

B.Sc. NUTRITION LAB MANUAL  
4th Semester



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Nutrition

**MIDNAPORE CITY COLLEGE**



## **C8P Diet and Diseases (Practical)**

### **Guideline for Diarrhea diet**

1. Choose and prepare foods and drinks with less salt and added sugars. Use the nutrition information on food level to help you make healthy choices. The percent of daily value listed on the food level tells you whether a food is low or high in certain nutrients. A percent daily value of 20% or more means that the food is high in a nutrient.
2. Get 2 hours and 30 minutes or more of physical activity that each week. Such as brisk walking get 1 hour and 15 minute of physical activity each effort such as running speed physical activity throughout the week. Talk to your care given about the best exercise plan to you.

Some example of hospital diets is given free diet, dairy free diet, sugar constituent, vegetarian diet, wheat free diet, low fat diet, low sodium diet.

## Personal data

Name -

Age –

Sex -

Body weight -

Body height -

BMI -

Occupation –

Socioeconomic condition –

Food habit –

Physical activity –

Disease type -

## Nutritional Requirement

**Energy requirement:**

**REE (Resting Energy Expenditure):**

**Male:**

$$\{(10 \times \text{body in kg}) + (6.25 \times \text{height in cm}) - (5 \times \text{age in year})\} + 5$$

**Female:**

$$\{(10 \times \text{body weight in kg}) + (6.25 \times \text{height in cm}) - (5 \times \text{age in year}) + 5\} - 161$$

**Micro and Macro-nutrient (According to RDA, 2010)**

## **Nutrient analysis of consumed food stuff**

## **Balance sheet**

## **Menu planning**

## **Interpretation**



## Planning and preparation of diet for Steatorrhea patient

What is steatorrhea?

Steatorrhea is the medical term for fat in stool. Fat in the stool can cause bulky stool that floats, has an oily or greasy appearance, and smells foul. Fat in the stool is fat that the digestive tract was unable to absorb. Temporary steatorrhea may result from dietary changes or intestinal infections. Steatorrhea that is persistent may result from diseases of the biliary tract, pancreas, or intestines.

Fat absorption is dependent upon bile (which is produced in the liver and stored in the gallbladder), pancreatic lipases (enzymes that break down fat), and normal intestine function. Absence of bile is often due to blockage of the biliary tract and can result in pale colored fatty stool and jaundice. Absence of pancreatic lipases is uncommon, but can occur as a result of a diseased pancreas, cystic fibrosis, or an abnormality that is present at birth.

Inflammation of the lining of the intestines, which may occur with conditions such as ulcerative colitis (inflammation of the colon and rectum), Crohn's disease (inflammation of the bowels), and celiac disease (a severe sensitivity to gluten in the diet), can interfere with absorption of fats. Also, fat absorption may be affected by surgical removal of a portion of the intestines.

Often, steatorrhea is a short-lived problem related to diet or infection; however, if it lasts for more than a couple of weeks, becomes more severe, or is accompanied by other symptoms, it may be due to a more serious condition. Seek immediate medical care if you have bloody stool, black or tarry stool, stool with pus, severe abdominal pain or cramping, or high fever (higher than 101 degrees Fahrenheit).

### Digestive tract symptoms that may occur along with steatorrhea

Steatorrhea may accompany other symptoms affecting the digestive tract including:

- Abdominal pain or cramping
- Abdominal swelling, distension or bloating
- Abnormally foul-smelling stools
- Bloody stool (the blood may be red, black or tarry in texture)
- Diarrhea
- Gas
- Nausea with or without vomiting
- Pale feces

### Other symptoms that may occur along with steatorrhea

Steatorrhea may accompany symptoms related to other body systems including:

- Cough
- Dark urine

- Frequent infections
- Itchy skin
- Unexplained weight loss
- Yellowing of the skin and whites of the eyes (jaundice)

**Serious symptoms that might indicate a life-threatening condition**

In some cases, steatorrhea may be a symptom of a life-threatening condition that should be immediately evaluated in an emergency setting. Seek immediate medical care (call 911) if you, or someone you are with, have any of these life-threatening symptoms including:

- Bloody stool (blood may be red, black, or tarry in texture)
- Change in level of consciousness or alertness, such as passing out or unresponsiveness
- Chest pain, chest tightness, chest pressure, or palpitations
- High fever (higher than 101 degrees Fahrenheit)
- Not producing any urine, or an infant who does not produce the usual amount of wet diapers
- Respiratory or breathing problems, such as shortness of breath, difficulty breathing, or labored breathing
- Rigidity of the abdomen
- Severe abdominal pain or sharp pain that comes on suddenly\

**Dietary guideline:** A diet low in fat and high in protein and carbohydrates is recommended, especially in patients with steatorrhea. The degree of fat restriction depends on the severity of fat malabsorption; generally, an intake of 20 g/day or less is sufficient.

**Analysis of diet chart same as above**

## **Planning and preparation of diet for Diverticular disease patient**

What is diverticulitis?

Diverticulitis is a condition that affects the digestive tract. It causes inflamed pouches in the lining of the intestine. These pouches are called diverticula.

Diverticula develop when weak spots in the intestinal wall give way under pressure, causing sections to bulge out.

When diverticula develop, the person has diverticulosis. When the diverticula become inflamed or infected, this is called diverticulitis.

Diverticulosis becomes more common as you age, occurring in around 58% of Americans over age 60. Fewer than 5% of people with diverticulosis will develop diverticulitis.

Diverticulitis may lead to health problems or complications, including:

- nausea
- fever
- severe abdominal pain
- bloody bowel movements
- an abscess, or an inflamed pocket of tissue
- fistula

### **Dietary guideline:**

Eating a high-fibre diet may help ease the symptoms of diverticular disease and stop you developing diverticulitis.

Generally, adults should aim to eat 30g of fibre a day.

Good sources of fibre include fresh and dried fruits, vegetables, beans and pulses, nuts, cereals and starchy foods.

Fibre supplements, usually in the form of sachets of powder that you mix with water, are also available from pharmacists and health food shops.

Gradually increasing your fibre intake over a few weeks and drinking plenty of fluids can help prevent side effects associated with a high-fibre diet, such as bloating and farting.

**Analysis of diet chart same as above**

## Planning and preparation of diet for Ulcerative Colitis patient

Ulcerative colitis is an idiopathic chronic inflammatory disease of the colon that follows a course of relapse and remission. In a small number of cases, ulcerative colitis is associated with extra-intestinal features. Disease extent can be divided into:

**Distal disease (left-sided colitis):** colitis confined to the rectum (proctitis) or rectum and sigmoid colon (proctosigmoiditis).

**More extensive disease includes:** left-sided colitis (up to the splenic flexure, 40% of patients), extensive colitis (up to the hepatic flexure) and pancolitis (affecting the whole colon, 20% of patients).

Some patients with pancolitis may have involvement of the terminal ileum due to an incompetent ileocaecal valve.

It is sometimes difficult to distinguish between ulcerative colitis and isolated colonic Crohn's disease. These patients can be described as having indeterminate colitis.

### Aetiology

The aetiology is unknown. Ulcerative colitis is probably an autoimmune condition triggered by colonic bacteria causing inflammation in the gastrointestinal tract.

The risk of ulcerative colitis is greatest in first-degree relatives but is also raised in second-degree and third-degree relatives of people with ulcerative colitis.

There is concern that non-steroidal anti-inflammatory drugs (NSAIDs) may increase the risk of relapse or exacerbation of inflammatory bowel disease (IBD) - ulcerative colitis and Crohn's disease.

The risk of ulcerative colitis is decreased in smokers.

### Symptoms

The cardinal symptom is bloody diarrhoea.

Associated symptoms include colicky abdominal pain, urgency, or tenesmus (a feeling of incomplete defecation with an inability or difficulty to empty the bowel at defecation).

Disease limited to the rectum (proctitis) may present with constipation and rectal bleeding.

There may be symptoms of systemic upset, including malaise, fever, weight loss and symptoms of extra-intestinal (joint, cutaneous and eye) manifestations.

The presentation may mimic gastrointestinal infection and the history should include recent foreign travel in considering the possibility of an infective cause.

Recent medication history is also important in considering other possible causes of the presenting gastrointestinal upset.

### Dietary guideline:

While there is no one-size-fits-all for meal planning, these tips can help guide you toward better daily nutrition:

- Eat four to six small meals daily.
- Stay hydrated — drink enough to keep your urine light yellow to clear — with water, broth, tomato juice, or a rehydration solution
- Drink slowly and avoid using a straw, which can cause you to ingest air, which may cause gas.
- Prepare meals in advance, and keep your kitchen stocked with foods that you tolerate well (see list below).
- Use simple cooking techniques — boil, grill, steam, poach.
- Low-fiber foods are easy for your body to digest. They can help slow your bowel movements and limit diarrhea. You can still eat a lot of the foods that you'd normally eat, while keeping your fiber consumption down to around 10 to 15 grams per day.
- Gluten free and specific carbohydrate diet should be recommended.

**Analysis of diet chart same as above**

## **Planning and preparation of diet for Flatulence patient**

### **What is flatulence?**

Flatulence (also called as farting, or passing wind), is the passing of gas out of the back passage (rectum/anus) through the digestive system.

Passing wind is a normal process for everyone, when odorless and limited between 5-15 times a day. However, frequent and foul-smelling flatulence can be socially embarrassing, especially so in a crowded elevator or at an important meeting.

### **What are the symptoms of flatulence?**

Symptoms of excessive flatulence include:

- Passing wind frequently
- Smelly and loud passing of gas
- Abdominal swelling and discomfort
- Rumbling in the lower abdomen

**Causes**

Flatulence can be the result of normal bodily processes, or it may stem from a condition that affects the digestive system.

Exogenous sources are those that come from outside. We swallow air when we eat, drink, or swallow saliva, especially when excess saliva is produced, due to nausea or acid reflux.

Endogenous sources are inside the gut. Gas may arise as a by-product of digestion of certain foods, or when foods are not completely digested.

If any food is not digested completely by the stomach or the small intestine, flatulence can occur when it reaches the large intestine.

**Dietary guideline:**

Eating smaller meals: Symptoms often improve if the person eats four to six smaller meals each day, rather than three large ones. Peppermint tea may help.

Eating slowly: Digestion starts in your mouth, so food should be chewed thoroughly before swallowing.

Avoiding gum and carbonated drinks: Chewing gum makes people swallow more air. This can increase flatulence.

Not smoking: Smoking causes people to swallow more air, and it can also irritate the digestive system.

