Immunology Practical – week 13

Laboratory diagnosis of immunodefficiences

Approach to Assessing Immune Deficiency

When assessing a patient for possible immune deficiency, consider the following:

- Number of infections
- Severity of infections
- Type of infections
- Location of infections
- Need for antibiotics
- Need for hospitalization
- Failure to thrive
- School or work attendance and performance
- Is there a family history of immune disorders?
- Are the parents related? (e.g., first cousin marriages increase likelihood of a recessive condition)
- History of atopic disease (children with allergies often present with recurrent rhinositis, even pneumonia in asthmatics)
- In adults, fatigue rather than significant infection may be a presenting feature

Laboratory Assessment of Immune Deficiency

Laboratory testing has become increasingly sophisticated, including molecular/genetic assessments. If initial screening tests suggest concern for an underlying immune disorder, then referral to a specialist with expertise may be indicated to reduce costs and focus the evaluation.

Tests for immune deficiency usually include:

- Complete blood count (in addition to other parameters, provides absolute neutrophil and absolute lymphocyte numbers)
- Quantitative serum immunoglobulins (IgG, IgA, IgM)

Of note:

- A complete blood count and quantitative immunoglobulins are usually sufficient to diagnose >90 percent of patients with a primary immunodeficiency.
- Most patients diagnosed clinically with sinusitis really only have rhinitis.
- The most common cause of recurrent otitis media and rhinosinusitis is underlying allergy.

Types of immunodeficiency disorders:

1- Primary: Causes in immune system component:

i. Complements. **ii. Phagocytic.** **iii. B cells.** **iv. T Cells.**

2- Secondary: Non Immunogenic causes:

a. Prematurity. **b. Malnutrition.**

c. Hodgkin`s and others malignancy. **d. Injury, Burns, Splenectomy.**

e. Drugs. **f. HIV**

The primary immunodeficiency diseases are a group of disorders in which the primary defect appears to be intrinsic to one or more components of the immune system.

Primary IDD – stem cell defects:

Disease	Inheritance	Gene	Chromosome	Consequences
Adenosine deaminase (ADA) deficiency	Autosomal-recessive	*ADA* (adenosine deaminase)	20	Very susceptible to infection; impaired purine metabolism; T and B cells numbers and functions decreased due to toxic metabolites; immunoglobulin levels decreased
Immuno-deficiency with ataxia-telangiec-tasia	Autosomal-recessive	*ATM* (ataxia telangiec-tasia mutated)	11	Increased susceptibility to infection; frequent sinopul-monary infections; DNA repair affected and variable signs, including ataxia and telangiectasia (problems with balance and widened small capillaries); occurs at varying ages and in varying functions; T cell numbers and functions and immunoglobulin levels (especially IgG, IgA, and IgE) may decrease; B cell numbers may be normal; autoantibodies and chromosomal abnormal-ities are frequently found
Purine nucleoside phospho-rylase deficiency	Autosomal-recessive	*NP* (nucleo-side phos-phorylase)	14	Increased susceptibility to infection; impaired purine metabolism; declining T cell numbers over time (more susceptible than B cells to accumulated toxic metabo-lites); declining immunoglobulin levels due to decreased T cell help
Severe combined immune deficiency (SCID)	Autosomal-recessive	*RAG1* and/or *RAG2* (recombi-nation-activating genes)	11	High susceptibility to infection; unable to rearrange DNA to form variable regions of immunoglobulins and T cell receptors; T and B lymphocyte numbers/functions reduced or absent; immunoglobulin levels reduced or absent
	X-linked-recessive	*IL2RG* (common cytokine receptor γ chain, a component of the re-ceptor com-plexes for IL-2, IL-4, IL-7, IL-9, and IL-15)	X	Multiple effects because common γ chain is a component of receptors for several cytokines; increased suscepti-bility to infection; T cell numbers and immunoglobulin levels decreased; B cell numbers normal or increased
	Autosomal-recessive	*JAK3* (janus kinase 3)	19	Increased susceptibility to infection; defective intracellu-lar signaling; T cell numbers and immunoglobulin levels decreased; B cell numbers normal or increased
Wiskott-Aldrich syndrome	X-linked-recessive	*WAS* (Wiskott-Aldrich syndrome)	X	Increased susceptibility to infection, especially by *S. aureus*, develops during infancy and early childhood; T and B cell numbers and functions reduced, as are immunoglobulin levels; platelets abnormal and reduced in number

