BILIRUBIN TOTAL

Diagnostic reagent for determination of Total bilirubin concentration.

Liquid. Dual Reagents. Store at 2°C - 8°C. For in Vitro Diagnostic Use. Do not freeze

Ref No	Pack	Ref No	Pack	Ref No	Pack	Ref No	Pack
A2040	5 X 100	R2040	2885 Tests	BY2041	4091 Tests	K2040	5455 Tests
A2041	5 X 50	R2041	962 Tests	M2040	2512 Tests	K2041	3636 Tests
A2042	5 X 25	S2040	2609 Tests	M2041	1675 Tests	L2040	3750 Tests
T2040	3000 Tests	S2041	1565 Tests	N2040	800 Tests	L2041	2000 Tests
T2041	1556 Tests	BY2040	6136 Tests	N2041	400 Tests	DM2040	720 Tests

INTENDED USE

The test is applied for the quantitative determination of total bilirubin in nonhemolized serum.

TEST PRINCIPLE

Bilirubin reacts with diazotized sulphanilic acid to produce an intensely colored diazo dye (490-520 nm). The intensity of color of this dye in solution is proportional to the concentration of total bilirubin. Free bilirubin is not soluble in acqueous media, but this reagent contains an association of surfactant and accelerators in order to provide an accurate measurement of total bilirubine. The absence of dimethylsulphoxyde and urea allows a clean implementation on the majority of analyzers.

TEST PARAMETERS

Method	: Colorimetric, Increasing Reaction, Endpoint (Sample Blank)
Wavelength	: 510 nm (490-520)
Temperature	: Room temperature, 37°C
Sample	: Non-hemolyzed serum or plasma, (store light-protected)
Linearity	: 0.15 mg/dL - 20 mg/dL

REAGENT COMPOSITION

Reagent I

Sodium benzoate	≤ 0.30 M,
Sodium acetate	≤ 0.50 M,
Caffeine	≤ 0.15 M,
Surfactant.	

Reagent II

Sulphanilic acid	≤ 33 mM,
Hydrochloric acid	≤ 0.20 M.

REAGENT PREPARATION

Use separate reagent ready to use.

Stable of reagent is 30 days at 2-8°C.
Caution: keep well refrigerated.
Stability of unmixed reagents is up to expiration date on labels at 2-8°C.

Working reagents are stable at 2-8ºC in case of closed vials and avoiding contamination after preparation.

For manual working procedures; if working reagent will be used; shake the Reagent 2 vial gently before pouring its contents into the Reagent 1 bottle. It is advisable to wash the Reagent 2 vial with a small volume of the prepared mixture in order to completely rinse the vial and avoid any losses.

REAGENT STABILITY AND STORAGE

Once opened vials are stable minimum 60 days at 2-8°C at optimum conditions. On board stability is strongly related to auto analyzers cooling specification and carry-over values.

SAMPLE

Samples are collected by standard procedures.
Use only clear, unhemolyzed serum. Bilirubin is unstable in the samples and the assay should be completed within 2 hours from collection. If longer delay is unavoidable, refrigerate the samples.
Protect samples from direct solar and artificial light.

Bilirubin total in serum is stable for 15 days at 20-25°C, 7 days at 2-8ºC, 6 months at -20°C and 6 months at -80°C.

TEST PROCEDURE

Sample Start
There have many ready application procedures dedicated to different kind of photometers and ready manual working process can be supplied on request.

There have many ready application procedures dedicated to different kind of biochemistry auto analyzers can be supplied on request.

Substrate Start
There have many ready application procedures dedicated to different kind of biochemistry auto analyzers can be supplied on request.

CALCULATION

For the calculation of Bilirubin Total in mg/dl sample, perform Bilirubin Total assay and calculate as follows:

Direct Bilirubin Concentration = Fx ΔA (S-SB)

Unit Conversion

mg/dL x 17.1 = µmol/L

REFERENCE INTERVAL (NORMAL VALUES) (Based on CLSI C28-P Document)*

Adults: 0.2 - 1.0 mg/dL (3.4 - 17.1 µmol/L)
Newborns:
Up to 24 h: 2.0 - 6.0 mg/dL (34 - 103 µmol/L)
Up to 48 h: 6.0 - 10.0 mg/dL (103 - 171 µmol/L)
Days 3-5: 4.0 - 8.0 mg/dL (68 - 137 µmol/L)

*It is recommended that each laboratory establish its own reference range.

QUALITY CONTROL AND CALIBRATION

Commercially available control material with established values determined by this method may be used.
We recommend:

"ARCON N", Assayed Control Serum Normal
Cat.No. A3910

"ARCON P", Assayed Control Serum Abnormal
Cat.No. A3920

The assay requires the use of a Bilirubin Total Standard or a Bilirubin Calibrator. We recommend:

ARCAL Calibrator ("Arcal Auto")
Cat. No. A39050

Any commercially available Standard or Calibrator suitable for this method may be used.

*Calibration Stability: It is strongly depend of application to auto analyzers and auto analyzers specification. Calibration stability is 30 days.

*Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

Quality control is recommended every morning. Calibration is not recommended if QC control values are acceptable. Reagent should be calibrated after lot changes.

