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Editorial

An eruption of the skin, taking various names, according to its form, or the part affected; especially, an eruption of vesicles in small distinct clusters, accompanied with itching or tingling, including shingles, ringworm, and the like; -- so called from its tendency to creep or spread from one part of the skin to another. How do you describe these features in one word? You are right, it is called HERPES.

Herpes simplex virus 1 and 2 (HSV-1 and HSV-2), also known as Human herpes virus 1 and 2 (HHV-1 and -2), are two members of the herpes virus family, Herpesviridae, that infect humans. Both HSV-1 (which produces most cold sores) and HSV-2 (which produces most genital herpes) are ubiquitous and contagious. They can be spread when an infected person is producing and shedding the virus.

Symptoms of herpes simplex virus infection include watery blisters in the skin or mucous membranes of the mouth, lips or genitals. Lesions heal with a scab characteristic of herpetic disease. Sometimes, the viruses cause very mild or atypical symptoms during outbreaks. However, as neurotropic and neuroinvasive viruses, HSV-1 and -2 persist in the body by becoming latent and hiding from the immune system in the cell bodies of neurons. After the initial or primary infection, some infected people experience sporadic episodes of viral reactivation or outbreaks. In an outbreak, the virus in a nerve cell becomes active and is transported via the neuron's axon to the skin, where virus replication and shedding occur and cause new sores. **"DISEASE DIAGNOSIS"** segment unveils the clinico-diagnostic mysteries of Herpes simplex viral infections for you.

"INTERPRETATION" segment of this issue carries forward the **"INTERPRETATION OF LABORATORY TESTS IN RHEUMATIC DISEASE"** from the previous issue. An exhaustive spread out that was laid out to simplify understanding related investigations is concluded here. All tests old and new have been presented in ample details.

"TROUBLE SHOOTING" outlines the **"Quality control issues as related to sero-immunological assays"**. If followed honestly, one would always get truthful, correct and reproducible results. QC compliance is a must for a fair practice.

Have you laughed today? Did a wise thought pass through your brain? Did a few simple questions amaze you? NO! **"BOUQUET"** shall let you do all this. TRY!

DISEASE DIAGNOSIS

HERPES SIMPLEX

Background

The herpes simplex viruses comprise 2 distinct types of DNA viruses: herpes simplex virus 1 (HSV-1) and herpes simplex virus 2 (HSV-2). The epidemiology of herpes infection has dramatically changed over the past several decades. HSV-1 causes oral lesions in approximately 80% of cases and genital lesions in 20% of cases. In adolescents, as many as 30-40% of genital herpes is caused by HSV-1, as this proportion is thought to be increasing in the developed world, due to increased oro-genital contact. The reverse is true for HSV-2, which causes genital lesions in 80% and oral lesions in 20%. Cutaneous herpes is shown in the image below.



Cutaneous vesicles characteristic of herpes simplex virus infection

Herpes viruses cause a wide range of diseases, including the following: Gingivostomatitis, Keratoconjunctivitis, Encephalitis, Genital disease, Newborn infection

Primary infection

Primary infections usually are mild and, in many cases, asymptomatic. Patients who are immunocompromised may develop severe infections involving multiple organ systems. Immunocompetent individuals also may have severe primary infections.

Latency and recurrence

After the patient begins to produce antibodies, the infection becomes latent in the sensory ganglia. HSV-1 infection remains latent in the trigeminal ganglia and HSV-2 in the sacral ganglia. The viruses become reactivated secondary to certain stimuli, including fever, physical or emotional stress, ultraviolet light exposure, and axonal injury.

Recurrent infections tend to be less severe because of existing cellular and humoral immunity from prior exposures. Although many persons are seropositive for HSV-1, the recurrence rates range from 10-40% after the primary infection. Infection by HSV requires a break in the skin's barrier; intact skin is resistant to the virus.

Pathophysiology

HSV-1 infections are spread via respiratory droplets or direct exposure to infected saliva. HSV-2 usually is transmitted via genital contact. The contact must involve mucous membranes or open or damaged skin. The incubation period may last from 2-12 days, and vesicles typically erupt 6-48 hours after

the onset of a prodrome.

