Morphological Methods in Diagnostics

DualDur® reagent & Dark-Field Microscopy

SZEROLÓGIA

Lyme borreliosis folyamán
Diagnosis & Pathogenesis

Dr. Bózsik Béla Pál

Diagnosis

Kórokozó kimutatás/Direkt Diagnosis

- **Tenyésztés**
- ***Natív vizsgálat (DFM)***
- ***Natív immuncytologia (IFA)***
- ❖ **Festés**
- ❖ **Immuncytológia**
- ❖ **Molecularis módszerek*(DNA,RNA)***

Indirect diagnosis

- **Savópár vizsgálat**
  - **Egyidejű, egyedi: TTT, mTTT, bWB**
  - **Kétidejű: EM-Control**
- **Egyedi vizsgálat**
  - **IFA, HA, ELISA, WB – LTT**
  - **Összehasonlító, módszerközi**
- **Kiértékelés – Lelet értelmezés!**

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– A megszilárdult folyékony szövet, a vér, alvadékától elkülönült savó, vagy valamelyik testfolyadék punctatumának vizsgálata –

az apró módosítások gyakorlati tudománya

Takátsy Gyula mikro-titrator és eljárás, Mandula Ferenc antigen és sero-epidemiologiai alkalmazása

A szervezet idegen, elsősorban pathogen anyagokra adott válaszának tudományos és diagnosztikus vizsgálata:

az ellenanyagok,

a kórokozók kimutatása

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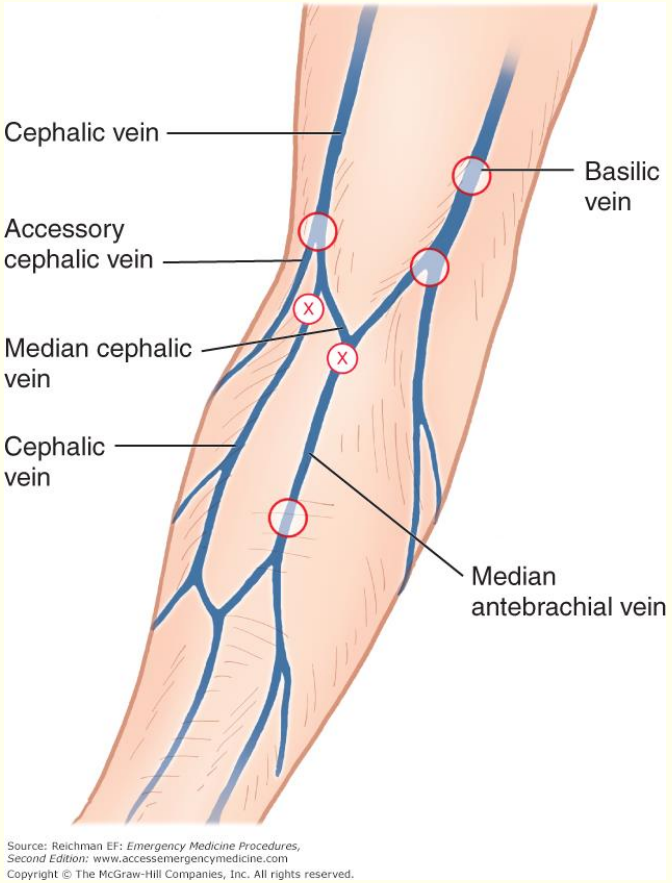
- **Szűrővizsgálat** gyors, nagyon érzékeny módszerrel, amely a legkisebb gyanú esetén is jelez
- **Diagnosztikus** vizsgálat
  - egyes savó,
  - savó-pár, javasolt ECM idején – Tünetesen
  - savó-liquor cerebrospinalis/CSF mintapár vizsgálattal.
- **Megerősítő** vizsgálat **azonnal!** beérkezett savómintából
- **Specificus vizsgálat** – vércsoport meghatározás, gyulladás- illetve tumor markerek, etc.
- **Tudományos vizsgálat:** oltás hatékonyság ellenőrzés.

Vérvétel

Preanalitika & Mintavétel alapvető jelentőségű

Minta típusa	Cső típusa	Mely immunológiai vizsgálatokban használjuk?
Natív vér (szérum)	**Piros** kupakos natív, vagy **sárga** kupakos gélszeparátoros cső	Szerológiai vizsgálatok
Heparinos vér	**Zöld** kupakos cső	Celluláris vizsgálatokhoz
EDTA-s vér	**Lila** kupakos cső	Celluláris vizsgálatokhoz
Citrátos vér	**Kék** kupakos cső	Celluláris vizsgálatokhoz

PLASMA



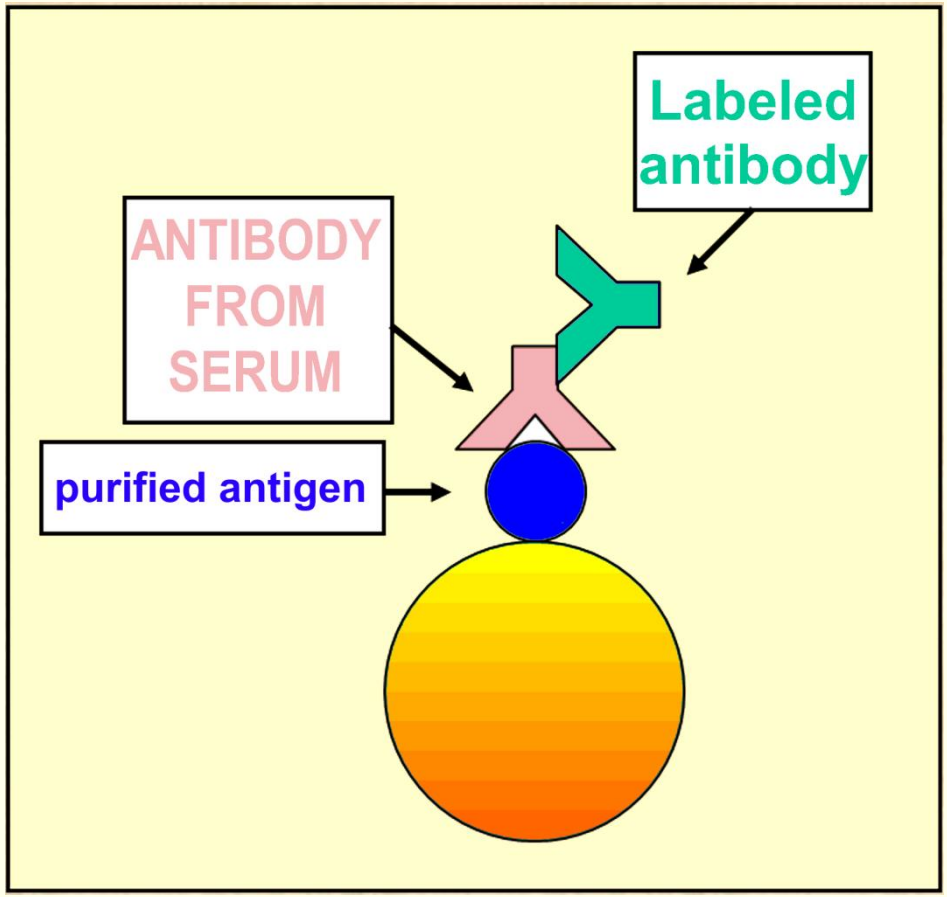
Vérvétel

Preanalitika & Mintavétel alapvető jelentőségű

- **Mikor történjen? –** Reggel-Délelőtt **éhgyomorra**
- **Milyen csőbe? –** Natív, steril, **PIROS kupakos csőbe,** amelyben nincs clot-activator *(tömör az alvadék,* **nincs idegen anyag***, nem géles)*; üveg a jobb – **Bowen RA, Remaley AT, 2014**
- **Szerológiához 2-4 ml éhgyomri teljes vér szükséges**
- **Hányszor forgassuk meg a csövet? – Legalább 5x** Max 10x
- **Mit határozunk meg? –** *Borrelia burgdorferi sensu lato* egyes összetevőihez kötődő **ellenanyagokat**
  - **Összegezve az IgM és IgG típusúakat**
  - **Legalább kétféle, IgM és IgG típusra vonatkozóan**
- **Miért kettős jelentőségű az ellenanyag reaktivitás? –**
  - **Diagnosztikus:** a Lyme borreliosis szerológiai igazolása
  - **A szervezet védettségét** jelzi Betegnek! Orvosnak!

SZEROLOGIA

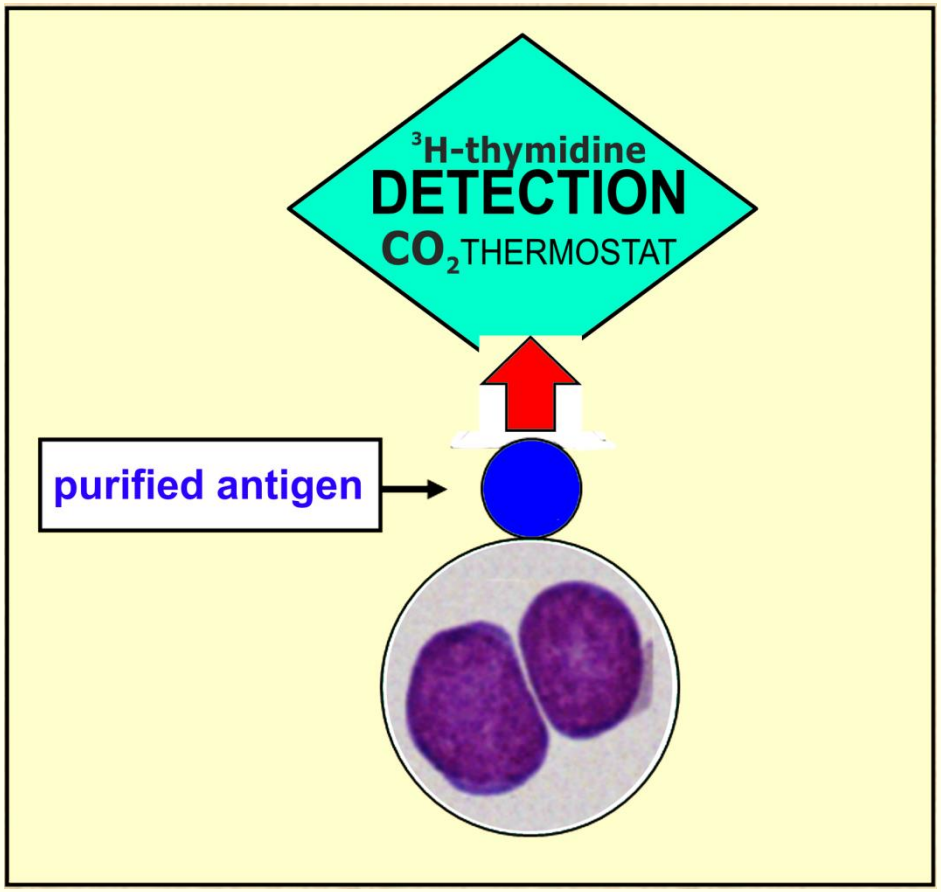
a leggyakoribb, jól kidolgozott diagnosztika



- INDIRECT eljárás
- SPECIFICUS
  - rec-ANTIGENS
  - Double-triple immune reaction
- SENSITIVE
  - Reactiók molecular szinten
  - Alapvetően reagens-függő, ezért mindig a reaktivitást mutatókkal ellenőrizzünk
- SZŰRŐVIZSGÁLATOKRA ALKALMAS
- ANTITEST elfogyhat kórfolyamatban
- ANTITEST élettartama 1-6 hónap
- KÓROKOZÓ, BORRELIA különösen megváltoztathatja geno- és pheno-typusát
- Sok „házi” és többféle reagens-kit miatt nehezen összevethető reakció

SENSITIZED LYMPHOCYTE REACTIVITY

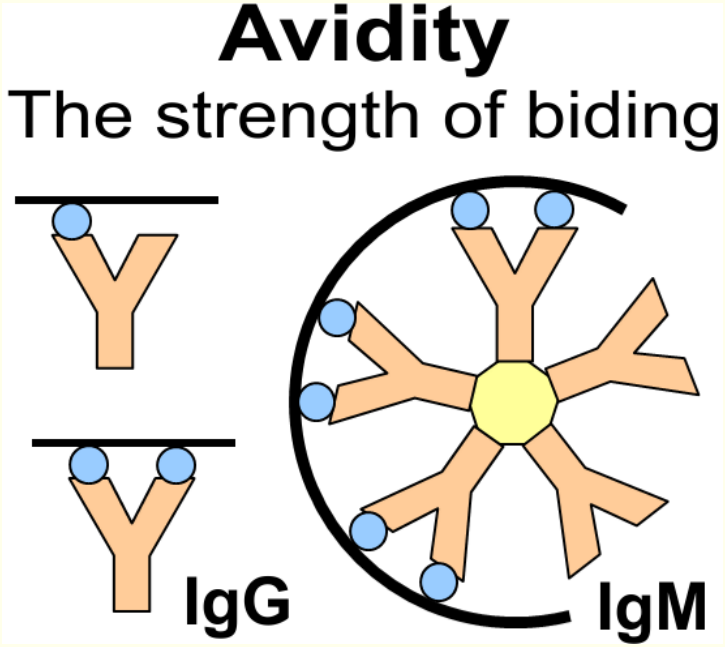
IS THE NEW WAYS OF DIAGNOSTICS

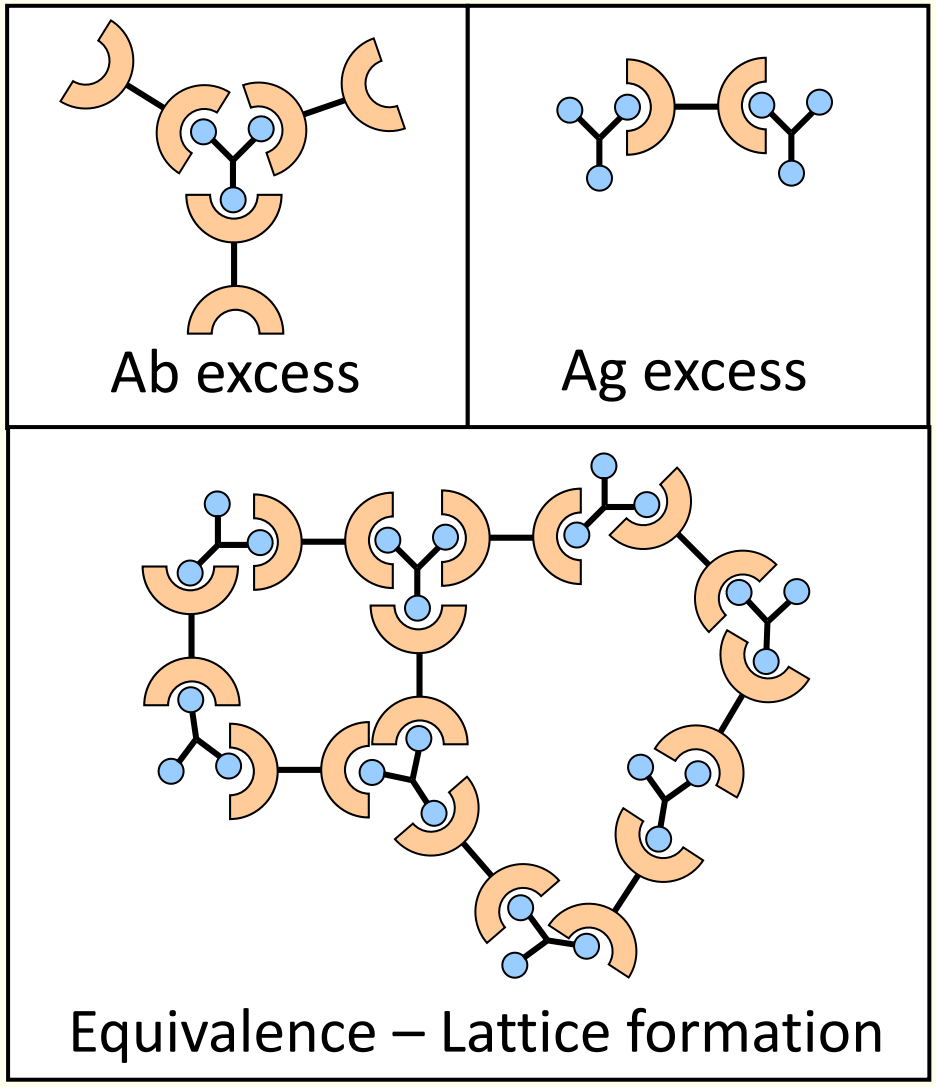


- INDIRECT MEASURE
- SPECIFIC
  - rec-ANTIGENS
  - Specific immune raction
  - triplicate reaction
- SENSITIVE
  - Reaction at molecular level
  - Depending on the reagent
- HIGH-TECH laboratory is needed
- Lymphocyte reactivity can be inhibited
- Quickly follow the clinical pathogenesis
- CAUSATIVE AGENT can be transformed
- PHYSICIANS HARDLY FOLLOWED its specific evaluation

Factors Affecting Measurement of Ag/Ab Reactions

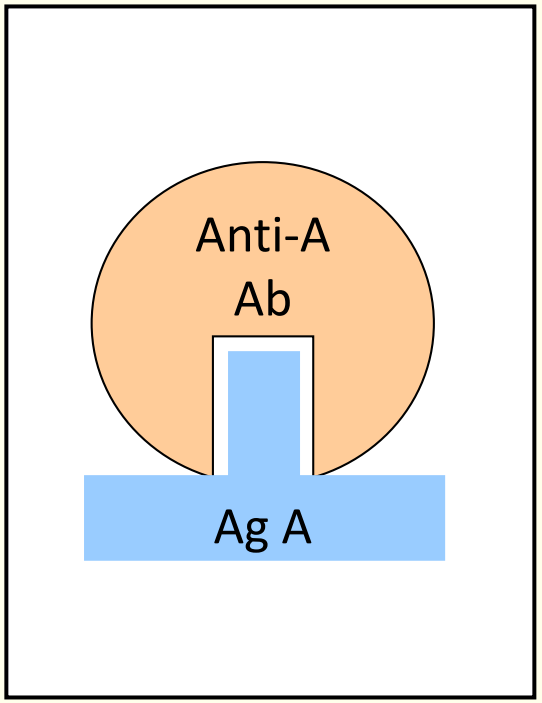
- Affinity
- Avidity
- Ag:Ab ratio
- Physical form of Ag
- Steric inhibition to AB

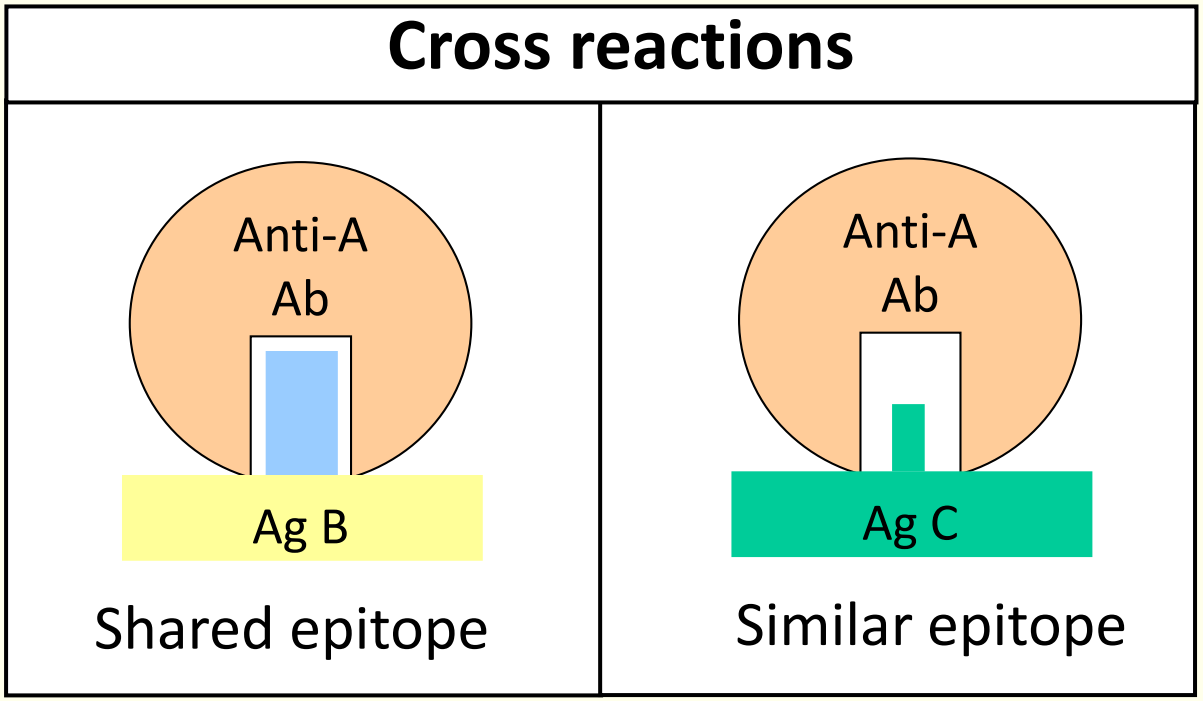




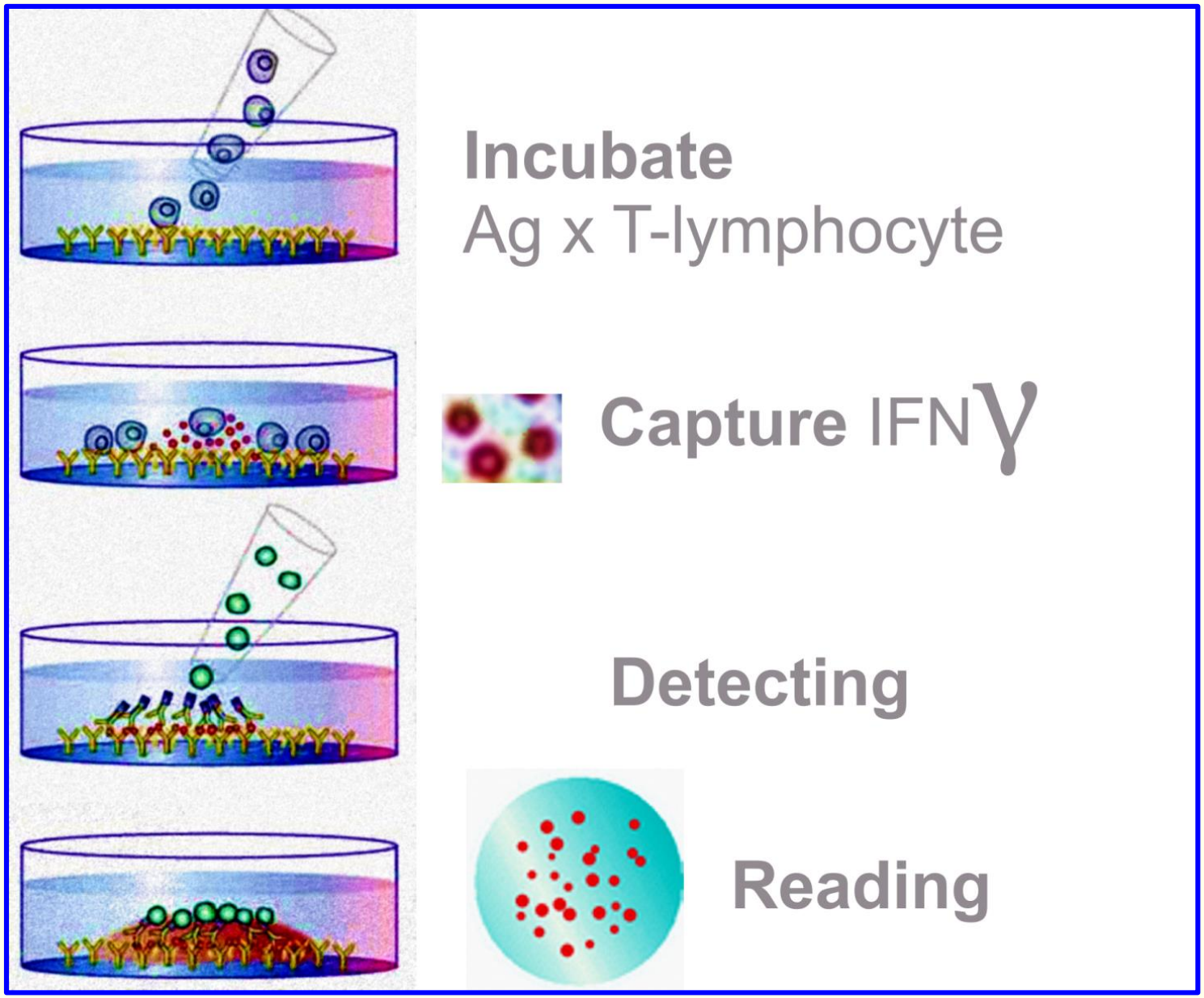
Cross Reactivity

- The ability of an individual Ab combining site to **react with more than one antigenic determinant**.
- The ability of a population of Ab molecules to **react with more than one Ag**
- **Lyme-Spirochete** detected in CSF of the Hungarian first case in 1984 proved with the help of cross-reaction with IFA – The same was applied with HA in USA.





