Unravelling Some of the Complexities of Laboratory Testing

Testing for viral and bacterial infections in multi-system diseases
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Agenda

Antibody (B cell) tests vs. using T cells

- B cells: IgG, IgM, IgA
- T cells: EliSpot (LTT-Interferon Gamma Release Assay)

The benefits of using a CD3/CD57 assay

How to decide what to test for

Where to find further information

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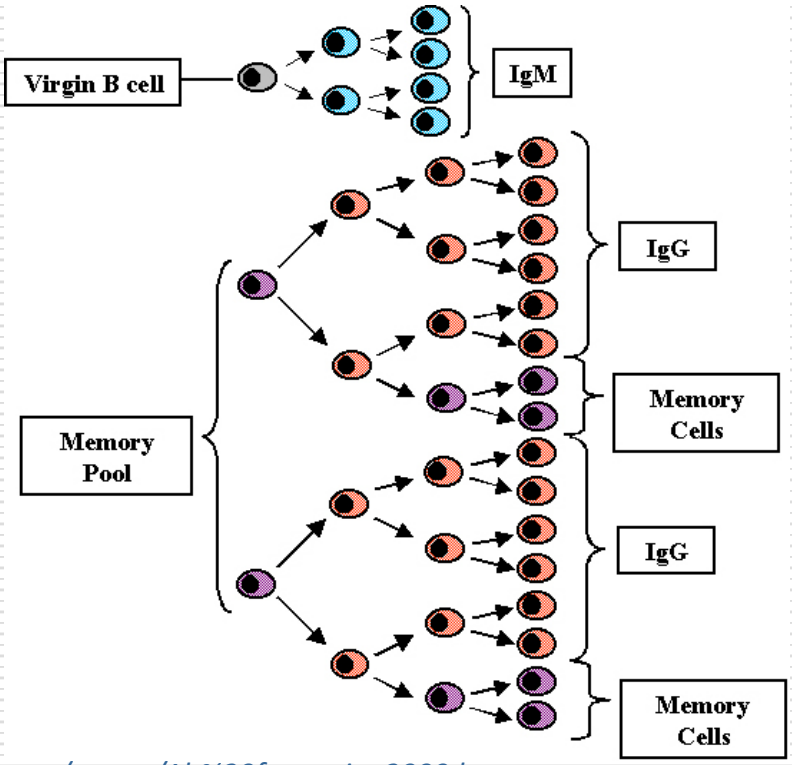
IgM (Immunoglobulin M) vs. IgG (Immunoglobulin G)

"In the primary response, the major class of antibody produced is IgM, whereas in the secondary response it is IgG (or IgA or IgE). The antibodies that persist in the secondary response are the IgG antibodies."



Source: http://www.microbiologybook.org/mayer/Ab%20formation2000.htm; http://www.microbiologybook.org/mayer/ab1-8.jpg

IgM antibodies are the first type of antibody produced, and usually do not persist

"Detection of IgM antibodies tends to indicate a recent initial exposure to an antigen, whereas detection of total or IgG antibodies indicates exposure some time ago."[2]

IgM Antibody Functions and its Role in Disease

During infection, innate or "natural immunity" is provided by poly-reactive IgM antibody made by (B1a) B cells. IgM antibody acts to quickly recognize and initiate an immune response by directly neutralizing pathogens or clearing novel antigens. The three components of the IgM antibody-mediated immune response are activation of complement (C1qR and Fcα/μR), recruitment of phagocytic cells, and opsonization. Current research suggests that B1b B cells which make IgM antibodies may provide memory to certain pathogens and support T-cell independent immune responses. IgM antibody also acts as an educator of the immune system by transporting antigens to lymph tissues where memory is induced. Read more »

"The time required for the development of IgG antibodies following HSV infection varies from 21 to over 42 days with most individuals having detectable IgG 21–28 days after exposure to the infection and probably lasting for life.[7–,9] **IgM antibodies are usually detectable 9–10 days after exposure and last 7–14 days**, although they may remain detectable for up to 6 weeks in a minority of individuals.[9–,11] IgM antibodies may be detectable during recurrences of the infection, particularly with some of the commercial ELISAs."[2]

Source: 1. https://www.labtestsonline.org.au/learning/test-index/antibody-tests; 2. https://www.genscript.com/IgM-antibody.html

The difficulties of evidencing chronic (continuing) disease using IgG and IgM

In chronic disease, IgG may be there, but will be discounted as "past"; IgM probably will not be

ENDOCRINOLOGY

Cytomegalovirus Ab(IgG)	183.0	AU/ml
	< 6.0 AU/mL is considered non-reactive	
	>=6.0 AU/mL is considered reactive	
Cytomegalovirus Ab(IgM)	Negative	
Comment	Result suggestive of previous CMV infection.	

IMMUNOLOGY

Epstein-Barr virus screen			
EBNA IgG antibody	* 36	U/ml	(< 5 U/ml Negative)
EBV Early Ag ab.(IgG)	<5	U/ml	(<10 U/ml Negative)
EBV VCA ab.(IgM)	<10	U/ml	(<20 U/ml Negative)
Comment	Results suggestive of past (latent) EBV infection.		

"IgG is produced in a delayed response to an infection and can be retained in the body for a long time …. Detection of IgG usually indicates a prior infection or vaccination."

Source: http://www.microbiologybook.org/mayer/Ab%20formation2000.htm

Studies can be found using "at least fivefold rise" in IgG level as a sign of viral reactivation

Lupus. 2018 Jul;27(8):1271-1278. doi: 10.1177/0961203318770535. Epub 2018 Apr 18.

Longitudinal analysis of varicella-zoster virus-specific antibodies in systemic lupus erythematosus: No association with subclinical viral reactivations or lupus disease activity.

Rondaan C[1], van Leer CC[2], van Assen S[3], Bootsma H[1], de Leeuw K[1], Arends S[1], Bos NA[1], Westra J[1].

Author information

Abstract

Systemic lupus erythematosus (SLE) patients are at high risk of herpes zoster. Previously, we found increased immunoglobulin (Ig)G levels against varicella-zoster virus (VZV) in SLE patients compared to controls, while antibody levels against diphtheria and cellular immunity to VZV were decreased. We aimed to test our hypothesis tha
caused by stress because of lupus disease activity or imm
IgG and VZV-DNA were longitudinally determined in the se
polymerase chain reaction. Clinical data were retrieved fro
VZV-IgG or presence of VZV-IgM or VZV-DNA. Generalize
between antibody levels, lupus disease activity and medica
stranded DNA and complement levels were used as indicators of lupus disease activity. Results A VZV reactivation was determined in 11 patients (33%). In at least five of them, herpes zoster was clinically overt. No association between SLE disease activity or medication use and VZV-specific antibody levels was found. There was a weak association between total IgG and VZV-IgG. Conclusions Our results indicate that increased VZV-IgG levels in SLE do not result from frequent subclinical VZV reactivations, and are not associated with lupus disease activity. Increased VZV-IgG can only partially be explained by hypergammaglo

KEYWORDS: Systemic lupus erythematosus; herpes zoster; humoral immunity

"Reactivation of VZV was defined as an at least fivefold rise in VZV-IgG or presence of VZV-IgM or VZV DNA."[1]

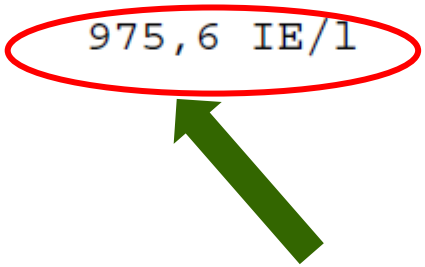
"...reactivation of VZV (defined as a fivefold increase in the IgG antibody titer)"[2]

Source: 1. Rondaan, Christien & C van Leer, C & van Assen, S & Bootsma, H & de Leeuw, K & Arends, S & Bos, Nicolaas & Westra, J. (2018). Longitudinal analysis of varicella-zoster virus-specific antibodies in systemic lupus erythematosus: No association with subclinical viral reactivations or lupus disease activity. Lupus. 27. 2. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.858.3795&rep=rep1&type=pdf;

Example: Varicella Zoster Virus reactivation? (The shingles virus, from former chicken pox)

VZV IgG-/IgA-/IgM-antibodies

3	VZV IgG antibodies (ELISA)	positive
		975,6 IE/l
	<80 IE/l	negative
	>80 - < 110 IE/l	weak
	>110 IE/l	positive



This is around 10x the upper reference range

Titres are used in some antibody tests

Analysis		Result	Units	Reference Range	Chart
HHV6-IgG-/IgM-antibodies					
4 HHV6 IgG-antibodies (IFT)	**+**	**1:1000**		**< 1:10**	**[..... *>**
4 HHV6 IgM-antibodies (IFT)		<1:10		< 1:10	[....*...]
The specific Human Herpes Virus 6 (HHV6)-IgG-antibodies indicate humoral immune-response against Human Herpes Virus 6 (HHV6).					
validated by Dr.Armin Schwarzbach					

Titres indicate increasing dilution. Dilution of less than one to ten for HHV6 IgM means negative, over (e.g. 1:100) would be positive

Coxsackie-Virus antibodies				
Coxsackie-Virus Type A7-IgG (IFT)	+	1:1000	Titer	< 1:100
Coxsackie-Virus Type B1-IgG (IFT)	+	1:1000	Titer	< 1:100

Some antibody tests show IgA status: this does show current activity

Am J Kidney Dis. 1988 Nov;12(5):384-7.