Herpes viruses cause cytolytic infections; therefore, pathologic changes are due to cell necrosis as well as inflammatory changes. Fluid accumulates between the dermis and the epidermal skin layers, causing vesicle formation. The fluid then is absorbed, scabs are formed, and healing is completed without evidence of scarring. Shallow ulcers form after the vesicles rupture on mucous membranes.

Lesions from primary herpes infection typically take longer to form and usually persist for a longer duration of time.

The virus travels from the site of infection in the skin or mucosa to the sensory dorsal root and remains latent until a recurrent outbreak. Outbreaks are usually due to some sort of stress including ultraviolet radiation, trauma, emotional or psychological stress, or immunosuppression.

Epidemiology

Frequency - International Greater than one third the world's population has recurrent clinical HSV infections. Reportedly, 13-40% of the world's population is seropositive for HSV-2 and 56-85% is seropositive for HSV-1, varying by country. Women are infected more often than men.

Mortality/Morbidity

Most patients with herpetic infection experience short-term local pain and irritation, with mild constitutional symptoms. **Infection** occasionally may become life threatening. **Immunocompromised** patients are at increased risk of developing severe HSV infections. **HSV-1** is a common cause of fatal encephalitis in the US, with a mortality rate 60-80%. Fewer than 10% of patients are left without significant neurologic sequelae. **Keratoconjunctivitis** may be caused by HSV-1. It is second only to trauma as a cause of corneal blindness in the US.

Sex Men are 20% more likely to develop recurrences of HSV-2 than are women.

Age Highest incidence of HSV-1 occurs in children aged 6 months to 3 years. HSV-2 most commonly occurs in those aged 18-25 years.

Clinical History

The typical incubation period from exposure to development of symptoms is 4 days but can range from 1-26 days. Prodromal symptoms of local pain, tingling, itching, and burning often precede development of the rash. Constitutional symptoms of fever, fatigue, myalgias, and headache often accompany the primary herpes simplex virus (HSV) infection. **Herpetic** lesions usually begin as clusters of small bumps, then blisters, followed by open sores or ulcers. Lesions coalesce and usually heal over several weeks.

Many times these classically described lesions in the genital area may not present in all patients and may be difficult to differentiate from other conditions such as syphilis and chancroid. **Local pain** is a prominent and common complaint. Patients with genital herpes may also complain of pain in the groin area secondary to local adenopathy. Women often present with complaints of genital swelling, discharge, and dysuria. **Many primary** infections are asymptomatic. Up to 80% of women with HSV-2 antibodies have no clinical history of infection. However, when primary infections are symptomatic, they are usually more severe than recurrent infections. Persons with asymptomatic genital HSV-2 infections still shed virus but less frequently than persons with symptomatic infections. **Recurrent** lesions are common.

Patients may give a history that includes the following:

Occupational exposure Herpetic whitlow, found in health care workers (especially medical or dental), Herpes gladiatorum on bodies of wrestlers, - Previous history of herpetic diseases, Apparently undiagnosed episodes

Immune status HIV, Malnourishment, Hematological malignancies., Bone marrow, Renal transplant, Cardiac transplant

Neurologic symptoms Headache, Confusion, Fever

Lesions Location varies, May be very painful, Tenesmus, itching with anal/perianal lesions, Dysuria with genital lesions, Sore throat with oral lesions

Constitutional symptoms (usually present with development of herpes lesions) Anorexia, General malaise, Fever, Headache, Myalgias

Prodromal symptoms (present in advance of herpes lesions) Burning, Itching, Tingling, Pain

Physical Findings

Physical examination findings of HSV vary depending on location of the lesions.

General findings

Lesions usually are vesicular or ulcerative on an erythematous base, as shown in the image below Cutaneous vesicles characteristic of herpes simplex virus infection, **Lesions** coalesce and then heal over the next several weeks. **Tender** bilateral lymphadenopathy occurs with genital lesions.

Skin infections (HSV-1 or HSV-2)

Herpetic whitlow or paronychia on the fingers of health care workers (not to be confused with abscess). This is usually due to infection with HSV-1, but HSV-2 infections may be seen with digital-genital contact. **Herpes gladiatorum** on the bodies of wrestlers and other sports that involve close physical contact. It has been estimated that in Division I National Collegiate Athletic Association (NCAA) wrestling, the incidence of herpes gladiatorum can be as high as 20-40%.