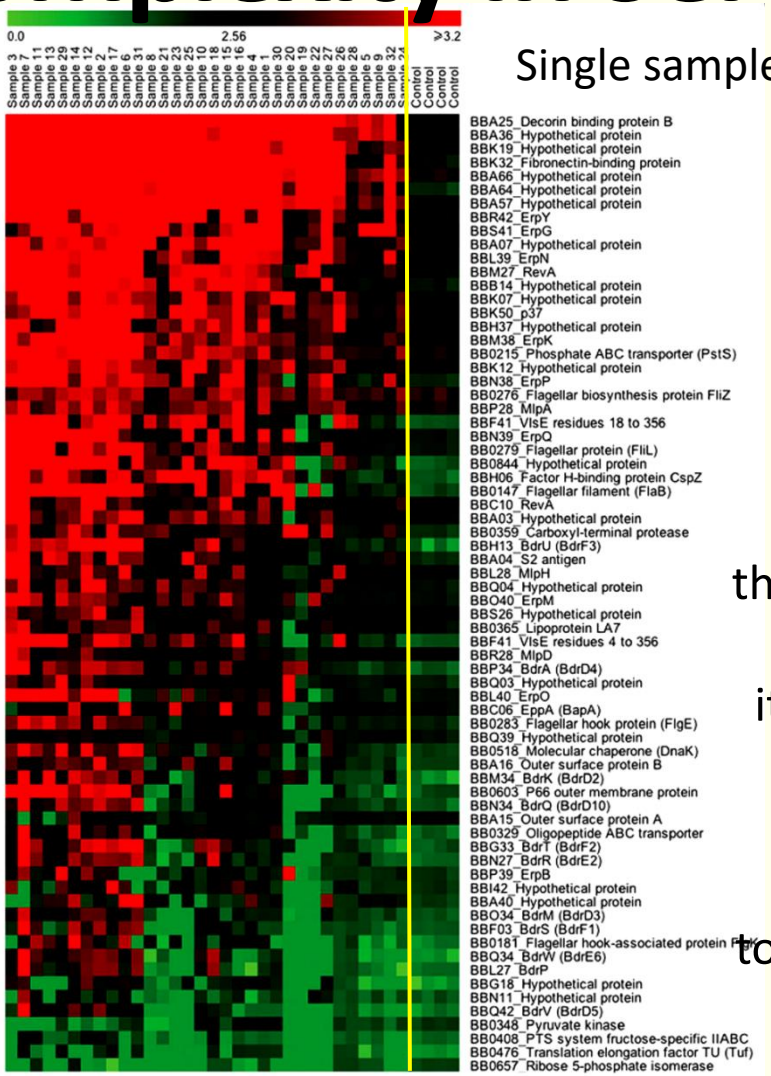
Lymphocyte Transformation Test



Further Complexity In Serodiagnosis.

There were applied
69 recombinant antigens
printed in duplicate in four dilutions over the range of 30 to 900 g/ml, in volumes of 1.5 nl / spot
The individual intensity is among 1,537 -2,510 pixels/spot.

Single samples & their reactivity



Two-color display of an analysis of intensity of antibody **binding to 69 proteins of *B.burgdorferi*** by sera from infected and control dogs. The gradient heatmap depicts the log10-transformed signal intensity values of serum antibody binding to *B. burgdorferi* proteins; gradient colors indicate the range of binding intensity values from **0 (green) to3.2 (red)**, with the median value of **2.56 in black**. Individual dog serum samples are arranged by the total sums of pixel values for binding to the proteins in the array, while proteins are listed by averaged intensities of responses among infected serum samples. Where known, the gene name for the ORF of each protein is shown.
Baum E – Grosenbaugh DA – Barbour AG *Clin. Vaccine Immunol.* 2014, 21(6):838.

The two dimensions are:
the sero-reactivity of singles to the recombinant antigens;
it was composing a **3rd one:** individuals to all antigens;
4th one: the distribution of these singles intensity to all antigens as a population.
Comparing to the Controls.

Lyme borreliosis serodiagnosis

CDC által ajánlott módszerei

Two-tier method

1994-2018

- ELISA-válogatott antigének
- **Western-blot**

Two-ELISA 2019-

Modified two-tiered testing

(**MTTT in 2019**)

- ELISA-válogatott antigének
- C6-ELISA-selected antigen
- (Western-blot, ha szükséges)

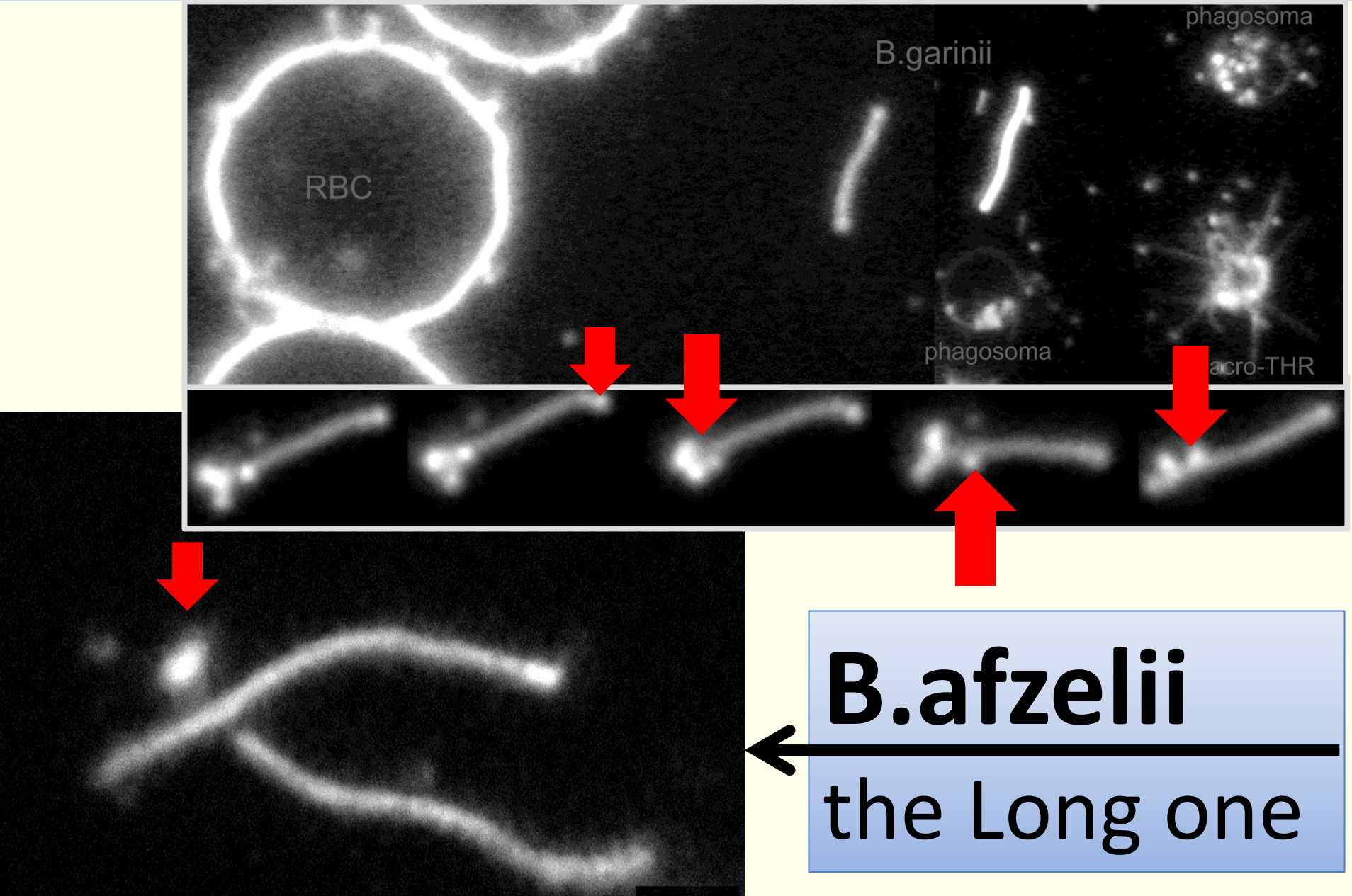
Föl kell ismerni, hogy létezik Lyme borreliosis kimutatható ellenanyag nélkül:
lekötődik-nem termelődik specifikus ellenanyag!

Az egyik módszerből nem következik a másik reaktivitása, mert

a szervezeti reakció pedig antigénenként, kontinensenként eltérő, amit egyik eljárás kimutat, míg a másik nem.

– A gyakorlatban azzal a módszerrel kell ellenőrizni a kezelés hatékonyságát, amely reaktiv. –

B.garinii–the Short+IC causes Se-neg!

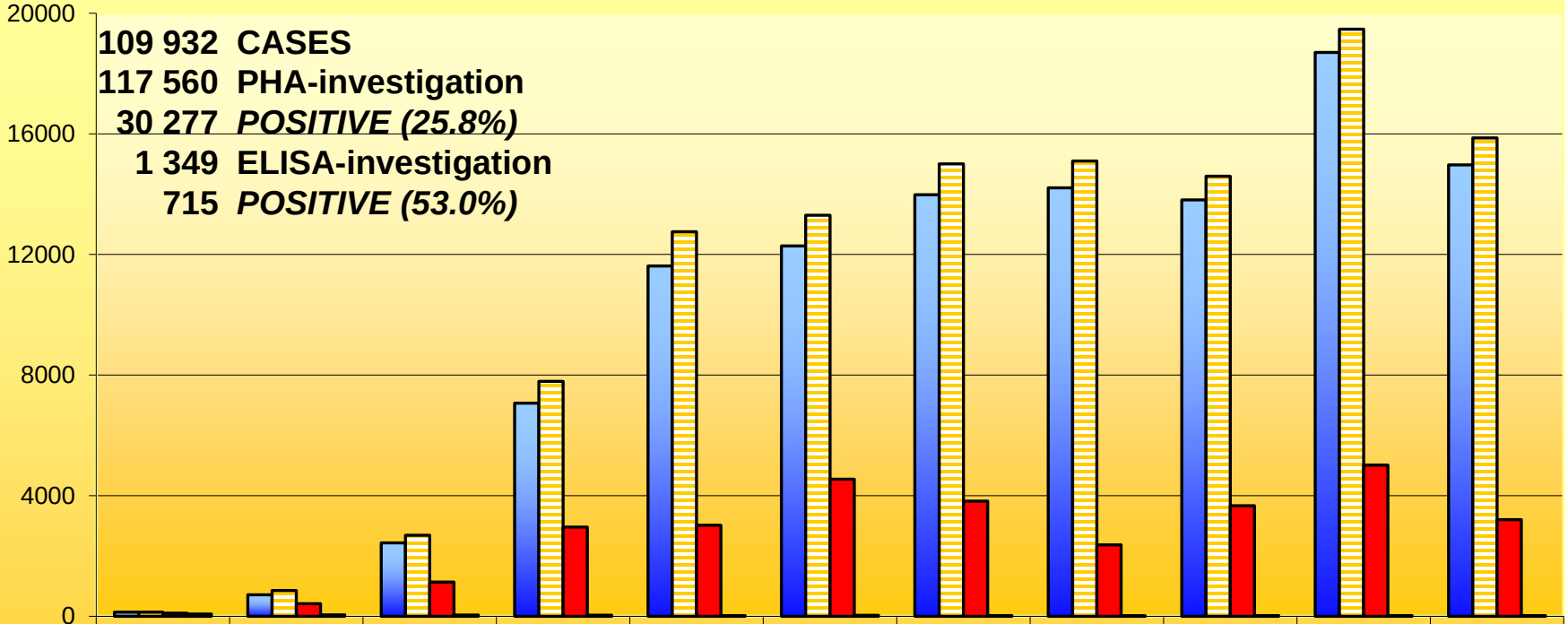


B.afzelii

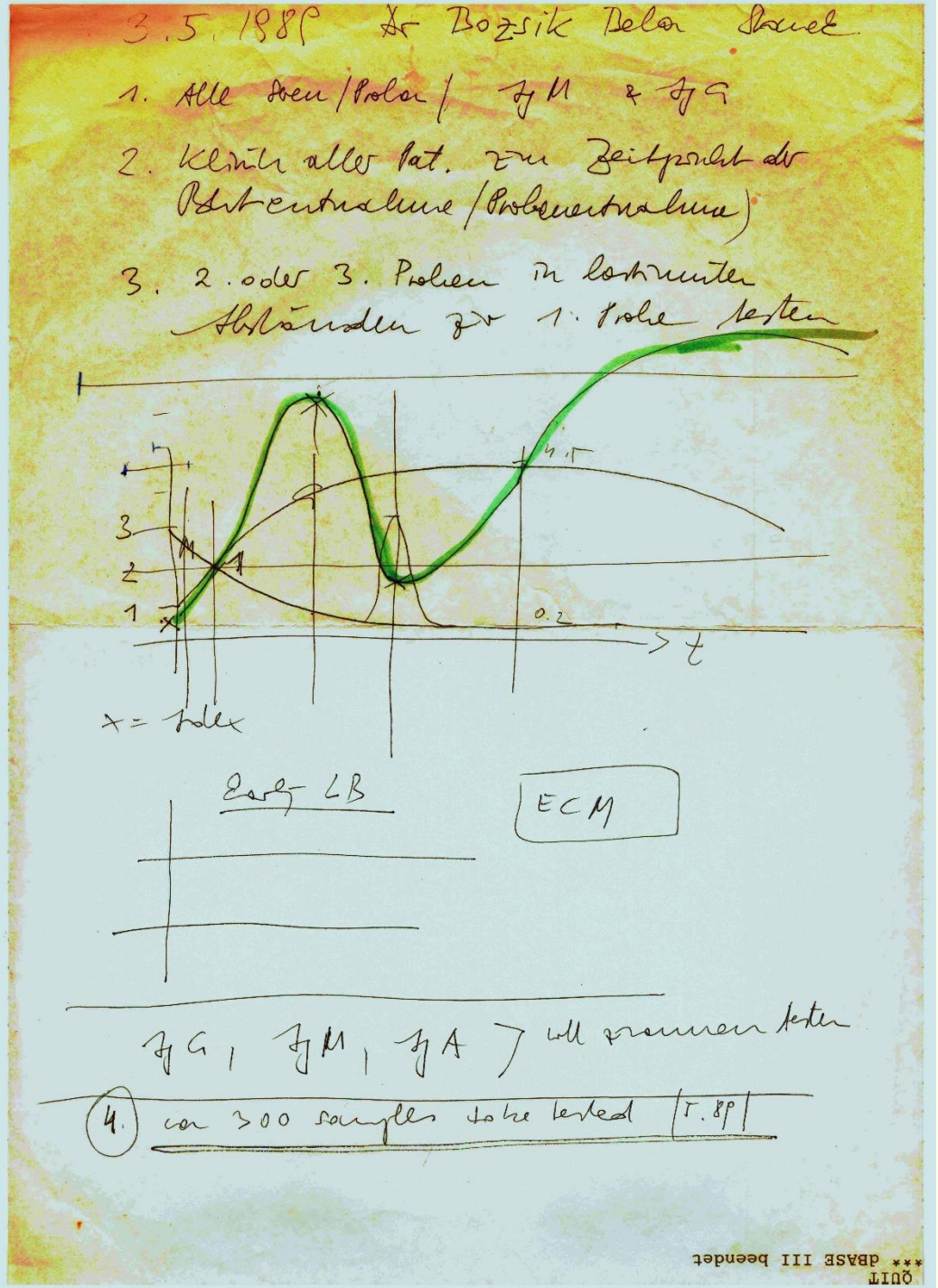
the Long one

Vizsgálatok 1984-1998 között

‘Johan Béla’ OKI Szerológiai Laboratórium



	1987-Vienna	1988-Vienna	89.	90.	91.	92.	93.	94.	95.	96.	97.
ESETSZÁM	139	715	2437	7071	11617	12283	13983	14207	13811	18698	14971
Vizsgálatszám	139	857	2689	7793	12752	13299	15003	15101	14596	19468	15863
POZITIV	106	420	1138	2958	3024	4549	3823	2369	3671	5013	3206
POZITIVITÁS %	76.3	49	42.3	38	23.7	34.2	25.5	15.7	25.2	25.8	20.2



Szero-dinamika

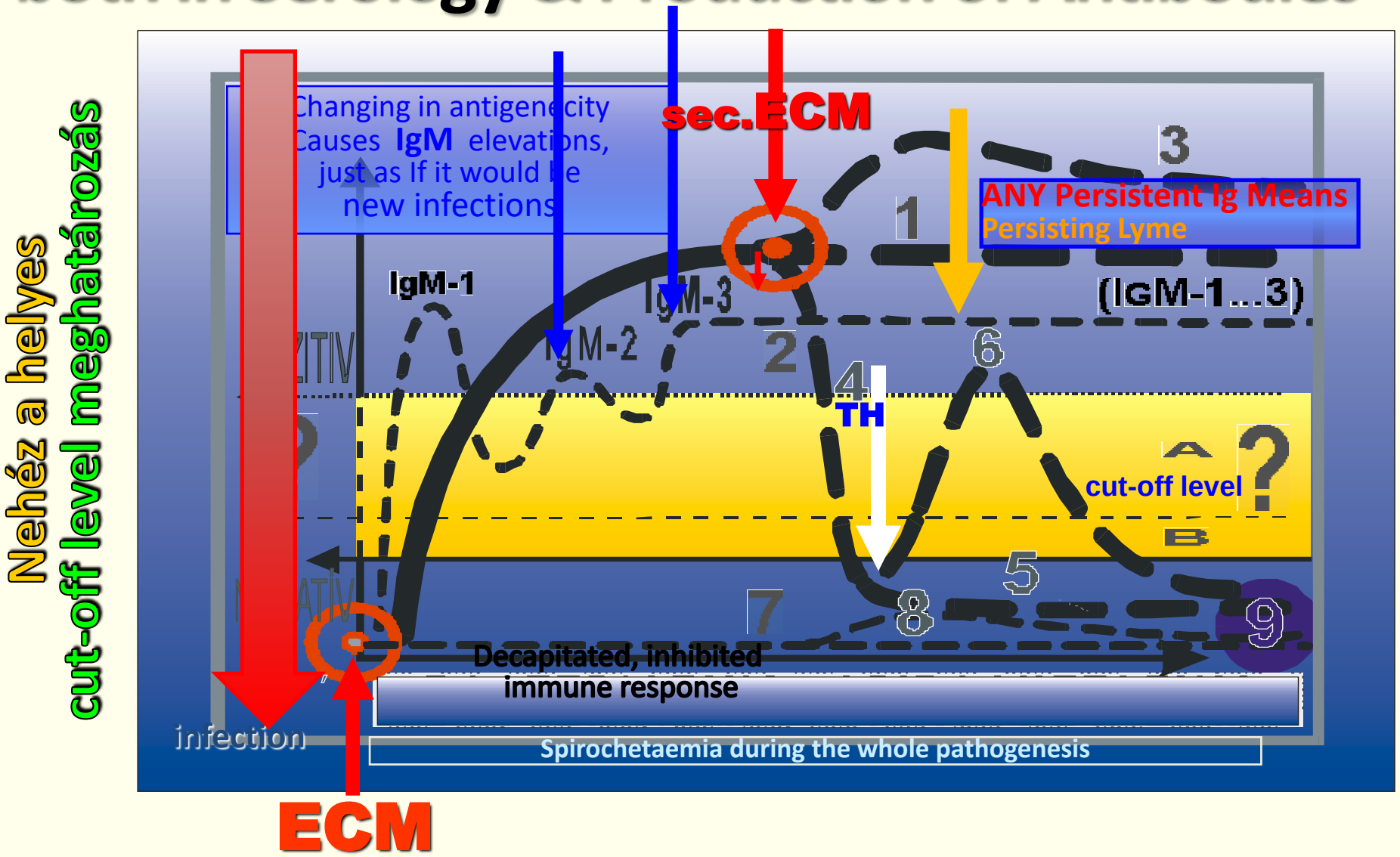
ahogy 1989-ben láttuk

Dr.med. Gerold Stanek vázlata szerint

- **A sero-válasz késése ismert**
- **Szükséges az ismerete**
  - IgM
  - IgG
  - IgA talán
- **A sero-válasz hullámzása elismert**
- **Booster effect** kezelés, ismételt(!) kezelés esetén is megjelenhet
- **Decapitatio** (korai, elégtelen kezelés)
- **Tartós IgG**(sero-heg) ismert