Primary IDD atributable to T cell defects:

Disease	Inheritance	Gene	Chromosome	Consequences
CD3 deficiency	Autosomal-recessive	*CD3G* or *CD3E*	11	Increased susceptibility to infection; defects in CD3γ (CD3G) or CD3ε (CD3E) proteins; variable effects on T cell functions
DiGeorge syndrome	Autosomal-dominant or sponta-neous	Unknown Defects in embryonic thymic de-velopment	22 (when genetic)	Increased susceptibility to infections; T cell numbers and functions intrinsically normal but reduced and variable owing to abnormal development of thymus from third and fourth brachial arches; variable immunoglobulin levels; deletions in chromosome 22 frequently seen; often accompanied by other defects (e.g., facial fea-tures, palate, aorta, and parathyroid glands and cal-cium metabolism)
MHC class II deficiencies (bare lym-phocyte syndrome)	Autosomal-recessive	*CIITA* or *RFX5*	16 or 1	Increased susceptibility to infection; defective intra-cellular signaling; $CD4^+$ T cell numbers reduced; immunoglobulin levels decreased owing to defective T cell help
Purine nucleoside phospho-rylase deficiency	Autosomal-recessive	*NP* (nucleo-side phos-phorylase)	14	Increased susceptibility to infection; impaired purine metabolism; T cell numbers decline over time (more susceptible than B cells to accumulated toxic metabo-lites); immunoglobulin levels decline due to decreased T cell help
Transporter associated with anti-gen pre-sentation (TAP) -1 or -2 deficiency	Autosomal-recessive	*TAP1* or *TAP2*	6	Increased susceptibility to viral infections and to some intracellular bacteria; decreased MHC I expression and antigen presentation; $CD8^+$ T cell numbers and functions decreased
ZAP-70 deficiency	Autosomal-recessive	*ZAP70* (ζ chain associated protein kinase)		Recurrent severe infections; defective signaling from TCR; $CD8^+$ T cells absent; $CD4^+$ T cells present in normal numbers but nonfunctional

Primary IDD atributable to defects in B cells and Ig:

Disease	Inheritance	Gene Locus	Chromosome	Consequences
Autosomal-recessive agamma-globu-linemia	Autosomal-recessive	Various genes involved in early differ-entiation	Various	Increased susceptibility to infection; failure in early differentiation of B cells
Bruton's agamma-globu-linemia	X-linked-recessive	*BTK* (Bruton agamma-globuline-mia tyro-sine kinase)	X	Increased susceptibility to infection; increased suscepti-bility to encapsulated bacteria (e.g., *H. influenzae*, *staphylococci*, and *streptococci*); drastic decrease in B cell numbers and immunoglobulin levels
Common variable immuno-deficiency (CVI or CVID)	Multiple forms	Unknown	?	Increased susceptibility to pyogenic infection; variable symptoms; varying isotypes (or combinations of isotypes) reduced or absent
Immuno-deficiency with hyper-IgM	X-linked-recessive Autosomal-recessive	*CD40LG* (CD40 ligand, CD154)	X	Increased susceptibility to pyogenic infection; inability of B cells to undergo isotype switching or somatic hypermutation; elevated IgM with decreased/absent IgG, IgA, and IgE; 70% of cases due to X-linked defect
Ig heavy chain gene deletions	Autosomal-recessive	Heavy chain constant genes	14	Increased susceptibility to infection (patients with IgG1 deficiency have increased susceptibility to pyogenic infections, while those with IgG2 or IgG3 are susceptible to encapsulated bacteria); various immunoglobulin isotypes absent (dependent upon the affected heavy chain gene); IgG most frequently affected; B cell numbers frequently reduced
Kappa chain deficiency	Autosomal-recessive	κ chain genes	2	Decreased or absent immunoglobulin containing κ chains; little or no effect on susceptibility to infection
Selective IgA deficiency	Multiple forms	Multiple genes	Various	Although patients with this deficiency display no increase in infections, an increased susceptibility to infections may be seen in some, especially recurrent pyogenic bacterial infections in patients also deficient in IgG2; IgA-expressing B cells decreased or absent; serum IgA reduced and often accompanied by IgG subclass deficiency; frequent allergic or autoimmune disorders; frequency of 1 to 2 per thousand individuals makes it one of the most common immune deficiency diseases

