INTERPRETING COMMONLY USED RAPID DIAGNOSTIC TESTS

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Identification of the organism in infection depends upon a positive interaction between the clinician and the microbiologist, the clinician must be aware of the complexity of the tests, the microbiologist must appreciate the nature of the patient's condition and be able to assist the clinician in interpreting the laboratory result

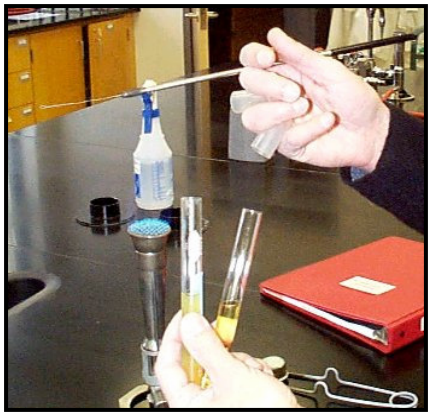
This is an ideal set-up but this is not the reality!

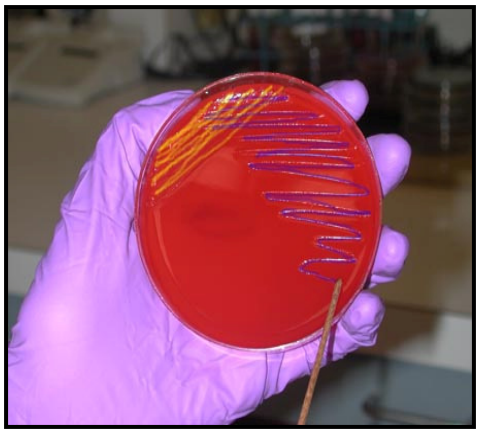
It is the clinician's choice of an "appropriate specimen" and when the result is available it is our responsibility (**not** the laboratory personnel) to properly interpret laboratory results.

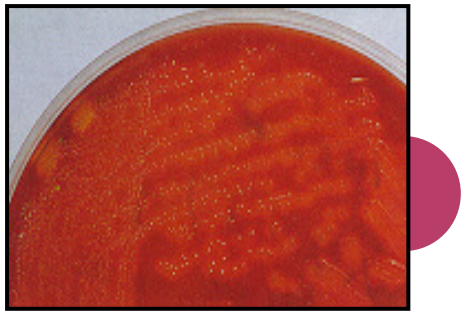
IDENTIFICATION OF THE CAUSATIVE PATHOGEN

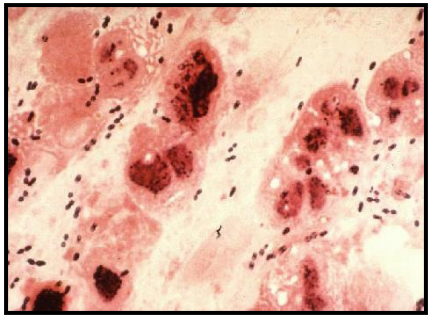
Three Main Categories

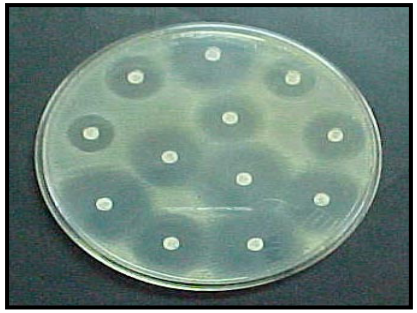
- **Identification of microorganism by isolation and culture**
- **Identification of specific microbial products (cell wall antigen, toxins)**
- **Detection of specific antibodies to a pathogen (IgM, IgG antibodies)**





