Media

1. Natural Media	a) Coagulant, such as plasma clots	-Blood before coagulation; Plasma can also be prepared in the laboratory by taking out blood and adding heparin to prevent blood coagulation.
-natural sources of nutrient sufficient for growth and proliferation of animal cells and tissues	b) Biological fluids such as serum	-Blood after coagulation; serum: components of animal cell culture which is the source of various nutrients.
		-serum: Contains growth factors which promotes cell proliferation, cell attachment and adhesion factors
		-other forms of biological fluids used are coconut water, amniotic fluid, pleural fluid, insect haemolymph serum, culture filtrate, aqueous humour, from eyes etc.
	c) Tissue extracts for example Embryo extracts	-Extracts from tissues such as embryo, liver, spleen, leukocytes, tumour, bone marrow etc. are also used for culture of animal cells.
2. Synthetic Media	a) Serum containing media	

Media (cont)

-prepared artificially	b) Serum-free media

-Recipe: organic and inorganic nutrients, vitamins, salts, serum proteins, carbohydrates, co-factors, etc.

Base Media

-many different types of base medium available

-Additional supplements are added to this bottle

-Some cell types prefer one type of medium to another, be sure to know all this information ahead of time.

Types of Base Medium

Types of Base Medium

Dulbecco's Modified Eagle's Medium Low Glucoase/High Glucose
Iscove's Modified Dulbecco's Medium
Medium 199 Earle's
Minimum Essential Medium (MEM)
Nutrient Micture F10 (F12) Hams
Roswell Park Memorial Institute 1640 (RPMI1640)
Dulbecco's Phosphate Buffered Saline
Earle's Balanced Salt Solution (EBSS)
Hank's Balanced Salt Solution (HBSS)

Basic Constituents of Media

Constituents	Function
1. Inorganic Salts	-retain the osmotic balance of the cells
	-regulate membrane potential by provision of sodium, potassium and calcium ions
	-required in the cell matrix for cell attachment and as enzyme cofactors
2. Carbohydrates (glucose)	-main source of energy
major sugars used are glucose and galactose, however, some media contain maltose or fructose	-media containing the higher concentration of sugars are able to support the growth of a wider range of cell types
3. Amino Acids	-building blocks of proteins
	-'Essential' amino acids must be added to culture media as cells are not able to synthesize these themselves

Basic Constituents of Media (cont)	
	-The concentration of amino acids in the culture medium will determine the maximum cell density that can be achieved
	-once depleted the cells will no longer be able to proliferate
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	-Commonly the necessary amino acids include cysteine and tyrosine, but some non-essential amino acids may be needed.
	-Adding supplements of non-essential amino acids to media both stimulates growth and prolongs the viability of the cells in culture.
4. Vitamins	-precursors for numerous co-factors
	-Many vitamins, especially B group vitamins, are necessary for cell growth and proliferation and for some lines the presence of B12 is essential.
	-Many vitamins, especially B group vitamins, are necessary for cell growth and proliferation and for some lines the presence of B12 is essential.
5. Fatty Acids and Lipids	-important in serum free media since they are normally present in serum
	-E.g. cholesterol and steroids essential for specialised cells.

Basic Constituents of Media (cont)	
6. Proteins and Peptides	-particularly important in serum free media
	-most common proteins and peptides include albumin, transferrin, fibronectin and fetuin and are used to replace those normally present through the addition of serum to the medium
7. Buffering Systems - pH	-Close control of pH is essential for optimum culture conditions
	-Most cells require pH conditions in the range 7.2-7.4
	-Phenol red indicator to monitor pH (pH 6.5 = yellow, pH 7.0 = orange, pH 7.4 = red, pH 7.8 = purple)
	-Could monitor contamination
8. Trace Elements	-E.g. zinc, copper, selenium and tricarboxylic acid intermediates
	-Selenium is a detoxifier and helps remove oxygen free radicals
9. Antibiotics and Anti-mycotics (anti--fungal)	-Unless good sterile conditions can be maintained (e.g., using laminar flow hoods) it is necessary to incorporate antibiotics and antimycotics into the media.
	-To prevent contamination in culturing
	-Specific antibiotics - penicillin/streptomycin solutions
	-Broad spectrum antibiotics - kanamycin or amphotericin B
	-should not be toxic to the cells in culture

