Pointe Microprotein Reagent Set

HORIBA Instruments Incorporated
5449 Research Drive, Canton, MI 48188
Phone: 734-487-8300; (800) 445-9853

Intended Use
For the quantitative determination of total protein in urine. **Rx Only**.

Clinical Significance
The presence of protein in urine is a very sensitive indicator of renal disorders. There are four ways by which increased amounts of protein can occur: increased glomerular permeability; defective tubular re-absorption; increased plasma concentration of an abnormal, low molecular weight protein; and abnormal secretion of protein into the urinary tract.[1] Albuminuria, increased amounts of albumin in urine, has been recognized as an early indicator of renal damage in diabetes that can be reversed if detected and treated early.[2]

Method History
Various methods have been described for the determination of protein concentrations in biological fluids. These methods are based on colorimetric, turbidimetric, electrophoretic, or immunologic procedures.[3,4] The dye binding methods are characterized as having good precision and sensitivity. The Coomassie Blue method[5] is very sensitive, but the reagents stain glassware and plastic.
This method is based on the procedure developed by Fujita[6] and Watanabe.[7] It is a sensitive dye binding, colorimetric method employing Pyrogallol Red. The method seldom stains cuvettes or plastic tubing, and can be automated.

Principle
Pyrogallol Red is combined with molybdenum acid at a low pH. When the complex is combined with protein, a blue-purple color is formed. The increase in absorbance at 600 nm is directly proportional to the protein concentration in the sample.

Reagents
1. MICROPROTEIN REAGENT: Contains Buffer, Pyrogallol Red 0.067 mmol/L, sodium molybdate stabilizer 0.153 mmol/L, surfactants, and preservative.
2. PROTEIN STANDARD SOLUTION: Contains Albumin 50 mg/dl in saline with sodium azide 0.05% as a preservative.

Reagent Preparation
Microprotein Reagent and Protein Standard Solution are provided ready to use.

Reagent Storage
Store Microprotein Reagent and Protein Standard refrigerated (2-8°C). Reagent and standard are stable until the expiration date shown on the labels.

Precautions
1. Microprotein Reagent is for *in vitro* diagnostic use only.
2. Normal precautions exercised in handling laboratory reagents should be followed.
3. Protein Standard contains sodium azide. Do not ingest. May react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build up.

Reagent / Standard Deterioration
The reagent and standard should be clear. Do not use if turbid. Failure to achieve assayed control values may indicate deterioration of reagent and or standard. Do not use if the reagent absorbance at 600nm is less than 0.100.

Specimen Collection and Storage
It is recommended that specimen collection be carried out in accordance with NCCLS Document M29-T2. Specimens containing visible particulate matter should be clarified by centrifugation prior to testing.
URINE: Tests are performed on 24-hour samples. The urine should not be collected during periods of exercise because of its effect on albumin concentration. Protein determinations should be performed with fresh specimens. If test cannot be performed with fresh urine, specimens may be stored at –20°C for up to one year.[8] NOTE: Hemoglobin can increase recovered total protein values, DO NOT USE samples containing blood.

Interferences
It is recommended not to use urine specimens with added preservatives since some added preservatives such as HCL and benzoic acid have been shown to interfere in the protein assay, giving false low results.[7] Bilirubin to a level of 20 mg/dl and Ascorbic acid to a level of 3.0 mg/dl have been found not to interfere with the assay. (Less than 3.0% deviation for samples in the range of 130.0-132.0 mg/dl and less than 17% for samples measured in the range of 9.2-12.5 mg/dl) Hemoglobin being a protein, will increase recovered total protein values. pH variation was found to have no effect on the total protein determination. The effects of specific gravity variation were not evaluated. Some drugs and medications may interfere, see Fujita.[6]

Materials Provided
1. Microprotein Reagent
2. Protein Standard Solution

Materials Required but not Provided
1. Accurate Pipetting devices.
2. Test Tubes/Rack
3. Water Bath or Heat Block (37°C)
4. Timer.
5. Spectrophotometer able to read at 600nm.

Procedure (Automated)
Application procedures are available for various discrete automated instruments. Please contact Technical Service.

Procedure (Manual)
1. Label test tubes “Blank”, “Standard”, “Control”, “Samples”, etc.
2. Pipette 1.0ml of Microprotein reagent to each tube.
3. Allow the tubes to warm to 37°C.
4. Pipette 0.02ml (20ul) deionized water, standard, controls, and samples to the appropriately labeled tubes.
5. Allow tubes to incubate at 37°C for 5 minutes.
6. After 5 minutes, set the spectrophotometer to 600nm and zero the instrument with the BLANK tube.
7. Read and record the absorbance (Abs) of the standard, controls, and samples.
8. To determine Microprotein concentration of the samples, refer to the “Calculations” section.

Calibration
Use an aqueous protein standard (traceable to NIST SRM 927c).

Quality Control
Standard practice for Quality Control should be applied to this procedure. Commercially available Urine controls (2 levels) should be used to monitor the daily acceptable variations. A satisfactory level of performance is achieved when the analyte values obtained are within the acceptable range established by the laboratory.

