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Direct Bilirubin Reagent Set

Intended Use
For the quantitative determination of direct bilirubin in serum using the Mindray BS-200 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).

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

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

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

Precautions
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.

Reagent Preparation
Reagents are supplied ready to use.

Reagent Storage
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. Do not freeze reagents.
3. 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 100 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]

Materials Provided
Direct bilirubin reagents R1 and R2

Materials Required but not Provided
1. Mindray BS-200 Analyzer.
2. BS-200 Operation manual.
3. Chemistry Calibrator, catalog number C7506-50
4. Chemistry control, catalog number C7592-100

Mindray BS-200 Test Parameters

Test :	DBIL	R1 :	180
No.:	006	R2 :	47
Full Name:	Direct Bilirubin	Sample Volume:	4
Standard No.:		R1 Blank:	
Reac. Type:	Endpoint	Mixed Rgt. Blank:	
Pri. Wave:	546nm	Linearity Range:	0.0 - 10.0
Sec. Wave	670nm	Linearity Limit:	
Direction:	Increase	Substrate Limit:	
Reac. Time:	-1 / 19	Factor:	
		Compensate: Slope 1.0	Intercept: 0
Incuba. Time:	3	□ Prozone check	
Unit:	mg/dl	q1: q2: q3: q4:	
Precision:	0.1	PC:	Abs:

Calibration Parameters

Rule:	Two-point linear	Calibrator 1:	Deionized Water
Sensitivity:		Calibrator 2:	Chem Cal
Replicates:	2	Calibrator 3:	
Interval (day):		Calibrator 4:	
Difference Limit:		Calibrator 5:	
SD:		Calibrator 6:	
Blank Response:			
Error Limit:			
Coefficient:	0		

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Calibration
Use an NIST-traceable serum calibrator. 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.

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.

Calculations (Example)
Abs. = Absorbance
Unk. = Unknown
Cal. = Calibrator

$$\frac{\text{Abs. Unk.} - \text{Abs. Unk. Blank}}{\text{Abs. Cal.} - \text{Abs. Cal. Blank}} \times \text{Conc. of Cal. (mg/dl)} = \text{Direct Bilirubin (mg/dl)}$$

Sample: If Abs. of Unknown = 0.35, Abs. of Unknown Blank = 0.01, Abs. of Calibrator 0.25, Abs. of Calibrator Blank = 0.01, Concentration of Calibrator = 4.0 mg/dl

Then: $\frac{0.35 - 0.01}{0.25 - 0.01} \times 4 = \frac{0.34}{0.24} \times 4 = 5.7$ mg/dl

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 100 mg/dl and triglyceride to 500 mg/dl do not interfere with results.

Performance
1. Linearity: 10.0 mg/dl
2. Limit of Detection (Sensitivity): 0.1 mg/dl
3. Comparison: A study was performed between the Mindray BS-200 and a similar analyzer using this method, resulting in a correlation coefficient of 0.999 with a regression equation of y=0.985x - 0.12.
4. Precision: Precision studies were performed using the Mindray BS-200 analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.[10]

Within Day			Day to Day		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
0.48	0.05	10.1	0.39	0.05	12.8
1.92	0.09	4.5	1.91	0.11	5.8

References

1. Ehrlich, P., Charite Ann. 8:140(1883).
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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)
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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)
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Use by (YYYY-MM) | LOT Lot and batch code | REF Catalog number | Manufacturer | IVD *In vitro* diagnostic medical device
Temperature limitation | Consult instructions for use | CE mark | EC REP Authorized representative in the European Community