estimates of false positivity or false-negative results in the test assays.

In conclusion, among the three CrAg-EIAs within the study, the Bordier and Euroimmun kits appear to exhibit similar performance characteristics as competitive serological tools compared to the DRG assay. The introduction of the InBios EIA as a more specific alternative to replace the existing CrAg-based EIAs remains difficult in this first-time evaluation, due to the lack of well-defined cut-off values in different populations. Importantly, the data presented here are indicative of the need for further prospective studies to be conducted on a large scale, well-characterised population, with an emphasis on the rNiE antigen, provided that studies broadly support its enhanced specificity. This would meet the objective of improving the accuracy of *Strongyloides* serodiagnosis in the Australian population.

Acknowledgement: We thank the laboratory scientists of the Serology and Virology Division (SAViD) at the NSW Health Pathology SEALS Randwick for technical assistance, and the NSW Organ and Tissue Donation Service (OTDS) for collaboration on this study.

Conflicts of interest and sources of funding: The study is supported by the NSW Organ and Tissue Donation Service (OTDS). The authors state that there are no conflicts of interest to disclose.

**Yonghui Lu¹, Vidiya Ramachandran²,
Siddhartha Mahanty³, William D. Rawlinson^{1,2,*}**

¹Serology and Virology Division (SAViD), NSW Health Pathology Randwick, Level 4, Prince of Wales Hospital, Randwick, NSW, Australia; ²School of Medical Sciences and School of Women's and Children's Health, Faculty of Medicine, School of Biotechnology and Biomolecular Sciences, Faculty of Science, University of New South Wales, Randwick, NSW, Australia; ³The Peter Doherty Institute for Infection and Immunity, Department of Infectious Diseases, Melbourne Medical School, University of Melbourne, Melbourne, Vic, Australia; *joint senior author

Contact Prof William D. Rawlinson.
E-mail: w.rawlinson@unsw.edu.au

1. Vasquez-Rios G, Pineda-Reyes R, Pineda-Reyes J, *et al.* Strongyloides stercoralis hyperinfection syndrome: a deeper understanding of a neglected disease. *J Parasit Dis* 2019; 43: 167–75.
2. Buonfrate D, Formenti F, Perandin F, *et al.* Novel approaches to the diagnosis of Strongyloides stercoralis infection. *Clin Microbiol Infect* 2015; 21: 543–52.
3. Krolewiecki AJ, Ramanathan R, Fink V, *et al.* Improved diagnosis of Strongyloides stercoralis using recombinant antigen-based serologies in a community-wide study in northern Argentina. *Clin Vaccine Immunol* 2010; 17: 1624–30.
4. Requena-Mendez A, Chiodini P, Bisoffi Z, *et al.* The laboratory diagnosis and follow up of strongyloidiasis: a systematic review. *PLoS Negl Trop Dis* 2013; 7: e2002.
5. Fradejas I, Herrero-Martinez JM, Lizasoain M, *et al.* Comparative study of two commercial tests for Strongyloides stercoralis serologic diagnosis. *Trans R Soc Trop Med Hyg* 2018; 112: 561–7.
6. Ravi V, Ramachandran S, Thompson RW, *et al.* Characterization of a recombinant immunodiagnostic antigen (NIE) from Strongyloides stercoralis L3-stage larvae. *Mol Biochem Parasitol* 2002; 125: 73–81.
7. Ramanathan R, Burbelo PD, Groot S, *et al.* A luciferase immunoprecipitation systems assay enhances the sensitivity and specificity of

diagnosis of Strongyloides stercoralis infection. *J Infect Dis* 2008; 198: 444–51.

8. Bisoffi Z, Buonfrate D, Sequi M, *et al.* Diagnostic accuracy of five serologic tests for Strongyloides stercoralis infection. *PLoS Negl Trop Dis* 2014; 8: e2640.
9. Ruantip S, Eamudomkarn C, Techasen A, *et al.* Accuracy of urine and serum assays for the diagnosis of strongyloidiasis by three enzyme-linked immunosorbent assay protocols. *Am J Trop Med Hyg* 2019; 100: 127–9.
10. Van Doorn HR, Koelewijn R, Hofwegen H, *et al.* Use of enzyme-linked immunosorbent assay and dipstick assay for detection of Strongyloides stercoralis infection in humans. *J Clin Microbiol* 2007; 45: 438–42.
11. Bon B, Houze S, Talabani H, *et al.* Evaluation of a rapid enzyme-linked immunosorbent assay for diagnosis of strongyloidiasis. *J Clin Microbiol* 2010; 48: 1716–9.
12. Anderson NW, Klein DM, Dornink SM, *et al.* Comparison of three immunoassays for detection of antibodies to Strongyloides stercoralis. *Clin Vaccine Immunol* 2014; 21: 732–6.