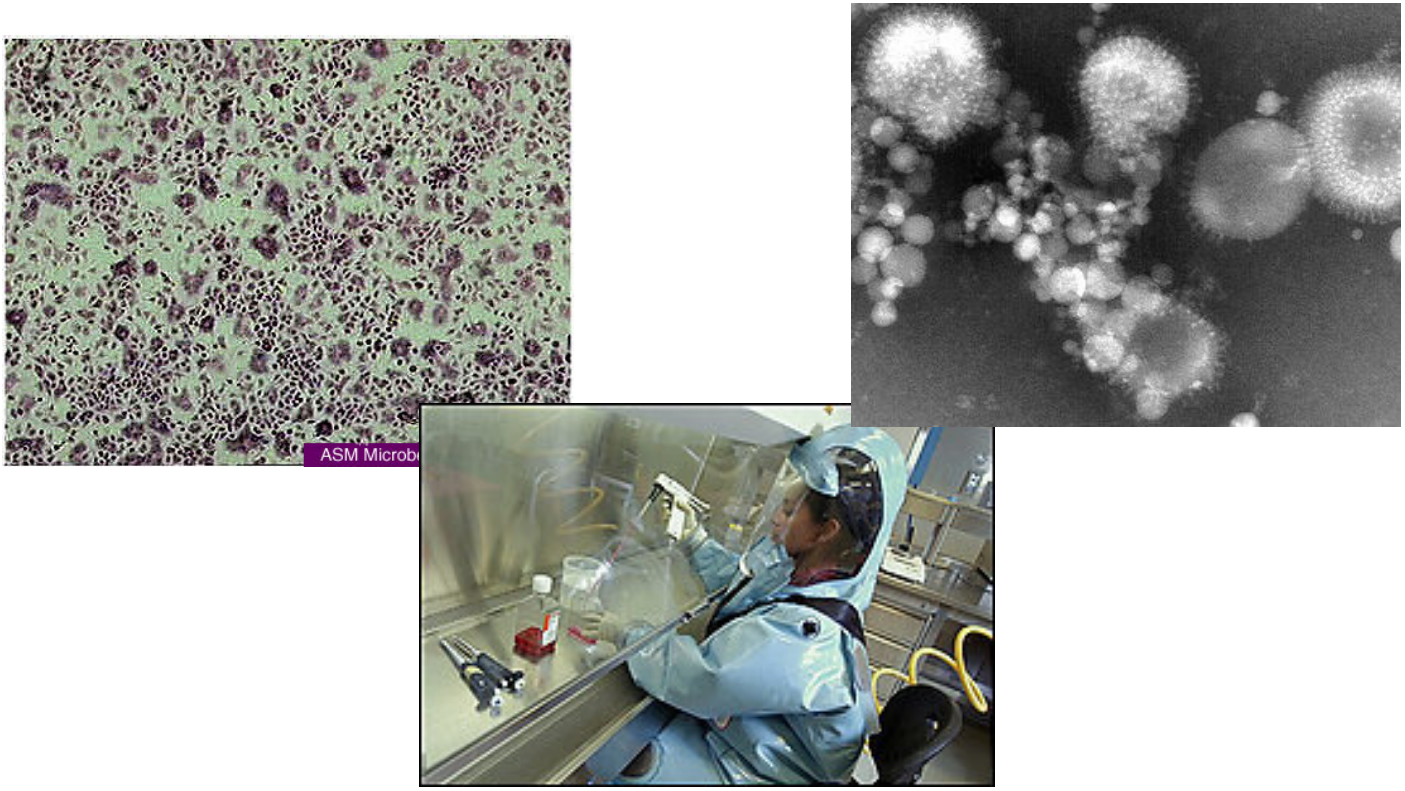






BACTERIAL CULTURE
AND ANTIBIOTIC
SUSCEPTIBILITY
REQUIRES
18 - 48 HOURS

VIRUS DETECTION



Viral culture result available 2 to 4 weeks, depending on the viruses suspected

IDENTIFICATION OF THE CAUSATIVE PATHOGEN

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COMMERCIALY AVAILABLE RAPID DIAGNOSTIC TESTS (RDTs)

Currently Available Rapid Diagnostic Tests (RDTs) for Infectious Agents (Bacteria, Virus, Parasite)

A. Latex Particle Agglutination Test

- *S. pneumoniae*
- *H. influenzae*
- Cryptococcus
- Malaria
- *E. histolytica*

B. Immunochromatographic Assay

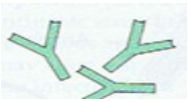
- Salmonella typhi
- Leptospira
- S. Pneumoniae
- Strep A
- TB
- Syphilis
- HIV
- Dengue
- Influenza
- RSV
- Adenovirus
- Rotavirus
- Rubella
- Malaria

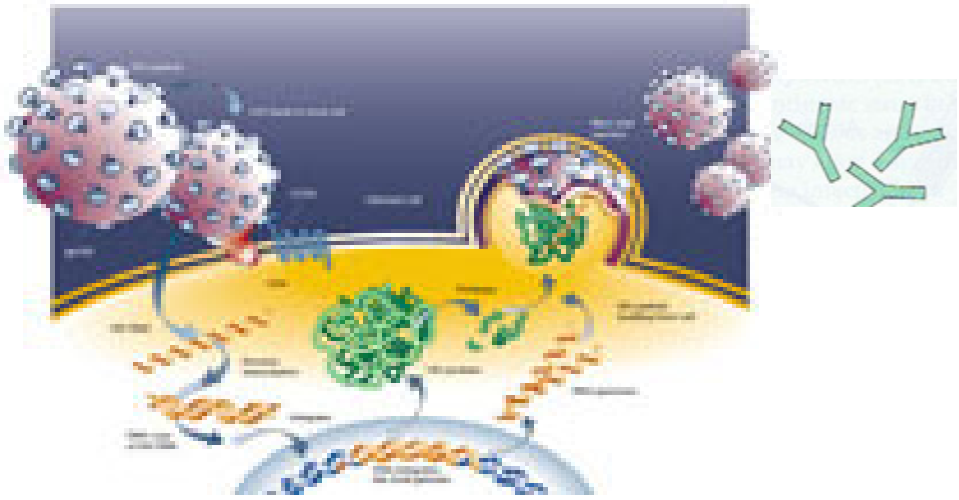
C. Enzyme linked Immunosorbent Assay (ELISA)

- Leptospira
- Dengue
- JEV
- HIV
- Syphilis
- Malaria
- *E. histolytica*

D. PCR

- Dengue
- TB

Immunological methods (Particle Agglutination, ELISA, Immunochromatographic assay) use antigen (Ag)  and antibodies (Ab)  as tools to detect microorganisms. Antigen are microbial products/ polysaccharide capsule/ foreign substances that elicit the production of antibodies



IMMUNOLOGICAL METHODS OF RDTs

PARTICLE AGGLUTINATION ASSAY

S. pneumoniae, H. influenzae, N. meningitidis

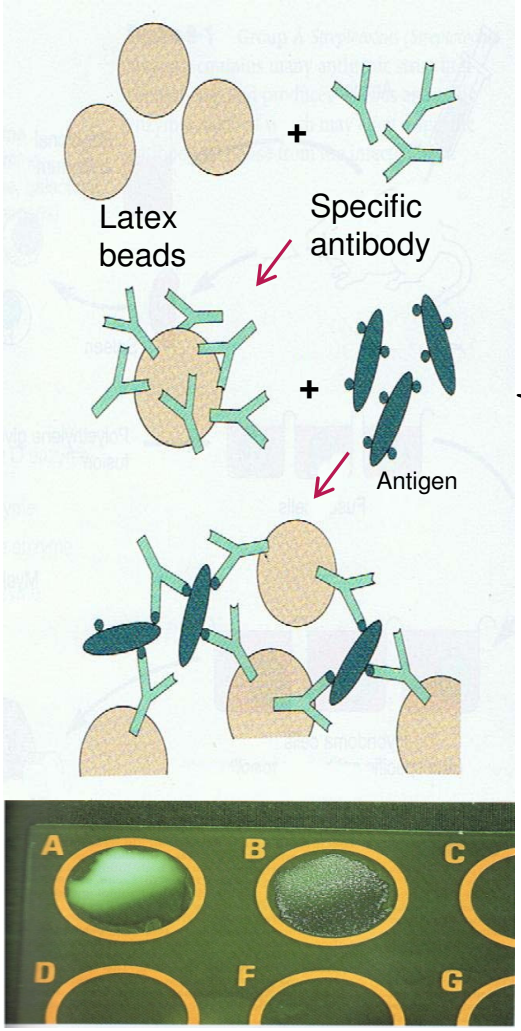
causative agents of bacterial meningitis

