Common Serological tests

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What is Serology

- **Serology** is the scientific study of serum and other body fluids
- Is involves the detection of antibodies in serum.
- Traditionally applies to detection of antibodies against infections
- RA (Rheumatoid Arthritis)
- ASO (Anti Streptolysin O)
- CRP (C reactive protein)
- Widal
- VDRL
- HIV , HBsAg, HCV and other viruses and bacteria

Common terms

- Qualitative test (Yes / No)(Positive / Negative)
    - VDRL test is positive
- Semi Quantitative
    - VDRL test is positive Titer (1:8)
    - (1:8) means test is positive after serum diluted 8 times but negative after it was diluted 16 times
- Quantitative test (units in IU/ml, mg/dl)
    - RA test = 88 IU/ml
- WHO reference material available for RA,ASO, CRP
- Latex test -> Semi Quantitative results
- Nephelometry / Turbidimetry → Quantitative results

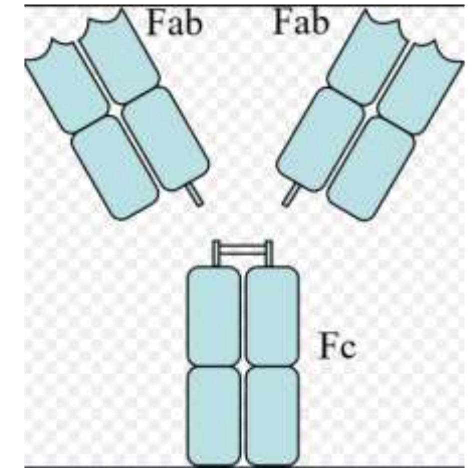
Rheumatoid Arthritis – Typical presentation

- 35 yr old Women
- Has been healthy till now
- Given history of joint pains
- Ankle, Knee and joints of the fingers pain
- Pain relieved by pain killers but does not go away completely
- Pain in the knee has increased, unable to perform daily routine
- Goes to a doctor for treatment

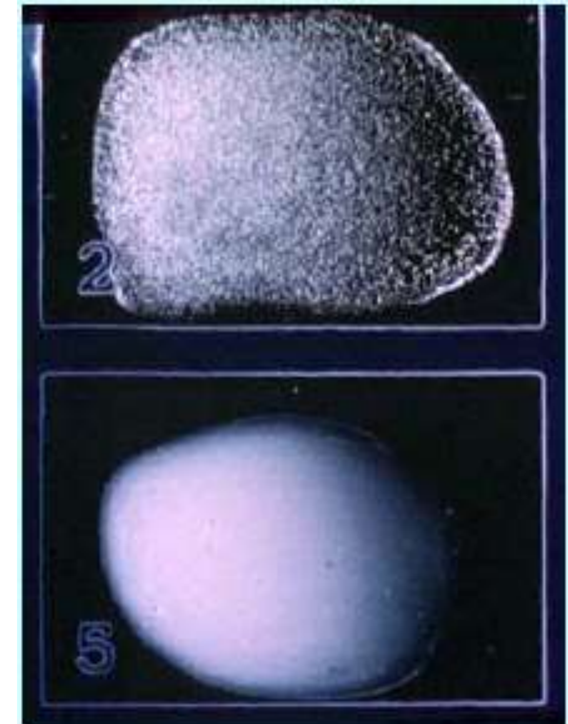
RA (Rheumatoid Arthritis)

- Rheumatoid arthritis first described by Dr. Garrod
- Rheumatoid arthritis (RA) is a symmetric, inflammatory, peripheral polyarthritis of unknown etiology **(Auto-Immune disease)**
- RA test first described by Waaler and Rose in 1940 (Rose Waller test)
- Rheumatoid factors are antibodies directed against the Fc portion of immunoglobulin G (IgG), usually IgM but can be IgG or IgA
- Test for RA factor commonly used in the diagnosis of rheumatoid arthritis
- Other auto-immune disease may mimic RA