DOI: <https://doi.org/10.1016/j.pathol.2021.03.002>

HMGCR autoantibody testing: two tiers required

Sir,

Autoimmune myopathies are a group of immune-mediated muscle diseases classifiable by clinicopathological patterns. The different patterns have been increasingly shown to associate with various antigen-specific autoantibodies.¹ Recently, the 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) autoantigen was added to others in a qualitative commercial line blot (Euroline Autoimmune Inflammatory Myopathies 16 Ag et cN-1A et HMGCR IgG; Euroimmun, Germany) allowing more convenient screening for 18 different myositis-specific autoantibodies.² Before introduction, all laboratories are required to verify assay performance in their local population using methods and analyses relevant to the assay's intended use.^{3,4}

In practice the detection of myositis-specific antibodies (MSA) is used in the diagnostic work-up of suspected autoimmune myositis. The original labour-intensive immunoprecipitation assays⁵ have been largely replaced by technically simpler automatable assays amenable to high throughput. Line blots of multiple individual antigens adsorbed to a solid phase matrix allow rapid and simultaneous screening.

Autoantigens adsorbed to solid matrices may be presented in a distorted conformation when compared with tissue-expressed autoantigens and those that remain in fluid phase. This may impact assay performance. The manufacturer of the Euroline blot has recommended that, where available and depending on the antigen, users seek further evidence of positivity using indirect immunofluorescence (IIF) staining on Hep-2 cells and/or primate tissues.² While lacking analytical specificity, the finding of an IIF staining pattern known to be consistent with the cellular distribution of the relevant myositis-specific autoantigen adds weight to the detection of disease-specific autoantibodies. However, data regarding their frequency of detection using these methods is generally lacking, which may limit diagnostic utility.¹ Alternative methods for HMGCR autoantibody detection include IIF staining on liver substrate (HMGCR associated liver IFL pattern, or HALIP)⁶ and immunoprecipitation.^{7,8}

Previously we described a counter-immunoelectrophoresis (CIEP) precipitation method for the detection of HMGR autoantibodies.⁷ Until recently our laboratory, Canterbury Health Laboratories, was the only laboratory in New Zealand (population circa 5 million) testing for HMGR autoantibodies and received samples from all regions. We used an in-house ELISA assay developed in 2014.⁸

In this verification study of the new Euroline blot we compared the performance of the newly added HMGR autoantigen with our in-house HMGR ELISA. In addition, as is recommended for a qualitative diagnostic test,⁴ we performed a case control study to determine the appropriateness of the manufacturer's recommended clinical decision limit and the utility of secondary testing using CIEP.

Archived sera from 51 patients formed the study population. Twenty-five case sera from clinically confirmed HMGR autoantibody positive (in-house ELISA) myositis patients were used. Cases were defined by the authors after consideration of clinical and laboratory evidence. Details such as myalgia, weakness, elevated creatinine kinase (CK), radiology, histology (biopsy cases $n=12$) and treatment response were collected along with the diagnostic opinion of the referring clinician. Thirteen cases were not biopsied but were included based on available clinical evidence. We cannot discount a degree of circular bias in the case definitions given the result of HMGR autoantibody testing was known to treating clinicians who were contacted retrospectively.

Twenty-six control sera were selected from patients with clinically overlapping disorders. Samples referred from patients with myalgia and/or weakness but no serum CK elevation, those with other autoimmune myositides, and those with interstitial lung disease, all with or without statin use, were included.

The in-house ELISA used human recombinant HMGR (Cayman Chemical, USA). A standard curve was generated from a known high titre sample which was serially diluted with a reporting interval of 0–210 AU/L. The cut-off for positivity (3 AU/L) had been previously determined using 101 healthy blood bank donors and 58 disease control sera.

