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BIMONTHLY FORUM FOR THE LABORATORIANS

CONTENTS

Editorial

IN MEDICINE THERE IS NOTHING WHICH IS NEVER AND NOTHING WHICH IS ALWAYS. Gone are the days when Diabetes mellitus presents with the classical triad of symptoms. Here today, Gone tomorrow! No, no, not vanished but shifted to another place somewhere on the Globe!

A disease presently rampant in West Africa can suddenly emanate globally. We all must be aware about all its intricacies. Though not in the Indian subcontinent, we hereby present this potentially outrageous often fatal disease called **EVD**. If it goes on unabated, the disease is likely to inflict 1.5 million patients by the year end. BEWARE!! **DISEASE DIAGNOSIS** outlines this disease in a simplistic manner for you.

The Ebola virus causes an acute, serious illness which is often fatal if untreated. Ebola virus disease (EVD) first appeared in 1976 in 2 simultaneous outbreaks, one in Nzara, Sudan, and the other in Yambuku, Democratic Republic of Congo. The latter occurred in a village near the Ebola River, from which the disease takes its name.

The current outbreak in west Africa, (first cases notified in March 2014), is the largest and most complex Ebola outbreak since the Ebola virus was first discovered in 1976. There have been more cases and deaths in this outbreak than all others combined. It has also spread between countries starting in Guinea then spreading across land borders to Sierra Leone and Liberia, by air (1 traveller only) to Nigeria, and by land (1 traveller) to Senegal.

The most severely affected countries, Guinea, Sierra Leone and Liberia have very weak health systems, lacking human and infrastructural resources, having only recently emerged from long periods of conflict and instability. On August 8, the WHO Director-General declared this outbreak a Public Health Emergency of International Concern.

A separate, unrelated Ebola outbreak began in Boende, Equateur, an isolated part of the Democratic Republic of Congo.

The virus family Filoviridae includes 3 genera: Cuevavirus, Marburgvirus, and Ebolavirus. There are 5 species that have been identified: Zaire, Bundibugyo, Sudan, Reston and Taï Forest. The first 3, Bundibugyo ebolavirus, Zaire ebolavirus, and Sudan ebolavirus have been associated with large outbreaks in Africa. The virus causing the 2014 west African outbreak belongs to the Zaire species. The article presented was written in the month of September 2014.

The **INTERPRETATION** segment outlines a very simple but significant test as urinalysis and **TROUBLE SHOOTING** takes urinalysis by defining the right sample collection, transportation techniques etc.

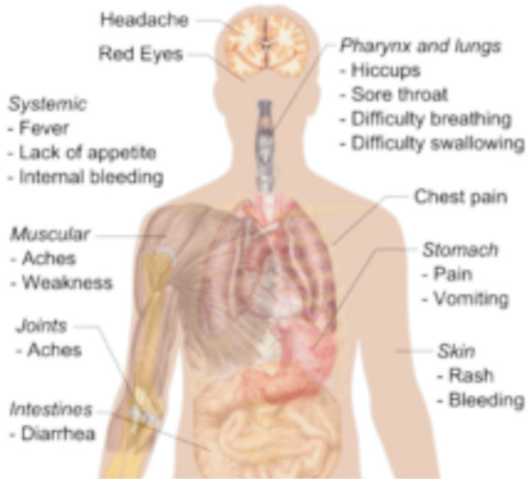
Amidst all this **BOUQUET** hasn't been forgotten!

DISEASE DIAGNOSIS

Ebola virus disease

Ebola virus disease (**EVD**) or **Ebola hemorrhagic fever** (**EHF**) is the human disease caused by the Ebola virus. Symptoms typically start two days to three weeks after contracting the virus, with a fever, sore throat, muscle pains, and headaches. Typically nausea, vomiting, and diarrhea follow, along with decreased functioning of the liver and kidneys. At this point, some people begin to have bleeding problems. The virus may be acquired upon contact with blood or bodily fluids of an infected animal (commonly monkeys or fruit bats). Spread through the air has not been documented in the natural environment. Fruit bats are believed to carry and spread the virus without being affected. Once human infection occurs, the disease may spread between people as well. Male survivors may be able to transmit the disease via semen for nearly two months. In order to make the diagnosis, typically other diseases with similar symptoms such as malaria, cholera and other viral hemorrhagic fevers are first excluded. To confirm the diagnosis blood samples are tested for viral antibodies, viral RNA, or the virus itself. Prevention includes decreasing the spread of disease from infected monkeys and pigs to humans. This may be done by checking such animals for infection and killing and properly disposing of the bodies if the disease is discovered. Properly cooking meat and wearing protective clothing when handling meat may also be helpful, as are wearing protective clothing and washing hands when around a person with the disease. Samples of bodily fluids and tissues from people with the disease should be handled with special caution. There is no specific treatment for the disease; efforts to help persons who are infected include giving either oral rehydration therapy (slightly sweet and salty water to drink) or intravenous fluids. The disease has high mortality rate: often killing between 50% and 90% of those infected with the virus. EVD was first identified in Sudan and the Democratic Republic of the Congo. The disease typically occurs in outbreaks in tropical regions of Sub-Saharan Africa. From 1976 (when it was first identified) through 2013, fewer than 1,000 people per year have been infected. The largest outbreak to date is the ongoing 2014 West Africa Ebola outbreak, which is affecting Guinea, Sierra Leone, Liberia and Nigeria. As of August 2014 more than 1750 suspected cases have been reported. Efforts are ongoing to develop a vaccine; however, none yet exists.

Signs and symptoms



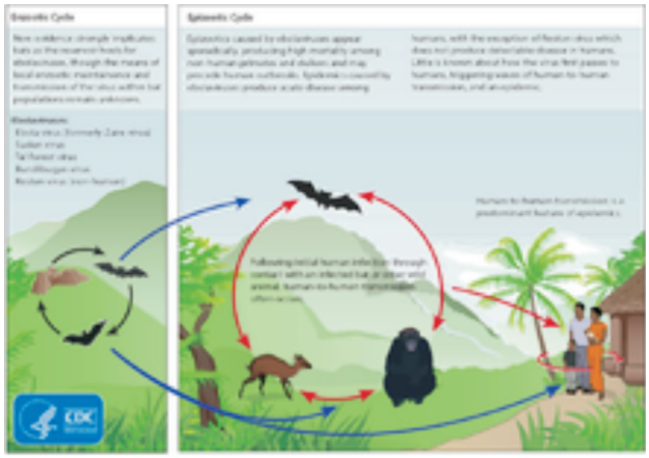
Symptoms of Ebola.

Signs and symptoms of Ebola usually begin suddenly with a flu-like stage characterized by fatigue, fever, headaches, and joint, muscle, and abdominal pain.[9][10] Vomiting, diarrhea and loss of appetite are also common.[10] Less common symptoms include the following: sore throat, chest pain, hiccups, shortness of breath and trouble swallowing. The average time between contracting the infection and the start of symptoms is 8 to 10 days, but it can vary between 2 and 21 days. Skin manifestations may include a maculopapular rash (in about 50% of cases). Early symptoms of EVD may be similar to those of malaria, dengue fever, or other tropical fevers, before the disease progresses to the bleeding phase.

Bleeding

All people infected show some symptoms of circulatory system involvement, including impaired blood clotting. In 40–50% of cases, bleeding from puncture sites and mucous membranes (e.g. gastrointestinal tract, nose, vagina and gums) has been reported. In the bleeding phase, internal and subcutaneous bleeding may present itself through reddening of the eyes and bloody vomit. Bleeding into the skin may create petechiae, purpura, ecchymoses, and hematomas (especially around needle injection sites). Types of bleeding known to occur with Ebola virus disease include vomiting blood, coughing it up or blood in the stool. Heavy bleeding is rare and is usually confined to the gastrointestinal tract. In general, the development of bleeding symptoms often indicates a worse prognosis and this blood loss can result in death

Causes



Life cycles of the Ebolavirus

EVD is caused by four of five viruses classified in the genus *Ebolavirus*, family *Filoviridae*, order *Mononegavirales*. These four viruses are Bundibugyo virus (BDBV), Ebola virus (EBOV), Sudan virus (SUDV), Taï Forest virus (TAFV). The fifth virus, Reston virus (RESTV), is not thought to be disease-causing in humans. During an outbreak, those at highest risk are health care workers and close contacts of those with the infection.

Transmission

It is not entirely clear how Ebola is spread. EVD is believed to occur after an ebola virus is transmitted to an initial human by contact with an infected animal's body fluids. Human-to-human transmission can occur via direct contact with blood or bodily fluids from an infected person (including embalming of an infected dead person) or by contact with contaminated medical equipment, particularly needles and syringes. Semen is infectious in survivors for up to 50 days. Transmission through oral exposure and through conjunctiva exposure is likely and has been confirmed in non-human primates. The potential for widespread EVD infections is considered low as the disease is only spread by direct

contact with the secretions from someone who is showing signs of infection. The quick onset of symptoms makes it easier to identify sick individuals and limits a person's ability to spread the disease by traveling. Because dead bodies are still infectious, some doctors disposed of them in a safe manner, despite local traditional burial rituals. Medical workers who do not wear appropriate protective clothing may also contract the disease. In the past, hospital-acquired transmission has occurred in African hospitals due to the reuse of needles and lack of universal precautions. Airborne transmission has not been documented during previous EVD outbreaks. They are, however, infectious as breathable 0.8–1.2 micrometre laboratory generated droplets; because of this potential route of infection, these viruses have been classified as Category A biological weapons. Recently the virus has been shown to travel without contact from pigs to non-human primates. Bats drop partially eaten fruits and pulp, then land mammals such as gorillas and duikers feed on these fallen fruits. This chain of events forms a possible indirect means of transmission from the natural host to animal populations, which has led to research towards viral shedding in the saliva of bats. Fruit production, animal behavior, and other factors vary at different times and places that may trigger outbreaks among animal populations.

