Clinical Chemistry

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REAGENT 99 mL

IVD CE

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ABX Pentra Albumin CP

- Pentra C400

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

Application Release

Serum, plasma: Alb

1.xx

Intended Use

ABX Pentra Albumin CP reagent is intended for the quantitative *in vitro* diagnostic determination of albumin in human serum and plasma by colorimetry.
Albumin measurements are used in the diagnosis and treatment of numerous diseases involving primarily the liver or kidneys.

Clinical Interest (1)

Albumin is the main component of plasmatic proteins. Its essential role is the maintenance of osmotic pressure. It also assures the fixation and transport of a large number of products. The albumin serum constitutes a predictive factor in the alteration of the transport of bilirubin, calcium and hormones due to a deteriorated functioning of the liver and/or due to inflammations.
A relative increase of plasmatic albumin is observed in states of dehydration. The decreases are the result of malnutrition, synthesis alteration (hepatic pathologies) or a serious loss of albumin by the organism (traumas, burns, haemorrhages, diarrhea, nephrotic syndromes).

Method

Colorimetric test for the quantitative determination of albumin in serum and plasma using Bromocresol Green dye-binding procedure.
This method allows to measure out simply and quickly albumin, unlike electrophoresis or salt fractionation determinations which are not very convenient in laboratories.
The principle of this test has been discovered by Klotz and Walker (1947) (2), while they were studying the link between Bovine Serum Albumin and Bromocresol green.
Rodkey, in the 1965s, after having transposed his works on the Human Serum Albumin (3), had proposed a methodology in which the optical density (OD) variation was directly proportional to the albumin concentration (4).
But the OD of the reagent, which was too high, made this determination out of reach of most spectrophotometers.
Furthermore, interferences with globulin fractions could be responsible of albumin overestimations in the low concentration range with the initial methodology (5).
Later, new methodologies at different pH (6, 7), faster time reading (8) and the use of Brij35 (9) has allowed the development of reliable, more specific (8) and precise manual or automatic methods within the reach of many analysers (7, 9, 10).

At pH 4.20, in succinate buffer and with a nonionic surfactant Brij35, the Bromocresol Green (BCG) fixes itself selectively to the albumin of the sample, producing a blue colour which is measured at 628 nm. The intensity of the colouring is directly proportional to the albumin concentration (10, 11).

$$\text{Albumin} + \text{BCG} \xrightarrow{\text{pH} = 4.20} \text{Albumin-BCG complex}$$

Reagents

ABX Pentra Albumin CP is ready-to-use.

Reagent:	
Succinate buffer	87 mmol/L
Bromocresol green	0.2 mmol/L
Brij 35	7.35 mL/L

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ABX Pentra Albumin 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 C400
- 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

- 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 (12)
Albumin, in serum, is reported stable for 1 week at room temperature (18-30°C) and approximately 1 month when stored in the refrigerator (2-8°C) and protected against evaporation.

Reference Range (13) [c]

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

0 - 4 days:	2.8 - 4.4 g/dL	28 - 44 g/L
4 days - 14 years:	3.8 - 5.4 g/dL	38 - 54 g/L
14 - 18 years:	3.2 - 4.5 g/dL	32 - 45 g/L
20 - 60 years:	3.5 - 5.2 g/dL	35 - 52 g/L
60 - 90 years:	3.2 - 4.6 g/dL	32 - 46 g/L
> 90 years:	2.9 - 4.5 g/dL	29 - 45 g/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.

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

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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".

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 non-hazardous in compliance with regulation (EC) N°.1272/2008.
- Do not pipette by mouth.
- Do not replenish the reagents.
- Do not swallow. Avoid contact with skin and mucous membranes.
- 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 [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: < 8%.

Serum, plasma

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

Number of tests: 327 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 XEC083 (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 83 days.

Sample volume: 2 µL/test

Detection Limit [f]

The detection limit is determined according to CLSI (NCCLS), EP17-A2 protocol (14) and equals 7.35 µmol/L (0.05 g/dL).

Limit of Quantitation [g]

The limit of quantitation is determined according to CLSI (NCCLS), EP17-A2 protocol (14) and equals 13 µmol/L (0.09 g/dL).

Accuracy and Precision

Repeatability (within-run precision)

Repeatability according to the recommendations found in the Valtec protocol (15) with samples tested 20 times:

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

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

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	Mean value µmol/L	Mean value g/dL	CV %
Control specimen 1	514.0	3.39	0.59
Control specimen 2	505.9	3.34	0.84
Specimen 1	348.4	2.30	0.44
Specimen 2	628.4	4.15	0.47
Specimen 3	848.0	5.60	0.83

Reproducibility (total precision)

Reproducibility according to the recommendations found in the CLSI (NCCLS), EP5-A2 protocol (16) 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 g/dL	CV %
Control specimen 1	514.8	3.40	1.3
Control specimen 2	501.6	3.31	1.0
Specimen 1	356.5	2.35	1.7
Specimen 2	643.4	4.25	1.9

Measuring Range [h]

The assay confirmed a measuring range from 13 µmol/L (0.09 g/dL) to 848.0 µmol/L (5.60 g/dL).
The measuring range is extended up to 1696.0 µmol/L (11.20 g/dL) with the automatic post-dilution.
The reagent linearity has been assessed up to 848.0 µmol/L (5.60 g/dL) according to the recommendations found in the CLSI (NCCLS), EP06-Ed2 protocol (17).

