Serological HIV and STI tests in surveillance surveys

Oktavija Đaković Rode, MD, MSc, microbiologist
University Hospital for Infectious Diseases
"Dr. Fran Mihaljević", Zagreb

SEROLOGICAL ASSAYS

- Determination of immune status for **epidemiological** purposes
- Methods with **highest possible sensitivity** level should be employed

TEST QUALITY

- **SENSITIVITY**
  - defined as the proportion of subjects with the disease who have a positive test for the disease
  - describes ability of an immunologic reagent to detect small amounts of antigen
- **SPECIFICITY**
  - the proportion of subjects without the disease who have a negative test
  - describes the selective reaction between antigen and its corresponding antibody

SENSITIVITY, SPECIFICITY & SEROLOGICAL ASSAYS

- The sensitivity and specificity of the assays depend greatly on the **antigen** used
- Assays that use **recombinant protein** or **synthetic peptide** antigens tend to be more specific than those using whole or disrupted virus particle

PREDICTIVE VALUE

- Predictive value is determined by sensitivity & specificity of the test and the prevalence of disease in the population being studied
- **POSITIVE PREDICTIVE VALUE**
  - the probability of disease in a patient with positive test result
- **NEGATIVE PREDICTIVE VALUE**
  - the probability of not having the disease if the test result is negative or normal

Positive predictive value (PPV) and **negative predictive value (NPV)** of a serologic test, based on an assay assumed to be 95% sensitive and 90% specific & assuming disease prevalence of 0.01%, 0.1%, 1%, 5%, 10%, and 50%

Prevalence assumption	Test results	Disease present	Disease absent	Total	PPV %	NPV %
0.01%	Positive Negative Total	95 5 100	99.990 899.910 999.900	100.085 899.915 1.000.000	**0.09**	**99.9994**
0.1%	Positive Negative Total	95 5 100	9.990 89.910 99.900	10.085 89.915 100.000	**0.94**	**99.994**
1%	Positive Negative Total	95 5 100	990 8.910 9.900	1.085 8.915 10.000	**8.8**	**99.9**
5%	Positive Negative Total	475 25 500	950 8.550 9.500	1.425 8.575 10.000	**33**	**99.7**
10%	Positive Negative Total	950 50 1000	900 8.100 9.000	1.850 8.150 10.000	**51.4**	**99.4**
50%	Positive Negative Total	950 50 1000	100 900 1.000	1.050 950 2.000	**90.5**	**94.7**

SEROLOGICAL METHODS

CLINICAL TECHNIQUES

- Complement fixation tests (CFT)
- Haemaglutination inhibition tests
- Imunfluorescence techniques (IF)
- Neutralization tests
- Counter-immunoelectrophoresis

NEWER TECHNIQUES

- Radioimmunoassay (RIA)
- Enzyme-linked immunosorbent assay (ELISA)
- Particle agglutination
- Western blot (WB)
- RIBA, Line immunoassay (LIA)

LABORATORY QUALITY CONTROL

- **Quality Control** - QC refers to the measures that must be included during each assay run to verify that the test is working properly
- **Quality Assurance** - QA is defined as the overall program that ensures that the final results reported by the laboratory are correct
- "The aim of quality control is simply to ensure that the results generated by the test are correct. However, quality assurance is concerned with much more: that the right test is carried out on the right specimen, and that the right result and right interpretation is delivered to the right person at the right time"

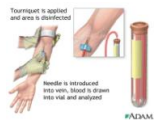
VARIABLES THAT AFFECT THE QUALITY OF RESULTS

- The educational background and training of the laboratory personnel
- The condition of the specimens
- The controls used in the test runs
- Reagents
- Equipment
- The interpretation of the results
- The transcription of results
- The reporting of results

SPECIMENT HANDLING AND TRANSPORT

- Quality laboratory results begin with proper collection and handling of the specimen submitted for analysis
- Correct patients preparation, specimen collection, specimen packaging, and transportation are of vital importance

SPECIMEN FOR SEROLOGICAL DIAGNOSTIC



HUMAN SERUM

- **Blood** should be collected aseptically by venipuncture, allowed to clot, and serum separated from clot after centrifugation
- As a general rule, the volume of blood drawn should equal 2-1/2 times the amount of serum / plasma required
  - For example, to obtain 4 mL serum or plasma, draw at least 10 mL blood

QUALITY SAMPLE

Hyperlipemic, hemolysed, heat-inactivated samples as well as samples containing particulate matter or exhibiting obvious microbial contamination may cause erroneous results!

