
ME, MS, Fibromyalgia, Alzheimer's, Parkinsonism, Autism...

Tailored Testing Protocols

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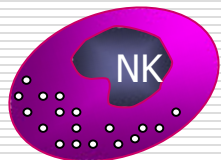
Aims of the presentation today

- Give an overview of the infections/viruses that it is important to consider testing for in chronic conditions such as ME, MS, RA, Fibromyalgia, Alzheimer's, Parkinsonism and Autism
- Offer tailored testing protocols for these conditions beyond Lyme Disease

Overview of the different immune components and infections/viruses tested for that may be relevant

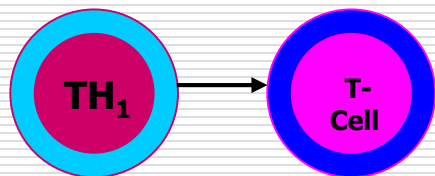
- CD3-/CD57+ T-Lymphocytes
- Elispot
- Seraspot
- Chlamydia pneumoniae
- Mycoplasma
- Ehrlichia/Anaplasma
- Bartonella
- Babesia
- Rickettsia
- Epstein Barr Virus (EBV)
- Cytomegalovirus (CMV)
- HSV1/2, HHV6
- Coxsackie virus

Aims of immune-competent cells



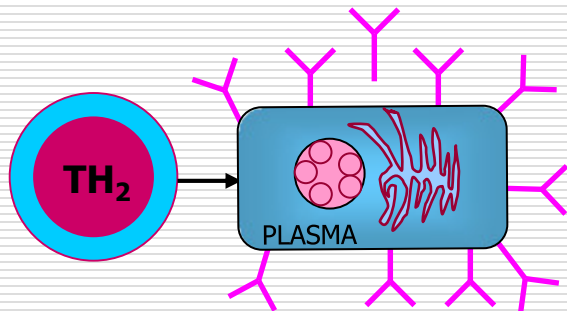
CD57+ NK cells

- Destruction of antigen-antibody complexes



EliSpot (T-cells):

- Borrelia burgdorferi
- Chlamydia pneumoniae
- Anaplasma/Ehrlichia
- Yersinia, EBV, CMV



Antibodies (B cells: IgG/IgA/IgM)

- Borrelia burgdorferi
- Chlamydia, Mycoplasma
- Ehrlichia/Anaplasma, Bartonella, Babesia, Rickettsia, Coxsackie Virus...

CD3-/CD57+ T-Lymphocytes

1. Subpopulation of the CD56+ NK cells
2. Reduction indicates **chronic activity** of Lyme disease (symptoms > 1 year)
3. Reduction in untreated and inadequately treated Lyme disease
4. After the end of therapy for chronic Lyme disease: their normalization represents therapeutic success
5. Not highly specific: Also low in other bacterial infections, esp. Chlamydia pneumonia and Mycoplasma pneumoniae

CD3-/CD57+ T-Lymphocytes

Reference range (mean/range)

Lyme patient: 46 /ul / 8 – 160 /ul

Healthy: 164 /ul / 60 – 354 /ul

Source: J.J.Burrascano JR., MD, R. Stricker, MD, 2006 ILADS, Crowne Plaza Hotel, Center City Philadelphia

Reflecting the actual T-cellular activity: The EliSpot Established



The established Borrelia EliSpot (T-Cell-Spot)

- ✓ reflects the actual activity of chronic and recent infections
- ✓ sensitivity is estimated at 84%, and the specificity is 94%
- ✓ is approved by the FDA in May 2011 for *M. tuberculosis*
- ✓ Available for: *Borrelia burgdorferi*, *Ehrlichia*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Yersinia*, EBV, CMV
- ✓ covers the following Antigens for *Borrelia* subspecies:

Borrelia burgdorferi Fully Antigen: *Borrelia* b. B31-reference strain (*Borrelia* b. sensu stricto)

Borrelia b. Peptide-Mix: OspA from *Borrelia* b. sensu stricto, *Borrelia afzelii*, *Borrelia garinii* + OspC native + DbpA recombinant

