Case Presentation
A 28-year-old male presented to a dermatologist with fever and a one week history of a rash. The rash was first noted on his face but it later moved to his trunk and extremities. The patient described the lesions as red, mildly tender and occasionally itchy.

The patient was employed at a computer retail store as a salesman and also related recent weight loss and night sweats. He had no other positive findings and an unremarkable previous medical history.

The patient reported vacationing in some of the Caribbean Islands about two months previous to presentation. During that trip he had multiple male sexual partners, although previously he had been in only three stable monogamous relationships with other men.

Physical exam showed a rash comprised of macules, papules and some pustules covering his affected body. His temperature was 38.5 °C and a CBC was unremarkable.

A serum RPR was reactive with a very high titer and a treponemal test was also positive. An HIV ELISA screen was positive and confirmed by Western Blot.

The patient was treated initially with penicillin and a referral to an infectious disease specialist was obtained.

INTRODUCTION
The diverse clinical manifestations of the two patients (presented in the Case Presentation above and in the 2010 CM-B Body Fluid Identification Challenge) illustrate the complexity of this venereal disease. This education activity will emphasize the importance of clinical suspicion in establishing the diagnosis of syphilis and review the available and most commonly used laboratory tests to support or exclude the diagnosis of syphilis. An overview of the different clinical stages with emphasis on neurosyphilis will also be presented.

According to some, syphilis was introduced to Europe in the 15th century most likely by returning explorers from the Caribbean and American continent. However, others believe that the disease existed in Europe but was unrecognized. Syphilis quickly spread thereafter and imposed a respectable degree of suffering to humankind. Syphilis has a variety of different clinical presentations and may mimic other infections and disorders and consequently earned the labels of “the great impostor”, “the great mimicker”, and “the great imitator”, among many others.

One of the most revered and famous clinicians Sir William Osler, who died in 1919, said “The physician who knows syphilis knows medicine”.

1 - Education
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ORGANISM

In 1905 Fritz Schaudinn and Erich Hoffmann identified the causative agent of syphilis. The etiologic organism is a spirochete Treponema pallidum, subspecies pallidum and a member of the Spirochaetales family. This organism is distinct from the subspecies causing yaws (T. pallium subspecies pertenue) and bejel (T. pallidum subspecies endemicum). The organism that causes pinta (T. crateum) is closely related but is currently considered a separate species. Yaws, pinta and bejel are nonsexual transmitted infections that mainly occur in tropics and subtropics and affect the skin, cartilage and bone. Molecular signatures by polymerase chain reaction (PCR) can be used to differentiate these T. pallidum subspecies.

The Spirochaetales order also includes Leptospira species and Borrelia species, including B. burgdorferi, the organism responsible for Lyme disease.

T. pallidum measures 6-20 µm has characteristic corkscrew motility and is easily killed by heat, cold, desiccation and most disinfectants. The organism can be visualized by silver stains (Warthin-Starry stain), dark field examination and immunofluorescence techniques. The organism cannot be cultured in the clinical laboratory and there are no good animal models for syphilis.

EPIDEMIOLOGY

Syphilis is a public health problem worldwide. There are about 12 million new cases of syphilis each year, with more than 90% occurring in developing nations.

Transmission is by direct contact with active lesions and primarily through sexual contact, with man being the only natural host for the Treponema pallidum.

On average there is a 30-50% probability of transmission among sexual partners but it is higher among men having sex with men (MSM). Vertical transmission across the placenta is the second most common mode of infection and is a reminder of the importance of appropriate laboratory testing as part of prenatal care for pregnant women to protect the unborn child.

Although transmission can occur by parenteral routes like blood transfusion and accidental needle sticks, this is extremely rare as the organism does not survive the refrigerated conditions of blood storage.

The incidence of syphilis in the United States significantly declined after the availability of penicillin for treatment starting in the late 1940s. Efforts to reduce the incidence of syphilis culminated in the year 2000, with an all time low incidence of 2.2 per 100,000 individuals. Unfortunately, a significant increase in cases was noted thereafter. In the United States for the year 2006, the CDC tabulated over 36,000 cases of syphilis including 9,756 cases of primary and secondary syphilis (P&S). This represented an increase of 11.8% as compared to the previous year. The incidence of primary and secondary syphilis was highest in women 20 to 24 years of age and in men 35 to 39 years of age.
For the year 2008, the incidence of syphilis was tabulated by the CDC as 4.5 per 100,000 individuals, with a total number of cases over 46,000, showing a significant increase in a two year period. The majority of the new P&S cases of syphilis reported in 2006 and 2008 were among MSM. In 2008, there were 13,500 of P&S syphilis reported to the CDC.

