

Direct Bilirubin Reagent

Acid Diazo Method

PRODUCT SUMMARY

Stability	:	Until Expiry at 2-25°C
Linear Range	:	Up to 340 µmol/L (20 mg/dL)
Specimen Type	:	Serum
Method	:	Endpoint
Reagent Preparation	:	Supplied ready to use.

IVD

INTENDED USE

This reagent is intended for the in vitro quantitative determination of direct bilirubin in human serum.

CLINICAL SIGNIFICANCE

Red blood cells at the end of their circulating life are broken down in the reticulo-endothelial system, mainly the spleen. The resulting haem, once the iron is removed, is then converted to bilirubin. This process accounts for about 80 percent of the 500 µmol (300 mg) of bilirubin formed daily. Other sources of bilirubin include the breakdown of myoglobin and cytochromes and the catabolism of immature red blood cells in the bone marrow.

Once formed, bilirubin is transported to the liver bound to albumin as it is water insoluble. This fraction of bilirubin is referred to as indirect or unconjugated bilirubin. In the liver bilirubin is conjugated to glucuronic acid (mono and di glucuronides) to form conjugated bilirubin by the enzyme uridyl diphosphate glucuronyl transferase. Conjugated bilirubin or direct bilirubin is excreted via the biliary system into the intestine where it is metabolised by bacteria to a group of products known collectively as stercobilinogen. Elimination of it is almost complete and plasma levels are normally negligible.

Total Bilirubin is the sum of the unconjugated and conjugated fractions. Total bilirubin is elevated in conditions causing obstruction of the bile duct, hepatitis, cirrhosis, in haemolytic disorders and several inherited enzyme deficiencies.

Indirect bilirubin is elevated by pre-hepatic causes such as haemolytic disorders or liver diseases resulting in impaired entry, transport or conjugation within the liver.

Monitoring of bilirubin in the newborn, particularly if premature is of particular importance. Since the hepatic handling of bilirubin in such cases is often immature, jaundice due to a rise in unconjugated bilirubin is common. Unconjugated bilirubin if not bound to albumin is able to cross the blood brain barrier more easily, increasing the danger of cerebral damage.¹

METHODOLOGY

Most methods currently used for assaying bilirubin are based on the reaction between bilirubin and diazotised sulphanilic acid solutions. In aqueous solution only the direct (conjugated) bilirubin will react in this manner.

This Direct Bilirubin reagent utilises an acid diazo method. Conjugated bilirubin reacts with diazotised sulphanilic acid to produce an acid azobilirubin, the absorbance of which is proportional to the concentration of direct bilirubin in the sample and can be measured at 550 nm. For bichromatic analyzers the blank reading should be taken at 660 nm.

REAGENT COMPOSITION

Active Ingredients	Concentration
Direct Bilirubin Reagent:	
Hydrochloric Acid	100 mmol/L
Sulphanilic acid	10 mmol/L
Sodium Nitrite Reagent:	
Sodium Nitrite	144 mmol/L

WARNING: DO NOT mouth pipette. If spilt, thoroughly wash affected areas with water. For further information consult the Direct Bilirubin Reagent Material Safety Data Sheet.

Direct Bilirubin Reagent: Irritant!

R38	Irritating to skin.
R41	Risk of serious damage to eyes.
S24/25	Avoid contact with skin and eyes.
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

Sodium Nitrite Reagent: Harmful!

R22	Harmful if swallowed.
S45	In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorized Representative	Temperature Limitation
IVD	For in vitro diagnostic use	Use by/Expiration Date
LOT	Batch code/Lot number	CAUTION. CONSULT INSTRUCTIONS FOR USE.
REF	Catalogue number	Manufactured by
Consult instructions for use		
Xi - Irritant		Xn - Harmful
REAG	Direct Bilirubin Reagent	NIT Sodium Nitrite Reagent

REAGENT PREPARATION

Test Reagent:

Add Sodium Nitrite Reagent to Direct Bilirubin Reagent in the ratio of 1:100. For example, to 10 mL of Direct Bilirubin Reagent add 0.1 mL of Sodium Nitrite (This is approximately equal to 3 drops but it is recommended that this be verified by the operator).

Blank Reagent:

Use Direct Bilirubin Reagent as supplied.

STABILITY AND STORAGE

When stored at 2-25°C the reagent is stable until the expiry date stated on the bottle and kit box label.

Working Reagent:

Working reagent is stable for at least 21 days at 2-8°C.

Indications of Reagent Deterioration:

- Turbidity;
- Reagent absorbance > 0.1 AU at 550 nm; and/or
- Failure to recover control values within the assigned range.

SPECIMEN COLLECTION AND HANDLING

Serum: Use non-haemolysed serum.

Storage: Specimens should be protected from bright light as bilirubin is photolabile. Specimens may be stored refrigerated for 3 days.²

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

- If required, pipettes for accurately dispensing measured volumes.
- A clinical chemistry analyzer capable of maintaining constant temperature (30/37°C) and measuring absorbance at 550 nm.
- Analyzer specific consumables; e.g. sample cups.
- Normal and abnormal control material.
- Calibrator or a suitable aqueous Direct Bilirubin Standard.

ASSAY PROCEDURE

It is recommended that a sample blank be performed on each sample. A sample blank can be obtained by simply using Direct Bilirubin Reagent as supplied without the addition of Sodium Nitrite.

The following system parameters are recommended. Individual instrument applications are available upon request from the Technical Support Group.

