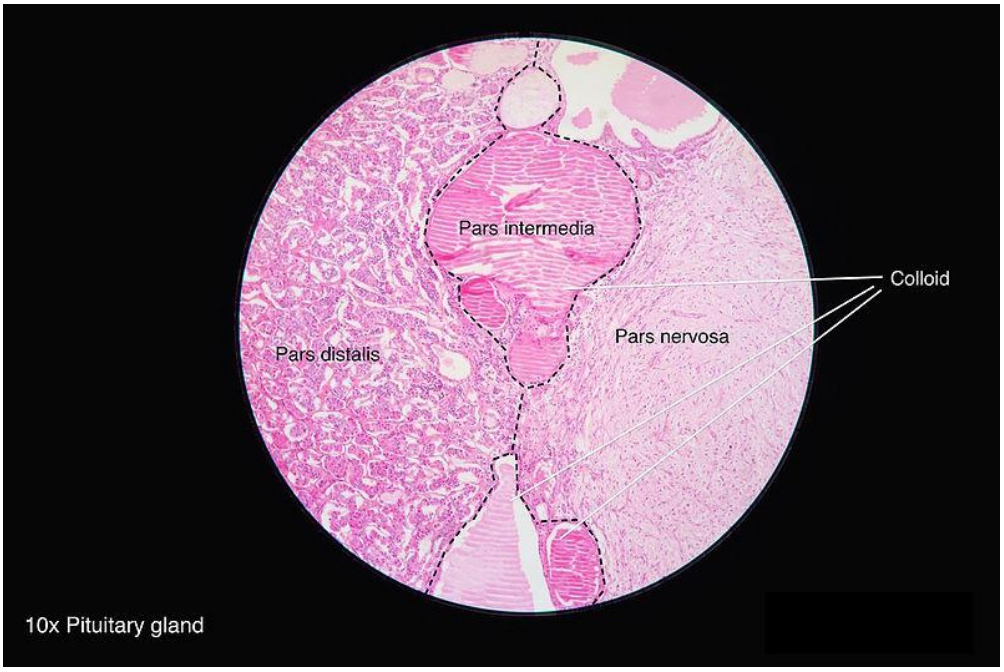
Pituitary Gland



Histology of the Adenohypophysis

The bulk of the adenohypophysis is **pars distalis.** That tissue is composed of winding cords of epithelial cells flanked by vascular sinusoids. In sections stained with dyes such as hematoxylin and eosin, three distinct cell types are seen among epithelial cells:

- **Acidophils** have cytoplasm that stains red or orange
- **Basophils** have cytoplasm that stains a bluish color
- **Chromophobes** have cytoplasm that stains very poorly

The figure below shows pars distalis from a cat at two magnifications (H&E stain).

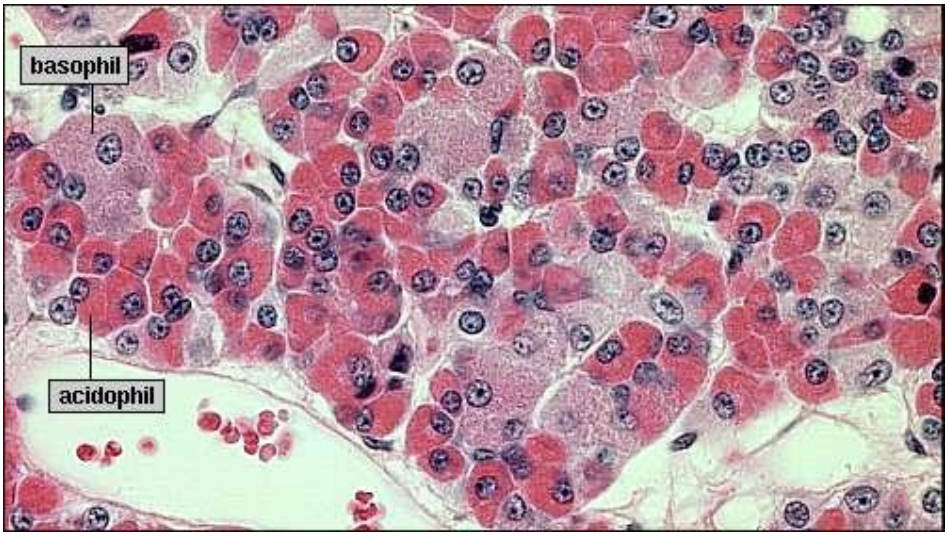


Fig: Cell types of adenohypophysis

The differential staining pattern described above is a reflection of the type of hormonal content of the cells.

Acidophils	Cells that contain the **polypeptide hormones**: • **Somatotropes** which produce growth hormone • **Lactotropes** which produce prolactin
Basophils	Cells that contain the **glycoprotein hormones**: • **Thyrotropes** which produce thyroid stimulating hormone • **Gonadotropes** which produce luteinizing hormone or follicle-stimulating hormone • **Corticotropes** which produce adrenocorticotrophic hormone Due the high carbohydrate content of the hormones within acidophils, they also stain bright purple with PAS stains.
Chromophobes	These are cells that have minimal or no hormonal content. Many of the chromophobes may be acidophils or basophils that have degranulated and thereby are depleted of hormone. Some chromophobes may also represent stem cells that have not yet differentiated into hormone-producing cells.

In addition to differential staining characteristics, the size of secretory granules varies among different types of cells in the anterior pituitary. Somatotropes and lactotropes tend to have the largest size granules. The electron microscopic image below of ovine adenohypophysis depicts cells with several densities and sizes of granules.

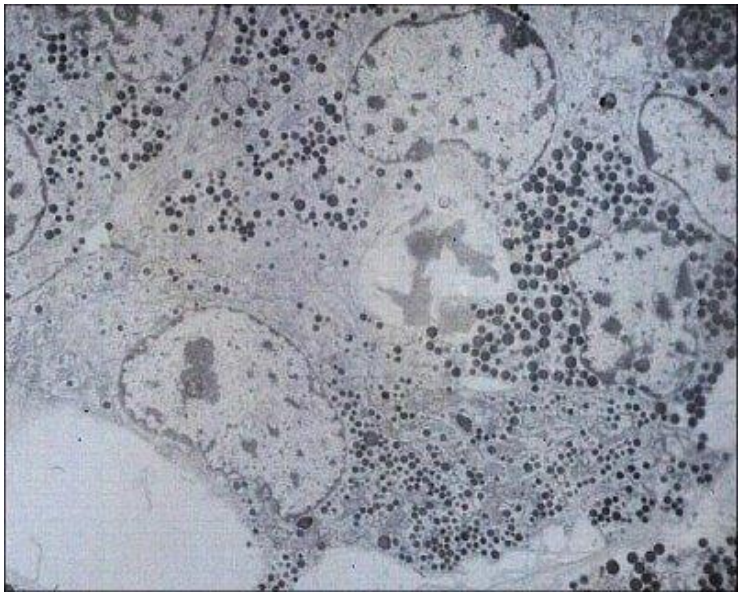
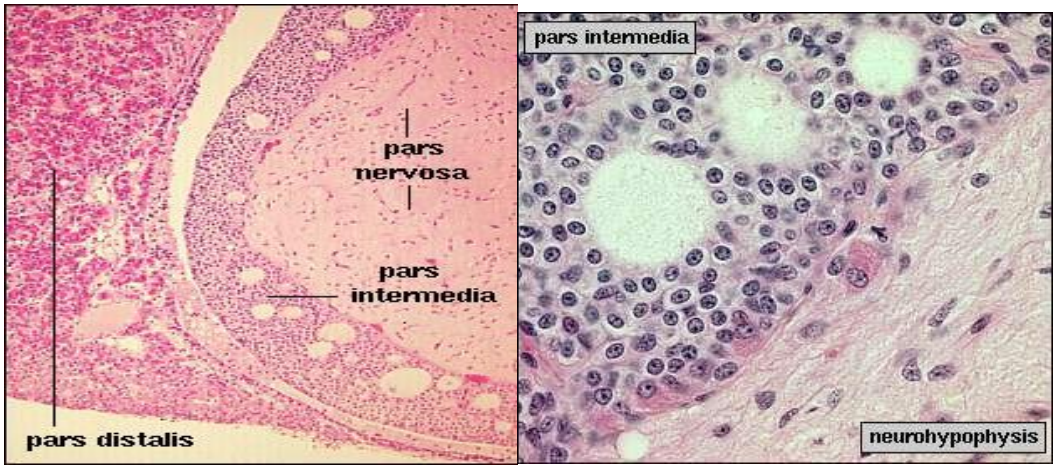


