III B.SC BIOCHEMISTRY

CLINICAL BIOCHEMISTRY (16SCCBC9)

SEMESTER: VI

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Expressing concentrations and solutions

There are several different ways to quantitatively describe the concentration of a solution. Concentration is a very common concept used in chemistry and related fields. It is the measure of how much of a given substance there is mixed with another substance

Mass/Weight Percentage or Percentage by Mass/Weight

It is the amount of solute in grams present in 100 grams of the solution. Therefore, the formula will be:

$$Mass\ Percentage = \frac{Mass\ of\ Solute}{Mass\ of\ Solution} \times 100$$

$$= \frac{Mass\ of\ Solute}{Mass\ of\ Solute + Mass\ of\ Solvent} \times 100$$

$$= \frac{Mass\ of\ Solute}{Volume\ of\ Solution + Density\ of\ Solution} \times 100$$

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The ratio mass of solute to the mass of the solvent is the mass fraction. Thus, the mass percentage of solute = Mass fraction × 100. 10% solution of sugar by mass means that 10 grams of sugar is present in 100 grams of the solution, i.e., we have dissolved 10 grams of sugar in 90 grams of water

2) Volume Percentage

It is the volume of solute in mL present in 100 mL solution. The formula will be:

$$Volume\ Percentage = \frac{Volume\ of\ Solute}{Volume\ of\ Solution} \times 100$$

10% solution of HCl by volume means that 10 mL of liquid HCl is present in 100 mL of the solution.

3) Mass by Volume Percentage

It is the mass of solute present in 100 mL of solution. We can calculate the mass of the solute using the volume percentage. The formula would be:

$$Mass\ by\ Volume\ Percentage = \frac{Mass\ of\ Solute}{Volume\ of\ Solution} \times 100$$

A 10% mass by volume solution means that 10 gm solute is present in 100 mL of solution.

4) Molarity

The molarity of a solution gives the number of gram molecules of the solute present in one litre of the solution.

$$Molarity(M) = \frac{Number\ of\ moles\ of\ solute}{Volume\ of\ Solution\ in\ L}$$

For example, 1 mol L^{-1} solution of KCl means that 1 mol of KCl is dissolved in 1 L of water. Unit of molarity: mol L^{-1}

5) Molality

Molality of a solution is the number of moles of solute dissolved in 1 Kg of the solvent.

$$Molality\ (m) = \frac{Number\ of\ moles\ of\ solute}{Mass\ of\ Solvent\ in\ kg}$$

Thus, if one gram molecule of a solute is present in 1 kg of the solvent, the concentration of solutions is said to be one molal. The unit of molarity is mol

kg^{-1}. Molality is the most convenient method to express the concentration of solutions because it involves the mass of liquids rather than their volumes. It is also independent of the variation in temperature.

6) Normality

The normality of a solution gives the number of gram equivalents of the solute present in one litre of the solution.

$$Normality\ (N) = \frac{Number\ of\ gram\ equivalents\ of\ solute}{Volume\ of\ Solution\ in\ L}$$

Thus, if one gram equivalent of a solute is present in one litre of the solution, the concentration of solutions is said to be 1 normal.

- 1N = Normal = One gram equivalent of the solute per litre of solution = Normality is 1
- N/2 = Seminormal = 0.5 g equivalent of the solute per litre of solution = Normality is 0.5
- N/10 = Decinormal = 0.1 g equivalent of the solute per litre of solution = Normality is 0.1
- N/100 = Centinormal = 0.01 g equivalent of the solute per litre of solution = Normality is 0.01
- N/1000 = Millinormal = 0.001 g equivalent of the solute per litre of solution = Normality is 0.001

7) Mole Fraction

The mole fraction of any component in a solution is the ratio of the number of moles of that component to the total number of moles of all components. The total mole fraction of all the components of any solution is 1. For a binary solution of A and B