SYSTEM PARAMETERS

Temperature	30/37°C
Primary Wavelength	550 nm
Secondary Wavelength	660 nm
Assay Type	Endpoint
Direction	Increase
Sample : Reagent Ratio	1 : 20
e.g. Sample Vol	10 µL
Reagent Vol	200 µL
Incubation Time	300 seconds
Reagent Blank Limits	Low 0.0 AU
(550 nm, 1 cm lightpath)	High 0.1 AU
Linearity	340 µmol/L (20 mg/dL)
Sensitivity	2.5 ΔmAbs per µmol/L
(550 nm, 1 cm lightpath)	(43 ΔmAbs per mg/dL)

CALCULATIONS

Results are calculated, usually automatically by the instrument, as follows:-

$$\text{Direct Bilirubin} = \frac{\text{Absorbance of Unknown}}{\text{Absorbance of Calibrator}} \times \text{Calibrator Value}$$

Example:

Absorbance of calibrator (Test) = 0.038
 Absorbance of calibrator (Blank) = 0.003

Absorbance of unknown (Test) = 0.029
 Absorbance of unknown (Blank) = 0.01
 Value of calibrator = 15 µmol/L (0.88 mg/dL)

Absorbance of calibrator = 0.038 - 0.003 = 0.035
 Absorbance of unknown = 0.029 - 0.01 = 0.019

Direct Bilirubin = $\frac{0.019}{0.035} \times 15 = 8.1 \mu\text{mol/L}$

Direct Bilirubin = $\frac{0.019}{0.035} \times 0.88 = 0.48 \text{ mg/dL}$

NOTES

- Specimens with values greater than 340 µmol/L (20 mg/dL) should be diluted and reassayed. Multiply the results by the dilution factor.
- Unit Conversion: µmol/L x 0.0585 = mg/dL

CALIBRATION

Calibration is required. A suitable aqueous standard or serum based calibrator is recommended.

For Calibration Frequency on automated instruments refer to the instrument manufacturers specifications. However, calibration stability is contingent upon optimum instrument performance and the use of reagents which have been stored as recommended in the stability and storage section of this package insert. Recalibration is recommended at anytime if one of the following events occurs:-

- The lot number of reagent changes.
- Preventative maintenance is performed or a critical component is replaced.
- Control values have shifted or are out of range and a new vial of control does not rectify the problem.

QUALITY CONTROL

To ensure adequate quality control, normal and abnormal controls with assayed values should be run as unknown samples:-

- At least every eight hours.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.

Control results falling outside the upper or lower limits of the established ranges indicate the assay may be out of control.

The following corrective actions are recommended in such situations:-

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results are still out of control, recalibrate with fresh calibrator, then repeat the test.
- If results are still out of control, perform a calibration with fresh reagent, then repeat the test.
- If results are still out of control, contact Technical Services or your local distributor.

LIMITATIONS

- Studies to determine the level of interference from haemoglobin and lipaemia were carried out. The following results were obtained.

Haemoglobin: Avoid the use of haemolysed samples.

Lipaemia: No interference from lipaemia, measured as triglycerides, up to 8.8 mmol/L (780 mg/dL).

- Young DS³ has published a comprehensive list of drugs and substances which may interfere with this assay.

EXPECTED VALUES²

Adults and Infants (>1 month old): 0-3 µmol/L (> 0.2 mg/dL)

The quoted values are representative of the expected range for this method and should serve as a guide only. It is recommended that each laboratory verify this range or derives a reference interval for the population that it serves.⁴

PERFORMANCE DATA

The following data was obtained using the Direct Bilirubin reagent on a well maintained automated clinical chemistry analyzer. Users should establish product performance on their specific analyzer used.

IMPRECISION

Imprecision was evaluated using two levels of commercial control and following the NCCLS EP5-T procedure.⁵

Within run:	LEVEL I	LEVEL II
Number of data points	80	80
Mean (µmol/L / mg/dL)	20.5 / 1.2	35.9 / 2.1
S.D. (µmol/L / mg/dL)	1.9 / 0.11	1.9 / 0.11
C.V. (%)	<1	2.9
Total:	LEVEL I	LEVEL II
Number of data points	80	80
Mean (µmol/L / mg/dL)	20.5 / 1.2	35.9 / 2.1
S.D. (µmol/L / mg/dL)	1.9 / 0.11	1.9 / 0.11
C.V. (%)	8.7	5.2

ACCURACY

Comparison studies were carried out using a similar commercially available Direct Bilirubin reagent as a reference. Serum samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained.

Number of sample pairs	51
Range of sample results	5 - 488 µmol/L (0.3 - 28.6 mg/dL)
Mean of reference results	107 µmol/L (6.3 mg/dL)
Mean of Direct Bilirubin results	111 µmol/L (6.5 mg/dL)
Slope	0.96
Intercept	8.5 µmol/L (0.5 mg/dL)
Correlation coefficient	0.99

LINEARITY


When run as recommended the assay is linear to 340 µmol/L (20 mg/dL).

SENSITIVITY

When run as recommended the sensitivity of this assay is 2.5 ΔmAbs per µmol/L or 43 ΔmAbs per mg/dL (1cm light path, 550nm).

REFERENCES

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- Young DS. Effects of Drugs on Clinical Laboratory Tests, Third Edition. AACC Press, 1990.
- Watchel M et al, Creation and Verification of Reference Intervals. Laboratory Medicine, 1995; 26: 593-7.
- National Committee for Clinical Chemistry Standards. User evaluation of Precision Performance of Clinical Laboratory Devices NCCLS; 1984, NCCLS Publication EP5-T.

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REF	Reorder Information		
	Catalogue No.	REAG	NIT
	TR33321	2 x 125 mL	1 x 10 mL
	TR33326	2 x 250 mL	1 x 10 mL
	TR33398	2 x 500 mL	1 x 20 mL
	1225-200H (Hitachi)	4 x 50 mL	1 x 8 mL
	TL33301 (iLab 600)	6 x 100 mL	1 x 20 mL