SPECTRUM

The Creative Approach to Bioscience

Bilirubin (TOTAL AND DIRECT)
Jendrassik Grof

p-21,22

REF: 222 001 (255ml)	100 Test	REF: 222 002 (750ml)	300 Test
R1 Sulphanilic Acid	1 x 45 ml	R1 Sulphanilic Acid	2 x 65 ml
R2 Nitrite	1 x 10 ml	R2 Nitrite	2 x 15 ml
R3 Caffeine	1 x 100 ml	R3 Caffeine	3 x 100 ml
R4 Tartarate	1 x 100 ml	R4 Tartarate	3 x 100 ml

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorised Representative		Use by/Expiration Date
IVD	For in-vitro diagnostic use		CAUTION. Consult instructions for use
LOT	Batch Code/Lot number		Manufactured by
REF	Catalogue Number		(Xi) - Irritant
	Consult instructions for use		
	Temperature Limitation		

Intended Use

Spectrum Diagnostics bilirubin reagent is intended for the in-vitro quantitative, diagnostic determination of bilirubin in human serum on both automated and manual systems.

Background

The average level of the bilirubin produced in humans from different sources ranges between 250 to 300 mg/day, of which 85% is derived from the heme moiety of the haemoglobin released from senescent erythrocytes that are destroyed in the reticuloendothelial system. The remaining 15 % is produced from erythrocytes destroyed in the bone marrow and from catabolism of other heme containing proteins such as cytochromes and myoglobin.
After it is produced in the peripheral tissues, bilirubin is transported to the liver in association with albumin. In the liver, bilirubin is conjugated with glucuronic acid for solubilization and subsequent transport through the bile duct and elimination via the digestive tract. Disease or conditions which, through hemolytic processes, produce bilirubin faster than the liver can metabolize it, cause the levels of unconjugated (indirect) bilirubin to increase in the circulation. Bile duct obstruction or damage to hepatocellular structure causes increases in the levels of both conjugated (direct) and unconjugated (indirect) bilirubin in the circulation.

Method

Colorimetric Diazo method.

Assay Principle

The total bilirubin concentration is determined in presence of caffeine by the reaction with diazotized sulphanilic acid to produce an intensely colored diazo dye (560-600 nm). The intensity of color of this dye formed is proportional to the concentration of total bilirubin.
Direct bilirubin is determined in absence of caffeine by the direct reaction with diazotized sulphanilic acid to form red-colored azobilirubin, the color intensity of which measured at 546 nm is proportional to the concentration of the direct bilirubin in the sample.

$$\text{Sulfanilic acid} + NaNO_2 \xrightarrow{HCL} \text{Diazotized sulfanilic acid}$$

$$\text{Bilirubin} + \text{Diazotized sulfanilic acid} \xrightarrow{pH\ 1.4} \text{Azobilirubin}$$

Reagents

Reagent 1 (R1)	
Sulfanilic acid	31.0 mmol/l
HCL	0.20 N
Reagent 2 (R2)	
Sodium nitrite	28.0 mmol/l
Reagent 3 (R3)	
Caffeine	0.28 mol/l
Sodium benzoate	0.55 mol/l
Reagent 4 (R4)	
Tartarate	0.99 mol/l
Sodium hydroxide	2.0 N

Reagent 4 contains caustic material.
Corrosive (C)
R35 Causes severe burns.
R41 Risk of serious damage to eyes.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S28 After contact with skin, wash immediately with plenty of soap and water.
For further information, refer to the Bilirubin reagent material safety data sheet.

Digitally signed by Ceaicovschi Tudor
Date: 2023.12.12 16:21:46 EET
Reason: MoldSign Signature
Location: Moldova

Precautions and Warnings

Do not ingest or inhalate. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.

Reagent Preparation, Storage and Stability

Spectrum bilirubin reagents are supplied ready-to-use and stable up to the expiry date labeled on the bottles when stored at room temperature.The opened vials are stable for 6 months at the specified temperature if contamination is avoided.

Deterioration

Do not use the Spectrum bilirubin reagents if precipitate forms. Failure to recover control values within the assigned range may be an indication of reagent deterioration.

