Recent developments in early detection strategies and population-based screening: the perspectives of cervical cancer and COVID-19

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Abstract

Early detection strategies and population-based screening are important public health tools in early detection of disease and population surveillance. This work aimed to examine cervical cancer and COVID-19 with a focus on new strategies for early detection and population-based screening data; these approaches can help detect pre-cancerous lesions before they develop into cervical cancer and can help to better understand the spread of the SARS-CoV-2 virus in the general population. While cervical cancer screening is a long-standing program, requiring review of existing programs and new methodologies, the emergence of the COVID-19 requires an initial evaluation of new test methodologies. Cervical cancer data was collected through testing enrolled patient specimens and programmatic data, and COVID-19 data was collected from testing de-identified patient specimens. This dissertation is comprised of three studies (4 manuscripts). The first study reviewed the Newfoundland and Labrador (NL) cervical screening program to assess positivity and clinical disease endpoint and reviewed programmatic indicators to determine the ability of the program to detect pre-cancerous lesions. The second study evaluated the diagnostic indices and properties of CINtec PLUS and cobas HPV tests among those referred to colposcopy with a history of low-grade squamous intraepithelial lesions (LSIL) to identify those at increased risk of pre-cancerous lesions and cervical cancer and potentially reduce the proportion requiring further follow-up in all age groups, for those <30 years of age, and those $\geq$ 30 years of age. Finally, the

third study evaluated three (2 different IgG and 1 IgA) serological tests' abilities to detect prior infection with SARS-CoV-2 from laboratory-confirmed COVID-19.

The findings indicate in the first study that while there have been attempts to improve cervical screening participation, high rates of abnormalities, pre-cancerous lesions, and invasive cancers are troubling. In the second study, high sensitivity (93.2%) and negative predictive value (NPV, 98.1% for CINtec PLUS, 97.0% for cobas) were observed in patients referred to colposcopy with a history of LSIL cytology for CINtec PLUS cytology and the cobas HPV test (CIN3+). However, the reduced sensitivity of CINtec PLUS for detection of CIN2+ in general (81.8% for CINtec PLUS, 93.9% for cobas), and CIN 2, especially in patients <30 years, needs to be considered in risk assessments if choosing LSIL-CINtec PLUS triage. Nevertheless, CINtec PLUS was consistently more specific than the HPV test. In the third study, observed sensitivities ranged from 91.3-100.0% and specificities of 90.8-98.2%; cross-reactivity was observed in the IgA test. A two-tiered approach was observed to improve performance in low prevalence settings.

In conclusion, based on the review of local cervical screening programs, there are opportunities for improvement. Either test examined could serve as a predictor of CIN3+ with high sensitivity in patients referred to colposcopy with a history of LSIL regardless of age while significantly reducing the number of LSIL referral patients requiring further investigations and follow-up in colposcopy clinics. For COVID-19, IgG tests may serve as practical tools in helping detect past SARS-CoV-2 infection.

General Summary

Early detection strategies and population-based screening are important public health tools in early detection of disease and population surveillance. This work aimed to examine cervical cancer and COVID-19 with a focus on new strategies for early detection and population-based screening data; these approaches can help detect pre-cancerous lesions before they develop into cervical cancer and can help to better understand the spread of the SARS-CoV-2 virus in the general population. Our study looked at new ways to detect and manage cervical cancer screening and how new tests could help detect past COVID-19 infection. This dissertation is comprised of three studies (4 manuscripts). These studies were performed using test results and data from programs. The first study reviewed the Newfoundland and Labrador (NL) cervical screening program to assess the current tests' performance and to look at possible improvements. The second study assessed how a new type of test that looks at cellular changes (CINtec PLUS) and a test that looks for HPV (cobas test) performed for patients referred to for additional follow up after a history of low-grade cervical abnormalities (LSIL) to identify those at increased risk and potentially reduce the proportion requiring further follow-up. The third study evaluated three different blood tests to detect previous infection with SARS-CoV-2 from laboratory-confirmed COVID-19.