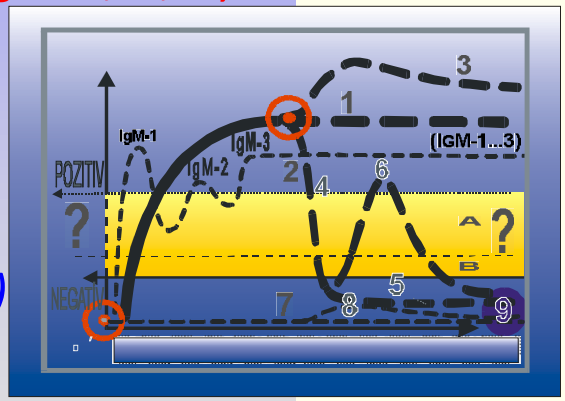
Dinamic Changing

both in Serology & Production of Antibodies



Szervezeti válasz & Lyme borreliosis

1. IgM **korai**, de **késett válasz, Bbsl** minden változására*(IgM-1,-2,-3)*
2. **Specifikus IgM-IgG switching gátlás** *(2)*
3. **Lyme borreliosis** esetében az **IgM-titer EMELKEDETT maradhat** az idült folyamat stimulus+gátlással együtt*(1-3)*
4. **A specifikus IgG titer**
   - i. **Emelkedett reinfection/***effective kezeléseknél (3+6+8)*
   - ii. Lyme borreliosis fennállásakor magas *(1+3)*
   - iii. **Lecsökken** effective kúránál *(4+5)*, or
   - iv. **kimutathatatlan IgG** mert a circulating immuncomplexhez kötődött, a plazmában nincs, vagy minimálisan van jelen sero-negatív *(nem álnegatív_see: 4+5)*
   - v. Lyme borreliosis idült formájában **nem termelődik** sero-negatív *(4+5)*
   - vi. Decapitált korai**&**elégtelen kezelés hatására *(7+8+9)*
5. Sero-positive eredmény alapvetően a **cut-off level** meghatározásától függ *(see: A or B lehetőséget)*



A complex Lyme borreliosis

I. **<u>Lyme borreliosis</u> (a kezdet)**

II. **<u>KULLANCS -kór</u>**

Lyme borreliosis

+Bartonella,

+babesia,

+Anaplasmosis/Ehrlichiosis,

III. **<u>Komplex KULLANCS –kór</u>** *<u>(quasi immunsupprimált „Lyme-beteg”)</u>*

TBD + ad hoc „co-infections”:

- Macro-biom(populatios germs)
- Microbiom (personal germs)

IV. **<u>Patho-reactions:</u>**

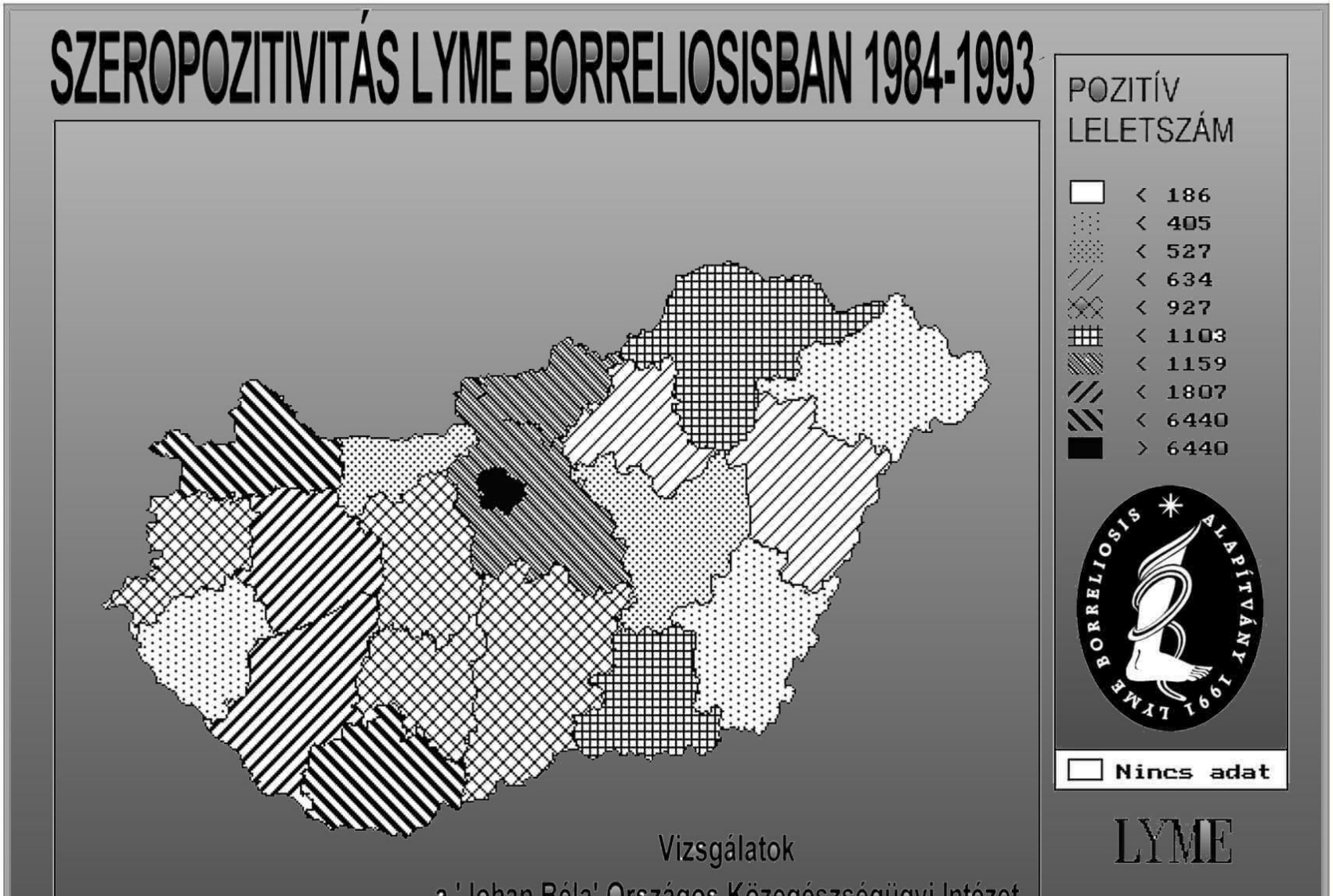
– Borrelia burgdorferi sensu lato & szöveti antigének

– Bio-film közösség

–

Lyme borreliosis endemiás Hazánkban

Meghatározások a ‘Johan Béla’ Országos Közegészségügyi Intézet Szerológiai Laboratóriumában

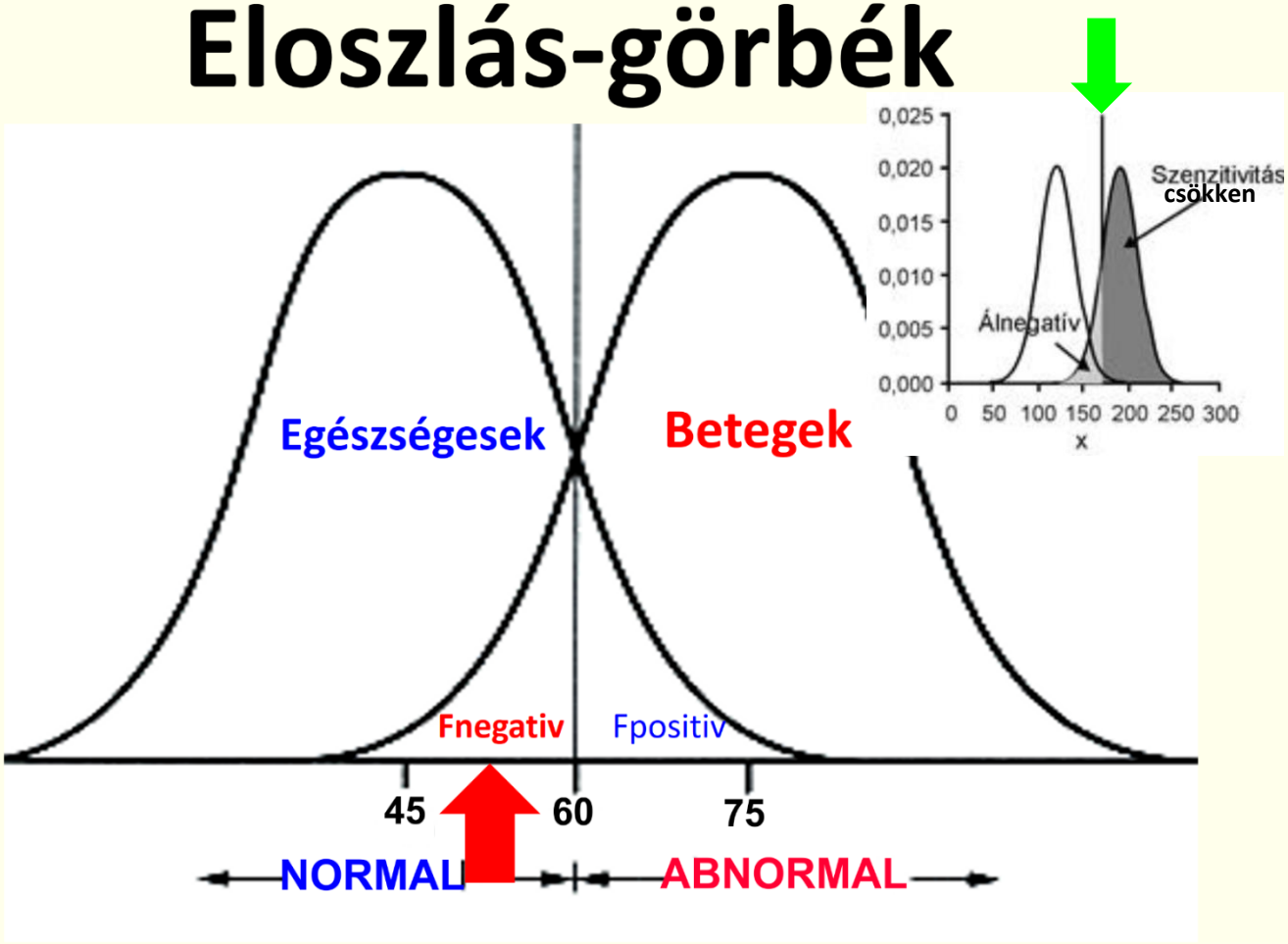


Cut-off level meghatározás

120,000 vérminta 150,000
meghatározása alapján
a Cut-off level optimális értéke
meghatározható az eredmények
Tail-cutting analysise alapján

– Ne veszítsünk el betegeket (I)
– Ne tegyünk beteggé embereket (II)

Eloszlás-görbék

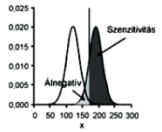


Vizsgálatok elemzése

Vizsgált Feltétel

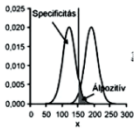
Vizsgálat	Beteg	Egészséges	Emberek		
Positiv összes positiv	TP	FP	$\frac{TP}{TP+FP}$	*PPV*	?Vpos Beteg
Negativ összes negativ	FN	TN	$\frac{TN}{TN+FN}$	*NPV*	?Vneg egészséges
	összes beteg	összes egészséges			
Eredmény	$\frac{TP}{TP+FN}$	$\frac{TN}{TN+FP}$		Predictive Value	

Sensitivitás



Biztosan nem NEG, de több betegről nincs döntés(FN)

Specificitás



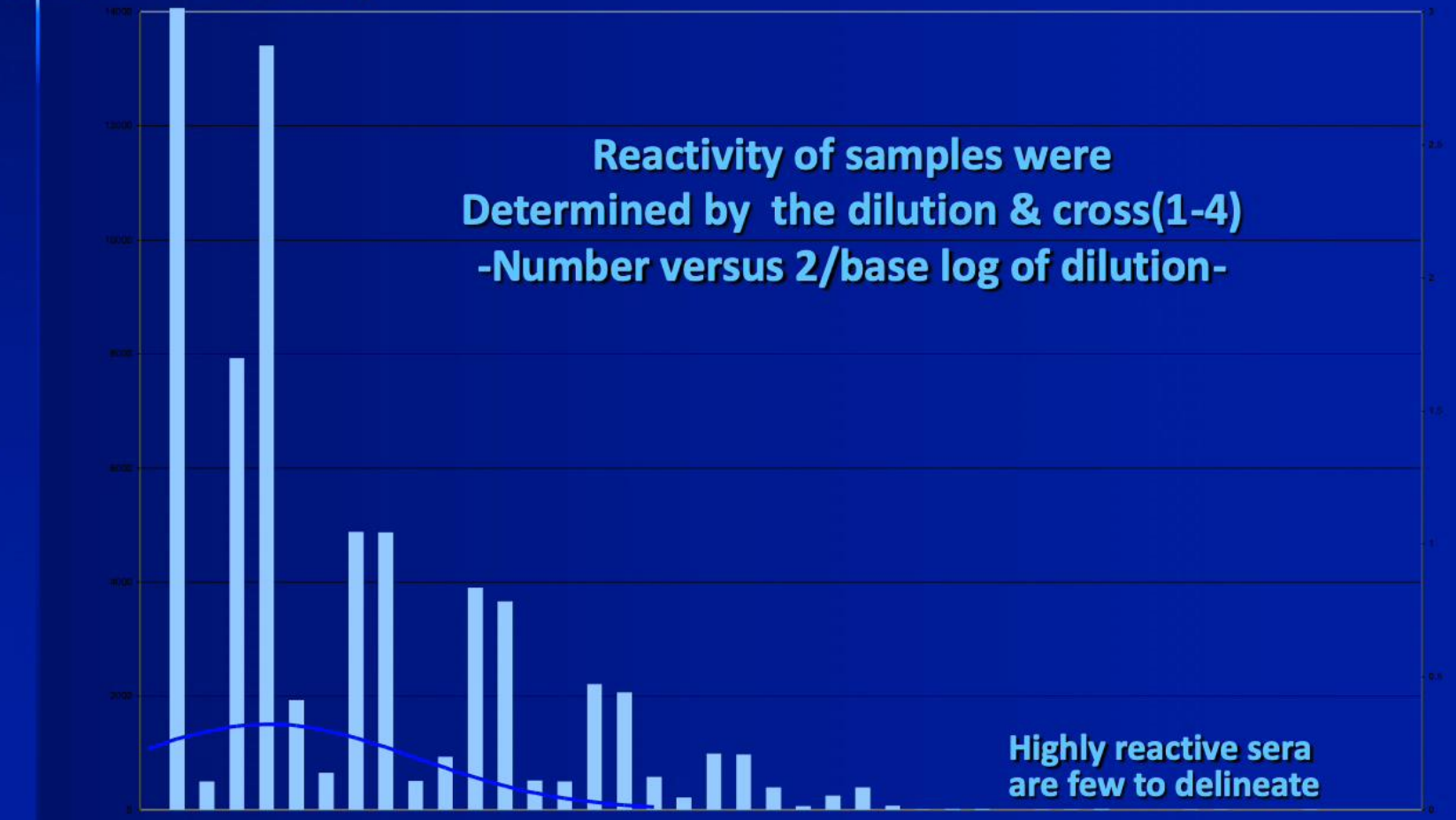
Biztosan nem POS, de sok egészséges ál-pozitív(FN)

Kulcskérdés
Határ-érték – Cut-off level megállapítása

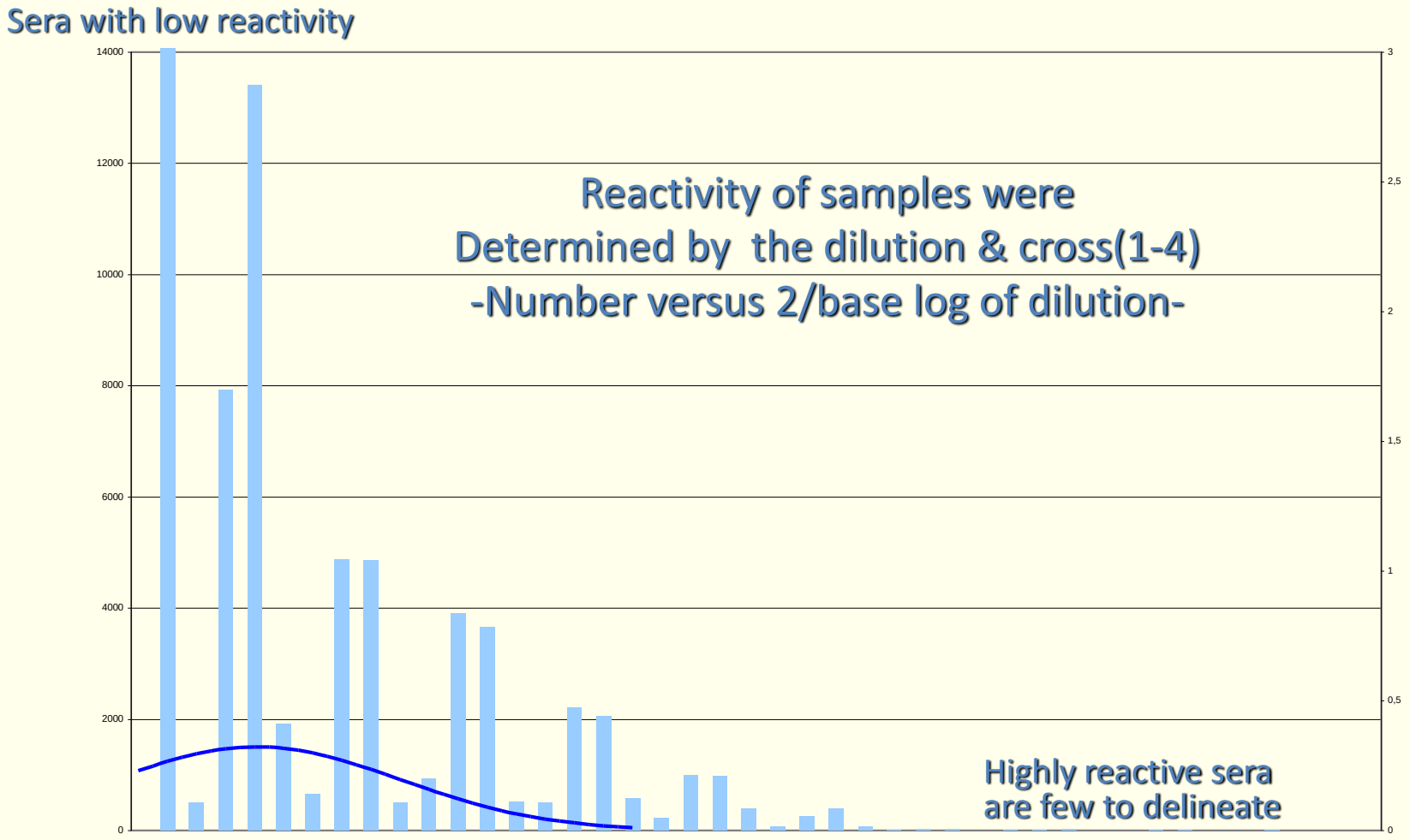
Tail-cutting

for evaluating serology

Sera with low reactivity



Tail-cutting for cut-off level



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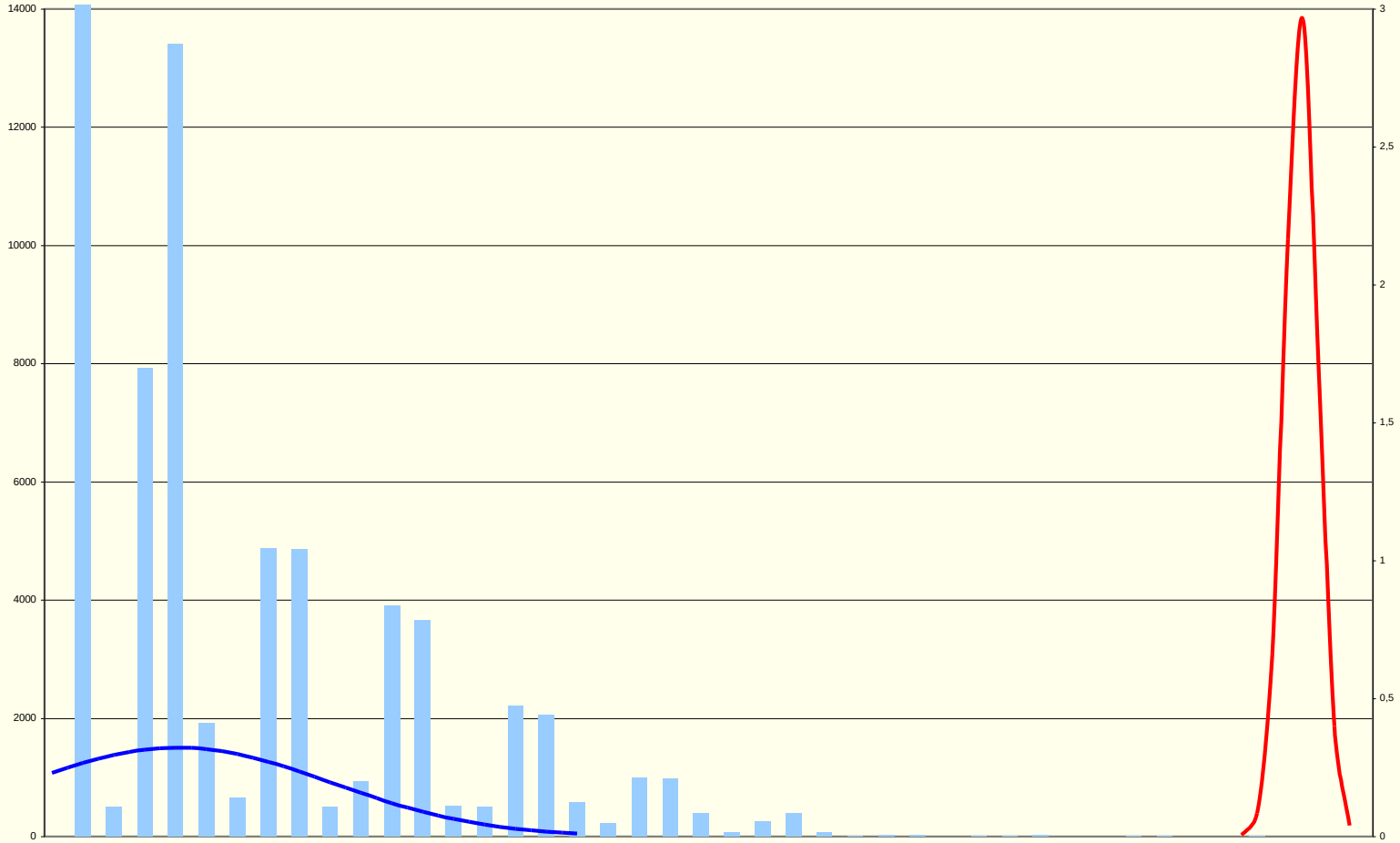

