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The best testing strategies for pathogens

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DIAGNOSING TICK-BORNE DISEASES


COLLEGE of AMERICAN PATHOLOGISTS

CERTIFICATE OF ACCREDITATION

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Agenda

- **The different types of test for pathogens**
  - **B cells: Antibody testing**
  - **T cells: EliSpot testing**
- Testing panels for Viral Reactivation Post-Covid
- New ArminLabs tests

In recent infection, antibody testing is generally reliable if the antigens are specific: you can hope to see an IgM

"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

However, in chronic (continuing) disease, using conventional IgG/IgM antibodies means patients fall between the cracks

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

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

The most useful antibody in a chronic infection is Immunoglobulin A

IgA is an excellent immunoglobulin as it indicates current, ongoing or very recent infection, as well as chronic persistent infection, reactivation or reinfection

"IgA antibody is the most abundant antibody class in human serum and has a unique role in mediating immunity. IgA is a polyvalent antibody that is translocated to mucosal surfaces as the first line of defense against infections. Most of the secreted IgA lines the mucosal surfaces including respiratory, digestive and genitorurinary tracts to protect against pathogens while maintaining gut homeostasis."

The persistence of IgA antibodies in Yersinia, as an example ➡

JOURNAL ARTICLE

Persistence of IgM, IgG, and IgA Antibodies to *Yersinia* in Yersinia Arthritis

Get access

Kaisa Granfors ✉, Matti Viljanen, Anja Tiilikainen, Auli Toivanen

The Journal of Infectious Diseases, Volume 141, Issue 4, April 1980, Pages 424–429,
https://doi.org/10.1093/infdis/141.4.424
Published: 01 April 1980 **Article history** ▾

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Abstract

IgA antibodies to *Yersinia enterocolitica* were demonstrated in the sera of 13 patients with severe yersinia arthritis who were studied six to eight months after an acute infection with *Yersinia*. Four of the patients were monitored for two to three years, and they continued to demonstrate these antibodies. Only one of 12 control patients (individuals with yersinia infection without arthritis) had IgA antibodies specific to *Yersinia* six to eight months after the acute infection. The persistence of IgO antibodies was also in direct correlation to the occurrence of arthritis, but not as clearly as was the persistence of IgA antibodies. Antibodies of the IgM class persisted in most cases for only one to three months and always disappeared during the first six months after the onset of the infection. Thus, the demonstration of IgA antibodies to *Yersinia* is important in the diagnosis of yersinia arthritis, and the occurrence of IgM

Source: https://www.genscript.com/IgA-antibody.html; Granfors K, Viljanen M, Tiilikainen A, Toivanen A. Persistence of IgM, IgG, and IgA antibodies to Yersinia in yersinia arthritis. J Infect Dis. 1980 Apr;141(4):424-9.

IgA is available for CPn, Mycoplasma, HSV1/2, VZV, Coxsackie, Echovirus, Campylobacter and others

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.

ArminLabs is a specialist in precision testing: we also use the other arm of the immune system – T cells

Immunoglobulin A is not available when the infection does not live in the mucosal membranes: EBV (Epstein Barr Virus, glandular fever), CMV (Cytomegalovirus), Parvo Virus B19, etc.

So how to test chronic infection in infections where there is no IgA available?

There is another arm to the immune system that can be tested, too: not just B cells, but T cells. Tests of cellular T-cell immunity are called EliSpots (enzyme-linked immunosorbent spot). This is a lymphocyte transformation test using an Interferon Gamma Release Assay.

"Accuracy, sensitivity, reproducibility, and robustness – a gold standard"

"Enzyme-linked immune absorbent spot (Elispot) is a quantitative method for measuring relevant parameters of T cell activation. The sensitivity of Elispot allows the detection of low-frequency antigen-specific T cells that secrete cytokines and effector molecules, such as granzyme B and perforin. Cytotoxic T cell (CTL) studies have taken advantage with this high-throughput technology by providing insights into quantity and immune kinetics. Accuracy, sensitivity, reproducibility, and robustness of Elispot resulted in a wide range of applications in research as well as in the diagnostic field. Actually, CTL monitoring by Elispot is a gold standard for the evaluation of antigen-specific T cell immunity in clinical trials and vaccine candidates where the ability to detect rare antigen-specific T cells is of relevance for immune diagnostic."

Source: Ranieri E, Popescu I, Gigante M. CTL ELISPOT assay. *Methods Mol Biol.* 2014;1186:75-86.

