when compared to culture.
Evidence shows that keeping the viral load levels as low as possible for as long as possible decreases the complications of HIV disease and prolongs life. Public health guidelines state that treatment should be considered for asymptomatic HIV-infected people who have viral loads more than 55,000 copies using an RT-PCR test. The risk of HIV infection developing into AIDS depends on the CD4+ cell count and the viral load (how much virus is found in the blood).

**Diagnostic Recommendations**

**HIV-1 RNA Quant Sensitive V-Load**

This test is not intended for primary detection of HIV infections. It is intended to monitor known HIV positive infections. National guidelines state that the goal of therapy is undetectable viral load. It is used to monitor HIV-1 disease progression and to determine viral loads in patients before initiating anti-HIV-1 therapy or to detect drug resistance, and noncompliance with therapy, or to monitor for evidence of HIV-1 resistance during drug therapy. The range of this assay is from 400 to 1,000,000 copies/ml.

**HIV-1 PCR viral load Ultra-sensitive**

This test is used to monitor HIV-1 disease progression and to determine viral loads in patients who have initiated and are compliant with highly active anti-retroviral therapy (HAART). It may also be used to detect HIV-1 RNA after infection but before seroconversion. The range of this assay is 50 to 100,000 copies/ml in plasma.

**Specimen Requirements**

EDTA whole blood is preferred but ACD specimens are also acceptable. Centrifuge tubes and separate within 4 hours of collection. Split into 2 plastic vials and freeze the plasma (EDTA or ACD) to ensure accuracy.

**Test Result Interpretation**

**8001 HIV-1 RNA Quant Sensitive V-Load**

Turnaround time is 2 to 7 days

Positive example:

| HIV-1 RNA (ultraSens) | 321,000 H copies/ml |
| HIV-1 RNA (log 10) | 5.5 H |

Viral load tests are reported as the number of HIV copies in a milliliter of blood. If the viral load measurement is high, it indicates that HIV is reproducing and that the disease will likely progress faster than if the viral load is low. A high viral load can be anywhere from 5,000 to 10,000 copies and can range as high as 1 million or more. A low viral load is usually between 200 to 500 copies. This result indicates that HIV is not actively reproducing and that the risk of disease progression is low.

< 50 IU/ML = is reported as undetectable

A viral load result that reads “undetectable” means that the level of HIV virus in the blood is below the threshold needed for detection. Change in viral load is also a very important measurement. A rising count indicates an infection that is getting worse, while a falling count indicates improvement and suppression of the HIV infection.
Hepatitis C virus (HCV) infection is the most common chronic bloodborne infection in the U. S.; approximately 2.7 million persons are chronically infected. HCV infection can lead to cirrhosis, hepatocellular carcinoma (HCC), and ultimately liver transplantation. Monitoring of HCV viral load, expressed in IU/ml, predicts the level of responsiveness to treatment in patients receiving ribavirin and pegylated interferon.

Although HCV is not efficiently transmitted sexually, persons at risk for infection through injection-drug use might seek care in STD treatment facilities, HIV counseling and testing facilities, correctional facilities, drug treatment facilities, and other public health settings where STD and HIV prevention and control services are available.

Persons newly infected with HCV typically are either asymptomatic or have a mild clinical illness. HCV RNA can be detected in blood within 1–3 weeks after exposure to the virus and weeks before the onset of alanine aminotransferase (ALT) elevations or the appearance of anti-HCV. The average time from exposure to antibody to HCV (anti-HCV) seroconversion is 8–9 weeks, and anti-HCV can be detected in >97% of persons by 6 months after exposure. Chronic HCV infection develops in 60%–85% of HCV-infected persons; 60%–70% of chronically infected persons have evidence of active liver disease. The majority of infected persons might not be aware of their infection because they are not clinically ill. However, infected persons serve as a source of transmission to others and are at risk for Chronic Liver Disease or other HCV-related chronic diseases for decades after infection.

HCV is most efficiently transmitted through large or repeated percutaneous exposure to infected blood (e.g., through transfusion of blood from unscreened donors or through use of injecting drugs), although less efficient, occupational, perinatal, and sexual exposures also can result in transmission of HCV.