Primary idd atributable in accessory cells:

Disease	Inheritance	Gene Locus	Chromosome	Consequences
Chediak-Higashi syndrome	Autosomal-recessive	*LYST* (lyso-somal traf-ficking regulator; also called CHS1)	1	Increased susceptibility to infection by pyogenic bacte-ria; defective fusion of lysosomes and phagosomes due to defect in organelle membranes; reduced ability to kill ingested microbes; decreased NK and T cell functions; frequent albinism of eyes and skin and other defects of organelle membranes; giant granules in neutrophils and other cells
Chronic granulo-matous disease (CGD)	X-linked-recessive	*CYBB* (β chain of cytochrome b; also called gp91phox)	X	Increased susceptibility to infection, especially *Staphylo-coccus aureus*, *Salmonella enteric*, *Typhimurium*, *Serratia marcescens*; macrophages and neutrophils affected; unable to produce superoxide metabolites
	Autosomal-recessive	*NCF1* (p47phox)	7	Increased susceptibility to infection; unable to produce superoxide metabolites for killing of ingested microbes; macrophages and neutrophils affected; NCF1 and NCF2 encode components of the NADPH oxidase complex; CYBA encodes the α chain of cytochrome b
		NCF2 (p67phox)	1	
		CYBA (p22phox)	16	
IFN-γ receptor deficiency	Autosomal-recessive	*IFNGR1* (IFN-γ receptor)	6	High susceptibility to mycobacterial infections; macrophages, neutrophils, NK cells, and Th1 cells are affected
Leukocyte adhesion defect 1 (LAD-1)	Autosomal-recessive	*ITGB2* (also known as CD18)	21	Increased susceptibility to recurrent infection by bacte-ria; frequent nonresolving abscesses; defective chemo-taxis and adherence to endothelial surfaces by macrophages, neutrophils and NK cells
Leukocyte adhesion defect 2 (LAD-2)	Autosomal-recessive	*GDP-fucose transporter1*	11	Increased susceptibility to recurrent infection by bacteria and nonresolving abscesses; impaired synthesis of CD15s, a carbohydrate adhesion molecule; defects in ability of leukocytes to adhere to endothelial surfaces; reduced ability of leukocytes to move from vasculature into tissues; also causes Bombay blood group phenotype

Primary idd – defects of complement system:

