

EBV and CMV: When Common Viruses Do Uncommon Things.

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Marek Smieja, MD PhD FRCPC

Professor, McMaster University

Head of Microbiology

Hamilton Regional Lab Medicine Program



Financial Disclosures:

Investigator-Initiated Grants:

Copan Italia, Brescia Italy

Grants/Clinical Trials:

Luminex (RVP, GPP)

Grand Challenges Canada-Botswana

GenMark (Respiratory, Gastrointestinal Panels)

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All grants and honoraria support costs of research or student research and travel.

- **When you have eliminated the impossible, whatever remains, *however improbable*, must be the truth**

- **When you have eliminated the impossible, whatever remains, *however improbable*, must be the truth**

– Sherlock Holmes

Sir Arthur Connan Doyle,

The Sign of the Four, 1890



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Outline

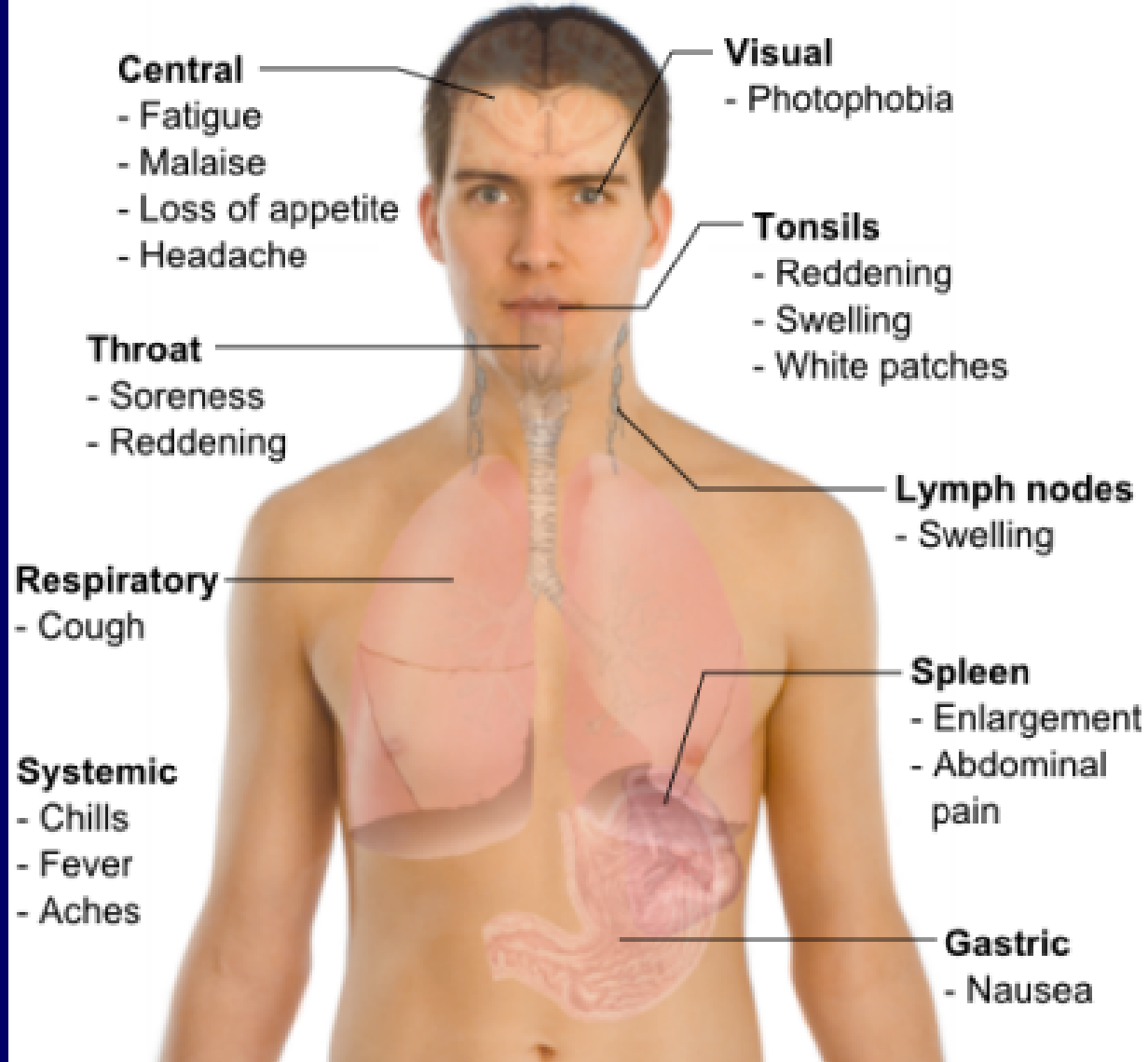
1. Review EBV and CMV testing
2. Examine diagnosis of atypical illness