The findings indicated that while there have been some improvements, there are still high rates of abnormalities, pre-cancerous lesions, and invasive cancers in NL. In the second study, there was high sensitivity and negative predictive value (NPV) for detecting

CIN3+ in both methods, but the reduced sensitivity of CINtec PLUS for detection of CIN2+ in general, and CIN 2, especially in patients <30 years, needs to be considered in any risk assessments if considering LSIL-CINtec PLUS triage. Nevertheless, CINtec PLUS was consistently more specific than the HPV test. In the third study, high sensitivities and specificities were observed for IgG tests, but cross-reactivity was observed in the IgA test making it a less desirable choice. The utilization of multiple IgG tests together was observed to improve performance in low prevalence settings.

In conclusion, based on the review of local cervical screening programs, there are opportunities for improvement. Either test examined could serve as a predictor of CIN3+ with high sensitivity in patients referred to colposcopy with a history of LSIL regardless of age while significantly reducing the number of LSIL referral patients requiring further investigations and follow-up in colposcopy clinics. For COVID-19, IgG tests may serve as practical tools in helping detect past SARS-CoV-2 infection.

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Thank you to Nespresso. Without your coffee this would not have been possible.

And, Dolly Parton… always.

Glossary

AGC	Atypical Glandular Cells
A-IgG	Abbott Architect SARS-CoV-2 Immunoglobulin G
AIS	Adenocarcinoma in situ
ALTS	ASCUS-LSIL Triage Study
ASC-H	Atypical Squamous Cells cannot exclude HSIL
ASCUS	Atypical Squamous Cells of Unknown Significance
ATHENA	Addressing the Need for Advanced HPV Diagnostics
BC	British Columbia
BD	Becton, Dickinson and Company
CACMID	Canadian Association for Clinical Microbiology and Infectious Diseases
CCPN	Cervical Cancer Prevention Network
CI	Confidence Interval
CIN	Cervical intraepithelial neoplasia
CIS	Carcinoma in situ
CMIA	Chemiluminescent microparticle immunoassay

COVID-19	Coronavirus disease of 2019
CPHLN	Canadian Public Health Laboratory Network
DALY	Disability-adjusted Life Year
DHCS	Department of Health and Community Services
DNA	Deoxyribonucleic acid
EBV	Epstein-Barr Virus
EBNA	EBV Nuclear Antigen
HER	Electronic Health Record
EI	EuroImmun
EI-IgA	EuroImmun Anti-SARS-CoV-2 ELISA Immunoglobulin A
EI-IgG	EuroImmun Anti-SARS-CoV-2 ELISA Immunoglobulin G
ELISA	Enzyme-linked immunosorbent assay
FTP	Federal, Territorial, and Provincial
HCMV	Human Cytomegalovirus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus

HPV	Human Papillomavirus
HREB	Health Research Ethics Board
hr-HPV	High-risk HPV (genotypes)
HSIL	High-grade squamous intraepithelial lesion
HSV-1/2	Herpes simplex virus 1/2
ICU	Intensive Care Unit
IgA	Immunoglobulin A
IgG	Immunoglobulin G
It-RNA	in-vitro transcribed RNA
LBC	Liquid-Based Cytology
LLOD	Lower Limit of Detection
LOD	Limit of Detection
lr-HPV	Low-risk HPV (genotypes)
LSIL	Low-grade squamous intraepithelial lesion
LSIL-H	LSIL cannot exclude HSIL
MCM-TOP2a	Mini-chromosome Maintenance protein and DNA topoisomerase IIA

NAAT	Nucleic Acid Amplification Test
NB	New Brunswick
NL	Newfoundland and Labrador
NLCHI	Newfoundland and Labrador Centre for Health Information
NPV	Negative Predictive Value
OHR	Other High Risk (genotypes)
ON	Ontario
ORF	Open Reading Frame
PAP	Papanicolaou
PEI	Prince Edward Island
PCCSN	Pan-Canadian Cervical Cancer Screening Network
PCR	Polymerase Chain Reaction
PHML	Public Health and Microbiology Laboratory
PPV	Positive Predictive Value
QC	Quebec
RBD	Receptor-binding Domain

RPAC	Research Proposal Approval Committee
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SCC	Squamous Cell Carcinoma
SDH	Social Determinants of Health
SES	Socioeconomic Status
SIN	Squamous Intraepithelial Neoplasia
ROC	Receiver Operating Characteristic
RNA	Ribonucleic acid
RT-qPCR	Quantitative Reverse Transcription PCR
TP	*Treponema pallidum*
UK	United Kingdom
URR	Upstream Regulatory Region
USA	United States of America
VCA	Viral Capsid Antigen
VIA	Visual Inspection with Acetic Acid
VOC	Variant of Concern

VOI	Variant of Interest
VUM	Variant Under Monitoring
VZV	Varicella Zoster Virus
WHO	World Health Organization

List of Tables

List of Figures

Table of Contents

Preamble

The dissertation work presented was conducted in manuscript format and is presented as such.

