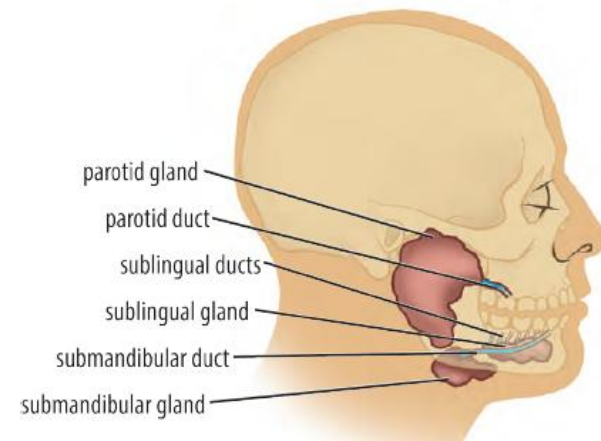


Saliva & Feces analysis

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

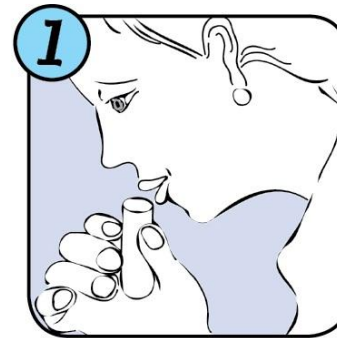


Saliva analysis

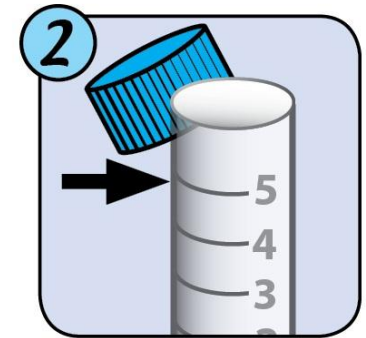
- **Sample collection**
- Collection method and transport depends on tests to be done
- For chemical analysis preferably frozen before analysis
- For cellular analysis fresh sample needed – smoking effects
- Harsh collection methods (oral brush collection methods) can give false +ve results
- Aggressive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

Sputum analysis

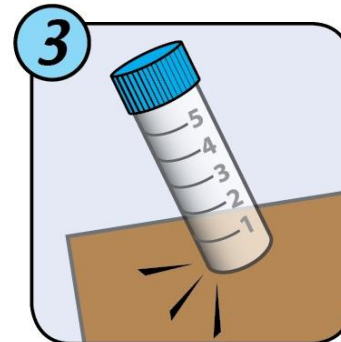
- Sample collection



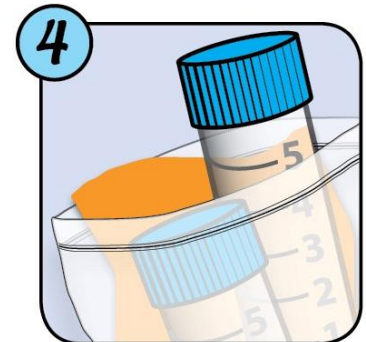
Collect liquid saliva directly into the vial.



Fill to the fifth line from the bottom excluding foam, approximately $\frac{3}{4}$ full.



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



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

Saliva analysis

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

Chemical analysis

Primary area includes endocrinology and toxicology

Presence of unusual components is of preference

As normal reference values differ for healthy individuals also

Hormone or drug concentration measured in saliva is considered to be free of binding proteins and thus can reflect biological active concentration in blood

Cortisol, Sex steroids, to limited extent pituitary & thyroid secretions – major endocrine applications of saliva analysis