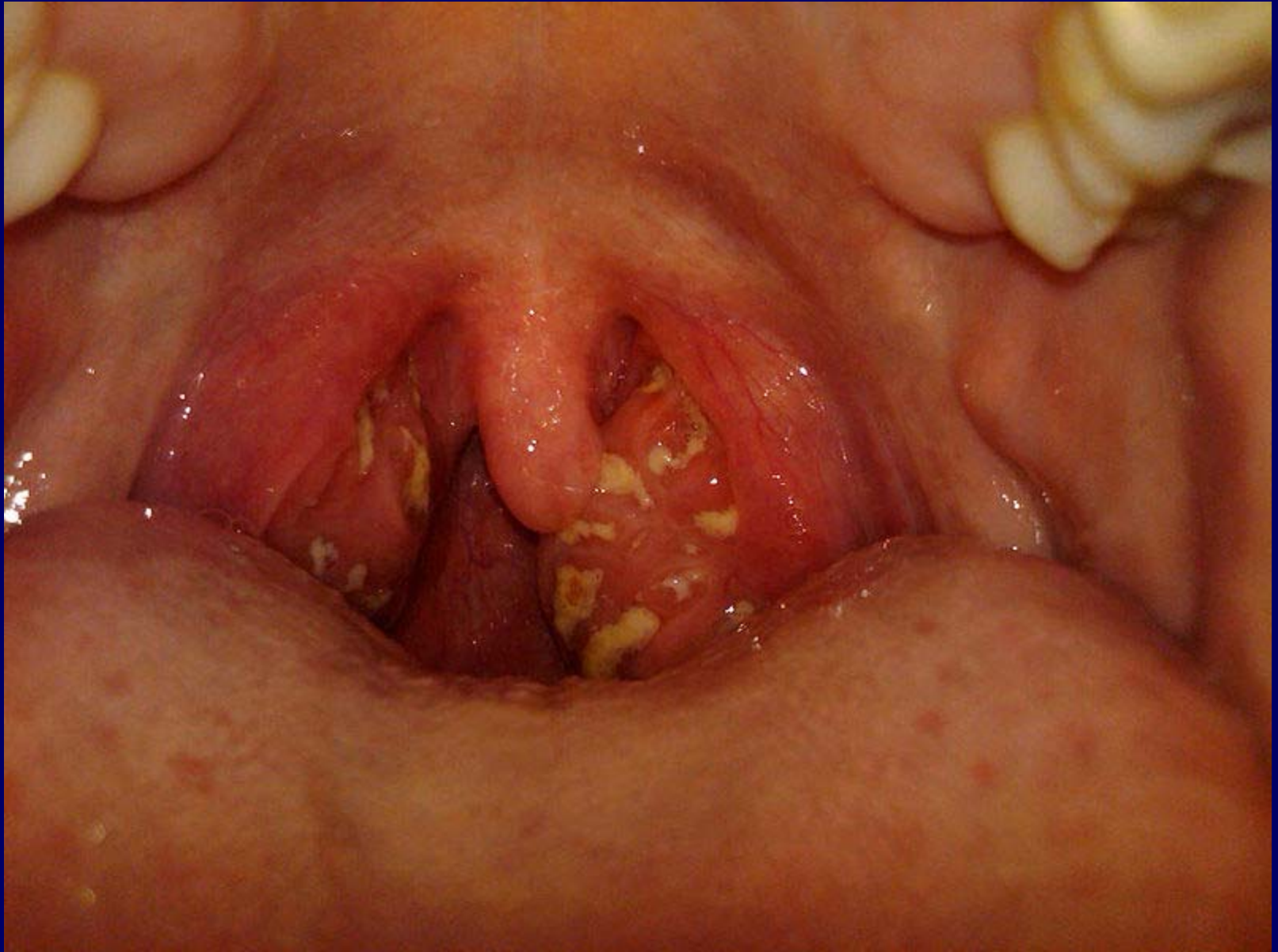
EBV

Epstein-Barr Virus (EBV)

- Herpesvirus 4
- Spread through saliva
- >75% of population
- Mononucleosis
