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The Crux

BIMONTHLY FORUM FOR THE LABORATARIANS

CONTENTS

Editorial

We have in our previous issues considered epidemiologically significant vector borne diseases as related to the Indian subcontinent and the world at large - viz., Malaria, Dengue fever, Typhus; this issue, at the request of our readers, presents an equally menacing disease - CHIKUNGUNYA. Needless to say it is a contemporary problem being faced by as many as 151 districts in 8 states spread diffusely all over the country even as on date. Chikungunya virus (CHIKV) is an insect-bourne virus, of the genus, *Alphavirus*, that is transmitted to humans by virus-carrying *Aedes* mosquitoes. There have been recent outbreaks of CHIKV associated with severe morbidity. CHIKV causes an illness with symptoms similar to dengue fever. CHIKV manifests itself with a prolonged arthralgic disease that affects the joints of the extremities. The acute febrile phase of the illness lasts only two to five days. The pain associated with CHIKV infection of the joints persists for weeks or month. The DISEASE DIAGNOSIS section outlines all clinico-diagnostic aspects of Chikungunya in ample detail. Prevention is the best and the cheapest cure! For personal protection, individuals should take measures to protect themselves against the bites of the mosquitoes which transmit the virus (including *Aedes aegypti*), which are active during the daytime. Elimination of breeding sites can contribute to the reduction of mosquito densities.

Again, at the persistent request of our valued readers, we have taken up a simple but sometimes cumbersome procedure of semen analysis (seminogram) under INTERPRETATION and the process of actual sperm counting under TROUBLE SHOOTING segments of this issue. Within its covers, you will find all details pertaining to interpretation of various results/ values obtained in relation to detailed semen analysis. The Global incidence of infertility is about 13-18%. Male infertility is found to have a role in approximately 50% of infertile couples. Semen analysis being the cheapest and non-invasive investigation is the first one to be requisitioned for in all infertility cases that come for medical opinion. You will find normal reference ranges that are usually considered while conducting a seminogram.

BOUQUET has its place, as usual. Few jokes, words of wisdom passed on by generations and a few questions in relation to the reticuloendothelial system are adorning this issue. We would like to thank you - the reader, for enhancing the popularity of our endeavour The Crux, truly, it has become an international requirement. We are getting more and more requests for this free service rendered to our our fellow Laboratarians. Happy reading!

Tulip Group

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DISEASE DIAGNOSIS

CHIKUNGUNYA

Introduction

Chikungunya is a virus that can be transmitted to humans by mosquitoes. The resulting illness is also called Chikungunya. It was originally described in the early 1950s after an outbreak in a Swahili village on the Makonde plateau that lies between Tanzania and Mozambique. The translation of Chikingunya from Makonde means "illness of the bended walker," "that which bends up," stooped over, walking bent over, or "bended walker." In India, it is known as Aakyda, meaning "stiff man" and Maakyda meaning "monkey-like". These words refer to the arthritic condition that occurs in some patients which gives rise to a stooped posture. The virus was first found in Asia, isolated in Bangkok, Thailand in 1958.

The Chikungunya Virus

Virus classification

Group: Group IV ((+)ssRNA)
Family: *Togaviridae*
Genus: *Alphavirus*
Species: *Chikungunya virus*

Chikungunya virus (CHIKV) is an arbovirus, of the genus, *Alphavirus*, that is transmitted to humans by virus-carrying *Aedes* mosquitoes. There have been recent outbreaks of CHIKV associated with severe morbidity. CHIKV causes an illness with symptoms similar to dengue fever. CHIKV manifests itself with a prolonged arthralgic disease that affects the joints of the extremities. The acute febrile phase of the illness lasts only two to five days. The pain associated with CHIKV infection of the joints persists for weeks or months.

Epidemiology

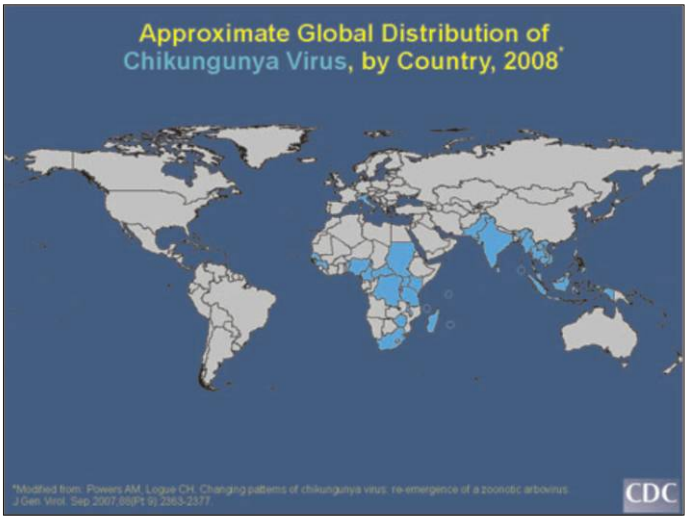
Chikungunya virus is an alphavirus closely related to the O'nyong'nyong virus, the Ross River virus in Australia, and the viruses that cause eastern equine encephalitis and western equine encephalitis.