Congenital syphilis is also on the rise in the United States. In 2006, a total of 372 cases of congenital syphilis were reported to the CDC, but in 2008 the number of reported cases increased to 431.

**PATHOGENESIS**

Syphilis can be divided into four stages including *primary, secondary, latent and tertiary*. Each one has distinct clinical and pathologic manifestations.

**Primary**

Syphilis is most commonly transmitted from person to person through direct contact with a primary syphilis sore or lesion, referred to as a chancre. The chancre is a painless hard, eroded papule that occurs at the point of spirochete inoculation. It usually manifests 2-3 weeks after exposure. Chancre are mainly present in male and female genitalia, as well as anal and rectal areas. They can also be present in the mouth and lips.

Genital ulcers and ulcers in general may be caused by a variety of infectious agents and conditions. Herpes simplex virus (HSV) is probably the most common agent responsible for genital ulcers.

*Treponema pallidum* may penetrate an intact mucous membrane or may gain access to tissue, usually through abraded skin. It multiplies at the inoculation site before entering the lymphatic and circulatory system allowing for systemic spread.

Clinically identifiable lesions appear when a critical number of organisms are reached and consequently there is variability in the incubation period with a range of 3 days to 3 months from infection and a median of 3 weeks. A primary lesion does not develop in all patients and as it is painless, it may not be noticed by the patient. The chancre lasts from 3 to 6 weeks and will heal without treatment. Local adenopathy is often present. In the absence of treatment, the infection progresses to the secondary stage.

**Secondary**

The healing of the chancre lesion landmarks the initiation of the secondary stage, which is characterized by dissemination of the organism via the bloodstream and the development of mucocutaenous lesions and organ involvement as well as the presence of constitutional symptoms. This usually occurs 6-12 weeks after initial inoculation of the organism.
Skin rash and mucous membrane lesions are characteristic for this stage. Patients develop a rash that begins on the trunk and extremities. The rash characteristically appears as small macules that progress to papules and in some patients to pustules over a period of weeks. The typical rash appears as rough, red or reddish brown spots in the trunk and extremities and characteristically on the palms of the hands and the bottoms of the feet. The rash is usually non-pruritic and symmetric. The rash of secondary syphilis also can be pustular, annular, or follicular.

When the mucocutaneous lesions coalesce in the genital area, they produce the pale plaques referred to as condyloma lata. Patients also have generalized lymphadenopathy.

Non specific symptoms may include fever, sore throat, patchy hair loss, headaches, weight loss, muscle pain, and fatigue.

Secondary syphilis is a systemic process and can cause neurologic, renal, ophthalmic, gastrointestinal and hepatic disease.

Signs and symptoms of secondary syphilis will resolve with or without treatment but in the absence of treatment, the infection will progress to the latent and possibly tertiary stages of the disease.

Latent
During the latent stage, the patient shows no manifestations of infection. This stage can only be diagnosed by positive laboratory tests. The first four years of this period are termed the early latent period and relapses of secondary syphilis may occur, usually during the first year. This is followed by the late latent period, during which relapses usually do not occur.

Tertiary
Anywhere from 5-25 years after the primary stage, one-third of untreated patients develop tertiary syphilis, with the most common manifestations occurring in the cardiovascular system, the central nervous system (CNS), and the so-called benign tertiary syphilis. These clinical expressions may occur alone or in combination.

Cardiovascular syphilis is characterized by an inflammation of the aorta, which leads to a dilation that causes aortic valve insufficiency and aneurysms of the proximal aorta.
CNS syphilis involvement is referred to as neurosyphilis. It may be symptomatic or asymptomatic. This will be further discussed below.