Choosing low-lactose dairy products: Eliminating foods high in lactose may improve symptoms.

Choosing beans that are fermented before cooking: These have less soluble fiber and a higher nutritional content and may decrease Trusted Source flatulence.

Doing exercise: Activity enhances the functioning of the digestive system, and this can help reduce gas and bloating.

Charcoal pads: Placed inside clothing, these absorb released gas and reduce the impact of foul-smelling gas. These are available to purchase online.

Probiotics: These may reduce symptoms in some people. Probiotic supplements are available to purchase online from different brands.

**Analysis of diet chart same as above**

## Planning and preparation of diet for Constipation patient

Having fewer than three bowel movements a week is, technically, the definition of constipation. However, how often you “go” varies widely from person to person. Some people have bowel movements several times a day while others have them only one to two times a week. Whatever your bowel movement pattern is, it’s unique and normal for you – as long as you don’t stray too far from your pattern.

Regardless of your bowel pattern, one fact is certain: the longer you go before you “go,” the more difficult it becomes for stool/poop to pass. Other key features that usually define constipation include:

- Stools are dry and hard.
- Bowel movement is painful and stools are difficult to pass.
- Have a feeling that you have not fully emptied your bowels.

**Cause:** There are many causes of constipation – lifestyle choices, medications, medical conditions, and pregnancy.

### Common lifestyle causes of constipation include:

Eating foods low in fiber.

Not drinking enough water (dehydration).

Not getting enough exercise.

Changes in your regular routine, such as traveling or eating or going to bed at different times.

Eating large amounts of milk or cheese.

Stress.

Resisting the urge to have a bowel movement.

### Medications that can cause constipation include:

Strong pain medicines, like the narcotics containing codeine, oxycodone (Oxycontin®) and hydromorphone (Dilaudid®).

Nonsteroidal anti-inflammatory drugs, like ibuprofen (Advil®, Motrin®) and naproxen (Aleve®).

Antidepressants, including the selective serotonin reuptake inhibitors (like fluoxetine [Prozac®]) or tricyclic antidepressants (like amitriptyline [Elavil®]).

Antacids containing calcium or aluminum, such as Tums®.

Iron pills.

Allergy medications, such as antihistamines (like diphenhydramine [Benadryl®]).

Certain blood pressure medicines, including calcium channel blockers (like verapamil [Calan SR], diltiazem [Cardizem®] and nifedipine [Procardia®]) and beta-blockers (like atenolol [Tenormin®]).

Psychiatric medications, like clozapine (Clozaril®) and olanzapine (Zyprexa®).

Anticonvulsant/seizure medications, such as phenytoin and gabapentin.

Antinausea medications, like ondansetron (Zofran®).

Many drugs can cause constipation. Ask your doctor or pharmacist if you have any questions or concerns.

**Medical and health conditions that can cause constipation include:**

Endocrine problems, like underactive thyroid gland (hypothyroidism), diabetes, uremia, hypercalcemia.

Colorectal cancer.

Irritable bowel syndrome (IBS).

Diverticular disease.

Outlet dysfunction constipation. (A defect in the coordination of pelvic floor muscles. These muscles support the organs within the pelvis and lower abdomen. They are needed to help release stool.)

Neurologic disorders including spinal cord injury, multiple sclerosis, Parkinson's disease, and stroke.

Lazy bowel syndrome. The colon contracts poorly and retains stool.

Intestinal obstruction.

Structural defects in the digestive tract (like fistula, colonic atresia, volvulus, intussusception, imperforate anus, or malrotation.)

Multiple organ diseases, such as amyloidosis, lupus, and scleroderma.

Pregnancy.

**Symptom:** Symptoms of constipation include:

- Have fewer than three bowel movements a week.
- Stools are dry, hard and/or lumpy.
- Stools are difficult or painful to pass.
- Have a stomach ache or cramps.
- Feel bloated and nauseous.
- Feel that you haven't completely emptied your bowels after a movement.

**Dietary management:**

- Increasing your intake of fiber and fluid may help you feel less constipated and bloated. It's important to understand that there are two types of fiber in the diet: Soluble Fiber and Insoluble Fiber.
- Drink plenty of liquids.



- Choose whole fruit instead of juice.
- Eat the skins and seeds for extra fiber.
- Try to have a fruit or vegetable with each meal or snack.
- Choose foods that promote regularity.
- Eat cereals, breads, and pastas that are made with 100% whole grain.
- Have brown or wild rice in place of white rice or potatoes.
- Choose hot cereals like oatmeal or cold cereals with at least 5 grams of fiber.
- Choose whole wheat breads, whole corn or wheat tortillas, and whole grain crackers instead of refined products.
- Eat more beans, lentils, and peas. Add them to soups and casseroles, or have as a main entrée.
- Beans are also a great source of protein, so you can use them as a substitute for meat at mealtimes.
- Beans can be gas forming, so add them gradually. If you experience bloating or discomfort, you may want to limit them in your diet.
- Try to include exercise or physical activity in your daily routine.

**Analysis of diet chart same as above**

## Planning and preparation of diet for Irritable Bowel Syndrome patient

Irritable bowel syndrome (IBS) is a common disorder that affects the large intestine. Signs and symptoms include cramping, abdominal pain, bloating, gas, and diarrhea or constipation, or both. IBS is a chronic condition that you'll need to manage long term.

### Symptoms

The signs and symptoms of IBS vary but are usually present for a long time. The most common include:

- Abdominal pain, cramping or bloating that is related to passing a bowel movement
- Changes in appearance of bowel movement
- Changes in how often you are having a bowel movement
- Other symptoms that are often related include bloating, increased gas or mucus in the stool.

### Causes

The precise cause of IBS isn't known. Factors that appear to play a role include:

- **Muscle contractions in the intestine.** The walls of the intestines are lined with layers of muscle that contract as they move food through your digestive tract. Contractions that are stronger and last longer than normal can cause gas, bloating and diarrhea. Weak intestinal contractions can slow food passage and lead to hard, dry stools.
- **Nervous system.** Abnormalities in the nerves in your digestive system may cause you to experience greater than normal discomfort when your abdomen stretches from gas or stool. Poorly coordinated signals between the brain and the intestines can cause your body to overreact to changes that normally occur in the digestive process, resulting in pain, diarrhea or constipation.
- **Severe infection.** IBS can develop after a severe bout of diarrhea (gastroenteritis) caused by bacteria or a virus. IBS might also be associated with a surplus of bacteria in the intestines (bacterial overgrowth).
- **Early life stress.** People exposed to stressful events, especially in childhood, tend to have more symptoms of IBS.

Changes in gut microbes. Examples include changes in bacteria, fungi and viruses, which normally reside in the intestines and play a key role in health. Research indicates that the microbes in people with IBS might differ from those in healthy people.

### Dietary management:

- Patients with IBS should be advised to establish a regular meal pattern (breakfast, lunch and dinner with snacks as appropriate) and to avoid skipping meals, leaving long gaps between eating or eating late at night and IBS patients should be recommended to avoid large meals as well as to take time to eat, to sit down to eat, and to chew food thoroughly.

- IBS patients should be advised to consume alcohol in compliance with recommended safe limits, while having at least two alcohol free days a week.
- Caffeine intake should be assessed in IBS patients and, if related to symptoms, daily intake should be restricted to 400 mg caffeine, which is the safe limit for most adults.
- Spicy food intake should be assessed in IBS patients and, if related to IBS symptoms, the intake should be restricted.
- According to FAO/WHO dietary recommendations, the total fat intake for an adult should range from 30% to 35% of total energy, without being under 15% of total energy and patients with IBS should be advised to limit fat intake to no more than 40-50 g/d.
- A gradual increase in total dietary fiber (naturally occurring and supplemented) to a target dose of 20-30 g/d should be suitable.
- IBS patients should be advised to use a low-lactose diet only if they have a positive lactose hydrogen breath test.
- IBS patients should be advised to drink up to 1.5-3 L/d of fluids (about 35 mL/kg), especially water or other caffeine-free and alcohol-free non-carbonated drinks.
- If IBS patients decide to restrict gluten, they should be warned that an inappropriate gluten-free diet may be detrimental.
- Low FODMAP diet needs to be provided by a healthcare professional with training in medical nutrition therapy and knowledge of food composition, such as a specialized dietitian.

**Analysis of diet chart same as above**

## Planning and preparation of diet for Haemorrhoids patient

Hemorrhoids, also called piles, happen when clusters of veins in your rectum or anus get swollen (or dilated). When these veins swell, blood pools and causes the veins to expand outward into the membranes around your rectal and anal tissue. This can become uncomfortable or painful.

There are four types of hemorrhoids:

Internal, external, prolapsed, thrombosed

Most hemorrhoids aren't serious and you may not notice them. In fact, less than 5 percent of people who get hemorrhoids have symptoms. Even less need treatment.

Hemorrhoids aren't that uncommon. At least three of every four adults will get them at one point in their life. But see your doctor right away if your hemorrhoids are causing you pain, or disrupting your normal activities and bowel movements.

**Symptom:** Symptoms of hemorrhoids include:

- extreme itching around the anus
- irritation and pain around the anus
- itchy or painful lump or swelling near your anus
- fecal leakage
- painful bowel movements
- blood on the tissue after having a bowel movement

Although hemorrhoids are painful, they aren't life threatening and often go away on their own without treatment.

If you ever have bleeding or black bowel movements, however, you should see your doctor. Bleeding can be caused by something other than hemorrhoids and must be evaluated.

**Cause:**

**Causes**

**Enlargement of the veins around the anus causes hemorrhoids.**

**They can occur for the following reasons:**

**Pregnancy:** They occur more commonly in pregnant women because, as the uterus enlarges, it presses on the vein in the colon, causing it to bulge.

**Ageing:** Hemorrhoids are most common among adults aged 45 to 65 years. This does not mean, however, that young people and children do not get them.

**Diarrhea:** Hemorrhoids can occur after cases of chronic diarrhea.

Chronic constipation: Straining to move stool puts additional pressure on the walls of the blood vessels.

**Sitting for too long:** Staying in a seated position for long periods of time can cause hemorrhoids, especially on the toilet.

**Heavy lifting:** Repeatedly lifting heavy objects can lead to hemorrhoids.

Anal intercourse: This can cause new hemorrhoids or worsen existing ones.