Borrelia b. LFA-1 (Lymphocyte Function Antigen 1): Own body protein + *Borrelia burgdorferi* sensu stricto (shared epitope)


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DIAGNOSING TICK-BORNE DISEASES

Borrelia antigens in the Borrelia EliSpot

- Borrelia burgdorferi full antigen: Borrelia burgdorferi B31 reference strain (Borrelia burgdorferi sensu stricto)
- Borrelia burgdorferi peptide mix: OspA from Borrelia b. sensu stricto, Borrelia afzelii, Borrelia garinii + OspC native + DbpA recombinant
- Borrelia burgdorferi LFA-1 (Lymphocyte Function Antigen 1): Own body protein + Borrelia burgdorferi sensu stricto (shared epitope). Often associated with autoimmune diseases: collagenosis, Rheumatoid Arthritis, vasculitis (ANA, CCP antibodies, ANCA)

Explanation: Native = cultured antigens; Recombinant: produced using genetic technology

ELISPOT: New T-Cell Test a Game Changer for Lyme Disease

- ... The sensitivity of the ELISPOT is estimated at 84%, and the specificity is 94%...
- ... ELISPOT assays provide robust, highly reproducible data...
- ... ELISPOT can be retested to gain additional information in follow-up assays...
- ... the tests in the two-assay system (ELISPOT + CD57 cell count) complement each other in the quest to understand T cell-mediated immunity in vivo....

Lehman PV et al.: Unique Strengths of ELISPOT for T Cell Diagnostics in: Kalyuzhny AE. Handbook of ELISPOT: Methods and Protocols, Methods in Molecular Biology, Vol. 792. 2nd Ed: Springer; 2012: 3-23

94 % **Specificity of Borrelia Elispot-LTT**

84 % **Sensitivity of Borrelia Elispot-LTT**



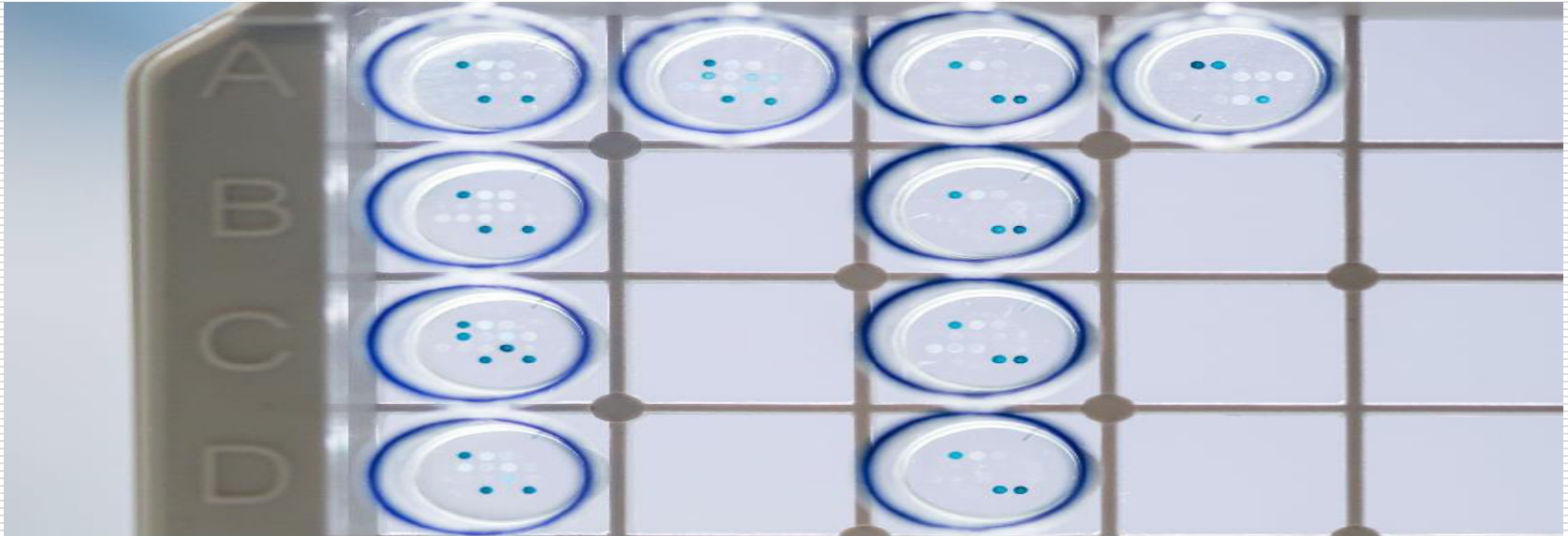
Currently the EliSpot is available for:

