
Principle

Intended Use

BUNm reagent, when used in conjunction with UniCel® DxC System(s) and SYNCHRON® Systems AQUA CAL 1, 2 and 3, is intended for quantitative determination of urea nitrogen in human serum, plasma or urine.

Clinical Significance

Urea nitrogen (UN) or urea measurements are used in the diagnosis and treatment of certain renal and metabolic diseases. Increased plasma UN levels suggest impaired kidney function that may be due to acute or chronic kidney disease, damage or failure. It may also be due to a condition that results in decreased blood flow to the kidneys, such as congestive heart failure, shock, stress, recent heart attack, or severe burns, conditions that cause obstruction of urine flow, or to dehydration. UN concentrations may be elevated when there is excessive protein catabolism, significantly increased protein in the diet, or gastrointestinal bleeding (because of the proteins present in the blood).

Low plasma UN levels are not common and not usually a cause for concern. They may be seen in severe liver disease, malnutrition, and sometimes when a person is overhydrated.

Both increased and decreased plasma UN concentrations may be seen during a normal pregnancy.

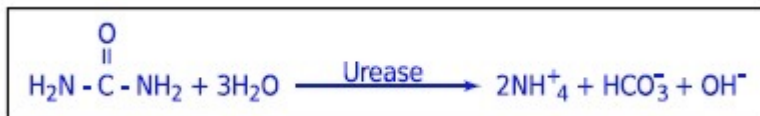
Total urinary nitrogen (TUN) is used to determine nitrogen balance (nitrogen ingested – nitrogen excreted). Determination of TUN can be accomplished by either a direct method (Dumas combustion) or estimated based on urinary urea nitrogen (UUN) from a 24-hour urine specimen. Results help clinicians guide nutrition, especially in patients with increased protein catabolism (e.g., trauma, surgery, infection, burns). (1)

Methodology

The SYNCHRON® System(s) determines urea nitrogen concentration by means of an enzymatic conductivity rate method.

A precise volume of sample (10 microliters) is injected in a reaction cup containing a urease solution. The ratio used is one part sample to 76 parts reagent. The reaction converts the non ionic species (urea) to one which is ionic (ammonium ion and bicarbonate). During the reaction, the timed rate of increase of solution conductivity is directly proportional to the concentration of urea present in the reaction cup.(2,3,4)

Chemical Reaction Scheme



Specimen

Acceptable Sample Containers

13 x 75 PST, SST and Red Top BD tubes

PST, SST and Red Top BD microtainers

Spot urines should be aliquoted into a 13 x 75 clear cap BD tube

24 hour urine collections are usually 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.(5) Freshly drawn serum, plasma or properly collected urine (random/timed) are the preferred specimens. **Whole blood is not recommended for use as a sample.**

Specimen Storage and Stability

1. Tubes of blood are to be kept closed at all times and in a vertical position. It is recommended that the serum or plasma be physically separated from contact with cells within two hours from the time of collection.(6)
2. Separated serum or plasma should not remain at room temperature longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at +2°C to +8°C. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -15°C to -20°C. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.(6)
3. It is recommended that urine assays be performed within 2 hours of collection. For timed specimens, the collection container is to be kept in the refrigerator or on ice during the time period. No preservative is required.(7)
4. **Urine samples aspirated by the IRIS IQ are not acceptable for urine chemistry testing.**
5. **Urine collected in a BD UA Preservative Tube is not acceptable for urine chemistry testing.**

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](#).

Unacceptable Specimens

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 # 472482**

Two Urease Concentrate Bottles (2 X 200 mL)
Two Diluent Bottles (2 X 1800 mL)
Two Wetting Agent Bottles (2 X 10 mL)*
Instruction insert

Volumes per Test

Sample Volume	10 µL
ORDAC Sample Volume	5 µL
Total Reagent Volume	765 µL

Reactive Ingredients

Reagent Constituents	
Jack Bean Urease	25 U/mL

Also non-reactive chemicals necessary for optimal system performance.

CAUTION: Avoid skin contact with reagent. Use water to wash reagent from skin.