Disease	Inheritance	Gene Locus	Chromosome	Consequences
C1q, C1r deficiency	Autosomal-recessive	*C1QA, C1QB, C1QC* (A, B, and C chains of C1q)	1	Increased incidence of infections; systemic lupus erythematosus (SLE) -like syndromes (type III hypersensitivities; see Chapter 8); impaired removal of immune complexes
		C1R or *C1S* (C1r and C1s)	12	
C2 deficiency	Autosomal-recessive	*C2*	6	SLE-like syndromes; vasculitis; impaired removal of immune complexes
C3 deficiency	Autosomal-recessive	*C3*	19	Recurrent pyogenic infections; impaired opsonization
C4 deficiency	Autosomal-recessive	*C4*	6	Increased incidence of infections; SLE-like syndromes; impaired removal of immune complexes
C5, C6, C7 deficiency	Autosomal-recessive	*C5, C6* or *C7*	9, 5, or 5	Increased susceptibility to *Neisseria*; unable to form membrane attack complex; SLE-like syndromes
C8 deficiency	Autosomal-recessive	*C8A* or *C8B* (α, β CD8 chains)	2	Increased susceptibility to *Neisseria*; unable to form membrane attack complex; SLE-like syndromes
C9 deficiency	Autosomal-recessive	*C9*	5	Increased susceptibility to *Neisseria*; unable to form membrane attack complex
Factor H deficiency	Autosomal-recessive	*CFH (Factor H gene)*	1	Recurrent pyogenic infections; increased activation of alternative pathway
Factor P (Properdin) deficiency	X-linked recessive	*PFC* (properdin factor, complement)	X	Increased susceptibility to infection, particularly by *Neisseria* ssp.; impaired alternative pathway; reduced stability of C3bBb convertase on microbial surfaces
Hereditary angio-edema	Autosomal-dominant	*SERPING1* (C1 inhibitor)	11	Excessive spontaneous activation of classical complement pathway (especially C2) causing local inflammation; swelling of tracheal and bronchial passages that can be life-threatening
Paroxysmal nocturnal hemoglobinuria	X-linked-recessive	*PIGA* (phosphatidylinositol glycan)	X	Impaired synthesis of phosphatidylinositol glycan (PIG); absence of PIG prevents fixation of DAF and CD59 to the host cell membrane; unable to break down complement complexes on the host cell; excessive lysis of erythrocytes

Secondary immunodeficiencies

Secondary immunodeficiencies result from a variety of factors that can affect a host with an intrinsically normal immune system, including infectious agents, drugs, metabolic diseases, and environmental conditions. These deficiencies of immunity are clinically manifested by an increased frequency or unusual complications of common infections and occasionally by the occurrence of opportunistic infections

Cause	Examples	Mechanisms
Physiologic sequelae	General malnutrition	High impact on functions with high energy requirements
	Energy metabolism	Deficiencies of amino acids crucial for energy metabolism
	Trace metal deficiencies	Deficiencies of critical cofactors
	Vitamin deficiencies	Deficiencies of critical cofactors
Therapeutic treatment	Ionizing radiation	Damages replicating cells; induces oxidative stress
	Cytotoxic drugs (including many used for cancer treatment)	Damage/kill replicating cells
	Anti-inflammatory drugs (e.g., corticosteroids)	Interfere with production of some cytokines
	Immunosuppressive drugs (e.g., cyclosporine, tacrolimus, rapamycin)	Interfere with production of some cytokines
Infection	Human immunodeficiency virus (HIV)	Kills $CD4^+$ T cells, monocytes, and even $CD8^+$ T cells; the viral *nef* gene product also redirects pMHC I molecules from the cell surface and into lysosomes where they are degraded
	Epstein-Barr virus	Produces analog of interleukin-10
	Schistosoma	Secretes enzymes capable of cleaving immunoglobulins
	Herpesvirus	Inhibits MHC class I maturation within the endoplasmic reticulum
	Human cytomegalovirus (HCMV)	Interferes with transport of peptides into ER through TAP; redirects MHC class I molecules into cytoplasm rather than to cell surface
	Chlamydia	Interferes with phagocytic function by preventing fusion of phagosomes and lysosomes
	Staphylococcus	Produces toxin that kills phagocytic cells; produces protein that interferes with FcR-driven opsonization
	Yersinia	Produces toxin that kills phagocytes
	Streptococcus	Produces toxin that kills phagocytes
	Mycobacterium	Produces toxin that kills phagocytes; inhibits acidification within phagosomes by preventing fusion with lysosomes; inhibits oxidative degradation within phagosomes
	Salmonella	Inhibits oxidative degradation within phagosomes
	Leishmania	Inhibits oxidative degradation within phagosomes
Cancer	Multiple myeloma	Increasingly oligoclonal immune response
	Burkitt's lymphoma	Epstein-Barr virus (causative agent) produces an analog of IL-10
	Waldenström's macroglobulinemia	Excessive production of immunoglobulins; increased blood viscosity
	Chronic lymphocytic leukemia (CLL)	Reduced production of immunoglobulins
	Small lymphocytic lymphoma (SLL)	Reduced production of immunoglobulins