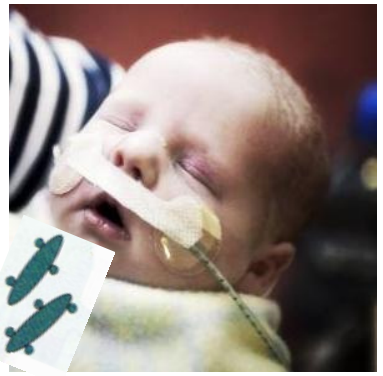
Latex particle coated with Ab

Ag adsorbed to carrier surface

Ag-Ab reaction form visible complexes (clumping)

Visible agglutination (positive test)





IMMUNOLOGICAL METHODS OF RDTs

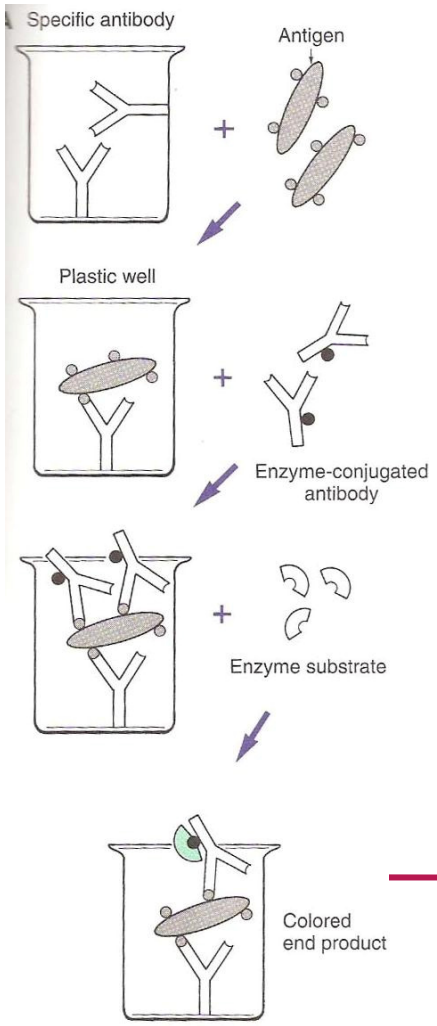
ELISA (ENZYME-LINKED IMMUNOSORBENT ASSAY)

