Minimum performance requirements in autoimmunity proficiency testing: lessons learned from abroad

experience from ANA proficiency testing in the german INSTAND e.V. External Quality Assessment program (EQA) 2001 - 2013

Martin Blüthner, Karlsruhe, Germany

Stichting Kwaliteitsbewaking
Medische Laboratoriumdiagnostiek

Hoe goed moet het?

SKML congres
De ReeHorst Ede, 6 juni 2017

Disclosure

- The speaker has no financial relationship with any IVD industry

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Affiliation

LIMBACH GRUPPE

MVZ Laboratory PD Dr. Volkmann & Colleagues, Karlsruhe, Germany

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INSTAND e.V.
Gesellschaft zur Förderung der Qualitätssicherung in medizinischen Laboratorien e.V.

INSTAND e.V. (Society for the advancement of quality assurance in medical laboratories), Düsseldorf, Germany

- Introduction:

  History and range of the INSTAND autoantibody QA programme
- Background:

  the german healthcare system
- Procedure:

  realization of the proficieny tests
- Problems:

  major obstacles

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INSTAND e.V.

"INSTAND e.V. is one of three reference institutions appointed by the German Medical Association. It is responsible for the organisation of the EQA for proficiency testing in medical laboratories."

www.instand-ev.de

- INSTAND provides the framework
- appoints specialists as counselors

History

INSTAND e.V.
Gesellschaft zur Förderung der Qualitätssicherung in medizinischen Laboratorien e.V.

The INSTAND QA program for ANA proficiency testing started as an inter-laboratory comparision with both ANA-IFT and non-IFT screens (+ dsDNA antibody testing) designed and organized by Hans-Peter Seelig (assisted by Hans Ehrfeld) via INSTAND e.V. (since 1994 WHO collaborating Centre for Quality Assurance and Standardization in Laboratory Medicine)

In its current form:

2001 – 2008	Hans-Peter Seelig
2008 – 2012	Hans-Peter Seelig Martin Blüthner
2013 – today	Martin Blüthner (Hans-Peter Seelig)

Autoantibody proficieny testing by INSTAND e.V.

Ctlg.# ***Programme***

- 251 Autoimmune Diseases 01 – Connective Tissue Diseases (ANA, DNA)
- 253 Autoimmune Diseases 02 – Hepatic Syndromes (AMA, SMA, PCA)
- 255 Autoimmune Diseases 03 – Connective Tissue Diseases (ENA)
- 257 Autoimmune Diseases 04 – Vasculitis/Glomerulopathy (ANCA, GBM)
- 259 Autoimmune Diseases 05 – Autoimmune Dermatitis (Pemphigus/-oid)
- 261 Autoimmune Diseases 06 – Diabetes mellitus (type I)
- 263 Autoimmune Diseases 07 – Autoimmune Endocrinopathies (ACA)
- 265 Autoimmune Diseases 08 – Paraneoplastic Neuropathies (ONA)
- 267 Autoimmune Diseases 09 – Peripheral Neuropathies (MAG, Gangl.)
- 269 Autoimmune Diseases 10 – Myasthenia Gravis (ACR)
- 271 Autoimmune Diseases 11 – Glutensensitive Enteropathies (EMA, TG)
- 273 Autoimmune Diseases 12 – Rheumatoid Arthritis (CCP, RF)
- 275 Autoimmune Diseases 13 – Antiphospholipid Syndrome (ACA, B2GP)

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RiliBÄK revised version from 2008

Guideline of the German Medical Association

- Instrument to control the quality of medical diagnostic procedures
- Appendices: lists of parameters, that MUST be controlled by external proficiency testing
- Some Autoantibodies are listed in appendix B2: qualitative external proficiency testing (ANA, dsDNA, ANCA, rheumatoid factor, tissue transglutaminase)

No valid certificate for >1yr: no reimbursement

Principle of German Healthcare System

- Any medical treatment or diagnosis is listed with a unique number/identifier in two catalogues
- Costs for treatment/diagnostic procedures are reimbursed by health insurance companies (public/national or private)
- Prices are fixed according to catalogues (EBM/GOÄ)

Healthcare costs

Reimbursement:

Public/national health insurance companies

- EBM (standard tariff rate)

Private health insurance companies

- GOÄ (tariff rate of the medical associations)

Reimbursement according to EBM

ANA, DNA

- #32490 qualitative and/or quantitative analysis of antibodies against self-antigens ("autoantibodies") by means of indirect immunofluorescence, immunoassay, or immunoblot

Reimbursement according to GOÄ

ANA

- #3813: Analysis by means of indirect immunofluorescence or a comparable method
- #3854: Analysis with a similar method

DNA antibodies

- #3857: Analysis of subforms of antinuclear antibodies by ligand assay, westernblot or a comparable method
- #3864: Analysis with a similar method

Conclusion

Clinical laboratories are free to choose whatever testsystem they see fit for ANA (or anti-DNA) screening !!!