DIAGNOSIS OF IMMUNODEFICIENCY DISEASE

1- Clinical Features.

2- laboratory investigation:

1- **C.B.C**: increase PMNL suspect phagocyte deficiency.

2- **Cuture**: to know the organism and chose the antibodies.

3- **ESR**: to follow up.

3- **X- rays**:

a. Chest pneumonia---------B cells deficiency.

b. Ribs and scapula ---------ADA.

c. Chronic sinusitis --------- Karatagener syndrome.

d. Long bone ---------- short limbs dwarfism (cartilage hair hypoplasia)

Suspected cells has specific tests:

a. B-cells:

Total Ig, IgM, G, ...

b. T cells:

i. Lymphocyte count.

ii. Delayed hypersensitivity reaction

iii. T cells and macrophage function test.

c. Phagocyte:

Neutrophil count

d. Complement: Total and specific complement count.

LABORATORY DIAGNOSIS OF IMMUNODEFICIENCY

- EVALUATION OF B-LYMPHOCYTE FUNCTION: The initial screening test for B-lymphocyte function is the measurement of serum immunoglobulines.
- Quantitative measurements of serum IgG, IgA and IgM will identify patients with panhypogammaglobulinemia as well as patients who have a deficiency of an individual class of immunoglobulin, such as selective IgA deficiency.

LABORATORY DIAGNOSIS OF IMMUNODEFICIENCY

- EVALUATION OF T-LYMPHOCYTE FUNCTION:

- Delayed type hypersensitivity (DTH) skin tests using a panel of ubiquitous antigens can be used as a screening test in older children and adults.
- The presence of a positive DTH skin test generally indicates intact T-cell function and cell mediated immunity.
- ELISPOT – production of cytokines

LABORATORY DIAGNOSIS OF IMMUNODEFICIENCY

EVALUATION OF PHAGOCYTIC FUNCTION

- reductions in phagocytic cell number in the peripheral blood and, therefore, can be detected by using a white blood cell count and differential.
- Phagocytic activity
- Phagocytic index

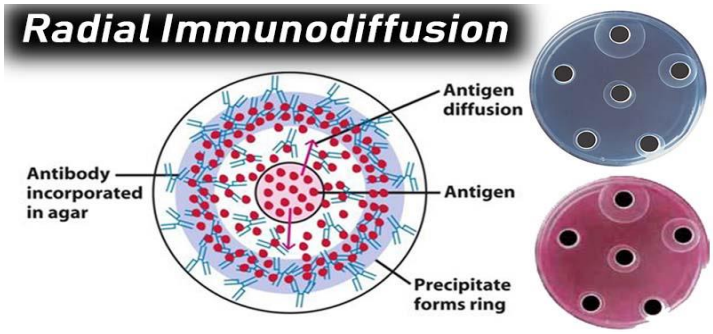
EVALUATION OF THE COMPLEMENT SYSTEM

- CH50 assay , this assay requires the functional integrity of C1 through C9.
- The identification of the individual component which is deficient rests on specialized functional and immunochemical tests which are specific for each component
- Detections of C3, C4 complement components – single radial immunodifussion

