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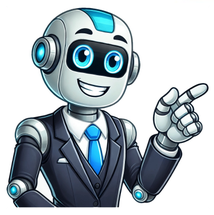

Bilirubin test in infants

Photo by: Marzky Ragsac Jr. A bilirubin test is a diagnostic blood test performed to measure levels of bile pigment in an individual's blood serum and to help evaluate liver function. The bilirubin test is an important part of routine newborn (neonatal) diagnostic screening tests. The level of bilirubin in a newborn's blood serum is measured to determine if the circulating level of bilirubin is normal or abnormal. Bilirubin is a yellow-orange bile pigment produced during the breakdown of hemoglobin, the iron-bearing and oxygen-carrying protein in red blood cells. All individuals produce bilirubin daily as part of the normal turnover of red cells. A higher than normal (elevated) bilirubin test can reflect accelerated red blood cell destruction or may indicate that bilirubin is not being excreted as it should be, suggesting that liver function problems or other abnormalities may be present. Neonatal bilirubin screening often reveals an elevated bilirubin (hyperbilirubinemia). The bilirubin test will determine if hyperbilirubinemia is present and, along with other diagnostic tests, help determine if the condition is relatively normal (benign) or possibly related to liver function problems or other conditions. Usually all newborns (neonates) delivered in the hospital will have total serum bilirubin (TSB) measured in the Blood taken from the heel of a newborn to test the level of bilirubin. (© Ted Horowitz/Corbis.) clinical laboratory on one or more blood samples as requested by attending pediatricians. To obtain a blood sample for TSB, a phlebotomist takes blood from the infant's tissue (usually the heel) rather than from a vein, as the veins of newborns are extremely small and easily damaged. After sterilizing the surface of the site with alcohol and/or an antibacterial solution such as betadine, a heel puncture is made and blood from the puncture is drawn into a tiny capillary tube about 2 inches (5 cm) long that is stoppered at each end when full. This tube is spun down in a special centrifuge in the laboratory to separate serum, the liquid part of blood, from red cells. In the TSB test, spectrophotometry is used to identify and quantify the amount of bilirubin in a specific amount of serum by measuring the amount of ultraviolet light absorbed by bilirubin pigment in the sample. The test method requires only minutes and a very small amount of blood serum to produce accurate results, measuring the results in milligrams per desiliter (mg/dL). The amount of total bilirubin in circulating blood can be calculated from the results of a single bilirubin test. Results are compared to known normal values to determine if the individual has normal or abnormal levels. All newborn infants begin to destroy fetal red blood cells (RBCs) in their first few days of life, replacing them with new red blood cells. The rapid destruction of red blood cells and subsequent release of fetal hemoglobin into the bloodstream results in the production of bilirubin. As a waste product, bilirubin is filtered out of blood (cleared) by the liver and excreted in bile, eliminated normally in stool produced by the large intestine. However, immediately after birth, more bilirubin is produced than the infant's immature liver can handle, and the excess remains circulating in the blood. This situation results in jaundice in over 60 percent of newborns, usually due to the presence of fetal hemoglobin released into the blood during the normal destruction of fetal red blood cells. Even healthy infants may appear to have a yellow stain in their skin (physiological jaundice or icterus) and the whites of the eyes (sclerae) in the first week after birth. This may first be noticed by pediatric nurses as they care for the infant. Visual evaluation of jaundice is not considered a reliable way, however, to determine its cause or the risk of continued rising of bilirubin and possible complications. Performing bilirubin tests is the first step in making sure that normal degrees of jaundice do not become more severe and that liver dysfunction or other causative conditions, if present, are identified and treated early. Besides normal red cell destruction after birth, neonatal hyperbilirubinemia may also be