laboratory methods in diagnosis of viral infections in immunocompromised patients

Masoud Parsania, Ph.D.
Department of Microbiology, Tehran Medical Sciences Branch,
Islamic Azad University, Tehran, Iran.

complications such as infection and allograft rejection, which are related by immunosuppressive therapy, remain major causes of morbidity and mortality following solid organ transplantation.

Epidemiologically, some viral infections are the result of community exposures (influenza, adenovirus), whereas some are commonly transmitted with the allograft (cytomegalovirus, Epstein-Barr virus), and others are the result of more distant exposures reactivated in the setting of immune suppression (chicken pox and varicella zoster as shingles)

Timing of Pathogens Post-BMT

	Phase I, Pre-engraftment <30 days	Phase II, Post-engraftment, 30-100 days	Phase III, Late Phase, >100 days
Sources	Neutropenia, mucositis and acute GVHD	Impaired cellular immunity And acute and chronic GVHD	Impaired cellular and humoral immunity and chronic GVHD
• Reactivation	HSV	CMV+	
		BK virus	
		EBV Lymphoprolferative disease	
		HHV-6	
		HHV-7	
			VZV
Community Acquired	HHV-6		
		Parvo B-19	
		Respiratory and Enteric Viruses	
Environmentally Acquired	Facultative GNR		
	GI Tract Streptococci sp.		
	Staphylococcus epidermidis		
			Encapsulated bacteria
	All Candida sp.		
	Aspergillus sp.		
			Pneumocystic carinii
		Toxoplasma gondii	

Correlate of immunity to be checked, reached and documented

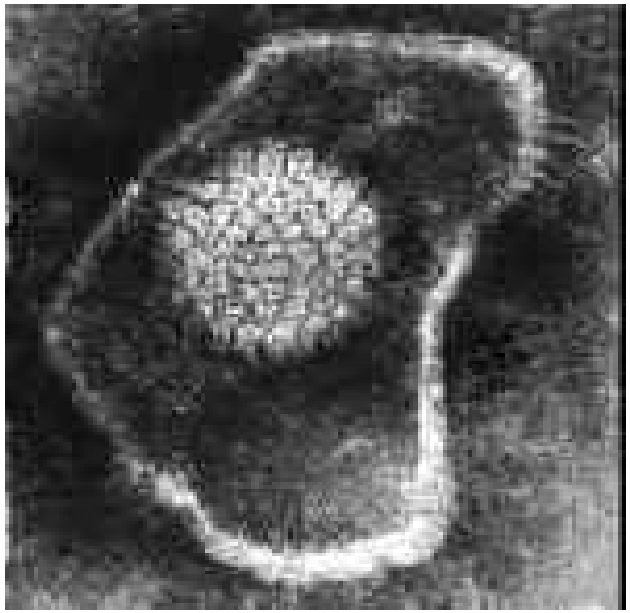
Vaccine	Indication for determination of specific antibody titres				Specific antibody (IgG) and unit	Interpretation of serological analyses		
	During end-stage organ disease	At pre-transplant (listing)	After catch-up immunization (pre- or post-trans-plant)	Post trans-plant		Suscepti-ble	Some protection	Long term protection
Tetanus	Yes, if history unclear (§)	If unknown serology	Yes	At 12 months	Anti-tetanus toxoid (IU/l)	< 100	≥ 100	≥ 1000
***Haemophilus influenzae* type b**	Yes (children < 5 years)(§)	Yes, (If unknown serology in children < 5 years)	Yes (children < 5 years)	At 12 months	Anti-PRP IgG (mg/l)	< 0.15	≥ 0.15	≥ 1
Hepatitis B	Yes (#, &)	Yes, if unknown serology	Yes (#)	Every 12 months (¥)	Anti-HBs IgG (IU/l)	< 10	≥ 10 (¥)	≥ 100 (¥)
Measles	Yes	Yes, if unknown serology	Yes	At 12 months	Measles IgG, by EIA (IU/l)	< 50 (*)	50-149 (*)	≥ 150 (**)
Rubella	Yes	Yes, if unknown serology	Yes	Not if immune before SOT	Rubella IgG (IU/ml)	< 10	≥ 10	
Varicella	Yes	Yes, if unknown serology	Yes	At 12 months	VZV IgG or VZV-gp (IU/l)	< 50 (*)	50–149 (*)	≥ 150 (*, **)

§ If history unclear, check antibody titre 4 weeks after a booster dose to define whether further doses are needed.
# Check anti-HBs IgG titre if last dose given <5 years ago, or 4–12 weeks after completion of primary series or a booster dose;
& Include HBsAg and anti-HBc to exclude current/past infection.
¥ In immunosuppressed SOT patients, the unknown contribution of immune memory requires regular booster doses to maintain anti-HBs titers ≥ 10 IU/l at all time in patients at risk of exposure.
* Measles and VZV IgG, by commercially used tests; if positive= immune, if negative or doubtful: send serum for analysis by a more sensitive test [30] to the Laboratoire de Vaccinologie des Hôpitaux Universitaires de Genève.
** Loss of pre-existing immunity to measles / VZV may occur in SOT patients.

Herpesviridae

Subfamily	Growth & Cytopathology	Latent infections	Genus		Common name
Alphaherpesvirinae	Short, cytolytic	Neurons	Simplexvirus		HSV-1 HSV-2
			Varicellvirus		VZV
Betaherpesvirinae	Long, cytomegalic	Glands, kidneys	Cytomegalovirus		CMV
	Long, lymphoproliferative	Lymphoid tissue	Roseolovirus		HHV-6 HHV-7
Gammaherpesvirinae	Long, lymphoproliferative	Lymphoid tissue	Lymphocryptovirus		EBV
			Rhadinovirus		Kaposi' sarcoma virus

- **Primary infection** -first contact with HSV
- **Latent infection** -persistent virus in root ganglia
- **Reactivation** -production of infective virus by latently infected cell
- **Recurence** -clinically apparent disease produced by reactivation



HSV-1

- Primary infection occurs in oral mucosa
  - 30% people get clinically apparent cold sores
  - 90% healthy people have been infected with HSV-1
- Virus then travels along trigeminal nerve to ganglion in most those infected
- 70% cases of HSV-1 encephalitis already have antibody present suggesting reactivation of virus most common mechanism
- Why HSV-1 reactivates not known
- In children, HSV-1 encephalitis occurs during primary infection

Complications

- **Meningitis**-- infection of the sheaths and membranes (meninges) covering the brain and the spinal cord.
- **Encephalitis**-- acute inflammation of the brain, commonly caused by a viral infection by insect bites or food and drink
- **Eczema herpetiform**-- widespread herpes across the skin)
- **Keratoconjunctivitis**-- Infection of the eye
- **Prolonged, severe infection** in immunosuppressed individuals
- **Pneumonia**
- **Infection of the trachea**
- **Keratitis**-- Corneal infection, irritations, and inflammations