Mole Fraction of A (X_A) = $\frac{n_A}{n_A + n_B}$

Mole Fraction of B (X_B) = $\frac{n_B}{n_A + n_B}$

And, $X_A + X_B = 1$

8) Parts per million (ppm)

When a solute is present in trace quantities, it is convenient to express the concentration of solutions in parts per million (ppm). The formula is as follows:

$$ppm = \frac{Number\ of\ parts\ of\ the\ component}{Total\ number\ of\ parts\ of\ the\ components\ in\ the\ solution} \times 10^6$$

In case of mass, we may express it as: (Mass of solute/Mass of solution)× 106

In case of volume, we may express it as: (Volume of solute/Volume of solution) × 106

So, we can express the concentration of solutions in parts per million as mass to mass, volume to volume and mass to volume form. Atmospheric pollution in cities is also expressed in ppm by volume. It refers to the volume of the pollutant in 10^6 units of volume. 10 ppm of SO_2 in the air means 10 mL of SO_2 is present in 10^6 mL of air.

9) Formality

It is the number of mass in grams present per litre of solution. In case, formula mass is equal to molecular mass, formality is equal to molarity. Like molarity and normality, the formality is also dependent on temperature. It is used for ionic compounds in which there is no existence of a molecule. A mole of ionic compounds is called formal and molarity as the formality.

$$Formality = \frac{Weight\ of\ solute(gm)}{Formula\ weight\ of\ solute} \times \frac{1}{Volume\ of\ solutions(L)}$$

$$F = \frac{w}{f} \times \frac{1}{V\ (L)}.(i)$$

$$F = \frac{w}{f} \times \frac{100}{V\ (mL)}.(ii)$$

$$F = n_f \times \frac{1}{V\ (L)}.(iii)$$

Where,

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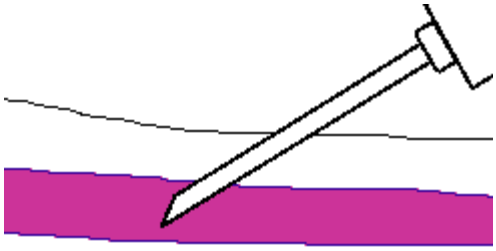
- w = weight of solute,
- f = formula weight of solute
- V= volume of solution
- n_f = no. of gram formula weight

Blood Specimen Collection and Processing

The first step in acquiring a quality lab test result for any patient is the specimen collection procedure. The venipuncture procedure is complex, requiring both knowledge and skill to perform. Several essential steps are required for every successful collection procedure:

Venipuncture Procedure:

1. A phlebotomist must have a professional, courteous, and understanding manner in all contact with all patients.
2. The first step to the collection is to positively identify the patient by two forms of identification; ask the patient to state and spell his/her name and give you his/her birth date. Check these against the requisition (paper or electronic).
3. Check the requisition form for requested tests, other patient information and any special draw requirements. Gather the tubes and supplies that you will need for the draw.
4. Position the patient in a chair, or sitting or lying on a bed.
5. Wash your hands.
6. Select a suitable site for venipuncture, by placing the tourniquet 3 to 4 inches above the selected puncture site on the patient.
7. Do not put the tourniquet on too tightly or leave it on the patient longer than 1 minute.
8. Next, put on non-latex gloves, and palpate for a vein.
9. When a vein is selected, cleanse the area in a circular motion, beginning at the site and working outward. Allow the area to air dry. After the area is cleansed, it should not be touched or palpated again.
10. Grasp the patient's arm firmly using your thumb to draw the skin taut and anchor the vein. Swiftly insert the needle through the skin into the lumen of the vein. The needle should form a 15-30 degree angle with the arm surface. Avoid excess probing.



 When the last tube is filling, remove the tourniquet.
 Remove the needle from the patient's arm using a swift backward motion.
 Place gauze immediately on the puncture site. Apply and hold adequate pressure to avoid formation of a hematoma. After holding pressure for 1-2 minutes, tape a fresh piece of gauze or Band-Aid to the puncture site.