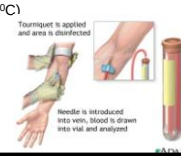
SAMPLE COLLECTION: supplies, collection procedure, storage & shipment

A. SUPPLIES

1. 5 to 7 ml red top or serum separator blood collection tubes
2. Venipuncture supplies
3. 2 mL non-glass serum storage tube

B. COLLECTION PROCEDURES

1. Use of universal precautions is recommended when collecting any biological specimen
2. Properly label a blood collection tube with patient ID and collection date
3. Using acceptable venipuncture technique, collect 5-7 ml whole blood
4. Allow a minimum of 15 minutes to allow clot form
5. Centrifuge sample at ~ 3000 rpm for 15 minutes to separate serum from clot
   - this can also be accomplished by storing the whole blood sample, in an upright position, overnight in the refrigerator (2-6^{0}C)
6. Properly label a 2 mL plastic storage tube with:
   a. Complete patient ID
   b. Serum collection date
7. Transfer 1-2 mL of serum to the storage tube



C. SAMPLE STORAGE

- Serum samples can be stored in the refrigerator at **2-6^{0}C** for up to one week
- Serum samples can be stored frozen at **-20^{0}C** or lower
- For longer storage sera should be **aliquoted** because repeatedly frozen and thawed samples may produce erroneous results
- Performance is not affected by sample that have undergone up to 3-4 freeze-thaw cycles

D. SAMPLE PACKING AND SHIPMENT

Transportation of hazardous materials
(materials known or suspected to contain biological agents)

- Diagnostic samples are shipped as "Dangerous goods"
  - They do not need to be shipped as "Infectious agent". Dangerous goods and dry ice shipping regulations must be followed for any diagnostic sample
- Refer to the following web pages for regulated shipping instructions:
  - http://www.cdc.gov/od/ohs/biosfty/shipregs.htm
- Before packing, ensure that the submission form is filled out complete and legible
- **PRIMARY, SECONDARY AND OUTER SHIPPING CONTAINER**
- Ship serum with a minimum of 5 pounds dry ice or with cold packs that are placed between secondary container and the outer shipping container
- Use extra dry ice or extra frozen cold packs for Friday, weekend or holiday shipments
- Pack carefully to avoid sample breakage and leaks
- Keep paperwork dry and separate from specimens

PACKAGING OF DIAGNOSTIC SPECIMENS

- *Such material must be packaged to withstand leakage of contents, shocks, pressure changes, and other conditions incident to ordinary handling in transportation*
- This should be interpreted to mean that the contents should not leak to the outside of the shipping container, even if there should be leakage of the primary container(s) during transit, unless the package is severely damaged, e.g. like being run over by a transport vehicle

17

PLEASE DO NOT!

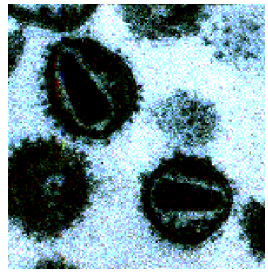
1. Ship serum in glass
2. Freeze or ship frozen whole blood samples for serum antibody testing
3. Ship samples with incomplete labeling
4. Label tubes with unnecessary (and confusing) information such as investigation's name, study numbers, cage numbers, etc.
5. Allow samples to freeze-thaw before shipping

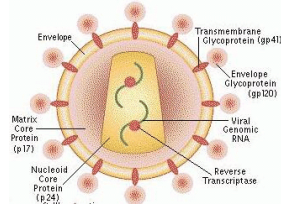
Serological diagnostic in the study

- Human immundeficiency virus
- Hepatitis B
- Hepatitis C
- Herpes simplex type 2
- Syphilis

19

Human Immunodeficiency Virus





20

HIV

- HIV antigens and antibodies appear and are detectable at different stages of the seroconversion and infection
- Risk of transmitting viral infection is linked to the window period, which takes place after infection and before the serologic markers detection

21

CHALLENGES OF HIV TESTING

- Sensitivity→ Early diagnostic (window period)
- Specificity→ Cross reactivity
- Detection of HIV-1 and HIV-2 and discrimination between the two viruses
- Easy to perform, low cost

==> One test can not fulfill these requirements
Need to perform a combination of HIV tests for screening and confirmation

22

Laboratory Diagnosis of HIV

- Serology is the usual method for diagnosing HIV infection.
- Serological tests can be divided into **screening** and **confirmatory** assays.
- Screening assays should be as sensitive whereas confirmatory assays should be as specific as possible.

