

Immunology: Is machine learning leading the way to harmonisation of ANA testing?

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20th October 2023

EQALM Symposium 2023, Lyon, France

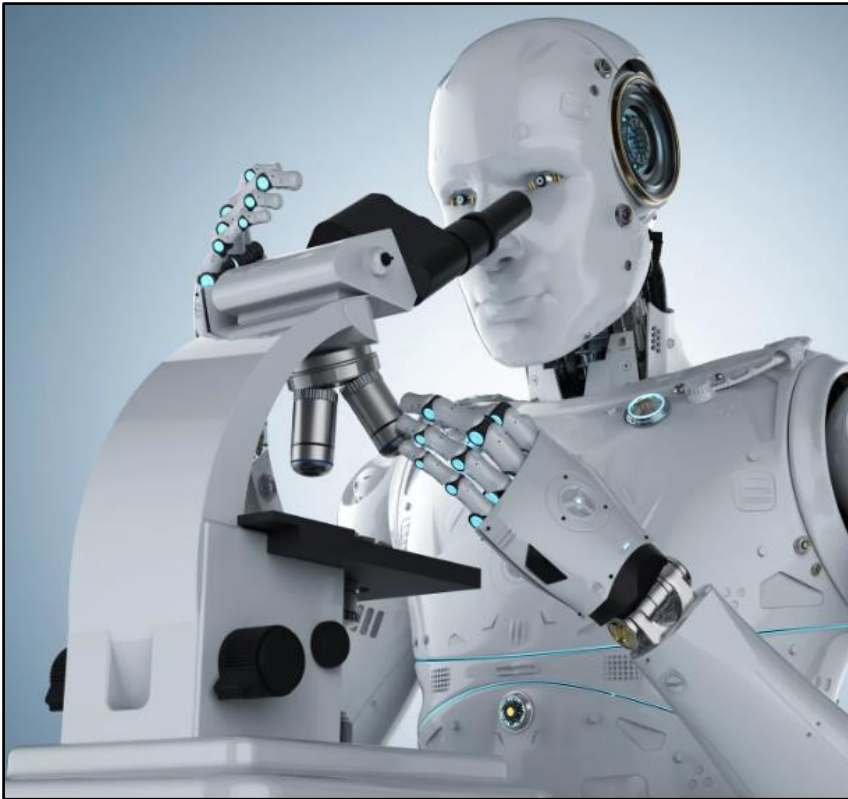
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Overview



- Review of EQA data to summarise current situation
- Consider potential improvements using AI
- Next steps, what's the future

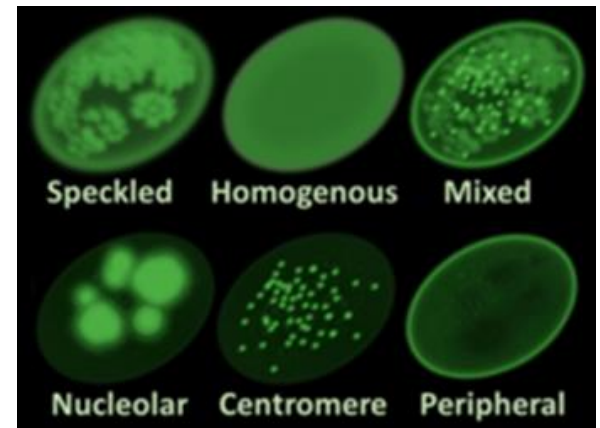
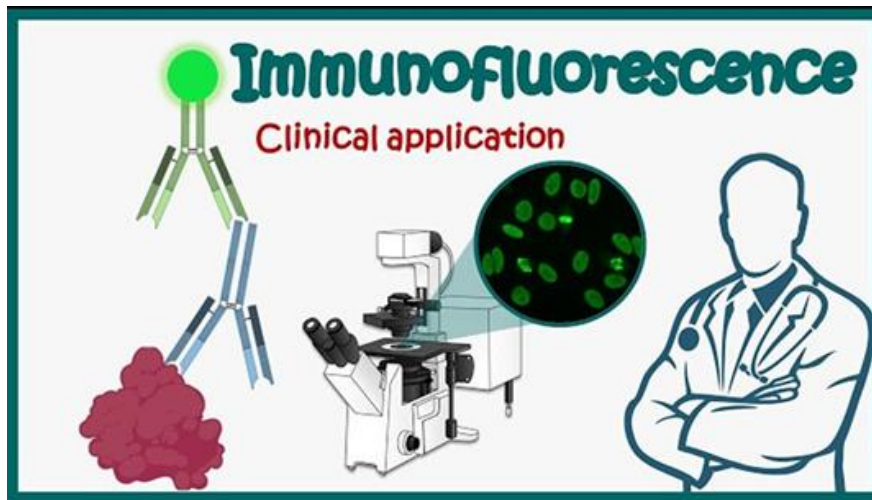
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ANA-staple test of an immunology lab

