

## Introduction

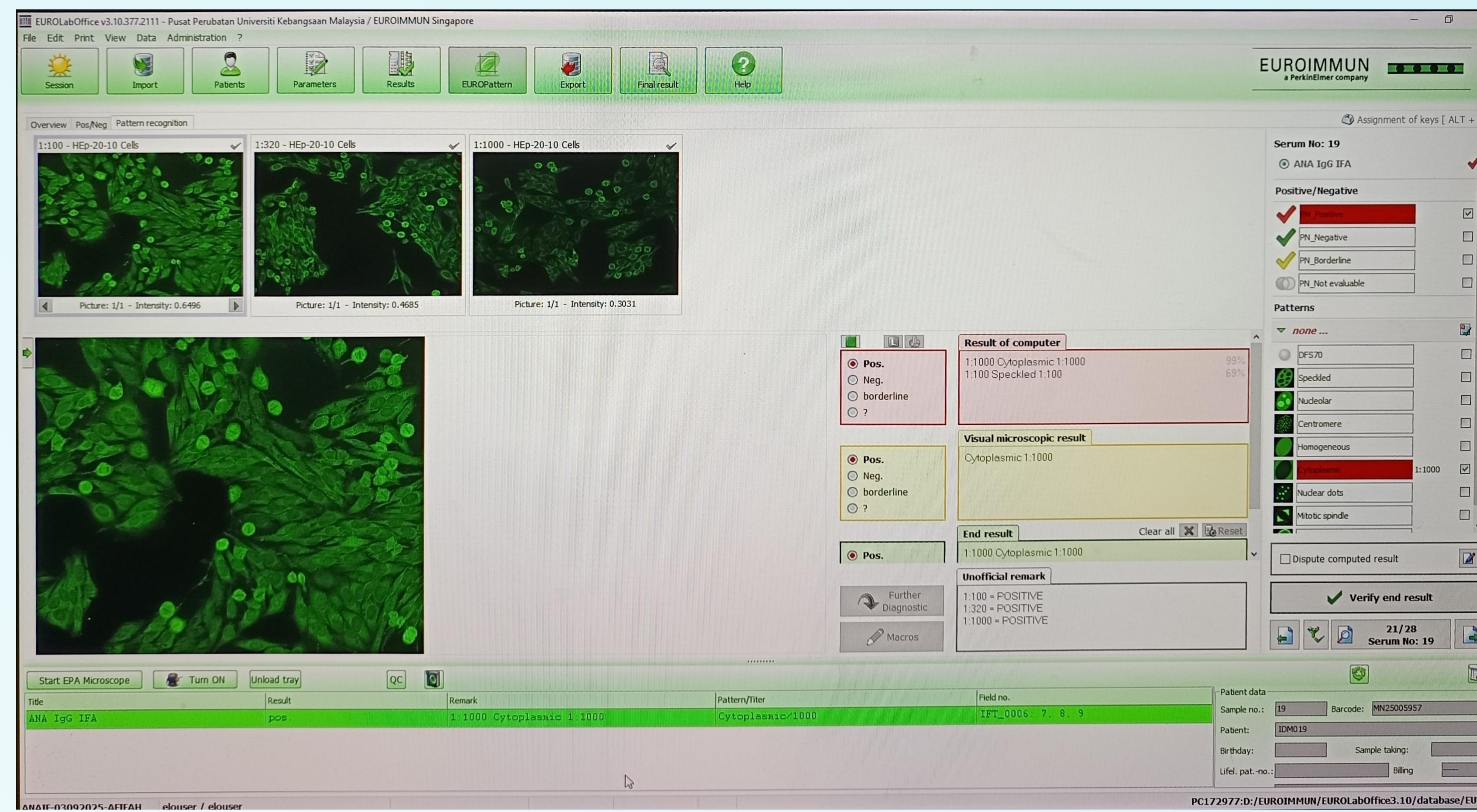
Indirect immunofluorescence (IIF) is the gold standard technique for antinuclear antibody (ANA) testing and plays a crucial role in guiding clinicians for management of patients suspected of systemic autoimmune rheumatic diseases (SARD). To address high sample volumes, automation of ANA IIF test using automated image analysers has been introduced. However, several recent studies have reported mixed findings regarding the accuracy of these analysers in pattern recognition. It can be as low as 48.8% to as high as 72.4%, depending on the type of pattern and its complexity, whether single pattern detected or mixed.

## Results

The essential agreement (i.e., exact pattern match) ranged from 0% to 100%, with an overall agreement of 50.4%. Centromere pattern had the highest agreement (based on a small sample size), while more common patterns such as homogeneous and speckled showed moderate agreement (61.1% and 65.8%, respectively). Notably, 46.5% of samples were discordant with manual readings due to the machine interpreting them as mixed patterns. However, if mixed patterns (where the true pattern is included) are considered acceptable, the overall agreement increased to 90.7%. Interpretation of the DFS70 pattern remained particularly challenging, with only 33.3% agreement.

## Methodology

Our study evaluated 129 positive ANA IIF samples with a single pattern, using the EUROPattern Suite (EUROIMMUN, Lübeck, Germany). These samples were sent to Immunology Laboratory of Hospital Canselor Tuanku Muhriz between May and July 2025 for routine ANA test and comparison were made with manual IIF readings as the gold standard, by two independent laboratory personnel. Samples were titrated to 1:100, 1:320, and 1:1000. Data were recorded for positivities, and their respective patterns. Patterns were reported as per suggested competent-level reporting by International Consensus on ANA Patterns (ICAP), except for cytoplasmic patterns, whereby they are all grouped under cytoplasmic.