Fig: Secretory granules

The portion of the adenohypophysis known as the pars tuberalis contains cords of epithelial cells and is filled with hypophyseal portal vessels. It reportedly contains gonadotropes and thyrotropes, plus other secretory cells of unknown function.

The pars intermedia is closely associated with pars nervosa and separated from the pars distalis by the hypophyseal cleft. This lobe of the pituitary shows considerable variation in size among species. It is small in man, but much larger in species such as amphibians. The pars intermedia contains large pale cells that often surround follicles filled with ill-defined "colloid". Melanocyte-stimulating hormone is the predominant hormone secreted by the pars intermedia. The images below show pars intermedia from a cat at low and higher magnification. The hypophyseal cleft is seen in the middle of the left image. In the right image, the three round, clear areas are follicles characteristic of this tissue.



Pineal Gland

The pineal body in humans consists of a lobular parenchyma of pinealocytes surrounded by connective tissue spaces. The gland's surface is covered by a pial capsule.

The pineal gland consists mainly of pinealocytes, but four other cell types have been identified. As it is quite cellular (in relation to the cortex and white matter), it may be mistaken for a neoplasm.

Cell type	Description
Pinealocytes	The pinealocytes consist of a cell body with 4–6 processes emerging. They produce and secrete melatonin. The pinealocytes can be stained by special silver impregnation methods. Their cytoplasm is lightly basophilic. With special stains, pinealocytes exhibit lengthy, branched cytoplasmic processes that extend to the connective septa and its blood vessels.
Interstitial cells	Interstitial cells are located between the pinealocytes. They have elongated nuclei and a cytoplasm that is stained darker than that of the pinealocytes.
Perivascular phagocyte	Many capillaries are present in the gland, and perivascular phagocytes are located close to these blood vessels. The perivascular phagocytes are antigen presenting cells.
Pineal neurons	In higher vertebrates neurons are usually located in the pineal gland. However, this is not the case in rodents.
Peptidergic neuron-like cells	In some species, neuronal-like peptidergic cells are present. These cells might have a paracrine regulatory function.