This has to be considered by a QA programme

Prerequisites

- The QA programme organizers assume the role of a requesting physician
- In routine screening for a suspected disease, usually tests for given parameters are requested but NOT for specific methods
- Results for a given parameter thus have to be equaly valid irrespective of the method used

Procedure ANA proficiency testing

- Schedule: twice per year (spring and fall)
- Sample number: two independent samples (300µl) with different specificities are sent out to the participants
- Sample source: external company as sub-contractor
- Sample composition: serum or plasma, single donation or pooled samples from a single donor or from multiple donors (depending on availability), maybe diluted
- Sample selection: target antigen(s), titer, diagnostic relevance (plausibillity check)
- Reference labs for cross-check
- Dispatch: samples are dispatched at ambient temperature
- Time for analysis: 2 weeks

Sample selection

Parameter	result sample 21	result sample 22	Ref.val.	units	Method
ANA	1:2560, nucl. speckled	1:1280 nucl. speckled	1:<80	Titer	IFT
centromere	1:<80	1:<80	1:<80	Titer	IFT
dsDNA (Farr)	<2,5	<2,50	<7	U/ml	RIA (FARR)
dsDNA (C.luc.)	1:<10	1:<10	1:<10	Titer	CLIFT
ssDNA	7,08	30,09	<20	RE/ml	Elisa
histones	<1,00	<1,00	<20	RE/ml	Elisa
DFS70	negativ	negativ	negativ		Elisa
U1-70k (nRNP)	0,30	0,40	<7<10	U/ml	FEIA
CENPB	0,50	0,60	<7<10	U/ml	FEIA
SMD	0,90	1,00	<7<10	U/ml	FEIA
SS-A/Ro52	301,00	0,70	<7<10	U/ml	FEIA
SS-A/Ro60	>282,00	>282,00	<7<10	U/ml	FEIA
SS-B/La	>320,00	>320,00	<7<10	U/ml	FEIA
Scl 70	0,60	<0,40	<7<10	U/ml	FEIA
nucleosomes	<1,00	<1,00	<20	RE/ml	Elisa
PM/Scl	0,80	1,40	<7<10	U/ml	FEIA
fibrillarin	0,70	1,00	<7<10	U/ml	FEIA
SP100	negativ	negativ	negativ		IB
PCNA	7,69	9,45	<10<15	AK-Ratio	RIA (TnT)
JO1	0,40	0,50	<7<10	U/ml	FEIA

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result submission

Analyte	Method	Results sample A				Results sample B				Reference range
		U/titer	FM-1	FM-2	Val.	U/titer	FM-1	FM-2	Val.	
ANA (IgG, -A, -M)*	IIFT									
ANA (IgG)**	IIFT									
ANA***										
anti-ds-DNA										

Method:

01 = indirect immunfluorescence test (IFT)
02 = radioimmunoassay (RIA)
03 = enzyme linked immuno sorbent assay (Elisa)
08 = westernblot/dot-blot (WB)
99 = other methods

Membranous nuclear patterns
a1 Smooth membranous
a2 Punctate membranous

Nucleoplasmic patterns
b1 Homogeneous/positive nucleoli
b2 Homogeneous/negative nucleoli
b3 Large speckled (nuclear matrix)
b4 Coarse speckled
b5 Fine speckled
b6 Grainy Scl-70-like
b7 Pleomorphic speckled (PCNA)
b8 Centromere
b9 Multiple nuclear dots (NSp-I)
b10 Coiled body

Nucleolar patterns
c1 Homogeneous nucleolar
c2 Clumpy nucleolar
c3 Punctate nucleolar

Spindle apparatus patterns
d1 Centriole
d2 Spindle pole (NuMa)
d3 Spindle fibre
d4 Midbody (MSA-2)
d5 CENP-F (NSp-II, MSA-3)

Cytoplasmic fluorescence patterns
e1 Fine speckled cytoplasmic
e2 Diffuse cytoplasmic
e3 Mitochondrial-like
e4 Lysosomal-like
e5 Golgi-like
e6 Actin-like
e7 Vimentin-like

f1 **Negative**
g1 **Unknown**

Certification (both samples correct):

IFT titer:
Mean titer ±3 endpoint titers (pattern irrelevant)
or
semi-quantitative evaluation
Mean values 0-4 (± 1-2 steps, depending on SD) with
0=neg 1=bl 2-4=pos +-+++

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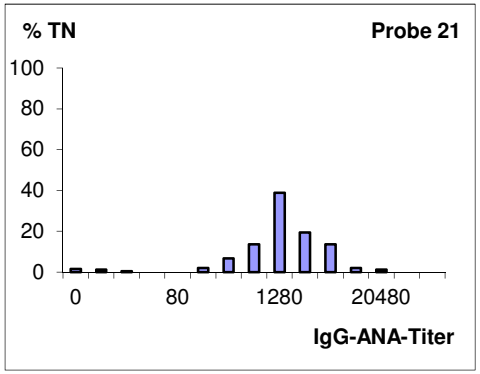
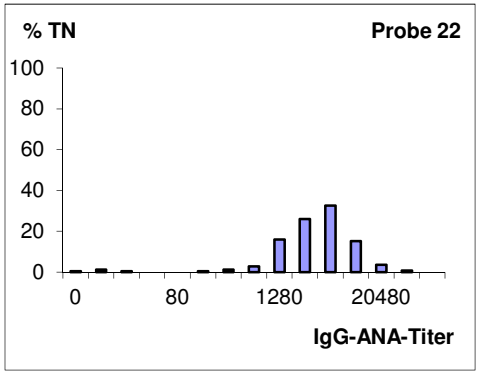
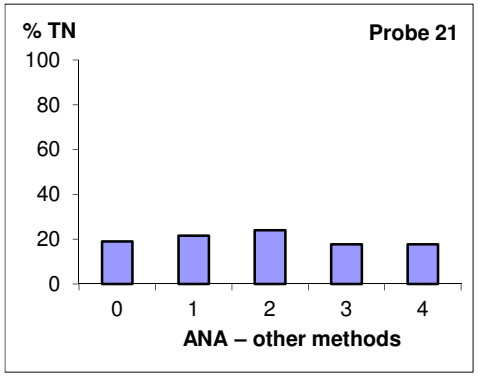
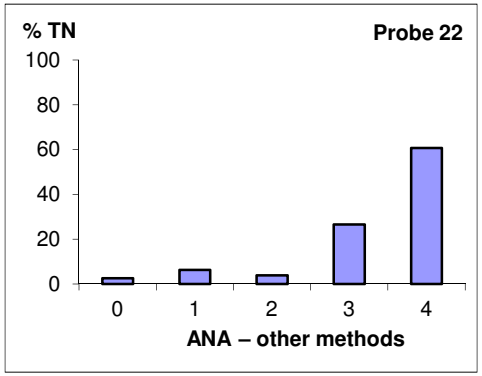
Example results I









