



PRESENTED BY

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Queen Savang Vadhana Memorial Hospital &

SciSpec Co., Ltd.

# What is a heavy metal in clinical test?

- Heavy metals and some toxic elements such as **As, Pb, Hg, Cd, Mn, Cr, Co, Ni** and **Al** are dangerous substances and can cause some diseases or negative impacts on our health
- Trace element analysis is also important for identification and monitoring of disease resulting from chronic elemental accumulations and potentially toxic exposures, such as through an occupational or environmental setting
- Metal exposure can be occurred by several sources, especially contaminated food and drink as well as occupation
- **As** is absorbed into the human body; the major portion is excreted in the urine (~60%)
- Whole blood samples is preferred for some elements such as **Pb** and **Cd** because most of these circulating elements (>90%) are found within erythrocytes
- Serum or plasma samples are preferred in the analysis of elements that bind to serum proteins such as **Al** and **Cu**. Elements that are excreted by the kidneys and that are rapidly cleared from circulating blood, such as **As** (half-life: 4 to 6 hours), are best detected in urine because of a longer window of detection (up to 3 weeks)



# Biomarker of exposure

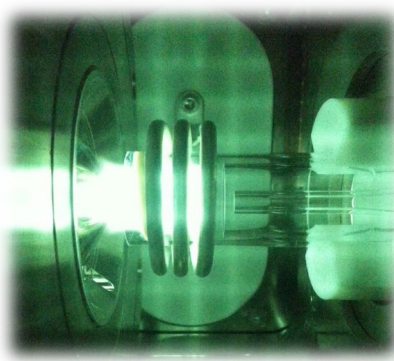
National Institute of Health (NIH) of Thailand		
Elemental	Sample	NIH
Arsenic (As)	Urine	< 40 µg/g creatinine
Cadmium (Cd)	Whole blood	< 2.0 µg/L. ( In smokers, it should not exceed 6.0 µg/L )
Lead (Pb)	Whole blood	< 10 µg /dL
Manganese (Mn)	Whole blood	4 – 15 µg/L
Mercury (Hg)	Whole blood	< 4.0 µg/L
	Urine	< 5.0 µg /g creatinine
Nickel (Ni)	Urine	30 µg/g Creatinine
Zinc	Serum	0.7 – 1.7 µg/mL



Thai Biological Exposure Indices : Thai BEIs		
Elemental	Sample	Thai BEIs
Arsenic (As)	Urine	35 µg As/L
Cadmium (Cd)	Urine	5 µg/g Creatinine
	Whole blood	5 µg/L
Lead (Pb)	Whole blood	30 µg/dL
Manganese (Mn)	Urine	50 µg/L
Mercury (Hg)	Urine	20 µg/g Creatinine
Nickel (Ni)	Urine	30 µg/g Creatinine
Chromium (Cr)	Urine	<input type="checkbox"/> Total chromium in urine 25 µg/L (End of shift at end of workweek)
		<input type="checkbox"/> Total chromium in urine 10 µg/L (Increase during Shift)



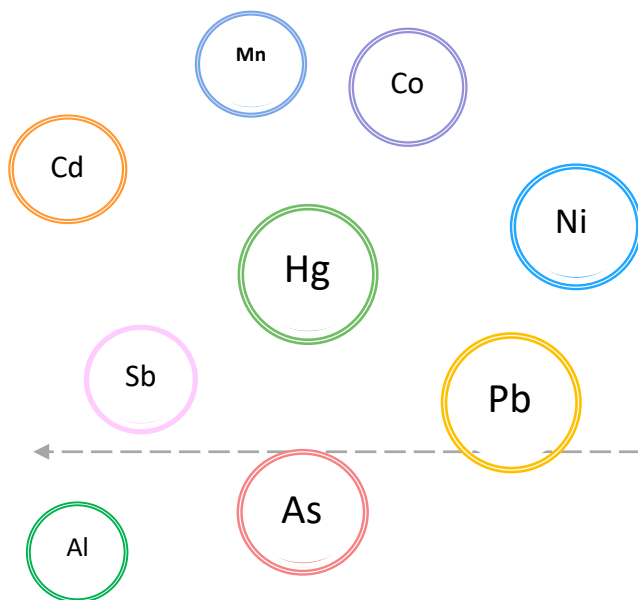
# Inductively coupled plasma Mass spectrometer (ICP-MS)



- ❑ Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) which is the most reliable analytical method for metal determination including **high accuracy, precision, specificity and sensitivity**
- ❑ Efficient low-level **quantitation of multiple** trace and toxic elements



iCAP RQ™ ICP-MS  
Thermo Scientific



- ✓ **Fast, easy operation with quick-connect sample introduction components**
- ✓ **Easy to maintenance**
- ✓ **Excellent interference removal with high ion transmission**
- ✓ **Measure all analytes in a single helium Kinetic Energy Discrimination (He KED) mode**
- ✓ **High sensitivity and low detection limit**
- ✓ **'Get Ready' one-click set-up for easy, reproducible analysis**