 Dispose of contaminated materials/supplies in designated containers.

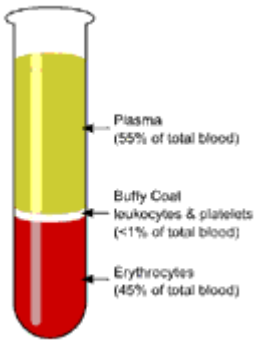
Blood Sample Handling and Processing:

Pre-centrifugation Handling - The first critical step in the lab testing process, after obtaining the sample, is the preparation of the blood samples. Specimen integrity can be maintained by following some basic handling processes:

- Fill tubes to the stated draw volume to ensure the proper blood-to-additive ratio. Allow the tubes to fill until the vacuum is exhausted and blood flow ceases.
- Vacutainer tubes should be stored at 4-25°C (39-77°F).
- Tubes should not be used beyond the designated expiration date.
- Mix all gel barrier and additive tubes by gentle inversion 5 to10 times immediately after the draw. This assists in the clotting process. This also assures homogenous mixing of the additives with the blood in all types of additive tubes.
- Serum separator tubes should clot for a full 30 minutes in a vertical position prior to centrifugation. Short clotting times can result in fibrin formation, which may interfere with complete gel barrier formation.

Blood Sample Centrifugation – It is recommended that serum be physically separated from contact with cells as soon as possible, with a maximum time limit of 2 hours from the time of collection.

- Complete gel barrier formation (gel barrier tubes) is time, temperature and G-force dependent. The uniformity of the barrier is time dependent; an incomplete barrier could result from shortened centrifugation times.



In general, for a horizontal, swing-bucket centrifuge, the recommended spin time is 10 minutes. For a fixed-angle centrifuge, the recommended spin time is 15 minutes.

Types of urine specimens:

Over the course of a 24-hour period, the composition and concentration of urine changes continuously. For this reason, various types of specimens may be collected, including:

- First morning specimen
- Single random specimen
- Timed short-term specimens
- Timed long term specimens: 12 or 24 hours
- Double voided specimens (test for sugar and acetone)
- Clean-catch (midstream) specimen for urine culture and cytological analyses

The first voided morning specimen is particularly valuable because it is more concentrated and abnormalities are easier to detect. An early morning specimen is also relatively free of dietary influences and changes due to physical activity. In collecting any urine specimen, it is always important for the nurse to observe

specific agency protocols, to check with the laboratory regarding the need for refrigeration or preservation of specimens, and to follow universal precautions. Single random specimens may be taken at any time of the day or night. Timed specimens range from short-term 2-hour collections to 24-hour collections.

A 24-hour urine specimen is an extremely important diagnostic test because it reveals how the kidney adjusts to changing physiologic needs over a long period. Substances excreted by the kidney are not excreted at the same rate or in the same amounts during different periods of day and night; therefore, a random urine specimen does not accurately represent the processes taking place over a 24-hour period. However, a 24-hour urine specimen is useful only when all the patient's urine is collected for 24 hours. Even if just one sample is discarded, the results will be inaccurate. The nurse must ensure that the patient and all assistive personnel understand the importance of saving all the urine. To begin the 24-hour collection, the person voids and discards the urine already in the bladder. All urine starting with the next voiding is collected for the next 24 hours and put into a large collection bottle. To prevent breakdown of urinary components, the collection has a preservative added to it or is refrigerated.