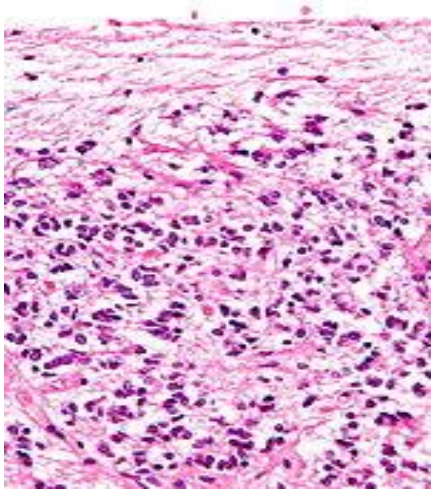


Fig: Pineal gland

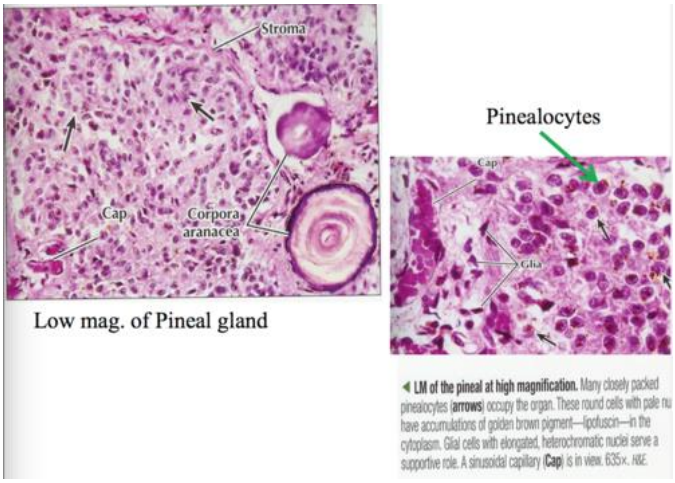


Fig: Cell constituents of pineal gland

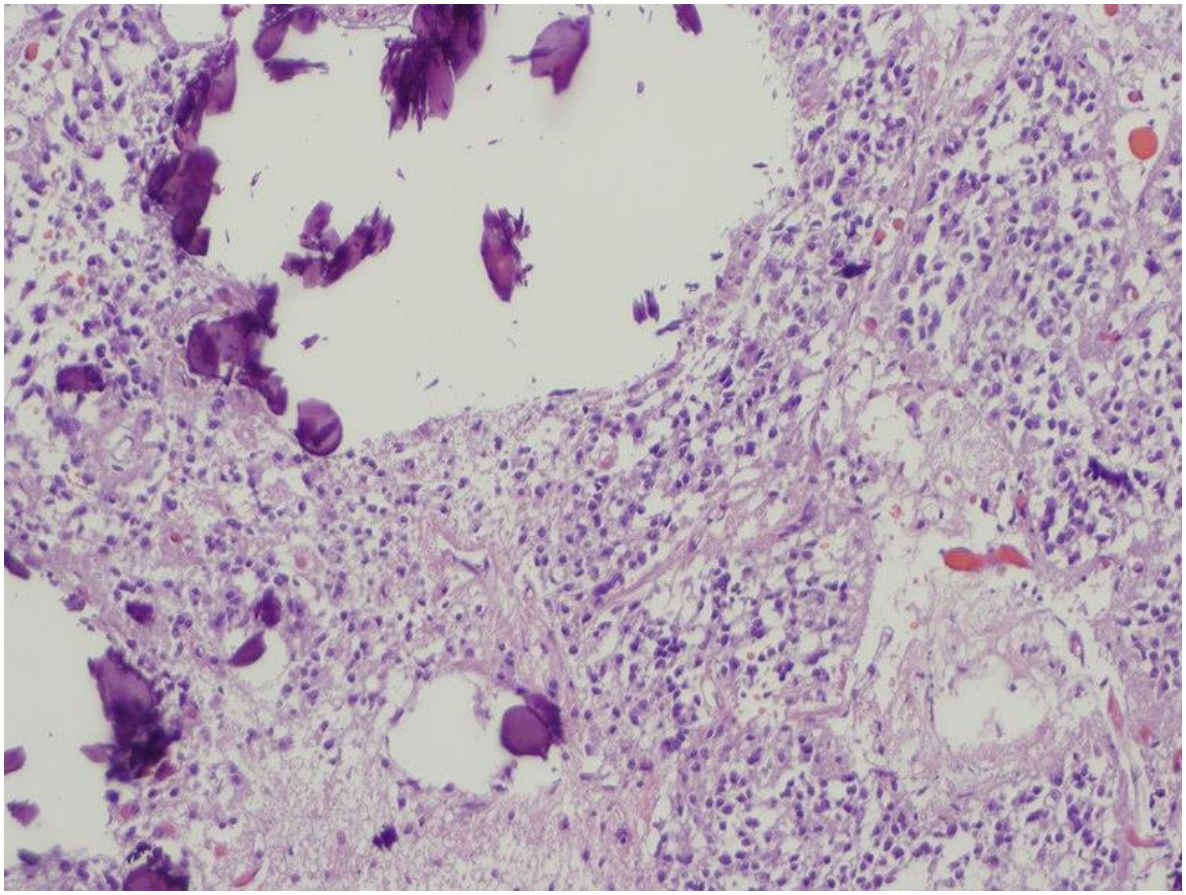
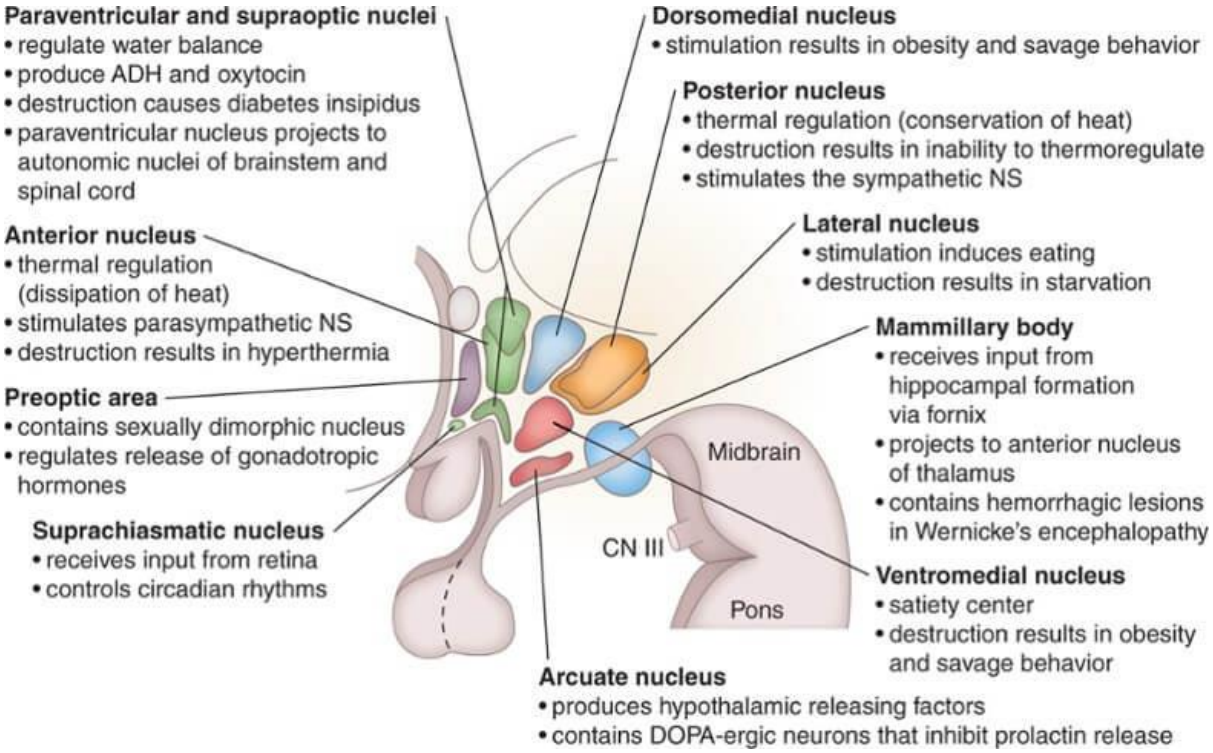


Fig: Pineal gland parenchyma with calcification

Hypothalamic Centres



Hypothalamus

The hypothalamus is a small but crucial part of the dien-cephalon that lies below the thalamus and surrounds the lower part of the third ventricle.

From ventral to dorsal, the undersurface of the hypothalamus is marked by the optic chiasm, the tuber cinereum, the infundibulum, and the mammillary bodies.

The rostral border comprises the anterior commissure and the lamina terminalis; the caudal border merges with the tegmentum of the midbrain.

Laterally, the hypothalamus is flanked by the internal capsule; medially, it is flanked by the third ventricle.

The hypothalamus exerts its influence through three major systems:

(1) The limbic,

(2) The autonomic, and

(3) The endocrine.

It is composed of numerous nuclei of ill-defined boundaries that make connections with many parts of the central nervous system (CNS), including (1) limbic structures, (2) autonomic nuclei of the brainstem and spinal cord, and (3) the pituitary gland.

Hypothalamic Nuclei

The hypothalamus may be divided into a medial hy-pothalamic region that contains the majority of nuclei and a lateral hypothalamic region that contains the major fiber tracts (e.g., the medial forebrain bundle) and a group of diffuse nuclei.

The medial hypothalamic area is further subdivided into three regions:

(1) The supraoptic region, which lies farthest anterior and includes the *supraoptic*, suprachias-matic, and *paraventricular nuclei*;

(2) The tuberal region, which lies just posterior to the supraoptic region and includes the *ventromedial*, *dorsomedial*, and *infundibular nuclei*; and

(3) The mammillary region, which lies farthest posterior and includes the *mammillary body* to the *posterior nucleus*.