- EBV-associated lymphoproliferative disease
- EBV-positive lymphomas
- Nasopharyngeal carcinoma

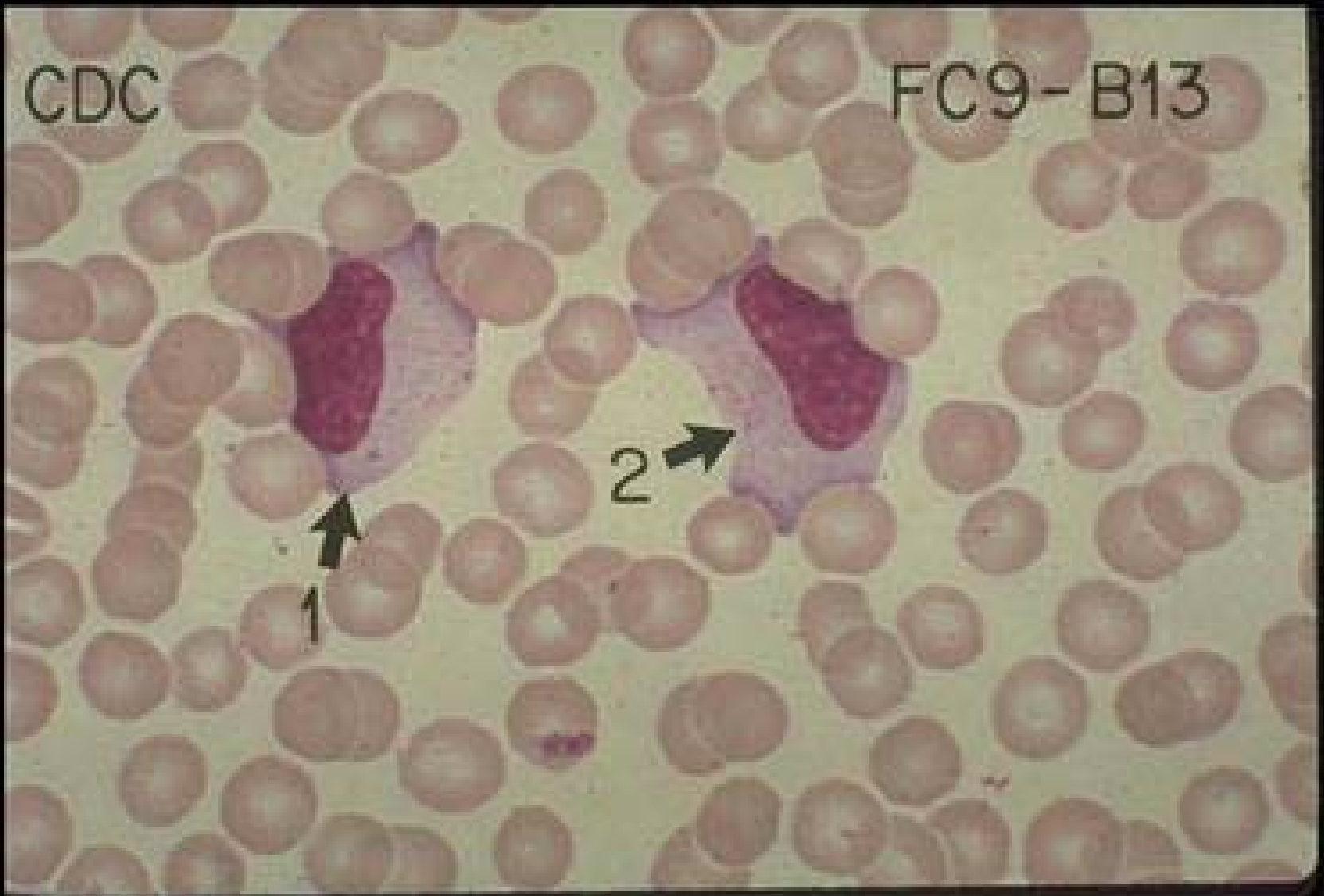
Main symptoms of Infectious mononucleosis





