

8. APPENDIX

Recommended Methods

8.1 Microalbumin in urine

8.1.1 Reagents

Glycine buffer (230 mmol/l, pH 3.0) Dissolve 17.27 gm. of glycine in about 800 ml. distilled water, adjust to pH 3.0 with 5N HCl, keep in refrigerator.

Bromphenol blue, stock solution (1.25 mmol/l) Dissolve 125.7 mg. of bromphenol blue in 3 ml. of 0.1 mol/l NaOH and dilute to 150 ml. with distilled water.

Bromphenol blue, working solution (0.188 mmol/l, pH 3.0) mix 150 ml. of BPB stock solution with glycine buffer to 1,000 ml. Add 4 ml. of surfactant solution, (30% Brij 35). Absorbance at 610 nm. should be 0.20-0.29.

Standard Human Albumin: Dissolve 0.20 gm. of human serum albumin (Cohn fraction V; Sigma Chemical Co.) in 0.85 % NaCl to 100 ml. The standard curve was formed from six standard dilutions covering the assay range 0-100 mg/dl.

8.1.2 Test parameters. (Automated chemistry analyzer, Abbott CCx.)

TEST DEFINITION

ENTRY NAME	MICROALBUMIN	SAMPLE	UL	NORMAL
20.0				
REPORT NAME	MA	LOW	HIGH	.000
TEST NUMBER	82	UNITS PRIN	M/MOL	SEC

TEST TYPED CALIBRATED SEC. UNITS FACTOR 0.000
 MATH LIN REG END PT PRINT DIGITS 0
 REACTION DIRECTION UP
 REAGENTS 1 INST MUL 1.000 INT
 0.000
 TEMPERATURE(C) 37 NORMAL [C] 0.000 TO
 3.000
 TEST BLANK TYPE REAGENT BLANK

TEST PARAMETER FILE : REAGENT DEFINITION

REAGENT NUMBER 1 FOR TEST MICROALBUMIN
 REAGENT NAME MA LINEARITY [C] 0.000 TO
 100.000
 LOT ID 32 INITIAL (Ad) 0.200 ABS LIMIT (Ad)
 0.300
 REAGENT VOLUME (UL) NORM 200.0 LOW 0.000 HIGH 0.000
 BEFORE WASH CYCLES 18 ALTER WASH CYCLES 18
 FIRST READ TIME (SEC) 60 LAST READ TIME (SEC) 180
 NUMBER OF READS 2 MIX TIME (SEC) 1.00
 READ INTERVAL (SEC) 60.0 CONSTANT INTERCEPT 0.000
 AUX REAG DISP (SEC) 0. CORRECTION LIMIT 0.000
 RSM 0 SPECTRAL CORRECTION
 RPIM SEC USE IN CONST E.F LOW HIGH
 610/MA A 1.000 .000 0.000 0.000
 610/MA A 1.000 .000 0.000 0.000
 610/MA A 1.000 .000 0.000 0.000
 610/MA LE 1.000 .000 0.000 0.000

LINEAR MODEL CALIBRATION DEFINITION

TEST NAME MICROALBUMIN TEST TYPE CALIBRATED
 MATH MODEL LIN REG END PT CAL ON CMD CAL
 INTERVAL (HR) 255
 INT CPT TOL [C] 1000.00 - -TO -1000.00 REF CAL FACTOR 0.000
 % TOL OF CAL FACTOR 20 % TOL OF CAL 20

