KIDNEY STONE ANALYSIS

Ready to use kit for the semi-quantitative colorimetric determination of carbonate, calcium, magnesium, ammonium, oxalate, phosphate, uric acid and cystine on kidney and urinary stones

INTENDED USE

Urinary Stones are made of insoluble organic and inorganic substances such as calcium oxalate and/or phosphate, cystine, uric acid, etc. Most urinary stones consist of several components.
Etiology varies and includes metabolic dysfunctions, obstructions, infections, hyper-saturation, lack of inhibitors, etc.
Almost all urinary (or kidney) stones consist of several components, due to co-precipitation or following precipitation.

TEST SUMMARY

A small amount of urinary (or kidney) stones is crushed and pulverized. On this sample, as homogenous as possible, the following semi-quantitative determinations can be carried out: calcium, magnesium, ammonium, oxalate, phosphate, uric acid and cystine
The percentage of each component is determined by visual comparison with the colour scale contained in the kit. The composition of the urinary stone is obtained from the results of these determinations using the enclosed slide rule.

SAMPLES

Kidney or urinary stone
Samples collection in compliance with CLSI (NCCLS)[17]

REAGENTS/KIT CONTENTS

CODE	CC04100	(50 TESTS x 7 parameters)
Reagent 1	Sulfuric acid	1 x 11 mL
H314 - Causes severe skin burns and eye damage.		
Reagent 2	Sodium hydroxide	1 x 13 mL
H314 - Causes severe skin burns and eye damage.		
Reagent 3	Calconcarboxilic acidin in Alcohol	1 x 16 mL
H225 - Highly flammable liquid and vapour.		
Reagent 4	EDTA	1 x 26 mL
Reagent 5	Borate Buffer	1 x 16 mL
H315 - Causes skin irritation.		
H319 - Causes serious eye irritation.		
Reagent 6	Iron Chloride	1 x 15 mL
Reagent 7	Sulfosalicyclic acid	1 x 15 mL
Reagent 8	Nessler reagent	1 x 10 mL
H300+H310+H330 - Fatal if swallowed, in contact with skin or if inhaled		
H314 - Causes severe skin burns and eye damage.		
H373 - May cause damage to organs through prolonged or repeated exposure.		
H410 - Very toxic to aquatic life with long lasting effects. (Acute toxicity M-factor = 0)		
Reagent 9	Ammonium molybdate	1 x 16 ml
H315 - Causes skin irritation.		
H319 - Causes serious eye irritation.		
Reagent 10	Sodium meta-bisulfite	1 x 16 mL
H412 - Harmful to aquatic life with long lasting effects.		
Reagent 11	Borate Buffer	1 x 13 mL
H360FD - May damage fertility. May damage the unborn child.		
H314 - Causes severe skin burns and eye damage.		
Reagent 12	Xylidyl blue	1 x 26 mL
H225 - Highly flammable liquid and vapour.		
Reagent 13	Phosphomolybdic acid	1 x 10 mL
H314 - Causes severe skin burns and eye damage.		
Reagent 14	Ammonia	1 x 26 mL
H314 - Causes severe skin burns and eye damage.		
H335 - May cause respiratory irritation.		
H336 - May cause drowsiness or dizziness.		
Reagent 15	Sodium sulphite	1 x 25 g
Reagent 16	Sodium nitroprusside	1 x 25 g
Control 1	Powder Control Standard for Carbonate, Calcium, Magnesium, Ammonium, Oxalate, Phosphate, Uric acid *Expected values on the attached sheet*	1 x 0,5 g
H300 - Fatal if swallowed.		
H315 - Causes skin irritation.		
H319 - Causes serious eye irritation.		
H335 - May cause respiratory irritation.		
Control 2	Powder Control Standard for Cystine *Expected values on the attached sheet*	1 x 0,1 g
Stirrer		2 x 25 pieces
Reusable test-tubes		4 x 10 mL / 1 x 50 mL
Reusable Petri dish		1 piece
White Micro-spatula 225 mg		2 pieces
Blue Micro-spatula 15 mg		3 pieces
Color scale		
Slide rule		

MATERIAL REQUIRED BUT NOT SUPPLIED

White porcelain pestle and mortar. 5 ml pipette. Distilled water.

PRECAUTIONS

It is recommended to handle carefully the reagents, avoiding ingestion and contact with eyes, mucous membranes and skin; to use reagents according to good laboratory practice. The material safety data sheet (MSDS) details the operating procedures for the manipulation of these products. pay attention to the Hazard statement and pictograms on labels.