caused by the following: low birth weight feeding or nutrition problems glucose 6-phospho-dehydrogenase (G6PD) deficiency insufficient intestinal bacteria incompatibility of major blood groups (ABO) between mother and baby blood type (Rh) incompatibility (rare due to treatment of Rh negative mothers) genetic abnormalities linked to a history of jaundice among siblings liver dysfunction From 8 to 9 percent of newborns develop severe hyperbilirubinemia. Severe hyperbilirubinemia is of great concern to pediatricians because it may lead to bilirubinrelated brain damage (kernicterus). Persistent elevated levels of bilirubin in the body can place infants at risk of neurotoxicity or bilirubin-induced neurologic dysfunction (BIND). The risk of liver dysfunction has been shown to be higher in infants who were born before term (less than 37 weeks' gestation) or who have other abnormalities in addition to an elevated total serum bilirubin. Some pediatricians order bilirubin tests at defined times within 24 to 48 hours after birth to monitor the rate of increase of bilirubin and to help determine associated risks on an individual basis. Infants with a low rate of rise in bilirubin (less than 17mg/dL per hour) are considered lower risk and are likely to be discharged without further testing or treatment. Those who show visual jaundice at birth or within several hours after birth and whose rate of bilirubin rises more rapidly are considered at higher risk for severe hyperbilirubinemia and associated kernicterus, especially if the bilirubin level is still rising at time of discharge. Some newborns are placed under special lamps (phototherapy) to help correct the jaundice caused by elevated bilirubin levels and to bring down the bilirubin level. Supervision of breastfeeding and supplemental nutritional support may be needed to help infants who are not getting their nutritional needs met. Exchange transfusions may be given for high-risk infants, especially those with blood group (ABO) or type (Rh positive infants born to Rh negative mothers) incompatibilities. Additional tests may be required to evaluate G6PD deficiency, genetic abnormalities, or liver function. After discharge from the hospital, about 25 percent of otherwise healthy infants who are still showing signs of jaundice may continue to be tested for bilirubin levels. An elevated bilirubin usually goes down on its own if the hyperbilirubinemia is benign; if liver dysfunction or other abnormalities exist, bilirubin levels may remain elevated or continue to rise, indicating that further diagnostic testing, clinical evaluation, and treatment are needed. Performance of the bilirubin test itself is a precaution against the serious consequences that can occur when bilirubin levels continue to rise in jaundiced infants. Visual jaundice present at birth may predict rapid rises in bilirubin and risk of liver dysfunction or other abnormalities. No preparation is needed before performing bilirubin tests on infants' blood samples. Proper identification and careful handling of the infant are important when a blood sample is being obtained for testing. A site, usually on the infant's heel, is chosen by the phlebotomist who draws the infant's blood sample. The area is prepared by wrapping the baby's foot in a warm cloth for a few minutes to bring blood to the surface and allow it to flow more easily. The heel is then wiped with alcohol and/or an antibacterial solution such as betadine to sterilize the surface. The heel is then punctured with a lancet, avoiding the center of the heel, in order to prevent inflammation of the bone. The blood sample is drawn in tiny capillary tubes, properly labeled, and taken to the laboratory for testing. In rare instances, a phlebotomist is not able to draw sufficient blood from a heel puncture, and a physician may draw venous blood from a femoral vein in the groin area, which is larger than veins in an infant's arms. Bilirubin —A reddish yellow pigment formed from the breakdown of red blood cells, and metabolized by the liver. When levels are abnormally high, it causes the yellowish tint to eyes and skin known as jaundice. Levels of bilirubin in the blood increase in patients with liver disease, blockage of the bile ducts, and other conditions. Glucose-6-phosphate dehydrogenase (G6PD) deficiency —A sex-linked