**Urinary calculi analysis**

**Reagent kit for semi-quantitative determination of the most important components of urinary calculi, calcium, oxalate, phosphate, magnesium, ammonium, uric acid and cystin**

**Order Information**
Cat. No. 1 3139 99 90 351 Reagent kit for 100 determinations each Laboratory equipment

**Principle**
Dissolve a sample as homogeneous as possible of the urinary calculi to be analysed. From this solution the various components of the calculus are determined semi-quantitatively, the titrimetric method being used for calcium and a colorimetric method (i.e. visual colour comparison) being used for oxalate, phosphate, magnesium, ammonium, uric acid and cystine. The composition of the urinary calculus is obtained from the results of these determinations with the help of the enclosed calculation aid. The reagent kit also contains a specimen calculus consisting of genuine material for checking purposes.

**Storage Instructions and Reagent Stability**
The reagents are stable up to the end of the indicated month of expiry, if stored at 15 - 25 °C.

**Warnings and Precautions**
1. Reagent 1 is corrosive. R35: Causes severe burns. S1/2: Keep locked up and out of the reach of children. S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S30: Never add water to this product. S45: In case of accident or if you feel unwell seek medical advice immediately (show the label where possible).
2. Reagent 2 is corrosive. R35: Causes severe burns. S1/2: Keep locked up and out of the reach of children. S25: Avoid contact with eyes. S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S27/28: Take off immediately all contaminated clothing. After contact with skin, wash immediately with plenty of water. S33/39: Wear suitable gloves and eye/face protection. S39: Wear eye/face protection. S45: In case of accident or if you feel unwell seek medical advice immediately (show the label where possible). S64: If swallowed, rinse mouth and allow to stand for 2 min (also see Note).
4. Reagent 6 is irritant. R36: Irritating to eyes. S2: Keep out of the reach of children. S25: Avoid contact with eyes. S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
5. Reagent 8 is very toxic and dangerous to the environment. R26/27/28: Very toxic by inhalation, in contact with skin and if swallowed. R33: Danger of cumulative effects. S1/1: Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment. S1/2: Keep locked up and out of the reach of children. S36/37: Wear suitable protective clothing and gloves. S45: In case of accident or if you feel unwell seek medical advice immediately (show the label where possible). S61: Avoid release to the environment. Refer to special instructions/safety data sheet.
11. Please refer to the appropriate safety data sheets.
12. Material of human origin was used for the production of the control calculus. The control material was found to be non-reactive when tested with approved methods for HBsAg, anti-HIV 1-2 and anti-HCV. As there is no possibility to exclude definitely that products derived from human source transmit infectious agents, it is recommended to handle the control calculus with the same precautions used for patient specimens.
13. Take the necessary precautions for the use of laboratory reagents.

**Sample Preparation**
Reagents: 1. Sulfosalicylic acid conc. 95 – 97 %. Highly corrosive! Avoid contact with skin!

Finely triturate the calculus to be analysed in a mortar. Mix the resulting powder thoroughly and using the enclosed spatula, transfer a sample (in the amount of a level spatula-tipful) to the plastic boat, add 5 drops of Reagent 1 and stir with the spatula to achieve complete dissolution.

**Evolution of gas during dissolution indicates carbonate.**

Transfer the solution into the 100-ml graduate filled to one-third with distilled water, make up to the 50-ml mark with distilled water, and mix well with the plastic boat.

Transfer this solution into the small reaction vessel (up to the calibration mark) for each determination envisaged, with the exception of magnesium. Carry out the determinations in the reaction vessels.

**Calcium**

**Principle:**
Carry out the titrimetric determination with Titriplex III (ethylendinitrietraacetic acid disodiumsalt). A titration of calciuncarboxylic acid is used as an indicator.

