Emerging New Tests for Detecting Autoantibodies

Autoimmune Diseases and Autoantibodies

Autoimmune diseases (AID) are a heterogeneous group of diseases in which structural and functional damage to one or more components of the body is generated by the action of immunologically competent cells or antibodies (known as autoantibodies) against the normal components of the body [1]. Underpinning AID pathogenesis is the immune system’s loss of self-tolerance, where the immune system attacks the host’s own cells, tissues, organs, or organ systems. AID can range from organ-specific disorders, such as autoimmune thyroiditis, type 1 Diabetes Mellitus (DM), to whole-body (systemic) disorders like Systemic Lupus Erythematosus (SLE), Rheumatoid Arthritis (RA). AID include more than 70 different disorders affecting approximately 5% of the population of the Western countries [2]. A recent study on systemic autoimmune rheumatic disease (SARD) across 7 Canadian provinces estimated the prevalence of SARD between 2–5 cases per 1000 Canadians, overall, and prevalence approaches or exceeds 1% in older women [3].

Although the etiology of AID is largely unknown, studies have shown that genetic, hormonal and environmental factors contribute to their pathogenesis. Most AID are associated with a specific major histocompatibility complex (MHC), like class I or class II human leukocyte antigen (HLA) molecule. For example, class II HLA-DRB1 genes are strongly associated with RA in individuals of northern European ancestry. In
addition to genetic factors, environmental factors, like infectious and non-infectious agents, can cause an immune reaction that precipitates an autoimmune reaction and leads to an AID. For example, Epstein-Barr Virus (EBV), which causes mononucleosis, has been associated with the development of SLE. As for hormonal factors estrogen and progesterone can promote humoral autoreactivity [4]. Complex interactions of genetic, hormonal and environmental factors contribute to the development of AIDs.

Autoantibodies are a hallmark of many AID and the presence of autoantibodies is a key characteristic of AID. For some AID, such as SLE and scleroderma, the presence in serum of certain autoantibodies represents one of the classification criteria. These autoantibodies may be directed against a variety of substrates including intra-cellular, cell surface and extracellular antigens.

How are autoantibodies used in the diagnosis of autoimmune diseases?

The diagnosis of AID is difficult and often requires a detailed clinical history, physical examination, and multiple laboratory tests which include routine complete blood count, inflammatory markers, complements, flow cytometry, and autoantibodies [5]. Complicating the diagnosis of AID is the fact that biological autoimmunity is not always pathologic. For example, anti-nuclear antibodies (ANAs), which are used in the diagnosis of SLE and other overlap syndromes, can be found in as much as 25% of the general population [6]. Generally, the detection of autoantibodies is important in the diagnostic criteria for AID, as in the case of SLE. Autoantibodies can also be used as prognostic indicators to anticipate the future clinical manifestation of an AID in addition to assessing the overall disease activity in a patient.

How do we test for autoimmune diseases?

Laboratory testing for AID involve the use of several different methodologies. The most common methodologies include indirect immunofluorescence (IIF), enzyme-linked immunosorbent assay (ELISA), and immunoblotting. In IIF, the patient sample (serum or cerebrospinal fluid) is incubated with a fixed animal tissue or cells. The autoantibodies in the patient sample will bind to the specific antigens and after a washing step, the bound autoantibodies will be visualized and analyzed under a microscope and the staining patterns and titers will be reported. For enzyme immunoassay methods, antigen coated reagent wells are incubated with the patient sample. Following a wash step, a detection antibody is added to visualize the autoantibody bound to the antigen. Finally, immunoblotting methods utilized recombinant antigens that are covalently attached to an absorbent membrane. Following incubating with the patient sample and washing, a detection antibody is added to visualize the autoantibodies.

All of these methods have advantages and disadvantage that must be considered carefully [Table 1]. In the case of IIF, it is the gold standard in the assessment of autoantibodies in many AID and is highly sensitive. However, this method is highly time consuming, requires experienced staff, has a high false positive rate due to non-specific binding of the autoantibodies, and the interpretation is subjective. Enzyme immunoassay (EIA) methods, like ELISA, are less subjective than IIF and can be automated. A disadvantage of EIA includes the use of recombinant antigens in various assays, which may cause false negatives. Further, non-specific binding can cause false positives. Immunoblot methods are to identify multiple antibodies concurrently and, similar to EIA, is both less subjective and more sensitive and specific relative to IFA. Contemporary methods utilize the multiplex immunoassays, where multiple antigens are coated on beads that contain a bead-specific fluorescent signal. Following incubation of the sample with the beads, they are simultaneously analyzed and reported.
Table 1. Brief description of common autoantibody testing methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
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<tbody>
<tr>
<td>IIF</td>
<td>Sensitive test for screening</td>
</tr>
<tr>
<td>EIA</td>
<td>Sensitive and Quantitative; Can be automated</td>
</tr>
<tr>
<td>Immunoblot</td>
<td>Very Sensitive, Specific, Detect Multiple Analytes simultaneously</td>
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<tr>
<td>Multiplex</td>
<td>Rapid, Sensitive, Specific, Detect Multiple Analytes simultaneously</td>
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Autoantibody testing at the Clinical Chemistry and Immunology Laboratory