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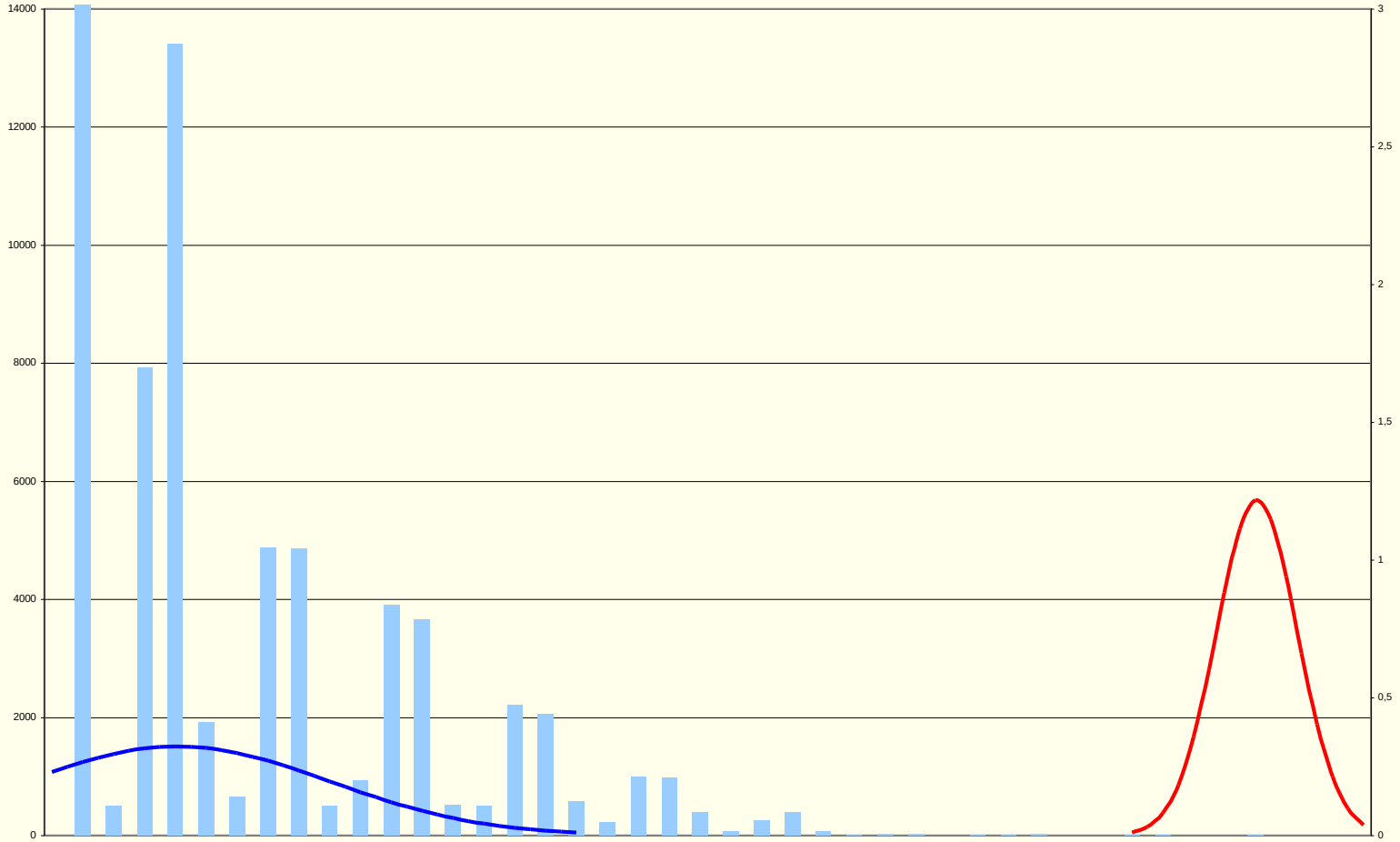

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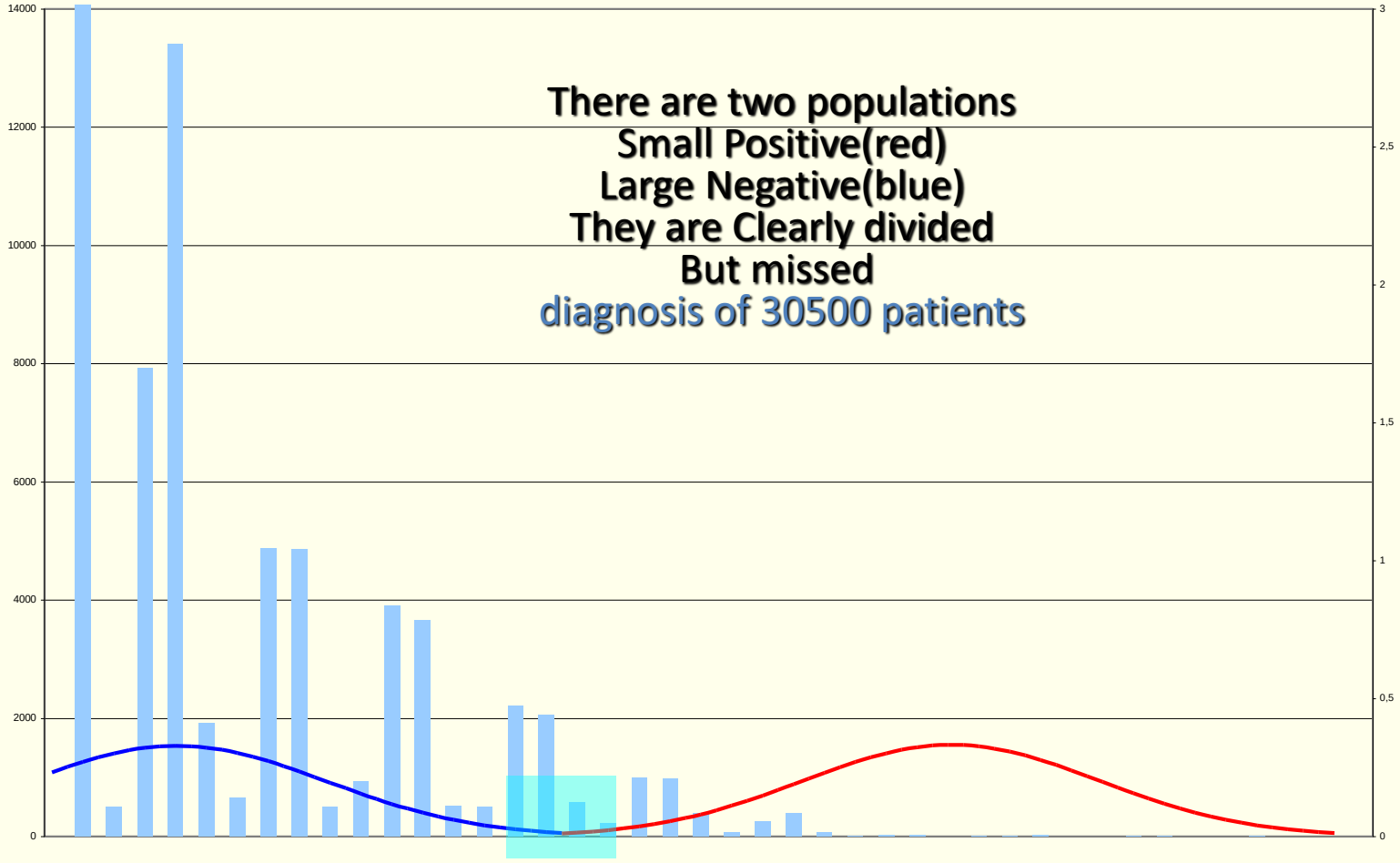

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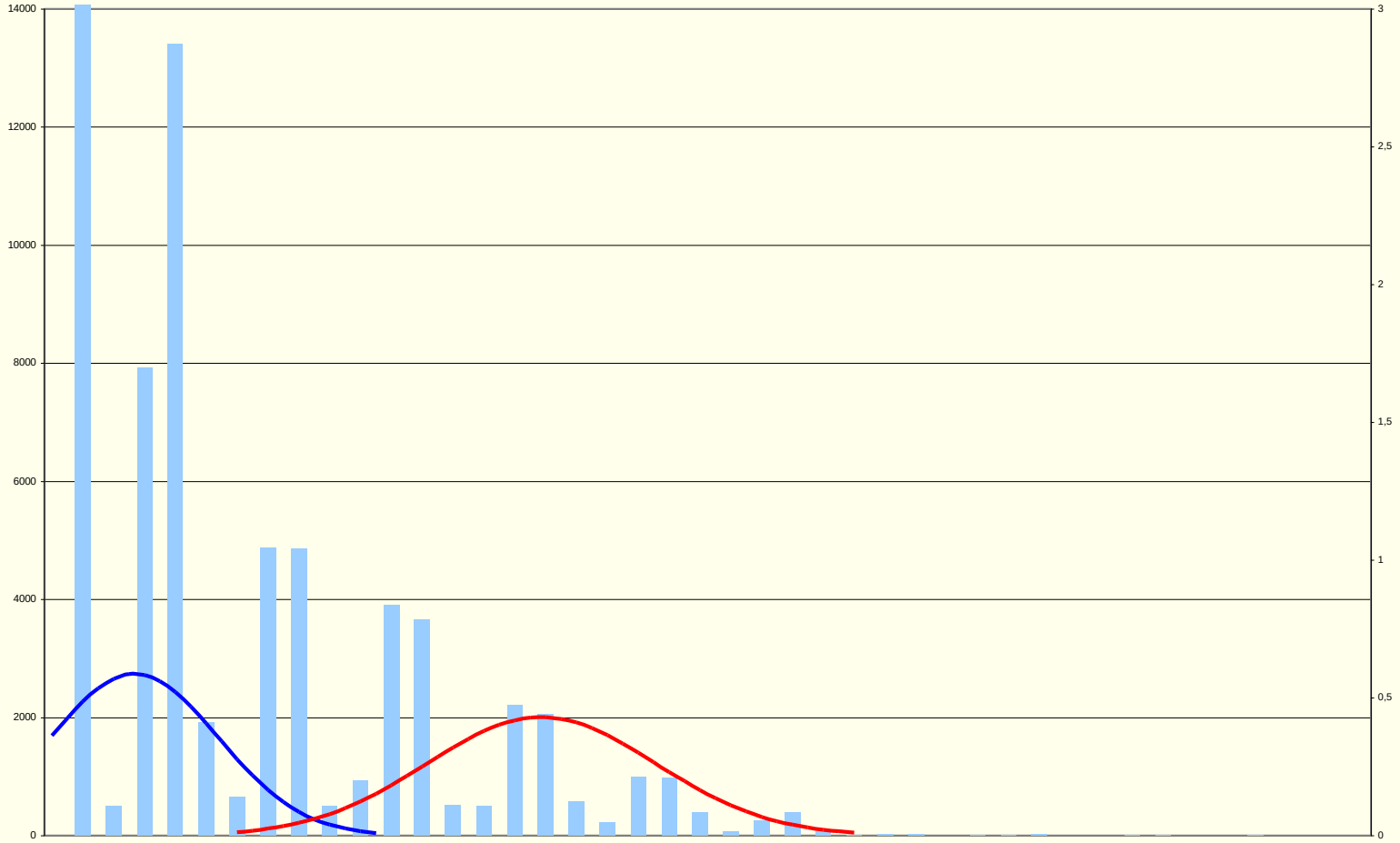

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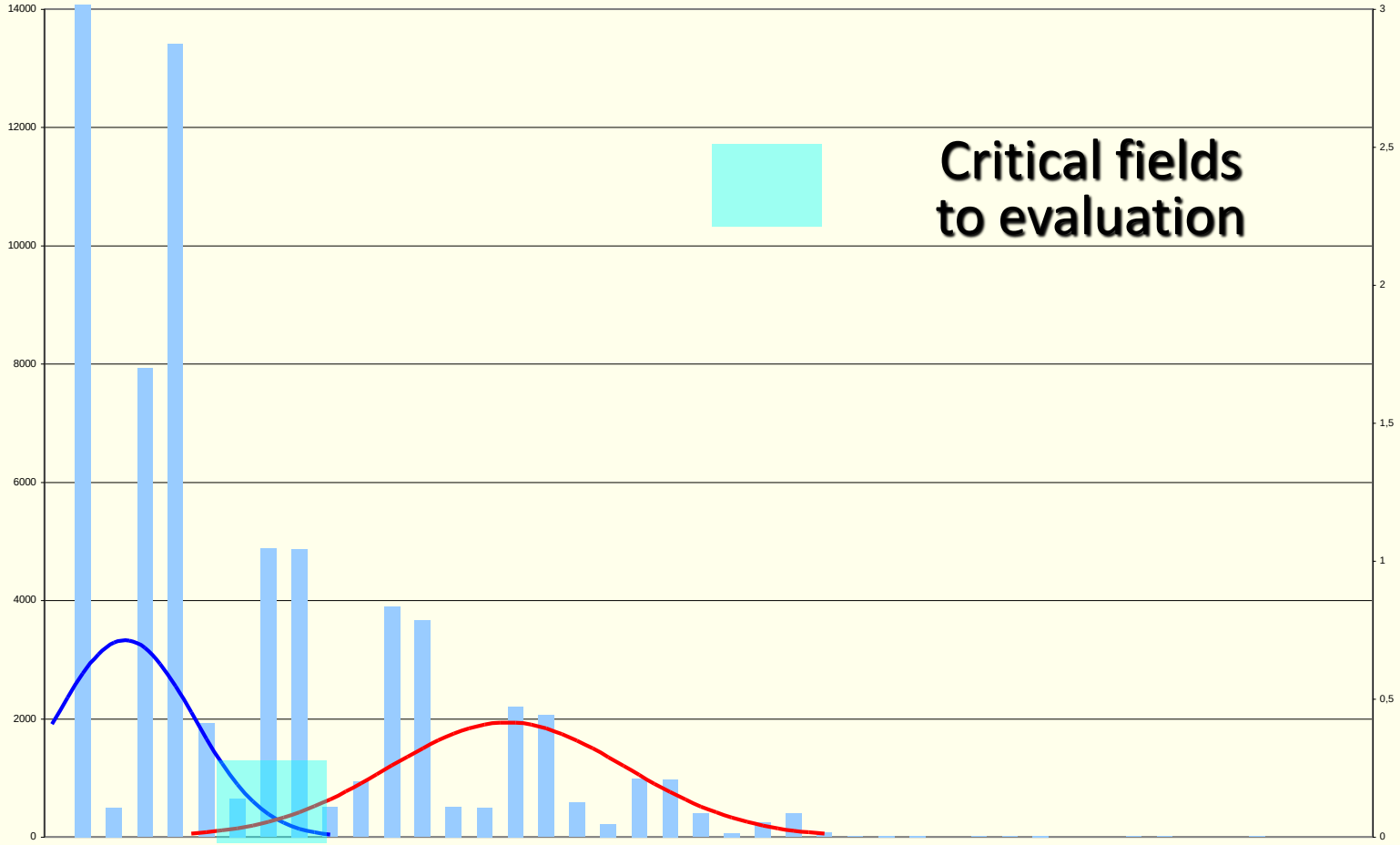

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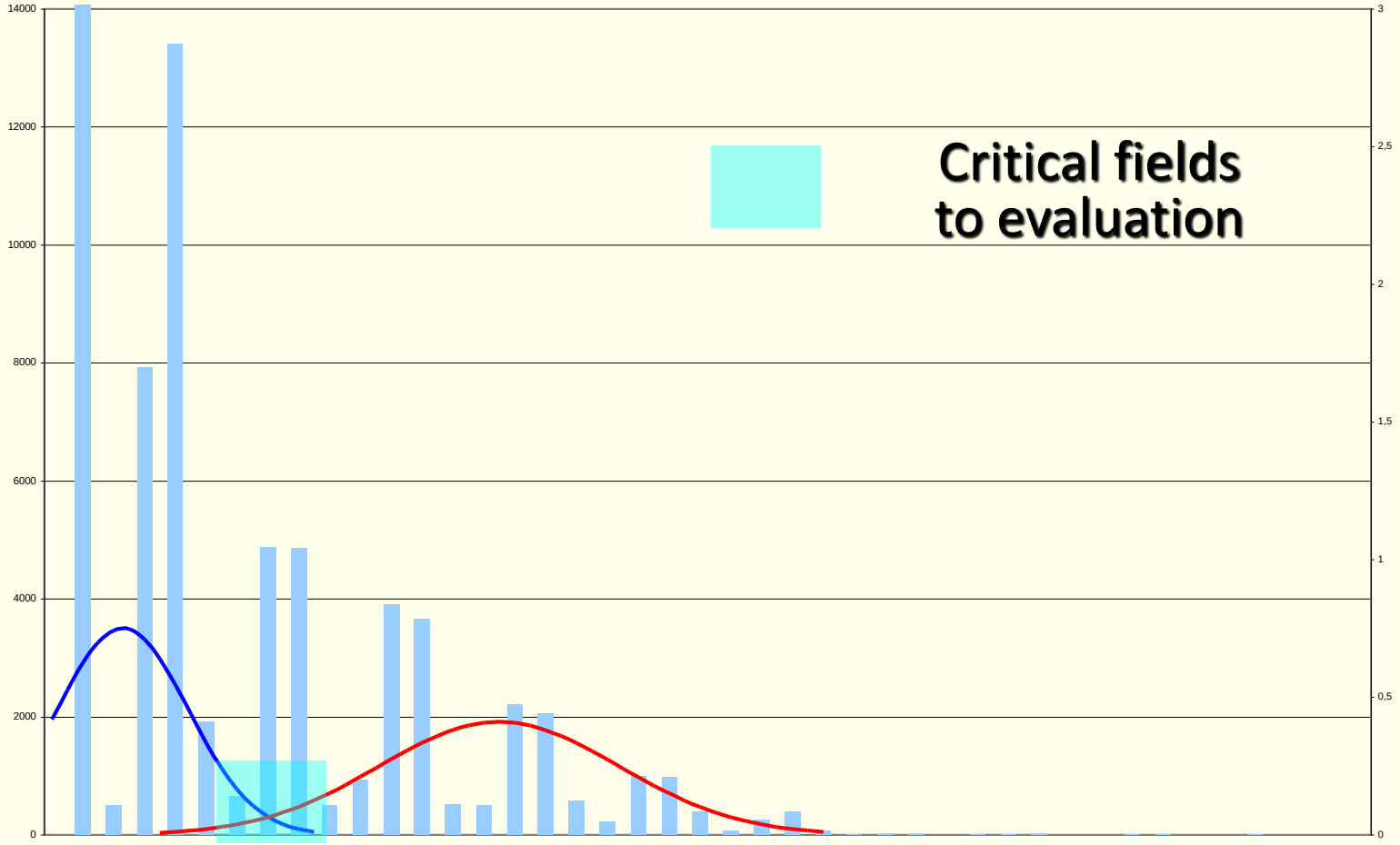

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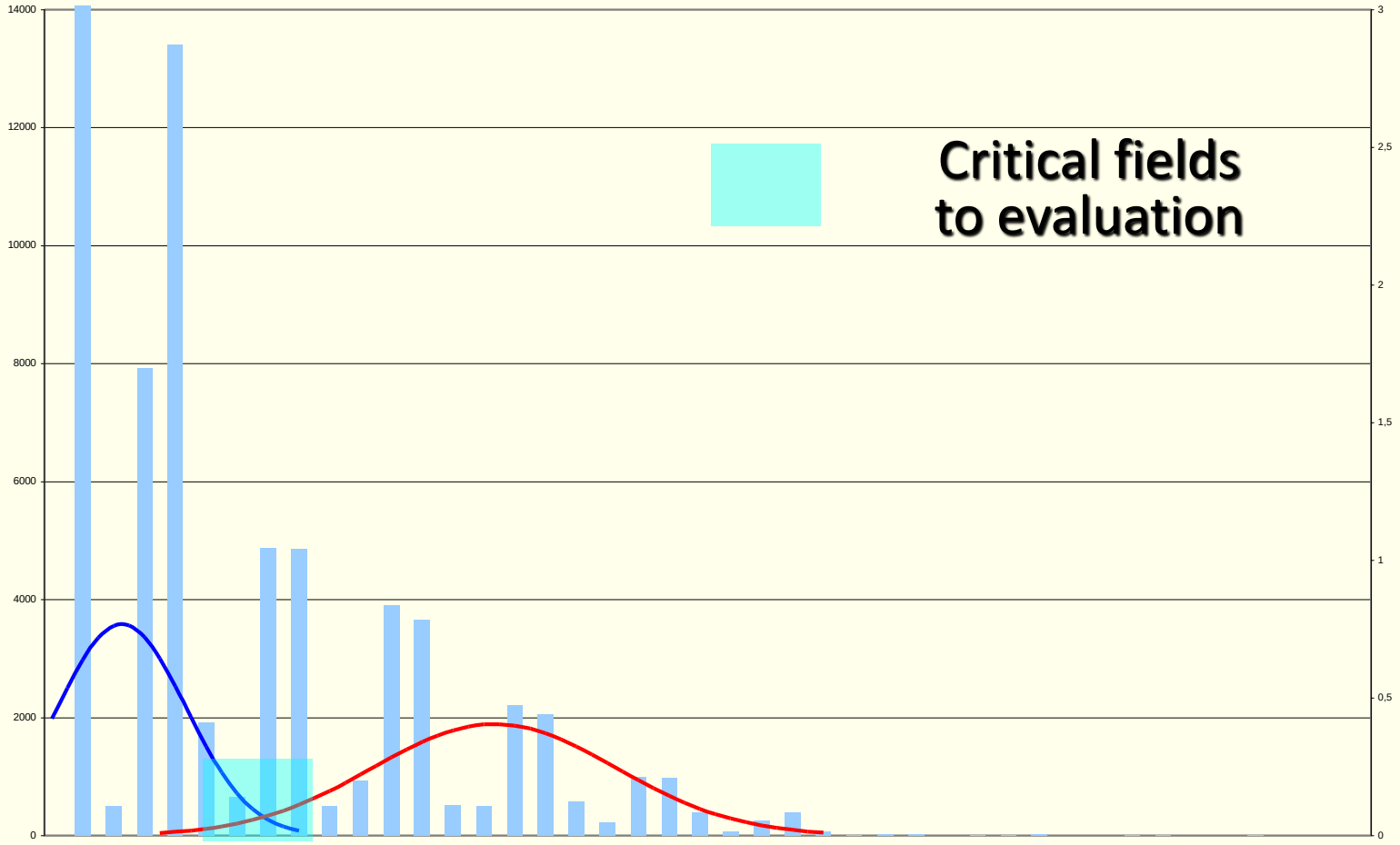