- Borrelia burgdorferi (3 subspecies)
- Chlamydia pneumoniae
- Chlamydia trachomatis
- Ehrlichia
- Yersinia species
- Epstein Barr Virus (EBV)
- Cytomegalovirus (CMV)
- Herpes Simplex Virus 1 / 2

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DIAGNOSING TICK-BORNE DISEASES

Introducing the modern MicroArray: **The SeraSpot®**



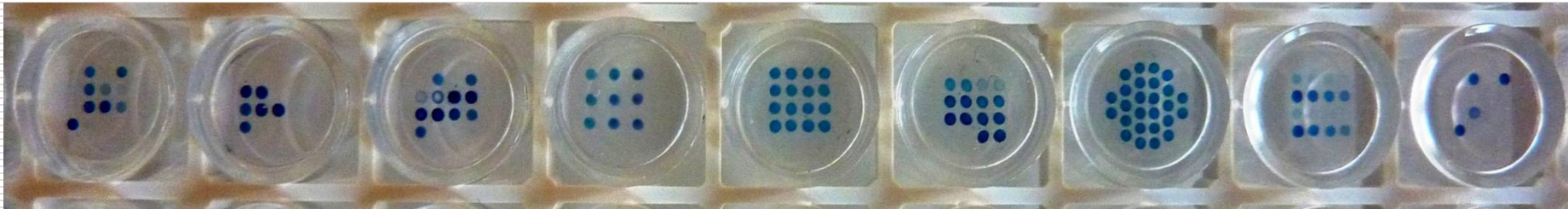
Replaces the Immunoblot at ArminLabs as it

- ✓ offers a better standardisation and more controls
- ✓ combines established ELISA-technique with improved sensitivity of MicroArray analytics
- ✓ covers the following Antigens for subspecies:

VlsE(B.b. afzelii), p39(B.b. afzelii), p58(B.b. garinii), p100 (B.b. afzelii), OspC (B.b. afzelii + B.b. garinii + B.b. sensu stricto), DbpA (B.b. afzelii + B.b. garinii + B.b. sensu stricto)

SeraSpot MicroArray

- Microplates are coated with several antigen spots
- ...processed like an ELISA
- ...read in a special MicroSpot Reader
- ...interpreted using multiplex software
- ...tests for 3 different European Borrelia subspecies:
B.b.s.s. + B.b. garinii + B.b. afzelii (i.e., ArminLabs tests for all 3 European subspecies; IGeneX and some other labs do not)



Borrelia burgdorferi antigens in test systems

Combination of specific Borrelia markers

Recombinant antigens

ArminLabs uses these; IGeneX and some other labs do not:
Higher sensitivity than native antigens that are not expressed in bacterial cultures or expressed only in insufficient amounts, e.g. VlsE has over 99% specificity

