

Editorial

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Every year, approximately 10 million people worldwide are infected with the virus. It is most commonly transmitted by the fecal-oral route via contaminated food or drinking water. The time between infection and the appearance of the symptoms, (the incubation period), is between two and six weeks and the average incubation period is 28 days.

In developing countries, and in regions with poor hygiene standards, the incidence of infection with this virus approaches 100% and the illness is usually contracted in early childhood. The infection causes no clinical signs and symptoms in over 90% of these children and since the infection confers lifelong immunity, the disease is of no special significance to the indigenous population. In Europe, the United States and other industrialized countries, on the other hand, the infection is contracted primarily by susceptible young adults, most of whom are infected with the virus during trips to countries with a high incidence of the disease.

It does not have a chronic stage and does not cause permanent organ damage. Following infection, the immune system makes antibodies against the virus that confer immunity against future infection. The disease can be prevented by vaccination and has been proved effective in controlling outbreaks worldwide. Which disease are we talking about? Perhaps you have guessed it right. It is Hepatitis A, (formerly known as *infectious hepatitis*), is an acute infectious disease of the liver caused by Hepatitis A virus. DISEASE DIAGNOSIS delves deep into its all clinico-diagnostic aspects.

INTERPRETATION has the left over paras from the previous issue. Hereafter you should always be in a position to identify common as well as rarer urine crystals. May be sometimes you could identify what drugs the patient is on!

TROUBLESHOOTING segment talks about Glycated Haemoglobin. Starting from terminologies (confusing sometimes!), underlying principle, methodologies available, correlation with mean blood glucose levels, reference guidelines and indications are all spread out for your consumption. Hope you'll appreciate this much often requisitioned for analyte discussed threadbare for you.

Like always, BOUQUET glorifies this issue too.

DISEASE DIAGNOSIS

HEPATITIS A

INTRODUCTION

Hepatitis A, (formerly known as *infectious hepatitis*), is an acute infectious disease of the liver caused by Hepatitis A virus, which is most commonly transmitted by the fecal-oral route via contaminated food or drinking water. Every year, approximately 10 million people worldwide are infected with the virus. The time between infection and the appearance of the symptoms, (the incubation period), is between two and six weeks and the average incubation period is 28 days. In developing countries, and in regions with poor hygiene standards, the incidence of infection with this virus approaches 100% and the illness is usually contracted in early childhood. Hepatitis A infection causes no clinical signs and symptoms in over 90% of these children and since the infection confers lifelong immunity, the disease is of no special significance to the indigenous population. In Europe, the United States and other industrialized countries, on the other hand, the infection is contracted primarily by susceptible young adults, most of whom are infected with the virus during trips to countries with a high incidence of the disease. Hepatitis A does not have a chronic stage and does not cause permanent liver damage. Following infection, the immune system makes antibodies against the hepatitis A virus that confer immunity against future infection. The disease can be prevented by vaccination and hepatitis A vaccine has been proved effective in controlling outbreaks worldwide.

Hepatitis A

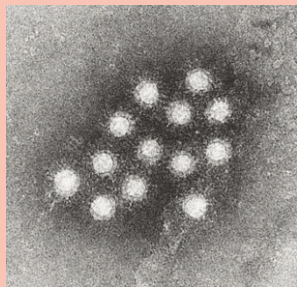
Electron micrograph of hepatitis A virions.

Virus classification

Group: Group IV ((+)ssRNA)

Family: *Picornaviridae*

Genus: *Hepatovirus*



The causative virus

The Hepatitis virus (HAV) is a Picornavirus; it is non-enveloped and contains a single-stranded RNA packaged in a protein shell. There is only one type of the virus.

Hepatitis A Transmission

The virus spreads by the fecal-oral route and infections often occur in conditions of poor sanitation and overcrowding. Hepatitis A can be transmitted by the parenteral route but very rarely by blood and blood products. Food-borne outbreaks are not uncommon, and ingestion of shellfish cultivated in polluted water is associated with a high risk of infection. Approximately 40% of all acute viral hepatitis is caused by HAV. Infected individuals are infectious prior to onset of symptoms, roughly 10 days following infection. The virus is resistant to detergent, acid (pH 1), solvents (e.g., ether, chloroform), drying, and temperatures up to 60°C. It can survive for months in fresh and salt water. Common-source (e.g., water, restaurant) outbreaks are typical. Infection is common in children in developing countries, reaching 100% incidence, but following infection there is life-long immunity. HAV can be inactivated by: chlorine treatment (drinking water), formalin (0.35%, 37°C, 72 hours), peracetic acid (2%, 4 hours), beta-propiolactone (0.25%, 1 hour), and UV radiation (2 iW/cm²/min).

- The hepatitis A virus is found in the stools (feces) of people with hepatitis A. It is transmitted when a person puts something in his or her mouth that has been contaminated with the feces of an affected person. This is referred to as fecal-oral transmission.
 - If food or drinking water becomes contaminated with stool from an infected person (usually because of inadequate hand washing or poor sanitary conditions), the virus can quickly spread to anyone who drinks

or swallows the contaminated food or water.

- The virus can also be spread by eating raw or undercooked shellfish collected from water that has been contaminated by sewage.
- The hepatitis A virus can be transmitted through blood transfusions, although this is extremely rare.

- People who are infected can start spreading the infection about 1 week after their own exposure. People who do not have symptoms can still spread the virus. Infection with HAV is known to occur throughout the world.

- The risk of infection is greatest in developing countries with poor sanitation or poor personal hygiene standards.
- Infection rates are also higher in areas where direct fecal-oral transmission is likely to occur, such as daycare centers, prisons, and mental institutions.

