


Bilirubin Auto Total FS*

Order Information

Cat. No.	Kit size	Instrument	
1 0811 99 10 972	R1 3 x 15.8 mL	BX-3010	375 (3 x 125)
		BX-4000	261 (3 x 87)
	R2 3 x 6.5 mL	BX-3010	375 (3 x 125)
		BX-4000	261 (3 x 87)

Intended Use

Diagnostic reagent for quantitative in vitro determination of total bilirubin in human serum or heparin plasma on automated Sysmex BX-Series.

Summary

Bilirubin is a breakdown product of hemoglobin. Free, unconjugated bilirubin is extremely apolar and nearly insoluble in water, thus forming a complex with albumin for the transport in the blood from the spleen to the liver. In the liver, bilirubin is conjugated with glucuronic acid and the resulting water soluble bilirubin glucuronic acid is excreted via the bile ducts. Hyperbilirubinemia can be caused by increased bilirubin production due to hemolysis (pre-hepatic jaundice), by parenchymal damages of the liver (intra-hepatic jaundice) or by occlusion of bile ducts (post-hepatic jaundice). A chronic congenital (predominantly unconjugated) hyperbilirubinemia called Gilbert's syndrome is quite frequent in the population. High levels of total bilirubin are observed in 60 – 70% of neonates due to an increased postpartal breakdown of erythrocytes and because of delayed function of enzymes for bilirubin degradation. Common bilirubin methods detect either total bilirubin or direct bilirubin. Determinations of direct bilirubin measure mainly conjugated, water soluble bilirubin. Therefore, the value of unconjugated bilirubin may be estimated from the difference between total bilirubin and direct bilirubin. [1,2]

Method

Photometric test using 2,4-dichloroaniline (DCA)

Direct bilirubin in presence of diazotized 2,4-dichloroaniline forms a red colored azocompound in acidic solution. A specific mixture of detergents enables a safe determination of the total bilirubin [3].

Reagents

Components and Concentrations

R1:	Phosphate buffer	50 mmol/L
	NaCl	150 mmol/L
R2:	2,4-Dichloroaniline	5 mmol/L
	HCl	130 mmol/L

Storage and Stability

Reagents are stable up to the date of expiry indicated on the kit, if stored at 2 – 8°C and contamination is avoided. Do not freeze reagents and protect them from light.

Warnings and Precautions

- ⚠ Reagent 1: Warning. H290 May be corrosive to metals. H315 Causes skin irritation. H319 Causes serious eye irritation. H410 Very toxic to aquatic life with long lasting effects. P234 Keep only in original packaging. P264 Wash hands and face thoroughly after handling. P273 Avoid release to the environment. P280 Wear protective gloves/protective clothing/eye protection. P337+P313 If eye irritation persists: Get medical advice/attention. P391 Collect spillage.
- ⚠ Reagent 2: Warning: H290 May be corrosive to metals. H319 Causes serious eye irritation. P234 Keep only in original packaging. P264 Wash hands and face thoroughly after handling. P280 Wear protective gloves/protective clothing/eye protection. P305+P351+P338 If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P337+P313 If eye irritation persists: Get medical advice/attention. P390 Absorb spillage to prevent material damage.
- In very rare cases, samples of patients with gammopathy might give falsified results [4].
- Eltrombopag medication leads to falsely low or high results in patient samples.
- Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
- For professional use only.

Waste Management

Refer to local legal requirements.

Reagent Preparation

The reagents are ready to use. The bottles are placed directly into the reagent rotor.

Materials Required

General laboratory equipment

Specimen

Human serum or heparin plasma

Protect sample from light.

Stability [5]:

1 day	at	20 – 25°C
7 days	at	4 – 8°C
6 months	at	-20°C

in case of immediate freezing.

Only freeze once. Discard contaminated specimens.

Calibrators and Controls

DiaSys TruCal U is recommended for calibration. TruCal U calibrator values have been made traceable to the NIST SRM 916 reference material. Use DiaSys TruLab N and P for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat. No.	Kit size
TruCal U	5 9100 99 10 063	20 x 3 mL
	5 9100 99 10 064	6 x 3 mL
TruLab N	5 9000 99 10 062	20 x 5 mL
	5 9000 99 10 061	6 x 5 mL
TruLab P	5 9050 99 10 062	20 x 5 mL
	5 9050 99 10 061	6 x 5 mL

Performance Characteristics

Exemplary data mentioned below may slightly differ in case of deviating measurement conditions.