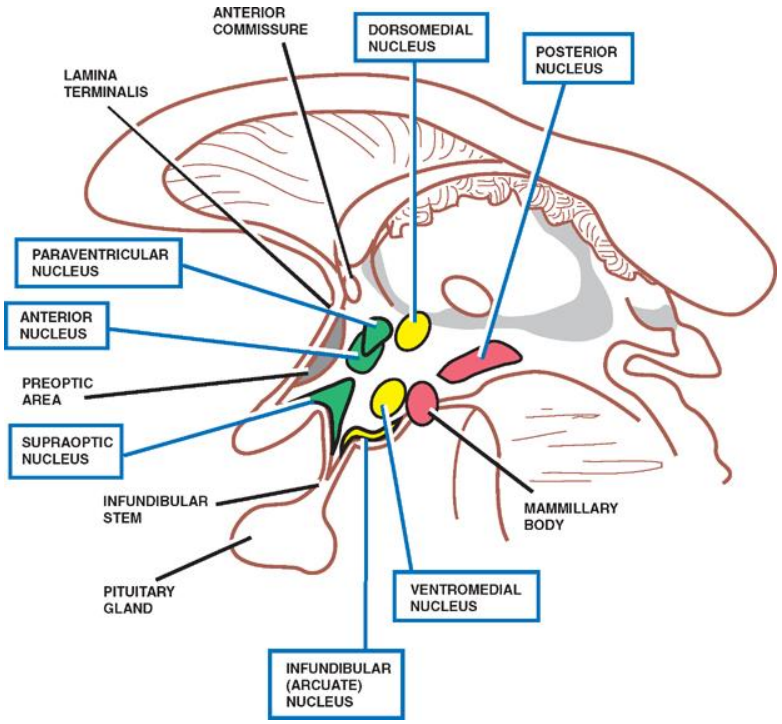


Fig. **Hypothalamic nuclei.**

Afferent Connections

The hypothalamus is located in the center of the lim-bic system and receives numerous projections from lim-bic system structures. In addition, the hypothalamus receives ascending afferents from the brainstem reticular formation and descending afferents from the thalamus and cerebral cortex.

The major afferent connections of the hypothalamus include the following:

- *Olfactory and septal areas* these areas are concerned with smell and basic emotional drives. They send axons to the hypothalamus via the medial forebrain bundle.

- *Hippocampus* This limbic system structure is probably involved in a variety of behaviors, including learning and memory. The hippocampus sends axons to the *mammillary bodies* of the hypothalamus in a large fiber bundle called the *fornix*.

- *Amygdaloid nucleus* like the hippocampus, the amygdaloid nucleus is associated with complex behaviors. It sends axons to the hypothalamus via the stria terminalis.

- *Midbrain tegmentum (reticular formation)* this includes a diffuse network of neurons concerned with a variety of autonomic functions. It sends axons to the hypothalamus through the *medial forebrain bundle*. Two additional brainstem nuclei, closely related to the reticular formation, also send axons to the hypothalamus. They are the raphe nucleus, which projects serotonin-containing fibers, and the nucleus ceruleus, which projects norepinephrine-containing fibers. Both fiber types project to the hypothalamus in the dorsal longitudinal fasciculus.

- *Dorsomedial and midline thalamic nuclei* These are concerned with emotional states and autonomic functions. They project axons to the hypothalamus via the thalamohypothalamic tract.

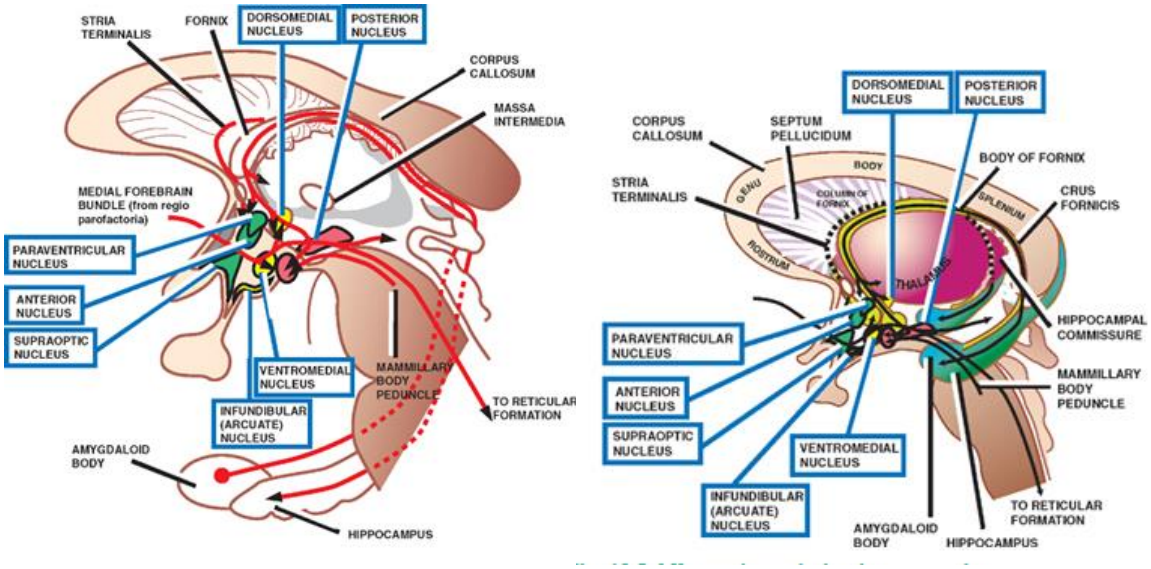


Fig. **Afferent hypothalamic connections.**

Efferent Connections

The hypothalamus is a major output pathway of the limbic system. The efferent connections of the hypothalamus are largely reciprocal to the afferent projections. There are ascending connections to the cortex and thalamus as well as descending connections to the autonomic nuclei of the brainstem and spinal cord. Separately, two hypothalamic pathways project to the pituitary gland.

The major efferent connections of the hypothalamus include the following:

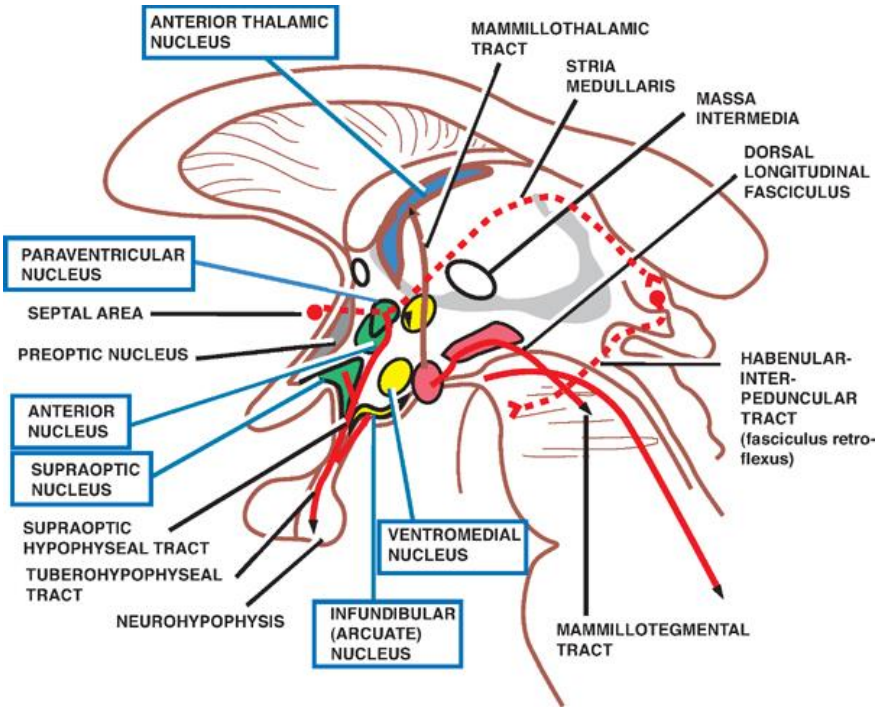
- *Olfactory and septal areas* these areas are concerned with smell and basic emotional drives. They receive hypothalamic axons via the medial forebrain bundle.