Laboratory diagnosis of HSV

Direct staining

Tzanck test

Immunostaining

HSV isolation

Serology

PCR

Tzanck test

Cell scrape from base of the lesion

smear on slide

↓

Staining

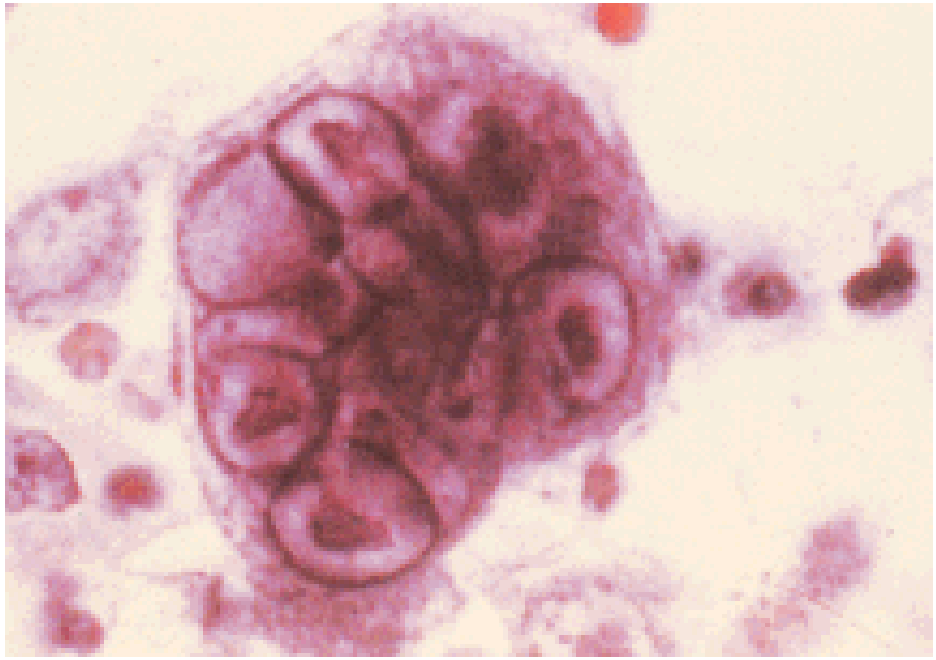
Wright-Giemsa, Giemsa

↓

Ballooning cell with intranuclear inclusion

multinucleated cell

Tzanck test



Multinucleated cell

Immunofluorescent staining

Cell scrape, smear
fix in cold acetone

↓

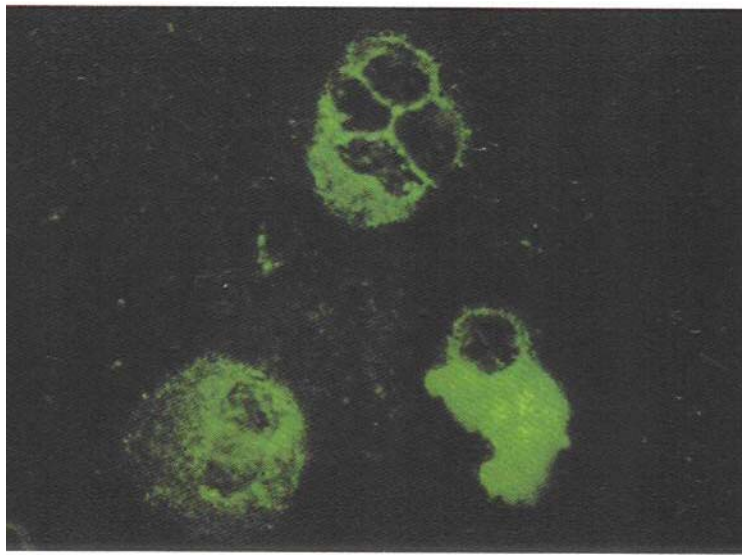
rabbit anti-HSV Ig

↓

goat anti-RaIg conjugated
with fluorescein dye

↓

mount with glycerine buffer



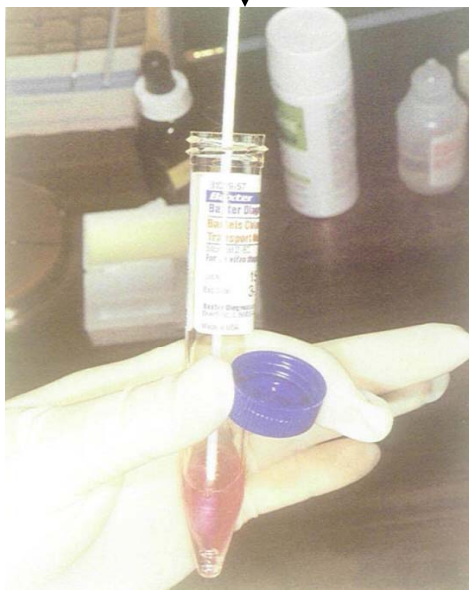
Virologic Tests
(continued)

- Antigen detection (DFA or EIA)
  - Fairly sensitive (>85%) in symptomatic shedders
  - Rapid (2-12 hours)
  - May be better than culture for detecting HSV in healing lesions
- Cytology (Tzanck)
  - Insensitive and nonspecific and should not be relied on for HSV diagnosis

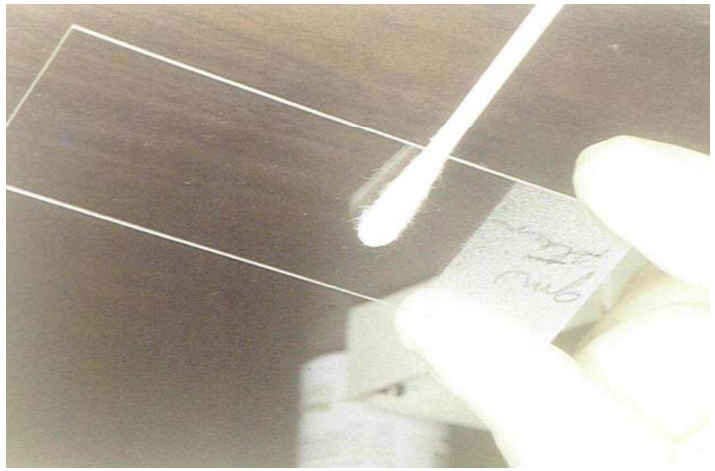
Specimen collection

Samples :

