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The Crux

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CONTENTS

Editorial

Shigellosis, also known as **bacillary dysentery** or **Marlow syndrome**, in its most severe manifestation, is a foodborne illness caused by infection by bacteria of the genus *Shigella*. Shigellosis rarely occurs in animals other than humans.

The causative organism is frequently found in water polluted with human feces, and is transmitted via the fecal-oral route. The usual mode of transmission is directly person-to-person hand-to-mouth, in the setting of poor hygiene among children.

Shigella is one of the leading bacterial causes of diarrhea worldwide and is a top pathogen causing moderate-to-severe diarrhea in African and South Asian children. It is also a major cause of illness in military personnel and travelers.

Signs and symptoms may range from mild abdominal discomfort to full-blown dysentery characterized by cramps, diarrhea, with slimy-consistent stools, fever, blood, pus, or mucus in stools or tenesmus. Onset time is 12 to 96 hours, and recovery takes 5 to 7 days.

Infections are associated with mucosal ulceration, rectal bleeding, and drastic dehydration. Reactive arthritis and hemolytic uremic syndrome are possible sequelae that have been reported in the aftermath of shigellosis.

Shigella can be transmitted through food, including salads (potato, tuna, shrimp, macaroni, and chicken), raw vegetables, milk and dairy products, and meat. Contamination of these foods is usually through the fecal-oral route. Fecally contaminated water and unsanitary handling by food handlers are the most common causes of contamination. Apart from hand-to-mouth infection, shigellosis is transmitted through fomites, water and mechanical vectors like houseflies.

The most common neurological symptom includes seizures.

For a complete in-depth clinico-diagnostic approach to Shigellosis, please turn over.

Aptly so, the INTERPRETATION segment deals with Stool Analysis. The TROUBLE SHOOTING component highlights the correct approach to preparing a semen specimen for wash. This remains a big variable in IUI/ infertility work up. Followed properly this could become a constant instead of a variable.

BOUQUET exists within in its natural funny, thought provoking and scalp scratching format!

DISEASE DIAGNOSIS

DYSENTRY (Shigellosis)

Background

Shigella organisms are a group of gram-negative, facultative intracellular pathogens. They were recognized as the etiologic agents of bacillary dysentery or shigellosis in the 1890s. *Shigella* were discovered over 100 years ago by a Japanese microbiologist named Shiga, for whom the genus is named. *Shigella* was adopted as a genus in the 1950s. These organisms are members of the family Enterobacteriaceae and tribe Escherichieae; they are grouped into 4 species: *Shigella dysenteriae, Shigella flexneri, Shigella boydii,* and *Shigella sonnei,* also known as groups A, B, C, and D, respectively. They are nonmotile, non – spore forming, rod shaped, and nonencapsulated. Subgroups and serotypes are differentiated from each other by their biochemical characteristics (e.g., ability to ferment D-mannitol) and antigenic properties. Group A has 15 serotypes, group B has 8 serotypes, group C has 19 serotypes, and group D has 1 serotype.

Geographic distribution and antimicrobial susceptibility varies with different species. *S. dysenteriae* serotype 1 causes deadly epidemics, *S. boydii* is restricted to the Indian subcontinent, and *S. flexneri* and *S. sonnei* are prevalent in developing and developed countries, respectively. *S. flexneri,* enteroinvasive gram-negative bacteria, is responsible for the worldwide endemic form of bacillary dysentery.

Pathophysiology

Shigella infection is a major public health problem in developing countries where sanitation is poor. Humans are the natural reservoir, although other primates may be infected. No natural food products harbor endogenous *Shigella* species, but a wide variety of foods may be contaminated. Shigellosis is spread by means of fecal-oral transmission. Other modes of transmission include ingestion of contaminated food or water (untreated wading pools, interactive water fountain), contact with a contaminated inanimate object, and certain mode of sexual contact. Vectors like the housefly can spread the disease by physically transporting infected feces. The infectivity dose (ID) is extremely low. As few as 10 *S. dysenteriae* bacilli can cause clinical disease, whereas 100-200 bacilli are needed for *S. sonnei* or *S. flexneri* infection. The reasons for this low-dose response are not completely clear. One possible explanation is that virulent *Shigellae* can withstand the low pH of gastric juice. Most isolates of *Shigella* survive acidic treatment at pH 2.5 for at least 2 hours. The incubation period varies from 12 hours to 7 days but is typically 2-4 days; the incubation period is inversely proportional to the load of ingested bacteria. The disease is communicable as long as an infected person excretes the organism in the stool, which can extend as long as 4 weeks from the onset of illness. Bacterial shedding usually ceases within 4 weeks of the onset of illness; rarely, it can persist for months. Appropriate antimicrobial treatment can reduce the duration of carriage to a few days.

Virulence

Virulence in *Shigella* species involves both chromosomal-coded and plasmid-coded genes. Virulent *Shigella* strains produce disease after invading the intestinal mucosa; the organism only rarely penetrates beyond the mucosa.[1] The characteristic virulence trait is encoded on a large (220 kb) plasmid responsible for synthesis of polypeptides that cause cytotoxicity. Shigellae that lose the virulence plasmid are no longer pathogenic. *Escherichia coli* (*E. coli* O157:H7) that harbor this plasmid clinically behave as *Shigella* bacteria. Siderophores, a group of plasmid-coded genes, control the acquisition of iron from host cells from its protein-bound state. In the extra intestinal phase of infection by gram-negative bacteria, iron becomes one of the major factors that limit further growth. This limitation occurs because most of the iron in human body is sequestered in hemoproteins (i.e., hemoglobin, myoglobin) or iron-chelating proteins involved in iron transport (transferrin and lactoferrin). Many bacteria can secrete iron chelating compounds, or siderophores, which chelate iron from the intestinal fluids and which bacteria then take up to obtain iron for its metabolic needs. These siderophores are under the control of plasmids and are tightly regulated by genes such that, under low iron conditions, expression of the siderophore system is high. Regulatory genes control expression of virulence genes. Shiga toxin (Stx) is not essential for virulence of *S. dysenteriae* type 1 but contributes to the severity of dysentery. Both plasmid-encoded virulence traits and chromosome-encoded factors are essential for full virulence of shigellae. Regarding chromosomally encoded enterotoxin, many pathogenic features of *Shigella* infection are due to the production of potent cytotoxins known as Stx, a potent protein synthesis–inhibiting exotoxin. *Shigella* strains produce distinct enterotoxins. These are a family of cytotoxins that contain 2 major immunologically non–cross-reactive groups called Stx1 and Stx2. The homology sequences between Stx1 and Stx2 are 55% and 57% in subunits A and B, respectively. These toxins are lethal to animals; enterotoxic to ligated rabbit intestinal segments; and cytotoxic for vero, HeLa, and some selected endothelial cells (human renal vascular endothelial cells) manifesting as diarrhea, dysentery, and hemolytic-uremic syndrome (HUS). Stx1 is synthesized in significant amount by *S. dysenteriae* serotype 1 and S flexneri 2a and *E. coli* (*Shigella* toxin–producing *E. coli* [ShET]). Stx1 and Stx2 are both encoded by a bacteriophage inserted into the chromosome. Stx1 increases inflammatory cytokine production by human macrophages, which, in turn, leads to a burst of interleukin (IL)-8. This could be relevant in recruiting neutrophils to the lamina propria of the intestine in hemorrhagic colitis and accounts for elevated levels of IL-8 in serum of patients with diarrhea-associated HUS. Stxs have 2 subunits. Stx is transported into nucleoli. Stx nucleolar movement is carrier-dependent and energy-dependent. Subunit A is a 32-kD polypeptide that, when digested by trypsin, generates A1 with a 28-kD fragment and another small fragment, A2, which is 4 kD. A1 fraction acts like *N*-glycosidase; it removes single adenine residue from 28S rRNA of ribosome and inhibits protein synthesis. The A2 fraction is a pentamer polypeptide of 7.7-kD protein and is required to bind the A1 fraction to the B subunit. The main function of the B subunit is the binding of toxins to the cell surface receptor, typically globotriaosylceramide (Gb3), on the brush border of intestinal epithelial cells.