Oropharyngeal disease

Gingivostomatitis (herpes labialis on the lips, shown in the image)



Herpes labialis

Submandibular lymphadenopathy, **Fever**

Genital herpes

Painful vesicular or ulcerative lesions may appear similar to chancroid or

syphilis (vesicular lesions as shown in the image below)



Penile infection with herpes simplex virus type 2

Inguinal lymphadenopathy, **Genital** lesions, especially urethral lesions, may cause transient urinary retention in women.

Keratoconjunctivitis

Dendritic keratitis found with slit lamp (dendritic ulcer shown below)



Herpes simplex virus dendritic ulcer with fluorescein staining.

Corneal ulcers, **Vesicles** on eyelids

Neurologic

New psychiatric symptoms (indicative of encephalitis)-Confusion; seizures; meningeal signs (Recurrent lymphocytic meningitis [benign form of meningitis/encephalitis that may occur during primary HSV-2 infection]) Bell palsy (possible relationship with HSV-1)

Anal/perianal involvement

Discharge, Vesicles, Ulcerations, Inguinal adenopathy

Causes

HSV-1 is transmitted through direct contact with infected saliva or direct contact with contaminated utensils. **HSV-2** is usually acquired as an STD. **Maternal-fetal** transmission-risk of transmission is greater in primary outbreak (30-50%) than with recurrent outbreaks (< 1%). **Recurrent** disease (reactivation) due to certain stimuli: fever, physical or emotional stress,

ultraviolet light exposure, or axonal injury

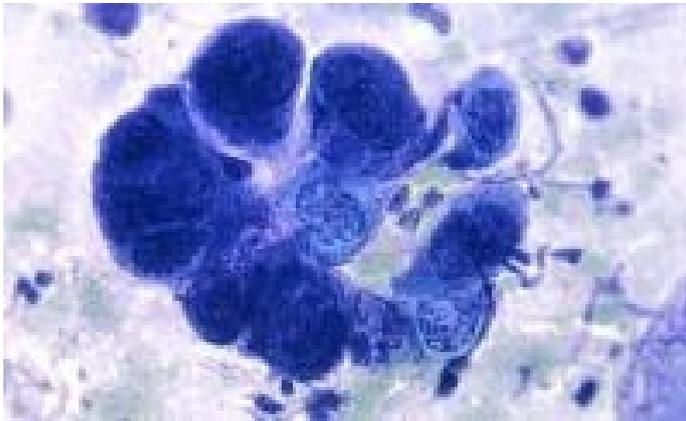
Differential Diagnosis

Chancroid, Erythema Multiforme, Herpes Zoster, Pediatrics, Hand-Foot-and-Mouth Disease, Pediatrics, Meningitis and Encephalitis, Pediatrics, Pharyngitis, Pharyngitis, Proctitis, Syphilis, Urethritis, Male

Laboratory Studies

Scrapings from suspected lesions of herpes simplex (**Tzanck smear**). This is not a reliable screening test, with a reported sensitivity of 65%. It also does not identify the type of herpes simplex virus (HSV) present.

Multinucleated giant cells, as shown below



Tzanck smear showing a multinucleated giant cell and intranuclear inclusions

Viral culture from skin vesicles (more sensitive than Tzanck smear but dependent on duration of viral shedding)

Monoclonal antibody testing, Rapid ICT, ELISA, PCR formats are available.

Serology

Cerebrospinal fluid (CSF) analysis for lymphocytic pleocytosis
Bloody CSF, Polymerase chain reaction (PCR) detects HSV DNA

Imaging Studies

CT scan and MRI for differentiation of encephalitis from other entities

Procedures

Slit-lamp examination for dendritic keratitis with ocular involvement, **Lumbar** puncture, if concerned about encephalitis, **Brain biopsy**, if encephalitis is considered

Emergency Department Care

ED care consists of diagnosis and appropriate treatment. Most patients may be treated in the outpatient setting. Identification of patients that need inpatient treatment (ie, encephalitis) and initiation of antiviral and supportive therapy is imperative

