

# KIDNEY STONES

## Improved method

Semi quantitative colorimetric determination of calcium, magnesium, cystine, uric acid, oxalate, phosphate, ammonium on kidney and urinary stones

50 tests x 7 parameters

REF CM05-50T

### INTENDED USE

Kidney/urinary stones are crystalline concretions composed of insoluble organic and inorganic substances that can form in the urinary tract. Identification of their components is of primary importance to determine their origin and etiology. Primary stones that form in acidic urine (without inflammation) are mainly formed by uric acid, urates and oxalates and rarely by cystine and calcium. Secondary stones that form in alkaline urine (following inflammation) whose common constituent is ammonium-magnesium phosphate.

### TEST SUMMARY

One or more kidney or urinary stones are crushed in a mortar. A semi-quantitative determination of its components can be carried out with this kit. The percentage of the components is estimated by comparison with the color scale supplied with the kit.

Improvements of our kit compared to the traditional method:

1. three milligrams of kidney stone are enough to perform one test,
2. just one powder reagent in the kit,
3. no titration for the analytes determination,
4. our color scales are better defined,
5. two steps for a more precise determination of the percentages of magnesium, uric acid and oxalate,
6. Simplified and accurate reading of the results without using the ruler.

### SAMPLE

Kidney and/or urinary stones.  
For IVD use only.

### REAGENTS

Reagents are ready to use.

(\* **Dangerous reagents are marked by an asterisk.**

**Refer to MSDS** (material safety data sheet).

### Kit composition

* <b>Reagent 1</b> (carbonate)	
Sulfuric acid	1x11 mL
* <b>Reagent 2</b> (calcium)	
Arsenazo III	1x300 drops
* <b>Reagent 3</b> (magnesium)	
Tris Buffer	1x300 drops
* <b>Reagent 4</b> (magnesium)	
Xylidyl blue	1x200 drops
* <b>Reagent 5</b> (cystine)	
Ammonia	1x300 drops
<b>Reagent 6</b> (cystine)	
Sodium sulfite	1x12,5 g
<b>Reagent 7</b> (cystine)	
Sodium nitroprusside	1x150 drops
* <b>Reagent 8</b> (uric acid)	
Phosphomolybdic acid	1x50 drops
* <b>Reagent 9</b> (uric acid)	
NaOH concentrated	1x210 drops
* <b>Reagent 10</b> (oxalate)	
Glycine buffer	1x20 mL
* <b>Reagent 11</b> (oxalate)	
Iron chloride	1x150 drops
* <b>Reagent 12</b> (oxalate)	
Sulfosalicylic acid	1x150 drops
* <b>Reagent 13</b> (phosphate)	
Ammonium molybdate	1x300 drops
* <b>Reagent 14</b> (phosphate)	
Sodium meta-bisulphite	1x150 drops
* <b>Reagent 15</b> (ammonium)	
Nessler reagent	1x150 drops
<b>Synthetic Control A</b>	1x0,3 g
Powder Control Standard for Carbonate, Calcium, Ammonium, Oxalate.	
<b>Synthetic Control B</b>	1x0,3 g
Powder Control Standard for Magnesium, Cystine, Uric acid, Phosphate.	

STABILITY: when stored at room temperature and tight sealed reagents and controls are stable up to the expiration date on the label.

### ADDITIONAL ITEMS INCLUDED WITH THE KIT

Stirrer (disposable)	2x25 pcs
Test tubes (reusable)	7x4 mL
Sample solution test tube (reusable)	1x50 mL
Petri dish (reusable)	1 piece
White micro-spatula 200 mg	1 piece
Blue micro-spatula 5 mg	1 piece
Color scale	

### REQUIRED BUT NOT PROVIDED

White porcelain mortar and pestle.  
Two variable micro pipettes (20-200 uL and 200-1000 uL).  
Double distilled water.

### PRECAUTION IN HANDLING REAGENTS

#### Read the MSDS before performing the test.

Use the reagents according to good laboratory practice.  
Pay attention to the hazard statement and pictograms on the labels.  
Mix gently by inversion the bottles before use.  
Close the bottles immediately after use.  
Do not to contaminate the bottles.  
The dispensed drops should not contain air, if in doubt, discard the drop.