In summary, events that occur on exposure to *Shigella* toxin are as follows:

- The B subunit of holotoxin binds to the Gb3 receptor on the cell surface of brush-border cells of the intestines.
- The receptor-holotoxin complex is endocytosed.
- The complex moves to Golgi apparatus and then to the endoplasmic reticulum.

The A1 subunit is released and it targets 28S RNA of the ribosome, inhibiting protein synthesis. Stxs may play a role in the progression of mucosal lesions after colonic cells are invaded, or they may induce vascular damage in the colonic mucosa. Stx adheres to small-intestine receptors and blocks the absorption of electrolytes, glucose, and amino

acids from intestinal lumen. The B subunit of Stx binds the host's cell glycolipid in the large intestine and in other cells, such as renal glomerular and tubular epithelia. The A1 domain internalized by means of receptor-mediated endocytosis and causes irreversible inactivation of the 60S ribosomal subunit, inhibiting protein synthesis and causing cell death, microvascular damage to the intestine, apoptosis in renal tubular epithelial cells, and hemorrhage (as blood and mucus in the stool). Chromosomal genes control lipopolysaccharide (LPS) antigens in cell walls. LPS plays an important role in resistance to nonspecific host defense encountered during tissue invasion. These genes help in invasion, multiplication, and resistance to phagocytosis by tissue macrophages. LPS enhances the cytotoxicity of Stx on human vascular endothelial cells. *Shigella* chromosomes share most of their genes with *E. coli* K12 strain MG1655, and the diversity of putative virulence genes acquired by means of bacteriophage-mediated lateral gene transfer is extensive. As a result of convergent evolution involving the gain and loss of functions, *Shigella* species have become highly specific human pathogens with variable epidemiologic and pathologic features. A 3-kb plasmid that harbors information for the production of bacteriocin by *S. flexneri* strains has been described. The production of this bacteriocin may be related to dysenteric diarrhea these bacterial strains produce.

Intestinal adherence factor

Intestinal adherence factor favors colonization in vivo and in animal models. This is 97-kD outer-membrane protein (OMP) encoded by each gene on chromosomes. This codes for intimin protein, and an anti-intimin response is observed in children with HUS.

Pathology

The host response to primary infection is characterized by the induction of an acute inflammation, which is accompanied by polymorphonuclear cell (PMN) infiltration, resulting in massive destruction of the colonic mucosa. Apoptotic destruction of macrophages in subepithelial tissue allows survival of the invading shigellae, and inflammation facilitates further bacterial entry. Gross pathology consists of mucosal edema, erythema, friability, superficial ulceration, and focal mucosal hemorrhage involving the rectosigmoid junction primarily. Microscopic pathology consists of epithelial cell necrosis, goblet cell depletion, PMN infiltrates and mononuclear infiltrates in lamina propria, and crypt abscess formation. *Shigella* bacteria invade the intestinal epithelium through M cells and proceed to spread from cell to cell, causing death and sloughing of contiguously invaded epithelial cells and inducing a potent inflammatory response resulting in the characteristic dysentery syndrome. In addition to this series of pathogenic events, only *S. dysenteriae* type 1 has the ability to elaborate the potent Shiga toxin that inhibits protein synthesis in eukaryotic cells and that may lead to extraintestinal complications, including hemolytic-uremic syndrome and death. Invasion of M cells, the specialized cells that cover the lymphoid follicles of the mucosa, overlying Peyer patches, may be the earliest event.

Epidemiology

Frequency

International: Worldwide, the incidence of shigellosis is estimated to be 164.7 million cases per year, of which 163.2 million were in developing countries, where 1.1 million deaths occurred. About 60% of all episodes and 61% of all deaths attributable to shigellosis involved children younger than 5 years. The incidence in developing countries may be 20 times greater than that in developed countries. Although the relative importance of various serotypes is not known, an estimated 30% of these infections are caused by *S. dysenteriae*. Case-fatality rates for *S. dysenteriae* infections may approach 30%. Patients with malnutrition are at increased risk of having complicated course. *Shigella* infection in malnourished children often causes a vicious cycle of further impaired nutrition, recurrent infection, and further growth retardation.

Mortality/Morbidity

Although shigellosis-related mortality is rare in developed countries, *S. dysenteriae* infection is associated with substantial morbidity and mortality rates in the developing world. Case fatality is as high as 15% among patients with *S. dysenteriae* type 1 who require hospitalization; this rate is increased by delayed arrival and treatment with ineffective antibiotics. Infants, non-breastfed children, children recovering from measles, malnourished children, and adults older than 50 years have a more severe illness and a greater risk of death. The overall mortality rate in developed countries is less than 1%. In the Far East and Middle East, the mortality rates for *S. dysenteriae* infections may be as high as 20-25%.

Race

No racial predilection is known.

Sex

No sexual predilection is known.

Clinical Presentation

History

Populations that are at high-risk for shigellosis include the following:

- Children in daycare centers (< 5 y) and their caregivers
- Persons in custodial institutions
- International travelers
- Homosexual men
- People living in crowded conditions with poor sanitary facilities and inadequate clean water supply (eg, refugee camps, shelters for displaced people)
- People with human immunodeficiency virus (HIV) infection

Symptoms include the following:

- Sudden onset of severe abdominal cramping, high-grade fever, emesis, anorexia, and large-volume watery diarrhea. Seizures may be an early manifestation.
- Abdominal pain, tenesmus, urgency, fecal incontinence, and small-volume mucoid diarrhea with frank blood (fractional stools) may subsequently occur.

Signs include the following:

- Elevated temperatures (as high as 106 ºF) are documented in approximately one third of cases, and a generally toxic appearance is noticed.
- Tachycardia and tachypnea may occur secondary to fever and dehydration. Depending on the degree of dehydration, dry mucous membranes, hypotension, prolonged capillary refill time, and poor skin turgor may be present.
- Abdominal tenderness is usually central and lower, although it may be generalized.

Extra intestinal manifestations are as follows:

- CNS symptoms include severe headache, lethargy, meningismus, delirium, and convulsions lasting less than 15 minutes, especially with *S. dysenteriae*. Severe toxic encephalopathy is rare, but lethal complications occur when initial symptoms are followed by sensory obtundation, seizures, coma, and death in 6-48 hours. The pathogenesis of neurologic manifestations during shigellosis is unclear. However, data now clearly demonstrate that Stx is not responsible.