The IgA mucosal immune system.

Lamm ME[1].

Author information

"IgA is quantitatively the most important of the immunoglobulins, having a synthetic rate exceeding that of all other immunoglobulins combined when secretory as well as circulating IgA is taken into account."[1]

Abstract

This report reviews the immunophysiology of the mucosal immune system, the principal antibody of which is a special form of IgA, termed secretory IgA. This IgA is produced locally by mucosal plasma cells that are descended from precursors initially stimulated in organized, mucosal lymphoid organs designed for antigen sampling. After the initial triggering, the precursor cells pass via regional lymph nodes, lymph, and blood to disseminate widely among mucosal sites. After secretion from a local plasma cell, IgA binds to an epithelial cell surface receptor and the complex passes through the epithelial cell into the secretions where it serves as a nonphlogistic immunologic barrier to inhibit uptake of antigens. The production of IgA is facilitated by particular regulatory T cells. At the same time, the synthesis of other classes of antibody, such as the phlogistic IgG, is dampened. This differential regulation of individual antibody classes after exposure to mucosal antigen plus the interrelatedness of the various mucous membranes of the body have important implications for host defense, pathogenesis of a variety of diseases including IgA nephropathy, and strategies of immunization.

PMID: 3055963

"The major antibodies found on mucous membranes are secretory IgA, which function primarily by binding microorganisms and thereby preventing their contact with the host tissues."[2]

Source: 1. Mucosal Immunity, Stephen P. James, in Encyclopedia of Immunology (Second Edition), 1998, https://www.sciencedirect.com/topics/neuroscience/immunoglobulin-a; 2. Hanson, L., Andersson, B., Carlsson, B. et al. Infection (1985) 13(Suppl 2): S166.

IgA is available for CPN, HSV1/2, VZV, Coxsackie, Echovirus, and various others (always worth asking)

Chlamydia pneum. IgG-/IgA-AB

4 Chlam.pneum. IgG-AB (ELISA) positive negative
! 1,525 Ratio
Ratio < 0,8 = negative
Ratio 0,8 - 1,1 = weak
Ratio >= 1,1 = positive

4 Chlam.pneum. IgA-AB (ELISA) positive negative
! 1,628 Ratio
Ratio < 0,8 = negative
Ratio 0,8 - 1,1 = weak
Ratio >= 1,1 = positive

Coxsackie IgG-/IgA-antibodies

3 Coxsackie-Virus IgG A7 (IFT)	**+**	**1:100**	**< 1:100**	**[**	**. . . . . *>**
3 Coxsackie-Virus IgG B1 (IFT)	**+**	**1:1000**	**< 1:100**	**[**	**. . . . . *>**
3 Coxsackie-Virus IgA A7 (IFT)		<1:10	< 1:10	[	....*...]
3 Coxsackie-Virus IgA B1 (IFT)	**+**	**1:100**	**< 1:10**	**[**	**. . . . . *>**

The specific Coxsackie-Virus Type B1-IgG-/IgA-antibodies indicate current humoral immune response against Coxsackie-Virus Type B1.
The specific Coxsackie-Virus Type A7-IgG-antibodies indicate humoral immune-response against Coxsackie-Virus Type A7.
The test system is highly specific for Coxsackie Virus antibodies. Other Enterovirus antibodies (f.e. Echovirus antibodies) are not detectable.

Agenda

Antibody (B cell) tests vs. using T cells

- B cells: IgG, IgM, IgA
- T cells: EliSpot (LTT-Interferon Gamma Release Assay)

Testing the other arm of the immune system: T-cells

Using T-cells to show a cellular response against antigens is much more sensitive, and **indicates active infection (in contrast to antibodies, which can remain for months or years long after an infection is gone)**. EliSpot (enzyme-linked immunosorbent spot) technology has long been used in Germany to do exactly this: it quantifies T-cells that secrete signature proteins (such as a given cytokine) against a specific antigen. The Borrelia EliSpot evaluates the number of spot-forming units using a stimulation index (SI) based on IGRA (Interferon Gamma Release Assay).

Humana Press; 3rd ed. 2018 edition (14 July 2018)

The Elispot technique

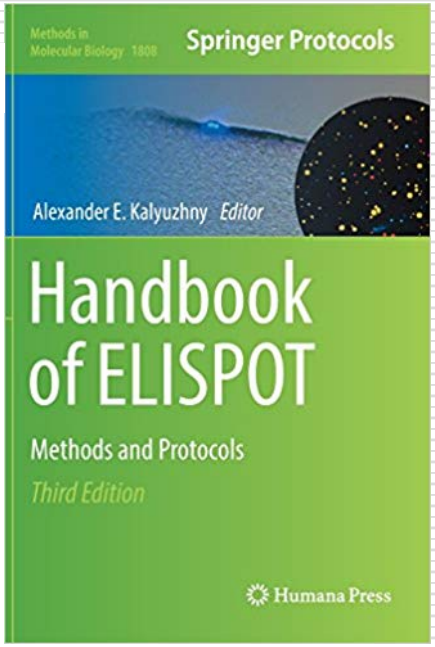
Chapter 1

Unique Strengths of ELISPOT for T Cell Diagnostics

Paul V. Lehmann and Wenji Zhang

Abstract

The T cell system plays an essential role in infections, allergic reactions, tumor and transplant rejection, as well as autoimmune diseases. It does so by the selective engagement of different antigen-specific effector cell lineages that differentially secrete cytokines and other effector molecules. These T cell subsets may or may not have cytolytic activity, can preferentially migrate to different tissues, and display variable capabilities to expand clonally. The quest of T cell immune diagnostics is to understand which specific effector function and T cell lineage is associated with a given clinical outcome, be it positive or adverse. No single assay can measure all of the relevant parameters. In this chapter, we review the unique contributions that ELISPOT assays can make toward understanding T cell-mediated immunity. ELISPOT assays have an unsurpassed sensitivity in detecting low frequency antigen-specific T cells that secrete effector molecules, including granzyme and perforin. They provide robust, highly reproducible data – even by first time users. Because
cytometry, ELISPOT is ideally su
ditions. These include defining (
establishing the fine–specificity
concentrations of the antigen in se
secretory products released by T
because T cells survive ELISPOT



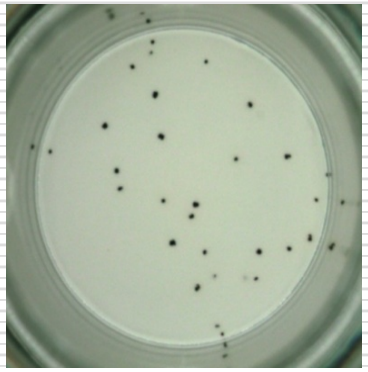
"The quantification of single cell interferon-gamma (IFN-γ) release for assessing cellular immune responses using the Enzyme-linked immunospot (ELISPOT) assay is an invaluable technique in immunology."[1]

Source: 1 Sedegah M. The Ex Vivo IFN-γ Enzyme-Linked Immunospot (ELISpot) Assay Methods Mol Biol. 2015;1325:197-205; Humana Press; 3rd ed. 2018 edition (14 July 2018)