+

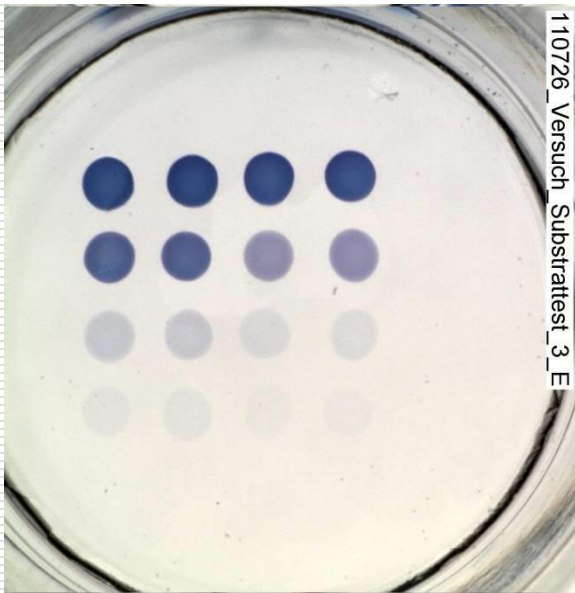
Native antigens: ArminLabs uses these, too

High specificity but lower sensitivity than recombinant antigens

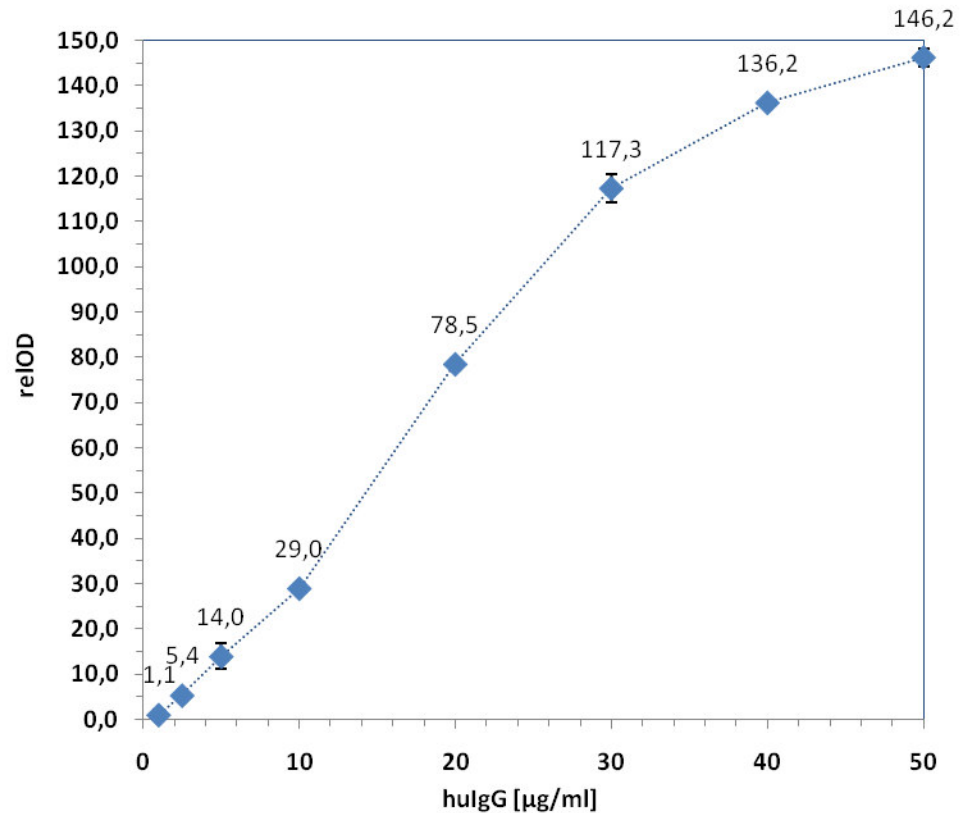
1. Isolated natively, e.g. OspC
2. Cut from a Western blot membrane, e.g. BmpA

Combination of recombinant antigens + native antigens should be used (ArminLabs does; IGeneX and some other labs do not)

It is possible to quantify the SeraSpot MicroArray, but not the Immunoblot



Quantification



Basic diagnostic tests for chronic Lyme Borreliosis

1. **Borrelia IgM and IgG antibodies** by the Microarray (**SeraSpot**) technique, incl. VlsE: Sensitivity around 60%, specificity around 99%
2. **Borrelia Elispot (LTT): current Borrelia activity:** Sensitivity around 84%, specificity 82-100%
3. **CD3-/CD57+ cells: chronic Borrelia activity:** Sensitivity around 70%, specificity ? (i.e. also low in Chlamydia or Mycoplasma infections)

