SYNCHRON® System(s)
Chemistry Information Sheet

M-TP
Microprotein
REF 445860

For *In Vitro* Diagnostic Use

ANNUAL REVIEW

Reviewed by:	Date	Reviewed by:	Date

PRINCIPLE

INTENDED USE

M-TP reagent, when used in conjunction with SYNCHRON LX® System(s), UniCel® DxC 600/800 System(s) and SYNCHRON® Systems Microprotein Calibrator, is intended for the quantitative determination of total microprotein in human urine and cerebrospinal fluid (CSF).

CLINICAL SIGNIFICANCE

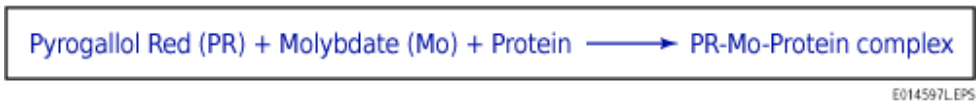
An increase in spinal fluid protein is seen in a variety of disease states. Among them are meningitis, polyneuritis, and some tumors. Increases in urinary proteins are associated with a number of conditions among them are nephrosis hypergammaglobulinemia, pregnancy, and destructive lesions of the kidney.

METHODOLOGY

M-TP reagent is used to measure the protein concentration by a timed endpoint method.[1,2] Protein in the sample reacts with the pyrogallol red (PR) and molybdate (Mo) to form a purple color complex that has a maximum absorbance at 600 nanometers.

The SYNCHRON® System(s) automatically proportions the appropriate sample and reagent volumes into a cuvette. The ratio used is one part sample and 60 parts reagent for cerebrospinal fluid and one part sample and 30 parts reagent for urine. The system monitors the change in absorbance at 600 nanometers. This change in absorbance is directly proportional to the concentration of protein in the sample and is used by the System to calculate and express the protein concentration.

CHEMICAL REACTION SCHEME



SPECIMEN

TYPE OF SPECIMEN

Biological fluid samples should be collected in the same manner routinely used for any laboratory test.[3] Freshly collected spinal fluid or urine are the preferred specimens. Whole blood, serum or plasma are not recommended for use as a sample.

SPECIMEN STORAGE AND STABILITY

1. It is recommended that urine assays be performed within 2 hours of collection. For timed specimens, the collection container should be kept in the refrigerator or on ice during the timed period. Preservatives are not recommended.[4]
2. CSF specimens should be centrifuged and analyzed without delay. Specimens may be refrigerated or frozen for 7 to 10 days for repeat determinations.[5]

ADDITIONAL SPECIMEN STORAGE AND STABILITY CONDITIONS AS DESIGNATED BY THIS LABORATORY:

SAMPLE VOLUME

The optimum volume, when using a 0.5 mL sample cup, is 0.3 mL of sample. For optimum primary sample tube volumes and minimum volumes, refer to the Primary Tube Sample Template for your system.

CRITERIA FOR UNACCEPTABLE SPECIMENS

Refer to the PROCEDURAL NOTES section of this chemistry information sheet for information on unacceptable specimens.

CRITERIA FOR SAMPLE REJECTION AS DESIGNATED BY THIS LABORATORY:

PATIENT PREPARATION

SPECIAL INSTRUCTIONS FOR PATIENT PREPARATION AS DESIGNATED BY THIS LABORATORY:

SPECIMEN HANDLING

SPECIAL INSTRUCTIONS FOR SPECIMEN HANDLING AS DESIGNATED BY THIS LABORATORY:

REAGENTS

CONTENTS

Each kit contains the following items:
Two Microprotein Reagent Cartridges (2 x 50 tests)

VOLUMES PER TEST

Sample Volume	
Urine	10 µL
CSF	5 µL
Total Reagent Volume	300 µL
Cartridge Volumes	
A	– –
B	300 µL
C	– –

REACTIVE INGREDIENTS

REAGENT CONSTITUENTS

Pyrogallol Red	0.058 mmol/L
Sodium Molybdate	0.12 mmol/L

Also non-reactive chemicals necessary for optimal system performance.

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

SYNCHRON® Systems Microprotein Calibrator
At least two levels of control material

REAGENT PREPARATION

No preparation is required.

ACCEPTABLE REAGENT PERFORMANCE

The acceptability of a reagent is determined by successful calibration and by ensuring that quality control results are within your facility's acceptance criteria.