vesicle fluid, lesion swab



Transport media

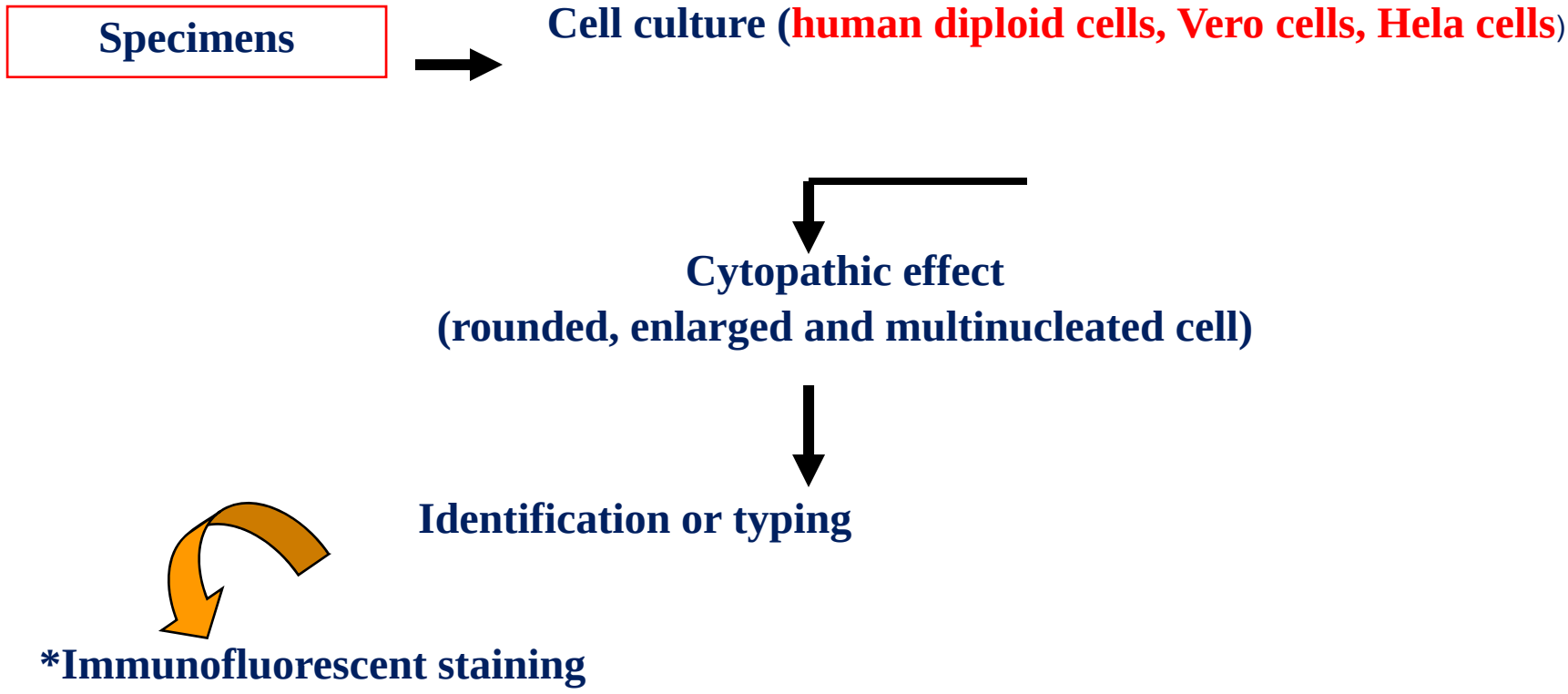


Smear on slide

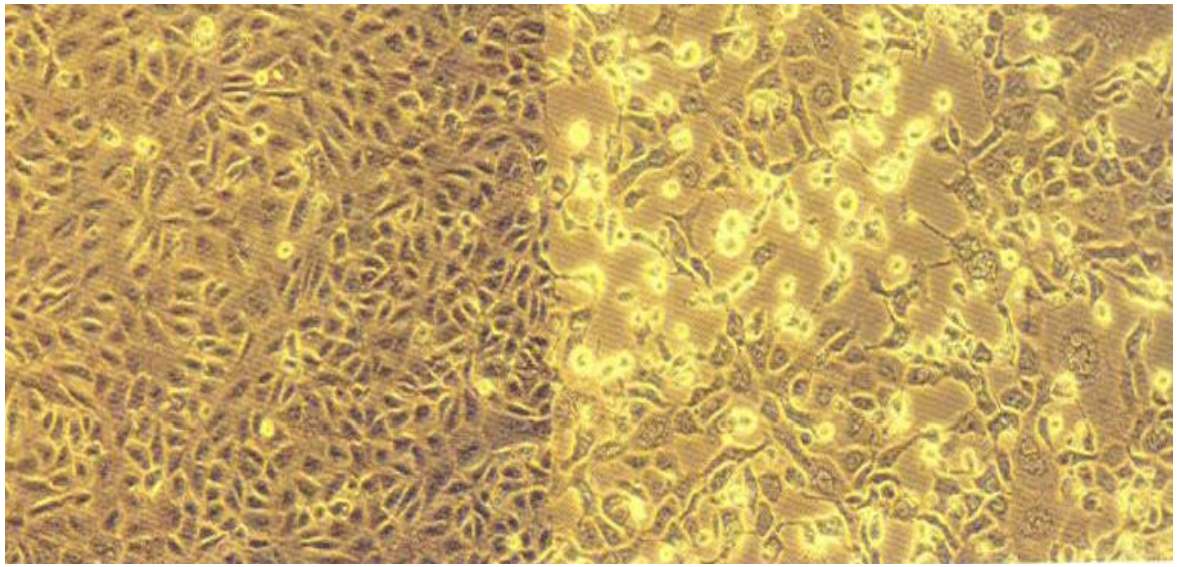
Virologic Tests

- Viral culture (gold standard)
  - Preferred test if genital ulcers or other mucocutaneous lesions are present
  - Highly specific (>99%)
  - Sensitivity depends on stage of lesion; declines rapidly as lesions begin to heal
  - Positive more often in primary infection (80%–90%) than with recurrences (30%)
  - Cultures should be typed
- Polymerase Chain Reaction (PCR)
  - More sensitive than viral culture; has been used instead of culture in some settings
  - Preferred test for detecting HSV in CNS

Viral isolation



HSV Cytopathic effect



Normal cells **CPE**

Who Is a Candidate for HSV Serologic Testing? Special populations

- Pregnant women
- Patients prior to transplant or starting immunosuppressive therapy
- Patients with HIV infection