Laboratory diagnosis of immunodeficiency states

Immune system defense mechanism	Functional component	Screening tests	Other diagnostic tests
Humoral immunity	B-cell function	Quantitative immunoglobulins	IgG subclass levels Immunization/antibody titers (pneumococcal vaccine, tetanus/diptheria toxoid)
	Complement system	Total hemolytic complement assay	Evaluation/measurement of individual complement components
Cell-mediated immunity	T-cell function	CBC count with differential	
		Delayed-type hypersensitivity skin tests	Lymphocyte quantitation (T-cells and B-cells)
Innate immunity	Phagocytic function	CBC count with differential	Nitroblue tetrazolium test
			Measurement of chemotaxis, phagocytosis, bacterial killing (research assays)
			Flow cytometry for surface markers and adhesion molecules

Examples of laboratory test used for diagnosis of immunodeficiency



Immuno-diffusion is a technique for the detection or measurement of antibodies and antigens by their precipitation which involves diffusion through a substance such as agar or gel agarose. Simply, it denotes precipitation in gel. It refers to any of the several techniques for obtaining a precipitate between an antibody and its specific antigen.

This can be achieved by:

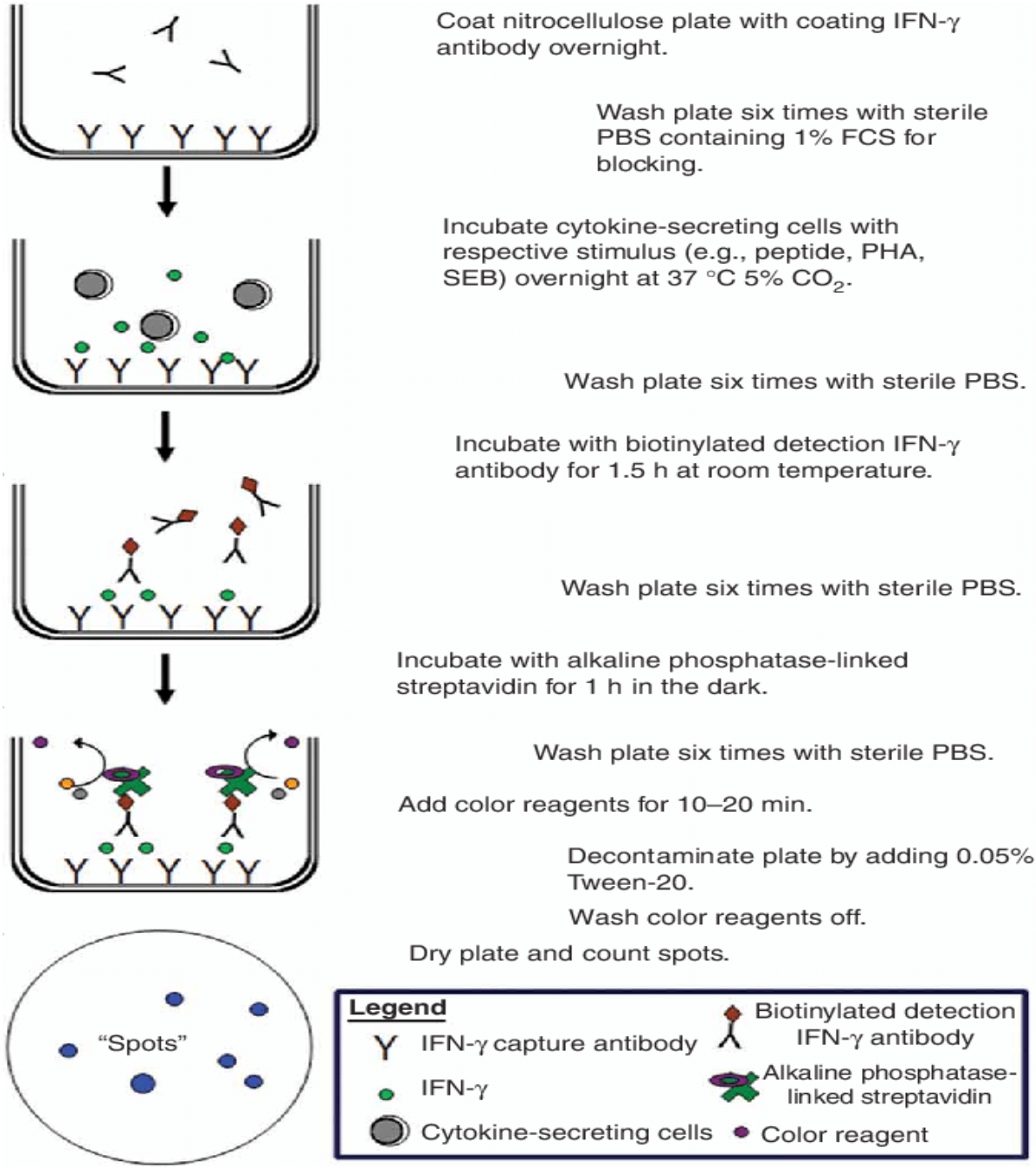
1. a) Suspending antigen/antibody in a gel and letting the other migrate through it from a well or,
2. b) Letting both antibody and antigen migrate through the gel from separate wells such that they form an area of precipitation.