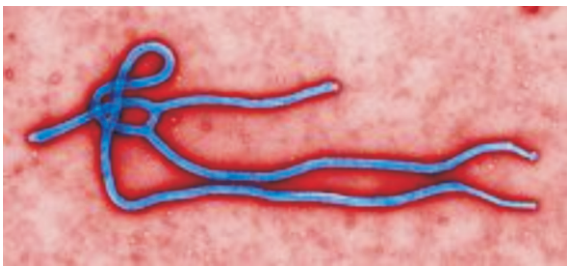
Reservoir

Bushmeat being prepared for cooking in Ghana, 2013. Human consumption of equatorial animals in Africa in the form of bushmeat has been linked to the transmission of diseases to people, including Ebola.

Bats are considered the most likely natural reservoir of the Ebola virus (EBOV); plants, arthropods, and birds have also been considered. Bats were known to reside in the cotton factory in which the first cases for the 1976 and 1979 outbreaks were employed, and they have also been implicated in Marburg virus infections in 1975 and 1980. Of 24 plant species and 19 vertebrate species experimentally inoculated with EBOV, only bats became infected. The absence of clinical signs in these bats is characteristic of a reservoir species. In a 2002–2003 survey of 1,030 animals including 679 bats from Gabon and the Republic of the Congo, 13 fruit bats were found to contain EBOV RNA fragments. As of 2005, three types of fruit bats (*Hypsignathus monstrosus*, *Epomops franqueti*, and *Myonycteris torquata*) have been identified as being in contact with EBOV. They are now suspected to represent the EBOV reservoir hosts. Antibodies against Ebola Zaire and Reston viruses have been found in fruit bats in Bangladesh, thus identifying potential virus hosts and signs of the filoviruses in Asia. Between 1976 and 1998, in 30,000 mammals, birds, reptiles, amphibians, and arthropods sampled from outbreak regions, no *ebolavirus* was detected apart from some genetic traces found in six rodents (*Mus setulosus* and *Praomys*) and one shrew (*Sylvisorex ollula*) collected from the Central African Republic. Traces of EBOV were detected in the carcasses of gorillas and chimpanzees during outbreaks in 2001 and 2003, which later became the source of human infections. However, the high lethality from infection in these species makes them unlikely as a natural reservoir. Transmission between natural reservoir and humans is rare, and outbreaks are usually traceable to a single case where an individual has handled the carcass of gorilla, chimpanzee, or duiker. Fruit bats are also eaten by people in parts of West Africa where they are smoked, grilled or made into a spicy soup.

Virology

Genome



Electron micrograph of an Ebola virus virion

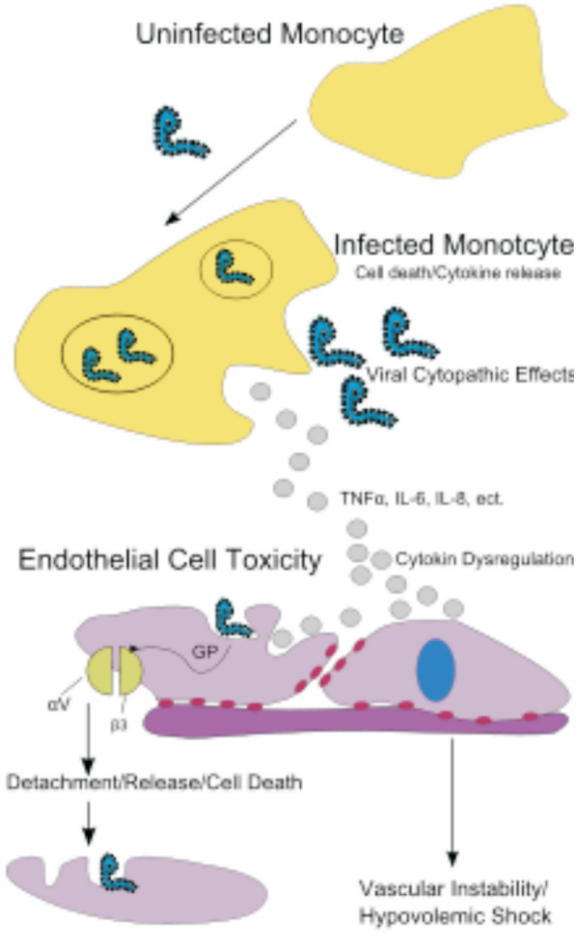
Like all mononegaviruses, ebolavirions contain linear nonsegmented, single-strand, non-infectious RNA genomes of negative polarity that possesses inverse-complementary 3' and 5' termini, do not possess a 5' cap, are not polyadenylated, and are not covalently linked to a protein. Ebolavirus genomes are approximately 19 kilobase pairs long and contain seven genes in the order 3'-UTR-*NP*-*VP35*-*VP40*-*GP*-*VP30*-*VP24*-*L*-5'-UTR. The genomes of the five different ebolaviruses (BDBV, EBOV, RESTV, SUDV, and TAFV) differ in sequence and the number and location of gene overlaps.

Structure: Like all filoviruses, ebolavirions are filamentous particles that may appear in the shape of a shepherd's crook or in the shape of a "U" or a "6", and they may be coiled, toroid, or branched. In general, ebolavirions are 80 nm in width, but vary somewhat in length. In general, the median particle length of ebolaviruses ranges from 974 to 1,086 nm (in contrast to marburgvirions, whose median particle length was measured at 795–828 nm), but particles as long as 14,000 nm have been detected in tissue culture.

Replication: The ebolavirus life cycle begins with virion attachment to specific cell-surface receptors, followed by fusion of the virion envelope with cellular membranes and the concomitant release of the virus nucleocapsid into the cytosol. The viral RNA polymerase, encoded by the L gene, partially uncoats the nucleocapsid and transcribes the genes into positive-strand mRNAs, which are then translated into structural and nonstructural proteins. Ebolavirus RNA polymerase (L) binds to a single promoter located at the 3' end of the genome. Transcription either terminates after a gene or continues to the next gene downstream. This means that genes close to the 3' end of the genome are transcribed in the greatest abundance, whereas those toward the 5' end are least likely to be transcribed. The gene order is, therefore, a simple but effective form of transcriptional regulation. The most abundant protein produced is the nucleoprotein, whose concentration in the cell determines when L switches from gene transcription to genome replication. Replication results in full-length, positive-strand antigenomes that are, in turn, transcribed into negative-strand virus progeny genome copy. Newly synthesized structural proteins and genomes self-assemble and accumulate near the inside of the cell membrane. Virions bud off from the cell, gaining their envelopes from the cellular membrane they bud from. The mature progeny particles then infect other cells to repeat the

cycle.The Ebola Virus genetics are difficult to study due to its virulent nature

Pathophysiology



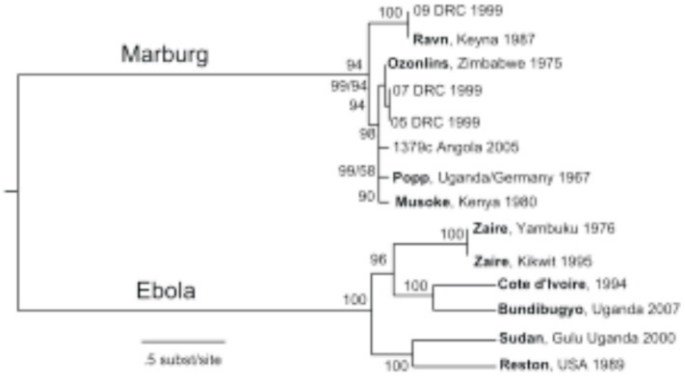
Pathogenesis schematic

Endothelial cells, mononuclear phagocytes, and hepatocytes are the main targets of infection. After infection, a secreted glycoprotein (sGP) known as the Ebola virus glycoprotein (GP) is synthesized. Ebola replication overwhelms protein synthesis of infected cells and host immune defenses. The GP forms a trimeric complex, which binds the virus to the endothelial cells lining the interior surface of blood vessels. The sGP forms a dimeric protein that interferes with the signaling of neutrophils, a type of white blood cell, which allows the virus to evade the immune system by inhibiting early steps of neutrophil activation. These white blood cells also serve as carriers to transport the virus throughout the entire body to places such as the lymph nodes, liver, lungs, and spleen. The presence of viral particles and cell damage resulting from budding causes the release of cytokines (to be specific, TNF-α, IL-6, IL-8, etc.), which are the signaling molecules for fever and inflammation. The cytopathic effect, from infection in the endothelial cells, results in a loss of vascular integrity. This loss in vascular integrity is furthered with synthesis of GP, which reduces specific integrins responsible for cell adhesion to the inter-cellular structure, and damage to the liver, which leads to coagulopathy.

