بسم الله الرحمن الرحيم

Immune summary

Lec 1 : Immunoassays-and-serology

* إن شاء الله الملخص شامل كل المحاضرات *

هذا العمل صدقة جارية عن روح الزميل عمر عطية

قبل ما تبدأ:

"الباقياتُ الصالحاتُ خيرٌ وابقي."

-سبحان الله

-الحمد لله

-الله اكبر

-لا اله الا الله

-سبحان الله وبحمده

-سبحان الله العظيم

-استغفر الله واتوب إليه

-اللّٰهُمَّ صلي علي نبينا محمد

-لا اله الا انت سبحانك اني كنت من الظالمين

Serology

- What?
  - In vitro Ag–Ab reactions
- Principle
  - Immune specificity (key–lock)
- Problem
  - Cross-reaction → ↓ specificity
- Uses
  - Infections (syphilis, hepatitis viruses, Rickettsia)
  - Autoimmune (Anti-DNA antibodies in lupus or rheumatoid factor)
  - Blood / tissue typing

Tests

- Agglutination → particulate
- Precipitation → soluble
- ELISA / RIA → high sensitivity
- Immunofluorescence → see antigen on cells
- Complement fixation → complement consumption
- Western blot → confirmation

Titer: Highest dilution still positive

1/64 vs 1/4
↑Ab
Immune ideal

- Higher denominator = more Ab
- Antibodies

1. IgM → current / Acute
2. IgG → past / immunity/ current (compare results btw 2 weeks & must be increased by 4 folds) / between acute and convalescent

Patient Ag
Ab = serum

Agglutination

Ab (multivalent) + particulate Ag → lattic/شبكة → visible clumping

- Particulate Ag
1. Bacteria
2. RBCs / heme
3. ABO
4. Latex beads

- Clinical Applications

1- ABO blood typing → RBC (specific Ag) + antisera (serum with specific Ab)

2- Latex agglutination → beads + serum → pathogen detection

3- Hemagglutination → RBC clumping (≠ clotting)

✅ Simple, rapid, minimal equipment, visual results fast

Precipitation

Soluble, Multivalent Ag + multivalent Ab → lattice → precipitate

- Zone of Equivalence : Optimal ratio (Ag-Ab) → max lattice
- Prozone → Ab excess → false negative → Labs dilute serum to fix this issue
- Postzone → Ag excess → small complexes

Nephelometry (solution):
→ measured by optical density (light refraction / scattering → measure IgG/IgM

Immunodiffusion (agar):
1- **Single radial** → ring size → concentration
2- Double (**Ouchterlony**) = compare antigens → lines → identity:

A- Identity: line merges / Ag identical
B- Non-identity: crossing lines / NO shared epitopes
C- Partial identity: merged line + spur / shared + unique epitopes
-spur points to more complex antigen

Immunodiffusion (Agar)
Electrophoresis → separate proteins by electricity → Ab diffusion → precipitin **arcs**
✅ Detect abnormal globulins (myeloma)

Counter-immunoelectrophoresis (السريع)
same as 👆 but instead of waiting slow diffusion of Ab we use electric again

Radioimmunoassay = RIA

- Competitive assay btw
Radio-labeled antigen (كمية معروفة)
Patient antigen (كمية مجهولة)
On limited # of Ab

- Inverse relationship:
↓ radioactivity = ↑ patient Ag

✅ highly sensitive so measures:
1. antigen concentration (quantitative method)
2. hormones and drugs.

- **Radioallergosorbent test = RAST :** specialized RIA for IgE antibodies (allergy diagnosis)

↑ Ag patient → بمسك الحشري Ab ال
→ المشع سيك اقل
→ اقل اشعاع يقيم

Immunofluorescence

Direct:
Biopsy with specific Ag → fluorescent-labeled Ab → Fluorescence microscope.

✅ "one-step" - rapid diagnosis. It detects Ag in biopsy specimens

Indirect:
Ag + patient serum → **fluorescent-labeled anti-human IgG**

✅ "two-step" + **more sensitive than direct**

Indirect IF	Direct IF	Feature
2	1	Steps
Secondary antibody	Primary antibody	Fluorescent on
أعلى	أقل	Sensitivity
Antibody (patient)	Antigen	Target
When searching for Ab ↳ Anti nucleur Ab ↳ Double stranded DNA Ab → ⊕ in Autoimmune Disease	Autoimmune ↳ Skin ↳ kidney	Uses

