Clinical Chemistry

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A93A01299HEN

REF A11A01639
REAGENT 1 29.5 mL
REAGENT 2 9.8 mL

IVD CE

HORIBA ABX SAS
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FRANCE

ABX Pentra Bilirubin, Total CP

- **Pentra C400**

Diagnostic reagent for quantitative *in vitro* determination of Total Bilirubin in serum or plasma by colorimetry.

Application Release

Serum, plasma: Bili-T

1.xx (neonates: not for use in the USA)

Intended Use

ABX Pentra Bilirubin, Total CP reagent is intended for the quantitative *in vitro* diagnostic determination of total bilirubin in human serum and plasma based on a photometric test using 2,4-dichloroaniline (DCA).
Clinical laboratories use.
Measurements of the levels of bilirubin (direct or total), an organic compound formed during the normal and abnormal destruction of red blood cells, are used to aid in the diagnosis and monitoring of liver, red cell and metabolic disorders.

Clinical Interest (1, 2)

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 glucoronic acid and the resulting water soluble bilirubin glucoronides are 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. Unconjugated bilirubin can therefore be estimated as the difference between total bilirubin and direct bilirubin.

Method (3)

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.

Reagents

ABX Pentra Bilirubin, Total CP is ready-to-use.

Reagent 1:	
Phosphate buffer	50 mmol/L
NaCl	150 mmol/L
Reagent 2:	
2,4-Dichlorophenyl-diazonium salt	5 mmol/L
HCl	130 mmol/L

ABX Pentra Bilirubin, Total CP should be used according to this notice. The manufacturer cannot guarantee its performance if used otherwise.

Handling

1. Remove both caps of the cassette.

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2. If present, remove foam by using a plastic pipette.
3. Place the cassette into the refrigerated reagent compartment.

Calibrator

For calibration, use:
ABX Pentra Multical (A11A01652) (not included)
10 x 3 mL (lyophilisate)

Control

For internal quality control, use:

- **ABX Pentra N MultiControl** (1300054414) (not included)
  10 x 5 mL (lyophilisate)
- **ABX Pentra P MultiControl** (1300054415) (not included)
  10 x 5 mL (lyophilisate)

Each control should be assayed daily and/or after a calibration.
The frequency of controls and the confidence intervals should correspond to laboratory guidelines and country-specific directives. You should follow federal, state and local guidelines for testing quality control materials. The results must be within the range of the defined confidence limits. Each laboratory should establish a procedure to follow if the results exceed these confidence limits.

Materials Required but not Provided

- Automated clinical chemistry analyzer: Pentra C400
- Calibrator: **ABX Pentra Multical** (A11A01652)
- Controls:
  ABX Pentra N MultiControl (1300054414)
  ABX Pentra P MultiControl (1300054415)
- Standard laboratory equipment.

Specimen

This device intended testing population is general population.

- Serum.
- Plasma in lithium heparin.

Anticoagulants other than those listed have not been tested by HORIBA Medical and are therefore not recommended for use with this assay.

Stability (1, 4)**:**

- At 20-25°C: 1 day
- At 4-8°C: 7 days
- At -20°C: 6 months (if frozen immediately)

Freeze only once!
Discard contaminated specimens.
It is very important to store the sample protected from light!
In the case of intensive sun irradiation: decrease in total bilirubin by up to 30% after 1 hour.

Reference Range [a] (1)

Each laboratory should establish its own reference ranges. The values given here are used as guidelines only.

	[mg/dL]	[µmol/L]
Neonates (not for use in the USA):		
24 hours	< 8.7	< 150
2nd day	1.3 - 11.3	22 - 193
3rd day	0.7 - 12.7	12 - 217
4th - 6th days	0.1 - 12.6	2 - 216
Adults:	0.1 - 1.2	1.7 - 21

Clinical sensitivity and specificity, positive predictive value and negative predictive value are not commonly reported for this analyte. This is largely attributed to the fact that this analyte is not sole indicator for the intended purpose and patient treatment decision making. To arrive at a diagnosis and a course of treatment, results from others routine clinical chemistry tests should be used in conjunction with other diagnostic information and the attending health-care professional's evaluation of the patient's condition.

Storage and Stability

Stability before opening:

Stable up to the expiry date on the label if stored at 2-8°C.

Stability after opening:

Refer to the paragraph "Performance on Pentra C400".

Do not freeze.