Diagnosis

The medical history, especially travel and work history along with exposure to wildlife are important to suspect the diagnosis of EVD. The diagnosis is confirmed by isolating the virus, detecting its RNA or proteins, or detecting antibodies against the virus in a person's blood. Isolating the virus by cell culture, detecting the viral RNA by polymerase chain reaction (PCR) and detecting proteins by enzyme-linked immunosorbent assay (ELISA) is effective early and in those who have died from the disease. Detecting antibodies against the virus is effective late in the disease and in those who recover. During an outbreak, virus isolation is often not feasible. The most common diagnostic methods are therefore real time PCR and ELISA detection of proteins, which can be performed in field or mobile hospitals. Filovirions can be seen and identified in cell culture by electron microscopy due to their unique filamentous shapes, but electron microscopy cannot tell the difference between the various filoviruses despite there being some length differences.

Classification



Phylogenetic tree comparing the Ebolavirus and Marburgvirus. Numbers indicate percent confidence of branches.

The genera *Ebolavirus* and *Marburgvirus* were originally classified as the species of the now-obsolete *Filovirus* genus. In March 1998, the Vertebrate Virus Subcommittee proposed in the International Committee on Taxonomy of Viruses (ICTV) to change the *Filovirus* genus to the *Filoviridae* family with two specific genera: *Ebola-like viruses* and *Marburg-like viruses*. This proposal was implemented in Washington, DC, on April 2001 and in Paris on July 2002. In 2000, another proposal was made in Washington, D.C., to change the "-like viruses" to "-virus" resulting in today's *Ebolavirus* and *Marburgvirus*. Rates of genetic change are 100 times slower than influenza A in humans, but on the same magnitude as those of hepatitis B. Extrapolating backwards using these rates indicates that Ebolavirus and Marburgvirus diverged several thousand years ago. However, paleoviruses (genomic fossils) of filoviruses (Filoviridae) found in mammals indicate that the family itself is at least tens of millions of years old. Fossilized viruses that are closely related to ebolaviruses have been found in the genome of the Chinese hamster.

Differential diagnosis

The symptoms of EVD are similar to those of Marburg virus disease. It can also easily be confused with many other diseases common in Equatorial Africa such as other viral hemorrhagic fevers, falciparum malaria, typhoid fever, shigellosis, rickettsial diseases such as typhus, cholera, gram-negative septicemia, borreliosis such as relapsing fever or EHEC enteritis. Other infectious diseases that should be included in the differential diagnosis include the following: leptospirosis, scrub typhus, plague, Q fever, candidiasis, histoplasmosis, trypanosomiasis, visceral leishmaniasis, hemorrhagic smallpox, measles, and fulminant viral hepatitis. Non-infectious diseases that can be confused with EVD are acute promyelocytic leukemia, hemolytic uremic syndrome, snake envenomation, clotting factor deficiencies/platelet disorders, thrombotic thrombocytopenic purpura, hereditary hemorrhagic telangiectasia, Kawasaki disease, and even warfarin poisoning.

Prevention

A researcher working with the Ebola virus while wearing a BSL-4 positive pressure suit to avoid infection

Behavioral changes

Ebola viruses are contagious, with prevention predominantly involving behavior changes, proper full-body personal protective equipment, and disinfection. Techniques to avoid infection involve not contacting infected blood or secretions, including from those who are dead. This involves suspecting and diagnosing the disease early and using standard precautions for all patients in the healthcare setting. Recommended measures when caring for those who are infected include isolating them, sterilizing equipment, and wearing protective clothing including masks, gloves, gowns and goggles. Hand washing is important but can be difficult in areas where there is not even enough water for drinking. Due to lack of proper equipment and hygienic practices, large-scale epidemics have occurred mostly in poor, isolated areas without modern hospitals or well-educated medical staff. Traditional burial rituals, especially those requiring embalming of bodies, should be discouraged or modified. Airline crews who fly to these areas of the world are taught to identify Ebola and isolate anyone who has symptoms.

Quarantine: Quarantine, also known as enforced isolation, is usually effective in decreasing spread. Governments often quarantine areas where the disease is occurring or individuals who may be infected. In the United States the law allows quarantine of those infected with Ebola. The lack of roads and transportation may help slow the disease in Africa. During the 2014 outbreak Liberia closed schools.

Vaccine: No vaccine is currently available for humans. The most promising candidates are DNA vaccines or vaccines derived from adenoviruses, vesicular stomatitis Indiana virus (VSIV) or filovirus-like particles (VLPs) because these candidates could protect nonhuman primates from ebolavirus-induced disease. DNA vaccines, adenovirus-based vaccines, and VSIV-based vaccines have entered clinical trials. Vaccines have protected nonhuman primates. Immunization takes six months, which impedes the counter-epidemic use of the vaccines. Searching for a quicker onset of effectiveness, in 2003 a vaccine using an adenoviral (ADV) vector carrying the Ebola spike protein was tested on crab-eating macaques. Twenty-eight days later they were challenged with the virus and remained resistant. A vaccine based on attenuated recombinant vesicular stomatitis virus (VSV) vector carrying either the Ebola glycoprotein or the Marburg glycoprotein in 2005 protected nonhuman primates, opening clinical trials in humans. The study by October completed the first human trial, over three months giving three vaccinations safely inducing an immune response. Individuals for a year were followed, and, in 2006, a study testing a faster-acting, single-shot vaccine began; this new study was completed in 2008. Trying the vaccine on a strain of Ebola that more resembles one that infects humans is the next step. On 6 December 2011, the development of a successful vaccine against Ebola for mice was reported. Unlike the predecessors, it can be freeze-dried and thus stored for long periods in wait for an outbreak. An experimental vaccine made by researchers at Canada's national laboratory in Winnipeg was used in 2009 to pre-emptively treat a German scientist who might have been infected during a lab accident. However, actual EBOV infection could never be demonstrated without a doubt. Experimentally, recombinant vesicular stomatitis Indiana virus (VSIV) expressing the glycoprotein of EBOV or SUDV has been used successfully in nonhuman primate models as post-exposure prophylaxis.

Laboratory: Ebola viruses are World Health Organization Risk Group 4 pathogens, requiring biosafety level 4-equivalent containment. Laboratory researchers must be properly trained in BSL-4 practices and wear proper personal protective equipment.

Treatment

A hospital isolation ward in Gulu, Uganda, during the October 2000 outbreak

No ebolavirus-specific treatment exists. Treatment is primarily supportive in nature and includes minimizing invasive procedures, balancing fluids and electrolytes to counter dehydration, administration of anticoagulants early in infection to prevent or control disseminated intravascular coagulation, administration of procoagulants late in infection to control bleeding, maintaining oxygen levels, pain management, and the use of medications to treat bacterial or fungal secondary infections. Early treatment may increase the chance of survival. A number of experimental treatments are being studied.

Prognosis

The disease has a high mortality rate: often between 50 percent and 90 percent. If an infected person survives, recovery may be quick and complete. Prolonged cases are often complicated by the occurrence of long-term problems, such as inflammation of the testicles, joint pains, muscle pains, skin peeling, or hair loss. Eye symptoms, such as light sensitivity, excess tearing, iritis, iridocyclitis, choroiditis and blindness have also been described. EBOV and SUDV may be able to persist in the semen of some survivors for up to seven weeks, which could give rise to infections and disease via sexual intercourse.

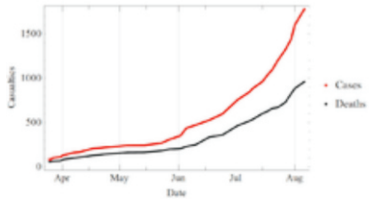
Epidemiology

CDC worker incinerates medical waste from Ebola patients in Zaire in 1976

The disease typically occurs in outbreaks in tropical regions of Sub-Saharan Africa. From 1976 (when it was first identified) through 2013, fewer than 1,000 people per year have been infected. The largest outbreak to date is the ongoing 2014 West Africa Ebola outbreak, which is affecting Guinea, Sierra Leone, and Liberia. As of August 2014 it is also affecting Nigeria. As of the end of July 2014 more than 1320 cases have been identified.

2007 to 2013: At various times, varying intensity outbreaks have been reported in Sub-Saharan Africa

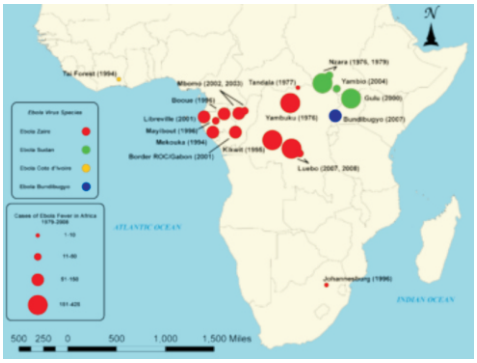
2014 outbreak: 2014 West Africa Ebola outbreak



Increase over time in the cases and deaths during the 2014 outbreak.