Individuals at increased risk for hepatitis A infection

- Household contacts of people infected with HAV
 - Sexual partners of people infected with HAV
 - International travelers, especially to developing countries
 - Military personnel stationed abroad, especially in developing countries
 - Men who have sex with other men
 - Users of illegal drugs (injected or non-injected)
 - People who may come into contact with HAV at work
- Workers in professions such as health care, food preparation, and sewage and waste water management are not at greater risk of infection than the general public.
 - People who live or work in close quarters, such as dormitories, prisons, and residential facilities, or work in or attend daycare facilities are at increased risk only if strict personal hygiene measures are not observed.

Pathogenesis

Following ingestion, HAV enters the bloodstream through the epithelium of the oropharynx or intestine. The blood carries the virus to its target, the liver, where it lives and multiplies within hepatocytes and Kupffer cells (i.e., liver macrophages). There is no apparent virus-mediated cytotoxicity, and liver pathology is likely immune-mediated. Virions are secreted into the bile and released in stool. HAV is excreted in large quantities approximately 11 days prior to appearance of symptoms or anti-HAV IgM antibodies in the blood. The incubation period is 15-50 days, and mortality is less than 0.5%.

Hepatitis A Symptoms

Many people with HAV infection have no symptoms at all. Sometimes symptoms are so mild that they go unnoticed. Older people are more likely to have symptoms than children. People who do not have symptoms can still spread the virus.

- Symptoms of hepatitis A usually develop between 2 and 6 weeks after infection. The symptoms are usually not too severe and go away on their own, over time. The most common symptoms are as follows:

■ Nausea	■ Vomiting
■ Diarrhea, especially in children	■ Low-grade fever
■ Loss of appetite	■ Rash
■ Tiredness, fatigue	
■ Jaundice - A yellow discolouration of the skin and the whites of the eyes	
■ Urine is dark brownish in colour, like cola or strong tea.	
■ Pain in area of liver - On the right side of the abdomen, just under the rib cage	
- If the vomiting is severe, dehydration may occur. The symptoms of dehydration include the following:
 - Feeling weak, tired, or "blah"
 - Feeling confused or unable to concentrate
 - Rapid heartbeat
 - Headache
 - Urinating less frequently than usual
 - Irritability
- Symptoms usually last less than two months, although they may last as long as nine months. About 15% of people infected with hepatitis A have symptoms that come and go for 6-9 months.

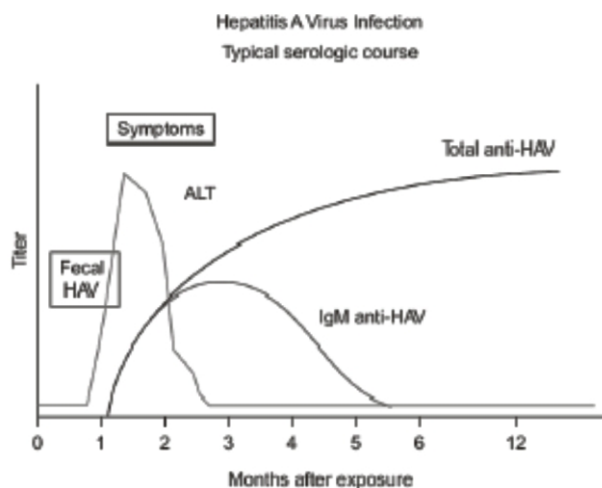
- Hepatitis does not occur simply from being near someone who has the disease at work or at school.

Diagnosis of Hepatitis A

Relevant History - Ask about: **Abrupt** onset of fatigue. **Abdominal** pain, particularly in the right upper quadrant. **Nausea**. **Vomiting**. **Anorexia**. **Diarrhea**. **Fever**. **Weight** loss. **Malaise**. **Myalgias**. **Pruritus**. **Symptoms** of icteric disease, including skin or sclera colour change, colour change of urine or stool, and pruritic skin. **Risks** for hepatitis A: **Recent** travel to endemic country. **Young** children who attend day care living in the house. **Contact** with a person with hepatitis A. **Occupation**. **Receipt** of clotting factor concentrates. **Men** who have sex with men. **Illicit** drug use. **Underlying** liver disease. **History** of hepatitis. **Pregnancy**. **Hydration** status. **Alcohol** use. **Medications** (prescription, over the counter, herbal preparations). **Vaccine** history.

Diagnosis:

Hepatitis A Virus Infection: Typical Serologic Course



Serum IgG, IgM and ALT following Hepatitis A virus infection.

Although the virus is excreted in the feces towards the end of the incubation period, specific diagnosis is made by the detection of Hepatitis A virus specific IgM antibodies in the blood. IgM antibody is only present in the blood following an acute hepatitis A infection. It is detectable from one to two weeks after the initial infection and persists for up to 14 weeks. The presence of IgG antibody in the blood means that the acute stage of the illness is past and the person is immune to further infection. IgG antibody to HAV is also found in the blood following vaccination and tests for immunity to the virus are based on the detection of this antibody. **During** the acute stage of the infection, the liver enzyme alanine transferase (ALT) is present in the blood at levels much higher than is normal. The enzyme comes from the liver cells that have been damaged by the virus. **Hepatitis A** virus is present in the blood, (viremia), and feces of infected people up to two weeks before clinical illness develops. **Rapid** ICTs and ELISA/ CLIA formats are available for serologic diagnosis.

