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The Crux

BIMONTHLY FORUM FOR THE LABORATORIANS

CONTENTS

Editorial

Plague is a deadly infectious disease that is caused by the enterobacteria *Yersinia pestis*, named after the French-Swiss bacteriologist Alexandre Yersin. Until June 2007, plague was one of the three epidemic diseases specifically reportable to the World Health Organization (the other two being cholera and yellow fever).

Depending on lung infection, or sanitary conditions, plague can be spread in the air, by direct contact, or by contaminated undercooked food or materials. The symptoms of plague depend on the concentrated areas of infection in each person: bubonic plague in lymph nodes, septicemic plague in blood vessels, pneumonic plague in lungs, and so on. It is treatable if detected early. Plague is still endemic in some parts of the world.

The epidemiological use of the term *"plague"* is currently applied to bacterial infections that cause *buboes*, although historically the medical use of the term "plague" has been applied to pandemic infections in general. Plague is often synonymous with "bubonic plague", but this describes just one of its manifestations. Other names have been used to describe this disease, such as "The Black Plague" and "The Black Death"; the latter is now used primarily by scholars to describe the second, and most devastating, pandemic of the disease.

The etymology of the word *"plague"* is believed to come from the Latin word *plāga* ("blow, wound") and *plangere* ("to strike, or to strike down"), cf. German *Plage* ("infestation").

Transmission of *Y. pestis* to an uninfected individual is possible by any of the following means.[2]

- droplet contact – coughing or sneezing on another person
- direct physical contact – touching an infected person, including sexual contact
- indirect contact – usually by touching soil contamination or a contaminated surface
- airborne transmission – if the microorganism can remain in the air for long periods
- fecal-oral transmission – usually from contaminated food or water sources
- vector borne transmission – carried by insects or other animals.

For the complete clinico-diagnostic details just flip over to the next page. DISEASE DIAGNOSIS discusses plague in complete entirity.

INTERPRETATION outlines for you a very commonly asked for investigation that doesn't raise an eyebrow usually. Many a times it is ill understood. Yes we are talking about **urinalysis** (**UA**), also known as **routine and microscopy** (**R&M**), is an array of tests performed on urine, and one of the most common methods of medical diagnosis. The word is a portmanteau of the words *urine* and *analysis*.

- The target parameters that can be measured or quantified in urinalysis include many substances and cells, as well as other properties, such as specific gravity.
- A part of a urinalysis can be performed by using urine test strips, in which the test results can be read as color changes. Another method is light microscopy of urine samples.

The whole battery of tests (most) are covered for better assimilation.

TROUBLE SHOOTING too springs a common surprise! All grey areas while staining a Pap's smear are presented for isolation of problem points!

BOUQUET is non pictorial this time but equally interesting. Happy reading!!!

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DISEASE DIAGNOSIS

PLAGUE

Background

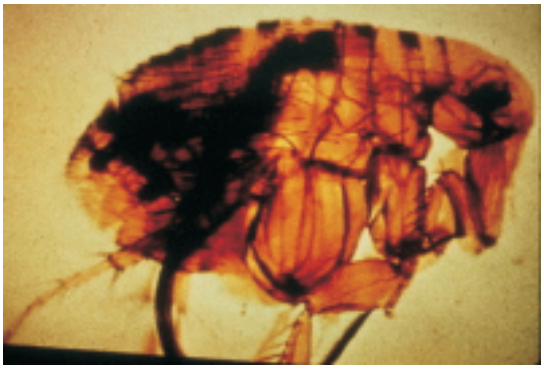
Plague, first described in the Old Testament, has persisted into the modern era. Plague has caused large-scale epidemics, thereby changing the course of history in many nations. The first pandemic was believed to have started in Africa and killed 100 million people over a span of 60 years. In the Middle Ages, plague killed approximately one fourth of Europe's population. The pandemic that began in China in the 1860s spread to Hong Kong in the 1890s and was subsequently spread by rats transported on ships to Africa, Asia, California, and port cities of South America. In the early twentieth century, plague epidemics accounted for about 10 million deaths in India. As reported in *National Geographic*, mass graves of plague victims were recently discovered in an area of Venice called "Quarantine Island." Plague is worldwide in distribution, with most of the human cases reported from developing countries. This disease is an acute, contagious, febrile illness transmitted to humans by the bite of an infected rat flea. Human-to-human transmission is rare except during epidemics of pneumonic plague. The disease is caused by the plague bacillus, rod-shaped bacteria referred to as *Yersinia pestis*. *Yersinia* is named in honor of Alexander Yersin, who successfully isolated the bacteria in 1894 during the pandemic that began in China in the 1860s. Three studies have shown that this bacterium emerged from the gut pathogen *Yersinia pseudotuberculosis* shortly after the first epidemic. Three biovars (with minor genetic variations) have been identified within the *Y pestis* clone—Antiqua, Medievalis, and Orientalis. One theory is that these biovars emerged before any of the plague epidemics. In fact, as reported by Drancourt et al (2004), genotyping performed on bacteria derived from the remains of plague victims of the first two epidemics revealed sequences similar to that of Orientalis. The virulence of this bacterium results from the 32 *Y pestis* chromosomal genes and two *Y pestis* –specific plasmids, constituting the only new genetic material acquired since its evolution from its predecessor. These acquired genetic changes have allowed the pathogen to colonize fleas and to use them as vectors for transmission. Plague is a zoonotic disease that primarily affects rodents; humans are incidental hosts. Survival of the bacillus in nature depends on flea-rodent interaction, and human infection does not contribute to the bacteria's persistence in nature. Of the 1500 flea species identified, only 30 of them have been shown to act as vectors of plague. The most prominent of these vectors is *Xenopsylla cheopis* (oriental rat flea); however, *Oropsylla montana* has been incriminated as the primary vector for this disease in North America.

Oriental rat flea (Xenopsylla cheopis), the primary vector of plague, engorged with blood.

Host fatality has been known to be the harbinger of an epidemic. Whether susceptibility and fatality are related is unknown. However, rats, mice, ground squirrels etc have been known to be highly susceptible to plague, whereas others have been known to be either moderately susceptible or absolutely resistant to infection. The last known epidemic in India took place at Surat (Gujarat) in 1994.