CDC

FC9-B13



[cdc.gov](https://www.cdc.gov)

Table 2: Complications of Infectious Mononucleosis

Cardiac	Myocarditis, pericarditis, arrhythmias
Dermatologic	Ampicillin-associated rash, oral hairy leukoplakia
Hematologic	Hemolytic anemia, thrombocytopenia, neutropenia, aplastic anemia
Hepatic	Hepatitis, Reye's syndrome
Immunologic	Decreased cell-mediated immunity, lymphoproliferative syndromes, Burkitt's and non-Hodgkin's lymphomas
Neurologic	Encephalitis, Guillain-Barré syndrome, Bell's palsy, psychosis, optic neuritis, transverse myelitis, seizures
Renal	Glomerulonephritis, interstitial nephritis
Respiratory	Upper airway obstruction, interstitial pneumonitis, pneumonia
Splenic	Spleen rupture both spontaneous or after mild abdominal trauma

Source: References 4, 19.

Non-Specific tests for EBV

- **CBC**
 - Lymphocytosis
 - Atypical lymphocytes
- **Elevated LFTs**
 - AST, ALT 50-250
 - More common cause of hepatitis than HAV
- **Unusual:**
 - Myocarditis, pancreatitis, meningitis, encephalitis

Mononucleosis: Differential

- 1. EBV
- 2. CMV
- 3. Hepatitis A, B, C
- 4. HIV
- 5. Toxoplasmosis
- 6. Rubella
- 7. Streptococcal pharyngitis

Monospot Test

- Heterophile antibody test
 - RBC agglutination (horse)
 - 2- 6 weeks after infection
 - Low level antibodies persist for up to 1 year
 - 70-90% sensitive, 96-100% specific
- False negative: early, young children
- False positive: toxoplasma, rubella, lymphoma, leukemia, HIV
- Not recommended by CDC
- EBV can give false-positive Lyme serology