Radial immunodiffusion (RID) or **Mancini method** is also known as **Mancini immunodiffusion** or **single radial immunodiffusion assay**. It is a single diffusion technique whereby a solution containing the antigen is placed into wells in a gel or agar surface evenly impregnated with antibody. The diameter of the ring that precipitates around the well as a result of antigen antibody reaction corresponds to the amount of antigen in the solution.

- Immuno-diffusion techniques are mostly used in immunology to determine the quantity or concentration of an antigen in a sample.
- Estimation of the immunoglobulin classes and complement molecules in sera,

Schematic drawing of the principle of the Elispot assay

The enzyme-linked immunospot (ELISpot) assay is a highly sensitive immunoassay that measures the frequency of cytokine-secreting cells at the single-cell level. In this assay, cells are cultured on a surface coated with a specific capture antibody in the presence or absence of stimuli. Proteins, such as cytokines, that are secreted by the cells will be captured by the specific antibodies on the surface. After an appropriate incubation time, cells are removed and the secreted molecule is detected using a detection antibody in a similar procedure to that employed by the ELISA. The detection antibody is either biotinylated and followed by a streptavidin-enzyme conjugate or the antibody is directly conjugated to an enzyme. By using a substrate with a precipitating rather than a soluble product, the end result is visible spots on the surface. Each spot corresponds to an individual cytokine-secreting cell.



Nitroblue tetrazolium test

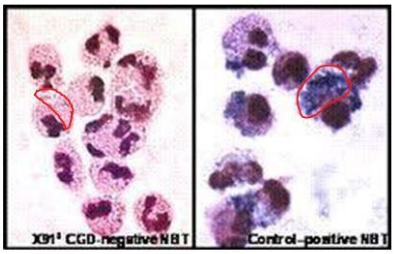
Nitroblue tetrazolium test is a blood test that measures the ability of the immune system to convert the colorless nitroblue tetrazolium (NBT) to a deep blue. This test is performed as a screen for chronic granulomatous disease (CGD). If an individual has CGD, the white cells in their blood will not turn blue when exposed to the NBT.

Chronic granulomatous disease (CGD)

- **NITROBLUE TETRAZOLIUM (NBT) DYE TEST**

- Leukocytes in a test tube are incubated with the NBT dye, which turns blue if superoxide FRs are present, indicating that the respiratory (oxidative) burst is intact

- The NBT dye test is **negative in the X-linked type of CGD** (NBT dye is not converted to a blue dye), because the NADPH oxidase enzyme complex is dysfunctional



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