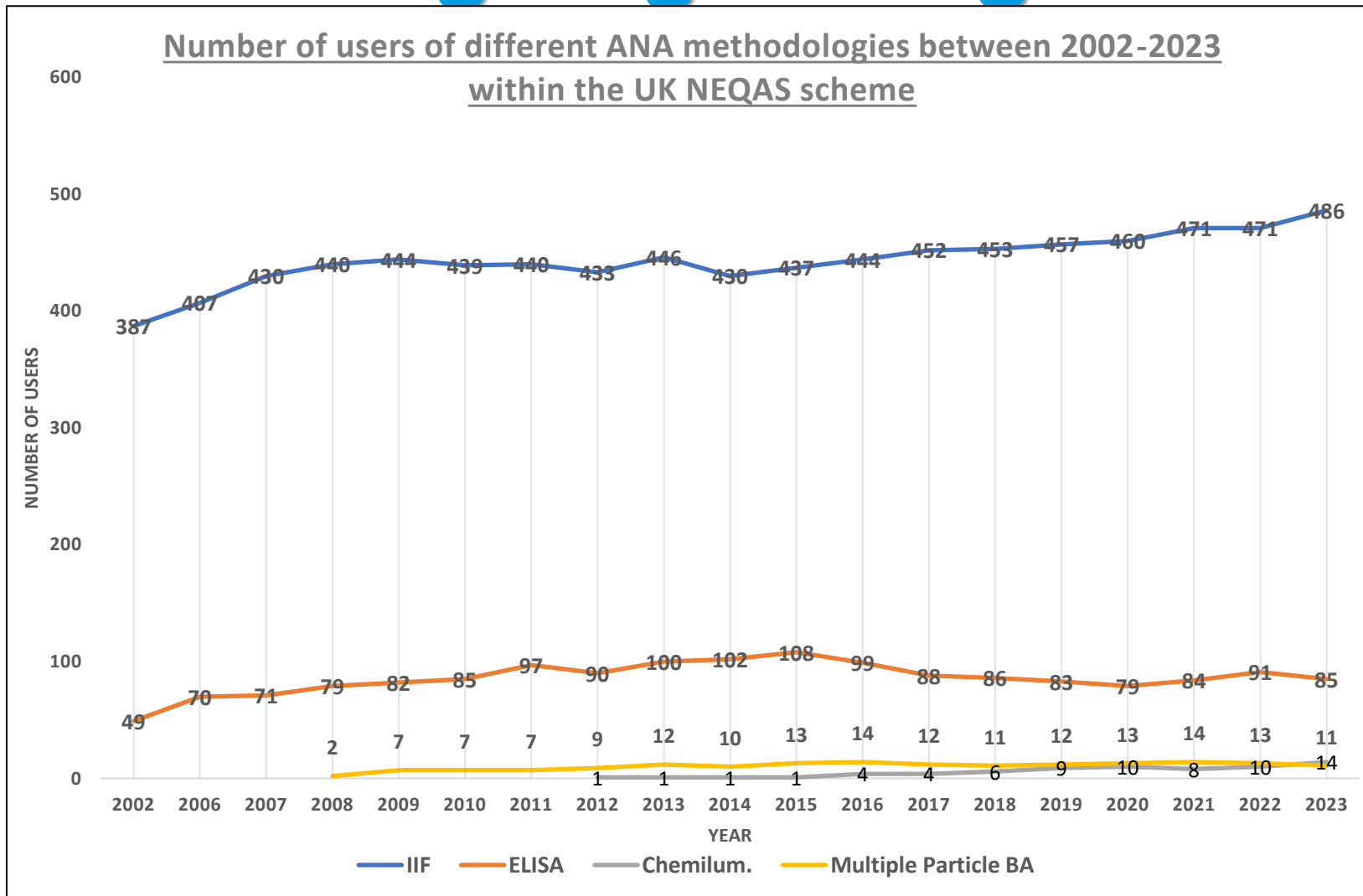


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Still going strong-IIF



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UK NEQAS for Antibodies to Nuclear and Related Antigens

Distribution :	192	March 2019	Participant :	INFO
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654 out of 737 participants returned data for this distribution. 89% response rate.

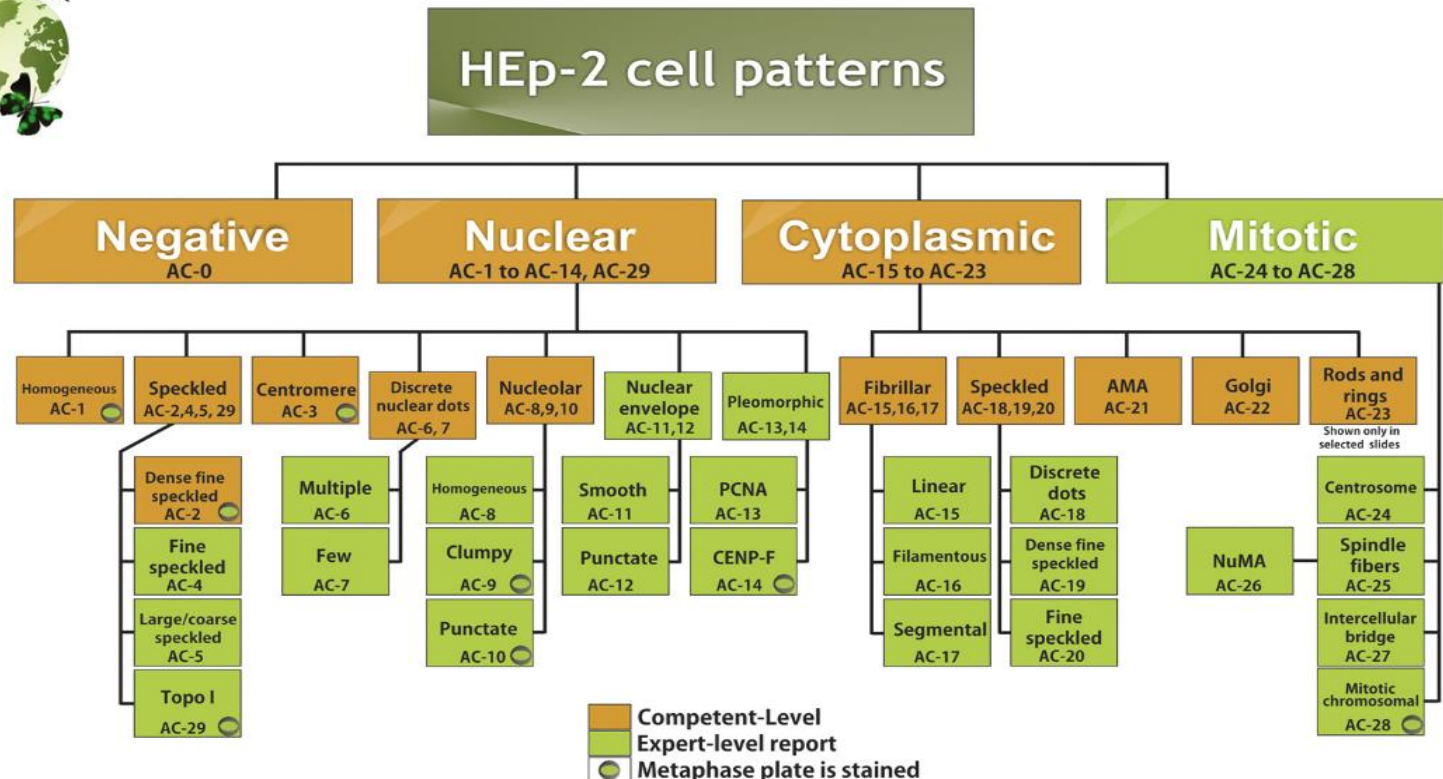
HEp2 Cell Pattern Source	HEp2 Cell Pattern Category (ICAP)	Count
Nuclear	Homogeneous (AC-1)	250
Nuclear	Discrete nuclear dots (AC-6, AC-7)	1
Nuclear	Nucleolar (AC-8, AC-9, AC-10)	62
Nuclear	Speckled (AC-2, AC-4, AC-5, AC-29)	151
Nuclear	Dense fine speckled (AC-2)	25
Nuclear	Fine speckled (AC-4)	27
Nuclear	Large/coarse speckled (AC-5)	2
Nuclear	Topo I (AC-29)	90
Nuclear	Homogeneous nucleolar (AC-8)	19
Nuclear	Large/coarse speckled (AC-10)	5
Cytoplasmic	Reticular / AMA (AC-21)	1
Cytoplasmic	Fibrillar (AC-15, AC-16, AC-17)	2
Cytoplasmic	Speckled (AC-18, AC-19, AC-20)	9
Cytoplasmic	Discrete dots (AC-18)	1
Cytoplasmic	Dense fine speckled (AC-19)	1
Cytoplasmic	Fine speckled (AC-20)	4
Mitotic	Mitotic chromosomal envelope (AC-28)	2
Other		7
Negative	Negative (AC-0)	1