Test Numbers & Ordering Info for HCV

8218- HCV Quantitative V-Load
8062- HCV Qualitative

Turnaround time is 1 to 5 days

Diagnostic Recommendations

TaqMan real-time PCR assay, performed at Sparrow Regional Laboratories, is an excellent tool for qualitatively detecting HCV RNA because of low sensitivity, 10 IU/ml, and therapeutically monitoring HCV RNA because of its broad measurable range and is linear up to 22 million IU/ml

HCV Quantitative V-Load:

Sparrow Regional Laboratories Hep C virus (HCV) viral load quantitative measures the number of viral RNA particles in blood. Viral load tests are often used before and during treatment to help determine response to treatment by comparing the amount of virus before and after treatment.

Continued on page 15
HCV Test Result Interpretation

Diagnostic Recommendations

Quantitative measurement of HCV RNA viral load is based on the reverse transcription of HCV genomic RNA followed by real-time PCR amplification and detection in the presence of a quantitative standard. This test quantitates viral loads above 50 IU/mL.

HCV Qualitative:

HCV qualitative test identifies whether the virus in the blood, indicating an active infection with HCV and diagnoses HCV infection.

The qualitative HCV assay may be used to confirm a positive HCV Antibody (Test # 1400, EIA method) or to diagnose HCV in patients who are immunocompromised.

Specimen Requirements- Quantitative & Qualitative

Lavender top EDTA, 5.0ML (Min. Vol: 4 EDTA (LAV) rockets)
Processing: Aseptically spin down, remove plasma from cells within 4 hours.
Split plasma into 2 screw-capped vials, and freeze.

Test Result Interpretation

Results between 10 to 50 IU/mL will be reported as Detected. Detected; viral load is between 10 and 50 IU/mL. Samples giving a viral load result above 50 IU/ml are reported quantitatively.

Assay methodology is real-time polymerase chain reaction using the COBAS TaqMan® HCV ASR. The analytic measurement range of this assay is 50 - 22,000,000 IU/mL.

HCV Qualitative

Test Result Interpretation

<10 IU/ML (Not Detected)
Deep vein thrombosis (DVT) is a common cause of morbidity and mortality. In hospitalized patients, DVT results in fatal pulmonary embolism in up to 0.8% of general surgical patients. Acquired disorders such as vascular disease, obesity, trauma, infection, use of oral contraceptives, pregnancy and labor, nephritic syndrome, myeloproliferative syndromes and immobilization following surgery are the most common causes of DVT. Deficiencies in one of the main inhibitors of the clotting system are seen in families with a hereditary tendency to form venous thrombosis. Individuals with hereditary abnormalities tend to suffer from their first hypercoagulable event at an early age (<40 years) and experience multiple thrombotic episodes. Patients with this polymorphism have a substitution of the arginine (R) at position 506 by glutamine (Q). Because of the mutant factor V cannot be cleaved and inactivated by activated protein C, carriers of this mutation are at significantly increased risk of venous thrombosis. The Factor V Leiden mutation is carried in heterozygous form by about 5 to 15% of the caucasian population; it is rarer in Hispanic-Americans, rarer still in African-Americans and Asian-Americans, and virtually absent in Africans and Asians. Homozygous carriers of this mutation, have an 80 – 100 fold increased risk. Other genetic disorders causing deficiency of antithrombin, protein C or Protein S, or hyperhomocsteinemia are independently associated with Venous thromboembolism. Interaction of these genetic disorders with the Factor V Leiden allele markedly increases the risk for Venous thromboembolism. The Venous thromboembolism risk is increased among women with are heterozygous Factor V Leiden carriers receiving oral contraceptives, during pregnancy or post-partum period.