Pathophysiology

Y pestis is a nonmotile, pleomorphic, gram-negative coccobacillus that is nonsporulating. The bacteria elaborate a lipopolysaccharide endotoxin, coagulase, and a fibrinolysin, which are the principal factors in the pathogenesis of plague. The pathophysiology of plague basically involves two phases—a cycle within the fleas and a cycle within humans. The key to the organism's virulence is the phenomenon of "blockage," which aids the transmission of bacteria by fleas. After ingestion of infected blood, the bacteria survive in the midgut of the flea owing to a plasmid-encoded phospholipase D that protects them from digestive juices. The bacteria multiply uninhibited in the midgut to form a mass that extends from the stomach proximally into the esophagus through a sphincter like structure with sharp teeth called the proventriculus.

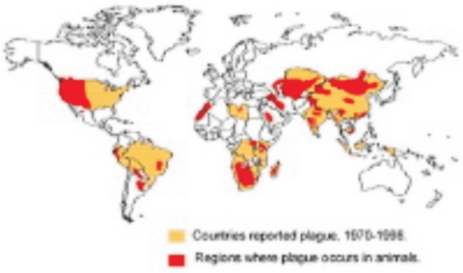


Pictured is a flea with a blocked proventriculus, which is equivalent to the gastroesophageal region in a human. In nature, this flea would develop a ravenous hunger because of its inability to digest the fibrinoid mass of blood and bacteria. If this flea were to bite a mammal, the proventriculus would be cleared, and thousands of bacteria would be regurgitated into the bite wound. It has been shown that this property requires the presence of hemin-producing genes, which are needed for the formation of a biofilm that permits colonization of the proventriculus. In fact, as described by Jarrett et al (2004), this mutation in hemin genes allows colonization in the midgut without extension to the proventriculus. Consequently, the "blockage phenomenon" does not occur, thereby leading to failure of transmission. This blockage causes the flea to die of starvation and dehydration. As a desperate measure, the flea then repeatedly tries to obtain a meal by biting a host, managing only to regurgitate the infected mass into host's bloodstream. However, the concept that the flea must be engorged before becoming infectious loses support when trying to explain the rapid rate of spread of disease during a plague epidemic. Studies of vectors such as *O montana* clearly indicate the redundancy of the aforementioned hypothesis, since this vector does not die of blockage and remains infectious for a long period, unlike its counterpart. Once the flea bites a susceptible host, the bacilli migrate to the regional lymph nodes, are phagocytosed by polymorphonuclear and mononuclear phagocytes, and multiply intracellularly. Survival and replication within macrophages is probably of greatest importance in early stages of the disease. Involved lymph nodes show dense concentrations of plague bacilli, destruction of the normal architecture, and medullary necrosis. With subsequent lysis of the phagocytes, bacteremia can occur and may lead to invasion of

distant organs in the absence of specific therapy. The following are the modes of plague transmission in humans: Bites by fleas, Exposure to humans with pneumonic plague, Handling of infected carcasses, Scratches or bites from infected domestic cats, and Exposure to aerosols containing plague-causing bacilli.

Epidemiology

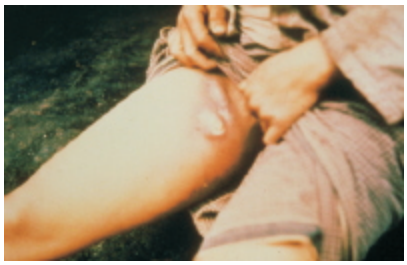
Frequency: ***International:*** Most cases of plague reported outside of the United States are from developing countries in Africa and Asia. During 1990-1995, a total of 12,998 cases of plague were reported to the World Health Organization (WHO), particularly from countries such as India, Zaire, Peru, Malawi, and Mozambique. The following countries reported more than 100 cases of plague: China, Congo, India, Madagascar, Mozambique, Myanmar, Peru, Tanzania, Uganda, Vietnam, and Zimbabwe. Several foci are located in the semi-arid regions of northeastern Brazil, and outbreaks have also been reported from Malawi and Zambia. Australia is the only continent that is considered free of plague. The largest enzootic plague area is in North America—the southwestern United States and the Pacific coastal area. The WHO reports that, in 2003, 9 countries reported a total of 2118 plague cases and 182 deaths, 98.7% and 98.9% of which were reported from Africa, respectively.



1998 world distribution of plague.

Mortality/Morbidity

The risk of plague-related death depends on the type of plague and whether the infected individual receives appropriate treatment. The following are the mortality rates associated with the different types of plague: Pneumonic plague: Untreated - 100%, Treated - 50%. Bubonic plague: Untreated - 50%-90%, Treated - 10%-20%. Septicemic plague: 20%-25%. **Race:** Humans are exposed in the domestic or outdoor environment. No special racial predeliction is observed. **Sex:** Plague has no sexual predilection. **Age:** Most cases of plague occur in persons younger than 20 years. **History:** Travel to endemic areas within and outside the United States, history of a flea bite, close contact with a potential host, or exposure to dead rodents or rabbits should raise suspicion for plague. **Bubonic plague:** This is the most common presentation of plague. The incubation period varies but usually ranges 2-6 days. There is a sudden onset of high fever, chills, and headache. Patients with this type also experience body aches, extreme exhaustion, weakness, abdominal pain, and/or diarrhea. Painful, swollen lymph glands (buboes) arise, usually in the groin (most common site), axilla, or neck.