ICAP ANA Harmonisation

ICAP nomenclature system aims to promote consensus in reporting patterns observed by IIF on HEp-2 cells-introduced within EQA scheme in 2016.



www.anapatterns.org



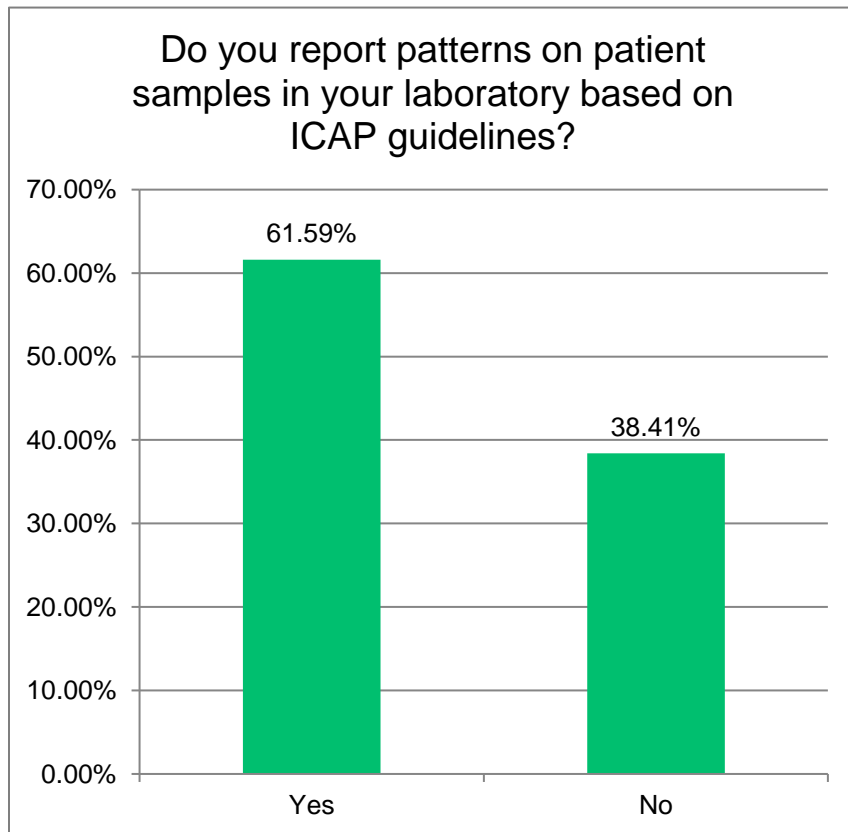
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Are ICAP guidelines used?



- EQALM survey sent to laboratories in June 2023
- Majority of labs report ANA patterns based on ICAP guidelines

Is there Harmonisation of ANA testing?

- The same sample has been circulated within 2 separate distributions within the UK NEQAS Nuclear and Related antigens scheme
 - 2017: Sample 173-2 (pre ICAP harmonisation initiative)
 - 2022: Sample 221-2 (post ICAP harmonisation initiative)
- Sample from a SLE patient with following result profile
 - ANA positive
 - dsDNA positive
 - Centromere negative
 - ENA Ro/La positive

