Evaluation of a virtual ANA External Quality Assurance Challenge

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Introduction

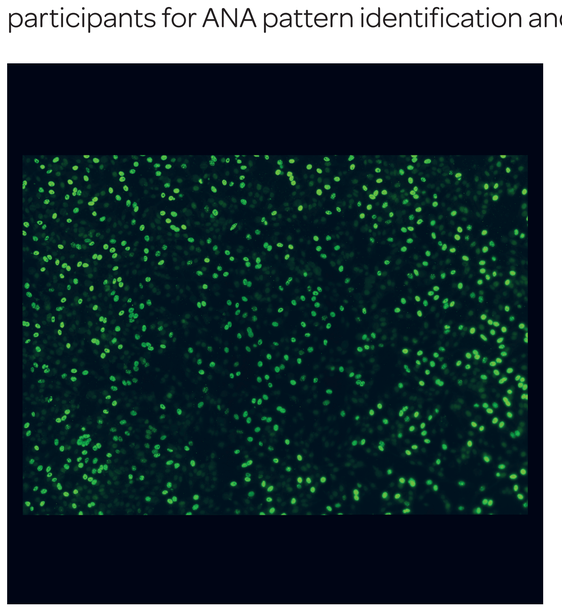
Since 1980 The Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP) has been providing an Antinuclear Antibodies (ANA) External Quality Assurance (EQA) program. Difficulty in accessing sufficient donor serum, especially for rare auto antibodies can be challenging. To ensure coverage of these uncommon patterns, in 2019 The RCPAQAP introduced a "virtual sample" in its ANA program with the aim of widening the scope of patterns scheduled to ensure inclusion of patterns classified as both competent-level and expert-level (ICAP standards).[1]

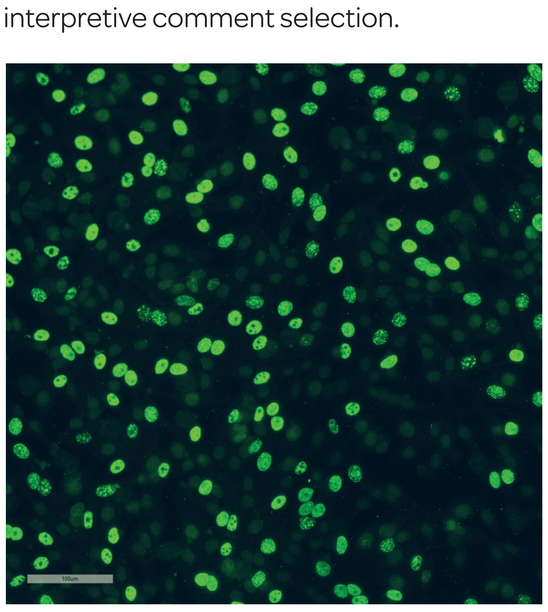
Methods

A commercial control, Euroimmun IIFT Control for antibodies against PCNA Cyclin 2 IgG, was sent to a local reference laboratory to prepare slides using an Immuno Concepts HEp 2000® kit. The slides were prepared and returned to RCPAQAP on the same day and scanned in-house using a Zeiss Axio Imager Z2 microscope with MetaSystems scanning and imaging platform. A pdf of the scanned image was then loaded to the myQAP portal for participating laboratories to access (Figure 1). Result submission for the ANA pattern and interpretive comment (titre not required) was done as usual using the myQAP Result Entry portal.

As part of the normal report generation process, all results were reviewed for continuity and any redundancy removed. Target setting for RCPAQAP Antinuclear Antibodies reports was based on primary pattern group reported by 80% of participants. An in-depth review of results (post report publication) was then conducted to review the performance with the use of a virtual sample.

Figure 1. Sample AN39.20, virtual ANA. Scanned images of indirect immunofluorescence of a commercial control for anti-PCNA were scanned and provided as a pdf document to participants for ANA pattern identification and interpretive comment selection.





