Microprotein (M-TP) – CSF, Urine, Fluids
Beckman UniCel DxC Systems

Technical Procedure 3142

Principle

Intended Use

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

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 absor- bance 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

Pyrogallol Red (PR) + Molybdate (Mo) + Protein → PR-Mo-Protein Complex

Specimen

Acceptable Sample Containers

Spot urine and fluid samples should be sent to the laboratory in a 13 X 75 Clear Cap BD tube.

CSF should be submitted in the LP sterile collection tubes. Tube #1 is the preferred tube for chemistry testing.

24 hour urine collections are received in 3000 ml plastic urine collection jugs.

Type of Specimen

Biological fluid samples should be collected in the same manner routinely used for any laboratory test.(3)

Freshly collected spinal fluid, urine or fluid are the preferred specimens.

Whole blood, serum or plasma are not recommended for use as a sample.

Specimen Storage and Stability

It is recommended that random urine assays be performed within 2 hours of collection. (4)

For timed specimens, the collection container is to be kept in the refrigerator or on ice during the collection period. No preservative is required. Upon receipt in the laboratory, the collection container must be stored refrigerated until testing is performed. **Testing must be performed within 4 days (96 hours) of start of collection**.(5,6,7)

CSF specimens should be centrifuged and analyzed without delay. Specimens may be refrigerated or frozen for 7 to 10 days for repeat determinations.

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.

Criteria for Unacceptable Specimens

Urine samples aspirated by the IRIS IQ are not acceptable for urine chemistry testing.

Urine collected in a BD UA Preservative Tube is not acceptable for urine chemistry testing.

Refer to the ***Procedural Notes*** section of this chemistry information sheet for information on unacceptable specimens.

Reagents

Contents

Each kit contains the following items: **Kit Reorder # 445860**

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.

Date and initial cartridge and document in reagent log before loading each new cartridge.

Acceptable Reagent Performance

The acceptability of this reagent is determined by successful calibration and by ensuring that quality control results are within acceptance criteria, as defined in the Clinical Chemistry Quality Control Procedure #3000.T.

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.**

Equipment

This test is performed on the Beckman UniCel DxC 800 systems; Beckman-Coulter, Brea, California. For technical assistance, call the Beckman-Coulter hotline: 1-800-854-3633.

Refer to the UniCel DxC 800 systems *Reference Manual* for detailed instructions.

Calibration

Calibrator Required

SYNCHRON® Systems Microprotein Calibrator **Reorder #445930**

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 Administra- tion (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)

Calibration Information

The system must have a valid calibration curve in memory before controls or patient samples can be run.

Under typical operating conditions the M-TP reagent cartridge must be calibrated every 14 days or with each new bottle of reagent and also with certain parts replacements or maintenance procedures, as defined in the UniCel DxC 800 Systems *Instructions for Use* (IFU) manual. This assay has within-lot calibration available. Refer to the UniCel DxC 800 System Instructions For Use (IFU) manual for information on this feature.

For detailed calibration instructions, refer to the UniCel DxC 800 System *Instructions For Use* (IFU) manual.

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 print out with error codes and the system will alert the operator of the failure. The explanation of these error codes can be found in the UniCel DxC 800 System *Instructions For Use* (IFU) manual.

Traceability

Total Protein in this Calibrator is traceable to the NIST* SRM 927. Traceability process is based on prEN ISO 17511.

**NIST=National Institute of Standards and Technology*

Quality Control

At least two levels of control material should be analyzed each shift. 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 UniCel DxC 800 System *Instructions For Use* manual. More frequent use of controls or the use of additional controls is left to the discretion of the user based on workload and workflow.

The following controls should be used in accordance with the package instructions for use inserts. Quality control results should be evaluated and handled with respect to the Clinical Chemistry Quality Control Procedure #3000.T. Controls are compiled statistically in the LIS and reagent lot changes are documented on DxC Reagent Log sheets.