Chikungunya is generally spread through bites from *Aedes aegypti* mosquitoes, but recent research by the Pasteur Institute in Paris has suggested that chikungunya virus strains in the 2005-2006 Reunion Island outbreak incurred a mutation that facilitated transmission by *Aedes albopictus* (Tiger mosquito). Concurrent studies by arbovirologists at the University of Texas Medical Branch in Galveston Texas confirmed definitively that enhanced chikungunya virus infection of *Aedes albopictus* was caused by a point mutation in one of the viral envelope genes (E1). Enhanced transmission of chikungunya virus by *Aedes albopictus* could mean an increased risk for chikungunya outbreaks in other areas where the Asian tiger mosquito is present. A recent epidemic in Italy was likely perpetuated by *Aedes albopictus*.
In Africa, chikungunya is spread via a sylvatic cycle in which the virus largely resides in other primates in between human outbreaks.
Since its discovery in Tanganyika, Africa in 1952, chikungunya virus outbreaks have occurred occasionally in Africa, South Asia, and Southeast Asia, but recent outbreaks have spread the disease over a wider range.

Common breeding places for the vectors of Chikungunya

Chikungunya Distribution and Global Map

The geographic range of chikungunya virus is mainly in Africa and Asia. Given the current large chikungunya virus epidemics and the worldwide distribution of *Aedes aegypti* and *Aedes albopictus* mosquitoes, there is a risk of importing chikungunya virus into new area through infected travelers.



Countries where people have become infected with chikungunya virus.

Benin	Burundi	Cambodia
Cameroon	Central African Republic	Comoros
Congo, DRC	East Timor	Guinea
India	Indonesia	Italy
Kenya	Laos	Madagascar
Malawi	Malaysia	Mauritius
Mayotte	Myanmar	Nigeria
Pakistan	Philippines	Reunion
Senegal	Seychelles	Singapore
South Africa	Sudan	Taiwan
Tanzania	Thailand	Uganda
Vietnam	Zimbabwe	

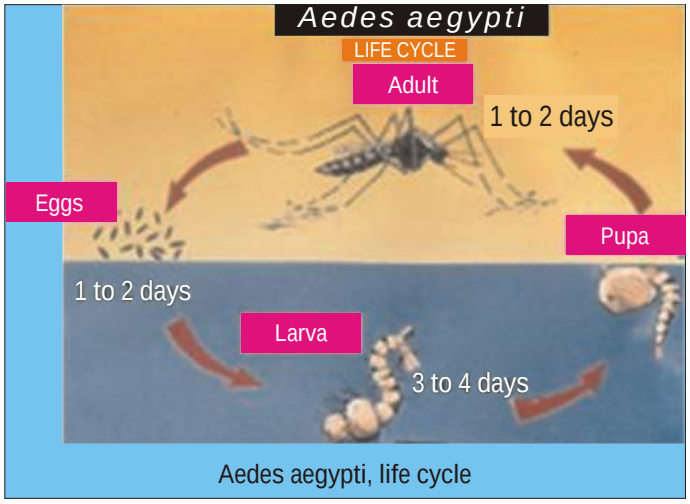
Chikungunya Transmission

Chikungunya virus is spread by the bite of an infected mosquito. Mosquitoes become infected when they feed on a person infected with chikungunya virus. Infected mosquitoes can then spread the virus to other humans when they bite. Monkeys, and possibly other wild animals, may also serve as reservoirs of the virus.

Aedes aegypti (the yellow fever mosquito), a household container breeder and aggressive daytime biter which is attracted to humans, is the primary vector of chikungunya virus to humans. *Aedes albopictus* (the Asian tiger mosquito) has also played a role in human transmission in Asia, Africa, and Europe. Various forest-dwelling mosquito species in Africa have been found to be infected with the virus.

The *Aedes aegypti* mosquito is the principle vector responsible for transmitting the chikungunya virus to humans.

Aedes albopictus female.



Aedes aegypti, life cycle

Chikungunya - Signs and Symptoms

Chikungunya virus infection can cause a debilitating illness, most often characterized by fever, headache, fatigue, nausea, vomiting, muscle pain, rash, and joint pain. The term 'chikungunya' means 'that which bends up' in the Kimakonde language of Mozambique.