Laboratory Investigations for Hepatitis A

Hepatitis A Virus Test

Hepatitis A virus (HAV) test is a blood test that looks for proteins (antibodies) made by the body in response to the virus that causes hepatitis A. These proteins will be present in the blood has hepatitis A infection now or has had one in the past. It is important to identify the type of hepatitis virus causing the infection to prevent it from spreading and to start the proper treatment. **HAV** infection is spread through food or water that has been contaminated by the feces (stool) of an infected person.

- IgM anti-HAV antibodies indicate a recent infection with hepatitis A virus. IgM anti-HAV antibodies generally can be detected in the blood as early as 2

weeks after the initial HAV infection. These antibodies disappear from the blood 3 to 12 months after the infection.

- IgG anti-HAV antibodies means that the patient has had a hepatitis A viral infection. About 8 to 12 weeks after the initial infection with hepatitis A virus, IgG anti-HAV antibodies appear and remain in the blood for lifelong protection (immunity) against HAV.

Hepatitis A vaccine is available to prevent an HAV infection. If the patient takes this vaccine and has anti-HAV antibodies, this means the vaccination was effective.

Indications for serologic tests

Hepatitis virus testing is done to: **Identify** the type of hepatitis virus causing a hepatitis infection. **Screen** people (such as doctors, dentists, and nurses) who have an increased chance of getting or spreading hepatitis A. **Screen** potential blood donors and donor organs to prevent the spread of hepatitis A. **Find** out whether a person has antibodies after getting a hepatitis A vaccine. If you had this vaccine and you now have antibodies to the hepatitis A virus (anti-HAV antibodies) in your blood, this means the vaccination was effective (you are immune to hepatitis A). **Find** out if a hepatitis A infection is the cause of abnormal liver function tests.

Differential Diagnosis: Screen all persons with unexplained acute hepatitis for hepatitis A, B, and C and consider other causes of acute hepatitis, including: **Other** infectious agents. **Autoimmune** hepatitis. **Alcoholic** liver disease. **Drug-induced** hepatitis. **Ischemic** hepatitis. **Metabolic** liver diseases. **Biliary** obstruction.

Differential Diagnosis of Hepatitis A

Disease	Characteristics	Notes
Acute hepatitis B	Clinical features of viral hepatitis in a person with a history of injection drug use, blood or body fluid exposure to an infected individual or blood product, sexual contact with a person with hepatitis B, or a new sexual partner in the previous 4 weeks to 3 months	Disease incubation period is longer; anti-HAV IgM is negative, whereas HBsAg and/or IgM antibody to hepatitis B core antigen are positive
Hepatitis D superinfection or coinfection in a person with acute or chronic hepatitis B	Clinical features of viral hepatitis in a person with a history of chronic hepatitis B or acute hepatitis B	Patient is positive for HBsAg, as well as anti-HDV anti-HAV IgM negative
Exacerbation of chronic hepatitis B	Clinical features of viral hepatitis in a person who is chronically infected with hepatitis B	Sudden elevation of aminotransferases without other evidence of cause (e.g., HCV, HDV, HAV) in a person with chronic hepatitis B
Acute hepatitis C	Clinical features of viral hepatitis with a history of an occupational or hemodialysis exposure in the previous 6 months, recent initiation of injection drug use, or other plausible exposure; acute hepatitis C is uncommonly symptomatic at presentation	Anti-HCV positive by ELISA and confirmed by RIBA or positive HCV RNA; anti-HCV may be negative in acute HCV infection; thus, test for HCV RNA if anti-HCV is negative, the patient has HCV-associated risk factors, and acute HCV infection is suspected
Exacerbation of chronic hepatitis C	Clinical features of viral hepatitis in a person with a history of blood transfusion, any history of injection drug use, exposures to contaminated blood or body fluids	Sudden elevation of aminotransferases in a person with hepatitis C without other cause; anti-HAV IgM negative

Hepatitis E	Clinical features of viral hepatitis in a person with a history of recent travel to endemic areas or exposure to an infected person or contaminated water	Test for anti-HEV IgM in acute-phase sera. Hepatitis E is often more severe and fulminant in pregnant women
Other infectious diseases, including Epstein-Barr virus, cytomegalovirus, HSV, Coxsackie virus (A and B), spirochetal diseases, toxoplasmosis, brucellosis, rickettsial diseases	Clinical features of viral hepatitis in a person with negative serology results for all types of viral hepatitis and exposure to other organisms that can cause hepatitis; classic lesions of specific pathogen (e.g., vesicular lesions of HSV, lymphadenopathy and splenomegaly in EBV, buccal or pharyngeal lesions with Coxsackie viruses)	Test for causative organism or antibody to pathogen. Atypical lymphocytes tend to be present
Alcoholic hepatitis	History of excessive alcohol consumption; hepatomegaly and jaundice are found in approximately 95% and 55% of persons presenting with alcoholic hepatitis, respectively	Up to 80% of persons with alcoholic hepatitis have an AST/ALT ratio of at least 2:1. Anemia is present in 50%-70% of persons with alcoholic hepatitis and leukopenia, and thrombocytopenia is present in 10%-15%.
Drug-induced hepatitis	History of excessive acetaminophen ingestion or therapeutic amounts of acetaminophen in a patient with alcoholic liver disease or alcohol ingestion; other therapeutic drugs may cause hepatitis (e.g., isoniazid, NSAIDs, beta-lactam antibiotics, sulfa-containing compounds, insulin-sensitizing drugs); may affect men and women of all ages but more common in women and the elderly	History of drug consumption; improvement usually occurs with discontinuation
Ischemic hepatitis	History of injury or hypotension, postoperative patient	Rapid increase and decrease in ALT and AST with disproportionate LDH elevation
Autoimmune hepatitis	Ninety percent of cases occur in women. Other autoimmune disorders may be present; 25%-40% of cases can present with acute hepatitis. HAV has been described as a trigger for autoimmune hepatitis	Antinuclear antibodies, smooth muscle antibodies, IgG, characteristic liver biopsy
Metabolic liver disease, including Wilson's disease	Wilson's disease prevalence is 1 in 30,000 and is rarely present after age 40 without neurologic symptoms; rarely presents as acute hepatitis	Obtain 24-hour urine for copper, serum ceruloplasmin, quantitative hepatic copper for definitive diagnosis
Alpha-1 antitrypsin disease	Uncommonly presents as acute hepatitis	Obtain serum level and phenotyping. Stain liver biopsy for periodic acid-Schiff-positive inclusions
Biliary obstruction	Jaundice, right upper quadrant pain, negative serologic markers for other causes of acute hepatitis	The alkaline phosphatase level is generally >3 times the ALT or AST levels. ERCP may be needed, especially if bile ducts are dilated on ultrasound