REAGENT PREPARATION

Reagents are ready to use and are stable, stored at 15-25°C, until expiration date on label, also after first opening. Mix kindly before use and let the reagents reach room temperature before use.
Reagent 8 appears as of slightly turbid suspension.
Precipitate eventually present in the reagent 13 does not influence the final result of the analysis..
Be careful to use the reagents in a proper way in order to avoid contamination.

PROCEDURE

PRELIMINARY PROCEDURE PHASE - ***CARBONATE DETERMINATION***
After an examination of the stone to record shape, colour, smoothness, size, etc. crush the urinary stone in the mortar and reduce to powder.
Put approximate 15 mg (or 1 level spoonful using the blue spatula) of the powdered stone to be analysed in the supplied Petri dish or in another transparent polystyrene container. Add 5 drops of Reagent 1: a formation of bubbles/foam indicates the presence of carbonate.
Warning: Reagent 1 (sulphuric acid) is highly corrosive, carefully avoid contact with skin.

Stir with the included plastic stirrer for a few minutes, until complete dissolution. Possible traces of undissolved material does not influence the continuation of the analysis.
Transfer in the 50 mL test-tube and using distilled water, take care to rinse well the Petri disk. Add distilled water up to 50 ml. and mix well.
Withdraw 5 ml. of the solution for each parameter to be determined and carry out the tests in the supplied 10 mL test-tubes.

CALCIUM DETERMINATION
Calcium is determined by means of a complexometric titration with Calconcarboxilic acid as an indicator.
-Procedure-
Add 2 drops of Reagent 2 and 3 drops of Reagent 3 to the sample tube. Mix well.
Possibly maintaining a continuous shaking (also manually) add, drop after drop, Reagent 4 until the colour changes from red to blue. Count in the same moment the numbers of added drops: the percentage of Calcium in the stone is calculated multiplying the number of drops per 5.

OXALATE DETERMINATION
Oxalate decolours the mixture of Iron and Sulfosalicyclic acid.
-Procedure-
Add to the sample tube, respecting the following sequence, 2 drops of Reagent 5, 3 drops of Reagent 6 and 3 drops of Reagent 7.
Let the solution rest for 2 minutes and then compare the colour with the relative colour scale, identifying the relative oxalate percentage.

AMMONIUM DETERMINATION
The ammonium ion forms yellow-brown solutions together with the Nessler reagent.
-Procedure-
Add to the sample tube, respecting the following sequence, 3 drops of Reagent 8 and 3 drops of Reagent 2 and then compare the colour with the relative colour scale, identifying the relative ammonium percentage.

PHOSPHATE DETERMINATION
The phosphomolybdic acid formed in the reaction is reduced to molybdenum blue.
-Procedure-
Add to the sample tube, respecting the following sequence, 5 drops of Reagent 9 and 5 drops of Reagent 10. Let the solution rest for 5 minutes and then compare the colour with the relative colour scale, identifying the relative phosphate percentage.

MAGNESIUM DETERMINATION
Magnesium reacts with the chemical components and forms a red complex.
-Procedure-
In a glass test-tube add 1 ml of the sample and 4 ml of distilled water. Shake and then add, respecting the following sequence, 1 drops of Reagent 11 and 5 drops of Reagent 12. Let the solution rest for 1 minute and then compare the colour with the relative colour scale, identifying the relative magnesium percentage.

URIC ACID DETERMINATION
Uric acid reduces the phosphomolybdic acid to molybdenum blue.
-Procedure-
Add 3 drops of Reagent 13 to the sample test-tube. Shake and let the solution rest for 2 minutes, then add 2 drops of Reagent 5. Then compare the colour, as quickly as possible, with the relative colour scale, identifying the relative uric acid percentage.
The comparison must be made promptly because the obtained colour is unstable and tends to change to blue.

CYSTINE DETERMINATION
Cystine, in presence of sodium sulphite, is reduced to Cysteine, which together with nitroprusside, forms a red colour.
-Procedure-
Add 10 drops of Reagent 14 and 450 mg (or 2 level spoonfuls using of the white spatula) of Reagent 15 to the sample test-tube. Stir until completely dissolved. Wait 1 minute and then add 450 mg (or 2 level spoonfuls using the white spatula) of Reagent

16. Stir until completely dissolved. After 30 seconds compare the colour with the relative colour scale, identifying the relative cystine percentage.

