

Total Bilirubin Reagent Set

Intended Use

For the quantitative determination of total 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 1883¹, several modifications have been proposed to enhance the reaction. The Malloy and Evelyn method² 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 A detergent is used to accelerate the reaction and to avoid protein precipitation. The diazo reagent is 2,5-dichlorophenyldiazonium tetrafluoroborate (DPD) that reacts very rapidly in coupling with blirubin under acidic conditions. The resulting procedure is simple, yet exhibits good correlation when compared with the method of Jendrassik and Grof.

Principle

Total bilirubin is coupled with a diazonium salt (DPD) in a strongly acid medium (pH 1 - 2).

Acid, surfactant

Bilirubin + DPD -----> Azobilirubin

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

Reagents

- 1. Total bilirubin R1 reagent: acid buffer 50 mmol/L, Surfactant.
- 2. Total bilirubin R2 reagent: acid buffer >30 mmol/L, >2.0 mmol/L DPD and stabilizers.

Reagent Preparation

Reagents provided as ready to use liquids.

Reagent Storage

- 1. Packaged reagents are stored at 2-8°C. The reagents are 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 contamination (turbidity)
- 2. The R2 may develop very slight precipitation that does <u>not</u> affect performance and will re-dissolve if the R2 is warmed gently.
- 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.

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.

Specimen Collection and Storage

- 1. Fresh, unhemolyzed serum is recommended.
- 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.⁵
- 3. Bilirubin in serum is stable for three months when stored frozen (-20°C) and protected from light.⁵
- 4. Direct sunlight may cause up to a 50% decrease in bilirubin within one hour.⁶
- 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

- All interference studies were performed according to the procedures recommended in NCCLS guideline No. EP7-P for interference testing in clinical chemistry.⁷
- 2. Serum hemoglobin levels up to 500 mg/dl do not interfere with results.
- 3. Serum Triglycerides up to 1000 mg/dl do not interfere with results.
- 4. A number of drugs and substances affect bilirubin results. See Young, et al.8

Materials Provided

- 1. Total Bilirubin R1 reagent
- 2. Total Bilirubin R2 reagent

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:	TBILI	R1:	180	
No.:	007	R2:	47	
Full Name:	Total Bilirubin	Sample Volume:	3	
Standard No .:		R1 Blank:		
Reac. Type:	Endpoint	Mixed Rgt. Blank:		
Pri. Wave:	546nm	Linearity Range: 0.0 -	30.0	
Sec. Wave:	630nm	Linearity Limit:		
Direction:	Ascending	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.

Calculations (Example)

Abs. = Absorbance Unk. = Unknown

Cal. = Calibrator

<u>Abs. Unk. – Abs. Unk. Blank</u> x Conc. of Cal. (mg/dl) = Total Bilirubin (mg/dl) Abs. Cal. – Abs. Cal. Blank

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 = 5.0 mg/dl

Then: $\frac{0.35 - 0.01}{0.25 - 0.01} \times 5 = \frac{0.34}{0.24} \times 5 = 7.1 \text{ mg/dl}$

Quality Control

The validity of the reaction should be monitored by use of the control sera with known normal and abnormal total bilirubin values. These controls should be run at least with every working shift in which total 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.

Limitations

- 1. Samples with values above 30 mg/dl must be diluted 1:1 with isotonic saline, re-assayed and the final answer multiplied by two.
- Serum hemoglobin levels of up to 500 mg/dl and triglycerides up to 1000 mg/dl do not interfere with results.

Performance

- 1. Linearity: 30.0 mg/dl
- 2. Limit of Detection (Sensitivity): 0.15 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=0.902x + 0.02.
- Precision: Precision studies were performed using the Mindray BS-200 analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.⁹

Within Day		Day to Day			
<u>Mean</u>	<u>S.D.</u>	C.V.%	<u>Mean</u>	S.D.	C.V.%
0.84	0.08	10.1	0.67	0.04	6.0
5.99	0.14	2.4	5.98	0.25	4.2

Expected Values¹⁰

Total: Adults and infants older than 1 month: 0.2 – 1.0 mg/dl Infants: Full Term Newborn

Full Term Newborn Up to 24hrs: 2.0-6.0 mg/dl Up to 48hrs: 6.0-10.0 mg/dl Days 3-5: 4.0-8.0 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).

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REF Catalog number

CE CE mark

Manufacturer

IVD In vitro diagnostic medical device

EC REP Authorized representative in the European Community

- 4. Wahlefeld AW, et al. Scand J Clin Lab Invest. 29 Supplement 126(1972).
- 5. Martinek, R.G., Clin. Chim. Acta 13:161 (1966).
- Tietz, N.W., Fundamentals of Clinical Chemistry, Philadelphia, W.B. Saunders, p.1028 (1976).
- NCCLS document, "National Evaluation Protocols for Interference Testing", Evaluation Protocol Number 7, Vol. 4, No. 8, (June 1984).
- Young, D.S., Effects of Preanalytical Variables on Clinical Laboratory Tests, Washington DC, AACC Press, (1997)
- NCCLS document "Evaluation of Precision Performance of Clinical Chemistry Devices", 2nd Ed. (1992).
- Tietz, Textbook of Clinical Chemistry, Philadelphia, W.B. Saunders, 3rd Ed., p. 1170 (1999)