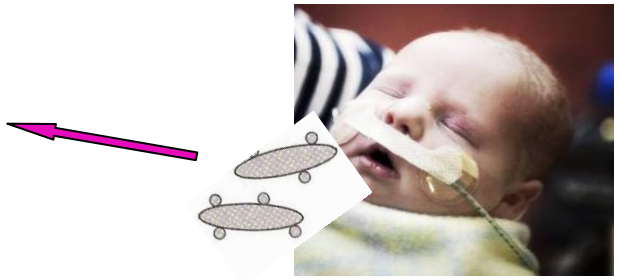
Ab bound to solid phase

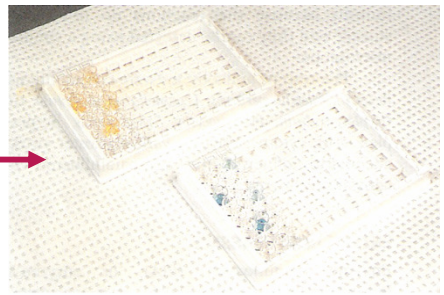
Unknown Ag added

Ag bound with Ab

Ag bound with enzyme-labled Ab

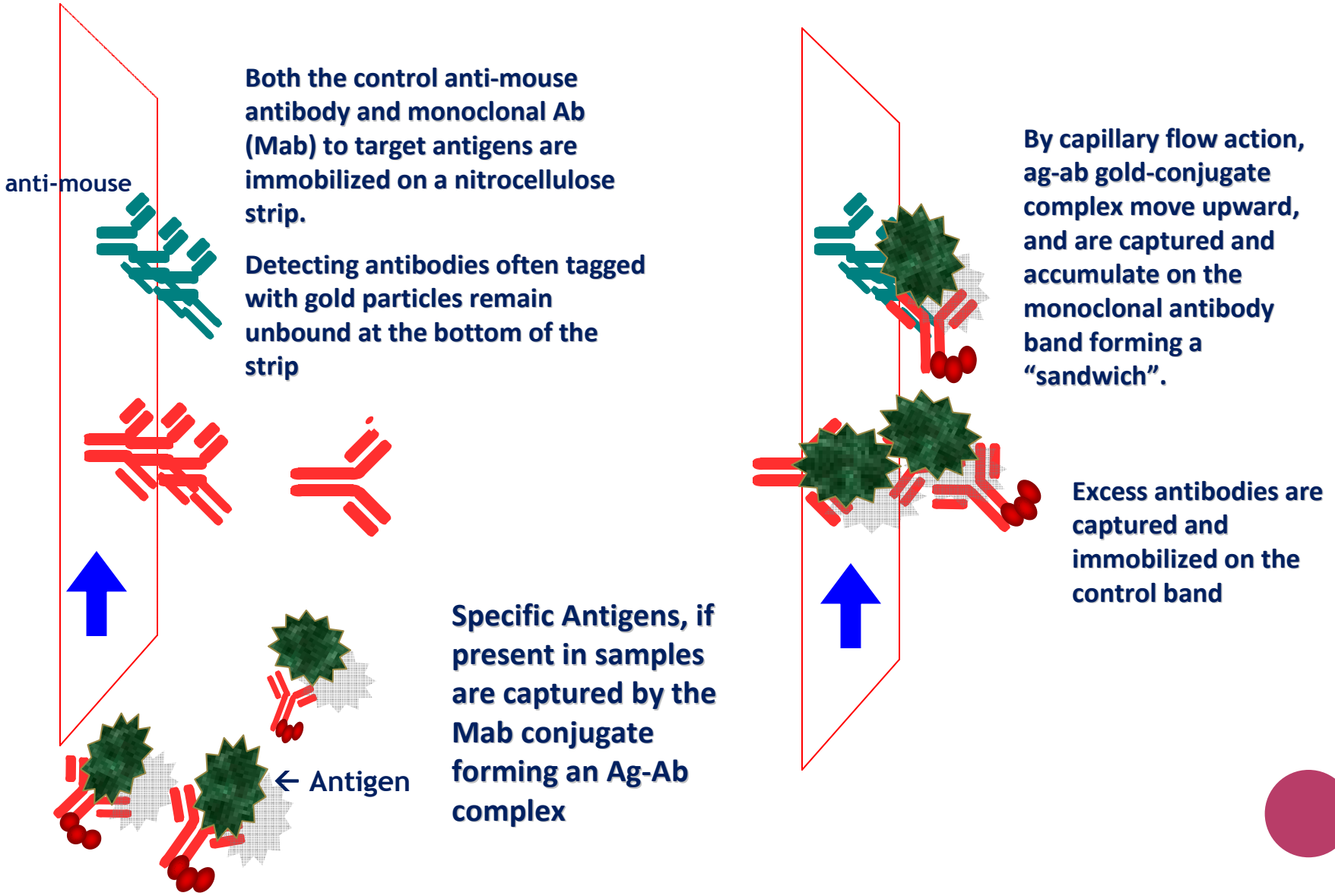






IMMUNOLOGICAL METHODS OF RDTs

IMMUNOCHROMATOGRAPHIC ASSAY



Both the control anti-mouse antibody and monoclonal Ab (Mab) to target antigens are immobilized on a nitrocellulose strip.

Detecting antibodies often tagged with gold particles remain unbound at the bottom of the strip

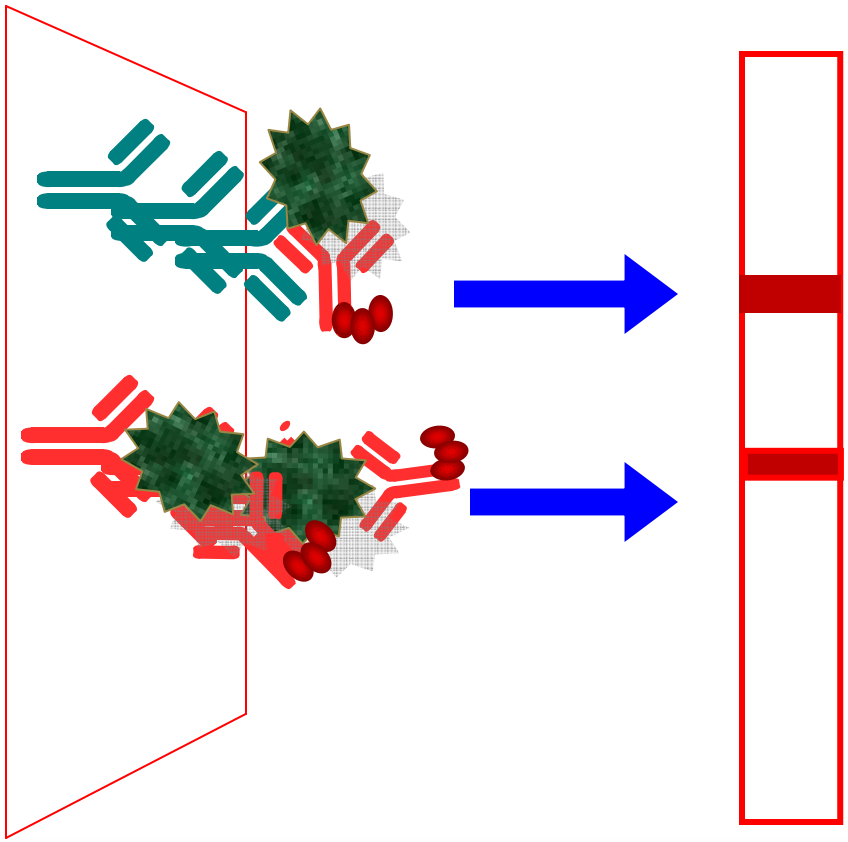
Specific Antigens, if present in samples are captured by the Mab conjugate forming an Ag-Ab complex

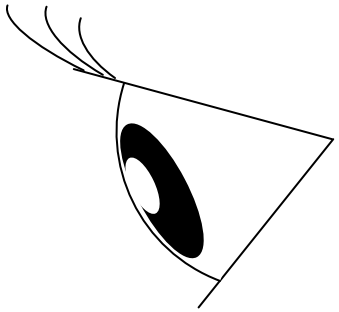
By capillary flow action, ag-ab gold-conjugate complex move upward, and are captured and accumulate on the monoclonal antibody band forming a "sandwich".

