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Mini Review

A Decade of Progress and Persistent Challenges in Autoimmune Diagnostics: Insights from UK NEQAS (2012–2021)

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Abstract: External Quality Assessment (EQA) programs play a crucial role in monitoring laboratory performance, identifying inconsistencies, and promoting the adoption of best practices in autoimmune diagnostics. Over the past decade, this field has undergone substantial evolution, driven by technological advances, enhanced standardisation, and increasing efforts toward harmonisation. This review examines findings from two recent studies assessing the decade-long evolution of autoimmune testing through data from the UK National External Quality Assessment Service (UK NEQAS) programmes. The results highlight considerable progress in the adoption of novel immunoassay technologies and improvements in test standardisation. However, challenges persist, particularly in achieving full harmonisation across laboratories and methodologies.

Keywords: quality assurance; autoimmunity; laboratory testing; autoimmune diagnostics

1. Introduction

Autoimmune diseases encompass a diverse group of disorders characterised by immune system dysregulation and the presence of autoantibodies. The accurate detection and interpretation of autoantibodies is crucial for diagnosis and disease management. This aids the application of precision medicine to autoimmune diseases improving the patient care while reducing health care costs. The UK NEQAS Immunology, Immunochemistry & Allergy (IIA) EQA programmes have provided comprehensive insights into the evolution of autoimmune diagnostics. Over the past decade, significant technological advancements have been introduced within patient testing laboratories, alongside increased efforts to harmonise diagnostic practices.

2. Methods

This paper summarises key findings from two recent studies: Garrafa et al. [1], which evaluated the technological evolution in autoimmune testing, and Infantino et al. [2], which focused on the harmonisation of anti-nuclear antibody (ANA) testing by indirect immunofluorescence (IIF). Data for these studies were drawn from several UK NEQAS EQA programmes, covering a broad spectrum of autoantibody assays, including ANA, anti-dsDNA, anti-centromere, anti-extractable nuclear antigen (ENA), anti-phospholipids, anti-neutrophil cytoplasm (ANCA), anti-proteinase 3 (PR3), anti-myeloperoxidase (MPO), anti-glomerular basement membrane (GBM), rheumatoid factor (RF), anti-citrullinated protein antibodies (ACPA), mitochondrial (AMA), liver-kidney-microsomal (LKM), smooth muscle (ASMA), gastric parietal cell (APCA), and coeliac disease antibodies. EQA programmes for each analyte consists of six cycles annually, with two distinct samples per cycle.

3. Results

The technological evolution in autoimmune diagnostics has been extensively reviewed by Garrafa et al. [1], who provide a comprehensive overview of advances in testing methods using data from the UK NEQAS EQA programmes. In addition, Infantino et al. review the increasing harmonisation observed across a broad spectrum of autoantibody assays between 2012 and 2021 [2].

3.1. Technological Evolution

Over the examined period, there has been a marked shift in the immunoassay technologies employed by laboratories (Table 1). Traditional ELISA methods have seen a steady decline, replaced by more advanced platforms such as fluoroenzyme immunoassay (FEIA), chemiluminescence immunoassay (CLIA), multiple particle based assays (MPBA), and immunoblot (IB). Among these, FEIA has emerged as the dominant method for several key autoantibodies, including anti-dsDNA, ANCA, anti-ENA, anti-GBM, anticardiolipin (aCL), and anti-β2 glycoprotein I (anti-β2GPI).

Table 1. Immunoassay technologies used within laboratories.

Immunoassay Technology	Abbreviation	Description
Chemiluminescence immunoassay	CLIA	A chemiluminescent label attached to either the antibody or antigen, produces light when triggered by a chemical reaction. The amount of emitted light is measured by a luminometer and is directly proportional to the concentration of the target analyte in the sample
Enzyme linked immunosorbent assay	ELISA	Uses an enzyme-labelled antibody or antigen complex. When a substrate is added, the enzyme catalyses a colour-producing reaction. The intensity of the colour change, measured using a spectrophotometer, is proportional to the amount of analyte present in the sample.
Fluoroenzyme immunoassay	FEIA	Uses an immunoassay method that uses an enzyme label to generate a fluorescent rather than colorimetric signal
Immunoblot	IB	Proteins are first separated by gel electrophoresis, transferred onto a membrane, and then probed with antibodies that bind the target protein. A labelled secondary antibody produces a visible signal, allowing identification and semi-quantification of the protein of interest.
Multiple particle based assays	MPBA	Use tiny beads or particles, each coated with a specific antibody or antigen, to detect several analytes simultaneously in a single sample. When the sample is added, target molecules bind to their corresponding particles, and a labelled detection antibody generates a measurable signal (often fluorescent)

Interestingly, despite the availability of solid-phase assays (SPA), IIF on HEp-2 cell substrates has remained the primary method for ANA testing, used by approximately 80% of participating laboratories (Figure 1). By 2021, the use of older, less sensitive substrates such as rat and mouse tissues for ANA-IIF had been completely phased out.

