Exp. WASTE DISPOSAL IN BIOMEDICAL LABROTARY Date

 www.FirstRanker.com

Page No.:

Aim :- Waste disposal in Biochemical Laboratory.

Biomedical Waste :- Discarded biological material from teaching clinical and research laboratories and operation theaters.

(i) Animal waste :- Tissue, body parts, fluid like blood amputated limbs etc.

(ii) Biological Labwaste :- cutters live or attennated vaccines, human or animal cell structure.

(iii) Human Anatomical waste :- Any part of human body like extracted tooth, nails, bones, amputated limbs organs etc.

(iv) Sharpes :- Needles, syringes with needles scalpal, blades, contaminated broken glass from pipettes, test tube etc.

Laboratory Incharge :-

It should be insured that all lab staff technicians have received proper training for biomedical waste handling and disposal and also to ensure that lab is properly equipped with containers and bags in proper place.

Student and Lab Technicians :-

It is mandatory to wear apron as well as wear gloves while disposing off biomedical waste.

Experiment..... Date.....
www.FirstRanker.com
..... Page No.:

Log book of lab waste disposal :-

A log should be maintained regarding the waste pick up in our hospital sembronky maintenance of records of the amount of waste generated.

General Guidelines :

(A) Segregation :- Biomedical waste must be stored in a secure enviroment. It must not be mixed with chemicals, radioactive or other laboratory trash. Various lab waste must be segregated, solid, liquid.

(B) Containment and labelling :- Different colored bags to contain different categories of biomedical waste.

eg. Sharps in Shapps leis. All containers must be covered and contain the biohazard symbol.

(C) Packaging of waste :- The following are some guidelines to remember while Packaging waste.

(i) Double bag if necessary to perforation.
(ii) Absorbent material added when carrying large amount of liquids.
(iii) To be ensured that bags are well sealed.
(iv) Bags and containes should not be overfilled.

(D) Treatment :- All biomedical waste should be decontaminated prior to disposal.

Experiment Date
Page No.:

Waste decontamination results in the destruction or removal of micro-organism to a lower level, such that their is no danger of infection to others main choices are autoclaving or chemical disinfectant. Waste that has been decontaminated or disinfected is no longer considered as biomedical waste. So after treatment it can be dispersed off in the regular waste stream.

(E) Handling :- Untreated waste should be handled as little as possible. Exterior surfaces of the containers should not be contaminated, Untreated waste should not be contaminated transported through high traffic corridors. Secondary contains should be used while transporting liquids and the secondary containers should be decontaminated after use carts with raised sides for transport. It should be ensured that containes or bags are tightly closed or tored shut during transport.

(F) Collector :-

In our hospital collection of biomedical waste and further disposal after terminal disinfectio has been outsourced to sumbranky.

(G) Storage :-

Sharps in Puncture, Proof Sharp containes are boxes. Anatomical waste should be stored in sealed drums and early disposed.

(H) Disposal Time

In small institution the pick up van arrives weekly where as in our hospital it is daily pick up.

(I) Records

Sharps waste container disposal log - log is kept at the biomedical waste cage.

Radioactive carcasses disposal log - not kept in our lab.

CYTOTOXIC WASTE

(i) Must be segregated from other biomedical waste.

(ii) Sharp containers or red biohazard bags used to contain cytotoxic material must be labelled cytotoxic waste.

(iii) Packaging for disposal (bones or barrels) must also be labelled by 'cytotoxic waste'

(iv) cytotoxic waste must not be refrigerated.

Experiment: Preparation of carbonate buffer and estimation of PH with PH meter. Date

Page No.:

Aim :- Preparation of carbonate buffer and estimation of PH with PH meter.

PH and significance

The term PH was introduced in 1909 by Sorenson was defined PH as the negative log of the hydrogen ion concentration.

$$PH = -\log[H^+]$$

Low PH value (below 7.0) correspond to high conc. of H^+ (acidic solution) and high PH value (above 7.0) correspond to low concentration of H^+ (basic solution).