Example results II

sample 21

The serum sample originated from a patient of unknown gender with collagenosis. Indirect immunofluorescence revealed a speckled pattern due to antibodies against S-A/Ro-52, SS-A/Ro-60, and SS-B/La in the plasma. The antibody specificity could be detected correctly by almost all participating laboratories both with pattern and titer. Also, when using other methods, hardly any problems were seen.

The serum did not contain antibodies against double stranded DNA, or against DFS70 which is in agreement with the results of the reference laboratories and most participating laboratories.

sample 22

The serum originated from a 56-yr female patient with mixed connective tissue disease. An indirect immunofluorescence assay revealed a speckled nuclear staining pattern, presumably due to antibodies against Ro/SS-A (Ro-60) and La/SS-B. The antibodies could be detected correctly by almost all participating laboratories both with pattern and titer. When using other methods, hardly any problems were seen.

The serum contained no antibodies against double stranded DNA, but contained antibodies against single stranded DNA. This caused considerable problems when reporting dsDNA antibodies. These discrepancies were also reported by the reference labs. Therefore, all results were accepted as correct.

No antibodies against DFS70 were detectable.

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Example results III

Resultate und Beurteilung

Teilnehmer [redacted]

Probe 51			Ihre Resultate			
Testparameter	**Methode**	**Ref.Bereich**	**Titer 1**	**BK 1**	**BQ 1**	**Bewertung**
ANA IIFT Flum	275	>=320 <=40960	10240			richtig
ANA quantitativ						
ANA qualitativ						
anti-DSF70 qualitativ	9999	=0			0	richtig
anti-ds-DNA IIFT						
anti-ds-DNA quantitativ	66	>=0 <=4		0		richtig
anti-ds-DNA qualitativ						

Probe 52			Ihre Resultate			
Testparameter	**Methode**	**Ref.Bereich**	**Titer 2**	**BK 2**	**BQ 2**	**Bewertung**
ANA IIFT Flum	275	>=320 <=40960	10240			richtig
ANA quantitativ						
ANA qualitativ						
anti-DSF70 qualitativ	9999	=0			0	richtig
anti-ds-DNA IIFT						
anti-ds-DNA quantitativ	66	=0		0		richtig
anti-ds-DNA qualitativ						

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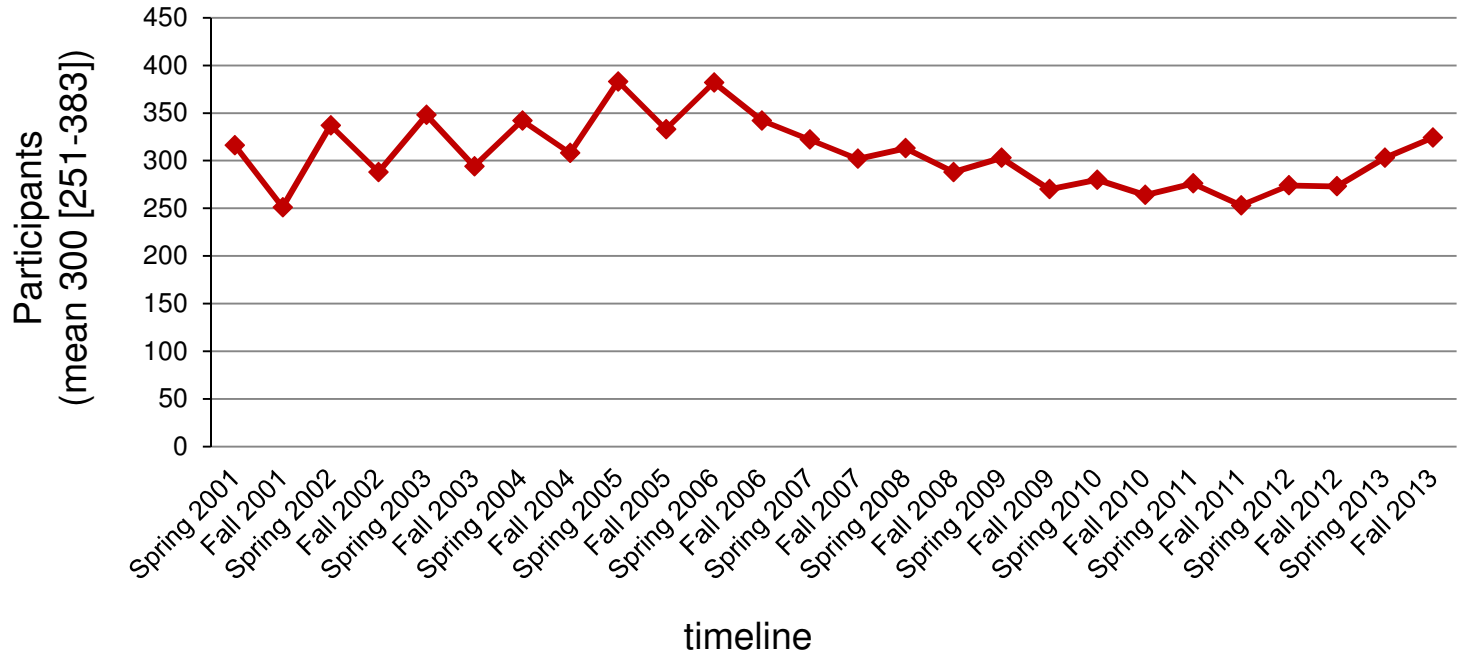
Participating laboratories 2001 - 2013