# Objective



ศูนย์เวชศาสตร์อุตสาหกรรม

เครือข่ายโรงพยาบาลสมเด็จพระบรมราชเทวี ณ ศรีราชา สภาเภสัชกรรม

Analysis of Arsenic, Lead and Mercury in Whole blood and Urine using iCAP RQ ICP-MS



A rapid, highly sensitivity, Easy operation and Accurate High Throughput and ICP-MS method was developed for detection and quantitation of Elemental impurities in Clinical product



# Sample Preparation



Examination and reporting of results				
Analytical method	Specimen	Amount	Delivery	Reporting
AAS, ICP/MS  (depending on the type of toxic metal)	Serum	2 ml of serum per type	Place in a plastic tube that has been washed with 10% nitric acid and rinsed 3-4 times with distilled water. Close the cork tightly.	The results are reported in numbers in units of µg/dl, µg/ml or µg/l depending on the type of toxic metal
	whole blood	2-10 ml per type (depending on the type of metal being examined)	It is placed in a plastic vacutainer tube containing the anticoagulant heparin or EDTA	The results are reported in numbers in units of micrograms per deciliter, micrograms per milliliter or micrograms per liter depending on the type of toxic metal
	Urine	20 ml per type	Place in a plastic container washed with 10% nitric acid and rinse 3-4 times with distilled water, tightly closing the lid	The results are reported in numbers in units of micrograms per deciliter, micrograms per milliliter or micrograms per liter depending on the type of toxic metal.
	Should be taken to the laboratory immediately. If this is not possible, store in the refrigerator 4-8 ° C.			

A microscopic view of whole blood, showing numerous red blood cells (erythrocytes) and a plasma background. The red blood cells are biconcave discs, appearing as large, round, pinkish-red structures with darker centers. They are densely packed in some areas and more sparse in others. The plasma is a light pinkish-red fluid. The overall image has a soft, slightly blurred quality, typical of a microscopic photograph.

Whole Blood



# Standard Method and Blood Control

## ClinChek® - Control

Whole Blood Control, Level I, II, III

Analyte / Analyt	Unit / Einheit	Mean Value / Sollwert	Control Range / Kontrollbereich
<b>Arsenic / Arsen</b>			
Level I	µg/l	5.25	4.20 - 6.30
Level II	µg/l	10.1	8.11 - 12.2
Level III	µg/l	19.4	15.5 - 23.3
<b>Cadmium / Cadmium</b>			
Level I	µg/l	1.19	0.948 - 1.42
Level II	µg/l	2.93	2.35 - 3.52
Level III	µg/l	6.40	5.12 - 7.68
<b>Chromium / Chrom</b>			
Level I	µg/l	1.25	0.941 - 1.57
Level II	µg/l	5.49	4.39 - 6.59
Level III	µg/l	10.9	8.74 - 13.1
<b>Cobalt / Kobalt</b>			
Level I	µg/l	1.53	1.14 - 1.91
Level II	µg/l	7.05	5.64 - 8.46
Level III	µg/l	13.1	10.5 - 15.7

### Lead / Blei

Level I	µg/l	59.1	47.3 - 70.9
Level II	µg/l	228	182 - 274
Level III	µg/l	446	357 - 535

### Mercury

Level I	µg/l	1.44	1.01 - 1.87
Level II	µg/l	6.47	4.86 - 8.09
Level III	µg/l	12.1	9.64 - 14.5

Level III	µg/l	12.9	10.4 - 15.5
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## Standard Reference Material® 955c

## Thai BEIs Thai Biological Exposure Indices : Thai BEIs

Elemental	Sample	Thai BEIs
Arsenic (As)	Urine	35 µg As/L

NIH National Institute of Health (NIH) of Thailand		
Elemental	Sample	NIH
Arsenic (As)	Urine	< 40 µg/g creatinine
Cadmium (Cd)	Whole blood	< 2.0 µg/L. ( In smokers, it should not exceed 6.0 µg/L )
<b>Lead (Pb)</b>	<b>Whole blood</b>	<b>&lt; 10 µg / dL</b>
Manganese (Mn)	Whole blood	4 - 15 µg/l
<b>Mercury (Hg)</b>	<b>Whole blood</b>	<b>&lt; 4.0 µg/L</b>
	Urine	< 5.0 µg /g creatinine
Nickel (Ni)	Urine	30 µg/g Creatinine
Zinc	Serum	0.7 - 1.7 µg/mL

5 µg/g Creatinine

**300 µg/L**

**30 µg/dl**

50 µg/L

**20 µg/g Creatinine**

**µg/L. , ขึ้นกับ Creatinine**

(End of shift at end of workweek)

☐ Total chromium in urine 10 µg/L (Increase during Shift)