سؤال متوقع جدا

Enzyme-Linked Immunosorbent Assay (ELISA)	Western Blot (Immunoblot)	Complement Fixation Test
Ag (fixed) → Patient Ab (if present) → **Enzyme-linked anti human IgG = secondary Ab** → Substrate → Color → spectrophotometer (optical density OD / **absorbance**) => Darker the colour => ↑Ab ✅ quantifies Ab without ~~radiation~~ uses **enzyme-linked conjugates** instead. as **sensitive** as RIA standard tool for screening, safe, widely available أعلى OD قياس كمية الضوء المبعثر أو المتص = Optical density كلما زادت OD زادت كمية ال Ag:Ab	Viral proteins separated by electrophoresis → transferred onto filter paper → add Antisera (patient) → Ab bind to specific bands → **Enzyme-labeled anti-IgG** → visualizes the band → confirms specific Ab are present ✅ Confirmatory test confirms positive screening results	Patient's serum heated = stop patient complement (Endogenous) 1. Patient serum = Ab 2. Known Ag 3. Complement Step1 ✅ if Ab exist → Ag-Ab complex →complement is FIXED ❎ if Ab missed → complement is FREE Step2 Interpreting Complement Fixation: Add Sensitized RBCs (surround by Ab) ➖ FREE complement attack RBCs → hemolysis ➕ FIXED complement NO attack → NO hemolysis **- Test = hemolysis to FREE RBC = NO Ab** **+ Test = NO Hemolysis to FIXED RBC = Ab exist**

ELISA: Ab هل في

Western blot: Ab against which protein

Neutralization: is the Ab functional or not

النتيجة	معنى
No hemolysis	positive → patient antibodies موجودة → complement تم استهلاكه
Hemolysis	negative → patient antibodies غير موجودة → complement حر

Neutralization Tests	Hemagglutination Tests	Antiglobulin (Coombs) Test
Have to know: Ab block toxins + viral effect on cells Ab prevent viral damage in cell culture(inhibition of cytopathic effects • In cell culture: Known virus 🦠 → patient serum (Ab) → into a living cell ✅ if neutralizing Ab exist → NO cell death (+ test) ❎ if neutralizing Ab missed → cell death (- test) • In Host animals: Ab neutralized toxin = mice 🐁 survive No Ab = No neutralization = toxin effect = mice death Ex: Plaque Reduction Neutralization Test (PRNT): conformational test for arbo virus ✅ **highly specific** (so used when there is cross reactivity in previous test)	*Have to know:* *Active **Hemagglutination:** some viruses / like **Influenza virus** clumb RBCs by a protein called **Hemagglutinin** So agglutination will happen.* **Hemagglutination Inhibition (HI test):** هل المريض عنده Ab ضد الفيروس ولا لأ ؟ Known virus 🦠 → patient serum (Ab) → add RBC ✅ if Ab exist → Ab prevent virus from binding to RBC → NO agglutination (+test) ❎ if Ab missed → virus bind to RBC → agglutination (- test) **NO clumping = antibody موجود** **Passive Hemagglutination (HI test):** RBCs coated by sluble Ag → add patient serum ✅ if Ab exist → RBC bind Ab → Agglutination (+test) ❎ if Ab missed →no binding → NO agglutination(- test) **Clumping = antibody موجود**	Have to know: Normally NO clumbing in RBC Diseases: 1. Hemolytic disease of newborn[Rhincompatibility] 2. Drug-related hemolytic anemias Ab against RBC (sensitized) => NO SYMPTOMS NO Hemlolysis NO Agglutination (so making agglutination happen will prove that Ab bound to RBC) **By (different names= same thing):** • **Antibody against Human Ab** • **Anti-human immunoglobulin** • **Coombs reagent** • **Antiserum to human immunoglobulin** • **Direct Coombs:** detects Ab on washed RBCs in vivo 1. Autoimmune hemolytic anemia 2. Hemolytic disease of newborn • **Indirect Coombs:** detects Ab in serum patient's serum + normal RBC + antiserum to human immunoglobulins is added ✅ if Ab exist in serum → Agglutination (+test) ❎ if Ab missed in serum → NO agglutination (- test) 1. Blood transfusion / prevents transfusion reactions 2. cross-matching

Flow Cytometry

Mix of cells & I need to know (how much + how many)
Flow = تيار / سيل Cyto = خليه Metry = قياس

Label cells with **fluorescent monoclonal Ab**
Each Ab bind to specific marker on specific cell (CD4 cells)
laser analysis of single cells (cells pass one by one – laser beats each cell - light 💡- send a signal)

✅ counts cells in a fluid stream.
The machine analyzes cell size & fluorescence
Quantifies specific immune cell populations

• **fluorescence-activated cell sorter (FACS)** :
specialised form of this technology.
It physically **sorts** cells based on markers

ABO Blood Groups

ABO (Antigens):
• each RBC has specific surface sugars (genetically encoded) & determine blood type

H antigen is the base structure
Group A: H Ag + we = A Ag
Group B: H Ag + galactose = B AG
Group AB: precursor for A + B Ag
Group O: only H Ag

ABO (Antibodies):
Landsteiner's Law: we have Ab against missing Ag.
These are **"natural" IgM Ab**
detectable in (early life) first 3-6 months of age,
develop against cross-reacting bacterial antigens.
They activate complement & cause hemolysis.

Type A: Anti-B Ab
Type B: Anti-A Ab
Type AB: nothing
Type O: Anti-A + Anti- B

Transfusion Compatibility

Transfusions must match donor + recipient Incompatibility causes immediate cell lysis
(Ab in recipient serum attack donor RBC)
Mismatches trigger shock and hemolysis
(due to complement activation)

Group O: universal red cell donor (NO A,B Ag)
Group AB: universal recipient (plasma NO A,B Ab)

Rh Blood Group System: defined by the D antigen / They are **IgG antibodies**.