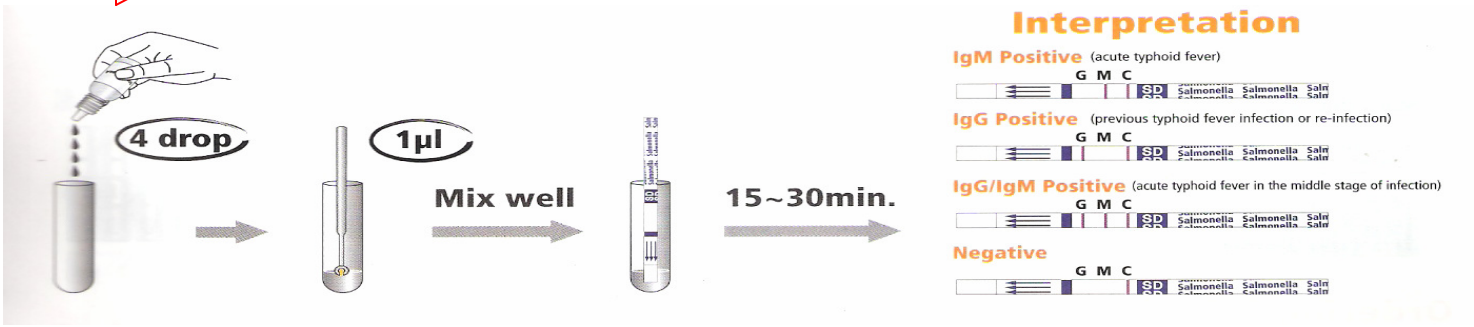
Excess antibodies are captured and immobilized on the control band

IMMUNOLOGICAL METHODS OF RDTs

IMMUNOCHROMATOGRAPHIC ASSAY

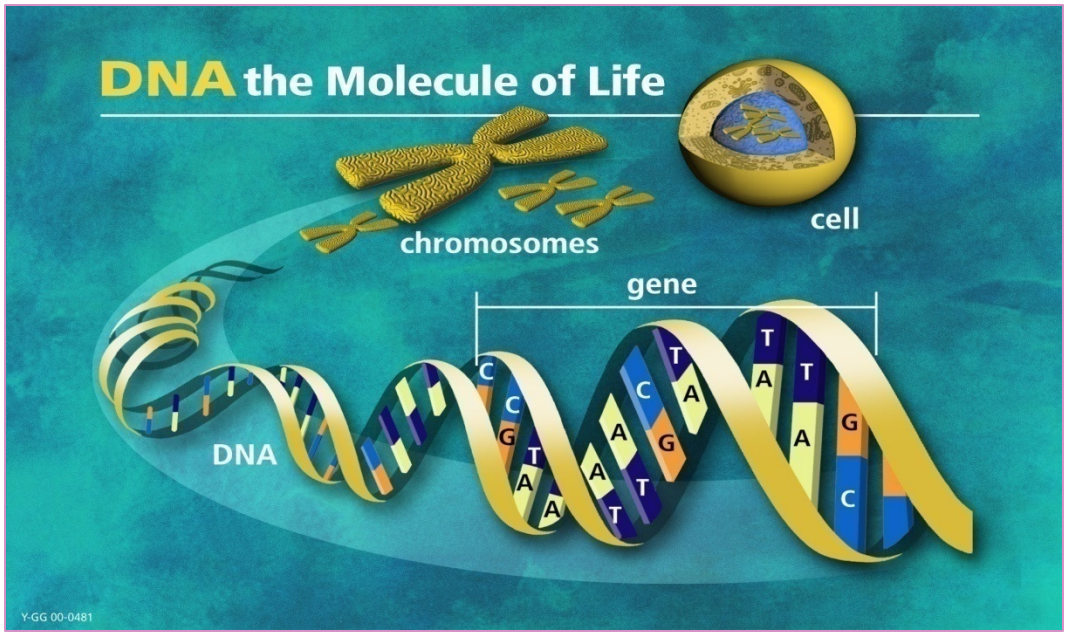






Polymerase Chain Reaction (PCR)

- Commonly used among the nucleic-acid based tests
- Ability to detect and identify organisms that cannot be grown in culture or are extremely difficult to grow or grow slowly
- The sequence of nucleotide bases in the DNA of a microorganism is ultimately its most defining feature



Polymerase Chain Reaction (PCR)

Reverse Transcription (RT) - PCR

Sample
RNA

Reverse
Transcription

cDNA

PCR

Detection

- The technique amplify fragments in clinical specimens using enzymes and DNA fragments through repeated thermal cycles
- Can amplify a single copy of DNA by million fold in < 2 hours

Anything looked at closely becomes wonderful

APPLICATIONS OF RDTs

- **To diagnose infections for organisms that are slow or difficult to grow (leptospira, legionella, *T. pallidum*)**
- **To identify microorganisms and their products (PS capsule of *S. pneumoniae*, *H. influenzae*, *N. meningitidis*, *Strep. pyogenes*, *Cryptococcus neoformans*)**
- **Provide an early or presumptive diagnosis (malaria, TB)**
- **To identify antibodies to determine acute or past infection (typhoid fever, dengue)**

TYPHOID FEVER

SALMONELLA TYPHI IgM/IgG

- **Typhidot, first known qualitative Ab detection test against *S. typhi***
- **Rapid detection of specific IgM/IgG against specific OMP of *S. typhi***
- **Use in early diagnosis of typhoid fever**
- **Indicate stages of typhoid infection(acute, convalescence, previous exposure)**
- **Can diagnose with a single serum specimen**

Typhidot IgM

Blood Culture			
	(+) N= 56	(-) N= 56	No typhoid N= 57
IgM (+)	52	44	0
IgG (-)	4	12	57
Sensitivity	93%	79%	
Specificity	100%	100%	

Typhidot IgG

Blood Culture			
	(+) N= 56	(-) N= 56	No typhoid N= 57
IgM (+)	41	44	36
IgG (-)	15	10	21
Sensitivity	73%	82%	
Specificity	63 %	73%	False + 63%

Collantes and Velmonte, Phil J Microbiol Infect Dis, 1997 .