**Obesity:** Diet-related obesity can cause hemorrhoids.

**Genetics:** Some people inherit a tendency to develop hemorrhoids.

**Dietary management of hemorrhoids:** The best way to prevent hemorrhoids is to keep your stools soft, so they pass easily. To prevent hemorrhoids and reduce symptoms of hemorrhoids, follow these tips:

**Eat high-fiber foods.** Eat more fruits, vegetables and whole grains. Doing so softens the stool and increases its bulk, which will help you avoid the straining that can cause hemorrhoids. Add fiber to your diet slowly to avoid problems with gas.

**Drink plenty of fluids.** Drink six to eight glasses of water and other liquids (not alcohol) each day to help keep stools soft.

**Consider fiber supplements.** Most people don't get enough of the recommended amount of fiber — 20 to 30 grams a day — in their diet. Studies have shown that over-the-counter fiber supplements, such as psyllium (Metamucil) or methylcellulose (Citrucel), improve overall symptoms and bleeding from hemorrhoids.

If you use fiber supplements, be sure to drink at least eight glasses of water or other fluids every day. Otherwise, the supplements can cause or worsen constipation.

**Don't strain.** Straining and holding your breath when trying to pass a stool creates greater pressure in the veins in the lower rectum.

**Go as soon as you feel the urge.** If you wait to pass a bowel movement and the urge goes away, your stool could dry out and be harder to pass.

**Exercise.** Stay active to help prevent constipation and to reduce pressure on veins, which can occur with long periods of standing or sitting. Exercise can also help you lose excess weight that might be contributing to your hemorrhoids.

**Avoid long periods of sitting.** Sitting too long, particularly on the toilet, can increase the pressure on the veins in the anus.

**Analysis of diet chart same as above**

## Planning and preparation of diet for Celiac sprue patient

Celiac disease is an autoimmune disorder that's triggered when you eat gluten. It's also known as celiac sprue, no tropical sprue, or gluten-sensitive enteropathy.

Gluten is a protein in wheat, barley, rye, and other grains. It's what makes dough elastic and gives bread its chewy texture.

### Symptoms

**The signs and symptoms of celiac disease can vary greatly and differ in children and adults. Digestive signs and symptoms for adults include:**

Diarrhea

Fatigue

Weight loss

Bloating and gas

Abdominal pain

Nausea and vomiting

Constipation

**However, more than half the adults with celiac disease have signs and symptoms unrelated to the digestive system, including:**

Anemia, usually from iron deficiency

Loss of bone density (osteoporosis) or softening of bone (osteomalacia)

Itchy, blistering skin rash (dermatitis herpetiformis)

Mouth ulcers

Headaches and fatigue

Nervous system injury, including numbness and tingling in the feet and hands, possible problems with balance, and cognitive impairment

Joint pain

Reduced functioning of the spleen (hyposplenism)

Children

**Children with celiac disease are more likely than adults to have digestive problems, including:**

Nausea and vomiting

Chronic diarrhea

Swollen belly

Constipation

Gas

Pale, foul-smelling stools

**The inability to absorb nutrients might result in:**

Failure to thrive for infants

Damage to tooth enamel

Weight loss

Anemia

Irritability

Short stature

Delayed puberty

Neurological symptoms, including attention-deficit/hyperactivity disorder (ADHD), learning disabilities, headaches, lack of muscle coordination and seizures.

**Causes**

Your genes combined with eating foods with gluten and other factors can contribute to celiac disease, but the precise cause isn't known. Infant-feeding practices, gastrointestinal infections and gut bacteria might contribute, as well. Sometimes celiac disease becomes active after surgery, pregnancy, childbirth, viral infection or severe emotional stress.

When the body's immune system overreacts to gluten in food, the reaction damages the tiny, hairlike projections (villi) that line the small intestine. Villi absorb vitamins, minerals and other nutrients from the food you eat. If your villi are damaged, you can't get enough nutrients, no matter how much you eat.

**Dietary management:**

- Avoid all products with barley, rye, triticale (a cross between wheat and rye), farina, graham flour, semolina, and any other kind of flour, including self-rising and durum, not labeled gluten-free.
- Be careful of corn and rice products. These don't contain gluten, but they can sometimes be contaminated with wheat gluten if they're produced in factories that also manufacture wheat products. Look for such a warning on the package label.
- Go with oats. Recent studies suggest you can eat oats as long as they are not contaminated with wheat gluten during processing. You should check with your healthcare provider first.
- Substitute potato, rice, soy, amaranth, quinoa, buckwheat, or bean flour for wheat flour. You can also use sorghum, chickpea or Bengal gram, arrowroot, and corn flour, as well as tapioca starch extract. These act as thickeners and leavening agents.

**Analysis of diet chart same as above**

## **Planning and preparation of diet for Viral Hepatitis patient**

Viral hepatitis is an infection that causes liver inflammation and damage. Inflammation is swelling that occurs when tissues of the body become injured or infected. Inflammation can damage organs. Researchers have discovered several different viruses NIH external link that cause hepatitis, including hepatitis A, B, C, D, and E.

### **Hepatitis A**

Hepatitis A is caused by an infection with the hepatitis A virus (HAV). This type of hepatitis is most commonly transmitted by consuming food or water contaminated by feces from a person infected with hepatitis A.

### **Hepatitis B**

Hepatitis B is transmitted through contact with infectious body fluids, such as blood, vaginal secretions, or semen, containing the hepatitis B virus (HBV). Injection drug use, having sex with an infected partner, or sharing razors with an infected person increase your risk of getting hepatitis B.

It's estimated by the CDC Trusted Source that 1.2 million people in the United States and 350 million people worldwide live with this chronic disease.

### **Hepatitis C**

Hepatitis C comes from the hepatitis C virus (HCV). Hepatitis C is transmitted through direct contact with infected body fluids, typically through injection drug use and sexual contact. HCV is among the most common bloodborne viral infections in the United States. Approximately 2.7 to 3.9 million Americans Trusted Source are currently living with a chronic form of this infection.

### **Hepatitis D**

Also called delta hepatitis, hepatitis D is a serious liver disease caused by the hepatitis D virus (HDV). HDV is contracted through direct contact with infected blood. Hepatitis D is a rare form of hepatitis that only occurs in conjunction with hepatitis B infection. The hepatitis D virus can't multiply without the presence of hepatitis B. It's very uncommon in the United States.

### **Hepatitis E**

Hepatitis E is a waterborne disease caused by the hepatitis E virus (HEV). Hepatitis E is mainly found in areas with poor sanitation and typically results from ingesting fecal matter that contaminates the water supply. This disease is uncommon in the United States. However, cases of hepatitis E have been reported in the Middle East, Asia, Central America, and Africa, according to the CDC.

### **Common symptoms of hepatitis**

If you have infectious forms of hepatitis that are chronic, like hepatitis B and C, you may not have symptoms in the beginning. Symptoms may not occur until the damage affects liver function.



**Signs and symptoms of acute hepatitis appear quickly. They include:**

fatigue

flu-like symptoms

dark urine

pale stool

abdominal pain

loss of appetite

unexplained weight loss

yellow skin and eyes, which may be signs of jaundice

Chronic hepatitis develops slowly, so these signs and symptoms may be too subtle to notice.

**Dietary management:** The following nutrition considerations apply to prevention and treatment of viral hepatitis:

**Hygiene and sanitation.** Persons who travel internationally are at higher risk for hepatitis A (HAV) through the consumption or handling of contaminated uncooked fruits and vegetables. Boiling or cooking food and water for  $\geq 1$  minute to  $85^{\circ}\text{C}$  ( $185^{\circ}\text{F}$ ) is necessary to inactivate HAV.

**Avoiding contaminated shellfish and game meats.** Most acute HAV infections are due to contaminated shellfish consumption. Shellfish are often taken from wastewater-polluted areas of the sea and can concentrate the microbial pathogens in seawater. Those taken from near the shoreline (e.g., clams and oysters) are particularly likely to be pathogenic. Hepatitis E (HEV) has been identified in contaminated shellfish and produce, as well as animal meats, particularly wild game, and contaminated pork.

**Avoiding high-iron foods and iron supplements.** Hepatitis C progression occurs in patients as a result of accelerated hepatic iron uptake and the oxidative stress caused by iron-catalyzed free radical production. Along with phlebotomy, a low-iron diet helps lower the risk for hepatocellular carcinoma (HCC) in these patients.

**Nutritional supplementation may be required.** Treatment with interferon (IFN) can cause digestive complaints with a subsequent reduction in appetite and food intake and has been reported to result in weight loss in 11-29% of treated patients.

**A low-fat, low-cholesterol diet may be helpful.** Chronic hepatitis C (CHC) infection increases the risk for hepatic steatosis.[23],[26] A higher intake of dietary cholesterol contributes to this problem and is associated with the progression of hepatitis C-related liver disease. Individuals on a dietary regimen that is reduced in fat (23% of calories) and cholesterol (185 mg/d) experienced a reduction in liver enzyme elevations and an improvement in immunological abnormalities (TH17/Treg balance) known to contribute to liver inflammation in patients with CHC.

**Adequate vitamin D status.** Vitamin D deficiency is common in patients with chronic liver disease, and these patients may have a reduced ability to convert vitamin D to its active form.[28] An inverse relationship seems to exist between vitamin D concentrations and viral load in patients with CHC. Deficiency significantly lowers the chance for a sustained virological response to pegylated interferon and ribavirin, and vitamin D supplementation improves the probability of response to treatment.

**Avoidance of extremes in B12 status.** Adequate B12 status helps with clearance of hepatitis C from the circulation of infected patients. However, overly high serum B12 levels may also foster viral replication and are associated with concentrations of hepatitis C RNA levels.

**Coffee consumption and chronic hepatitis C.** Coffee consumption may be helpful, reducing oxidative DNA damage, increasing death of virus-infected cells, stabilizing chromosomes, and reducing fibrosis. Moderate daily unsweetened coffee ingestion is a reasonable adjunct to therapy for NAFLD patients.

**Analysis of diet chart same as above**

## **Planning and preparation of diet for Cirrhosis of liver patient**

Cirrhosis is a complication of liver disease that involves loss of liver cells and irreversible scarring of the liver.

### **Symptoms**

Cirrhosis often has no signs or symptoms until liver damage is extensive. When signs and symptoms do occur, they may include:

- Fatigue
- Easily bleeding or bruising
- Loss of appetite
- Nausea
- Swelling in your legs, feet or ankles (edema)
- Weight loss
- Itchy skin
- Yellow discoloration in the skin and eyes (jaundice)
- Fluid accumulation in your abdomen (ascites)
- Spiderlike blood vessels on your skin
- Redness in the palms of the hands
- For women, absent or loss of periods not related to menopause
- For men, loss of sex drive, breast enlargement (gynecomastia) or testicular atrophy
- Confusion, drowsiness and slurred speech (hepatic encephalopathy)

### **Causes**

A wide range of diseases and conditions can damage the liver and lead to cirrhosis.