2017- Sample 173-2

Summary Page for Sample 173-2

Distribution :	173	May 2017	Participant :	INFO
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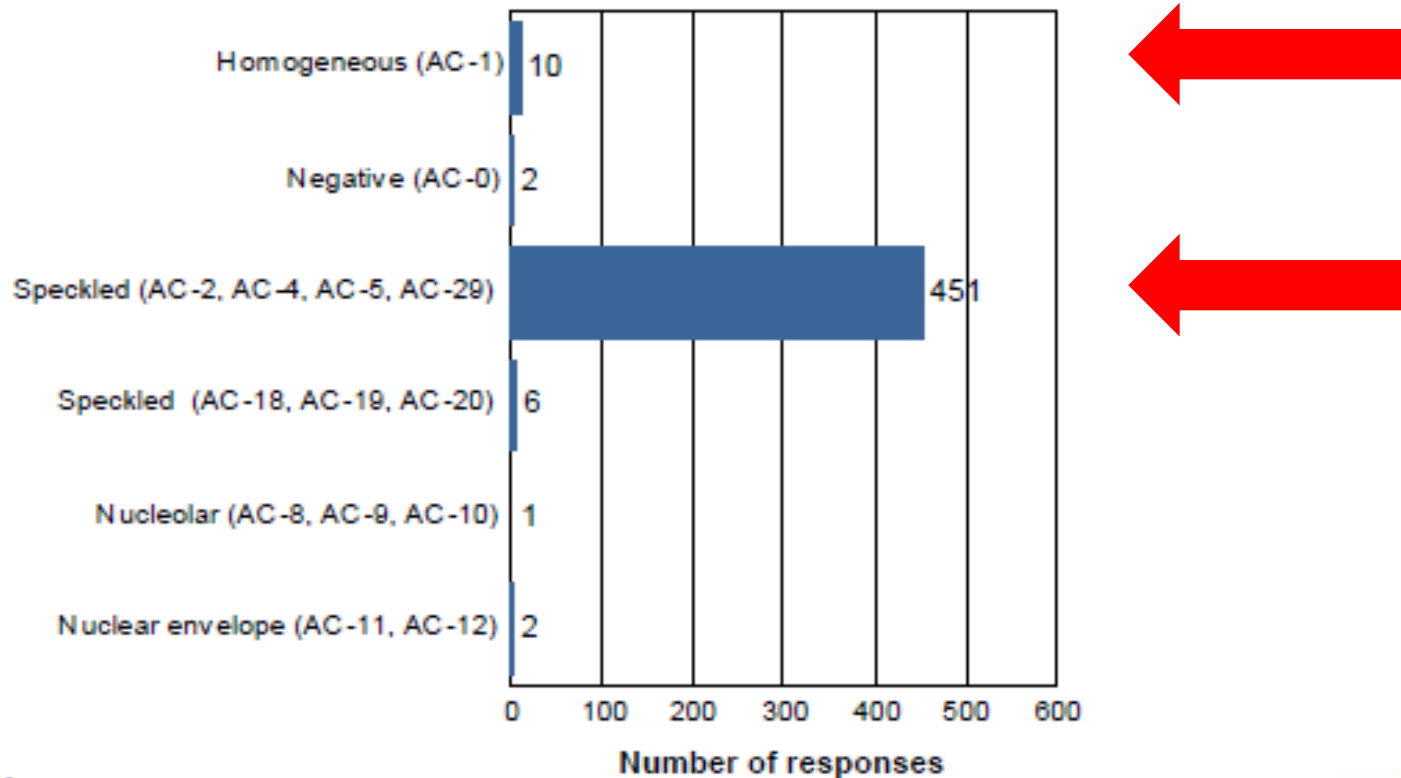
Pattern Source	Pattern Category (ICAP)	Count
Nuclear	Homogeneous (AC-1)	65
Nuclear	Centromere (AC-3)	2
Nuclear	Nucleolar (AC-8, AC-9, AC-10)	3
Nuclear	Speckled (AC-2, AC-4, AC-5)	385
Nuclear	Dense fine speckled (AC-2)	15
Nuclear	Fine speckled (AC-4)	157
Nuclear	Large/coarse speckled (AC-5)	15
Nuclear	Homogeneous nucleolar (AC-8)	1
Nuclear	Large/coarse speckled (AC-10)	1
Cytoplasmic	Fibrillar (AC-15, AC-16, AC-17)	1
Cytoplasmic	Speckled (AC-18, AC-19, AC-20)	3
Cytoplasmic	Fine speckled (AC-20)	2
Other		5
Negative		1

2022- Sample 221-2

ANA (nuclear, cytoplasmic, mitotic) Responses for Sample 221-2

Distribution :	221	January 2022	Participant :	INFO
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ANA- IIF Staining patterns



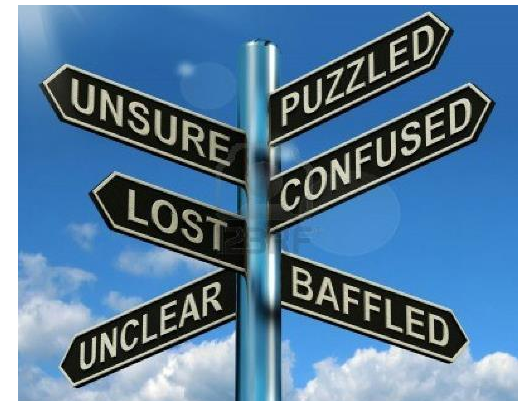
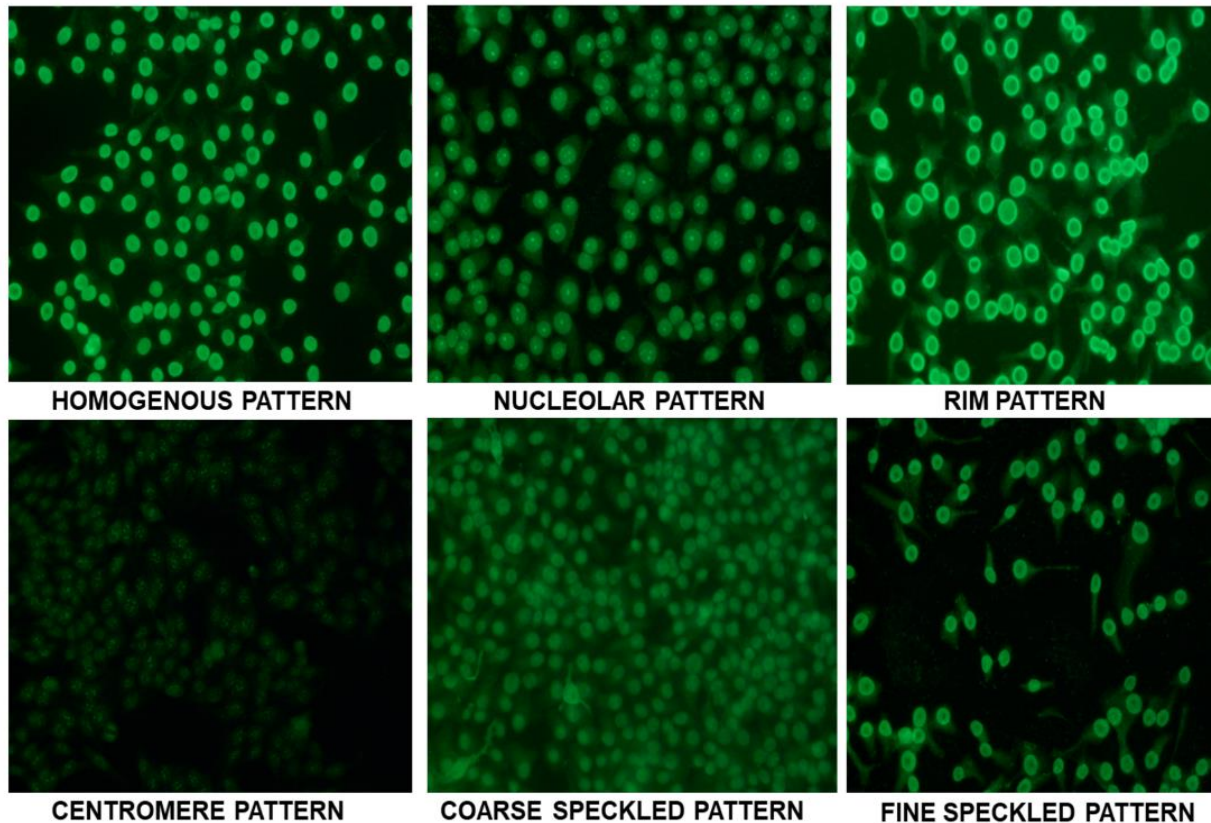
Improved consensus but why?