The initial focus of my work was on cervical cancer screening programs, opportunities for improvement, and potential applications for new tests. The manuscripts covered by this work are presented in Chapters 3, 4, and 5.

As the world dramatically changed due to the COVID-19 pandemic, so too did my work. Consequently, Chapter 6 represents my work surrounding the SARS-CoV-2 virus and serological screening tests.

The work's distribution is based chiefly on cervical cancer screening; therefore, you will see this reflected in the introductory, background, and conclusions. Methodology is included in each manuscript (Chapters 3-6). In addition, the rationale for the inclusion of both topics is included in section 1.1.

Chapter 1 : Introduction

1.1 Public health screening: connection between cervical cancer and COVID-19

Public health and epidemiology have been lifelong passions of mine. My essays in secondary school seemed to always go back to medicine. My public speaking topics were always about equitable access to health care[1] and public health. My free time was filled with books about infectious diseases and epidemiology.

Over a decade ago, I was given the opportunity to work for Dr. Ratnam at the Public Health Laboratory; that was when I was introduced to epidemiology and public health screening. Over the years, I have been lucky enough to assist and participate in cervical cancer screening research, public health epidemiology, and disease surveillance activities. Potential improvements to existing programs are both fascinating and critical to systemic improvement. It was a natural fit for my dissertation work to include cervical cancer screening programs and applications of new tests.

However, working in epidemiology and public health means recognizing and adapting to new challenges in one's work. The coronavirus disease 2019 (COVID-19) pandemic has truly illustrated the necessity of such flexibility and resilience. In December 2019, public health professionals became aware of a growing threat of severe acute respiratory

[1] My favorite was in Grade 9 and stressed the importance of access to anti-retroviral therapy in sub-Saharan Africa.

syndrome of unknown cause in Wuhan, China (McKenna, 2020). By January 30, 2020, the World Health Organization (WHO) declared the situation a Public Health Emergency of International Concern (Cucinotta & Vanelli, 2020) (Coronaviridae Study Group of the International Committee on Taxonomy of Viruses, 2020). On February 11, 2020, WHO named COVID-19, an acronym for Coronavirus disease of 2019 (Lovelace Jr, 2020). From the rapid spread of COVID-19 disease to the lightning speed of the discovery of the causative virus, SARS-CoV-2, and developments of molecular tests, epidemiologists and public health professionals began to prepare for the worst. The World Health Organization had declared COVID-19 a pandemic on March 11, 2020 (Cucinotta & Vanelli, 2020). As of January 23, 2022, there have been over 346 million cases and over 5.5 million deaths globally (World Health Organization, 2022).

In a crisis, an "all hands-on deck" and collaborative approach is critical to a cohesive and effective response plan. Professionally, in my role as Public Health Informatician at the Newfoundland and Labrador Public Health Microbiology Laboratory, I was faced with one of the most challenging experiences of my career when our work completely shifted to address the growing pandemic provincially. As a result, changes have been made across many aspects of our organization, including implementing new methodologies, validation, and verification of new tests, reporting 24/7/365 work schedule and reporting, and complete redevelopment of our staffing, organizational structure, and physical space. So naturally, the current global pandemic has also impacted

my work academically. The additional workload has meant many overtime hours for the past 14 months, depending on outbreaks and surges at the provincial level.

The basic principles of public health screening are interchangeable, balancing individual care needs and population health assessment of risk, and appropriate selection of tests and strategies logically. As such, the inclusion of SARS-CoV-2 serological work along with my work in cervical cancer screening is appropriate and reflective of real-world necessity and practice.