Acute chikungunya fever typically lasts a few days to a few weeks, but as with dengue, West Nile fever and other arboviral fevers, some patients have prolonged fatigue lasting several weeks. Additionally, some patients have reported incapacitating joint pain, or arthritis which may last for weeks or months. The prolonged joint pain associated with chikungunya virus is not typical of dengue. No hemorrhagic cases related to chikungunya virus infection have been conclusively documented in the scientific literature. Co-circulation of dengue fever in many areas may mean that chikungunya fever cases are sometimes clinically misdiagnosed as dengue infections, therefore the incidence of chikungunya fever could be much higher than what has been previously reported.

The incubation period (time from infection to illness) can be 2-12 days, but is usually 3-7 days. “Silent” chikungunya virus infections (infections without illness) do occur; but how commonly this happens is not yet known. Chikungunya virus infection (whether clinically apparent or silent) is thought to confer life-long immunity. Fatalities related to chikungunya virus are rare.

Pregnant women can become infected with chikungunya virus during all stages of pregnancy and have symptoms similar to other individuals. Most infections occurring during pregnancy will not result in the virus being transmitted to the fetus. The highest risk for infection of the fetus/child occurs when a woman has virus in her blood (viremic) at the time of delivery. There are also rare reports of first trimester abortions occurring after chikungunya infection. Pregnant women should take precautions to avoid mosquito bites. Products (repellants) containing DEET can be used in pregnancy without adverse effects. Currently, there is no evidence that the virus is transmitted through breast milk.

There is no vaccine or specific antiviral treatment currently available for chikungunya fever. Treatment is symptomatic and can include rest, fluids, and medicines to relieve symptoms of fever and aching such as ibuprofen, naproxen, acetaminophen, or paracetamol. Aspirin should be avoided. Infected persons should be protected from further mosquito exposure (staying indoors in areas with screens and/or under a mosquito net) during the first few days of the illness so they can not contribute to the transmission cycle.

Joint swelling in Chikungunya

Diagnosis

Arboviral Diagnostic Testing

Preliminary diagnosis is often based on the patient's clinical features, places and dates of travel (if patient is from a non-endemic country or area), activities, and epidemiologic history of the location where infection occurred.

Laboratory diagnosis of arboviral infections is generally accomplished by testing of serum or cerebrospinal fluid (CSF) to detect virus-specific IgM and neutralizing antibodies. Serological tests, such as enzyme-linked immunosorbent assays (ELISA), may confirm the presence of IgM and IgG anti-chikungunya antibodies. IgM antibody levels are highest three to five weeks after the onset of illness and persist for about two months.
ICT (RDTs) formats give the result within 15 minutes to an hour.

During an acute infection, certain viruses can be isolated through culture or detected by nucleic acid amplification. Various reverse transcriptasepolymerase chain reaction (RTPCR) methods are available but are of variable sensitivity. Some are suited to clinical diagnosis. RTPCR products from clinical samples may also be used for genotyping of the virus, allowing comparisons with virus samples from various geographical sources.

In fatal cases, nucleic acid amplification, histopathology with immunohistochemistry and virus culture of autopsy tissues can also be useful. Only a few state laboratories or other specialized laboratories are capable of doing this specialized testing.

Test results are normally available 4 to 14 days after specimen receipt. Initial serological testing will be performed using IgM capture ELISA, MIA (Microsphere-Based Immunoassay) and IgG ELISA. If the initial results are positive, further confirmatory testing may delay the reporting of final results.

Chikungunya Prevention

The best way to prevent chikungunya virus infection is to avoid mosquito bites. There is no vaccine or preventive drug currently available. Prevention tips are similar to those for other viral diseases transmitted by mosquitoes, such as dengue or West Nile.

- Use insect repellent containing DEET, Picaridin, oil of lemon eucalyptus, or IR3535 on exposed skin. Always follow the directions on the package.
- Wear long sleeves and pants (ideally treat clothes with permethrin or another repellent).
- Have secure screens on windows and doors to keep mosquitoes out.
- Get rid of mosquito sources in your yard by emptying standing water from flower pots, buckets and barrels. Change the water in pet dishes and replace the water in bird baths weekly. Drill holes in tire swings so water drains out. Keep children's wading pools empty and on their sides when they aren't being used.

Additionally, a person with chikungunya fever should limit their exposure to mosquito bites to avoid further spreading the infection. The person should use repellents when outdoors exposed to mosquito bites or stay indoors in areas with screens or under a mosquito net.