hereditary disorder in which the body lacks an enzyme that normally protects red blood cells from toxic chemicals. When people with this condition take certain drugs, their red blood cells break down, causing anemia. Hyperbilirubinemia —A condition characterized by a high level of bilirubin in the blood. Bilirubin is a natural byproduct of the breakdown of red blood cells, however, a high level of bilirubin may indicate a problem with the liver. Kernicterus —A potentially lethal disease of newborns caused by excessive accumulation of the bile pigment bilirubin in tissues of the central nervous system. Neurotoxic — Refers to a substance that is harmful to the nervous system. Phlebotomist —A person who draws blood from a vein. Spectrophotometry —A testing method that measures the amount of ultraviolet light absorbed by specific substances such as bilirubin pigment. A spectrophotometer can accurately measure how much bilirubin is in a blood sample and the result can be compared to known normal values. The site from which blood is withdrawn must be kept clean after the procedure and must be checked regularly for bleeding. A small adhesive patch may be used to protect the site. The performance of bilirubin tests carries no significant risk. Drawing blood for the test may involve light bleeding or bruising at the site of puncture, or blood may accumulate under the puncture site (hematoma), requiring that a new location be found for subsequent tests. Not performing bilirubin tests, however, may have significant risks for some infants. Infants with rising bilirubin levels are at risk of neurotoxicity and developing kernicterus, making the monitoring of bilirubin in the first week of life critical for these infants. At birth, a newborn's TBS is normally 1 or 2 mg/dL, peaking at 6 mg/dL in three or four days. In 10 days to two weeks, a healthy infant's TBS is expected to be less than 0.3 mg/dL. During the first seven days of the infant's life, TBS results are rated for risk of bilirubin toxicity or bilirubinrelated brain damage within percentile ranges representing degrees of hyperbilirubinemia. TBS values less than 20 mg/dL are lower-risk percentile ranges below the 95th percentile, with an incidence of one in nine infants. TBS values greater than 20 mg/dL are in the 98th percentile, with an incidence of one in 50 infants; greater than 25 mg/dL are in the 99.9 percentile, with an incidence of one in 700 infants; and TBS values greater than 30 ng/dL are at the highest level of risk at 99.99 percentile, indicating almost certain neurotoxicity. One in 10,000 infants are in the 99.99 percentile. Parents will usually be informed by the pediatrician about any risks associated with an elevated bilirubin, such as liver dysfunction or possible kernicterus. Parents concerned about these risks can be made aware that bilirubin levels usually return to normal in most infants (more than 60%) and the related jaundice goes away gradually. Testing after the baby is discharged is sometimes necessary (in 25% of infants) and is a preventive measure rather than a cause for concern. Repeat testing is necessary to monitor bilirubin levels. Parents should be aware that, although the baby's heel may be bruised, elevated bilirubin levels can cause serious complications, and testing is critical to help prevent them. Resources Maisels, M. Jeffrey. Neonatal Jaundice. London: CRC Press, 2000. "Jaundice." MedicineNet , March 2001. Available online at October 28, 2004). "Newborn Jaundice." Caring for Your Baby. Available online at (accessed October 28, 2004). Wong RJ, et al. Clinical manifestations of unconjugated hyperbilirubinemia in term and late preterm infants. . Accessed April 2, 2018.Maisels MJ, et al. Hyperbilirubinemia in the newborn infant ≥ 35 weeks' gestation: An update with clarifications. Pediatrics. 2009;124:1193.American Academy of Pediatrics Subcommittee on Hyperbilirubinemia. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. Pediatrics. 2004;114:297.Subcommittee on Hyperbilirubinemia. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. 