- Has the ICAP classification system assisted with harmonisation?
- Has subjectivity of reading been reduced?

Standardise variables

- Screening dilution
 - End point titres
 - Substrate
 - Conjugate (and dilution)
- Or is this due to something else?

Subjectivity- What do you see?



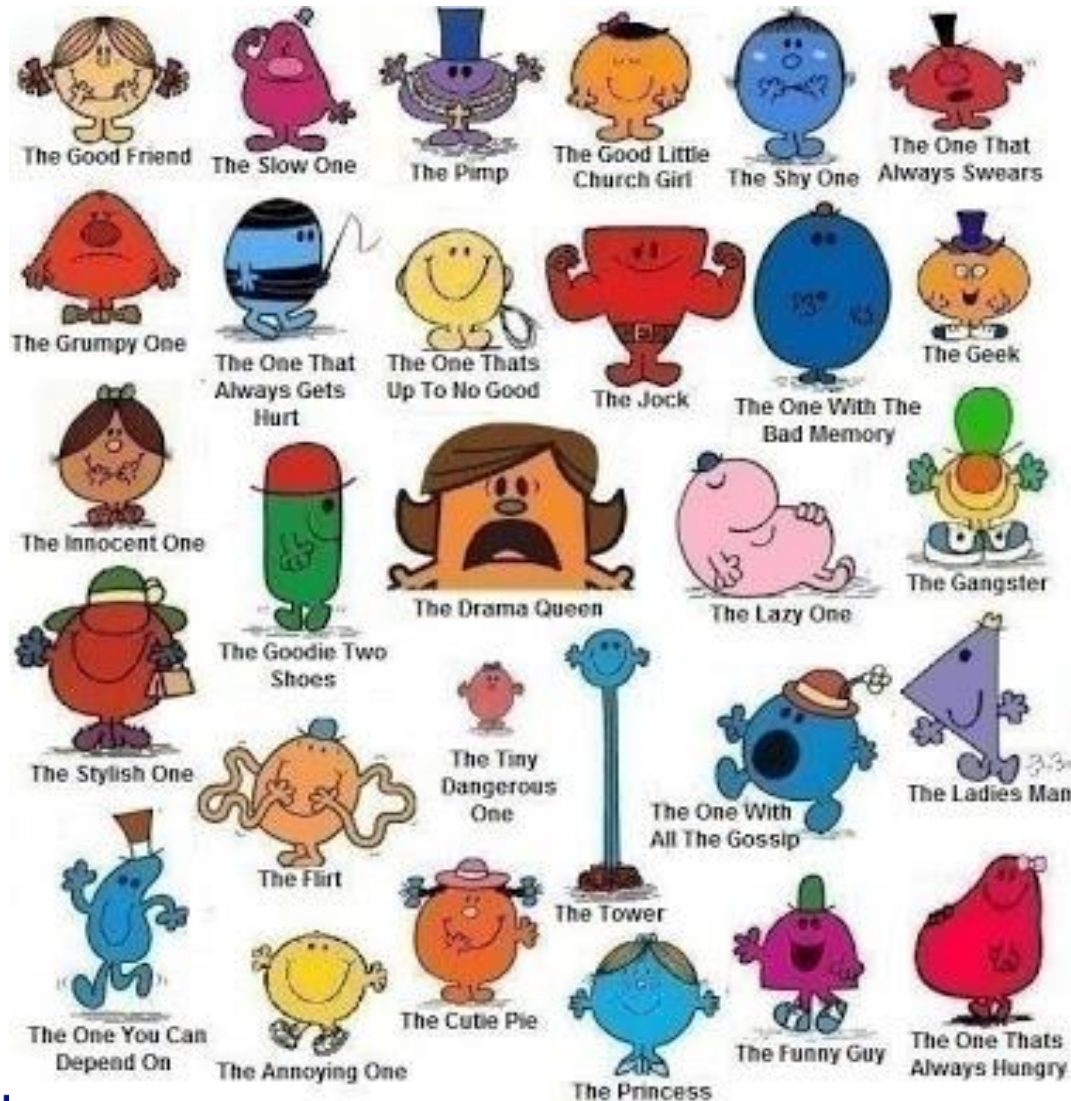
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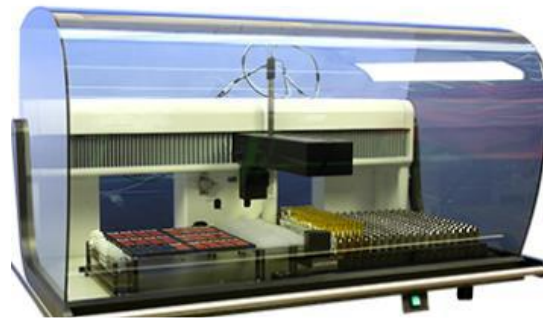
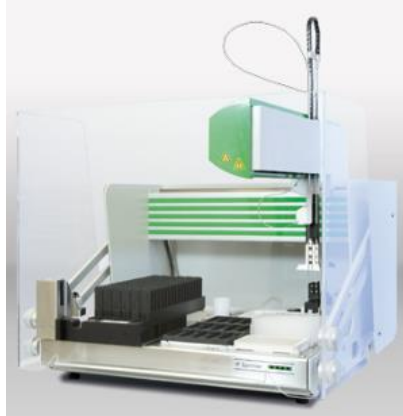
The Problems



- Do we all see the same thing?
- How do you know?
- Are you consistent in your image reading?