- Regarding HUS, microangiopathic hemolytic anemia, thrombocytopenia, and renal failure have been reported with *S. dysenteriae* because of vasculopathy mediated by Stx. The principal organ affected in Stx1-mediated HUS is the kidney. This is presumed to be the consequence of the high renal blood flow and abundant baseline expression and high inducibility of the Stx glycolipid receptor Gb_e in the glomerular microcirculation. Manifestations of the disease arise due to 2 primary pathogenetic mechanisms: (1) direct Stx-mediated injury to vascular endothelial cells that leads to tissue ischemia and dysfunction and (2) a systemic inflammatory response triggered by Stx-mediated release of a wide range of cytokines and chemokines, including IL-6, IL-8, and tumor necrosis factor-alpha.
- Septicemia is rare, except in malnourished children with *S. dysenteriae* infection. Septicemia is sometimes caused by other gram-negative organisms and is related to loss of mucosal integrity by *Shigella* infection.
- Profound dehydration and hypoglycemia is more common with *S. dysenteriae* infection.
- *Shigella*sepsis may be complicated with disseminated intravascular coagulation (DIC), bronchopneumonia, and multiple organ failure in lethal cases.
- Arthritis, urethritis, conjunctivitis syndrome is commonly observed in adults carrying human leukocyte antigen (HLA)-B27 histocompatibility antigen.
- Cholestatic hepatitis, if present, is usually mild.
- Myocarditis is identified with cardiogenic shock, arrhythmias, and heart block.
- Rectal prolapse, toxic megacolon, and intestinal obstruction may be present.
- Shigellosis in the first 6 months of life is rare probably due to presence of antibodies to both virulence plasmid-coded antigens and lipopolysaccharides in the breast milk. Shigellosis in the neonatal period results from mother-to-infant fecal-oral transmission during labor and delivery, usually from asymptomatic mothers.
- Symptoms usually begin on the third day of life.
- Septicemia and chronic diarrhea are common.
- Fever may be absent.
- Diarrhea is not usually bloody.
- Intestinal perforation and mortality are more common in this group than in older children.

Shigellosis in patients with HIV infection is often a protracted, chronic, relapsing disease (even when treated with antibiotics). Bacteremia is rare, although it can occur in immunocompromised or malnourished patients.

Physical

- Physical examination during acute illness reveals a febrile ill-appearing child. Fever with a temperature as high as 39-40 ºC may be noted.
- The patient's hydration status should be carefully assessed. Especially note dryness of the oral mucosa, lack of tears, decreased urine output, and loss of skin turgor.
- Abdominal examination may reveal generalized mild-to-moderate tenderness with no guarding or rigidity.
- In a child who presents with febrile seizures, careful neurologic examination is mandatory to exclude meningitis.

Causes

- The primary mode of transmission of *Shigella* infection is fecal-oral contamination by the gram-negative aerobic bacilli.
- Contaminated food usually looks and smells normal. Food may become contaminated by infected food handlers who forget to wash their hands with soap after using the bathroom. Vegetables can become contaminated if they are harvested from a field with sewage in it.
- Outbreaks of shigellosis have also occurred among men who have sex with men.
- Travellers from developed to developing regions and soldiers serving under field conditions are also at an increased risk to develop shigellosis.
- Shigellosis can be caused by exposure to contaminated treated water and, more likely, from untreated recreational water.

Differential Diagnoses

- Pediatric Campylobacter Infections
- Pediatric Crohn Disease
- Pediatric Salmonella Infection
- Shigatoxin-producing Escherichia coli (eg, E. coli 0157:H7)
- Ulcerative Colitis in Children
- Yersinia Enterocolitica Infection

Workup

Laboratory Studies

- Hematology
  - The total WBC count reveals no consistent findings. A shift to the left (increased number of band cells) in the differential WBC count in a patient with diarrhea suggests bacillary dysentery. Leukopenia or leukemoid reactions are occasionally detected.
  - In HUS, anemia and thrombocytopenia occur.
  - Bacteremia is rare, even in severe disease, possibly due to the superficial nature of *Shigella* infection; the organism rarely penetrates beyond the mucosa.
  - Blood culture should be obtained in children who appear toxic, very young, severely ill, malnourished, or immunocompromised because of their increased risk of bacteremia.
- Stool examination
  - Isolation of *Shigella* from feces or rectal swab specimen is diagnostic but lacks specificity. Routine microscopy may reveal sheets of leukocytes on methylene-blue stained stool smear, which is a sensitive test for colitis but not specific for *Shigella* species.
  - In approximately 70% of patients with shigellosis, fecal blood or leukocytes (confirming colitis) are detectable in the stool.
- Stool culture
  - A sample for stool culture should be obtained in all suspected cases of shigellosis.
  - The yield from stool cultures is greatest early in the course of disease. Guidelines for obtaining specimens to improve the yield are as follows:
    - Process specimens immediately after collection.
    - If processing is delayed, use a transport medium (eg, buffered glycerol saline).

- Collect more than one stool or rectal (not anal) swab and inoculate them promptly on at least 2 different culture media.
- Specimens should be plated lightly onto MacConkey, xylose-lysine-deoxycholate, Hektoen enteric, or *Salmonella-Shigella,* or eosin-methylene blue agars.

- If processing is delayed, a rectal-swab sample can be placed in Cary-Blair transport medium or buffered glycerol saline.
- After overnight incubation, colorless, nonlactose-fermenting colonies may be tested by means of latex agglutination to establish a preliminary identification of *Shigella* infection.
- Antimicrobial susceptibility tests of all confirmed isolates should be performed by using the agar diffusion technique. The agar and broth-dilution methods are also widely used. The new Epsilometer strip method (E test) is used to accurately determine the minimum inhibitory concentration (MIC).
- Despite meticulous care in obtaining and processing specimens from patients infected with *Shigella* species, approximately 20% may fail to yield *Shigella* organisms.

- Enzyme immunoassay: An enzyme immunoassay for Stx is used to detect *S. dysenteriae* type 1 in the stool.
- Rapid techniques: With rapid techniques, gene probes or polymerase chain reaction (PCR) primers are directed toward virulence genes (invasion plasmid locus).
- Other testing modalities, such as fluorescent antibody test and enzyme-linked DNA probes, are available in research laboratories.

Other Tests

Additional diagnostic tools, such as gene probes and PCR analysis of stool for specific genes such as *ipaH*, *virF,* or *virA* can detect cases not diagnosed by culture but are usually available in research laboratories.

Treatment & Management

Medical Care

The clinician should rapidly assess the patient's fluid and electrolyte status and institute parenteral or oral hydration along with antipyretics as needed. Prompt recognition and treatment of seizures and raised intracranial pressure are essential. Nutritional supplementation of vitamin A (200,000 IU) can hasten clinical resolution in malnourished children.

Zinc supplementation (20 mg elemental zinc for 14 d) has been shown to reduce the duration of diarrhea, improve weight gain during recovery, and result in better immune response to the *Shigella* along with decreased incidence of diarrheal illness in the subsequent 6 months in malnourished children.

Surgical Care

Surgical care may be required for complications (eg, intestinal perforation).

Consultations

- Consult a neurologist if seizures and altered sensorium predominate.
- Consult a nephrologist if HUS is suspected (eg, for patients with anemia, thrombocytopenia, oliguria, and renal failure).

Diet

The diet may need to be restricted according to the severity of the disease.

Activity

No restrictions are necessary.