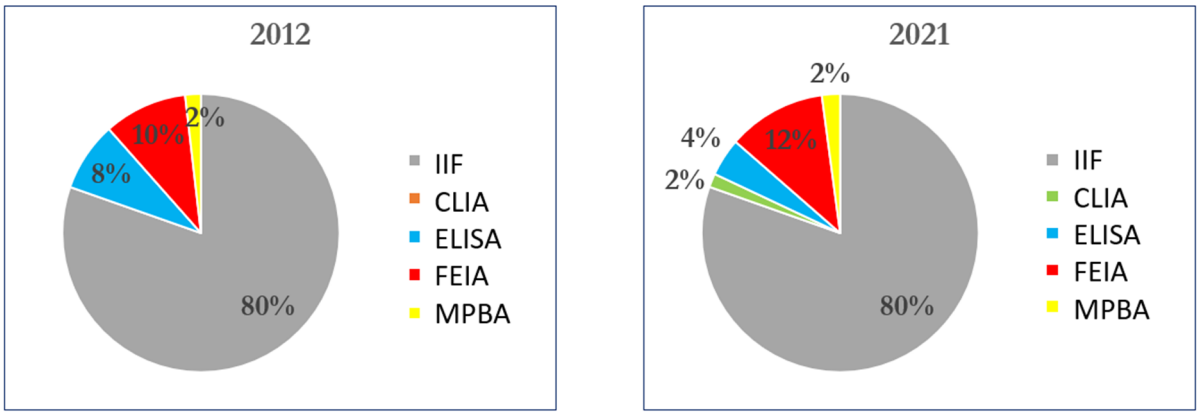

Figure 1. Methodologies used for ANA testing over time.

Another notable change is immunoblotting, which has gained increased prominence, particularly for the confirmation of specific autoantibodies such as AMA, anti-LKM, and anti-centromere antibodies, supporting its growing role as a confirmatory tool following initial screening.

In addition, quantitative agreement also improved significantly, especially for assays with established international standards (e.g., anti-dsDNA and RF), with notable reductions in the coefficient of variation (CV%) observed. This change can be attributed to increased automation and a gradual consolidation of platforms. Additionally, the accuracy of qualitative (positive/negative) results has also shown a modest but consistent improvement across most autoantibodies.

3.2. Challenges in Harmonisation

Despite notable progress, significant challenges remain in achieving full harmonisation of autoantibody testing. Although there has been a modest improvement in inter-laboratory agreement, particularly across different testing methodologies, statistically significant harmonisation continues to be limited [2]. One of the most persistent issues lies in ANA-IIF testing, which remains particularly complex due to its reliance on subjective visual interpretation. Variability in reading patterns, the use of different nomenclature systems, variations in screening dilutions, and inconsistent endpoint titration all contribute to ongoing discrepancies between laboratories [3].

Pattern recognition within ANA-IIF testing presents further difficulties. Lower consensus rates have been observed in some ANA-related EQA distributions or cycles, which may stem from limitations in certain assays—for instance, difficulties detecting cytoplasmic antigens with solid-phase assays or challenges identifying anti-Jo1 and anti-SSA/Ro60 with HEp-2 IIF. Additionally, some laboratories continue to incorrectly classify cytoplasmic patterns as ANA-negative, contrary to established International Consensus on ANA Patterns (ICAP) guidelines. This has led to ongoing performance scoring issues, for participants within the programme.

However, standardisation challenges are not limited to ANA testing. In coeliac disease antibody testing, consensus has also proven elusive, particularly for borderline samples. High rates of equivocal results were observed for tests such as anti-gliadin antibodies (AGA) IgA and anti-deamidated gliadin peptide (DGP) IgA, and a concerning number of laboratories were still using the outdated AGA assay as recently as 2021 despite diagnostic guidelines and expert consensus making it clear that testing for native anti-gliadin antibodies (AGA) is no longer recommended for diagnosing coeliac disease. The EQA provider is in the process of assessing usage by laboratories with the aim to remove the analyte from the EQA programme which would assist with ensuring testing practices are standardised across laboratories. This highlights the variability in assay choice and interpretation practices that continues to affect the reliability of autoimmune diagnostics across the board.

Further insights into the harmonisation of ANA-IIF testing were provided in a 2025 study by [2], which assessed the impact of international standardisation efforts over a ten-year period.

They observed notable improvements in ANA-IIF harmonisation from 2013 to 2023 [2], with correct positive/negative classifications increasing from 64.0% to 90.9% ($p < 0.001$).

Despite this, pattern recognition remained variable. Homogeneous patterns were associated with the lowest agreement rates (70.5 ± 16.0), while centromere, speckled, and negative patterns demonstrated higher consensus, with the speckled pattern showing the highest agreement (90.3 ± 12.3). The increasing prevalence of speckled patterns in EQA samples may have contributed to this upward trend in consensus in later years.

Titre levels also influenced agreement rates. The highest consistency was observed for titres between 1:80 and 1:320, whereas titres above 1:320 were more prone to discrepancies. The adoption of the ICAP nomenclature in late 2016, led to a significant improvement in pattern recognition and reporting. In fact, the comparison of pre- and post-ICAP periods showed an increase in consensus from 62.7% ± 7.7 to 73.7% ± 14.3 ($p < 0.001$), underscoring the positive impact of standardised pattern nomenclature and reporting [2].