Sir Alfred Baring Garrod



RA test – Detection methods

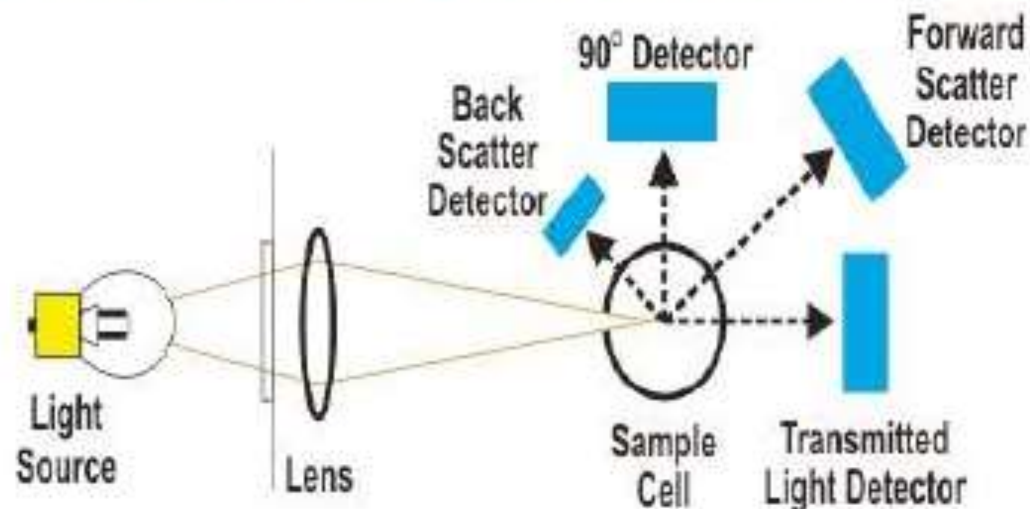


- Agglutination (Semi – quantitative)
    - Rose Waller test : Sensitized Sheep RBC’s
    - Latex test
    - Can provide a semi quantitative measurement
    - Sensitivity 10 IU/ml
    - Sample diluted 1:4 → Positive RA => 40 IU/L
- **Immunoturbidimetry / Nephelometry (Quantitative)**
- Currently done on Biochemistry analysers
    - Antibody in liquid form mixed with patient’s sample
    - Turbidity measured
    - Quantification done using a calibrator
    - International reference material from WHO available
    - RA test reported in exact quantity.
    - RA test values more than **3 times normal adds +3** to the score for the diagnosis of RA
- **Samples with positive RA test may give false positive results for other Immunoassay tests**

Immuno-Turbidimetry / Nephelometry

Scattered light may be measured by

- Turbidimetry
- Nephelometry
- In turbidimetry, the intensity of light transmitted through the medium, the unscattered light, is measured.



Diagnosis of RA

Definitions

JOINT DISTRIBUTION (0-5)	
1 large joint	0
2-10 large joints	1
1-3 small joints (large joints not counted)	2
4-10 small joints (large joints not counted)	3
>10 joints (at least one small joint)	5
SEROLOGY (0-3)	
Negative RF AND negative ACPA	0
Low positive RF OR low positive ACPA	2
High positive RF OR high positive ACPA	3
SYMPTOM DURATION (0-1)	
<6 weeks	0
≥6 weeks	1
ACUTE PHASE REACTANTS (0-1)	
Normal CRP AND normal ESR	0
Abnormal CRP OR abnormal ESR	1

≥6 = definite RA

Definition of "SEROLOGY"

Negative: ≤*ULN* (for the respective lab)

Low positive: >*ULN but ≤3xULN*

High positive: >*3xULN*

SEROLOGY (0-3)	
Negative RF AND negative ACPA	0
Low positive RF OR low positive ACPA	2
High positive RF OR high positive ACPA	3

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2 large joint : +1
1 small joint : +1
RA test 160 IU/ml : +3
History > 6 weeks : +1
ESR high : +1

Total score : 7

RA test : challenges

The major nonrheumatic diseases associated with rheumatoid factor (RF)-positivity