Medication Summary

Various antimicrobial agents are effective in the treatment of shigellosis, although options are becoming limited because of globally emerging drug resistance. Resistance of *Shigella* species to sulfonamides, tetracyclines, ampicillin, and trimethoprim-sulfamethoxazole (TMP-SMX) has been reported worldwide, and these agents are not recommended as empirical therapy. The World Health Organization (WHO) recommends that all suspected cases of shigellosis based on clinical features be treated with effective antimicrobials (antibiotics). The choice of antimicrobial drug has changed over the years as resistance to antibiotics has occurred, with different patterns of resistance being reported around the world. Evidence is insufficient to consider any class of antibiotic superior in efficacy in treating *Shigella* dysentery. The following antibiotics are used to treat *Shigella* dysentery:

- Beta-lactams: Ampicillin, amoxicillin, third-generation cephalosporins (cefixime, ceftriaxone), and pivmecillinam (not available in the United States)
- Quinolones: Nalidixic acid, ciprofioxacin, norfioxacin, and ofioxacin
- Macrolides: Azithromycin
- Others: sulfonamides, tetracycline, cotrimoxazole, and furazolidone.

Most clinical infections with *S. sonnei* are self-limited (48-72 h) and may not require antimicrobial therapy. If an ampicillin and TMP-SMX resistant strain is isolated or if susceptibility is unknown, parenteral ceftriaxone sodium, fluoroquinolone (eg, ciprofloxacin, ofloxacin), azithromycin dihydrate (off-label indication), or oral cefixime are the drugs of choice. Amoxicillin is less effective than ampicillin for treatment of ampicillin-sensitive strains. Oral first- and second-generation cephalosporins are inadequate despite in vitro susceptibility. Recently, *Shigella* isolates with decreased susceptibility to azithromycin (DSA-*Shigella*), with minimum inhibitory concentration (MIC) greater than 16 μ g/mL has been described by the CDC. In June 2015, the Centers for Disease Control and Prevention (CDC) warned that they received reports of infections with Shigella strains that are not susceptible to ciprofloxacin and/or azithromycin. CDC is seeing resistance to ciprofloxacin in 1.6% of the Shigella cases tested and resistance to azithromycin in approximately 3%. The CDC added that most cases have been reported among gay, bisexual, and other men who have sex with men in Illinois, Minnesota, and Montana and among international travelers, but cases are also occurring among other populations. Because shigellosis is self-limiting, some authorities recommend withholding antibiotic therapy. When an effective antibiotic is given, clinical improvement is anticipated within 48 hours. This lessens the risk of serious complications and death, shortens the duration of symptoms, and hastens the elimination of *Shigella* and the subsequent spread of infection. The risk of continued shedding of organisms in stool increases the risk of transmission of further disease among contacts argues against withholding antimicrobial treatment. Antimicrobial therapy is typically administered for 5 days. Antibiotic treatment decreases the duration of illness, person-to-person spread, and cases in household contacts. Treatment in malnourished children (eg, in developing countries) is likely to reduce the risk of worsening malnutrition morbidity after shigellosis. In persons infected with *S. dysenteriae* type 1, early administration of effective antibiotics decreases Shiga toxin (Stx) concentrations in the stool and lowers HUS risk. However, the risk of HUS caused by *E. coli* O157-H7 may be increased with the early administration of antibiotics. Prophylactic antibiotics are not recommended for contacts.

INTERPRETATION

Stool Analysis

Test Overview

A stool analysis is a series of tests done on a stool (feces) sample to help diagnose certain conditions affecting the digestive tract. These conditions can include infection (such as from parasites, viruses, or bacteria), poor nutrient absorption, or cancer. For a stool analysis, a stool sample is collected in a clean container and then sent to the laboratory. Laboratory analysis includes microscopic examination, chemical tests, and microbiologic tests. The stool will be checked for color, consistency, amount, shape, odor, and the presence of mucus. The stool may be examined for hidden (occult) blood, fat, meat fibers, bile, white blood cells , and sugars called reducing substances. The pH of the stool also may be measured. A stool culture is done to find out if bacteria may be causing an infection.

Why It Is Done

Stool analysis is done to:

- Help identify diseases of the digestive tract, liver, and pancreas. Certain enzymes (such as trypsin or elastase) may be evaluated in the stool to help determine how well the pancreas is functioning.
- Help find the cause of symptoms affecting the digestive tract, including prolonged diarrhea, bloody diarrhea, an increased amount of gas, nausea, vomiting, loss of appetite, bloating, abdominal pain and cramping, and fever.
- Screen for colon cancer by checking for hidden (occult) blood.
- Look for parasites, such as pinworms or GiardiaGiardia.
- Look for the cause of an infection, such as bacteria, a fungus, or a virus.
- Check for poor absorption of nutrients by the digestive tract (malabsorption syndrome). For this test, all stool is collected over a 72-hour period and then checked for fat (and sometimes for meat fibers). This test is called a 72-hour stool collection or quantitative fecal fat test.

How to Prepare

Many medicines can change the results of this test. You will need to avoid certain medicines depending on which kind of stool analysis you have. You may need to stop taking medicines such as antacids, antidiarrheal medicines, antiparasite medicines, antibiotics, laxatives, or nonsteroidal anti-inflammatory drugs (NSAIDs) for 1 to 2 weeks before you have the test. Be sure to tell your doctor about all the nonprescription and prescription medicines you take.

Be sure to tell your doctor if you have:

- Recently had an X-ray test using barium contrast material, such as a barium enema or upper gastrointestinal series (barium swallow). Barium can interfere with test results.
- Traveled in recent weeks or months, especially if you have traveled outside the country. This helps your doctor look for the parasites, fungi, viruses, or bacteria that may be causing a problem.

If your stool is being tested for blood, you may need to avoid certain foods for 2 to 3 days before the test. This depends on what kind of stool test you use. And do not do the test during your menstrual period or if you have active bleeding from hemorrhoids. If you aren't sure about how to prepare, ask your doctor. Do not use a stool sample for testing that has been in contact with toilet bowl cleaning products that turn the water blue.

How It Is Done

Stool samples can be collected at home, in your doctor's office, at a medical clinic, or at the hospital. If you collect the samples at home, you will be given stool collection kits to use each day. Each kit contains applicator sticks and two sterile containers. You may need to collect more than one sample over 1 to 3 days. Follow the same procedure for each day.

Collect the samples as follows:

- Urinate before collecting the stool so that you do not get any urine in the stool sample.
- Put on gloves before handling your stool. Stool can contain germs that spread infection. Wash your hands after you remove your gloves.
- Pass stool (but no urine) into a dry container. You may be given a plastic basin that can be placed under the toilet seat to catch the stool.
  - Either solid or liquid stool can be collected.
  - If you have diarrhea, a large plastic bag taped to the toilet seat may make the collection process easier; the bag is then placed in a plastic container.
  - If you are constipated, you may be given a small enema.
  - Do not collect the sample from the toilet bowl.
  - Do not mix toilet paper, water, or soap with the sample.
- Place the lid on the container and label it with your name, your doctor's name, and the date the stool was collected. Use one container for each day's collection, and collect a sample only once a day unless your doctor gives you other directions.

Take the sealed container to your doctor's office or the laboratory as soon as possible. You may need to deliver your sample to the lab within a certain time. Tell your doctor if you think you may have trouble getting the sample to the lab on time. If the stool is collected in your doctor's office or the hospital, you will pass the stool in a plastic container that is inserted under the toilet seat or in a bedpan. A health professional will package the sample for laboratory analysis. You will need to collect stool for 3 days in a row if the sample is being tested for quantitative fats. You will begin collecting stool on the morning of the first day. The samples are placed in a large container and then refrigerated. You may need to collect several stool samples over 7 to 10 days if you have digestive symptoms after traveling outside the country. Samples from babies and young children may be collected from diapers (if the stool is not contaminated with urine) or from a small-diameter glass tube inserted into the baby's rectum while the baby is held on an adult's lap. Sometimes a stool sample is collected using a rectal swab that contains a preservative. The swab is inserted into the rectum, rotated gently, and then withdrawn. It is placed in a clean, dry container and sent to the lab right away.