Calculations
Protein values are expressed as mg/dl.

$$\text{Protein (mg/dl)} = \frac{\text{Abs Unk}}{\text{Abs Std.}} \times \text{Conc. of Std.}$$

Where:
Abs Unk = The absorbance of the unknown sample
Abs Std. = The absorbance of the standard
Conc. of Std. = Concentration of standard (50 mg/dl)

Example:
Abs Unk = 0.085
Abs Std = 0.195
Conc. of Std. = 50 mg/dl

Protein (mg/dl) = $\frac{0.085}{0.195}$ x 50 = 21.8 mg/dl

To determine the 24-Hour Urinary Protein, measure the 24-hour urine total volume in ml (TV) and assay the urine protein content (mg/dl). Calculate the 24-Hour Urinary Protein using the following formula:

Protein (mg/day) = Protein (mg/dl) x $\frac{TV}{100}$

Where: TV = 24-hr. urine total volume in ml
100 = converts ml/day to dl/day

S.I. Units

To convert the results into S.I. units, multiply the microprotein concentration (mg/dl) by 0.0100. For example, microprotein concentration = 21.8 mg/dl x 0.0100 = 0.218 g/L.

Expected Values [6, 7]

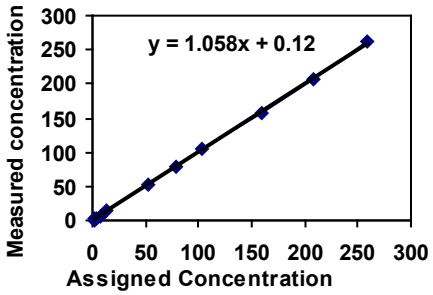
24-Hour Urinary Protein	28 - 141 mg/day
Random Urine	Under 10 mg/dl

NOTE: Each laboratory should confirm the validity of the interval ranges listed for the population it serves.

Performance

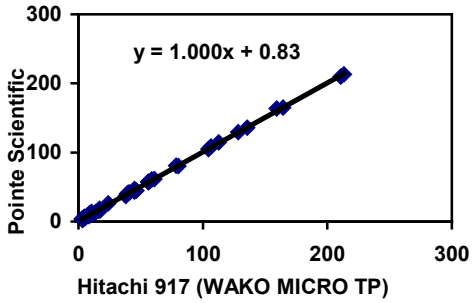
1. Assay Range: The Microprotein procedure has an assay range of 2.0 - 250 mg/dl. Samples that exceed 250 mg/dl should be diluted with an equal volume of isotonic saline and re-assayed. Multiply the result by 2 to compensate for the dilution.
2. Linearity was performed using eleven known concentrations ranging from 1.23 to 259.43 mg/dl. Study was performed on a Roche Hitachi 917 analyzer. Graphical recovery is displayed.

Linearity Graph



3. Sensitivity: Based on an instrument absorbance resolution of 0.001 this procedure has a sensitivity of 0.250 mg/dl. The Limit of Detection was found to be 2.0 mg/dl when the absorbance was read bichromatically at 600 / 700 nm (Hitachi 917 chemistry analyzer).
4. Comparison: Studies between the present method and a similar method (Wako Autokit Micro TP performed on the Hitachi 917) yielded a correlation coefficient of 0.9997 and a regression equation of y=1.000x +0.83.
55 samples with a range of 2.5 – 213.3 mg/dl were used in the study. Graphical recovery is displayed.

Correlation Graph



5. Precision: Studies performed on a Roche Hitachi 917 analyzer. The precision of the assay was evaluated following a modification of NCCLS protocol EPT-T2. The within Run precision data was obtained by running three samples in replicates of 20 on the same day. The Run to Run data was obtained by running three samples in replicates of five over a three day period.

Within Run Urine (N=20)			Run to Run Urine (N=20)		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
8.7	0.7	8.6	10.2	0.9	8.6
121.8	2.1	1.7	127.0	1.6	1.2
240.3	1.7	0.7	242.5	2.4	1.0

References

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8. Tietz, N.W.; Clinical Guide to Laboratory Tests, W.B. Saunders, Phil. P.470, 1990.

Symbol Key

Use by (YYYY-MM-DD)	LOT Lot and batch code
REF Catalog number	Manufacturer
IVD *In vitro* diagnostic medical device	Temperature limitation
Consult instructions for use	**Rx Only:** Prescription Use Only
CE mark	EC REP Authorized representative in the European Community

REF P7582

P7582-240

Manufactured by
HORIBA Instruments Incorporated
5449 Research Drive
Canton, MI 48188

2°C 8°C IVD

Manufactured by HORIBA Instruments Incorporated: Pointe Brand
5449 Research Drive, Canton, MI 48188

European Authorized Representative:
Obelis s.a.
Boulevard Général Wahis 53
1030 Brussels, BELGIUM
Tel: (32)2.732.59.54 Fax:(32)2.732.60.03 email: mail@obelis.net

Certified to Perform Reagents

The Pointe reagents are certified to be manufactured according to specified parameters. Any Pointe reagent product not meeting specifications through its listed expiration date will be remedied immediately without charge.

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