Treatment

There are no specific treatments for Chikungunya. There is no vaccine currently available. A Phase II vaccine trial, sponsored by the US Government and published in the *American Journal of Tropical Medicine and Hygiene* in 2000, used a live, attenuated virus, developing viral resistance in 98% of those tested after 28 days and 85% still showed resistance after one year.

A serological test for Chikungunya is available currently and is being used at various locations the world over, especiallt the South East Asia.
Chloroquine is gaining ground as a possible treatment for the symptoms associated with chikungunya, and as an antiviral agent to combat the Chikungunya virus. A study found that for arthritis-like symptoms that are not relieved by aspirin and non-steroidal anti-inflammatory drugs (NSAID), chloroquine phosphate (250 mg/day) has given promising results. Research by an Italian scientist, Andrea Savarino, and his colleagues together with a French government press release in March 2006 have added more credence to the claim that chloroquine might be effective in treating chikungunya. Unpublished studies in cell culture and monkeys show no effect of chloroquine treatment on reduction of chikungunya disease. The fact sheet on Chikungunya advises against using aspirin, ibuprofen, naproxen and other NSAIDs that are recommended for arthritic pain and fever.

Infected persons should limit further exposure to mosquito bites, stay indoors and under a mosquito net. Further, "supportive care with rest is preferred during the acute joint symptoms. Movement and mild exercise tend to improve stiffness and morning arthralgia, but heavy exercise may exacerbate rheumatic symptoms." Arthralgia remains troublesome even after 8 months. In Kerala, patients use honey-and-lime mix. Some people cite relief from consuming turmeric in low volumes.

DNA vaccine

DNA vaccination is a technique for protecting an organism against disease by injecting it with genetically engineered DNA to produce an immunological response. Nucleic acid vaccines are still experimental, and have been applied to a number of viral, bacterial and parasitic models of disease, as well as to several tumour models. DNA vaccines have a number of advantages over conventional vaccines, including the ability to induce a wider range of immune response types. A recent study report that a novel consensus-based approach to vaccine design for Chikungunya virus, employing a DNA vaccine strategy. The vaccine cassette was designed based on CHIKV Capsid and Envelope specific consensus sequences with several modifications, including codon optimization, RNA optimization, the addition of a Kozak sequence, and a substituted immunoglobulin E leader sequence. Analysis of cellular immune responses, including epitope mapping, demonstrates that these constructs induces both potent and broad cellular immunity in mice. In addition, antibody ELISAs demonstrate that these synthetic immunogens are capable of inducing high titer antibodies capable of recognizing native antigen. Taken together, these results support further study of the use of consensus CHIKV antigens in a potential vaccine cocktail.

Prognosis

Recovery from the disease varies by age. Younger patients recover within 5 to 15 days; middle-agers recover in 1 to 2.5 months. Recovery is longer for the elderly. The severity of the disease as well as its duration is less in younger patients and pregnant women. In pregnant women, no untoward effects are noticed after the infection.
Ocular inflammation from Chikungunya may present as iridocyclitis, and have retinal lesions as well.

Pedal oedema (swelling of legs) is observed in many patients, the cause of which remains obscure as it is not related to any cardiovascular, renal or hepatic abnormalities.

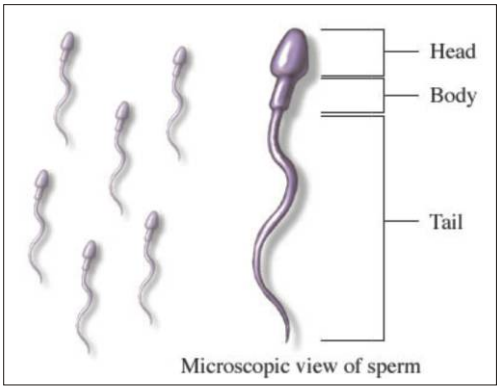
INTERPRETATION

SEMINOGRAM

Semen Analysis

A semen analysis measures the amount of semen a man produces and determines the number and quality of sperm in the semen sample.

Spermatozoa:Spermatozoa (sperm) are the male sex cells that carry a man's genetic material. Pregnancy occurs when a sperm penetrates a woman's egg (ovum). Sperm develop in a man's testicles and finish maturing in the epididymides. During ejaculation, the sperm move out of the epididymides through the vas deferens and into the urethra, the tube that runs through the penis. Semen, the thick liquid produced in the seminal vesicles and prostate gland, is added to the sperm before ejaculation. A semen analysis is usually one of the first tests done to help determine whether a man has a problem fathering a child (infertility). A problem with the semen or sperm affects more than one-third of the couples who are unable to have children (infertile).



