DIRECT BILIRUBIN

Diagnostic reagent for determination of Direct Bilirubin concentration.
Liquid. Dual reagents. Store at +2/+8°C. For in Vitro Diagnostic Use (IVD). **Do not freeze.**

Ref No	Package	Ref No	Package	Ref No	Package	Ref No	Package
A2050N	500 mL	D2050	788 mL	L2052	200 mL	PL2050	150 mL
A2051N	250 mL	D2051	375 mL	L2053	200 mL	RD2050	300 mL
A2052N	125 mL	HN050	675 mL	MD050	250 mL	RD2051	150 mL
A2053N	200 mL	HN051	300 mL	M2051	350 mL	S2052	125 mL
BB035	200 mL	K2051	300 mL	M2052	350 mL	TB2050	250 mL
BY2050	675 mL	LB050	300 mL	M3050	250 mL	TB2051	150 mL
BY2051	450 mL	LM50	150 mL	M3051	200 mL	T2050	660 mL
BZ2035	375 mL	LM51	300 mL	M3052	75 mL	T2051	350 mL
DM2050	277,5 mL	L2050	675 mL	M4051	350 mL	8A2050	675 mL
		L2051	300 mL	M4052	490 mL	8A2051	450 mL

Changes made in the instructions for use are marked as grey.

INTENDED USE

The test is applied for the quantitative determination of direct bilirubin in serum and plasma.

GENERAL INFORMATION

Bilirubin was discovered by Virchow in 1849; he called this yellow pigment "hematidine". The term bilirubin was coined by Stadeler in 1864 and in 1874 Tarchanoff showed the direct relationship of bile pigments to Hb. Bilirubin is an orange-yellow pigment derived mainly from heme, a product of the red blood cell (RBC) cycle.[1] Important chemical properties of the bilirubin molecule are its insolubility in water and solubility in various non-polar solvents. Bilirubin from natural sources is almost entirely (99%) composed of the IXα isomer. The bilirubins IXβ and IXδ, resulting from cleavage of β- and δ-methene bridges, constitute less than 0.5% of bilirubin isolated from bile. Approximately 85% of the total bilirubin produced is derived from the heme moiety of Hb released from senescent erythrocytes that are destroyed in the reticuloendothelial cells of the liver, spleen, and bone marrow. The remaining 15% is produced from RBC precursors destroyed in the bone marrow (so-called ineffective erythropoiesis) and from catabolism of other heme-containing proteins such as myoglobin, cytochromes and peroxidases.[1]

Unconjugated (indirect) bilirubin, formed in reticuloendothelial cells as a result of heme catabolism, is transported to the liver by the carrier molecule albumin, where it dissociates from albumin and enters hepatocytes by facilitated diffusion; and binds to intracellular proteins, particularly the ligandin protein. Here the solubility of bilirubin is increased by the addition of two molecules of glucuronic acid.[2] "Uridine diphosphate (UDP)-glucuronyltransferase" is the enzyme that catalyzes the reaction. The reaction results in the formation of bilirubin diglucoronide (conjugated=direct bilirubin) which returns to the cytosol, probably via a transporter, where it binds to its ligand and diffuses to the canalicular pole for secretion into bile or to the sinusoidal pole for secretion back into plasma.[1]

Direct bilirubin is hydrolyzed by bacteria in the intestine to form urobilinogen, a colorless compound. Most urobilinogen is oxidized by intestinal bacteria to sterkobilin, which gives the stool its characteristic brown color. However, some of the urobilinogen is reabsorbed from the intestine and enters the portal blood. Part of this urobilinogen participates in the enterohepatic urobilinogen cycle, where it is taken up by the liver and then re-secreted into bile. The rest of the urobilinogen is transported through the blood to the kidney, where it is converted to urobilin, which gives urine its characteristic yellow color, and excreted in the urine.[3]

In the type of jaundice generally defined as post-hepatic jaundice, direct bilirubin level increases. In this type of jaundice, there is a disorder in the biliary part of the hepatobiliary system. The most important cause of post-hepatic jaundice is extrahepatic biliary obstruction.[4] Therefore, it is also known as obstructive jaundice.[5] The causes of obstruction are of two types: congenital and acquired. Biliary atresia, cystic fibrosis, idiopathic dilatation of bile ducts, pancreatic biliary dysfunction and choledochal duct cyst are congenital causes of post-hepatic jaundice.[5,6] Portal biliopathy, cholecystitis, trauma, pancreatitis, pancreatitis, citricures, choledocholithiasis, AIDS, intra-abdominal tuberculosis, tumors and bile duct obstructions are examples of the causes of acquired post-hepatic jaundice.[6-13] Clinical manifestations of obstructive jaundice include dark urine, pale stools and generalized pruritus. A history of febrile biliary colic, weight loss, abdominal pain and abdominal mass are also signs of obstructive jaundice.[6] Obstructive jaundice may lead to various complications including cholangitis, pancreatitis, renal and hepatic failure.[4] Increased direct bilirubin is rarely seen in congenital defects in bilirubin excretion and in impaired bilirubin excretion occurring in sepsis or other acute diseases.[1] Dubin-Johnson (DJS) and Rotor syndrome are examples of posthepatic jaundice due to congenital defects causing direct bilirubin elevation.