# Whole blood control & Urine control

## Seronorm™ Trace Elements Whole Blood



**BIO-RAD**

## Lyphochek Urine Metals Control



## Seronorm™ Trace Elements Urine

Lyophilized urine control for toxicology and environmental analysis of trace elements and heavy metals

2 clinically relevant levels  
Product documentation with a list of elements and toxic organic components  
Human-based urine



### Components

Aluminium, Antimony, Arsenic, Barium, Beryllium, Bismuth, Boron, Bromine, Cadmium, Calcium, Cerium, Cesium, Chromium, Cobalt, Copper,

## NIST Standard Reference Materials®



Health &  
Clinical

Clinical  
Health &

**BIO-RAD**

## Lyphochek Whole Blood Metals Control



## RECIPE

**ClinChek®**

PRODUCTS / CLINCHEK® CONTROLS

### TRACE ELEMENTS

**Analytes:** Aluminium, Antimony, Arsenic, Barium, Beryllium, Cadmium, Chromium, Cobalt, Copper, Fluoride, Gold, Iodide, Iron, Lead, Magnesium, Manganese, Mercury, Molybdenum, Nickel, Palladium, Platinum, Selenium, Thallium, Tin, Vanadium, Zinc

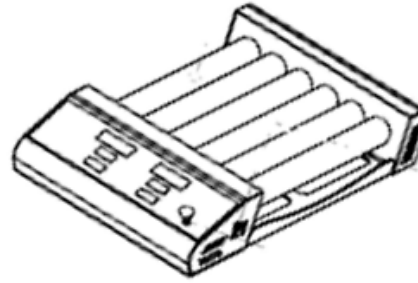


# Whole Blood Control Preparation Procedures

## ClinChek® - Whole Blood Control



Add 5 ml of deionized water to the vial

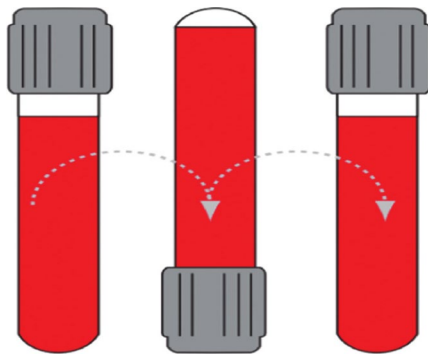


Roller mixer for 60 mins



Incubate the samples for 2 hours at room temperature by shaking it carefully

One mixing cycle for mix the blood



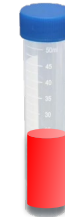
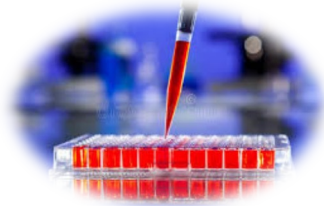
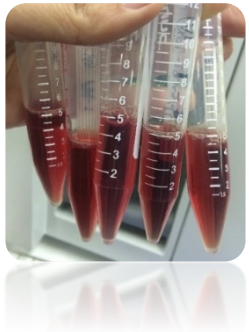
Prior to use, mixing the controls well again.



Make sure the RM has the same preparation as the sample

# Sample Preparation Procedures

Method No. **ITB001A** The Centers for Disease Control and Prevention (CDC), USA : This method directly measures **lead**, cadmium and **mercury** in whole blood specimens using ICP-MS after a simple dilution sample preparation step.



Sample and Calibration Standard is  
50 -fold diluted with diluent

Vortex mixer

Analysis by iCAP RQ ICP-MS

## Diluent

- 1.0 g of disodium ethylenediamine tetraacetate (EDTA)  
In 1% v/v tetramethylammonium hydroxide (TMAH)
- 10% ethyl alcohol
- 0.05% v/v Triton X-100
- 10ug/L Au ( For reduce intrinsic Hg memory effects)

## Internal Standard

- add Bi for internal standardization of Hg and Pb





Urine

# Standard Method and Urine Control

ClinChek® - Control  
Urine Control lyophilised  
FOR TRACE ELEMENTS

**ClinChek® - Control**

Urine Control, Level I, II

ClinChek® - Control  
Urine Control, Level I, II  
Kontrollurin, Level I, II

REF 8847-8849  
LOT 1227  
2021-06

## Thai BEIs Thai Biological Exposure Indices : Thai BEIs

Elemental	Sample	Thai BEIs
Arsenic (As)	Urine	35 µg As/L

<b>Arsenic / Arsen</b>					
Level I	µg/l	43.5	34.8	-	52.2
Level II	µg/l	82.3	65.9	-	98.8
<b>Barium / Barium</b>					
Level I	µg/l	10.8	8.65	-	13.0
Level II	µg/l	51.3	41.1	-	61.6
<b>Beryllium / Beryllium</b>					
Level I	µg/l	0.057	0.040	-	0.073
Level II	µg/l	0.246	0.184	-	0.307
<b>Cadmium / Cadmium</b>					
Level I	µg/l	2.47	1.98	-	2.96
Level II	µg/l	14.3	11.4	-	17.2
<b>Chromium / Chrom</b>					
Level I	µg/l	4.04	3.23	-	4.85