All biochemical reaction invivo or invitro are greatly influenced by the hydrogen ion concent. of surrounding medium.

The PH value of the important biological fluids are as follows :-

Fluid	PH
Pancreatic Juice	8.8
Bile	7.6
Blood	7.35
Saliva & Human milk	6.7
Gastric Juice	1.77

Buffers :-

A buffer has the capacity to resist the change in PH of solution after the addition of small amount on acid or an alkali buffer solution.

Date
Page No.:

Buffer solutions are prepared by mixing a "weak acid with its salt of strong base or weak base with its salt of strong acid.
The pH of the buffer solution can be calculated by Henderson - Hasselbatch equation -

$$pH = pK_a + \log \frac{[Acid]}{[salt]}$$

, where pK_a = negative log of the dissociation constant.

Buffer System of Blood :-

1. Bicarbonate - Carbonic acid ($BHCO_3$, H_2CO_3)
2. Haemoglobin - Haemoglobin (BHb, HHb)
3. Oxyhaemoglobin - Oxyhaemoglobin ($BHbO_2$, $HHbO_2$)
4. Phosphate Buffer - Phosphate (B_2HPO_4, BH_2PO_4)
5. Protein Buffer - Protein (B Protein, H Protein)

The most important buffer of Plasma is Bicarbonate - Carbonic acid Buffer system. It is of great importance in the acid base balance of the extracellular fluid and in the maintenance of the blood pH within normal physiological limits.

Determination of pH :-

1. By using indicators :- Indicators are various organic dyes of natural and synthetic origin, they change their color accordingly to the pH of the solution. They are useful in determination of pH of solution and as end point indicators in the tritations of acids and bases. Some common indicators useful for biological pH range are,

Litmus Paper - to know the solution is acidic or basic.

Indicator pH - to determine approximate pH range of solution.

2. By using Universal Indicator Solutions - It shows different colors accordingly to pH of solution. The commonly used indicators solution are - Methyl Blue, Methyl Yellow, Methyl orange, Methyl Red Phenol Red etc.

3. Electronic Determination by using pH meter

Principle :- A pH meter will be made up of a probe, which itself is made up of 2 electrodes. This Probe Passes electric signals to a metre which displays the reading in pH unit. The glass Probe has 2 electrodes because one is glass sensor electrode and the other is a reference electrode. Some pH meters do have two separate probes in which case one would be the sensor electrode and the other the reference Point.

> Both electrodes are hollow bulbs containing a KCl solution with a AgCl wire suspended into it. The glass sensing electrode has a bulb made up of a very special glass coated with silica and metal salts. This glass sensing electrodes measures the pH as the conc^{n}. of hydrogen ions surrounding the tip of the thin walled glass bulb. The reference electrode has a bulb made up of a non conductive glass or plastic.

> When one metal is brought in contact with another, a voltage difference occurs due to their difference in electron mobility.

Similar is the case with two liquids. A PH meter measures essentially the electro chemical Potential between a known liquid inside the glass electrode (membrane) and an unknown liquid outside. Because the thin glass bulb allow mainly the agile and small H^+ ions to interact with the glass, the glass electrode measures the electro chemical potential of H^+ ions or the potential of hydrogen. To complete the electrical circuit, also reference electrode is needed.

Procedure :-

(1) Put the electrode in distilled water first and put the plug of electrode in socket, in such condition the function of Knob was in stand by mode.

(2) Set the functional knob and put in solution and wait to get the value, after dipping for 30 s

(3) Put in standby mode again and remove the electrode and tap with a tissue papes to remove the liquid drop stuck.

(4) Put the electrode again in given (prepared liquid to check and follow step (2) to get the value, after keeping for 30 sec.

(5) Again clean with a tissue paper and put in distilled water.

(6) Note down the values properly to get the PH of solution.

Precautions :-

(1) As the electrode is very sensitive, use tissue paper to tap and remove the stuck liquid after every dip.