- *Anterior thalamic nucleus* this nucleus is the tha-lamic part of the limbic system. It is concerned with emotional states and memory. It receives hypotha-lamic axons via the *mammillothalamic tract* and projects in turn to the cingulate gyrus.

- *Preganglionic autonomic neurons of the brainstem and spinal cord* these are concerned with various autonomic functions. They include the dorsal nucleus of the vagus (brainstem) and the intermediolateral cell column (spinal cord). Both receive input from the hypothalamus via the dorsal longitudinal fasciculus; the hypothalmic projections to the intermediolateral cell column are relayed in reticulospinal pathways.

- *Posterior pituitary gland* the posterior pituitary gland receives direct axonal projections from large neurosecretory cells in the *paraventricular* and *su-praoptic nuclei* of the hypothalamus. They are carried in the *supraoptic hypophyseal tract*. Neurons of the paraventricular and supraoptic nuclei synthesize and secrete the hormones oxytocin and antidiuretic hormone (ADH), which are involved in reproductive functions and water balance, respectively. They are discussed in greater detail later in the chapter.

- *Anterior pituitary gland* Neurosecretory cells in the *infundibular nucleus* of the hypothalamus produce releasing and inhibiting factors that influence the secretion of pituitary hormones. There are no direct axonal connections between the hypothalamus and the anterior pituitary gland. These factors are carried by axoplasmic transport in the *tuberoinfundibular tract* and are secreted into a capillary bed in the median eminence. From the median eminence, they are transported in *hypophyseal portal veins* to a second capillary bed in the anterior pituitary gland, where they modulate the secretion of trophic hormones, such as thyroid-stimulating hormone (TSH), adreno-corticotropic hormone (ACTH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), growth hormone (GH), melanocyte-stimulating hormone (MSH), and prolactin.



Nucleus	Zone(s)	Region(s)	Functions
Paraventricular	Periventricular, Medial	Anterior,Tuberal	Fluid balance, milk let-down, parturition, autonomic & anterior pituitary control
Preoptic	Medial, Lateral	Anterior	Lateral anterior thermoregulation, sexual behavior
Anterior	Medial	Anterior	Lateral anterior thermoregulation, sexual behavior
Suprachiasmatic	Medial	Anterior	Biological rhythms
Supraoptic	Medial, Lateral	Anterior	Fluid balance, milk let-down, parturition
Dorsomedial	Medial	Tuberal	Emotion (rage)
Ventromedial	Medial	Tuberal	Appetite, body weight, insulin regulation
Arcuate	Periventricular, Medial	Tuberal	Control of anterior pituitary, feeding
Posterior	Medial	Posterior	Thermoregulation
Mammillary	Medial	Posterior	Emotion and short-term memory
Lateral Complex	Lateral	Tuberal	Appetite and body weight control

Preparation of Sperm Slide:

¬Take a drop of Bull's semen on a cleaned, prewashed microscopic slide.

¬ Spread the semen evenly over the slide using needle and let it air dry.

¬ Fix using methanol and let it air dry.

¬ Stain with eosin for 10 minutes and wash off the excess stain.

¬ Examine under microscope.

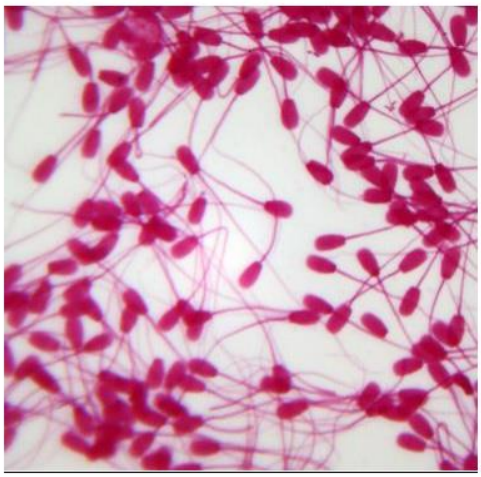


Fig: Bull sperm under microscope

ELISA

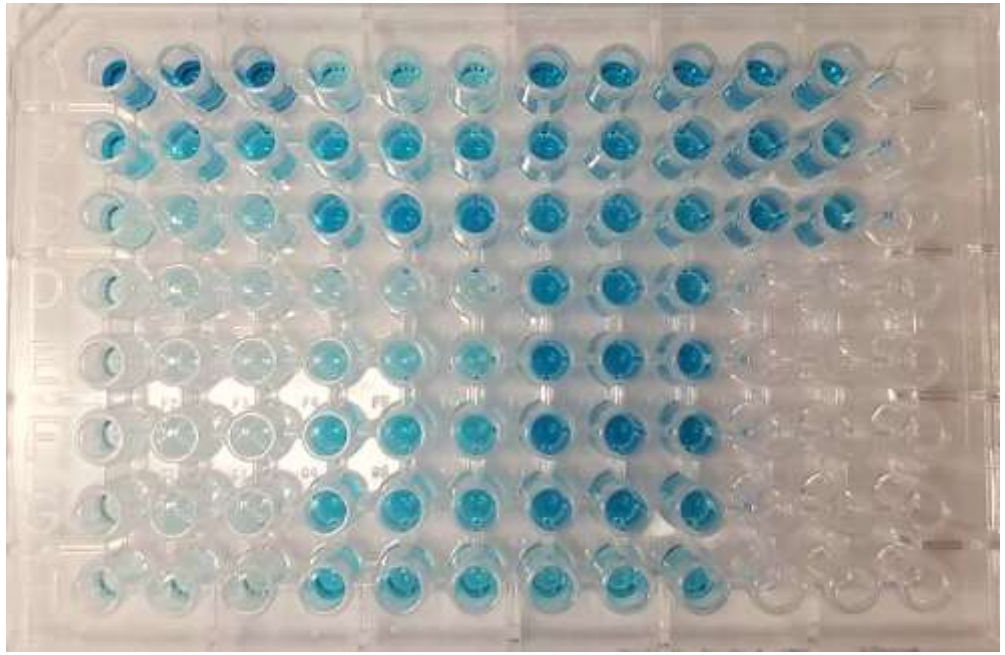
Enzyme Linked Immunosorbant Assay

- **ELISA is a plate based assay technique which is used for detecting and quantifying substances such as peptides, proteins, antibodies and hormones.**

- **An enzyme conjugated with an antibody reacts with colorless substrate to generate a colored product. Such substrate is called chromogenic substrate.**

- **A number of enzymes have been used for ELISA such as alkaline phosphatase, horse radish peroxidase and beta galactosidase.**