Level I	µg/l	1.54	1.15	-	2.14	nmol/l	15.4	10.8	-	20.1
Level II	µg/l	9.72	7.29	-	12.1	nmol/l	91.3	68.5	-	114



## Laboratory Procedure Manual

Analytes: Antimony, Arsenic, Barium, Beryllium, Cadmium, Cesium, Cobalt, Lead

Standard Method CDC No.: 3018.3 (15 element panel)  
3018A.2 (total arsenic)

Analytes: Antimony, Arsenic, Barium, Beryllium, Cadmium, Cesium, Cobalt, Lead, Manganese, Molybdenum, Platinum, Strontium, Thallium, Tin, Tungsten, and Uranium

Matrix: Urine

3018A.2 (total arsenic)

Revised: March 19, 2012

As performed by: Inorganic Radionuclides and Toxicology  
Division of Laboratory Sciences  
National Center for Environmental Health

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Phone: 770-488-7990  
Fax: 770-488-4097  
Email: [KCaldwell@cdc.gov](mailto:KCaldwell@cdc.gov)

James L. Pirkle, M.D., Ph.D.  
Director, Division of Laboratory Sciences

### Important Information for Users

The Centers for Disease Control and Prevention (CDC) periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

# Sample Preparation Procedures

Method No. [3018A.2](#) , The Centers for Disease Control and Prevention (CDC), USA : This method directly measures **total arsenic** in urine specimens using ICP-MS



Sample is 10- fold diluted with 2 %  $\text{HNO}_3$  + 1.5% ethanol (In the case of arsenic)  
\*if multi-element do not fill 1.5% ethanol

Vortex mixer

Filter samples through a 0.45- $\mu\text{m}$  cellulose ester membrane filter



Analysis by ICP-MS



1. add Ga for internal standardization of As
2. Rinse Solution: 2% Triton X-100™ in 5% (v/v)  $\text{HNO}_3$  + 1.5% EtOH  
(In the case of arsenic, add 1.5% ethanol)

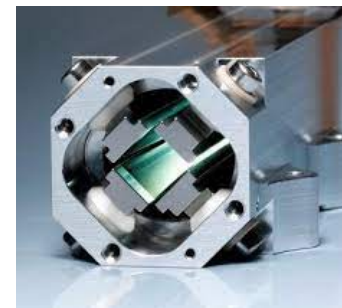
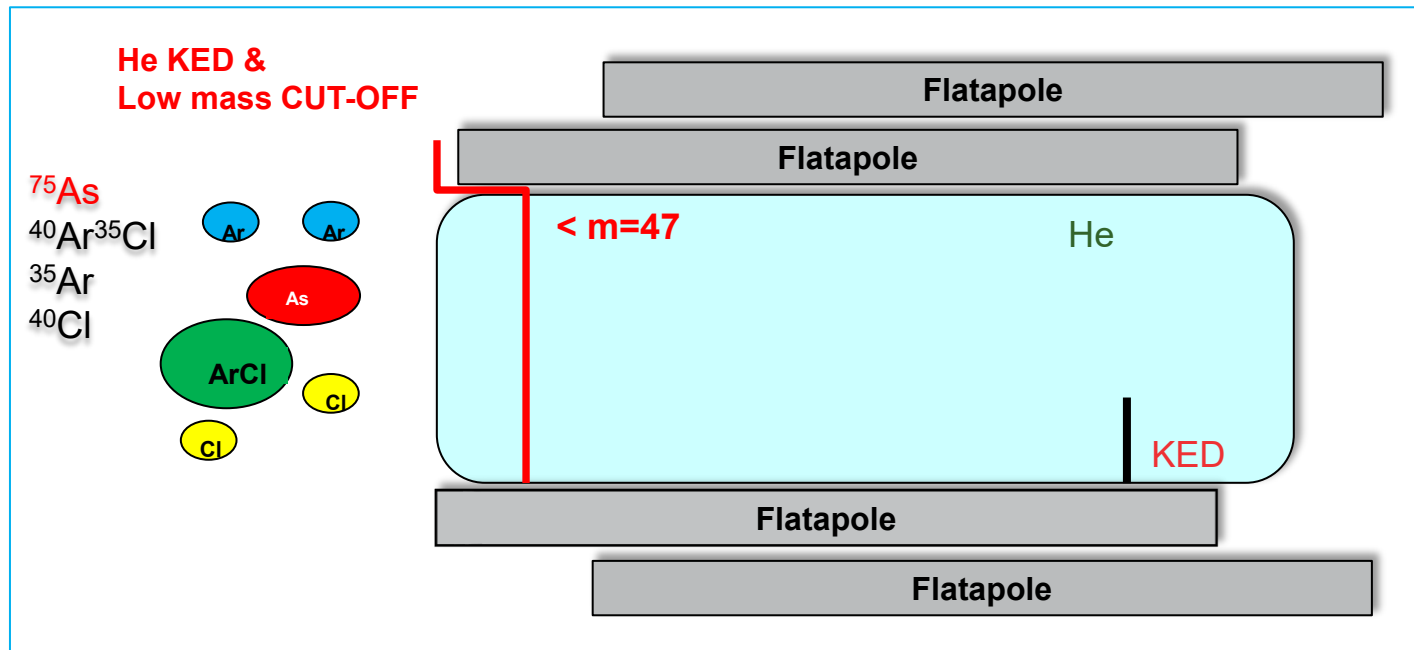