Samples were incubated on Euroline autoimmune inflammatory myopathies 16 Ag et cN-1A et HMGR IgG test strips (i.e., containing 18 antigens) as per the manufacturer's instructions.² The density of the colour reaction was read on a CanoScan LiDE 200 scanner (Canon, Japan), which was calibrated with the EurolineScan insert and reported on a continuous scale. The manufacturer defined >10 AU as positive for all antigens with a reporting interval of zero to an undefined upper limit.

Immunoprecipitating HMGR autoantibodies were detected using CIEP as previously described.⁷ Undiluted patient sera were added to pre-punched circular wells in an agarose gel plate (SeaKem ME agarose; Lonza, Switzerland). Recombinant HMGR antigen (Cayman Chemical) was added to an adjacent trough before a counter current was applied. Coomassie blue was used to reveal the characteristic immunoprecipitating bands for visual inspection.

In the method comparison study 48 of the 51 samples were concordant ($\kappa=0.88$) between line blot and ELISA with a significant correlation for ranking ($\rho=0.815$, $p<1\times 10^{-13}$). Of the three discordant results, one control serum was positive by line blot but negative by ELISA and two control sera were positive by ELISA but negative by line blot.

In the case control study, the line blot had a clinical sensitivity of 25/25 (100%), and clinical specificity of 23/26 (88.5%). For dual positivity (both line blot and immunoprecipitation positive) the clinical sensitivity was 24/25 (96%) and specificity 26/26 (100%). One control sample (Fig. 1, gel 2 sample 17) produced a precipitin; however, this migrated closer to the antigen well and did not show a line of identity with adjacent positive sera, thus deemed negative.

We have shown that detection of HMGR autoantibodies using the Euroline blot had good concordance with our established in-house ELISA. We also showed that the line blot had a high estimated clinical sensitivity. However, it is diagnostic specificity that is primarily required by the requesting clinician. As a stand-alone assay we found the estimated diagnostic specificity was 88.5%. Dual positivity enhanced the diagnostic specificity.

Fig. 1 Counter-current immunoelectrophoresis of 51 patients (cases annotated with *). Gel 1 shows results of 19 samples. Gel 2 shows results of 32 samples. Gel 2 includes duplication of sample 15 (as labelled) and two different patient post-treatment samples not included in the 51 study samples (sample 6 and 27). Sample 3 and sample 17 on gel 2 (both control samples) show precipitin bands of undefined specificity.

The Euroline package insert quotes a study of the line blot giving an estimated false positive rate of 4% for HMGR autoantibodies in a population of healthy blood donors ($n=120$). In a comparison study of three other technologies all using HMGR antigen adsorbed to various solid phases the false positive rate in healthy controls ($n=517$) was 0.6% whereas in a disease control group ($n=117$) it was 2.6%.⁹ The disease control group used in our study estimated the false positive rate for the line blot at 11.5%.

Immunoprecipitation in agarose maintains analytes in fluid phase. The method also better replicates the method originally used to make the association between HMGR autoantibodies and immune necrotising myositis.⁵ Whilst it is relatively labour intensive, agarose gel immunoprecipitation is not technically demanding. As the rate of positives is relatively low, additional testing can be relatively easily accommodated into laboratory workflows.

Clinicians working in peripheral centres have difficulty obtaining muscle biopsy. Pragmatically they place reliance on detection of autoantibodies in making a diagnosis. In doing so, they risk a common error of diagnostic reasoning: the inverse fallacy, i.e., the conditional probability (MSA positive given myositis) erroneously 'inverts' to (myositis given MSA positive).¹⁰ Therefore, it behoves adopting laboratories to apply as much rigor as is feasible to verify the manufacturer's quoted clinical decision limits^{4,11} as suitable for a laboratory's catchment. For low prevalence diseases such as autoimmune myositis this may prove challenging.

There are several limitations to this study. Firstly, only 12 of the 25 cases had undergone muscle biopsy. Eleven of the 12 biopsies reported a necrotising pattern and one reported features consistent with an inclusion body pattern (gel 2, patient 18). However, the latter patient was on statin therapy at disease onset, had a CK in the 12000s and responded to immunosuppressive therapy. Despite the biopsy report, the treating clinician considered the patient's disease was more in keeping with immune-necrotising myositis associated with statin use.