Condition	Frequency of RF, percent
Aging (>age 60)	5 to 25
Infection	
Bacterial endocarditis*	25 to 50
Hepatitis B or hepatitis C*	20 to 75
Tuberculosis	8
Syphilis*	Up to 13
Parasitic diseases	20 to 90
Leprosy*	5 to 58
Other viral infection*	15 to 65
Pulmonary disease	
Sarcoidosis*	3 to 33
Interstitial pulmonary fibrosis	10 to 50
Silicosis	30 to 50
Asbestosis	30
Miscellaneous diseases	
Primary biliary cholangitis*	45 to 70
Malignancy*	5 to 25
After multiple immunizations	10 to 15

RA test Challenges
RA test can be positive in other Rheumatic diseases

- Sjögren's syndrome – 75 to 95 percent
- Mixed connective tissue disease – 50 to 60 percent
- Mixed cryoglobulinemia (types II and III) – 40 to 100 percent
- Systemic lupus erythematosus – 15 to 35
- Polymyositis or dermatomyositis – 5 to 10 percent

RA test - challenges

- RA test sensitivity : About 69 %
    - Out of 100 patients with RA 31 patients may have normal RF
- RA negative Rheumatoid arthritis
    - Anti CCP antibody may help
- RA test specificity
    - Health population 4% positivity
    - Varying degree of false positive rate in other rheumatic diseases
- Possible to have patients with positive RA and negative CCP and vice versa

RA test – Important points

- RA test can be negative in patients with RA.
- Patients with positive RA test may have aggressive disease
- High Titre / Concentration of RA increase the specificity of the test
- No role for serial measurements for prognosis or treatment
- C Reactive protein used for follow up
- Anti CCP test used as an additional test
- RA test may be positive in healthy patients

SEROLOGY (0-3)	
Negative RF AND negative ACPA	0
Low positive RF OR low positive ACPA	2
High positive RF OR high positive ACPA	3

C Reactive Protein (CRP)

Dr. William Tillett

- Produced by the liver
- Discovered in sera from patients with acute phase of pneumococcal pneumonia by Dr. Tillett
- Sera of patients who had suffered from pneumococcal pneumonia reacted with C fraction extracted from Pneumococci
- Part of Acute Phase Reactants
  - ESR
  - Interleukin 6
  - TNF
  - Pro-Calcitonin

C Reactive Protein (CRP) – clinical utility

- CRP increase with increased infection, decreases as patient becomes well.
- Monitor progress of chronic disease by serial measurements
- Used in Pediatric/Neonatal age as a sign of infection
- May co-relate with ESR
- There is a lag period between infection and raised CRP
- Pro-calcitonin has replaced CRP in detection of septicemia
- CRP a Marker of Atherosclerosis in the western world
- CRP response may be muted in SLE
- CRP useful in other rheumatic diseases

C Reactive Protein – Reference intervals

- 0 – 3 mg/L : Considered normal
- 3 – 10 mg/L : Low grade inflammation (e.g. atherosclerosis)
- More then 10 mg/L : High grade inflammation
    - Latex methods have a detection limit of 5-6 mg/L
    - Cannot accurately measure below 10 mg/L
- What is HS CRP (High Sensitive CRP)
    - CRP with Sensitivity of 0.1 mg/L
    - Can measure accurately from 1 mg/L
    - HS CRP based on immunoturbidimetry / Nephelometry
    - HS CRP is a CRP assay with improved sensitivity

WIDAL TEST

- Agglutination test (F Widal in 1896) : Detect antibody to Salmonella antigens
- H (flagellar) and O (somatic) antigens of *Salmonella typhi, Para typhi A,B*
- Has been used in diagnose of typhoid fever
- Test Variants
  - Slide test
  - Tube test
  - Rapid tests
- Rising titer significant
- Doubling dilutions of patient's serum as a paired test 1 week apart recommended