Swollen lymph glands, termed buboes, are a hallmark finding in bubonic plague. Image courtesy of Centers for Disease Control and Prevention (CDC), Atlanta, Ga. Axillary, cervical, and epitrochlear buboes are almost always seen in cat-associated plague. Without intervention, this stage may lead to secondary pneumonic plague or meningitis or may disseminate and manifest as a sepsis picture. **Meningeal plague:** This is characterized by fever, headache, and nuchal rigidity. Buboes are common in meningeal plague. Axillary buboes are associated with an increased incidence of the meningeal form. **Pharyngeal plague:** Pharyngeal plague results from ingestion of the plague bacilli. Patients experience sore throat, fever, and painful cervical lymph nodes. Marshall et al (1967) has described an asymptomatic pharyngeal carrier state of *Y pestis* infection in patients with bubonic plague. **Pneumonic plague:** Pneumonic plague is highly contagious and transmitted by aerosol droplets. This is often secondary to bubonic or septicemic plague. However, primary pneumonic plague may be seen in laboratory workers, individuals exposed to an infected person, or those who have been exposed to a cat with pneumonic plague. There is an abrupt onset of fever and chills, accompanied by cough, chest pain, dyspnea, purulent sputum, or hemoptysis. Buboes may or may not be associated with pneumonic plague. The ability for plague to be spread by aerosols makes *Y pestis* a potential agent of bioterrorism. **Septicemic plague:** Septicemic plague is observed in elderly patients and causes a rapid onset of symptoms. Patients experience nausea, vomiting, abdominal pain, and diarrhea. (Diarrhea may be the predominant symptom.) Patients exhibit a toxic appearance and soon become moribund. Buboes are uncommon in septicemic plague, making the diagnosis elusive. Septicemic plague carries a high mortality rate and is associated with disseminated intravascular coagulation (DIC), multiorgan failure, and profound hypotension. Plague initially occurred as a flea-borne septicemic disease. However, over its evolutionary course, it acquired the plasminogen activator gene, giving rise to the bubonic form of disease. **Genitourinary/gastrointestinal plague:** This was reported as the sole presentation of *Y pestis* infection in 4 of 27 patients in a case series published in 1992. **Cutaneous plague:** This manifests as purpura.

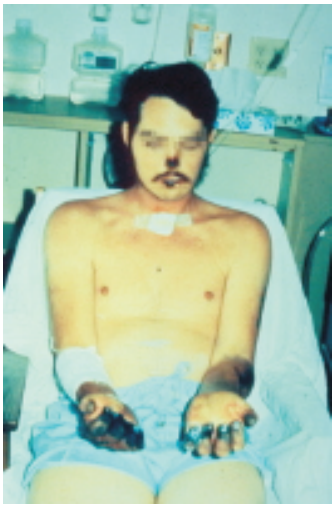
Physical

Bubonic plague: Vesicles may be observed at the site of the infected flea bite. With advanced disease, papules, pustules, carbuncles, or an eschar may be observed in areas of the skin drained by the involved lymph nodes. A generalized papular rash of the hands and feet may be observed. Buboes are unilateral, oval, extremely tender lymph nodes and can vary from 2-10 cm in size. Femoral lymph nodes are most commonly involved. Patients with an inguinal bubo walk with a limp, and the affected limb may be in a position of flexion, abduction, and external rotation. Patients resist any attempt to examine the involved lymph nodes. Enlargement of the buboes leads to rupture and discharge of malodorous pus. Hepatomegaly and splenomegaly often occur and may be tender.

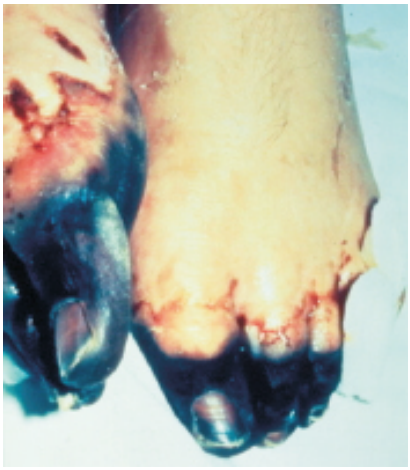
Pharyngeal plague causes pharyngeal erythema and painful and tender anterior cervical nodes.

Pneumonic plague causes fever, lymphadenopathy, productive sputum, and/or hemoptysis.

Septicemic plague: Because of an overwhelming infection with the plague bacillus, patients with septicemic plague have a toxic appearance and may present with tachycardia, tachypnea, and hypotension. Hypothermia is common. Generalized purpura may be observed and can progress to necrosis and gangrene of the distal extremities.



Acral necrosis of the nose, the lips, and the fingers and residual ecchymoses over both forearms in a patient recovering from bubonic plague that disseminated to the blood and the lungs. At one time, the patient's entire body was ecchymotic.



Acral necrosis of the toes and residual ecchymoses over both forearms in a patient recovering from bubonic plague that disseminated to the blood and the lungs. At one time, the patient's entire body was ecchymotic. No evidence of lymphadenitis or bubo formation is apparent. Patients may die of a high-grade bacteremia.

Causes

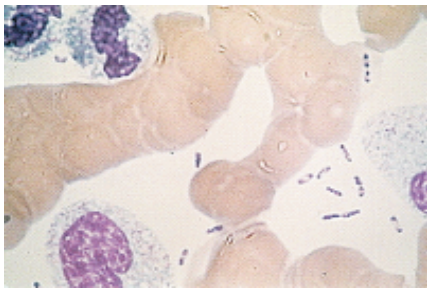
Y pestis is the cause of plague. Risk factors: Flea bite, Contact with a patient or a potential host, Contact with sick animals or rodents, Residence in an endemic area of plague (eg, southwestern United States), Presence of a food source for rodents in the immediate vicinity of the home, Camping, hiking, hunting, or fishing, Occupational exposure (eg, researchers, veterinarians), Direct handling or inhalation of contaminated tissue or tissue fluids.

Differential Diagnoses

Anthrax, Brucellosis, Catscratch Disease, Cellulitis, Chancroid, Dengue Fever, Disseminated Intravascular Coagulation, Lymphadenitis, Lymphadenopathy, Lymphogranuloma Venereum (LGV), Lymphoma, B-Cell, Malaria, Pasteurella Multocida Infection, Pharyngitis, Bacterial, Pneumonia, Bacterial, Pneumonia, Community-Acquired, Rocky Mountain Spotted Fever, Sepsis, Bacterial, Septic Shock, Syphilis, Systemic Inflammatory Response Syndrome, Tonsillitis and Peritonsillar Abscess, Tularemia, Typhus.