Serological test for HSV infection

Immunofluorescent staining

Complement fixation test

ELISA : **IgM capture test**

IgG test

HSV serology

Primary infection

Pair serum: **acute & convalescent serum**

IgG assay ***rising titer** → **≥ ٤ times**

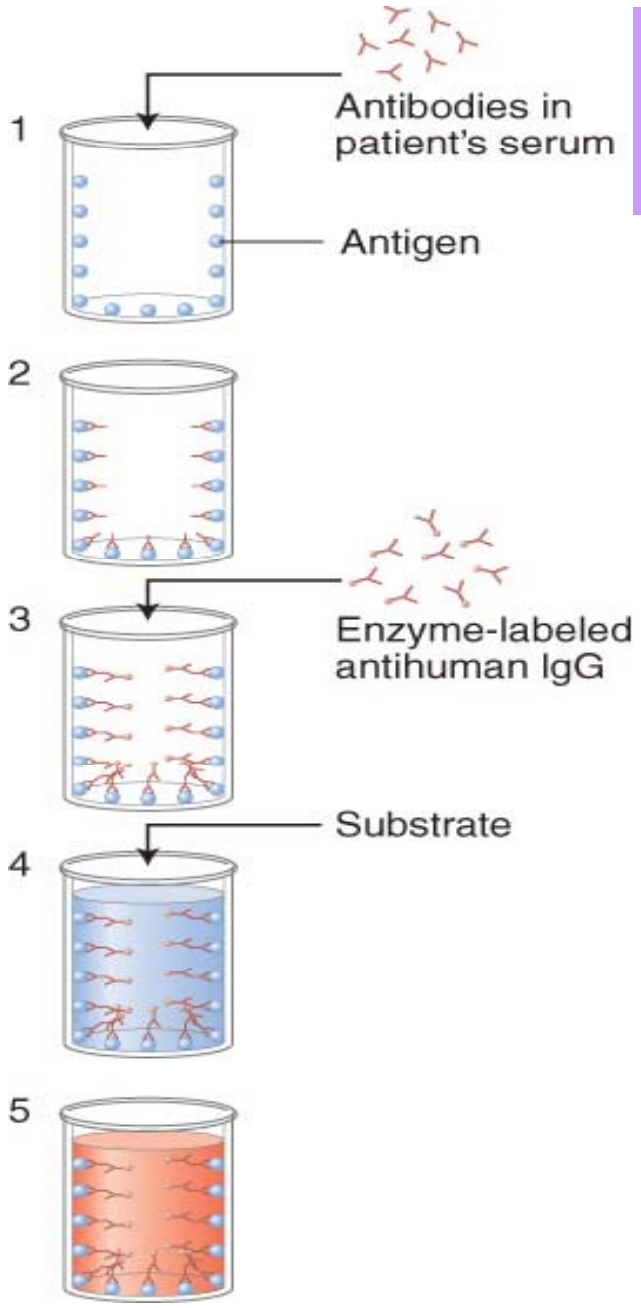
***seroconversion**

Single serum: **IgM assay**

Recurrent infection

not useful; **multiple reactivation**

IgM capture ELISA



Substrate+chromogen

Enzyme labeled anti-viral antibody

HSV antigens

Tested sera (IgM)

Anti-m chain capture Ab

Polymerase chain reaction

Samples

infected cell, vesicle fluid,CSF

↓

DNA extraction

↓

PCR solution

(buffer, dNTP,Taq DNA pol, primres)

↓

Amplify ๒๐-๓๐ cycles

Multiplex primers;

- **cutaneous group; HSV, VZV**
- **lymphotropic group; CMV,**

Detection:

- **gel electrophoresis**
- **dot blot hybridization**
- ***restriction fragment length polymorphism**

Cytomegalovirus

- Member of the herpes virus family (EBV, varicella-zoster, herpes simplex)
- Worldwide seroprevalence 30-100%
- Found in body fluids
  - Blood, saliva, urine, breast milk

Types of CMV Infection

- Primary infection

  (asymptomatic to mononucleosis like syndrome in immune competent individuals)
- Latent infection (presence of viral genome in mononuclear leukocytes, endothelial cells, and organs in the absence of active replication of infectious virus)
- Reactivation
- Reinfection

Laboratory Diagnosis

1. CMV antigenaemia test - widely used in many European countries. CMV antigens at the surface of polymorphonuclear leukocytes are detected by immunoperoxidase or immunofluorescence techniques. A result can be obtained within 4 to 6 hours but the technique is very tricky.
2. Polymerase chain reaction - becoming the method of choice in a few laboratories, had been reported to carry a higher prognostic value for CMV disease than the Detection of Early Antigen Fluorescent. Foci (*DEAFF)* test. Potential problems with sensitivity.
3. Serology - not reliable in general but occasionally, rises in IgG titre and the presence of IgM may be seen.
4. viral culture can be insensitive
5. Histopathology

Detection of CMV Infection

- Immune status: serology (IgG)
- Active infection (viremia)
  - Histology
  - Viral culture
  - Shell vial culture
  - Antigenemia assay
  - CMV PCR (qualitative/quantitative)

Laboratory Diagnosis

- Serology
  - Serologic tests for antibody to CMV are useful for determining whether a patient had CMV infection in the past, a determination of great clinical importance for organ and blood donors, and in the pretransplant evaluation of prospective transplant recipients.
  - Antibody tests are not useful in the diagnosis of CMV disease in the immunocompromised host

Treatment of CMV Infection in Allogeneic Bone Marrow Transplant Patients

- Ganciclovir/Foscarnet
- Two major treatment approaches
  - Prophylactic treatment – treat all patients at engraftment
  - Pre-emptive treatment – monitor patients for viremia and treat when infection detected
- Goal: prevent CMV disease

CMV Monitoring

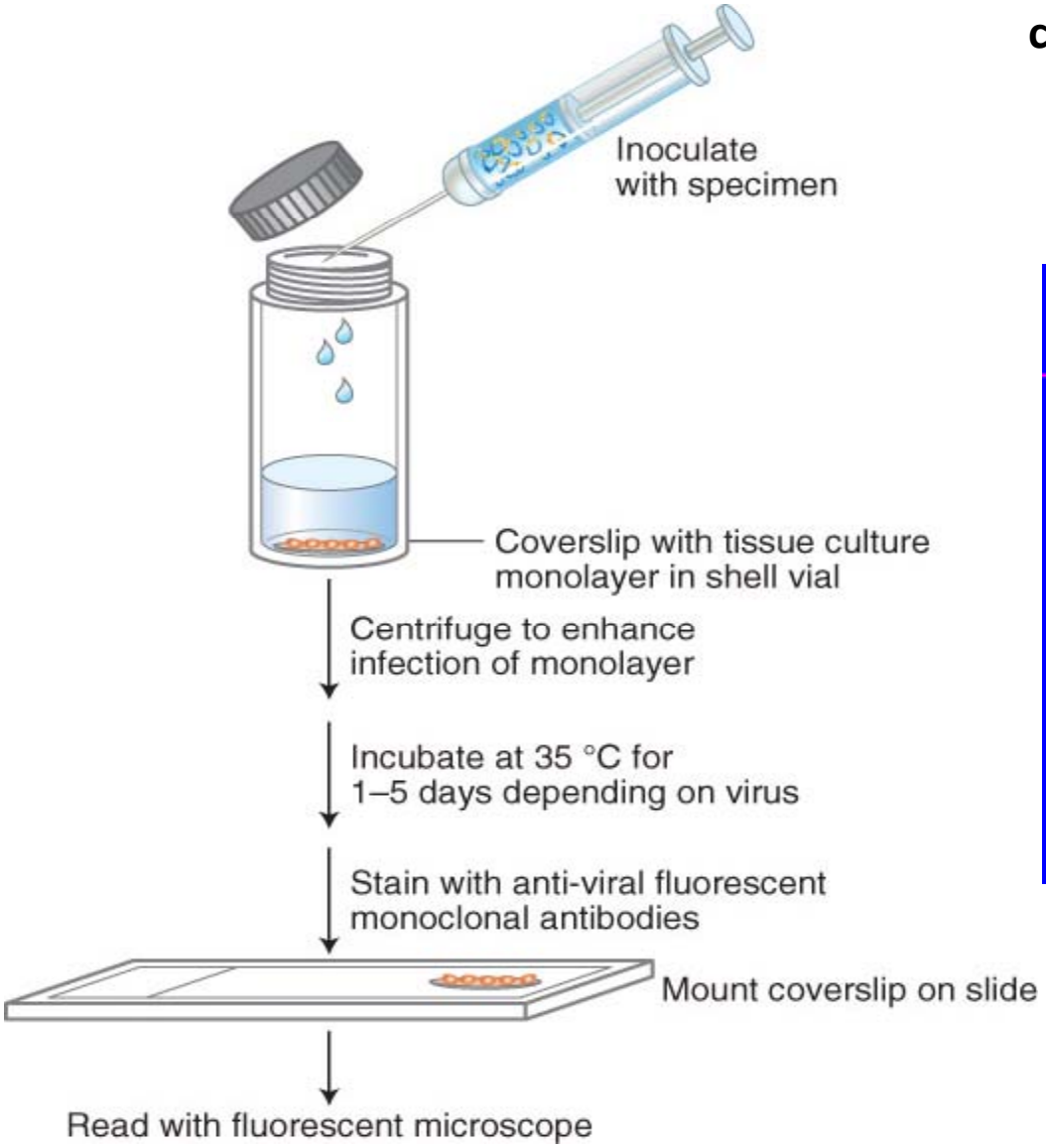
- Patients monitored every 1-2 weeks for CMV viremia
  - Shell vial cultures
  - Antigenemia assays
  - CMV PCR
- Incidence of CMV viremia may vary depending on monitoring strategy