EliSpot (Interferon-Gamma Release Assay)

Reflects the **current T-cellular activity** of bacteria and viruses

- **T-Cell-Spot/IGRA was approved by the FDA in May 2011 for M. tuberculosis**



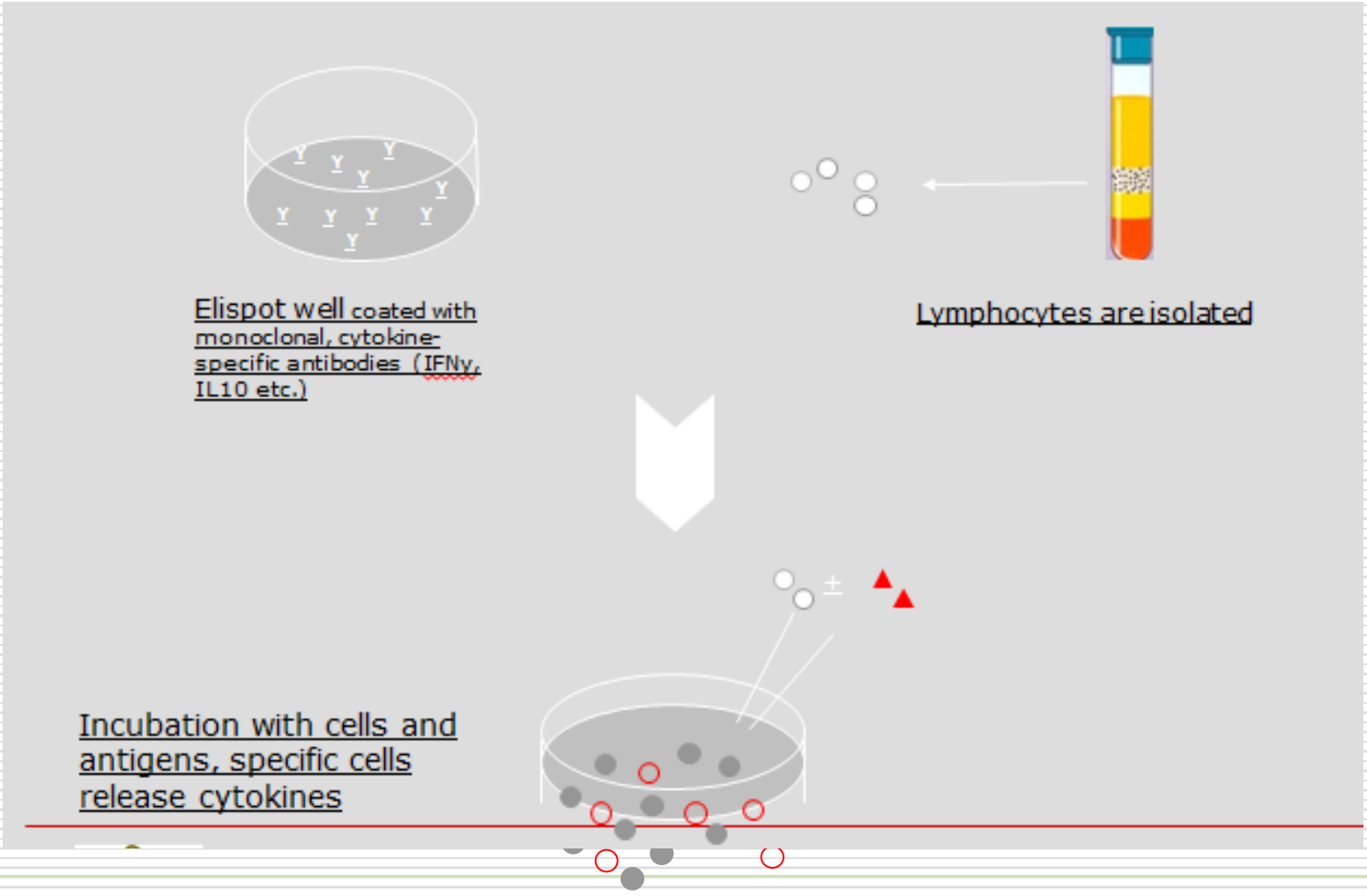
- **"… A positive result suggests that an infection is likely, a negative result suggests that an infection is unlikely…."**
  "…Results can be available within 24 hours…"

… ELISPOT assays provide robust, highly reproducible data, and can be retested to gain additional information in follow-up assays…

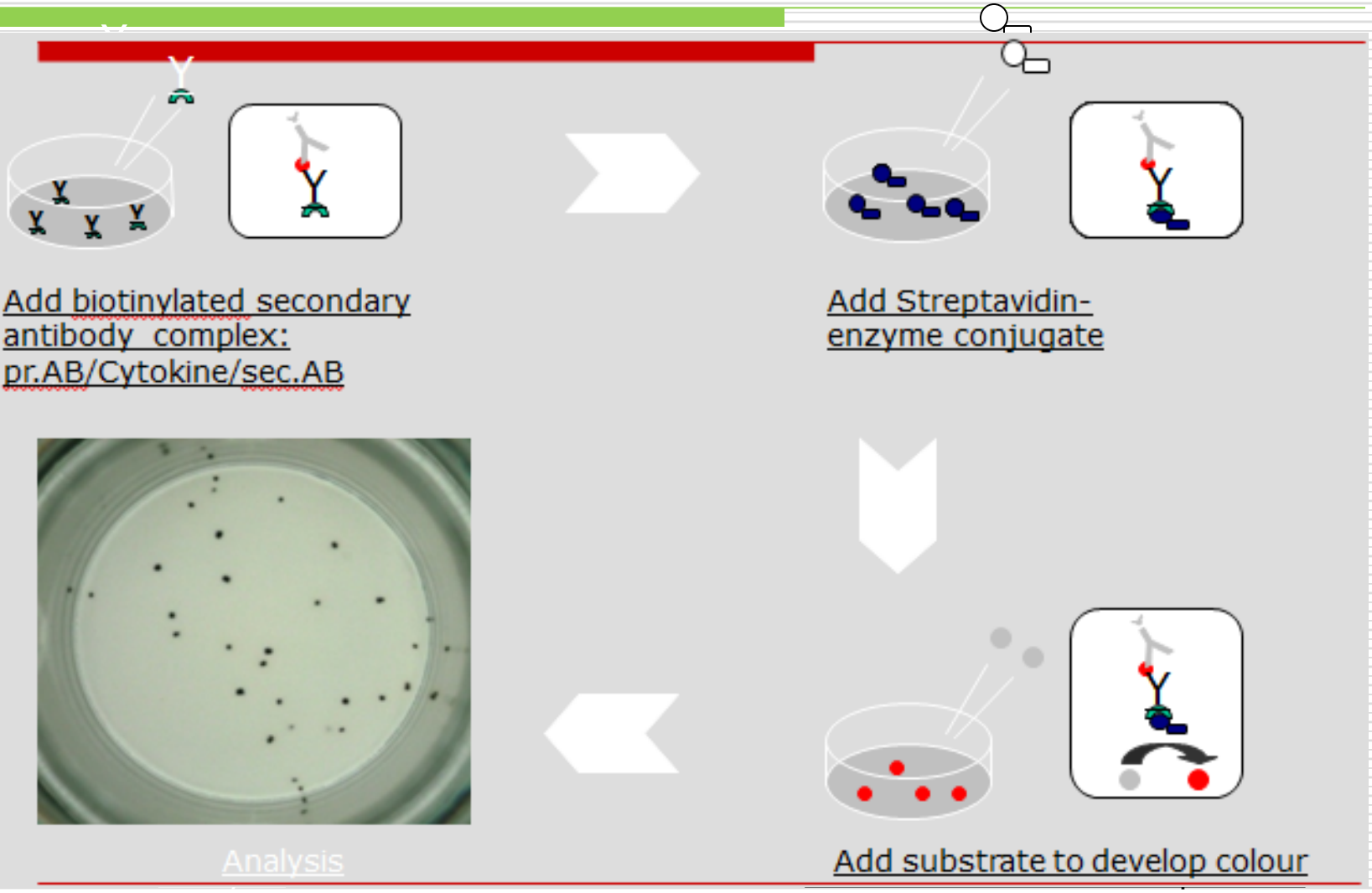
… the tests in the two-assay system (ELISPOT + CD57 cell count) complement each other in the quest to understand T cell-mediated immunity in vivo….