New "Springer Protocols" book (2024) with a chapter on EliSpots

Book | © 2024

Chapter 6

Adaptive Immune Response Investigation in Lyme Borreliosis

Mihail Pruteanu, Armin Schwarzbach, and Markus Berger

Abstract

To diagnose Lyme Borreliosis, it is advised to use an enzyme-linked immunosorbent test to check for serum antibodies specific for Lyme and all tests with positive or ambiguous enzyme-linked immunosorbent assay (ELISA) results being confirmed by immunoblot. This method of measuring the humoral immunity in human fluids (e.g., by ELISA) has provided robust and reproducible results for decades and similar assays have been validated for monitoring of B cell immunity. These immunological tests that detect antibodies to *Borrelia burgdorferi* are useful in the diagnosis of Borreliosis on a routine basis. The variety of different *Borrelia* species and their different geographic distributions are the main reasons why standards and recommendations are not identical across all geographic regions of the world. In contrast to humoral immunity, the T cell reaction or cellular immunity to the *Borrelia* infection has not been well elucidated, but over time with more studies a novel T cell-based assay (EliSpot) has been developed and validated for the sensitive detection of antigen-specific T cell responses to *B. burgdorferi*. The EliSpot Lyme assay can be used to study the T cell response elicited by *Borrelia* infections, which bridges the gap between the ability to detect humoral immunity and cellular immunity in Lyme disease. In addition, detecting cellular immunity may be a helpful laboratory diagnostic test for Lyme disease, especially for seronegative Lyme patients. Since serodiagnostic methods of the *Borrelia* infection frequently provide false positive and negative results, this T cell-based diagnostic test (cellular assay) may help in confirming a Lyme diagnosis. Many clinical laboratories are convinced that the cellular assay is superior to the Western Blot assay in terms of sensitivity for detecting the underlying *Borrelia* infection. Research also suggests that there is a dissociation between the magnitude of the humoral and the T cell-mediated cellular immune responses in the *Borrelia* infection. Lastly, the data implies that the EliSpot Lyme assay may be helpful to identify *Borrelia* infected individuals when the serology-based diagnostic fails to do so. Here in this chapter the pairing of humoral and cellular immunity is employed to evaluate the adaptive response in patients.

The Elispot technique reflects the current T-cellular activity of bacteria and viruses

"The EliSpot Lyme assay can be used to study the T cell response elicited by Borrelia infections, which bridges the gap between the ability to detect humoral immunity and cellular immunity in Lyme disease. **Many clinical laboratories are convinced that the cellular assay is superior to the Western Blot assay in terms of sensitivity for detecting the underlying Borrelia infection..** Research also suggests that there is a dissociation between the magnitude of the humoral and the T cell-mediated cellular immune responses in the Borrelia infection."

Examples: Borrelia burgdorferi/Mycoplasma

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.

Mycoplasma pneum.EliSpot

1 Mycoplasma pneum. EliSpot ! 7 SI

SI = Stimulation Index
0-1 = negative
2-3 = weak positive
> 3 = positive

The result of the EliSpot test indicates current cellular activity against Mycoplasma pneumoniae.

EliSpots for Epstein Barr Virus and Cytomegalovirus show both lytic and latent values

CMV EliSpot

1 CMV lytic	!	355 SI
0-1 = negative		
2-3 = weak positive		
> 3 = positive		
1 CMV Latent	!	106 SI
0-1 = negative		
2-3 = weak positive		
> 3 = positive		

The result of the EliSpot test indicates current celluar activity against Cytomegalo Virus (CMV).

Explanation of CMV antigens:
CMV-lytic antigen: sign for replication of infectious CMV virions
CMV-latent antigen: sign for CMV latency with no production of infectious CMV virions

Lytic = currently replicating

Latent = dormat, but suppressing immunity, and can unfold again with any new assault to the immune system

Particularly high EBV results post COVID, backed up by thousands of lab tests and scientific studies

EBV EliSpot (lytic+latent)