Benign tertiary syphilis is characterized by granuloma-like nodular lesions in tissues that probably develop as a consequence of delayed hypersensitivity to the spirochete and are called gummas. Due to the widespread use of antibiotics, not only for syphilis treatment but for treatment of multiple conditions, these lesions are now rarely seen and when present they are usually in patients with HIV infection. On histologic examination the gummas have centers of coagulated, necrotic material with margins made of palisading macrophages, fibroblasts and a large number of mononuclear inflammatory cells that are predominantly plasma cells. Treponemes are rarely visualized in these lesions.

**LABORATORY TESTING**

The diagnosis of syphilis is made and / or confirmed by laboratory testing.

Since the organism cannot be cultured, the traditional laboratory methodologies have included visualization of the spirochete and serology. Serologic testing can be divided between nontreponemal, non specific antibody testing (also known as tests for reagin antibodies) and more specific treponemal-based antibody tests.

**Test Targeted to the Direct Identification of Spirochetes**

Dark-field microscopy exam is of use only if a chancre or condyloma lata lesions are present. It is one of the oldest, quickest and most direct methods for diagnosing primary and secondary syphilis. It is based on the direct visualization of the organism from moist mucous membrane lesions. Recognition of the thin, delicate, corkscrew-shaped organisms and their characteristic mobility requires the availability of the proper equipment and an experienced examiner. Most laboratories and clinical practices in the United States currently do not offer or use this test. Because of the lack of sensitivity, a negative dark-field microscopic exam does not exclude the diagnosis of syphilis.

In lieu of dark-field microscopy some laboratories offer direct fluorescent antibody testing. This also has the advantage of allowing the identification of the organism when smears cannot be examined immediately. Again, a negative test does not exclude the diagnosis of syphilis.

**Serologic Testing**

*Nontreponemal Tests*

The first available nontreponemal antibody test was described by Wasserman in 1906 and was based on a complement fixation methodology. It is no longer in use.

The currently used nontreponemal tests are rapid plasma reagin (RPR), Venereal Disease Research Laboratory (VDRL) and the automated reagin test, which is as the name suggests an automated adaptation.
of nontreponemal tests. These tests measure antibodies against a cardiolipin-lecithin-cholesterol antigen. They are inexpensive, rapid, and highly sensitive except in the early infection stage. However, they are non specific and generate a large amount of false positive results when they are used for screening populations. Biologic false positive results occur in a variety of inflammatory and infectious conditions. Therefore, a positive nontreponemal test is traditionally followed by a treponemal assay for confirmation.

Nontreponemal tests become positive within weeks to months after infection. A positive result is reported as a titer and titers rise with dissemination of the infection, especially during the secondary stage. Titers tend to decrease over time even without treatment but successful therapy accelerates the timing of antibody decline. The reactivity of these nontreponemal tests may progressively decrease over decades and become negative in some patients.

Nontreponemal tests are used not only for screening but also have a critical role in the monitoring of response to treatment. Physicians should use the same type of test and preferentially the same laboratory for monitoring response to treatment.

A fourfold decline in titer, equivalent to a change of two dilutions is usually considered a significant change reflective of a response to treatment. (Example 1:16 to 1:4 or 1:32 to 1:8). Failure to respond to treatment should only be considered when the titer rises, as slow decline is not sufficient in the absence of clinical evidence.

Nontreponemal tests have high sensitivity (Table 1. on the following page) but poor specificity as they detect antibodies that react with a lipoprotein reagent. This antibody is also known as reagin (different from the IgE related antibody against allergens), an antibody that forms against cardiolipin, a component of most cell membranes. The antibodies detected by RPR and VDRL usually develop 1-4 weeks after the appearance of the chancre. Consequently, sensitivity of the test is related to the time of patient testing, and very early in the course of infection the test may be negative.

The VDRL test is qualitative and quantitative and utilizes serum as the specimen but can also be performed on the cerebrospinal fluid (CSF). The test is simple but requires an experienced technologist as the reagent is prepared daily following a standardized protocol. The procedure requires heating of the serum to inactivate complement. The test method is flocculation, a specific type of precipitation of the antigen that is read microscopically. In situations of antibody excess, a nonreactive pattern that is granular or rough in appearance may be seen. This is a prozone phenomenon caused by the presence of antibody excess that should be recognized by laboratories. If not, a false negative result may be reported if the specimen is not appropriately diluted.
The RPR is a modified VDRL using visual agglutination as the endpoint reading. The antigen is bound to charcoal particles and the antigen reagent includes a modification to inactivate complement so there is no need to heat the patient’s serum. Because this test is easier to perform, it is the most popular nontreponemal test used. However, it is not used for CSF specimens. Positive results are also reported as a titer.

