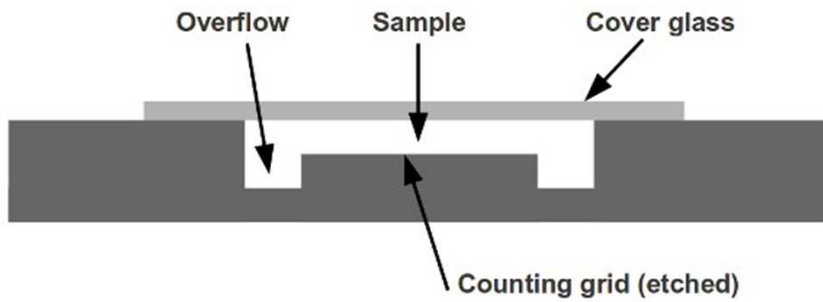
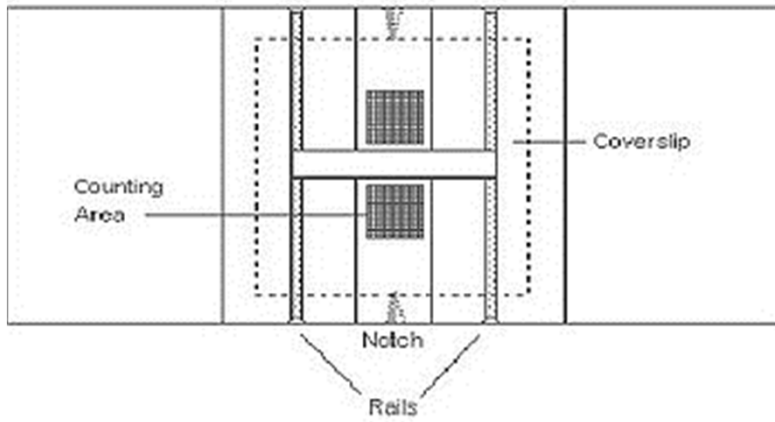
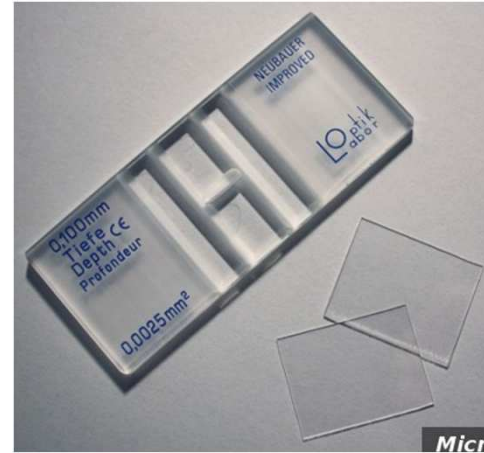


## **EXERCISE - 5**

- **Preparation of chick embryo fibroblast primary cell culture**

### **REQUIREMENT –**

- 10 days old embryonated egg,
- egg candler,
- tincture iodine,
- incubator,
- forceps,
- petridish,
- BSS – Balanced Salt Solution-Hank's,
- two scissors straight and curved,
- Beaker,
- waterbath,
- trypsin,
- trypsinizing flask,
- magnetic bar,
- magnetic stirrer,
- centrifuge tube,
- funnel,
- MEM (Minimum essential medium)
- FCS (Fetal calf serum)
- growth medium (MEM with 5% FCS),
- maintenance medium (MEM with 2% FCS)
- cell culture flask
- Inverted microscope
- Neubauer's cell counting chamber



PROCEDURE –

Collect 10 days old fertile eggs



Select proper one by candling  
(only well developed, active embryos with good blood supply)



Apply tincture iodine on the entire surface of the egg



Keep the egg's air sac end uppermost



Break the egg shell at the air sac with the help of sterile forceps



Remove the shell, the shell membrane and the chorioallantoic membrane



Take out the embryo, catching it by the beak using the curved forceps and place it in a petridish containing BSS