Source: 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.

Elispot LTT: Methodology (I)



Elispot LTT: Methodology (II)



Currently the EliSpot is available for:

- Borrelia burgdorferi (3 subspecies: B.b. sensu stricto + B.b. garinii + B.b. afzelii)
- Borrelia myamotoi
- **Bartonella henselae (new)**
- **Babesia microti (new)**
- Chlamydia pneumoniae
- Chlamydia trachomatis
- **Mycoplasma pneumoniae (new)**
- Ehrlichia
- Yersinia species
- Epstein Barr Virus (EBV)
- Cytomegalovirus (CMV)
- Herpes Simplex Virus 1 / 2
- **Varicella Zoster Virus (VZV) (new)**

Also new:
Candida
Aspergillus niger

Examples: Borrelia/EBV

Borrelia burgdorferi Elispot

Borrelia burgdorferi Full Antigen	+	32	SI
Borrelia b. OSP-Mix (OSPA/OSPC/DbpA)	+	29	SI
Borrelia burgdorferi LFA-1	(+)	2	SI

>3 = positive

2-3 = weak positive

<2 = negative

The results of the EliSpot-Tests indicate current cellular activity against Borrelia burgdorferi.

Epstein-Barr-Virus EliSpot

EBV-EliSpot (lytic)	+	30	SI
EBV-EliSpot (latent)	+	21	SI

>3 = positive

2-3 = weak positive

<2 = negative

The result of the EBV-EliSpot-Test indicates current cellular activity against Epstein-Barr-Virus.

References on the Elispot: examples

Navarrete MA ELISpot and DC-ELISpot Assay to Measure Frequency of Antigen-Specific IFNγ-Secreting Cells, in Hnasko R (Editor), Elisa Methods and Protocols 2015.

Navarrete MA, Bertinetti-Lapatki C, Michelfelder I et al (2013) Usage of standardized antigen-presenting cells improves ELISpot performance for complex protein antigens. J Immunol Methods 391:146–153

Czerkinsky CC, Nilsson LA, Nygren H et al (1983) A solid-phase enzyme-linked immunospot (ELISPOT) assay for enumeration of specifi c antibody-secreting cells. J Immunol Methods 65:109–121

Keilholz U, Weber J, Finke JH et al (2002) Immunologic monitoring of cancer vaccine therapy: results of a workshop sponsored by the Society for Biological Therapy. J Immunother 25:97–138
Scheibenbogen C, Lee KH, Mayer S et al (1997) A sensitive ELISPOT assay for detection of CD8+ T lymphocytes specifi c for HLA class I-binding peptide epitopes derived from infl uenza proteins in the blood of healthy donors and melanoma patients. Clin Cancer Res 3:221–226

Sedegah M. **The Ex Vivo IFN-γ Enzyme-Linked Immunospot (ELISpot) Assay**
Methods Mol Biol. 2015;1325:197

Nehete PN, Gambhira R, Nehete BP et al (2003) Dendritic cells enhance detection of antigen-specifi c cellular immune responses by lymphocytes from rhesus macaques immunized with an HIV envelope peptide cocktail vaccine. J Med Primatol 32:67–73

Elispot references (contd.)

Moller I, Michel K, Frech N et al (2008) Dendritic cell maturation with poly(I:C)-based versus PGE2-based cytokine combinations results in differential functional characteristics relevant to clinical application. J Immunother 31:506–519

Warncke M, Dodero A, Dierbach H et al (2006) Murine dendritic cells generated under serumfree conditions have a mature phenotype and effi ciently induce primary immune responses. J Immunol Methods 310:1–1

Malyguine A, Strobl SL, Shafer-Weaver KA et al (2004) A modifi ed human ELISPOT assay to detect specifi c responses to primary tumor cell targets. J Transl Med 2:9

Moodie Z, Price L, Gouttefangeas C et al (2010) Response defi nition criteria for ELISPOT assays revisited. Cancer Immunol Immunother 59: 1489–1501

Janetzki, S. & Britten, C.M. The impact of harmonization on ELISPOT assay performance. *Methods Mol. Biol.* **792**, 25–36 (2012)

Zhang, W. & Lehmann, P. Objective, user-independent ELISPOT data analysis based on scientifically validated principles. *Methods Mol. Biol.* **792**, 155–171 (2012)

Calarota SA. Enumeration and characterization of human memory T cells by enzyme-linked immunospot assays. Clin Dev Immunol. 2013;2013:637649

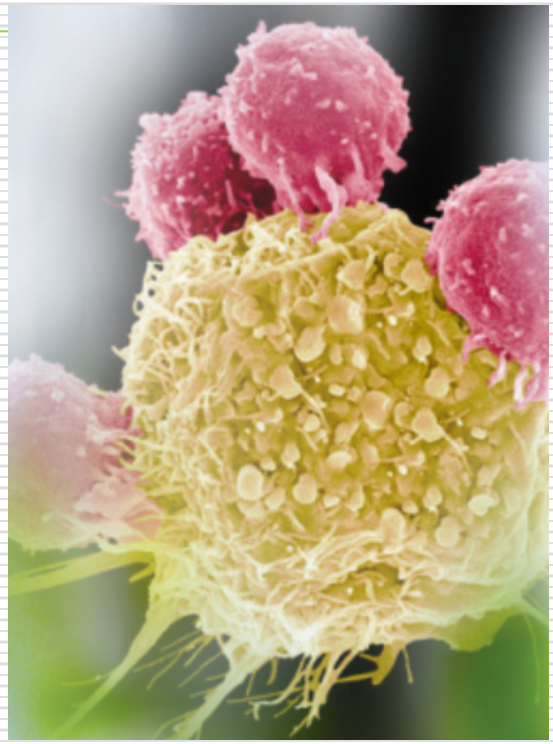
Agenda

Antibody (B cell) tests vs. using T cells

- B cells: IgG, IgM, IgA
- T cells: EliSpot (LTT-Interferon Gamma Release Assay)

The benefits of using a CD3/CD57 assay

CD57 is a stable marker of human natural killer (NK) cell subsets



Source: Differential activation of CD57-defined natural killer cell subsets during recall responses to vaccine antigens. White MJ, Nielsen CM, McGregor RH, Riley EH, Goodier MR - Immunology (2014)