Some of the causes include:

- Chronic alcohol abuse
- Chronic viral hepatitis (hepatitis B, C and D)
- Fat accumulating in the liver (nonalcoholic fatty liver disease)
- Iron buildup in the body (hemochromatosis)
- Cystic fibrosis
- Copper accumulated in the liver (Wilson's disease)
- Poorly formed bile ducts (biliary atresia)
- Alpha-1 antitrypsin deficiency

- Inherited disorders of sugar metabolism (galactosemia or glycogen storage disease)
- Genetic digestive disorder (Alagille syndrome)
- Liver disease caused by your body's immune system (autoimmune hepatitis)
- Destruction of the bile ducts (primary biliary cirrhosis)
- Hardening and scarring of the bile ducts (primary sclerosing cholangitis)
- Infection, such as syphilis or brucellosis
- Medications, including methotrexate or isoniazid

**Dietary management:**

- Provision of Adequate Nutrition
- Sodium restriction
- Fluid restriction
- Protein restriction
- Low-Fat diets
- Antioxidants and B-vitamins

**Analysis of diet chart same as above**

## Planning and preparation of diet for Cholelithiasis patient

Cholelithiasis is the medical name for hard deposits (gallstones) that may form in the gallbladder. Cholelithiasis is common in the United States population. Six percent of adult men and 10% of adult women are affected.

The cause of cholelithiasis is not completely understood, but it is thought to have multiple factors. The gallbladder stores bile and releases it into the small intestine when it is needed for digestion. Gallstones can develop if the bile contains too much cholesterol or too much bilirubin (one of the components of bile), or if the gallbladder is dysfunctional and cannot release the bile.

### Symptom:

Cholelithiasis may cause irritation and inflammation of the gallbladder (cholecystitis) that can result in a number of symptoms. The symptoms can vary in intensity among individuals.

Common symptoms of cholelithiasis

You may experience cholecystitis symptoms daily or just once in a while. At times, any of these common symptoms can be severe:

- Abdominal pain (typically localized to the right upper quadrant of the abdomen)
- Abdominal swelling, distension or bloating
- Abdominal tenderness
- Clay-colored stools
- Fever and chills
- Loss of appetite
- Nausea with or without vomiting
- Pain that radiates from the abdomen to the right shoulder or back
- Sweating
- Yellowing of the skin and whites of the eyes (jaundice)

### Serious symptoms that might indicate a life-threatening condition

In some cases, cholelithiasis can be life threatening. Seek immediate medical care (call 911) if you, or someone you are with, have any of these life-threatening symptoms including:

- Abdominal swelling, distension or bloating
- High fever (higher than 101 degrees Fahrenheit)
- Nausea with or without vomiting
- Severe abdominal pain

**Cause:** In most cases, cholelithiasis is caused by excessive amounts of cholesterol in the bile that is stored in the gallbladder. The cholesterol hardens to form stone-like substances. Increased body weight and older age are associated with increased levels of cholesterol in the bile. Thus, gallstones are more likely to occur in women, in people who are obese, and in older individuals.

Some gallstones develop because the bile contains too much bilirubin, a waste product of the liver that is a component of bile. Gallstones that develop from excess bilirubin are called pigment stones.

**Dietary management:**

The following factors are associated with reduced risk of gallstones:

**Plant-based diets.** Both animal fat and animal protein may contribute to the formation of gallstones. Up to 90% of gallstones are cholesterol stones ( $\geq 20\%$  cholesterol composition), suggesting the possibility that dietary changes (e.g., reducing dietary saturated fat and cholesterol and increasing soluble fiber) may reduce the risk of gallstones.

**Replacement of sugars and refined starches with high-fiber foods.** The cholesterol saturation index of bile, a known risk factor for gallstone formation, is higher with diets that provide carbohydrates in a refined, as opposed to unrefined, form.

**Avoidance of excess weight and a healthful approach to weight control.** Women with a BMI  $\geq 30$  kg/m<sup>2</sup> have at least double the risk for gallstone disease compared with women with a BMI  $< 25$  kg/m<sup>2</sup>. The same degree of risk exists for men with a BMI  $\geq 25$  kg/m<sup>2</sup>, compared with a BMI  $< 22.5$  kg/m<sup>2</sup>. Risk rises with increased weight.

**Moderate alcohol intake.** Compared with infrequent consumption or abstinence, moderate alcohol intake was found to be inversely associated with the risk for gallstones. However, given the health risks (e.g., breast or colorectal cancer) associated with alcohol consumption, caution regarding alcohol use is warranted.

**Analysis of diet chart same as above**

## Planning and preparation of diet for Pancreatitis patient

Pancreatitis is a disease in which your pancreas becomes inflamed.

The pancreas is a large gland behind your stomach and next to your small intestine. Your pancreas does two main things:

It releases powerful digestive enzymes into your small intestine to help you digest food.

It releases insulin and glucagon into your bloodstream. These hormones help your body control how it uses food for energy.

Your pancreas can be damaged when digestive enzymes begin working before your pancreas releases them.

### Types of Pancreatitis

The two forms of pancreatitis are acute and chronic.

**Acute pancreatitis** is sudden inflammation that lasts a short time. It can range from mild discomfort to a severe, life-threatening illness. Most people with acute pancreatitis recover completely after getting the right treatment. In severe cases, acute pancreatitis can cause bleeding, serious tissue damage, infection, and cysts. Severe pancreatitis can also harm other vital organs such as the heart, lungs, and kidneys.

**Chronic pancreatitis** is long-lasting inflammation. It most often happens after an episode of acute pancreatitis. Another top cause is drinking lots of alcohol for a long period of time. Damage to your pancreas from heavy alcohol use may not cause symptoms for many years, but then you may suddenly have severe pancreatitis symptoms.

### Symptoms:

Signs and symptoms of pancreatitis may vary, depending on which type you experience.

#### Acute pancreatitis signs and symptoms include:

- Upper abdominal pain
- Abdominal pain that radiates to your back
- Abdominal pain that feels worse after eating
- Fever
- Rapid pulse
- Nausea
- Vomiting
- Tenderness when touching the abdomen

**Chronic pancreatitis signs and symptoms include:**

- Upper abdominal pain
- Losing weight without trying
- Oily, smelly stools (steatorrhea)

**Causes:**

Pancreatitis occurs when digestive enzymes become activated while still in the pancreas, irritating the cells of your pancreas and causing inflammation.

With repeated bouts of acute pancreatitis, damage to the pancreas can occur and lead to chronic pancreatitis. Scar tissue may form in the pancreas, causing loss of function. A poorly functioning pancreas can cause digestion problems and diabetes.

**Conditions that can lead to pancreatitis include:**

- Abdominal surgery
- Alcoholism
- Certain medications
- Cystic fibrosis
- Gallstones
- High calcium levels in the blood (hypercalcemia), which may be caused by an overactive parathyroid gland (hyperparathyroidism)
- High triglyceride levels in the blood (hypertriglyceridemia)
- Infection
- Injury to the abdomen
- Obesity
- Pancreatic cancer

Endoscopic retrograde cholangiopancreatography (ERCP), a procedure used to treat gallstones, also can lead to pancreatitis.

Sometimes, a cause for pancreatitis is never found.

**Dietary management:**

Though acute pancreatitis usually heals within one to two weeks, solid foods are usually not advised during this period, to minimize the load on the pancreas. Physicians usually recommend eating small amounts of food once the diagnosis of pancreatitis is made; however, observations show that most people start eating little as soon as the symptoms get relieved.

Solid foods are safe once pancreatitis is relieved; however, it is advisable to consume foods that are easy to digest and have a low-fat content.



Physicians usually recommend a diet which is high in protein and contains only moderate amounts of animal fat and sugars. The following list shows foods which are safe during pancreatitis:

- Protein-rich foods like lentils or beans
- Skimmed or non-fat milk or milk products
- Fresh fruits and vegetables
- Foods like berries, green leafy vegetables, nuts or berries which are high in antioxidants
- Whole grains

However, it is advisable to consume lower amounts of foods like olive oil, fatty fruits like avocado, nuts and fatty fish because of their high fat content. The Mediterranean-type diet is recommended during pancreatitis as it is easy to digest, especially if while convalescing from acute or mild pancreatitis.

The consumption of vegetables and fruits increases the fiber intake and reduces the overall cholesterol intake. Reduced cholesterol consumption minimizes the chances of gallstones and high triglyceride levels, which are among the risk factors for developing acute pancreatitis. An antioxidant-rich diet also aids in eliminating free radicals from the body, which further reduces the inflammation of the pancreas.

A few basic dietary and lifestyle changes can facilitate recovery from acute pancreatitis. Below are various simple steps which may be incorporated into the process of recovery to speed it up:

- Consuming frequent meals with small portions; dividing the total food intake into six to eight small meals a day reduces the load on the pancreas
- Adding one to two tablespoons of MCTs daily to meals helps in recovering from moderately severe or severe chronic pancreatitis.
- A multivitamin supplement containing A, D, E, K, B12, zinc and folic acid is useful
- Consuming less than 30 grams per day of fats; cutting out saturated fats
- Refrain from smoking and alcohol
- Drink copious amounts of water daily

**Analysis of diet chart same as above**

## Planning and preparation of diet for Anaemia patient

Anemia is a condition in which you lack enough healthy red blood cells to carry adequate oxygen to your body's tissues. Having anemia can make you feel tired and weak.

There are many forms of anemia, each with its own cause. Anemia can be temporary or long term, and it can range from mild to severe. See your doctor if you suspect that you have anemia. It can be a warning sign of serious illness.

Treatments for anemia range from taking supplements to undergoing medical procedures. You might be able to prevent some types of anemia by eating a healthy, varied diet.

### Types

Aplastic anemia

Iron deficiency anemia

Sickle cell anemia

Thalassemia

Vitamin deficiency anemia

### Symptoms

Anemia signs and symptoms vary depending on the cause. If the anemia is caused by a chronic disease, the disease can mask them, so that the anemia might be detected by tests for another condition.

Depending on the causes of your anemia, you might have no symptoms. Signs and symptoms, if they do occur, might include:

- Fatigue
- Weakness
- Pale or yellowish skin
- Irregular heartbeats
- Shortness of breath
- Dizziness or lightheadedness
- Chest pain
- Cold hands and feet
- Headaches
- At first, anemia can be so mild that you don't notice it. But symptoms worsen as anemia worsens.