23

SEROLOGICAL DIAGNOSTIC

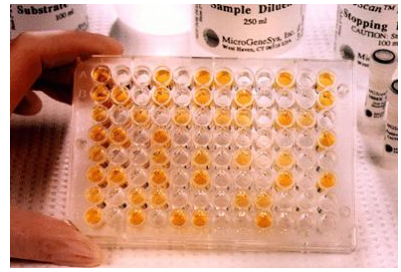
- **Screening assays**
  - **EIA**s are the most frequently used screening assays.
    - The sensitivity and specificity of the presently available commercial systems now approaches 100% but false positive and negative reactions occur.
- **Confirmatory assay**
  - **Western blot (WB)** is regarded as the gold standard for serological diagnosis.
    - However, its sensitivity is lower than screening EIAs.
  - **Line immunoassays (LIA)** incorporate various HIV antigens on nitrocellulose strips.
    - The interpretation of results is similar to WB. It is more sensitive and specific.

24

Evolution of HIV ELISAs

- First generation
  Ag : Purified lysates of HIV
  Poor sensitivity and specificity
  - Second generation
    Ag : HIV-recombinant proteins and/ or peptides
    Detection of HIV-1 and HIV-2
    Poor sensitivity, improved specificity
- Third generation
  Ag : HIV-recombinant proteins and/ or peptides
  Detection of IgM and IgG, improved sensitivity
  Detection of HIV group O
- Fourth generation
  Capacity to detect p24 Ag and antibodies

ELISA for HIV antibody



Microplate ELISA for HIV antibody: coloured wells indicate reactivity

Ag-Ab EIA

- In order to reduce the window period time of HIV infection and laboratory detection, a new generation screening EIA had been developed
- HIV Ag-Ab test allows the simultaneous detection of anti-HIV-1 (M&O groups) and anti-HIV-2 antibodies, and antigens in human serum or plasma
- A significantly higher sensitivity than previous assays
- **Genscreen Ultra HIV Ag-Ab, BioRad**
- **The diagnostic window is reduced by about 2.7-2.8 days**

Genscreen Ultra HIV Ag-Ab BioRad

- Solid phase is coated with:
  - Monoclonal Ab against p24 HIV-1 Ag
  - Purified Ag: gp160 recombinant protein, a synthetic peptide mimicking a totally artificial (i.e. encoded by no existing virus) HIV-1 group O-specific epitope and a peptide mimicking the immunodominant epitope of the HIV-2 envelope protein
  - **Sensitivity 100%**
  - **Specificity**
    - **Blood donors 99.95%**
    - **Clinical samples 99.75%**
    - **Different pathologies (not linked to HIV) 98.72%**
      - pregnant women, RF, autoimmune diseases, chronic renal failure, dialysis, other viral or bacterial diseases (HAV, HBV, HCV, Rubella, Toxoplasmosis, Mumps, Measels, CMV, HSV, EBV, VZV, HTLV1, Malarial, Flu vaccinated patients)

Genscreen Ultra HIV Ag-Ab, BioRad
LIMITS OF THE TEST

- Very low titre of HIV antigen or antibodies may not be detectable during the first stage of the infection, consequently a negative result indicates that the tested sample does not contain detectable HIV antigen or anti-HIV antibodies. However, such a result does not prelude the possibility of exposure to an HIV-1 / HIV-2 infection.
- The variability of HIV-1 (group M and O) and HIV-2 allows the possibility of false negative reactions. No known test method can offer complete assurance that HIV virus is absent.
- Highly sensitive ELISA may produce false positive results.
- To verify the specificity of the reaction, every positive result should be **confirmed** with an appropriate method (western blot).