CALIBRATOR		LEVEL [C]	REPLICATES				
WATER		0.000	1				
MA1		12.5	1				
MA2		50.0	1				
MA3		100.0	1				

CALIBRATOR STATUS							
TEST NAME		MICROALBUMIN		LAST CAL ; 3.38 AM 01 - MAR - 00			
		LIN REG END PT		CAL STATUS		O.K	ACCEPT
CAL							
CAL MODE		CAL ON CMD		CAL LEVEL		0	
		ENTERED		ACCEPTED		NEW	
LEVEL	NAME	CONC	CONC	DELTA Ad	CONC	DELTA Ad	
1	WATER	0.000	-1.597	-0.031	-0.996	0.018	SELECT
2	MA 1	12.50	10.324	0.055	13.280	0.125	A NOTHER
3	MA 2	50.00	57.002	0.393	50.626	0.405	TEST
4	MA 3	100.00	96.771	0.681	99.589	0.771	EXAMINE
CALIBRATOR							
CAL FACT			138.141		133.648	LOADLIST	
INTERCEPT (Ad)			0.00	-0.0198	0.00	0.0258	
INTERCEPT (C)			2.7454	0.00	-3.4490	0.00	
C. OF C.			0.9945		0.9998	RECALIBRATE	

8.2 Urinary N-acetyl-beta-D-glucosaminidase

8.2.1 Reagents

Citrate buffer (100 mmol/l) Dissolve 9.67 gm. citric acid (MW.210.14), 15.88 gm. Trisodium citrate dihydrate (MW. 294.12) in 700 ml. distilled water, adjust pH to 4.8 and filled up to 1,000 ml. with distilled water.

CNP-NAG substrate (2.0 mmol/l) : Dissolve 0.0377 gm. CNP-NAG in citrate buffer approximately 40 ml. The solution was warmed up to 60 °C while mix with magnetic

stirrer for 30 minutes then 0.1017 gm $MgCl_2 \cdot 6H_2O$. was added, the volume was adjusted to 50 ml. with citrate buffer, The substrate can be kept in refrigerator for 2 weeks.

Working standard NAG (6.6 unit /mg. protein) : Dilute stock standard NAG (6.6 units /mg. protein) 200 μ l with distilled water to get NAG 64.52, 32.26 and 16.13 U/l, respectively.

8.2.2 Test parameters (Automated chemistry analyzer, Abbott CCx.)

TEST DEFINITION

ENTRY NAME	NAG 2	SAMPLE	UL	NORMAL	20.00
REPORT NAME	NAG 2	LOW		HIGH	0.000
TEST NUMBER	59	UNITS PRIN	M/MOL	SEC	
TEST TYPED	CALIBRATED	SEC. UNITS FACTOR			0.000
MATH	LIN REG END PT	PRINT DIGITS			0
REACTION DIRECTION	UP				
REAGENTS	1	INST MUL	1.000	INT	0.000
TEMPERATURE(C)	37			NORMAL [C]	0.000 TO 11.000
TEST BLANK TYPE	REAGENT BLANK				

TEST PARAMETER FILE : REAGENT DEFINITION

REAGENT NUMBER	1	FOR TEST	NAG 2
REAGENT NAME	CNP	LINEARITY [C]	0.000 TO 25.000
LOT ID	32	INITIAL (Ad)	0.400
		ABS LIMIT (Ad)	2.40
REAGENT VOLUME (UL)	NORM 200.0	LOW 0.000	HIGH 0.000
BEFORE WASH CYCLES	18	ALTER WASH CYCLES	18
FIRST READ TIME (SEC)	100	LAST READ TIME (SEC)	240
NUMBER OF READS	2	MIX TIME (SEC)	1.00
READ INTERVAL (SEC)	60.0	CONSTANT INTERCEPT	0.000
AUX REAG DISP (SEC)	0.	CORRECTION LIMIT	0.000
RSM	0	SPECTRAL CORRECTION	
RPIM SEC	USE IN	CONST	E.F
			LOW HIGH

404/MA	A	1.000	.000	0.000	0.000
404/MA	A	1.000	.000	0.000	0.000
404/MA	A	1.000	.000	0.000	0.000
404/MA	LE	1.000	.000	0.000	0.000