Picture 1: Screenshot of EUROPattern automated image analyser interface. ANA IIF images can be viewed directly on the screen and manual reading can be done and recorded.

Manual IIF readings	Automated Image Analyser readings												Essential agreement (%)	Agreement with mixed pattern (true pattern included) (%)
	Homogenous	Speckled	Nucleolar	Nuclear dots	Centromere	DFS70	Centromere	Nuclear envelope	Mitotic spindle	Cytoplasmic	Mixed pattern (with true pattern included)	Mixed pattern (with true pattern excluded)		
Homogenous (n=18)	<u>11</u>	2	-	-	-	-	-	-	-	-	5	-	61.1	88.9
Speckled (n=73)	1	<u>48</u>	-	-	-	-	-	-	-	-	23	1	65.8	97.2
Nucleolar (n=11)	-	-	<u>1</u>	-	-	-	-	-	-	-	9	1	9.1	90.9
Nuclear dots (n=1)	-	1	-	-	-	-	-	-	-	-	-	-	0	0
Centromere (n=2)	-	-	-	-	<u>2</u>	-	-	-	-	-	-	-	100	100
DFS70 (n=9)	-	-	-	-	-	<u>1</u>	-	-	-	-	2	6	11.1	33.3
Cytoplasmic (n=15)	-	-	-	-	-	-	-	-	<u>2</u>	-	13	-	13.3	100
Nuclear envelope (n=0)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mitotic spindle (n=0)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pleomorphic (n=0)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>Total (n=129)</b>	12	51	1	-	2	1	-	-	-	2	52	8	<b>50.4</b>	<b>90.7</b>

Table 1: Tabulated data on the agreements between the patterns individually (numbers highlighted and underlined), and collectively (darker green). Agreement of manual reading with mixed pattern (where the true pattern is included) was also calculated at the rightmost column.

## Discussions and Conclusion

Our findings suggest that the automated system tends to overinterpret patterns, emphasizing the need for caution in reporting. Nevertheless, its ability to include the true pattern within a mixed result may assist laboratory personnel in improving diagnostic accuracy. DFS70 pattern can be masked as homogenous and/or speckled patterns as demonstrated by other studies, which makes it difficult.

### Acknowledgements

This study is approved by Research Ethics Committee of UKM, ref. no : JEP-2025-040.

This study is also approved for conduct by Faculty of Medicine, UKM, project code : FF-2025-182

### References

- Asrul Abdul Wahab (2023). Aplikasi Pengujian Antibodi Anti-Nuklear dalam Menentukan Penyakit Autoimun Reumatik Sistemik dan Penyakit Berkaitan. Sains Malaysia, 52(3), 887-898. <https://doi.org/10.17576/jsm-2023-5203-16>
- Park, Y., Kim, S. Y., Kwon, G. C., Koo, S. H., Kang, E.-S., & Kim, J. (2019). Automated Versus Conventional Microscopic Interpretation of Antinuclear Antibody Indirect Immunofluorescence Test. Annals of Clinical and Laboratory Science, 49(1), 127-133. <http://www.ncbi.nlm.nih.gov/pubmed/30814088>
- Choi, H. W., Kwon, Y. J., Park, J. H., Lee, S. Y., Chun, S., Won, E. J., Lee, J. H., Choi, H. J., Kim, S. H., Shin, M. G., Shin, J. H., & Kee, S. J. (2020). Evaluation of a Fully Automated Antinuclear Antibody Indirect Immunofluorescence Assay in Routine Use. Frontiers in Immunology, 11. <https://doi.org/10.3389/fimmu.2020.607541>
- Irure-Ventura, J., & López-Hoyos, M. (2022). The Past, Present, and Future in Antinuclear Antibodies (ANA). In Diagnostics (Vol. 12, Issue 3). Multidisciplinary Digital Publishing Institute (MDPI). <https://doi.org/10.3390/diagnostics12030647>
- Li, X., Pan, J., Zhou, H., He, M., Li, W. X., Chen, Z. Z., Dai, W. L., Wang, F., Wei, Q. J., Lao, X. Bin, Zhang, L., Li, L., Hu, H., Li, M., Qiu, Y., & Hou, T. (2020). A multi-centre study for standardization of antinuclear antibody indirect immunofluorescence screening with automated system. Journal of Immunological Methods, 477, 112701. <https://doi.org/10.1016/j.jim.2019.112701>
- Li, Z. Y., Han, R. L., Yan, Z. L., Li, L. J., & Feng, Z. R. (2019). Antinuclear antibodies detection: A comparative study between automated recognition and conventional visual interpretation. Journal of Clinical Laboratory Analysis, 33(1). <https://doi.org/10.1002/jcla.22619>
- Wu, S., Liu, F., Wang, F., Huang, J., Yin, B., Huang, M., Wang, T., & Wei, N. (2020). Evaluation of the Automated Indirect Immunofluorescence Test for Antinuclear Antibodies. Annals of Clinical and Laboratory Science, 50(2), 282-286. <http://www.ncbi.nlm.nih.gov/pubmed/32366571>