### Table 1  Factor V Leiden & Risk of Deep Vein Thrombosis

<table>
<thead>
<tr>
<th>Condition or contributing Factor</th>
<th>Relative Risk</th>
<th>Mean Onset of Thrombosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterozygous for FV R506Q</td>
<td>7.90%</td>
<td>44 years (17 - 69 years)</td>
</tr>
<tr>
<td>Homozygous for FV R506Q</td>
<td>91%</td>
<td>31 years (22 - 55 years)</td>
</tr>
<tr>
<td>Women using oral contraceptives</td>
<td>3.80%</td>
<td></td>
</tr>
<tr>
<td>Women using oral contraceptives who are Heterozygous (FV R506Q)</td>
<td>35%</td>
<td></td>
</tr>
</tbody>
</table>

### Test No. & Ordering Info

**6784**

### Specimen Requirements

5 ml EDTA or 10 ml ACD whole blood plus a 3.0 ml or 1.8 ml Blue top tube properly filled. Draw a separate tube if another coagulation test is ordered.

### Test Result Interpretation

Currently, Factor V Leiden requests are automatically screened by testing for Activated Protein C Resistance (APCR). A Negative APCR test results in cancellation of the Factor V Leiden test. APCR screening for Factor V Mutation will be replaced by direct genetic testing for the mutation due to poor PPV of the APC for Factor V Leiden. The APCR test will still be offered to test for abnormalities other than Factor V Leiden. Results are reported as **Present** or **Absent**. Patient is heterozygous for the Factor V Leiden mutation, Patient is homozygous for Factor V Leiden mutation.
The Prothrombin G20210A mutation is the substitution of guanine with adenine at nucleotide 20210. The Prothrombin mutation is associated with about a 3-fold increased risk of venous thromboembolism (VTE) because of increased plasma prothrombin activity among carriers. Interaction of other genetic disorders with the Prothrombin mutation markedly increases the risk for VTE.

Women Prothrombin mutation carriers who receive oral contraceptive therapy also are at increased risk for venous thromboembolism. Recurrent venous thromboembolism is increased 2-3 fold among Prothrombin mutation carriers, especially among carriers of both the Prothrombin mutation and Factor V Leiden mutation.

Prothrombin mutation G20210A gives rise to slowed mRNA degradation and to an increase in circulating prothrombin levels. This appears to create a hypercoagulable state.

The mutation is inherited in an autosomal dominant manner. Someone with the mutation, has a 50% chance that each of their sibs and children also received the mutation. Testing for prothrombin mutation G20210A is therefore useful in determining a person’s predisposition to thrombosis and can assist in determining the need for anticoagulant therapy.

Prothrombin mutations have also been linked with thrombotic events other than DVT, including recurrent miscarriage and interactions with other risk factors for thrombosis, including pregnancy, the use of oral contraceptives, and immobilization.

**Clinical Background**

**Diagnostic Recommendations**

The recommendations concerning the criteria for testing for the prothrombin G20210A mutation include testing in the initial evaluation of suspected inherited thrombophilia. The recommendations for testing for the prothrombin mutation parallel those for the factor V Leiden mutation and include patients with a history of recurrent VTE, a first episode of VTE before the age of 50 years, a history of an unprovoked VTE at any age, thromboses in unusual anatomic sites, or an affected first-degree relative with VTE. A history of VTE related to pregnancy or estrogen use and unexplained pregnancy loss during the second or third trimesters were also considered to be indications for testing.

**Test No. & Ordering Info**

6656

**Specimen Requirements**

5 ml EDTA or 10 ml ACD whole blood- keep refrigerated. Draw a separate tube if another coagulation test is ordered.
Enteroviruses (EVs) are single-stranded RNA viruses and are members of the Picornaviridae family. The enterovirus group includes polioviruses, coxsackieviruses, echoviruses and other enteroviruses. EVs cause an estimated 10 to 30 million infections annually in the United States. These infections, which peak July through October, most commonly affect young children, in whom the severity of infection is dependent on age. The EVs are an interesting group because of the broad range of diseases they cause. Common syndromes include nonspecific febrile illness with or without rash, acute hemorrhagic conjunctivitis, hand-foot-and-mouth disease, sepsis syndrome in newborns, myocarditis, hepatitis, and a variety of central nervous system infections. The latter syndromes are the most troublesome in terms of their confusion with infections with other viral and bacterial pathogens, which may lead to unnecessary treatments and diagnostic tests. J Clin Microbiol. 1998 November; 36(11): 3408–3409.