# Condition of iCAP RQ ICP-MS

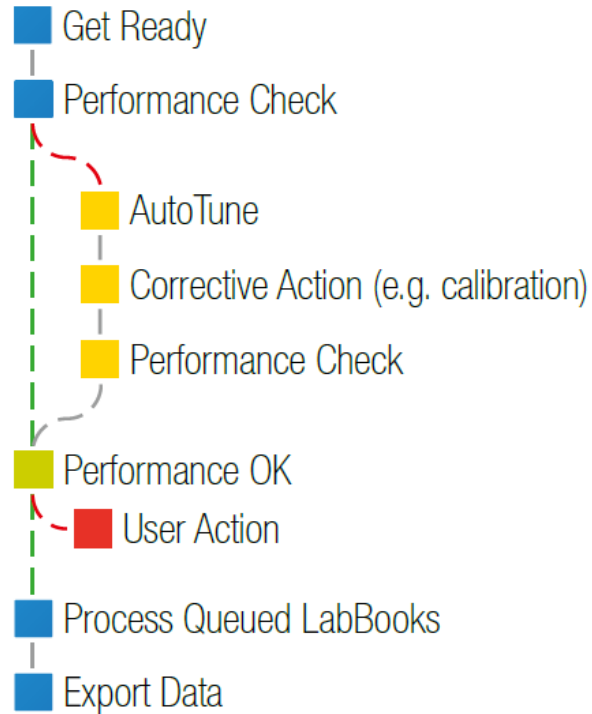
**KED (Kinetic energy discrimination)** : Technique to reduce polyatomic ion interferences derived from the plasma or vacuum interface in collision cell ICP-MS.

Nebulizer	Micro mist Nebulizer (400 $\mu$ L/min)
Nebulizer flow	1.0 L/min
Spray chamber	Cyclonic Quartz
Injector	Quartz 2.5 mm ID
Interface	Ni Sample and Skimmer cone
RF power	1550 W
CRC conditions	4.2 mL/min pure He
Scan settings	0.05 sec per analyte, 3 Main runs
Number of Replicates	3
Total analysis time per sample	45 sec

Typical operating parameters iCAP RQ ICP-MS



# Sequential for Analysis



## Batch Start

- Run Calibration Standards 1-6
- Run CRM's ClinChek® - Control of Urine Control or whole blood Control Level I, Level II and Level III

80-120 %

## Sample Analysis

- Sample Analysis with IS monitorization
- QC sample (Calibration Standard) every 10 samples

## Batch End

- Run CRM's ClinChek® - Control Urine or blood Control lyophilized Level I, Level II and Level III