Specimen Collection and Preservation

Avoid exposure of the specimen to light. If plasma is used, only heparin and oxalate plasma are suitable. Other anticoagulants should not be used. The average half-life of total bilirubin and direct bilirubin in serum is 17 days and few hours respectively.

Stability:

	-20 °C	4 – 8 °C	20 – 25 °C
Total	6 months	7 days	1 day
Direct	6 months	7 days	2 days

Procedure

Total Bilirubin

	Sample blank	Sample
Reagent 1	200 µl	200 µl
Reagent 2	-----	1 drop
Reagent 3	1.0 ml	1.0 ml
Sample	200 µl	200 µl
Mix and incubate for 10 minutes at 20 – 25 °C. then add;		
Reagent 4	1.0 ml	1.0 ml

Mix and incubate for 5 minutes at 20 – 25 °C. Measure absorbance of sample (A_{sample}) against sample blank at 578 nm(560 - 600 nm) The color intensity is stable for 30 minutes.

Direct Bilirubin

	Sample blank	Sample
Reagent 1	200 µl	200 µl
Reagent 2	-----	1 drop
Saline 0.9% NaCl	2.0 ml	2.0 ml
Sample	200 µl	200 µl

Mix and incubate for exactly 5 minutes at 20 – 25 °C. Measure absorbance of sample (A_{sample}) against sample blank at 546 nm (530 - 560 nm).

Calculation

Total bilirubin (mg/dl) = A_{Sample} x 10.8

Direct bilirubin (mg/dl) = A_{Sample} x 14.4

Quality Control

Normal and abnormal commercial control serum of known concentrations should be analyzed with each run.

Performance Characteristics

Precision
Within run (Repeatability)

	Total		Direct	
	Level 1	Level 2	Level 1	Level 2
n	20	20	20	20
Mean (mg/dL)	0.79	4.37	0.299	0.77
SD	0.016	0.18	0.016	0.057
CV%	2.03	4.12	5.35	7.4

Run to run (Reproducibility)

	Total		Direct	
	Level 1	Level 2	Level 1	Level 2
n	20	20	20	20
Mean (mg/dL)	0.82	4.52	0.32	0.82
SD	0.02	0.17	0.023	0.062
CV%	2.44	3.76	7.19	7.56

Methods Comparison

A comparison between Spectrum Diagnostics Bilirubin and a commercial reagent of the same methodology was performed on 20 human sera. A correlation of 0.975 was obtained.

Sensitivity

When run as recommended, the sensitivity of this assay is 0.1 mg/dL (1.7 µmol/L) for both total and direct bilirubin.

Linearity

The reaction is linear up to a total bilirubin concentration of 30 mg/dL (513 µmol/L) and a direct bilirubin concentration of 10 mg/dL (171 µmol/L). Specimens showing higher concentration should be diluted 1+4 with physiological saline and repeat the assay (result×5).

Interfering substances

Haemolysis
Avoid haemolysis since it interferes with the test.

Lipemia
Lipemic specimens interfere with the test.

Drugs
Theophylline and propranolol may cause artificially low total bilirubin levels.

Expected Values

Total Bilirubin

Adults and infants >1 month	< 0.2-1.0 mg/dL	(3.4 -17 µmol/l)
Newborns premature (3-5 d)	10-14 mg/dL	(171-239 µmol/l)
Newborns:		
(3-5 d)	4.0 - 8.0 mg/dL	(68-137 µmol/l)
(<48 h)	6.0 - 10.0 mg/dL	(103-171 µmol/l)
(<24 h)	2.0 - 6.0 mg/dL	(34-103 µmol/l)
Direct Bilirubin	0 - 0.3 mg/dL	(0 - 51 µmol/L)

Spectrum Diagnostics does not interpret the results of a clinical laboratory procedure; interpretation of the results is considered the responsibility of qualified medical personnel. All indications of clinical significance are supported by literature references.