[a]Modification: modification of reference range.

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Waste Management

Please refer to local legal requirements.

General Precautions [b]

- This reagent is for professional *in vitro* diagnostic use only.
  For laboratory use.
- For prescription use only.
- This reagent is classified as hazardous in compliance with regulation (EC) N°.1272/2008.
- **Reagent 1 and 2 (R1 and R2):**
  Danger
  H290: May be corrosive to metals.
  H314: Causes severe skin burns and eye damage.
  H318: Causes serious eye damage.
  P234: Keep only in original container.
  P260: Do not breathe dust/fume/gas/mist/vapours/spray.
  P264: Wash hands thoroughly after handling.
  P280: Wear protective gloves/protective clothing/eye protection/face protection.
  P301 + P330 + P331: IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
  P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower].
  P304 + P340: IF INHALED: Remove person to fresh air and keep comfortable for breathing.
  P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
  P310: Immediately call a POISON CENTER or doctor/physician.
  P390: Absorb spillage to prevent material damage.
  P405: Store locked up.
  P501: Dispose of contents and container in accordance with all local, regional, national and international regulations.
- **Reagent 1 (R1):**
  H400: Very toxic to aquatic life.
  H412: Harmful to aquatic life with long lasting effects.
  P273: Avoid release to the environment.
  P391: Collect spillage.
  Contains: Hydrochloric acid and Cetrimonium bromide.
- **Reagent 2 (R2):**
  Contains: Hydrochloric acid and Dodecan-1-ol, ethoxylated.
- Observe the standard laboratory precautions for use.
- The reagent cassettes are disposable and should be disposed of in accordance with the local legal requirements.
- Please refer to the SDS associated with the reagent.
- Do not use the product if there is visible evidence of biological, chemical or physical deterioration.
- Do not use the product if the recommended storage conditions, including temperature, are not followed.
- User must be trained by a HORIBA Medical representative before attempting to operate the device.
- It is the user's responsibility to verify that this document is applicable to the reagent used.
- For technical assistance, you can call +33 (0)4 67 14 15 16.
- Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the country in which the user and/or the patient is established.

Performance on Pentra C400

Lot to Lot Variability [c]

The recovery of samples (serum and plasma) done during QC release of three consecutive lots of reagent shows that the lot to lot variability is within specification:

Sample value	Specification
< 20 µmol/L	< 2 µmol/L
> 20 µmol/L	< 10%

Serum, plasma

The performance data listed below are representative of performance on HORIBA Medical Systems.

Number of tests: 130 tests
If the number of tests requested is low and the Pentra C400 user intends to utilise the cassette to the maximum on board stability, it is the recommendation of HORIBA Medical, to utilise the consumable part XEC232 (Kit membrane) to achieve the number of tests stated in this notice.

On Board Reagent Stability

Once opened, the reagent cassette placed in the refrigerated Pentra C400 compartment is stable for 25 days.

Sample volume: 8 µL/test

[b]Modification: general precautions modification.
[c]Modification: lot to lot variability specification added.

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Detection Limit

The detection limit is determined according to CLSI (NCCLS), EP17-A2 protocol (5) and equals 1.49 µmol/L (0.09 mg/dL).

Limit of Quantitation

The limit of quantitation is determined according to CLSI (NCCLS), EP17-A2 protocol (5) and equals 2.4 µmol/L (0.14 mg/dL).

Accuracy and Precision

Repeatability (within-run precision)

Repeatability according to the recommendations found in the Valtec protocol (6) with samples tested 20 times:

- 2 controls
- 5 specimens (low / medium / high levels)

	Mean value µmol/L	Mean value mg/dL	CV %
Control specimen 1	16.6	1.0	2.14
Control specimen 2	87.6	5.1	0.99
Specimen 1	10.3	0.6	3.09
Specimen 2	14.6	0.9	2.23
Specimen 3	37.7	2.2	1.33
Specimen 4	142.8	8.4	0.83
Specimen 5	312.0	18.7	0.51

Reproducibility (total precision)

Reproducibility according to the recommendations found in the CLSI (NCCLS), EP5-A2 protocol (7) with samples tested in duplicate for 20 days (2 series per day):

- 2 controls
- 3 specimens (low / medium / high levels)

	Mean value µmol/L	Mean value mg/dL	CV %
Control specimen 1	16.9	1.0	4.04
Control specimen 2	94.1	5.5	1.70
Specimen 1	13.6	0.8	5.97
Specimen 2	49.0	2.9	2.78
Specimen 3	156.1	9.1	2.20

Measuring Range

The assay confirmed a measuring range from 2.4 µmol/L (0.2 mg/dL) to 450.0 µmol/L (26.3 mg/dL).
The measuring range is extended up to 1350.0 µmol/L (79.0 mg/dL) with the automatic post-dilution.
The reagent linearity has been assessed up to 450.0 µmol/L (26.3 mg/dL) according to the recommendations found in the CLSI (NCCLS), EP06-Ed2 protocol (8).