Materials Needed But Not Supplied With Reagent Kit

SYNCHRON® Systems AQUA CAL 1, 2 and 3
At least two levels of control material
Saline

Reagent Preparation

1. Pour the contents of the BUN Reagent Concentrate bottle (200 mL) into the 2000 mL BUN Reagent Diluent bottle.
2. Replace the cap and **MIX GENTLY BY INVERTING TEN (10) TIMES.**
3. Record the preparation date and time on the top of the bottle.
4. Allow the reagent to warm to room temperature. This will require 2-3 hours if the Diluent was stored at room temperature. This will require 8-12 hours if the Diluent was stored refrigerated. A 32°C or 37°C water bath or incubator may be used to speed up the equilibration to room temperature. Loosen the cap slightly to allow for out gassing.
5. Date and initial reagent container and document in reagent log before loading each new carboy.

NOTICE

Do not reuse old reagent or mix fresh reagent with old reagent.

*** BUNm Wetting Agent is currently not used when preparing the BUNm reagent and is only used for troubleshooting BUNm problems at UCDMC.**

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

1. Urea Nitrogen Reagent (BUN) Concentrate stored unopened at +2°C to +8°C is stable until the expiration date indicated on each bottle.
2. Urea Nitrogen (BUN) Diluent stored unopened at the **RECOMMENDED ROOM TEMPERATURE** (+18°C to +30°C) is stable until the expiration date indicated on each bottle.
3. Once mixed and loaded onto the instrument, Urea Nitrogen Reagent is stable for 15 days or until the expiration date, whichever is sooner.
4. Reagent frozen in transit will lose urease activity and may fail to calibrate. If frozen reagent calibrates, it will not have claimed on-instrument or unopened bottle stability. Frozen reagent should be discarded.

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 AQUA CAL 1, 2 and 3 ([Kit Reorder #s 471288, 471291, 471294](#))

Calibrator Preparation

No preparation is required.

Calibrator Storage and Stability

1. Unopened calibrators should be stored at +2°C to +8°C until the expiration date printed on the calibrator bottle. Once opened, the calibrators are stable at room temperature for 30 days.

2. Repetitive refrigeration of the aqueous calibrators may facilitate crystal formation. Once removed from refrigerated storage, these calibrators should remain at room temperature.

Calibration Information

1. The system must have a valid calibration in memory before controls or patient samples can be run.
2. Under typical operating conditions the BUNm assay must be calibrated every 72 hours 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.
3. For detailed calibration instructions, refer to the UniCel DxC 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 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

Urea Nitrogen (analyte) in this calibrator is traceable to NIST* 912a.

*NIST=National Institute of Standards and Technology

The set point values were established based upon the gravimetric addition of specific quantities of the measurand to achieve the appropriate concentration.

The values are verified using representative samples from each lot of calibrator and are specific to the assay methodologies of the SYNCHRON systems. Values determined by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

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.

NOTICE

Do not use controls containing diethylamine HCl.

The following controls should be used in accordance with the package instructions for use inserts. Copies of these inserts can be found in the [Control IFUs](#) folder on the S drive ([S:\APS\ClinLab\PoliciesandProcedures\1000-8999CLINICALPATHOLOGY\3000-3999Chemistry\3000-3499AutomatedChemistry\ControlIFUs](#)) 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
MAS ChemTrak 1	+2°C to +8°C*
MAS ChemTrak 3	+2°C to +8°C*

*Controls are received frozen and stored at -15°C to -25°C. Bottles of controls in use are thawed and stored at +2°C to +8°C and are good for 14 days.

Testing Procedure

1. If necessary prepare reagent as defined in the Reagent Preparation section of this chemistry information sheet and load the reagent onto the system.

2. After reagent load is completed, calibration is 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 automatically performs internal calculations 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 Remisol, the final calculated result from a dilution will not be calculated by the UniCel DxC system but by Remisol.

