Clinical Chemistry

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REF A11A01635
REAGENT 1 24 mL
REAGENT 2 7 mL

IVD CE Rx Only

HORIBA ABX SAS
Parc Euromédecine
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FRANCE

ABX Pentra Bilirubin, Direct CP

- Pentra C400

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

Application Release

Serum, plasma: Bili-D

1.xx

Intended Use [a]

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.

[a]Modification: new leaflet form.

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Handling

1. Remove both caps of the cassette.
2. If present, remove foam by using a plastic pipette.
3. Position the protective cap (GBM0969) on the cassette.
4. Place the cassette into the refrigerated reagent compartment.

Calibrator

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

Control [b]

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 [b]

- 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 [c]

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 (4, 5, 1)

- 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) [d]

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).

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.

[b]Modification: control removed.
[c]Modification: modification of "Specimen".
[d]Modification: information added.

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Stability after opening:

Refer to the paragraph "Performance on Pentra C400".

Do not freeze.

Waste Management

Please refer to local legal requirements.

General Precautions [e]

- 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.
- **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.
- 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 [f]

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.

Serum, plasma

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

Number of tests: 100 tests

On Board Reagent Stability

Once opened, the reagent cassette placed in the refrigerated Pentra C400 compartment is stable for 30 days.

Sample volume: 25 µL/test

Detection Limit [g]

The detection limit is determined according to CLSI (NCCLS), EP17-A2 protocol (6) and equals 0.41 µmol/L (0.02 mg/dL).

Limit of Quantitation [h]

The limit of quantitation is determined according to CLSI (NCCLS), EP17-A2 protocol (6) and equals 2.70 µmol/L (0.16 mg/dL).

Accuracy and Precision

Repeatability (within-run precision)

Repeatability according to the recommendations found in the Valtec protocol (7) 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	15.33	0.90	0.67
Control specimen 2	31.64	1.85	0.44

[e]Modification: general precautions modification.
[f]Modification: chapter added.
[g]Modification: modification of detection limit.
[h]Modification: data added.

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	Mean value µmol/L	Mean value mg/dL	CV %
Specimen 1	4.01	0.23	3.23
Specimen 2	25.92	1.52	0.59
Specimen 3	134.63	7.88	2.69

Reproducibility (total precision)

Reproducibility according to the recommendations found in the CLSI (NCCLS), EP5-A2 protocol (8) with samples tested in duplicate for 20 days (2 series per day):

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

	Mean value µmol/L	Mean value mg/dL	CV %
Control specimen 1	16.0	0.94	4.26
Control specimen 2	34.9	2.02	4.22
Specimen 1	11.7	0.69	3.27
Specimen 2	65.4	3.83	2.98

Measuring Range [i]

The assay confirmed a measuring range from 2.7 µmol/L (0.16 mg/dL) to 116.0 µmol/L (6.79 mg/dL).
The measuring range is extended up to 580 µmol/L (33.90 mg/dL) with the automatic post-dilution.
The reagent linearity has been assessed up to 116.0 µmol/L (6.79 mg/dL) according to the recommendations found in the CLSI (NCCLS), EP06-Ed2 protocol (9).

Correlation [j]

Patient samples: Serum and plasma
Number of patient samples: 92
Specimens are correlated with a commercial reagent taken as reference according to the recommendations found in the CLSI (NCCLS), EP09c protocol (10).
Values ranged from 2.75 µmol/L (0.16 mg/dL) to 115.13 µmol/L (6.74 mg/dL).
The equation for the allometric line obtained using Passing-Bablok regression procedure (11) is:
Y = 1.026 X + 1.3 (µmol/L)
Y = 1.026 X + 0.076 (mg/dL)
with a correlation coefficient r^2 = 0.992.

Interferences [k]

Haemoglobin:	Do not use hemolysed samples.
Triglycerides:	No significant influence is observed up to a triglyceride concentration of 4.51 mmol/L (395 mg/dL).
N-acetyl-p-benzoquinone imine (NAPQI):	No significant influence is observed up to 125 µmol/L (1.86 mg/dL).
Eltrombopag:	Do not use this assay if patient is under treatment with Eltrombopag.

Other limitations are given by Young as a list of drugs and preanalytical variables known to affect this methodology (12, 13).

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

1. Thomas L. ed. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft (1998): 192-202.
2. Tolman KG, Rej R. Liver function. In: Burtis C.A., Ashwood E.R., editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: WB Saunders Company (1999): 1125-1177.
3. Rand RN, Di Pasqua A. A new diazo method for the determination of bilirubin. Clin. Chem. (1962) **6**: 570-8.
4. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag, (2001): 18-19.
5. Use of Anticoagulants in diagnostics laboratory investigations. WHO publication WHO/DIL/LAB/99.1 Rev.2 (2002): 24.
6. Evaluation of detection capability for clinical laboratory measurement procedures. Approved Guideline, 2nd ed., CLSI (NCCLS) document EP17-A2 (2012) **32** (8).

[i]Modification: modification of measuring range.
[j]Modification: modification of correlation.
[k]Modification: modification of interferences.

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7. Vassault A, Grafmeyer D, Naudin C et al. Protocole de validation de techniques (document B). Ann. Biol. Clin. (1986) **44**: 686-745.
8. Evaluation of Precision Performance of Quantitative Measurement Method. Approved Guideline, CLSI (NCCLS) document EP5-A2 (2004) **24** (25).
9. Evaluation of Linearity of Quantitative Measurement Procedures. 2nd Edition, CLSI (NCCLS) guideline EP06-Ed2 (2020) **40** (16).
10. Measurement Procedure Comparison and Bias Estimation Using Patient Samples. Approved Guideline, 3rd ed., CLSI (NCCLS) document EP09c (2018) **38** (12).
11. 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.
12. Young DS. Effects of Drugs on Clinical Laboratory Tests. 4th Edition, Washington, DC, AACC Press (1997) **3**: 143-163.
13. Young DS. Effects of Preanalytical Variables on Clinical Laboratory Tests. 2nd Edition, Washington, DC, AACC Press (1997) **3**: 120-132.