Microscopic view of sperm

Tests that may be done during a semen analysis include:

Volume: This is a measure of how much semen is present in one ejaculation. Liquefaction time: Semen is a thick gel at the time of ejaculation and normally becomes liquid within 20 minutes after ejaculation. Liquefaction time is a measure of the time it takes for the semen to liquefy. Sperm count: This is a count of the number of sperm present per milliliter (mL) of semen in one ejaculation. Sperm morphology: This is a measure of the percentage of sperm that have a normal shape. Sperm motility: This is a measure of the percentage of sperm that can move forward normally. The number of sperm that show normal forward movement in a certain amount of semen can also be measured (motile density). pH: This is a measure of the acidity (low pH) or alkalinity (high pH) of the semen. White blood cell count: White blood cells are not normally present in semen. Fructose level: This is a measure of the amount of a sugar called fructose in the semen. The fructose provides energy for the sperm.

Indications: A semen analysis is done to determine whether: A man has a reproductive problem that is causing infertility. A vasectomy has been successful. The reversal of a vasectomy has been successful.

Preparation: Ask the client to avoid any sexual activity that results in ejaculation for 2 to 5 days before a semen analysis. This helps ensure that his sperm count will be at its highest, and it improves the reliability of the test. If possible, he should not avoid sexual activity for more than 1 to 2 weeks before this test, because a long period of sexual inactivity can result in less active sperm. Ask him to avoid drinking alcohol for a few days before the test. Do inquire regarding the medications, the client is curerently on.

Method: The client need to produce a semen sample, usually by ejaculating into a clean sample cup. He can do this in a private room or in a bathroom at your office or clinic. If he lives close to your office or clinic, he may be able to collect the semen sample at home and then transport it to the office or clinic for testing. The most common way to collect semen is by masturbation, directing the semen into a clean sample cup. He can collect a semen sample during sex by withdrawing his penis from his partner just before ejaculating (coitus interruptus). He then ejaculates into a clean sample cup. This method can be used after a vasectomy to test for the presence of sperm, but other methods will likely be recommended if one are testing for infertility. He can also collect a semen sample during sex by using a condom. If he uses a regular condom, he will need to wash it thoroughly before using it to remove any powder or lubricant on it that might kill sperm. He may also be given a special condom that does not contain any substance that kills sperm (spermicide). After the client has ejaculated, carefully remove the condom from the penis. He should then tie a knot in the open end of the condom and place it in a container that can be sealed in case the condom leaks or breaks. If he collects the semen sample at home, the sample must be received at the laboratory or clinic within 1 hour (ideally within 20 minutes or so). Keep the sample out of direct sunlight and do not allow it to get cold or hot. If it is a cold day, carry the semen sample container against the body to keep it as close to body temperature as possible. Do not refrigerate the semen sample. Since semen samples may vary from day to day, 2 or 3 different samples should be evaluated within a 3-month period for accurate testing. A semen analysis to test the effectiveness of a vasectomy is usually done 6 weeks after the vasectomy. Producing a semen sample does not cause any discomfort. However, one may feel embarrassed about the method used to collect it. If masturbation is against one's religious beliefs, discuss alternate methods of collection with your client. There are no risks associated with collecting a semen sample.

Results: A semen analysis measures the amount of semen a man produces and determines the number and quality of sperm in the semen sample. Results of a semen analysis are usually available within a day. Normal values may vary from lab to lab.

Semen analysis:

Semen volume	Normal:	1.0 - 6.5 milliliters (mL) per ejaculation
	Abnormal:	An abnormally low or high semen volume is present, which may sometimes cause fertility problems.
Liquefaction time	Normal:	Less than 60 minutes
	Abnormal:	An abnormally long liquefaction time is present, which may indicate an infection.
Sperm count	Normal:	20 - 150 million sperm per milliliter (mL) 0 sperm per milliliter if the man has had a vasectomy
	Abnormal:	A very low sperm count is present, which may indicate infertility. However, a low sperm count does not always mean that a man cannot father a child. Men with sperm counts below 1 million have fathered children.
Sperm shape (morphology)	Normal:	At least 70% of the sperm have normal shape and structure.
	Abnormal:	Sperm can be abnormal in several ways, such as having two heads or two tails, a short tail, a tiny head (pinhead), or a round (rather than oval) head. Abnormal sperm may be unable to move normally or to penetrate an egg. Some abnormal sperm are usually found in every normal semen sample. However, a high percentage of abnormal sperm may make it more difficult for a man to father a child.
Sperm movement (motility)	Normal:	At least 60% of the sperm show normal forward movement. At least 8 million sperm per milliliter (mL) show normal forward movement.
	Abnormal:	Sperm must be able to move forward (or "swim") through cervical mucus to reach an egg. A high percentage of sperm that cannot swim properly may impair a man's ability to father a child.
Semen pH	Normal:	Semen pH of 7.1 - 8.0
	Abnormal:	An abnormally high or low semen pH can kill sperm or affect their ability to move or to penetrate an egg.