1 EBV EliSpot (lytic) ! 657 SI

0-1 = negative
2-3 = weak positive
> 3 = positive

1 EBV EliSpot (latent) ! 65 SI

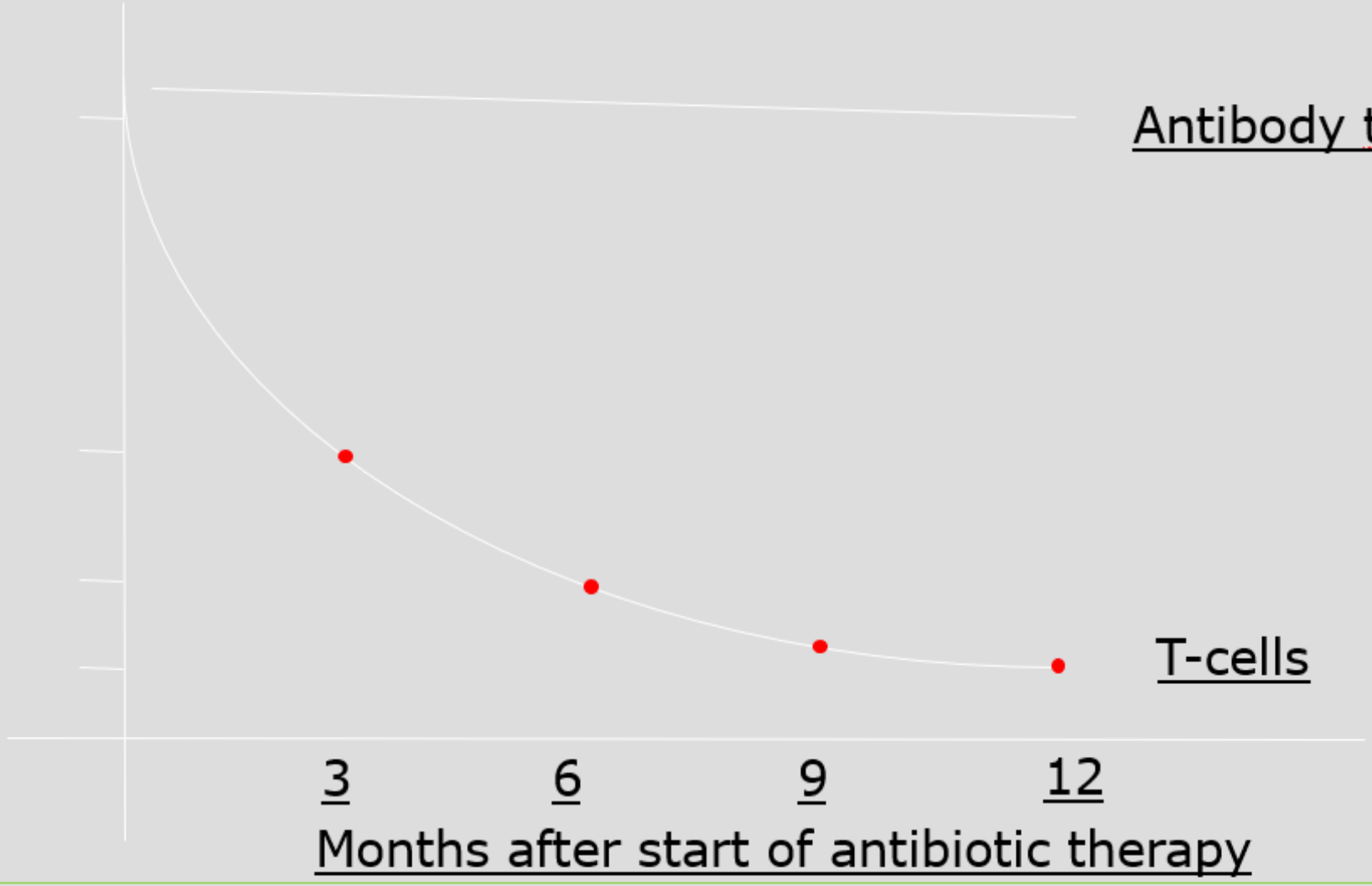
0-1 = negative
2-3 = weak positive
> 3 = positive

The result of the EliSpot test indicates current celluar activity against Epstein-Barr-Virus (EBV).

Explanation of EBV antigens:
EBV-lytic antigen: sign for replication of infectious EBV virions
EBV-latent antigen: sign for EBV latency with no production of infectious EBV virions

Source: Arminlabs laboratory results

EliSpot during antibiotics: "Staging" process



Next generation EliSpot = Lyme iSpot

AUTOIMMUN DIAGNOSTIKA GMBH

Next generation antigens for cellular immune response against Lyme coinfections in routine diagnostics

Next generation EliSpot = Lyme iSpot

AUTOIMMUN DIAGNOSTIKA GMBH

Interpretation

IFNy negative
IL-2 positive
→ Latent or cured state of Borrelia Infection
No indication for treatment, Monitoring if clinical symptoms remain