Laboratory Studies

The possibility of plague should be strongly considered in febrile patients from endemic areas who have history of exposure to rodents. Rapid recognition of the classic symptoms of this disease and laboratory confirmation are essential to instituting lifesaving therapy. Expertise in testing for plague bacilli is limited to reference laboratories in plague-endemic states and the CDC. Leukocytosis with a predominance of neutrophils is observed, and the degree of leukocytosis is proportional to the severity of illness. Peripheral blood smear shows toxic granulations and Dohle bodies. Thrombocytopenia is common, and levels of fibrin degradation products may be elevated. Serum transaminase and bilirubin levels may be elevated. Proteinuria may be present, and renal function test findings may be abnormal. Hypoglycemia may be observed. Twenty-seven percent to 96% of blood cultures are positive for *Y pestis* in patients with bubonic plague and septicemic plague. Microbiology staff should be informed of the possibility of *Y pestis* agents in samples so that they can take adequate precautions when handling specimens. *Y pestis* may be observed on a peripheral blood smear. Smear stained with Wright-Giemsa reveals rod-shaped bacteria. A Wayson stain demonstrates the typical "safety pin" appearance (bipolar staining) of the bacterium. Gram stain shows small gram-negative coccobacilli.



Wayson stain showing the characteristic "safety pin" appearance of Yersinia pestis, the plague bacillus.

Lymph node aspirates often demonstrate *Y pestis*. In patients with pharyngeal plague, *Y pestis* is cultured from throat swabs. Cerebrospinal fluid (CSF) analysis in meningeal plague may show pleocytosis with a predominance of polymorphonuclear leukocytes. Gram stain of CSF may show plague bacilli. Limulus test of CSF demonstrates the presence of endotoxin. Gram stain of sputum often reveals *Y pestis*.

Imaging Studies

Chest radiography reveals patchy infiltrates, consolidation, or a persistent cavity in patients with pneumonic plague. ECG reveals sinus tachycardia and ST-T changes. Nuclear imaging may help localize areas of lymphadenitis and meningeal inflammation.

Other Tests

Direct immunofluorescence testing of fluid or cultures may aid in rapid diagnosis. A novel rapid diagnostic test capable of detecting miniscule amounts of *Y pestis* F1 antigen within 15 minutes has been developed and field tested in Madagascar. This test yields 100% sensitivity and specificity for *Y pestis* and other *Yersinia* species. A passive hemagglutination test (performed on serum from a patient in acute or convalescent stages) with a 16-fold or greater increase in titer (single titer) suggests plague infection. A 4-fold rise in antibody titers to the F-1 antigen of *Y pestis* also confirms infection. A polymerase chain reaction (PCR) using primers derived from *Y pestis* plasminogen activator gene has been used to detect the pathogen in fleas, but the application of this

method in humans is still a matter of speculation.

Procedures

Aspiration of lymph node (bubo): Inject 1 mL of sterile saline into the bubo with a 20-gauge needle; after withdrawing several times, aspirate the fluid. Gram stain of the aspirate reveals gram-negative coccobacilli and polymorphonuclear leucocytes. Wayson stain of the aspirate shows plague bacilli as light-blue bacilli with dark-blue polar bodies. Examination of the aspirate of the fluid from the inguinal lymph nodes shows a characteristic bipolar appearance that resembles a closed safety pin. Lumbar puncture for CSF analysis.

Medical Care

Precautions: All patients with suspected plague and signs of pneumonia should be placed in strict respiratory isolation for 48-72 hours after antibiotic therapy is initiated and kept there until pneumonia has been ruled out or until sputum culture have shown negative findings. Report patients thought to have plague to the local health department and to the WHO. Alert laboratory personnel to the possibility of the diagnosis of plague. All fluid specimens must be handled with gloves and mask to prevent aerosolization of the infected fluids.

Supportive therapy: Hemodynamic monitoring and ventilatory support are performed as appropriate. Intravenous fluids, epinephrine, and dopamine are implemented as necessary for correction of dehydration and hypotension.

Postexposure prophylaxis: Presumptive therapy consists of a 7-day course of oral doxycycline and ciprofloxacin. Chloramphenicol may be used as an alternative. Levofloxacin may be prescribed as a 10-14 day regimen for either treatment or postexposure prophylaxis. In a community experiencing a pneumonic plague epidemic, individuals with a temperature of 38.5°C or higher or newly onset cough should promptly receive parenteral antimicrobial therapy.

Surgical Care: Enlarging or fluctuant buboes require incision and drainage.

Consultations: Infectious disease specialist, Pulmonary and critical care specialist, General surgeon, Neurologist.

Medication Summary: Untreated plague can progress to a fulminate illness with a high risk of mortality. Thus, early and appropriate antibiotic treatment is essential. Historically, streptomycin (15 mg/kg, up to 1 g intramuscularly every 12 h) has been the drug of choice. However, in the United States, supplies of streptomycin are scarce. An in vitro comparison and a murine model trial demonstrated that gentamicin (5 mg/kg intravenously or intramuscularly once daily) is comparable to or superior than streptomycin. Gentamicin has been used successfully in the treatment of human plague, is inexpensive, and can be dosed once daily. Doxycycline (as dosed for anthrax) is a recommended alternative in patients who cannot take aminoglycosides or in the event of a mass casualty scenario, making parenteral therapy unachievable. Studies in murine models have shown that fluoroquinolones demonstrate efficacy similar to that of the aminoglycosides. Fluoroquinolones are a reasonable alternative therapy, with ciprofloxacin (as dosed for anthrax) the most studied and therefore preferred. However, no trials of fluoroquinolone therapy in human plague have been conducted. Because chloramphenicol attains high CSF concentrations, it has been used to treat CNS infections associated with plague, although no studies have been conducted for substantiation. The FDA has approved levofloxacin for the treatment of plague. It is also approved for use as a prophylactic treatment following the exposure to *Yersinia pestis.* Trimethoprim-sulfamethoxazole has been used to treat bubonic plague; however, it is not considered first-line therapy. Beta-lactam antibiotics and macrolides should not be used. Patients with advanced plague have a presentation of typical gram-negative sepsis and need antibiotic treatment for 10 days, along with other supportive measures.

Transfer: Patients with plague who are critically ill and require transfer to another facility should be transported under strict isolation precautions.