ALT = alanine aminotransferase; anti-HCV = antibody to hepatitis C virus; anti-HDV = antibody to hepatitis D virus; anti-HEV = antibody to hepatitis E virus; AST = aspartate aminotransferase; EBV = Epstein-Barr virus; ELISA = enzyme-linked immunosorbent assay; ERCP = endoscopic retrograde cholangiopancreatography; HAV = hepatitis A virus; HBsAg = hepatitis B surface antigen; HCV = hepatitis C virus; HDV = hepatitis D virus; HSV = herpes simplex virus; IgG = immunoglobulin G; IgM = immunoglobulin M; LDH = lactic dehydrogenase; NSAID = nonsteroidal anti-inflammatory drug; RIBA = recombinant immunoblot assay; RNA = ribonucleic acid.

Prevention: **Vaccinate** all children between 12 and 35 months of age, with catch-up vaccination through the preschool years. **Administer** hepatitis A vaccine or immunoglobulin to persons traveling to endemic areas of the world. **Administer** hepatitis A vaccine to persons at increased risk for hepatitis A. **Administer** hepatitis A vaccine to persons at increased risk for severe outcomes from hepatitis A. **Administer** immunoglobulin or hepatitis A vaccine for postexposure prophylaxis to persons within 2 weeks of exposure to HAV.

Hospitalization: Hospitalize persons with severe dehydration or those with signs of severe disease or liver failure, including elevated prothrombin time, encephalopathy, or hepatorenal syndrome.

Hospitalize for: **Inability** to maintain oral hydration and nutrition due to nausea, vomiting, and anorexia. **Mental** status changes. **Elevated** prothrombin time due to acute liver failure. **Azotemia** (dehydration vs. hepatorenal syndrome). **Gastrointestinal** bleeding. **Evaluation** for liver transplantation.

Treatment: There is no specific treatment for hepatitis A. Sufferers are advised to rest, avoid fatty foods and alcohol (these may be poorly tolerated for some additional months during the recovery phase and cause minor relapses), eat a well-balanced diet, and stay hydrated. Approximately 15% of people diagnosed with hepatitis A may experience one or more symptomatic relapse(s) for up to 24 months after contracting this disease.

Prognosis: **Low** mortality rate for hepatitis A of 4 deaths per 1000 cases for the general population but a higher rate of 17.5 per 1000, in those aged 50 and over has been reported for Hepatitis A. Death usually occurs when the patient contracts Hepatitis A while already suffering from another form of Hepatitis, such as Hepatitis B or Hepatitis C or AIDS. **Young** children who are infected with hepatitis A typically have a milder form of the disease, usually lasting from 1-3 weeks, whereas adults tend to experience a much more severe form of the disease.

Prevention: Hepatitis A can be prevented by vaccination, good hygiene and sanitation.

Vaccine: **Hepatitis A** vaccines protect against the virus. Vaccines contain inactivated. **Hepatitis A** virus providing active immunity against a future infection.

Patient Education: **Emphasize** the need for preventing HAV transmission to close contacts of persons with acute HAV infection. **Provide** persons with hepatitis A with information to decrease the risk of dehydration and further damage to the liver. **Provide** persons with hepatitis A with information regarding disease monitoring and worsening symptoms.

Complications of hepatitis A: Consider referral to a gastroenterologist or hepatologist for: **Suspected** HAV-associated cholestasis (decreasing aminotransferase levels, normal prothrombin time, bilirubin level >20 mg/dL) for confirmation and possible administration of steroids. **Continued** or recurrent elevation of transaminases possibly due to relapsing hepatitis A or underlying liver disease. **Elevated** prothrombin time or mental status changes suggesting worsening liver failure and possible need for transplantation. **Aplastic** anaemia can sometimes be a complication of Hepatitis A.

Monitor patients with uncomplicated hepatitis A for clinical improvement. Ask about: **Fatigue**, return to normal activities. **Weight** loss. **Improvement** in acute symptoms, including nausea, vomiting, abdominal pain, anorexia, and skin and sclerae colour. **Look** for resolution of jaundice, icterus, hepatomegaly, and splenomegaly. **Monitor** AST and ALT levels until they return to normal.

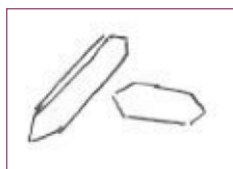
INTERPRETATION

Continued...

ABNORMAL CRYSTALS THAT ARE OF METABOLIC ORIGIN.