Cervical cancer screening programs are well-established, long-standing, and successful public health programs. Over the decades, many lessons have been learned while these programs have been in place. When new pathogens emerge, public health professionals do not need to re-invent the wheel: using firm, pragmatic approaches in implementing new population health screening programs makes sense financially, clinically, and operationally.

Evaluation of new tests and the identification of how they can fit into an active public health context is paramount. This is no different in the case of new tests like CINtec PLUS or Human Papillomavirus (HPV) testing in the case of long-standing Papanicolaou (PAP) test cervical screening programs or SARS-CoV-2 tests in an unknown and constantly evolving situation. When COVID-19 emerged, the priority was for timely and reliable diagnostics and molecular methodologies to meet that need. As needs evolved during the pandemic, the need to better identify active symptomatic disease versus past exposure

and asymptomatic infections from a public health perspective also became necessary. While SARS-CoV-2 and HPV tests and their applications are different, it is important to remember that their net impact and end results on public health systems are similar.

1.2 Overall Impact of Infectious Diseases

The idea that some lives matter less is the root of all that is wrong in the world.

Paul Farmer

The burden of infectious diseases globally is enormous and disproportionate. In 2016, almost 10 million people died of infectious diseases globally, accounting for 1/5 of all deaths (Furuse, 2019). To illustrate the overall impact and importance, in 2019, six infectious diseases (lower respiratory infections, diarrheal diseases, malaria, meningitis, whooping cough, and sexually transmitted infections) were still among the top ten causes of disability-adjusted life years (DALYs) in children under ten years of age (GBD 2019 Diseases and Injuries Collaborators, 2020). Over time we have seen a shift from infectious diseases being the urgent health care focus to an increased emphasis on cardiovascular disease, cancer, and chronic illness (McFee, 2013).

The burden of infectious diseases also disproportionately affects developing countries in comparison with wealthy or more developed countries (Farmer, Infections and inequalities, 1999) (Farmer, Pathologies of Power: Health, human rights, and the new war on the poor, 2005) (Moss, Liu, & Feuer, 2017) and between socioeconomic groups within nations (Pini, et al., 2019). For example, in 2001, 76.4% of all infectious disease deaths occurred in sub-Saharan Africa and South Asia (Michaud, 2009). Additionally, children, women, and vulnerable persons and populations are also more likely to be

negatively impacted by infectious disease morbidity and mortality (Pini, et al., 2019) (Farmer, Infections and inequalities, 1999) (Budgell, 2018).

From a Canadian perspective, there have been general decreases in infectious disease rates due to improvements in public health, such as vaccination programs, increased health promotion and communication, improved drinking water, etc. (Reported cases from 1924 to 2019 in Canada - Notifiable diseases on-line, 2021). However, infections are still rising, and marginalized communities are disproportionately impacted by higher than average rates (Jetty, 2020) (Ontario HIV Treatment Network, 2019). From a Newfoundland and Labrador perspective, the province had very high rates of infectious diseases until the middle of the twentieth century (Collier, 2011). Unfortunately, there is limited, recent publicly available data on the status of infectious diseases in the province (Government of Newfoundland and Labrador, n.d.). However, certain regions have disproportionately high rates of infectious diseases, such as *Mycobacterium tuberculosis*, at 50X the national rate (Mercer, 2020).

While it has been reported that the burden of infectious disease pathogens has been on the decline in recent years, now is not the time to be complacent, particularly as the risk of emerging infectious disease remains ever-present and in light of the SARS-CoV-2 pandemic[2]. To address the impact of infectious disease, we need to holistically

[2] There has also been a re-emergence of diphtheria, trench fever, measles, and scarlet fever (Venkatesan, 2021). In addition, Ebola has reemerged several times over the past four decades with a large outbreak in 2014-2016, and in some countries, polio still rears its ugly head.

understand the physical and psychosocial effects, the epidemiology of the pathogen, and risk factors for programmatic planning logistics to best serve communities in need.