# Results

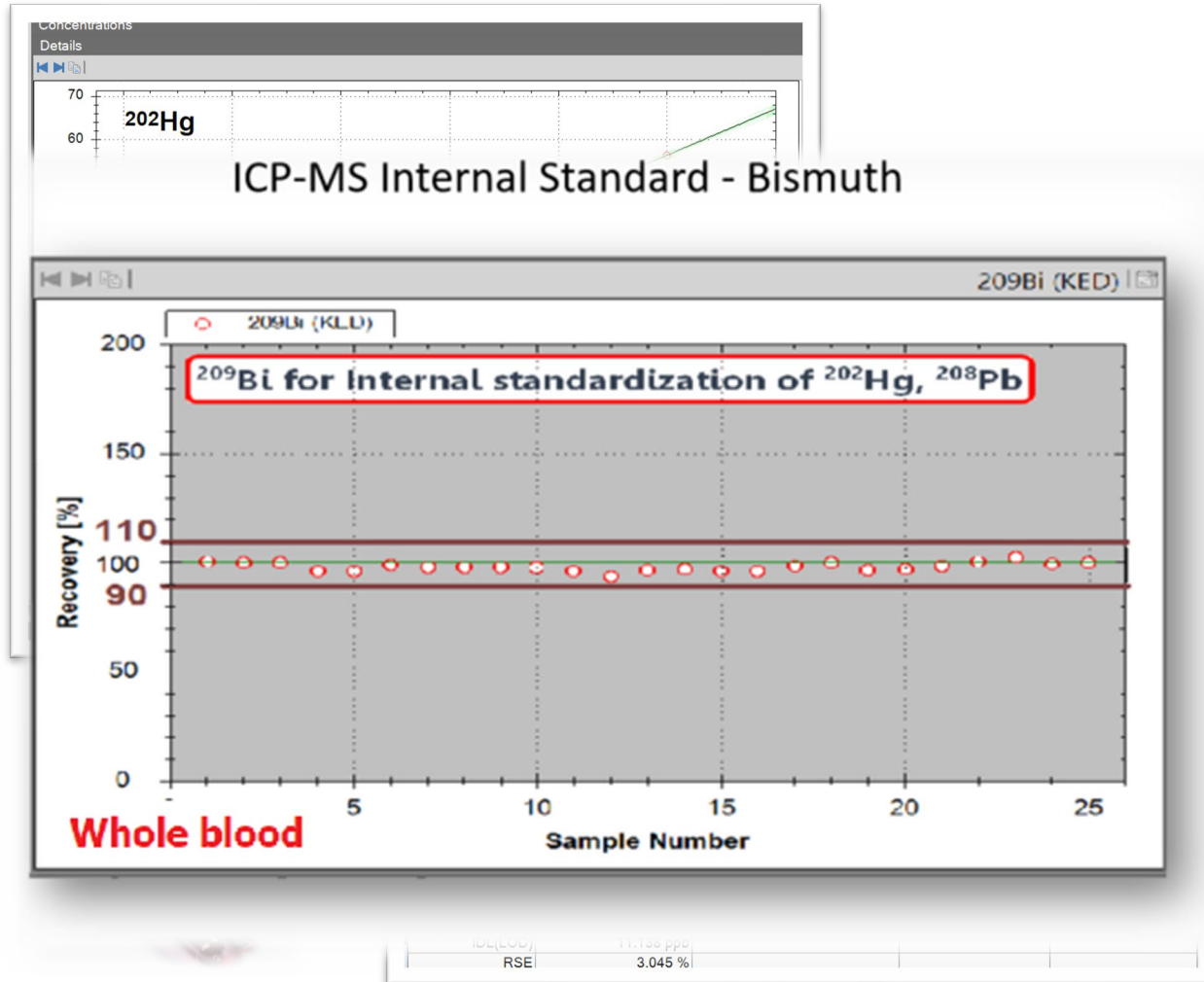
Whole Blood and Urine





# Whole Blood - Calibration curve

Linear calibrations with low (sub ng.ml<sup>-1</sup>) blanks were obtained for Hg and Pb



Sample Typ ▾	Label ▾	202Hg (KED) [pp ▾	208Pb (KED) [ppb] ▾
AVERAGE B		0.000	0.000
STD			
STD	STD1	0.999 (1.000)	10.650 (10.000)
STD	STD1	1.026 (1.000)	10.653 (10.000)
STD	STD2	10.303 (10.000)	100.854 (100.000)
STD	STD2	10.120 (10.000)	100.669 (100.000)
STD	STD3	20.308 (20.000)	200.370 (200.000)
STD	STD3	20.073 (20.000)	200.273 (200.000)
STD	STD4	29.539 (30.000)	297.295 (300.000)
STD	STD4	29.986 (30.000)	298.895 (300.000)
STD	STD5	99.996 (100.000)	999.864 (1,000.000)
STD	STD5	100.027 (100.000)	1,000.985 (1,000.000)
Calibratio			
Sample Typ ▾	Label ▾	202Hg (KED) [pp ▾	208Pb (KED) [ppb] ▾

# Whole Blood - Control

Analyte	Mean Value (µg/l)	Control Range (µg/l)	Measure (µg/l)	
			#1	#2
ClinChek® Control - Whole blood Control lyophilized				
Lead				
Level I	59.1	47.3 – 70.9	65.130	60.377
Level II	228.0	182 – 274	245.532	242.271
Level III	446.0	357 – 535	470.917	508.248
Mercury				
Level I	1.44	1.01 – 1.87	1.293	1.087
Level II	6.47	4.86 – 8.09	5.162	5.434
Level III	12.1	9.64 – 14.5	10.116	10.013



ClinChek®

Label
AVERAGE B
STD

## Thai BEIs

Elemental	
Arsenic (As)	
Cadmium (Cd)	
Lead (Pb)	
Manganese (Mn)	
Mercury (Hg)	
Nickel (Ni)	
Chromium (Cr)	

NIH National Institute of Health (NIH) of Thailand		
Elemental	Sample	NIH
Arsenic (As)	Urine	< 40 µg/g creatinine
Cadmium (Cd)	Whole blood	< 2.0 µg/L. ( In smokers, it should not exceed 6.0 µg/L )
<b>Lead (Pb)</b>	<b>Whole blood</b>	<b>&lt; 10 µg /dL</b>
Manganese (Mn)	Whole blood	4 – 15 µg/l
<b>Mercury (Hg)</b>	<b>Whole blood</b>	<b>&lt; 4.0 µg/L</b>

