Clinical Chemistry

2023/09/28
A93A01238JEN

ABX Pentra Iron CP

REF A11A01637
REAGENT 1 60 mL
REAGENT 2 20 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 Iron in serum or plasma by colorimetry.

Application Release

Serum, plasma: IRON

01.xx

Intended Use

ABX Pentra Iron CP reagent is intended for the quantitative *in vitro* diagnostic determination of iron (non-heme) in human serum and plasma based on a photometric test (Ferene method). Iron (non-heme) measurements are used in the diagnosis and treatment of diseases such as iron deficiency anemia and hemochromatosis.

Clinical Interest (1, 2)

Iron exists in the body as a component of hemoglobin and myoglobin as well as bound to transferrin for the transport in plasma and stored in ferritin. Increased iron concentrations occur in hemochromatosis and liver damage. Decreased iron levels can be caused by anemia due to malabsorption as consequence of gastrointestinal diseases or by blood loss as a result of gastrointestinal lesions or heavy menstrual bleeding. For the estimation of the body iron status the measurement of transferrin and ferritin can provide more detailed information.

Method (3, 4)

Photometric test using Ferene.
Iron bound to transferrin is released in an acidic medium as ferric iron and is then reduced to ferrous iron in the presence of ascorbic acid. Ferrous iron forms a blue complex with Ferene. The absorbance at 595 nm is directly proportional to the iron concentration.

$$\text{Transferrin}(Fe^{3+})_2 \xrightarrow{\text{Ascorbic acid, Buffer}} 2\,Fe^{2+} + \text{Transferrin}$$

$$Fe^{2+} + 3\,\text{Ferene} \longrightarrow \text{Ferrous Ferene (blue complex)}$$

Reagents [a]

ABX Pentra Iron CP is ready-to-use.

Reagent 1 (R1):	
Acetate buffer pH 4.5	1 mol/L
Thiourea	120 mmol/L
Reagent 2 (R2):	
Ascorbic acid	240 mmol/L
Ferene	3 mmol/L
Thiourea	120 mmol/L

ABX Pentra Iron 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.
2. If present, remove foam by using a plastic pipette.
3. Place the cassette into the refrigerated Pentra C200 reagent compartment.

[a]Modification: § "Reagents": modification.

ABX Pentra Iron CP

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 C200
- 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.

Specimen types

- Serum.
- Plasma in lithium heparin (Do not freeze).

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

Separate serum at the latest 2 hours after blood collection to minimize hemolysis.
Centrifuge the heparinized blood for at least 15 minutes at 2000 to 3000 g (5).

Stability (6)

- At 20-25°C: 7 days
- At 4-8°C: 3 weeks
- At -20°C: 1 year

Reference Range (7) [d]

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

Children:	**µg/dL**	**µmol/L**
2 weeks	63 - 201	11 - 36
6 months	28 - 135	5 - 24
12 months	35 - 155	6 - 28
2 -12 years	22 - 135	4 - 24

Women:	**µg/dL**	**µmol/L**
25 years	37 - 165	6.6 - 29.5
40 years	23 - 134	4.1 - 24.0
60 years	39 - 149	7.0 - 26.7

Pregnant women:	**µg/dL**	**µmol/L**
12th gestational week	42 - 177	7.6 - 31.6
At term	25 - 137	4.5 - 24.5
6 weeks postpartum	16 - 150	2.9 - 26.9

Men:	**µg/dL**	**µmol/L**
25 years	40 - 155	7.2 - 27.7
40 years	35 - 168	6.3 - 30.1
60 years	40 - 120	7.2 - 21.5

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

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

ABX Pentra Iron CP

attending health-care professional's evaluation of the patient's condition.

Storage and Stability [e]

Stability before opening:

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

Stability after opening:

Refer to the paragraph "Performance on Pentra C200".

Do not freeze.

Waste Management

Please refer to local legal requirements.

General Precautions [f]

- 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: Danger**
  H315: Causes skin irritation.
  H318: Causes serious eye damage.
  P264: Wash hands thoroughly after handling.
  P280: Wear protective gloves/protective clothing/eye protection/face protection.
  P310: Immediately call a POISON CENTER or doctor/ physician.
  P302 + P352: IF ON SKIN: Wash with plenty of soap and water.
  P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
  Contains: Acetic acid, Dodecan-1-ol, ethoxylated and Alcohols, C9-11-iso-, C10-rich, ethoxylated.
- Use only disposable material to avoid iron contamination. Rinse glass material with diluted HCl and copious distilled water.
- In very rare cases, samples of patients with gammopathy might give false results (8).
- 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 C200

Lot to Lot Variability [g]

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
< 15 µmol/L	+/- 2 µmol/L
> 15 µmol/L	+/- 10%

Serum, plasma

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

Number of tests: approximately 354 tests

On Board Reagent Stability

Once opened, the reagent cassette placed in the refrigerated Pentra C200 compartment is stable for 99 days.

Sample volume: 22 µL/test

[e]Modification: modification of storage and stability.
[f]Modification: general precautions modification.
[g]Modification: chapter added.