Can automation help?

- Number of manufacturers now provide laboratories with an automated option for
 - ANA slide processing
 - and /or image capture with pattern interpretation



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Does automation provide the answers?

- Will the subjective nature of ANA testing by IIF disappear with the advent of machine reading systems which require minimal or no intervention?
- Will this lead to more consistent reading of IIF results?
- What does the EQA data show?

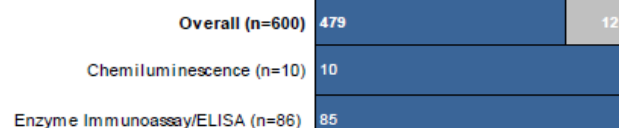
ANA (nuclear, cytoplasmic, mitotic) Responses for Sample 231-2

Distribution : 231	January 2023	Participant : INFO
Your Results		
Pos/Neg : No Response	Method : None Selected	
Quantitative:	Manufacturer : None Selected	Substrate : None Selected
Unit : None Selected	Assay Name : None Selected	Interpretation: N/A

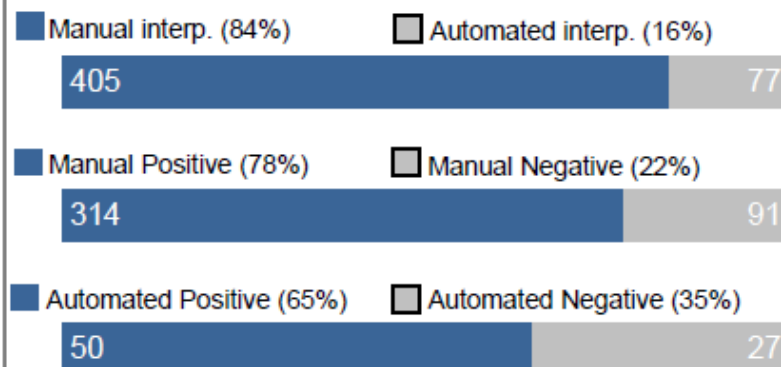
Overall response (all methods) - ANA



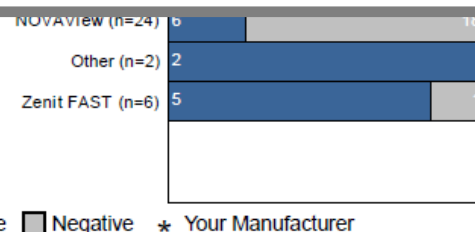
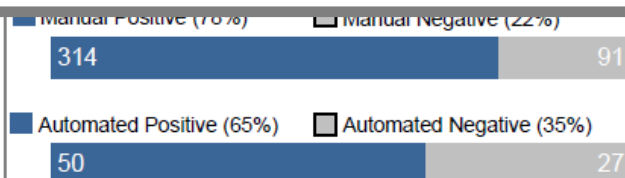
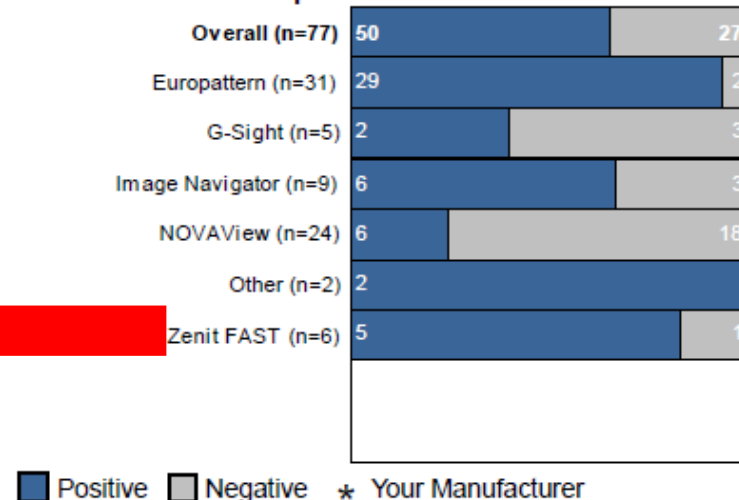
Methodology response - ANA