At present, the Clinical Chemistry & Immunology (CCI) Division of the Hamilton Regional Laboratory Medicine Program (HLRMP) employ the use of IIF, EIA and Multiplexing assays in the investigations of various autoantibodies which include anti-smooth muscle antibody, anti-neuronal antibody, anti-LKM antibody and anti-nuclear antibody. Currently, inflammatory myopathy (dermatomyositis/polymyositis), autoimmune encephalitis, and paraneoplastic neurological syndrome (PNS) antibody testing are sent out to other centers. CCI has procured an automated Immunoblot System (EuroBlotMaster) [Figure 1] and is currently validating the immunoblot testings for detection of inflammatory myopathies (Myositis 12 Ag panel) and PNS (12 Ag panel). CCI is also validating a cell-based IIF assay for autoimmune encephalitis panel by utilizing newly procured automated IIF system (IF Sprinter; Euroimmune) [Figure 2].

Substantial progress has been achieved in the field of both AID and AID diagnostic testing. The increasing characterization of the nature and biochemical structure of autoantibodies along with the availability of purified antigen preparations and technological analytical advances has substantially increased the analytical and diagnostic sensitivity and accuracy of laboratory investigations. Emerging new autoantibody tests, in turn, have led to increased testing requests from physicians in order to correlate clinical and other laboratory findings. Clinical Immunology Laboratory plays an important role in the diagnosis, monitoring, and prediction of AID clinical outcomes. Finally, the close collaboration between laboratory professional staff and clinicians, particularly around the advantages and limitations of each methodology, is a critical component in establishing new tests and their appropriate clinical utilization.

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References
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News from Administration

Dear HRLMP Faculty and Staff:

**RE: National Medical Laboratory Week**

In recognition of National Medical Laboratory Week, we would like to thank each of you for the work you do each day in support of our patients. Your commitment to patient care, patient safety, and each other in a challenging time is demonstrated every day and so very much appreciated!

We really value the ongoing work of the HRLMP to support all of the clinical services and patients throughout the year. HRLMP is an integral part of the care for both HHS and SJ’s.

With the remarkable dedication and contributions of all the HRLMP laboratory professionals, the HRLMP has processed over 15 million tests in the past year! This exemplifies your commitment and dedication to healthcare and our vital role in the healthcare system. Since the beginning of the pandemic, over 530,000 COVID tests have been performed.

HRLMP laboratory staff continue to provide quality, timely and accurate patient results and are proud of the work they do each and every day. Our unprecedented virtual Accreditation visit in October 2020 was very successful. Staff are committed and dedicated to providing exceptional patient care by adhering to the Accreditation Canada Diagnostics (ACD – previously IQMH) requirements.

We have much to look forward to after the pandemic is over. This transformational year will include the rollout and implementation of our Strategic Plans, including the relocation of Genetics and the redevelopment of Pathology.

We hope that you have an opportunity to celebrate NMLW this week. We look forward to the announcement of the winners of this year’s HRLMP Medical Laboratory Excellence Awards. Thank you again for your unwavering commitment to patient care.

Sincerely,

**Gail Johnson**, Executive Director, HRLMP

**Dr. Marek Smieja**, Interim Medical Director, HRLMP; Interim Chief, Laboratory Medicine, Hamilton Health Sciences and St. Joseph’s Healthcare

**Sandra Fazari**, Director of Laboratory Operations, HRLMP

**Mr. Rick Badzioch**, VP Clinical Programs, St. Joseph’s Healthcare

**Leslie Gauthier**, Vice President, Clinical Support Services and Surgery, Hamilton Health Sciences
Dear Colleagues:

In recognition of National Medical Laboratory Week, and on behalf of the Department of Pathology and Molecular Medicine, I would like to thank all our HRLMP colleagues who contribute day after day to the academic mission of our department. Whether it is teaching a medical student or resident at the bench or answering their clinical queries, helping with undergraduate and graduate student projects, working on research samples just as diligently as clinical samples, or leading research and development projects, your contributions are an essential part of our university activities.