Hippuric acid (pH: mostly alkaline, sometimes in acidic and neutral urine), cystine (pH: acidic), tyrosine (pH: acidic), leucine (pH: acidic), cholesterol (pH: acidic), bilirubin (pH: acidic), and hemosiderin (pH: acidic).

HIPPURIC ACID CRYSTALS AND THEIR SIGNIFICANCE.

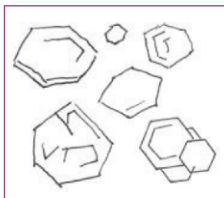


This crystal is not discussed in most textbooks. It is considered to be an abnormal, but non-pathogenic crystal. It is formed as a colorless crystal, and like uric acid, it has an affinity for urinary pigments to take on a yellowish color. It occurs most often as an elongated prism with variable lengths and widths, the ends being triangular or pointed. Other shapes

reported are needles (which can arrange in clusters), and rhombic plates. Hippuric acid can be a uric acid look-a-like. It is soluble in acetic acid (uric acid is not soluble), hot water, alkali, and ether. It has been reported to be observed in the urine of "glue-sniffers".

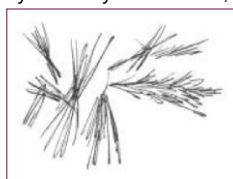
CYSTINE CRYSTALS AND THEIR SIGNIFICANCE.

A rarely observed crystal that appears in the urine as a hexagonal plate and has the tendency to laminate to other cystine plates. Sides of the hexagon may be of unequal lengths and will vary greatly in size. This crystal is soluble in dilute HCl, ammonia, dilute bases; but is insoluble in acetic acid, alcohol, boiling water and ether. Cystine can crystallize out in the kidney to form stones and cause urinary blockage. It is rapidly destroyed by bacteria. When searching for these crystals, use in fresh urine. Bacteria will rapidly destroy cystine. Avoid letting the slide dry, crystals will wrinkle and change shape as the urine evaporates. The presence is due to an inborn error of metabolism in which there is a defect in the transport mechanisms for cystine, ornithine, lysine and arginine. Cells cannot reabsorb these amino acids and thus appear in the urine in large quantities and of these four, only cystine precipitates as crystals. The appearance of these crystals are clinically significant and indicate some type of pathology. Cystine crystals are also observed in pyelonephritis, heavy metal nephrotoxicity, Wilson's disease, and renal tubule acidosis.



TYROSINE CRYSTALS AND THEIR CLINICAL SIGNIFICANCE.

Tyrosine crystals are fine, delicate needles that may be arranged in sheaves or clumps. Sometimes these crystals may have a fine, silky appearance. They are formed first as colorless crystals and will take on a pale yellow color if bilirubin (or some other strong dye, Sternheimer-Malbin stain will dye the crystals purple) is present. When focusing with a microscope, these crystals may appear black. These crystals are soluble in alkali, mineral acids, acetic acid, or heat; but insoluble in acetone, alcohol, or water. Because of their insolubility in water, these crystals may be seen more often than others. These crystals are observed with severe liver disease, Oasthouse urine disease, hereditary tyrosinosis, bilirubinuria, or if leucine crystals are present.



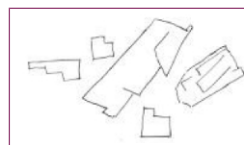
LEUCINE CRYSTALS AND THEIR CLINICAL SIGNIFICANCE.

Often described as yellow to brown, oily appearing spheres; with variable sizes, having the appearance of concentric rings (with or without radial striations), and a central nidus. Their presence is a serious prognosis. These crystals may be observed in severe liver disease, but more likely to be found associated with metabolic disorders. These crystals are soluble in hot alkali, hot alcohol, and boiling glacial acetic acid. They are



insoluble in dilute HCl and warm dilute acetic acid. They may be found in urine with tyrosine crystals.

CHOLESTEROL CRYSTALS AND THEIR CLINICAL SIGNIFICANCE.



If observed in urine, it is pathologically significant. Cholesterol crystallizes out as broad, flat plates, often characterized by a notched corner. These crystals will appear colorless or take on a pale-green to yellow coloration. They are soluble in chloroform, ether, and boiling alcohol; but

insoluble in warm alcohol. If cholesterol crystals are truly present, look for a specific gravity < 1.035 and a positive protein test along with the presence of fat globules, oval fat bodies, and/or fatty casts. If these are not present then another crystal should be considered. Be cautious in identifying these crystals since they can resemble radiographic dye crystals. It is recommended that cholesterol crystals should not be reported if the confirmatory findings are not present. Cholesterol crystals are observed in lymph gland disorders, chyluria, severe UTI, and nephrotic syndrome.

BILIRUBIN CRYSTALS AND THEIR SIGNIFICANCE.

Bilirubin crystals have been reported in a variety of forms: fine needles (that may form clusters), rhombic plates, cubes, and granules. Colors range from yellow-brown to reddish-brown. These crystals are soluble in alkali, acetone, chloroform, and acids; but insoluble in alcohol and ether. Bilirubin is a strong dye and if present in urine, will stain other crystals (especially uric acid) along with cells and casts. The appearance of precipitated bilirubin has the same significance as a positive reagent strip test or Ictotest.



HEMOSIDERIN CRYSTALS AND THEIR SIGNIFICANCE.

Hemosiderin, if it precipitates out, will appear as amorphous urates. If hemosiderin crystals are present, they may be confirmed with a Prussian blue stain. The presence of hemosiderin in the urine, either free or crystalline form, is an indicator of an intravascular hemolytic episode.

CRYSTALS OF IATROGENIC OR DRUG ORIGIN AND THE pH AT WHICH THEY ARE FOUND.