DJS is characterized by chronic, predominantly conjugated non-hemolytic hyperbilirubinemia and its phenotype is similar to Rotor syndrome. Unlike Rotor, biliary excretion of organic anions other than bile acids is also impaired in DJS.[14]

TEST PRINCIPLE

Colorimetric diazo method

Direct bilirubin in the sample to be measured reacts with diazotized 2,4-dichloroaniline in the reagent to form azobilirubin, a diazo molecule with an intense red color in acidic medium. This color is measured photometrically by absorbance reading at a wavelength of 546 nm (520-560 nm) and is directly proportional to the concentration of direct bilirubin in the sample.

REAGENT COMPONENTS

Reaktif 1

Sodium chloride	: ≤ 0.01 M
EDTA	: ≤ 0.30 M

Reaktif 2

Diazotized 2,4-dichloroaniline	: ≤ 0.12 M
Hydrochloric acid	: ≤ 0.22 M
EDTA	: ≤ 0.01 M

REAGENT PREPARATION

Reagent is ready for use.

REAGENT STABILITY AND STORAGE

Reagents are stable at +2/+8°C till the expiration date stated on the label which is only for closed vials.

Once opened vials are stable for 30 days at +2/+8°C in optimum conditions. On board stability is strongly related to auto analyzers' cooling specification and carry-over values.

Reagent stability and storage data have been verified by using Clinical and Laboratory Standards Institute (CLSI) EP25-A protocol.[15]

SAMPLE REQUIREMENTS

Serum and plasma can be used and are collected according to the standard procedures. For plasma, sample collection tubes with Li heparin, Na heparin, K2-EDTA or K3-EDTA must be preferred. Contact with light must be avoided. Non-lipemic samples must be used. Multiple sample freezing and thawing should be avoided.

Direct bilirubin activity stability in serum and plasma[30,31]:

2 days at +20/+25 ºC
7 days at +2/+8ºC
3 months at -20°C

Annotation:

- Significant reductions in serum bilirubin concentrations have been reported in serum samples not protected from light, with more noticeable changes in samples with normal or low bilirubin concentrations.[16,17]

Unit Conversion:

mg/dL x 17.1 = µmol/L

CALIBRATION AND QUALITY CONTROL

Calibration: The assay requires the use of an Arcal Auto Calibrator.

Arcal Auto Calibrator-Lyophilized
Ref.No: A39052
Ref.No: A39054
Ref.No: A39055 (For Olympus AU series.)

Calibration stability is 30 days.Calibration stability depends on the application characteristics and cooling capacity of the autoanalyzer used.

Control: Commercially available control material with established values determined by this method can be used. We recommend:

Arcon N Level 1 Control- Lyophilized
Ref.No: A3910
Ref.No: A3912 (For Olympus AU series.)
Ref.No: A3913 (For BS series.)
Ref.No: A3914 (For Erba.)

Arcon P Level 2 Control- Lyophilized
Ref.No: A3920
Ref.No: A3922 (For Olympus AU series.)
Ref.No: A3923 (For BS series.)
Ref.No: A3924 (For Erba.)

At least two level controls must be run once in every 24 hours. Each laboratory should determine its own quality control scheme and procedures. If quality control results are not within acceptable limits, calibration is required.

REFERENCE INTERVALS / MEDICAL DECISION LEVELS

Adults : ≤ 0.40 mg/dL

Annotation:

- Reference ranges for serum bilirubin concentrations vary among a variety of different populations, being lowest in African Americans.[16,18]

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference range.

Reference interval has been verified by using CLSI EP28-A3c protocol.[19]

PERFORMANCE CHARACTERISTICS

Measuring Interval

According to CLSI EP34-ED1:2018, "Measuring Interval" refers to the interval where the analyte concentration is measured with intended accuracy in terms of medical and laboratory requirements without dilution, concentrating or any kind of pre-treatment that is between the analyte's lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ).[20]

The determined analytic measuring interval for Bilirubin Direct is 0.09 – 13 mg/dL.

Detection Capability

Limit of Detection (LoD): 0.04 mg/dL

Limit of Quantitation (LoQ): 0.09 mg/dL

Note: LoQ values are based on Coefficient of Variation Percentage (CV) ≤ 20%.

LoD and LoQ values have been verified by using CLSI EP17-A2:2012 protocol.[21]

Linearity

This method shows measurement linearity in the activities up to 13 mg/dL. Autoanaylzer's auto-dilution system can be used if the concentrations have higher values. See device manual for further information.