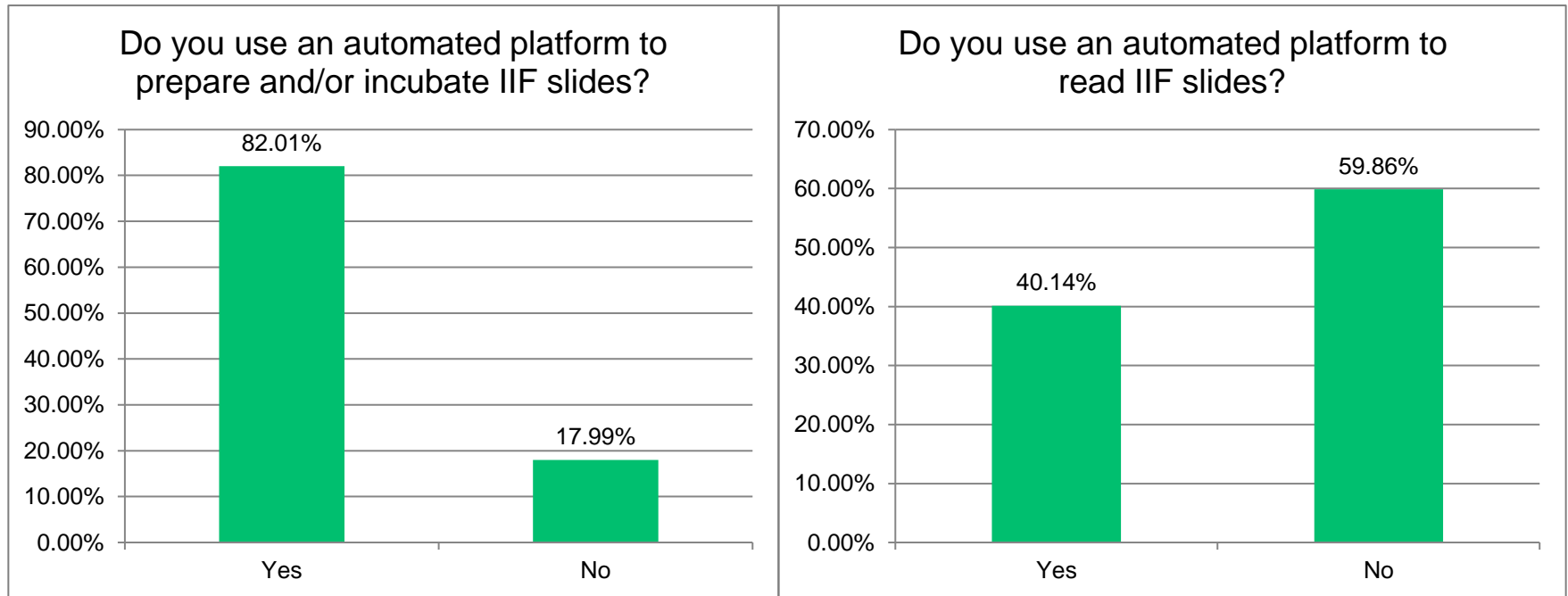
IIF - HEp-2, HEp-2000 and HEp 20-10 Automated vs Manual slide interpretation