Table 1. Sensitivity of Nontreponemal Tests

<table>
<thead>
<tr>
<th></th>
<th>Primary(%)</th>
<th>Secondary(%)</th>
<th>Latent(%)</th>
<th>Late/Tertiary(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VDRL</td>
<td>78 (74-87)</td>
<td>100</td>
<td>95 (88-100)</td>
<td>71 (37-94)</td>
</tr>
<tr>
<td>RPR</td>
<td>86 (77-100)</td>
<td>100</td>
<td>98 (95-100)</td>
<td>73</td>
</tr>
</tbody>
</table>

Numbers in parentheses are ranges of sensitivity. Adapted from LA Sala, PR, Smith M. Henry’s Clinical Diagnosis and Management by Laboratory Methods. 21st ed. Philadelphia, PA: 2007.

Treponemal Tests

These tests detect antibody directed to T. pallidum and/or treponemal-related antigens. The most commonly used include the fluorescent treponemal antibody absorption (FTA-ABS) and agglutination tests like microhemagglutination test for antibodies to Treponema pallidum (MHA-TP), the Treponema pallidum particle agglutination assay (TP-PA) and the Treponema pallidum enzyme immunoassay (TP-EIA).

FTA-ABS is an indirect fluorescent antibody test and consequently requires a specific infrastructure and skill. Specially manufactured slides and specific protocols are followed as part of the execution of this test. Results are reported as intensity of fluorescence and graded as 0 to 4+. A 1+ positive result usually requires a reset or a new specimen as it may represent a false positive. This test is highly sensitive and specific but is very labor intensive.

As part of the treponemal-based tests there have been a variety of passive hemagglutination-based tests. Microhemagglutination tests for antibodies to T. pallidum (MHA-TP) were used by laboratories for the same confirmatory approach as the FTA-ABS and/or as next line testing for inconclusive FTA-ABS. However, MHA-TP is not used for CSF specimens. Table 2. on the following page lists the sensitivities of treponemal tests.

Many of the microagglutination tests have been replaced with TP-PA. In this test, patient serum or plasma is diluted in microtiter plates and incubated with T. pallidum-sensitized gel particles along with unsensitized gel particles as a control. Particles agglutinate in the presence of T. pallidum antibodies.
Table 2. Sensitivities of Treponemal Tests

<table>
<thead>
<tr>
<th></th>
<th>Primary(%)</th>
<th>Secondary(%)</th>
<th>Latent(%)</th>
<th>Late/Tertiary(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FTA-ABS</td>
<td>84 (70-100)</td>
<td>100</td>
<td>100</td>
<td>96</td>
</tr>
<tr>
<td>MHA-TP</td>
<td>76 (69-90)</td>
<td>100</td>
<td>97 (97-100)</td>
<td>94</td>
</tr>
</tbody>
</table>

Numbers in parentheses are ranges of sensitivity. Adapted from LA Sala, PR, Smith M. Henry’s Clinical Diagnosis and Management by Laboratory Methods. 21st ed. Philadelphia, PA: 2007.

New Tests

Several enzyme immunoassay (EIA) tests have become available for the serodiagnosis of syphilis. Some replicate the nontreponemal test and use cardiolipin-like antigens. This approach has the advantage of handling a larger number of tests and possible automation but still has the same or higher rate of false positive tests as the VDRL and RPR.

Some EIA tests have been developed to specifically capture an IgG or IgM class of antibody against a treponemal antigen. Many vendors have provided EIA options for the diagnosis of syphilis. As expected, these new tests are proving to be very sensitive and specific and also have the advantage of a measured objective endpoint result as compared to the FTA-ABS that has a subjective microscopic endpoint reading. These tests also have the advantage of automation and consequently are a more palatable option for the screening of large populations. Nevertheless, false positives may still occur.

Availability of the new EIA-specific treponemal tests created the opportunity for laboratories to reverse the traditional approach of screening with nontreponemal tests (VDRL or RPR) and confirming with treponemal tests (FTA-ABS or TP-PA).