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

$$\text{Urine Urea Nitrogen} \frac{\text{mg}}{\text{dL}} \times \frac{\text{g}}{1000\text{mg}} \times \frac{\text{dL}}{100 \text{ mL}} \times \text{Total volume collected (mL)} = \text{g/24hr}$$

24-hour Total Urine Nitrogen results are obtained by multiplying 24-hour UUN from the above calculation by a factor of 1.25 (1)

**Calculations are only performed on 24 hour collections (±15 minutes) and reported as g/24 hr.
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 – Urea Nitrogen

Intervals	Sample Type	Conventional Units (Urea Nitrogen)		S.I. Units (Urea)
Literature	Serum or Plasma	6 - 20 mg/dL		2.1 - 7.1 mmol/L
	Urine (timed)	12 - 20 g/24 hrs		0.43 - 0.71 mol/24 hrs
SYNCRHON	Serum or Plasma	8 - 20 mg/dL		2.9 – 7.1 mmol/L
UCDMC	Serum or Plasma	0 days to 1 yr	3 - 7 mg/dL	1.8 – 5.0 mmol/L
	Serum or Plasma	1 yr to 2 yrs	5 – 14 mg/dL	1.1 – 2.5 mmol/L
	Serum or Plasma	2 yrs to 16 yrs	7 – 17 mg/dL	2.5 – 6.1 mmol/L
	Serum or Plasma	16 yrs to adult	8 – 22 mg/dL	2.9 – 7.9 mmol/L
	Urine (timed)	12 – 20 g/24 hrs		0.43 – 0.71 mmol/24 hrs

Reference Intervals – Total Urine Nitrogen

Literature	Urine (timed)	< 16 yrs	Not established	Not established
	Urine (timed)	≥16 yrs	7 – 20 g/24 hrs	147.8-714 mmol/24 hrs

Refer to References (1,8,9,10) for guidelines on establishing laboratory-specific reference intervals.

Pediatric reference intervals are cited from literature. No in-house studies were performed. This information should serve as a general guideline.

24-hour urine reference interval is cited from literature. No in-house studies were performed.

Calculated 24-hour urea nitrogen results less than 7 g/24 hours will be reported as **< 7 g/24 hours**.

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

Procedural Notes

Anticoagulant Test Results

- If plasma is the sample of choice, the following anticoagulants were found to be compatible with this method based on a study of 20 healthy volunteers:

Compatible Anticoagulants

Anticoagulant	Level Tested for In Vitro Interference	Average Plasma-Serum Bias (mg/dL)
Ammonium Heparin	14 Units/mL	NSI ^a
Lithium Heparin	14 Units/mL	NSI
Sodium Heparin	14 Units/mL	NSI
Potassium Oxalate/ Sodium Fluoride	2.0 / 2.5 mg/mL	NSI

^a NSI = No Significant Interference (within ± 3 mg/dL of urea nitrogen or 6%).

Limitations

None identified

Interferences

- The following substances were tested for interference with this methodology:

Interferences

Substance	Source	Level Tested	Observed Effect ^a
Bilirubin (unconjugated)	Bovine	30 mg/dL	NSI ^b
Hemoglobin	RBC Hemolysate	500 mg/dL	NSI
Lipemia	Intralipid ^c	500 mg/dL	NSI
L-Dopa	NA ^d	40 μ g/mL	-3 mg/dL
Methylbenzethonium Chloride	NA	0.5 mg/dL	-5 mg.dL

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

^b NSI = No Significant Interference (within ± 3 mg/dL of urea nitrogen or 6%)

^c Intralipid is a registered trademark of KabiVitrum, Inc., Clayton, NC 27250.

^d NA = Not applicable.

- If urine samples are cloudy or turbid, it is recommended that they be centrifuged before dilution and analysis.
- Grossly lipemic samples should be ultracentrifuged and the analysis performed on the infranate.
- Refer to References (12,13,14) for other interferences caused by drugs, disease and preanalytical variables.