Analytical Range

Total bilirubin	: 0.1 – 30 mg/dL	(1.7 – 513 µmol/L)
Direct bilirubin	: 0.1 – 10 mg/dL	(1.7 – 171 µmol/L)

Waste Disposal

This product is made to be used in professional laboratories.
Please consult local regulations for a correct waste disposal.
S56: dispose of this material and its container at hazardous or special waste collection point.
S57: use appropriate container to avoid environmental contamination.
S61: avoid release in environment. refer to special instructions/safety data sheets.

References

1. Balistreri WF, Shaw LM. Liver function. In: Tietz NW, ed. Fundamentals of clinical chemistry.3 rd ed. Philadelphia:WB Saunders: 1987:729-761.
2. Malloy HT, Evelyn KA. The determination of bilirubin with the photoelectric colorimetric method.J Biol Chem.1937:119:481-490.
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CATALOG NO.	**QUANTITY**
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222 002	300 test

Egyptian Company for Biotechnology (S.A.E)
Obour city industrial area. block 20008 piece 19 A. Cairo. Egypt.
Tel: +202 4489 2248 - Fax: +202 4489 2247
www.spectrum-diagnostics.com
E-mail:info@spectrum-diagnostics.com

EC REP **MDSS GmbH**
Schiffgraben 41
30175 Hannover, Germany

CE

TÜV SÜDWEST CERTIFIED ISO 13485:2016

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Jendrassik Grof

p. 25

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Reagents

Reagent 1 (R1)	
Sulfanilic acid	31.0 mmol/l
HCL	0.20 N
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Sodium hydroxide	2.0 N

Reagent 4 contains caustic material.
Corrosive (C)
R35 Causes severe burns.
R41 Risk of serious damage to eyes.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
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Reagent Preparation, Storage and Stability

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Deterioration

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Specimen Collection and Preservation

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Total	6 months	7 days	1 day
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Procedure

Total Bilirubin

	Sample blank	Sample
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Reagent 3	1.0 ml	1.0 ml
Sample	200 µl	200 µl
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Direct Bilirubin

	Sample blank	Sample
Reagent 1	200 µl	200 µl
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Saline 0.9% NaCl	2.0 ml	2.0 ml
Sample	200 µl	200 µl

Mix and incubate for exactly 5 minutes at 20 – 25 °C. Measure absorbance of sample (A_{sample}) against sample blank at 546 nm (530 - 560 nm).

Calculation

Total bilirubin (mg/dl) = $A_{Sample} \times 10.8$

Direct bilirubin (mg/dl) = $A_{Sample} \times 14.4$

Quality Control

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Performance Characteristics

Precision

Within run (Repeatability)

	Total		Direct	
	Level 1	Level 2	Level 1	Level 2
n	20	20	20	20
Mean (mg/dL)	0.79	4.37	0.299	0.77
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Direct Bilirubin

	Sample blank	Sample
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Saline 0.9% NaCl	2.0 ml	2.0 ml
Sample	200 µl	200 µl

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EC REP **MDSS GmbH**
Schiffgraben 41
30175 Hannover, Germany

CE

TÜV SÜDWEST CERTIFIED ISO 13485:2016

SPECTRUM

The Creative Approach to Bioscience

Calcium
O-CPC

REF: 226 001 (2 x 30 ml) 60 test
REF: 226 002 (2 x100 ml) 200 test
REF: 226 003 (4 x100 ml) 400 test
REF: 226 004 (2 x 50 ml) 100 test

D. 32,35

SYMBOLS IN PRODUCT LABELLING

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Intended Use

Spectrum Diagnostics calcium reagent is intended for the in-vitro quantitative, diagnostic determination of calcium in human serum on both automated and manual systems.

Background

Calcium is the fifth most common element in the body, most of which (98 %) is present in the skeleton. One half of the remaining calcium is found in extracellular fluid and the rest in tissues. Calcium has a crucial role in bone mineralization and is also vital for basic physiological processes such as blood coagulation, neuromuscular conduction, and normal muscle tone. Calcium is constantly lost from the body through excretion in faeces, urine and to a small extent in sweat. The determination of serum calcium is useful for monitoring myeloma, renal failure, acid base balance,and cirrhosis. Both serum and tissue calcium in the body are controlled by parathyroid hormone, calcitonin and vitamin D. Hypocalcemia may be observed in hypoparathyrodism, steatorrhea, pancreatitis and nephrosis. Increased levels may be associated with multiple myeloma and other neoplastic diseases.