Deterrence/Prevention

Prophylactic antibiotic therapy: Most organisations recommend short-term prophylactic antibiotic therapy in people who have been bitten by potentially infected rodent fleas during a plague outbreak. Prophylactic antibiotic therapy is recommended in persons who have handled an animal known to be infected with the plague bacterium. Prophylactic antibiotic therapy is recommended in persons who have had close exposure to a person or an animal thought to have pneumonic plague. Sulfadoxine prophylaxis has been effective in outbreaks of pneumonic plague. The infection rate in contacts was 8.4% with this strategy. Recent studies have shown that doxycycline can be used as an alternative for sulfadoxine. Preferred antibiotics for prophylaxis against plague include doxycycline 100 mg PO q12h for 14-21 days (for patients >8 y) or full-dose ciprofloxacin for 7 days. Chloramphenicol may be used as an alternative. To be effective, chemoprophylaxis must be initiated within 7 days of exposure.

Plague vaccine: Plague vaccination is of limited use and is not mandatory for entry into any country. The vaccine is not effective against the pneumonic form of plague. Plague vaccine is recommended for field workers in endemic areas and for scientists and laboratory personnel who routinely work with the plague bacterium. The vaccine is composed of killed whole cells. It needs to be taken as 2 injections 1-3 months apart followed by the booster every 6 months until the patient is no longer considered to be at risk. Live vaccines are in development. Animal studies have conclusively established that certain antibodies are protective against plague. Murine antibodies to fraction (FI) protein and/or fraction V antigen have been shown to be protective against bubonic and pneumonic plague in murine models. The F1-V (fusion protein) vaccine protected mice for a year against an inhalation challenge and is now being tested in primates.

Environmental sanitation: Efforts to control the animal reservoir and flea population may be effective in reducing transmission of plague bacteria. Remove food sources used by rodents. Rodent-proof homes, buildings, and warehouses. Trained professionals should apply chemicals to kill fleas and rodents. Trained professionals should fumigate cargo areas of ships and docks.

Complications

Acute respiratory distress syndrome, Chronic lymphedema from lymphatic scarring, DIC, Septic shock, Superinfections of the buboes by *Staphylococcus* and *Pseudomonas* species.

Prognosis

Untreated plague carries a mortality rate of approximately 50%; however, with appropriate therapy, the mortality rate drops to approximately 5%.

Patient Education

Report sick or dead animals to the local health department or law enforcement officials and wear gloves when handling potentially infected animals. Eliminate food sources and nesting places for rodents around homes, workplaces, and recreation areas and make homes rodent-proof. Personal protective measures include wearing protective clothing and applying insect repellents to clothing and skin to prevent flea bites. Restrain pet dogs and cats in areas endemic to plague and regularly treat pets to control fleas. Spraying of appropriate chemicals by health authorities may be necessary to kill fleas at selected sites during animal plague outbreaks.

INTERPRETATION

Urine Test

The kidneys take out waste material, minerals, fluids, and other substances from the blood to be passed in the urine. Urine has hundreds of different body wastes. What you eat and drink, how much you exercise, and how well your kidneys work can affect what is in your urine.

More than 100 different tests can be done on urine. A regular urinalysis often includes the following tests:

Qualitative

Color. Many things affect urine color, including fluid balance, diet, medicines, and diseases. How dark or light the color is tells you how much water is in it. Vitamin B supplements can turn urine bright yellow. Some medicines, blackberries, beets, rhubarb, or blood in the urine can turn urine red-brown.

Clarity. Urine is normally clear. Bacteria, blood, sperm, crystals, or mucus can make urine look cloudy.

Odor. Urine does not smell very strong, but it has a slightly "nutty" odor. Some diseases cause a change in the odor of urine. For example, an infection with *E. coli* bacteria can cause a bad odor, while diabetes or starvation can cause a sweet, fruity odor.

Specific gravity. This checks the amount of substances in the urine. It also shows how well the kidneys balance the amount of water in urine. The higher the specific gravity, the more solid material is in the urine. When you drink a lot of fluid, your kidneys make urine with a high amount of water in it, which has a low specific gravity. When you do not drink fluids, your kidneys make urine with a small amount of water in it, which has a high specific gravity.

pH. The pH is a measure of how acidic or alkaline (basic) the urine is. A urine pH of 4 is strongly acidic, 7 is neutral (neither acidic nor alkaline), and 9 is strongly alkaline. Sometimes the pH of urine is affected by certain treatments. For example, your doctor may instruct you how to keep your urine either acidic or alkaline to prevent some types of kidney stones from forming.

Protein. Protein normally isn't found in the urine. Fever, hard exercise, pregnancy, and some diseases, especially kidney disease, may cause protein to be in the urine.

Glucose. Glucose is the type of sugar found in blood. Normally there is very little or no glucose in urine. When the blood sugar level is very high, as in uncontrolled diabetes, the sugar spills over into the urine. Glucose can also be found in urine when the kidneys are damaged or diseased.

Nitrites. Bacteria that cause a urinary tract infection (UTI) make an enzyme that changes urinary nitrates to nitrites. Nitrites in urine show a UTI is present.

Leukocyte esterase (WBC esterase). Leukocyte esterase shows leukocytes (white blood cells [WBCs]) in the urine. WBCs in the urine may mean a UTI is present.

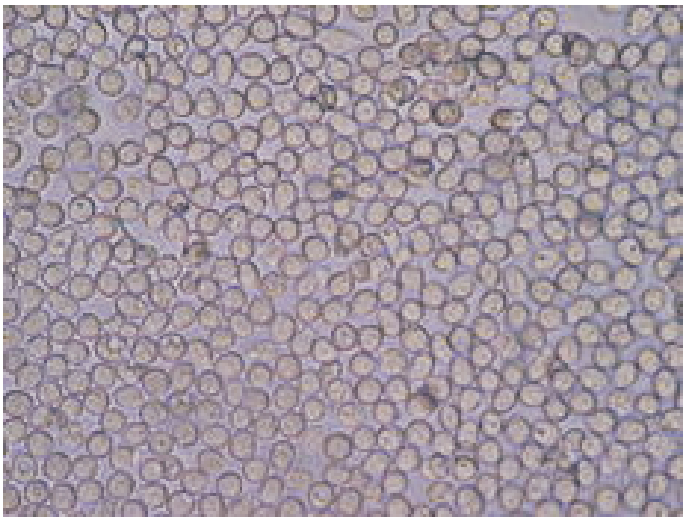
Ketones. When fat is broken down for energy, the body makes substances called ketones (or ketone bodies). These are passed in the urine. Large amounts of ketones in the urine may mean a very serious condition, diabetic ketoacidosis, is present. A diet low in sugars and starches (carbohydrates), starvation, or severe vomiting may also cause ketones to be in the urine.