In March 2014, an outbreak of the Ebola virus occurred in the Western African nation of Guinea. This is the first Ebola virus outbreak registered in the region. As of 10 April, 157 suspected and confirmed cases and 101 deaths had been reported in Guinea, 22 suspected cases in Liberia including 14 deaths, 8 suspected cases in Sierra Leone including 6 deaths, and 1 suspected case in Mali. By late June 2014, the death toll had reached 390 with over 600 cases reported. By 23 July 2014, the World Health Organization had reported 1201 confirmed cases including 672 deaths since the epidemic began in March. On 31 July 2014, WHO reports the death toll has reached 826 from 1440 cases. Emory University Hospital was the first US hospital to care for people exposed to Ebola. Two American medical providers, Kent Brantly and Nancy Writebol, were exposed while treating infected patients in Liberia. Arrangements were made for them to be transported to Emory via speciality aircraft. Emory Hospital has a specially built isolation unit set up in collaboration with the CDC to treat people exposed to certain serious infectious diseases. On 2 August 2014, Brantly was flown in to Dobbins Air Force Base in Marietta, Georgia, and transferred to Emory Hospital. On 8th August 2014, the World Health Organization declared it to be an international public health emergency. On 6 August 2014, a 40-year old Saudi man suspected of having Ebola died at a Jeddah hospital in Saudi Arabia. The man had recently traveled to Sierra Leone.

History



Cases of ebola fever in Africa from 1979 to 2008.

Society and culture: Given the lethal nature of Ebola, and since no approved vaccine or treatment is available, it is classified as a biosafety level 4 agent, as well as a Category A bioterrorism agent by the Centers for Disease Control and Prevention. It has the potential to be weaponized for use in biological warfare.

Other animals: In general, outbreaks of EVD among human populations result from handling infected wild animal carcasses. In general, declines in animal populations precede outbreaks among human populations. Since 2003, such declines have been monitored through surveillance of animal populations with the aim of predicting and preventing EVD outbreaks in humans. Recovered carcasses from gorillas contain multiple Ebola virus strains, which suggest multiple introductions of the virus. Bodies decompose quickly and carcasses are not infectious after three to four days. Contact between gorilla groups is rare, suggesting transmission among gorilla groups is unlikely, and that outbreaks result from transmission between viral reservoir and animal populations. Ebola has a high mortality among primates. Frequent outbreaks of Ebola may have resulted in the deaths of 5,000 gorillas. Outbreaks of EVD may have been responsible for an 88% decline in tracking indices of observed chimpanzee populations in 420 square kilometer Lossi Sanctuary between 2002 and 2003. Transmission among chimpanzees through meat consumption constitutes a significant risk factor, while contact between individuals, such as touching dead bodies and grooming, is not. **Domestic animals:** Ebola virus can be transmitted to dogs and pigs. While dogs may be asymptomatic, pigs tend to develop symptomatic disease.

Research

Medications: Favipiravir looks like it may be useful in a mouse model of the disease. Estrogen receptor drugs used to treat infertility and breast cancer (clomiphene and toremifene) inhibit the progress of Ebola virus in infected mice. Ninety percent of the mice treated with clomiphene and fifty percent of those treated with toremifene survived the tests. Given their oral availability and history of human use, these drugs would be candidates for treating Ebola virus infection in remote geographical locations, either on their own or together with other antiviral drugs.

Antibodies

Researchers looking at slides of cultures of cells that make monoclonal antibodies. These are grown in a lab and the researchers are analyzing the products to select the most promising of them.

During an outbreak 1999 in the Democratic Republic of the Congo, seven of eight people who received blood transfusions from individuals who had previously survived the infection survived themselves. However, this potential treatment is considered controversial. Intravenous antibodies appear to be protective in non-human primates who have been exposed to large doses of ebola. On July 31, 2014, an experimental drug, ZMapp, was first tested on humans. It was administered to two Americans who had been infected with Ebola. Both people appeared to have had positive results.

Other treatments: Other promising treatments rely on antisense technology. Both small interfering RNAs (siRNAs) and phosphorodiamidate morpholino oligomers (PMOs) targeting the Zaire Ebola virus (ZEBOV) RNA polymerase L protein could prevent disease in nonhuman primates. TKM-Ebola is a small-interfering RNA compound, currently tested in a phase I clinical trial in people.

INTERPRETATION

ROUTINE AND MICROSCOPIC URINE EXAMINATION

The urine specimen is analyzed in 3 main parts.

Gross visual examination

Normal urine color is due to the presence of a pigment called urochrome. Urine color varies based on the urine concentration and chemical composition. Normal urine can vary from pale light yellow to a dark amber color. Highly concentrated urine has a darker yellow appearance. This may be seen in patients who are volume depleted. In contrast, dilute urine has a lighter yellow appearance. This may be seen in patients with diabetes insipidus due to impaired urine concentrating ability. Urine color may vary due to certain medications, foods, and medical conditions.

Colors of urine may indicate the following:

Red:

Foods – Beets, blackberries, rhubarb

Drugs – Propofol, chlorpromazine, thioridazine, Ex-lax

Medical conditions – Urinary tract infections (UTIs), nephrolithiasis, hemoglobinuria (rhabdomyolysis), porphyrias (urine color, port wine)

Orange:

Foods – Carrot, vitamin C

Drugs – Rifampin, phenazopyridine

Green:

Food – Asparagus

Drugs – Vitamin B, methylene blue, propofol, amitriptyline

Medical condition – UTI

Blue:

Drugs – Methylene blue, indomethacin, amitriptyline, triamterene, cimetidine (intravenous), promethazine (intravenous)

Medical condition – Blue diaper syndrome (also known as tryptophan malabsorption)

Purple:

Medical condition – Bacteriuria in patients with urinary catheters (purple urine bag syndrome)

Brown:

Food – Fava beans

Drugs – Levodopa, metronidazole, nitrofurantoin, primaquine, chloroquine, methocarbamol, senna

Medical conditions – Gilbert syndrome, tyrosinemia, hepatobiliary disease

Black:

Medical conditions – Alkaptonuria, malignant melanoma

White:

Drug – Propofol

Medical conditions – Chyluria, pyuria, phosphate crystals

Urine clarity or turbidity refers to how clear the urine is. It is determined by substances in urine, such as the amount of cellular debris, casts, crystals, bacteria, or significant proteinuria. Vaginal discharge, sperm, and prostatic secretions may also influence the outcome. Urine clarity is typically classified as clear, mildly cloudy, cloudy, or turbid.

Chemical examination/urine dipstick

In most individuals, urine pH is usually lower, representing a slightly acidic environment. This is due to the obligate renal H+ ion excretion, due to the normal daily average endogenous acid production of 1 mEq/kg required to maintain acid-base balance in the body. Therefore, any abnormalities in the acid-base balance in the body has a direct effect on urinary pH levels. Diet can also affect urinary pH levels. Cranberries and high-protein diets create a more acidic urinary environment, whereas citrus fruits and low-carbohydrate diets create a more alkaline urine environment. Urinary pH levels are particularly useful in the evaluation of stones, infection, and renal tubular acidosis (RTA). For example, in a patient with nephrolithiasis, the urinary pH level is helpful when trying to distinguish between different types of calculi. Calcium oxalate/calcium phosphate, magnesium-ammonium phosphate, and staghorn calculi are associated with alkaline urine. Conversely, uric acid and cystine calculi are associated with acidic urine (although pH is more important for treatment than formation of cystine stones). Also, patients with a UTI due to urea-splitting organisms, such as proteus and klebsiella, typically have alkaline urine. Urinary pH can also help to distinguish between the different types of RTA. Additionally, urinary pH levels can be useful in gauging the response to treatment in patients with rhabdomyolysis or medication/drug overdoses. Alkaline urine is associated with calcium oxalate/calcium phosphate calculi and struvite (magnesium ammonium phosphate) calculi. Acidic urine is associated with uric acid calculi and cystine calculi. Specific gravity is a measurement of urine concentration and is representative of the kidney's ability to concentrate urine. The specific gravity is a comparison of the amount of solutes in urine as compared with pure water. Specific gravity may also be used as a rough estimate of urine osmolality. For each rise in the specific gravity by 0.001 above 1, the urine osmolality increases by about 30-35 mosmol/kg. For example, a urine specific gravity of 1.010 usually corresponds to a urine osmolality of about 300-350 mosmol/kg. However, in the setting of substances such as glucose and radiocontrast media, the specific gravity is increased more than the urine osmolality. Often specific gravity is reflective of hydration status; however, it can be inaccurate. Low specific gravity is seen in patients with impaired urinary concentrating ability (eg, diabetes insipidus, sickle cell nephropathy, acute tubular necrosis). A specific gravity of 1.003 or less is indicative of maximally dilute urine. In addition, low values may be seen due to glucose, urea, or alkaline urate. High values may be due to significant amounts of protein or ketoacids.[1] Glucose present in the urine is termed glucosuria. Most commonly, this indicates diabetes mellitus but is also often seen in pregnancy. It is due to either a high blood glucose level or a decreased kidney threshold concentration. When blood glucose levels exceed approximately 180 mg/dL, the proximal tubules become overwhelmed and cannot reabsorb the excess glucose. As a result, glucose is then excreted in the urine. Additionally, because urinary dipstick tests detect the presence of glucose only, the Clinitest and Benedict qualitative test should be used for patients with suspected inborn errors of metabolism.[1] Ketones in the urine are abnormal. Ketones accumulate when carbohydrates are insufficient and the body must get its energy from fat metabolism. Acetone, acetoacetic acid, and B-hydroxybutyric acid are the common ketones formed. Ketonuria may be seen with uncontrolled diabetes, diabetic ketoacidosis, severe exercise, starvation, vomiting and pregnancy. Nitrate testing is specific, but not sensitive, in detecting UTIs. Normally no nitrites are detected in the urine. Urinary nitrates are converted to nitrites by bacteria in the urine. A positive nitrite result signifies that bacteria capable of this conversion (eg, *Escherichia coli*, *Klebsiella, Proteus, Enterobacter, Citrobacter, Pseudomonas*) are present in the urinary tract. However, some bacteria are not capable of converting nitrates to nitrites (eg, *Staphylococcus, Streptococcus*, *Haemophilus*), and these bacteria may still be present in the urinary tract despite a negative test result. Therefore, a positive test suggests a UTI (typically due to Enterobacteriaceae), but a negative test result does not rule out a UTI.