White blood cells	Normal:	No white blood cells or bacteria are detected.
	Abnormal:	Bacteria or a large number of white blood cells are present, which may indicate an infection.
Fructose level	Normal:	300 milligrams (mg) of fructose per 100 milliliters (mL) of ejaculate
	Abnormal:	The absence of fructose in the semen may indicate that the man was born without seminal vesicles or has blockage of the seminal vesicles.

Certain conditions may be associated with a low or absent sperm count. These conditions include orchitis, varicocele, Klinefelter syndrome, radiation treatment to the testicles, or diseases that can cause shrinking (atrophy) of the testicles (such as mumps). If a low sperm count or a high percentage of sperm abnormalities are found, further testing may be done. Other tests may include measuring hormones, such as testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), or prolactin. Sperm morphology is routinely evaluated as part of a standard semen analysis. Morphology refers to the size and shape of the sperm. The results of a sperm morphology exam indicate the percentage of sperm that appear normal when semen is viewed under a microscope. Abnormal sperm morphology may be a contributing factor in infertility. Normal sperm have an oval head with a long tail. Abnormal sperm may have head or tail defects - such as a large or misshapen head or a crooked or double tail. These defects may impair the ability of the sperm to reach and fertilize an egg. Causes of abnormal morphology include: Testicular abnormalities that are present at birth (congenital). Enlargement of veins within the scrotum (varicocele). High fever. Illicit drug use. Infections. The best recommendation is to repeat the semen analysis in four to six weeks to determine if the changes in morphology are temporary or permanent. Even with poor morphology, pregnancy may still be possible. Intrauterine insemination or in vitro fertilization methods are sometimes needed. A small sample (biopsy) of the testicles may be needed for further evaluation if the sperm count or motility is extremely low.

Factors that affect the test: Factors that can interfere with semen test or the accuracy of the results include: Medicines, such as cimetidine (Tagamet), male and female hormones (testosterone, estrogen), sulfasalazine, nitrofurantoin, and some chemotherapy medicines. Caffeine, alcohol, cocaine, marijuana, and smoking tobacco. Herbal medicines, such as St. John's wort and high doses of echinacea. A semen sample that gets cold. The sperm motility value will be inaccurately low if the semen sample gets cold. Exposure to radiation, some chemicals (such as certain pesticides or spermicides), and prolonged heat exposure. An incomplete semen sample. This is more common if a sample is collected by methods other than masturbation. Not ejaculating for several days. This may affect the semen volume.

Do not forget: A semen sample collected at home must be received at the laboratory or clinic within 1 hour. Keep the sample out of direct sunlight and do not allow it to get cold or hot. If it is a cold day, carry the semen sample container against your body to keep it as close to body temperature as possible. Do not refrigerate the semen sample. Consistently detecting sperm in the semen of a man who has had a vasectomy indicates that his surgery was not successful, and another form of birth control should be used to prevent pregnancy. A low number of sperm may be present in a semen sample taken initially after a vasectomy. However, sperm should not be present in subsequent samples. A man whose mother took the medicine diethylstilbestrol (DES) during her pregnancy with him has a greater-than-normal risk of being unable to father a child (infertile). Additional tests may include measuring hormone levels, such as testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), or prolactin. For more information, see the medical tests Testosterone, Luteinizing Hormone, Follicle-Stimulating Hormone, and Prolactin. Other fertility testing, including sperm penetration, the presence of antisperm antibodies, or analysis after sexual intercourse (postcoital), may be recommended for infertility problems. For more information, see the medical test under Infertility Testing.


Sperm fertilising an ovum

BOUQUET

In Lighter Vein

I guess some things will never change. I hired a temp while my secretary was on maternity leave. Trying to arrive at an agreeable wage, I asked what she expected to earn.

She said, "Well... the minimum I could work for is four hundred a week." I told her I'd give her that much with pleasure.

She shook her head and replied, "With pleasure, it'll be $600 a week."

A doctor vacationing on the Riviera met an old lawyer friend and asked him what he was doing there. The lawyer replied, "Remember that lousy real estate I bought? Well, it caught fire, so here I am with the fire insurance proceeds. What are you doing here?"

The doctor replied, "Remember that lousy real estate I had in Mississippi? Well, the river overflowed, and here I am with the flood insurance proceeds."

The lawyer looked puzzled. "Gee," he asked, "how do you start a flood?"

