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A93A01248DEN

ABX Pentra Bilirubin, Direct CP

REF A11A01635
REAGENT 1 24 mL
REAGENT 2 7 mL

IVD CE

HORIBA ABX SAS
Parc Euromédecine
Rue du Caducée
BP 7290
34184 Montpellier Cedex 4
FRANCE

- Pentra C200

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

Application Release

Serum, plasma: DBIL

01.xx

Intended Use

ABX Pentra Bilirubin, Direct CP reagent is intended for the quantitative *in vitro* diagnostic determination of direct bilirubin in human serum and plasma based on a photometric test using 2,4-dichloroaniline (DCA). 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 in the diagnosis and treatment of liver, hemolytic hematological, and metabolic disorders, including hepatitis and gall bladder block.

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.

Reagents

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

Reagent 1:	
EDTA-Na_2	0.1 mmol/L
NaCl	150 mmol/L
Sulfamic acid	100 mmol/L
Reagent 2:	
2,4-Dichlorophenyl-diazonium salt	0.5 mmol/L
HCl	900 mmol/L
EDTA-Na_2	0.13 mmol/L

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

ABX Pentra Bilirubin, Direct CP

Handling

1. Remove both caps of the cassette.
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 [a]

For internal quality control, use:

- **ABX Pentra N Control / ABX Pentra N MultiControl** (A11A01653 / 1300054414) (not included)
  10 x 5 mL (lyophilisate)
- **ABX Pentra P Control / ABX Pentra P MultiControl** (A11A01654 / 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 [a]

- Automated clinical chemistry analyzer: Pentra C200
- Calibrator: **ABX Pentra Multical** (A11A01652)
- Controls:
  ABX Pentra N Control / ABX Pentra N MultiControl (A11A01653 / 1300054414)
  ABX Pentra P Control / ABX Pentra P MultiControl (A11A01654 / 1300054415)
- Standard laboratory equipment.

Specimen

- 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 (4)**:**

- At 20-25°C: 2 days
- At 4-8°C: 7 days
- At -20°C: 6 months (in case of immediate freezing)

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.
Freeze only once.

Reference Range (1)

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

Adults and children: ≤ 0.2 mg/dL (≤ 3.4 µmol/L).

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 C200".

Do not freeze.

Waste Management

Please refer to local legal requirements.

General Precautions

- This reagent is for professional *in vitro* diagnostic use only.
- For prescription use only.
- This reagent is classified as hazardous in compliance with regulation (EC) N°.1272/2008.

[a]Modification: new control.

S.A.S. au capital de 23.859.980 € - RCS Montpellier 328 031 042 - SIRET 328 031 042 000 42 - APE 332 B

ABX Pentra Bilirubin, Direct CP

- **Warning**
  H290: May be corrosive to metals.
  P234: Keep only in original container.
  P390: Absorb spillage to prevent material damage.
  P406: Store in corrosive resistant container with a resistant inner liner.
- 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.
- It is the user's responsibility to verify that this document is applicable to the reagent used.

Performance on Pentra C200

Serum, plasma

The performance data listed below have been obtained on the Pentra C200 analyzer.

Number of tests: approximately 119 tests

On Board Reagent Stability

Once opened, the reagent cassette placed in the refrigerated Pentra C200 compartment is stable for 34 days.

Sample volume: 15 µL/test

Limit of Quantitation

The limit of quantitation is determined according to CLSI (NCCLS), EP17-A protocol (5) and equals 1.62 µmol/L (0.09 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
- 3 specimens (low / medium / high levels)

	Mean value µmol/L	Mean value mg/dL	CV %
Control specimen 1	13.41	0.78	1.77
Control specimen 2	35.89	2.10	2.61
Specimen 1	17.78	1.04	0.95
Specimen 2	55.61	3.25	0.94
Specimen 3	152.23	8.91	0.29

Reproducibility (total precision)

Reproductibility 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	11.57	0.68	2.32
Control specimen 2	38.83	2.27	2.12
Specimen 1	18.11	1.06	2.60
Specimen 2	53.59	3.13	3.00
Specimen 3	95.79	5.60	2.44

Measuring Range

The assay confirmed a measuring range from 1.62 µmol/L (0.09 mg/dL) to 116 µmol/L (6.79 mg/dL).
The measuring range is extended up to 580 µmol/L (33.9 mg/dL) with the automatic post-dilution.
The reagent linearity has been assessed up to 116 µmol/L (6.79 mg/dL) according to the recommendations found in the CLSI (NCCLS), EP6-A protocol (8).

Correlation

Patient samples: Serum
Number of patient samples: 106
Specimens are correlated with a commercial reagent taken as reference according to the recommendations found in the CLSI (NCCLS), EP9-A2 protocol (9).
Values ranged from 3.36 µmol/L (0.20 mg/dL) to 106.86 µmol/L (6.25 mg/dL).
The equation for the allometric line obtained using Passing-Bablock regression procedure (10) is:
$Y = 0.99\ X + 0.78$ (µmol/L)
$Y = 0.99\ X + 0.04$ (mg/dL)
with a correlation coefficient $r^2 = 0.9945$.

Interferences

Haemoglobin: Do not use hemolysed samples.

ABX Pentra Bilirubin, Direct CP

Triglycerides: No significant influence is observed up to an Intralipid® concentration (representative of lipemia) of 7 mmol/L (612.5 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 13 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. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag, (2001): 18-9.
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6. Vassault A, Grafmeyer D, Naudin C et al. Protocole de validation de techniques (document B). Ann. Biol. Clin. (1986) **44**: 686-745.
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9. Method Comparison and Bias Estimation Using Patient Samples. Approved Guideline, 2nd ed., CLSI (NCCLS) document EP9-A2 (2002) **22** (19).
10. Passing H, Bablock W. A new biometrical procedure for testing the equality of measurements from two different analytical methods. J. Clin. Chem. Clin. Biochem. (1983) **21**: 709-20.
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