WBCs contain an enzyme known as leukocyte esterase, which is released when WBCs undergo lysis. Normally, too few WBCs are

present in the urine for the test to be positive. However, when the number of WBCs in the urine increases, the result becomes positive. A positive leukocyte esterase test result indicates pyuria. Pyuria typically implies a UTI. Sterile pyuria is seen in analgesic nephropathy and UTIs due to organisms that do not grow by standard culture techniques (eg, *Chlamydia, Mycobacterium tuberculosis, Ureaplasma urealyticum*).[1] Bilirubin should not be present in the urine. In obstructive hepatobiliary conditions and in certain liver diseases, such as hepatitis, conjugated (water-soluble) bilirubin is excreted in the urine. Often, this may occur prior to the development of clinical symptoms (ie. jaundice). Urinary bilirubin may be present in low amounts in the urine. Bilirubin excreted into the intestine is metabolized by bacteria and forms urobilinogen. Urobilinogen is reabsorbed via the portal circulation and a small amount is excreted in the urine. Increased urobilirubin levels are associated with excessive hemolysis, liver parenchymal diseases, constipation, and intestinal bacterial overgrowth. Decreased urobilirubin levels are associated with obstructive biliary disease and severe cholestasis. Normal urinary proteins values are less than 150 mg/d and are undetectable using urinary dipstick. The urinary dipstick only detects the presence of albumin and no other proteins. When urinary protein values exceed 300-500 mg/d, the dipstick test result becomes positive. Thus, it is a very specific, but not sensitive, test for proteinuria. This is especially important to note in patients with diabetes because the urine dipstick is insensitive for microalbuminuria. The urine should not be tested within 24 hours after a contrast study because contrast (many iodinated radiocontrast agents) may produce false-positive results. In addition, the measurement of the proteinuria on the dipstick depends on the urine concentration. For example, on a small volume of concentrated urine, the dipstick may overestimate the amount of proteinuria. Whereas, on a large volume of dilute urine, the dipstick may underestimate the amount of proteinuria. This is why dipstick protein results must be quantified, as follows: Trace proteinuria - Approximately 10-30 mg/dL, 1+ - Approximately 30 mg/dL, 2+ - Approximately 100 mg/dL, 3+ - Approximately 300 mg/dL, 4+ - 1000 mg/dL or more. Because the urinary dipstick protein results can be inaccurate for the above stated reasons, a more accurate test is the sulfosalicylic acid test (SSA). This test detects all proteins in the urine at any amounts, including albumin, globulin, and Bence Jones proteins. However, just as with the urinary dipstick, contrast can cause false-positive results. The test is performed by mixing 3 parts of 3% sulfosalicylic acid with one part urine supernatant. Then, assess the turbidity of the solution. Sulfosalicylic acid test results are as follows: 0 - No turbidity (proteinuria, 0 mg/dL), Trace - Slight turbidity (proteinuria, 20 mg/dL), 1+ - Print visible through specimen (proteinuria, 50 mg/dL), 2+ - Print invisible (proteinuria, 200 mg/dL), 3+ - Flocculation (proteinuria, 500 mg/dL), 4+ - Dense precipitate (proteinuria, ≥1000 mg/dL). Patients with significant or persistent proteinuria should undergo a quantitative measurement of protein excretion. This can be accomplished by performing a 24-hour urine collection or by calculating the total urine protein to creatinine ratio using a single random urine specimen. A 24-hour urine collection can be difficult for elderly patients or those with incontinence and is especially cumbersome in the outpatient setting. Hence, the urine protein to creatinine ratio is typically the preferred method and has been shown to have a good correlation with the 24-hour urine protein determination. In addition to detection and quantification, identifying the etiology of proteinuria is important. Proteinuria is typically classified as transient or persistent. Overall, proteinuria can be classified as transient or persistent. Transient proteinuria is often benign and self-limited . Persistent proteinuria is then further classified into glomerular, tubular, or overflow. Transient proteinuria due to transient changes in glomerular hemodynamics (increased excretion of urinary protein) may have the following etiologies: Congestive heart failure, Fever, Strenuous exercise, Seizure disorders, Stress, Orthostatic proteinuria. Glomerular proteinuria due to disruption of filtration barrier (an increased filtration of albumin across the glomerular capillary wall) may have the following etiologies: Nephrotic syndrome (ie, diabetic nephropathy), Glomerulonephritis, Orthostatic proteinuria (rather benign course, lesser degrees of proteinuria [typically < 2 g/d]), Exercise-induced proteinuria (rather benign course, lesser degrees of proteinuria [typically < 2 g/d]). Tubular proteinuria due to defective reabsorptive capacities in the proximal tubules of freely filtered proteins, mostly low&molecular weight proteins such as immunoglobulin light chains (excretion of normally reabsorbed proteins), may be caused by tubulointerstitial diseases (ie, ATN, acute interstitial nephritis, Fanconi syndrome). Overflow proteinuria due to overproduction of immunoglobulin light chains in multiple myeloma (amount produced exceeds maximum amount for reabsorption in the tubules) may have the following etiologies: Multiple myeloma, Myoglobinuria. The dipstick test for blood detects the peroxidase activity of RBCs.[1] If more than 3 RBCs are present, then the urinary dipstick test result is positive for blood. However, the urine dipstick does not detect where the blood is coming from. A positive blood result on the urine dipstick can represent hematuria, hemoglobinuria, myoglobinuria, false-positive results, or contamination. False-positives may be seen with alkaline urine (pH >9), semen in the urine, and urine contaminated with oxidizing agents used to cleanse the perineum. In addition, remember that positive results can also represent contamination with blood from a nonurinary source, such as hemorrhoids or vaginal bleeding. In general, clear supernatant and red urinary sediment is due to hematuria. A red supernatant that is heme positive on dipstick testing is due to hemoglobinuria or myoglobinuria. A red supernatant that is heme negative on dipstick is due to beets, food dyes, porphyria, hydroxocobalamin, phenazopyridine, or various other medications. Additionally, if the urinary dipstick is positive for blood and urine microscopy is positive for RBCs, hematuria is confirmed. If the dipstick result is positive for blood but no RBCs are found in the urinary sediment when analyzed on urine microscopy, then that indicates myoglobinuria (caused by rhabdomyolysis or myoglobinuric renal failure) or hemoglobinuria (caused by infections such as *Plasmodium falciparum* or *Clostridium welchii*, transfusion-related reactions, or paroxysmal nocturnal hemoglobinuria).

Microscopic examination/urine sediment

WBCs, RBCs, epithelial cells, and, rarely, tumor cells are the cellular elements found in the urinary sediment. The number of WBCs considered normal is typically 2-5 WBCs/hpf or less. A high number of WBCs indicates infection, inflammation, or contamination.[1] Typically most of the WBCs found are neutrophils. Urinary eosinophils and lymphocytes may also be found and can been seen with a Wright stain of the sediment. If found, urinary eosinophils may help diagnose acute interstitial nephritis (AIN). Eosinophiluria is seen with AIN, but the absence of eosinophiluria does not rule out AIN. Urinary lymphocytes are often associated with tubulointerstitial diseases. Hematuria can be gross or microscopic. Gross hematuria is the presence of red/brown urine. As little as 1 mL of blood per liter of urine can produce a visible color change; therefore, gross hematuria does not automatically indicate a large amount of blood. In addition, as previously discussed in above sections, the red/brown color change could be due to contamination from other sources of blood (menstruation/hemorrhoids) and can also be seen without the presence of any RBCs (certain medications, beets,