### CRUSHING OF THE KIDNEY STONE

#### Preliminary Assay Procedure

After an examination of the kidney/urinary stone to record shape, color, smoothness, size, etc., crush it in the mortar and reduce it to powder.

### PREPARATION OF THE SAMPLE SOLUTION and DETECTION OF THE PRESENCE OF CARBONATES

Put **10 mg** (or **2 spoonful of the blue spatula**) of the powdered kidney stone to be tested in the Petri dish.

Pipette on the powder **exactly 200 uL** of R1.

The formation of bubbles/foam indicates the presence of carbonate.  
Stir with the plastic stirrer until complete dissolution.

Traces of undissolved material does not influence the result of the test.  
Using the pipette, add **slowly 4 mL** of double distilled water on the Petri dish and mix well.

Transfer all the contents into the 50 mL sample solution test tube.

Rinse well the Petri dish with double distilled water and transfer the contents into the sample solution test tube.

Brings the sample solution to a final volume of 25 mL with double distilled water.

Cap and shake by inversion.

**This is the sample solution to be used for all seven tests.**

**Note 1:** If the kidney/urinary stone is less than 10 mg, dissolve the powder with a proportional amount of R1 and of double distilled water.

Example: 8 mg of sample with 160 uL of R1 and double distilled water up to a final volume of 20 mL.

Test can be carried out with very small quantity of sample (3 mg of sample are enough, adding 60 uL of R1 and double distilled water up to a final volume of 7.5 mL).

**Note 2:** If it's not possible to weigh the kidney stone, use the blue spatula that contains 5 mg.

The quantity of 5 mg per spatula is an average estimate, much depends on the composition and on the grain size of the kidney stone.

**Note 3:** All plastic reusable items must be washed carefully with double distilled water and dried after use.

**Note 4:** The sample solution may be cloudy, depending on the composition of the kidney stone

Shake well the tube by inversion before taking the sample solution.

### CALCIUM DETERMINATION

Add to a test tube respecting the sequence:

**40 uL** of the **sample solution**,  
**1 mL** of **double distilled water** and  
**6 drops** of **R2**.

Cap the test tube and shake well by inversion.

Let the solution rest for 1 minute.

Then compare the color with the **Calcium Color Scale**, identifying the respective calcium percentage.

### MAGNESIUM DETERMINATION

#### "Step I"

Add to a test tube respecting the sequence:

**50 uL** of the **sample solution**,  
**3 mL** of **double distilled water**,  
**6 drops** of **R3** and  
**2 drops** of **R4**.

Cap the test tube and shake well by inversion.

Let the solution stand for 1 minute.

Compare the color with the **Magnesium Color Scale "Step I"** identifying the respective magnesium percentage. The chromatic differences are evident for the low but not for the high percentages (>6%).

In the second case, if necessary, go to **"Step II"**

#### **"Step II"**

Add to the test tube of **"Step I"**:

**2 drops of R4.**

Cap the test tube and shake well by inversion.

Let the solution stand for 1 minute.

Compare the color with the **Magnesium Color Scale "Step II"** identifying the respective magnesium percentage.

The chromatic differences are now evident for the high but not for the low percentages (<3%).

#### **CYSTINE DETERMINATION**

Add to a test tube respecting the sequence:

**1 mL of the sample solution,**

**6 drops of R5 and**

**200 mg of R6 (1 spoonful using of the white spatula).**

Cap the test tube and shake well by inversion until complete dissolution.

Let the solution stand for 1 minute and then add:

**3 drops of R7.**

Wait **exactly** 30 seconds and compare the color with the **Cystine Color Scale**, identifying the respective cystine percentage.

Compare the color quickly because the color is unstable.

#### **URIC ACID DETERMINATION**

##### **"Step I"**

Add to a test tube respecting the sequence:

**1 mL of the sample solution,**

**1 drop of R8.**

Cap the test tube and shake well by inversion.

Let the solution stand for 2 minutes, then add:

**1 drop of R9.**

Cap the test tube and shake well by inversion.

Compare the color, **as quickly as possible**, with the **Uric Acid Color Scale "Step I"**, identifying the respective uric acid percentage. If in doubt about the colors, go to "Step II".