- Number of participating labs varies between ~250 – 380
- Mostly german labs, some from other european countries (! anonymous reports !)
- Some manufacturers



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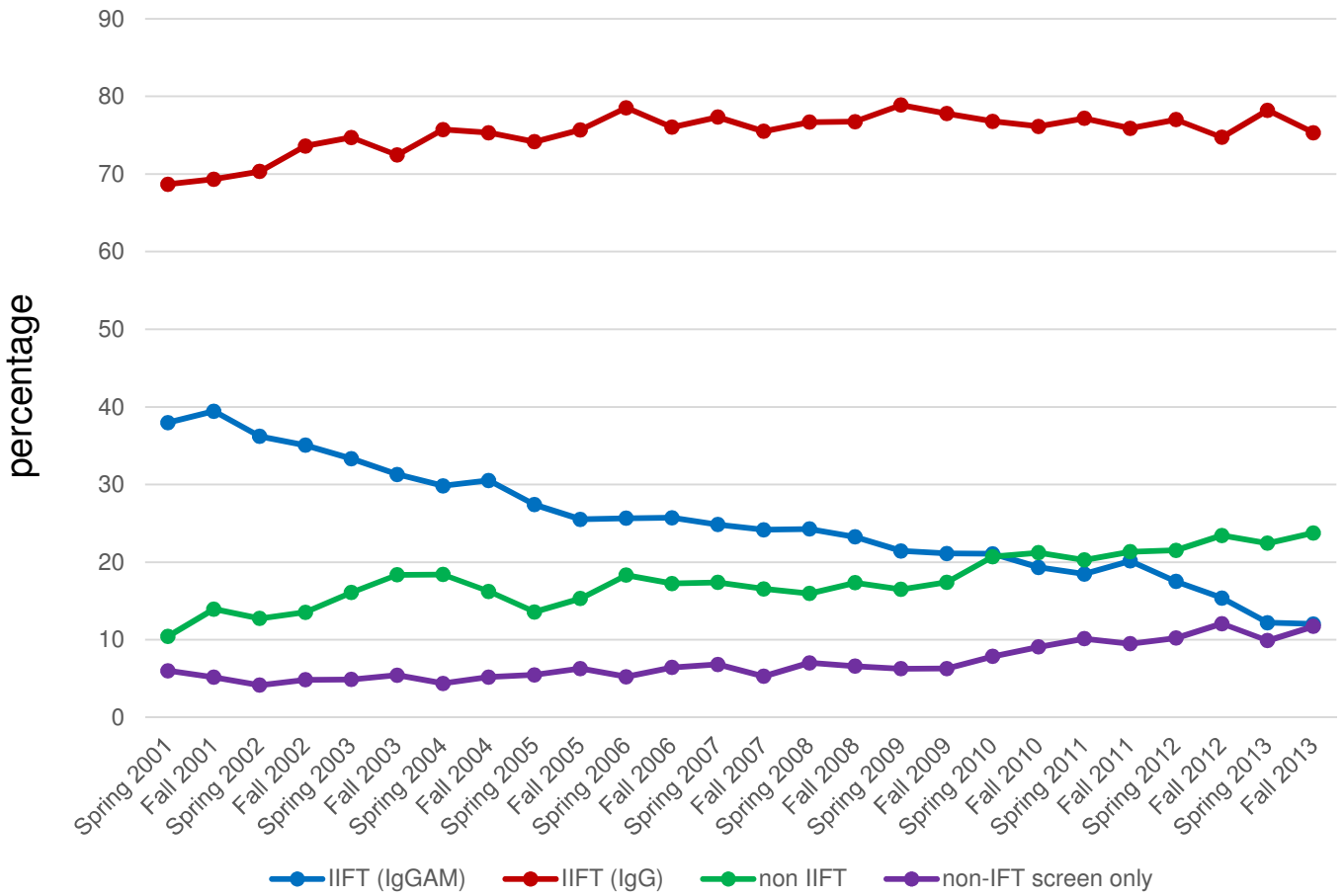
Method distribution (ANA-IFT / Non-IFT screens)