myoglobinuria, or hemoglobinuria). Normally, less than 2 RBCs/hpf are observed. Microscopic hematuria is defined as the presence of 3 RBCs/hpf or more in 2 of 3 urine samples.[1] Hematuria may also be transient or persistent. Transient hematuria in young patients is fairly common and is typically benign. However, in older patients (>50 y), hematuria, even when transient, can be serious and warrants a full workup for possible underlying malignancy. On the other hand, persistent hematuria should always warrant a full evaluation. The causes of hematuria are often categorized as renal versus extrarenal. If the cause is thought to be renal, it is further categorized into glomerular versus nonglomerular. The hallmark findings of hematuria of glomerular origin include red cell casts, proteinuria (>500 mg/d), and dysmorphic RBCs.[1] Hematuria of glomerular origin is also commonly described as "cola-colored."[3] The first step in the evaluation of a patient with hematuria is a detailed history. This may provide the clinician with important diagnostic clues. For example, hematuria with acute onset flank pain radiating to the ipsilateral groin with nausea/vomiting suggests nephrolithiasis, whereas dysuria suggests a UTI or pyelonephritis, if fevers/chills are also present. A patient who notes a recent upper respiratory infection should be evaluated for possible postinfectious glomerulonephritis or immunoglobulin (Ig)A nephropathy. The timing is also important. Hematuria at the start of urination may indicate a distal urethra origin; hematuria at the end of urination may indicate bladder neck/posterior urethra/prosthetic urethra origin; and hematuria throughout urination suggests upper urinary tract/upper bladder origin. A patient's family history is also important to gather because hematuria may also be due to familial disorders (ie, polycystic kidney disease, Alport syndrome, sickle cell nephropathy, thin basement membrane nephropathy, benign familial hematuria). Glomerular causes of hematuria are associated with the following: Proteinuria - >500 mg/d, RBC casts, Dysmorphic RBCs. The causes of glomerular-based hematuria include the following: Thin basement membrane nephropathy (benign familial hematuria), Alport syndrome, IgA nephropathy. Nonglomerular causes of hematuria are associated with the following: Proteinuria - >500 mg/d, No RBC casts, No dysmorphic RBCs. Causes of nonglomerular-based hematuria include the following: Tubulointerstitial nephritis, Pyelonephritis, Polycystic kidney disease, Renal cell carcinoma, Sickle cell disease or trait, Renovascular disease (eg, atheroembolic renal disease, renal vein thrombosis, arteriovenous malformations, “nutcracker syndrome”). Extrarenal-based hematuria may be caused by the following: Tumors/malignancies (prostate, ureteral, bladder), Stones (kidney, bladder), Benign prostatic hyperplasia, Infections (pyelonephritis, cystitis, prostatitis, urethritis), Schistosomiasis, Foley trauma, Anticoagulants, Chemotherapeutic agents (mitotane, ifosfamide, cyclophosphamide). Epithelial cells that may be found in the urinary sediment include squamous epithelial cells (from the external urethra) and transitional epithelial cells (from the bladder). Generally 15-20 squamous epithelial cells/hpf or more indicates that the urinary specimen is contaminated. Hyaline casts may be seen in healthy individuals. Other types of casts are not normally found and are suggestive of renal disease. In particular, the finding of cells within a cast is diagnostic of an intrarenal origin. Casts are cylindrical particles that are formed from coagulated protein secreted by tubular cells. The organic matrix is mainly composed of Tamm-Horsfall mucoprotein (which glues or cements casts together). They are usually cylindrical with regular margins, as they are formed in the long, thin, hollow renal tubules and typically take the shape of the tubule in which they are formed. They are only formed in the distal convoluted tubule or the collecting duct and not in the proximal convoluted tubule or in the loop of Henle. Low urine pH, low urine flow rate, and high urinary salt concentration promote cast formation (by favoring protein denaturation and precipitation).[1] Hyaline casts are found in healthy individuals and are relatively nonspecific. They may be increased after strenuous exercise. They are often seen in small volumes of concentrated urine or with diuretic therapy. Red cell casts are nearly diagnostic of glomerulonephritis or vasculitis. White cell casts and pyuria are most commonly seen with tubulointerstitial nephritis and acute pyelonephritis. WBC casts are also seen with renal tuberculosis and vaginal infections. "Muddy-brown" granular casts are diagnostic of acute tubular necrosis. Waxy and broad casts are consistent with advanced renal failure. Fatty casts and lipiduria, with the typical "maltese-cross" appearance on polarized microscopy, are commonly seen with nephrotic syndrome. Crystals are solid forms of a particular dissolved substance in the urine. Identifying factors of crystals include shape, color, and urine pH. Crystal formation is determined by the urine pH, the supersaturation of the molecules, and the presence of possible inhibiting factors. Crystalluria may be normal when the crystals are composed of solutes that are usually found in the urine. However, the observation of certain urinary crystals can diagnostically significant. For example, calcium oxalate crystals ("envelope-shaped") and acute kidney injury is seen with ethylene glycol ingestion. The presence of large amounts of uric acid crystals ("diamond" or "barrel" shaped) and acute kidney injury is seen in tumor lysis syndrome. Uric acid crystals may also be seen with other causes of hyperuricosuria, such as gout. In addition, cystine crystals ("hexagonal") areseen with cystinuria. Finally, magnesium ammonium phosphate and triple phosphate crystals (struvite) are "coffin-lid" shaped and seen with UTIs caused by urea-splitting organisms (ie, *Proteus, Klebsiella*). Bacteria in the urine sediment are generally due to infection or contamination. Normally no bacteria should be seen in the urinary sediment. However, given the abundance of normal microbial flora in the vagina and/or external urethral meatus, this is not an unusual finding. In addition, bacteria multiply rapidly if the urine specimen is left standing for too long in room temperature. A urinalysis with positive tests for nitrites, leukocyte esterase, and bacteria is highly suggestive of a urinary tract infection. However, if a significant amount of squamous epithelial cells ($\geq$15-20/hpf) are present as well, these findings may primarily indicate a contaminated specimen and the urinalysis should be repeated. Although, even if no squamous cells are present and true bacteriuria is found, these findings should be correlated clinically with the presence of symptoms consistent with a urinary tract infection. If the patient does not have concomitant symptoms consistent with a UTI, then it is termedasymptomatic bacteriuria. Except for in certain circumstances, asymptomatic bacteriuria is generally not treated. If bacteriuria is found and a UTI is suspected, a urine culture with sensitivities is recommended. In catheterized patients or with urine obtained from a suprapubic tab, any organism on the urine culture is considered significant. Otherwise, generally, 100,000/mL or more of a single organism reflects significant bacteriuria. The presence of multiple organisms, especially at less than 100,000/mL, typically suggests polymicrobial contamination. Yeast cells are not normally found in the urine specimen. They can be distinguished from red cells and amorphous crystals by their tendency to bud. Commonly the yeast cells are of the *Candida* species, which can colonize the vagina, urethra, or bladder. Yeast cells may signify true infection or contamination (often due to contamination by vaginal secretions in women with a yeast infection).

BOUQUET

In Lighter Vein

How to choose right person for the job?
Put about 100 bricks in some Particular order in a closed room with an open window, Then send 2 or 3 candidates in the room and close the door. Leave them alone and come back after 6 hours and then analyze the situation.
If they are counting the Bricks, Put them in the accounts Department.
If they are recounting them....Put them in auditing .
If they have messed up the whole place with the bricks, then Put them in engineering.
If they are arranging the bricks in some strange order, Put them in planning.
If they are throwing the Bricks at each other, Put them in operations .
If they are sleeping, Put them in security.
If they have broken the bricks Into pieces, Put them in information technology.
If they are sitting idle, Put them in human resources.
If they say they have tried different combinations, yet not a brick has Been moved. Put them in sales.
If they have already left for the day, Put them in marketing.
If they are staring out of the Window, Put them on strategic Planning.
And then last but not least.....If they are talking to each other and not a single brick Has been Moved, Congratulate them and put them In top management.

The English language. Did you know?
The most commonly used letter in the alphabet is 'E.'
The least used letter in the alphabet is 'Q.'
Skiing is the only word with double 'i.'
Dreamt is the only word that ends in 'mt.'
The first letters of the months July through to November spell JASON.
There are only 4 words in the English language which end in 'dous' (they are: hazardous, horrendous, stupendous and tremendous).
The oldest word in the English language is 'town.'
'Bookkeeper' and 'bookkeeping' are the only 2 words in the English language with three consecutive double letters.
The word 'Strengths' is the longest word in the English language with just one vowel.
The dot on top of the letter 'i' is called a tittle.
The past tense for the English word 'dare' is 'durst.'
The word 'testify' derived from a time when men were required to swear on their testicles.
The first English dictionary was written in 1755.
The word old English word 'juke' meaning dancing lends its name to the Juke Box.
1 out of every 8 letters written is an 'e.'
The longest one syllable word in the English language is 'screeched.'
All pilots on international flights identify themselves in English regardless of their country of origin.
The expression to 'knuckle down' originated from playing marbles (players used to put their knuckles to the ground for their best shots).
The word 'almost' is the longest in the English language with all the letters in alphabetical order.
The most commonly used word in English conversation is 'I.'

Wisdom Whispers

Quotes:

Our greatest weakness lies in giving up. The most certain way to succeed is always to try just one more time.
--Thomas A. Edison

You are never too old to set another goal or to dream a new dream.
--C. S. Lewis

Keep your eyes on the stars, and your feet on the ground.
--Theodore Roosevelt

Start where you are. Use what you have. Do what you can.
--Arthur Ashe

A creative man is motivated by the desire to achieve, not by the desire to beat others.
--Ayn Rand

I don't believe you have to be better than everybody else. I believe you have to be better than you ever thought you could be.
--Ken Venturi

If you can dream it, you can do it.
--Walt Disney

Brain Teasers

1. **The active coenzyme form of cobalamin is-**
   A. Methyl cobalamin
   B. Deoxyadenosylcobalamin
   C. Both of the above
   D. None of the above.

2. **All are one carbon donors except-**
   A. Serine
   B. S-adenosylmethionine
   C. Choline
   D. Thiamine.

3. **All are needed as coenzyme for alpha keto glutarate dehydrogenase complex except-**
   A. Niacin
   B. Folic acid
   C. Riboflavin
   D. Lipoic acid.