Measuring range up to 25 mg/dL (428 µmol/L). In case of higher activities re-measure samples after manual dilution with NaCl solution (9 g/L) or use rerun function.	
Limit of detection***	0.1 mg/dL (1.71 µmol/L)
Onboard stability	6 weeks
Calibration stability	7 days

Interfering substance	Interferences ≤ 10% up to	Analyte concentration
Ascorbic acid	30 mg/dL	0.771 mg/dL (13.2 µmol/L)
Hemoglobin	125 mg/dL	0.254 mg/dL (4.35 µmol/L)
	500 mg/dL	0.776 mg/dL (13.3 µmol/L)
Lipemia (triglycerides)	1000 mg/dL	0.355 mg/dL (6.07 µmol/L)

For further information on interfering substances refer to Young DS [6,7].

Precision BX-3010			
Within run (n=20)	Sample 1	Sample 2	Sample 3
Mean [mg/dL]	0.483	1.33	5.91
Mean [µmol/L]	8.26	22.8	101
CV [%]	1.50	1.33	0.628
Between day (n=20)	Sample 1	Sample 2	Sample 3
Mean [mg/dL]	0.509	1.36	6.50
Mean [µmol/L]	8.70	23.3	111
CV [%]	2.60	2.78	1.30

Method comparison (n=124)	
Test x	Bilirubin Auto Total FS (BioMajesty 6010C)
Test y	Bilirubin Auto Total FS (BX-3010)
Slope	1.04
Intercept	-0.010 mg/dL (0.176 µmol/L)
Coefficient of correlation	0.9998

** lowest measurable concentration which can be distinguished from zero; mean + 3 SD (n = 20) of an analyte free specimen.

Conversion Factor

Bilirubin [mg/dL] x 17.1 = Bilirubin [µmol/L]

Reference Range [1]

	[mg/dL]	[µmol/L]
Neonates		
24 h	< 8.8	< 150
2nd day	1.3 – 11.3	22 – 193
3rd day	0.7 – 12.7	12 – 217
4th – 6th day	0.1 – 12.6	1.7 – 216
Children		
>1 month	0.2 – 1.0	3.4 – 17
Adults	0.1 – 1.2	1.7 – 21

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Literature

1. Thomas L ed. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft, 1998: p. 192-202.
2. Tolman KG, Rej R. Liver function. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999. p. 1125-77.
3. Rand RN, di Pasqua A. A new diazo method for the determination of bilirubin. Clin Chem 1962; 6: 570-8.
4. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 2007;45(9):1240-1243.
5. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001; p. 18-9.
6. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
7. Young DS. Effects on Clinical Laboratory Tests - Drugs Disease, Herbs & Natural Products, <https://clinfx.wiley.com/aaccweb/aacc/>, accessed on May 2020. Published by AACC Press and John Wiley and Sons, Inc.



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* Fluid Stable

Chemistry Parameters 1				Sysmex BX-3010 Chemistry Analyzer Analytical Parameters			
Method No.	*	Method Name	TBIL	Reagent Name	Reagent (µL)	Water (µL)	
Print Name	Bilirubin total	MethodColor		R1	TBIL	100	
Sample Type	Serum			R2	TBIL	25	
Unit	mg/dL			Diluent	Disable		
Assay Type	End			Sample Ppt. Wash	Disable		
Measuring points		Start	End	Stirring Speed R1	Low	R2 Low	
		1	22 - 23				
		2	45 - 46				
Wave Length	Prim. 546	Sec. 660		Normal Range			
				No.	Normal Range Name	Min	Max
				1	Male-G1	*	*
				2	Male-G2	*	*
				3	Male-G3	*	*
				4	Female-G1	*	*
Normal	Sample Volume (µL)	Diluted Sample (µL)	Diluent (µL)	Technical Range	(Conc)	0.1	30.0
<input type="checkbox"/>	Low 0.0 < 2.5	Normal 2.5 < 0.0	High 0.0		(mAbs/10)	*	*
<input type="checkbox"/>	Diluent 0.0 < 2.5	< 0.0		Previous Result Comparison (%)	*	*	%
<input type="checkbox"/>	Diluent 0.0 < 2.5	< 0.0		Abnormal Range	(Conc)	*	*
<input type="checkbox"/>	Diluent 0.0 < 2.5	< 0.0		Panic Range	(Conc)	*	*
				Decimal Point	2	Profile SI	Disable