Sulfonamides (pH: acid to neutral), radiographic (pH: acid to neutral), penicillin (pH: acid to neutral), and acyclovir (pH: acid to neutral).

SULFONAMIDE CRYSTALS AND THEIR SIGNIFICANCE.

Sulfa's or sulfonamides are important findings in urine specimens. The crystal that is encountered is determined by the drug administered. When sulfa's were first discovered and prescribed, they tended to be insoluble and could precipitate out in kidney, posing a risk for renal damage. Current manufacturing technology has essentially eliminated this problem and such crystals are seldom observed in the urine, however a potential risk is still present if the patient is allowed to become dehydrated. Crystal formation in the tubules may cause hematuria or oliguria (by blockage). The variety of shapes for sulfa's include: dumbbells, rosettes, sheaves (with central or lateral bindings), fans, hexagonal plates, rectangular plates, arrowheads, rhomboids, and spheres. The following are four examples of sulfa's that have been prescribed.

A. Sulfadiazine:

(a) appears as spheres with irregular striations, but may look like a dense brown spheres or bundles of needles similar to sheaves of wheat. (b) it has been reported as a look-a-like to the ammonium biurate crystal.



B. Sulfamethoxazole:

(a) Also called "Gantrisin", this is a very soluble sulfa and is rarely seen. (b) When observed as a crystal, it appears as a brown sphere that may be unevenly divided or rosettes.

C. Sulfasalazine:

(a) A poorly absorbed sulfa that is used to treat enteric diseases. If there is tissue

damage in the intestine, it may be absorbed into the blood stream. (b) It is excreted as sulfapyridine or acetylsulfapyridine. (c) Crystals will appear as rhomboids.

D. Acetylsulfadiazine"

(a) This is a seldom used medication. (b) Its crystals appear as yellow-brown sheaves of wheat with eccentric bindings.

Sulfa's are soluble in acetone. They precipitate out as colorless crystals, but have an affinity to absorb urinary pigments and which will contribute to a yellow coloration. If a urine is allowed to stand and the pH becomes alkaline, sulfa crystals tend to dissolve. Fresh urine should be used to look for these crystals. To help identify suspected sulfa crystals, review the patients medication charts. Note the illustrations of other sulfa crystals.



Sulfisoxazole

PENICILLIN CRYSTALS AND THEIR CLINICAL SIGNIFICANCE.

Penicillin-type antibiotics are seldom observed in urine. If they should be present, it will be due to a high dosage when the physician is aggressively treating for an infection (example: meningitis, septicemia). Ampicillin crystals are long, thin, colorless prisms or needles that may appear singly or in clusters. Penicillin-G crystals tend to be rectangular, oblong, and with pointed ends. These types of crystals tend to form when refrigerated. See the following example of ampicillin crystals.

ACYCLOVIR CRYSTALS AND THEIR CLINICAL SIGNIFICANCE.

Acyclovir is an anti-viral medication. When given high doses of this drug, the urine may demonstrate fine, slender needles that closely resemble sodium urate crystals. These crystals are most likely to be observed in neutral or slightly alkaline urine.

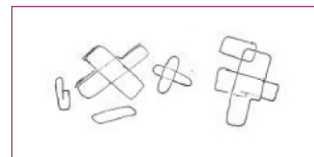


NOTE: When medications are administered in high doses, there is a chance that crystals may appear in urine. When encountering unknown crystals in a sediment examination, refer to the patients chart as a strategy to identify the crystal.

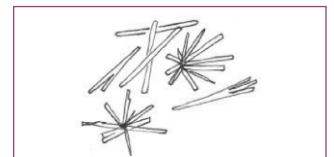
RADIOGRAPHIC DYE CRYSTALS AND THEIR CLINICAL SIGNIFICANCE.

These are water soluble radio-opaque chemicals that are readily excreted by the kidney. These dyes are derived from triiodobenzoic acid. If the dye is designated as a meglumine, it is a triiodobenzoic acid conjugated with a synthetic organic compound to form a more water-soluble and less-toxic chemical. Triiodobenzoic acid based dyes are a variety of mixtures (with different inorganic anions) and are known by trade names as Hypaque, Renograffin, Cystograffin, and Renovist. Crystals of Renograffin (meglumine diatrizoate) will appear in urine shortly after injection and may be mistaken for cholesterol crystals. One characteristic of the presence these radio-opaque chemicals in urine is a positive sulfosalicylic acid test. The importance of recognizing radiographic dye crystals is that of false identification. Things to remember about these type of crystals are (1) they may appear in urine for up to four hours after injection, (2) the strip reagent test for protein is negative, but the 3% SSA test is likely to be positive, and (3) specific gravity will be elevated, usually over 1.040. There is usually no clinical significance associated with these crystals. If a patient has a kidney disorder or is dehydrated, then the patient may experience a problem. If the crystals are unusually abundant, the appearance of the urine specimen may be cloudy.

Note the following illustrations of x-ray dye crystals.



renograffin



hypaque

BOUQUET

In Lighter Vein

A farmer buys several pigs, hoping to breed them for ham and bacon. After several weeks, he notices that none of the pigs are getting pregnant, and calls a vet for help.

The vet tells the farmer that he should try artificial insemination. The farmer doesn't have the slightest idea what this means but, not wanting to display his ignorance, he only asks the vet how he will know when the pigs are pregnant. The vet tells him that when pregnant, they will stop standing around and will, instead, lay down and wallow in the mud. The farmer hangs up and gives it some thought. He comes to the conclusion that artificial insemination means he has to impregnate the pigs.