Correlation [i]

Patient samples: Serum
Number of patient samples: 136
Specimens are correlated with a commercial reagent taken as reference according to the recommendations found in the CLSI (NCCLS), EP09c protocol (18).
Values ranged from 69.7 µmol/L (0.46 g/dL) to 818.2 µmol/L (5.40 g/dL).
The equation for the allometric line obtained using Passing-Bablok regression procedure (19) is:
Y = 0.9475 X + 4.121 (µmol/L)

Y = 0.9475 X + 0.02724 (g/dL)
with a correlation coefficient r^2 = 0.989.

Interferences [j]

Haemoglobin: No significant influence is observed up to 174 µmol/L (300 mg/dL).
Triglycerides: No significant influence is observed up to a triglyceride concentration of 5.42 mmol/L (474.25 mg/dL).
Total Bilirubin: No significant influence is observed up to 615 µmol/L (36 mg/dL).
Direct Bilirubin: No significant influence is observed up to 615 µmol/L (36 mg/dL).
Ampicillin has been found to seriously interfere with BCG methods (20).

Other limitations are given by Young as a list of drugs and preanalytical variables known to affect this methodology (21, 22).

Calibration Stability

The reagent is calibrated on Day 0. The calibration stability is checked by testing 2 control specimens.
The calibration stability is 14 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 0.066 = g/L
µmol/L x 0.0066 = g/dL

Reference

1. Thomas L. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: THBooks Verlagsgesellschaft (1998): 652-653.
2. Klotz IM and Walker FM, J. Phys. Colloid. Chem. (1947) **51**: 666.
3. Rodkey FL. Arch. Biochem. Biophys. (1964) **108**: 510.
4. Rodkey FL, Clin. Chem. (1965) **11**: 478.
5. Webster D. A study of the interaction of bromocresol Green with isolated serum globulin fractions. Clin. Chim. Acta (1974) **53**:109-115.
6. Bartholomew RJ and Delaney AM. Proc. Austral. Assoc. Clin. Biochem. (1966) **1**: 214.
7. Hernandez O, Murray L and Doumas B. Clin. Chem. (1967) **13**: 701.

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

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8. Gustafsson Jan EC. Improved specificity of serum albumin determination and estimation of «Acute phase reactants» by use of the bromocresol green reaction. Clin. Chem. (1976) **22**: 616-622.
9. Dow D and Pinto PVC. Clin. Chem. (1969) **15**: 1006.
10. Doumas BT, Watson WA and Biggs HG. Albumin standards and the measurement of serum albmin with bromocresol green. Clin. Chim. Acta (1971) **31** (1): 87-96.
11. Drupt F. Dosage de l'albumine sérique par le vert de bromocrésol. Pharm. Biol. (1974) **9**: 777.
12. Doumas BT, Biggs HG. Standard Methods of Clinical Chemistry, Academic Press, NY. (1972) **7**: 175.
13. Roberts WL, McMillin GA, Burtis CA, Bruns DE. Reference Information for the Clinical Laboratory. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th Ed., Burtis CA, Ashwood ER, Bruns DE. (Elsevier Saunders eds. St Louis USA) (2006): 2254.
14. Evaluation of detection capability for clinical laboratory measurement procedures. Approved Guideline, 2nd ed., CLSI (NCCLS) document EP17-A2 (2012) **32** (8).
15. Vassault A, Grafmeyer D, Naudin C et al. Protocole de validation de techniques (document B). Ann. Biol. Clin. (1986) **44**: 686-745.
16. Evaluation of Precision Performance of Quantitative Measurement Method. Approved Guideline, CLSI (NCCLS) document EP5-A2 (2004) **24** (25).
17. Evaluation of Linearity of Quantitative Measurement Procedures. 2nd Edition, CLSI (NCCLS) guideline EP06-Ed2 (2020) **40** (16).
18. Measurement Procedure Comparison and Bias Estimation Using Patient Samples. Approved Guideline, 3rd ed., CLSI (NCCLS) document EP09c (2018) **38** (12).
19. 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.
20. Beng CG, Lim KL. An improved automated method for determination of serum albumin using bromocresol green. Am. J. Clin. Path. (1973) **59**:14.
21. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th Edition, Washington, DC, AACC Press (2000).
22. Young DS. Effects of Preanalytical Variables on Clinical Laboratory Tests. 2nd Edition, Washington, DC, AACC Press (1997) **3**: 120-132.