*Entered by user

Chemistry Parameters 2				Sysmex BX-3010 Chemistry Analyzer Analytical Parameters		
Method No.	*	Method Name	TBIL	Sample	Serum	
Limit Checks				Blank measurement	Blank measurement:	Disable reagent blank and C1 blank
<input checked="" type="checkbox"/>	Duplicate Limit	50	mAbs/10	Measurement of Reagent Blank during Run:	None	
<input checked="" type="checkbox"/>	Sensitivity Limit	200	mAbs/10	Reagent blank measurement at calibration:	Reagent blank (No sample)	
<input checked="" type="checkbox"/>	Linearity Limit		%	The number of measurement:	Duplicate	
			(mAbs/10)/min	Reagent blank limit checks:	<input checked="" type="checkbox"/>	Duplicate Limit 20 mAbs/10
<input type="checkbox"/>	Prozone Limit	Higher	%	Instrument Factor	a	1.00
					b	0.00
		SL1-S	SL1-F			
		SL2-S	SL2-F			
	Sensitivity		mAbs/10			
<input checked="" type="checkbox"/>	Absorbance Limit					
	Abs. in reaction	Increase				
	Limit	25000	mAbs/10			

Calibration Registration

**Sysmex BX-3010 Chemistry Analyzer
Analytical Parameters**

Method No.

Method Name

Sample Type

Replication

Check Interval

Test without calibration

Calibration Type

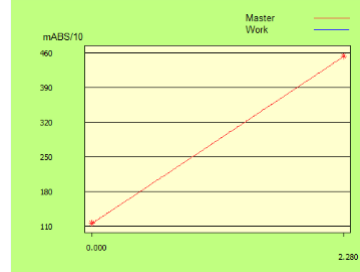
Reagent Lot

Calibrator Name

	Conc.	WORK	MASTER	Calibr. Lot No.	<input type="checkbox"/> All
C1	0	Automatic entry	Automatic entry	*	
C2	*	Automatic entry	Automatic entry	*	
C3	*				
C4	*				
C5	*				
C6	*				
C7	*				

K C1 Blank
 Reagent Blank for C1

Reagent Lot No.
(R1) Last
(R2)



The calibration curve is lot dependent

Reagent blank mAbs/10 Last

Blank mAbs/10 Last

Calibration Curve Conc.

Absorbance mAbs/10

*Entered by user

Chemistry Parameters		Sysmex BX-4000 Chemistry Analyzer Analytical Parameters																							
Method	* <input type="text"/>	Name	<input type="text" value="TBIL"/>																						
Print Name	<input type="text" value="Bilirubin total"/>	R1	<input type="text" value="TBIL"/>	<input type="text" value="150"/>	<input type="text"/>																				
Sample	<input type="text" value="Serum"/>	R2	<input checked="" type="checkbox"/> Enable	<input type="text" value="TBIL"/>	<input type="text" value="38"/>																				
Unit	<input type="text" value="mg/dL"/>																								
Assay Type	<input type="text" value="End"/>	Diluent	<input type="checkbox"/> Enable	<input type="text"/>	<input type="text"/>																				
Measuring points		Start	End	Decimal Points	<input type="text" value="2"/>																				
		1	<input type="text" value="33"/>	-	<input type="text" value="34"/>																				
<input type="checkbox"/> Enable		2	<input type="text" value="67"/>	-	<input type="text" value="68"/>																				
Wave Length	Prim. <input type="text" value="546"/>	Sec	<input type="checkbox"/> Disable	<input type="text" value="660"/>																					
<table border="1" style="width:100%; border-collapse: collapse;"> <thead> <tr> <th>No.</th> <th>Normal Range Name</th> <th>Min</th> <th>Max</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>Male-G1</td> <td>*</td> <td>*</td> </tr> <tr> <td>2</td> <td>Male-G2</td> <td>*</td> <td>*</td> </tr> <tr> <td>3</td> <td>Male-G3</td> <td>*</td> <td>*</td> </tr> <tr> <td>4</td> <td>Female-G1</td> <td>*</td> <td>*</td> </tr> </tbody> </table>						No.	Normal Range Name	Min	Max	1	Male-G1	*	*	2	Male-G2	*	*	3	Male-G3	*	*	4	Female-G1	*	*
No.	Normal Range Name	Min	Max																						
1	Male-G1	*	*																						
2	Male-G2	*	*																						
3	Male-G3	*	*																						
4	Female-G1	*	*																						
<input type="checkbox"/> Dilution	<input type="text" value="3.8"/>	Sample (µL)	<input type="text"/>	Diluent (µL)	<input type="text"/>																				
<input type="checkbox"/> Rerun (High/Prozone)	<input type="text"/>																								
<input type="checkbox"/> Dilution	<input type="text" value="3.8"/>																								
<input type="checkbox"/> Rerun (Low)	<input type="text"/>																								
<input type="checkbox"/> Dilution	<input type="text" value="3.8"/>																								
		Technical Range		(Conc)	<input type="text" value="0.1"/>																				
				(mAbs/10)	- <input type="text" value="30.0"/>																				
		SPT Wash		<input type="checkbox"/> Enable	<input type="text"/>																				
		Stirring Speed		R1	<input type="text" value="Low"/>																				
				R2	<input type="text" value="Low"/>																				