How It Feels

There is no pain while collecting a stool sample. If you are constipated, straining to pass stool may be painful. If your health professional uses a rectal swab to collect the sample, you may feel some pressure or discomfort as the swab is inserted into your rectum.

Risks

Any stool sample may contain germs that can spread disease. It is important to carefully wash your hands and use careful handling techniques to avoid spreading infection.

Results

A stool analysis is a series of tests done on a stool (feces) sample to help diagnose certain conditions affecting the digestive tract . The normal values listed here-called a reference range-are just a guide. These ranges vary from lab to lab, and your lab may have a different range for what's normal. Your lab report should contain the range your lab uses. Also, your doctor will evaluate your results based on your health and other factors. This means that a value that falls outside the normal values listed here may still be normal for you or your lab. Stool analysis test results usually take at least 1 to 3 days.

Stool analysis

Normal:
The stool appears brown, soft, and well-formed in consistency.
The stool does not contain blood, mucus, pus, undigested meat fibers, harmful bacteria, viruses, fungi, or parasites.
The stool is shaped like a tube.
The pH of the stool is 7.0-7.5.[1]
The stool contains less than 0.25 grams per deciliter (g/dL)[less than 13.9 millimoles per liter (mmol/L)] of sugars called reducing factors.[1]
The stool contains 2-7 grams of fat per 24 hours (g/24h).[1]
Abnormal:
The stool is black, red, white, yellow, or green.
The stool is liquid or very hard.
There is too much stool.
The stool contains blood, mucus, pus, undigested meat fibers, harmful bacteria, viruses, fungi, or parasites.
The stool contains low levels of enzymes, such as trypsin or elastase.
The pH of the stool is less than 7.0 or greater than 7.5.
The stool contains 0.25 g/dL (13.9 mmol/L) or more of sugars called reducing factors.
The stool contains more than 7 g/24h of fat (if your fat intake is about 100 g a day).

Many conditions can change the results of a stool analysis. Your doctor will talk with you about any abnormal results that may be related to your symptoms and past health.

Abnormal values

- High levels of fat in the stool may be caused by diseases such as pancreatitis, sprue (celiac disease), cystic fibrosis, or other disorders that affect the absorption of fats.
- The presence of undigested meat fibers in the stool may be caused by pancreatitis.
- A low pH may be caused by poor absorption of carbohydrate or fat. Stool with a high pH may mean inflammation in the intestine (colitis), cancer, or antibiotic use.
- Blood in the stool may be caused by bleeding in the digestive tract.
- White blood cells in the stool may be caused by inflammation of the intestines, such as ulcerative colitis, or a bacterial infection.
- Rotaviruses are a common cause of diarrhea in young children. If diarrhea is present, testing may be done to look for rotaviruses in the stool.
- High levels of reducing factors in the stool may mean a problem digesting some sugars.
- Low levels of reducing factors may be caused by sprue (celiac disease), cystic fibrosis, or malnutrition. Medicine such as colchicine (for gout) or birth control pills may also cause low levels.

What Affects the Test

Reasons you may not be able to have the test or why the results may not be helpful include:

- Taking medicines such as antibiotics, antidiarrheal medicines, barium, bismuth, iron, ascorbic acid, nonsteroidal anti-inflammatory drugs (NSAIDs), and magnesium.
- Contaminating a stool sample with urine, blood from a menstrual period or a bleeding hemorrhoid, or chemicals found in toilet paper and paper towels.
- Exposing the stool sample to air or room temperature or failing to send the sample to a laboratory within 1 hour of collection.

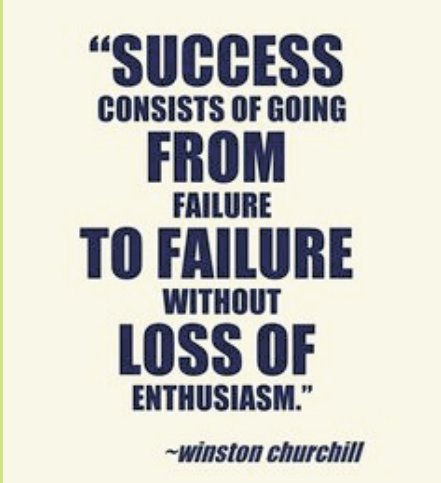
What To Think About

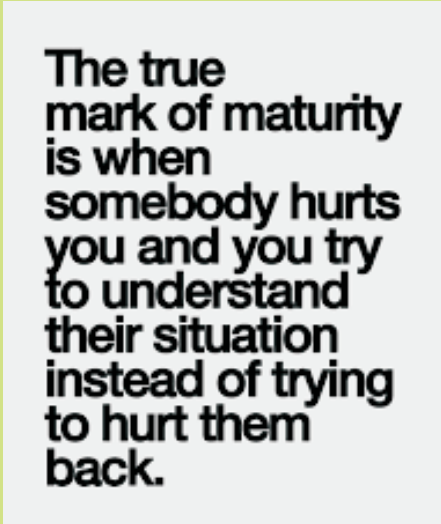
- Stool may be checked for hidden (occult) blood. To learn more, see the topic Stool Tests for Colorectal Cancer.
- A stool culture is done to find the cause of an infection, such as bacteria, a virus, a fungus, or a parasite. To learn more, see the topic Stool Culture.
- A bowel transit time test is done to help find the cause of abnormal movement of food through the digestive tract. To learn more, see the topic Bowel Transit Time.
- The D-xylose absorption test is done to help diagnose problems that prevent the small intestine from absorbing nutrients in food. This test may be done when symptoms of malabsorption syndrome (such as chronic diarrhea, weight loss, and weakness) are present. To learn more, see the topic D-Xylose Absorption Test.
- A stool analysis to measure trypsin or elastase is not as reliable as the sweat test to detect cystic fibrosis. To learn more, see the topic Sweat Test.

BOUQUET

In Lighter Vein

Wisdom Whispers





Brain Teasers

1. **How many types of epitopes are there?**
   A. Sequential
   B. Conformational
   C. Both of the above
   D. None of the above.

2. **What is not correct regarding fluorescence and chemiluminescence?**
   A. Fluorescence is a phenomenon where molecule absorbs light in one wavelength and emits in another wavelength
   B. In fluorescence there is a source of excitation
   C. Chemiluminescence is the production of light by a chemical reaction
   D. Definitely fluorescence is a better technology for use in immunoassays.

3. **What is true about apoptosis?**
   A. Cell death most often, however, is a planned process
   B. Apoptosis is sometimes called cellular suicide
   C. It is important, for example, in development, in the immune response
   D. All of the above.

4. **What is true regarding secondary immune response?**
   A. It involves an amplified population of memory cells
   B. The response is more rapid than primary response
   C. Higher levels of antibodies are formed than primary response
   D. All of the above.

ANSWERS: 1.C, 2.D, 3. D, 4. D

TROUBLESHOOTING

1.

Sperm preparation techniques and culture media

2.