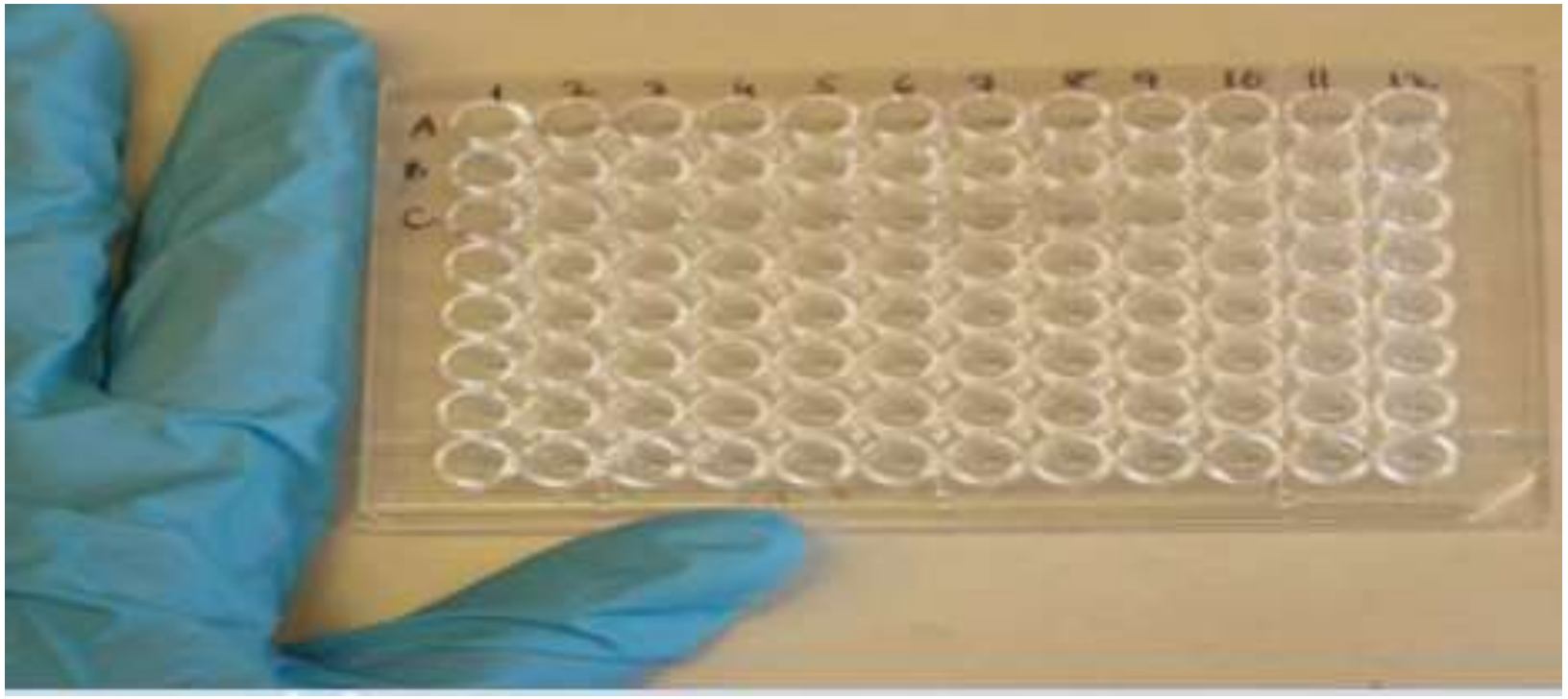
- **Specific substrate such as ortho-phenyldiamine dihydrochloride (for peroxidase), paranitrophenyl phosphate (for alkaline phosphatase) are used which are hydrolysed by above enzymes to give colored end product.**



Basic terms

Solid phase:

- **Usually a microtitre plate well having 8*12 well format**



Adsorption:

The process of adding an antigen/antibody, diluted in buffer , so it attaches to the solid phase on incubation.

Washing:

The flooding or emptying the wells with a buffered solution to separate bound from unbound reagents in ELISA

Antigen:

Any molecule that elicits the production of antibodies when introduced into body.

Antibodies:

Proteins produced in response to antigenic stimuli.

Enzyme conjugate:

An enzyme that is attached irreversibly to an antibody.

Chromogen:

A chemical alters color as a result of an enzyme interaction with substrate

Stopping:

The process of stopping the action of the enzyme on substrate

Reading:

Spectrophotometric measurement of color developed in ELISA

Principle

- ELISAs are typically performed in 96-well polystyrene plates.
- The serum is incubated in a well, and each well contains a different serum.
- A positive control serum and a negative control serum would be included among the 96 samples being tested. Antibodies or antigens present in serum are captured by corresponding antigen or antibody coated on to the solid surface.
- After some time, the plate is washed to remove serum and unbound antibodies or antigens with a series of wash buffer.
- To detect the bound antibodies or antigens, a secondary antibodies that are attached to an enzyme such as peroxidase or alkaline phosphatase are added to each well.
-
- After an incubation period, the unbound secondary antibodies are washed off. When a suitable substrate is added, the enzyme reacts with it to produce a color.
- This color produced is measurable as a function or quantity of antigens or antibodies present in the given sample. The intensity of color/ optical density is measured at 450nm.
- The intensity of the color gives an indication of the amount of antigen or antibody.