PERFORMANCE CHARACTERISTICS

Low linearity: 0.15 mg/dL.

High Linearity: The method is linear up to 20 mg/dL.
If the limit value is exceeded, it is suggested to dilute sample 1+9 with distilled water and to repeat the test, multiplying the result by 10.

Linearity may considerably vary depending on the instrument used.

Precision Studies (Based on CLSI EP5 Doc.):

Repeatibility (within run) (Intra-assay)

Mean conc.	SD	CV	n
1.02 mg/dL	0.02	1.5 %	10
4.21 mg/dL	0.04	0.9 %	10

Reproducibility (run to run) (Inter-assay)

Mean conc.	SD	CV	n
1.05 mg/dL	0.02	2.1 %	10
4.28 mg/dL	0.07	1.5 %	10

Sensitivity (LOD) (Based on CLSI EP17 document): The limit of detection is 0.1 mg/dL.

Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.

Methods comparison: A comparison between Archem and a commercially available product gave the following results:
Bilirubin total Archem = x
Bilirubin total competitor = y
n = 100
Y = 1.179X - 0.184 MG/DL $r^2 = 0.96$

Interferences: No interference was observed by the presence of:
Hemoglobin ≤ 50 mg/dL
Lipids ≤ 500 mg/dL

These performance characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure is used.

NOTES

1. For in vitro diagnostic use only. Do not pipette by mouth. Avoid contact with skin and mucous membranes.
2. All the calibrators, controls and some reagents must be considered as human&animal sample, so potentially infectious; all the protection actions must be applied to avoid any potential biological risk.
3. Material safety data sheet will be supplied on request.
4. Exercise the normal precautions required for handling laboratory reagents.
5. After measurements are taken, reagent bottles should cap and kept at 2-8°C. Caps of the

reagents bottles cannot be used between two different kind of reagent and between R1 & R2.
6. Reagents with different lot numbers should not be interchanged or mixed.
7. The linearity limit depends on the sample to reagent ratio.

PRECAUTIONS AND WASTE DISPOSAL

This product is made to be used in professional laboratories and by professional operators. Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.
R36/38 : Irritating to eyes and skin.
S20/21 : When using, do not eat, drink or smoke.
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 water.
S36/37/39: Wear suitable protective clothing, gloves and eye/face protection.
S45 : In case of accident or if you feel unwell, seek medical advice immediately.
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.
Please consult local regulations for a correct waste disposal.

ABBREVIATIONS

CLSI : Clinical and Laboratory Standards Institute
CV% : Coefficient of Variation Percentage
EP : Evaluation Protocols
GLP : Good Laboratory Practice
IU : International Unit
mA : miliabsorbance
mL : mililiter
NCCLS : National Committee for Clinical Laboratory Standards
QC : Quality Control

REFERENCES

1. Tietz, N.W., Fundamentals of Clinical Chemistry, p. 940, W.B. Saunders Co., Philadelphia, 1987.
2. Wu AHB. Tietz Clinical Guide to Laboratory Tests, 4th edition, Saunders Elsevier, St. Louis, MO: 2006:316.
3. Young DS. Effects of Drugs on Clinical Laboratory Tests. 3rd ed. Washington: AACC Press; 1990.
4. Tokuda K, Tanimoto K. New method of measuring serum bilirubin using vanadic acid. Jpn J Clin. Chem. 1993; 22(2):116-122.
5. Henry, R.J., Editor, Clinical Chemistry, Principles and Technics, p.1058, Harper and Row, Publishers, Hagerstown, Maryland, 1974.
6. Tietz Fundamentals of Clinical Chemistry. Burtis CA, Ashwood ER, eds. 5th ed. Philadelphia, PA: WB Saunders Company; 2001:605.
7. Young, D.S., Effects of Drugs on Clinical Laboratory Testing, p. 3.71 -3.72, AACC Press, Washington, D.C., 1990.
8. Clinical and Laboratory Standards Institute (formerly NCCLS). Protocol for Determination of Limits of Detection and Limits of Quantitation – Approved Guideline. Wayne, PA: Clinical and Laboratory Standards Institute; 2004. NCCLS Document EP17-A, Vol. 24, No. 34.
9. Van den Bergh, A.A.H. and Snapper, J., Deut. Arch. Klin. Med. 110, 540, 1913.
10. Clinical and Laboratory Standards Institute (formerly NCCLS). Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Second Edition. Wayne, PA: Clinical and Laboratory Standards Institute; 2004. NCCLS Document EP5-A2.

SYMBOLS

IVD	Only for invitro diagnostic use
LOT	Lot of manufacturing
R1	Reagent 1
R2	Reagent 2
CONC	Concentration
INGRED	Reagent Ingredients
REF	Reference Number (Catalog No)
SN	Serial Number
	Expiration date
	Storage temperature interval
	Read the directions
	Biological risk

CE

Archem Diagnostics Industry LTD. ŞTİ.
Organize Sanayi Bölgesi, Mutsan Sanayi Sitesi
M8 Blok No: 48 Başakşehir / ISTANBUL TURKEY
Tlf: + 90 212 444 08 92
Fax: +90 212 629 98 89
info@archem.com.tr
www.archem.com.tr