Correlation (adult samples)

Patient samples: Serum
Number of patient samples: 101
Specimens are correlated with a commercial reagent taken as reference according to the recommendations found in the CLSI (NCCLS), EP09c protocol (9).
Values ranged from 5.75 µmol/L (0.34 mg/dL) to 441.90 µmol/L (25.85 mg/dL).
The equation for the allometric line obtained using Passing-Bablok regression procedure (10) is:
Y = 1.024 X - 2.402 (µmol/L)
Y = 1.024 X - 0.1405 (mg/dL)
with a correlation coefficient r^2 = 0.995.

Correlation (neonatal samples (not for use in the USA))

Patient samples: serum
Number of patient samples: 111
Specimens are correlated with a commercial reagent taken as reference according to the recommendations found in the CLSI (NCCLS), EP09c protocol (9).
Values ranged from 3.40 µmol/L (0.20 mg/dL) to 432.75 µmol/L (25.32 mg/dL).
The equation for the allometric line obtained using Passing-Bablok regression procedure (10) is:
Y = 0.953 X + 0.2755 (µmol/L)
Y = 0.953 X + 0.01612 (mg/dL)
with a correlation coefficient r^2 = 0.992.

Interferences [d]

Haemoglobin:	No significant influence is observed up to 290 µmol/L (500 mg/dL).
Triglycerides:	No significant influence is observed up to a triglyceride concentration of 4.40 mmol/L (385 mg/dL).
Ascorbic Acid:	No significant influence is observed up to 340 µmol/L (5.98 mg/dL).

[d]Modification: modification of interferences.

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Ibuprofen:	No significant influence is observed up to 2.43 mmol/L (50.10 mg/dL).
Acetaminophen:	No significant influence is observed up to 1324 µmol/L (20 mg/dL).
Acetylsalicylic Acid:	No significant influence is observed up to 3.62 mmol/L (65 mg/dL).
N-acetyl-p-benzoquinone imine (NAPQI):	No significant influence is observed up to 250 µmol/L (3.73 mg/dL).
Eltrombopag:	No significant influence is observed up to 30 mg/dL.

Other limitations are given by Young as a list of drugs and preanalytical variables known to affect this methodology (11, 12).

Calibration Stability

The reagent is calibrated on Day 0. The calibration stability is checked by testing 2 control specimens.
The calibration stability is 10 days.
Note: A recalibration is recommended when reagent lots change, and when quality control results fall outside the range established.

Conversion Factor

µmol/L x 0.585 = mg/L
µmol/L x 0.0585 = mg/dL

Reference

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4. Use of Anticoagulants in diagnostics laboratory investigations. WHO publication WHO/DIL/LAB/99.1 Rev.2 (2002): 24.
5. Evaluation of detection capability for clinical laboratory measurement procedures. Approved Guideline, 2nd ed., CLSI (NCCLS) document EP17-A2 (2012) **32** (8).
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8. Evaluation of Linearity of Quantitative Measurement Procedures. 2nd Edition, CLSI (NCCLS) guideline EP06-Ed2 (2020) **40** (16).
9. Measurement Procedure Comparison and Bias Estimation Using Patient Samples. Approved Guideline, 3rd ed., CLSI (NCCLS) document EP09c (2018) **38** (12).
10. Passing H, Bablok W. A new biometrical procedure for testing the equality of measurements from two different analytical methods. J. Clin. Chem. Clin. Biochem. (1983) **21**: 709-720.
11. Young DS. Effects of Drugs on Clinical Laboratory Tests. 4th Edition, Washington, DC, AACC Press (1997) **3**: 143-163.
12. Young DS. Effects of Preanalytical Variables on Clinical Laboratory Tests. 2nd Edition, Washington, DC, AACC Press (1997) **3**: 120-132.