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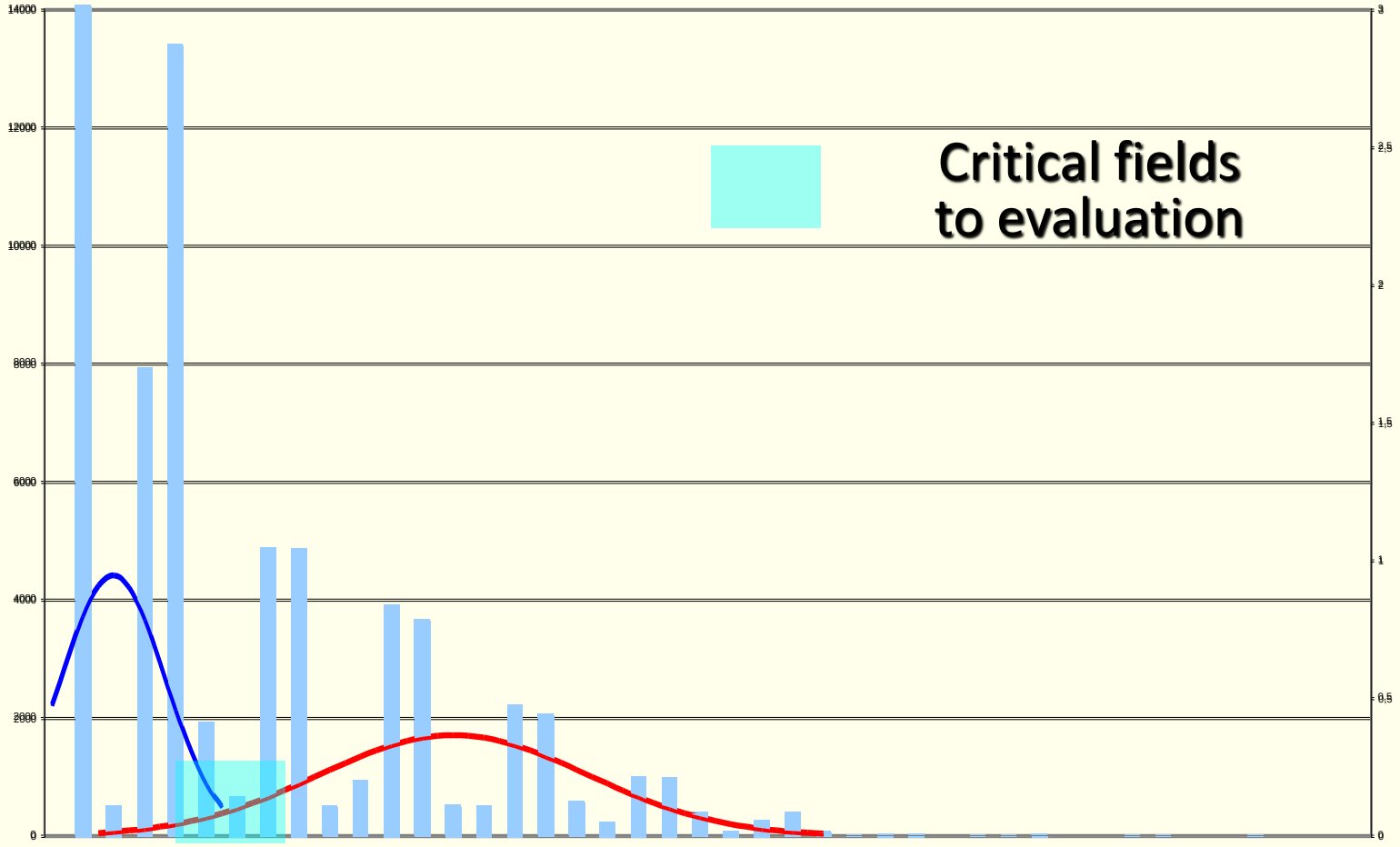

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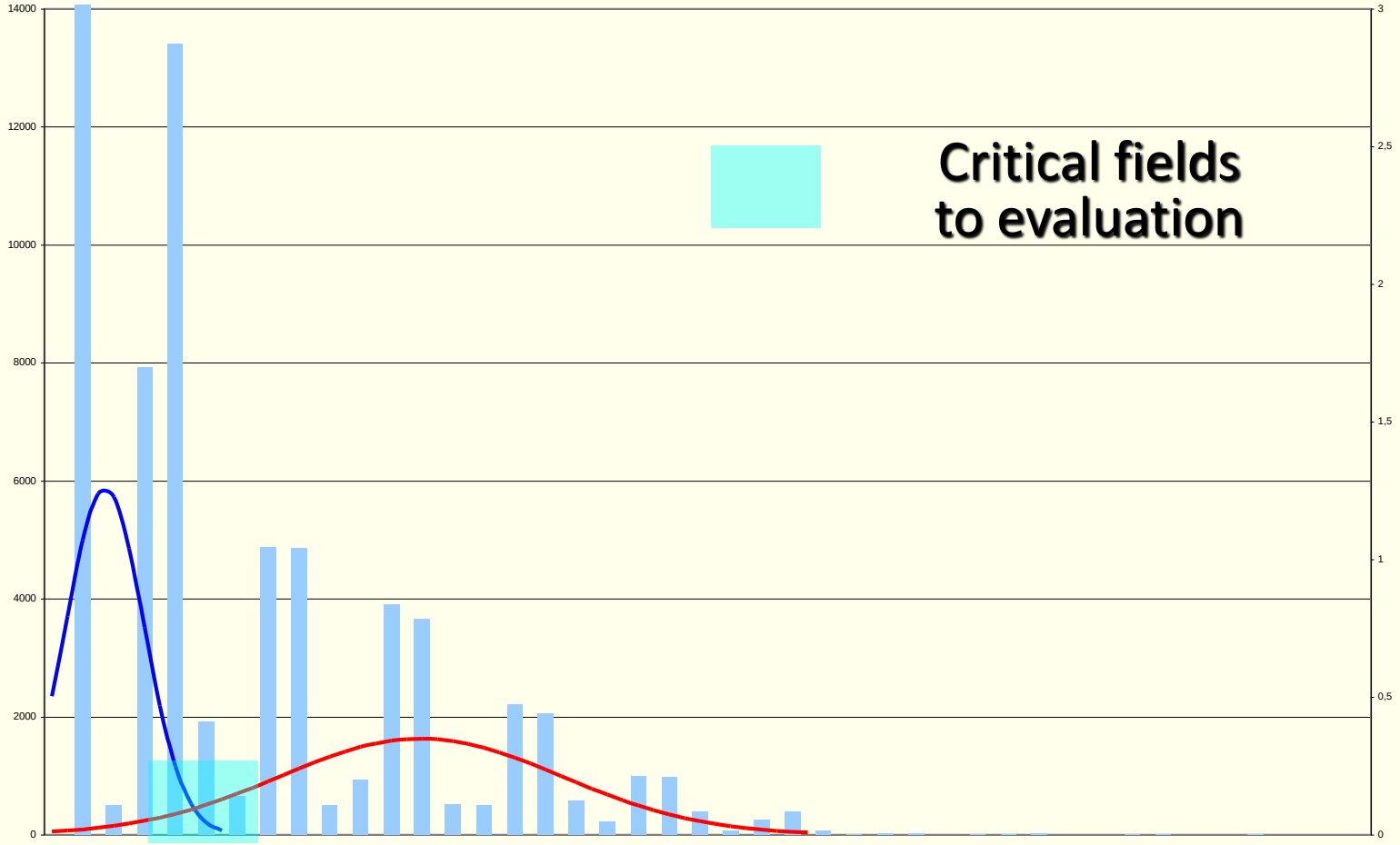

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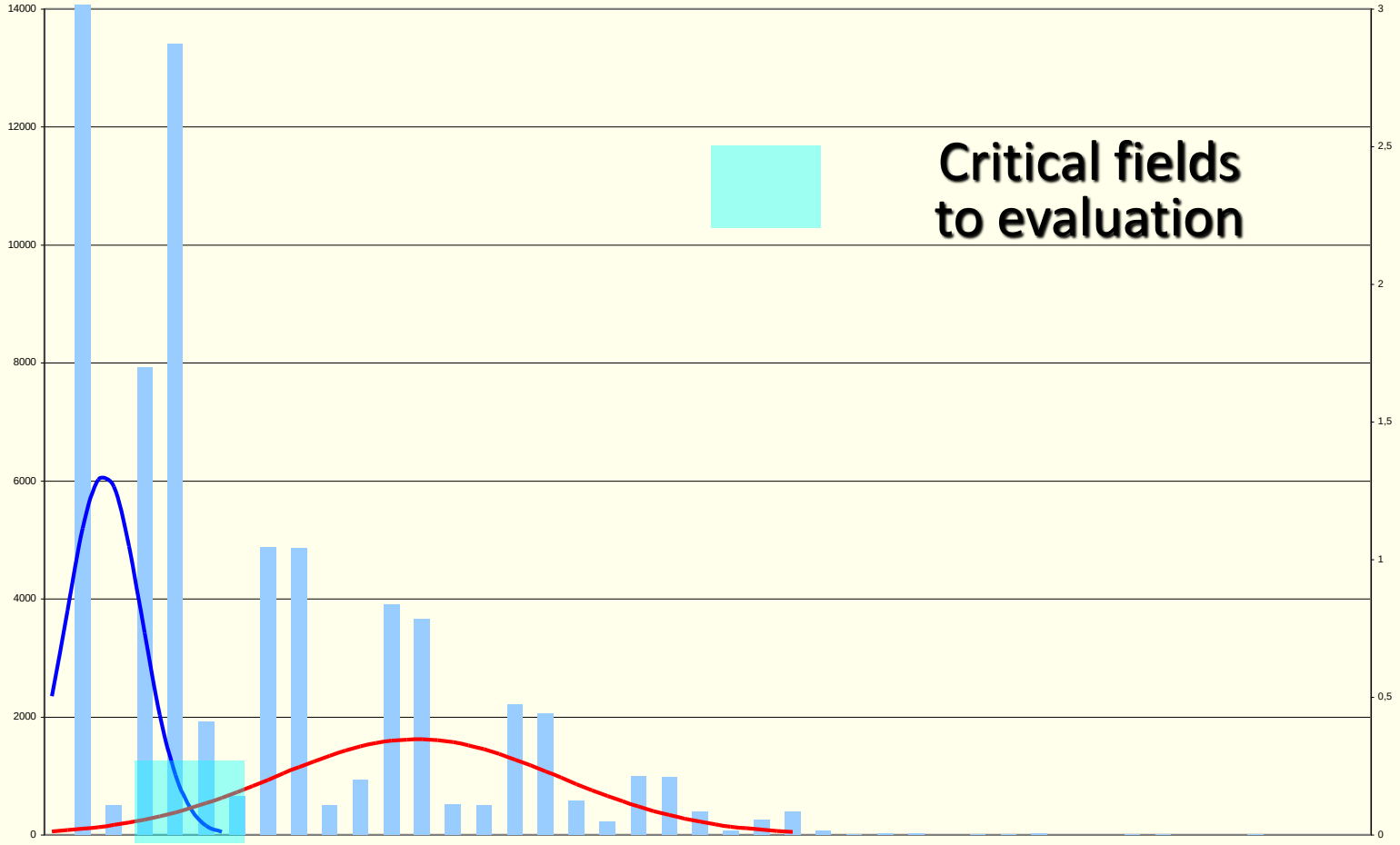
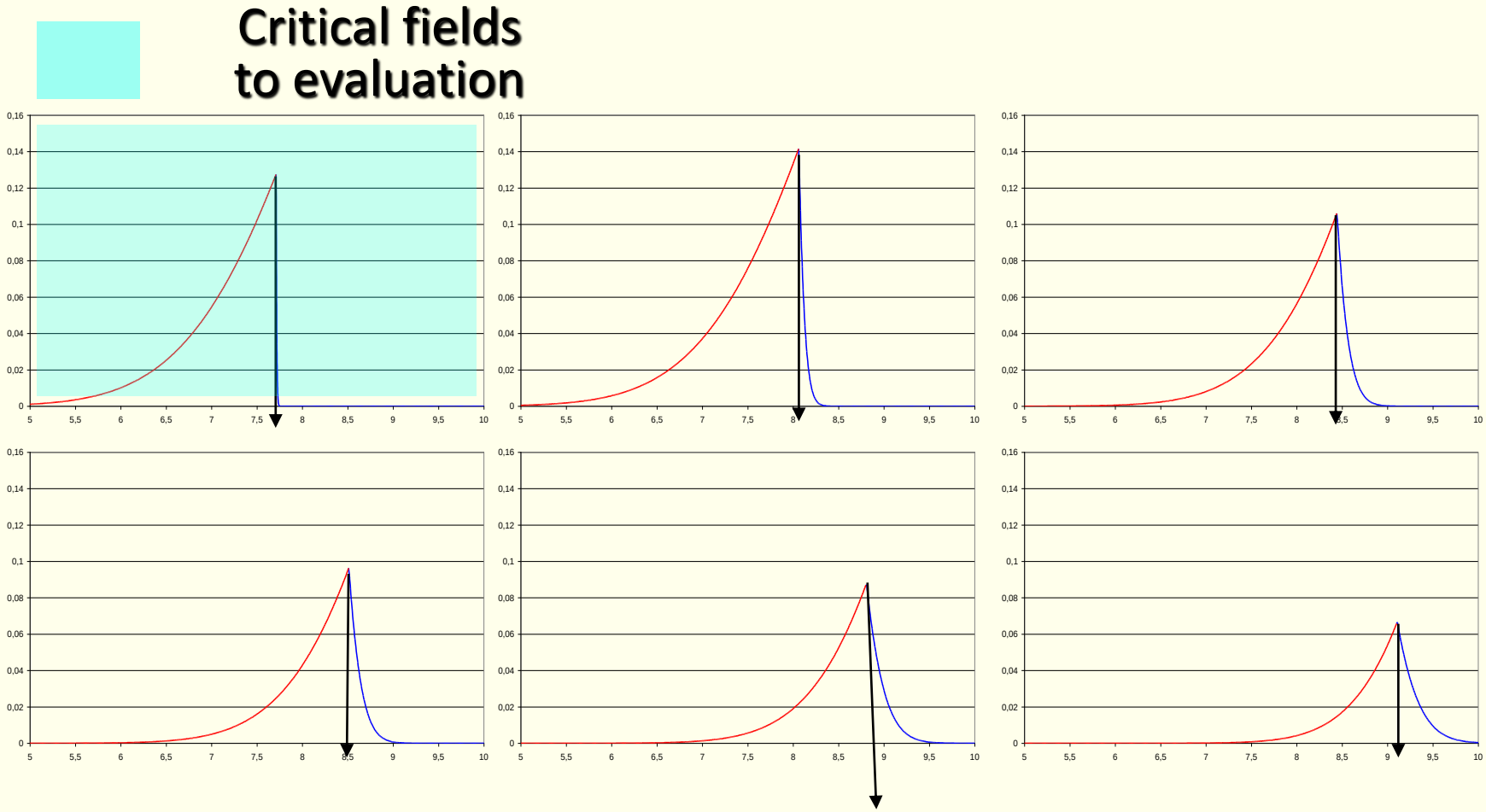
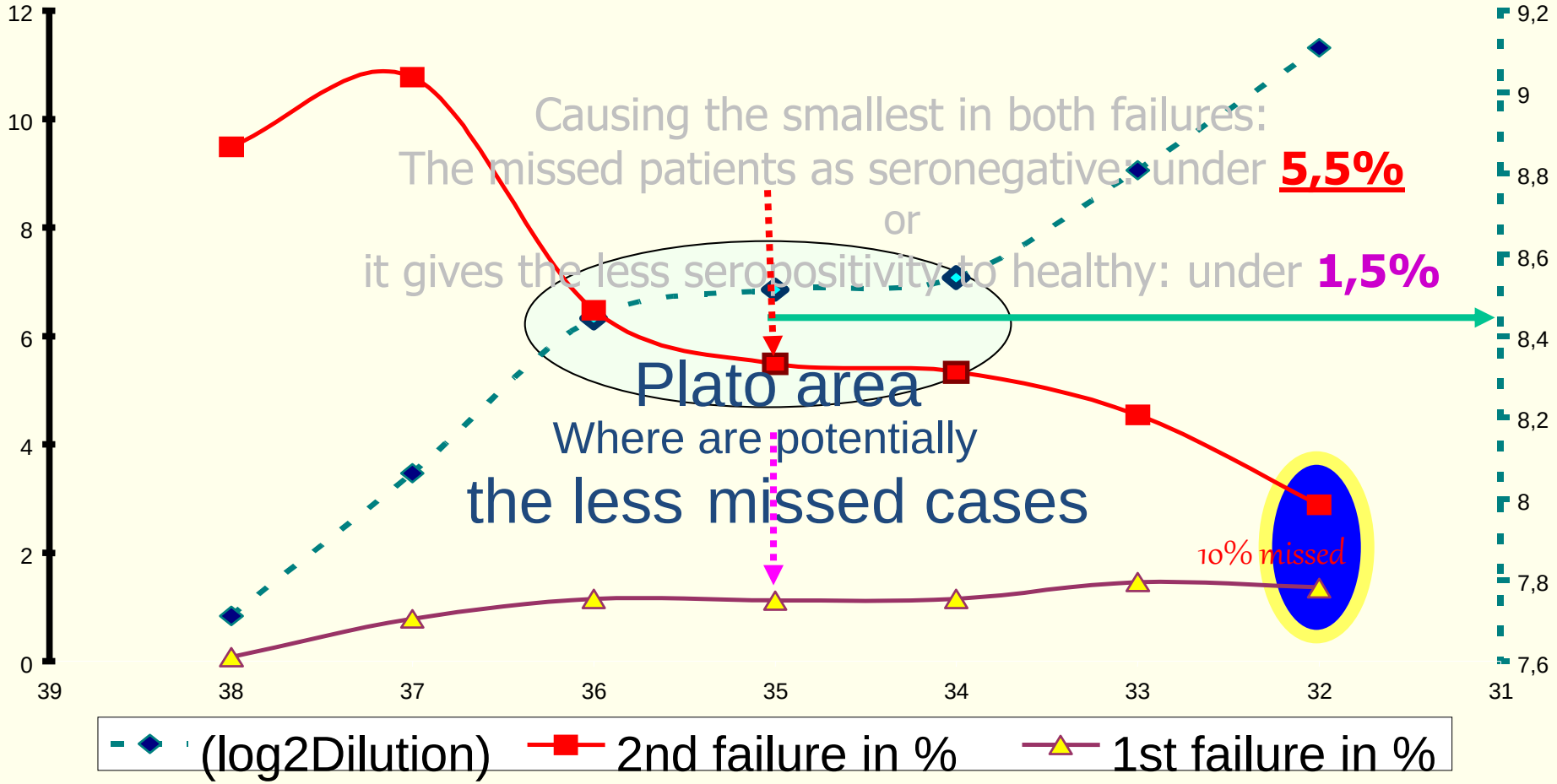


Cut-off level meghatározás



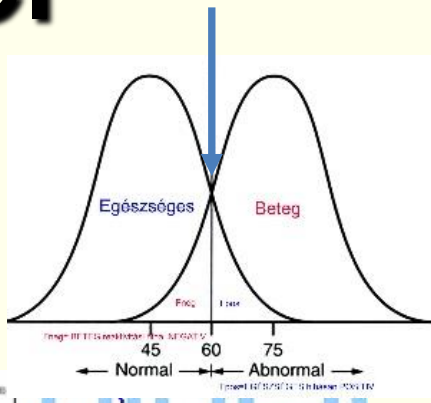
Cut-off level meghatározás

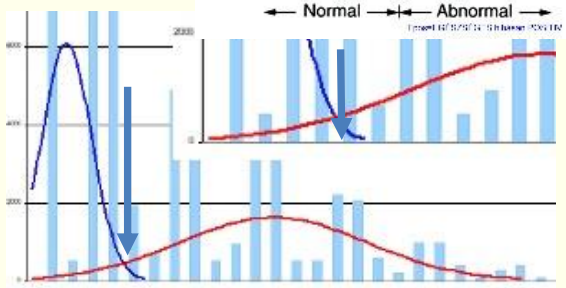
The cut-off level **1:200 dilution 3+** reactivity



First failure under 2.0 %!

Tail-cutting módszerrel meghatározott cut-off level & meghatározási hibák





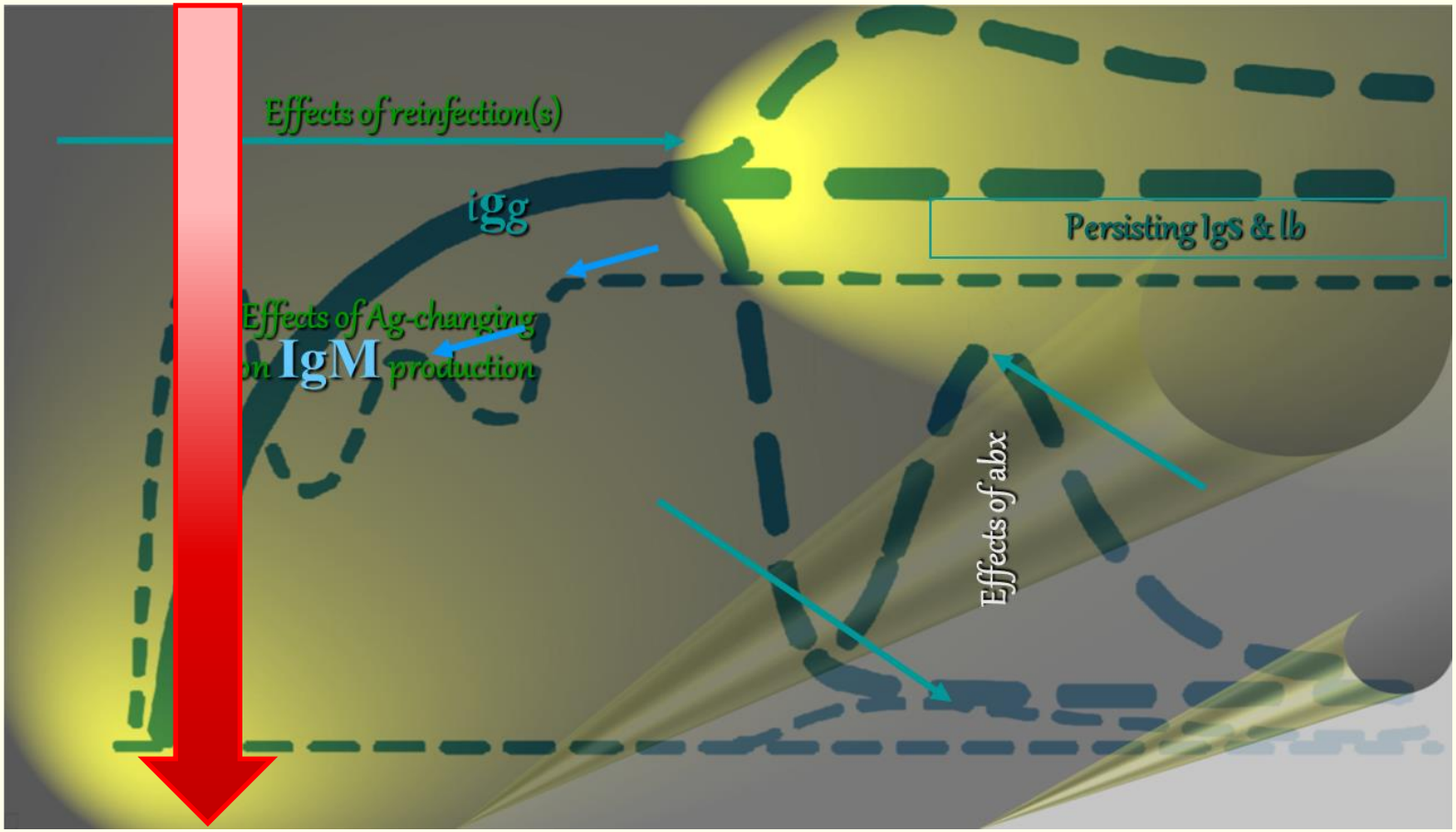
Egészségesek tévesen szeropositiv aranya nem több, mint 1,5%

Lyme beteg, aki tévesen seronegativ legalább a meghatározás 5,5%-a

HÁROMSZOR GYAKORIBB A BETEGEK TÉVES EREDMÉNYE

Ellenanyag termelődés dinamikája

MINDEN ÖSSZETEVŐNEK HASONLÓ



VIZSGÁLATOK CSAK EGY SZELETET LÁTNAK

Female 34 yo, 54 kg; **napi 30 perc tréning**

ECM.07-10.06.2015

EuroLine RN-AT IgM sorozatvizsgálat

			2015			
			06.07 - 06.10.	06.24.	07.07	08.04	10.09.
VlsE		ECM Doxycycline 2x100mg 06.24-ig Tünetmentes 2017-ig; amikor két spontan abortus+neurologiai panaszok		1	1	1	2
Flagellin				2	42	21	20
BmpA-p39				1	2	1	9
OspC	B.afzelii			5	134	103	35
	B.b. s. stricto			8	130	102	31
	B.garinii			3	108	83	18
	B.spielmanii			5	116	96	34

Ellenanyag termelődés dinamikája
Három hetes kezelés után

Cluj-Kolozsvár 2015 IgM

Budapest 2016

ANTIGEN		BÖZSIK METHOD IgM	BÖZSIK METHOD IgG	STANDARD* IgM	STANDARD* IgG	POS. CTRL IgM	POS. CTRL IgG
P83/100 – *B.afzelii*		–	–	–	–	5	5
VlsE-p40-C6		–	–	–	–	5	5
p58 – *B.garinii*		–	–	–	–	4	4
p41 – *B.burgdorferi sensu stricto*		±	1	-	±	1	1
BmpA/p39 – *B.afzelii*		–	–	–	–	4	5
OspA/p31 – *B.afzelii*		–	–	–	–	5	5
OspC p25	*B.b. sensu stricto*	+	-	+	–	8	5
	B.afzelii	+	–	+	–		
	B.garinii	+	+	+	±		
	B.spielmanii	+	–	+	–		
p18	*B.b. sensu stricto*	–	–	–	–	5	5
	B.afzelii	–	–	–	–		
	B.bavariensis	–	–	–	–		
	B.garinii	–	–	–	–		
	B.spielmanii	–	–	–	–		
WB-EVALUATION *According to the manuscript*	*Results*			POS	neg	B=6	
	Points	8	6	8	0	*Borderline*	

OPINION: Tick-biting in Austria was reported followed ECM and monotherapy *(Doxycycline 2x100mg 3 weeks)*. The pathogenetic development was controlled with Western-blot analyses. It was demonstrated effective immune responses with both the continuously existing IgM and later the less expressed IgG that disappeared.