LINEAR MODEL CALIBRATION DEFINITION

TEST NAME	NAG 2	TEST TYPE	CALIBRATED
MATH MODEL	LIN REG END PT	CAL ON CMD	CAL INTERVAL (HR) 270
INT CPT TOL [C]	1000.00 --TO -1000.00	REF CAL FACTOR	350.00.
% TOL OF CAL FACTOR	20	% TOL OF CAL	20
CALIBRATOR	LEVEL [C]	REPLICATES	
WATER	0.000	1	
NAG 1	16.130	1	
NAG 2	32.260	1	
NAG 3	64.520	1	

CALIBRATOR STATUS

TEST NAME	NAG 2	LAST CAL ;	1.43 AM 01 - MAR - 00				
LIN REG END PT	CAL STATUS	O.K	ACCEPT				
CAL							
CAL MODE	CAL ON CMD	CAL LEVEL	0ENTEREDCCEPTED NEW				
LEVEL	NAME	CONC	CONC	DELTA Ad	CONC	DELTA Ad	
1	WATER	0.000	-0.523	-0.0117	-0.523	-0.012	SELECT
2	NAG 1	16.130	16.130	0.043	16.130	0.043	ANOTHER
3	NAG 2	32.260	32.023	0.090	32.023	0.089	TEST
4.	NAG 3	64.520	64.425	0.189	64.425	0.190	EXAMINE
							CALIBRATOR
CAL FACT	350.00	323.067		323.067			LOADLIST
INTERCEPT (Ad)		0.00	-0.0100	0.00	0.0258		
INTERCEPT (C)		3.260	0.00	3.260	0.00		
C. OF C.		0.99977		0.99977			RECALIBRATE

8.3 Fructosamine

8.3.1 Reagents

Stock 1 mmol/l Nitroblue tetrazolium (NBT) ;
NBT 0.0817 gm. was dissolved with 100 ml of carbonate buffer pH 10.35.

Standard DMF 10.00 mmol/l ; 1-Deoxy-1-morpholinofructose (DMF) 0.0125 gm. was dissolved with 40 gm/L human serum albumin to 10 ml.

Carbonate buffer 0.1 M pH 10.3 (Na₂CO₃ 5 mmol/l and 25 mmol/l NaHCO₃) ; Na₂CO₃ 0.795 gm. , NaHCO₃ 0.210 gm. were dissolved with 100 ml. distilled water.

Human serum albumin (40 gm./l) ; Human serum albumin 0.4 gm. was dissolved with 0.85 % NaCl to 10.0 ml.

8.3.2 Test parameters (Automated chemistry analyzer, Abbott CCx.)

TEST DEFINITION

ENTRY NAME	FRUC 2	SAMPLE	UL	NORMAL	1.00
REPORT NAME	FRUC 2		LOW 0.000	HIGH	.000
TEST NUMBER	84		UNITS PRIN	M/MOL	SEC
TEST TYPED	CALIBRATED		SEC. UNITS FACTOR		0.000
MATH	LIN REG END PT		PRINT DIGITS		0
REACTION DIRECTION	UP				
REAGENTS	1		INST MUL	1.000	INT 0.000
TEMPERATURE(C)	37			NORMAL [C]	0.000 TO 3.000
TEST BLANK TYPE	REAGENT BLANK				

TEST PARAMETER FILE : REAGENT DEFINITION

REAGENT NUMBER	1	FOR TEST	FRUC 2		
REAGENT NAME	FRUC 2	LINEARITY [C]	0.000 TO 10.000		
LOT ID	32	INITIAL (Ad)	0.200 ABS LIMIT (Ad)		
0.500					
REAGENT VOLUME (UL)	NORM 200.0	LOW 0.000	HIGH 0.000		
BEFORE WASH CYCLES	18	ALTER WASH CYCLES	18		
FIRST READ TIME (SEC)	60	LAST READ TIME (SEC)	240		
NUMBER OF READS	2	MIX TIME (SEC)	1.00		
READ INTERVAL (SEC)	60.0	CONSTANT INTERCEPT	0.000		
AUX REAG DISP (SEC)	0.	CORRECTION LIMIT	0.000		
RSM	0	SPECTRAL CORRECTION			
RPIM SEC	USE IN	CONST	E.F	LOW	HIGH
548/MA	A	1.000	.000	0.000	0.000
548/MA	A	1.000	.000	0.000	0.000
548/MA	A	1.000	.000	0.000	0.000
548/MA	LE	1.000	.000	0.000	0.000