One day a college professor of Psychology was greeting his new college class.

He stood up in front of the class and said, "Would everyone who thinks he or she is stupid please stand up?"

After a minute or so of silence, a young man stood up.

"Well, hello there sir. So you actually think you're a moron?" the professor asked.

The kid replied, "No sir, I just didn't want to see you standing there all by yourself."

Wisdom Whispers

- "No one gets into trouble without his own help."
- "You can't measure the whole world with your own yardstick."
- "To every problem there is already a solution whether you know it or not."
- "It rings, it is empty."
- "It is unwise to be too sure of one's own wisdom. It is healthy to be reminded that the strongest might weaken and the wisest might err."
- "An abundance of money ruins youth."
- "The law says what the king pleases."
- "The buyer has need of a hundred eyes. the seller but one."
- "Had you gotten up early, you wouldn't have needed to stay up late."

Brain Teasers

1. IgG, IgA and IgM, all may be reduced in
   A. Acute thermal burn B. Nephrotic syndrome C. Protein losing enteropathy
   D. All of the above
2. B cell area in a lymph node is
   A. Paracortical region B. Medullary region C. Secondary follicle D. Cortical region
3. Unencapsulated lymphoid tissue I man is seen in
   A. Spleen B. Lamina propria of GIT C. Lymph nodes D. Thymus
4. B cell is asscoiated with…
   A. Mycosis fungoides B. Lymphoblastic lymphoma C. CLL D. Sezary Syndrome
5. Null cells are commonly assocaite with…
   A. ALL B. Burkitt's lymphoma C. Multiple myeloma D. Hodgkin's disease
6. Type I amyloidosis may occur in
   A. Multiple myeloma B. Hodgkin's disease, C. Familial Medirerranean fever
   D. Rheumatoid arthritis

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

TROUBLESHOOTING

SPERM COUNTS

Safety Precautions

Safety precautions should be observed when handling seminal fluid. The following guidelines should be followed:

- If non-disposable items are used, soak contaminated items (e.g. hemacytometers and coverslips) in 70% alcohol or Alconox.
- All disposable items should be placed in a biohazard bag for autoclaving.
- Gloves must be worn and hands thoroughly washed when the examination is completed.
- Seminal fluids that are to be discarded should be placed in biohazard bags for autoclaving.

Sperm counting methods

Sperm can be counted either manually or by automated methods. Although automated counting has some advantages for assessment of motility parameters, manual counting is still performed by most laboratories. There are several manual counting methods available for semen.

These include:

- Neubauer hemacytometer.
- Makler chamber.
- CellVu.
- MicroCell.

The Makler, CellVu, and MicroCell methods have the advantage of requiring no dilution of the semen. Since semen is viscous, accurate dilution can be problematic. These methods also allow counting of motile and non-motile sperm at the same time and thus avoid the need for separate assessment via wet mount. Each laboratory should determine the best most reproducible method for their own situation, equipment, and expertise.

Calculating sperm count on a hemacytometer:

The formula for calculating the sperm count when 5 small squares within the large center square are counted is:

- Number of sperm counted in 25 squares on each of 2 sides x dilution factor/volume x 1000 = sperm/ml.
- Example: 100 sperm are counted in the five small squares of one side of the hemacytometer, 110 sperm are counted in 5 small squares of the other. The dilution is 1:20.
- Number of sperm in 25 squares on 2 sides = 210 x 5 = 1050 Sperm/ml = 1050 x 20 (dilution factor) divided by 0.2 mm^3 x 1000 = 105 million sperm/ml.

Diluting a specimen for counting on a hemacytometer

Following liquefaction (20-30 minutes), mix the sample manually by swirling the container several times. Thorough mixing is essential for accurate counting. Calibrated automatic pipettes are used to prepare a dilution. Because of the viscosity of semen, the semen should be added to the diluent using a positive pressure pipettor.

The dilution often used for routine sperm counts is 1:20 but the actual dilution factor will vary depending on the total sperm count. For high concentration specimens a greater dilution will be necessary. For low concentrations an undiluted or minimally diluted specimen may be required. The appropriate dilution is determined by estimating the concentration needed to do a count of at least 100 cells per side of the loaded hemacytometer.

The diluent that may be used for sperm counts on a hemacytometer can be as follows: 5 gm of sodium bicarbonate in 100 ml of distilled water, plus 1ml of formalin (neutral).

Other counting chambers

Some professionals believe that sperm counts done by hemacytometer are not accuate because of the need to dilute the viscous semen prior to counting. There are several other counting methods available to assess sperm concentration.

The advantages of the following methods are:

- The specimen does not have to be diluted.
- Motile and non-motile sperm can both be counted avoiding the need for wet mount evaluation of motile cells.

