MEDTEST DX
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Direct Bilirubin Reagent Set

Intended Use
For the quantitative determination of direct bilirubin in serum using the Mindray BS-480 analyzer. For *in vitro* diagnostic use only.

Method History
Since the introduction of the diazo method for bilirubin determination by Ehrlich in 1883,[1] several modifications have been proposed to enhance the reaction. The Malloy and Evelyn method[2] employs methanol to catalyze the azo-coupling reaction of the indirect Bilirubin, as well as to keep the azobilirubin in solution. A serious disadvantage of this method lies in the fact that protein may be precipitated by the methanol solution to yield falsely lowered results.

In 1938, Jendrassik and Grof.[3] presented an assay that gave reliable results. The method is, however, cumbersome and involves several pipetting steps.

The method presented here was developed by Wahlefeld et al.[4]The diazo reagent is 2,5-dichlorophenyldiazonium tetrafluoroborate (DPD) which reacts very rapidly in coupling with Bilirubin under acidic conditions. The resulting procedure is simple, yet exhibits good correlation when compared with the method of Jendrassik and Grof.

Principle
Direct Bilirubin is coupled with a diazonium salt (DPD) in a strongly acid medium (pH 1 – 2).

$$\text{Bilirubin + DPD} \xrightarrow{\text{acid}} \text{Azobilirubin}$$

The intensity of the color of the azobilirubin produced is proportional to the Direct Bilirubin concentration and can be measured photometrically.

Reagents
Direct Bilirubin R1 reagent: acid buffer 50 mmol/L, Direct Bilirubin R2 reagent: acid buffer >30 mmol/L, >2.0 mmol/L DPD and stabilizers

Precautions and Hazards
1. Reagents are toxic and corrosive. Do not pipette by mouth. Avoid contact with skin and clothing.
2. This reagent is for *in vitro* diagnostic use only.

Hazards:

R1 and R2: Hazard Classifications: Skin Corrosion/Irritation (Category 1), Serious eye damage/eye irritation (Category 1)
Hazard Statements: H314: Causes severe skin burns and eye damage, H318: Causes serious eye irritation
Precautionary Statements: **Prevention:** P260: Do not breathe dust/fume/gas/mist/vapors/spray. P264: Wash skin thoroughly after handling. P280: Wear protective gloves/protective clothing/eye protection/face protection. **Response:** P310: Immediately call a POISON CENTER or doctor/physician. P363: Wash contaminated clothing before reuse. P301+P330+P331: If SWALLOWED: Rinse mouth. Do NOT induce vomiting. P303+P361+P353: IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse SKIN with water/shower. P304+P340: IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. **Storage:** P404: Store in a closed container. **Disposal:** P501: Dispose of contents into sewer system after diluting with large volumes of water, if in accordance with local regulations. **Refer to the Safety Data Sheet for this product (SDS-DIB600) available at www.medtestdx.com.**

Signal Word: Danger

Reagent Preparation
Reagents are supplied ready to use.

Reagent Storage and Stability
1. Packaged reagents may be stored at 2-8°C. The reagent is stable until the expiration date appearing on the label when stored as directed.
2. Manufacturer studies have shown reagent is stable for 30 days once placed in the refrigerated reagent carousel (2-10°C), however reagent stability may vary based on individual laboratory conditions.
3. Do not freeze reagents.
4. Avoid exposure to direct sunlight.

Reagent Deterioration
1. Do not use if reagents show evidence of microbial contamination (turbidity).
2. If the R2 develops very slight precipitation that re-dissolves when the R2 is warmed gently, the reagent may be used.
3. R2 reagent containing a precipitate that does not re-dissolve and results in product discoloration should not be used.
4. Do not use if reagent fails to achieve assigned assay values of fresh control sera.

Specimen Collection and Storage
1. Fresh, unhemolyzed serum is recommended.[5]
2. Samples should be analyzed within two hours of collection if kept at room temperature in the dark and within twelve hours if kept refrigerated (2-8°C) and protected from light.[6]
3. Bilirubin in serum is stable for three months when stored frozen (-20°C) and protected from light.[6]
4. Direct sunlight may cause up to a 50% decrease in bilirubin within one hour.[7]
5. Specimen collection should be carried out in accordance with NCCLS M29-T2. No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood samples should be considered potentially infectious.

Interferences
1. All interference studies were performed according to the procedures recommended in NCCLS guideline No. EP7-P for interference testing in clinical chemistry.[8]
2. Serum hemoglobin levels up to 66 mg/dl do not interfere with results.
3. Serum Triglycerides up to 500 mg/dl do not interfere with results.
4. A number of drugs and substances affect bilirubin results. See Young, et al.[9]

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Materials Provided
Direct bilirubin reagents R1 and R2

Materials Required but not Provided
1. Mindray BS-480 Analyzer.
2. BS-480 Operation manual.
3. Chemistry Calibrator, catalog number CHEC480
4. Chemistry Control, catalog number CHEQ480

Calibration
Use MedTest DX Chemistry Calibrator (Catalog Number CHEC480). Follow instrument application instructions for calibration. Refer to instrument manual instructions for calibration procedures and frequency. It is recommended that each laboratory determine its own frequency of calibration. If control results are found to be out of range, the test may need to be re-calibrated. Under typical operating conditions manufacturer calibration stability studies have shown the calibration curve will be stable for at least 14 days.

Quality Control
The validity of the reaction should be monitored by use of the control sera with known normal and abnormal direct bilirubin values. These controls should be run at least with every working shift in which direct bilirubin assays are performed. It is recommended that each laboratory establish its own frequency of control determination. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

Expected Values (Direct)[7,11]
Adults and infants (over one month): 0 – 0.5 mg/dl
It is strongly recommended that each laboratory establish its own normal range.