Results

A total of 77 participants reported results for the virtual sample. The primary ANA pattern group set as the target set for sample AN39.20 was 'Other Speckled' (or 'Atypical Speckled').

Following an in-depth review of the results (post report publication), one result was excluded from further analysis as the general comment indicated that this participant didn't realise this was a virtual sample.

The following is a breakdown of the remaining 76 responses for ANA pattern for the virtual sample AN39.20.

- 65/76 (87%) reported the specific pattern Cell Cycle related (PCNA-like) in isolation or combination with other patterns
- 1/76% (1%) reported the primary ANA pattern (Other/Atypical Speckled) in combination with other patterns
- 2/76 (3%) misidentified the specific Other/Atypical Speckled pattern as large/coarse Speckled or CENP-F
- 5/76 (7%) identified Speckled in isolation or in combination with other patterns
- 3/76 (4%) identified SSA/Ro (including one non-Hep 2000 user) [NB. 42 labs report use of Immuno Concepts Hep 2000 slides].

When assessing the results against the primary ANA pattern target group, 68/76 (89%) of participants identified patterns consistent with Other/Atypical Speckled. This was a better than expected outcome given the 2019 Antinuclear Antibodies program showed an average consensus of 79% (Range: 42 – 100%) (Table 1).

Lack of consensus for samples with lower reported titres is possibly due to interlaboratory variation in cut off titres.

Table 1. Target patterns and consensus rates for the 2019 RCPAQAP Antinuclear Antibodies program.

Program Sample	Target ANA Pattern	Median Titre	Percentage Consensus
AN 39.01	Speckled	1:2560	93
AN 39.02	NO TARGET SET (Homogeneous/Speckled)	1:640	71
AN 39.03	Not Detected	1:40	80
AN 39.04	NO TARGET SET (Nucleolar)	1:80	66
AN 39.05	Dense Fine Speckled	1:640	60
AN 39.06	Centromere	1:2560	99
AN 39.07	Not Detected (Speckled)	1:40	62
AN 39.08	Speckled	1:160	94
AN 39.09	Nuclear Envelope	1:1280	81
AN 39.10	Not Detected	1:40	95
AN 39.11	Speckled	1:2560	94
AN 39.12	Homogeneous	1:1280	98
AN 39.13	NO TARGET SET (Nuclear Envelope)	1:160	42
AN 39.14	Centromere	1:2560	100
AN 39.15	Nucleolar	1:320	94
AN 39.16	Dense Fine Speckled	1:1280	57
AN 39.17	NO TARGET SET (Homogeneous)	1:80	54
AN 39.18	NO TARGET SET (Not Detected)	1:80	52
AN 39.19	Nucleolar	1:160	95
AN 39.20	Other Speckled	Not Applicable	90

Discussion

Analysis of the patterns reported for the virtual ANA sample indicated a high level of consensus among responders. This level of consensus was sufficient to set a target based on the RCPAQAP guidelines of 80% consensus.

The prevalence of anti-PCNA antibodies has been reported at 1 – 6% in disease cohorts (systemic lupus erythematosus, systemic sclerosis, rheumatoid arthritis, mixed connective tissue disease)[2]. As interlaboratory practices and internal cut offs can introduce variation into ANA interpretation, the use of a virtual ANA using a commercial control can be an indicator of a laboratory's ability to correctly identify ANA patterns, particularly patterns that are not frequently seen in routine laboratory practice.

Conclusion

The use of virtual samples allows for inclusion of a wider variety of ANA patterns that should be part of a laboratory's routine reporting repertoire for ANA testing. The virtual ANA can also serve as a valuable educational resource to ensure the competency of staff performing ANA testing across a broad range of ANA patterns.

References

1. Hep-2 cell patterns: Nomenclature and classification tree. https://www.anapatterns.org/trees-full.php
2. Mahler, M., Miyachi, K., Peebles, C., Fritzler, M.J., (2012) The clinical significance of autoantibodies to the proliferating cell nuclear antigen (PCNA). *Autoimmunity Reviews 11: 771- 775.*

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