LINEAR MODEL CALIBRATION DEFINITION

TEST NAME	FRUCT 2	TEST TYPE	CALIBRATED
MATH MODEL	LIN REG END PT	CAL ON CMD	CAL INTERVAL (HR) 255
INT CPT TOL [C]	1000.00 --TO -1000.00	REF CAL FACTOR	0.00
% TOL OF CAL FACTOR	20	% TOL OF CAL	20
CALIBRATOR	LEVEL [C]	REPLICATES	
WATER	0.000	1	
DMF 1	1.25	1	
DMF 2	2.50	1	
DMF 3	5.00	1	

CALIBRATOR STATUS

TEST NAME	FRUC 2	LAST CAL ;	3.38 AM 01 - MAR - 00				
	LIN REG END PT	CAL STATUS	O.K	ACCEPT			
CAL							
CAL MODE	CAL ON CMD	CAL LEVEL	0				
	ENTERED	ACCEPTED	NEW				
LEVEL	NAME	CONC	CONC	DELTA Ad	CONC	DELTA Ad	
1	WATER	0.000	-0.088	0.056	-0.996	0.018	SELECT
2	DMF 1	1.25	1.409	0.155	13.280	0.125	ANOTHER
3	DMF 2	2.50	2.4373	0.224	50.626	0.405	TEST

4	DMF 3	5.00	4.991	0.393	99.589	0.771	EXAMINE
							CALIBRATOR
	CAL FACT		15.046		15.046		LOADLIST
	INTERCEPT (Ad)		0.00	0.0616	0.00	0.0258	
	INTERCEPT (C)		-0.9264	0.00	-0.9264	0.00	
	C. OF C.		0.9987		0.9998		RECALIBRATE

8.4 HbA_{1c}

Glyco-Tek Affinity Columns : Each column contains cellulose resin covalently bonded to dihydroxyboryl groups, in a low ionic strength buffer.

Glyco-Tek Hemolysate Reagent contains magnesium chloride, surfactant, buffer and 0.02% sodium azide as a preservative.

Glyco-Tek Developer A contains magnesium chloride buffer.

Glyco-Tek Developer B contains sorbitol, buffer and 0.1 % sodium azide, as a preservative; pH 6.0.

8.5 Glucose (Trace package insert book : Hexokinase method)

<u>Active ingredients</u>	<u>Concentration</u>
Triethanolamine	50 mmol/l
ATP	1.30 mmol/l
NAD	0.65 mmol/l
Hexokinase	> 1500 U/l
G-6-PDH	> 2500 U/l

pH 7.5 ± 0.2 at 20 °C

8.6 BUN (Trace, package insert book : Enzymatic urea reagent)

<u>Active ingredients</u>	<u>concentration</u>
α- ketoglutarate	7.5 mmol/l
NADH	0.28 mmol/l
Urease	> 8000 U/l
GLDH	> 650 U/l
ADP	1.2mmol/l
Tris buffer	115 mmol/l

Also contains non-reactive fillers and stabilizers, pH 7.9 ± 0.1 at 25 °C.

8.7 Creatinine (Trace, package insert book : Creatinine reagent, Picric acid method)

<u>Active ingredients</u>	<u>concentration</u>
Picric acid	16 mmol/l
Sodium hydroxide	150 mmol/l

9. CURRICULUM VITAE

NAME Kanokwan Mohprasit
DATE OF BIRTH October 19, 1959
PLACE OF BIRTH Chiang Mai, Thailand
INSTITUTE ATTENDED
Faculty of Associated Medical Sciences,
Chiang Mai University, Chiang Mai
March, 1981 : Bachelor of Science
(Medical Technology)