MATHEMATICAL PROCESSING OF RESULTS

The single elements and/or the compounds determined with this reagent kit and found in urinary stones are usually part of the following chemical compounds:

- Calcium oxalate (Whewellite)
- Magnesium ammonium phosphate (Struvite)
- Calcium hydrogen phosphate (Brushite)
- Tri-Calcium phosphate (Apatite)
- Ammonium urate
- Uric acid
- Cystine

These compounds have been used as a base for the included slide rule.
The composition of a urinary stone is obtained from the percentage values of its individual components with the help of the slide rule.
Matching requires two steps:

1. For each cation the possible compounds (see list above) are compiled, considering the actually present anions.
2. Then the compounds which are probably present and their relative quantities are determined by means of the slide rule.

1. Calcium oxalate (Whewellite)
a) Set the percentage of oxalate content obtained in the test on the oxalate scale and read the associated calcium oxalate value on the calcium oxalate scale.
b) Check for the amount of calcium consumed in the chemical process on the calcium scale.
If more calcium was found in the analysis than what would correspond to the amount indicated, calculate by subtraction the amount of calcium, which hasn't probably been combined with oxalic acid (f.e. calcium phosphate)

2. Magnesium ammonium phosphate (Struvite)
a) Set the percentage of magnesium content obtained in the test on the magnesium scale and read the associated magnesium ammonium phosphate value on the struvite scale.
b) Check for the amounts of ammonium and phosphate consumed in the chemical process on the ammonium or phosphate scale, respectively.
If more ammonium or phosphate was found in the analysis than what would correspond to the amounts indicated, calculate by subtraction the amount of ammonium or phosphate which hasn't probably been combined to form magnesium ammonium phosphate (f.e. Ammonium urate or calcium phosphate, respectively).

3. Ammonium urate
a) Set the ammonium content obtained in the test [or the one obtained by subtraction as described in point 2.b. of the magnesium ammonium phosphate (Struvite) paragraph] on the ammonium scale. Read the associated ammonium urate value on the ammonium urate scale.
b) Check for the amount of uric acid consumed in the process on the uric acid scale.
If more uric acid was found in the analysis than would correspond to the amount indicated, calculate by subtraction the residual amount of uric acid.

4. Calcium phosphate
a) Set the calcium content obtained in the test [or the one obtained by subtraction as described in point 1.a. of the Calcium oxalate (Whewellite) paragraph] on the calcium scale. At the same time check on the phosphate scales (Brushite or Apatite), which of these values corresponds best either to the actually found value or to the amount obtained by subtraction as described in point 2.b. of the Magnesium ammonium phosphate (Struvite) paragraph.
b) Read the Brushite or the Apatite content on the next scale below in either case.

CALCULATION EXAMPLE BASED ON OBTAINED RESULTS

From the analysis of a stone it has been determined that this contains, for example, only the following components:

Calcium 35% Oxalate 15% Phosphate 40%

It is possible that this stone contains calcium oxalate and calcium phosphate, therefore we will verify the different percentages of calcium bound to oxalates and phosphates:

- 15% of oxalate on the slide rule corresponds to 25% of calcium oxalate
- 25% of calcium oxalate on the slide rule corresponds to 7% of calcium (bound to oxalate)
- Therefore the calcium not bound to oxalates (presumably bound to phosphates) is obtained by the following calculation: 35% - 7%= 28%
- On the slide rule 28% of calcium corresponds to 40% phosphate, which means on the lower phosphate scale 70% of Apatite

So the final conclusion is that the stone consists of about 25% calcium oxalate and 70% apatite.

NB: For methodological reasons, the addition of the component percentage does not always give exactly 100%.

NOTE

- DO NOT mix Reagents from different Production lots.
- As with any diagnostic procedure, if the results are incompatible with the medical records, the physician should evaluate obtained data using this test considering other clinical information.
- Only for IVD use.
- As part of an internal Q.C., it is recommended to use the controls standard contained in the kit just like a sample according to the operating procedure. The relative expected values for the various components are recorded in the included control sheet.
  Use Control 1 for Carbonate, Calcium, Magnesium, Ammonium, Oxalate, Phosphate, Uric acid and Control 2 for cystine.

WASTE DISPOSAL

This product is intended for professional laboratories.
Waste products must be handled as per relevant security cards and local regulations.

SENSITIVITY

The method is capable of detecting minimum amount of each component , as shown in a following table:

Detection limits		
Component	**Measurement unit**	**Limit**
Ammonium	mg	0.3
Magnesium	mg	0.1
Calcium	mg	0.75
Phosphates	mg	1
Uric acid	mg	0.1
Sodium Oxalate	mg	0.2
Carbonates	mg	0.4
Cystine	mg	0.25

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SYMBOLS

IVD	Only for IVD use		Expiration date
LOT	Lot of manufacturing		Warning, read enclosed documents
REF	Code number		Read the directions
	Storage temperature interval		Biological risk

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