REAGENT STORAGE AND STABILITY

M-TP reagent, when stored unopened at +2°C to +8°C, will remain stable until the expiration date printed on the cartridge label. Once opened, the reagent cartridge is stable for 30 days unless the expiration date is exceeded. DO NOT FREEZE.

REAGENT STORAGE LOCATION:

CALIBRATION

CALIBRATOR REQUIRED

SYNCHRON® Systems Microprotein Calibrator

CALIBRATOR PREPARATION

No preparation is required.

CALIBRATOR STORAGE AND STABILITY

SYNCHRON® Systems Microprotein Calibrator when stored unopened at -15°C to -20°C will remain stable until the expiration date printed on the label. Once opened, resealed calibrators are stable for 60 days at +2°C to +8°C unless the expiration date is exceeded.

⚠ CAUTION

Because this product is of human origin, it should be handled as though capable of transmitting infectious diseases. Each serum or plasma donor unit used in the preparation of this material was tested by United States Food and Drug Administration (FDA) approved methods and found to be negative for antibodies to HIV and HCV and nonreactive for HbsAg. Because no test method can offer complete assurance that HIV, hepatitis B virus, and hepatitis C virus or other infectious agents are absent, this material should be handled as though capable of transmitting infectious diseases. This product may also contain other human source material for which there is no approved test. The FDA recommends such samples to be handled as specified in Centers for Disease Control's Biosafety Level 2 guidelines.[6]

CALIBRATOR STORAGE LOCATION:

CALIBRATION INFORMATION

1. The system must have a valid calibration curve in memory before control or patient samples can be run.
2. Under typical operating conditions the M-TP reagent cartridge must be calibrated every 14 days and also with certain parts replacements or maintenance procedures, as defined in the SYNCHRON LX *Maintenance Manual and Instrument Log*, or the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual. This assay has within-lot calibration available. Refer to the SYNCHRON LX *Operations Manual*, or the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual for information on this feature.
3. For detailed calibration instructions, refer to the SYNCHRON LX *Operations Manual*, or the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.
4. The system will automatically perform checks on the calibration and produce data at the end of calibration. In the event of a failed calibration, the data will be printed with error codes and the system will alert the operator of the failure. For information on error codes, refer to the SYNCHRON LX *Diagnostics and Troubleshooting Manual*, or the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

TRACEABILITY

For Traceability information refer to the Calibrator instructions for use.

QUALITY CONTROL

At least two levels of control material should be analyzed daily. In addition, these controls should be run with each new calibration, with each new reagent cartridge, and after specific maintenance or troubleshooting procedures as detailed in the appropriate system manual. More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws.

The following controls should be prepared and used in accordance with the package inserts. Discrepant quality control results should be evaluated by your facility.

TABLE 1 QUALITY CONTROL MATERIAL

CONTROL NAME	SAMPLE TYPE	STORAGE

TESTING PROCEDURE(S)

1. If necessary, load the reagent onto the system.
2. After reagent load is completed, calibration may be required.
3. Program samples and controls for analysis.
4. After loading samples and controls onto the system, follow the protocols for system operations.

For detailed testing procedures, refer to the SYNCHRON LX *Operations Manual*, or the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

CALCULATIONS

The SYNCHRON® System(s) performs all calculations internally to produce the final reported result. The system will calculate the final result for sample dilutions made by the operator when the dilution factor is entered into the system during sample programming.

REPORTING RESULTS

Equivalency between the SYNCHRON LX and UniCel DxC 600/800 Systems has been established. Chemistry results between these systems are in agreement and data from representative systems may be shown.

REFERENCE INTERVALS

Each laboratory should establish its own reference intervals based upon its patient population. The following reference intervals were taken from literature.[7]

TABLE 2 REFERENCE INTERVALS

INTERVALS	SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS

INTERVALS	SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS
Literature	CSF	15 – 45 mg/dL	0.15 – 0.45 g/L
	Urine (random)	< 10 mg/dL	< 0.1 g/L
	Urine (24 hour)	50 – 100 mg/24 hrs	0.05 – 0.1 g/24 hrs
	Urine (average)	1 – 14 mg/dL	0.01 – 0.14 g/L

INTERVALS	SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS
Laboratory			

Refer to References (5, 8, 9) for guidelines on establishing laboratory-specific reference intervals.