### Dietary management:

- Food rich in iron, folic acid like pulses, green leafy vegetable, cluster bean, ladies finger, liver and eggs should be included in the diet.
- Balanced diet rich in protein, vitamin and minerals should be consumed.

**Analysis of diet chart same as above**

## C9P: Food Microbiology (Practical)

### Study of Equipments in A Microbiology Lab

#### 1. Analytical Balance

An analytical balance is a type of balance that is commonly used for the measurement of mass in the sub-milligram range.

#### Working Principle

- These types of balances are made with a measuring pan enclosed in a transparent covering that prevents small particles or air currents from getting collected on the pan.
- An electric analytical balance uses the force necessary to counteract the mass rather than measuring the mass itself.
- An electromagnet is used to create a force required to achieve a balance with the mass of the substance, and the resulting force is displayed.

#### Uses

As they are highly precise and based on advanced technology, analytical balances are explicitly used in laboratories for the effective completion of tasks like weighing test materials and sampling amounts, formulation, density determination, purity analysis, quality control testing, and material and conformance testing.



#### 2. Autoclave

An autoclave is a pressurized chamber used for the process of sterilization and disinfection by combining three factors: time, pressure and steam

#### Working Principle

- Autoclaves use steam as their sterilization agent. The basic principle of an autoclave is that all the items within the autoclave come in direct contact with the steam for a particular period irrespective of the nature of the material- whether it is liquid, plastic ware, or glassware.
- The amount of time and the temperature depends on the type of material being sterilized and the increase in temperature of the cycle allows for shorter periods.

### Uses

- Autoclaves are mostly used for the sterilization of medical or laboratory equipment with the capacity of sterilizing a large number of materials at once.
- They are commonly used for the preparation of culture media during laboratory applications.



### 3. Centrifuge

- A centrifuge is a device that allows the rotation of an object about a single axis, where an outward force is applied perpendicularly to the axis.
- A laboratory centrifuge is motor-based and allows the rotation of a liquid sample resulting in the separation of the components of the mixture.

#### Working Principle

- A centrifuge works on the principle of sedimentation, where the high speed of the rotation causes the denser particles to move away from the center while smaller, less dense particles are forced towards the center.
- Thus, the denser particles settle at the bottom while the lighter particles are collected at the top.
- In a laboratory tabletop centrifuge, the sample tubes are aligned at an angle so that the particles have to travel a shorter distance before they hit the bottom.

### Uses

The primary application of a centrifuge is the separation of particles suspended in a suspension. It can be used for the separation of cell organelles, nucleic acid, blood components, and separation of isotopes.



#### 4. Colony Counter

A colony counter is used to estimate the density of a liquid culture by counting the number of CFU (colony forming units) on an agar or culture plates.

##### Working Principle

This instrument can accommodate different sizes of plates which are scanned on top with UV, white light and/or fluorescent illumination.

One can accomplish the counting either manually with the touch pressure or with a digital counter.

##### Uses

A colony counter is primarily used for counting the number of colonies present on a culture plate to estimate the concentration of microorganisms in liquid culture.



#### 5. Deep Freezer

##### Working Principle

- Deep freezers are based on the principle that under extremely low temperatures, there is minimum microbial growth which allows for the protection and preservation of different substances.
- Based on this principle, we can even preserve cultures over a long period of time without any change in the concentration of the microorganisms.

## Uses

A deep freeze can be used for the preservation of different things used in the laboratories for a very long period of time. Deep freezers are used in laboratories to store and preserve medical equipment, food items, blood samples, medicines, and injections, etc. for a more extended period of time.



## 6. Homogenizer

Homogenizer is a device used in laboratories for the mixing of various liquids and materials like tissue, plant, food, soil, and many others.

### Working Principle

- This instrument is based on the principle that when large globules in coarse emulsion are passed under high pressure through a narrow orifice, they break down into smaller particles giving a more uniform and stable mixture.
- A homogenizer has a metal rod with narrow parallel openings in the form of a comb at the end which acts as the orifice for the homogenization process.

### Uses

- A homogenizer is primarily used to disrupt cells to acquire cell organelles for different microbiological processes.
- It is used in the preparation step before the extraction and purification of different macromolecules like proteins, nucleic acids, and lipids.



## 7. Hot plate

A hot plate is a stand-alone appliance used in microbiology laboratories as a tabletop heating system.

### Working Principle

- Unlike the traditional ways of producing heat through the fire, a hot plate produces heat by the flow of electricity.
- On a hot plate, electricity runs through the coils which have a high level of electrical resistance. The resistance in the coils converts the electrical energy into heat energy which causes the coils to release heat.

### Uses

- In a laboratory, hot plates are used to heat glassware and their components.
- They are used over water baths as in water baths might be hazardous in case of any spills or overheat.



## 8. Hot air oven

A hot air oven is an electrical device that is used for sterilization of medical equipment or samples using dry heat.

### Working Principle

- Hot air oven is a type of dry heat sterilization which is performed on dry materials and on substances that do not melt or catch fire under high temperature.
- There are two types of hot air oven based on the working principle
- Forced air hot air oven: In this type of hot air oven, the heated air inside the oven is distributed throughout the oven with a fan. This prevents the rising of hot air towards the top while keeping the cold air at the bottom. This allows for the adequate heating of materials inside the oven.
- Static air hot air oven: In this type of oven, the heat is produced by coils present at the bottom of the oven with no fan. The hot air rises and doesn't allow the effective sterilization of the materials.

- The equipment inside the oven acquire heat and pass the heat towards the center, one layer at a time which allows for effective dry heat sterilization.

#### Uses

- Hot air oven can be used to sterilize materials like glassware, metal equipment, powders, etc.
- It allows for the destruction of microorganisms as well as bacterial spores.



#### 9. Incubator

- An incubator is a device that is used in the laboratories for the growth and maintenance of microorganisms and cultures.
- Incubator provides an optimal temperature, pressure, moisture, among other things required for the growth of microorganisms.

#### Working Principle

- The incubator is based on the principle of maintaining a proper atmosphere for the growth of microorganisms.
- Incubators have a heating system that allows for the temperature within the incubator to be adjusted according to the type of organism cultivated inside.
- Similarly, they are provided with adjustments for maintaining the concentration of CO<sub>2</sub> to balance the pH and humidity required for the growth of the organisms.
- Variation of the incubator like a shaking incubator is also available, which allows for the continuous movement of the culture required for cell aeration and solubility studies.

#### Uses

- Incubators have a wide range of applications including cell culture, pharmaceutical studies, hematological studies, and biochemical studies.
- Incubators can also be used in the stem cell research area.





### 10. Laminar Air Flow/ Laminar Hood

Laminar Hood is a closed device primarily for processes or instruments sensitive to microbial contamination.

#### Working Principle

- A Laminar Hood is made up of stainless steel, avoiding joints and corners to prevent the accumulation of bacterial spores.
- This device creates a sterile environment with the flow of sterile air through a High-Efficiency Particulate Air (HEPA) filter and shortwave ultraviolet germicidal lamp that sterilizes the workstation.
- Laminar Air Flow has to turn on 15 minutes before to ensure complete sterilization and the workstation should be cleaned with ethanol before and after use.

#### Uses

- Laminar Hood is commonly used to conduct processes that are sensitive to contamination.
- It is used for experiments related to plant tissue culture and for the experiments of genetic transformation.



## 11. Magnetic Stirrer

Magnetic Stirrer is a device commonly used in microbiology laboratories for the purpose of mixing liquids.

### Working Principle

- This device consists of a rotating magnetic or an electromagnet creating a rotating magnetic field that allows the stir bar (a piece of heavy metal) to move around in the vessel.
- It is coupled with a heating system to heat the liquid while it mixed.

### Uses

- It is usually used for mixing various liquid components in a mixture in a chemical or microbiology laboratory.
- This device is used in place of other stirrers as it is noise-free and because the size of the stir bar is so tiny, there is less chance of contamination.



## 12. Microscope

Microscopes are devices that allow the observer to an exceedingly close view of minute particles.

### Working Principle

- There are many different types of microscopes, each of which works on their respective principles. However, there is some commonality in them.
- The basic principle in a microscope is magnification. Based on the relative position of the object from the lens or electromagnets, different positions, nature, and magnification of the image can be achieved.
- Different types of microscopes are developed to cater to the specific needs of the observation. However, the common theme is magnification.

### Uses

Based on the type of microscopes, different microscopes are used for different purposes.

They are primarily used for the observation of minute particles which cannot be observed with naked eyes.



### 13. pH Meter

- pH meter is a device used in laboratories that measure the H-ion concentration in water-based solutions to determine the acidity or alkalinity of the solution.
- A pH meter is often termed as “potentiometric pH meter” as it measures the difference in electric potential between the reference and a pH electrode.

#### Working Principle

- In a potentiometric pH meter, single or multiple glass electrodes, connected to a bulb selective to hydrogen ions, are attached to a metal rod.
- When the bulb with the electrodes is dipped into a solution, hydrogen ions in the solution exchange with positive charges on the electrode generating an electrochemical potential which is displayed in terms of pH units on display.

#### Uses

- A pH meter is primarily used to measure the acidity of pharmaceutical chemicals, cultures, soil, and water treatment plant.
- It can be used to measure the acidity level in wine and cheese during their production.



### 14. Spectrophotometer

- The spectrophotometer is an optical instrument for measuring the intensity of light in relation to the wavelength.
- Based on the amount of light absorbed by a colored solution, a quantitative analysis of the solution can be done.

### Working Principle

- Spectrophotometry is based on the Beer-Lambert Law, which states the absorbance of light by a solution (of a particular wavelength) is directly proportional to the concentration of the substance.
- Different wavelengths of lights are passed through a solution as different substances have better absorbance at different wavelengths. Based on the absorbance of a particular wavelength, the quantitative analysis of a solution can be done.

### Uses

In a microbiology laboratory, a spectrophotometer is applied for the measurement of substance concentration of protein, nucleic acids, bacterial growth, and enzymatic reactions.



## 15. Vortex Mixture/ Vortexer

A vortex mixture is one of the basic technologies used for the mixing of samples in glass tubes or flasks in laboratories.

### Working Principle

- It is based on the simple principle of causing reactions and homogenization by agitating the mixture.
- Motorized draft shafts present on the mixer oscillates and transfers the movement to the sample tubes causing the sample fluids to undergo turbulent flow.

### Uses

Vortex mixer is mostly used for the mixing of various sample fluids in the sample tubes and also allows for the homogenization of cells and cell organelles.



