CALCULUS ANALYSIS II

QUALITATIVE COLORIMETRIC DETERMINATION of CARBONATE, CALCIUM, MAGNESIUM, AMMONIUM, OXALATE, PHOSPHATE, URIC ACID and CYSTINE on RENAL and URINARY STONES by VISUAL INSPECTION

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. The aetiologies vary and include metabolic dysfunctions, obstructions, infections, hyper-saturation, lack of inhibitors, etc..

PRINCIPLE
A small amount of urinary (or kidney) stones is shattered and dust. Different rates are added to different solutions; the chemical reactions that develop allow qualitative means of identifying the presence/absence of individual components. The tests are qualitative, Visual.

ATTENTION!
Material safety data sheet should be supplied on request.

PRECAUTIONS FOR USE
1. This product has been formulated for in vitro diagnostic use.
2. A proportional variation of the reaction volumes does not change the result.
3. DO NOT mix Reagents from different Production lots.
4. It is recommended to handle the reagents carefully, avoiding ingestion and contact with eyes, mucous membranes and skin; to use reagents according to good laboratory practice. On the material safety data sheet are detailed the operating procedures for the manipulation of this product.
Material safety data sheet should be supplied on request.

ATTENTION!
A) The reagent must be used ONLY for the intended destinations, by expert and trained people and in according to good laboratory practice.
B) The clinical diagnosis cannot be done correctly using the result of only one test, but have to be done integrating critically the results of different laboratory tests and clinical data.
C) A lot of factors, as ambient temperature, the working reagent temperature, may affect the tests performances.
D) All the precautions normally used in the laboratory must be respected for reagents handling.
E) Follow exactly what described here to have right results.

REAGENTS
Components of the kit:

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1 - HCl</td>
<td>1 x 26 mL</td>
</tr>
<tr>
<td>R2 - C2H8N2O4</td>
<td>1 x 5 mL</td>
</tr>
<tr>
<td>R3 - NaOH</td>
<td>1 x 10 mL</td>
</tr>
<tr>
<td>R4 - C12H9N3O4</td>
<td>1 x 5 mL</td>
</tr>
<tr>
<td>R5 - KO</td>
<td>1 x 5 mL</td>
</tr>
<tr>
<td>R6 - MnO2</td>
<td>1 x 0.6 g</td>
</tr>
<tr>
<td>R7 - H24Mo7NaO24</td>
<td>1 x 4 mL</td>
</tr>
<tr>
<td>R8 - Na2CO3</td>
<td>1 x 2 mL</td>
</tr>
<tr>
<td>R9 - Na2O4W</td>
<td>1 x 4 mL</td>
</tr>
<tr>
<td>R10 - NH4OH</td>
<td>1 x 2 mL</td>
</tr>
<tr>
<td>R11 - BUFFER</td>
<td>1 x 4 mL</td>
</tr>
<tr>
<td>R12 - Na2SO3</td>
<td>1 x 0.6 g</td>
</tr>
<tr>
<td>R13 - C5FeN6Na2O</td>
<td>1 x 5 mL</td>
</tr>
<tr>
<td>R14 - SOLF.</td>
<td>1 x 1 g</td>
</tr>
<tr>
<td>R15 - NaX</td>
<td>1 x 1 g</td>
</tr>
<tr>
<td>R16 - CONTROL</td>
<td>1 x 2 g</td>
</tr>
</tbody>
</table>

STABILITY: the Reagents are stable up to the expiry date mentioned on the labels, stored at 15-25°C, if closed and kept in their intact primary container; if not exposed to heat sources and/or pressure variations.
In case of damaging of the primary container organize the waste disposal.

AUXILIARY REAGENTS FOR QUALITY CONTROL
Using the control contained in the kit, as they are all analytes can be determined with this device, you can verify the chemical reaction for your reference.
CALCULUS ANALYSIS II

PREPARATION OF THE WORKING REAGENT
Ready-to-use. Mix kindly before use and let the reagent reach the room temperature before use. Close immediately after handling. The Reagents have to be used correctly, to avoid contamination. An incompetent handling relieves us from any responsibility.