Introduction

- Spermatozoa may need to be separated from seminal plasma for a variety of purposes:
- Diagnostic tests of function

  Therapeutic recovery for insemination and assisted reproductive technologies (ART)
- If tests of sperm function are to be performed, it is critical that the spermatozoa are separated from the seminal plasma within 1 hour of ejaculation, to limit any damage from products of non-sperm cells.

3.

Separation of sperm from seminal plasma

Reasons:

- **Components (e.g. prostaglandins, zinc):**

  are obstacles to the achievement of pregnancy when natural barriers are by passed in ART, such as intrauterine insemination (IUI) or in-vitro fertilization (IVF).
- **To yield a final preparation containing a high percentage of :**

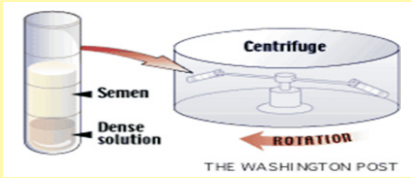
1. Morphologically normal.
2. Motile cells.
3. Free from debris, non-germ cells, dead spermatozoa.

4.

- **Diluting semen** with culture media and centrifuging is still used for preparing normozoospermic specimens for IUI (Boomsma et al. , 2004).
- **Density-gradient centrifugation** and **direct swim-up** are generally preferred for specimens with one or more abnormalities in semen parameters e.g. Morshedi et al., 2003).
- **Glass-wool columns** are reported to be as effective as density-gradients for the separation of spermatozoa from semen with suboptimal characteristics (Rhemrev et al., 1989; Johnson et al.,1996).

5.

Methods



- Filtration (Glass-wool columns)
- Swim-up
- Density gradient centrifugation Continuous, Discontinuous, mini percoll
- Flow cytometry

6.

Choice of method

- The choice of sperm preparation technique is dictated by the nature of the semen sample (see Canale et al., 1994).
- **Direct swim-up technique:**

  is often used when the semen samples are considered to be largely normal
- **Density-gradients**

  severe oligozoospermia, teratozoospermia or asthenozoospermia.

  The greater total number of motile spermatozoa recovered. The centrifugation time can be increased for specimens with high viscosity.

7.

- Each laboratory should determine the centrifugal force and centrifugation time necessary to form a manageable sperm pellet.
- When sperm numbers are extremely low, it may be necessary to modify the centrifugal force or the time, in order to increase the chances of recovering the maximum number of spermatozoa.

8.

Efficiency of sperm separation from seminal plasma and infectious organisms

The efficiency of a sperm selection technique is usually expressed as :

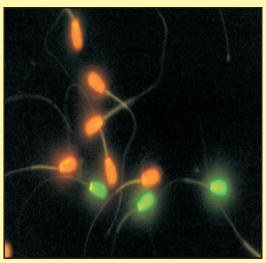
- *The absolute sperm number,*
- The total number of motile spermatozoa,
- The recovery of morphologically normal motile spermatozoa.

9.

- **swim-up:**
  - produces a lower recovery of motile spermatozoa (<20%)
  - simple
- **Density-gradient centrifugation**
  - produces a higher recovery of motile spermatozoa(>20%)
  - Inflamation risk
  - Fast

10.

- **Flowcytometry:**
- Separation of spermatozoa containing y chromosome
- Prevention of X-linked diseases
- Prevention of inborn diseases
- Using in repeated miscarriage
- Damage of DNA?



11.

General Principles

- Semen samples may contain harmful infectious agents, and technicians should handle them as a biohazard with extreme care.
- Sperm preparation techniques cannot be considered 100% effective in removing infectious agents from semen.

12.

- **For all of methods**,
  The culture medium suggested is a balanced salt solution supplemented with protein and containing a buffer appropriate for the environmental conditions in which the spermatozoa will be processed.
- **For assisted reproduction procedures:**
  Such as intracytoplasmic sperm injection (ICSI), in-vitro fertilization (IVF), artificial insemination (AI) or gamete intrafallopian transfer (GIFT),
  it is imperative that the human serum albumin is highly purifed and free from viral, bacterial and prion contamination.
- Albumins specifcally designed for such procedures are commercially available.

13.

Incubator

- If the incubator contains only atmospheric air and the temperature is 37 °C, the medium should be buffered with Hepes or a similar buffer, and the caps of the tubes should be tightly closed.
- If the incubator atmosphere is 5% (v/v) CO_2 in air and the temperature is 37 °C, then the medium is best buffered with sodium bicarbonate or a similar buffer, and the caps of the test-tubes should be loose to allow gas exchange.

14.

Simple Washing

- This simple washing procedure provides the highest yield of spermatozoa and is adequate if semen samples are of good quality.
- It is often used for preparing spermatozoa for intrauterine insemination.

15.

Reagents

- **BWW, Earle's, Ham's F-10 or human tubal fluid (HTF)** , supplemented preferably with human serum albumin (HSA), or serum, as described below.
- **HSA**, highly purifed and free from viral, bacterial and prion contamination and endotoxins.
- **HSA supplement**: to 50 ml of medium add 300 mg of HSA, 1.5 mg of sodium pyruvate, 0.18 ml of sodium lactate (60% (v/v) syrup) and 100 mg of sodium bicarbonate.
- **Serum supplement**: to 46 ml of medium add 4 ml of heat-inactivated (56 °C for 20 minutes) client's serum, 1.5 mg of sodium pyruvate, 0.18 ml of sodium lactate (60% (v/v) syrup) and 100 mg of sodium bicarbonate.

16.

Procedure

- Mix the semen sample well.
- Dilute the entire semen sample 1 + 1 (1:2) with medium to promote removal of seminal plasma.
- Transfer the diluted suspension into multiple centrifuge tubes, with preferably not more than 3 ml per tube.
- Centrifuge at 300–500g for 5–10 minutes.
- Carefully aspirate and discard the supernatant.
- Resuspend the combined sperm pellets in 1 ml of medium by gentle pipetting.

17.

- Centrifuge again at 300–500g for 3–5 minutes.
- Carefully aspirate and discard the supernatant.
- Resuspend the sperm pellet, by gentle pipetting, in a volume of medium appropriate for final disposition, e.g. insemination, so that concentration and motility can be determined.
- **Note:**
- The number of washings to remove seminal plasma can be reduced by using fewer tubes and increasing the volume in each tube.

18.

Direct Swim-up

- Spermatozoa may be selected by their ability to swim out of seminal plasma and into culture medium.
- The semen should preferably not be diluted and centrifuged prior to swim-up, because this can result in peroxidative damage to the sperm membranes.

19.

The Direct Swim-up Technique:

- can be performed either by layering culture medium over the liquefied semen or by layering liquefied semen under the culture medium.
- Motile spermatozoa then swim into the culture medium.
- This procedure gives:

a lower yield of spermatozoa than washing, but selects them for their motility and is useful where the percentage of motile spermatozoa in semen is low, e.g. for IVF and ICSI.

20.

Reagents

- **BWW, Earle's, Ham's F-10 or HTF** supplemented preferably with HSA, or serum, as described below.
- **HSA**, highly purified and free from viral, bacterial and prion contamination and endotoxins.
- **HSA supplement**: to 50 ml of medium add 300 mg of HSA, 1.5 mg of sodium pyruvate, 0.18 ml of sodium lactate (60% (v/v) syrup) and 100 mg of sodium bicarbonate.
- **Serum supplement**: to 46 ml of medium add 4 ml of heat- nactivated (56 °Cfor 20 minutes) client's serum, 1.5 mg of sodium pyruvate, 0.18 ml of sodium lactate (60% (v/v) syrup) and 100 mg of sodium bicarbonate.