Types of ELISA

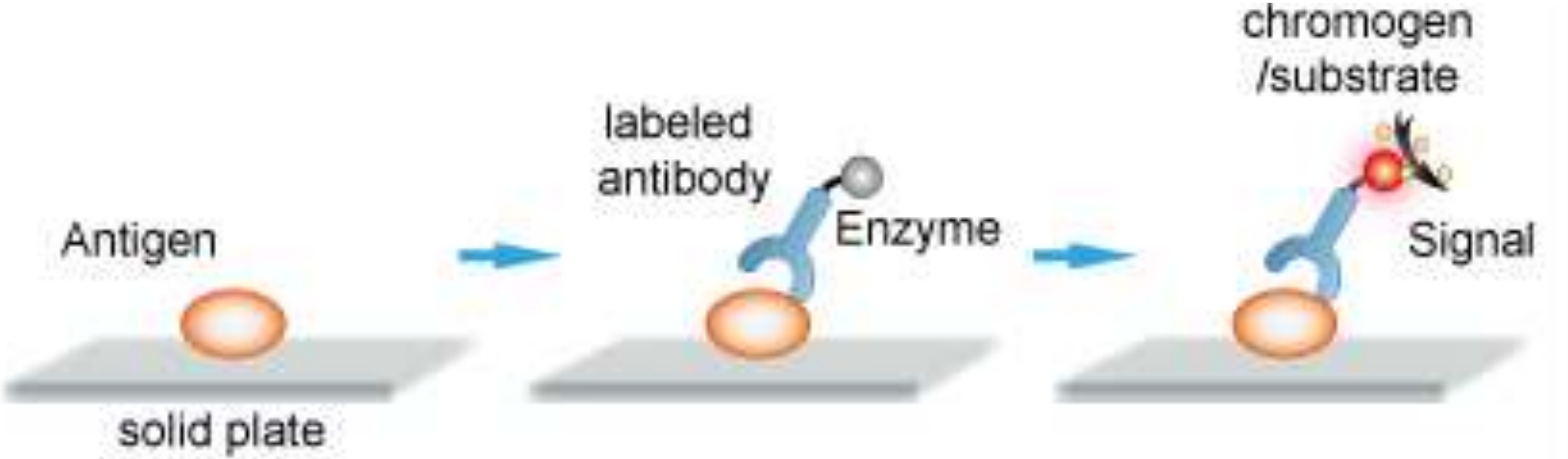
1. Direct ELISA

2. Indirect ELISA

3. Sandwich ELISA

4. Competitive ELISA

1. Direct ELISA

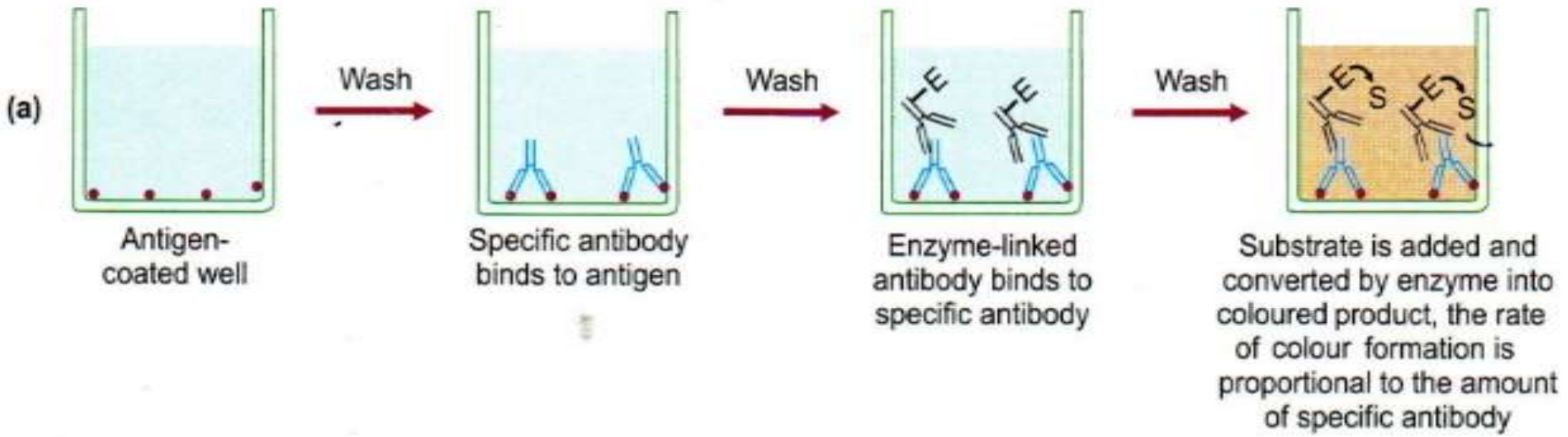


1. Antigen is coated onto wells by passive adsroption
2. Antibody conjugated with enzyme is added and incubated with antigen.and incubation.
3. Substrate / chromophore is added and colour develops.

2. Indirect ELISA

- **Antibody can be detected or quantitatively determined by indirect ELISA.**
- In this technique, antigen is coated on the microtiter well. Serum or some other sample containing primary antibody is added to the microtiter well and allowed to react with the coated antigen.
- Any free primary antibody is washed away and the bound antibody to the antigen is detected by adding an enzyme conjugated secondary antibody that binds to the primary antibody.
- Unbound secondary antibody is then washed away and a specific substrate for the enzyme is added.
- Enzyme hydrolyzes the substrate to form colored products.
- The amount of colored end product is measured by spectrophotometric plate readers that can measure the absorbance of all the wells of 96-well plate.

INDIRECT ELISA

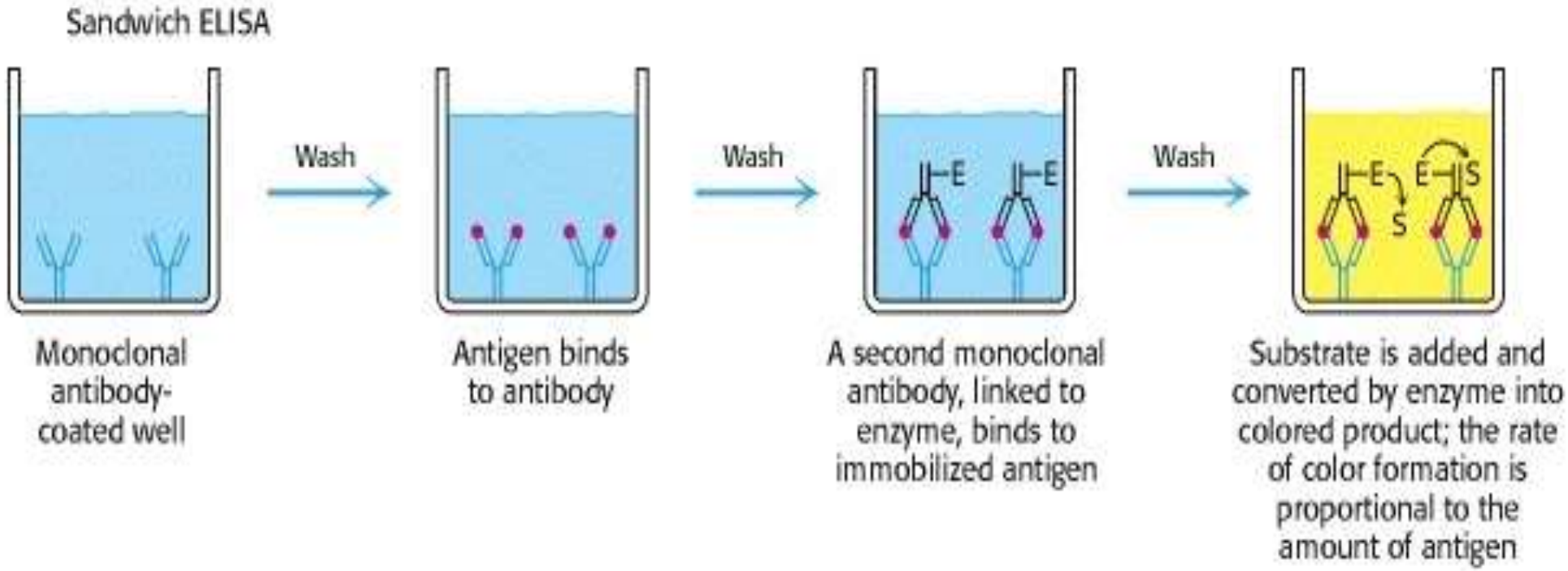


Procedure of Indirect ELISA

1.Coat the micro titer plate wells with antigen.
2.Block all unbound sites to prevent false positive results.
3.Add sample containing antibody (e.g. rabbit monoclonal antibody) to the wells and incubate the plate at 37°c.
4.Wash the plate, so that unbound antibody is removed.
5.Add secondary antibody conjugated to an enzyme (e.g. anti- mouse IgG).
6.Wash the plate, so that unbound enzyme-linked antibodies are removed.
7.Add substrate which is converted by the enzyme to produce a colored product.
8.Reaction of a substrate with the enzyme to produce a colored product.