Before the investigations in Synlab it was proved IgG negativity and not determined the IgM at Lyme Diagnostic. Beside the specific serology for *Lyme borreliosis chronica* it was also demonstrated seropositivity for *Bartonella henselae, Toxoplasma gondii, Rubella, CMV, HSV1, Parvovirus B19, VZV/HHV-3*, which can contribute to be inhibiting the immune response for *B.burgdorferi sensu lato* and provoke similar signs and symptoms.

2017-ig panaszmentes, majd tünetes beteg
Ismételt kombinált antibiotikumra gyógyult
IgG-válasz 2017-2018-ban sem alakult ki

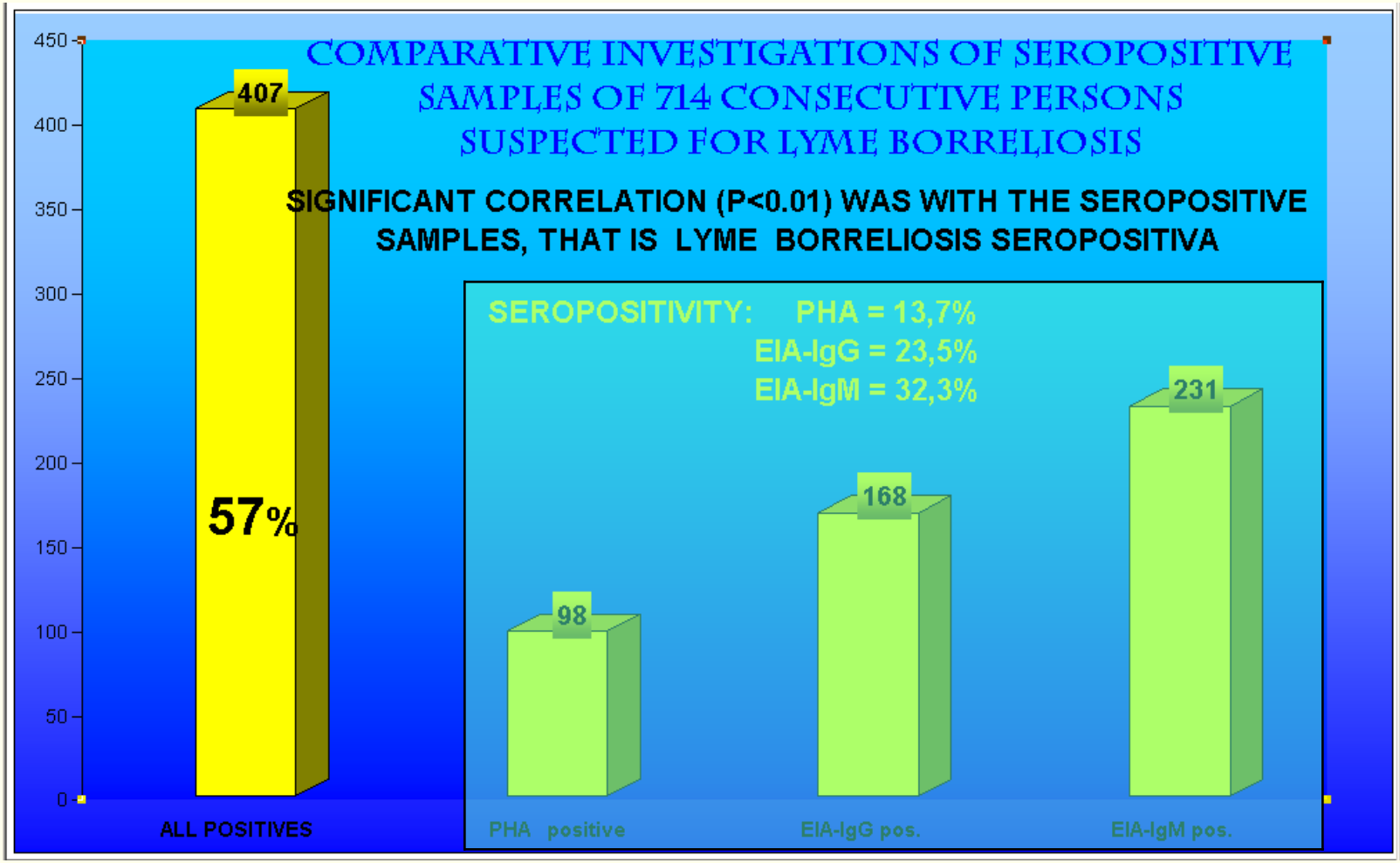
Jellegzetes „decapitatio” Lyme borreliosis kapcsán

Az OspC-re adott immunválaszt nem érinti a **promt doxycyclin***(2x100 mg)*

Pathogenitás marad, két év „tünetmentesség” után jelentkezett a LB

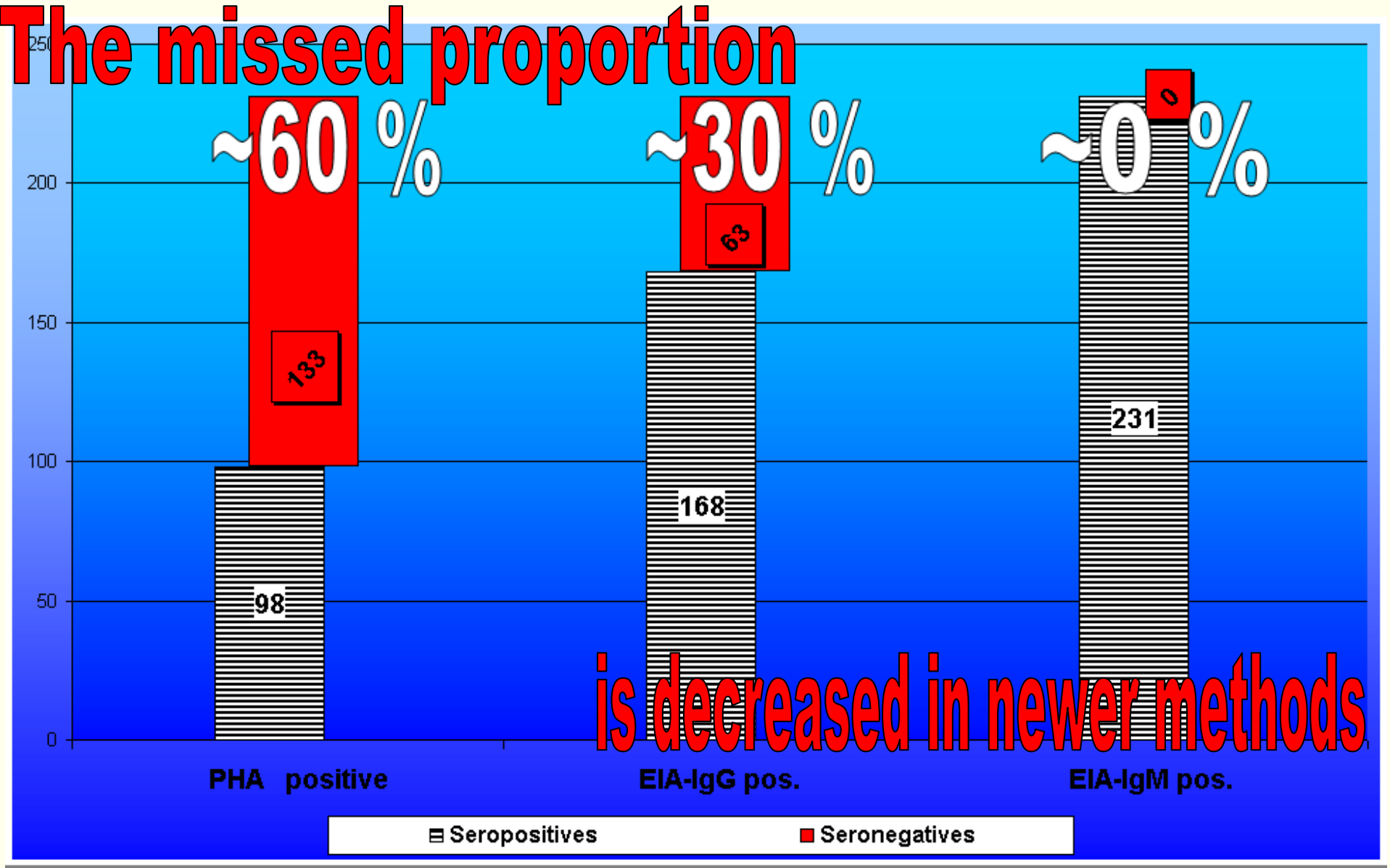
Which Method Could Became The Golden Standard?

Compare with Newer methods



Lyme borreliosis seronegativa

the relation in different methods



TAKING THE RESULTS
TO REFER 100 PARALLEL INVESTIGATIONS

Investigations at the NIH Hungary

METHODS	Sensitivity per cent	Specificity per cent	SEROLOGICALLY DETECTED CASES**	So called SERONEGATIVE
Enzygnost-M *	**100**	92	92	8
Enzygnost-G	72	90	65	35
PHA*(117.560)*	42	**90**	38	62!

* Enzygnost-M accepted as if it could detect all seropositivity

** (Sensitivity%) * Specificity relative frequency

TAKING THE RESULTS*
TO REFER 100 PARALLEL INVESTIGATIONS

Marangoni A, et al.: J Med Microbiol 2005, 54: 361-367

METHODS	Sensitivity per cent	Specificity per cent	SEROLOGICALLY DETECTED CASES**	THE MISSED ONES So called SERONEGATIVE
Enzygnost-M	71	92	**65**	**35/29!**
C-6	62	97	**60**	**40/38!!**
rec-WELL-M	56	99	**55**	**45/44**
rec-WELL-G	58	97	**56**	**44/42**
Enzygnost-G	37	90	**33**	**67/63!**
PHA in NIH, HU *117.560 serum*	**25,8**	**98/93[1]**	**25/24**	**75/74!**

** (Sensitivity%) x Specificity = relative frequency

There is a great difference between biological & biostatistic error

- Although 1.5 % first order/fals-positive and 5.5 % second order error/fals negative are excellent results for any laboratory it is astonishing that the developments of science give possibility to determine further 60 % or more cases as people, who are in bad need of treatment.
- This result is
  - **neither over-diagnosis,**
  - nor under-estimation,
  - especially not fals/untrue determinations

they really belong to both the development of methodology and the epidemic character of Lyme borreliosis.

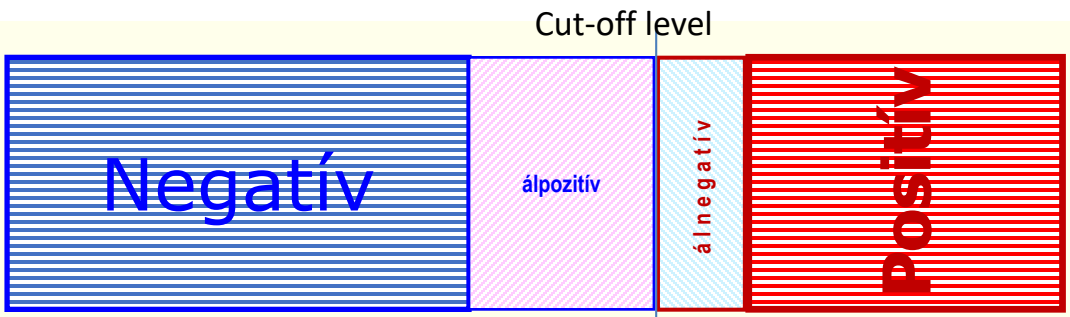
TAKING THE RESULTS
TO REFER 100 PARALLEL INVESTIGATIONS

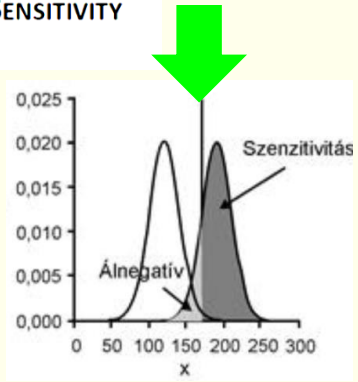
Marangoni A, et al.: Microbiol. 2005 Jan; 28(1):37-43.

METHODS	Sensitivity	Specificity	Sero-	
			Positive	Negative
Anti-Borrelia plus VlsE IgM, **Euroimmun**	85,0	78,5	**67**	**33**
Anti-Borrelia plus VlsE ELISA IgG, **Euroimmun**	56,6	98,5	**56**	**44**
Quick ELISA **C6, Immunetics**	33,3!	?	**34?**	**66?!**
Euroline-WB against Borrelia, **Euroimmun**	68.3	80.0	**55**	**45**
Qualicode B. burgdorferi WB, **Immunetics**	26.6	100	**27**	**73**
multispecies "home made" WB	71.7	100	**72**	**28**

A módszerek áttekintése

ANTIGEN CONTENT			METHOD	EVALUATION			SPECIFICITY
Recombinant	**Preferred list Solely antigens**		**Western-blot**	Informed INDIVIDUALLY & SUMMED UP		INDIVIDUALLY & SUMMED UP	
Purified	Selected	*Solely/mixed*	*ELISA*	informs	More specific	OD summation	
		complete	*Western-blot(elfo)*	informs	More specific	INDIVIDUALLY & SUMMED UP	
	Mixed		*ELISA*	summation	More specific	OD summation	
Solved	Crude solved		PHA, *ELISA*	summation	specific	OD summation	
Spirochete	Whole fixed one		IFA	summation	specific	Semi-quantitative	SENSITIVITY





Cut-off level CSÖKKENTVE

Cut-off level EMELVE

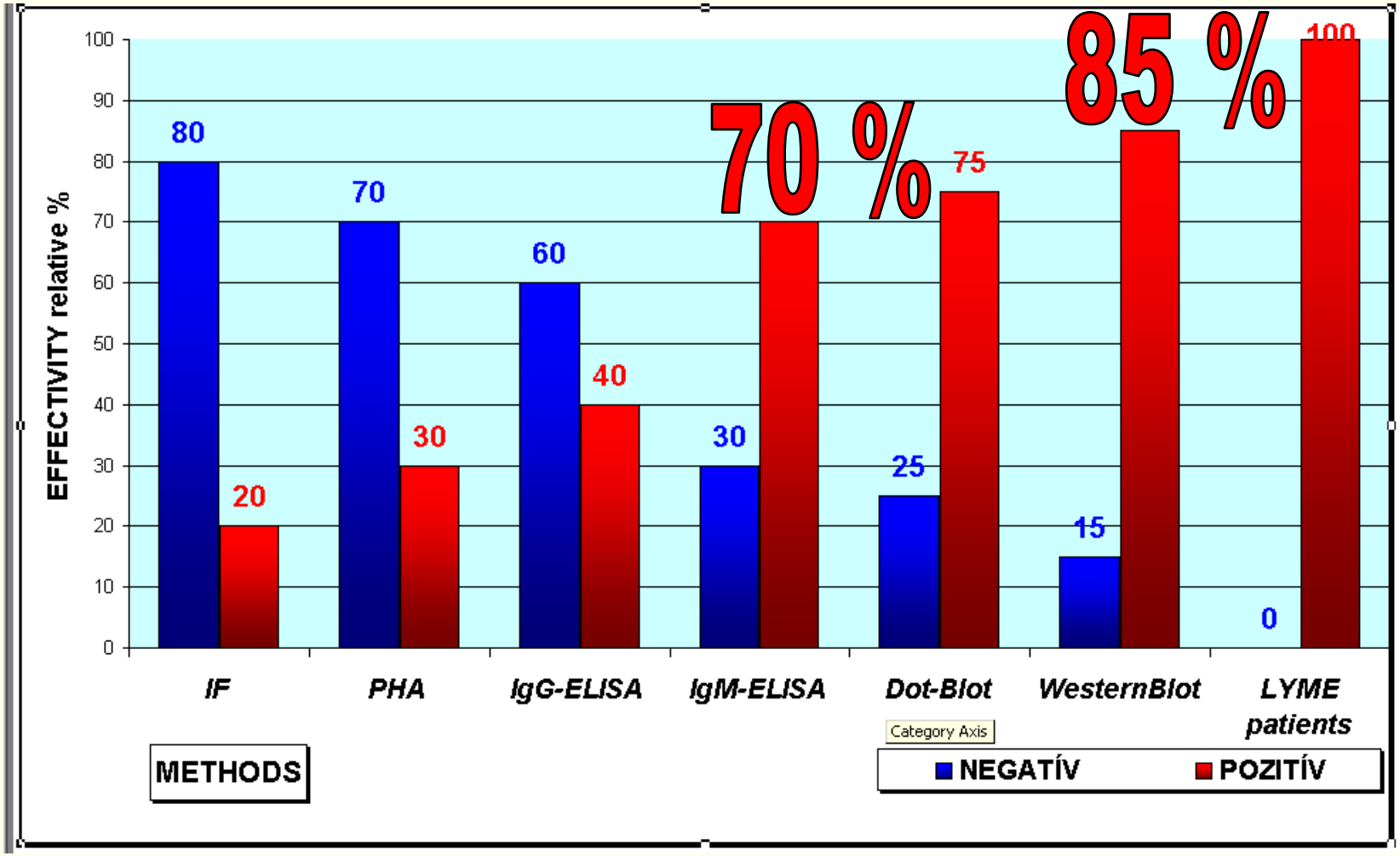
Nő a SENSITIVITÁS, **de több álpozitív**
Csökken a SPECIFICITÁS
SOK EGÉSZSÉGEST KELL **ELLENŐRIZNI**

Nő a SPECIFICITÁS, **de több álnegatív:**
Csökken SENSITIVITÁS, kevesebb positive,
SOK BETEG MARAD KEZELETLEN

The BLUE COLUMNS

related to the missed proportions of patients

theoretical graph!



