Clinical Chemistry

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REAGENT 61 mL

IVD CE

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ABX Pentra Total Protein CP

- **Pentra C200**

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

Application Release

Serum, plasma: TP

01.xx

Intended Use

ABX Pentra Total Protein CP reagent is intended for the quantitative *in vitro* diagnostic determination of total protein in serum and plasma by colorimetry.
Measurements obtained by this device are used in the diagnosis and treatment of a variety of diseases involving the liver, kidney, or bone marrow as well as other metabolic or nutritional disorders.

Clinical Interest (1, 2)

Blood plasma is a concentrated solution of proteins, 60% of which is albumin. Considered in their entirety, plasma proteins perform very different tasks ranging from the maintenance of oncotic pressure to the transport of various molecules. They are involved in the complex mechanisms of blood coagulation and immunological reactions against antibodies. Enzymes, contained at low levels, constitute one group of the various proteins. An increase in their activity is a reliable indicator for cell injuries.
The variations of the global level of proteins thus present a value of diagnostic orientation, which however should be completed with a more specific balance.
Hypoproteinaemias reflect low levels of albumin linked to an abnormal renal protein escape, a protein synthesis defect (hepatic insufficiency) or a deficiency disease.
Hyperproteinaemias are notably observed in connection with dehydration symptoms, but they can also result from dysglobulinaemia or a myeloma.

Method

Colorimetric test for the quantitative determination of total proteins in serum and plasma. This end point methodology, simple, rapid and precise, has been developed and improved upon by Gornall *et al.* (1949) (3) by using the Biuret reaction.
This Biuret reaction has been previously studied, simplified using a single working reagent (4) and improved by increasing the stability of the Biuret reagent with the addition of ethylene glycol (5), tartrate (6) or citrate (7).
This methodology is based on the formation, in alcaline solution, and in the presence of copper ions, of a caracteristic purple colored complex between the Biuret (NH_2-CO-NH-CO-NH_2) and two consecutive peptidic connections.
The resulting coordination complex obtained absorbs mostly in the blue colour. The intensity of the colouring is directly proportional to the protein concentration.

Proteins + Cu^{2+} ⟶ Coloured complex

NB: Sodium and potassium tartrate prevent the precipitation of copper hydroxide and potassium iodide prevents self-reduction of copper from happening.

Reagents

ABX Pentra Total Protein CP is ready-to-use.

Reagent:	
Potassium iodide	6 mmol/L
Potassium sodium tartrate	21 mmol/L
Copper sulphate	6 mmol/L
Sodium hydroxide	58 mmol/L

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

Handling

1. Remove the cap 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 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 [a]

- 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 [b]

This device intended testing population is general population.

Specimen types

- Non-haemolysed 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.
Note: The reference range chosen depends on the user's choice of matrix.
Refer to Reference Range paragraph.

Stability (1)

- In closed tube at room temperature: up to 1 week
- At 4-8°C: up to 1 month
- In deep-frozen state: > 1 year

Reference Range [c]

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

Values for serum specimens (2):

Ambulatory patients:	64 - 83 g/L
	6.4 - 8.3 g/dL
Recumbent patients:	60 - 78 g/L
	6.0 - 7.8 g/dL

Serum and plasma can be used for total protein determination. Due to fibrinogen, the mean total protein concentration in plasma is higher than in serum, and specifically as shown below (1):

Blood origin	**Protein concentration increase from serum to plasma**
Blood donors:	+ 2.5 g/L
Nonhospitalized patients:	+ 3.6 g/L
Hospitalized patients:	+ 4.6 g/L
Hospitalized patients with CRP >50mg/dL:	+ 6.6 g/L

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

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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 C200".

Waste Management

Please refer to local legal requirements.

General Precautions [d]

- 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.
  H412: Harmful to aquatic life with long lasting effects.
  P234: Keep only in original container.
  P273: Avoid release to the environment.
  P390: Absorb spillage to prevent material damage.
  P406: Store in corrosive resistant container with a resistant inner liner.
- 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 [e]

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: < 10%.

Serum, plasma

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

Number of tests: approximately 258 tests

On Board Reagent Stability

Once opened, the reagent cassette placed in the refrigerated Pentra C200 compartment is stable for 21 days.

Sample volume: 2 µL/test

Detection Limit [f]

The detection limit is determined according to CLSI (NCCLS), EP17-A2 protocol (8) and equals 0.72 g/L (0.07 g/dL).

Limit of Quantitation

The limit of quantitation is determined according to CLSI (NCCLS), EP17-A2 protocol (8) and equals 4.1 g/L (0.41 g/dL).

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[d]Modification: general precautions modification.
[e]Modification: chapter added.
[f]Modification: data added.

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Accuracy and Precision

Repeatability (within-run precision)

Repeatability according to the recommendations found in the Valtec protocol (9) with samples tested 20 times:

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

	Mean value g/L	Mean value g/dL	CV %
Control specimen 1	68.95	6.90	0.79
Control specimen 2	50.98	5.10	1.51
Specimen 1	42.75	4.27	1.17
Specimen 2	64.65	6.46	1.05
Specimen 3	82.92	8.29	0.95
Specimen 4	99.18	9.92	0.58

Reproducibility (total precision)

Reproducibility according to the recommendations found in the CLSI (NCCLS), EP5-A2 protocol (10) with samples tested in duplicate for 20 days (2 series per day):

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

	Mean value g/L	Mean value g/dL	CV %
Control specimen 1	68.25	6.83	2.2
Control specimen 2	50.09	5.01	2.4
Specimen 1	42.58	4.26	2.9
Specimen 2	63.96	6.40	2.3
Specimen 3	83.66	8.37	2.2

Measuring Range [g]

The assay confirmed a measuring range from 4.1 g/L (0.41g/dL) to 118 g/L (11.8 g/dL).
The measuring range is extended up to 236 g/L (23.6 g/dL) with the automatic post-dilution.
The reagent linearity has been assessed up to 118 g/L (11.8 g/dL) according to the recommendations found in the CLSI (NCCLS), EP06-Ed2 protocol (11).

Correlation [h]

Patient samples: Serum and plasma

Number of patient samples: 103
Specimens are correlated with a commercial reagent taken as reference according to the recommendations found in the CLSI (NCCLS), EP09c protocol (12).
Values ranged from 5.32 g/L (0.53 g/dL) to 93.75 g/L (9.38 g/dL).
The equation for the allometric line obtained using Passing-Bablok regression procedure (13) is:
Y = 0.9739 X + 2.132 (g/L)
Y = 0.9739 X + 0.2132 (g/dL)
with a correlation coefficient r^2 = 0.987.

Interferences [i]

Haemoglobin:	No significant influence is observed up to 100 µmol/L (172 mg/dL).
Haemoglobin:	Do not use hemolysed samples.
Triglycerides:	No significant influence is observed up to a triglyceride concentration of 2.95 mmol/L (258 mg/dL).
Total Bilirubin:	No significant influence is observed up to 750 µmol/L (43.9 mg/dL).
Direct Bilirubin:	No significant influence is observed up to 600 µmol/L (35.1 mg/dL).

Other limitations are given by Young as a list of drugs and preanalytical variables known to affect this methodology (14, 15).

Calibration Stability

The reagent is calibrated on Day 0. The calibration stability is checked by testing 2 control specimens.
The calibration stability is 7 days.
Note: A recalibration is recommended when reagent lots change, and when quality control results fall outside the range established.

Reference

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[g]Modification: modification of measuring range.
[h]Modification: modification of correlation.
[i]Modification: modification of interferences.

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