Typhidot IgM highly sensitive and specific test

Typhidot IgG has lower sensitivity and specificity reflecting the high endemicity of typhoid fever

TYPHOID FEVER

Asian Studies on Typhidot

Author/year	Country	Sensitivity	Specificity
Choo/1994	Malaysia	95%	74.6%
Karamat/1998	Pakistan	92%	96%
Jackson/1995	Malaysia	90%	91%
Membrebe/1999	Philippines	72%	52%
Collantes/1997	Philippines	93%	79%
Gopalakrishnan/2002	Malaysia	73%	68.1%
Sherwal/2004	India	79%	-
Rizvi/2007	Pakistan	98%	-

TYPHOID FEVER

Non-typhoidal illness positive for tpyhidot	Number	Percent
UTI	3	4.3
Hepatitis A	2	4.3
ATP	1	2.2
DFS/DHF	3	4.3
URTI	4	8.7
Pneumonia	1	2.2
SVI	31	67.4
Hepatic granuloma (probable TB)	1	2.2

Membrebe and Chua, Phil J Microbiol Infect Dis, 1999

Typhidot should not be used as a screening tests for all febrile illness, Request should be limited to those with high clinical suspicion of typhoid fever

LEPTOSPIROSIS
Leptospirosis IgM

- **Mortality up to 15% in severe leptospirosis, early diagnosis can lead to appropriate antibiotic therapy and prevent complications**
- **Most assays detect IgM antibodies to a broad range of serovars positive by MAT, the gold standard**
- **Some assays use only a single serovar (eg. interrogans, patoc)**
- **The time between onset of Sx and collection of acute sample greatly affects the sensitivity**

LEPTOSPIROSIS
Leptospirosis IgM

- **Sensitivity is lowest during the first 3 days of illness, maximum sensitivity between 8 and 14 days of illness, 43% when the duration of infection <1 week and 68% when the duration >2 weeks**
- **Cross reactions with syphilis, lyme disease legionella, dengue, viral hepatitis**
- **Presumptive diagnosis of leptospirosis**
- **A practical alternative test in hospitals where MAT is not performed because of its technical complexity**

IMMUNOLOGICAL RESPONSE TO ***PRIMARY*** DENGUE INFECTION

NSI antigens

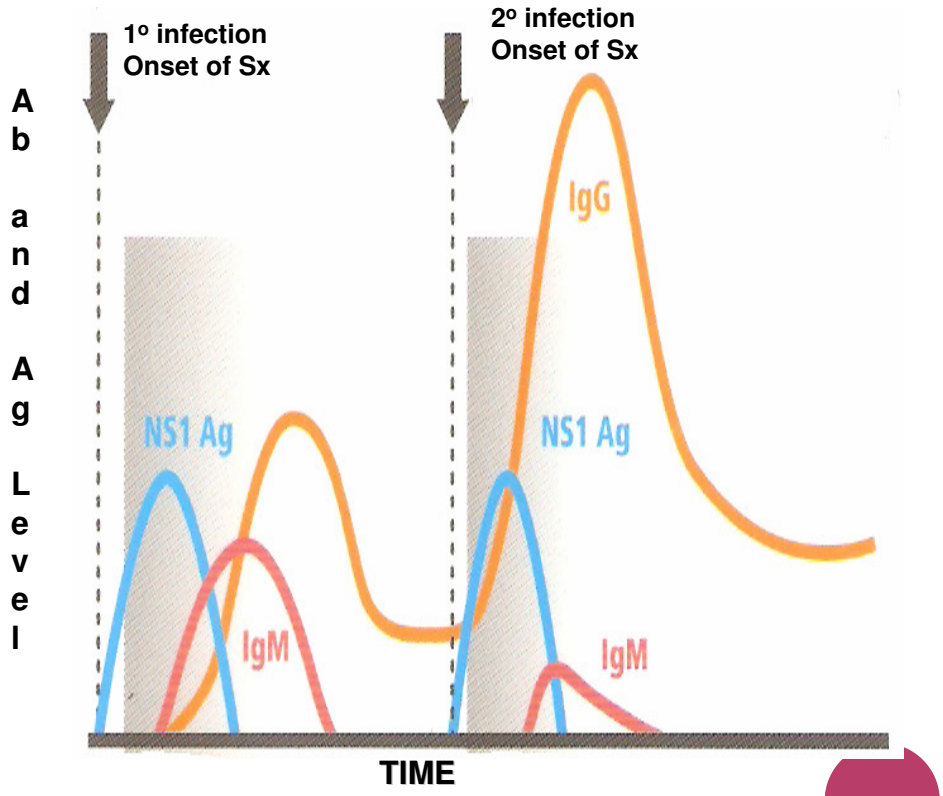
- A glycoprotein essential for viral replication and viability
- Appears from Day 1 after onset of fever and up to Day 6
- Circulate at high levels in serum during the entire clinical illness and in the first fever days of convalescence
- Not detectable once anti NS1 IgG Ab are produced (corresponds to defervescence)

IgM antibodies

- Produced approximately 5 days after Sx appear
- Rise for 1-3 weeks, may persist for up to 60 days
- May be detectable for up to 6 months