1.3 Cervical Cancer & HPV

Cancer begins and ends with people. In the midst of scientific abstraction, it is sometimes possible to forget this one basic fact…

Siddhartha Mukherjee (Mukherjee, 2010)

Worldwide 275,000 lives are lost annually due to cervical cancer (Safaeian & Sherman, 2013). Nationally, cervical cancer incidence and mortality have decreased despite increasing risk factors (Dickenson, et al., 2012). While cytology refers to the general examination of cells, Papanicolaou or Pap cytology tests or smears are screening tests that involve collecting cells from a cervix and examining the cells for abnormalities under microscope. Nationally, it was reported that in 2017, 74.0% of women aged 25-69 years did have a Pap test in the past three years (Statistics Canada, 2018). While uptake of cervical screening services in the Newfoundland and Labrador has increased, a fraction of the population (~23%) is still not availing of the service (Cancer Care, Eastern Health, 2018).

Further details about cervical abnormalities and progression to cancer is included in Chapter 2. As a brief introduction, Pap cytology can identify various pap results. The

following results range from the most benign to abnormal growth of cervical cells or dysplasia to higher cellular involvement; atypical squamous cells of undetermined significance (ASCUS), atypical squamous cells cannot rule out high grade squamous intraepithelial lesion (ASC-H), atypical glandular cells (AGC) which includes several different categories, low grade squamous intraepithelial lesion (LSIL), LSIL cannot rule out high grade squamous intraepithelial lesion (LSIL-H), high grade squamous intraepithelial lesion (HSIL), and adenocarcinoma in situ (AIS). For each of these results, various approaches and strategies exist based on the risk of cervical cancer; the higher the grade, the higher the risk of developing into cancer; this is described in greater detail in Chapter 2. Cervical intraepithelial neoplasia (CIN) refers to the presence of cells in layers of the epithelium; CIN is graded from 1 to 3 and refers to the progression of abnormal cells. CIN2 and greater (CIN2+) is often used as the clinical endpoint in evaluating cervical screening tests. HSIL refers to a higher level of cellular abnormalities and includes both CIN2 and CIN3.

Pap cytology-based screening programs have dramatically reduced the burden of cervical cancer worldwide. As the cost of healthcare balloons, the demand for accountability grows, and newer technologies emerge, it is prudent to evaluate potential new approaches and algorithms in cervical screening strategies. Although cytology has been a well-established standard of cervical cancer screening programs, it has limitations. Assay sensitivity ranges from 30-87% for the detection of dysplasia (Morantz, 2006) while others report sensitivity of 55.4% and specificity of 96.8% for the detection of CIN2+

(Kripke, 2008). Thus, the need to repeat it periodically, partly to detect those at risk who might have been missed in the previous rounds, and more importantly, follow a large number of patients diagnosed with borderline and low-grade cytological abnormalities, only a small fraction of which are genuinely at increased risk[3]. In the management of the latter, a triaged approach can allow for better risk stratification and thus improve patient care and ensure overall efficiency. In 2007, NL was the first province in Canada to implement one type of triage called ASCUS-HPV for a low-grade cytological abnormality for women 30 years of age and older. This triage program will be described in greater detail in the following chapters.

Low-grade squamous intraepithelial lesion (LSIL) is the second most common cytological abnormality found in routine Pap cervical screening. In most cases, LSILs regress meaning most are not at risk of cervical cancer (Cuzick, et al., 2013). However, a small fraction of those with LSIL have high-grade squamous intraepithelial lesions (HSIL) or could be at risk of progression to HSIL and cervical cancer. Due to this risk, those found to have LSIL in routine screening are either referred to colposcopy directly or conservatively managed cytologically, with those having persistent abnormalities being referred to colposcopy (Cancer Care Ontario, 2016), (Wright Jr, et al., 2007), (Munoz, et al., 2003). In colposcopy clinics, LSIL cases are typically followed with cytology, colposcopy, and potentially biopsy, for an extended period, until they obtain two consecutive negative

[3] In 2012, Pap cytology grades were broken down as follows:
ASCUS/LSIL, 7.7%; AGC, 0.3%; ASC-H, 0.3%; HSIL, 0.5% (NL Cervical Screening Initiatives Program, 2013)

cytology results before being returned to routine screening, sometimes never returning to routine cytology. Given that the majority are not at risk, this referral to colposcopy is excessive and unnecessary for most people and is associated with considerable costs to the system, physically, and psychologically. However, an effective triage of LSILs can identify those at increased risk who need to remain under care and return those not at immediate risk to routine screening (Cuzick, et al., 2013), (Thrall, Smith, & Mody, 38).