A low CD57+ indicates chronic immune suppression

CD3-/CD57+ Cells

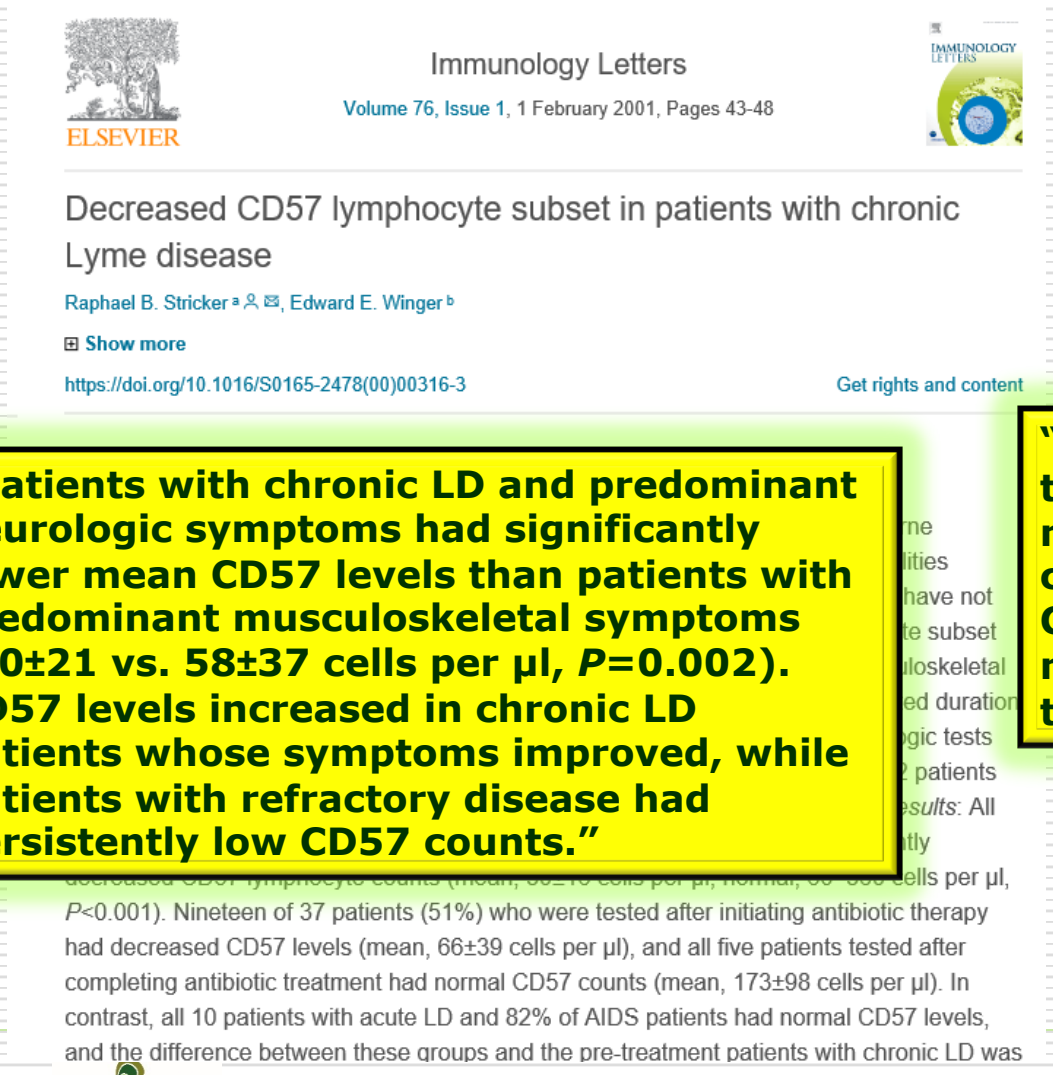
5 CD3-/CD56+ Flow Cytometry					
5 T cells CD3+ (%)	**+**	**82,18 %**	**62,00 - 80,00**	[.....	*>
5 T cells CD3+ (absolute)		1225 /ul	900 - 1900	[..*.....	]
5 NK cells CD56+ CD3- (%)	**-**	**4,75 %**	**6,00 - 29,00**	<*	]
5 NK cells CD56+ CD3- (absolute)		71 /ul	60 - 700	[*.....	]
5 CD57+ NK-cells (%)		18,27 %	2,00 - 77,00	[.*.....	]
5 CD57+ NK-cells (absolute)	**-**	**13 /ul**	**100 - 360**	<*	]

The result of the CD57-cell count indicates chronic immune-suppression, which can be caused by Borrelia burgdorferi or other bacteria like Chlamydia pneumoniae or Mycoplasma pneumoniae.

Suppression = generally bacterial causes: Borrelia, Chlamydia pneumoniae, Mycoplasma

Source (partly, rest Dr. Schwarzbach): Ginger Saveley PhD, http://www.publichealthalert.org/everything-you-always-wanted-to-know-about-the-cd-57-test-but-were-too-sick-to-ask.html

A low CD57+ count has particularly been observed in patients with neurological symptoms



Immunology Letters
Volume 76, Issue 1, 1 February 2001, Pages 43-48

Decreased CD57 lymphocyte subset in patients with chronic Lyme disease

Raphael B. Stricker [a], Edward E. Winger [b]

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ells per µl,

P<0.001). Nineteen of 37 patients (51%) who were tested after initiating antibiotic therapy had decreased CD57 levels (mean, 66±39 cells per µl), and all five patients tested after completing antibiotic treatment had normal CD57 counts (mean, 173±98 cells per µl). In contrast, all 10 patients with acute LD and 82% of AIDS patients had normal CD57 levels, and the difference between these groups and the pre-treatment patients with chronic LD was

"Patients with chronic LD and predominant neurologic symptoms had significantly lower mean CD57 levels than patients with predominant musculoskeletal symptoms (30±21 vs. 58±37 cells per µl, *P*=0.002). CD57 levels increased in chronic LD patients whose symptoms improved, while patients with refractory disease had persistently low CD57 counts."

"*Conclusions*: A decrease in the CD57 lymphocyte subset may be an important marker of chronic LD. Changes in the CD57 subset may be useful to monitor the response to therapy in this disease" ....

"The CD57 lymphocyte subset appears to be a useful marker of long-term infection with the Lyme disease spirochete." (Stricker, Burrascano, 2002)

CD57+ numbers tend to rise in patients with viral burdens …

frontiers in Immunology

Front Immunol. 2013; 4: 422.
Published online 2013 Dec 9. doi: 10.3389/fimmu.2013.00422
PMCID:

Functional Significance of CD57 Expression on Human NK Cells Relevance to Disease

Carolyn M. Nielsen,[1] Matthew J. White,[1] Martin R. Goodier,[1] and Eleanor M. Riley[1,*]

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Abstract

Go to:

Historically, human NK cells have been identified as $CD3^{-}CD56^{+}CD16^{\pm}$ lymphocytes. More recently it has been established that CD57 expression defines functionally discrete sub-populations of NK cell cells, CD57 expression has been regarded as a marker of terminal differentiation and (perhaps wron anergy and senescence. Similarly, CD57 expression seems to identify the final stages of peripheral maturation; its expression increases with age and is associated with chronic infections, particularly cytomegalovirus infection. However, $CD57^{+}$ NK cells are highly cytotoxic and their presence seem beneficial in a number of non-communicable diseases. The purpose of this article is to review our c understanding of CD57 expression as a marker of NK cell function and disease prognosis, as well as to outline areas for further research.

Keywords: CD57, NK cells, HCMV infection, ageing, chronic infection, cancer, autoimmune diseases, T cells

"Chronic viral infections such as HCMV (104), human immunodeficiency virus (HIV) (105), hepatitis C virus (106), and Epstein–Barr virus (EBV) (107) infections offer some of the clearest examples of expansion of $CD57^{+}CD8^{+}$ T cells, presumably as a result of persistent antigenic stimulation"

"Similar skewing of NK cells toward the $CD57^{+}$ phenotype is now reported in a variety of viral infections"

Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3856678/

… whereas the CD3+ count tends to be low

CD 57 Flow Cytometry

T cells CD3 + (%)		76.58	%	62-80
T cells CD3 + (absolute)	-	659	/ul	900-1900
NK cells CD56+CD3- (%)		16.91	%	6-29
NK cells CD56+CD3- (absolute)		146	/ul	60-700

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There is also one sign for a viral infection, this can be seen with the low CD3+cells, which should be looked at with CMV-Elispot, EBV-Elispot, HSV 1/2-Elispot, VZV-antibodies, Coxsackie Virus antibodies.

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- B cells: IgG, IgM, IgA
- T cells: EliSpot (LTT-Interferon Gamma Release Assay)

The benefits of using a CD3/CD57 assay

How to decide what to test for

- Checklists
- Tailored testing protocols

Where to find further information

Checklists help decide which infections to test for; history and physical signs/symptoms also vital (1/2)

Short Symptom Checklist for Lyme Borreliosis

Name, first name ..... Date: .....

►	Actual and former symptoms: Please mark with a cross	X
1	Former or recent tick bite	☐
2	Former or recent bull´s eye rash	☐
3	Summer flu after tick bite	☐
4	Fatique/Malaise/Lethargy	☐
5	Loss of physical/mental capacity, general weakness	☐
6	Neck-pain, neck stiffness	☐
7	Headache	☐
8	Painful joints, swollen joints	☐
9	General aches and pains, tendon problems	☐
10	Muscle pain, muscle weakness	☐
11	Fever, feverish feeling, shivering	☐
12	Ears: intermittent red, swollen earlap	☐
13	Heart problems, disturbance of cardiac rhythm	☐
14	Cough, expectoration, breathlessness	☐
15	Night sweat	☐
16	Sleeplessness, waking up around ..... p.m.	☐
17	Tinnitus	☐
18	Swollen lymph nodes	☐
19	Numbness of the skin	☐
20	"Burning" or "pins and needles" skin sensations, painful sole or foot	☐
21	Back pain, back stiffness	☐
22	Occasional muscle twitching in the face, arms, legs	☐
23	Shivering, chill	☐
24	Blurred, foggy, cloudy, flickering, double vision	☐
25	Aggressiveness, drowsiness, panic attacks, anxiety, mood swings	☐
26	Concentration problems, short-term memory loss, forgetfulness	☐
27	Skin partly thin, paper-like, transparent, dry	☐
	Total number of symptoms for Lyme Borreliosis	

Antibiotics? When? Which one(s)? How long?