Medication Summary

Antiviral drugs with activity against viral DNA synthesis have been effective against HSV infections. These drugs inhibit virus replication and may suppress clinical manifestations but are not a cure for the disease. Since HSV remains latent in sensory ganglia, the rates of relapse are similar in treated and untreated patients. **The 2006 CDC guidelines** for STD treatment recommend that all initial genital herpes infections be treated with

antivirals to reduce any potential complications. **Acyclovir** (Zovirax) provides initial, recurrent, and suppressive therapy for genital HSV. It is effective for mucocutaneous HSV in an immunocompromised host as well as HSV encephalitis. Little evidence supports the routine use of acyclovir for primary oral-labial HSV. Oral acyclovir has been shown to be effective in suppressing herpes labialis in immunocompromised patients with frequent recurrent infections. Begin use during the prodromal period. **Daily suppressive** therapy has shown to be 80% effective in preventing recurrences and should be considered in patients who suffer from frequent recurrences. **Administer** famciclovir (Famvir) or valacyclovir (Valtrex) for recurrent episodes of genital HSV. Herpes simplex keratoconjunctivitis is treated with topical 1% trifluridine (Viroptic). **In pregnancy**, the use of antiviral agents such as valacyclovir and acyclovir has been shown to be safe with no increased risk of birth defects. **Use pain** medication as needed. Many patients may require narcotics for the relief of severe pain from the lesions.

Further Inpatient Care

Admission for patients with herpes simplex is necessary in the following instances: Encephalitis, **Severe** gingivostomatitis causing decreased ability to tolerate oral fluids, **Immunocompromised** patients with severe or disseminated disease.

Further Outpatient Care

Oral medication (see Medication): Topical acyclovir is only minimally helpful in patients with primary disease and is probably ineffective in recurrent episodes.

Deterrence/Prevention

HSV-2 is an STD. Patients and all sexual contacts should be tested and treated for accompanying STDs. **Practice** abstinence when lesions are present. **Always** use condoms because of the potential for asymptomatic viral shedding. **Health care** personnel (especially medical, dental) should use universal precautions (eg, gloves) to prevent herpetic whitlow. **Experimental** vaccines are currently in clinical trials. **Use sunscreen** to decrease herpes labialis recurrences.

Complications

Encephalitis: Rare complication of herpetic infection; commonly HSV-1 (hypothesized to spread to the brain via neural routes after primary or recurrent infection), **Neonatal** infections: Range from mild localized infection to a fatal disseminated disease; HSV-2 usually spread via the maternal genital tract; congenital infections possible, **Compromised host:** Progressive and disseminated disease possible, **Genital infection:** Acute urinary retention

Prognosis

Genital HSV-2 infection has a high recurrence rate. More than 85% of patients with one symptomatic episode will experience another. Recurrences may be frequent; 38% of the population with genital herpes have more than 6 recurrences per year; 20% have more than 10 recurrences per year.

Patient Education

Antiviral therapy may decrease the clinical manifestations of the disease but does not cure it. **Initiate** antiviral therapy as soon as possible after the patient notes symptoms. **Consider** prophylaxis for patients who have more than 6 recurrences per year. **Educate** patient that HSV-2 is an STD. Follow deterrence measures.

INTERPRETATION

INTERPRETATION OF LABORATORY TESTS IN RHEUMATIC DISEASE

(continued from last issue)

RHEUMATOID FACTOR Rheumatoid factors (RF) are autoantibodies directed at the Fc portion of IgG molecules. Currently used tests measure IgM RF, but IgG and IgA subtypes exist. The presence of RF can be detected by agglutination of IgG-sensitised sheep red cells (SCAT) or latex particles coated with human IgG (Rose Waaler), ELISA and nephelometry.

Table II. Conditions other than RA associated with a positive RF test

	Prevalence of RF %
Young healthy individuals	5
Age > 60 years	5 - 25
Other rheumatic diseases	
SLE	20 - 30
Sjögrens syndrome	75 - 90
Mixed connective tissue disease	50 - 60
Scleroderma	20 - 30
Polymyositis	5 - 10
Infections	
Bacterial endocarditis	25 - 50
Tuberculosis	10 - 20
Syphilis	10
Viral infections (including HIV)	15 - 65
Miscellaneous	
Interstitial pulmonary fibrosis	10 - 50
Primary biliary cirrhosis	50 - 70
Sarcoidosis	5 - 33
Malignancy	5 - 25