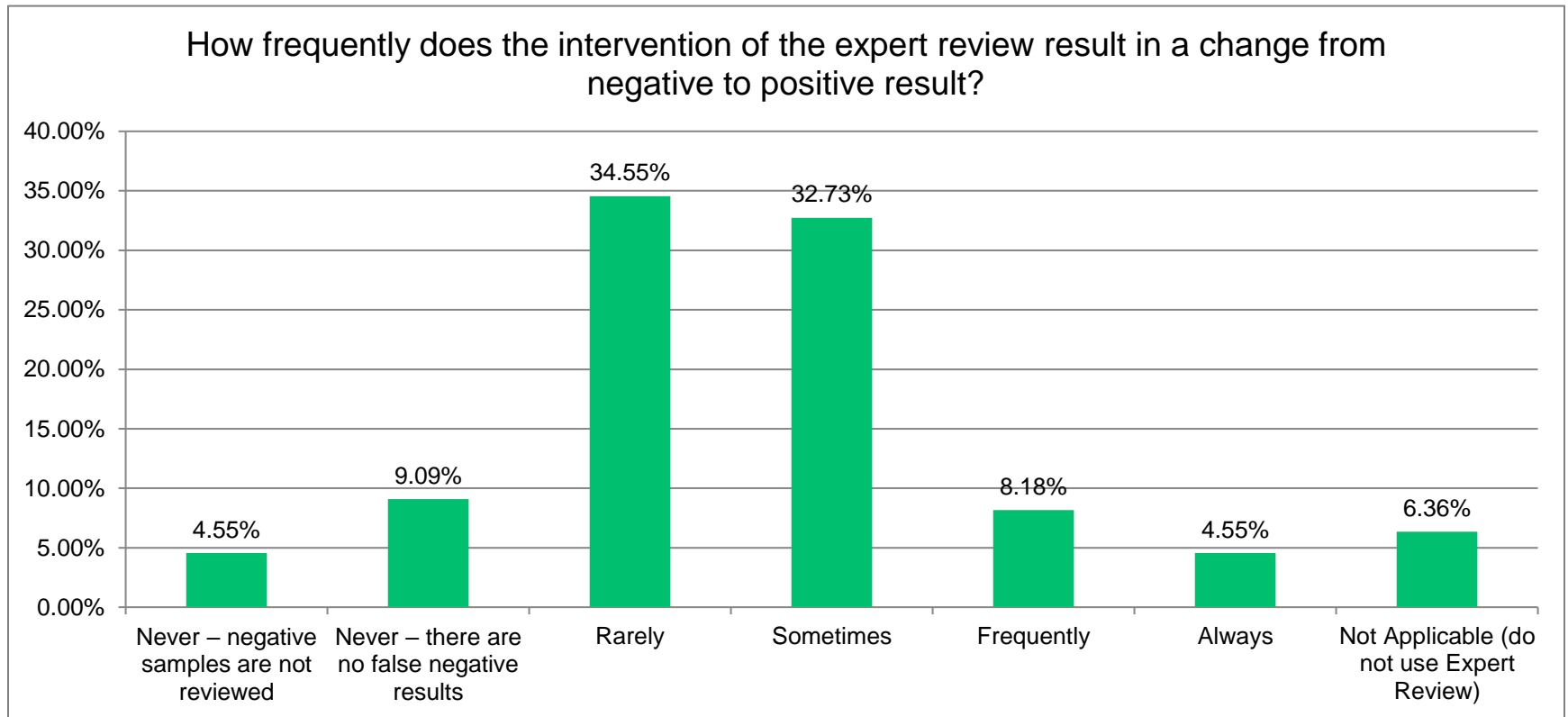
IIF Automated slide interpretation



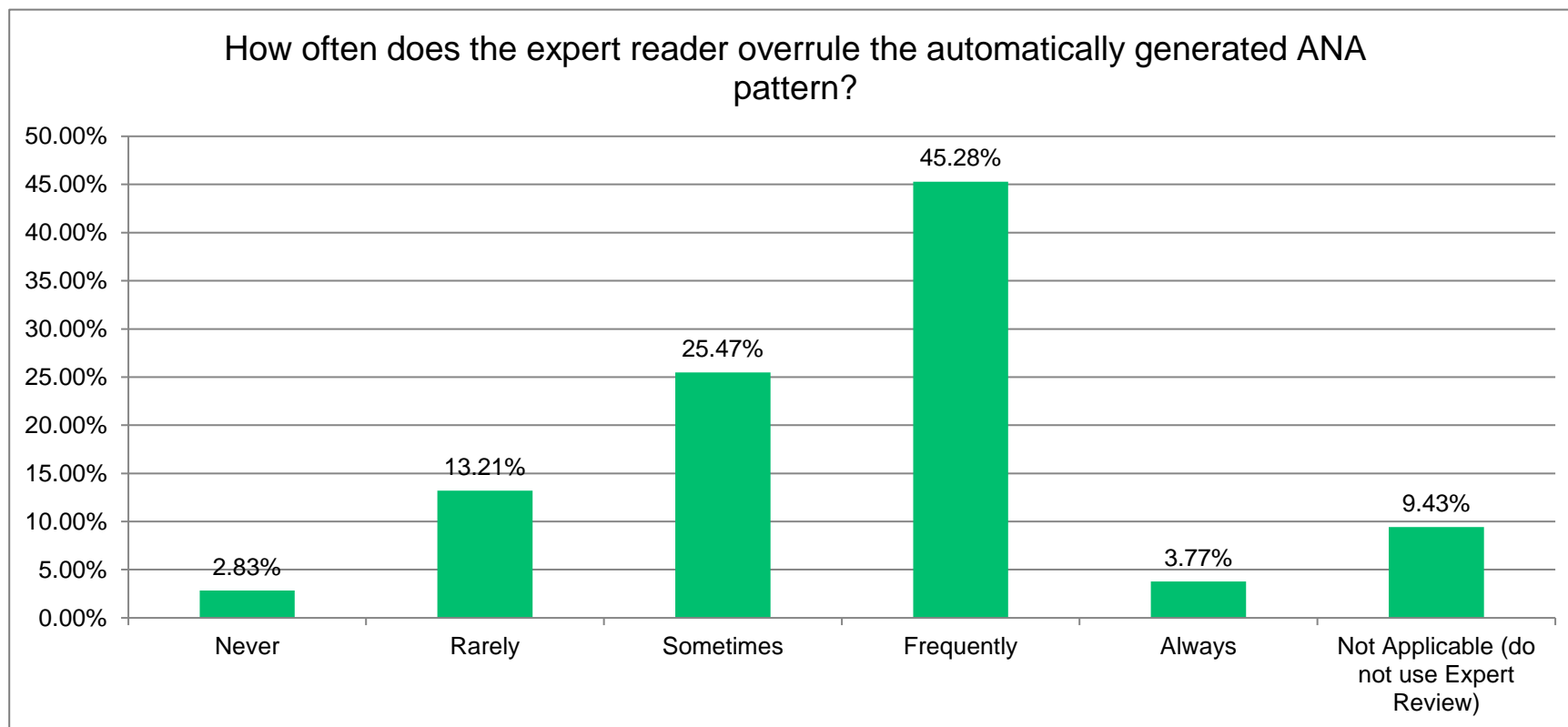
Are Labs using automation?



Impact of using automation?



Overruling automation



ANA recommendations

European Federation of Laboratory Medicine (EFLM) Working Group
The European Autoimmune Standardization Initiative (EASI)
International Consensus on Antinuclear Antibody Patterns (ICAP)

Carolien Bonroy, Martine Vercammen, Walter Fierz, Luis E.C. Andrade, Lieve Van Hoovels, Maria Infantino, Marvin J. Fritzler, Dimitrios Bogdanos, Ana Kozmar, Benoit Nespola, Sylvia Broeders, Dina Patel, Manfred Herold, Bing Zheng, Eric Y.T. Chan, Raivo Uibo, Anna-Maija Haapala, Lucile Musset, Ulrich Sack, Gabor Nagy, Tatjana Sundic, Katarzyna Fischer, Maria-José Rego de Sousa, Maria Luisa Vargas, Catharina Eriksson, Ingmar Heijnen, Ignacio García-De La Torre, Orlando Gabriel Carballo, Minoru Satoh, Kyeong-Hee Kim, Edward K.L. Chan, Jan Damoiseaux, Marcos Lopez-Hoyos and Xavier Bossuyt* for the European Federation of Laboratory Medicine (EFLM) Working Group "Autoimmunity Testing," the European Autoimmune Standardization Initiative (EASI) and International Consensus on Antinuclear Antibody Patterns (ICAP)

Detection of antinuclear antibodies: Recommendations from EFLM, EASI and ICAP

- A computer-aided diagnosis system (CAD) can support HEp-2 IFA, but expert review remains recommended for positive/negative discrimination.
- A CAD can support HEp-2 IFA, but expert review remains mandatory for pattern recognitions.

Bonroy et al.: Detection of antinuclear antibodies: Recommendations from EFLM, EASI and ICAP. Clin Chem Lab Med 2023;61(7):1167-1198

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What impact do you feel the use of automated microscopes to read ANA IIF slides has on ANA IIF reading skills for scientists/technicians

Answer Choices	Responses	
Automated microscopes assist with the training of IIF reading skills	77%	89
Automated microscopes assist with workload and reduced turnaround times	72%	84
Automated microscopes assist with harmonisation of test results	53%	61
Automated microscopes enable ability to produce a bank of images for ease of follow up of patients	75%	87
Automated microscopes assist with sharing of images from other laboratories	44%	51
Automated microscopes enable ability to centralise the interpretation of images from a group of laboratories	22%	25
Automated microscopes are cost prohibitive	5%	6
Automated microscopes have led to the deskilling of IIF reading by scientists/technicians	3%	4
Other, please state:	4%	5

Summary

- ICAP is providing a route to partial harmonisation in ANA testing but this is not a complete solution
- Automation of reading can assist but this is not optimised yet so human intervention is required in some instances
- EQALM Immunology WG are reviewing the data from a recent survey
- Further discussions on how this information can be utilised to assist harmonisation and implementation of automated slide reading systems