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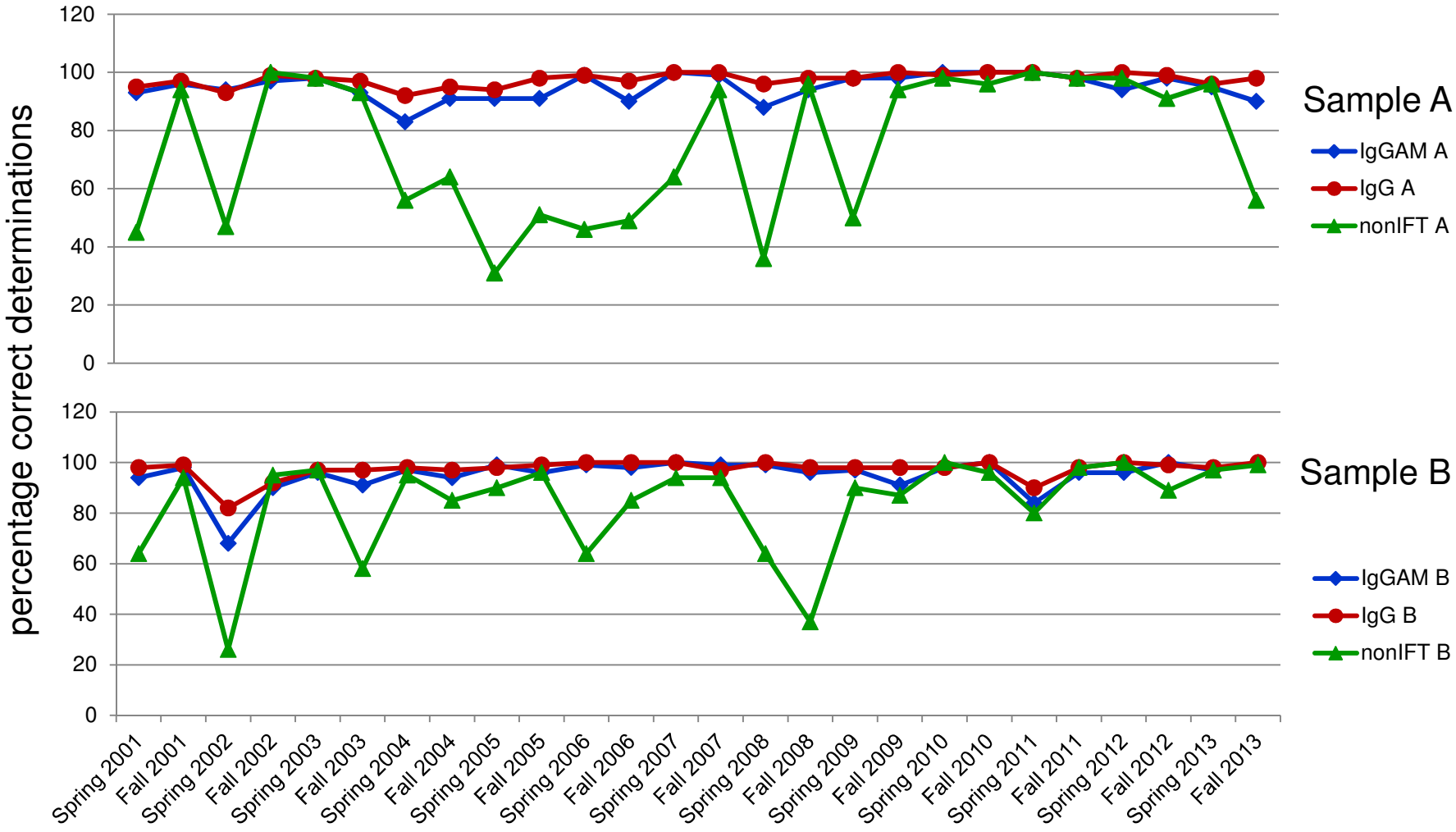
development of ANA-IFT vs. non-IFT screens

- use of IgG conjugate: increase (70% - 80%)
- use of IgG/A/M conjugate: decrease (~40% - 12%)
- non-IFT screens: increase (10% - 25%)
- **non-IFT screen as sole „ANA“-test: increase (5% - 12%) !!!**
- Success rate of non-IFT screens shows tremendous level of heterogeneity

note: some participants report results in several categories

Percentage correct determinations



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Influence of assay type on success rates

- No difference in success rates between use of IgG alone and combined IgG/A/M conjugates
- Tremendous variation in success rates when non-IFT screens are used

non-IFT screens: antigen composition

antigen / assay	dsDNA	Histones	nRNP/Sm	U1-70k	U1-A	U1-C	SS-A	Ro52	Ro60	SS-B	Scl-70	Sm	RibP	Jo-1	CENP-A	CENP-B	Ku	PM/Scl-100	PM/Scl-75	Fibrillarin	RNA-Pol.	Mi-2	Jo-1	PL7	PL12	SRP-54	PCNA	sp100,	gp2010	M2-PDC	Actin	nativ extract	nucleosomes
test A	■	■	■				■			■	■	■	■	■		■																	
test B																																**1)**	
test C			■	■				■	■	■	■	■				■							■				■						
test D	■	■	■					■	■	■	■	■	■			■	■	■	■			■	■							■			■
test E			■	■				■	■	■	■	■				■							■										
test F	**2)**	■	■	■	■	■		■	■	■		**3)**				■		■					■										■
test G	■			■	■	■		■	■	■	■	■	■	■		■		■		■	■	■	■				■						
test H	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■				■	■	■	■	■	■	■	■	■	■		■