## 16. Water Bath

Water Bath is a conventional device that is used for chemical reactions that required a controlled environment at a constant temperature.

### Working Principle

A sensor in the device transfers water temperature to a reference value which is then amplified and a control system generates a signal for the heating system which heats the water to the desired temperature.

### Uses

- Water baths are primarily used for heating samples under a controlled temperature.
- These are suitable for heating chemicals that might be flammable under direct ignition.



## 17. Water Distiller

- A water distiller is a device that purifies water by the process of distillation.
- This instrument is commonly used in medical laboratories, microbiology laboratories, organic chemistry laboratories and medical industries.

### Working Principle

- A water distiller is based on the principle of distillation.
- According to this process, water is first brought to a boil and then condensed into liquid form to obtain pure distilled water.

### Uses

It is used to obtain distilled water required for many lab tests as well as for the preparation of culture media.



## Preparation of different culture media

### Culture media

The method for the preparation of basic microbiology media is given below. In situations where preparation is uneconomic in time, prepared, sterilized media (liquid and solid) are available from the major school science equipment suppliers. Sterilization is at 121 °C (15 lb in  $\bar{2}$ ) for 15 minutes. pH values are 7.0 unless stated otherwise.

Note: Allow 15 cm<sup>3</sup> of agar for each Petri dish and 5-10 cm<sup>3</sup> of broth for each McCartney bottle. All cotton wool plugs should be made of non-absorbent cotton wool. Plastic or metal caps may also be used.

### Nutrient agar

Suspend 28 g of nutrient agar powder in 1 litre of distilled water. Bring to the boil to dissolve completely. Dispense as required and sterilize.

### Nutrient broth

Add 13 g of nutrient broth powder to 1 litre of distilled water. Mix well. Dispense as required and sterilize.

### Malt extract agar

Suspend 18g agar powder in 1 litre of distilled water. Bring to the boil to dissolve completely. Add 15g malt extract per litre. Mix well. Dispense as required and sterilize.

### Mannitol yeast extract agar

Suspend 10 g agar in 1 litre of distilled water. Heat to dissolve. Add 0.5 g K<sub>2</sub>HPO<sub>4</sub> , 0.2g MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.2 g NaCl, 0.2 g CaCl<sub>2</sub>.6H<sub>2</sub>O, 10 g mannitol and 0.4 g yeast extract. Dispense as required and sterilize.

### Mannitol yeast extract broth

As above, without agar.

### Glucose nutrient broth

Make up nutrient broth as already directed and add 10 g per litre of glucose.

### Milk agar

Make up nutrient agar as above but using only 900 cm<sup>3</sup> of distilled water. Dissolve 20 g of dried skimmed milk in 100 cm<sup>3</sup> of distilled water. Sterilize separately. Transfer the milk to the agar aseptically after cooling to 45-50 °C. Dispense aseptically.

### Starch agar

Suspend 15 g of nutrient agar in 100 cm<sup>3</sup> distilled water. Bring to the boil to dissolve completely. Heat 40 g of soluble starch in 100 cm<sup>3</sup> of distilled water to form a suspension. Allow to cool and then mix with the nutrient agar solution. Dispense and sterilize.

## Gram Staining

**Principle:** Differential staining requires the use of at least three chemical reagents that are applied sequentially to a heat fixed smear. The first reagent is called the primary stain. Its function is to impart its colour to all cells. In order to establish a colour contrast the second reagent is the decolourising agent. Based on the chemical composition of cellular components the decolourising agent may or may not remove the primary stain from the entire cells or only from certain cell structures. The final reagent, the counter stain has a contrasting colour to that of the primary stain.

Following decolourisation, if the primary stain is not washed out, the counter stain can't be observed and the cells or their components will retain the colour of the primary stain. If the primary stain is removed, it accepts the contrasting colour of counter-stain. In this way cell type or their structures can be distinguished from each other on the basis of the stain that cells retained.

**Purposes:** To become familiar with-

- i) The chemical and theoretical basis for differential staining procedures.
- ii) The chemical basis of gram-stain.
- iii) Performance of the procedure for differentiating between the two principal groups of bacteria-
  - a. Gram positive (+ve) bacteria.
  - b. Gram negative (-ve) bacteria.

### Materials:

- **Culture:** Twenty-four hours old culture

• **Reagents:** Crystal violet- Primary stain

Gram Iodine- Mordant

Decolourising agent- 70% ethyl alcohol

Counter Stain- Safranin

• **Equipment:** Bunsen burner, inoculating loop, staining tray, glass slide, lens paper and microscope.

**Procedure:**

- A clean glass slide was obtained.
- The smear was prepared by placing a drop of culture by using sterile inoculating loop.
- The smear was allowed to air dry and then heat fixed by using Bunsen-burner.
- The smear was covered with several drops of crystal violet and incubated for 30 seconds to 1 minute.
- The slide was gently washed with drops of tap water.
- The smear was then flooded with the Gram's iodine and incubated for one minute.
- The slide was gently washed with drops of tap water.
- The slide was then decolourized with 90% ethyl alcohol.
- The slide was air dried followed by counter staining with safranin for 45 seconds.
- The slide was gently washed with drops of tap water.
- The slide was air dried and observed under oil immersion microscope (100x).

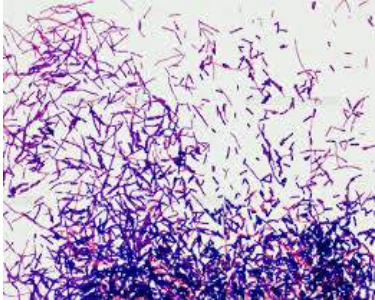
**Caution:** Don't over decolourise, add reagent drop by drop until alcohol runs almost clear, showing a blue colouration.

Gently wash it with tap water is when done, when the next step is to apply gently or counter stain gently with safranin for 45sec.

Bolt dry with bibulous paper and examine under oil immersion microscope.

**Observation and Result:**



<b>Draw a Representative field:</b>	
<b>Cell Morphology:</b>	
<b>Shape:</b> <b>Arrangement:</b> <b>Cell Colour:</b> <b>Gram Reaction:</b>	

**Comment:** Hence the supplied bacterial sample was Gram Positive rod shaped bacteria.  
Hence it is Gram Positive Bacteria.

### **Microbiological examination of milk (Methylene blue reduction test)**

The methylene blue reduction test is based on the fact that the color imparted to milk by the addition of a dye such as methylene blue will disappear more or less quickly. The removal of the oxygen from milk and the formation of reducing substances during bacterial metabolism causes the color to disappear. The agencies responsible for the oxygen consumption are the bacteria. Though certain species of bacteria have considerably more influence than others, it is generally assumed that the greater the number of bacteria in milk, the quicker will the oxygen be consumed, and in turn the sooner will the color disappear. Thus, the time of reduction is taken as a measure of the number of organisms in milk although actually it is likely that it is more truly a measure of the total metabolic reactions proceeding at the cell surface of the bacteria.

The methylene blue reduction test has lost much of its popularity because of its low correlation with other bacterial procedures. This is true particularly in those samples which show extensive multiplication of the psychrotropic species.

**Apparatus**—The necessary equipment consists of test tubes with rubber stoppers, a pipette or dipper graduated to deliver 10 ml of milk and a water bath for maintaining the samples at 35o to 37oC. The bath should contain a volume of water sufficient to heat the samples to 35o C within 10 minutes after the tubes enter the water and should have some means of protecting the samples from light during the incubation period. If a hot-air chamber is used, the samples should be heated to 35o C in a water bath since warm air would heat the milk too slowly.

The dry tablets contain methylene blue thiocyanate and may be obtained from any of the usual laboratory supply houses. They should be certified by the Commission on Standardization of Biological Stains. The solution is prepared by autoclaving or momentarily boiling 200 ml of distilled water in a light resistant (amber) stoppered flask and then adding one methylene blue tablet to the flask of hot water. The tablet should be completely dissolved before the solution is cooled. The solution may be stored in the stoppered, amber flask or an amber bottle in the dark. Fresh solution should be prepared weekly.

**Procedure in Testing**—The following procedures are recommended.

- Sterilize all glassware and rubber stoppers either in an autoclave or in boiling water. Be sure all glassware is chemically clean.
- Measure 1 ml of the methylene blue thiocyanate solution into a test tube.
- Add 10 ml of milk and stopper.
- Tubes may be placed in the water bath immediately or may be stored in the refrigerator at 0o to 4o C for a more convenient time of incubation. When ready to perform the test, the temperature of the samples should be brought to 35o C within 10 minutes.
- When temperature reaches 36o C, slowly invert tubes a few times to assure uniform creaming. Do not shake tubes. Record this time as the beginning of the incubation period. Cover to keep out light.

- Check samples for decolorization after 30 minutes of incubation. Make subsequent readings at hourly intervals thereafter.
- After each reading, remove decolorized tubes and then slowly make one complete inversion of remaining tubes.
- Record reduction time in whole hours between last inversion and decolorization. For example, if the sample were still blue after 5 hours but was decolorized (white) at the 2.5 hour reading, the methylene blue reduction time would be recorded as 2 hours. Decolorization is considered complete when four-fifths of the color has disappeared.

**Classification**—The suggested classification is listed.

Class 1. Excellent, not decolorized in 8 hours.

Class 2. Good, decolorized in less than 8 hours but not less than 6 hours.

Class 3. Fair, decolorized in less than 6 hours but not less than 2 hours.

Class 4. Poor, decolorized in less than 2 hours.

**Factors Affecting the Test**—Many factors affect the methylene blue reduction test and therefore the steps of operation should be uniform.

Since the oxygen content must be used up before the color disappears, any manipulation that increases the oxygen affects the test. Cold milk holds more oxygen than warm milk; pouring milk back and forth from one container to another increases the amount, and at milking time much oxygen may be absorbed.

The kind of organisms affect the rate of reduction. The coliforms appear to be the most rapidly reducing organisms, closely followed by *Streptococcus lactis*, some of the faecal *Streptococci*, and certain micrococci. Thermophilic and psychrotrophic bacteria reduce methylene blue very slowly if at all. A large number of leucocytes affect the reduction time materially.

Light hastens reduction and therefore the tests should be kept covered. The concentration of the dye should be uniform as an increased concentration lengthens the time of reduction. Increasing the incubation temperature augments the activity of the bacteria and therefore shortens the reduction time.

The creaming of the test samples causes a number of organisms to be removed from the body of the milk and brought to the surface with the rising fat. This factor causes variations in the reduction time, since the bacteria are not evenly distributed. The accuracy of the test is increased, reduction time shortened and decolorization more uniform if the samples are periodically inverted during incubation.