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By clicking "Subscribe Now", I agree to the MedicineNet Terms and Conditions and Privacy Policy. I also agree to receive emails from MedicineNet and I understand that I may opt out of MedicineNet subscriptions at any time. Red pigment of the bile "Billy Rubin" redirects here. For the American art scholar, see William Rubin. Bilirubin Names IUPAC name 3,3′-(2,17-Diethenyl-3,7,13,18-tetramethyl-1,19-dioxo-10,19,21,22,23,24-hexahydro-1H-biline-8,12-diyl)dipropanoic acid Systematic IUPAC name 3,3′-([12(2)Z,6(72)Z]-13,74-Diethenyl-14,33,54,73-tetramethyl-15,75-dioxo-11,15,71,75-tetrahydro-31H,51H-1,7(2),3,5(2,5)-tetrapyrrolaheptaphane-12(2),6(72)-diene-34,53-diyl)dipropanoic acid Other names Bilirubin IXα Identifiers CAS Number 635-65-4 Y 3D model (JSmol) Interactive imageInteractive image ChEBI CHEBI:16990 Y ChEMBL ChEMBL501680 Y ChemSpider 4444055 Y ECHA InfoCard 100.010.218 IUPHAR/BPS 4577 PubChem CID 5280352 UNII RFM9X3LJ49 Y CompTox Dashboard (EPA) DTXSID8060905 DTXSID90239827, DTXSID8060905 InChI InChI=1S/C33H36N4O6/c1-7-20-19(6)32(42)37-27(20)14-25-18(5)23(10-12-31(40)41)29(35-25)15-28-22(9-11-30(38)39)17(4)24(34-28)13-26-16(3)21(8-2)33(43)36-26/h7-8,13-14,34-35H,1-2,9-12,15H2,3-6H3,(H,36,43)(H,37,42)(H,38,39)(H,40,41)/b26-13-,27-14- YKey: BPYKTIZUTYGOLE-IFADSCNNSA-N YKey: BPYKTIZUTYGOLE-IFADSCNNBS SMILES CC1=C(/C=C2C(C)=C(C=C)C(N/2)=O)NC(CC3=C(CCC(O)=O)C(C)=C(/C=C4C(C=C)=C(C)C(N/4)=O)N3)=C1CCC(O)=OCc1c(c([nH]c1/C=C\2/C(=C(C(=O)N2)C=C)C)Cc3c(c(c([nH]3)/C=C4/C(=C(C(=O)N4)C)C=C)C)CCC(=O)O)CCC(=O)O Properties Chemical formula C33H36N4O6 Molar mass 584.673 g·mol−1 Density 1.31 g·cm-3[1] Melting point 235°C[2] Except where otherwise noted, data are given for materials in their standard state (at 25 °C [77 °F], 100 kPa). Y verify (what is YN ?) Infobox references Chemical compound Bilirubin (BR) (adopted from German, originally bili—bile—plus ruber—red—from Latin) is a red-orange compound that occurs in the normcomponent of the straw-yellow color in urine.[3] Another breakdown product, stercobilin, causes the brown color of feces. Although bilirubin is usually found in animals rather than plants, at least one plant species, Strelitzia nicolai, is known to contain the pigment.[4] Bilirubin consists of an open-chain tetrapyrrole. It is formed by oxidative cleavage of a porphyrin in heme, which affords biliverdin. Biliverdin is reduced to bilirubin. After conjugation with glucuronic acid, bilirubin is water-soluble and can be excreted.[5] Bilirubin is structurally similar to the pigment phycobilin used by certain algae to capture light energy, and to the pigment phytochrome used by plants to sense light. All of these contain an open chain of four pyrrolic rings.[6] Like these other pigments, some of the double-bonds in bilirubin isomerize when exposed to light. This isomerization is relevant to the phototherapy of jaundiced newborns: the E,Z-isomers of bilirubin formed upon light exposure are more soluble than the unilluminated Z,Z-isomer, as the possibility of intramolecular hydrogen bonding is removed.[7] Increased solubility allows the excretion of unconjugated bilirubin in bile.[citation needed] Some textbooks and research articles show the incorrect geometric isomer of bilirubin.[8] The naturally occurring isomer is the Z,Z-isomer. Bilirubin is created by the activity of biliverdin reductase on biliverdin, a green tetrapyrrolic bile pigment that is also a product of heme catabolism. Bilirubin, when oxidized, reverts to become biliverdin once again. This cycle, in addition to the demonstration of the potent antioxidant activity of bilirubin,[9] has led to the hypothesis that bilirubin's main physiologic role is as a cellular antioxidant.[10][11] Consistent with this, animal studies suggest that eliminating bilirubin results in endogenous oxidative stress.[12] Bilirubin's antioxidant activity may be particularly important in the brain, where it prevents excitotoxicity and neuronal death by scavenging superoxide during N-methyl-D-aspartic acid neurotransmission.[13] Heme metabolism Heme and bilirubin metabolism Bilirubin in plasma is mostly produced by the destruction of erythrocytes. Heme is metabolized into biliverdin (via heme oxygenase) and then into bilirubin (via biliverdin reductase) inside the macrophages.