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DIAGNOSING TICK-BORNE DISEASES

Basic testing panel for Borreliosis

1. Borrelia SeraSpot (modern Western blot)
2. Borrelia-EliSpot (current T-cell activity)
3. CD57-cells (chronic immune suppression)
4. New option: Tickplex Basic, includes round bodies (persisters)

Checklists help decide which infections to test for; history and physical signs/symptoms also vital (2/2)

Coinfections-Checklist

Name, first name ..... Date (DD/MM/YYYY) .....

	Actual and former symptoms Please mark with a cross	X	Score-Points (filled in by physician/naturopath)	Ranking
1	Stomach ache, gut problems	☒	Ehrlichia&Anaplasma: 5	4
2	Anaemia	☐	Babesia: 4	5
3	Diarhoea intermittent	☐	Rickettsia: 4	5
4	Fever or feverish feeling	☒	Bartonella: 7	2
5	Lack of concentration, memory disturbance, forgetfulness	☒	Chl.pneumoniae: 6	3
6	Encephalitis/Inflammation of the brain (NMR)	☐	Chl.trachomatis: 2	7
7	Yellowish colour of the skin/eyes	☐	Yersinia: 3	6
8	Painful joints, swollen joints	☐	Mycoplasma: 5	4
9	General aches and pains, tendon problems	☐	Coxsackie-/Echo-Virus: 8	1
10	Flu-like symptoms intermittent	☒	EBV/CMV/HSV/VZV: 8	1
11	Rash(es)	☒		
12	Small red/purple spots of the skin	☐		
13	Heart problems, disturbance of cardiac rhythm	☒		
14	Cough, expectoration	☐		
15	Headache	☒		
16	Impaired liver function/ liver laboratory values	☒		
17	Pneumonia, bronchitis	☐		
18	Swollen lymph nodes	☒		
19	Tonsilitis	☒		
20	Enlargement of the spleen	☐		

Electronic version fills automatically

Coinfections-Checklist

Name, first name ..... Date (DD/MM/YYYY) .....

	Actual and former symptoms Please mark with a cross	X	Score-Points (filled in by physician/naturopath)	Ranking
1	Stomach ache, gut problems	☒	Ehrlichia: 7	3
2	Anaemia	☐	Babesia: 4	6
3	Diarhoea intermittent	☐	Rickettsia: 6	4
4	Fever or feverish feeling	☒	Bartonella: 8	2
5	Lack of concentration, memory disturbance, forgetfulness	☒	Chl.pneumoniae: 9	1
6	Encephalitis/Inflammation of the brain (NMR)	☐	Chl.trachomatis: 5	5
7	Yellowish colour of the skin/eyes	☐	Yersinia: 5	5
8	Painful joints, swollen joints	☒	Mycoplasma: 7	3
9	General aches and pains, tendon problems	☒	Coxsackie-Virus: 9	1
10	Flu-like symptoms intermittent	☒	EBV/CMV/HSV: 7	3
11	Rash(es)	☒		
12	Small red/purple spots of the skin	☐		
13	Heart problems, disturbance of cardiac rhythm	☒		
14	Cough, expectoration	☐		
15	Headache	☒		
16	Impaired liver function/ liver laboratory values	☐		
17	Pneumonia, bronchitis	☐		
18	Swollen lymph nodes	☒		
19	Tonsilitis	☐		
20	Enlargement of the spleen	☐		
21	Fatigue / exhaustion, intermittent or chronic CFS	☒		
22	Muscle pain, muscle weakness	☒		
23	Shivering, chill	☐		
24	Blurred, foggy, cloudy, flickering, double vision	☒		
25	Nausea, vomiting	☒		
26	Dark urine	☐		
27	Itching or pain when urinating	☐		

Ranked in order of priority – draw for first place here: Chlamydia pneumoniae (CPN) and Coxsackie

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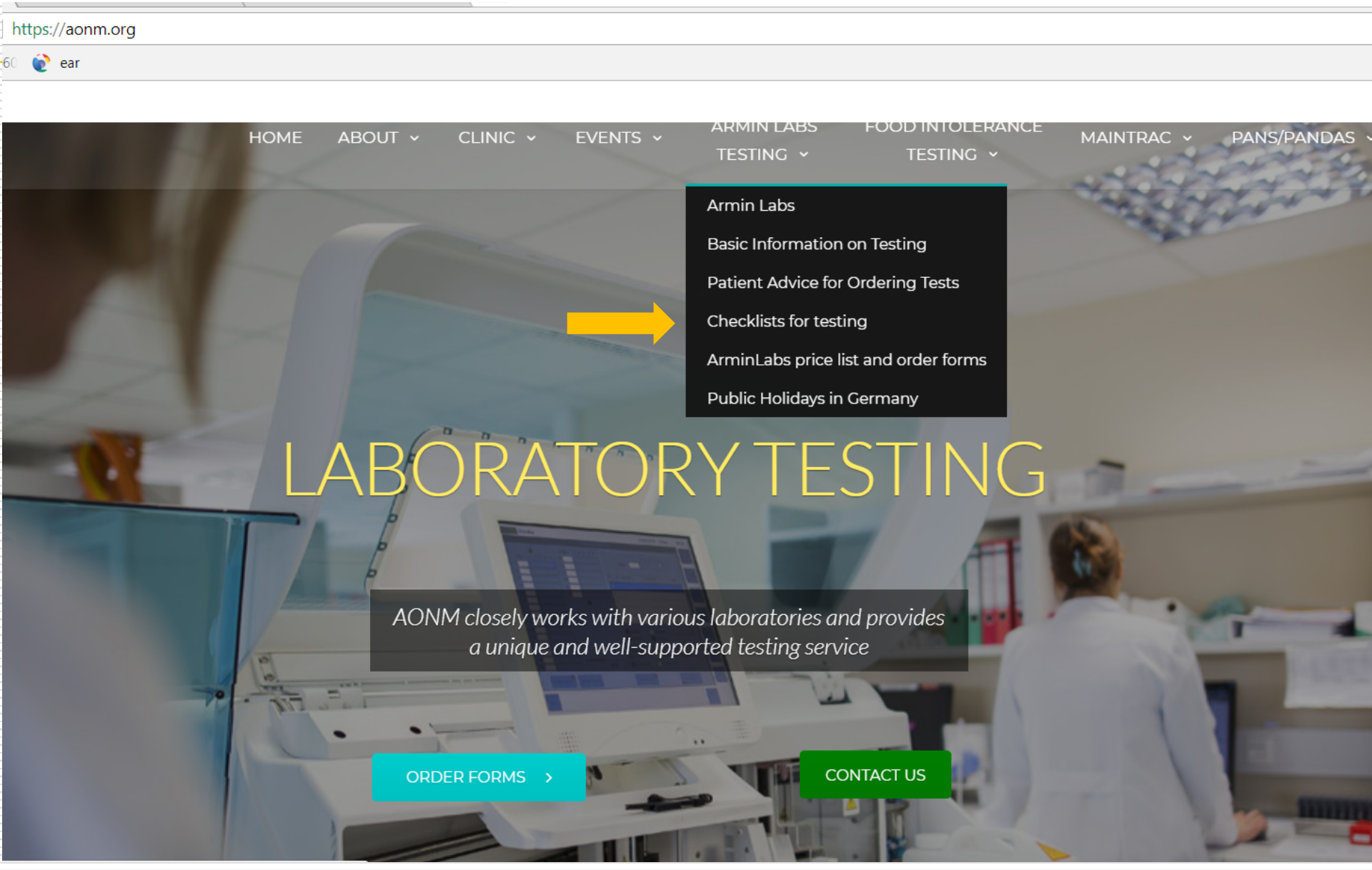
Where to find the checklists:

www.aonm.org – ArminLabs tab



Agenda

Antibody (B cell) tests vs. using T cells

- B cells: IgG, IgM, IgA
- T cells: EliSpot (LTT-Interferon Gamma Release Assay)

The benefits of using a CD3/CD57 assay

How to decide what to test for

- Checklists
- Tailored testing protocols

Where to find further information

Suggestions on what to test for have been collated for a large number of conditions (1/2)