Performance Characteristics

Analytical Measurement Range

The SYNCHRON® System(s) method for the determination of this analyte provides the following analytical ranges:
 Analytical Measurement Range (AMR)

Sample Type	Conventional Units (Urea Nitrogen)	S.I. Units (Urea)
Serum or Plasma	1 - 150 mg/dL	0.4 – 53.6 mmol/L
Serum or Plasma (ORDAC)	130 – 300 mg/dL	46.4 – 107.1 mmol/L
Urine	10 – 1500 mg/dL	3.57 – 535.7 mmol/L
Urine (ORDAC)	1300 – 3000 mg/dL	464.3 – 1071.4 mmol/L

Clinical Reportable Range:

Clinical Reportable Range (CRR)

Sample Type	Conventional Units (Urea Nitrogen)	S.I. Units (Urea)
Serum/Plasma/Urine	1 - diluted result mg/dL	0.4 – diluted result mmol/L

Serum/plasma/urine samples with concentrations below the AMR and CRR (< 1 mg/dL) should be reported as "**None detected**". Samples with concentrations greater than the AMR (> 150 mg/dL) should be 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 the urea nitrogen or urea determination is 1 mg/dL (0.4 mmol/L) for serum or plasma and 10 mg/dL (3.57 mmol/L) for urine.

Equivalency

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

As determined by Beckman

Serum or Plasma (urea nitrogen in the range of 3.0 to 126.0 mg/dL):

Y (SYNCHRON LX Systems)	= 0.949X + 1.13
N	= 79
MEAN (SYNCHRON LX Systems)	= 34.2
MEAN (SYNCHRON CX7 DELTA)	= 34.9
CORRELATION COEFFICIENT (r)	= 0.9986

Diluted Urine (urea nitrogen in the range of 35.0 to 1539 mg/dL):

Y (SYNCHRON LX Systems)	= 0.952X - 8.51
N	= 80
MEAN (SYNCHRON LX Systems)	= 748.8
MEAN (SYNCHRON CX7 DELTA)	= 795.4
CORRELATION COEFFICIENT (r)	= 0.9981

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Serum or Plasma (urea nitrogen in the range of 3 to 150 mg/dL):

Y (UniCel DxC Systems)	= 0.985X + 0.31
N	= 111
MEAN (UniCel DxC Systems)	= 48
MEAN (SYNCHRON LX Systems)	= 48
CORRELATION COEFFICIENT (r)	= 1.000

Diluted Urine (urea nitrogen in the range of 12 to 1500 mg/dL):

Y (UniCel DxC Systems)	= 1.001X + 3.23
N	= 140
MEAN (UniCel DxC Systems)	= 603
MEAN (SYNCHRON LX Systems)	= 599
CORRELATION COEFFICIENT (r)	= 0.998

Refer to References (14) for guidelines on performing equivalency testing.

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

As determined at UCDCMC

Serum or Plasma (in the range of 4 to 97 mg/dL):

Y (UniCel DxC800-4118)	= 0.966X + 1.1
N	= 39
MEAN (UniCel DxC800-4118)	= 25.3
MEAN (UniCel DxC800-1805)	= 25.0
CORRELATION COEFFICIENT (r)	= 0.9988

Serum or Plasma (in the range of 4 to 97 mg/dL):

Y (UniCel DxC800-4427)	= 0.963X + 1.2
N	= 39
MEAN (UniCel DxC800-4427)	= 25.3
MEAN (UniCel DxC800-1805)	= 25.0
CORRELATION COEFFICIENT (r)	= 0.9992

Serum or Plasma (in the range of 4 to 97 mg/dL):

Y (UniCel DxC800-4449)	= 0.963X + 0.9
N	= 39
MEAN (UniCel DxC800-4449)	= 24.9
MEAN (UniCel DxC800-1805)	= 25.0
CORRELATION COEFFICIENT (r)	= 0.9989

Serum or Plasma (in the range of 5 to 93 mg/dL):

Y (UniCel DxC800-4427)	= 0.996X + 0.1
N	= 39
MEAN (UniCel DxC800-4427)	= 25.3
MEAN (UniCel DxC800-4118)	= 25.3
CORRELATION COEFFICIENT (r)	= 0.9996

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Serum or Plasma (in the range of 5 to 93 mg/dL):

Y (UniCel DxC800-4449)	= 0.997X - 0.3
N	= 39
MEAN (UniCel DxC800-4449)	= 24.9
MEAN (UniCel DxC800-4118)	= 25.3
CORRELATION COEFFICIENT (r)	= 0.9996

Serum or Plasma (in the range of 5 to 93 mg/dL):

Y (UniCel DxC800-4449)	= 1.001X - 0.4
N	= 39
MEAN (UniCel DxC800-4449)	= 24.9
MEAN (UniCel DxC800-4427)	= 25.3
CORRELATION COEFFICIENT (r)	= 0.9995

Precision

A properly operating UniCel DxC System(s) should exhibit imprecision values less than or equal to the maximum performance limits in the table below. Maximum performance limits were derived by an examination of the imprecision of various methods, proficiency test summaries, and literature sources.