(2) Never turn the other knobs without any particular role.

(3) Use the electric socket with dry hands

(4) The PH can vary by ± 0.1

(5) The black mark in electrode should be dipped in solution.

Result :-

The Prepared buffer (carbonate solution) is of PH = 9.2 (± 0.1) is measured laboratory condition.

Experiment: Analysis of Normal Constituent in urine Date

Page No.:

Aim :- Analysis of Normal constituents in urine.

Urine :- It is an ultrafiltrate of Plasma from which glucose, amino acids, water and other substances essential to body metabolism have been reabsorbed.
Urine carries waste products and excess water out of the body. Urine contains - 96% water and 4% dissolved salts.
In dissolved salts - 2% urea (half organic) and 2% other compounds.

Urine Analysis :- Examination of urine

1. Physical (Macroscopic) Properties

Appearence	-	Clear and Transparent
Volume	-	1.0 - 2.0 litre.
Color	-	Amber Yellow
Odour	-	Aromatic
Reaction to litmus	-	Acidic (5.5 - 6.5)
Specific Gravity	-	1.015 - 1.025

2. Chemical Properties.

(a) Organic :-

→ Urea - 25.0 - 35.0 gm — 60% - 90% of nitrogenous material; derived from the metabolism of amino acids into ammonia.

→ Creatinine - 1.5 gm — Common component derived from creatine, nitrogenous substances in muscle tissues.

Date

Page No.

→ Uric acid	0.4 - 1.0 gm	common component of kidney stones ; derived from catabolism of nucleic acid in food and cell destruction.
→ Hippuric acid	0.7 gm	Benzoic acid is eliminated from the body in this form; increases with high vegetable diets.
→ Other substances	2.9 gm	Carbohydrates, Pigments, fatty acids, mucin, enzymes, hormones: may be present in small amounts depending on diet and health.
(B) Inorganic		
→ Sodium chloride	15.0 gm	Principal salt, varies with intake.
→ Potassium (K^+)	3.3 gm	Occurs as chloride, sulphates and Phosphates salts.
→ Sulphate (SO_4^{2-})	2.5 gm	Derived from amino acids.
→ Phosphate (PO_4^{3-})	2.5 gm	Occurs primarily as sodium compounds that serve as Buffers in the blood.
→ Ammonium (NH_4^+)	0.7 gm	Derived from protein metabolism and glutamine in kidney ; amount varies depending on blood and tissue fluid acidity.
→ Magnesium (Mg^{2+})	0.1 gm	Occurs as chloride, sulphates phosphate salts.

Experiment..... Date.....

Page No.:

→ Calcium (Ca^{2+}) 0.3 gm Occurs as chloride, sulphates, phosphate salts.

3. Microscopic Examination

This test will involve an examination of the appearence, concentration, as well as content of the urine sample. In microscopy, a sample of urine is centrifuged to obtain some sediment which can then used to examine the presence of crystals, casts, White and/or RBCs or bacteria yeast infection.

Result :- Clear urine is a sign of good hydration and potential overhydration. Pale Yellow urine is an indicator of good hydration. Dark-brown urine can be a sigh of liver or kidney problems. White urine can occur when your body contains excess calcium or phosphate.

Thus the urine sample contains the organic and inorganic constituents.

Experiment... Analysis of Abnormal Constituents in urine ..... Date .....

Page No.:

Aim :- Analysis of Abnormal constituents in urine.

Abnormal constituents of urine :-

Reducing sugar, proteins, bile salts, bile pigments, Ketone bodies, blood etc.

Test for carbohydrates.

(1) Molisch Test.

Principle :- A polysaccharide and a disaccharide hydrolysed by conc. H_2SO_4 acid into monosaccharide. Monosaccharides are dehydrated by the acids to form furfural or one of its derivatives, condensed with α-napthol to form violet color complex.