Method

O-cresolphthalein complexone colorimetric method.

Assay Principle

Calcium ions react with O-cresolphthalein complexone (O-CPC) under alkaline conditions to form a violet colored complex.

$$Ca^{2+} + \text{O-CPC} \xrightarrow{\textit{Alkaline pH}} \text{calcium-O-CPC complex}$$

The color intensity of the complex formed is directly proportional to the calcium concentration. It is determined by measuring the increase in absorbance at 578 nm.

Reagents

Standard Calcium (ST)		
10 mg/dL	2.5	mmol/L
Reagent 1 (R1 Buffer)		
2-Amino-2-methyl-1-propanol (pH 10.5)	0.3	mol / L
Reagent 2 (R2 Chromogen)		
O-cresolphthalein complexone	0.16	mmol/L
8-hydroxyquinoline	7.0	mmol/L

Irritant (Xi)

R20/21/22 Harmful by inhalation, in contact with skin and if swallowed.
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.

For further information, refer to the Calcium reagent material safety data sheet.

Precautions and Warnings

Do not ingest or inhalate. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.

Reagent Preparation, Storage and Stability

Spectrum Calcium reagents are supplied ready-to-use and stable up to the expiry date labeled on the bottles when stored sealed at 15 – 25 °C.
Once opened, the reagent and standard are stable for 3 months at the specified temperature.

Deterioration

Do not use the Spectrum Calcium reagents if turbid. Failure to recover control values within the assigned range may be an indication of reagent deterioration.

Specimen Collection and Preservation

Serum and plasma
Use nonhemolyzed serum.Heparin is the only acceptable anticoagulant. No other anticoagulant can be used. Fresh serum collected in the fasting state is the perferred specimen. Serum or plasma should be separated from cells as soon as possible, because prolonged contact with the clot may cause lower calcium values. Sera from patients receiving EDTA (treatment of hypercalcemia) are unsuitable for analysis, since EDTA will chelate the calcium and render it unavailable for reaction with O-cresolphthalein complexone. The biological half-life of calcium in blood is few hours.

Urine
Specimens should be collected in acid washed bottles. 24 hour Specimens should be collected in containers containing 5 ml of 6 mol/L HCl. If the specimen is collected without acid, the pH should be adjusted < 3 with 6 mol/L HCl. Dilute urine specimen 2 times with bidistilled water (1volume urine + 1volume distilled water) before assay.

Stability (serum): 7 days at 15 – 25 °C; 3 weeks at 4 – 8 °C ; 8 months at -20 °C

Stability (urine): 2 days at 15 – 25 °C; 4 days at 4 – 8 °C ; 3 weeks at -20 °C

Stored serum or urine specimens must be mixed well prior to analysis.

System Parameters

Wavelength	578 nm
Optical path	1 cm
Assay type	End-point
Direction	Increase
Sample : Reagent Ratio	1 : 100
Temperature	15 - 25 °C
Zero adjustment	Reagent Blank
Reagent Blank Limits	Low 0.00 AU High 0.3 AU
Sensitivity	2 mg/dL (0.5 mmol/L)
Linearity	20 mg/dL (5 mmol/L)

Procedure

	Blank	Standard	Specimen
Standard	.....	10 µl	.....
Specimen	.....	.....	10 µl
Reagent 1	0.5 ml	0.5 ml	0.5 ml
Reagent 2	0.5 ml	0.5 ml	0.5 ml

Mix and incubate for 5 minutes at 20 - 25 °C. Measure absorbance of specimen ($A_{specimen}$) and standard ($A_{standard}$) against reagent blank.

Calculation

Serum calcium concentration (mg/dL) = $\frac{A_{specimen}}{A_{standard}}$ x 10

Urine calcium (mg/24 hrs)= $\frac{A_{specimen}}{A_{standard}}$ x10 x10*x 2**x V***

* The factor "10" converts mg/dl to mg/litre
** The factor "2" represents the dilution factor
*** "V" represents the 24-hour urine volume in litres

Quality Control

Normal and abnormal control serum of known concentrations should be analyzed with each run.

