Identification and qualitative analysis of kidney stones
Formation of Stones

- Kidney stones, also known as renal calculi or renal lithiasis, are small, hard deposits that form inside kidneys. The stones are made of mineral and acid salts.
- Kidney stones can affect any part of the urinary tract, from the kidneys to the bladder.
- Often, stones form when the urine becomes concentrated, allowing minerals to crystallize and stick together.
- Urinary stones may be composed of Calcium oxalate, Calcium phosphate, Calcium Carbonate, Ammonium phosphate, Carbonate Apatite, Uric acid and its salts (urates), Cystine, and Xanthine.
There are two basic aspects in the pathogenesis of renal stones:

1. **Increased urinary excretion of stone** forming elements like calcium, phosphorus, uric acid, oxalate, and cystine

2. **Low fluid intake** A low fluid intake results in the production of concentrated urine, causing supersaturation and crystallisation of stone-forming compounds. In addition, low urine flow rates favor crystal deposition on the urothelium.

Physio-chemical changes which influence stone formation like: pH of urine, stone matrix, and protective substances in the urine.
Removal of stones

• The doctors follow different operational procedures for the removal of kidney stones based on location of the stone and on size of the stone.
Investigation of Renal Calculi

1-Urine analysis and Urine culture
   It may show crystals, red blood cells, and/or pus cells in urine

2-Stone analysis
   Chemical analysis of stones is a simple test but is not an accurate method. (will be done in today’s lab)
   Better method is crystallography

3-Biochemical investigations
   Serum calcium, phosphorus, uric acid, and renal function tests.
   24-hour urine for calcium, phosphorus, uric acid, oxalate, citrate, and cystine.
   Investigations for special clinical situations like hyperparathyroidism, gout, should also be included.
## Types of Calculi

<table>
<thead>
<tr>
<th>Stone composition</th>
<th>Cause</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium Stone</td>
<td>• are the most common type of kidney stone and occur in two major forms: calcium oxalate and calcium phosphate. <strong>Calcium oxalate:</strong> stones are more common. Calcium oxalate stone formation may be caused by high calcium and high oxalate excretion. (some food eg. spinach, strawberries and large doses of Vitamin C may increase the amount of oxalate in your urine) <strong>Calcium phosphate</strong> stones are caused by the combination of high urine calcium and alkaline urine.</td>
<td>Hypercalciuria caused by: Hyperparathyrodisim Vitamin D toxicity</td>
</tr>
</tbody>
</table>
# Types of Calculi

<table>
<thead>
<tr>
<th>Stone composition</th>
<th>Cause</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uric acid stones</td>
<td>• Form when the urine is persistently acidic.</td>
<td>A diet rich in purines</td>
</tr>
<tr>
<td></td>
<td>• Excessive urinary uric acid</td>
<td></td>
</tr>
<tr>
<td>Carbonate apatite (calcium carbonate and calcium phosphate)</td>
<td>• These stones develop as a consequence of recurrent or chronic urinary tract infections caused by urease producing bacteria</td>
<td>Some urinary bacteria can split the urea in urine to form ammonium and also to make urine less acidic.</td>
</tr>
<tr>
<td>Struvite (magnesium ammonium phosphate)</td>
<td>• develop in patients with cystinuria</td>
<td>caused by mutations in the genes, encode for two parts of a transporter protein that is made primarily in the kidneys. These defects prevent proper reabsorption of amino acids</td>
</tr>
<tr>
<td>Cystine stone</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 1. Micrograph taken by Scanning Electron Microscopy, showing the calcium oxalate monohydrate “leaves”

Figure 2. Micrograph taken by Scanning Electron Microscopy, showing calcium oxalate dihydrate in "pyramids"
Objective:

Identification and qualitative analysis of kidney stones
MATERIALS

- Dilute hydrochloric acid (2mol/l)
- Dilute sulphuric acid (2mole/l)
- Concentrated nitric acid
- Acetic acid (30ml glacial acetic acid/100 ml water)
- Potassium hydroxide solution
- Concentrated ammonia solution (s.g 0.88)
- Dilute ammonia reagent (dilute reagent 6 fivefold with water)
- Ammonium molybdate solution, 50g/l freshly prepared.
- Ammonium oxalate solution, prepare a saturated solution.
- Potassium permanganate solution, 3g/l.
Experiments:

1. Test for uric acid
2. Test for carbonate
3. Test for oxalate
4. Test for phosphates
5. Test for calcium
(1) Test for Uric acid

Principle: Uric acid undergoes oxidation when treated with HNO3

Method:
1. Put a small amount of the sample
2. Add 5-7 drops of concentrated nitric acid
3. Heating in a water bath.

(yellow to orange color on the inner surface of the test tube)
(2) Test for carbonate

**Principle:**

2 HCl + CaCO$_3$ --> CaCl$_2$ + H$_2$O + CO$_2$

**Method:**

1- Add 0.5 ml diluted hydrochloric acid (2M HCL) to a small portion of the sample

*(Gas bubbles will indicate the presence of carbonate).*
**Test for oxalate:**

**Principle:**
In sulfuric acid solution, oxalate combines with hydrogen to form oxalic acid.

**Method:**
1- Heat a part of the sample with 2 ml dilute sulphuric acid (2M H2SO4) for 1 min.
2- Add 2 drops (one by one) of potassium permanganate (KMnO4) solution and Mix.

*(The decolonization and evolution of bubbles will confirm the presence of oxalate.)*
(4) Test for phosphates

**Principle:** Phosphate ions react with ammonium molybdate to produce a characteristic yellow precipitate, ammonium phosphomolybdate.

**Method:**
1. Dissolve a little of the sample 4 in about 1.5 ml of concentrated nitric acid HNO₃.
2. Add an equal volume (1.5 ml) of ammonium molybdate solution.
3. Heat to boiling.

*(If phosphates are present, a yellow precipitate of ammonium phosphomolybdate is obtained)*
5) Test for calcium

**Principle:** calcium is precipitated as calcium oxalate using ammonium oxalate

**Method:**
1. Dissolve small amount of the sample 5 by heating with 2 ml dilute hydrochloric acid (2M HCL)
2. Add 1 ml ammonium oxalate

*A white precipitate of calcium oxalate shows the presence of calcium.*
<table>
<thead>
<tr>
<th>Components</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uric acid</td>
<td></td>
</tr>
<tr>
<td>carbonate</td>
<td></td>
</tr>
<tr>
<td>oxalate</td>
<td></td>
</tr>
<tr>
<td>phosphates</td>
<td></td>
</tr>
<tr>
<td>calcium</td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSIONS:

Comment in each results you obtained and mention whether the sample contains these component or not?

And what the disease that cause each type of stone.
References

- http://numonthly.com/?page=article&article_id=835
- http://www.mayoclinic.org/diseases-conditions/kidney-stones/basics/definition/con-20024829

Medical Aspects of Renal Stones, REVIEW ARTICLE, KK Malhotra, 2008

Kidney stone disease: pathophysiology, investigation and medical treatment, Clinical Medicine 2012

- A Comparative Study of Two Renal Stone Analysis Methods Samira Charafi1, Mohamed Mbarki1, Antonia Costa-Bauza2, Rafael M. Prieto2, Abdelkhalek Oussama1, Felix