[5] Bilirubin is then released into the plasma and transported to the liver bound by albumin, since it is insoluble in water in this state. In this state, bilirubin is called unconjugated (despite being bound by albumin). [5] In the liver, unconjugated bilirubin is up-taken by the hepatocytes and subsequently conjugated with glucuronic acid (via the enzyme uridine diphosphate–glucuronyl transferase). In this state, bilirubin is soluble in water and it is called conjugated bilirubin. [5] Conjugated bilirubin is excreted into the bile ducts and enters the duodenum. During its transport to the colon, it is converted into urobilinogen by the bacterial enzyme bilirubin reductase.[14] Most of the urobilinogen is further reduced into stercobilinogen and is excreted through feces (air oxidizes stercobilinogen to stercobilin, which gives feces their characteristic brown color). [5] A lesser amount of urobilinogen is re-absorbed into portal circulation and transferred to the liver. For the most part, this urobilinogen is recycled to conjugated bilirubin and this process closes the enterohepatic circle. There is also an amount of urobilinogen which is not recycled, but rather enters the systemic circulation and subsequently the kidneys, where it is excreted. Air oxidizes urobilinogen into urobilin, which gives urine its characteristic color.[5][15] In parallel, a small amount of conjugated billirubin can also enter the systemic circulation and get excreted through urine. This is exaggerated in various pathological situations.[15] Hyperbilirubinemia is a higher-than-normal level of bilirubin in the blood. Hyperbilirubinemia may refer to increased levels of conjugated, unconjugated or both conjugated and unconjugated bilirubin. The causes of hyperbilirubinemia can also be classified into prehepatic, intrahepatic, and posthepatic. [16] Prehepatic causes are associated mostly with an increase of unconjugated (indirect) bilirubin.[16] They include: Hemolysis or increased breakdown of red blood cells (for example hematoma resorption) Intrahepatic causes can be associated with elevated levels of conjugated bilirubin, unconjugated bilirubin or both.[16] They include:[16] Neonatal hyperbilirubinemia, where the newborn's liver is not able to properly process the bilirubin causing jaundice Hepatocellular disease Viral infections (hepatitis A, B, and C) Chronic alcohol use Autoimmune disorders Genetic syndromes: Gilbert's syndrome – a genetic disorder of bilirubin metabolism that can result in mild jaundice, found in about 5% of the population Rotor syndrome: non-itching jaundice, with rise of bilirubin in the patient's serum, mainly of the conjugated type Dubin–Johnson syndrome Crigler–Najjar syndrome Pharmaceutical drugs (especially antipsychotic, some sex hormones, and a wide range of other drugs) Sulfonamides are contraindicated in infants less than 2 months old (exception when used with pyrimethamine in treating toxoplasmosis) as they increase unconjugated bilirubin leading to kernicterus.[17] Drugs such as protease inhibitors like Indinavir can also cause disorders of bilirubin metabolism by competitively inhibiting the UGT1A1 enzyme.[18] Post-hepatic causes are associated with elevated levels of conjugated bilirubin. [16] These include:[16] Unusually large bile duct obstruction, e.g. gallstone in common bile duct (which is the most common post-hepatic cause) Biliary stricture (benign or malignant) Cholangitis Severe liver failure with cirrhosis (e.g. primary biliary cirrhosis) Pancreatitis Cirrhosis may cause normal, moderately high or high levels of bilirubin, depending on exact features of the cirrhosis. To further elucidate the causes of jaundice or increased bilirubin, it is usually simpler to look at other liver function tests (especially the enzymes alanine transaminase, aspartate transaminase, gamma-glutamyl transpeptidase, alkaline phosphatase), blood film examination (hemolysis, etc.) or evidence of infective hepatitis (e.g., hepatitis A, B, C, delta, E, etc.).[citation needed] Main article: Jaundice Hemoglobin acts to transport oxygen which the body receives to all body tissue via blood vessels. Over time, when red blood cells need to be replenished, the hemoglobin is broken down in the spleen; it breaks down into two parts: heme group consisting of iron and bile, and protein fraction. While protein and iron are utilized to renew red blood cells, pigments that make up the red color in blood are deposited into the bile to form bilirubin.[19] Jaundice leads to raised bilirubin levels[20] that in turn negatively remove elastin-rich tissues.