1) spiked with recombinant antigens
2) dsDNA & ssDNA
3) SmB/B‘, SmD, SmE, SmF, SmG

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Highest scores

year	semester	sample	pattern	antigen(s)	% successfull IgG/A/M	IgG	non IFT
2011	fall	A	speckled	SS-A, SS-B	98	98	98
2011	fall	B	speckled	SS-A, SS-B	96	98	98
2010	spring	A	nuclear dots	sp100, CENP-B	100	99	98
2003	spring	A	centromeres FCS	-	98	98	98
2012	spring	A	centromeres	CENP-A, -B	94	100	98
2013	fall	B	centromeres	CENP-B	100	100	99
2003	fall	A	speckled	SS-A, SS-B	97	99	100
2011	spring	A	speckled	dsDNA, mono-nucleosomes	100	100	100
2010	spring	B	speckled	U_1-70k, -A, -C	98	98	100
2012	spring	B	speckled	SS-A	96	100	100

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Lowest scores

year	semester	sample	pattern	antigen(s)	% successfull IgG/A/M	IgG	non IFT
2002	spring	B	nucleolar	?	68	82	26
2005	spring	A	multiple nuclear dots	sp 100	91	94	31
2014	fall	B	multiple nuclear dots	sp 100	89	97	33
2008	spring	A	membraneous	gp 210	88	96	36
2008	fall	B	multiple nuclear dots	sp 100	96	98	37
2001	spring	A	centromeres	CENP-B	93	95	45
2006	spring	A	pleomorph nuclear	PCNA	99	99	46
2002	spring	A	homogeneous	? (no dsDNA abs)	94	93	47
2006	fall	A	multiple nuclear dots cytoplasm (AMA)	sp 100, M2 (PDC E2)	90	97	49
2009	spring	A	nucleolar	PM/Scl-100, -75	98	98	50

Summary ANA testing

- non-IFT screens are increasingly used in daily routine ANA-screening
- non-IFT screens do not reflect the full spectrum of detectable antibodies. They miss relevant antinuclear antibodies
- if the respective target antigens are represented in a non-IFT screening test, the results are comparable and equally sensitive to those obtained by ANA IFT

Current solution

Disclaimer added to report form sheet:

Important advice for quantitative and qualitative ANA tests:

Antibodies potentially present in the test probes may be directed against: Chromatin (DNA, histones, nucleosomes), SS-A/Ro, SS-B/La, Sm, U1nRNP, centromeres (CENP-B), topoisomerase (Scl-70), cyclin (PCNA), Mi-2, PM-Scl, RNA-polymerase, fibrillarin, NOR-90, proteins of the nuclear envelope (lamins, gp210), nuclear dots (Sp 100, coilin).

Using test kits, which do not contain the above mentioned antigens may possibly give rise to false negative results!

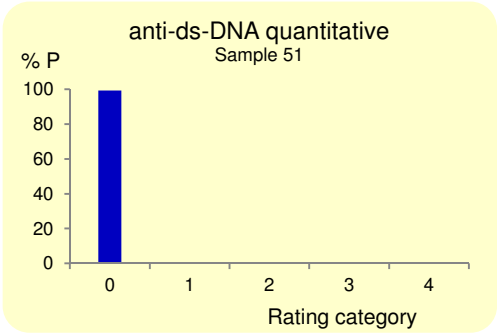
Summary recent changes

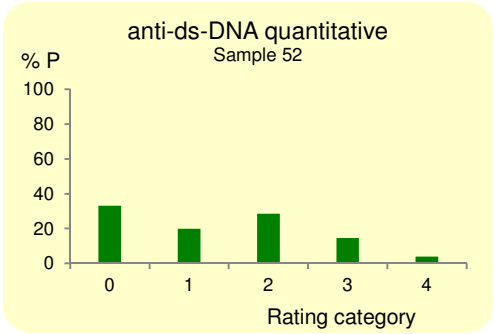
- Addition of disclaimer to report-sheet
- No longer discrimination between IgG and IgG/A/M
- Pattern description adjusted to recommendations by ICAP (International Consensus on Autoantibody Patterns)
- Inclusion of DFS

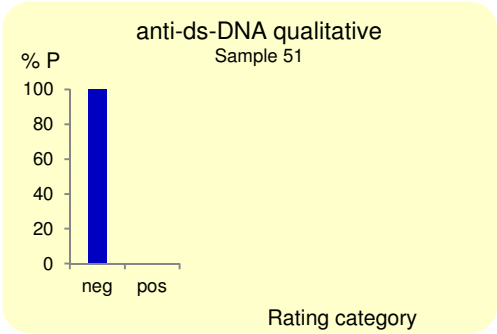
Agmon-Levin N et al. "International recommendations for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies". Ann Rheum Dis. 2014 Jan;73(1):17-23.