Testing protocols with substantiation from peer-reviewed medical journals exist for:

Conditions labelled CFS and ME
Fibromyalgia
Alzheimer's
Parkinson's Disease
ASD/Autism
Ehlers Danlos Syndrome (EDS)
Mast Cell Activation Syndrome (MCAS/MCAD)
Schizophrenia
Bipolar Disorder
Anxiety/panic attacks
OCD
Tourette's
PANS/PANDAS

Associations have been found in the scientific literature for autoimmune disorders and cancers, too

Autoimmune disorders:

Multiple Sclerosis
SLE
Ulcerative colitis
Sarcoidosis
Diabetes Type 1
Autoimmune thyroid disease/Hashimoto's, Grave's Disease
Vasculitis
Rheumatic fever/reactive arthritis/rheumatoid arthritis

Cancers:

Myelodysplastic syndrome
Leukaemia
Monoclonal gammopathy of undetermined significance (MGUS)
Non-Hodgkin's/Hodgkin's Disease/Mantle Cell Lymphoma
Breast/lung/prostate/brain cancer, glioblastoma

Fibromyalgia/Rheumatoid Arthritis: possible lab tests (correlate with checklist, history & symptoms)

1. Borrelia EliSpot
2. Chlamydia pneumoniae EliSpot
3. Mycoplasma pneumoniae EliSpot
4. Ehrlichia/Anaplasma EliSpot
5. Rickettsia Elispot
6. Yersinia EliSpot
7. Coxsackie Virus IgG/IgA antibodies
8. ANA (antinuclear antibodies) + CCP (cyclic citrullinated peptide) antibodies

Multiple Sclerosis: Laboratory tests suggested

1. Borrelia SeraSpot + Borrelia EliSpot + CD57-cells
2. Chlamydia pneumonia IgG/IgA antibodies + Chlamydia pneumoniae EliSpot
3. Mycoplasma pneumoniae IgG/IgA antibodies + EliSpot
4. Bartonella IgG/IgM antibodies + EliSpot
5. Coxsackie Virus IgG/IgA antibodies
6. EBV EliSpot
7. CMV EliSpot
8. HHV6 IgG/IgM antibodies

OCD/Tourette's Syndrome

1. Borrelia SeraSpot + Borrelia-EliSpot + Tickplex Basic + CD57-cells
2. Toxoplasma IgG/IgM
3. Mycoplasma pneumoniae IgG/IgA antibodies + Mycoplasma EliSpot
4. Toxoplasma IgG/IgM antibodies
5. Anti-streptolysin titer

Agenda

Antibody (B cell) tests vs. using T cells

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- T cells: EliSpot (LTT-Interferon Gamma Release Assay)

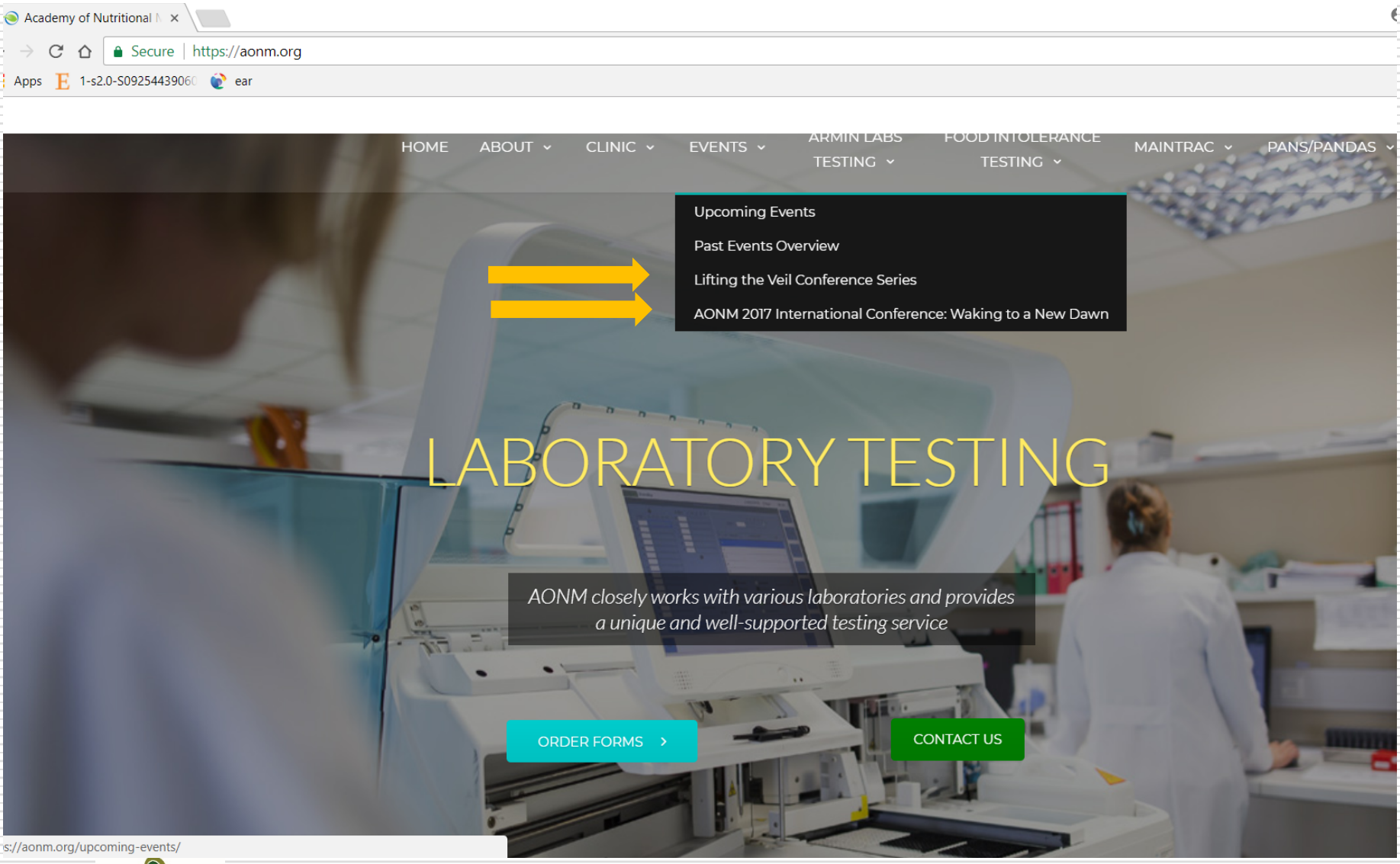
The benefits of using a CD3/CD57 assay

How to decide what to test for

- Checklists
- Tailored testing protocols

Where to find further information

For further information on testing: lots of downloadable presentations available on the AONM website



Presentations by Dr. Schwarzbach at each of the "Lifting the Veil" conferences, available as downloads

Secure | https://aonm.org/ltv-conference-series/

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LIFTING THE VEIL CONFERENCE SERIES

Home › Lifting the Veil Conference Series

During 2015 and 2016 AONM held a ground-breaking series of conferences which focused on different aspects of Lyme Disease and co-infections.

Speakers from around the world were invited to speak and 'lift the veil' on the misconceptions and lack of treatment for these conditions.

You can find here summaries of the conferences as well as the presentations given by speakers.

 Lifting the Veil I

 Lifting the Veil II

 Lifting the Veil III

The powerpoint presentations are provided for free and videos of these conferences are available to buy from AONM. Please contact info@aonm.org or call 03331 210 305

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"Lifting the Veil II" – my presentation is all about tailored protocols for numerous conditions, with full substantiation

ME, MS, Fibromyalgia, Alzheimer's, Parkinsonism, Autism... Tailored Testing Protocols

Holiday Inn Regents Park, 15th November 2015, London, UK

Armin Schwarzbach MD PhD

Specialist for laboratory medicine

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"Arminlabs Training" on the "Events" dropdown menu

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ARMINLABS TRAINING

On 6 March 2018 Dr. Armin Schwarzbach, CEO of ArminLabs, delivered a workshop designed specifically for health professionals, in order to help better understand the correct laboratory investigations needed to aid diagnosis as well as detailed discussion on how to interpret the results.

This event was held in 2 parts. The first part of the evening covered the basics for those who are new to ArminLabs, or those who would like to refresh their knowledge. The second part of the evening will be more advanced.

Please find the visual presentations provided by Dr. Schwarzbach that day.