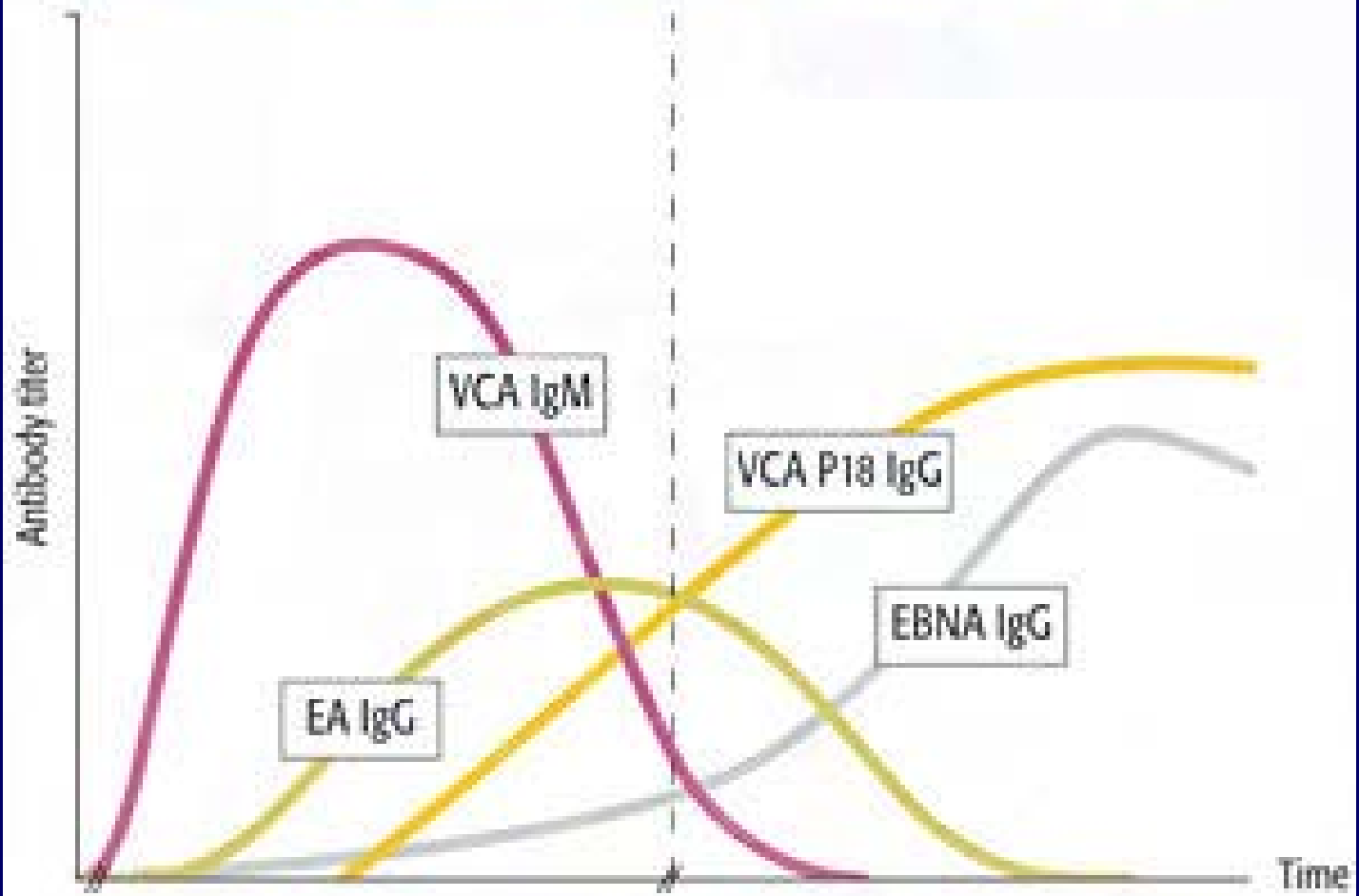
EBV Serology

- **Viral Capsid Antigen (VCA)**
 - IgM: last up to 12 months
 - Can cross-react with CMV, Parvovirus B19
 - IgG: elevated for life
- **Early antigen (EA)**
- **EBV nuclear antigen (EBNA)**
 - Appears later

EBV Infection Kinetics

Primary Infection

Convalescence



Performance of the Architect EBV Antibody Panel for Determination of Epstein-Barr Virus Infection Stage in Immunocompetent Adolescents and Young Adults with Clinical Suspicion of Infectious Mononucleosis

Alvaro Guerrero-Ramos, Mauli Patel, Kinjal Kadakia, Tanzina Haque

Department of Virology, Royal Free London NHS Foundation Trust, London, United Kingdom