PCR Based Screening Methods

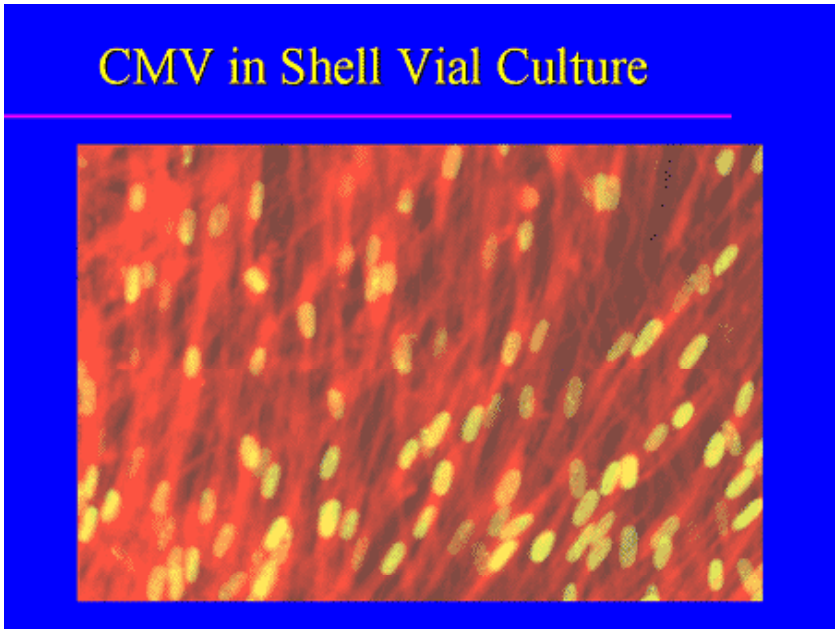
- PCR is more sensitive than shell vial or antigenemia assays
- Some patients may be pre-emptively treated unnecessarily using PCR strategies
- Quantitative PCR may be more sensitive than qualitative PCR

CMV Shell Vial Culture

Tissue culture cells are grown on coverslips on the bottom of shell vials.



Modified from J. H. Shelhamer, et. al., Ann. Intern. Med. 124 (1996): 585-599.



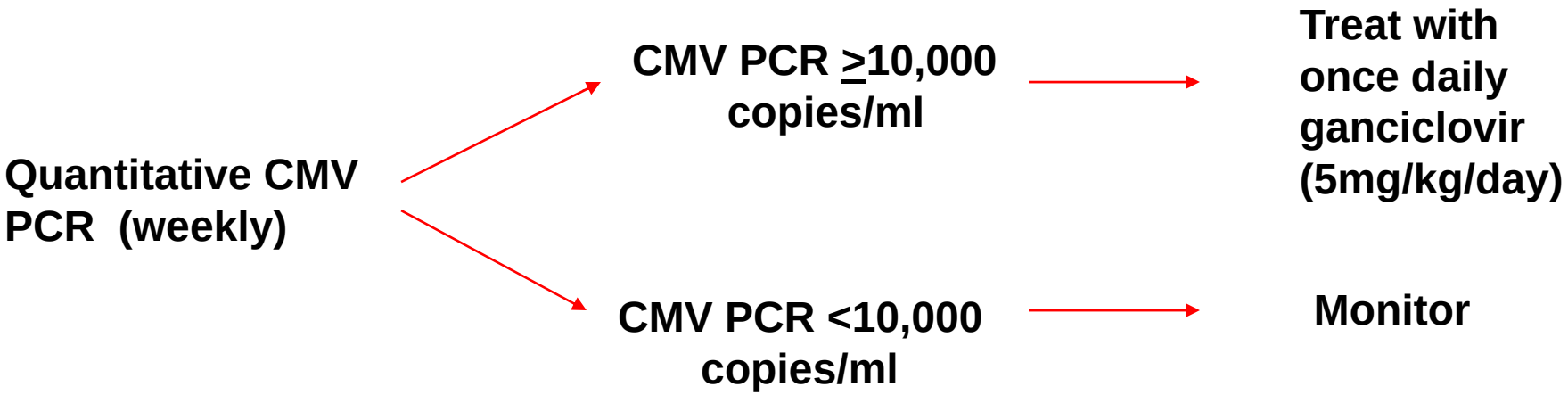
Laboratory Diagnosis

- Molecular Methods
  - Real-time PCR
  - PCR…
  - COBAS Ampliprep/Taqman CMV Test

  After treatment : < 137 IU/ml CMV DNA

  Specimen: Whole Blood with EDTA

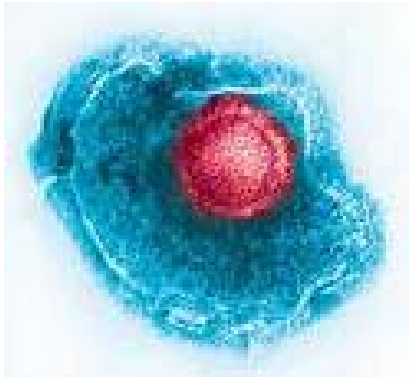
CMV Monitoring /Treatment Strategy

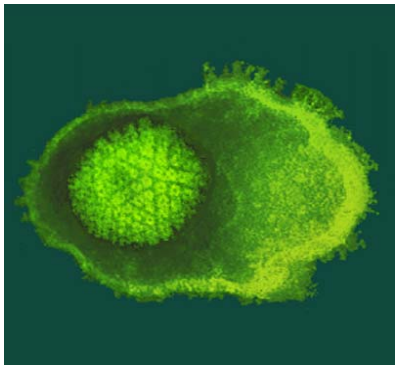


Comparing culture and molecular testing

	Culture	Molecular
Sensitivity	Fair	3- to 5-fold greater than culture
Specificity	Very high	Very high
Vulnerability to transport problems	Sensitive to extreme temperatures and drying	Viable specimen not required
Typing	2nd step often required	Incorporated in initial procedure
Turnaround time	2-3 days	4-8 hours

Varicella Zoster Virus





- VZV is a DNA virus
- belongs to the Herpes Virus Family

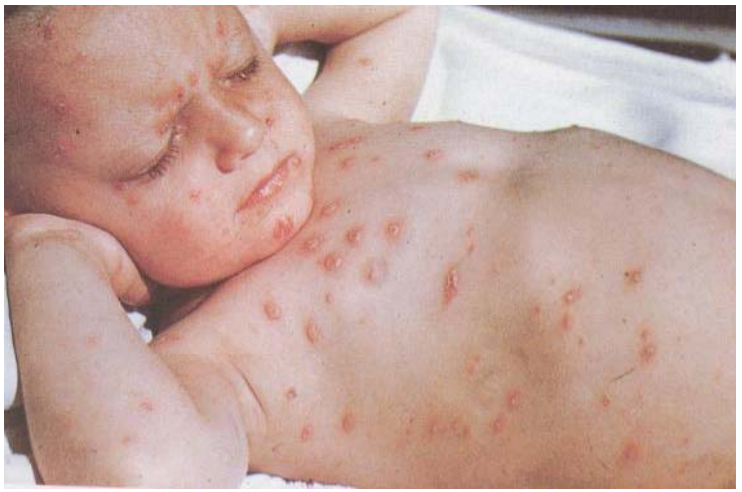
- Causes two clinically distinct forms of disease.
  - **Chicken-pox (Varicella)**
    - Primary infection
    - usually in childhood
  - **Herpes Zoster (shingles)**
    - secondary manifestation of an earlier infection
    - later in life