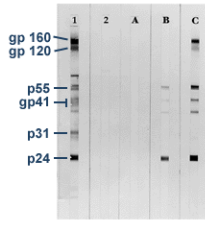
CONFIRMATORY TESTS

- **Western Blot (WB)**
  Purified antigenes from lysates of HIV on nitrocellulose bands
- **Line immuno assays (LIA)**
  HIV synthetic peptides on bands

WESTERN BLOT (WB)
for HIV antibody

HIV-1 Western Blot

- Lane1: Positive Control
- Lane 2: Negative Control
- Sample A: Negative
- Sample B: Indeterminate
- Sample C: Positive



Line immunoassays (LIA)
for HIV antibody

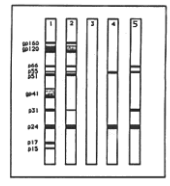


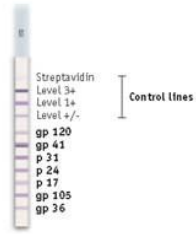
Figure:
Examples of reactions by an HIV-1 Western blot:
1. Positive control (strong)
2. Positive control (weak)
3. Negative control
4. Indeterminate profile
5. Indeterminate profile (highly suggestive)

- Important antibodies are those against the envelope glycoproteins gp120, gp160, gp41, gp105, gp36
- p24 antibody is usually present but may be absent in the later stages of HIV infection

HIV confirmation
INNO-LIA HIV I/II Score, Innogenetics

- A line immunoassay (LIA) to confirm the presence of antibodies against HIV-1, including group O, and HIV-2 in human serum or plasma
- Differentiate between HIV-1 and HIV-2
- In contrast with WB technique the Ag are fixed as fine lines on the membrane, avoiding difficult-to-control processes such as electrophoresis and blotting, and resulting in a highly reproducible assay

INNO-LIA HIV I/II Score



Positive for HIV-1 Ab:
•One HIV-1 Ag (sgp 120 or 41 gp) positive(≥1+): max reactivity of ± is allowed of one HIV-2 line (sgp 105 or gp 36)
•Both HIV-1 Ag (sgp 120 & gp 41) positive (≥1+): max reactivity of 1+ is allowed on one HIV-2 line (sgp 105 or gp 36)

Positive for HIV-2 Ab:
•One HIV-2 Ag (sgp 105 or gp36) positive(≥1+): max reactivity of ± is allowed on one HIV-1 line (sgp 120 or gp 41)
•Both HIV-2 Ag (sgp 105 & gp 36) positive (≥1+): max reactivity of 1+ is allowed on one HIV-1 line (sgp 120 or gp 41)

Positive for HIV antibodies (untypable):
•Different combination as the ones described above

HIV confirmation
INNO-LIA HIV I/II Score, Innogenetics

- All HIV strains detectable with one strip:
  - HIV-1, HIV-2, HIV-1 group O
- Recombinant proteins and synthetic peptides from HIV-1 and HIV-2, and a synthetic peptide from HIV-1 group O are coated as discrete lines on a nylon strip with plastic backing
  - **Specificity**
    - Blood donors **96.7%**
    - Clinical samples **96.1%**
    - Different pathologies (not linked to HIV) **94.4%**
  - **Sensitivity 100%**

RAPID HIV TESTS

CHARACTERISTICS
- Simple
- Provide same day results

Four immunologic principles
Particle agglutination
Immunodot (dipstick)
Immunofiltration (flow-through device)
Immunochromatography (lateral flow)

SUITABLE FOR LOW VOLUMES AND LIMITED RESSOURCES TESTING SITES

Rapid tests and ELISAs advantages

RAPID TESTS	ELISAs
Flexible	Batch capability good for ≥ 100 specimens at same time
Time to result (< 30 min)	Can be automated
Skilled staff not required	Centralized (QA/QC)
Very easy to interpret results	May be highly sensitive for seroconverters
On site testing	Cost per test less than cost per rapid test
Minimal equipment and reagents required	
Tests can be stored at room temperature	
Complexity 1-3	

37

Rapid tests and ELISAs disadvantages

RAPID TESTS	ELISAs
Small numbers for each test run	Less flexible (need a minimum numbers for maximum)
QA/QC at multiple sites	Time to result (>1.5h)
Some tests less sensitive (for seroconverters)	Complexity 4
May cost more per individual test than EIA	Skilled technician required
Interreader variability may provide inconsistent results (e.g., particle agglutination)	Refrigerated reagents
	Requires sophisticated equipments
	High equipment maintenance