**Reagents:**
Reagent 2: Sodium hydroxide solution 27 %
Reagent 3: Calciuncarboxylic acid Titration Reagent 4: Titriplex III solution

**Procedure:**
The sample solution add 2 drops of Reagent 2 and one spatula full of Reagent 3, and shake. Continue shaking, and while doing so add Reagent 4 drop by drop until the colour of the solution changes from red to blue. Count the drops required for the colour change to occur.

The number of drops required multiplied by 5 gives the percentage calcium content of the calculus.

**Oxalate**

**Principle:**
The colour complex formed by iron (III) and sulfosalicylic acid is discharged by oxalate.

**Reagents:**
Reagent 5: Borate buffer solution
Reagent 6: Iron (III) chloride solution
Reagent 7: Sulfosalicylic acid solution

**Procedure:**
To the sample solution add subsequently while shaking:
2 drops of Reagent 5
3 drops of Reagent 6
3 drops of Reagent 7
and allow to stand for 2 min (also see Note).

Compare the colour of the solution in the reaction vessel with the appropriate colour scale and choose the reference colour that most closely matches the colour of the solution looking through the solution from above in the process.

Intermediate values must be estimated. Read off the percentage oxalate content of the calculus.

**Ammonium**

**Principle:**
With Nessler’s reagent added, ammonium gives a yellow to brown solution.

**Reagents:**
Reagent 8: Dipotassium tetraiodomercurate solution
Reagent 2: Sodium hydroxide solution 27 %

**Procedure:**
Add subsequently to the sample solution, while shaking,
3 drops of Reagent 8 and 3 drops of Reagent 2.

Compare the colour of the solution in the reaction vessel with the appropriate colour scale and determine which of the reference colours most closely matches the colour of the solution, looking through the solution from above in the process.

Intermediate values must be estimated. Read off the percentage ammonium content of the calculus.
Phosphate

Principle:
The molybdotriphosphoric acid formed upon addition of ammonium molybdate is reduced to molybdenum blue by means of reducing agents.

Reagents:
Reagent 9: Ammonium molybdate solution
Reagent 10: Reduction solution (4-methylaminophenol sulfate, Sodium sulfite).

Procedure:
Add subsequently to the sample solution, while shaking:
5 drops of Reagent 9 and
5 drops of Reagent 10.
Allow to stand for 3 min. (also see Note)
Compare the colour of the solution in the reaction vessel with the appropriate colour scale and determine which of the reference colours most closely matches the colour of the solution, looking through the solution from above in the process. Intermediate values must be estimated. Read off the percentage phosphate content of the calculus.

Magnesium

Principle:
In a buffered solution magnesium reacts with a colour reagent (see Reagent 12) to form a red complex.

Reagents:
Reagent 11: Borate buffer solution
Reagent 12: Colour reagent (1-azo-2-hydroxy-3-(2,4-dimethyl-carboxoanilido)-naphthalene-1'- (2-hydroxybenzene-5-sodium sulfonate)

Procedure:
Pipette 1 mL of sample solution into a reaction vessel and make up to the calibration mark with distilled water. Add 10 drops of Reagent 11 and 10 drops of Reagent 12 while shaking.
After one minute compare the colour of the solution in the reaction vessel with the appropriate colour scale and determine which of the reference colours most closely matches the colour of the solution, looking through the solution from above in the process. Intermediate values must be estimated. Read off the percentage magnesium content of the calculus.

Uric acid

Principle:
In a buffered solution molybdotriphosphoric acid is reduced to form molybdenum blue by uric acid.

Reagents:
Reagent 13: Molybdotriphosphoric acid solution
Reagent 5: Borate buffer solution

Procedure:
Add 3 drops of Reagent 13 to the sample solution, shake, and allow to stand for 2 min. Then add 2 drops of Reagent 5 and shake.
Immediately compare the colour of the solution in the reaction vessel with the appropriate colour scale and determine which of the reference colours most closely matches the colour of the solution, looking through the solution from above in the process. Intermediate values must be estimated. The colour comparison should be performed within 10 seconds after addition of Reagent 5 to the sample solution because the colour is not stable and liable to change to blue.
Read off the percentage uric acid content of the calculus.