Microscopic analysis. In this test, urine is spun in a special machine (centrifuge) so the solid materials (sediment) settle at the bottom. The sediment is spread on a slide and looked at under a microscope. Things that may be seen on the slide include: **Red or white blood cells.** Blood cells aren't found in urine normally. Inflammation, disease, or injury to the kidneys, ureters, bladder, or urethra can cause blood in urine. Strenuous exercise, such as running a marathon, can also cause blood in the urine. White blood cells may be a sign of infection or kidney disease. **Casts.** Some types of kidney disease can cause plugs of material (called casts) to form in tiny tubes in the kidneys. The casts then get flushed out in the urine. Casts can be made of red or white blood cells, waxy or fatty substances, or protein. The type of cast in the urine can help show what type of kidney disease may be present. **Crystals.** Healthy people often have only a few crystals in their urine. A large number of crystals, or certain types of crystals, may mean kidney stones are present or there is a problem with how the body is using food (metabolism). **Bacteria, yeast cells, or parasites.** There are no bacteria, yeast cells, or parasites in urine normally. If these are present, it can mean you have an infection. **Squamous cells.** The presence of squamous cells may mean that the sample is not as pure as it needs to be. These cells do not mean there is a medical problem, but your doctor may ask that you give another urine sample.

Quantitative/ Semi-quantitative

Urinalysis

Intervention



White blood cells seen under a microscope from a urine sample.

A **urinalysis** (**UA**), also known as **routine and microscopy** (**R&M**), is an array of tests performed on urine, and one of the most common methods of medical diagnosis. The word is a portmanteau of the words *urine* and *analysis*. The target parameters that can be measured or quantified in urinalysis include many substances and cells, as well as other properties, such as specific gravity. A part of a urinalysis can be performed by using urine test strips, in which the test results can be read as color changes. Another method is light microscopy of urine samples.

Target parameters

Urine test results should always be interpreted using the reference range provided by the laboratory that performed the test, or using information provided by the test strip/device manufacturer. In addition to the substances mentioned in tables below, other tests include a description of color and appearance.

Ions and trace metals

Target	Lower limit	Upper limit	Unit	Comments
Nitrite	n/a	0 / negative		The presence of nitrites in urine, termed nitrituria, indicates the presence of coliform bacteria. Further information: Nitrite test
Sodium (Na) – per day	150	300	mmol / 24 h	A urinalysis is frequently ordered during the workup of acute renal failure. Full kidney function can be detected through the simple dipstick method. Urine K may be ordered in the workup of hypokalemia. In case of gastrointestinal loss of K, the urine K will be low. In case of renal loss of K, the urine K levels will be high. Decreased levels of urine K are also seen in hypoaldosteronism and adrenal insufficiency.
Potassium (K) – per day	40	90	mmol / 24 h	
Urinary calcium (Ca) – per day	15 100	20 250	mmol / 24 h mg / 24 hours	An abnormally high level is called hypercalciuria and an abnormally low rate is called hypocalciuria. Further information: Urinary calcium
Phosphate (P) – per day	n/a	38	mmol / 24 h	Phosphaturia is the hyperexcretion of phosphate in the urine. This condition is divided into primary and secondary types. Primary hypophosphatemia is characterized by direct excess excretion of phosphate by the kidneys, as from primary renal dysfunction, and also the direct action of many classes of diuretics on the kidneys. Additionally, secondary causes, including both types of hyperparathyroidism, cause hyperexcretion of phosphate in the urine. A sodium-related parameter is fractional sodium excretion, which is the percentage of the sodium filtered by the kidney which is excreted in the urine. It is a useful parameter in acute renal failure and oliguria, with a value below 1% indicating a prerenal disease and a value above 3%[7] indicating acute tubular necrosis or other kidney damage.

Proteins and enzymes

Target	Lower limit	Upper limit	Unit	Comments
Protein	0	trace amounts/ 20	mg/dl	Proteins may be measured with the Albustix test. Since proteins are very large molecules (macromolecules), they are not normally present in measurable amounts in the glomerular filtrate or in the urine. The detection of protein in urine, called proteinuria, may indicate the permeability of the glomerulus increased. This may be caused by renal infections or by other diseases that have secondarily affected the kidneys, such as hypertension, diabetes mellitus, jaundice, or hyperthyroidism. Further information: Proteinuria
Human chorionic gonadotropin |hCG	–	50	U/l	This hormone appears in the urine of pregnant women. Home pregnancy tests commonly detect this substance.

Blood cells

Target	Lower limit	Upper limit	Unit	Comments
Red blood cells (RBCs) / erythrocytes	0	2 – 3	per High Power Field (HPF)	May be present as intact RBCs, which indicate bleeding. Even trace amount of blood is enough to give the entire urine sample a red/pink hue, and it is difficult to judge the amount of bleeding from a gross examination. Hematuria may be due to a generalized bleeding diathesis or a urinary tract-specific problem (trauma, stone, infection, malignancy, etc.) or artefact of catheterization in case the sample is taken from a collection bag, in which case a fresh urine sample should be sent for a repeat test.

				If the RBCs are of renal or glomerular origin (due to glomerulonephritis), the RBCs incur mechanical damage during the glomerular passage, and then osmotic damage along the tubules, so get dysmorphic features. The dysmorphic RBCs in urine which are most characteristic of glomerular origin are called "G1 cells", which are doughnut-shaped rings with protruding round blebs sometimes looking like Mickey Mouse's head (with ears). Painless hematuria of nonglomerular origin may be a sign of urinary tract malignancy, which may warrant a more thorough cytological investigation. Further information: Hematuria
RBC casts	n/a	0 / negative		
White blood cells (WBCs) / leukocytes / (pus cells)	0 –	2 / negative 10	per µl or mm^3	Further information: Pyuria "Significant pyuria" at greater than or equal to 10 leucocytes per microlitre (µl) or cubic millimeter (mm^3)
"Blood" / (actually hemoglobin)	n/a	0 / negative	dip-stick qualitative scale of 0 to 4+	Hemoglobinuria is suggestive of *in vivo* hemolysis, but must be distinguished from hematuria. In case of hemoglobinuria, a urine dipstick shows presence of blood, but no RBCs are seen on microscopic examination. If hematuria is followed by artefactual *ex vivo* or *in vitro* hemolysis in the collected urine, then the dipstick test also will be positive for hemoglobin and will be difficult to interpret. The urine color may also be red due to excretion of reddish pigments or drugs.