Wash the embryo several times with BSS

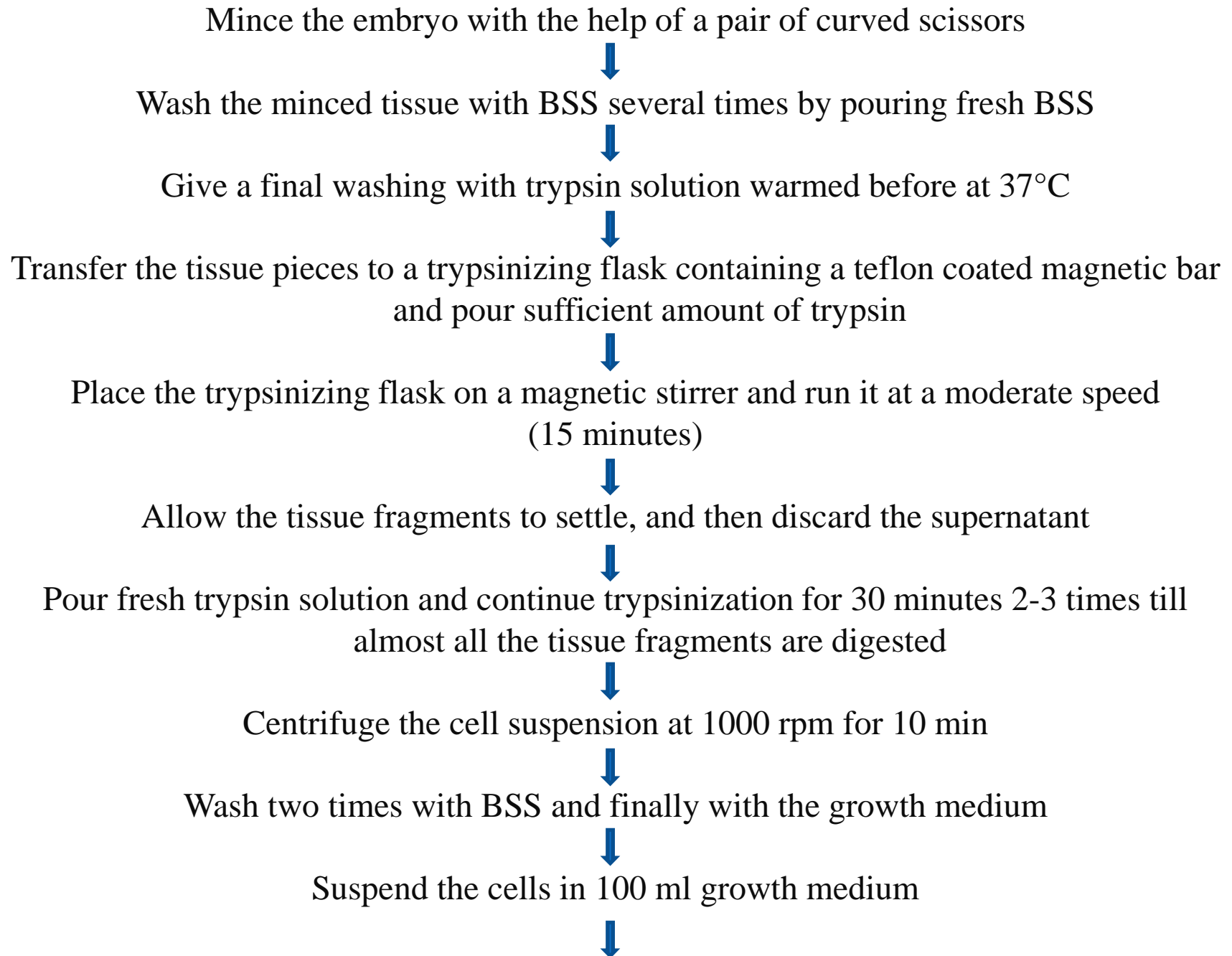


Remove the head and limbs using a pair of scissors and a forceps . Then remove the internal organ by tearing the abdominal wall with the scissors.



Transfer the embryo to a beaker containing BSS and wash it several times with BSS





Count the cells (Neubauer's cell counting chamber) and  
adjust the cell density at  $10^5$  cells / ml



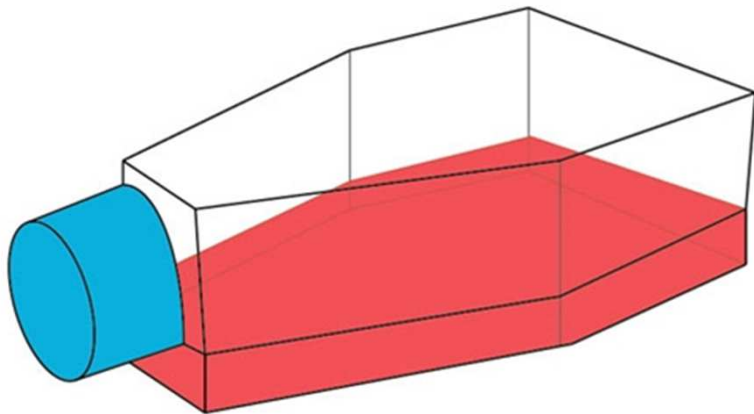
Distribute the cells in cell culture vessels



Incubate at  $37^{\circ}\text{C}$  in the incubator



Confluent monolayer forms in about 3-5 days.  
(If required, change the medium on the third day)



SN	Components	Use(s)
	BSS – Balanced Salt Solution-Hank's	<ul style="list-style-type: none"> <li>- A solution made to a physiological pH and isotonic salt concentration</li> <li>- Provide the cells with water and inorganic ions, while maintaining a physiological pH and osmotic pressure</li> <li>- Glucose is added as an energy source and phenol red is used as a pH indicator</li> </ul>
	Trypsin	<ul style="list-style-type: none"> <li>-Used to dissociate dissected tissue into cells</li> <li>-Used to resuspend cells adherent to the cell culture flask</li> </ul>
	Trypsinizing flask	<ul style="list-style-type: none"> <li>-Used for converting homogenous tissue samples into cell suspension by digestion of connective tissue proteins.</li> <li>- Deep baffles enhance vigorous agitation</li> </ul>
	Magnetic stirrer	-
	Magnetic bar	
	MEM (Minimum essential medium)	<ul style="list-style-type: none"> <li>-Used for growth and maintenance of cells of cell culture</li> <li>- Provides physical conditions such as pH, O<sub>2</sub>, CO<sub>2</sub>, osmotic pressure and nutrition to the cells in the form of chemical substances such as amino acids, carbohydrates and vitamins.</li> </ul>
	FCS (Fetal calf serum)	<ul style="list-style-type: none"> <li>- Used for protein requirement of the cell growth in cell culture.</li> </ul>

<b>SN</b>	<b>Components</b>	<b>Use(s)</b>
	Growth medium (MEM with 2% FCS)	- Medium used for growth of cells in cell culture
	Maintenance medium (MEM with 5% FCS)	- Medium used for maintenance of cells in cell culture
	Cell culture flask	
	Neubauer's cell counting chamber (Haemocytometer)	