Clinical Chemistry

2023/10/05
A93A01254FEN

REF A11A01642

REAGENT 29 mL

IVD CE

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

ABX Pentra Urinary Proteins CP

- **Pentra C200**

Diagnostic reagent for quantitative *in vitro* determination of Total Proteins in urine by colorimetry.

Application Release

Urine: TPU
01.xx

Intended Use

ABX Pentra Urinary Proteins CP reagent is intended for the quantitative *in vitro* diagnostic determination of urinary proteins in urine.
Identification of urinary protein is used in the diagnosis and treatment of disease conditions such as renal or heart diseases or thyroid disorders, which are characterized by proteinuria or albuminuria.

Clinical Interest (1, 2)

Elevated concentration of total protein in urine (proteinuria) can be detected in the majority of kidney diseases. Primary and secondary nephropathies may cause increased glomerular permeability or decreased tubular reabsorption. Post-renal causes of proteinuria are infections, bleedings or malignant diseases of the urinary tract. Elevated urine protein levels can also be related to other acute disorders like fever.

Method

The total protein test for urine is based on the procedure developed by Watanabe *et al.* (3) which is a dye-binding colorimetric method utilizing pyrogallol red-molybdate complex. This photometric test which provides good precision and linearity, has been modified to equalize the reactivity of albumin and gamma-globulin (4).
The pyrogallol red is combined with molybdenum acid, forming a red complex with maximum absorbance at 467 nm. When this complex is combined with protein in acidic conditions, a blue-purple color develops with an increase in absorption at 598 nm (3).
The color is directly proportional to the protein concentration.

Reagents

ABX Pentra Urinary Proteins CP is ready-to-use.

Reagent:	
Pyrogallol red	60 µmol/L
Sodium molybdate	40 µmol/L
Detergents	

ABX Pentra Urinary Proteins 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 TPU Cal (A11A01898) (not included)
3 x 3 mL

ABX Pentra Urinary Proteins CP

Control [a]

For internal quality control, use:

- **Yumizen C Urine Level 1 Control** (1300023946) (not included)
  6 x 5 mL
- **Yumizen C Urine Level 2 Control** (1300023947) (not included)
  6 x 5 mL

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 TPU Cal** (A11A01898)
- Controls:
  Yumizen C Urine Level 1 Control (1300023946)
  Yumizen C Urine Level 2 Control (1300023947)
- Standard laboratory equipment.

Specimen [b]

This device intended testing population is general population.

Specimen types

- Urine.

Stability (5)

- At 20-25°C: 1 day
- At 4-8°C: 7 days
- At -20°C: 1 month

Reference Range (6) [c]

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

Urine (Excretion):
Adults: < 100 mg/day (< 0.10 g/day)
Pregnancy: < 150 mg/day (< 0.15 g/day)

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".

Do not freeze.

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.
- **Warning:** This reagent is obtained from substances of animal origin. Consequently, it should be treated as potentially infectious and handled with the appropriate cautions in accordance with good laboratory practices (7).
- Do not pipette by mouth.
- Do not replenish the reagents.
- Do not swallow. Avoid contact with skin and mucous membranes.

---

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

ABX Pentra Urinary Proteins CP

- 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 [e]

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: +/- 10%.

Urine

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

Number of tests: approximately 112 tests

On Board Reagent Stability

Once opened, the reagent cassette placed in the refrigerated Pentra C200 compartment is stable for 30 days.

Sample volume: 4.7 µL/test

Detection Limit [g]

The detection limit is determined according to CLSI (NCCLS), EP17-A2 protocol (8) and equals 0.02 g/L (1.51 mg/dL).

Limit of Quantitation

The limit of quantitation is determined according to CLSI (NCCLS), EP17-A2 protocol (8) and equals 0.03 g/L (3 mg/dL).