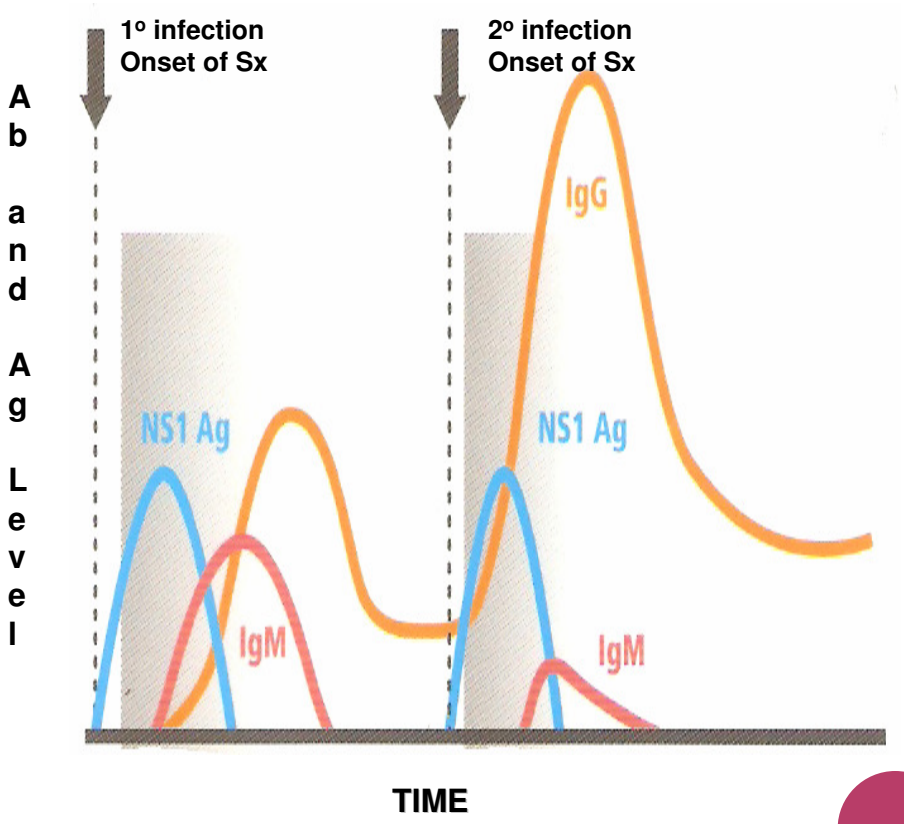
IgG antibodies

- Appear approximately 14 days after onset of symptoms
- Persist for life



IMMUNOLOGICAL RESPONSE TO ***SECONDARY*** DENGUE INFECTION

- ***NSI antigens***
- Similar response to primary infection
- ***IgM antibodies:***
- Kinetics of IgM response is variable
- 20-30% of patients do not produce IgM Ab by day 10, may not be detected until 20 days after onset of infection, some false negative results are observed
- May be produced as low or undetectable levels for a shorter period than in a primary infection
- ***IgG antibodies:***
- Rise rapidly 1-2 days after onset of symptoms
- Reach levels above those found in primary or past infection
- Persist at high levels for 30-40 days then decline to levels found in primary or past infection



DENGUE INFECTION

- **Different format of the test (ELISA, Immunochromatography take into considerations the host immnunological response to dengue**
- **Anti dengue IgM Ab cross – react with other flaviviruses (JE, St. louie encephalitis, yellow fever)**

DOH – Dengue Surveillance

RT-PCR Serotyping

Pampanga *- JBLMGH*
Dengue-3 34 (71%)
Dengue-2 11 (23%)
Dengue-1 2 (4%)
Dengue-4 1 (2%)

Region 1: *Vigan Polyclinic, Pangasinan Provincial Hospital, Region I Medical Center*

Dengue-3 6 **(100%)**

Metro Manila: *San Lazaro, RITM*
Dengue-3 165 (65%)
Dengue-2 82 (32%)
Dengue-1 7 (3%)

In summary…

Dengue-1	7%
Dengue-2	29%
Dengue-3	63%
Dengue-4	1%

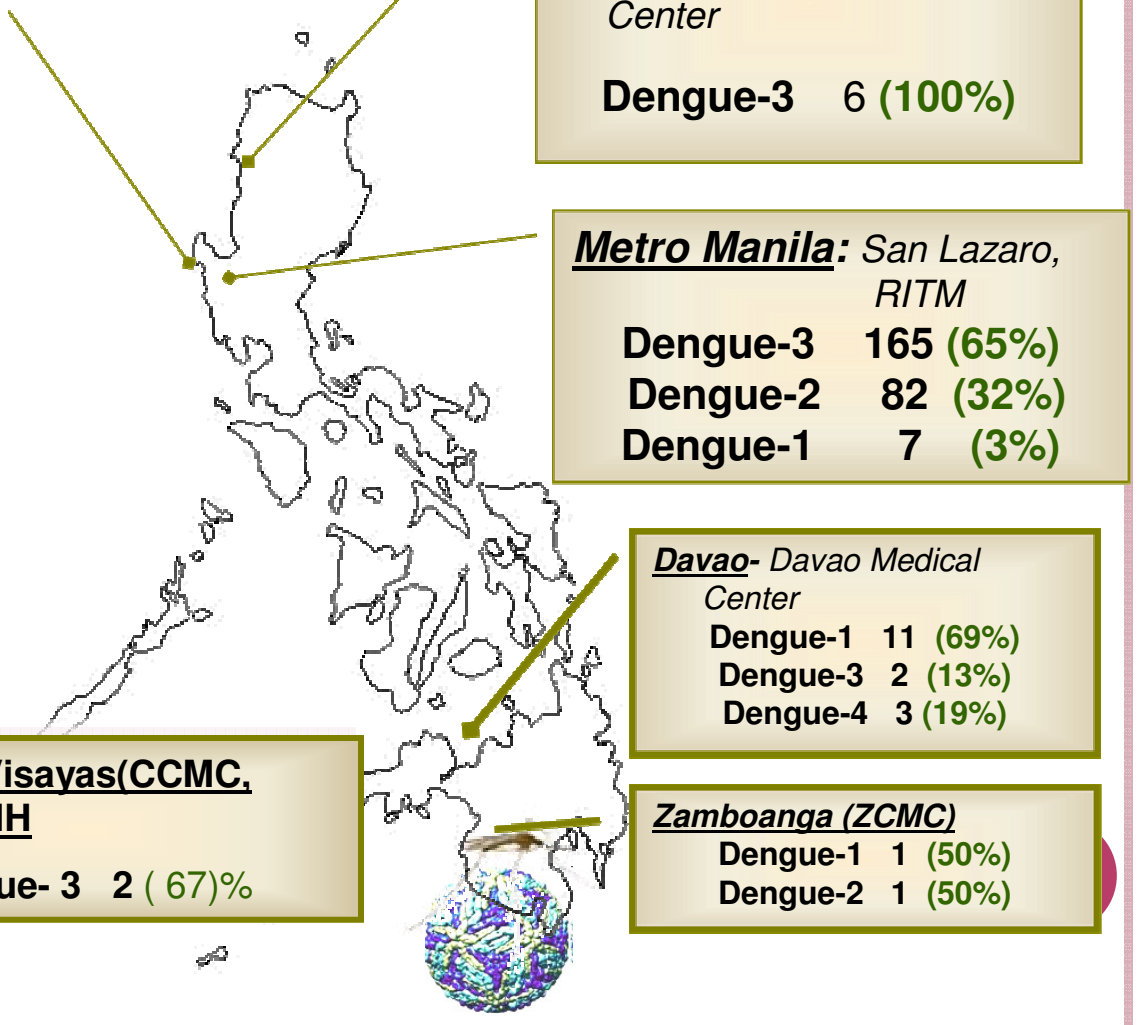
No. of pos samples= 576

Davao*- Davao Medical Center*
Dengue-1 11 (69%)
Dengue-3 2 (13%)
Dengue-4 3 (19%)