38

HEPATITIS

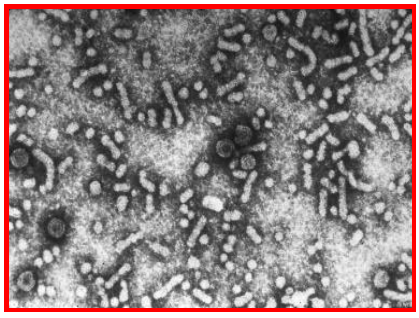
	A	B	C	D	E
Source of virus	feces	blood/ blood-derived body fluids	blood/ blood-derived body fluids	blood/ blood-derived body fluids	feces
Route of transmission	fecal-oral	percutaneous permucosal	percutaneous permucosal	percutaneous permucosal	fecal-oral
Chronic infection	no	yes	yes	yes	no
Prevention	pre/post-exposure immunization	pre/post-exposure immunization	blood donor screening; risk behaviour modification	pre/post-exposure immunization; risk behaviour modification	ensure safe drinking water

Serological diagnostics of hepatitis used in the study

- **Hepatitis B**
  - HBsAg ELISA
  - Anti-HBs ELISA
  - Anti-HBc ELISA
- **Hepatitis C**
  - Anti-HCV ELISA

40

Hepatitis B Virus



41

HBV SEROLOGICAL DIAGNOSTICS

- A battery of serological tests are used for the diagnosis of acute and chronic hepatitis B infection:

- **HBsAg** - used as a general marker of infection
- **Anti-HBs** - used to document recovery and/or immunity to HBV infection
- **anti-HBc IgM** - marker of acute infection
- **anti-HBc IgG** - past or chronic infection
- **HBeAg** - indicates active replication of virus and therefore infectiveness
- **Anti-HBe** - virus no longer replicating. However, the patient can still be positive for HBsAg which is made by integrated HBV

- ***HBV-DNA*** *- indicates active replication of virus, more accurate than HBeAg especially in cases of escape mutants. Used mainly for monitoring response to therapy*

42

HEPATITIS B – ELISA Ag

HBsAg:

•ETI-MAK-4 (DiaSorin)

for the qualitative determination of hepatitis B surface antigen in human serum or plasma

(wells coated with anti-HBs mouse monoclonal Ab)

Diagnostic specificity

- Blood donors **99.7%**
- Clinical samples (negative population) **98.8%**

Diagnostic sensitivity 100%

43

HEPATITIS B – ELISA Ab

anti-HBs:

•ETI-AB-AUK3 (DiaSorin)

for the qualitative/quantitative determination of total antibodies to hepatitis B surface antigen in human serum or plasma samples

Diagnostic specificity 98.2%- 99.2%

Diagnostic sensitivity 99.1%

44

HEPATITIS B – ELISA Ab

anti-HBc:

- **ETI-AB-COREK PLUS (DiaSorin)**

  for the qualitative determination of total antibodies to hepatitis B core antigen in human serum or plasma samples by competitive ELISA