No one technique has a clear advantage over another, however the ELISA and nephelometry are slightly more sensitive. Most laboratories will screen sera with a more sensitive technique and confirm the presence of an RF with a more specific technique. In an unselected population, the RF is 80% sensitive and 95% specific for rheumatoid arthritis. However, as the disease is relatively uncommon, the positive predictive value is low. Only 20 - 30% of unselected patients who have a positive test will actually have RA. Most of the positive tests will be false positive as there are a large number of other conditions that can give rise to a positive RF (Table II). In addition, the presence of a (false) positive RF test rises with age. These patients are more likely to have osteoarthritis, thus emphasizing the need for careful clinical evaluation. When used in a group of patients with rheumatic disease, the positive predictive value of the RF test increases to 80%. However, up to 50% of patients with RA will test negative for RF at disease onset, with some of these patients converting to a positive test over the first 2 years of disease. A persistently negative test is seen in 20% of patients with RA. The RF has been shown to be of no value in monitoring disease activity and serial monitoring of RFs is not indicated.

ANTI-CCP ANTIBODIES Anti-cyclic citrullinated peptide (CCP) antibodies are more recently discovered RA-specific antibodies targeting the modified amino acid, citrulline. The second-generation tests have sensitivity similar to IgM RF and a very good specificity of up to 97%. This test is valuable in

confirming the diagnosis of early RA when the diagnostic criteria are not yet fulfilled. Anti-CCP antibodies are of additional diagnostic value in the RF negative patient, being positive in up to a third of these patients. In other words, a positive test supports the diagnosis in ambiguous cases. The presence of this antibody has also been found to be associated with a more aggressive disease course. The IgM RF is still the most useful test for screening patients with suspected RA.

HLA B27 HLA B27 is a class I MHC allele strongly associated with the spondylarthropathies. This association varies among the different forms of spondylarthropathies (Table III). The HLA B27 test is not a routine diagnostic or screening test for ankylosing spondylitis (AS) in patients presenting with back pain or arthritis. The prevalence of AS correlates with the prevalence of HLA B27 in a given population. In South Africa, the prevalence of HLA B27 varies according to ethnic origins, and ranges from 8% to 11% in Caucasians to less than 1% in black Africans. In general, epidemiological surveys indicate that 4 - 7% of HLA B27-positive people will have the disease. This means 93 - 96% of patients who are HLA B27-positive will not have the disease. The predictive value of the test depends on the pre-test probability of AS. In patients in whom history and physical examination suggest AS (e.g. inflammatory back pain, other features of spondylarthropathies), but whose radiographic findings do not permit this diagnosis to be made, the HLA B27 test may minimise uncertainty. In patients with nonspecific back pain, with no other features suggestive of an inflammatory spondylitic disorder, HLA B27 testing is inappropriate, as a positive test would still not allow a diagnosis of AS. Routine screening of family members of an HLA B27-positive patient with AS is of limited practical value as it is not entirely predictive of the disease (20% of HLA-B27 positive 1st degree relatives develop the disease) and no preventive therapy is available. It does, however, define which family members are at risk. HLA B27-positivity in patients with reactive arthritis is associated with more severe and prolonged disease as well as the development of sacro-iliitis and spondylitis. Knowledge of HLA B27 status in these patients may predict long term prognosis.

Table III. Association of spondylarthropathies with HLA B27 in white persons (%)

Ankylosing spondylitis	90
Reactive arthritis	40 - 80
Enteropathic spondylarthritis	35 - 75
Psoriatic arthritis	40 - 50
Undifferentiated spondylarthropathy	70
Acute anterior uveitis	50

SYNOVIAL FLUID ANALYSIS Synovial fluid analysis is inexpensive and very important in confirming or excluding septic arthritis in a patient with a monoarthritis, or in the febrile patient with an acute flare of established arthritis. It can help to distinguish inflammatory from non-inflammatory arthritis and is diagnostic for the crystal associated arthritis. Fluid should be sent for cell count, crystals, Gram stain and culture. Fluid can also be assessed visually for colour, clarity and viscosity. Even a drop of fluid (in fluid phase) can be examined for the presence of crystals, and Gram stain can then be performed. In this scenario, communication with laboratory staff often facilitates a satisfactory result. Synovial fluid is categorised as normal, non-inflammatory, inflammatory, septic or haemorrhagic based on clinical and laboratory analysis (Table IV).