Other molecules

Target	Lower limit	Upper limit	Unit	Comments
Glucose	n/a	0 / negative		Glucose can be measured with Benedict's test. Although glucose is easily filtered in the glomerulus, it is not present in the urine because all of the glucose filtered is normally reabsorbed from the renal tubules back into the blood. Presence of glucose in the urine is called glucosuria. Further information: Glucosuria
Ketone bodies	n/a	0 / negative		With carbohydrate deprivation, such as starvation or high-protein diets, the body relies increasingly on the metabolism of fats for energy. This pattern is also seen in people with diabetes mellitus, when a lack of the hormone insulin prevents the body cells from using the large amounts of glucose available in the blood. This happens because insulin is necessary for the transport of glucose from the blood into the body cells. The metabolism of fat proceeds in a series of steps. First, triglycerides are hydrolyzed to fatty acids and glycerol. Second, the fatty acids are hydrolyzed into smaller intermediate compounds (acetoacetic acid, betahydroxybutyric acid, and acetone). Thirdly, the intermediate products are used in aerobic cellular respiration. When the production of the intermediate products of fatty acid metabolism (collectively known as ketone bodies) exceeds the ability of the body to metabolize these compounds, they accumulate in the blood and some end up in the urine (ketonuria). Further information: Ketonuria
Bilirubin	n/a	0 / negative		The fixed phagocytic cells of the spleen and bone marrow destroy old red blood cells and convert the heme groups of hemoglobin to the pigment bilirubin. The bilirubin is secreted into the blood and carried to the liver, where it is bonded to (conjugated with) glucuronic acid, a derivative of glucose. Some of the conjugated bilirubin is secreted into the blood and the rest is excreted in the bile as bile pigment that passes into the small intestine. The blood normally contains a small amount of

				free and conjugated bilirubin. An abnormally high level of blood bilirubin may result from an increased rate of red blood cell destruction, liver damage (as in hepatitis and cirrhosis), and obstruction of the common bile duct as with gallstones. An increase in blood bilirubin results in jaundice, a condition characterized by a brownish-yellow pigmentation of the skin and of the sclera of the eyes. Further information: Bilirubinuria
Urobilinogen	0.2	1.0	Ehrlich units or mg/dL	
Creatinine – per day	4.8	19	mmol / 24 h	
Free catecholamines, dopamine – per day	90	420	µg / 24 hours	
Free cortisol	28 or 30 10 or 11	280 or 490 100 or 176	nmol/24 h µg/24 h	Values below threshold indicate Addison's disease, while values above indicate Cushing's syndrome. A value smaller than 200 nmol/24 h (72 µg/24 h) strongly indicates absence of Cushing's syndrome.
Phenylalanine		30.0	mg/L	In neonatal screening, a value above the upper limit defines phenylketonuria.

Other urine parameters

Test	Lower limit	Upper limit	Unit	Comments
Urine specific gravity	1.003 [1][4]	1.030	no unit	This test detects the ion concentration of urine. Small amounts of protein or ketoacidosis tend to elevate the urine's specific gravity (SG). This value is measured using a urinometer and indicates hydration or dehydration. If the SG is under 1.010, the patient is hydrated; an SG value above 1.020 indicates dehydration.
Osmolality pH	400 5	n/a 7	mOsm/kg (unitless)	
Bacterial cultures	by urination	–	100,000 colony forming units per millilitre (CFU/mL)	Bacteriuria can be confirmed if a single bacterial species is isolated in a concentration greater than 100,000 CFU/ml of urine in clean-catch midstream urine specimens (one for men, two consecutive specimens with the same bacterium for women). Further information: Bacteriuria
	by bladder catheterisation	–	100	For urine collected via bladder catheterisation, the threshold is 100 CFU/ml of a single species. Further information: Bacteriuria

Illicit substances

Urine may be tested to determine whether an individual has engaged in recreational drug use. In this case, the urinalysis would be designed to detect whatever marker indicates drug use.

BOUQUET

In Lighter Vein

One night a teenage girl brought her new boyfriend home to meet her parents, and they were appalled by his appearance: leather jacket, motorcycle boots, tattoos and pierced nose.
Later, the parents pulled their daughter aside and confessed their concern. “Dear,” said the mother diplomatically, “he doesn't seem very nice.”
“Oh please, Mom,” replied the daughter, “if he wasn't nice, why would he be doing 500 hours of community service?”

Sale

“I see you went crazy at the big summer clearance sale,” Wanda comments, as she looks at all the bags of merchandise her friend, Carol just brought home from the store.
“You got that right … I almost bought their elevator 'cause it was marked down.”

Test By Puncture

Santa meets Banta in a hospital and expresses surprise, "What are you here for?"
Banta says, "I am here for blood test and these idiots are going to puncture my finger."
Santa started crying, "Oh my God, I am here for urine test and I am too young yet, what will happened to me?”

Gossiping Gurkhas

A Gurkha guard boasts to other, "You know, when I was small, that Victoria Tower fell down upon me."
So the second Gurkha guard inquired, "Hey why? Did it kill you then?"
The puzzled first one says, "I don't remember, I was too young then.”

Bush & Israeli Mossad

After numerous rounds of 'We don't even know if Osama bin Laden is still alive', Osama himself decided to send George Bush a letter in his own hand writing to let him know he was still in the game.
Bush opened the letter and it contained a single line of coded message: 370H-SSV-0773H. Bush was baffled, so he e-mailed it to Condoleezza Rice. Condi and her aides had not a clue either, so they sent it to the FBI.
No one could solve it at the FBI so it went to the CIA, and then to MI6.
Eventually they asked the Mossad (Israeli intelligence) for help.
Within a minute the Mossad emailed the White House with this reply: 'Tell the President he's holding the note upside down.

Brain Teasers

ANSWER THE FOLLOWING QUESTIONS

1. Which of the following analytes have higher values in the CSF than in the plasma?
A. Sodium
B. Chloride
C. Magnesium
D. All of the above.