Performance Characterstics

Precision

Within run (Repeatability)

	Level 1	Level 2
n	20	20
Mean (mg/dL)	9.58	13.97
SD	0.12	0.207
CV%	1.25	1.48

Run to run (Reproducibility)

	Level 1	Level 2
n	20	20
Mean (mg/dL)	9.62	14.15
SD	0.23	0.221
CV%	2.39	1.56

Methods Comparison

A comparison between Spectrum Diagnostics Calcium reagent and a commercial reagent of the same methodology was performed on 20 human sera. A correlation of 0.979 was obtained.

Sensitivity

When run as recommended, the minimum detection limit of this assay is 2.0 mg/dL.

Linearity

The reaction is linear up to calcium concentration of 20 mg/dl. Specimens showing higher concentration should be diluted 1+1 using physiological saline and repeat the assay (result × 2).

Interfering Substances:

Haemolysis
Avoid haemolysis.

Icterus
No significant interference.

Lipemia
No significant interference.

Anticoagulants
Complexing Anticoagulants such as citrate, oxalate and EDTA must be avoided.

Expected values

Serum, plasma		
Adults		
20 - 50 years	8.8-10.2 mg/dl	(2.20-2.55 mmol/L)
>50 years	8.4- 9.7 mg/dl	(2.09-2.42 mmol/L)
Children		
4 -18years	9.2-11.0 mg/dl	(2.30-2.75 mmol/L)
>4 weeks	7.2-11.2 mg/dl	(1.80-2.8 mmol/L)
Urine (24 h)		
Females	<250 mg/day	(<6.25 mmol/day)
Males	<300 mg/day	(<7.5 mmol/day)
Childern	<6 mg/Kg/day	(<0.15 mmol/day)

Spectrum Diagnostics does not interpret the results of a clinical laboratory procedure; interpretation of the results is considered the responsibility of qualified medical personnel. All indications of clinical significance are supported by literature references.

Analytical Range

2 – 20 mg/dl (0.5-5 mmol/L).

Waste Disposal

This product is made to be used in professional laboratories.
Please consult local regulations for a correct waste disposal.
S56: dispose of this material and its container at hazardous or special waste collection point.
S57: use appropriate container to avoid environmental contamination.
S61: avoid release in environment. refer to special instructions/safety data sheets.

References

1. Barnett RN: A scheme for the comparison of quantitative methods. AM J Clin Pathol 43: 562, 1965.
2. Fiereck EA: Appendix. Normal values. in:Fundamentals of clinical chemistry. NW Tietz, editor,Saunders, Philadelphia, p1208,1976.
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5. Tietz NW: Blood gases and electrolytes. In:Fundamentals of clinical chemistry, NW tietz, editor,Saunders, Philadelphia, 176,pp 903, 908.
6. Young DS, Effects of drugs on clinical laboratory tests. AACC press, Washington, D.C. 1990.

ORDERING INFORMATION	
CATALOG NO.	**QUANTITY**
226 001	2 x 30 ml
226 002	2 x 100 ml
226 003	4 x 100 ml
226 004	2 x 50 ml

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SPECTRUM

The Creative Approach to Bioscience

Urea/BUN - Liquizyme
(Modified Urease-Berthlot Method)

REF: 318 001	100 test	REF: 318 002	200 test
R1 Buffer	1 x 100 ml	R1 Buffer	2 x 100 ml
R2 Urease	1 x 6 ml	R2 Urease	2 x 6 ml
R3 Alkaline reagent	1 x 20 ml	R3 Alkaline reagent	1 x 45 ml
REF: 318 003	500 test	REF: 318 004	1000 test
R1 Buffer	5 x 100 ml	R1 Buffer	4 x 250 ml
R2 Urease	2 x 15 ml	R2 Urease	1 x 51 ml
R3 Alkaline reagent	2 x 55 ml	R3 Alkaline reagent	1 x 210 ml

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorised Representative		Use by/Expiration Date
IVD	For in-vitro diagnostic use		CAUTION. Consult instructions for use
LOT	Batch Code/Lot number		Manufactured by
REF	Catalogue Number		(Xi) - Irritant
	Consult instructions for use		
	Temperature Limitation		

Intended Use

Spectrum Diagnostics colorimetric urea reagent is intended for the in-vitro quantitative, diagnostic determination of urea in human serum,plasma or urine on both automated and manual systems.