Basic Constituents of Media (cont)

	-should depend on the type of contamination
	-Some antibiotics are used for selecting transfected animal cells, such as geneticin
	->Transfected cell acquire resistant selectable marker and able to survive on medium containing geneticin
	->Non-transfected cell could not survive
10. Serum	-supernatant of clotted blood
	-Many undefined growth factors are contained, including many proteins and metal ions.

Types of Serum

Types of Sera

Bovine Calf Serum	Standard sera used in our lab, a cost efficient alternative to FBS.
Fetal Calf Serum	Most commonly used sera in literature. However, FBS is more expensive than BCS.
Cosmic Calf Serum	BCS supplemented with extra nutrients, provided exclusively through HyClone.
Equine Serum	Serum from horses. Some select cell types prefer this serum.

Regulation of pH

Regulation of pH is usually achieved by one of two buffering systems

1. “natural” buffering system

-Gaseous CO2 balances with the CO3/HCO3 content of the culture medium

-Bicarbonate buffer requires 5-10 % CO2 (supplied in a CO2 incubator) to maintain pH 6.9-7.4

-Bicarbonate/CO2 is low cost, non-toxic and also provides other chemical benefits to the cells.

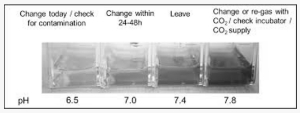
2. chemical buffering using a zwitterion called HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)

-HEPES has superior buffering capacity in the pH range 7.2-7.4

-but is relatively expensive and can be toxic to some cell types at higher concentrations (above ~100nMolar).

-HEPES buffered cultures do not require a controlled gaseous atmosphere.

Use of the phenol red indicator in media



Pros and Cons of Serum in Medium

Advantage	Disadvantage
1. Serum binds and neutralizes toxins	1. It is not chemically defined, its composition varies a lot
2. Serum contains a complete set of essential growth factors, hormones, attachment and spreading factors, binding and transport proteins	2. It is sometimes source of contamination by viruses, mycopl-asma, prions, etc.
3. Serum contains the protease inhibitors	3. Sera could contain inhibitors or toxins
4. Serum increases the buffering capacity	4. Some ingredients could cause unwanted reactions
5. Serum provides trace elements	

Serum-free media (SFM)

-A defined SFM is one in which a group of components of known purity, present at a known concentration, are formulated together to optimize performance for a given cell type

The two main approaches generally followed in designing a serum-free medium are:

1. Reduced serum

-concentration of serum in the basal medium is progressively reduced whilst other components, e.g. growth factors and hormones, are added to identify the factors capable of restoring growth to the level obtained in the presence of serum

2. Basal medium

-add components (singly or in combinations) to a basal medium in a stepwise manner until a medium is progressively ‘built up, to give a similar or equivalent cell growth to the serum-supplemented medium

Pros and Cons of Serum Free Media

Advantage	Disadvantage
1. Avoids qualitative and quantitative fluctuations of nutrients	1. Multiplicity of media-Each cell type appears to require a different recipe

Pros and Cons of Serum Free Media (cont)	
2. Growth conditions are defined and controlled	2. Selectivity-Some media may select a sublineage that is not typical of the whole population
3. Small chances of contam-ination	3. Reagent purity-The removal of serum also requires that the degree of purity of reagents and water and the degree of cleanliness of all apparatus be extremely high, as the removal of serum also removes the protec-tive, detoxifying action that some serum proteins may have
4. More consistent perfor-mance	4. Cell proliferation-Growth is often slower in serum-free media, and fewer generations are achieved with finite cell lines
5. Allows the possibility of studying the effects of one component present in serum, while eliminating the rest	
6. Avoid ethical issues	