Balance between
IL-2 and IFNy positive cells
→ Persistent state of Borrelia Infection
Diagnostic verification and monitoring if clinical Symptoms remain

IFNy positive
IL-2 negative
→ Active Immune answer to Borrelia Infection
Indication for Treatment, follow-up after treatment

IFNy negative
IL-2 negative
→ No Borrelia Infection
No Treatment

Borrelia iSpot – INF gamma and IL2/ also for SAR-CoV-2

Borrelia iSpot

1	Borr.iSpot INF gamma Full Ag.*	4 SI
1	Borr.iSpot INF gamma OSP-Mix	0 SI
1	Borr.iSpot INF gamma LFA-1	0 SI
1	Borr.iSpot IL2 Full antigen *	2 SI
1	Borr.iSpot IL2 OSP-Mix	0 SI
1	Borr.iSpot IL2 LFA-1	0 SI

SI = Stimulation Index

0-1 = negative
2-3 = weak positive
> 3 = positive

The result of the Borrelia iSpot test indicates positive cellular activity against Borrelia burgdorferi.

Explanation of antigens:
Borrelia-burgdorferi Full Antigen: Borrelia burgdorferi B

**SARS-CoV-2 iSpot *

1	SARS-CoV-2 iSpot light *	
1	Sars-CoV-2 iSpot INF gamma	15 SI
1	Sars-CoV-2 iSpot IL2	18 SI

SI = Stimulation Index

< 5 = negative
5-6 = weak positive
>= 7 = positive

The SARS-CoV-2 iSpots reflect cellular immune responses against SARS-CoV-2.

Explanation of the CoV-iSpot:
Isolated positive reactions of Interferon-Gamma-(IFN-G) activated T-cells reflect current cellular immune reactions in the case of SARS-CoV-2 infection or vaccination.
Similar numbers of IFN-G and IL-2 (Interleukin-2) producing T-cells reflect persistent infections with SARS-CoV-2.
No positive cellular immune reactions of IFN-G-producing effector cells, but positive cellular immune reactions of IL-2-producing memory cells reflect past SARS-CoV-2 infections or vaccinations.
Isolated positive reactions of IL-2-activated T-cells reflect presence of memory cells as a sign of past infection with SARS-CoV-2/Coronaviridae or vaccination with potential cellular immunity.

Agenda

- The different types of test for pathogens
  - B cells: Antibody testing
  - T cells: EliSpot testing
- **Testing panels for Viral Reactivation Post-Covid**
- New ArminLabs tests

Electronic checklist helps decide which coinfections to test for in Post-COVID; fills automatically

Post-Covid Checklist

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

	Your current and former symptoms Please click on the boxes next to the symptoms that you suffer from	X
1	Stomach ache, gut problems	☐
2	Anaemia	☐
3	Diarhoea intermittent, intestinal crampings/pain	☐
4	Fever or feverish feeling	☐
5	Lack of concentration, memory loss, forgetfulness	☒
6	Encephalitis/Inflammation of the brain	☐
7	Yellowish colour of the skin/eyes	☐
8	Painful joints or swollen joints	☒
9	General aches and pains, tendon problems	☐
10	Flu-like symptoms	☒
11	Rash(es), striae, exanthema	☐
12	Small red/purple spots of the skin	☐
13	Heart problems, disturbed cardiac rhythm	☐
14	Cough, expectoration, "air-hunger"	☐
15	Headache, dizziness	☐
16	Impaired liver function/ liver laboratory values	☐
17	Pneumonia, bronchitis	☐
18	Swollen lymph nodes	☒
19	Enlargement of the spleen	☐
20	Fatigue / exhaustion, intermittent or chronic CFS	☒
21	Muscle pain, muscle weakness	☐
22	Shivering, chill	☐
23	Blurred, foggy, cloudy, flickering, double vision	☐
24	Nausea, vomiting	☐
25	Dark urine	☐
26	Itching or pain when urinating	☐
27	Tingling, numbness, "burning" sensations	☐
28	Neck pain, neck stiffness	☐
29	Shoulder pain	☐