As determined by Beckman

Maximum Performance Limits

Type of Precision	Sample Type	1 SD		Changeover Value ^a		%CV
			mmol/L Urea	mg/dL	mmol/L Urea	
Within-run	Serum/Plasma	1.5	0.5	50	16.7	3.0
	Serum/Plasma (ORDAC)	NA ^b	NA	NA	NA	5.0
	Urine	3.0	1.1	100.0	37.0	3.0
	Urine (ORDAC)	NA	NA	NA	NA	5.0
Total	Serum/Plasma	2.3	0.8	51.1	17.8	4.5
	Serum/Plasma (ORDAC)	NA	NA	NA	NA	7.5
	Urine	4.5	1.7	100.0	37.1	4.5
	Urine (ORDAC)	NA	NA	NA	NA	7.5

^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.

^b NA = Not applicable

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Precision established at UCDCM

Type of Precision	Sample Type	n	Mean (mg/dL)	1 SD	%CV
DxC800-4118 Within-run	SYNCHRON 1	20	7.0	0.4	5.7
	SYNCHRON 3	20	60.3	0.6	1.1
DxC800-4427 Within-run	SYNCHRON 1	20	7.0	0.2	3.2
	SYNCHRON 3	20	60.8	0.4	0.7
DxC800-4449 Within-run	SYNCHRON 1	20	7.0	0.0	0.0
	SYNCHRON 3	20	60.1	0.7	1.1

Type of Imprecision	Sample Type	n	Mean (mg/dL)	SD	%CV
DxC800-4118 Day to Day	MAS ChemTrak 1	1339	13	0.9	6.9
	MAS ChemTrak 3	1327	65	1.3	2.0
DxC800-4427 Day to Day	MAS ChemTrak 1	1332	13	0.9	6.9
	MAS ChemTrak 3	1324	65	1.3	2.0
DxC800-4449 Day to Day	MAS ChemTrak 1	1327	12	0.7	5.8
	MAS ChemTrak 3	1336	65	1.2	1.8

Comparative Performance

Data for a SYNCHRON LX System evaluated using the NCCLS Proposed Guideline EP5-T2 appears in the table below.(16)

As determined by Beckman

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	Serum Control 1	1	80	7.7	0.4	5.0
	Serum Control 2	1	80	58.7	0.5	0.9
	Urine Control 1	1	80	384.7	4.7	1.2
	Urine Control 2	1	80	744.8	7.1	1.0
Total	Serum Control 1	1	80	7.7	0.7	8.9
	Serum Control 2	1	80	58.7	0.9	1.6
	Urine Control 1	1	80	384.7	8.1	2.1
	Urine Control 2	1	80	744.8	20.3	2.7

^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.

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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.

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Prepared By	Date Adopted	Supersedes Procedure #
Michael Inn	October, 2000	Reformatted

Revision Date	Type of Revision	Revised by	Review/Annual Review Date	Reviewed By
			11/27/2000	G. Kost
			12/28/2001	G.Kost
			10/16/2002	G. Kost
			10/10/2003	S. Devaraj
			10/25/2004	S. Devaraj
			11/28/2005	G. Kost
			09/26/2006	G. Kost
			11/05/2007	G. Kost
			06/16/2008	G. Kost
			09/15/2009	G. Kost
			10/12/2010	G. Kost
12/2010	update	M.Inn	11/16/2011	G. Kost
08/07/2013	No reference interval for spot/random urines	M. Inn	08/16/2013	G. Kost
			09/17/2013	G. Kost
12/11/2014	Added TUN calculation and reporting	kdagang	12/18/2014	L. Howell