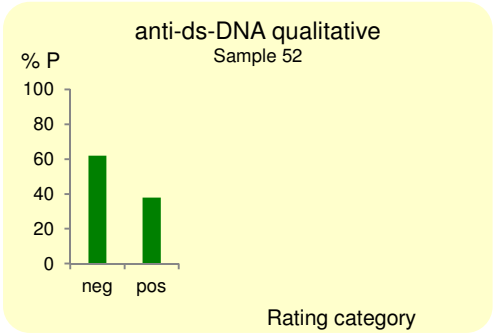
Chan EK et al. "Report of the First International Consensus on Standardized Nomenclature of Antinuclear Antibody HEp-2 Cell Patterns 2014–2015". Front Immunol. 2015; 6: 412.

DNA antibodies (spring 2017)









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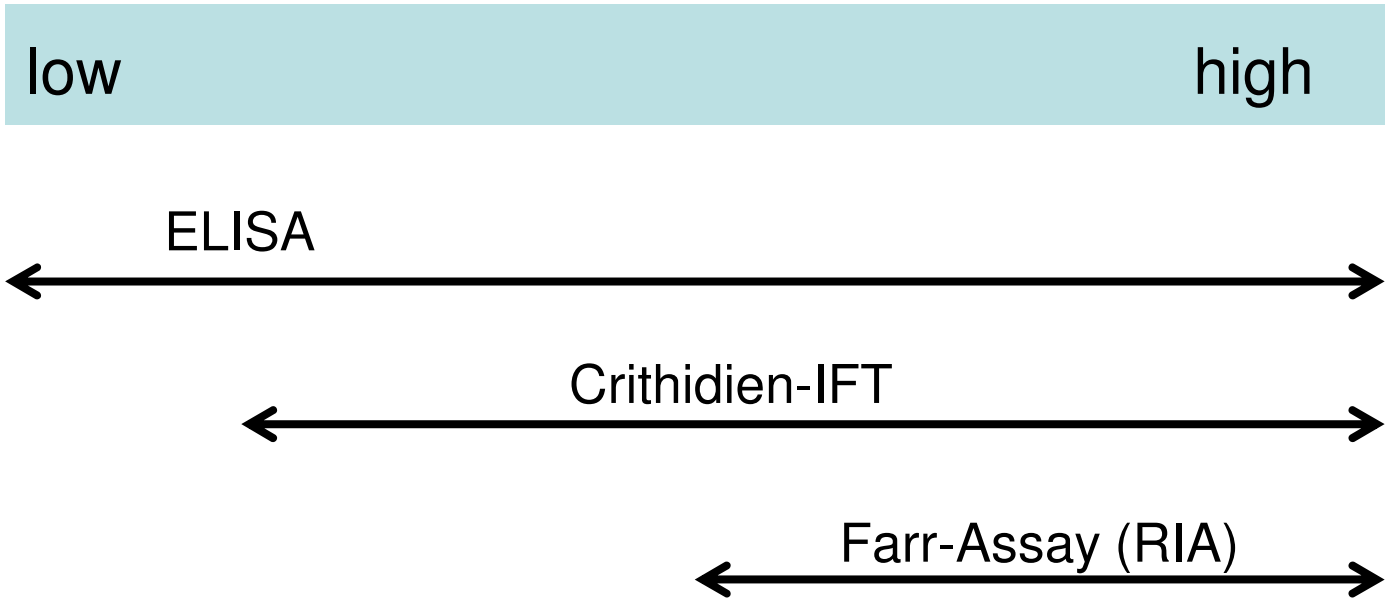
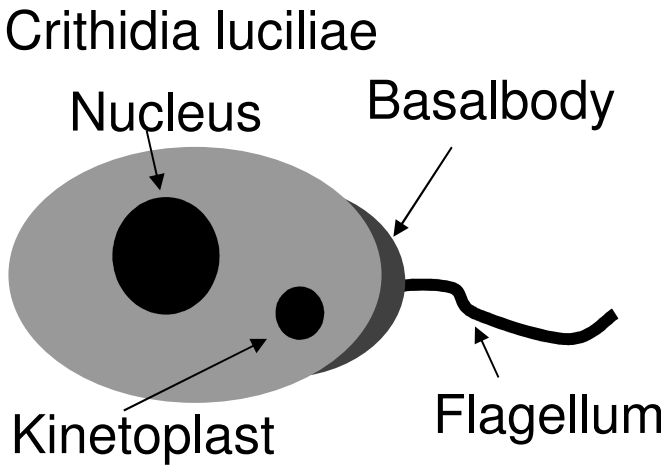
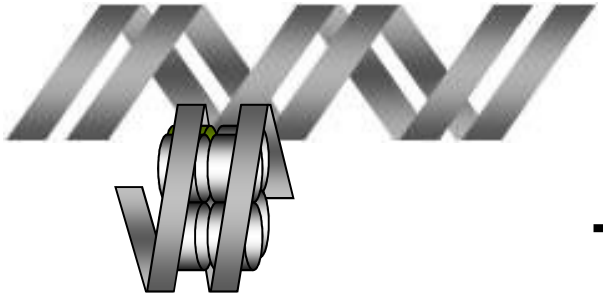
Anti dsDNA avidity



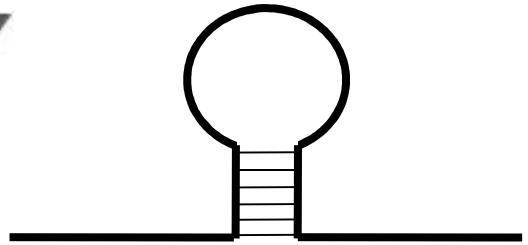
Hamann & Smeenk, in Shoenfeld at al. „Autoantibodies“, 2ed, 2007.