All 3 tests together: >90% sensitivity+99% specificity

Monitoring 1 month after the end of therapies to verify whether the therapy has been successful or not:

Laboratory STAGING process

Lyme Borreliosis: The great imitator

20-30% of autistic disorders can be caused by Borrelia and 58% by Mycoplasma
(Bransfield et al.: Med Hypotheses.2008; 70(5):967-74)

Multiple Sclerosis, myelopathies, polyneuropathies, brain tumor, encephalopathy.
(Neurosurgery.1992May;30(5):769-73)

Can cause meningitis, encephalitis, neuritis, mania, depression, schizophrenia, anorexia, dementia.
(Am J Psychiatry. 1994 Nov;151(11):1571-83)

"90% of chronic fatigue patients are Lyme positive."
(Informal study by American Lyme Disease Alliance at www.lymealliance.org)

"Most fibromyalgia patients are Lyme positive."
(Rheum Dis Clin North Am. 1998 May;24(2):323-51 & report of Lida Mattman,M.D.)

"Borrelia can cause Parkinsonism"
(Arch.of Path.& Lab.Med.127(9):1204-6)

Pure Lyme dementia exists and has a good outcome after antibiotics. It is advisable to do Lyme serology in demented patients.
(Blank et al.: Journal of Alzheimer's disease, Volume 4/2014, 1087-1093)

Chlamydia pneumoniae

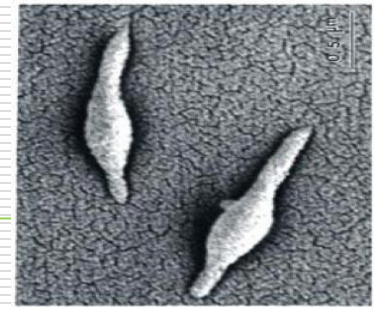
Bacteria: Chlamydophila pneumoniae (gram-negative, intracellular); cystic and aberrant forms, biofilms

Vector/transmission: airborne infection, human to human, ticks? Or reactivated in Lyme disease (horses, koalas, frogs are infected), aerogen transmission (cough) from horses to horse-riders?

Symptoms: cough, slight throat pain, hoarseness, sinusitis, atypical pneumonia, meningoencephalitis, bronchiolitis obliterans, myocarditis, Guillain-Barre Syndrome; arthritis, tendovaginitis (4 - 6 weeks)

Associations: Alzheimer's, Multiple Sclerosis, depression, Fibromyalgia, ME/CFS, heart attacks, acute ischemic stroke (AIS), arteriosclerosis, autism, Parkinsonism, Rheumatoid Arthritis, etc.

Mycoplasma infection



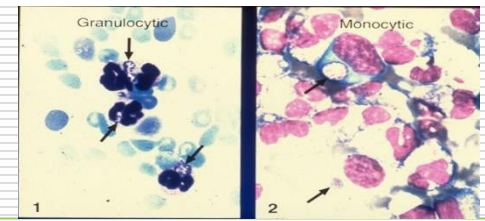
Bacteria: Mycoplasma pneumoniae/fermentans (gram-positive, intracellular)

Transmission: airborne infection, human to human, ticks?