[21] Jaundice may be noticeable in the sclera of the eyes at levels of about 2 to 3 mg/dl (34 to 51 μmol/L),[22] and in the skin at higher levels.[note 1] Jaundice is classified, depending upon whether the bilirubin is free or conjugated to glucuronic acid, into conjugated jaundice or unconjugated jaundice.[citation needed] Main article: Kernicterus Unbound bilirubin (Bf) levels can be used to predict the risk of neurodevelopmental handicaps within infants.[23] Unconjugated hyperbilirubinemia in a newborn can lead to accumulation of bilirubin in certain brain regions (particularly the basal nuclei) with consequent irreversible damage to these areas manifesting as various neurological deficits, seizures, abnormal reflexes and eye movements. This type of neurological injury is known as kernicterus. The spectrum of clinical effect is called bilirubin encephalopathy. The neurotoxicity of neonatal hyperbilirubinemia manifests because the blood–brain barrier has yet to develop fully,[dubious – discuss] and bilirubin can freely pass into the brain interstitium, whereas more developed individuals with increased bilirubin in the blood are protected. Aside from specific chronic medical conditions that may lead to hyperbilirubinemia, neonates in general are at increased risk since they lack the intestinal bacteria that facilitate the breakdown and excretion of conjugated bilirubin in the feces (this is largely why the feces of a neonate are paler than those of an adult). Instead the conjugated bilirubin is converted back into the unconjugated form by the enzyme β-glucuronidase (in the gut, this enzyme is located in the brush border of the lining intestinal cells) and a large proportion is reabsorbed through the enterohepatic circulation. In addition, recent studies point towards high total bilirubin levels as a cause for gallstones regardless of gender or age.[24] In the absence of liver disease, high levels of total bilirubin confers various health benefits.[25] Studies have also revealed that levels of serum bilirubin (SBR)[26] are inversely related to risk of certain heart diseases.[27][28] While the poor solubility and potential toxicity of bilirubin limit its potential medicinal applications, current research is being done on whether bilirubin encapsulated silk fibrin nanoparticles can alleviate symptoms of disorders such as acute pancreatitis.[29] In addition to this, there have been recent discoveries linking bilirubin and its ε-polylysine-bilirubin conjugate (PLL-BR), to more efficient insulin medication. It seems that bilirubin exhibits protective properties during the islet transplantation process when drugs are delivered throughout the bloodstream.[30] Bilirubin is degraded by light. Blood collection tubes containing blood or (especially) serum to be used in bilirubin assays should be protected from illumination.[31] For adults, blood is typically collected by needle from a vein in the arm.[32] In newborns, blood is often collected from a heel stick, a technique that uses a small, sharp blade to cut the skin on the infant's heel and collect a few drops of blood into a small tube. Non-invasive technology is available in some health care facilities that will measure bilirubin by using a bilirubinometer which shines light onto the skin and calculates the amount of bilirubin by analysing how the light is absorbed or reflected.[33] This device is also known as a transcutaneous bilirubin meter.[34] Bilirubin (in blood) is found in two forms: Abb. Name(s) Water-soluble Reaction "BC" "Conjugated bilirubin" Yes (bound to glucuronic acid) Reacts quickly when dyes (diazo reagent) are added to the blood specimen to produce azobilirubin "Direct bilirubin" "BU" "Unconjugated bilirubin" No Reacts more slowly, still produces azobilirubin, Ethanol makes all bilirubin react promptly, then: indirect bilirubin = total bilirubin – direct bilirubin Note: Conjugated bilirubin is often incorrectly called "direct bilirubin" and unconjugated bilirubin is incorrectly called "indirect bilirubin". Direct and indirect refer solely to how compounds are measured or detected in solution. Direct bilirubin is any form of bilirubin which is water-soluble and is available in solution to react with assay reagents; direct bilirubin is often made up largely of conjugated bilirubin, but some unconjugated bilirubin (up to 25%) can still be part of the "direct" bilirubin fraction. Likewise, not all conjugated bilirubin is readily available in solution for reaction or detection (for example, if it is hydrogen bonding with itself) and therefore would not be included in the direct bilirubin fraction.