Presence of D = Rh-positive (85% of humans)

Rh antibodies are not naturally occurring = not born with it — form after exposure to Rh-positive RBCs via transfusion or pregnancy.

• Rh incompatibility is a major clinical concern. It differs from ABO in antibody type

Hemolytic Disease of the Newborn –erythroblastosis fetalis (HDN): involves Rh incompatibility.

Rh- negative mother carries Rh-positive fetus. (baby positive)

• Maternal IgG An cross the placenta —> attack Rh positive fetal RBCs —> hemolysis in newborn (**the Direct Coombs test is positive**).

** Prevention: high titers **Anti-Rh immunoglobulins = RhoGAM injection at 28 weeks of gestation and upon delivery** . This prevents maternal sensitization

Immune Complexes in Diagnosis: Ag-Ab lattices.

Deposit in tissues (in kidney cause **glomerulonephritis) —> complement activation—> inflammatory disease**

Detected by **Fluorescent labeled complement**

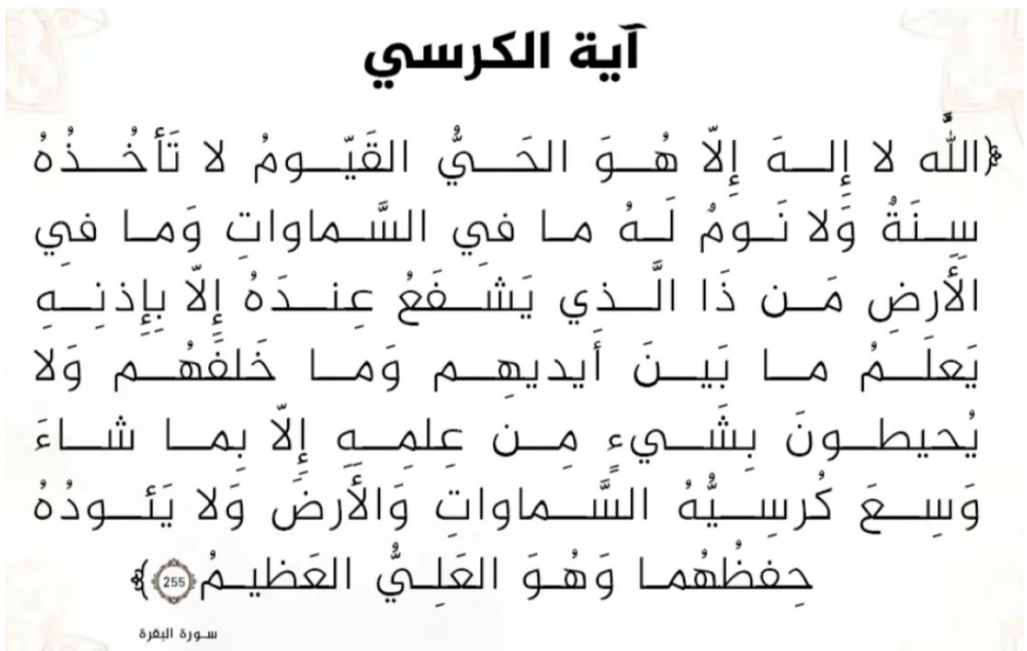
Serum complexes bind to complement as **C1q** or specific cells in culture.

Detection aids in diagnosing inflammatory diseases.

Just a small reminder: Hemolytic disease of the newborn does not occur during the first pregnancy. The first pregnancy usually leads only to maternal sensitization. During this initial exposure, the Rh-negative mother produces anti-D antibodies after contact with Rh-positive fetal red blood cells, typically at delivery.

In subsequent pregnancies, these preformed IgG anti-D antibodies can cross the placenta and attack the fetal red blood cells, leading to hemolysis. Therefore, prevention with anti-D immunoglobulin is essential to stop sensitization from occurring in the first place.

تم بفضل الله


آية الكرسي
﴿اللَّه لا إِلـهَ إِلّا هُـوَ الحَـيُّ القَيّـومُ لا تَأخُـذُهُ
سِـنَةٌ وَلا نَـومٌ لَـهُ مـا فِي السَّـماواتِ وَمـا فِي
الأَرضِ مَـن ذَا الَّـذي يَشـفَعُ عِنـدَهُ إِلّا بِإِذنِـهِ
يَعلَـمُ مـا بَيـنَ أَيديهِـم وَمـا خَلفَهُـم وَلا
يُحيطـونَ بِشَـيءٍ مِـن عِلمِـهِ إِلّا بِمـا شـاءَ
وَسِـعَ كُرسِـيُّهُ السَّـماواتِ وَالأَرضَ وَلا يَئـودُهُ
حِفظُهُما وَهُوَ العَلِـيُّ العَظيـمُ﴾
255
سورة البقرة