The new approach for testing starts with a treponemal EIA test. A negative test requires no further testing. However, if the EIA is positive it should be followed by an RPR or VDRL test. If EIA and nontreponemal tests are positive, this will be interpreted as positive evidence of syphilis. VDRL/RPR titers continue to be used for monitoring the treatment response. If the nontreponemal test is negative after a positive EIA, another treponemal test like FTA-ABS or TP-PA should be performed. In this situation a positive test will indicate the presence of late, latent or previous history of syphilis. Table 3. on the following page provides one possible approach / interpretation using the new serum EIA treponemal tests.
Table 3. Possible Test Interpretations

<table>
<thead>
<tr>
<th>IgM</th>
<th>IgG</th>
<th>RPR/VDRL</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>–</td>
<td>+</td>
<td>Acute primary syphilis</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Active or recently treated syphilis</td>
</tr>
<tr>
<td>–</td>
<td>+</td>
<td>+</td>
<td>Active or recently treated syphilis</td>
</tr>
<tr>
<td>–</td>
<td>+</td>
<td>–</td>
<td>*Past, successfully treated or latent syphilis</td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>Not done</td>
<td>*No evidence of active syphilis, or immunosuppressed, or successfully remote treated syphilis</td>
</tr>
</tbody>
</table>

*Other treponemal test may be required in some clinical circumstances. Adapted from Syphilis Testing Algorithms Using Treponemal Tests for Initial Screening – Four Laboratories, New York City, 2005-2006. MMWR. August 15, 2008.

Despite active syphilis, serologic tests may be negative in severely immunosuppressed patients, such as those with HIV.

Western blot and PCR tests on clinical specimens have also shown higher sensitivity and specificity compared to serologic testing and may be used also as a confirmatory test and/or with PCR as evidence of presence of the organism. A multiplex PCR consisting of targets for viruses, chancroid and syphilis and designed to be used in the differential diagnosis of genital ulcers has been developed but is not available in the United States.

Several rapid tests are also now available outside the United States. They are treponemal-based tests and use different methodologies. The expectation is that these tests will support the identification of syphilis when laboratory-based tests are non practical (sex workers) and/or not available (underdeveloped countries).

**NEUROSYPHILIS**

The patient presented in the 2010 CM-B Body Fluid Identification Challenge and accompanying this education activity reminds us that neurosyphilis may present as the initial manifestation of syphilis and clinical suspicion, and appropriate test ordering and interpretation are of utmost importance to establish the diagnosis.

Neurosyphilis is used as the catchall term for infections of the CNS by *Treponema pallidum*. It is important to point out at the start that involvement of the CNS can occur at any time - early or late, after initial infection. Only the late form is considered as tertiary syphilis. Most of the facts about neurosyphilis were gathered in the pre-antibiotic era. Neurosyphilis was relatively common in the pre-antibiotic period and was present in 25-35% of patients with syphilis, with the majority of the patients experiencing symptomatic and late syphilis manifestations.
It is the current understanding that invasion of the CSF by the *T. pallidum* occurs shortly after initial patient infection. It is also speculated that there may be a difference among *T. pallidum* strains for their CNS avidity of infection as spontaneous resolution without an inflammatory response and the absence of persistent infection occurs in some cases. Other patients may have transient meningitis, which is followed by spontaneous resolution.

Persistent meningitis is the result of failure to clear the organism from the CSF and results in what is described as asymptomatic neurosyphilis. However, it may evolve into symptomatic neurosyphilis. Early neurosyphilis is more frequently seen in patients with HIV, most likely related to their inability to clear the initial neuroinvasion. HIV infected persons with low CD4+ cell counts and RPR titers greater than 1:32 are also more likely to have symptomatic neurosyphilis.

**Early neurosyphilis** can be separated into the asymptomatic and symptomatic forms.

As expected, the asymptomatic form is characterized by no clinical symptoms or signs of CNS disease but patients have clinical evidence of primary or secondary syphilis.

The symptomatic early neurosyphilis form usually occurs within the first year after infection, but it can occur years later. Evidence of primary and secondary syphilis may also coexist. Patients complain of headache, confusion, nausea, vomiting and stiff neck. Visual acuity may be impaired if there is associated uveitis, vitritis, retinitis or optic neuritis. Inflammation of cranial nerves primarily optic, facial, and auditory nerves may also be present. Meningitis may cause hydrocephalus as well as arteritis of all sized vessels leading to ischemia or infarction of the brain and / or spinal cord.