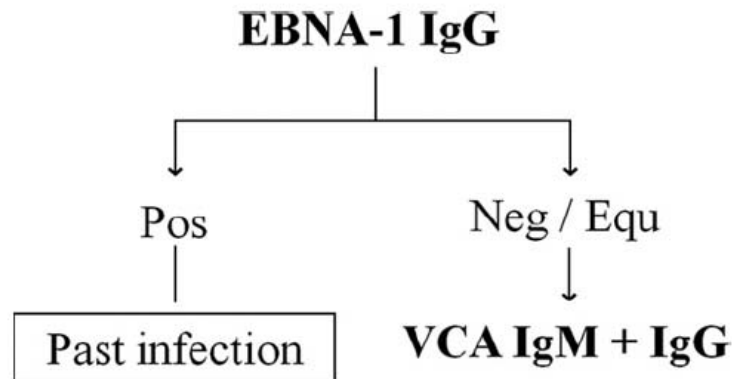


FIG 2 EBV sequential testing algorithm.

VCA-IgM False Positives

TABLE 5 Architect EBV VCA IgM results in selected primary viral infections

Architect VCA IgM result	Results (no. [%]) by virus type (total no. in group)		
	CMV (28)	HIV-1 (13)	Parvovirus B19 (19)
Negative	11 (39.3)	10 (76.9)	13 (68.4)
Equivocal	1 (3.6)	3 (23.1)	4 (21.1)
Positive	16 (57.1)	0 (0)	2 (10.5)

EBV PCR

- **qPCR for EBV**
- **Plasma, buffy coat or whole blood**
- **Monitor transplant patients for rising EBV levels**
 - **Serial levels to predict reactivation or new infection**
 - **Rising levels associated with lymphoproliferative disease**
 - **Decrease or stop immunosuppression, rituximab**
- **Primary mononucleosis**
 - **Limited data for blood, saliva**

Serum Epstein–Barr Virus DNA Load in Primary Epstein–Barr Virus Infection

Claudia C. Bauer,^{1*} Stephan W. Aberle,² Theresia Popow-Kraupp,² Magdalena Kapitan,³ Hanns Hofmann,¹ and Elisabeth Puchhammer-Stöckl²

¹Division of Clinical Virology, Institute of Virology, Medical University Vienna, Vienna, Austria

- **93/98 primary EBV had detectable DNA**
 - 38 to 64,000, median log 3.1
- **1/38 seropositive controls**
- **Sens 95%**
- **Spec 97%**
- **EBV-DNA in serum**
 - Decreases with time, age
 - Lasts <21 days

EBV-DNA in Mononucleosis

- **N=29: 10 monospot positive, 19 negative**
- **Mean log 3.7 (2.8-4.7)**

	Monospot +ve	Monospot -ve
EBV-DNA +ve	9	1*
EBV-DNA -ve	1	18

- **Sens: $9/10 = 90\%$**
- **Spec: $18/19 = 95\%$ (*discounting log 0.5)**

Oral EBV-DNA in Mononucleosis

- **N=3 McMaster students with acute mononucleosis**
- **Followed for 12 weeks**
 - Weekly self-collected oral swab
 - Monthly staff-collected swab
- **Baseline: 5.6 – 5.9 – 3.8**
- **12 week follow-up: 2.5 – 6.8 (occasional neg)**
- **9 healthy controls: 0/9 EBV-DNA**
- **CONCLUSION: oral swabs may yield persisting EBV-DNA for at least 12 weeks in primary EBV**

Case History #1

- 85-year-old man with fevers & lymphadenopathy
- PMH: A.fib, CAD, obstructive sleep apnea, osteoporosis, arthritis, benign pulmonary nodules
- Intermittent high fever, chills, malaise, night sweats since May 2015
- Sick contact: grand-daughter had positive monospot test same time.
- CT: mediastinal, axillary, retroperitoneal, cervical lymphadenopathy
- Lymph Node biopsy: negative for malignancy, atypical lymphoid proliferation with EBV +
- Bone marrow biopsy: no lymphoma