[citation needed] Total bilirubin (TBIL) measures both BU and BC. Total bilirubin assays work by using surfactants and accelerators (like caffeine) to bring all of the different bilirubin forms into solution where they can react with assay reagents. Total and direct bilirubin levels can be measured from the blood, but indirect bilirubin is calculated from the total and direct bilirubin.[citation needed] Indirect bilirubin is fat-soluble and direct bilirubin is water-soluble.[35] Total bilirubin = direct bilirubin + indirect bilirubin[36] Elevation of both alanine aminotransferase (ALT) and bilirubin is more indicative of serious liver injury than is elevation in ALT alone, as postulated in Hy's law that elucidates the relation between the lab test results and drug-induced liver injury[37] The measurement of unconjugated bilirubin (UCB) is underestimated by measurement of indirect bilirubin, as unconjugated bilirubin (without/yet glucuronidation) reacts with diazosulfanilic acid to create azobilirubin which is measured as direct bilirubin.[38][39] Direct bilirubin = Conjugated bilirubin + delta bilirubin[36] In the liver, bilirubin is conjugated with glucuronic acid by the enzyme glucuronyltransferase, first to bilirubin glucuronide and then to bilirubin diglucuronide, making it soluble in water: the conjugated version is the main form of bilirubin present in the "direct" bilirubin fraction. Much of it goes into the bile and thus out into the small intestine. Though most bile acid is reabsorbed in the terminal ileum to participate in enterohepatic circulation, conjugated bilirubin is not absorbed and instead passes into the colon.[40] There, colonic bacteria deconjugate and metabolize the bilirubin into colorless urobilinogen, which can be oxidized to form urobilin and stercobilin. Urobilin is excreted by the kidneys to give urine its yellow color and stercobilin is excreted in the feces giving stool its characteristic brown color. A trace (~1%) of the urobilinogen is reabsorbed into the enterohepatic circulation to be re-excreted in the bile.[41] Conjugated bilirubin's half-life is shorter than delta bilirubin.[42] Although the terms direct and indirect bilirubin are sometimes used interchangeably with conjugated and unconjugated bilirubin, the direct fraction actually includes both conjugated bilirubin and delta bilirubin.[43] Delta bilirubin is albumin-bound conjugated bilirubin.[36] In the other words, delta bilirubin is the kind of bilirubin covalently bound to albumin, which appears in the serum when hepatic excretion of conjugated bilirubin is impaired in patients with hepatobiliary disease.[44] Furthermore, direct bilirubin tends to overestimate conjugated bilirubin levels due to unconjugated bilirubin that has reacted with diazosulfanilic acid, leading to increased azobilirubin levels (and increased direct bilirubin).[citation needed] δ bilirubin = total bilirubin – (unconjugated bilirubin + conjugated bilirubin)[36] The half-life of delta bilirubin is equivalent to that of albumin since the former is bound to the latter, yields 2–3 weeks.[45][38] A free-of-bound bilirubin has a half-life of 2 to 4 hours.[45] Further information: Bilirubin glucuronide Originally, the Van den Bergh reaction was used for a qualitative estimate of bilirubin.[citation needed] This test is performed routinely in most medical laboratories and can be measured by a variety of methods.[46] Total bilirubin is now often measured by the 2,5-dichlorophenyldiazonium (DPD) method, and direct bilirubin is often measured by the method of Jendrassik and Grof.[47] The bilirubin level found in the body reflects the balance between production and excretion. Blood test results are advised to always be interpreted using the reference range provided by the laboratory that performed the test. The SI units are μmol/L.[48] Typical ranges for adults are:[49] 0–0.3 mg/dl – Direct (conjugated) bilirubin level 0.1–1.2 mg/dl – Total serum bilirubin level μmol/l = micromole/litre mg/dl = milligram/ decilitre total bilirubin