Serological test of VZV

ELISA with VZV specific antigen

IgG **seroconversion**
rising Ab titer ≥ ∝ times

IgM **detected both**
chickenpox & zoster



Laboratory diagnosis of VZV

Direct staining

Samples → **Infected cell scrape**

Tzanck test → **ballooning cell with intranuclear inclusion**

multinucleated cells

Immunostaining: **fluorescent staining**

Isolation of VZV

Nasal/throat washing vesicle fluid

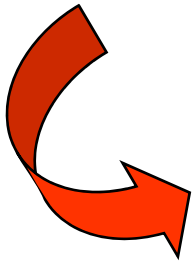
Inoculate promptly

Human diploid cell culture

๑-๕ weeks

CPE

ballooning,multinucleated cell



Identification: IF

Polymerase chain reaction

Single/Nested PCR

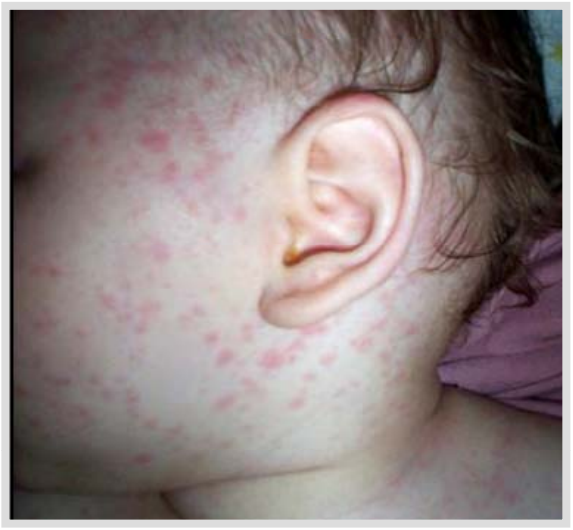
using primer common with HSV

detected both VZV & HSV

Multiplex PCR

using mix primers **HSV + VZV + ….**

HHV 6 and 7
Acute/primary infection



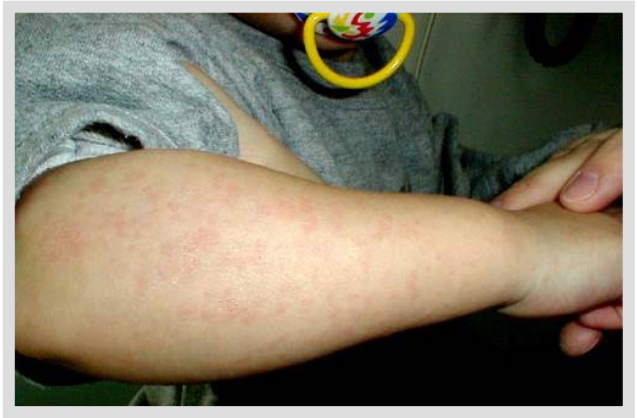
- new human herpes viruses (HHV)
- **HHV-6 and -7**
  - both members of the *Roseolovirus* genus of the β-herpesviruses.
  - T-lymphotropic but can infect other cell types
  - primary infections are associated with *roseola infantum* (*exanthem subitum* or 6th disease)

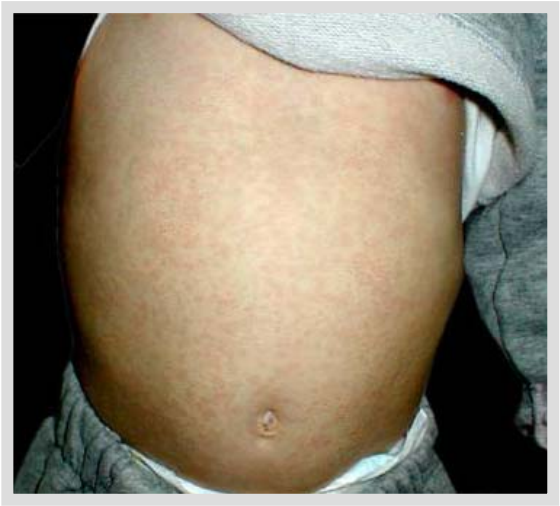
HUMAN HERPES VIRUSES-6

- **HHV6**
  - **Worldwide**
  - **virus replicates in T and B cells**
  - **infection occurs in first 3 years of life**
  - **Clinical** **Exanthem subitum (roseola infantosum)**
    - **mild acute febrile illness**
    - **incubation period of 2 weeks**
    - **fever lasts several days**
    - **macular papular rash appears within 2 days of fever**
  - **85% of adults carry virus in saliva**

HUMAN HERPES VIRUSE-7

- **HHV7**
  - **isolated from CD4 positive cells**
  - **virus present in saliva of >75% of adults**
  - **role in disease unclear**
  - **Evidence of infection present (seroconversion)**



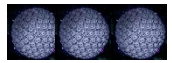


Human Herpes Virus 6 (HHV6)

- HHV6 associates with febrile convulsions in children under 2 years of age.
- It is a cause of meningitis and encephalitis in immuno-competent as well as immunocompromised patients.
- In the bone marrow transplant recipient, encephalitis presentation occurs between 10 days to 15 months (median 45 days) after transplantation.

Diagnosis of HHV-6/-7 Infection

- Virus Isolation
- Serological Assays
- Genomic Detection by PCR
  - Numerous PCR primer sets available for HHV-6
  - Quantitative PCR assay - persistence of a high HHV-6 load in the absence of apparent disease
  - Multiplex PCR method - simultaneous detection of HHV-6 and HHV-7

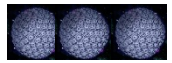


Studies of HHV-6 after BMT

TABLE 1. Prospective studies on HHV-6 reactivation after BMT

Detection method[a]	Sample[b]	No. of patients	Incidence of active HHV-6 infection (%)	Observed disease[d]	HHV-6 variant	Reference
PCR	PBMC, BALF	41	46	Vascular endothelial damage, GVHD	Not determined	389
PCR	PBMC, plasma	92	42.5	Myelosuppression, fever, delayed platelet and neutrophil engraftment	Not determined	177
PCR	PBL	22	60	Delayed platelet engraftment	B	266
PCR	PBL, plasma	61	28	Fever, engraftment failure	B	62
PCR	PBMC	37	Not given	Delayed platelet and granulocyte engraftment	B	421
PCR	PBL, oral lavage fluid, urine	57	60	Acute GVHD	90% B, 10% A	428
PCR, IHC	PBL, skin	57	Not given	GVHD	Not determined	15
qPCR	PBL, CSF	74	78	Delayed platelet engraftment	Not determined	245
qPCR	PBMC	20[c]	Not given	Rash and fever (2/20)	Not determined	79
VI	PBMC	82	38	Rash	B	447
VI	PBMC	22	Not given	Skin rash	Not determined	450
VI	PBMC	26	46	None	B	197
VI	PBMC and/or bone marrow	25	48	Skin rash	Not determined	456

[a] RT-PCR, reverse transcriptase PCR; qPCR, quantitative (real-time) PCR; VI, virus isolation; IHC, immunohistochemistry.
[b] PBL, peripheral blood lymphocytes; BALF, bronchoalveolar lavage fluid.
[c] Patients in this study were all HCMV seronegative.
[d] GVHD, graft-versus-host disease.