Cadmium (Cd)	Urine	5 µg/g Creatinine	240.633
	Whole blood	<b>300 µg/L</b>	505.681
<b>Lead (Pb)</b>	<b>Whole blood</b>	<b>30 µg/dl</b>	905.495
Manganese (Mn)	Urine	50 µg/L	60.377
<b>Mercury (Hg)</b>	<b>Urine</b>	<b>20 µg/g Creatinine</b>	242.271
Nickel (Ni)		<b>ประมาณ 32 µg/L. , ขึ้นกับ Creatinine</b>	508.248
Chromium (Cr)	Urine	(End of shift at end of workweek) <input type="checkbox"/> Total chromium in urine 10 µg/L (Increase during Shift)	148.248
			245.315
			514.681
			905.116

# Whole Blood – QC analysis

Thai BEIs Thai Biological Exposure Indices : Thai BEIs		
Elemental	Sample	Thai BEIs
Arsenic (As)	Urine	35 µg As/L
Cadmium (Cd)	Urine	5 µg/g Creatinine
	Whole blood	<b>300 µg/L</b>
<b>Lead (Pb)</b>	<b>Whole blood</b>	<b>30 µg/dl</b>
Manganese (Mn)	Urine	50 µg/L
<b>Mercury (Hg)</b>	<b>Urine</b>	<b>20 µg/g Creatinine</b>
Nickel (Ni)	<b>ประมาณ 32 µg/L. , ขึ้นกับ Creatinine</b>	
Chromium (Cr)	Urine	(End of shift at end of workweek) <input type="checkbox"/> Total chromium in urine 10 µg/L (Increase during Shift)

Certified Reference Material  
QC1187 Trace Metals-ICP Sample 1

Element	LOD ( µg/l )	BEC
Hg	1.138	2.419
Pb	11.138	20.544

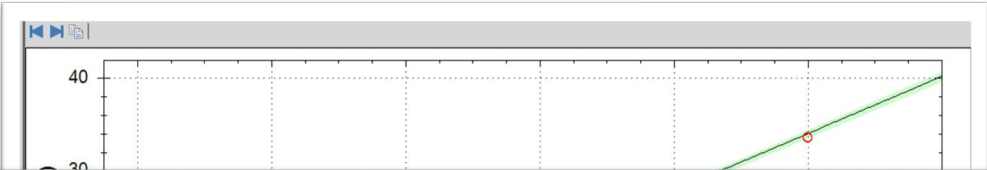
Analyte	Mean Value ( µg/l )	Control Range ( µg/l )	Measure ( µg/l )	
			#1	#2
QC Hg	90	87.58 – 92.42	90.698 (100.78 %)	90.059 (100.07 %)
QC Pb	900	720 - 1080	905.459 (100.61 %)	905.116 (100.57 %)

Result of the recovery of the **QC1187**



# Urine - Calibration curve of Arsenic in Urine

Linear calibrations with low (sub ng.ml<sup>-1</sup>) blanks were obtained As

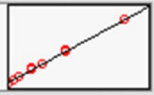


Label	69Ga (KE	75As (KED) [ppb]
	100.0%	0.000
std1	90.8%	5.421 (5.000)
std1	92.8%	5.618 (5.000)
	96.5%	10.611 (10.000)
	94.8%	10.398 (10.000)
	89.0%	20.517 (20.000)
	63.8%	20.087 (20.000)
	90.9%	30.966 (30.000)
	93.0%	30.711 (30.000)
	91.2%	50.660 (50.000)
	95.5%	50.988 (50.000)
	90.1%	98.613 (100.000)
	90.5%	98.987 (100.000)