Symptoms: Fatigue (100%), fever, joint pain, joint swelling , muscle pain, headache, insomnia, anxiety, emotional volatility, lack of concentration, memory loss, autism

ME, "Gulf War I syndrome"

Ehrlichia / Anaplasma



Source: CDC

Bacteria: Ehrlichia chaffeensis, Anaplasma phagocytophilum (gram-negative, obligatory intracellular in granulocytes or monocytes)

Human Granulocytic Ehrlichiosis (HGE) or

Human Monocytic Ehrlichiosis (HME)

Vector: Ixodes ricinus

Spectrum of hosts: game (e.g. deer), domestic animals, humans

Symptoms (incubation time: days up to 4 weeks): rapid onset of beginning illness with fever, headache and prostration, headaches are "sharp, knife-like and often located behind the eyes", muscle pain, not joint pain, neurological symptoms, psychiatric symptoms, rarely: diffuse vasculitic rash, including palms and soles (<10%)

Bartonella

Bacteria: *B. henselae* (cat scratch disease), *B. quintana* (Trench fever, bacillary angiomatosis), *B. bacilliformis* (Carrion 's disease/Oroya fever), 5 other subspecies known to be pathogens for humans (gram-negative, facultative intracellular bacterium in endothelial cells/erythrocytes)

Vector/transmission: cat-scratch surface wounds, *Ixodes ricinus* (Germany/Europe: up to 40% of ticks are contaminated), fleas, mosquitoes, sand flies

Symptoms (incubation time 3 - 38 days): tiredness (100%), headache (80%), muscle twitches, tremors, seizures, fever in the mornings (30%, in spates of up to 6 weeks, otherwise 1 - 3 weeks), swollen lymph nodes, arthralgia (often), myalgia, insomnia, depression, agitation, severe mood swings, lack of concentration and alertness, dizziness, anxiety, outbursts, antisocial behaviour, restlessness, gastritis, intestinal symptoms, sore soles (especially in the morning), tender subcutaneous nodules along the extremities, occasional lymphadenopathy and light sweats, striae; Complications: endocarditis, retinitis, epilepsy, aseptic meningitis, hepatosplenomegaly

Bartonella striae



Babesia

Bacteria: Babesia microti, Babesia divergens, Babesia duncani

Vector/transmission: Ixodes ricinus, Dermacentor reticulatus, blood transfusions

Hosts: game (e.g. deer), domestic animals, humans

Symptoms (incubation time 5 days – 9 weeks):

Rapid onset of beginning illness with severe fever, headache (can be severe/dull, global, involves the whole head, described like the head is in a vice), sweats (usually at night, but can be day-sweats as well), fatigue (worse with exercise), "air-hunger", need to sigh and take a deep breath, dry cough without apparent reason, stiffness of neck, nausea, diminished appetite, tiredness, feeling of weakness, permanent exhaustion even worse during stress, dizziness, haemolytic anaemia, hemoglobinuria, haemangiomas, (seldom) hepatosplenomegaly, muscle pain, dizziness, mental dullness and slowing of reactions and responses, hypercoagulability, stomach pain, emotional lability, "mental dullness", kidney problems, dyspnoea, influenza-like symptoms (could be lethal)

Risk factors: Splenectomy, HIV, organ transplantation, blood transfusions

Rickettsia

Bacteria: Rickettsia conorii (Boutonneuse Fever), R. rickettsia (RMSF), R. helvetica, R. slovaca, R. prowazekii (gram-negative, obligate intracellular in endothelial cells)

Vector/hosts: rodent, dogs, humans, Ixodes ricinus, Dermacentor reticulatus

Symptoms (incubation period 5 - 7 days): fever, nausea, vomiting, severe headache, lymphadenitis, exanthema

Complications (app. 13%): peri-/myocarditis, kidney insufficiency, pneumonia, encephalitis, gastrointestinal bleedings, anaemia, hepatitis, myalgia, meningitis

Epstein Barr Virus (EBV)

Virus: Epstein Barr Virus (obligate intracellular), double stranded DNA virus, one of the Herpesviruses, “Mononucleosis”

Transmission: “kissing disease”, saliva, drinking from the same glass, toothbrush, blood, sex, blood-transfusion, organ transplantation

Symptoms (incubation period several weeks): fatigue, fever, flu-like symptoms, nausea, loss of appetite, lymphadenitis (swollen lymph nodes in the neck), rash, sore throat, weakness, sore muscles

Complications: enlarged spleen, swollen liver, association with Non-Hodgkin Lymphoma