Cystine

Principle:
Cystine is reduced to cysteine by sodium sulfite. In an alkaline environment cysteine gives red colour together with sodium nitroprusside.

Reagents:
Reagent 14: Ammonia solution 9.5 %
Reagent 15: Reducing agent (sodium sulfite)
Reagent 16: Sodium nitroprusside trituration

Procedure:
To the sample solution add 10 drops of Reagent 14 and a red dosing-spoonful of Reagent 15, and swirl until dissolution is obtained. One minute after the addition of Reagent 15 add a black dosing-spoonful of Reagent 16, and again shake until dissolution is obtained.
Compare the colour of the solution in the reaction vessel with the appropriate colour scale 30 seconds after the addition of Reagent 16, and determine which of the reference colours most closely matches the colour of the solution, looking through the solution from above. Intermediate values must be estimated. Read off the percentage cystine content of the calculus.

Calcium oxalate (Whewellite)

Set the percentage oxalate content obtained on the oxalate scale and read the associated calcium oxalate value off the calcium oxalate scale.
Check for the amount of calcium consumed in the process on the calcium scale.
If more calcium was found in the analysis than would correspond to the amount indicated, determine by subtraction the differential amount of calcium.

Magnesium ammonium phosphate (Struvite)

Set the magnesium content obtained on the magnesium scale and read the associated magnesium ammonium phosphate value off the struvite scale.
Check for the amounts of ammonium and phosphate consumed in the process on the ammonium of phosphate scale, respectively.

Calculation

The components determined with this reagent kit are usually found in urinary calculi in the form of the following chemical compounds, on which the calculation aid has been based.

Calcium oxalate
Magnesium ammonium phosphate
Calcium hydrogen phosphate
Ammonium urate
Cystine

Calcium oxalate
Magnesium ammonium phosphate
Calcium hydrogen phosphate
Ammonium urate
Uric acid
Cystine

Calculation Example:
The following components were found:
35 % Calcium, 15 % oxalate, 40 % phosphate.
Calcium oxalate and calcium phosphates may be present.
15 % oxalate corresponds to 25 % calcium oxalate according to the calculation aid.
25 % Calcium oxalate corresponds to 7 % calcium.
The differential calcium value amounts to 28 % calcium.
28 % Calcium gives the best match on the calculation aid with 40 % phosphate on the lower phosphate scale, which means a result of 70 % apatite.
So, the calculus consists of about 25 % calcium oxalate and 70 % apatite.

Note
Addition of the component percentage values rarely gives exactly 100 % for methodological reasons.
Performance Characteristics

Measuring range
The test has been developed to determine

a. Calcium concentrations within a measuring range from 6–50 mg/100 mg urinary calculus
b. Oxalate concentrations within a measuring range from 5–70 mg/100 mg urinary calculus
c. Ammonium concentrations within a measuring range from 0.4–10 mg/100 mg urinary calculus
d. Phosphate concentrations within a measuring range from 9–50 mg/100 mg urinary calculus
e. Magnesium concentrations within a measuring range from 0.75–5 mg/100 mg urinary calculus
f. Uric acid concentrations within a measuring range from 1–80 mg/100 mg urinary calculus
g. Cystine concentrations within a measuring range from 10–100 mg/100 mg urinary calculus

Method comparison
A comparison between DiaSys Urinary calculi analysis and two physical methods (infrared spectrometry and x-ray examination) shows that the accomplished methods give almost identical results [1].

Cleaning of auxiliary devices
All auxiliary devices contained in the kit as eg spatula, measuring flasks, and reaction basins have to be cleaned thoroughly with distilled water after use, and then to be dried completely.

Waste Management
Please refer to the local legal requirements.

Literature

Manufacturer
DiaSys Diagnostic Systems GmbH
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