Quality Control Material

Control	Storage	Catalog No.
MAS UriChemTrak 1	+2°C to +8°C*	UR11001
MAS UriChemTrak 2	+2°C to +8°C*	UR22002

*Unopened vials are stable until the expiration date on the label. Once opened, vials of control are stable for 30 days when stored tightly capped at +2°C to +8°C. **Do not freeze**. Bacterial contamination produces an increase in turbidity and/or a characteristic odor. Discard vial if evidence of microbial contamination is observed.

Testing Procedure

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 operation. For detailed testing procedures, refer to the UniCel DxC 800 System *Instructions For Use* (IFU) manual.

Calculations

UniCel DxC Systems perform 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.

If the dilution was programmed in the DataLink, the final calculated result from a dilution will not be calculated by the UniCel DxC system but by the DataLink.

24 hr timed urine specimens are calculated from the following equation:

$$\text{Urine Protein}\ \frac{\text{mg}}{\text{dL}}\ \text{X}\ \frac{\text{dL}}{100\text{mL}}\ \text{X Total volume collected (mL)} = \text{mg/24hr}$$

Calculations are only performed on 24 hour collections (±15 minutes) and reported as mg/24hr.

Do not round off total collection time.

Reporting Results

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

Reference Intervals

The following reference intervals were taken from literature and a study performed on SYNCHRON Systems.(8)

Reference Intervals

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
UCDMC	CSF	15 – 45 mg/dL	0.15 to 0.45 g/L
	Urine (timed)	< 150 mg/24hrs*	< 1.50 g/24hrs

* Reference interval determined via a consensus survey at UCDMC.

Reference interval for a spot or random urine sample has not been established.

A reference interval for fluids has not been determined by UCDHS. This result may be less accurate than for the usual sample type, and should be interpreted in the context of the patient's clinical condition.

Refer to References (5,9,10) for guidelines on establishing laboratory-specific reference intervals.

Procedural Notes

Limitations

Do not use hemolyzed samples.

If serum protein carryover is suspected, a saline cup should be assayed prior to analysis of microprotein samples.

Samples containing light chains may produce falsely low results.

Interferences

The following substances were tested for interference with this methodology:

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

[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.

Refer to References (11,12,13) 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:

Specificity[a]

Albumin (mg/dL)	Globulin (mg/dL)
38.0	36.5
81.3	75.7

[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.

Performance Characteristics

Analytical Measurement Range (AMR)

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

Analytical Measurement Range (AMR)

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

Clinical Reportable Range (CRR)

Clinical Reportable Range (CRR)

Sample Type	Conventional Units	S.I. Units
CSF	6 - diluted result mg/dL	0.06 - diluted result g/L
Urine	6 - diluted result mg/dL	0.06 - diluted result g/L

Samples with concentrations below the AMR and CRR (6 mg/dL) are reported as "**< 6 mg/dL**".
Samples with concentrations greater than the AMR are diluted with saline and reanalyzed.

If the dilution was programmed in Remisol, the final calculated result from a dilution will not be calculated by the UniCel DxC system but by Remisol.

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.

As determined by Beckman.

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 (14) for guidelines on performing equivalency testing.

As determined at UCDMC

Urine (in the range of 6 to 140 mg/dL):

Y (UniCel DxC800-4118)	= 0.966X - 1.8
N	= 21
MEAN (UniCel DxC800-4118)	= 30.2
MEAN (SYNCHRON LX20-2194)	= 33.2
CORRELATION COEFFICIENT (r)	= 0.9989

Urine (in the range of 6 to 140 mg/dL):

Y (UniCel DxC800-4427)	= 0.985X - 2.2
N	= 21
MEAN (UniCel DxC800-4427	= 30.5
MEAN (SYNCHRON LX20-2194)	= 33.2
CORRELATION COEFFICIENT (r)	= 0.9992

Urine (in the range of 6 to 140 mg/dL):

Y (UniCel DxC800-4449)	= 0.990X - 2.0
N	= 21
MEAN (UniCel DxC800-4449)	= 30.9
MEAN (SYNCHRON LX20-2194)	= 33.2
CORRELATION COEFFICIENT (r)	= 0.9991

Urine (in the range of 5 to 131 mg/dL):