DNA sources





dsDNA (calf thymus)
w/o nucleosomes



ssDNA-Elisa

PCR-DNA (ALBIA)

Samples: single donors, single donations each

Parameter	result sample 21	result sample 22	Ref.val.	units	Method
ANA	1:2560, nucl. speckled.	1:1280 nucl. speckled.	1:<80	Titer	IFT
centromere	1:<80	1:<80	1:<80	Titer	IFT
dsDNA (Farr)	<2,5	<2,50	<7	U/ml	RIA (FARR)
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Scl 70	0,60	<0,40	<7<10	U/ml	FEIA
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fibrillarin	0,70	1,00	<7<10	U/ml	FEIA
SP100	negativ	negativ	negativ		IB
PCNA	7,69	9,45	<10<15	AK-Ratio	RIA (TnT)
JO1	0,40	0,50	<7<10	U/ml	FEIA

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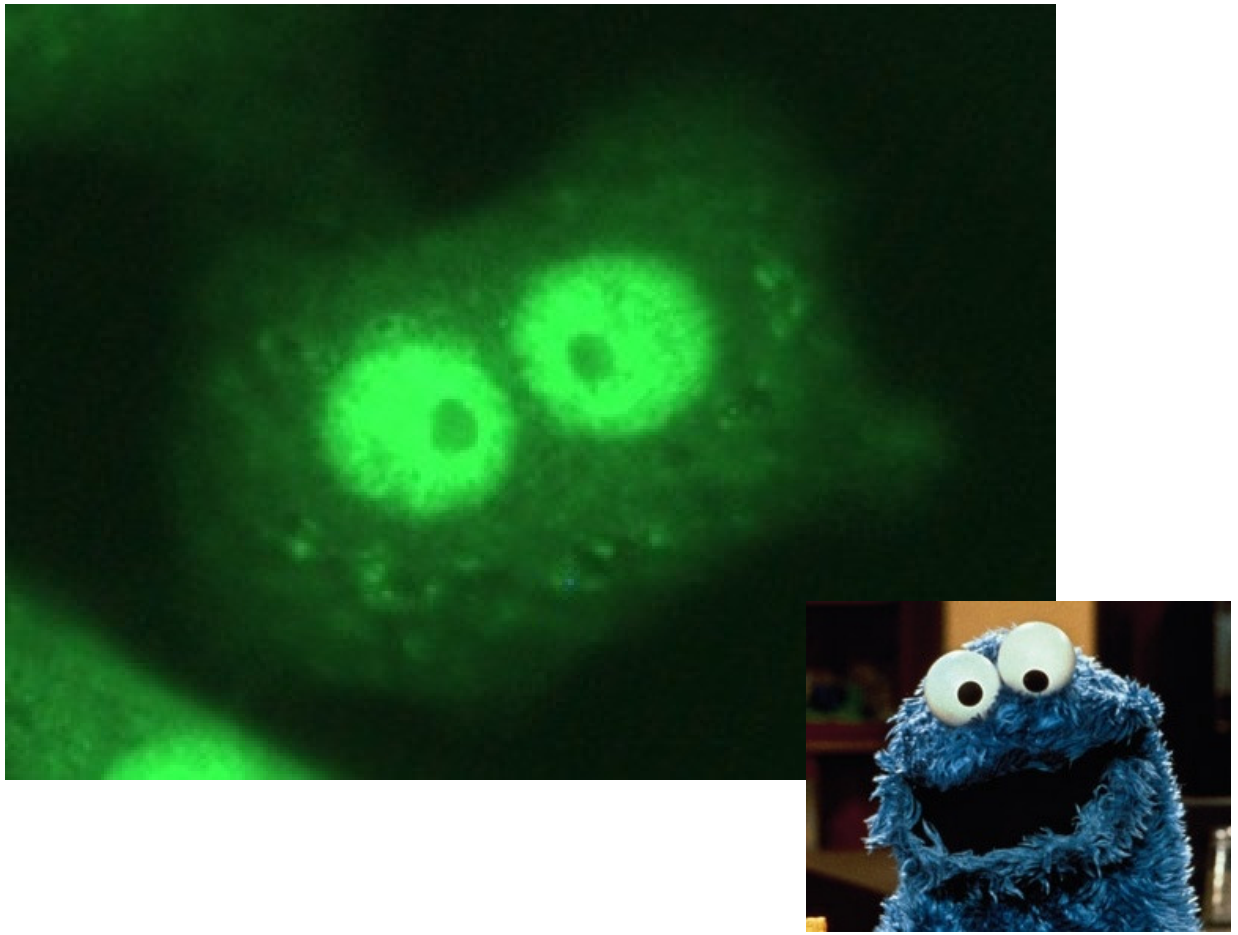
Conclusions

- Immunofluorescence remains the gold standard for ANA-screening
- Non-IFT screens do not adequately replace ANA-IFT (consistent with Agmon-Levin et al. 2014)
- The increasing use of non-IFT screens and the requirement of the revised guidelines of the german medical association (RiliBÄK) to participate in proficiency tests may indicate a possible need for a separate QA platform that allows for the inclusion of the non-IFT tests
- Potential solution for varying results in dsDNA antibody testing: independent tests for high avidity or low avidity dsDNA antibodies)

Thank you for your attention



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