So, he loads the pigs into his truck, drives them out into the woods, personally impregnates them all, brings them back and goes to bed. Next morning, he wakes and looks out at the pigs. Seeing that they are all still standing around, he concludes that the first try didn't take, and loads them in the truck again. He drives them out to the woods, impregnates each pig twice for good measure, brings them back and goes to bed.

Next morning, he wakes to find the pigs still standing around. One more try, he tells himself, and proceeds to load them up and drive them out to the woods. He spends all day working hard, and, upon returning home, falls listlessly into bed.

The next morning, he was woken up by his wife shaking him and saying "Wake up Dear, the pigs are acting strangely!". "What do you mean?" he asked excitedly, "Are they wallowing in the mud?" "No," she says, "they're all in the truck and one of them is honking the horn."

Wisdom Whispers

- "You must have good luck to catch hares with a drum."
- "He who blows in the fire will get sparks in his eyes."
- "We are not so much concerned if you are slow as when you come to a halt."
- "Every one can navigate in fine weather."
- "He may swim boldly who is held up by the chin."
- "A peasant between two lawyers is like a fish between two cats."
- "Though the wound be healed, a scar remains."
- "The names of fools are always written on walls."

Brain Teasers

CHOOSE THE MOST APPROPRIATE/ CORRECT ANSWER.

1. Tropical pulmonary eosinophilia is associated with
A. Microfilariae B. Tropical sprue C. Roundworms D. Pinworms
2. Hamman Rich syndrome is same as
A. Idiopathic pulmonary hemosiderosis B. Fibrosing alveolitis
C. Pulmonary alveolar proteinosis D. Goodpasture's syndrome
3. Vanishing lung syndrome is used for describing
A. Bronchogenic cysts B. Alveolar cysts
C. Emphysema D. Hyaline membrane disease
4. Adenocarcinomas of the lung constitute about ---% of all lung tumours
A. 40-60 B. 60-70 C. 5-10 D. 10-25
5. Eosinophilic granuloma primarily involves
A. Skin B. Lungs C. Bones D. Brain
6. The commonest intracranial neoplasm is
A. Medulloblastoma B. Glioma C. Astrocytoma D. Fibroma

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

TROUBLESHOOTING

GLYCOSYLATED HEMOGLOBIN

Introduction: Glycosylated (or glycated) hemoglobin (*hemoglobin A1c*, Hb_{1c} , or Hb_{A1c} , A1C) is a form of hemoglobin used primarily to identify the average plasma glucose concentration over prolonged periods of time. It is formed in a non-enzymatic pathway by hemoglobin's normal exposure to high plasma levels of glucose. Glycosylation of hemoglobin has been implicated in nephropathy and retinopathy in diabetes mellitus. Monitoring the Hb_{A1c} in type-1 diabetic patients may improve treatment.

History: Hemoglobin A1c was first separated from other forms of hemoglobin by Huisman and Meyering in 1958 using a chromatographic column. It was first characterized as a glycoprotein by Bookchin and Gallop in 1968. Its increase in diabetes was first described in 1969 by Samuel Rahbar and coworkers. The reactions leading to its formation were characterized by Bunn and his co-workers in 1975. The use of hemoglobin A1c for monitoring the degree of control of glucose metabolism in diabetic patients was proposed in 1976 by Koenig and coworkers.

Terminology: Glycohemoglobin (GHb, glycated hemoglobin, glycosylated hemoglobin) is a generic term for hemoglobin bound irreversibly (ketoamine form) to glucose. Often, the term is used to mean total glycated hemoglobin, and sometimes to mean hemoglobin A1c. **Total glycated hemoglobin** (Total GHb) refers to all the glycated hemoglobins, including glycated hemoglobin variants. Total glycated hemoglobin is usually determined by affinity chromatography or immunoassays. **Hemoglobin A1c** ($HbA1c$) is the major subfraction of the glycated normal hemoglobin ($HbA1$). Determination of $HbA1c$ is usually achieved by ion-exchange HPLC or gel electrophoresis.

Glycated Hemoglobins: Glycated hemoglobins are hemoglobin components formed through a two-step non-enzymatic reaction between hemoglobin and blood glucose. The first step consists of the formation of a reversible aldimine form of hemoglobin to glucose linkage. In the second step, the labile aldimine form is converted slowly to the stable and irreversible ketoamine form through an Amadori rearrangement. The level of glycated hemoglobins in the blood is directly related to the average blood glucose levels over the life span of the hemoglobin in the circulation. Since the half life of red blood cells is about 120 days, a single determination of glycated hemoglobin reflects the average blood glucose level during the preceding 8 to 12 weeks. The test is therefore a very good monitor for long-term (2 to 3 months) blood glucose control in patients with diabetes mellitus.

Underlying principle: In the normal 120-day life span of the red blood cell, glucose molecules join hemoglobin, forming glycated hemoglobin. In individuals with poorly controlled diabetes, increases in the quantities of these glycated hemoglobins are noted. Once a hemoglobin molecule is glycated, it remains that way. A buildup of glycated hemoglobin within the red cell reflects the average level of glucose to which the cell has been exposed during its life cycle. Measuring glycated hemoglobin assesses the effectiveness of therapy by monitoring long-term serum glucose regulation. The Hb_{A1c} level is proportional to average blood glucose concentration over the previous four weeks to three months. Some researchers state that the major proportion of its value is related to a rather short term period of two to four weeks.

Specimen Requirements: EDTA Whole Blood (Lavender top tube), 2.0 mL (minimum 1.0 mL). **Storage and Specimen Stability:** Stable at room temperature for up to 12 hours. Refrigerated below 4°C for up to 7 days. Frozen (below -20°C) for long-term storage. Glucose level over the past 60 days, and not the glucose value of the specimen obtained at the same time as the $HbA1c$.