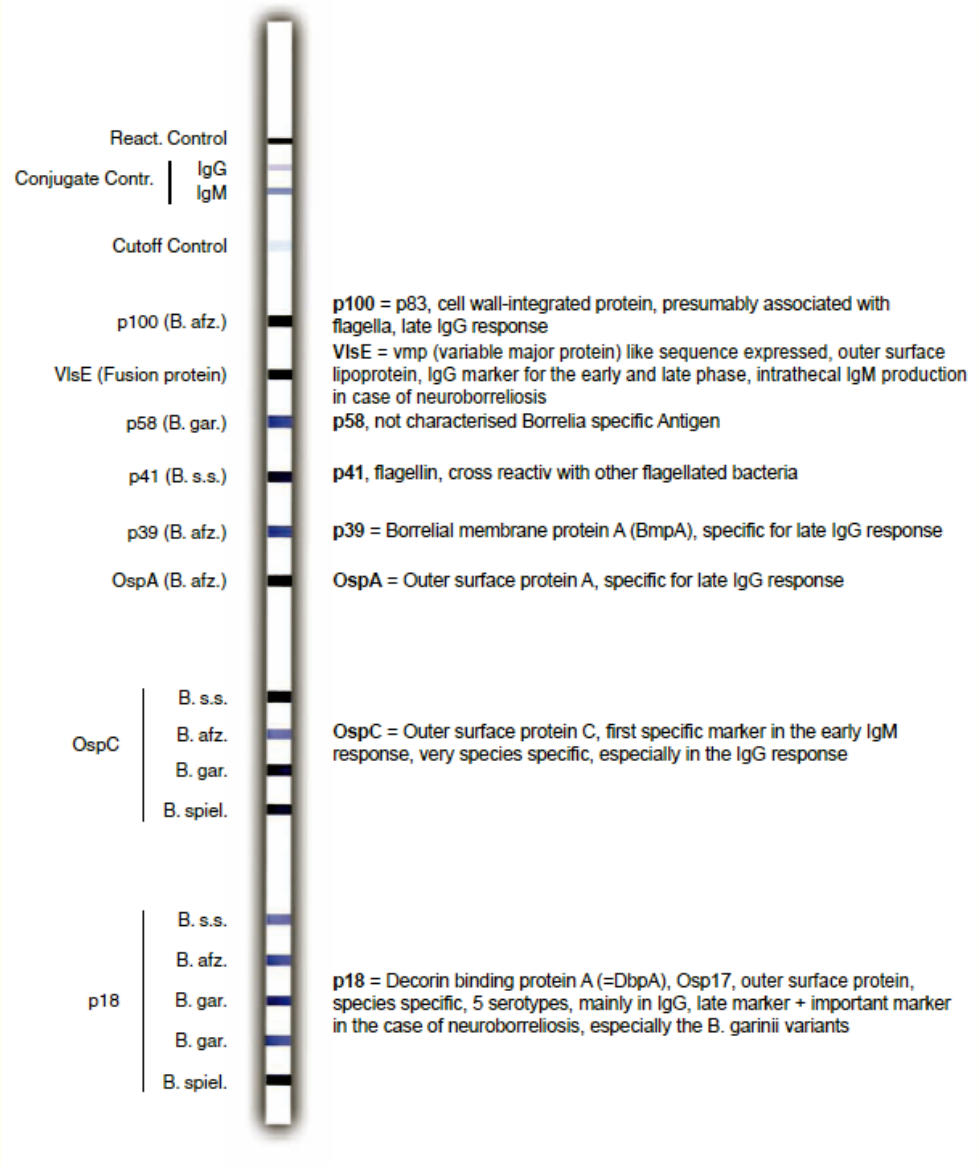
DIFFERENT DIAGNOSTIC GUIDELINES
FOR CHRONIC LYME

Ljøstad U, Mygland A _2012, but Modified 2013

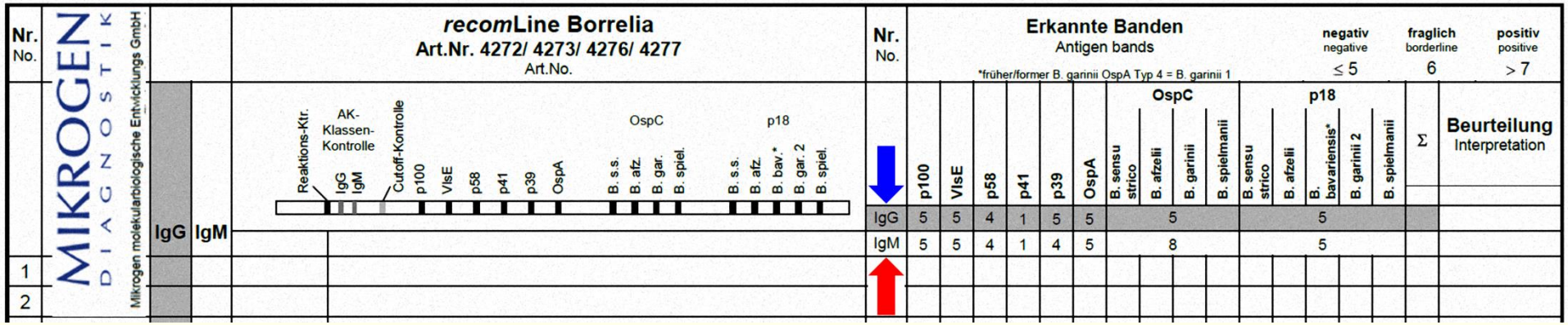
			EFNS_2012	EUCALB_2010	IDSA 2010(2006)	ILADS_2004 GERMAN_2010
CLINICS	NEUROLOGIC	PERIPHERAL	+	+	+	CLINICS
		CENTRAL				
	ARTHRITIC			+	+	
	DERMATOLOGIC			+	+	
	PSYCHOLOGIC				+	
DIAGNOSTICS	PLEOCYTOSIS		+	+	+	NO DEFINITIVE TESTS Positivity helps, but supersedes
	INTRATHECAL ANTIBODY		+	+	+	
	SEROLOGY			ESSENTIAL	IMPORTANT	
	SYNOVIAL			+	AB/PCR	
			ALL THREE	SEROLOGY	TWO-TIER	SYMPTOMS
CONSEQUENCE	SCIENTIFIC DATA (CDC epidemiological)					LONG TREATMENT FOR CURING
	MONOTHERAPY					COMBINED THERAPY frequently
	POST TREATMENT LYME SYNDROME					REPEATED TREATMENT
	NOT ANY OBJECT FOR RE-TREATMENT					
	Not selected according to either the strains of *B.burgdorferi sensu lato* their the sensitivity to the antibiotics					
						DualDur® *reangent*
FUTURE POSSIBILITY	In vitro antibiotic sensitivity investigations Especially to be determining THE INTRACELLULAR EFFECCTIVITY OF COMBINED ANTIBIOTICS WITH DIFFERENT STRAINS					

WESTERN-blot *line*-method

with recombinant Antigens



ADVANTAGE
in EVALUATION



Based on the intensivity and specificity of the serologic-reactivity:

Positive/Borderline >=6< *Negative*

Based on the recombinant Antigen-specificity
that can help to be developing personalized schedule(s).

Both IgG and IgM reactivity can be determined
with the same recombinant Antigen-selection

SEROLOGY READING

- P83/100 – B.afzelii : specific 83-100 kD protein
- VlsE-p40-C6 constant component of the causative agent
- p58 – B.garinii : specific 58 kD protein
- p41 – B.b. sensu stricto 41 kD generic protein (flagellar)
- BmpA/p39 – B.afzelii the first recombinant antigen, and it is highly specific.
- OspA/p31 – B.afzelii : specific Outer surface protein A, 31 kD the component of the first vaccines that produced RA-like inflammation & autoAT-LFA-1
- OspC p25 : specific Outer surface protein C, 25 kD that is the first appeared AntiBody, which should be disappearing after 2-3 months.
  - B.b. sensu stricto, B.afzelii, B.garinii, B.spielmanii
- P18 : specific 18 kD protein
  - B.b. sensu stricto, B.afzelii+B.spielmanii *and* B.bavariensis+B.garinii, the two big family
  - kD= kilo Dalton, molecular weight

CONTROL

*recom*Line Borrelia IgG/IgM

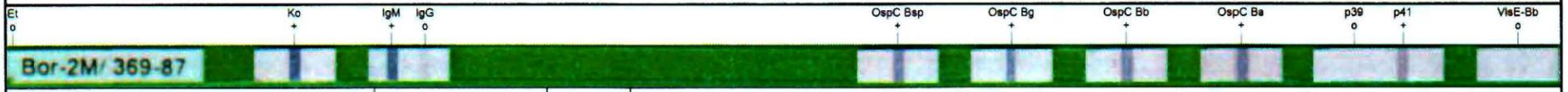
SPECIFICITY BLOOD DONORS (n = 200)	IgG	IgM
Negative	180	185
Positive/Borderline	20	15
Confirmed by WB	19	9
Prevalence	9.5 %	4.5 %
Specificity	99.4 %	96.7 %

SENSITIVITY	IgG	IgM	IgG+IgM
Arthritis (n = 28)	27 (96 %)	6 (21 %)	27 (96 %)
Acrodermatitis (n = 11)	11 (100 %)	1 (09 %)	11 (100 %)
Neuroborreliosis (n = 35)	29 (83 %)	18 (51 %)	33 (94 %)
Erythema migrans (n = 42)	18 (43 %)	30 (71 %)	33 (79 %)

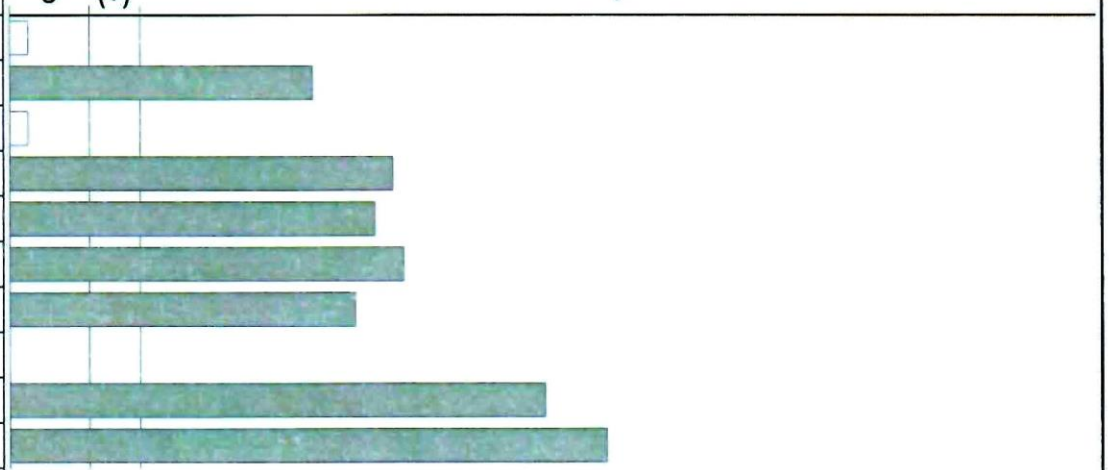
Patients IDs:	D	Elisa	**Test:**	Borrelia EUROLINE RN-AT adv IgM	
Name:	D	Elisa	**Strip number:**	369-87	
Date of birth:	13/01/2008		**Created on:**	26/08/2019	
Well:	9		**Results from:**	26/08/2019	
Sent from:	Dr Tatiana Rosca		**Receipt of sample:**	26/08/2019	

EUROIMMUN Medizinische Labordiagnostika AG

Automatic evaluation of test strips using the EUROLineScan software



Antigen	Intensity	Class
VlsE Borrelia burgdorferi	3	o
Flagellin Borrelia afzelii	42	+
BmpA Borrelia burgdorferi	3	o
OspC Borrelia afzelii	53	+
OspC Borrelia burgdorferi	51	+
OspC Borrelia garinii	55	+
OspC Borrelia spielmanii	48	+
Anti-human-IgG		
Anti-human-IgM	74	+
Control	83	+
Label		



Class	Explanation
o	Negative
(+)	Borderline
+	Positive

Test	Result
Borrelia EUROLINE RN-AT adv IgM	Positive

SPIROCHETALES

BACTERIA domain
SPIROCHAETALES phylum, *class, *ordo

***SPIROCHAETACEAE familia**

Borrelia genus

B.recurrentis

B.myamotoi complex

B.burgdorferi sensu lato

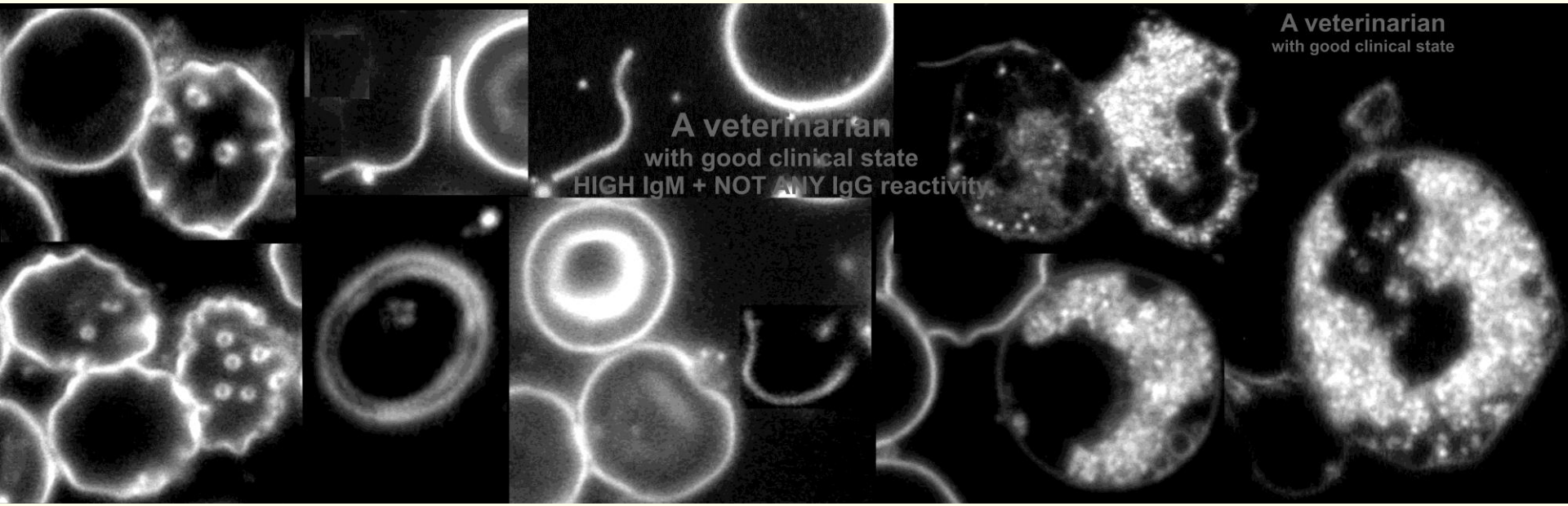
***TREPONEMATACEAE familia**

Treponema genus

T.pallidum

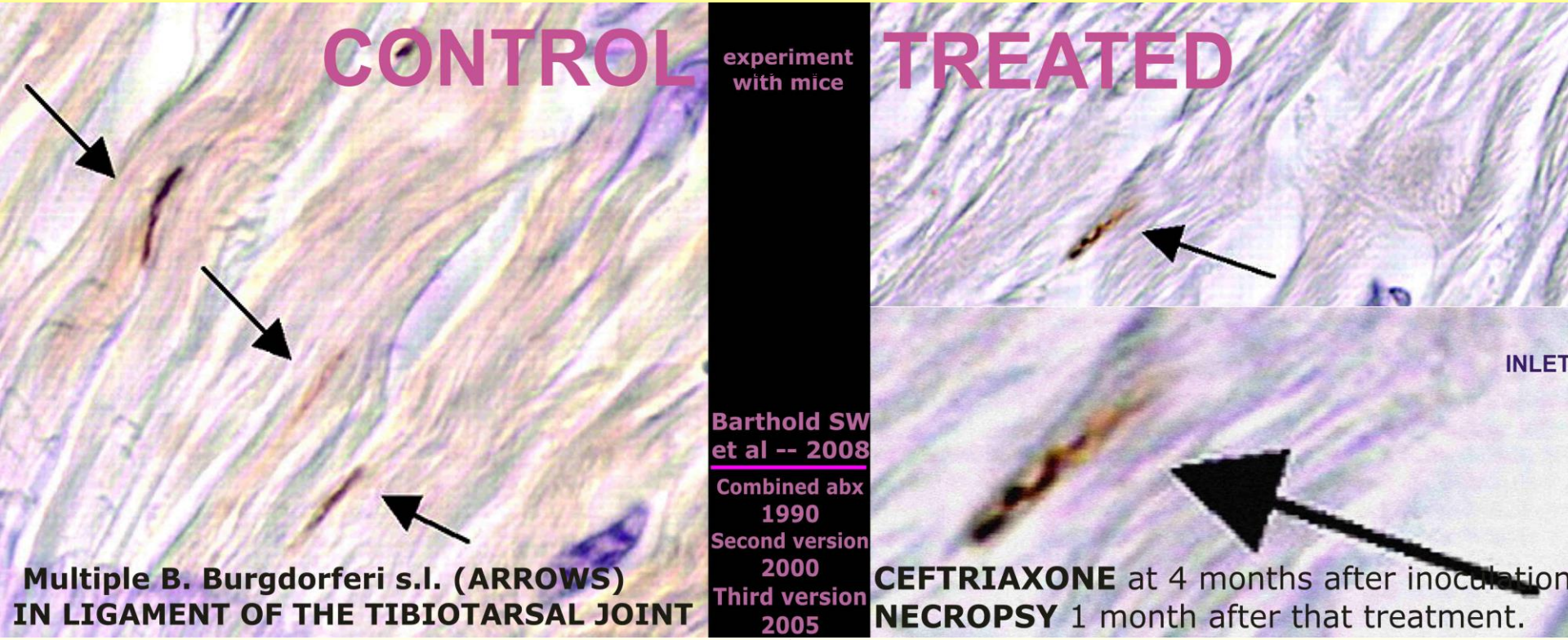
Leptospira genus

L.icterohaeorrhagiae



p100/83–*B.afzelii*		–	–	4	±	4	±	4	8
VlsE		–	–	3	–	3	–	3	4
p41–*Borrelia burgdorferi sensu stricto .*		–	–	1	±	1	–	1	1
BmpA/p39–*B.afzelii*		–	–	–	–	–	–	3	8
OspA–*B.afzelii*		–	–	–	–	–	–	4	4
OspC	*B.garinii-1*	–	–	+	–	+	–		
	Borrelia burgdorferi sensu stricto+B.afzelii	–	–	2 –	–	–	2 –	–	–	8 6
	B.garinii-2	–	–	–	–	–	–		
p41–*B.garinii*		–	–	3	–	3	–	3	1
p41–*B.afzelii*		–	–	1	–	1	–	1	1
P18–*B.afzelii*		–	–	–	–	–	–	4	8

Borrelia burgdorferi sensu lato facultative intracellularis parazita



Similar results were got with **TIGECYCLINE monotherapy.**

Lyme Borreliosis Foundation suggests **COMBINED ANTIBIOTIC TREATMENT since 1990** instead of the monotherapy debated.

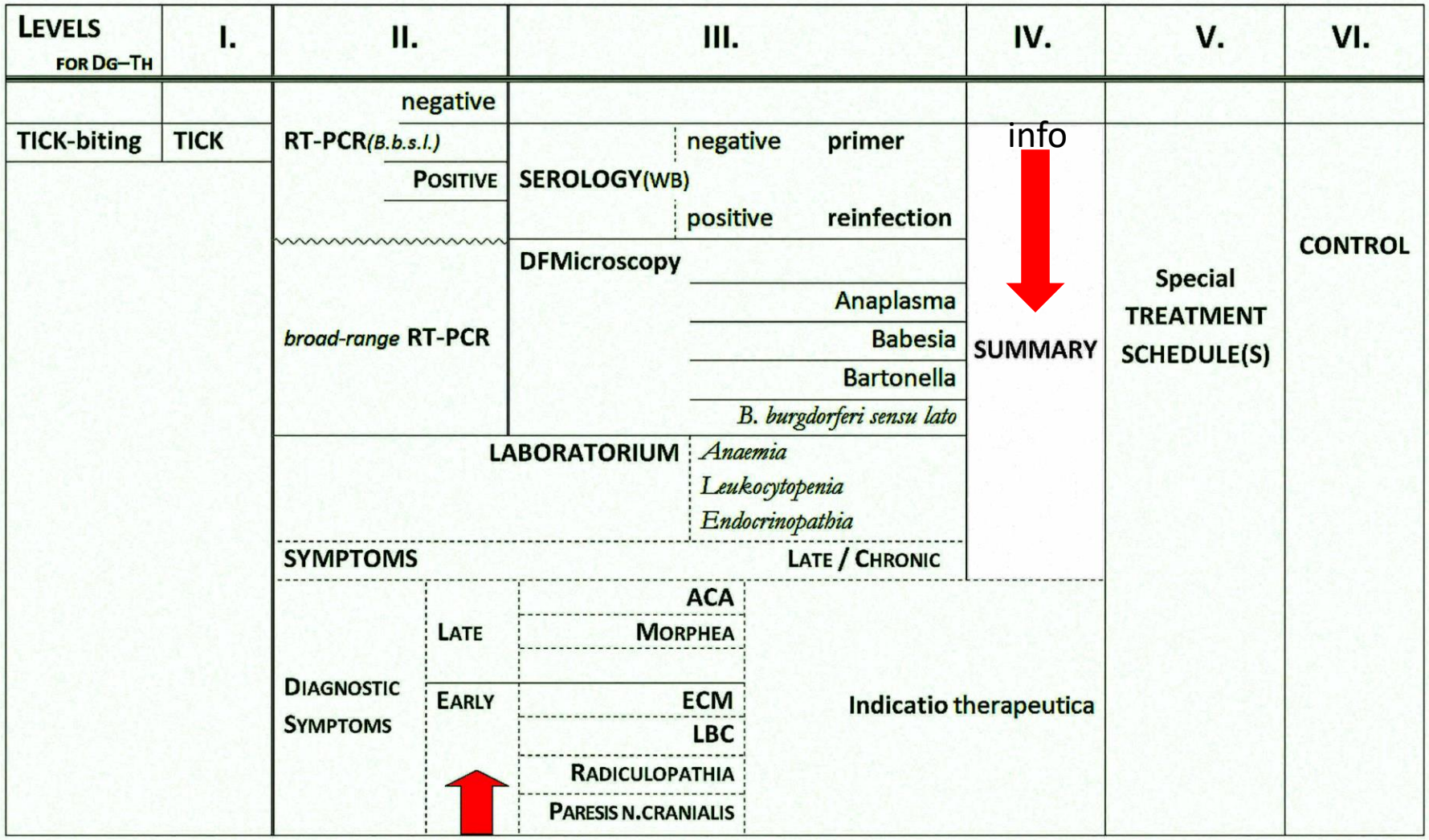
THE UNDERSTANDING HAS CHANGED about Lyme borrelisosis

- Lyme Spirochete became Borrelia burgdorferi sensu lato those have **great adaptation** character
- They are **sensitive** to several antibiotics, but in different manner
- These germs can hide **intracellular** and can be **wrapped** itself into unit membrane, or
- They can transform into **GEMMA** or produce colony in „cysts”
- They can specifically **inhibit the immune response** of the body from the beginning in different levels.