*Entered by user

Chemistry Parameters		Sysmex BX-4000 Chemistry Analyzer Analytical Parameters			
Method No.	* <input type="text"/>	Name	<input type="text" value="TBIL"/>	Sample	<input type="text" value="Serum"/>
Limit Checks					
<input checked="" type="checkbox"/> Duplicate Limit	<input type="text" value="50"/>	mAbs/10			
<input checked="" type="checkbox"/> Sensitivity Limit	<input type="text" value="200"/>	mAbs/10			
<input checked="" type="checkbox"/> Linearity Limit	<input type="text"/>	%	<input type="text"/>	(mAbs/10)/min	
<input type="checkbox"/> Prozone Limit	<input type="text"/>	%	<input type="text" value="Upper"/>		
	SL1-S	<input type="text"/>	-	SL1-F	<input type="text"/>
	SL2-S	<input type="text"/>	-	SL2-F	<input type="text"/>
	Sensitivity	<input type="text"/>	mAbs/10		
<input checked="" type="checkbox"/> Absorbance Limit	Reaction	<input type="text" value="Increase"/>			
	Limit	<input type="text" value="25000"/>	mAbs/10		
Blank measurement					
Blank measurement:					
<input type="text" value="Disable reagent blank and S1 blank"/>					
Measurement of Reagent Blank during Run:					
<input type="text" value="None"/>					
Reagent blank measurement at calibration:					
<input type="text" value="Reagent blank (No sample)"/>					
The number of measurement:					
<input type="text" value="Duplicate"/>					
Reagent blank limit checks:					
<input checked="" type="checkbox"/> Duplicate Limit	<input type="text" value="20"/>	mAbs/10			
Instrument Factor					
	a	<input type="text" value="1.00"/>	b	<input type="text" value="0.00"/>	

<u>Registration Calibration</u>		Sysmex BX-4000 Chemistry Analyzer Analytical Parameters																																																	
Method <input type="text" value="*"/> Name <input type="text" value="TBIL"/>	R Lot No. R1 <input type="text" value="*"/> Last <input type="text"/>																																																		
Sample <input type="text" value="Serum"/>	R2 <input type="text" value="*"/>																																																		
Sampling <input type="text" value="Duplicate"/>	<p style="font-size: small;">The calibration curve is lot dependent</p>																																																		
Check Interval <input type="text" value="7"/> days																																																			
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Material Name <input type="text" value="TruCal U"/>	Reagent blank <input type="text"/> mAbs/10 Last <input type="text"/>																																																		
<table border="1" style="width:100%; border-collapse: collapse; font-size: x-small;"> <thead> <tr> <th style="width: 5%;"></th> <th style="width: 15%;">Conc.</th> <th style="width: 15%;">WORK</th> <th style="width: 15%;">MASTER</th> <th style="width: 15%;">Lot No. (S)</th> <th style="width: 10%;"><input type="checkbox"/> All</th> </tr> </thead> <tbody> <tr> <td>S1</td> <td>0</td> <td>Automatic entry</td> <td>Automatic entry</td> <td></td> <td></td> </tr> <tr> <td>S2</td> <td>*</td> <td>Automatic entry</td> <td>Automatic entry</td> <td></td> <td></td> </tr> <tr> <td>S3</td> <td>*</td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <td>S4</td> <td>*</td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <td>S5</td> <td>*</td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <td>S6</td> <td>*</td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <td>S7</td> <td>*</td> <td></td> <td></td> <td></td> <td></td> </tr> </tbody> </table>					Conc.	WORK	MASTER	Lot No. (S)	<input type="checkbox"/> All	S1	0	Automatic entry	Automatic entry			S2	*	Automatic entry	Automatic entry			S3	*					S4	*					S5	*					S6	*					S7	*				
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K <input type="text" value="Automatic entry"/> <input type="checkbox"/> S1 Blank <input type="checkbox"/> Reagent Blank for S1																																																			
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