Saliva & Feces analysis
Saliva analysis

• What is Saliva?
• Watery substance present in humans and animals and secreted by salivary glands and aids in digestion
• It is a filtrate of plasma
• Contains electrolytes, enzymes, mucus, hormones, antibacterial compounds and cellular elements
Saliva analysis

- Sample collection
- Collection method and transport depends on tests to be done
- For chemical analysis preferably frozen before analysis
- For cellular analysis fresh sample needed – smoking effects
- Harsh collection methods (oral brush collection methods) can give false +ve results
- Aggressive brushing and flossing may also effect the sample consistency
Sputum analysis

- Sample collection

1. Collect liquid saliva directly into the vial.

2. Fill to the fifth line from the bottom excluding foam, approximately ¾ full.

3. If too much foam, place into freezer 2-3 minutes. Tap on solid surface to settle foam.

4. Recap vial tightly; place in zip lock bag with absorbent orange shipping pad and refrigerate. Mail within three days.
### Physical examination

**Appearance**

Normal saliva is colorless and clear

**pH** – ranging from 6.0-7.4

**Specific gravity** – 1.002 – 1.012

**Volume** – in humans it is estimated that 0.75 – 1.5 liters is produced per day. During night minimal production
## Saliva analysis

<table>
<thead>
<tr>
<th>Chemical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary area includes endocrinology and toxicology</td>
</tr>
<tr>
<td>Presence of unusual components is of preference</td>
</tr>
<tr>
<td>As normal reference values differ for healthy individuals also</td>
</tr>
<tr>
<td>Hormone or drug concentration measured in saliva is considered to be free of binding proteins and thus can reflect biological active concentration in blood</td>
</tr>
<tr>
<td>Cortisol, Sex steroids, to limited extent pitutory &amp; thyroid secretions – major endocrine applications of saliva analysis</td>
</tr>
</tbody>
</table>
# Saliva analysis

**Chemical analysis - Toxicology**

- Many exogenous analytes such as drugs can be detected.
- pH greatly effects drug concentration tested…. And thus can give errors.
- Can serve as qualitative test if not quantitative for many drugs in question.
- Immunoassays are often used in initial screening. Followed by chromatography & mass spectrometry methods for confirmation.
- Tests include monitoring therapeutic drug concentrations and detection of abused drugs.
- Correlation has to be established between plasma and saliva concentrations of a specific drug.
# Saliva analysis

## Microbiological testing

Not a recommended specimen as clinical utility in testing microbes. However, some tests do exist for detection of *helicobacter pylori* and *Borrelia* species.

Currently saliva testing is primarily used in the field of HIV and HCV.

Analogous to home pregnancy test, devices are available to test HIV or HCV at home.
Feces analysis
Feces analysis

- Most of the laboratory personnel categorize fecal analysis as “necessary evil”
- As an end product of metabolism, feces do provide valuable diagnostic information
- Routine analysis includes macroscopic, microscopic and chemical analysis
- Useful in early detection of GI bleeding, liver, pancreas diseases and identification of many pathogens and malabsorptions of nutrients
Feces analysis

- Normal feces contains bacteria, cellulose, undigested food, GI secretions, Bile pigments, cells from intestinal walls, electrolytes and water
Feces analysis

• Sample collection
• Fecal specimen / stool sample collection is not an easy task
• Detailed instructions and appropriate containers should be prescribed
• Dietary restriction applicable wherever necessary
• Should not be contaminated with urine
## Feces analysis

<table>
<thead>
<tr>
<th>Physical examination</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Color</strong> – 1&lt;sup&gt;st&lt;/sup&gt; indication of GI disturbances is often the changes in color and consistency of stool. Some cases abnormal color can be because of excess intake of pigmented foods and medications which is temporaty</td>
</tr>
<tr>
<td><strong>Brown</strong> color is because of action of intestinal bacteria which convert urobininogen to stercogilinogn</td>
</tr>
<tr>
<td>Stools that are pale in color may indicate blockage of bile duct</td>
</tr>
<tr>
<td>Presence of blood leads to different colors, <strong>red, bright red and black</strong>. Color depends on source of blood</td>
</tr>
<tr>
<td>Green stools can be observed in case of some antibiotic ingestions, ingestion of more green vegetables, food coloring etc……</td>
</tr>
</tbody>
</table>
Feces analysis

Physical examination – Appearance

Besides color, consistency changes can be observed in many cases

Watery – diarrhoea

Small hard – constipation

Slender, ribbon like – obstruction of passage

Fatty stool – “Steatorrhea” - pancreas problems

<table>
<thead>
<tr>
<th>Color/Appearance</th>
<th>Possible Cause</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black</td>
<td>Upper GI bleeding, Iron therapy, Charcoal</td>
</tr>
<tr>
<td>Red</td>
<td>Bismuth (antacids), Lower GI bleeding, Beets and food coloring, Rifampin</td>
</tr>
<tr>
<td>Pale yellow, white, gray</td>
<td>Bile-duct obstruction, Barium sulfate</td>
</tr>
<tr>
<td>Green</td>
<td>Biliverdin/oral antibiotics, Green vegetables</td>
</tr>
<tr>
<td>Bulk/frothy</td>
<td>Bile-duct obstruction, Pancreatic disorders</td>
</tr>
<tr>
<td>Ribbon-like</td>
<td>Intestinal constriction</td>
</tr>
<tr>
<td>Mucus- or blood-streaked mucus</td>
<td>Colitis, Dysentery, Malignancy, Constipation</td>
</tr>
</tbody>
</table>
Feces analysis