Central Visayas(CCMC, GCGMH

Dengue- 3 2 (67)%

Zamboanga (ZCMC)
Dengue-1 1 (50%)
Dengue-2 1 (50%)



*Results as of 15 Jan 2009, RITM

MALARIA RDTs

- **Can provide a useful guide to the presence of clinically significant malaria infection, particularly when good quality microscopy-based diagnosis is unavailable**
- **Qualitative presumptive detection to all species of malaria (P. falciparum, P.vivax, P. malariae, P. ovale)**
- **Qualitative and differential test for the detection of HRP-II (Histidine rich protein) specific to P. falciparum and LDH (Plasmodium lactate dehydrogenase) specific to other P. species, produced both in the sexual and asexual forms of parasite**

  > Useful for monitoring treatment efficacy

MALARIA RDTs

- **Accuracy has not been assessed systemically**
- **Few available studies with heterogenous results**
- **Maybe a useful diagnostic adjunct to microscopy in the private sector**
- **Not beneficial in areas of very high prevalence where demonstration of parasitemia will not contribute significantly to malaria management**
- **Expert microscopy is still required for specie identification and confirmation**

TB

TB PCR

- A major and relatively new advancement for TB diagnosis
- Detect presence of M. Tuberculosis and atypical mycobacteria in sputum, gastric lavage, CSF and other body fluids using a thermostabilized PCR with specific primers
- Shows great potential as a rapid and reliable test in the diagnosis of TB because of its high sensitivity and specificity

TB serology

- Detect IgM, IgG, IgA antibodies to MTB in serum, plasma or whole blood using immunochromatographic assay
- Problems with low sensitivity
- Need further evaluation on more samples

S. pneumoniae Antigen Test

- **A rapid ICT for detection of *S. pneumoniae* Ag in the urine of *adults* with pneumonia (moderate sensitivity and high specificity) and in CSF of patients of all ages with meningitis (low sensitivity and high specificity)**
- **Targets the C polysaccharide Ag common to 91 pnc serotypes**
- **Test on urine of children has low specificity, a positive test can result from nasopharyngeal colonization in the absence of disease**

PROPER SPECIMEN COLLECTION AND TRANSPORT FOR RDTs

- **Specimens**
  - **Whole blood, serum, plasma, CSF**
  - **Nasopharyngeal and throat swab**
- **Properly collected and transported**
- **Processing and transport**
  - **Collect blood in red top vacutainer or sterile test tube**
  - **Allow whole blood to clot before transport, refrigerate if transport is delayed for 1-2 days**

PROPER SPECIMEN COLLECTION AND TRANSPORT FOR RDTs

- Serum, plasma should be stored at 2-8^{o}C up to 5-7 days
- Finger stick samples are stable at ambient temperature for 1 day
- CSF should be transported in ambient temperature or preferably on wet ice
- Process all specimens without delay or store appropriately

RDTs and their VALUE in CLINICAL USE

Inf disease	*Method*	*Sensitivity*	*Specificity*	*Specimen*	*Turn-around-Time*
Typhoid fever	ICT ELISA	High	Moderate	Serum, plasma, WB	15-20 min 2-4 hrs
Leptospirosis	ICT ELISA	Moderate to High	Moderate to High	Serum,plasma, WB	15- 20 min 2-4 hrs
Dengue	ELISA PCR	High	High	Serum, plasma, WB	2-4 hrs 2-4 hrs
Malaria	ICT ELISA	Moderate to High	Moderate to High	Serum, plasma, WB	15-30 min 2-4 hrs
TB	PCR ICT	High Low to moderte	High Low to moderate	Serum, plasma, WB	1-2 hrs 15-30 min

Interpreting RDT's Results

1.Diagnostic accuracy of the test
- Sensitivity – ability to detect Ag even in small concentrations
- Specificity – cross reactions with other microbial components, may give rise to false positive results

2.Epidemiology of the disease
- In areas where disease is endemic, sensitivity and specificity and predictive values are valuable

3. Timing of sample collection
- Serum should be drawn during the acute phase of disease (when it is first discovered or suspected)
- if sample is obtained late, the peak of the titer is missed

Interpreting RDT's Results

4. Elicited IgM/IgG antibodies

- **IgM indicates primary or current infection**
- **Igm does not cross the placenta, any IgM detected in the newborn baby must have been produced by the baby itself**
- **IgG indicates past or previous infection**
- **IgM persist long after infection has run its course, complicate interpretation of RDT's**

5. Vaccination status

- **Antibodies detected maybe the result of immunization**

6. Presence of maternal antibodies may inhibit immune response

Interpreting RDT's Results

7. Immunocompetence of the patient

- **Patient's clinical state including history and PE**
- **Competency of the immune system to develop antibodies against the pathogen**

8. Care and experience of the performing laboratory

RECOMMENDATIONS ON THE USE OF RDTs

- **Rapid and accurate or presumptive diagnosis of infectious diseases through RDTs accelerates the initiation of appropriate management and may reduce unnecessary additional diagnostic testing and hospitalizations**
- **Exercise caution when selecting commercial kits to use, evaluate the tests thoroughly**
- **Some RDTs still need more validation studies to be recommended as routine diagnostic tests for specific infectious diseases**
- **RDTs should not replace the gold standard or reference diagnostic tests of proven microbiological methods**

Thank you!