The CSF abnormalities in symptomatic meningitis are usually more evident and severe than in asymptomatic meningitis, with CSF VDRL being almost always reactive.

**Late neurosyphilis** is what is considered tertiary syphilis and traditionally includes what is referred to as dementia paralytica or general paresis of the insane and tabes dorsalis.
The late classical clinical presentations of neurosyphilis have declined in the antibiotic era and they are now relatively uncommon. One of the explanations for this decline is the widespread use of antibiotics for unrelated illnesses.

General paresis is a progressive dementia that resulted in patient death in the preantibiotic era. General paresis is an acronym for Personality, Affect, Reflexes, Eye (Argyll Robertson pupil), Sensorial, Intellect, Speech clinical changes. It usually develops 10 to 25 years after the infection but it can occur as early as two years after initial infection. It is believed that in the early 1900s at least 10 percent of cases in psychiatric hospitals had syphilis as the underlying disease for the psychiatric malady. Symptoms early on may include forgetfulness, personality change and progress to clear dementia. In some patients, specific neurological deficit with abnormal physical exam may also be present.

Tabes dorsalis is a disease caused by demyelination of the posterior columns of the spinal cord and the dorsal nerve roots and ganglia. This creates sensory ataxia and bowel and bladder dysfunction. Patients characteristically have a wide-based gait. Optic atrophy is frequently associated with tabes dorsalis. The so-called Argyll-Robertson ocular pupil, is characteristically small, does not respond to light and has abnormal dilatation response.

**NEUROSYPHILIS AND THE LABORATORY**

The patient presentation in the 2010 CM-B Body Fluid Identification Challenge illustrates that testing of the CSF after proper clinical assessment is critical in establishing the diagnosis of neurosyphilis.

CSF abnormalities used for the diagnosis of neurosyphilis require contributions of more than one section of the laboratory. Necessary CSF tests include cell counts, total protein, glucose, VDRL, and FTA-ABS.

The examination of body fluids is a common procedure for clinical laboratories. CSF samples should be collected at least in three different sterile tubes. However, the number of tubes and quantity of fluid obtained as well as the type of tests ordered depends on the clinical differential diagnosis.

The processing and storing of CSF specimens is of critical importance as results may be affected if the handling deviates from standard laboratory protocol. As an axiom, body fluids and primarily CSF should be processed as quickly as possible.

CSF is an ultra-filtrate of plasma, secreted primarily by the choroid plexus and fills the space within the cerebral ventricles and the subarachnoid space. The CSF is not static and it circulates around the cerebral hemispheres and spinal cord and is reabsorbed into the venous sinuses (Figure 1. on the following page).
The total volume of CSF is replaced about 5 to 6 times in a 24 hour period. Under normal circumstances adults have a CSF total volume of 100 to 150 mL, children have a volume of 60 to 100 mL, and infants have a volume ranging from 10 to 60 mL.

The CSF protects the nervous system from abrupt changes in pressure and provides a means for the exchange of nutrients and waste for the central nervous system.

Figure 1.
CSF Specimen Collection
CSF is obtained by inserting a needle into the lumbar subarachnoid space, a procedure known as lumbar puncture. The needle is inserted between the third and fourth or fourth and fifth lumbar vertebrae. This is critical as the spinal cord usually stops near the second vertebrae and consequently prevents any spinal cord damage from the procedure. A bloody tap may result from injury to the small veins in the epidural space (Figure 2. below).

A manometer can be attached prior to fluid removal to measure the opening pressure. Changes in pressure may be related to postural changes, blood pressure, venous return, Valsalva maneuvers and alterations of cerebral blood flow. The normal opening adult pressure is 90 to 180 mmH$_2$O in the lateral decubitus position. Opening pressures above 250 mmH$_2$O are diagnostic of intracranial hypertension, which may be due to meningitis, intracranial hemorrhage or tumors. Obtaining the opening pressure is important because when the pressure is high, CSF fluid removal may be contraindicated.

CSF may also be obtained by cisternal and lateral cervical puncture or through ventricular cannulas or shunts.