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



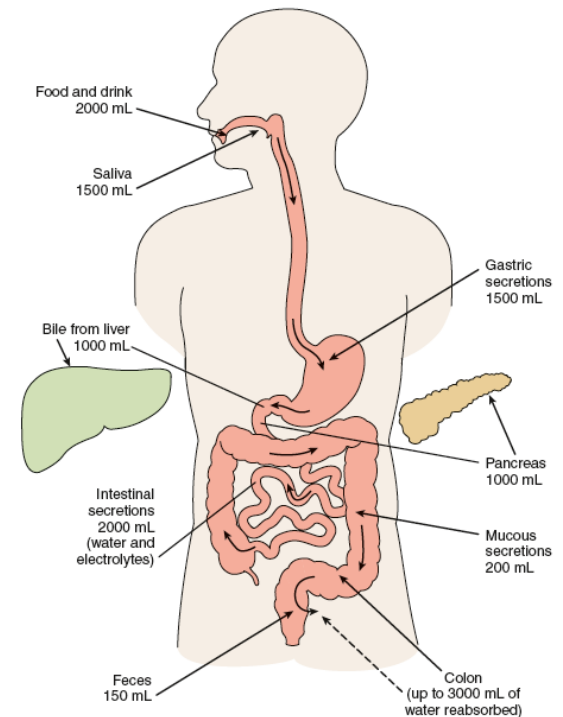
Feces analysis

Feces analysis

- Most of the laboratory personnel categorize fecal analysis as “necessary evil”
- As a 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

Physical examination

Color – 1st 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 temporary

Brown color is because of action of intestinal bacteria which convert urobilinogen to stercobilinogen

Stools that are pale in color may indicate blockage of bile duct

Presence of blood leads to different colors, **red, bright red and black.**
Color depends on source of blood

Green stools can be observed in case of some antibiotic ingestions, ingestion of more green vegetables, food coloring etc.....

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 14-3 Macroscopic Stool Characteristics^{12,26}

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

Feces analysis

Microscopic examination

Done to detect presence of leukocytes associated with microbial diarrhea and undigested fibers and fats associated with steatorrhea

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

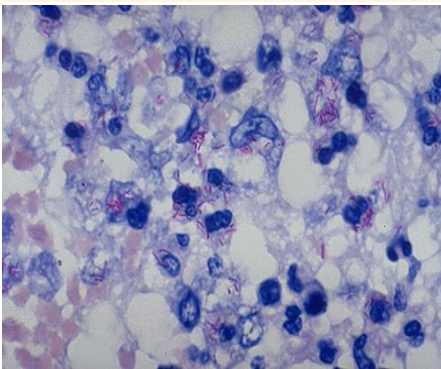
Methylene Blue Stain for Fecal Leukocytes

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

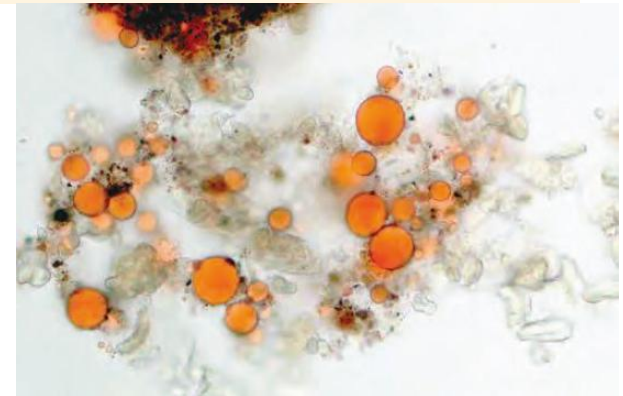
1. Emulsify a small amount of stool in two drops of 10% eosin in alcohol.
2. Apply cover slip and let stand 3 minutes.
3. Examine under high power for 5 minutes.
4. Count the number of undigested fibers.



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.



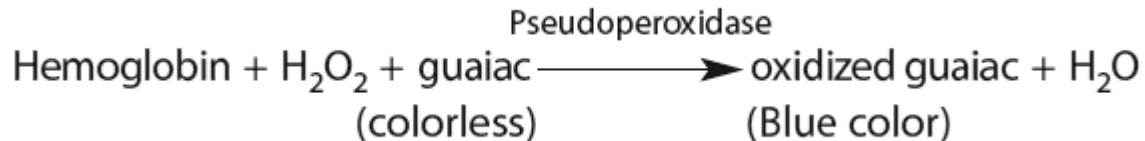
Feces analysis

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 gualac based FOBT (gFOBT)



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 14-5 Fecal Screening Tests

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

Comments.....

Feed back and comments will be helpful