Procedure	Observation	Interference
Take in a clean & dry test tube. Add 1-2 drops of Molisch reagent. Add 2 ml conc. H_2SO_4 along the side walls of the test tube.	A purple colored ring is formed at the interface of the solution.	Sugar present.

Conclusion :- Hence all carbohydrates give positive test.

Date

(2) Benedict's Test (Semi Quantitative Test)

Principle :- Reducing sugars when heated in the presence of alkali forms enediol which ~~results~~ reduces the cupric ion (Cu^{2+}) present in benedict's reagent to cuprous ion (Cu^{+}) which gets precipitated as insoluble red copper oxide.

Sugar → enediol → oxidised sugar acid

$Cu^{++} \leftarrow CuSO_4$ (Na citrate)

Reduced → $Cu^{+} \longrightarrow 2\,Cu(OH) \longrightarrow Cu_2O$

(Yellow) (Red).

Procedure	Observation	Interference
1. 5ml of Benedict's reagent was taken in test tube.	There was formation of a greenish-orange ppt.	Reducing sugar glucose is Present.
2. Add 8 drops of urine sample was added to it. The test tube was boiled over a flame for 2 min. Solution cooled.		

Conclusion :- Reducing sugar is present in the sample, it indicates the presence of glucose in urine sample.

Test for Protein.

(1) Heat coagulatory Test.

Principle :- Protein (Albumin) when heated, is coagulated i.e it's structure is changed by unfolding of the

polypeptide chains.
Then, Glacial Acetic acid is added, isoelectric point of the protein is reached, causing further precipitation which appears to us as turbidities.

Procedure	Observation	Interference.
Take 10ml of urine sample. Hold the test tube over a flame in slanting position and boil the upper portion of the solution. The lower soln. serves as controller. Add about 3 drops of 1% acetic acid	A cloudy white ppt. is seen in upper portion of test tube.	Proteins present.

Conclusion :- The given sample contains proteins.

(2) Sulphosalicylic Acid test.

Principle :- Negatively charged sulphosalicylic acid neutralises the +ve charge on proteins causing denaturation and hence precipitation of proteins.

Procedure	Observation	Interference.
Take 2ml of urine in a test tube and add a few drops of 25% sulphosalicylic acid.	White precipitate appear.	Albumin present.

Conclusion :- The given sample contains albumin.

Date.....

Page No..

Test for Ketone Bodies.

(1) Rothera's Test.

Principle :- Ketone Bodies react with sodium nitroprusside, to produce purple coloring at the junc^{n}. of two layers. $(NH_4)_2SO_4$ removes the impusities. Ammonia provides alkaline medium. B- hydroxy butyric acid cannot be examined.

Procedure	Observation	Interference.
Saturate 5ml of urine with Rothera's mixture and then add 1-2 ml of conc ammonia sol^{n}; gently mixed by rotation and allow it to stand.	Purple color is produced at the junc^{n}. of the both layers.	Ketone Bodies are present.

Test for Bile salts.

(1) Hay's Sulphur test.

Principle :- Bile salts decrease the surface tension of urine, so it allows sinking of light weight sulphur Powder in the urine.

Procedure	Observation	Interference.
1. In one test tube, 3ml of urine sample was taken. 2. In another test tube some amount of distilled	The sulphur Powder sinks into the	

water was taken.	urine sample	
3. In both the test tubes Sulphur Powder was sprinkled and the test tubes were kept undisturbed.	and floats into the control.	Bile salts are Present.

(2) Petenkoffer's test.

Principle :- Bile salt reacts with hydroxymethylfurfural to forms red solution. Hydroxymethylene pfurfural is formed of sugar that dehydrated with sulphuric acid.

Procedure	Observation	Inference
To 5ml of sample solution add a small crystal of glucose or 3-4 drops of glucose solution. Mix and then add 5ml of conc. H_2SO_4 along the side of test tube	A purple or brown color at the junc^n. of the two fluids.	Presence of Bile salt.

Conclusion :- Bile salts are present in the provided sample of urine.