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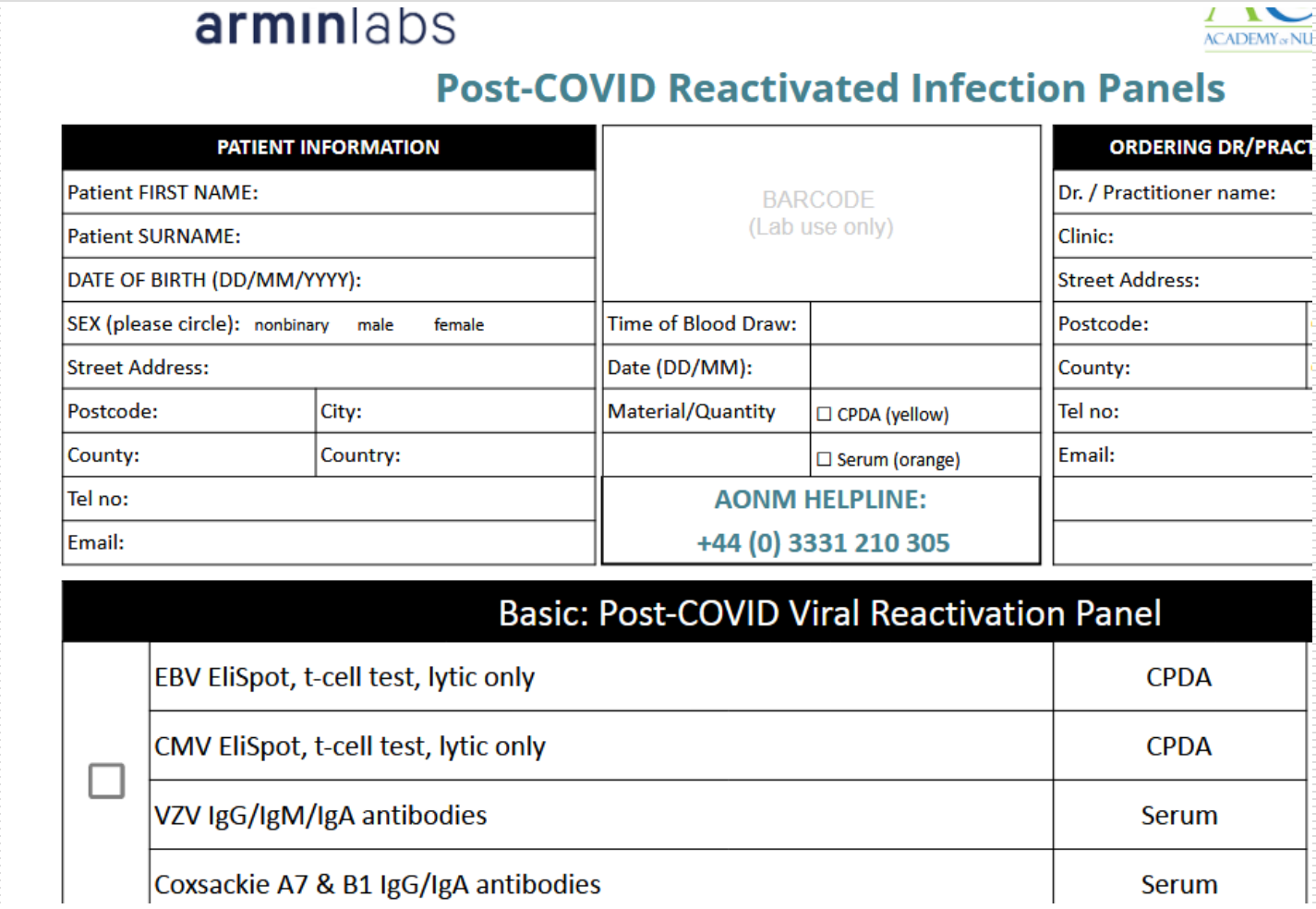
Ranked in order of priority:
CPn, Mycoplasma and the Herpesviruses draw for first place here ↓

Below you'll find the number of the symptoms for each of the infections that we test for and the ranking, in which order you should test for them

Ranking of the infections	No. of symptoms	Rank
Chlamydia pneumoniae	4	1
Mycoplasma pneumoniae	4	1
Yersinia	2	3
Campylobacter	2	3
HSV 1/2	4	1
EBV	4	1
CMV	4	1
VZV	3	2
HHV 6	4	1
Parvovirus	3	2
Coxsackie-Virus	3	2
Echovirus	2	3

NEW: ArminLabs Post-COVID Viral Reactivation Panels: Basic and Advanced



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Post-COVID Reactivated Infection Panels