21.

Procedure

I. Mix the semen sample well .

II. Place 1 ml of semen in a sterile 15-ml conical centrifuge tube, and gently layer 1.2 ml of medium over it. Alternatively, pipette the semen carefully under the culture medium.

III. Incline the tube at an angle of about 45°, to increase the surface area of the semen–culture medium interface, and incubate for 1 hour at 37 °C.

IV. Gently return the tube to the upright position and remove the uppermost 1 ml of medium. This will contain highly motile sperm cells.

V. Dilute this with 1.5–2.0 ml of medium.

22.

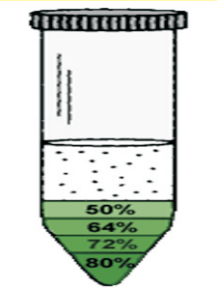
VI. Centrifuge at 300–500g for 5 minutes and discard the supernatant.

VII. Resuspend the sperm pellet in 0.5 ml of medium for assessment of sperm concentration, total motility and progressive motility .

VIII. The specimen may be used directly for therapeutic or research purposes.

23.

Discontinuous Density- gradients



24.

- Discontinuous density-gradients can provide the best selection of good-quality spermatozoa, giving good separation from other cell types and debris.
- It is easier to standardize than the swim-up technique, and thus results are more consistent.
- This technique is used to recover and prepare spermatozoa for use in IVF and ICSI.
- This method uses centrifugation of seminal plasma over density-gradients consisting of colloidal silica coated with silane, which separates cells by their density.

25.

- In addition, motile spermatozoa swim actively through the gradient material to form a soft pellet at the bottom of the tube.
- A simple two-step discontinuous density-gradient preparation method is most widely applied, typically with a 40% (v/v) density top layer and an 80% (v/v) density lower layer.
- Sperm preparation using density gradient centrifugation usually results in a fraction of highly motile spermatozoa, free from debris, contaminating leukocytes, non-germ cells and degenerating germ cells.

26.

- A number of commercial products are available.
- Most density-gradient media contain high relative molecular mass components that have inherently low osmolality, so they are usually prepared in medium that is iso-osmotic with female reproductive tract fluids.

27.

Reagents

- **BWW, Earle's, Ham's F-10 or HTF.**
- **HSA**, highly puri.ed and free from viral, bacterial and prion contamination and endotoxins.
- **HSA supplement**: to 50 ml of medium add 300 mg of HSA, 1.5 mg of sodium pyruvate, 0.18 ml of sodium lactate (60% (v/v) syrup) and 100 mg of sodium bicarbonate.
- **Serum supplement**: to 46 ml of medium add 4 ml of heat-inactivated (56 °C for 30-45 minutes) patient's serum, 1.5 mg of sodium pyruvate, 0.18 ml of sodium lactate (60% (v/v) syrup) and 100 mg of sodium bicarbonate.

28.

- **Isotonic density-gradient medium**: to 10 ml of 10× concentrated culture medium, add 90 ml of density-gradient medium, 300 mg of HSA, 3 mg of sodium pyruvate, 0.37 ml of sodium lactate (60% (v/v) syrup) and 200 mg of sodium bicarbonate.
- **Gradient 80% (v/v):** to 40 ml of isotonic gradient medium add 10 ml of supplemented medium.
- **Gradient 40% (v/v):** to 20 ml of isotonic gradient medium add 30 ml of supplemented medium.

29.

Procedure

1. Prepare the density-gradient medium in a test-tube by layering 1 ml of 40% (v/v) density-gradient medium over 1 ml of 80% (v/v) density-gradient medium.
2. Mix the semen sample well .
3. Place 1 ml of semen above the density-gradient media and centrifuge at 300–400g for 15–30 minutes. More than one tube per semen sample may be used, if necessary.
4. Remove most of the supernatant from the sperm pellet.

30.

5. Resuspend the sperm pellet in 5 ml of supplemented medium by gentle pipetting and centrifuge at 200g for 4–10 minutes.
6. Repeat the washing procedure (steps 4 and 5 above).
7. Resuspend the final pellet in supplemented medium by gentle pipetting so that concentration and motility can be determined.

31.

Preparing HIV-infected Semen Samples

- If the human immunodefciency virus (HIV) is present in semen, viral RNA and proviral DNA can be found free in seminal plasma and in non-sperm cells.
- As HIV receptors (CD4, CCR5, CXCR4) are expressed only by non-sperm cells, a combination of density-gradient centrifugation followed by swim-up has been proposed as a way of preventing infection of uninfected female partners.

32.

Preparing testicular and epididymal spermatozoa

33.

PESA

- The typical indication for epididymal aspiration is obstructive azoospermia rather than testicular dysfunction. Consequently, relatively large numbers of spermatozoa can be harvested for therapeutic purposes.
- Epididymal aspirates can often be obtained with minimal contamination from red blood cells and non-germ cells, making the isolation and selection of motile epididymal spermatozoa relatively straightforward.
- If large numbers of epididymal spermatozoa are obtained, density- gradient centrifugation is an effective method of preparing them for subsequent use.
- If sperm numbers are low, a simple wash can be performed.

34.

TESE

- Testicular spermatozoa can be retrieved by open biopsy (with or without microdissection) or by percutaneous needle biopsy.
- Testicular specimens are invariably contaminated with non-germ cells and large numbers of red blood cells, so additional steps are needed to isolate a clean preparation of spermatozoa.
- In order to free the seminiferous tubule-bound elongated spermatids ("testicular spermatozoa"), enzymatic or mechanical methods are needed.
- Testicular spermatozoa are prepared for ICSI, since sperm numbers are low and their motility is poor.

35.

Enzymatic Method

1. Incubate the testicular tissue with collagenase (e.g. 0.8 mg of Clostridium histolyticum, type 1A per ml of medium) for 1.5–2 hours at 37 °C, vortexing every 30 minutes.
2. Centrifuge at 100g for 10 minutes and examine the pellet.

36.

Mechanical Method

1. Macerate the testicular tissue in culture medium with glass coverslips until a fine slurry of dissociated tissue is produced.
2. Alternatively, strip the cells from the seminiferous tubules using fine needles bent parallel to the base of the culture dish.

37.

Processing sperm suspensions for intracytoplasmic sperm injection

1. Wash the specimens obtained by adding 1.5 ml of culture medium.
2. Centrifuge at 300g for 8–10 minutes.
3. Remove the supernatant and resuspend the pellet in 0.5 ml of fresh culture medium.
4. Estimate the motility and number of spermatozoa in the pellet. (Some specimens with a low number of spermatozoa may need to be resuspended in a lower volume of medium.)

38.

5. Place a 5–10 ul droplet of culture medium in a culture dish.
6. Cover it with mineral oil (pre-equilibrated with CO_2).
7. Introduce 5–10 ul of the sperm suspension into the culture medium.
8. Carefully aspirate the motile spermatozoa found at the interface between the culture medium and oil with an ICSI pipette.
9. Transfer them to a droplet of viscous solution, e.g. polyvinylpyrrolidone (7–10% (100 g/l) in medium).

39.

Preparing Retrograde Ejaculation Samples

- In some men, semen passes into the bladder at ejaculation, resulting in aspermia, or no apparent ejaculate.
- Confirmation of this situation is obtained by examining a sample of post-ejaculatory urine for the presence of spermatozoa.
- If pharmacological treatment is not possible or not successful, spermatozoa may be retrieved from the urine.
- Alkalinization of the urine by ingestion of sodium bicarbonate, for example, will increase the chance that any spermatozoa passing into the urine will retain their motility characteristics.