4. **Which of the following vitamins can act without phosphorylation?**
   A. Pyridoxine
   B. Lipoamide
   C. Niacin
   D. Thiamine.

Answers: 1. C, 2. D, 3. B, 4. B

TROUBLESHOOTING

URINALYSIS

Urine Specimens – an overview of collection methods, collection devices, specimen handling and transportation

This Focus Topic is the first of a two part series on urine specimen collection. Part 2 will cover sources of preanalytical artifact arising during urine collection, handling and transportation. Urine has a long history as a specimen for analysis in clinical laboratories. After blood, urine is the most commonly used specimen for diagnostic testing, monitoring of disease status and detection of drugs. Urine testing, using both automated and traditional manual methods, is growing rapidly. As with all clinical laboratory specimens, preanalytical error in urine specimens is often difficult to detect. Because of this, it is important for laboratories to have processes in place to ensure compliance with best practice in specimen collection, handling and transport – including the use of preservatives where appropriate.

Types of Urine Collection Methods

Urine specimens may be collected in a variety of ways according to the type of specimen required, the collection site and patient type. Randomly Collected Specimens are not regarded as specimens of choice because of the potential for dilution of the specimen when collection occurs soon after the patient has consumed fluids. First Morning Specimen is the specimen of choice for urinalysis and microscopic analysis, since the urine is generally more concentrated. Midstream Clean Catch Specimens are strongly recommended for microbiological culture and antibiotic susceptibility testing because of the reduced incidence of cellular and microbial contamination. Timed Collection Specimens may be required for quantitative measurement of certain analytes, including those subject to diurnal variation. Analytes commonly tested using timed collection include creatinine, urea, potassium, sodium, uric acid, cortisol, calcium, citrate, amino acids, catecholamines, metanephrines, vanillylmandelic acid (VMA), 5-hydroxyindoleacetic acid, protein, oxalate, copper, 17-ketosteroids, and 17-hydroxysteroids. Collection from Catheters (e.g. Foley catheter) using a syringe, followed by transfer to a specimen tube or cup. Alternatively, urine can be drawn directly from the catheter to an evacuated tube using an appropriate adaptor. Supra-pubic Aspiration may be necessary when a non-ambulatory patient cannot be catheterized or where there are concerns about obtaining a sterile specimen by conventional means. Pediatric Specimens present many challenges. For infants and small children, a special urine collection bag can be adhered to the skin surrounding the urethral area.

Urine Collection Devices

An extensive array of urine collection products is available on the market. Information on features, intended use and instructions for use should be obtained from the device manufacturer and reviewed before being incorporated into a specimen collection protocol.

Urine Collection Containers (cups for collection and transport): Urine collection container cups are available in a variety of shapes and sizes with lids that are either 'snap-on' or 'screw-on'. Leakage is a common problem with low quality products. To protect healthcare workers from exposure to the specimen and protect the specimen from exposure to contaminants, leak-proof cups should be utilized. Some urine specimen containers have closures with special access ports that allow closed-system transfer of urine directly from the collection device to the tube (further information).

Urine Collection Containers for 24-hour Collection: Urine collection containers for 24-hour specimens commonly have a 3liter capacity. As for the urine collection cups above ,closure types vary with some containers featuring anintegrated port for transfer of an aliquot of the specimen to an evacuated urine collection tube (further information). This provides the option for the laboratory to receive only the aliquot tube and specimen weight (with the large 24-hour container and contents discarded at the point of collection). Additional precautions need to be taken when a preservative is required (further information).

Urine Specimen Tubes: Urine specimens may be poured directly into tubes with 'screw-on' or 'snap-on' caps. Additionally, evacuated tubes, similar to those used in blood collection, are available. (further information)

Urine Specimen Collection and Transportation Guidelines

As for any type of clinical laboratory specimen, certain criteria for collection and transportation (further information) of urine specimens must be met to ensure high quality specimens free of preanalytical artifact are obtained consistently. Without this, accurate test results cannot be guaranteed.

Urine Specimen Preservation

For urinalysis and culture and sensitivity testing, CLSI Guidelines recommend testing within two hours of collection. Different time limits may apply to specimens required for molecular testing of infectious agents (e.g. testing for Neisseria gonorrhoeae, Chlamydia trachomatis). For this type of testing, laboratories should ensure they are able to comply with specimen transportation conditions prescribed by the assay manufacturers. Where compliance with these and/or CLSI recommendations is not possible, consideration should be given to the use of a preservative (further information). Specimen collection tubes with preservatives for chemical urinalysis (further information) and culture and antibiotic susceptibility are available (further information).

Urine Specimen Reception in the Laboratory

In addition to routine checks and precautions taken for all specimens received in the clinical laboratory, the following additional 'check items' apply to urine specimens. Labels, Volume, Collection Date and Time, Collection Method, Specimen Preservation, Light Protection.

Randomly Collected Specimens: Randomly collected specimens are suitable for urinalysis in the clinical chemistry laboratory and for microscopic analysis. However, they are not regarded as specimens of choice because of the potential for dilution of the specimen when collection occurs soon after the patient has consumed fluids. In this situation, analyte values may be artificially low. Of necessity, pediatric urine specimens for urinalysis and microscopy are often of this type.

First Morning Specimen is the Specimen: First morning specimens are the specimen of choice for urinalysis and microscopic analysis since the urine is generally more concentrated (due to the length of time the urine is allowed to remain in the bladder) and, therefore, contains relatively higher levels of cellular elements and analytes. Abnormal constituents are also likely to be present in higher concentration and, thus, more likely to be detected.

Midstream Clean Catch Specimens: Midstream specimens are strongly recommended for microbiological culture and antibiotic susceptibility testing because of the reduced incidence of cellular and microbial contamination. Following instruction from a healthcare professional, patients are required to follow a prescribed procedure commencing with cleansing the urethral area. The patient should then void the first portion of the urine stream into the toilet. These first steps significantly reduce the opportunities for contaminants to enter the urine stream during collection of the clinical specimen. The urine midstream is then collected into a clean container after which the remaining urine is voided

into the toilet. This method of collection can be conducted at any time of day or night.

Timed Collection Specimens: Timed specimens may be required for quantitative measurement of certain analytes, including those subject to diurnal variation. Analytes commonly tested using timed collection include creatinine, urea, potassium, sodium, uric acid, cortisol, calcium, citrate, amino acids, catecholamines, metanephrines, vanillylmandelic acid (VMA), 5-hydroxyindoleacetic acid, protein, oxalate, copper, 17-ketosteroids, and 17-hydroxysteroids. A timed collection allows measurement of the excretion of these substances in urine over a specified length of time, usually, but not always, 8 or 24 hours. In this collection method, the bladder is emptied prior to beginning the timed collection. Then, for the duration of the designated time period, all urine is collected and pooled into a collection container, with the final collection taking place at the very end of that period. Half an hour before the end of the collection period, it is helpful to ask the patient to drink a glass of water, so that the last urine specimen can be obtained. If no specimen is produced, then the total volume and time of collection cannot be determined. It is also important to caution the patient not to lose urine specimens to the toilet during defecation. When a 24-hour urine specimen is required for the assay of catecholamines, metanephrines and/or VMA, for the diagnosis of pheochromocytoma, which causes persistent or episodic hypertension, it is advisable to monitor the blood pressure of the patient and collect the urine specimen when the blood pressure is high, in order to improve the chance of a positive finding. Timed specimens should be refrigerated during the collection period, unless otherwise directed by the laboratory. Accurate timing is very important as this information forms a critical part of the calculations performed to determine urine clearance values (e.g. creatinine clearance). Interpretations based on faulty calculations can result in improper diagnoses or medical treatment.

Collection from Catheters: Urine specimens can be collected from catheters (e.g. Foley catheter) using a syringe, followed by transfer to a specimen tube or cup. Alternatively, urine can be drawn directly from the catheter to an evacuated tube using an appropriate adaptor.

Direct draw adaptor for urine specimen collection from Foley catheter

Supra pubic Aspiration: Supra-pubic aspiration may be necessary when a non-ambulatory patient cannot be catheterized or where there are concerns about obtaining a sterile specimen by conventional means. This procedure involves collection of the specimen by needle aspiration through the abdominal wall into the bladder.

Pediatric Specimens: Urine collection from pediatric patients presents many challenges. For infants and small children, a special urine collection bag can be adhered to the skin surrounding the urethral area. Once the collection is completed, the urine is poured into a collection cup or transferred directly into an evacuated tube with a transfer straw. Urine collected from a diaper is not recommended for laboratory testing since contamination from the diaper material may affect test results.

Urine Collection Containers

Urine collection containers with integrated port for transfer of specimen to evacuated urine collection tube.

24 Hour Urine Collection Container: 24 hour urine collection container with integrated port for transfer of specimen to evacuated urine collection tube. This provides the option for the laboratory to receive only the aliquot tube and specimen weight (with the large 24-hour container and contents discarded at the point of collection).