Table IV. Classification of synovial fluid findings

	Normal	Non-inflammatory	Inflammatory	Septic
Clarity	Transparent	Transparent	Translucent/opaque	Opaque
Colour	Colourless	Yellow/straw	Yellow	Yellow
Viscosity	High	High	Low	Variable
WBC (/mm ³)	< 200	200 - 2 000	2 000 - 50 000	> 50 000
Polymorphs (%)	< 25%	< 25%	> 50%	> 75%
Culture	Sterile	Sterile	Sterile	Positive

CONCLUSION

It must be stressed that the diagnosis of rheumatic diseases is still largely dependent on a thorough clinical evaluation, with specific tests that may support the diagnosis. Tests should be ordered selectively, depending on the clinical picture. Autoantibodies are not uncommon in healthy individuals, and unselective testing of autoantibodies by 'autoimmune screening panels' leads to a high number of false positive results with the resultant anxiety, expense of further evaluation, and inappropriate treatment. Results should always be interpreted in conjunction with the clinical scenario.

IN A NUTSHELL

- The presence of a laboratory abnormality does not always imply the presence of disease.
- The CRP is a sensitive and early indicator of inflammation.
- A negative ANA virtually excludes a diagnosis of SLE. Low-titre false positive ANAs are common in the normal population. A positive dsDNA or Sm antibody strongly suggests a diagnosis of SLE.
- Only 20 - 30% of unselected patients with a positive RF test will actually have RA. 20% of patients with RA will remain seronegative throughout their disease course.
- Anti-CCP antibodies are virtually diagnostic of RA.
- The HLA B27 test cannot be thought of as a routine, diagnostic or screening test for ankylosing spondylitis.
- Synovial fluid analysis is of utmost importance in excluding septic arthritis in patients with a monoarthritis.

BOUQUET

In Lighter Vein

A Brunette went to the doctors. "Doctor," she said. "Help me. I hurt all over."
 "Sit down," said the doctor, "And tell me exactly what is wrong."
 "If I touch here," she said, poking her thigh, "It hurts like hell. It also hurts here, here and here," she added, poking her chest, forehead and foot in turn.
 "Oh dear," said the doctor "This is serious. Tell me, when did you dye your hair?"
 "What on earth do you mean?" asked the young woman.
 "Well you are a natural blonde aren't you?" asked the doctor.
 "I am doctor," she said.
 "But how did you know?"
 The doctor replied, "You've broken your finger."

Bud and Jim were a couple of drinking buddies who worked as aircraft mechanics in Philadelphia. One day the airport was fogged in and they were stuck in the hangar with nothing to do. Bud said, "Man, I wish we had something to drink!"
 Jim says, "Me too. Y' know, I've heard you can drink jet fuel and get a buzz. You wanna try it?"
 So they pour themselves a couple of glasses of high octane hooch and get completely smashed. The next morning Bud wakes up and is surprised at how good he feels. In fact he feels GREAT! NO hangover! NO bad side effects. Nothing! Then the phone rings. It's Jim. Jim says, "Hey, how do you feel this morning?"
 Bud says, "I feel great. How about you?"
 Jim says, "I feel great, too. You don't have a hangover?"
 Bud says, "No, that jet fuel is great stuff -- no hangover, nothing. We ought to do this more often." "Yeah, well there's just one thing."
 "What's that?"
 "Have you answered nature's call yet?"
 "No"
 "Well, DON'T, 'cause I'm in Denver."

Wisdom Whispers

- 'Ego' is the only requirement to destroy any relationship, So be the bigger person skip the 'e' and let go
- Friends may come and go, but enemies accumulate
- A true friend is someone who thinks that you are a good egg even though he knows that you are slightly cracked.
- There are three faithful friends, an old wife, an old dog, and ready money
- Love is blind. Friendship tries not to notice
- It takes a long time to grow an old friend
- wise man hears one word and understands two
- "Education is what remains after one has forgotten everything he learned in school."
- "I've never been one who thought the Lord should make life easy; I've just asked Him to make me strong."
- "The best way to destroy your enemy, is to make him your friend."
- "Forbidden Fruit is the main ingredient in many Jams!"
- "Find something you love to do and you'll never have to work a day in your life"
- "Far and away the best prize that life offers is the chance to work hard at work worth doing."
- Success is the good fortune that comes from aspiration, desperation, perspiration and inspiration.
- Be not ashamed of thy virtues; honor's a good brooch to wear in a man's hat at all times.
- Screaming is bad for the voice but its good for the heart