Y (UniCel DxC800-4427)	= 1.019X - 0.3
N	= 21
MEAN (UniCel DxC800-4427)	= 30.5
MEAN (UniCel DxC800-4118)	= 30.2
CORRELATION COEFFICIENT (r)	= 0.9999

Urine (in the range of 5 to 131 mg/dL):

Y (UniCel DxC800-4449)	= 1.025X - 0.1
N	= 21
MEAN (UniCel DxC800-4449)	= 30.9
MEAN (UniCel DxC800-4118)	= 30.2
CORRELATION COEFFICIENT (r)	= 0.9999

Urine (in the range of 4 to 134 mg/dL):

Y (UniCel DxC800-4449)	= 1.006X + 0.2
N	= 21
MEAN (UniCel DxC800-4449)	= 30.9
MEAN (UniCel DxC800-4427)	= 30.5
CORRELATION COEFFICIENT (r)	= 0.9999

Precision

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

As determined by Beckman

Precision Values

Type of Precision	Sample Type	1 SD		Changeover Value[a]		%CV
		mg/dL	g/L	mg/dL	g/L	
Within-run	CSF/Urine	2.0	0.02	50	0.5	4.0
Total	CSF/Urine	3.0	0.03	50	0.5	6.0

[a] 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.

Precision established at UCDMC

Type of Precision	Sample Type	n	Mean (mg/dL)	1 SD	%CV
DxC800-4118 Within-run	MAS UriChemTrak 1	20	12.0	0.0	0.0
	MAS UriChemTrak 2	20	72.0	0.2	0.3
DxC800-4427 Within-run	MAS UriChemTrak 1	20	12.0	0.0	0.0
	MAS UriChemTrak 2	20	72.0	1.4	2.0
DxC800-4449 Within-run	MAS UriChemTrak 1	20	12.0	0.0	0.0
	MAS UriChemTrak 2	20	71.9	1.3	1.9

Analyzer	Type of Imprecision	Sample Type	n	Mean (mg/dL)	SD	%CV
DxC800-4118	Day to Day (Urine)	MAS UriChemTrak 1	24	12	0.6	5.0
		MAS UriChemTrak 2	22	73	0.8	1.1
DxC800-4427	Day to Day (Urine)	MAS UriChemTrak 1	394	12	0.7	5.8
		MAS UriChemTrak 2	391	74	1.3	1.8
	Day to Day (CSF)	MAS UriChemTrak 1	383	11	1.0	9.1
		MAS UriChemTrak 2	377	67	1.6	2.4
DxC800-4449	Day to Day (Urine)	MAS UriChemTrak 1	387	12	0.5	4.2
		MAS UriChemTrak 2	382	72	1.2	1.7
	Day to Day (CSF)	MAS UriChemTrak 1	382	11	0.7	6.4
		MAS UriChemTrak 2	379	67	1.4	2.1

Comparative performance

Data for a SYNCHRON LX System evaluated using the NCCLS Proposed Guideline EP5-T2 appears in the table below.(15)

NCCLS EP5-T2 Precision Estimate Method

Type of Imprecision	Sample Type	No. Systems	No. Data Points[a]	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

[a] The point estimate is based on the 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.

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 UniCel DxC Systems, refer to the *Instructions for Use* and *Reference* manual.

References

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16. M-TP, SYNCHRON® System(s) Chemistry Information Sheet A18528 AH, (4/2013), Instruction for use sheet, https://www.beckmancoulter.com/wsrportal/techdocs?docname=/cis/A18528/AH/EN_M-TP.pdf

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			11/27/2000	G. Kost
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			10/10/2003	S. Devaraj
			10/25/2004	S. Devaraj
			11/28/2005	G. Kost
			09/26/2006	G. Kost
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			06/16/2008	G. Kost
07/08/2009	Controls update	M. Inn		
			09/15/2009	G. Kost
			10/12/2010	G. Kost
06/10/2011	update	M.Inn		
9/26/2011	Reference interval update	M.Inn	11/16/2011	G. Kost
08/07/2013	No reference interval for spot/random urines	M. Inn	08/6/2013	G. Kost
			09/17/2013	G. Kost
04/03/2015	Updated 24-hour collection stability	kdagang	04/15/2015	J. Gregg