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STONE ANALYSIS SET

Chemical qualitative method

Reagent for qualitative determination of main individual components of urinary stones (urinary calculi).

REF 92315: 100 tests
R1 2 x 30 mL **R2** 2 x 30 mL **R3** 1 x 5 mL **R4** 1 x 5 mL **R5** 1 x 10 mL **R6** 1 x 5 mL **R7** 1 x 10 mL **R8** 1 x 5 mL **R9** 1 x 5 mL **R10** 10 g

REF 92330: 40 tests
R1 1 x 30 mL **R2** 1 x 30 mL **R3** 1 x 2 mL **R4** 1 x 2 mL **R5** 1 x 4 mL **R6** 1 x 2 mL **R7** 1 x 4 mL **R8** 1 x 2 mL **R9** 1 x 2 mL **R10** 4 g

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CE

IVD IN VITRO DIAGNOSTIC USE

CLINICAL SIGNIFICANCE (1) (2)

The presence of calculus in urines is due to the conglomeration and the crystallization of matter in supersaturation. There can be various reasons for this imbalance: nutritional, metabolical, genetical, anatomical, iatrogenical, neurological or infectious reasons. Several ones can occur, at the same time or successively, to the formation and the growth of the calculus.
The more frequently met chemical components are (in descending frequency order): calcium oxalates, calcium phosphate and magnesium ammonium phosphates, uric acids and urates, various proteins, cystine.

The qualitative analysis of a certain components (among the most frequent ones) of urinary calculus represents a first approach to the etiological diagnosis of urinary lithiasis and the direction of the patient's therapeutical follow-up. It is absolutely necessary to perform the test together with morphological analysis (low power stereo microscope, light microscopy) and/or with molecular and crystalline identification (X-ray diffraction, infrared spectophotometry). Because of its nature, the calculus has to be the subject of a precise and reliable analysis. Coupled and rationally used optical and chemical methods can, in most cases, give sufficient information about the composition and the etiology of the calculus.

PRINCIPLE (4) (5)

This method is used for identification of main mineral components and one organical component (cystine) of urinary calculi by easy chemical tests.

REAGENTS COMPOSITION

vial R1:	Hydrochloric Acid (HCl 1,65 M) **Corrosive, R34, S36/37/39**
vial R2	Sodium Hydroxyde (NaOH 6,25 M) **Corrosive, R35, S36/37/39**
vial R3	1er Reagent for Cystine determination (NaOH, Sodium Cyanide) **Corrosive, Toxic, Harmful for environment R34, R23/24/25, R31-52/53, S36/37/39, S46**
vial R4	2nd **REAGENT FOR CYSTINE DETERMINATION** (Sodium nitroprussiate) **XN: HARMFUL, R22, S21/26/37**
vial R5	Reagent for Phosphates determination (Sulfuric Acid, Ammonium Molybdate, Ferric Sulfate) **Corrosive, R34, S36/37/39**
vial R6	Reaget for Magnesium determination (NaOH, paranitrophenylazoréesorcinol)
vial R7	Reagent for Calcium determination (KOH, calcein) **Xi: Irritating, R34, S36/37/39**
vial R8	Reagent for Ammonia determination (Potassium Iodide, Mercuric Iodide) **Corrosive, R36/38, S36/37/39**
vial R9	Reagent for Uric Acid determination (Acetic Acid, neocuproïne, Copper Sulfate) **Xi: Irritating, R36/38, S36/37/39**
vial R10	Reagent for Oxalate determination (Manganese dioxide) **Xn: Harmful, R20/22, S36/37/39, S22-26-28$_1$**
SPATULA	**For use with R10 reagent only. Store and wipe away dust after use**

SAFETY CAUTIONS

See also § **REAGENTS COMPOSITON** and respect the symbol of security indicated on the label of the vial**.**
Refer to Material Safety Data Sheet to know the significance of R and S Phrases.
BIOLABO reagents are designated for professional, in vitro diagnostic use.

- Verify the integrity of the contents before use.
- Use adequate protections (overall, gloves, glasses).
- Do not pipette by mouth.
- In case of contact with skin and eyes, thoroughly wash affected areas with plenty of water and seek medical advice.
- Waste disposal: Respect legislation in force in the country.

All specimens should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

REAGENTS PREPARATION

Reagents are ready for use.

STABILITY AND STORAGE

Store away from light, well cap in the original vial at 18-25°C.

- When free from contamination, stored and used as described in the insert, reagents are stable until expiry date stated on the label of the kit.

SPECIMEN COLLECTION AND HANDLING

Morphological studies should be made with a whole calculus cleared out of possible impurities. Qualitative analysis and flame test should be realised on pulverised calculus using clean pestle and mortar to obtain finely ground powder.

INTERFERENCES (4)

✓Cobalt and nickel do not interfere with the determination of the magnesium because of their weak concentration in the organism.