ADDITIONAL REPORTING INFORMATION AS DESIGNATED BY THIS LABORATORY:

PROCEDURAL NOTES

LIMITATIONS

1. Do not use hemolyzed samples.
2. If serum protein carryover is suspected, a saline cup should be assayed prior to analysis of microprotein samples.
3. Samples containing light chains may produce falsely low results.

INTERFERENCES

1. The following substances were tested for interference with this methodology:

Table 3 Interferences[a]

SUBSTANCE	SOURCE	LEVEL TESTED	OBSERVED EFFECT[b]
Ascorbic Acid	NA[c]	500 mg/dL	≤ -2.0 mg/dL
Calcium	NA	130 mg/dL	≤ -3.2 mg/dL
Citrate	NA	50 mg/dL	≤ -2.0 mg/dL
Creatinine	NA	160 mg/dL	≤ +3.2 mg/dL
Glucose	NA	200 mg/dL	≤ -1.0 mg/dL
Magnesium	NA	400 mg/dL	≤ +1.0 mg/dL
Oxalate	NA	30 mg/dL	≤ -2.0 mg/dL
Urea	NA	140 mg/dL	≤ -2.0 mg/dL

2. Refer to References (10,11,12) for other interferences caused by drugs, disease and preanalytical variables.

SPECIFICITY

To determine specificity equivalent concentrations of albumin and globulin were assayed using this method. The results are listed below:

Table 4 Specificity[d]

ALBUMIN (mg/dL)	GLOBULIN (mg/dL)
38.0	36.5
81.3	75.7

PERFORMANCE CHARACTERISTICS

Analytic Range

The SYNCHRON® System(s) method for the determination of M-TP in CSF or urine provides the following analytical range:

TABLE 5 ANALYTICAL RANGE

SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS
CSF	6 – 300 mg/dL	0.06 – 3.00 g/L
Urine	6 – 150 mg/dL	0.06 – 1.50 g/L

CSF and urine samples with concentrations exceeding the high end of the analytical range should be diluted with normal saline and reanalyzed.

REPORTABLE RANGE (as determined on site):

TABLE 6 REPORTABLE RANGE

SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS

SENSITIVITY

Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for M-TP determination is 6 mg/dL (0.06 g/L).

EQUIVALENCY

Equivalency was assessed by Deming regression analysis of patient samples to accepted clinical methods.

CSF (in the range of 22 to 298 mg/dL):

Y (SYNCHRON LX Systems)	= 0.992X - 3.40
N	= 49
MEAN (SYNCHRON LX Systems)	= 128.4
MEAN (SYNCHRON CX7 DELTA)	= 132.9
CORRELATION COEFFICIENT (r)	= 0.9957

Urine (in the range of 12 to 148 mg/dL):

Y (SYNCHRON LX Systems)	= 0.944X - 1.72
N	= 73
MEAN (SYNCHRON LX Systems)	= 64.2
MEAN (SYNCHRON CX7 DELTA)	= 69.8
CORRELATION COEFFICIENT (r)	= 0.9949

Refer to References (13) for guidelines on performing equivalency testing.

PRECISION

A properly operating SYNCHRON® System(s) should exhibit precision values less than or equal to the following:

TABLE 7 PRECISION VALUES

TYPE OF PRECISION	SAMPLE TYPE	1 SD		CHANGEOVER VALUE[e]		% CV
		mg/dL	g/L	mg/dL	g/L	
Within-run	CSF/Urine	2.0	0.02	50.0	0.5	4.0
Total	CSF/Urine	3.0	0.03	50.0	0.5	6.0

Comparative performance data for a SYNCHRON LX® System evaluated using the NCCLS Proposed Guideline EP5-T2 appears in the table below.[14] Each laboratory should characterize their own instrument performance for comparison purposes.

TABLE 8 NCCLS EP5-T2 PRECISION ESTIMATE METHOD

TYPE OF IMPRECISION	SAMPLE TYPE		No. Systems	No. Data Points[f]	Test Mean Value (mg/dL)	EP5-T2 Calculated Point Estimates	
						SD	%CV
Within-run	CSF	Low Human Pool	1	80	47.55	0.87	1.82
	CSF	High Human Pool	1	80	263.43	2.24	0.85
	Urine	Control 1	1	80	16.85	0.47	2.82
	Urine	Control 2	1	80	67.38	0.63	0.94
Total	CSF	Low Human Pool	1	80	47.55	1.58	3.32
	CSF	High Human Pool	1	80	263.43	5.68	2.16
	Urine	Control 1	1	80	16.85	0.64	3.81
	Urine	Control 2	1	80	67.38	2.48	3.68

NOTICE

These degrees of precision and equivalency were obtained in typical testing procedures on a SYNCHRON LX® System and are not intended to represent the performance specifications for this reagent.