HHV-6 after SOT

TABLE 2. Prospective studies on HHV-6 reactivation after SOT

Transplant type	Detection method[b]	Sample[c]	No. of patients	Incidence of active HHV-6 infection (%)	Observed disease[e]	HHV-6 variant	Reference
Heart	PCR	PBMC	21	0	No disease		298
Kidney	PCR	PBMC	107	Not given[d]	No disease	Not determined	320
Kidney	qPCR	PBMC	52	23	No disease	Not determined	205
Kidney	VI, serology	PBMC	65	55	None	Not determined	455
Kidney and/or liver	VI, serology	PBMC	32	31	None (unless concomitant HCMV infection)	Not determined	162
Kidney and/or pancreas	PCR, serology	Urine, serum	30	50	Fever	Not determined	335
Liver	IHC	Liver tissue, PBMC	32	Not given	Acute liver failure	Not determined	155
Liver	IHC	PBMC	34	29	HCMV disease	Not determined	232
Liver	qPCR	PBMC	200	28	Opportunistic infections, HCMV disease, acute graft rejection	Not determined	171
Liver	qPCR, serology	PBMC	33	9	HCMV disease	Not determined	281
Liver	qPCR	PBMC	88	54	HCMV disease	Not determined	173
Liver	PCR, VI, serology	Plasma	47	49	Fever	B	452
Liver	VI	PBMC	80	39	CNS disease, fungal infections	Not determined	340
Liver	Serology, IHC	PBMC	51	22	Graft dysfunction	B	233
Liver	qPCR	PBMC	60	32	Graft rejection	Not determined	150
Liver	Serology	Serum	247	24	HCMV disease	Not determined	108
Liver	PCR	PBMC	46	Not given	None	10% A, 90% B	357
Liver[a]	qPCR	PBMC	66	54	Increased severity of HCV-related fibrosis or hepatitis	Not determined	172
Liver[a]	VI	PBMC	51	41	Increased severity of HCV-related fibrosis	Not determined	364
Liver heart/lung	VI, PCR	PBMC, BALF	30	27	Higher mortality rate, fungal infections	B	192

[a] Study population restricted to hepatitis C virus-positive patients undergoing liver transplantation.
[b] qPCR, quantitative PCR; VI, virus isolation; IHC, immunohistochemistry.
[c] BALF, bronchoalveolar lavage fluid.
[d] Unclear whether PCR was able to discriminate active from latent infection.
[e] HCV, hepatitis C virus.

Epstein-Barr Virus

- Epstein-Barr virus (EBV) is a human herpes DNA virus.
- It is estimated that 95 percent of the world population is exposed to the virus.
- In Infectious Mono the virus affects B-lymphocytes.
- There are two techniques used to identify EBV; immunofluorescence and complement fixation.

Epstein-Barr Viral Infection

- It is a systematic immune complex disease of soluble and tissue-fixed antigen involvement characterized by fever, fatigue, chills, headache, myalgia, skin rash, splenomegaly and cervical adenopathy.
- EBV infected B-lymphocytes express a variety of “new” antigens encoded by the virus. Infection with EBV results in expression of:
  1. Viral Capsid Antigen (VCA)
  2. Early Antigen (EA)
  3. Nuclear Antigen (NA)

  Each antigen expression has corresponding antibody responses.

Epstein-Barr Virus (VCA)

- Viral capsid antigen (VCA) is produced by infected B cells and can be found in the cytoplasm.
- Anti-VCA IgM is usually detectable early in the course of infection, 4 to 7 days after onset of signs and symptoms, but it is low in concentration and disappears within 2 to 4 months.

Epstein-Barr Virus (EA)

- Early antigen (EA) is a complex of two components, early antigen-diffuse (EA-D), which is found in both the nucleus and cytoplasm of the B cells, and early antigen-restricted (EA-R), which is usually found as a mass only in the cytoplasm.
- Anti-EA-D of the IgG type is highly indicative of acute infection, but it is not detectable in 10% to 20% of patients with IM. EA-D disappears in about 3 months; however, a rise in titer is demonstrated during reactivation of a latent EBV infection.
- Anti-EA-R IgG is not usually found in young adults during the acute phase. Anti-EA-R IgG appears transiently in the later convalescent phase. In general, anti-EA-D and anti-EA-R IgG are not consistent indicators of the disease stage.

Epstein-Barr Virus (EBNA)

- Epstein-Barr nuclear antigen (EBNA) is found in the nucleus of all EBV-infected cells. Although the synthesis of NA precedes EA synthesis during the infection of B cells, EBV-NA does not become available for antibody stimulation until after the incubation period of Infectious Mono, when activated T lymphocytes destroy the EBV genome-carrying B cells. As a result, antibodies to NA are absent or barely detectable during acute IM.
- Anti-EBNA IgG does not appear until a patient has entered the convalescent period. EBV-NA antibodies are almost always present in sera containing IgG antibodies to VCA of EBV unless the patient is in the early acute phase of IM. Patients with severe immunologic defects or immunosuppressive disease may not have EBV-NA antibodies, even if antibodies to VCA are present.

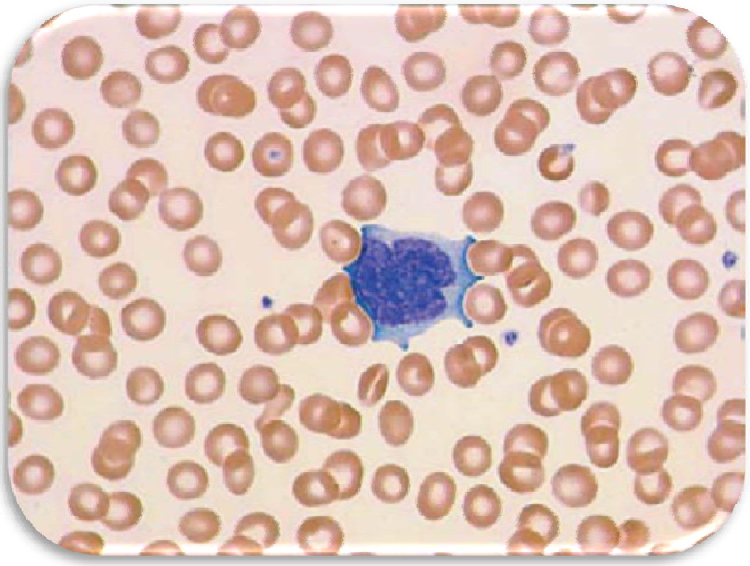
Epstein-Barr Virus (EBNA)

- Under normal conditions, antibody titers to NA gradually increase through convalescence and reach a plateau between 3 and 12 months postinfection. The antibody titer remains at a moderate, measurable level indefinitely because of the persistent viral carrier state established following primary EBV infection.
- Test results of antibodies to EBV-NA should be evaluated in relationship to patient symptoms, clinical history, and antibody response patterns to EBV-VCA and EA to establish a diagnosis.