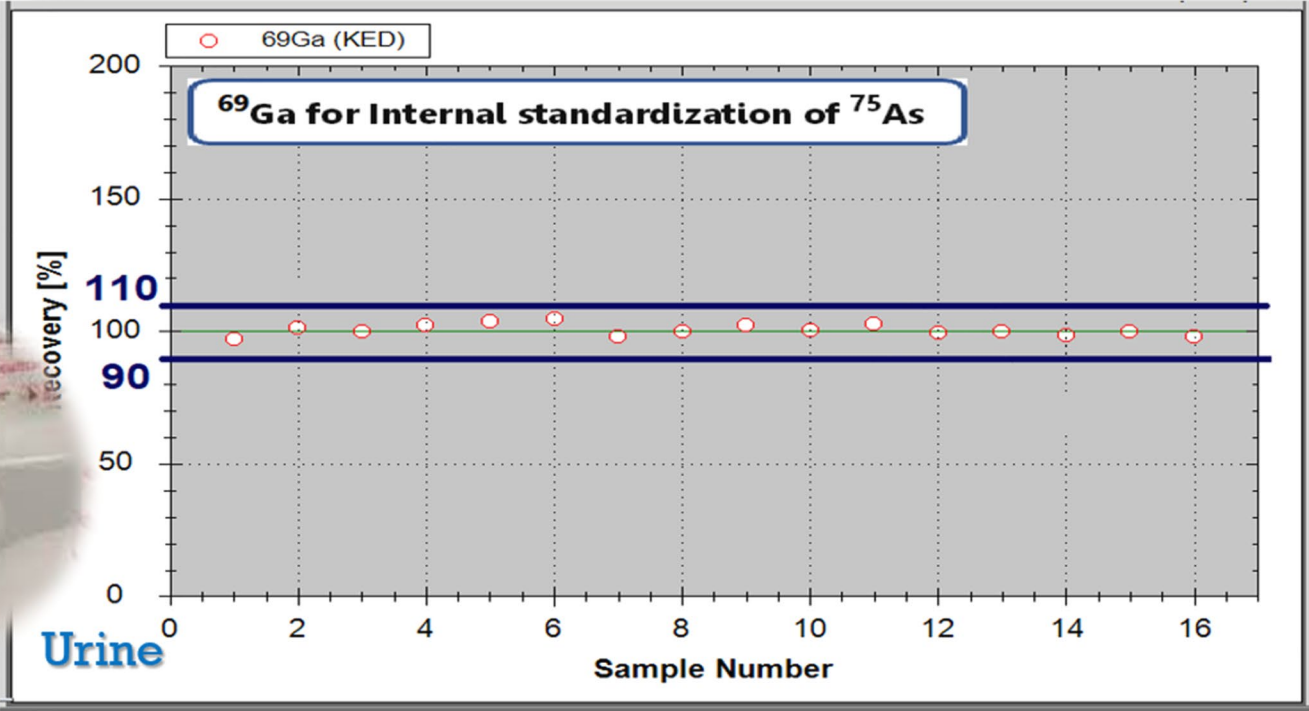
IS

69Ga (KE

75As (KED) [ppb]



## ICP-MS Internal Standard - Gallium



# Urine - Control

Analyte	Mean Value (µg/l)	Control Range (µg/l)	Measure (µg/l)	
			#1	#2
ClinChek® Control - Urine Control lyophilised				
Arsenic Level I	43.5	34.8 – 52.2	43.184	46.753
Arsenic Level II	82.3	65.9 – 98.8	85.214	87.442



Sample Typ ▾	Label ▾	72Ge (KED M ▾	75As (KED Mi ▾
: BLK		100.0%	0.119
: BLK		100.0%	0.073

Thai BEIs Thai Biological Exposure Indices : Thai BEIs		
Elemental	Sample	Thai BEIs
Arsenic (As)	Urine	35 µg As/L

: UNKNOWN	64Sxxx62	90.3%	41.464
: UNKNOWN	64Sxxx63	95.0%	57.787
: UNKNOWN	64Sxxx64	93.1%	47.570
: UNKNOWN	64Sxxx66	90.4%	28.708
: UNKNOWN	64Sxxx67	94.3%	23.818
: UNKNOWN	64Sxxx68	94.6%	31.757
: UNKNOWN	64Sxxx69	99.7%	54.102
: UNKNOWN	64Sxxx70	101.6%	74.057
: UNKNOWN	RM1 (34.8 - 52.2)	93.2%	46.753
: UNKNOWN	RM2 (65.9 - 98.8)	99.3%	87.442
: UNKNOWN	QC 300 ppb (270 - 330)	97.0%	306.327
: UNKNOWN	64Sxxx71 - xx	89.1%	130.312
: UNKNOWN	64Sxxx73 - xx	93.4%	42.904

Analyte	LOD ( µg/l )	BEC
As	0.013	11.156

Analyte	Quality Level ( µg/l )	Control Range ( µg/l )	Measure ( µg/l )	
			#1	#2
QC 1187	300	±20%	308.505 (102.84 %)	306.327 (102.11 %)

Thai BEIs Thai Biological Exposure Indices : Thai BEIs		
Elemental	Sample	Thai BEIs
Arsenic (As)	Urine	35 µg As/L

Result of the recovery of Trace Metals-ICP Sample 1

# Conclusion

- Heavy metal content was measured in blood samples, urine samples, Whole blood Control and Urine Control with the iCAP RQ ICP-MS.

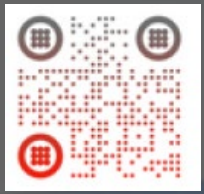
From the measurement results, lead and mercury values in Whole blood Control and Arsenic values in Urine Control are within the criteria of Certified and provides Method Detection Limits (MDL) in ppb level, demonstrating the iCAP RQ ICP-MS's performance in heavy metal detection. as well as providing fast measurement results reliable and use a small amount of sample to measure

- ICP-MS is a technique capable of analyzing heavy metals at ppb level or sub-ppb level, which this level are lower than BEIs standard of ACGIH.



**The iCAP RQ ICP-MS delivers the reliability, analytical performance and ease of use needed to meet the demands of the highest throughput routine laboratory**





Thank You