Brain Teasers

Choose the most appropriate answer

1. MacCallum's patch is found in:
 A. Left atrium B. Left Ventricle C. Right Atrium D. Superior ven cava
2. Aschoff bodies are seen in the heart in:
 A. Rheumatic Haert Disease B. Hypertension C. Uremia D. S.L.E
3. Most often which lymph nodes are affected in sarcoidosis?
 A. Pre-auricular B. Cervical C. Hilar and mediastinal D. Inguinal
4. Most carcinomas of pancreas arise from:
 A. Tail B. Head C. Body D. Ectopic pancreatic tissue
5. Multinucleated giant cells in kidney may be seen in:
 A. Chronic pyelonephritis B. Chronic glomerulonephritis
 C. Multiple myeloma D. Diabetic nephropathy
6. Chloromas are found in association with:
 A. ALL B. CLL C. AML D. Plasma cell leukemia

Answers: 1.A 2.A 3.C 4.B 5.C 6.C

TROUBLESHOOTING

QUALITY CONTROL OF SEROLOGICAL ASSAYS

Serological testing remains the bulk of the work carried out by a routine virus diagnostic laboratory. It is essential to have good quality control protocols in all sections of serology in order to ensure the validity of the test results. Strictly speaking, quality control refers to the measures that must be included during each assay to verify that the test is working properly. However, the term is often more loosely used to cover aspects of quality assurance and also quality assessment. Quality assurance is defined as the overall program that ensures that the final results reported by the laboratory are correct, and quality assessment (also known as proficiency testing) is a means to determine the quality of the results generated by the laboratory. It is usually an external evaluation of the laboratory's performance.

Quality Control: Monitoring the Testing Process As mentioned previously, quality control refers to those measures that must be included during each assay in order to verify that the test is working properly. The following items are essential elements of quality control that must be performed during every assay:

1. Each run must include one full set of controls
2. The controls for each test run must yield results within the limits of the manufacturer's criteria for acceptability and validity of the run.
3. All test kits must be used before the expiration date to ensure valid results
4. Physical parameters of the test such as incubation time and temperature must be followed to ensure proper performance. Ordinarily, each test kit has a set of positive and negative control that are to be included in each test run. These controls are considered to be internal controls, while any other controls included in the run are referred to as external controls. Internal controls are essential for QC measures for each run and are intended for use only with the lot number of the corresponding test kit. External controls can be included on a run to monitor consistent performance, lot to lot variation between kits, and to serve as an indicator of assay performance on samples that are borderline reactors.

Quality Assurance QA is an ongoing process that requires daily attention by all laboratory staff. Many variables can affect the quality of results so that the monitoring of which is essential in a quality assurance program.

1. Documentation - this is probably the most important part of quality assurance. There must be an up to date standard operating procedure of the test. The original source of the procedure, and all subsequent changes must be documented and traceable. All worksheets and testing records must be kept up to date and traceable, as are all computer records.
2. The condition of the specimens - the specimen should be clearly labeled with patient details using at least two different identifiers. The specimen to be tested should not be haemolysed or lipaemic. Utmost care must be taken during the splitting of the specimen so that the correct specimen is placed into the correctly labeled tube.
3. The educational background and training of the laboratory personnel. A continuing education program for laboratory workers should be included and individual laboratory personnel should be evaluated to identify areas for improvement
4. The controls used in the test runs - appropriate internal and possibly external controls should be used in each test run.
5. Up to date performance records of each test run and quality control procedures should be kept.
6. The interpretation of results - results should be interpreted according to the manufacturer's criteria. Where the interpretation of the test is potentially subjective, as in the case of haemagglutination-inhibition, complement-fixation, or single-radial haemolysis, it may be advisable for the results to be read and counterchecked by a second person.
7. The transcription of results - Transcriptional or clerical errors include mistakes made during the transfer of information from the test readout to the worksheet, and from the worksheet to the computer or report form. These type of errors probably account for the majority of errors in the laboratory. Possible mechanisms include having a second technologist to check the final result and the supervisor to check the results before releasing.
8. The reporting of results - the results should be reported in a timely manner to the appropriate individual. The report should be authorized by a suitably qualified individual.
9. Internal audit - regular audits should be carried out to look at the quality assurance program.