Limitations
1. Samples with values above 10 mg/dl must be diluted 1:1 with isotonic saline, re-assayed and the final answer multiplied by two.
2. Serum hemoglobin levels of up to 66 mg/dl and triglyceride to 500 mg/dl do not interfere with results.

Performance
1. Assay Range: 0.0-10.0 mg/dl
2. Comparison: A study was performed between the Mindray BS-480 and a similar analyzer using this method, resulting in the following:

Method	Direct Bilirubin
N	135
Mean Direct Bilirubin (mg/dL)	1.625
Range (mg/dL)	0.00-9.20
Standard Deviation	2.472
Regression Analysis	y = 1.097x – 0.065
Correlation Coefficient	0.9912

3. Precision: Precision studies were performed using the Mindray BS-480 analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.[10]

Within Day

Sample	LOW	MID	HIGH
N	20	20	20
Mean	0.74	5.08	9.80
Standard Deviation	0.05	0.04	0.00
Coefficient of Variation (%)	6.8%	0.8%	0.0%

Total

Sample	LOW	MID	HIGH
N	40	40	40
Mean	0.77	5.08	9.79
Standard Deviation	0.04	0.18	0.26
Coefficient of Variation (%)	6.6%	4.8%	2.7%

4. Sensitivity: 2SD Limit of Detection (95% Conf) = 0.0 mg/dL

References

1. Ehrlich, P., Charite Ann. 8:140(1883).
2. Malloy, H.T., Evelyn, K.A., J. Biol. Chem. 119:481 (1937).
3. Jendrassik, L., Grof, P., Biochem. Zeitschr. 297:81 (1938).
4. Wahlefeld AW, et al. Scand J Clin Lab Invest. 29 Supplement 126(1972).
5. Michaelsson, M. Scand. J. Clin. Lab. Invest (Suppl. 49) 13:1 (1961)
6. Martinek, R.G., Clin. Chem. Acta 13:161 (1966).
7. Tietz, N.W. Fundamentals of Clinical Chemistry, Philadelphia, W.B. Saunders, P. 1028 (1976).
8. NCCLS document, "National Evaluation Protocols for Interference Testing", Evaluation Protocol Number 7, Vol. 4, No. 8, (June 1984).
9. Young, D.S., Effects of Preanalytical Variables on Clinical Laboratory Tests, Washington DC, AACC Press, (1997)
10. NCCLS document, "Evaluations of Precision Performance of Clinical Chemistry Devices", 2nd Ed. (1992)
11. Gambino, S.R., et al, Bilirubin Assay (Revised), Commission on Continuing Education, Am. Soc. of Clin. Path., Chicago, (1968).

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CHEMISTRY PARAMETERS

Chem:	DBIL	No.:	205	Sample Type:	Serum
Chemistry:	Direct Bilirubin			Print Name:	DBIL
Reaction Type:	End Point			Reaction Direction:	Positive
Pri Wave:	546			Sec Wave:	660
Unit:	mg/dL			Decimal	0.1
Blank Time:	47	49		Reaction Time:	80 82

	Sample Vol.	Aspirated	Diluent	Reagent Vol.	Diluent
Standard:	2.7 ul	--- ul	--- ul	R1: 120 ul	--- ul
Decreased:	--- ul	--- ul	--- ul	R2: 31 ul	-- ul
Increased:	--- ul	--- ul	--- ul	R3: --- ul	-- ul
	☐ Sample Blank	☑ Auto Rerun		R4: --- ul	--- ul

Slope/Offset Adjustment

Slope: 1 Offset: 0

Linearity Range (Standard)	0	10		Linearity Limit:
Linearity Range (Decreased)	____	____		Substrate Depletion:
Linearity Range (Increased)	____	____		Mixed Blank Abs:
R1 Blank Abs:	____	____		Uncapping Time
Blank Response:	____	____		Reagent Alarm Limit:
Twin Chemistry:				☐ Enzyme Linear Extension

☐ Prozone Check ○ Rate Check ○ Antigen Addition

Q1: Q2: Q3: Q4:

PC: ABS:

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CALIBRATION PARAMETERS

Calibrator Definition

Calibrator:	*	Lot No.:	*
Exp Date:	*		

Carousel	**Pos**
Sample Carousel 1	*
Sample Carousel 2	
Sample Carousel 3	

Reagent/Calibration

Calibrator	Pos	Lot No	Exp Date	Chem	Conc	Unit
Water	W	*	*	DBIL	0	mg/dL
Chemistry Calibrator	*	*	*	DBIL	*	mg/dL

Calibration Setup

Chem: DBIL

Calibration Settings

Math Model:	Two-Point Linear		
Factor:		Replicates:	2

Acceptance Limits

Cal Time:	*	Hour	
Slope Diff:	---	SD:	---
Sensitivity :	---	Repeatability:	---
Deter Coeff:	---		

Auto Calib.

☐ Bottle Changed ☐ Lot Changed ☐ Cal Time

It is recommended that two levels of control material be assayed daily. * Indicates user defined parameter.

REF DIB480

Manufactured for MedTest DX
5449 Research Drive Canton, MI 48188

2°C 8°C

IVD

Symbol Key

Use by (YYYY-MM-DD)	LOT Lot and batch code	REF Catalog number	Manufacturer
Temperature limitation	Consult instructions for use	IVD *In vitro* diagnostic medical device	

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