COMBINED ANTIBIOTIC THERAPY BASED ON THE EXPERIMENTS IN VITRO

- *MINOCYCLINE(4-5x50mg)+CIPROFLOXACINE+TINIDAZOLE*
- DOXYCYCLINE*(3x100mg – 2x200mg)*+
- AMOXICILLIN*(3x2000mg)*+
- CEFTRIAXON*(2x2000mg)*+
- CEFTRIAXON+BICILLIN+
- CEFTRIAXON/AMOXICILLIN+
- KLARITHROMYCINE+BISEPTOL+CIPROFLOXACIN+PLAQUENYL
- AZITHROMYCINE+BISEPTOL+MALARONE+PLAQUENIL
- AZITHROMYCINE+CIPROFLOXACINE+TINIDAZOLE
- DOXYCYCLINE+CIPROFLOXACINE+RIFAMPIN+TINIDAZOLE

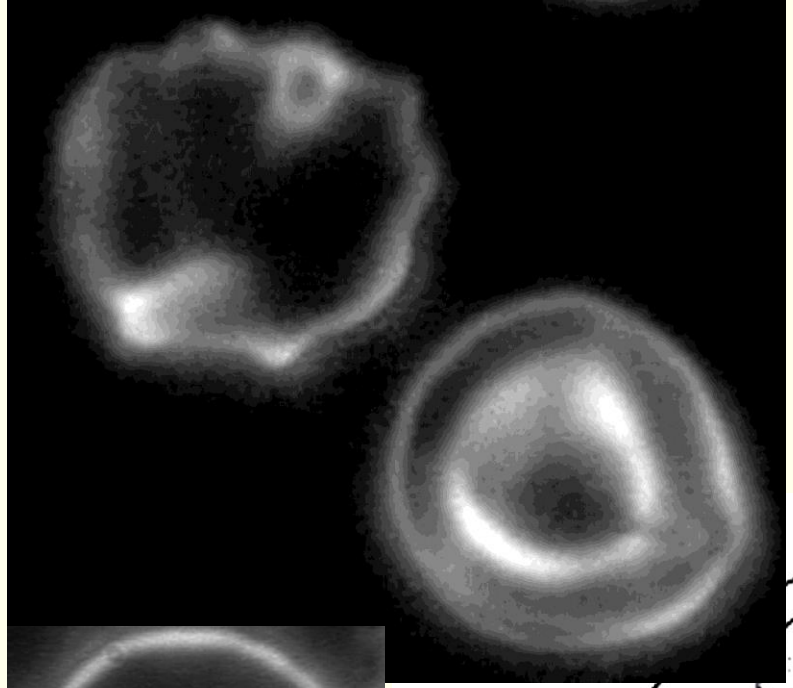
The suggested Flow-chart

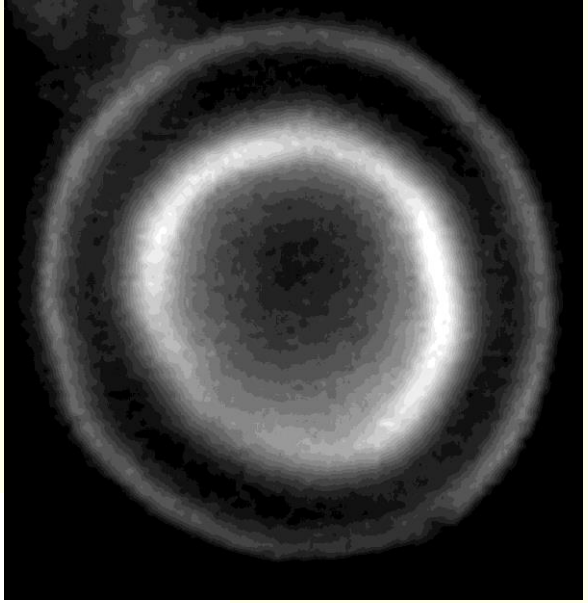


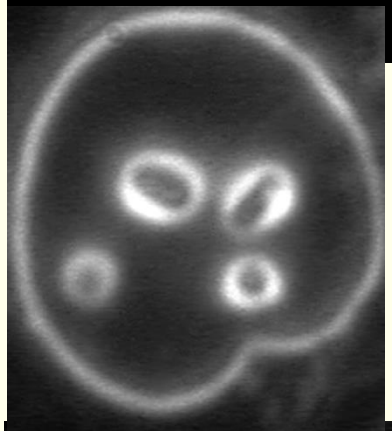
Babesiosis

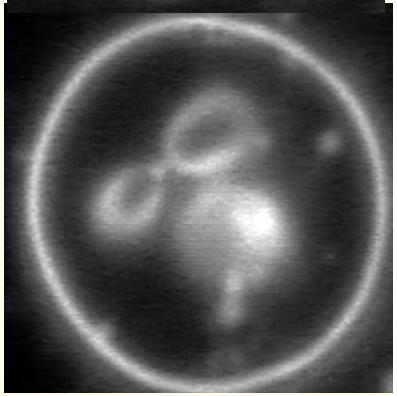
- Intraerythrocyter parasite
- Several strains exist
  - **There are over 100 species of *Babesia* identified**
  - B.microti, USA
  - B.divergens, EU
- Symptoms are different
  - Cephalalgia, neuropathia
  - hyperhidrosis nocturnalis
  - Cardiomyopathia, dyspnoe
  - Anaemia in chronic − anuria, death in acute form
- Transfusion associated Babesiosis rise reported

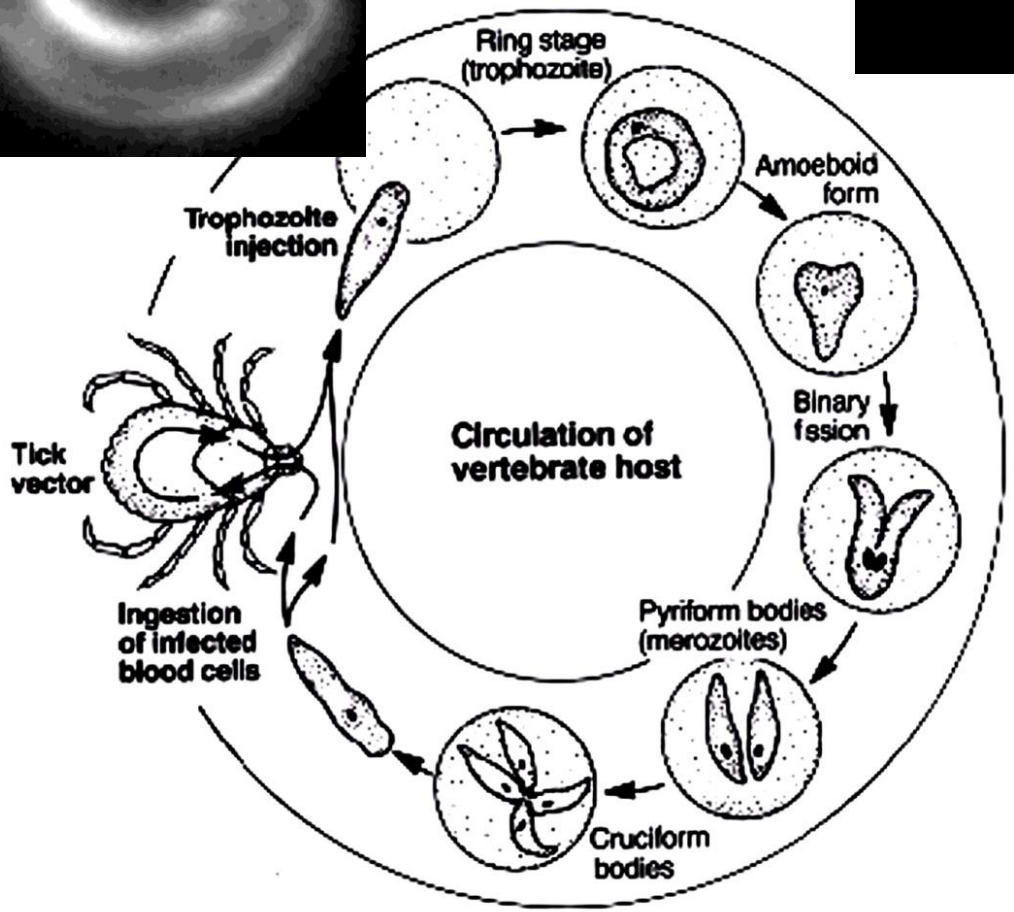
Babesia

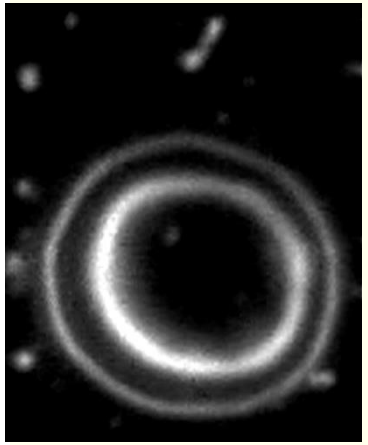


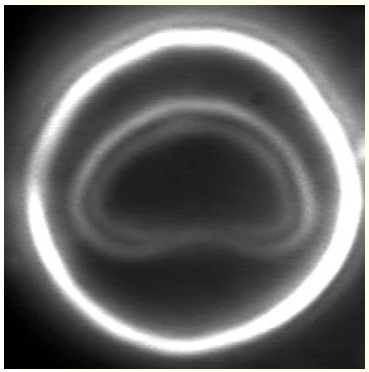






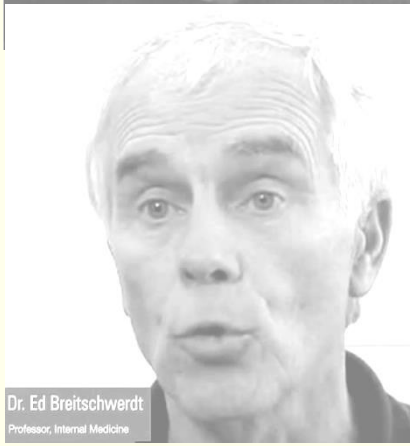

Ring stage
(trophozoite)
Amoeboid
form
Trophozoite
injection
Binary
fission
Tick
vector
Circulation of
vertebrate host
Ingestion
of infected
blood cells
Pyriform bodies
(merozoites)
Cruciform
bodies

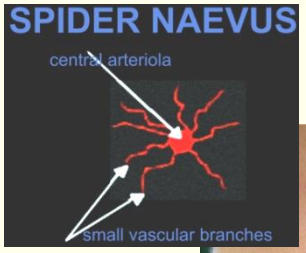




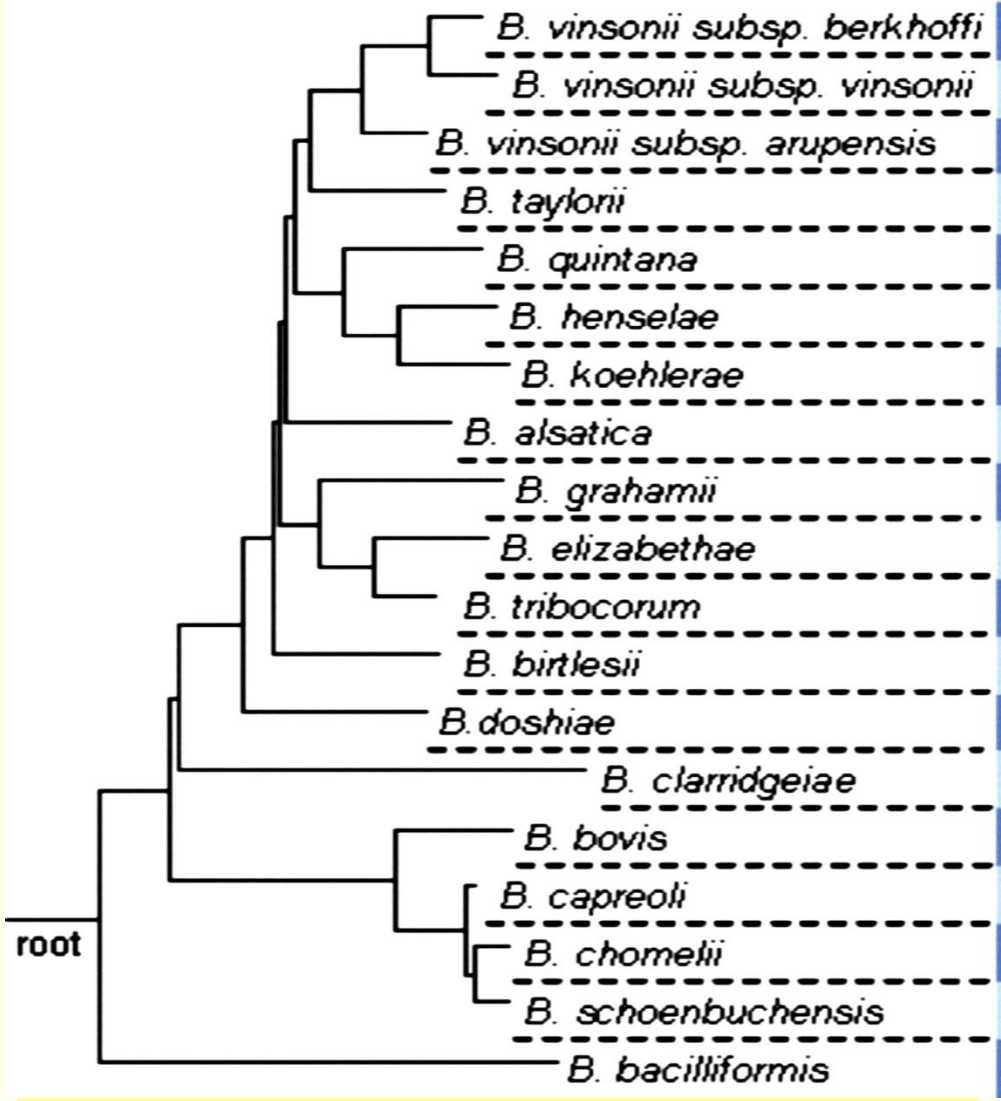
Bartonellosis

- Intraerythrocyter, hemotropic parasite
- Several strains, two(?) human pathogen
  - B.henselae
  - B.quintana
- Symptoms are different
  - **Dermatitis CSD, dyspigmentatio**
  - Angiopathia, **vasoproliferatio**
  - **Lymphadenopathia, lymphadenitis**
  - Cardiomyopathia, Endocarditis, dyspnoe
  - Cephalalgia, neuropathia….
- Transfusion assiciated possible





Phylogenetic tree of the genus Bartonella



	Arthropod host(s)	Mammalian host(s)	
		Reservoir	Incidental
B. vinsonii subsp. berkhoffi	Tick	Dog	Human
B. vinsonii subsp. vinsonii	Vole ear mite	Vole	
B. vinsonii subsp. arupensis	Tick	Mouse	Human
B. taylorii	?	Mouse, vole	
B. quintana	Body louse	Human	
B. henselae	Cat flea, tick	Cat	Human
B. koehlerae	?	Cat	Human
B. alsatica	?	Rabbit	Human
B. grahamii	Fleas	Mouse, vole	Human
B. elizabethae	?	Rat	Human
B. tribocorum	?	Rat	
B. birtlesii	?	Mouse	
B. doshiae	?	Vole	
B. clarridgeiae	?	Cat	
B. bovis	?	Cattle	
B. capreoli	?	Roe deer	
B. chomelii	?	Cattle	
B. schoenbuchensis	Deer ked	Roe deer	
B. bacilliformis	Sandfly	Human	

Dehio C: Cell. Microbiol. **(2008)** 10:1591–1598

ANAPLASMA/EHRLICHIA

- Intracellular/Intraleukocyter parasite
- Several strains, two(?) human pathogen
  - **Anaplasma phagocytophylum**
  - **A.monocytotica/E.chaffeensis**
- Symptoms are different
  - cephalalgia
  - hyperhidrosis nocturnalis
  - Cardiomyopathia, dyspnoe
  - Neuropathia–neutropenia relative %
  - Reccurrent, care especially during treatments
- Transfusion associated rise reported

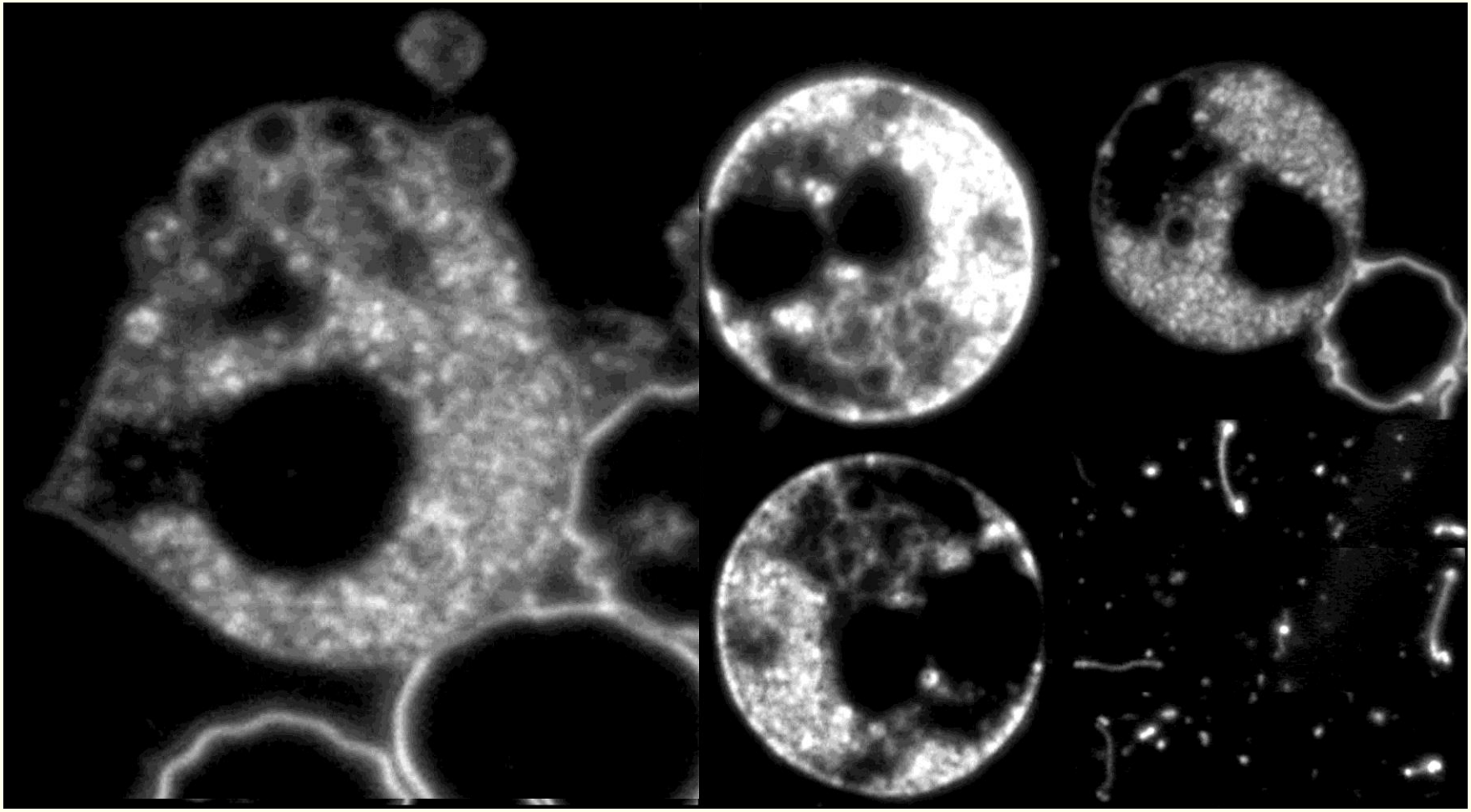
Well-known human pathogens in the family Anaplasmataceae

Species	Disease	Host	Host cells	Vector	Distribution
Ehrlichia chaffeensis	Human monocytic ehrlichiosis	Humans, deer and dogs	Monocytes and macrophages	*Amblyomma americanum*	USA, South America and Asia
Ehrlichia ewingii	Human ewingii ehrlichiosis	Humans, deer and dogs	Granulocytes	*Amblyomma americanum*	USA
Anaplasma phagocytophilum	Human granulocytic anaplasmosis and tick-borne fever	Humans, horses, ruminants, rodents, dogs, cats and deer	Granulocytes and endothelial cells	*Ixodes scapularis*, *Ixodes pacificus* and *Ixodes ricinus*	USA, Europe and Asia
Neorickettsia sennetsu	Sennetsu fever and glandular fever	Humans	Monocytes and macrophages	Unknown trematode	Japan and Southeast Asia

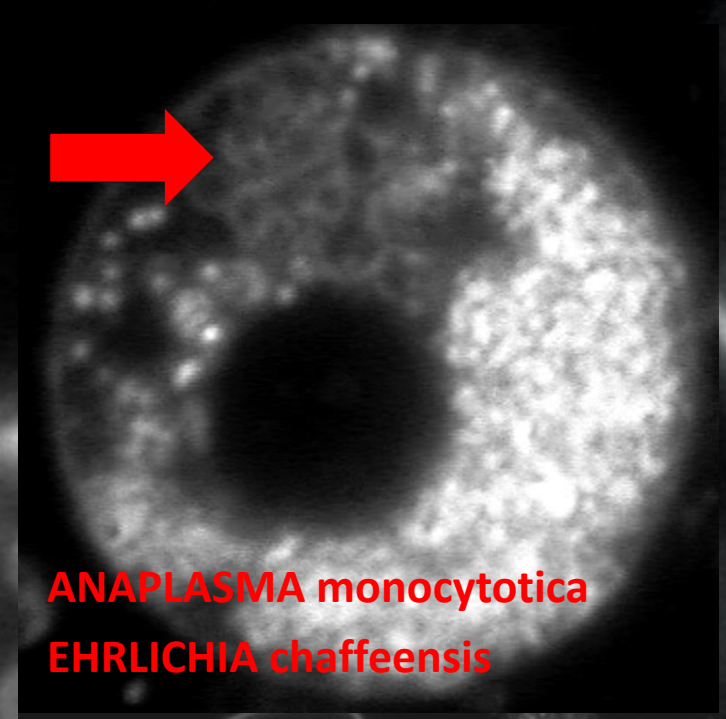
	NORMÁL	2011.05.	2011.03.	2010.12.	2010.11.
WBC	4,00 -10,00	2,47	2,81	3,27	2,85
granulocyta	1,80 - 7,00	1,20	1,39	1,35	1,33
lymphocyta	1,00 - 4,80	0,92	1,23	1,69	1,22
RBC	3,80 - 5,60	3,29	3,02	3,36	3,45
mcv	80,00 - 97,00	102,40	99,30	98,50	102,00
mch	26,00 - 34,00	35,00	35,40	34,80	35,10

Orsi, Gyopár

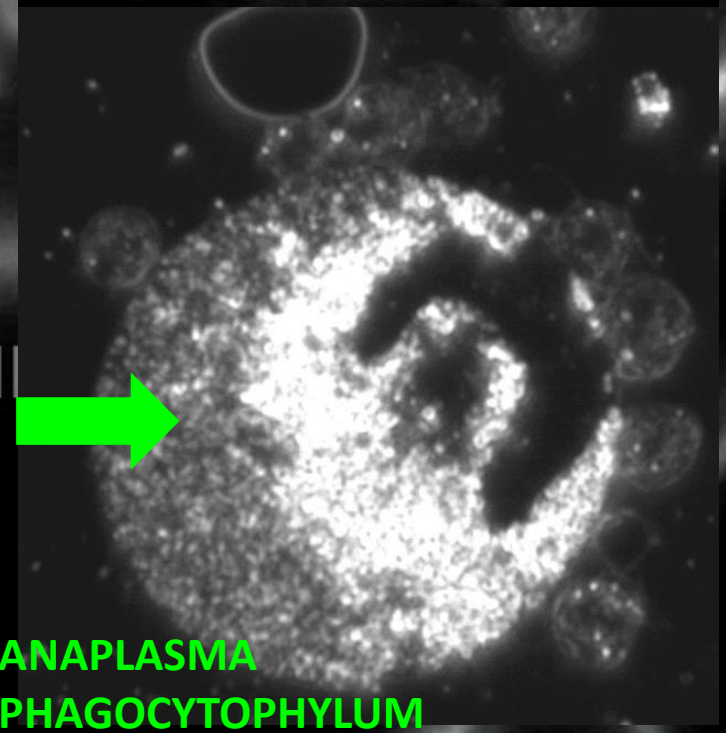
Anaplasma Human Blood-sample



Anaplasma in WBC



ANAPLASMA monocytotica
EHRLICHIA chaffeensis



ANAPLASMA
PHAGOCYTOPHYLUM

Ehrlichien Elispot LTT + **7 SI <2**
Mittels T-Cell-Test finden sich aktuell in vivo Ehrlichien-reaktive T-Lymphozyten.

PLEASE keep the HOPE

Nil nocere
Salus aegroti suprema lex, esto
Nil admirari


Justino Magalona