In the U.S., enteroviral infections occur primarily during the warm months of summer through early fall. Many of these infections are mild or asymptomatic. However, enteroviruses are responsible for a wide spectrum of acute diseases including nonspecific febrile illness, neonatal sepsis, aseptic meningitis, myocarditis, and conjunctivitis. Enteroviruses are the most common cause of meningitis in the U.S., with an estimated 50,000 to 75,000 cases each year.

**Clinical Background**

**Diagnostic Recommendations**

**Specimen Requirements**

Throat Swabs: The preferred specimen is a swab with a rayon tip, plastic shaft placed in viral transport. The swabs may be transported to the laboratory at room temperature. The throat swab is placed in viral transport medium. For longer storage, immediately freeze specimen on dry ice.

Specimens with evidence of potentially interfering substances (e.g., blood, excessive nasal secretions, etc.) should be tested within 24-36 hours. Otherwise, specimens can be stored up to 5 days at 2-8°C before testing. Specimens that can be tested within 36 hours can be kept at room temperature (15-30°C).

Note: Alginate swabs have been found to inhibit PCR and cannot be accepted.

Stool: Collect at least 1 gram random stool in a plastic, sterile container. Place 1 gram of stool in viral transport medium. Freeze immediately and ship frozen on dry ice.

CSF: 1.0 mL (min 0.5ml) of spinal fluid. Send specimen refrigerated in a screw-capped, sterile vial. Maintain sterility and forward promptly. Freeze specimen if test is not performed immediately.

**Test No. & Ordering Info**

8134

Testing available every Summer thru October or the end of the season
Roche partnership expands diagnosis, treatment capabilities

Lansing, MI — Sparrow Health System’s Department of Laboratories unveiled a new, state-of-the-art molecular laboratory on the St. Lawrence campus that greatly enhances the ability of physicians to diagnose and treat infectious diseases, hereditary disorders and cancers, leading to better care for patients.

Sparrow’s Department of Laboratories alliance with world-renowned diagnostic company Roche Diagnostics establishes Sparrow as a Molecular Center of Excellence. Sparrow joins a national network of laboratories working together to develop advanced molecular testing that can literally change the way diseases are discovered and treated.

“We are very excited to partner with Roche Diagnostics, a leader in the diagnostics industry,” said Walid T. Khalife, Ph.D., Sparrow’s technical director of microbiology. “With the combination of Roche’s state-of-the-art technologies and our expertise in clinical molecular diagnosis, we will be able to offer the most advanced diagnostic testing to assist Sparrow clinicians and ensure Sparrow patients receive the best care possible.”

As a Roche-designated Center of Excellence, one of only 32 nationwide, Sparrow’s Molecular Laboratory will offer physicians and patients the most advanced molecular diagnostics technologies such as Roche Diagnostics’ patented Polymerase Chain Reaction (PCR).

PCR is a Nobel Prize-winning technology recognized as one of the most important scientific developments of our generation. PCR allows scientists to copy a single segment of DNA billions of times, making it possible to take a specimen weighing only one trillionth of a gram and generate a test sample sufficient to perform a detailed analysis of a specific genetic question. Molecular laboratory technology can more accurately diagnose specific viral and bacterial pathogens, such as HIV or Hepatitis, determine their amount in the body, and assist physicians in prescribing more effective treatments. New technologies also can determine how aggressive a breast cancer tumor is likely to be, or screen for human papillomavirus, the causative agent for virtually all cervical cancer.

“This alliance establishes Sparrow as a leader in molecular diagnostics both in the menu of tests offered, and in the access to the latest breakthroughs,” Khalife said.

“Our new capabilities in molecular technology provide the medical community and the people of mid-Michigan with the very best modern medicine has to offer.”

Sparrow’s Department of Laboratories performs nearly 5 million medical laboratory procedures annually, serving clinicians and patients throughout the mid-Michigan region.