Figure 2. Lumbar Puncture

CSF Laboratory Evaluation
Gross evaluation of the fluid is important. The normal CSF is clear and of a watery consistency. Abnormal findings include a cloudy, turbid, purulent, bloody or xanthochromic CSF. Turbidity or cloudiness starts to show with WBC counts over 200 cells $\mu$/L or RBC of 400 $\mu$/L. A grossly bloody fluid usually has RBC counts greater than 6,000 $\mu$/L.
Many different tests can be performed on the CSF and consequently laboratories should have protocols addressing appropriate triage of requested tests when the amount of CSF is insufficient to complete all the tests ordered. Preferentially in these situations the ordering physician should be involved in the decision.

Cell counts and differential as well as routine chemistry testing including CSF glucose and protein are almost always part of the basic CSF evaluation.

Cell counts to differentiate RBC from nucleated cells have traditionally been performed by enumeration in a hemocytometer chamber by the manual method. Many laboratories with a relatively high volume of specimens have adopted the use of automated methods to perform the body fluid counts.

The review of a cytocentrifuge slide continues to be the preferred method for nucleated cells differential. This approach provides an enriched slide in low cell counts and the capability of performing a variety of stains. Cytocentrifuge slides allow for the proper differentiation of granulocytes (mature and young neutrophils, eosinophils and others) from lymphocytes and also mononuclear cells / macrophages, as well as plasma cells and ventricular lining cells.

The review of cytocentrifuge slides also contributes to the identification of malignancy (primary CNS or metastatic tumor) in some cases. This preparation also facilitates the proper identification of unexpected cells including bone marrow cells, cartilage cells and squamous cells usually present as a result of CSF contamination by the lumbar puncture procedure.

The normal CSF nucleated cell count in adults is 0-5 µ/L and in neonates 0-30 µ/L, with decreasing values until adolescence. No RBC should be present and their identification is usually an indication of a pathologic process or a traumatic tap. Although RBC counts in themselves have no diagnostic value they are useful in extrapolation formulas to derive a corrected CSF WBC when there is a coexistent peripheral blood CBC.

The presence of an increased component of neutrophils is seen in various conditions, and in bacterial meningitis it usually exceeds 60% of the total differential. However, in early infection some viral meningitis patients may present with the same proportion of neutrophils. Consequently, the differential and nucleated cell count should be interpreted in combination. When the WBC is over 2,000 µ/L with higher than 60% neutrophils there is a very high predictive value for bacterial meningitis. The other important caveat is that not all cases of neutrophil meningitis are caused by bacteria. Other organisms such as fungi may be the etiology.

Eosinophils, although rarely present in normal CSF, may be increased in a variety of CNS conditions. Frequently they are seen in association with shunts, foreign materials and drugs as well as part of inflammatory responses.
CSF lymphocytosis is also associated with various disorders and may also be present in early acute bacterial meningitis when the WBC is under 1000/µL. However, a lymphocytosis is most commonly associated with viral, tuberculous, fungal, or syphilitic meningitis as well as degenerative / demyelinating disorders of the CNS. In these situations, a spectrum of lymphocyte morphologies including reactive lymphocytes is usually present.

Plasma cells are not normally present in the CSF, most commonly appear in a variety of inflammatory conditions, and are usually associated with a component of reactive lymphocytes. Since the presence of plasma cells is an abnormal finding, the following conditions should be included in the differential diagnosis when plasma cells are reported as present in the CSF:

- Acute viral infections
- Multiple sclerosis
- Guillain-Barré syndrome
- Syphilitic meningoencephalitis
- Parasitic CNS infections
- Tuberculous meningitis

Appropriate reporting of reactive lymphocytes and plasma cells in CSF is critical as it may be the trigger to the ordering of specific serologic testing to establish a neurosyphilis diagnosis.

The patient presented in the 2010 CM-B Body Fluid Identification Challenge is an example of this clinical scenario.

Protein and glucose CSF results should be interpreted in correlation with the serum patient values. Over 80% of the CSF protein content is derived from plasma. Elevations of total protein are nonspecific and perhaps one of the most common abnormalities found in the CSF as an indicator of meningeal or CNS disease. However, increased IgG synthesis in the CNS has been documented in disorders like multiple sclerosis, neurosyphilis, and subacute sclerosing panencephalitis.