ABX Pentra Iron CP

Detection Limit [h]

The detection limit is determined according to CLSI (NCCLS), EP17-A protocol (9) and equals 0.47 µmol/L (2.62 µg/dL).

Limit of Quantitation [i]

The limit of quantitation is determined according to CLSI (NCCLS), EP17-A2 protocol (10) and equals 2.60 µmol/L (15 µg/dL).

Accuracy and Precision

Repeatability (within-run precision)

Repeatability according to the recommendations found in the Valtec protocol (11) with samples tested 20 times:

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

	Mean value µmol/L	Mean value µg/dL	CV %
Control specimen 1	20.2	112.86	1.36
Control specimen 2	29.3	163.72	0.68
Specimen 1	4.9	27.48	3.48
Specimen 2	20.2	112.80	1.37
Specimen 3	40.8	227.72	0.99

Reproducibility (total precision)

Reproducibility according to the recommendations found in the CLSI (NCCLS), EP5-A2 protocol (12) 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 µg/dL	CV %
Control specimen 1	20.57	114.78	4.7
Control specimen 2	29.94	167.09	4.2
Specimen 1	4.93	27.50	7.1
Specimen 2	20.63	115.11	4.4
Specimen 3	41.91	233.88	3.6

Measuring Range [j]

The assay confirmed a measuring range from 2.60 µmol/L (15 µg/dL) to 180 µmol/L (1004.4 µg/dL).
The measuring range is extended up to 900 µmol/L (5020 µg/dL) with the automatic post-dilution.
The reagent linearity has been assessed up to 180 µmol/L (1004.4 µg/dL) according to the recommendations found in the CLSI (NCCLS), EP06-Ed2 protocol (13).

Correlation [k]

Patient samples: Serum
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 (14).
Values ranged from 4.30 µmol/L (23.99 µg/dL) to 167.9 µmol/L (936.88 µg/dL).
The equation for the allometric line obtained using Passing-Bablok regression procedure (15) is:
Y = 1.044 X - 0.8378 (µmol/L)
Y = 1.044 X - 4.67 (µg/dL)
with a correlation coefficient r^2 = 0.998.

Interferences [l]

Haemoglobin: No significant influence is observed up to 100 µmol/L (172 mg/dL).
Triglycerides: No significant influence is observed up to a triglyceride concentration of 4.98 mmol/L (436 mg/dL).
Total Bilirubin: No significant influence is observed up to 500 µmol/L (29.3 mg/dL).
Direct Bilirubin: No significant influence is observed up to 350 µmol/L (20.5 mg/dL).
An interference was observed with patient samples treated with calcium heparinate.

Other limitations are given by Young as a list of drugs and preanalytical variables known to affect this methodology (16, 17).

Calibration Stability

The reagent is calibrated on Day 0. The calibration stability is checked by testing 2 control specimens.

[h]Modification: data added.
[i]Modification: modification of quantitation limit.
[j]Modification: modification of measuring range.
[k]Modification: modification of correlation.
[l]Modification: modification of interferences.

ABX Pentra Iron CP

The calibration stability is 63 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 5.58 = µg/dL
µmol/L x 0.0558 = mg/L

Reference

1. Wick M. Iron metabolism and its disorders. In: Thomas L., editor. Clinical laboratory diagnostics. 1st ed. Frankfurt: T.H.-Books Verlagsgesellschaft (1998): 268-73.
2. Fairbanks VF, Klee GG. Biochemical aspects of hematology. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company (1999): 1642-1710.
3. Higgins T. Novel chromogen for serum iron determinations. Clin. Chem. (1981) **27**: 1619.
4. Artiss JD, Vinogradov S, Zak B. Spectrophotometric study of several sensitive reagents for serum iron. Clin. Biochem. (1981) **14**: 311-15.
5. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag (2001): 8.
6. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag (2001): 34-35.
7. Thomas L. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: THBooks Verlagsgesellschaft (1998): 273-5.
8. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed, (2007) **45** (9): 1240-1243.
9. Protocols for determination of limits of detection and limits of quantitation. Approved Guideline, CLSI (NCCLS) document EP17-A (2004) **24** (34).
10. Evaluation of detection capability for clinical laboratory measurement procedures. Approved Guideline, 2nd ed., CLSI (NCCLS) document EP17-A2 (2012) **32** (8).
11. Vassault A, Grafmeyer D, Naudin C et al. Protocole de validation de techniques (document B). Ann. Biol. Clin. (1986) **44**: 686-745.
12. Evaluation of Precision Performance of Quantitative Measurement Method. Approved Guideline, CLSI (NCCLS) document EP5-A2 (2004) **24** (25).
13. Evaluation of Linearity of Quantitative Measurement Procedures. 2nd Edition, CLSI (NCCLS) guideline EP06-Ed2 (2020) **40** (16).
14. Measurement Procedure Comparison and Bias Estimation Using Patient Samples. Approved Guideline, 3rd ed., CLSI (NCCLS) document EP09c (2018) **38** (12).
15. 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.
16. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th Edition, Washington, DC, AACC Press (2000).
17. Young DS. Effects of Preanalytical Variables on Clinical Laboratory Tests. 2nd Edition, Washington, DC, AACC Press (1997) **3**: 120-132.