Cytomegalovirus (CMV)

Virus: Cytomegalovirus (obligate intracellular), double-stranded DNA virus, one of the Herpes viruses

Transmission: body fluids (urine, saliva, breast milk, sexual transmission), organ transplantation, blood transfusion

Symptoms (incubation period several weeks): fatigue, fever, flu-like symptoms, lymphadenitis (swollen cervical lymph nodes), sore throat, splenomegaly

Complications: congenital infection with hearing loss, vision loss, seizures, mental disabilities, lack of coordination; immune suppressed patients: hepatitis, colitis, retinitis, pneumonitis, esophagitis, polyradiculopathy, transverse myelitis, subacute encephalitis; arterial hypertension, arteroscleroris, aortic aneurysms; association with Non-Hodgkin Lymphoma

Herpes Simplex Virus 1 / 2 (HSV 1 / 2)

Virus: Herpes Simplex Virus (Human Herpes Virus HHV 1 / 2) (obligate intracellular), double-stranded DNA virus, one of the Herpes viruses

Transmission: Saliva, sharing drinks, sexually transmitted

Symptoms (incubation time 2-20 days): Watery blisters on the skin or mucous membranes of the mouth, lips, genitals, anus, flu-like symptoms (fever, muscle aches, swollen lymph nodes, problems urinating, herpes keratitis (pain, light sensitivity, discharge))

Complications: Multiple Sclerosis (neurovirulent), loss of vision, encephalitis, latent infection; reactivation by organ transplantation or HIV: encephalitis, pneumonitis, bone marrow suppression

Human Herpes Virus 6 (HHV6)

Virus: Human Herpes Virus 6 (obligate intracellular), double-stranded DNA virus, one of the Herpes viruses

Transmission: Saliva, latency in salivary glands, haematopoietic (blood-building) system

Symptoms: Exanthema subitum (roseola infantum, sixth disease) with high temperature followed by a rash

Complications: Multiple Sclerosis (neurovirulent), cofactor in CFS, fibromyalgia, AIDS, optic neuritis, cancer, temporal lobe epilepsy, Hashimoto thyroiditis, liver dysfunction, liver failure; reactivation by organ transplantation: encephalitis, pneumonitis, bone marrow suppression,

Coxsackie Virus

Virus: Coxsackie Virus (obligate intracellular), belongs to Picornaviridae/ enterovirus family, is a single-stranded RNA virus divided into group A and group B

Transmission: fecal-oral contamination, droplets, body fluids, utensils, toys, diaper-changing table

Symptoms: Group A: Herpangina, AHC (acute hemorrhagic conjunctivitis, HFM (hand-foot-and-mouth disease), Group B: myocarditis, pericarditis, pleurodynia, hepatitis; Group A and B: fever, rashes, sore throat, diahorrea, cough, fatigue, conjunctivitis, loss of appetite, headache, night sweats, aseptic meningitis

Complications: CNS disease similar to poliomyelitis, systemic neonatal disease, IDDM (insulin-dependent diabetes mellitus), Group A: generalized myositis with flaccid paralysis, Group B: focal muscle injury, degeneration of neuronal tissue with spastic paralysis

Tailored testing protocols

- Fibromyalgia/Rheumatoid Arthritis
- Multiple Schlerosis
- ME
- Dementia
- Yersinia species
- Epstein Barr Virus (EBV)
- Cytomegalovirus (CMV)

Fibromyalgia

**"Most fibromyalgia patients
are Lyme positive."**

(Rheum Dis Clin North Am. 1998 May;24
(2):323-51 & report of Lida Mattman, M.D.)

The oldest patient with "Fibromyalgia" (5,300 years ago): "Iceman" Ötzi



Ötzi's enemies: Ticks!
"Zink's team found almost two-thirds of the genome of *Borrelia burgdorferi*, a bacterium that causes Lyme disease. Zink speculates that tattoos on the iceman's spine and ankles and behind his right knee could have been an attempt to treat the joint pain that occurs when the condition goes untreated."