Hypoglycorrhachia (low glucose value in CSF) is a characteristic finding of bacterial, tuberculous and fungal meningitis. However, it also may be seen in a variety of clinical conditions including acute syphilitic meningitis.

The use of a positive CSF VDRL to confirm the diagnosis of neurosyphilis is well established. However, the VDRL is not very sensitive and a significant number of patients may have neurosyphilis with a negative CSF VDRL (the patient in this education activity is an example). Under these circumstances some advocate the use of CSF FTA-ABS since it is probably more sensitive than VDRL but less specific. Table 4. on the following page shows VDRL and FTA-ABS reactions in CSF.
Table 4. CSF VDRL and FTA-ABS

- A reactive serum FTA-ABS with a non reactive CSF rules out neurosyphilis.
- A reactive CSF VDRL makes neurosyphilis likely (requires non bloody specimen).
- A reactive CSF FTA-ABS test may indicate active, asymptomatic, treated neurosyphilis or false positive reaction.

TREATMENT

Penicillin is the preferred drug of treatment for all stages of syphilis. The dose and regimen used requires specific tailoring for each patient presentation by the treating physician. Other drugs are available if there is a documented allergy to penicillin. Some patients may experience the adverse Jarisch-Herxheimer reaction after treatment is initiated. This is a transient febrile reaction that occurs within the first 24 hours. Other clinical manifestations of this reaction include chills, headache, tachypnea, tachycardia, exacerbation of skin lesions and malaise. The circulating neutrophil count increases as well. The condition resolves by itself and is treated symptomatically.

In the absence of a microbiology test of cure, patients treated for syphilis are monitored using quantitative nontreponemal tests. Patients treated for P&S syphilis will have repeat RPR /VDRL usually at 3, 6 and 12 months after treatment and in some circumstances, one after 24 months may be indicated.

All patients with documented neurosyphilis are carefully monitored, including evaluations of the CSF at 6 month intervals over the first 2 years of treatment or until the CSF becomes normal.

It is important to remember that having syphilis once does not protect a person from getting it again. Therefore, after successful treatment, individuals are still susceptible to re-infection.

The laboratory also plays a critical and very important role in these situations.

SUMMARY

We have reviewed clinical presentations and the laboratory support required to make a diagnosis of syphilis. It is fair to say that often the diagnosis of syphilis may be complicated since its clinical manifestations are diverse or may be totally absent. As the incidence of this disease continues to increase, laboratory providers should be prepared to support their clinical colleagues and in some situations raise the possibility of syphilis as part of the differential diagnosis in complicated clinical scenarios. The natural course of syphilis may be altered in HIV positive patients. About one-fourth of HIV infected patients present with simultaneous lesions of primary and secondary syphilis at the time of diagnosis. Neurosyphilis may also present more frequently, progress more rapidly and have atypical signs in the presence of HIV infection.
References

William Koss, MD, FCAP: William Koss, MD, is Director of Clinical Pathology, Scott & White Memorial Hospital and Scott & White Clinic in Temple, TX. He is CP/AP and Hematology Board Certified by the American Board of Pathology. He is the Director of the Hematopathology Fellowship at Scott & White and Texas A&M Medical School where he is a Professor of Pathology and Laboratory Medicine. Dr. Koss is also a member of the Hematology and Clinical Microscopy Resource Committee for the College of American Pathologists (CAP).
Case History: A 42-year-old male admitted through the Emergency Department because of unusual and bizarre behavior over the past 1½ months.

Laboratory data shows:
CSF Cell Count: RBC = 290 Cells/µL; WBC = 14 cells/µL
CSF Chemistries: Glucose = normal; Protein = elevated.
CBC, urinalysis and comprehensive chemistry profile were all within range.
A VDRL was positive and was confirmed by a positive Treponema pallidum antibody testing.

Further laboratory testing on this patient revealed the following:
Serum glucose and protein were normal as well as CBC, comprehensive chemistry profile and urinalysis. A CSF VDRL was non reactive and a CSF FTA-ABS was reported as reactive 3+.
A serum VDRL was reactive with a titer of 1: 64 and confirmed by a reactive Treponema Particle Agglutination Antibody (TPA) test.