Collection of specimens:

The laboratory needs at least 10 ml of urine for a routine UA. The perineal area in women or the end of the penis in men should be cleaned before the urine is collected. For a female, collecting midstream urine lessens the contamination from vaginal secretions or menstrual flow. Wiping the genitalia with a sterile wipe may stimulate the voiding reflex in infants. Various collection bags can be also be attached to the genitalia of infants or small children. A cotton ball in a diaper can be used for quick collection of urine for dipstick testing. If a culture and sensitivity are to be completed in addition to the routine UA, the urine specimen must be placed in a sterile container. Urine specimens need to be examined within 2 hours. Urine that is left to standing too long becomes alkaline because bacteria begins to split the urea contained in urine into ammonia. Visualization of urine and other tests are inaccurate if the pH of the urine specimen has become highly alkaline. A urine specimen should be refrigerated if it cannot be sent to the laboratory within 2 hours.

Feces

Collection

- Ideally, feces should be processed as soon after passage from the animal as possible.
- Feces should be collected in airtight containers to prevent desiccation.
- If the processing of a fecal specimen must be delayed, it may be:
    - refrigerated (but not frozen) for several days (not recommended for samples with live larvae that you intend to examine using the Baermann technique).
    - fixed, e.g., 10% formalin (5% formalin-saline is better for protozoal cysts). Add fixative to feces at a ratio 3:1 (v:v) and mix well. (Not for Baermann technique.)
- If an animal has been treated with anti-diarrhea preparations containing bismuth or kaolin, mineral oil, oral contrast material (barium) for radiology (all of these materials float) or antibiotics, then parasites may be difficult or impossible to find. Therefore, repeat the fecal exam 5-10 days after treatment withdrawal.

Processing

- First, examine the feces for blood and other clinical signs, then examine the inside of container for tapeworm segments (which are motile and may move away from the fecal mass).
- Many techniques have been devised to increase the likelihood that parasites will be detected in a particular sample of feces. The merits and limitations of representative fecal processing techniques are summarized in the table on the next page. Step-by-step directions for performing the various methods are on the following pages.

Transportation of Samples

Transport of specimens takes place at following four temperatures:

- Frozen
- Refrigerated (2-8°C)
- 18-22°C
- Room Temperature

Use transport boxes supplied by LPL. All gel packs should be frozen for at least 24 hours < 0ºC prior to use. Use separate transport boxes for each of the temperature ranges e.g. all frozen samples in one box.

Frozen

- Empty transport (thermocol) box
- Place a layer of perforated sponge at bottom
- Place a pre-frozen gel pack over the perforated sponge
- Place specimens sealed in 'Zip lock bag' over the gel pack
- Place another pre-frozen gel pack over the samples
- Cover with second layer of perforated sponge
- Place un-perforated sponge and close the lid
- Seal the cardboard box and transport to laboratory immediately
- Indicate 'Frozen Samples' on cardboard box.

Refrigerated (2-8°C)

- Empty transport box
- Place a pre-frozen gel pack at bottom
- Place a layer of per-forated sponge over the pre-frozen gel pack
- Place specimens sealed in 'Zip lock bag' over the perforated sponge
- Cover specimens with second layer of perforated sponge
- Place another pre-frozen gel pack over the samples
- Place un-perforated sponge and close thermacol lid.
- Seal the cardboard box and transport to laboratory immediately
- Indicate 'Refrigerated Samples' on cardboard box

18-22°C

- Empty transport box
- Place a pre-frozen gel pack at bottom
- Place a layer of perforated sponge over the pre-frozen gel pack
- Place a second layer of perforated sponge
- Place another pre-frozen gel pack over the perforated sponge
- Place un-perforated sponge and specimens sealed in 'Zip lock bag' over the un-perforated sponge
- Seal the cardboard box and transport to laboratory immediately
- Indicate '18-22°C Samples' on cardboard box

Room Temperature

- Empty transport box
- Place a layer of perforated sponge at bottom
- Place specimens sealed in 'Zip lock bag' over the perforated sponge
- Cover specimens with second layer of perforated sponge
- Place un-perforated sponge and close the lid
- Seal the cardboard box and transport to laboratory immediately
- Indicate 'Room Temperature Samples' on cardboard box