Widal Test - Challenges

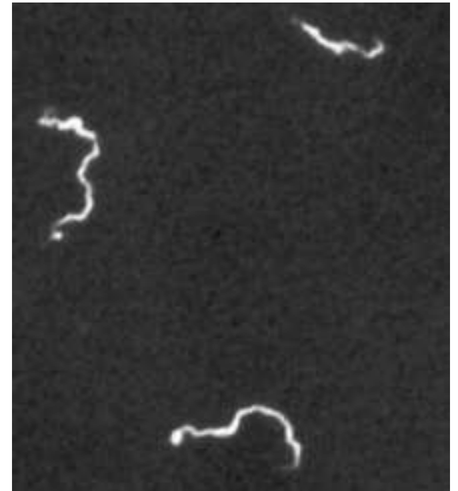
- Positive in endemic areas without fever
- Nobody waits for 1 week to do paired testing
- Tube test very laborious . TAT is next day
- Slide test not recommended
- Antigen preparations from different sources may yield discrepant results
- Significant titre : 1:160
- Blood Culture the preferred method of diagnosis
- WIDAL test should not be used to measure adequacy of treatment

Table 2. Sensitivity, specificity and predictive values of four rapid diagnostic tests for typhoid fever as determined by comparison with blood culture results

Kit	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
Cromotest® O : semiquantitative slide agglutination	95.2 (86.5–99.0)	3.6 (0.1–18.3)	25.0 (0.6–80.6)	68.6 (57.7–78.2)
Cromotest® H : semiquantitative slide agglutination	80.3 (68.2–89.4)	50.0 (30.6–69.4)	53.8 (33.4–73.4)	77.8 (65.5–87.3)
Cromotest® O: single tube Widal	87.3 (76.5–94.4)	6.9 (0.8–22.8)	20.0 (2.5–55.6)	67.1 (55.8–77.1)
Cromotest® H: single tube Widal	95.2 (86.5–99.0)	13.8 (3.9–31.7)	57.1 (18.4–90.1)	70.2 (59.3–79.7)
TUBEX®	73.0 (60.3–83.4)	69.0 (49.2–84.7)	54.1 (36.9–70.5)	83.6 (71.2–92.2)
Typhidot® IgM	75.0 (61.1–86.0)	60.7 (40.6–78.5)	56.7 (37.4–74.5)	78.0 (64.0–88.5)
Typhidot® IgG	69.2 (54.9–81.3)	70.4 (49.8–86.2)	54.3 (36.6–71.2)	81.8 (67.3–91.8)

Bulletin of the World Health Organization 2011;89:640-647. doi:10.2471/BLT.11.087627 Study done at two sub-Saharan African sites

Venereal disease research laboratory (VDRL) test



- Used in the diagnosis of Syphilis (Sexually transmitted disease)
- Syphilis → Causative agent is Treponema Pallidum
- VDRL is a nontreponemal test slide Flocculation test
- Detects antibodies to cardiolipin antigens (beta-2 glycoprotein independent)
- Extract of ox heart (diphosphatidyl glycerol) used in assay
- Pretreatment of serum required (57 ^{O}C for 30 mins)
- Rapid plasma reagin (RPR) is a variant : Does not require pre treatment
- Rapid card tests / ELISA also available

VDRL – Test Challenges

- False negative for few weeks after primary infection
  - Retesting advised at 3,6,12 months in clinically suspected individuals
- Pro-zone phenomenon seen. Testing in dilution advised
- Biological false positive seen , Titer higher than 1:8 less likely to be false positive
- Low specificity , Positive test at high titer increases specificity
- Positive test needs to be followed by specific tests
  - TPHA, FTA abs (Treponemal tests)
- Variable results in HIV positive patients
- Titers decrease after treatment (make take months or years)
- TPHA will remain positive for years in spite of treatment
- Single sample testing advised in pregnancy
- VDRL : Limited role in re-infection

ASO (Anti Streptolysin O) test

- Used to detect antibodies to Group A Streptococci
- High titers may be seen after infection with Group A Streptococal pharyngitis
- Associated with Rheumatic fever
- What is Rheumatic fever ?
  - Disease that licks the joints but bites the heart
  - Multiple transient joint involvement
- Immune mediated Inflammation of the heart → Mitral Stenosis
- ASO titre > 200 IU/ml significant