Secondly, the control group was made up of a heterogeneous sample of patient serum which may not reflect the type of patients within the diagnostic differential for myositis. Ideally for a diagnostic test, this control group should reflect the differential diagnosis of disease in patients referred from the local catchment population. An appropriate control group is critical in deciding the clinical decision limit that maximises diagnostic accuracy.

Finally, we acknowledge that interpretation of CIEP has an element of subjectivity and requires expertise in reading the gels.

Based on our study results we have introduced the new Euroline blot as our preferred screening method. Our current experience suggests that the clinical decision limit/cut-off for positivity on the HMGR line-blot lies between 10 and 30 AU but we are yet to decide if the local clinical decision limit for the HMGR antigen should differ from that recommended by the manufacturer. A two-tier testing system for HMGR autoantibodies testing has been adopted: screen by HMGR line blot with results >10 AU then tested for immunoprecipitating antibodies using CIEP. We and others¹² recommend a similar approach in all diagnostic laboratories.

Conflicts of interest and sources of funding: The authors state that there are no conflicts of interest to disclose.

Ke Li Chow, Paula Elizabeth Keating, Myfanwy Bailey Spellerberg, Ignatius Chua, John Liston O'Donnell

Department of Immunology, Canterbury Health Laboratories, Christchurch, New Zealand

Contact Dr John Liston O'Donnell.

E-mail: john.odonnell@cdhb.health.nz

1. Palterer B, Vitiello G, Carraresi A, *et al.* Bench to bedside review of myositis autoantibodies. *Clin Mol Allergy* 2018; 16: 5.
2. Euroline Autoimmune Inflammatory Myopathies 16 Ag et cN-1A et HMGR (IgG) (package insert). Lübeck: Euroimmun, 2019.
3. NZS/ISO 15189:2012 Medical laboratories — particular requirements for quality and competence. Wellington: Standards New Zealand, 2012.
4. Clinical and Laboratory Standards Institute. User protocol for evaluation of qualitative test performance; approved guideline—second edition. CLSI document EP12-A2. Wayne, PA: Clinical and Laboratory Standards Institute, 2008.
5. Christopher-Stine L, Casciola-Rosen LA, Hong G, *et al.* A novel autoantibody recognizing 200 and 100 kDa proteins is associated with an immune-mediated necrotizing myopathy. *Arthritis Rheum* 2010; 62: 2757–66.
6. Alvarado-Cardenas M, Marin-Sánchez A, Martínez MA, *et al.* Statin-associated autoimmune myopathy: a distinct new IFL pattern can increase the rate of HMGR antibody detection by clinical laboratories. *Autoimmun Rev* 2016; 15: 1161–6.
7. Keating P, Van Voorthuizen M, Liu J, Chua I, O'Donnell J. Response to: new immunoassays for anti-HMG-CoA reductase antibodies may lead to incorrect diagnosis in inflammatory myositis. *Pathology* 2018; 50: 588–96.
8. Kennedy N, Keating P, O'Donnell J. HMGR-associated myositis: a New Zealand case series and estimate of incidence. *Intern Med J* 2016; 46: 622–5.
9. Musset L, Miyara M, Benveniste O, *et al.* Analysis of autoantibodies to 3-Hydroxy-3-methylglutaryl-coenzyme A Reductase using different technologies. *J Immunol Res* 2014; 2014: 405956.
10. Rushdi R, Rushdi AM. Common fallacies of probability in medical context: a simple mathematical exposition. *J Adv Med Med Res* 2018; 26: 1–21.
11. Ceriotti F, Henny J. "Are my laboratory results normal?" Considerations to be made concerning reference intervals and decision limits. *eJIFCC* 2008; 19: 1–9.
12. Dronay L, Gillis D, Wong R. New immunoassays for anti-HMG-CoA reductase antibodies may lead to incorrect diagnosis in inflammatory myositis. *Pathology* 2017; 49: 638–9.

DOI: <https://doi.org/10.1016/j.pathol.2021.03.010>

Evidence of chromosomal damage in scleroderma

Sir,

Systemic sclerosis (scleroderma, SSc) is an uncommon heterogeneous connective tissue disease considered to have an autoimmune origin.¹ Cancer occurs more commonly in scleroderma² and we have previously documented acquired genetic damage at the glycophorin-A locus suggesting genomic instability.³

Seeking further evidence for genomic instability we examined peripheral blood lymphocytes (PBL) from scleroderma patients using the well validated and robust cytokinesis-