Note that counting moving sperm can be difficult and takes significant practice to avoid error.

For each of these methods accurate counts are best obtained when at least 100 sperm per replicate are counted.

Makler: An undiluted sample is placed on the chamber and covered with the coverglass. Ten squares on the grid contain 0.000001ml.

CellVu: Two sides of a special slide are loaded with a drop of undiluted semen. Coverslips with special grids are placed on top of the sperm according to manufacturer's directions. Sperm on both sides are counted.

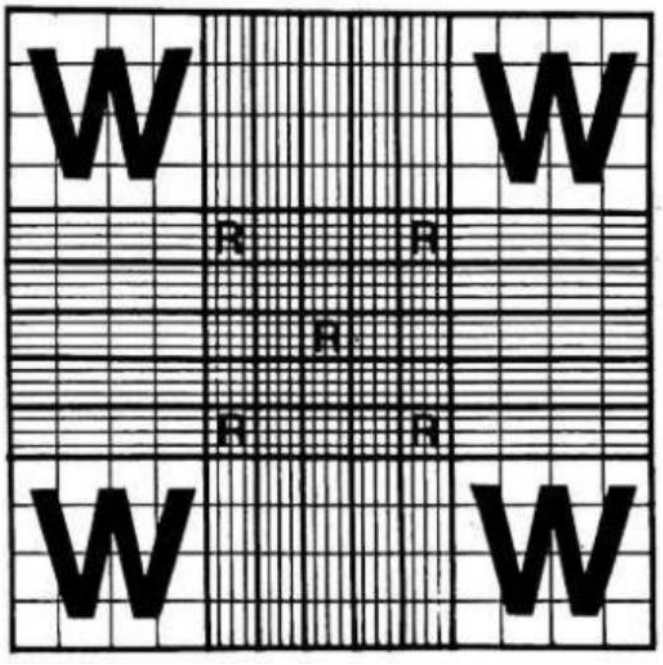
MicroCell: It has two chambers on a single, disposable slide. A special eyepiece with a grid is needed for counting.

Loading and counting using a hemacytometer

Fill both sides of the hemacytometer. Focus on the large center square with the 20X objective. The counting area consists of five small squares in the large center square. The squares usually counted are the four corner squares and the center square, all of which are marked R. A minimum of 100 sperm should be counted in the five small “R” squares. If the number of sperm is low then 10 squares or all squares may be counted to obtain the 100 per side. Count both sides of the hemacytometer and take the average of the two counts to calculate the actual count per ml.

Neubauer hemacytometer

The picture below shows the counting chamber of the Neubauer hemacytometer. This counting method is used to count many types of cells. To use this chamber for counting sperm the specimen will usually need to be diluted. Proper loading of the hemacytometer is also important for accurate sperm counts to be obtained.



TULIP NEWS

Tulip Group
INTRODUCES

AccuBas™ Ax1
BIOLOGICAL AIR SAMPLER

The air around us is teeming with microbes. Bacteria, fungi, algae, protozoa and viruses float in air currents. In the air of hospital wards or the rooms of persons suffering from infectious diseases, microbes such as tubercle bacilli, streptococci, pneumococci and staphylococci have been demonstrated. GMP, FDA, USP and ISO 14698 guidelines recommend air monitoring of sterile areas for environmental monitoring. Tulip Group has taken keen interest in developing a system for monitoring and detection of aerial microbes AccuBas™Ax1 based on Andersen Impaction principle. AccuBas™Ax1, is a user friendly, biological air sampler for hospital/operation theatres, pathological laboratories, medical research centres, public health centres, Blood donation centres, Blood banks, Dental institutes, Tissue culture laboratories, Biotechnology institutes, Nursing homes and Microbiology labs

FEATURES

- Non corrosive - since it is made of aviation aluminum body!
- It facilitates onsite - testing - since the battery operation capacity is upto 4 hours!- also it is portable & light weight!
- No turbulence created by testing person - since it is fitted with a feature called- programmable delay time
- Application oriented & convenient - since it is fitted with a feature called- programmable air speed!
- No need for setting the date & time before every run - since it comes with rtc (real time clock) battery for lifetime of about 10 years!
- Flexible usage - since it can accomodate commercially available 90 mm standard petriplate !
- Easy comparison with previous data - since it has capacity to store data upto 256 samples!
- Rechargeable battery operation & low battery indication - which provides 4 hours back - up !
- Easy to handle - since it has user friendly software !

ASSESS, CONTROL & MONITOR AERIAL MICROBES WITH AccuBas™ Ax1 BIOLOGICAL AIR SAMPLER

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