STABILITY AFTER THE FIRST OPEN
The product is stable up to the expiry date mentioned on the labels after the first open if stored at 15-25°C.

MATERIAL REQUIRED BUT NOT PROVIDED
Clean porcelain pestle and mortar.
Glass test-tubes and black glass/plastic slide.
Spatula.
Balance.
Centrifuge (for laboratory, low speed).
Water-bath or warm-plate.

SAMPLES
• Renal or urinary stone
  Samples collection in compliance with CLSI (NCCLS) (see References 2).

WASTE DISPOSAL
Observe all federal, state and local environmental regulations for waste disposal.

ANALYTICAL PROCEDURE
PRELIMINARY PHASE
NOTE: rem DO NOT USE all the powder of each sample of urinary stone to test STEP 1 - CARBONATE! Rem to keep an amount to test STEP 6-7-8.
After an examination of the stone to record shape, colour, smoothness, size, etc. crush the urinary stone (now called 'STO') and reduce to powder. Use the R16 - [CONTROL] as the powder of urinary stone.

STEP 1: TEST for CARBONATE
Put approximately 0.050 g (50 mg) powdered stone ‘STO’ in a glass test-tube. Add 15 drops of R1 - HCl; formation of bubbles/foam indicates a positive reaction.
POSITIVE: >>>>>> EFFERVESCENCE
At the end of any effervescence, centrifug; separate the supernatant SUR from the sediment SED.

TEST ONLY ON SUR (supernatant)

STEP 2: TEST for CALCIUM
On a black glass slide add 3 drops of SUR + 3 drops of R2 - C2H8N2O4.
POSITIVE: >>>>>> WHITE PRECIPITATE

STEP 3: TEST for MAGNESIUM
In a glass test-tube add 3 drops of SUR + 3 drops of R3 - NaOH + 3 drops of R4 - C12H9N3O4.
POSITIVE: >>>>>> BLUE/PURPLE PRECIPITATE

STEP 4: TEST for AMMONIUM
In a glass test-tube add 3 drops of SUR + 3 drops of R3 - NaOH + 3 drops of R5 - K I
POSITIVE: >>>>>> RUSTY PRECIPITATE

TEST ONLY ON SED (sediment)

STEP 5: TEST for OXALATE
Directly in the glass test-tube with only the SED add a trace of R6 - MnO2 on a spatula; neither MIX nor SHAKE. Warm gently to 37°C in a water-bath or with deep attention on a warm-plate.
POSITIVE: >>>>>> BUBBLES from the SEDIMENT

TEST ONLY ON STO (stone)

STEP 6: TEST for PHOSPHATE
Directly in a glass test-tube add a trace of the powdered STO using a spatula + 1 drop of R1 - HCl + 2 drops of R7 - H24Mo7N6O24. Mix gently. Then after 2-3 minutes add a little amount of R14 - SOLF.. Mix kindly for 2-3 minutes till a little dissolution. Add again a little amount of R15 - NaX and mix gently.
POSITIVE: >>>>>> BLUE COLOUR

STEP 7: TEST for URATE-URIC ACID
Directly in a glass test-tube add a trace of STO using a spatula + 1 drop of R8 - Na2CO3 + 2 drops of R9 - Na2O4W.
POSITIVE: >>>>>> IMMEDIATE, STRONG BLUE COLOUR (a pale colour is not positive)

STEP 8: TEST for CYSTINE
Directly in a glass test-tube add a trace of STO using a spatula + 1 drop of R10 - NH4OH + 2 drops of R11 – BUFFER + a trace of R12 - Na2SO3 using a spatula + 3 drops of R13 - C5FeN6Na2O.
POSITIVE: >>>>>> RED COLOUR

REFERENCES

EDMA (EDMS) CODE | 11 02 01 23 00