Murray Potter, MD, FRCPC, FCCMG
Chair and Professor
Department of Pathology and Molecular Medicine
McMaster University

Click on the links below to read the Thank you to Canadian and Ontario Laboratory Professionals from:

Prime Minister Justin Trudeau

CPHO Dr. Theresa Tam
https://twitter.com/CPHO_Canada/status/1381692588143611909

Doug Ford, Premier of Ontario
After 35 years with HRLMP, Julie Donnelly is retiring! Julie started out as a casual secretary in Lab Administration at HGH and has spent the last 20 years at SJHH working closely to support our administrative and quality teams.

Her last day with us will be on June 11, 2021. She plans to spend lots of time cycling with her husband and spending time with friends and family.

It has been a pleasure to get to know Julie over the past year and we all wish her the best of luck!

Submitted by: Amanda Hurdowar
Project and Office Operations Manager, HRLMP

Our Passport to HRLMP virtual celebration was a great success! Staff had a virtual passport to fill as they visited a different laboratory each day of the week for a virtual tour to see the lab, learn about what they do, and explore some interesting case studies.

A big thank you to our tour guides who took us through:

- **Clinical Chemistry and Immunology** to learn about trace metal testing;
- **Genetics Jungle** to learn about their vast array of testing;
- **Flow Cytometry** to learn that nothing in flow is black and white – it’s 10 colours;
- **Electron Microscopy** to see some amazing images of cellular structures;
- **Microbiology** for an interesting case study of a patient with a life-threatening infection.

In addition, on Wednesday April 14, 2021, we had a virtual retreat hosted by Michelle Cassidy, HHS Healthy Workplace Coordinator, who shared practical strategies to help us care for and promote our mental health.

Michelle’s talk was very timely as we continue to navigate through this pandemic. Thank you Michelle for an excellent talk, and for providing ways for us to practice self-compassion.

Education News

Congratulations to all Laboratory Professionals as we celebrated National Medical Laboratory Week from April 11 -17, 2021.
We would also like to congratulate the nominees of our 2021 Medical Laboratory Technology Excellence Awards.

We received over 30 nominations for outstanding individuals who go above and beyond to provide excellent patient care and truly demonstrate excellence in the profession.

**Congratulations** to all of the nominees!
The winners of the awards are:
- Teaching (Individual) – Karen Moffat
- Teaching (Team) – Cytology Laboratory
- Leadership – Kristina Wilson
- Quality Improvement – Jill Boreyko
- Innovation – Candy Rutherford
- Teamwork – Scott Phillips
- Teamwork – Michele Perry
- Interprofessional Practice – Ron Mackay
- Outstanding Achievement – Paula Costa
- Outstanding Achievement – Amie Black

**Thank you** to Marilyn Garzon, MLT, for providing a virtual tour of the SJHH Core Laboratory to the Bachelor of Medical Laboratory Science (BMLS) interns of San Pedro University in Davao City, Philippines on February 27, 2021.

The tour provided an opportunity for the MLT students to “see” our instrumentation and for Marilyn to share how our operations work.

They were especially captivated by our automated quality control practices.

Thank you Marilyn, for sharing your expertise and showcasing HRLMP … all the way to the Philippines!

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**News from Genetics**

The HRLMP Genetics Laboratory, in partnership with the HRLMP Virology Laboratory and McMaster University, has been chosen as a provincial site for clinical COVID sequencing.

This is a new network of four hospital-based laboratories, working with the Public Health Laboratory, to provide detailed sequencing data.

This will confirm variants of concern (VOC), monitor for newly emerging variants, and help Public Health to monitor COVID spread. We would like to thank Barry Eng, John Duck and Landry Nfonsam from the Genetics Lab for establishing testing protocols and a bioinformatics pipeline for VOC sequencing.

We would like to welcome back Anna Haasen who has accepted the temporary Technical Specialist role in the Genetics lab.

Anna has multiple years of experience as an MLT in Genetics and over the last three years has worked as a Quality Specialist in HRLMP Administration.

Anna is a great addition to our team due to her technical expertise and extensive knowledge of Quality and Accreditation.
Happy National Med Lab Week to my amazing lab team - the HRLMP Molecular Diagnostic Genetics Lab.

We reached an incredible milestone on March 22, 2021, when we received our 100 000th sample in our lab!

Dr. David Chui, with only one technologist, started the DNA lab back in 1989. Molecular genetic testing was in its infancy, as PCR had only been invented in 1985. Since that time, we have grown to four genetics technologists, three medical lab assistants, our technical specialist Barry Eng and our Director Dr. John Waye.

We are now the provincial reference lab for hemoglobinopathies and we test samples from across Ontario as well as across Canada.