40.

At the laboratory, the man should be asked to:

- urinate without completely emptying the bladder;
- produce an ejaculate by masturbation into a specimen container;
- urinate again into a second specimen vessel containing culture medium (to alkalinize the urine further).
- Both the ejaculate, if any, and urine samples should be analysed.
- Because a large volume of urine may be produced, it is often necessary to concentrate the specimen by centrifugation (500g for 8 minutes) The retrograde specimen, once concentrated, and the antegrade specimen, if produced, can be most effectively processed using the density-gradient preparation method.

41.

Preparing Assisted Ejaculation Samples

- Semen from men with disturbed ejaculation, or who cannot ejaculate, may be collected by direct vibratory stimulation of the penis or rectal electrical stimulation of the accessory organs.
- Ejaculates from men with spinal cord injury will frequently have high sperm concentrations, decreased sperm motility and red and white blood cell contamination.
- Specimens obtained by electro-ejaculation can be processed most effectively by density-gradient centrifugation . Regardless of the method of preparation, these types of ejaculates will often contain a high percentage of immotile sperm cells.

42.

Culture Media

BWW stock solution (Biggers et al., 1971)

1. To 1000 ml of purifed water add 5.54 g of sodium chloride (NaCl), 0.356 g of potassium chloride (KCl), 0.294 g of magnesium sulfate heptahydrate (MgSO4 .7H2O), 0.250 g of calcium chloride dihydrate (CaCl2 .2H2O) and 0.162 g of potassium dihydrogen phosphate (KH2PO4).
2. Adjust the pH to 7.4 with 1 mol/l sodium hydroxide (NaOH).
3. Add 1.0 ml (0.04%, 0.4 g/l) phenol red per litre.

- Note: This solution can be stored for several weeks at 4 °C.

43.

Dulbecco's Phosphate-Buffered Saline

1. **Dulbecco's glucose–PBS**: to 750 ml of purified water add 0.2 g of potassium chloride (KCl), 0.2 g of potassium dihydrogen phosphate (KH2PO4), 0.1 g of magnesium chloride hexahydrate (MgCl2 .6H2O), 8.0 g of sodium chloride (NaCl), 2.16 g of disodium hydrogen phosphate heptahydrate (Na2HPO4 .7H2O) and 1.00 g of D-glucose.
2. Dissolve 0.132 g of calcium chloride dihydrate (CaCl2. 2H2O) in 10 ml of purified water and add slowly to the above solution with stirring.
3. Adjust the pH to 7.4 with 1 mol/l sodium hydroxide (NaOH).
4. Make up to 1000 ml with purified water.

44.

Earle's Medium

1. To 750 ml of purified water add 6.8 g of sodium chloride (NaCl), 2.2 g of sodium bicarbonate (NaHCO3), 0.14 g of sodium dihydrogen phosphate monohydrate (NaH2PO4 .H2O), 0.4 g of potassium chloride (KCl), 0.20 g of magnesium sulfate heptahydrate (MgSO4 .7H2O) and 1.0 g of D-glucose.
2. Dissolve 0.20 g of anhydrous calcium chloride (CaCl2) slowly in the above solution with stirring.
3. Adjust the pH to 7.4 with 1 mol/l hydrochloric acid (HCl) or 1 mol/l sodium hydroxide (NaOH).
4. Make up to 1000 ml with purified water.

45.

Ham's F-10 Medium

1. To 750 ml of purified water add 7.4 g of sodium chloride (NaCl), 1.2 g of sodium bicarbonate (NaHCO3), 0.285 g of potassium chloride (KCl), 0.154 g of sodium monosodium phosphate (Na2HPO4), 0.153 g of magnesium sulfate heptahydrate (MgSO4 .7H2O), 0.083 g of potassium dihydrogen phosphate (KH2PO4), 0.044 of calcium chloride dihydrate (CaCl2 .2H2O) and 1.1 g of D-glucose.
2. Adjust the pH to 7.4 with 1 mol/l sodium hydroxide (NaOH).
3. Make up to 1000 ml with purified water.

46.

Hanks' Balanced Salt Solution

1. To 750 ml of purified water add 8.0 g of sodium chloride (NaCl), 0.4 g of potassium chloride (KCl), 0.35 g of sodium bicarbonate (NaHCO3), 0.185 g of calcium chloride dihydrate (CaCl2 .2H2O), 0.1 g of magnesium chloride hexahydrate (MgCl2 .6H2O), 0.1 g of magnesium sulfate heptahydrate (MgSO4 .7H2O), 0.06 g of potassium dihydrogen phosphate (KH2PO4), 0.048 g of sodium dihydrogen phosphate (NaH2PO4) and 1.0 g of D-glucose.
2. Adjust the pH to 7.4 with 1 mol/l sodium hydroxide (NaOH).
3. Make up to 1000 ml with purified water.

47.

Human Tubal Fluid

1. To 750 ml of purified water add 5.931 g of sodium chloride (NaCl), 0.35 g of potassium chloride (KCl), 0.05 g of magnesium sulfate heptahydrate (MgSO4 .7H2O), 0.05 g of potassium dihydrogen phosphate (KH2PO4), 2.1 g of sodium bicarbonate (NaHCO3), 0.5 g of D-glucose, 0.036 g of sodium pyruvate, 0.3 g of calcium chloride dihydrate (CaCl2 .2H2O) and 4.0 g of sodium DL-lactate (60% (v/v) syrup).
2. To 1 ml of the above medium add 10 g phenol red, 100 U penicillin and 50 g streptomycin sulfate.
3. Adjust the pH to 7.4 with 1 mol/l hydrochloric acid (HCl).

48.

Krebs–Ringer Medium

1. To 750 ml of purified water add 6.9 g of sodium chloride (NaCl), 2.1 g of sodium bicarbonate (NaHCO3), 0.35 g of potassium chloride (KCl), 0.32 g of calcium chloride dihydrate (CaCl2 .2H2O), 0.18 g of sodium dihydrogen phosphate dihydrate (NaH2PO4 .2H2O), 0.1 g of magnesium chloride hexahydrate (MgCl2 .6H2O) and 0.9 g of D-glucose.
2. Adjust the pH to 7.4 with 1 mol/l sodium hydroxide (NaOH).
3. Make up to 1000 ml with purifed water.

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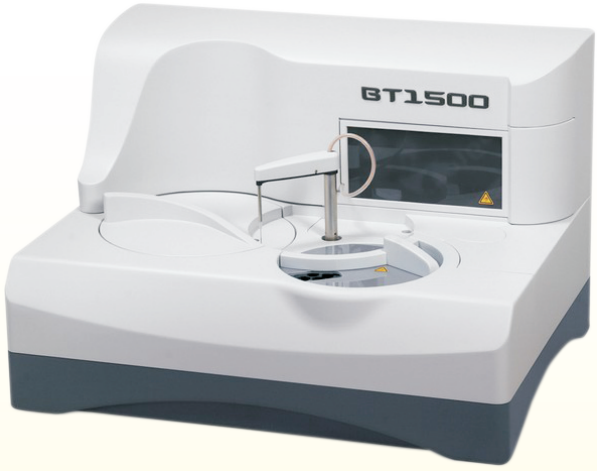

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