✓Carbonate testing: if there has been effervescence during the addition of R1 revealing the presence of carbonate, add R1 drop by drop until the end of the gas evolution. Then, shake vigorously ("Vortex") for at least one minute so as to get rid of all the carbon dioxide (or failing that, bring to the boil a few seconds in a Pyrex tube and bring back at room temperature). This process is necessary to avoid a false-positive result during the determination of oxalate.

MATERIAL REQUIRED BUT NOT PROVIDED

1. Low power stereo microscope
2. Clean porcelain pestle and mortar
3. Metallic seeding loop
4. mg scale (weighing of the calculus powder)
5. Pipette to dispense 1 drop (approx. 50µL) of reagents and mixture M1, M2.
6. Tubes, or glass/ceramic cored plate with a white bottom
7. REF 95315: stone analysis set positive and negative controls

QUALITY CONTROL

REF 95315: STONE ANALYSIS SET Positive and Negative Controls

When calculi show an unusual morphology or lead to negative or incoherent results during the chemical analysis, a more appropriate analysis that could highlight a particular composition or etiology should be carried out.

It is recommended that each laboratory establishes its own investigation procedure by techniques adapted to the diversity not only of the structure, but also of the molecular composition of the calculus studied.

Specificity: Each reaction is specific to the tested component. For oxalate, see also § **INTERFERENCES**.

PERFORMANCE CHARACTERISTICS

Detection limits:

Carbonate:	1 mg of Calcium carbonate
Cystine:	1 mg of L-Cystine
Phosphate:	1 mg of Calcium phosphate
Ammonium:	1 mg of Ammonium magnesium Phosphate
Magnesium:	3 mg of Ammonium magnesium Phosphate
Calcium:	0.1 mg of calcium (phosphate, carbonate, oxalate)
Uric acid:	0.1 mg of uric acid
Oxalate:	2.5 mg of Calcium Oxalate

Sensitivity: Reagents are very sensitive and so allow the detection of the different components even as trace

PROCEDURE (1)

Morphological analysis of calculus should be made before pulverizing the calculus for chemical test.

Flame test: Immerse a metallic seeding loop into distilled water, then into the calculus powder. Bring to the flame. The carbonization or the disappearance of the powder is the sign that the components are mostly of organic origin. The absence of carbonization shows that the calculus is of mineral origin.

Qualitative chemical tests: (see table below)

- **Step 1**: Weigh about 50 mg of the calculus powder, transfer into a test tube and add 10 drops of reagent R1. An effervescence shows the presence of carbonate (see § **INTERFERENCES**). In this case, shake vigorously for 1 minute. The remaining mixture is called M1 in the table below.
- Preparation of the mixture M2: Mix 50 µl of M1 and 5 ml of distilled water. Mix well and use only for the determination of calcium (**Step 5**).
- According to the step below, dispense 1 drop (approx. 50 µL) of the mixture M1 or M2 in each cavity of a ceramic cored plate or in tubes and carry out the next tests (**Step 2 to 8**):

	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Step 7	Step 8
	CARBONATE	**CYSTINE**	**PHOSPHATE**	**MAGNÉSIUM**	**CALCIUM**	**AMMONIUM**	**URIC ACID**	**OXALATE**
	CALCULUS POWDER 50 mg + R1 10 drops (500 µL) **= M1**	1 DROP **M1** + **R3** 1 DROP MIX, LET STAND FOR 5 MIN. **+ R4** 1 DROP	1 DROP **M1** + **R5** 2 DROPS (100 µL) MIX, LET STAND FOR 5 MIN.	1 DROP **M1** + **R6** 1 DROP + **R2** 5 DROPS (250 µL) MIX	1 DROP M2 + **R2** 1 DROP + **R7** 2 DROPS (100 µL) MIX	1 DROP **M1** + **R2** 1 DROP + **R8** 1 DROP MIX	1 DROP **M1** + **R2** 1 DROP + **R9** 1 DROP MIX	REMAINING MIXTURE **M1** + **R10** THE TIP OF A SPATULA Approximately. 60 mg WAIT FOR FEW SECONDS
POSITIVE RESULT	EFFERVESCENCE visible and audible	RED COLOUR	BLUE COLOUR	BLUE PRECIPITATE	YELLOW COLOUR	ORANGE-BROWN PRECIPITATE	YELLOW/ORANGE COLOUR	EFFERVESCENCE visible and audible
NEGATIVE RESULT	ABSENCE OF EFFERVESCENCE	YELLOW COLOUR	NO CHANGE IN COLOUR	ABSENCE OF PRECIPITATE PURPLE COLOUR	ORANGE COLOUR	YELLOW COLOUR	NO CHANGE IN COLOUR	ABSENCE OF EFFERVESCENCE

Notes:

1) Negative control: Use REF 95315 CONTROL 3 - .

2) Positive control: Use REF 95315 CONTROL 1+ and CONTROL 2 +

Treat as calculus powder

REFERENCES

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