I am proud to be a part of this amazing group, whose knowledge and expertise humbles me every day. Each person on our team works hard and always puts our patients first. A most heartfelt congrats to my world-class colleagues for a job well done.

Here’s to the next 100 000...

Submitted by: Meredith Hanna

Hematology News

HRLMP/HHS Stem cell laboratory plays key role in groundbreaking gene therapy study

Dr. Ronan Foley is an HHS clinical hematologist and Director of the Clinical Stem Cell Laboratory, HRLMP.

A Canadian research team, that included HHS, is the first to use gene therapy to treat patients with Fabry disease.

“This study – with multiple healthcare partners across the country – really is a success story that beat all expectations.” ~Dr. Foley

To read the full story, click on the link below: https://www.hamiltonhealthsciences.ca/share/fabrystudy/

Dr. Donald Arnold and Dr. Menaka Pai were interviewed on CTV news and discussed the risk of VITT / VIPIT and the AstraZenica vaccine and the work being done in the Platelet Immunology laboratory.

Click on the link below to watch the CTV video: https://www.ctvnews.ca/video?clipId=2171479&jwsource=e
To read more about the work being done in the Platelet Immunology laboratory, click on the link below:
https://brighterworld.mcmaster.ca/articles/mcmaster-lab-diagnoses-blood-clots-related-to-covid-19-vaccine/

Microbiology News

On February 8, 2021 the Microbiology laboratory took on the microbiology work for the new Cortellucci Vaughan hospital.

This hospital opened to help address the surge in COVID-19 cases in Ontario. The hospital’s main focus is to create additional space to care for critical and acute care patients.

The microbiology staff have been working hard to ensure that this new hospital receives the best service that we can provide. Their efforts are to be applauded.

McMaster Day in Hematology
Friday June 11, 2021

Please review the attached brochure for more information on the inaugural, virtual McMaster Day in Hematology.

News from Pathology

This year the Canadian Association of Pathologists has created an entire yearlong series of virtual education events that have been well received by laboratory doctors across the country. Dr. Catherine Ross, the CAP-ACP President, will be the moderator for these sessions.

The week of April 19th, 2021, there is a virtual course Applied Molecular Pathology being offered by CAP-ACP in conjunction with the Ontario Molecular Pathology Research Network (OMPRN).
Lab Connections

This promises to be a great offering for pathology and genetics, and one of the highlighted speakers this year is our own Dr. Asghar Naqvi from St. Joseph’s Healthcare who will be addressing molecular biomarkers in lung carcinoma on April 22, 2021.

Quality News

With a successful virtual peer assessment behind us, we carry on that momentum into preparing for our upcoming internal audit. We put a call out for MLT volunteers to join our audit team; and we had an overwhelming response. Our new internal assessors are currently completing their training and will soon be visiting labs for the internal assessments. From May 3rd to May 14th, welcome them and show them all the amazing work you are doing in your departments. The internal audit is an excellent way to identify opportunities for improvement and for us to become familiar with the new Version 8.0 requirements.

We would like to take this opportunity to thank Anna Haase for her drive and commitment to quality improvement these last 2.5 years. Anna has been an integral part of facilitating our Quality Leads team, updating our training processes, and among other projects, leading departments through risk management strategies. We know that Anna will bring that same drive to Genetics as the temporary Genetics Technical Specialist until November 2022.

We would like to welcome Jayne Clemens to the Quality Team! Jayne comes to Quality from Malignant Hematology where she has played an active role in various CQI initiatives. With a Certificate in Quality Improvement from The Institute for Healthcare Improvement and her involvement in other professional development activities, Jayne will bring a fresh perspective to this temporary Quality Specialist position.

Research News

lluminight is being reimagined as a virtual event.

From October 8 to 17, 2021 we invite you to “walk to shine a light on cancer” from the safety of your own community, while fundraising for the Cancer Program at Juravinski Hospital and Cancer Centre.

Click on the link below to register and/or donate: http://events.hamiltonhealth.ca/site/TR/Events/TeamraisersTheme1?fr_id=2413&pg=entry

We would like to thank Josephine Baldwin for her work as the temporary HRLMP Research...
Coordinator over the last year. Josephine’s last day with the HRLMP was April 8, 2021.

We would like to welcome back Mackensey Bacon as she returned from her leave on April 1, 2021.

Please email Mackensey (baconmack@hhsc.ca) with your research questions for how HRLMP can work with you and your team.

Choosing Wisely Canada

Choosing Wisely Canada, in collaboration with the CSTM Canadian Obstetrics and Pediatric Transfusion Network (COPTN), has released four new Perinatal recommendations.

To review these new recommendations, please click on the link below:
https://choosingwiselycanada.org/transfusion-medicine/