For the manual dilution procedure, dilute the sample 1:5 using 0.90% isotonic. After this process, multiply the result of the reworked sample by the dilution factor. Do not report the sample result after dilution if it is marked as lower than the linear lower limit. Rerun with a suitable dilution.

Linearity Studies data have been verified by using CLSI EP06-A:2003 protocol.[22]

Precision

Running system has been developed according to 20x2x2 "The Single Site" protocol. Repeatability and Within-Laboratory Precision/Within-Device values have been obtained according to the running results.

According to the protocol in use, 2 separate runs per day have been made for 20 days (no obligation for being consecutive days). This protocol has been applied to each low and high samples separately and 80 results have been obtained for each one. Statistically, the results have been obtained using 2-factor Nested-ANOVA model.[23]

Repeatability (Within Run) and Repeatability (Day to Day) SD (standard deviation) and CV% values of Direct Bilirubin have been given in the table 1 and 2 respectively.

Table 1. Direct Bilirubin Repeatability (Within Run) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
0.44 mg/dL	0.01	1.84	80
4.47 mg/dL	0.02	0.49	80

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.[24]

Table 2. Direct Bilirubin Repeatability (Day to Day) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
0.44 mg/dL	0.01	2.40	80
4.47 mg/dL	0.16	3.60	80

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.[24]

Method Comparison

As a result of the statistical evaluation of the method comparison data:

Passing-Bablok equation:[25]
y= 0.92x + 0.05 mg/dL
r= 0.998

Interference

Endogenous interferant and analyte concentrations that have been used in the Direct Bilirubin scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[26,27]

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from Direct Bilirubin interference scanning test is appropriate, is determined as ±10%.[28]

In Direct Bilirubin test results, no significant interaction has been observed in the determined endogenous interferant and analyte concentrations or between interferants and analyte.

Interferant-Concentration	Direct Bilirubin Target (mg/dL)	N*	Observed Recovery %
Hemoglobin 180 mg/dL	0,23	3	100

** Total acceptable error rate determined as interference limit and repeatability (within run) pre-detected for the related method were used for the calculations of how many times the control and test samples prepared as a serum pool are going to be run repetitively. In the calculations, the accepted error rate for type 1 (α error) was 5% and for type 2 (β error) was 10% (90% power).*[27]

It should be noted that endogenous interferants, as well as various medicines and metabolites, anticoagulants (e.g. Heparin, EDTA, citrate, oxalate) and preservatives (e.g. sodium floride, iodoacetate, hydrochloride acide) such as additives, materials that may contact with samples during collection and processing (serum separator devices, sample collection containers and contents, catheters, catheter wash solutions, skin disinfectants, hand cleaners and lotions, glass washing detergents, powder gloves), dietary substances known to affect some specific tests (caffeine, beta-carotene, poppy seeds, etc.), or some substances present in a sample that cause foreign proteins (heterophilic antibodies, etc.), autoimmune response (autoantibodies, etc.), or due to malignancy (for example, interference by paraproteins with phosphate testing and indirect ion selective electrode methods) may show some negative effects that will cause various attempts and some misjudgements.[27]

Annotation:

- Non- lipemic samples should be used.
- Some drugs, such as naproxen metabolites, may interfere with the diazo method and cause erroneous measurements.[29]

These performance characteristics have been obtained using an autoanalyzer. Results may vary slightly when using different equipment or manual procedures.

WARNINGS AND PRECAUTIONS

IVD: For in Vitro Diagnostic use only.
Do not use expired reagents.
Reagents with two different lot numbers should not be interchanged.
For professional use.
Follow Good Laboratory Practice (GLP) guidelines.

CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA (Occupational Safety and Health Administration) standards.

Danger

EUH032	:Releases a very toxic gas if contacts with acid.
H317	:May cause allergic skin reaction.

Precaution

P280	:Use protective gloves / clothes / glasses / mask.
P264	:Wash your hands properly after using.
P272	:Contaminated work clothes should not be allowed to be used outside of the workplace.

Intervention

P302+P352	:Wash with plenty of water and soap if it contacts with skin.
P333+P313	:Seek medical help if it irritates your skin or develops rash.
P362+P364	:Remove contaminated clothes and wash properly before using.

Disposal

P501	:Dispose the vials and contents according to the local regulations.

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SYMBOLS

IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
R1	Reagent 1
R2	Reagent 2
GTIN	Global Trade Item Number
REF	Reference Number
GLP	Good Laboratory Practices
FOR USE WITH	Identifies Products to Be Used Together
PRODUCT OF TURKEY	Product of Turkey
	Manufacturer
	Expiration Date
	Temperature Limits
	Consult Instructions for Use
	Caution
	Number of Tests