Finally, the study assessed the impact of manual versus digital interpretation in ANA-IIF testing. No statistically significant difference was observed in positive/negative classification accuracy between the two approaches (93.8% for manual vs. 92.4% for digital; $p = 0.078$). This suggests that digital interpretation is at least as reliable as manual methods for basic result classification at this time. However, computer-aided diagnostic (CAD) systems, at the current time, remain limited in their ability to accurately detect mixed or less common patterns, indicating that further development and validation are needed before these technologies can be fully relied upon for complex diagnostic tasks.

4. Discussion

EQA services, such as those delivered by UK NEQAS for Immunology, Immunochemistry, and Allergy (IIA), are instrumental in standardising laboratory methodologies and providing laboratories with objective

performance benchmarks. These programmes enable participants to assess their analytical accuracy and highlight areas for improvement. A review of UK NEQAS EQA data spanning approximately 2012 to 2021 offers important perspectives on the development of autoimmune diagnostics, illustrating both the field's progress and its ongoing challenges [1].

During this period, participation increased across nearly all EQA programmes assessing various autoantibodies, including ANA, anti-dsDNA, anti-ENA, ANCA, and those related to coeliac disease. This growing involvement suggests a heightened awareness among clinical immunology laboratories of the benefits EQA schemes offer in enhancing diagnostic quality.

A key development over the decade was the shift in technologies used for autoantibody detection. While the use of traditional ELISA methods gradually declined, there was a notable rise in the adoption of more advanced technologies, such as FEIA, CLIA, MPBA, and IB [1]. These automated and multiplex platforms gained traction due to their faster processing times, random access capability, broader measuring ranges, and ability to detect multiple antibodies simultaneously. Although solid-phase assays (SPA) have advanced significantly, indirect immunofluorescence on HEp-2 cell substrates has continued to be the primary method for ANA detection in most laboratories. Importantly, the use of rodent tissue substrates for ANA testing was entirely discontinued by 2021 [1].

With respect to harmonisation, there was a general trend toward improved inter-laboratory agreement across most autoantibody assays. However, in many cases, these improvements did not achieve statistical significance [2].

More substantial progress was observed in quantitative assays where international reference standards exist—specifically for anti-dsDNA and RF—with marked reductions in coefficient of variation (CV%) between 2012 and 2021 [2]. These gains are likely linked to the growing use of modern automated systems and a potential consolidation in the range of diagnostic platforms employed.

Despite these improvements, ANA testing via IIF remained a particularly challenging area for standardisation due to inherent subjectivity in visual interpretation. Factors such as inconsistent pattern nomenclature, variable screening dilutions, and discrepancies in endpoint titration continued to affect consensus.

A significant advancement came in 2016 with the implementation of the International ICAP nomenclature within UK NEQAS reporting. Subsequent EQA data demonstrated clear improvements in the consistency of ANA pattern reporting following this change [2]. Although manual and digital methods showed similar accuracy in positive/negative classification, the adoption of ICAP notably enhanced harmonisation. Nevertheless, pattern-specific variability persisted: homogeneous patterns consistently showed lower consensus compared to speckled, centromere, and negative patterns. In addition, higher agreement was observed for ANA titres in the 1:80–1:320 range, whereas titres above 1:320 were associated with greater inter-laboratory variability [2].

The EQA programs also highlighted specific areas needing further attention. For instance, some ANA exercises showed lower consensus, potentially due to assay limitations in recognizing certain cytoplasmic antibodies or to SSA/Ro60 which are not easily recognisable by HEp-2 IIF. Additionally, some participants continued to classify cytoplasmic ANA patterns as negative, despite ICAP guidelines recommending they be considered positive.

In conclusion, a decade of UK NEQAS data reveals notable advancements in autoimmune diagnostics, particularly in technological adoption and quantitative assay standardisation. While harmonisation has improved—especially following the introduction of ICAP—significant challenges remain in ANA-IIF interpretation and coeliac disease antibody testing. Ongoing collaboration among laboratories, manufacturers, and EQA providers is essential to address these challenges. Future research should focus on improving CAD systems, refining antibody detection methods, and promoting universal adherence to standardised nomenclature and interpretation guidelines. Adoption of the ICAP nomenclature and use of optimised CAD systems across all laboratories would assist with harmonisation of interpretation and reporting of ANA. This would minimise variability in results reported, regardless of location, ultimately benefitting patient care and clinical outcomes.

5. Limitations

There is acknowledgement that the data utilised for this paper relies exclusively on the data from one source, UK NEQAS IIA, and does not include comparative clinical data.

Author Contributions

D.P. and M.I. conceptualization, methodology, software; D.P.: data curation, writing—original draft preparation; D.P. and M.I.: visualization, investigation; D.P. and R.S.: supervision; D.P.: software, validation; D.P., R.S., N.B. and M.I.: writing—reviewing and editing. All authors have read and agreed to the published version of the manuscript.

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The authors declare no conflict of interest.

Use of AI and AI-Assisted Technologies

No AI tools were utilized for this paper.

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