Laboratory Diagnosis

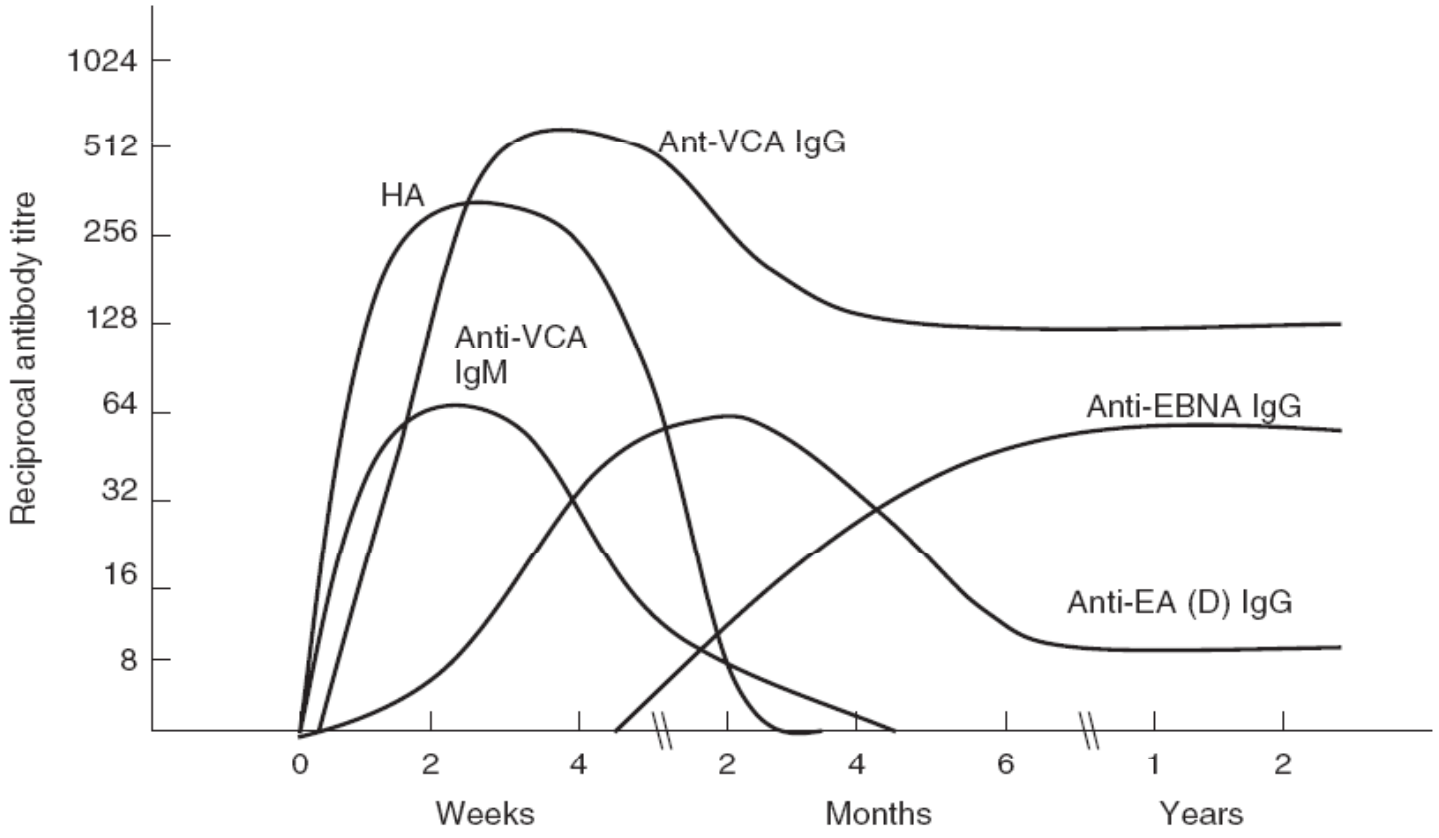
- The 3 classic criteria for laboratory confirmation
  1- lymphocytosis
  2- the presence of at least 10% atypical lymphocytes on peripheral smear
  3- a positive serologic test for Epstein-Barr virus (EBV).

- Common laboratory findings in patients with infectious mononucleosis include marked lymphocytosis (>50% leukocytes) with atypical lymphocytes. The detection of at least 10% atypical lymphocytes on a peripheral-blood smear in a patient with mononucleosis has a sensitivity of 75% and a specificity of 92% for the diagnosis of infectious mononucleosis.



- Complete blood count
  - 40-70%, Leukocytosis (WBC 10,000-20,000 cells per cm_{3})
  - 80-90% of patients have lymphocytosis, with greater than 50% lymphocytes. Lymphocytosis is greatest during 2-3 weeks of illness and lasts for 2-6 weeks.
  - 20-40% of the lymphocytes : atypical lymphocytes > 10% ;
  - Mild thrombocytopenia

- Liver function tests
  - 80-100% of patients : elevated LFT
  - Alkaline phosphatase, AST and bilirubin peak 5-14 days after onset
  - 95% of patients : elevated LDH
  - most liver function test results are normal by 3 months.



HA	+	+/–	–
Anti-VCA IgG	+	+	+
Anti-VCA IgM	+	+/–	–
Anti-EBNA IgG	–	+	+
Anti-EA IgG	+	+	–

جدول تغییرات سرولوژیک بیماری های مرتبط با EBV

تست‌ها		Anti-VCA		Anti-EA	
بیماری	هتروفیل	IgM	IgG	EA-D	EA-R	Anti-EBNA
مونونوکلئوز عفونی حاد	+	+	++	+	-	-
دوره نقاهت	±	-	+	-	±	+
عفونت قبلی	-	-	+	-	-	+
فعال شدن مجدد در اثر ضعف ایمنی	-	-	++	+	+	±
لنفوم بورکیت	-	-	+++	±	++	+
کارسینوم	-	-	+++	++	±	+

MONO-Spot TEST

The presence of IgM HA in IM serum causes agglutination of red blood cells from species other than humans.

- Heterophile antibody tests are negative in 25% of patients during the first week of infection and in 5 to 10% during or after the second week.
- Heterophile antibody tests are positive in only 25 to 50% of children under 12 years of age.

IM; laboratory diagnosis

– Real-time PCR

- Real-time PCR is when the amplified DNA is detected as the reaction progresses in real time. Test has 95% sensitivity and 97% specificity for primary EBV infection.
- Is expensive and not commonly used in clinical practice.
- Test can be useful for diagnosis of serologically indeterminate EBV infections.

HUMAN HERPES VIRUSE-8

- **HHV8**
- **Kaposi sarcoma- associated Herpes Virus (KSHV)**
  - **detected in epithelial cells of Kaposi sarcoma**
  - **also present in semen**
  - **postulated as cause of Kaposi sarcoma**

Diagnosis

- **Serological Assays (ELISA)**
- **Genomic Detection by PCR**
- **Indirect IF**