Case #1-Microbiology

■ Serology

VCA-IgG +

EA-IgG +

EBNA-IgG +

■ Blood

Aug 20: EBV 5.73 log copies/ml plasma

Sep 10: EBV 5.88 log copies/ml plasma

Sep 21: EBV 3.69 log copies/ml plasma

Case #1-Clinical follow-up

- Treated with valacyclovir from 08/19
- Switched to valgancyclovir from 09/08 till 10/19, stopped due to dizziness, generalized muscle pain.
- Completed 2m of treatment
- Most recent clinic visit 10/27, still intermittent fever.
- **FINAL DIAGNOSIS:**
EBV Reactivation/Re-infection vs. Primary EBV

Case #2-Clinical History

- 58-year-old male with severe hemolysis
- PMH: HTN, DM II, dyslipidemia, depression
- Sick contact with daughter and son with URTI symptoms.
- Extensive animal exposure
- New tattoo 3 weeks prior to disease onset
- 4-5 days of URTI prior to fever
- Daily fever, sweating, malaise x3 days
- Acute jaundice, drowsiness on admission

Case #2-On admission

- Elevated liver transamine enzymes
 - AST 592
 - ALT 296
 - total Bilirubin 136,
 - conjugated bilirubin 65.3
- WBC 12.4, neutrophil 10, monocyte 1.4
- Hemoglobin dropped from 101 to 63 in 24hours
- CRP 204

Case #2-Investigation

- CT abdomen/pelvis: hepatosplenomegaly, query cirrhosis, positive lymphadenopathy which are in keeping with hepatitis or inflammatory changes.
- Bone marrow examination compatible with hemolytic anemia

- HCV serology and PCR both negative
- Parvovirus B19 serology negative
- Q fever serology negative

Case #2-EBV Testing

■ SEROLOGY-EBV

VCA IgG: Reactive

EA IgG: Reactive

EBNA IgG: **Non-Reactive**

■ EBV qPCR: 13,860 copies/mL plasma.
(4.14 log copies/mL plasma).

■ EBV: log 5.4, 5.9, 3.7

Case #2-Hospital Course

- Treated with high-dose prednisone
- Rapid resolution of fevers
- Stabilization of hemoglobin
- At discharge:

AST 45

ALT 87

Conjugated Bilirubin 20.8

Hemoglobin 114

FINAL DIAGNOSIS:

Primary EBV infection and hemolytic anemia

Case History #3

- 19-year-old woman, travel to Central America
- Presents to ER in July 2013
- 2-week history of painful urination, fevers, right-sided tender inguinal “lumps”
- Nausea, pain with bowel movements
- Worsening inguinal pain, now bilateral
- 2 very tender lymph nodes right inguinal, 1-2 cm; 3 nodes on left

Case #3-Lab Testing

- WBC 6.2: neutrophils 1.3
 lymphocytes 5.8
- AST 160, ALT 161
- Cervical, urine: negative CT, NG
- LGV serology “unable to interpret due to cross-reactivity”
- HIV, syphilis serology negative
- HBsAg neg, HBc-Ab neg, HCV-IgG negative
- Lymphocytosis, **Monospot positive**

Case #3-EBV Serology & DNA

	JUL	AUG	SEPT	NOV
EBNA-IgG	NEG	NEG	NEG	NEG
EA-IgG	NEG	POS	NEG	NEG
VCA-IgG	NEG	NEG	IND	POS
Throat swab EBV-DNA		3.5 log	5.0 log	
Vagina swab EBV-DNA		5.1 log	NEG	

Case #3: 3-month Follow-Up

- Clinically resolved
- LGV, HIV, CMV, HBsAg, HCV, syphilis, toxoplasmosis all NEG
- Final diagnosis:
Primary vaginal/pelvic EBV
presenting as inguinal adenopathy