Background

Urea is the major end product of protein nitrogen metabolism. It is synthesized by the urea cycle in the liver and excreted through the kidneys. The circulating levels of urea depend upon protein intake, protein catabolism and kidney function. Elevated urea levels can occur due to renal impairment or in some diseases such as diabetes, infection, congestive heart failure and during different liver diseases. Determination of blood urea nitrogen is the most widely used screening test for renal function together with serum creatinine.

Method

Urease-colorimetric method.

Assay Principle

The reaction involved in the assay system is as follows:
Urea is hydrolyzed in the presence of water and urease to produce ammonia and carbon dioxide.

$$\text{Urea} + H_2O \xrightarrow{\textit{Urease}} 2NH_3 + CO_2$$

The free ammonia in an alkaline pH and in the presence of indicator forms coloured complex proportional to the urea concentration in the specimen.

Reagents

Standard urea (ST) Aqueous primary standard	
50 mg/dL	8.33 mmol/l
Reagent 1 (R1 Buffer)	
Phosphate buffer pH 8.0	100 mmol/l
Sodium salicylate	80 mmol/l
Sodium nitroprusside	6.0 mmol/l
EDTA	30.0 mmol/l
Reagent 2 (R2 Enzyme)	
Urease	>6000 U/l
Reagent 3 (R3 Alkaline Reagent)	
Sodium hydroxide	400 mmol/l
Sodium hypochlorite	20.0 mmol/l

Irritant (xi) R36/38: Irritating to eyes and skin. **S26:** In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. **S37/39:** Wear suitable gloves and eye/face protection.

For further information, refer to the Urea/BUN reagent material safety data sheet.

Precautions and Warnings

Do not ingest or inhalate. In case of contact with eyes or skin, rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.

Reagent Preparation, Storage and Stability

Spectrum colorimetric urea reagents are supplied ready-to-use and stable up to the expiry date labeled on the bottles at(2 – 8 °C).
Once opened, the reagent and standard are stable for 3 months at the specified temperature.

NB: For mega labs having high numbers of patient specimens,working buffer reagent can be prepared .(**Stability 1 week**)

REF:318 001: add 5 ml from R2 to one bottle of R1; mix gently.
REF:318 002: add 5 ml from R2 to one bottle of R1; mix gently.
REF:318 003: add 5 ml from R2 to one bottle of R1; mix gently.
REF:318 004: add 12.5 ml from R2 to one bottle of R1; mix gently.

Deterioration

Do not use the reagent if it is turbid. Failure to recover control values within the assigned range may be an indication of reagent deterioration.

Specimen Collection and Preservation

Serum and plasma
No special preparation of the patient is required. Use non haemolyzed serum or plasma.The only acceptable anticoagulants are heparin, EDTA and flouride.Do not use ammonium heparin plasma.
Stability: 7 days at 15 –25°C ; 7 days at 2 – 8 °C;
1 year at -20 °C

Urine
Urine samples are prediluted 1 : 50 with ammonium free water prior to assay.
Stability: 2 days at 15 –25 °C ; 7 days at 2 – 8 °C;
1 month at -20 °C

System Parameters

Wavelength	578 nm (578-623 nm)
Optical path	1 cm
Assay type	End-point
Direction	increase
temperature	15-25 °C or 37 °C
Zero adjustment	Against Reagent blank
Reagent Blank Limits	Low 0.02 AU High 0.2 AU
Sensitivity	0.6 mg/dL (0.1 mmol/l)
Linearity	200 mg/dL (33.3 mmol/l)

Procedure 1

	Blank	Standard	Specimen
R1(Buffer)	1.0 ml	1.0 ml	1.0 ml
R2(Enzyme)	one drop (50 µl)	one drop (50 µl)	one drop (50 µl)
Standard	-----	10 µl	-----
Sample	-----	-----	10 µl
Mix and incubate for at least 3 minutes at 37 °C or 5 minutes at 20-25 °C.			
R3(Alk.Reagent)	200 µl	200 µl	200 µl

Mix and incubate for 5 minutes at 37 °C or 10 minutes at 20-25 °C
Measure absorbance of specimen ($A_{specimen}$) and standard ($A_{standard}$) against reagent blank.