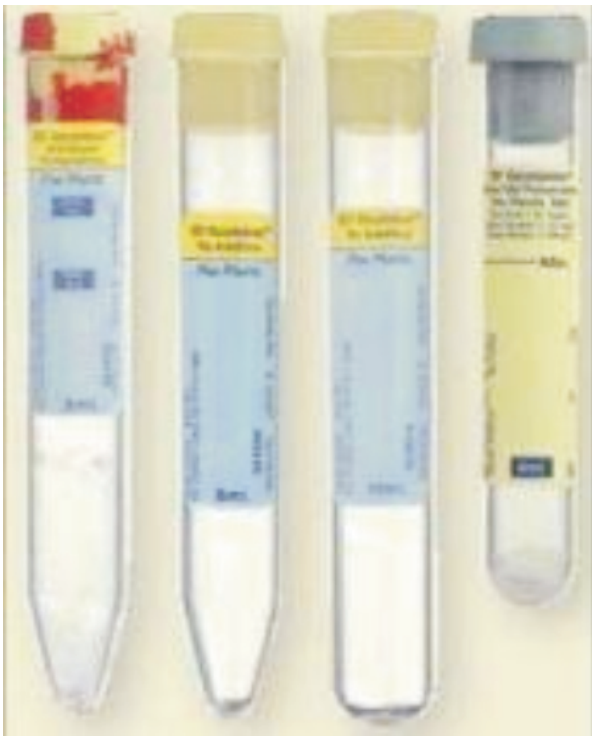
Preservatives for 24 Hour Specimens: When a preservative is required, it should be added to the collection container before the urine collection begins. Commonly used preservatives for 24 hour specimens are hydrochloric acid, boric acid, acetic acid, thymol and toluene. If more than one acceptable preservative is available for the analyte(s) being tested, the least hazardous one should, of course, be selected. Appropriate warning labels should be placed on the container to alert patients to possible harm arising from contact with the preservatives. This should be reinforced by appropriate instruction from the attending healthcare worker. A corresponding Material Safety Data Sheet (MSDS) should also be provided for the patient.

Urine Specimen Tubes: Evacuated tubes, similar to those used in blood collection, are available for urine collection. These can be filled using a straw device, from urine specimen containers with integrated transfer devices, or from direct sampling devices that are used to access catheter sampling ports.

Urine transfer 'straw' with adaptor for transfer of specimento evacuated urine collection tube

Urine collection containers with integrated port for transfer of specimen to evacuated urine collection tube

Urinalysis tubes are available in a variety of shapes: conical bottom, round bottom, or flat bottom. Conical bottom tubes offer advantages for microscopic examination of urine sediment. The laboratory's tube selection process must include consideration of centrifugation conditions and compatibility with automated instrument systems. Tube fill volumes are typically within the range of 4 to 10mL with dimensions of 13 x 75mm and 16 x 100mm.



Evacuated urine specimen collection tubes

Collection and Transport Guidelines

All urine collection and/or transport containers should be clean and free of particles or interfering substances. The collection and/or transport container should have a secure lid and be leak-proof. Leak-proof containers reduce specimen loss and risk of healthcare worker exposure to the specimen while also protecting the specimen from contaminants. The use of containers that are made from break-resistant plastic is strongly recommended. The container material should not leach interfering substances into the specimen. Specimen containers must not be re-used. Specimen tubes should be compatible with automated systems and instruments used by the laboratory. Collection containers and/or specimen tubes should be compatible with pneumatic tube systems where these are used for urine specimen transport. Use of leak-proof containers is essential in this situation.

The CLSI Guidelines makes the following recommendations for urine collection: Primary (routine) specimen containers to have a wide base and a capacity of at least 50 mL. 24 hour specimen containers to have a capacity of at least 3 litres. Sterile collection containers for all microbiology specimens. Specimen containers to have secure closures to prevent specimen loss and to protect the specimen from contaminants. Amber colored containers for specimens required for assay of light sensitive analytes such as urobilinogen and porphyrins.

Urine Tubes Preservatives

For chemical urinalysis and conventional (culture based) microbiological testing, unpreserved specimens exceeding the two hour limit that have not been refrigerated should not be accepted for analysis due to potential bacterial overgrowth leading to disintegration of cells and casts*, invalidation of bacterial colony counts and errors in chemical urinalysis. When specimens for such testing are directly transferred from a collection cup to a tube containing a suitable preservative, a stable environment is provided for the specimen until testing can be conducted. Preservatives are also available for some molecular tests (e.g. BD UPT urine specimen tube for use with BD ProbeTec™ ET assay system). When a decision to use a preservative is made – for any type of testing, potential interference with assay methods should be considered. Laboratories should validate all test procedures intended to be used for preserved specimens. Specimens may need to be split if various tests requiring different preservatives are requested.

* Bacterial growth increases the pH of the urine leading to lysis of red blood cells and white blood cells. Increased pH (alkalinity) can also cause casts to dissolve.

Chemical Urinalysis Preservatives: A variety of urine preservatives is available that allow urine to be maintained at room temperature while still providing urinalysis test results comparable to those achieved with fresh specimens or those stored under refrigerated conditions. Commonly used preservatives for chemical urinalysis specimens include tartaric acid, boric acid, chlorhexidine, ethyl paraben, thymol and sodium propionate (and 'cocktails' of these). Preservation times are typically within the range of 24 to 72 hours. Claims for the duration of stability for specific analytes should be obtained from the manufacturer.

Culture and Antibiotic Susceptibility Preservatives: Preservatives for culture and antibiotic susceptibility testing are designed to maintain the specimen in a state equivalent to that which would be achieved with refrigeration by deterring the proliferation of organisms that could result in a false positive culture or bacterial overgrowth. Careful attention must be given to the formulation of these preservatives to achieve this objective. There is evidence to suggest that non-pH buffered boric acid may be harmful to certain organisms and that buffered boric acid preservatives can reduce the harmful effects of the preservative on the organisms. Preserved urine specimens can be stored at room temperature until the time of testing. Product claims regarding duration of preservative potency should be obtained from the manufacturer.

Labels: If the collection container is used for transport, the label should be placed on the container and not on the lid, since the lid can be mistakenly placed on a different container. Note that some labels are unsuitable for specimens stored under refrigerated conditions because of a lack of adhesion at low temperatures.

Volume: It is important for specimen collection personnel to ensure there is sufficient volume to perform the required tests. For specimens in preservative tubes, the fill volume must be correct. As above, under-filling or over-filling these tubes may adversely affect test result accuracy.

Collection Time and Date: Collection time and date must be shown on the specimen label. For timed specimens, both the start and stop times of the collection must be shown. The time at which the specimen was received in the laboratory must also be documented for verification of proper handling and transport after collection.

Collection Method: The method of collection should be confirmed when the specimen is received in the laboratory to ensure the type of specimen submitted meets the needs of the required test(s). An example of an optimum specimen/test match would be a first morning specimen for urinalysis and microscopic examination.

Specimen Preservation: If the specimen is not received within two hours of collection, specimen reception personnel must confirm that a tube containing an appropriate preservative has been used. Confirmation that the specimen is received within the allowable time for the particular preservative tube used is required.

Light Protection: Specimens submitted for testing of light-sensitive analytes must be collected in containers that protect the specimen from light.

Euphoreia™ 4.1

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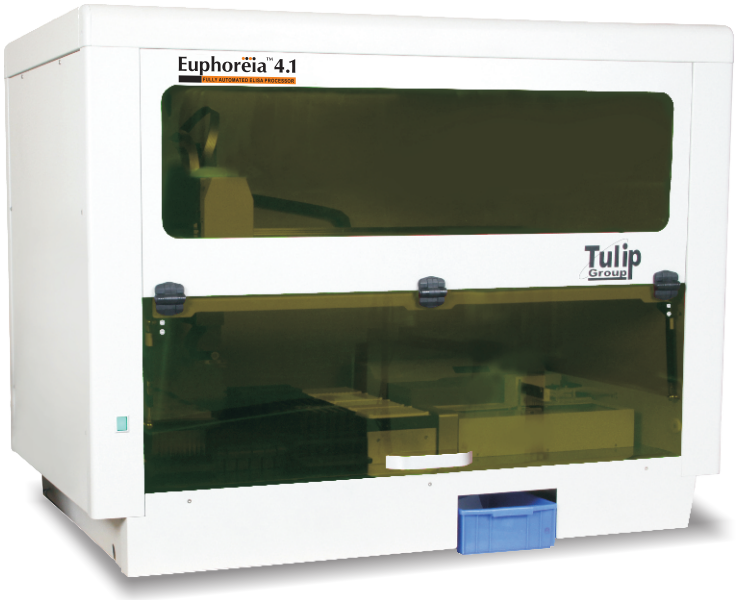

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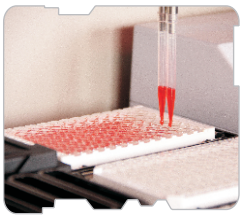
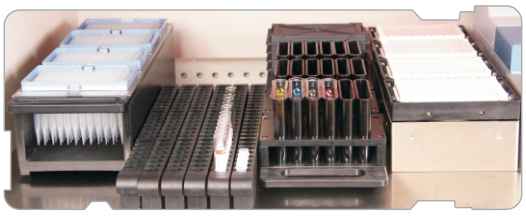


Two Channel Pipetting System



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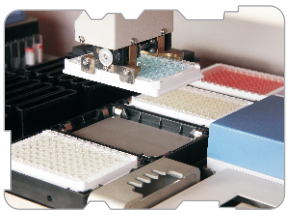

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