Accuracy and Precision [h]

Repeatability (within-run precision)

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

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

	Mean value g/L	Mean value mg/dL	CV %
Control specimen 1	0.20	19.6	1.86
Control specimen 2	0.80	80.0	2.23
Specimen 1	0.27	26.8	0.86
Specimen 2	0.66	66.5	1.71
Specimen 3	1.51	150.9	0.84

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):

- 1 control
- 3 specimens (low / medium / high levels)

	Mean value g/L	Mean value mg/dL	CV %
Control specimen 1	0.76	76.4	3.1
Specimen 1	0.26	25.9	3.0
Specimen 2	0.65	65.1	3.3
Specimen 3	1.45	145.3	3.9

Measuring Range

The assay confirmed a measuring range from 0.03 g/L (3.0 mg/dL) to 2.90 g/L (290 mg/dL).
The measuring range is extended up to 8.7 g/L (870 mg/dL) with the automatic post-dilution.
The reagent linearity has been assessed up to 2.90 g/L (290 mg/dL) according to the recommendations found in the CLSI (NCCLS), EP06-Ed2 protocol (11).

---

[e]Modification: unit modification.
[f]Modification: chapter added.
[g]Modification: data added.
[h]Modification: modification of accuracy and precision.

ABX Pentra Urinary Proteins CP

Correlation [i]

Patient samples: urine
Number of patient samples: 108
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 0.03 g/L (3.0 mg/dL) to 2.64 g/L (263.5 mg/dL).
The equation for the allometric line obtained using Passing-Bablok regression procedure (13) is:
Y = 1.050 x – 0.005 (g/L)
Y = 1.050 x – 0.509 (mg/dL)
with a correlation coefficient r^2 = 0.998.

Interferences [j]

Haemoglobin:	Do not use hemolysed samples.
Direct Bilirubin:	Do not use sample with Direct Bilirubin.
Ascorbic Acid:	No significant influence is observed up to 340 µmol/L (5.98 mg/dL).
pH:	Acidification or alcalinisation interfere with urinary protein determination by this test.

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 30 days.
Note: A recalibration is recommended when reagent lots change, and when quality control results fall outside the range established.

Conversion Factor:

g/L x 100.0 = mg/dL

Reference

1. Johnson AM, Rohlfs EM, Silverman LM. Proteins. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: WB Saunders Company (1999): 477-540.
2. Felgenhauer K. Laboratory diagnosis of neurological diseases. In: Thomas L. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: THBooks Verlagsgesellschaft (1998): 1308-26.
3. Watanabe N, Kamei S, Ohkubo A, Yamanaka M, Ohsawa S, Makino K, Tokuda K. Urinary protein as measured with a pyrogallol redmolybdate complex. Manually and in a Hitachi 726 automated analyzer. Clin. Chem. (1986) **32** (8): 1551-4.
4. Orsonneau JL, Douet P, Massoubre C, Lustenberger P, Bernard S. An improved pyrogallol red-molybdate method for determining total urinary protein. Clin. Chem. (1989) **35**: 2233-6.
5. Guder WG, Zawta B. The Quality of Diagnostics Samples. Samples: From the Patient to the Laboratory. 1st Ed. Guder WG, Narayanan S, Zawta B. (WHILEY-VCH, Darmstadt, Germany), (2001): 52-53.
6. 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): 2293.
7. Council Directive (2000/54/EC). Official Journal of the European Communities. No. L262 from October 17, 2000: 21-45.
8. Evaluation of detection capability for clinical laboratory measurement procedures. Approved Guideline, 2nd ed., CLSI (NCCLS) document EP17-A2 (2012) **32** (8).
9. Vassault A, Grafmeyer D, Naudin C et al. Protocole de validation de techniques (document B). Ann. Biol. Clin. (1986) **44**: 686-745.
10. Evaluation of Precision Performance of Quantitative Measurement Method. Approved Guideline, CLSI (NCCLS) document EP5-A2 (2004) **24** (25).
11. Evaluation of Linearity of Quantitative Measurement Procedures. 2nd Edition, CLSI (NCCLS) guideline EP06-Ed2 (2020) **40** (16).
12. Measurement Procedure Comparison and Bias Estimation Using Patient Samples. Approved Guideline, 3rd ed., CLSI (NCCLS) document EP09c (2018) **38** (12).
13. 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.
14. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th Edition, Washington, DC, AACC Press (2000).
15. Young DS. Effects of Preanalytical Variables on Clinical Laboratory Tests. 2nd Edition, Washington, DC, AACC Press (1997) **3**: 120-132.

[i]Modification: modification of correlation.
[j]Modification: modification of interferences.