  Diagnostic specificity

  - Blood donors **99.8%**
  - Clinical samples (negative population) **99.6%**

  Diagnostic sensitivity 100%

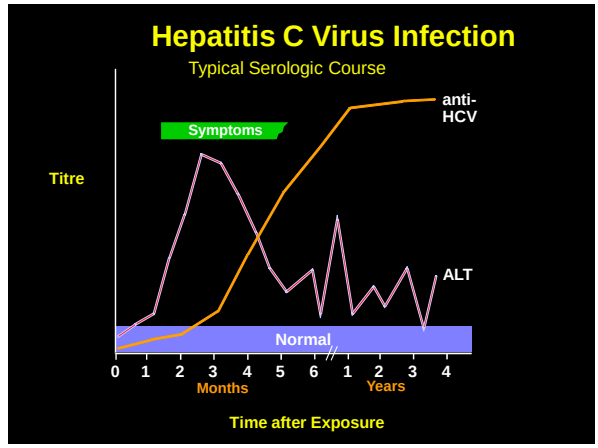
Interpretation of the Hepatitis B Panel

Tests	Results	Interpretation
HBsAg Anti-HBc Anti-HBs	Negative Negative Negative	Susceptible
HBsAg Anti-HBc Anti-HBs	Negative Positive Positive	Immune due to natural infection
HBsAg Anti-HBc Anti-HBs	Negative Negative Positive	Immune due to hepatitis B vaccination
HBsAg Anti-HBc IgM anti-HBc Anti-HBs	Positive Positive Positive Negative	Acutely infected
HBsAg Anti-HBc IgM anti-HBc Anti-HBs	Positive Positive Negative Negative	Chronically infected
HBsAg Anti-HBc Anti-HBs	Negative Positive Negative	Four interpretations possible*

*www.cdc.gov/ncidod/diseases/hepatitis/b/Bserology.htm

Hepatitis C Virus

- Genome resembled that of a **flavivirus** positive stranded RNA genome of around 10,000 bases
  - morphological structure remains unknown
- HCV has been classified into a total of six genotypes (type 1 to 6) on the basis of phylogenetic analysis



LABORATORY DIAGNOSIS OF HCV

- **HCV antibody** - generally used to diagnose hepatitis C infection
  - Not useful in the acute phase as it takes at least 4 weeks after infection before antibody appears
- **HCV-antigen** - EIA for HCV antigen is available
  - It is used in the same capacity as HCV-RNA tests but is much easier to carry out
- **HCV-RNA** - various techniques are available e.g. PCR and branched DNA
  - May be used to diagnose HCV infection in the acute phase. However, its main use is in monitoring the response to antiviral therapy

HEPATITIS C – ELISA Ab

anti-HCV:

•ETI-AB-HCVK-4 (DiaSorin)

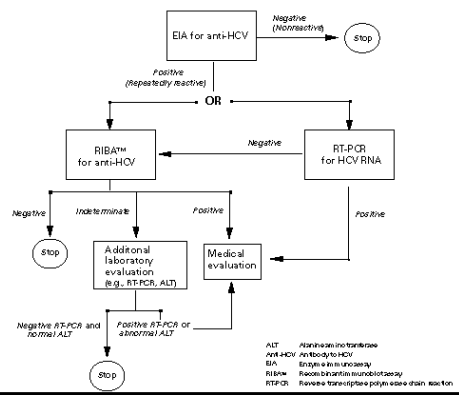
for the qualitative determination of antibodies to hepatitis C virus in human serum or plasma samples

Diagnostic specificity

- Blood donors **99.7%**
- Clinical samples (negative population) **100%**

Diagnostic sensitivity 100%

FIGURE 3. Hepatitis C virus (HCV)-infection–testing algorithm for asymptomatic persons



HERPESVIRUSES

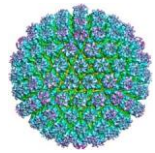
DESIGNATION	COMMON NAME	GENUS
HHV-1	Herpes simplex virus - 1 (HSV-1)	α_1
HHV-2	Herpes simplex virus - 2 (HSV-2)	α_1
HHV-3	Varicella zoster virus (VZV)	α_2
HHV-4	Epstein-Barr virus (EBV)	γ_1
HHV-5	Cytomegalovirus (CMV)	β_1
HHV-6	Human herpesvirus - 6 (HHV-6)	β_2
HHV-7	Human herpesvirus – 7 (HHV-7)	β_2
HHV-8	Human herpesvirus – 8 (HHV-8, KSHV)	γ_2

HERPESVIRUSES
GENERAL CHARACTERISTICS

- Ubiquitous in the population
  - except HSV-2, HHV-8
- Primary infections usually inapparent
- Causes latent and recurrent infections

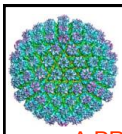
LATENCY

ESTABLISHMENT
MAINTENANCE
REACTIVATION



Properties of HSV

- The genome of HSV-1 and HSV-2 share 50 - 70% homology
- They also share several cross-reactive epitopes with each other. There is also antigenic cross-reaction with VZV

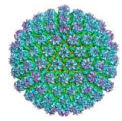


GENITAL HERPESVIRUSES

A PROBLEM THAT KEEPS ON GROWING

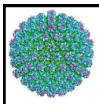
HSV-2, HSV-1

- ~45 million people ages 12 and older are infected with HSV-2
- 90% of those who test seropositive for HSV-2 have no history of genital lesion
- >1/3 experiencing >=6 recurrences in the first year following HSV-2 infection
- 10-20% of genital herpes are HSV-1