ADDITIONAL INFORMATION

For more detailed information on SYNCHRON LX Systems or UniCel DxC Systems, refer to the appropriate system manual.

SHIPPING DAMAGE

If damaged product is received, notify your Beckman Coulter Clinical Support Center.

REFERENCES

1. Fujita, Y., Mori, I., Kitano, S., "Color Reaction Between Pyrogallol Red-Molybdenum (VI) Complex and Protein", *Bunsaki Kagaku*, 32:E379 E386 (1983).

2. Watanaba, N., Kamel, S., Ohkubo, A., Yamanaka, M., Ojsawa, S., Makino, K., Tokudo, K., "Urinary Protein as Measured with a Pyrogallol Red-Molybdate Complex, Manually and in a Hitachi 726 Automated Analyzer", *Clin. Chem.*, 328:1551 (1986).

3. Tietz, N. W., "Specimen Collection and Processing; Sources of Biological Variation", *Textbook of Clinical Chemistry*, 2nd Edition, W. B. Saunders, Philadelphia, PA (1994).

4. National Committee for Clinical Laboratory Standards, *Routine Urinalysis and Collection, Transportation and Preservation of Urine Specimens*, Tentative Guideline, NCCLS publication GP16-T, Villanova, PA (1992).

5. Tietz, N. W., ed., *Fundamentals of Clinical Chemistry*, 3rd Edition, W. B. Saunders, Philadelphia, PA (1987).

6. CDC-NIH manual, *Biosafety in Microbiological and Biomedical Laboratories*, U.S. Government Printing Office, Washington, D.C. (1984).

7. Tietz, N. W., *Clinical Guide to Laboratory Tests*, 3rd Edition, W. B. Saunders, Philadelphia, PA (1995).

8. National Committee for Clinical Laboratory Standards, *How to Define, Determine, and Utilize Reference Intervals in the Clinical Laboratory*, Approved Guideline, NCCLS publication C28-A, Villanova, PA (1995).

9. Henry, J. B., *Clinical Diagnosis and Management by Laboratory Methods*, 18th Edition, W. B. Saunders Company, Philadelphia, PA (1991).

10. Young, D. S., *Effects of Drugs on Clinical Laboratory Tests*, 4th Edition, AACC Press, Washington, D. C. (1995).

11. Friedman, R. B., Young, D. S.,*Effects of Disease on Clinical Laboratory Tests*, 3rd Edition, AACC Press, Washington, D.C. (1997).

12. Young, D. S., *Effects of Preanalytical Variables on Clinical Laboratory Tests*, 2nd Edition, AACC Press, Washington, D. C. (1997).

13. National Committee for Clinical Laboratory Standards, *Method Comparison and Bias Estimation Using Patient Samples*, Approved Guideline, NCCLS publication EP9-A, Villanova, PA (1995).

14. National Committee for Clinical Laboratory Standards, *Precision Performance of Clinical Chemistry Devices*, Tentative Guideline, 2nd Edition, NCCLS publication EP5-T2, Villanova, PA (1992).

EC REP Beckman Coulter Ireland Inc., Mervue Business Park, Mervue, Galway, Ireland (353 91 774068)

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ENDNOTES

a Data shown was collected using SYNCHRON CX Systems. Equivalency between SYNCHRON LX Systems has been established by Deming regression analysis to SYNCHRON CX Systems.

b Plus (+) or minus (-) signs in this column signify positive or negative interference.

c NA = Not applicable.

d Data shown was collected using SYNCHRON CX Systems. Equivalency between SYNCHRON LX Systems has been established by Deming regression analysis to SYNCHRON CX Systems.

e When the mean of the test precision data is less than or equal to the changeover value, compare the test SD to the SD guideline given above to determine the acceptability of the precision testing. When the mean of the test precision data is greater than the changeover value, compare the test % CV to the guideline given above to determine acceptability. Changeover value = (SD guideline/CV guideline) x 100.

f The point estimate is based on the pooled data from one system, run for twenty days, two runs per day, two observations per run on an instrument operated and maintained according to the manufacturer's instructions.