##### **"Step II"**

Add to the test tube of **"Step I"**:

**1 mL of distilled water.**

Then compare the color, **as quickly as possible**, with the **Uric Acid Color Scale "Step II"**, identifying the respective percentage.

**Note 5:** Compare the color quickly because the color is unstable and tends to change to blue.

**Note 6: Very important.** Before taking 1 mL of the sample solution, shake the tube by inversion, especially in the presence of turbidity.

#### **OXALATE DETERMINATION**

##### **"Step I"**

Add a test tube respecting the sequence:

**1 mL of the sample solution,**

**200 uL of R10,**

**3 drops of R11 and**

**3 drops of R12.**

Cap the test tube and shake well by inversion.

Let the solution stand for 1 minute.

Compare the color with the **Oxalate Color Scale "Step I"**, identifying the respective oxalate percentage.

The chromatic differences are evident for low but not for the high percentages (<20%).

In the second case, if necessary, go to **"Step II"**.

##### **"Step II"**

Add to the test tube of **"Step I"**:

**1 mL of the sample solution,**

**200 uL of R10.**

Cap the test tube and shake well by inversion.

Let the solution stand for 1 minute.

Compare the color with the **Oxalate Color Scale "Step II"** identifying the respective oxalate percentage.

The chromatic differences are now evident for low but not for the high percentages (40 and 70%).

**Note 7:** The presence of oxalate cause a discoloration of solution and therefore, for high concentrations, it becomes almost or completely transparent.

#### **PHOSPHATE DETERMINATION**

Add to a test tube respecting the sequence:

**1 mL of the sample solution,**

**6 drops of R13 and**

**3 drops of R14.**

Cap the test tube and shake well by inversion.

Let the solution stand for 5 minutes.

Compare the color with the **Phosphate Color Scale**, identifying the respective phosphate percentage.

#### **AMMONIUM DETERMINATION**

Add to a test tube respecting the sequence:

**1 mL of the sample solution**

**3 drops of R15 and**

**3 drops of R9.**

Cap the test tube and shake well by inversion.

Then compare the color with the **Ammonium Color Scale**, identifying the respective ammonium percentage.

**Note 8:** Color comparisons with color scales should always be made against a white background.

#### **QUALITY CONTROL**

It is advisable to do the control using the Synthetic Controls.

**Synthetic Control A:** Lot nr. 0722 (Exp. 2025-07)

Calcium 18%, Oxalate 37%, Ammonium 7% and Carbonate presence.

**Synthetic Control B:** Lot nr. 0722 (Exp. 2025-07)

Magnesium 6%, Cystine 33%, Urate 44%, Phosphate 17%.

**Negative Control:**

Use the solution (80 uL of R1 in 10 mL of double distilled water) instead of sample solution.

#### **INTERPRETATION OF THE RESULTS**

In the kidney stones are present many ions. Most common are:

**Uric Acid:** Uric Acid, Ammonium Urate

**Cistyne:** Pure Cystine

**Carbonates:** Calcium Carbonate

**Oxalates:** Calcium Oxalate

**Phosphates:** Magnesium Ammonium Phosphate, Magnesium Phosphate, Calcium Phosphate, Calcium Magnesium Phosphate

**Calcium:** Calcium Carbonate, Calcium Oxalate, Calcium Phosphate

#### **NOTE**

1. Do not mix reagents from different lots.
2. 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.
3. Only for IVD use.
4. As part of an internal Q.C., it is recommended to use the controls contained in the kit, just like a sample according to the operating procedure.
5. The respective expected values for all the components are reported in the included control sheet.
6. For methodological reasons, the total of the component percentages does not always give 100%.

#### **WASTE DISPOSAL**

This product is intended for professional use only.

Dispose of the product according to national/international laws.

#### **SENSITIVITY**

##### **Detection limits**

Ammonium	0.3 mg	Magnesium	0.1 mg
Calcium	0.75 mg	Phosphates	1 mg
Uric acid	0.1 mg	Sodium Oxalate	0.2 mg
Carbonates	0.4 mg	Cystine	0.2 mg

#### **REFERENCES**

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\* For both Step I and Step II (Uric Acid) the coloring relative to the 0% concentration can be even lighter than the reported coloring (this is the limit coloring)