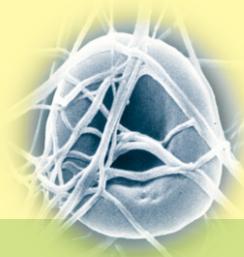
Quality Assessment Quality assessment is a means to determine the quality of results. It is usually an external evaluation of a laboratory's performance that relies on incorporating proficiency panels of well-characterized sera into the testing routine. External quality assessment (EQA) is now recognized as an essential component of quality assurance and is the only means to give the laboratory manager an independent means of ensuring that his routine quality control is adequate and effective. Increasingly, internal quality assessment schemes are being put in place in individual laboratories. These usually involve the use of internal control specimens. These o.d. values of the internal quality control specimens are plotted in a Shewhart-type chart (usually, it is the o.d of IQC/o.d. negative control which is used), and evaluated against Westgard rules. Westgard rules are based on statistical considerations and thus probability. An assay run where the IPC is >2 or 3 s.d., or there is a continual trend on one side of the chart, would raise suspicions of either or both random and systematic errors. It may be prudent to reject the run altogether.

Accreditation Accreditation is an external audit of an applicant department's organization and quality assurance program is an external audit of an applicant department's organization and quality assurance program. There is now a trend towards accepting ISO 15189 as the standard for all accreditation bodies in order to provide uniformity of standards and cross-recognition. Examples of accreditation authorities include the NBAL in India, CPA (College of American Pathologists) in the U.S., CPA (Clinical Pathology Accreditation) in the UK, and NATA in Australia. In addition the the whole department, individual assays may be accredited at the same time on an individual basis as well. Accurate and up to date documentation, with complete traceability forms a very important part of the accreditation process.

Quality control considerations for different serological tests. The following considerations apply to the following serological assays;- **Commercial EIAs and RIAs** - on the whole, currently available commercial assays are now of a high standard. It is important to ensure that the manufacturer's controls are included in each run and that the tests are interpreted according to the manufacturer's instructions. If wells or beads from more than one kit is used for the assay run, it is important to ensure that the kits come from the same batch, otherwise, a different set of controls should be put up for each different batch. **In house RIAs and EIAs** - on the whole, in house tests are considerably more difficult to perform and more likely to go wrong than commercial assays. The conditions specified for the test should be followed exactly each time to ensure that the test is reproducible. If possible, freshly made up reagents should be used each time to minimize the chance of deterioration of the reagent eg. fetal calf serum, affecting the result of the test. **Immunofluorescence (IFA)** - the interpretation of IFA result is subjective. Difficulty may arise in the interpretation of borderline positive results which may require an experienced reader to interpret. **Complement-fixation tests** - problems often arise with CFTs because of problems with one of the reagents such as the antigen, complement, the read blood cells or the VBS diluent. Vigilance must be exercised at all times. It is important to put up controls for each antigen and carry out a complement back titration. Each new batch of antigen must be titrated using chessboard titration method. **Haemagglutination-inhibition tests** - again problems are commonly seen with HAI. Any of the reagents used may be implicated. It is important to carry out an antigen titration prior to each test. **Single radial haemolysis** - as in the case of CFTs and HAIs, problems are relatively common. It is essential to use freshly made up reagents. **Agglutination tests** - Latex agglutination and particle agglutination tests are very simple to perform. However, there may be problems in the interpretation of results which is subjective. Care must be taken to avoid the contamination of one specimen with another specimen in an adjacent well when setting up the test. **Others** - the same principles apply to other serological tests such as western blots, radioimmunoprecipitation assays, virus neutralization assays and counter-immune electrophoresis. **To conclude**, quality control in serological assays is an on-going process and requires vigilance by the laboratory personnel at all times. Quality control of serological assay is a continuously evolving subject, but there is a general consensus that a laboratory should aim to be accredited. Where possible, the laboratory should aim for accreditation under ISO 15189 with all individual tests accredited.

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