2. Which of the following analytes have lower values in the CSF than in the plasma?
A. Uric acid
B. Iron
C. Thyroxine
D. All of the above.

3. What is the normal CSF pressure while the patient is lying on side?
A. 70–150 mm of water column
B. 10–50 mm of water column
C. 120–180 mm of water column
D. 200–300 mm of water column.

4. What is the normal volume of CSF in a human being?
A. 90–150 mL
B. 150–220 mL
C. 220–300 mL
D. 300–500 mL.

Answers: 1. D, 2. D, 3. A, 4. A

Wisdom Whispers

QUOTE: Success, Emerson

"To laugh often and much; to win the respect of intelligent people and the affection of children; to earn the appreciation of honest critics and endure the betrayal of false friends; to appreciate beauty, to find the best in others; to leave the world a little better; whether by a healthy child, a garden patch or a redeemed social condition; to know even one life has breathed easier because you have lived. This is the meaning of success."

-Ralph Waldo Emerson

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.'

TROUBLESHOOTING

THE PAP STAIN

PROBLEM	POSSIBLE REASON	REMEDY
DARK NUCLEI	Too much time in Harris' Hematoxylin. Not enough time in HCl or HCl concentration less than recommended.	Reduce time in HTX by 10,15,20,30 sec intervals Increase time in acid by 5,10 sec.
PALE NUCLEI	Polyethylene glycol coating not removed from cells prior to Hematoxylin. Concentration of HCl greater than recommended or too much time in HCl. Not enough time in Hematoxylin. Hematoxylin diluted by water (if water not properly drained from slides). Stain not changed frequently enough resulting in Hematoxylin exhausted	Extend prestaining soak with aqueous ethanol. Reduce time in acid by 5,10 sec and ensure correct amount of acid is added to the solution. Increase time in HTX by 10,15,20,30 sec intervals Ensure the arm of the staining machine is operating correctly. Ensure a set amount of slides are stained and then stains are changed.
CYTOPLASMIC COLOUR NOT CONSISTENT	Air drying prior to fixation. Polyethylene coating inadequately removed from cells. Solutions not at proper level within staining dishes. Excessive time in Hematoxylin or Hematoxylin not removed prior to OG and EA dyes. Slides left too long in ethanol rinses or clearing solutions following OG and EA. Inadequate rinsing of slides between solutions. Insufficient rinsing following staining solutions. pH of tap and distilled water not sufficiently alkaline. pH of EA needs to be controlled (pH 4.5 to 5 achieves maximum results).	Report the findings to the referring clinician Extend prestaining soak in aqueous ethanol. Check staining solution level Reduce time in HTX by 10,15,20,30 sec intervals Reduce ethanol rinse time Check if ethanol is changed regularly Increase ethanol rinse time Check pH Check pH Ensure a set amount of slides are EA dye exhausted stained and then stains are changed.
MACROSCOPICALLY ALL SLIDES ARE PINK, ORANGE OR YELLOW	Slide drying oven temperature too high	If this happens there is nothing that can be done to obtain a well-stained sample.
DULL PINK AND DEGENERATE APPEARANCE	This usually occurs to smears that accompany histology specimens. It is usually due to formalin fixation.	Ensure formalin pot and smear is transported in separate bags.
DULL, GREYISH APPEARANCE OF CELLS	Water contamination of dehydrating and clearing solutions. Polyethylene glycol coating not removed from cells prior to staining of filter.	Ensure dehydrating and clearing solutions are changed regularly. Extend the prestaining soak time.
OPAQUE/WHITE COLOUR ON BACK OF SLIDE	Bluing agent not rinsed from slides. water rinses following Scott's Tap	Use two separate but thorough Water substitute. (For progressive Pap staining)
STAIN DEPOSIT	Staining dyes not changed or filtered properly	Ensure staining dyes changed or filtered regularly
FUNGAL CONTAMINANTION	Slides contaminated by fungus during the staining process	Change staining solutions regularly and ensure the staining containers are disinfected with a dilute bleach solution.

TULIP NEWS

PUO SCREEN

Screening Test for Typhoid, Brucellosis & Scrub Typhus

Pyrexia of Unknown Origin (PUO – also known as Fever of Unknown Origin) can be observed in several febrile diseases. Among all the febrile cases, prevalence of Typhoid is remarkably high and well known. Febrile illness due to brucellosis and scrub typhus (rickettial) infections have also been reported. It has been estimated that true incidences of brucellosis and scrub typhus infections may be much higher than the reported incidences due to misdiagnosis and under reporting; largely due to lack of diagnostic facilities.

Screening test for above important parameters if performed initially on all febrile patients can help in early and conclusive diagnosis of febrile illness that aid to take appropriate treatment decision. To provide the complete picture of febrile diagnosis we have introduced a combined test for screening of Typhoid, Brucellosis and Scrub typhus infection, branded as **PUO SCREEN**.

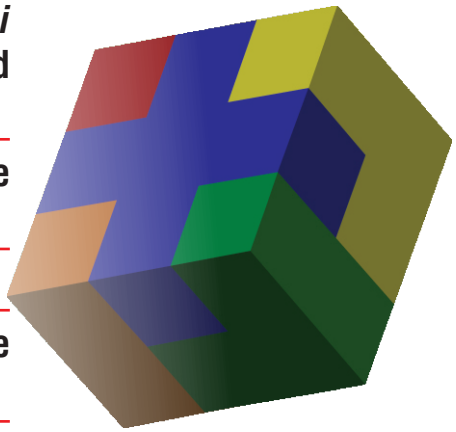
Screening test for three febrile diseases : Kit contains *S.typhi* 'O' antigen for typhoid, *Brucella abortus* for Brucellosis and *Proteus* OXK antigen for Scrub typhus detection.

Reagent can be validated using control : Polyspecific positive control provided in the kit.

Rapid turn around time : One minute test.

Simple and similar test method : Same test method for all the three reagents.

Convenient pack size, suitable for all class of customers : 3 x 2 ml pack size.



Complete Picture of Febrile Diagnosis !

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Fax: (0832) 2458544. E-mail: sales@tulipgroup.com Website: www.tulipgroup.com