Procedure 2 (Using working solution)

	Blank	Standard	Specimen
Working solution	1.0 ml	1.0 ml	1.0 ml
Standard	-----	10 µl	-----
Sample	-----	-----	10 µl
Mix and incubate for at least 3 minutes at 37 °C or 5 minutes at 20 -25 °C.			
R3(Alk.Reagent)	200 µl	200 µl	200 µl

Mix and incubate for 5 minutes at 37 °C or 10 minutes at 20-25 °C.
Measure absorbance of specimen ($A_{specimen}$) and standard ($A_{standard}$) against reagent blank.

Calculation

Serum urea concentration (mg/dl) = $\frac{A_{specimen}}{A_{standard}} \times n$

where n = 50.0 mg/dl (8.33 mmol/l)

Urine urea concentration is determined by multiplying the result by the dilution factor (50).

Urea Nitrogen: To convert the result from urea to urea nitrogen multiply the result by 0.467.

Quality Control

Normal and abnormal control serum of known concentrations should be analyzed with each run.

Performance Characteristics

Precision

Within run (Repeatability)

	Level 1	Level 2
n	20	20
Mean (mg/dL)	60	144
SD	1.87	2.1
CV%	3.12	1.46

Run to run (Reproducibility)

	Level 1	Level 2
n	20	20
Mean (mg/dL)	62	146
SD	1.92	2.5
CV%	3.10	1.71

Methods Comparison

A comparison between Spectrum Diagnostics Urea/BUN reagent and a commercial reagent of the same methodology was performed on 20 human sera. A correlation of 0.97 was obtained.

Sensitivity

When run as recommended, the minimum detection limit of the assay is 0.6 mg/dL.

Linearity

The reaction is linear up to a urea concentration of (200 mg/dl) 33.3 mmol/L. Specimens showing higher concentrations should be diluted 1+2 with physiological saline and repeat the assay (result×3).

Interfering Substances

Haemolysis
Erythrocyte contamination doesn't elevate results.

Icterus
No significant interference.

Lipemia
Lipemic specimens interfere with the method of Berthlot.

Anticoagulants
Ammonium heparin should not be used.

Others
Ammonium ions should be avoided since it may cause erroneously elevated results. Color development in the Berthlot reaction is suppressed by amines, thiols, steroids and ascorbic acid.

Expected Values

Urea(Serum)

Adults ≤ 65 years : 15 – 50 mg/dL (2.5-8.33 mmol/L)
Adults ≥ 65 years : ≤ 70 mg/dL (≤11.66 mmol/L)

BUN(Serum)

Adults ≤ 65 years : 7 – 23.5 mg/dL
Adults ≥ 65 years : 7 – 32.9 mg/dL
Children : 5 – 18 mg/dL

Urine (24) hours

Urea : 20 – 35 g/24hrs (330-580 mmol/24hrs)
BUN : 9.3 – 16.4 g/24hrs

Spectrum Diagnostics does not interpret the results of a clinical laboratory procedure; interpretation of the results is considered the responsibility of qualified medical personnel. All indications of clinical significance are supported by literature references.

Analytical Range

0.6 – 200 mg/dL (0.1 - 33.3 mmol/L).

Waste Disposal

This product is made to be used in professional laboratories.
Please consult local regulations for a correct waste disposal.
S56: dispose of this material and its container at hazardous or special waste collection point.
S57: use appropriate container to avoid environmental contamination.
S61: avoid release in environment. refer to special instructions/safety data sheets.

References

1. Batton, C. J & crouch, S.R : Anal. Chem., 1977,49:464-469.
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ORDERING INFORMATION	
CATALOG NO.	QUANTITY
318 001	100 Test
318 002	200 Test
318 003	500 Test
318 004	1000 Test

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