PATIENT INFORMATION		BARCODE (Lab use only)		ORDERING DR/PRACT
Patient FIRST NAME:				Dr. / Practitioner name:
Patient SURNAME:				Clinic:
DATE OF BIRTH (DD/MM/YYYY):				Street Address:
SEX (please circle): nonbinary male female		Time of Blood Draw:		Postcode:
Street Address:		Date (DD/MM):		County:
Postcode:	City:	Material/Quantity	☐ CPDA (yellow)	Tel no:
County:	Country:		☐ Serum (orange)	Email:
Tel no:		AONM HELPLINE: +44 (0) 3331 210 305		
Email:				

	Basic: Post-COVID Viral Reactivation Panel	
☐	EBV EliSpot, t-cell test, lytic only	CPDA
	CMV EliSpot, t-cell test, lytic only	CPDA
	VZV IgG/IgM/IgA antibodies	Serum
	Coxsackie A7 & B1 IgG/IgA antibodies	Serum

Advanced reactivated infection panel includes further viruses, and bacteria

	Advanced: Post-COVID Reactivated Infection Panel	
☐	EBV EliSpot, t-cell test, lytic only	CPDA
	CMV EliSpot, t-cell test, lytic only	CPDA
	VZV IgG/IgM/IgA antibodies	Serum
	Coxsackie A7 & B1 IgG/IgA antibodies	Serum
	HSV 1 & 2 IgG/IgM/IgA antibodies	Serum
	HHV6 EliSpot, t-cell test	CPDA
	Chlamydia pneumoniae IgG/IgA antibodies	Serum
	Mycoplasma pneumoniae IgG/IgA antibodies	Serum

Currently EliSpots and iSpots are available for:

- Borrelia burgdorferi (B.b. sensu stricto + garinii + afzelii)
- Borrelia myamotoi
- Ehrlichia/Anaplasma
- Bartonella henselae EliSpot
- Babesia microti EliSpot
- Rickettsia conorii/rickettsii/helvetica
- Chlamydia pneumoniae
- Chlamydia trachomatis
- Mycoplasma pneumoniae
- Yersinia species
- Epstein Barr Virus (EBV)
- Cytomegalovirus (CMV)
- Herpes Simplex Virus 1 / 2 (HSV 1 / 2)
- Varicella Zoster Virus (VZV)
- Candida
- Aspergillus
- SARS-CoV-2

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Direct test of Mycotoxins in serum now available: ToxiPlex

Direct immunochemical detection of multiple mycotoxins

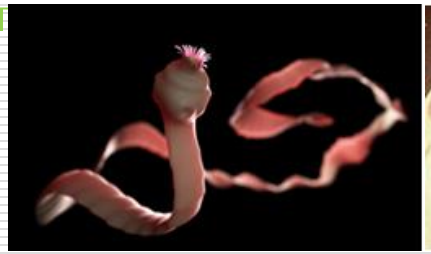
TOXIPLEX BASIC **DIRECTLY** detects Aflatoxin B1 (AFB1), Deoxynivalenol (DON), Fumonisin (FUM), Ochratoxin A (OTA), and Zearalenone (ZEA).

TOXIPLEX BASIC **DOES NOT** detect human antibody responses (IgA, IgG, IgE, etc.) against AFB1, DON, FUM, OTA, and ZEA.

TOXIPLEX BASIC **DOES NOT** measure mycotoxins in human urine because,

1. The use of human plasma or serum is five times more common than urine in literature (PubMed)
2. Variation in urine volume requires creatinine normalization
3. Daily mycotoxin intake variation demands 24hr sampling

Stool-based Parasite Multiplex PCR test



Two unique panels available:

For Intestinal Protozoa:
Giardia lamblia, Entamoeba histolytica, Crypto-sporidium spp., Blastocytis hominis, Dientamoeba fragilis, Cyclospora cayetanensis

For Intestinal Helminths (worms):

Ancylostoma spp., Ascaris spp., Enterobius vermicularis, Hymenolepis spp., Enterocytozoon spp./Encephalitozoon spp., Necator americanus, Strongyloides spp., Taenia spp., Trichuris trichiura

Accuracy = ≥ 99.9%, detection limit was 100 copies/reaction
Fast processing and easy sample handling – optimized for the detection of parasites in stool, does not need to be frozen and does not require large amounts of material
Swift return of results: 3-7 days after receipt of the sample

Checklist available for easier identification of which to test for:
https://aonm.org/wp-content/uploads/2024/02/english-parasite-coinfection-checklist-arminlabs.pdf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ENTER OUR RAFFLE TO WIN
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Thank you very much!
Q&A/Discussion

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