3. Sandwich ELISA

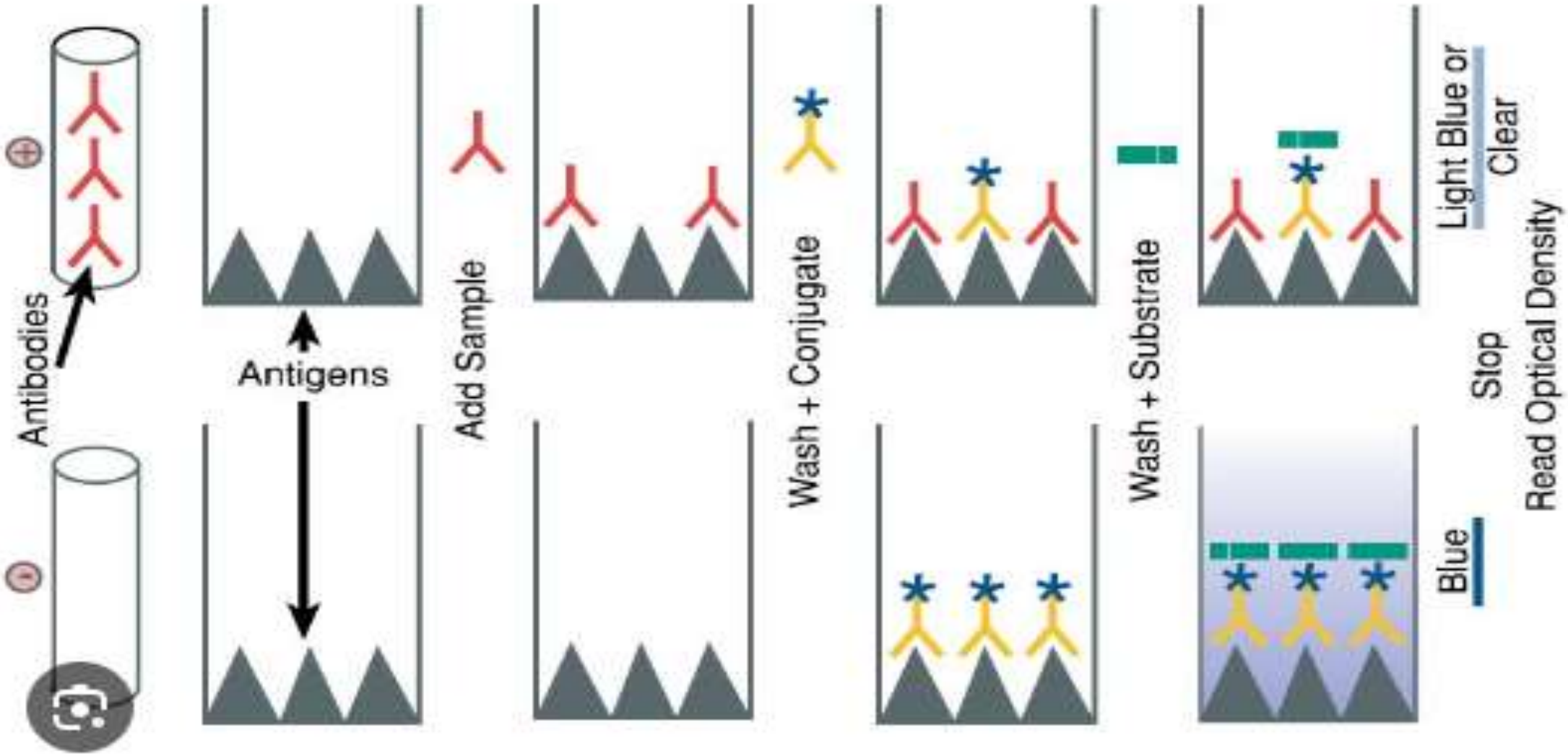
- Antigen can be detected by sandwich ELISA.
- In this technique, antibody is coated on the microtiter well.
- A sample containing antigen is added to the well and allowed to react with the antibody attached to the well, forming antigen-antibody complex.
- After the well is washed, a second enzyme-linked antibody specific for a different epitope on the antigen is added and allowed to react with the bound antigen.
- Then after unbound secondary antibody is removed by washing.
- Finally substrate is added to the plate which is hydrolyzed by enzyme to form colored products.

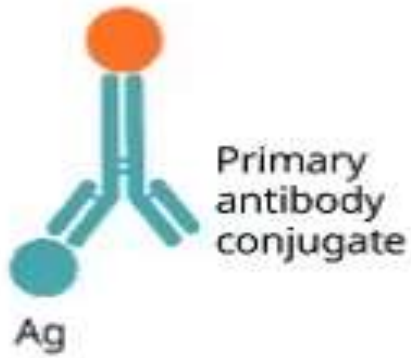


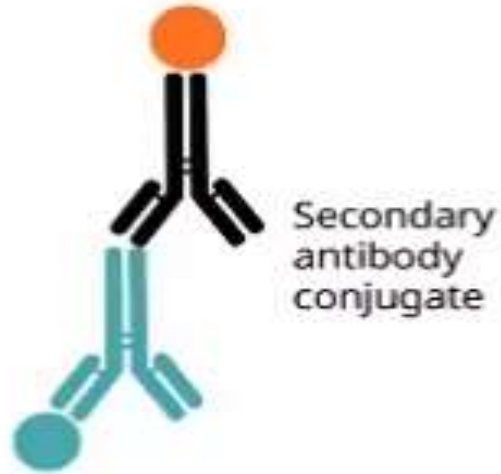
Procedure of sandwich ELISA

1.Prepare a surface to which a known quantity of antibody is bound.
2.Add the antigen-containing sample to the plate and incubate the plate at 37°c.
3.Wash the plate, so that unbound antigen is removed.
4.Add the enzyme-linked antibodies which are also specific to the antigen and then incubate at 37°c.
5.Wash the plate, so that unbound enzyme-linked antibodies are removed.
6.Add substrate which is converted by the enzyme to produce a colored product.
7.Reaction of a substrate with the enzyme to produce a colored product.

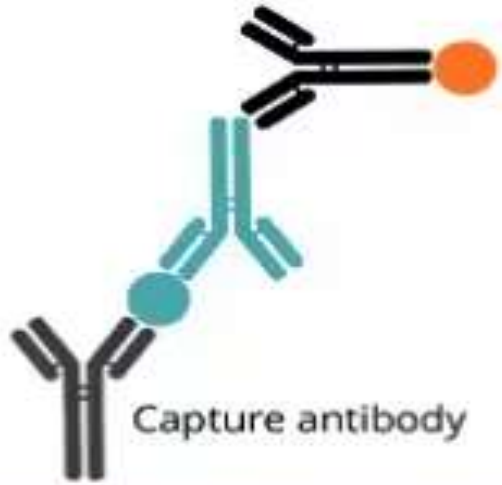
4. Competitive ELISA

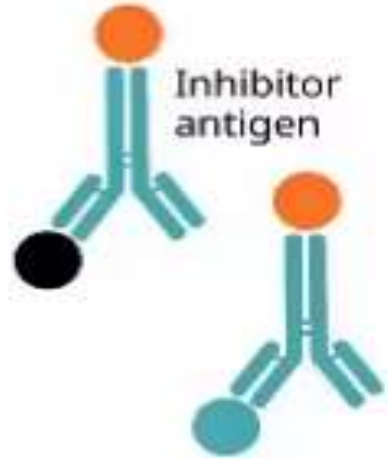






Direct ELISA | **Indirect ELISA**





Sandwich ELISA | **Competitive ELISA**