ARMIN LABS TESTING - THE BASICS | TAILORED TESTING PROTOCOLS | MEANING AND INTERPRETATION OF CD57+ TEST

Fibromyalgia, ME, Degenerative Disorders, EDS, MCAS, PANS/PANDAS ...: Tailored Testing Protocols

Holiday Inn Regents Park, 6th March 2018, London, UK

Armin Schwarzbach MD PhD

Specialist for Laboratory Medicine

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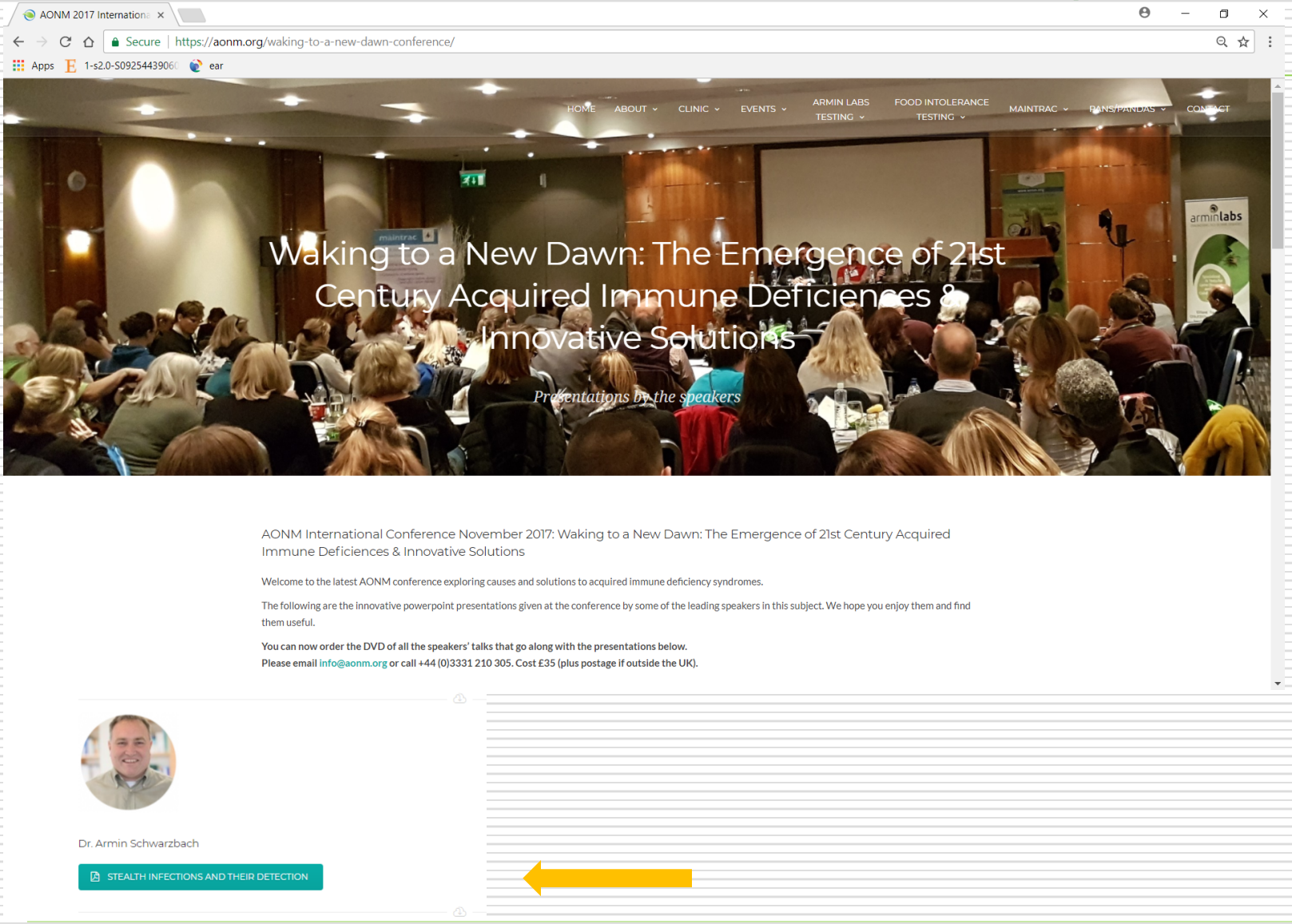
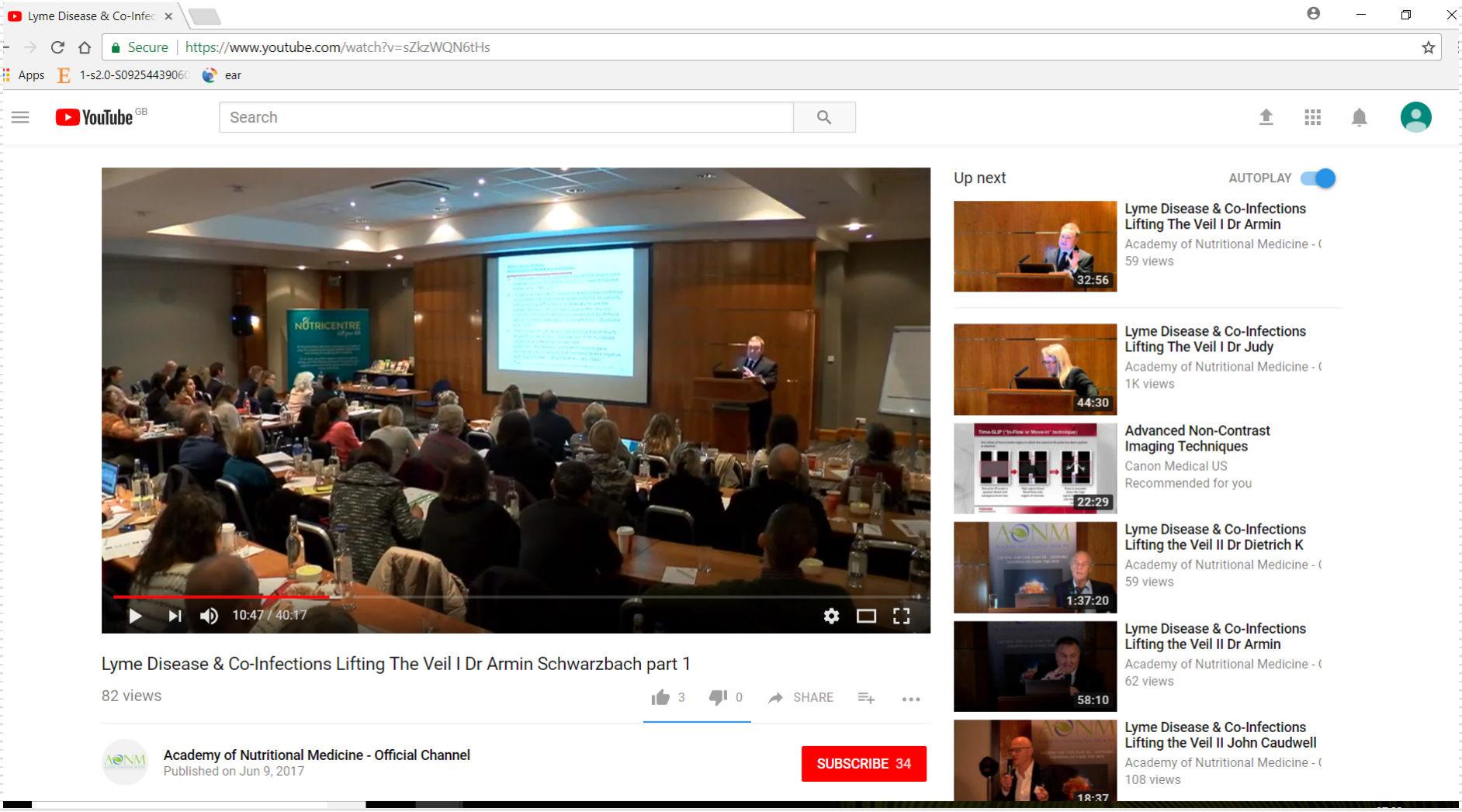
All presentations and video recordings from AONM's 2017 and 2018 Annual Conferences downloadable, too



AONM also has its own YouTube channel where previous conference videos are available free of charge



Thank you very much!

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