Measuring A1c: There are a number of techniques used to measure A1c. Laboratories use: high-performance liquid chromatography (HPLC) Immunoassay. Point of care (e.g. doctors surgery) devices use: Immunoassay. Boronate Affinity Chromatography. POC A1c tests are certified by the National Glycohemoglobin Standardization Program (NGSP) to standardise them against the results of the 1993 Diabetes Control and Complications Trial (DCCT).

Interpretation of results: Laboratory results may differ depending on the analytical technique, the age of the subject, and biological variation among individuals. Two individuals with the same average blood sugar can have A1C values that differ by as much as 1 percentage point. In general, the reference range (that found in healthy persons), is about 4 - 5.9%. Higher levels of Hb_{A1c} are found in people with persistently elevated blood sugar, as in diabetes mellitus. While diabetic patient treatment goals vary, many include a target range of Hb_{A1c} values. A diabetic person with good glucose control has a Hb_{A1c} level that is close to or within the reference range. The International Diabetes Federation and American College of Endocrinology recommend Hb_{A1c} values below 6.5%, while American Diabetes Association recommends that the Hb_{A1c} be below 7.0% for most patients. A high Hb_{A1c} represents poor glucose control. Persistent elevations in blood sugar (and therefore Hb_{A1c}) increase the risk for the long-term vascular complications of diabetes such as coronary disease, heart attack, stroke,

heart failure, kidney failure, blindness, erectile dysfunction, neuropathy (loss of sensation, especially in the feet), gangrene, and gastroparesis (slowed emptying of the stomach). Poor blood glucose control also increases the risk of short-term complications of surgery such as poor wound healing. Lower than expected levels of Hb_{A1c} can be seen in people with shortened red blood cell life span, such as with glucose-6-phosphate dehydrogenase deficiency, sickle-cell disease, or any other condition causing premature red blood cell death. Conversely, higher than expected levels can be seen in people with a longer red blood cell life span, such as with Vitamin B12 or folate deficiency. The approximate mapping between Hb_{A1c} values and average blood glucose measurements over the previous 4-12 weeks is shown in the table.

Correlation with Mean Blood Glucose Levels: A single fasting blood glucose measurement only gives an indication of the patient's immediate past (last 1 to 2 hours) condition, and may not represent the true status of blood glucose regulation. In contrast, the level of glycated hemoglobin is directly related to the average glucose concentration over the life-span of the hemoglobin in the circulation. Various formulae have been proposed to demonstrate the correlation between the mean blood glucose (MBG) and Hemoglobin A1c ($HbA1c$). The following is one from Nathan, et al, N Engl J Med (1984). $MBG = 33.3 * HbA1c - 86$. To verify the correlation, the mean blood glucose level for each patient was obtained as the average of up to 4 daily determinations over a period of 2 months (the average of over 200 glucose readings). Hemoglobin A1c was determined by ion-exchange HPLC at the end of the two month period. Note that the mean blood glucose value is the average.

HbA1c (%)	Avg. Blood Sugar	
	(mmol/L)	(mg/dL)
4	3.3	60
5	5.0	90
6	6.7	120
7	8.3	150
8	10.0	180
9	11.7	210
10	13.3	240
11	15.0	270
12	16.7	300
13	18.3	330
14	20.0	360

Reference Guidelines

Degree of glucose control	Total GHb	HbA1c
Normal (non-diabetic)	< 7%	< 6%
Near normoglycemic	7 to 8%	6 to 7%
DCCT therapeutic goal		Less than 7%
In good control	8 to 9%	7 to 8%
Actions suggested	9 to 11%	8 to 9%
Not in control	> 11%	> 9%

In patients with uncontrolled diabetes, the % of glycated hemoglobin is substantially higher than in diabetics in good control and in non-diabetics. The determination of a glycated hemoglobin level may therefore also assist in the initial diagnosis of diabetes, or it may be used to indicate the degree of long-term diabetic control in diabetic patients. The significance of a low glycated hemoglobin level has not been established.

Indications and use: Glycosylated hemoglobin is recommended for both (a) checking blood sugar control in people who might be pre-diabetic and (b) monitoring blood sugar control in patients with more elevated levels, termed diabetes mellitus. For a single blood sample, it provides far more revealing information on glycemic behavior than a fasting blood sugar value. That being said, fasting blood sugar tests are crucial in making treatment decisions. The American Diabetes Association guidelines are similar to others in advising that the glycosylated hemoglobin test be performed at least two times a year in patients with diabetes who are meeting treatment goals (and who have stable glycemic control) and quarterly in patients with diabetes whose therapy has changed or who are not meeting glycemic goals. Glycosylated hemoglobin measurement is not appropriate where there has been a change in diet or treatment within 6 weeks. Likewise the test assumes a normal red blood cell aging process and mix of hemoglobin subtypes (predominantly HbA in normal adults). Hence people with recent blood loss or hemolytic anemia, or genetic differences in the hemoglobin molecule (hemoglobinopathy) such as sickle cell disease and other conditions are not suitable for this test. The alternative fructosamine test may be used in these circumstances and it similarly reflects an average of blood glucose levels over the preceding 2 to 3 weeks. There is variation among laboratories and a lack of consensus on a diagnostic threshold for glycosolated hemoglobin. For these and other reasons, no medical organization recommends the use of this test alone to diagnose diabetes. Instead, fasting plasma glucose or an oral glucose tolerance test are used.

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