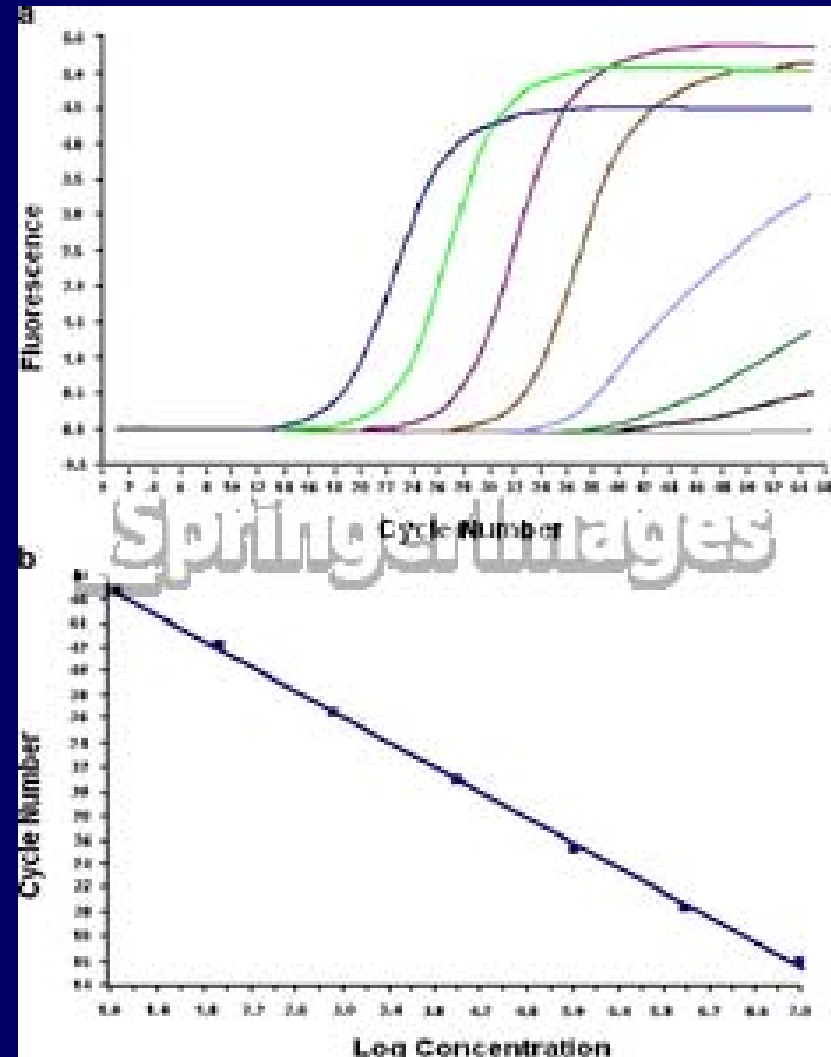
CMV

Cytomegalovirus (CMV)

- Herpesvirus 5
- Betaherpesvirus
- Mononucleosis, lymphadenopathy, hepatitis
 - Congenital CMV
 - CMV retinitis in HIV
 - CMV pneumonia, enterocolitis
- Diagnosis:
 - CMV IgG, IgM
 - CMV culture urine
 - CMV-DNA

CMV-DNA

- qPCR
 - Dynamic range
 - Reproducibility
 - Traceability
- Plasma, whole blood, buffy coat
- BAL
- Urine
- Vitreous fluid
- CSF
- Tissue



Case History #4

- 22-year-old woman
- Presents to ER in July 2014 with acute joint pains, fevers, hepatitis
- PMH: pancreatitis, ulcerative colitis

- AST: 196
- ALT: 179

Case #4-Laboratory

- CMV-IgM positive
 - CMV-DNA: 4.02 log (10,439 IU/ml)
- EBV
 - VCA, EA, EBNA reactive
 - EBV-DNA negative
- Parvo B19-IgG reactive, IgM indeterminate
- HIV, Syphilis negative
- Lyme disease: EIA, IgM-Western Blot +ve
 - IgG WB persistently negative
- **DIAGNOSIS: Primary CMV Infection**

CONCLUSIONS

- CMV-DNA and EBV-DNA testing well established in transplant population
- Validated
- Wide dynamic range
- Excellent reproducibility

- Expanding use into non-immunocompromised, atypical disease
- Need for multi-centre validation
 - Sample type, disease, extraction & assay type

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