## C10P: Food processing and Preservation (Practical)

### Preparation of selected common recipes

#### Experimental milk cookery

#### Milk Shake

#### Ingredient

**4 scoops** vanilla or chocolate ice cream (about 2 cups)

**1/2 cup** milk cold

**1/4 cup** HERSHEY'S Syrup

**4pcs** Ice cubes

Cashew nut



#### Procedure

1. Place ice cream, milk and syrup in blender container.
2. Poured the whole mixture in to a glass.
3. Now garnish with cashew nut.
4. Now it is ready to serve.

#### Nutritional importance

Chocolate milk provides important nutrients — such as calcium, protein, and vitamin D — which may benefit health. However, it's high in calories and added sugar, which can contribute to weight gain and may increase your risk of certain chronic diseases.

## Experimental cookery on eggs



Fresh eggs work best for poaching. Their whites hold together better than older eggs. Some people put a little vinegar in the poaching water—the vinegar helps the proteins in the egg whites coagulate. But the vinegar does affect the taste.

### Ingredients:

1 or more fresh eggs

### Procedure:

- Transfer eggs to a bowl of very hot tap water for 2 to 3 minutes. (Don't use boiling water or eggs might overcook). Top off the bowl with more hot water if needed.
- Gently scoop the eggs out of the water with a slotted spoon, blot them dry on a paper towel, and serve immediately.

### Nutritional importance:

- Eggs are high in a range of vitamins and minerals
- Proteins are the building blocks of life and a single egg contains about 6.3 grams of high-quality protein. The main functions of proteins in the body are to build, strengthen and repair or replace things, such as tissue. Eggs provide us with very high-quality protein that contains all nine essential amino acids in the right amounts needed by the body for optimum growth and maintenance.
- Eggs help increase levels of high-density lipoprotein (HDL), or “good” cholesterol as it’s commonly known. Higher levels of HDL can help reduce the risk of heart disease.
- Eggs are a good source of Omega-3s
- Eggs Are Filling and Help with Weight Loss
- Eggs Contain Antioxidants That Are Beneficial for the Eyes

## Recipes with Vegetables

### Mixed Veg Recipe



#### Procedure:

- Heat 3 tablespoons oil in a kadai (wok) or skillet or a pan. Then add 1 teaspoon cumin seeds and let them crackle.
- When the cumin seeds finish crackling, add ½ cup chopped onions.
- Mix well and begin to sauté onions on a low to medium flame.
- Sauté onions till they turn light golden.
- Add 1 teaspoon ginger-garlic paste.
- Mix well and sauté for until the aroma of raw ginger and garlic goes away, about 10 to 12 seconds
- Then add 1 tightly packed cup of finely chopped tomatoes.
- Mix well and begin to sauté tomatoes on a low heat. Stir often. If the ingredients starts sticking to the pan, add in some splashes of water. Mix and deglaze the pan removing any stuck particles or bits. Continue to sauté further.
- Sauté until the tomatoes turn pulpy, glossy and you see oil releasing from the sides.
- Add ½ teaspoon turmeric powder, ½ teaspoon Kashmiri chili powder or paprika, ½ teaspoon garam masala powder and 2 teaspoons coriander powder.
- Mix the ground spice powders very well with the onion-tomato mixture and sauté for some seconds or up to a minute.
- Add 2 cups of mixed chopped veggies and 1 green chili (about ½ teaspoon chopped chilies) to the onion tomato masala.
- Mix the veggies very well with the onion-tomato masala and sauté for a minute.

- Add 1.5 cups water. (For a thinner gravy add 2 cups water.)
- Add salt to taste.
- Mix well. Cover the kadai, skillet or pan with a lid and cook the veggies on a low to medium heat.
- Check periodically while the veggies are cooking.
- When the veggies are half cooked (still too firm to poke with a fork), add 2 tablespoons cream (light or heavy) or 3 tablespoons milk in the vegetable curry. Swap the cream with malai – the layer of cream that floats on top of boiled milk that has been cooled.
- Mix well. Cover the kadai, pan or skillet again and simmer till the veggies are cooked well and fork tender.
- Then add 3 tablespoons chopped coriander leaves (cilantro). Mix again.
- Serve mix vegetable curry steaming hot or warm.

### **Health Benefits**

Vegetables are full of essential vitamins, minerals, and antioxidants that provide many important health benefits to your body. For instance, carrots are known for being very high in vitamin A, which plays an important role in eye health, as you grow older.

Vegetables also offer many other health benefits like:

#### **Improved Digestive Health**

Vegetables are a good source of dietary fiber, a type of carbohydrate that helps pass food through your digestive system. Studies show that fiber may also improve vitamin and mineral absorption in the body, which could potentially raise your daily energy levels.

#### **Lower Blood Pressure**

Many green leafy vegetables like kale, spinach, and chard contain potassium. Potassium helps your kidneys filter sodium out of your body more efficiently, which can reduce your blood pressure.

#### **Lower Risk of Heart Disease**

Green leafy vegetables also contain vitamin K, which is believed to prevent calcium from building up in your arteries. This can lower your risk of arterial damage and help prevent many heart health complications in the future.

#### **Diabetes Control**

Vegetables are particularly high in fiber, which is needed for optimal digestion. They have a low glycemic index, so your blood sugar won't rise quickly after a meal. The American Diabetes Association recommends at least 3 to 5 servings per day of non-starchy vegetables like broccoli, carrots, or cauliflower.

### **Nutrition**

Vegetables are a rich source of f olate, a B vitamin that helps your body make new red blood cells. Folate is especially important for children’s health and may also reduce the risk of cancer and depression.

## Preparation of selected common recipes

### Mixed Fruit Salad



#### INGREDIENTS:

##### FOR THE DRESSING

1/4 c. honey

1/4 c. freshly squeezed orange juice

Zest of 1 lemon.

##### FOR THE SALAD

1 lb. strawberries, hulled and quartered

6 oz. blueberries

6 oz. raspberries

3 kiwis, peeled and sliced

1 orange, peeled and wedges cut in half

2 apples, peeled and chopped

1 mango, peeled and chopped

2 c. grapes



**DIRECTIONS**

In a small bowl whisk together honey, orange juice, and lemon zest. Add fruit to a large bowl and pour over dressing, tossing gently to combine. Chill until ready to serve.

**NUTRITIONAL IMPORTANCE:**

- One of the healthiest things that you can eat is a fruit salad. With the right kind and number of fruits, a fruit salad can be very good for you. It is high in fiber, vitamins, and anti-oxidants and has a low energy density level. There are a number of healthy food options to choose from, and fruits are among the best.
- The most common fruit that everyone likes to put in a fruit salad is a banana. If you put just one small chopped banana in your salad then it will improve the health of your bones, control your blood pressure and also prevent anemia.
- Everyone knows that apples are rich in antioxidants, it also consists of dietary fibers and flavonoids. The minerals in apples can reduce the risk of diseases like diabetes, hypertension and heart diseases.
- Oranges are packed with vitamin B1, vitamin C, calcium, copper and many others things. Vitamin C helps to lower blood pressure and it also reduces the risk of heart diseases.
- You can choose to add other fruits like pomegranates, kiwis, berries, and papayas. Berries like blueberries and strawberries are rich in Vitamin K and C, they are very good for your health and add a tangy flavor to the fruit salad. If you have high cholesterol levels in your blood then pomegranate will help to reduce those levels.

## Estimation of Sodium, Potassium, Calcium and Iron in different food staffs

### Determination of sodium and potassium by flame photometry

#### Introduction:

Potassium (K) is the major cation found inside of cells. The proper level of potassium is essential for normal cell function. An abnormal increase of potassium (hyperkalemia) or decrease of potassium (hypokalemia) can profoundly affect the nervous system and heart, and when extreme, can be fatal. The normal blood potassium level is 3.5 - 5.0 millimoles/liter (mmol/l). Sodium (Na) is the major extracellular cation and it plays a role in body fluid distribution. Concentration of sodium ions inside the plasma (extracellular) is 130-145 mmol/l. Higher and lower concentrations are referred to as hypernatremia and hyponatremia, respectively. When a solution containing cations of sodium and potassium is sprayed into flame, the solvent evaporates and ions are converted into atomic state. In the heat of the flame (temperature about 1800°C), small fraction of the atoms is excited. Relaxation of the excited atoms to the lower energy level is accompanied by emission of light (photons) with characteristic wavelength (Na: 589 nm, K: 766 nm). Intensity of the emitted light depends on the concentration of particular atoms in flame.

#### Instruments, reagents and glassware:

1. Flame photometer FLAPHO or Eppendorf.
2. Stock solutions of Na<sup>+</sup> and K<sup>+</sup>, c = 1 mg/ml.
3. 6 numbered 100 ml volumetric flasks.
4. Glass pipettes: 1, 2, 10 ml.

#### Analytical procedure:

##### Preparation of standard solutions

Standard solutions are prepared by dilution of stock solutions. Use different glass pipettes and numbered 100 ml volumetric flasks and prepare the solutions according to the following table:

Flask Number	1	2	3	4	5	6
Vol. of Pipette to use	1	1	2	10	10	10
Vol. of Na stock solution to pipette	0.5	1	2	4	6	8
Vol. of K stock solution to pipette	0.5	1	2	4	6	8
Conc. of solution obtained (µg/ml)	5	10	20	40	60	80

#### Sample preparation:

Test solution is given in 100 ml flask. Fill it up to the mark with distilled water and mix.

**Measurement procedure:**

**Attention:** Flame photometer uses flammable gases which can cause explosions if used improperly!

Switch the instrument on and off under supervision!

**Note:** Check the flame during work if it goes out, close the gas valve immediately!

**With Eppendorf flame photometer:**

1. Let the instrument warm up for 5-10 minutes.
2. Feed distilled water to the instrument.
3. Select the element Na by turning the selector "Elementwahl".
4. Turn the outer knob "Messbereich" into position "100". Pull the "Kompensaton I" knob slightly out and adjust readout to 0. Press the "Kompensation I" knob back. Readjust 0 reading with "Kompensation II" if necessary.
5. Aspirate the most concentrated standard solution (solution number 6) and adjust readout to approximately 350 (on uppermost scale) using inner "Messbereich" knob.
6. Aspirate distilled water – the instrument should read 0.
7. Aspirate standard solutions no. 1, 2, 3, test solution, and then standards 4, 5, 6. Record the results.
8. Repeat 3-7 for solutions of potassium.
9. Aspirate distilled water for at least 5 minutes to clean the system.