<table>
<thead>
<tr>
<th>Microscopic examination</th>
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<tbody>
<tr>
<td>Done to detect presence of leukocytes associated with microbial diarrhea and undigested fibers and fats associated with seatorrhea</td>
</tr>
</tbody>
</table>

**Presence of neutrophils** – ulcerative colitis, bacterial dysentery. Generally neutrophils are found in feces during infection with invasive bacterial pathogens like *salmonella*, *shigella*, *yersinia* and *E. Coli*. Toxin mediated and viral or parasitic diarrhea do not cause appearance of neutrophils

**Muscle fibers** – test for undigested muscle fibers can be helpful in diagnosis of **pancreatic insufficiency**

**Fat droplets** – steatorrhea – malabsorption disorders
Feces analysis

Microscopic examination

**PROCEDURE 14-1**

**Methylene Blue Stain for Fecal Leukocytes**
1. Place mucus or a drop of liquid stool on a slide.
2. Add two drops of Löffler methylene blue.
3. Mix with a wooden applicator stick.
4. Allow to stand for 2 to 3 minutes.
5. Examine for neutrophils under high power.

**PROCEDURE 14-2**

**Muscle Fibers**
1. Emulsify a small amount of stool in two drops of 10% eosin in alcohol.
2. Apply cover slip and let stand 3 minutes.
3. Examine under high power for 5 minutes.
4. Count the number of undigested fibers.

**PROCEDURE 14-3**

**Neutral Fat Stain**
1. Homogenize one part stool with two parts water.
2. Mix emulsified stool with one drop of 95% ethyl alcohol on slide.
3. Add two drops of saturated Sudan III in 95% ethanol.
4. Mix and apply cover slip.
5. Examine under high power.
6. Count orange droplets per high-power field.
# Feces analysis

## Chemical examination

**Most frequently done test is fecal occult (hidden) blood test (FOBT)**

Annual test is recommended for early detection of colon cancer

**Frequently used test is guaiac based FOBT (gFOBT)**

![Chemical reaction]

\[
\text{Hemoglobin} + H_2O_2 + \text{guaiac} \xrightarrow{\text{Pseudoperoxidase}} \text{oxidized guaiac} + H_2O \\
\text{(colorless)} \rightarrow \text{(Blue color)}
\]

**Immunochemical fecal occult blood test (iFBOT)**

Uses antibodies against human hemoglobin

APT test – fetal hemoglobin. Bloody stool or vomits can be observed in infants because of swallowing of blood during pregnancy
## Feces analysis

### Chemical examination

**APT test** – fetal hemoglobin. Bloody stool or vomits can be observed in infants because of swallowing of blood during pregnancy

**Fecal enzymes** – GI enzymes help in digestion of food
Main concern are of proteolytic enzymes like Trypsin, elastase I….
## Feces analysis

<table>
<thead>
<tr>
<th>Test</th>
<th>Methodology/Principle</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Examination for neutrophils</td>
<td>Microscopic count of neutrophils in smear stained with methylene blue, Gram stain, or Wright's stain</td>
<td>Three per high-power field indicates condition affecting intestinal wall</td>
</tr>
<tr>
<td>Qualitative fecal fats</td>
<td>Microscopic examination of direct smear stained with Sudan III</td>
<td>60 large orange-red droplets indicates malabsorption</td>
</tr>
<tr>
<td></td>
<td>Microscopic examination of smear heated with acetic acid and Sudan III</td>
<td>100 orange-red droplets measuring 6 to 75 μm indicates malabsorption</td>
</tr>
<tr>
<td>gFOBT</td>
<td>Pseudoperoxidase activity of hemoglobin liberates oxygen from hydrogen peroxide to oxidize guaiac reagent</td>
<td>Blue color indicates gastrointestinal bleeding</td>
</tr>
<tr>
<td>iFOBT</td>
<td>Uses polyclonal anti-human antibodies specific for the globin portion of human hemoglobin</td>
<td>Positive test and control lines indicate GI bleeding</td>
</tr>
<tr>
<td>APT test</td>
<td>Addition of sodium hydroxide to hemoglobin-containing emulsion determines presence of maternal or fetal blood</td>
<td>Pink color indicates presence of fetal blood</td>
</tr>
<tr>
<td>Trypsin</td>
<td>Emulsified specimen placed on x-ray paper determines ability to digest gelatin</td>
<td>Inability to digest gelatin indicates lack of trypsin</td>
</tr>
<tr>
<td>Elastase 1</td>
<td>Immunoassay using an ELISA test</td>
<td>Sensitive indicator of exocrine pancreatic insufficiency</td>
</tr>
</tbody>
</table>
Comments…..

Feed back and comments will be helpful ……