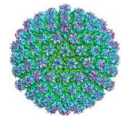
GENITAL HERPES

- Unrecognized and misdiagnosed
- Pregnancy
- Immunocompromised
- HSV coinfection
  - HIV + HSV-2
  - HHV-8 + HSV-2
- Transmission via asymptomatic viral shedding



LABORATORY DIAGNOSIS

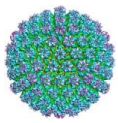
- Direct Detection
  - Electron microscopy of vesicle fluid - rapid result but cannot distinguish between HSV and VZV
  - Immunofluorescence of skin scrappings - can distinguish between HSV and VZV
  - PCR - now used routinely for the diagnosis of herpes simple encephalitis
- Virus Isolation
  - HSV-1 and HSV-2 are among the easiest viruses to cultivate. It usually takes only 1 - 5 days for a result to be available
- **Serology**
  - Not that useful in the acute phase
  - Patients recently infected with genital herpes may take several weeks/months to seroconvert!



GENITAL HERPES

We cannot answer
WHEN and **WHERE**
of viral shedding,
but can affirm the **WHO**
(nearly all infected individuals)!

Leone P. Medscape 2004.



FDA-Approved serologic tests available

- **Type-specific gG - based serology commercial kits**
- **Glycoprotein gG testing** is more sensitive and specific than crude antigen testing
- ELISA, Western blot, SIA (immunoblot) methods all show acceptable sensitivity and specificity

Herpes simplex type 2 (HSV-2)

- Anti-HSV-2 ELISA
  - **ETI-HSVK-G 2** (DiaSorin)
    - HSV-2 gG 2 recombinant glycoprotein

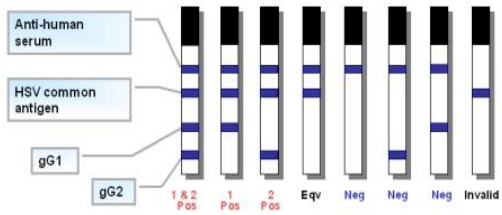
    Diagnostic specificity: **99.3%**

    Diagnostic sensitivity: **85.0%**
- Immunoblot (differentiate HSV-1 & HSV-2)
  - **HerpeSelect 1 and 2 immunoblot IgG** (FOCUS Diagnostics)

HerpeSelect 1 and 2 immunoblot IgG

FOCUS Diagnostics

Band Reactivity vs Patient Interpretation



HerpeSelect 1 and 2 immunoblot IgG

FOCUS Diagnostics

SENSITIVITY & SPECIFICITY

	HSV-1		HSV-2	
	Sensitivity %	Specificity %	Sensitivity %	Specificity %
Expectant mothers	100	93.1	100	93.7
Sexually active adults	99.3	95.1	97.3	98.1
Low prevalence	82.4	100	None	100

62

SYPHILIS

Serological Tests

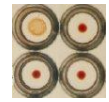
- **NON-TREPONEMIC TESTS**
  - **reagins (nonspecific IgM & IgG antibodies against cardiolipin); screening**
  - VDRL (Ag=mix of lecithin, cholesterol & purified cardiolipin); not specific
  - RPR (rapid plasma reagin test)
- **TREPONEMIC TESTS**
  - **Ab against *T.pallidum*; for diagnosis confirmation**
  - TPI (treponema immobilization test)
  - FTA (fluorescent treponemic antibody)
  - **TPHA** (haemaglutination assay)
  - EIA + WB – confirmatory methods
  - Immunochromatographic test (rapid treponemic test)

63

TPHA

Treponema pallidum haemaglutination assay

- Passive haemagglutination of sensitized erythrocytes (avian / sheep)
  - qualitative or quantitative
- Positive reaction is shown by agglutination of the cells
- Negative reactions by setting of the cells to a button or small ring



64

TPHA

Produced by Newmarket Laboratories Ltd, UK

Diagnostic specificity

- Blood donors **99.5%**
- Clinical samples (negative population) **100%**

Diagnostic sensitivity 100%