**With FLAPHO flame photometer:**

(FLAPHO is a dual channel instrument, which measures concentrations of Na and K simultaneously.)

Channel 1 (upper indicator) shows Na, and channel 2 (lower) K.

1. Let the instrument warm up for 5-10 minutes.
2. Feed distilled water to the instrument.
3. Using knobs adjust the indicators to 0 reading.
4. Aspirate the most concentrated standard solution (solution number 6) and adjust readout to approximately 90 (on uppermost scale) using the big knobs.
5. Aspirate distilled water – the instrument should read 0.
6. Aspirate standard solutions no. 1, 2, 3, test solution, and then standards 4, 5, 6. Record the results.
7. Aspirate distilled water for at least 5 minutes to clean the system.

**Calculation of the results:**

1. Draw calibration curves for sodium and potassium on a sheet of millimeter-paper. Use concentrations as abscissa and instrument readouts as ordinate values. Mind the units!
2. Find concentration of sodium and potassium ions in test solution from calibration curves.

**Determination of Iron****Preparation of stock solution**

Three stock solutions were made ready before the experiment and were stored in five 500 mL neatly labeled standard flasks. Firstly, the 0.001 M FeCl<sub>3</sub> stock solution was prepared by adding approximately 0.162 g of FeCl<sub>3</sub> in 500 mL distilled water followed by the addition of 5 mL concentrated HCl. The contents were diluted to 1 L and were mixed well before being transferred to the standard flask. This solution was only used for calibration purposes and was discarded after that. The 1.5 M KSCN solution was prepared by adding approximately 36.375 g of KSCN in 500 mL distilled water. The contents were mixed well before being transferred to the standard flask. This solution was the basis of the colorimetry involved in the analysis and was used till the end of the experiment. The 2 M HCl solution was prepared by adding 170mL of concentrated HCl to 500 mL distilled water and diluting the solution to 1 L with distilled water. The contents were mixed well before being transferred to the standard flask. This solution was used for dilution purposes and served as the blank in the spectrophotometric analysis.

**Apparatus and principle**

Thiocyanate spectrophotometry was carried out using a Lambda 25 UV/VIS Spectrophotometer of the PerkinElmer make. The spectrophotometer worked on the principle of the Beer-Lambert law and was operated in the visible range of the spectrum. The colorimetric reagent used for the analysis was potassium thiocyanate, for which the  $\lambda_{\max}$  value obtained was 480 nm. The basic reaction when thiocyanate reacts with iron(III) is as follows:  
$$\text{Fe}^{3+}(\text{aq}) + 6\text{SCN}^{-}(\text{aq}) \leftrightarrow [\text{Fe}(\text{SCN})_6]^{3-}(\text{aq})$$

The thiocyanate complex, [Fe(SCN)<sub>6</sub>]<sup>3-</sup> had a deep red colour and its intensity was directly related to the concentration of solution. The spectrophotometric analysis was used for its simplicity, convenience and availability in the institute.

**Calibration curve:**

Seven standard solutions were prepared each having a molarity of 0.5x10<sup>-4</sup> M, 1x10<sup>-4</sup> M, 1.5x10<sup>-4</sup> M, 2x10<sup>-4</sup> M, 2.5x10<sup>-4</sup> M, 3x10<sup>-4</sup> M and 4x10<sup>-4</sup> M. The first solution was prepared by diluting 0.5 mL of 0.001 M FeCl<sub>3</sub> solution with 9.5 mL of 2 M HCl solution. Similarly, the corresponding solutions are made by diluting 1 mL, 1.5 mL, 2 mL, 2.5 mL, 3 mL and 4 mL of 0.001 M FeCl<sub>3</sub> solution to 10 mL by 2 M HCl solution. After this, 5 mL of 1.5 M KSCN was added to each of the solution and mixed by swirling the test tubes. This step diluted the 10 mL solution to 15 mL causing the concentration to decrease by 2/3rd of its original molarity value. Thus, the values read by the spectrophotometer were for two-thirds of the actual concentration. After adding KSCN, the absorbance was measured immediately because absorbance value can be affected as the colour of the solution fades within 15-20 minutes. 2M HCl was used as the

blank. Using these solutions, the concentration vs absorbance curve was plotted as shown in Fig. 1, using the concentrations as enlisted in Table 2.

Original concentration (Mx10 <sup>4</sup> )	Concentration being measured (Mx10 <sup>4</sup> )	Absorbance
0.5	0.33	0.1849
1	0.67	0.4381
1.5	1	0.5478
2	1.3	0.7779
2.5	1.7	1.1221
3	2	1.2484
4	2.68	1.6847

**Table 2:** Concentration and absorbance values

Put the calibration curve using the standard solution

#### **Ashing of the samples:**

1-15 g of the edible portion of the food samples was weighed. They were finely chopped for the purpose of ashing. The weighed samples were finely chopped and heated in a stainless steel vessel over a hot induction plate at 200-240°C. This step was carried out in a well ventilated room. The heating time varied depending on the amount of sample and the rate at which the sample burned to ash. The samples were heated till a grayish ash was observed and then they were powdered using a mortar and pestle. After the samples were cooled, they were transferred to a small beaker of 100 mL capacity and the iron (III) in the ash was dissolved in 10 mL-30 mL of 2 M HCl. The ash solution was stirred using a glass stirring rod for about 5 minutes and then filtered.

#### **Analysis of the samples:**

5 mL of the filtered sample was transferred to a test-tube and then 5 mL of 1.5 M KSCN was added. The mixture was stirred by swirling the test tube. The absorbance was measured without delay as the colour of the solution faded within 15-20 minutes. The solution concentration was halved by adding 5 mL of KSCN, thus, the concentration values were multiplied by 2 during the calculations. The 2 M HCl solution served as the blank. The absorbance values were measured for all samples.

#### **Result and discussion:**

The absorbance values were determined by the spectrophotometer and the concentration was found out by interpolation or extrapolation using the calibration graph prepared earlier. After the calculations, the iron content determined in the different food samples was tabulated in an increasing order of amount of iron present as shown in below table:

## Determination of Calcium

### Principle:

In acid base titration the end point is marked by sudden change in pH and it can be detected by an indication. Here the determination of calcium in milk is based on a complexometric titration of calcium with an aqueous solution of EDTA bind to free calcium ion in the solution and the solution become calcium free. In presence of calcium in a particular pH, the calcon shows pink or purple colour. In absence of calcium calcon shows blue colour.

### Requirements:

- i. Alcoholic calcon solution
- ii. NaOH – 2 Molar (2.05 g of NaOH is added to 250ml of distilled water)
- iii. EDTA – 0.01 Molar (Dissolve 3.723 g of EDTA in 1 litre of distilled water in a volumetric flask.)
- iv. Burette with stand
- v. Conical flask
- vi. 5 ml pipette
- vii. 25ml measuring cylinder
- viii. Funnel
- ix. Milk sample

### Procedure:

- i. A 50ml conical flask was weighed and 1 ml supplied milk was taken. Then the conical is again weighed to get the weight of the supplied milk.
- ii. 25ml of distilled water and 4 ml of 2M NaOH was added to it.
- iii. Then 2-3 drops of calcon solution were added and mixed well. The solution will become purple colour.
- iv. Titrate the solution against 0.01M EDTA solution in a burette.
- v. When purple colour is turned into blue colour that time will be the end point of the titration and the volume of EDTA used was noted in the burette.

### Observation:

No of observation	Burette reading (ml)		Difference	Average
	Initial	Final		
1	15.5	19.5	4	4.15
2	19.5	23.8	4.3	

**Result:**

Volume of EDTA required for titration is- 4.15 ml= 0.0041 litre

Weight of milk- 0.98g

So,

$$\text{Calcium (mg\%)} = \frac{\text{Molarity of EDTA} \times \text{volume of EDTA (lit)} \times \text{Molecular weight of calcium}}{\text{Weight of milk (g)}} \times 100$$

Unit= mg/100g

**Interpretation:****Estimation of vitamin C content of food by biochemical method**

Vitamin C or ascorbic acid standard in metaphosphoric acid is titrated against 2,6-dichlorophenol indophenol salt. This salt is blue in colour in alkaline medium and became pink in acidic medium. Pink colour indicates the complete oxidation of ascorbic acid. The dye in this titration is coloured in the oxidised form and colourless in reduced form. Ascorbic acid is a strong reducing agent because of which it reduces the dye and converted to dehydro ascorbic acid (Oxidised form).

**Requirements:**

- i. 2,6, dichlorophenol indophenol was taken and added with 150 ml of distilled water. Then 42 mg of NaHCO<sub>3</sub> was added to it. Then 50 ml of distilled water was added with it or volume upto 200 ml with distilled water.
- ii. 6% meta phosphoric acid
- iii. 50mg of ascorbic acid was added with 100 ml of 6% meta phosphoric acid and the concentration is 50mg%.
- iv. 50ml conical flask
- v. 10 ml pipette, burette and beaker
- vi. Measuring cylinder
- vii. Lemon juice

**Procedure:**

- i. 1 ml of lemon juice was taken in a 50ml conical flask. Similarly 1ml of standard Vitamin C was taken in a conical flask
- ii. 9 ml of 6% metaphosphoric acid was added to each conical flask
- iii. Both standard vitamin C and sample was titrated against the 2, 6, dichlorophenol indophenol in a burette
- iv. Volume of the dye was recorded when a light pink colour persists for 30 seconds.

v. The total procedure of titration was repeated for 3 times.

**Observation of the sample:**

No of observation	Burette reading (ml)		Difference(ml)	Average (ml)
	Initial	Final		
1	0	3.3	3.3	3.15
2	3.3	6.3	3	

**Observation of the standard vitamin C:**

No of observation	Burette reading (ml)		Difference(ml)	Average (ml)
	Initial	Final		
1	0	2.5	1.5	1.5
2	2.5	5	1.5	

**Result:**

Titration value of standard vitamin C is 2.5 ml.

Titration value of lemon juice sample is 3.1ml

1 ml standard vitamin C solution contain 0.5mg vitamin C

So, 2.5 ml dye reduces 0.5 mg of vitamin C

1ml dye reduces  $0.5/2.5$  mg vitamin C

3.15 ml dye reduces  $0.5/2.5 \times 3.15 = 0.63$  mg

As 1 ml of lemon juice was taken so 1 ml lemon juice contain 0.63 mg vitamin C, So, 100ml lemon juice contain  $0.63 \times 100 = 63.0$  mg

**Interpretation:**