HPV is the aetiological agent of cervical cancer and, when combined with risk factors such as, having multiple and high-risk sexual partners and early onset of sexual activity (Emmett, et al., 2018)(Described in greater detail in Chapter 2), and persistent infections, can contribute to the development of precancerous and cancerous lesions (Munoz, Castellsague, Berrington de Gonzalez, & Gissmann, 2006). HPV is highly contagious and is now considered the most common sexually transmitted infection in most populations (Castellsague, 2008). It is estimated that 80% of sexually active people are infected with HPV at some point in their lives (Cleveland Clinic, 2018). However, in the majority, the infection is entirely asymptomatic, transient, and cleared within a year or two (Etude De Cohorte HITCH, 2022) as it is only when HPV integrates into the cell that it can cause disease. An additional consideration is that there are over 150 different genotypes of HPV, described further in Chapter 2. Only about 15 of these genotypes are cancer-causing or oncogenic; low-risk HPV (lr-HPV), which cause no or minimal disease or high-risk HPV (hr-HPV), which are disease-causing and can be oncogenic. Persistent HPV infections with these oncogenic genotypes can be problematic.

As such, the cobas HPV DNA test (Roche Diagnostics) is a PCR-based qualitative assay for the detection of 14 hr-HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 in cervical specimens and offers partial genotyping, identifying genotypes 16 and 18 (commonly referred to as 16/18 when paired together) individually and detecting 12 other high-risk (OHR) types collectively in one analysis. While those persistently infected with one or multiple of these hr-HPV genotypes are at risk for cervical pre-cancer and cancer, the attributable risk is far greater with genotypes 16/18 accounting for 70% of cervical cancers (Munoz, et al., 2003), (Khan, et al., 2005), (de Sanjose, et al., 2010). This attributable risk underscores a genotype-specific risk threshold in cervical screening strategy as evident in the interim US clinical guidance, which recommends direct referral to colposcopy for those testing positives for genotypes 16/18 in primary HPV screening (Huh, et al., 2015). In this context, the cobas HPV test can serve as an adjunct test for triaging the LSIL referral population through genotype 16/18-specific risk threshold.

The CINtec PLUS cytology (Roche Diagnostics) is a dual-stain immunocytochemical test that detects p16 and Ki-67 proteins over-expressed in cervical cells with transforming HPV infection. As the expression of p16 and Ki-67 is mutually exclusive in normal cells, the co-detection of p16 and Ki-67 simultaneously within the same cervical epithelial cell serves as a specific marker of HPV-mediated oncogenic transformation and predictor of cervical cancer risk. Co-detection of p16 and Ki-67 have been associated with significantly higher cumulative 5-year risk of CIN2+ in comparison with abnormal cytology (Clarke, et

al., 2019). As such, CINtec PLUS has the potential to serve as an adjunct test for triaging an LSIL referral.

A triaged screening program would reduce the number of patients requiring additional investigations and follow-up in colposcopy clinics, and thus, could aid in better patient care and resource management. In one of the studies making up the work of this dissertation, we found that both CINtec PLUS and HPV tests have the potential to identify those at increased risk for precancerous lesions and cervical cancer among those referred to colposcopy with a history of LSIL who need to be followed and spare the rest not at immediate risk from unnecessary colposcopy clinic visits and further tests.

HPV vaccination, which focuses on prevention of high-risk genotypes, is available in several different formats and is available through publicly funded programs across Canada and other jurisdictions. As such, it is prudent to consider the long-term impact of HPV vaccination. HPV vaccination has been shown to substantially reduce the risk (~88%) of invasive cervical cancer at the population level (Lei, et al., 2020) and contribute to a reduction in the rate of overall abnormalities. This reduction in prevalence would impact pre-test probability and should be considered in future program planning.

1.4 SARS-CoV-2 & COVID-19

This pandemic has magnified every existing inequality in our society – like systemic racism, gender inequality, and poverty.

Melinda French Gates

Since the emergence of SARS-CoV-2, the virus responsible for COVID-19, at the end of 2019. There have been over 346 million cases and over 5.5 million deaths worldwide, as of January 23, 2022 (World Health Organization, 2022). To stop the spread of infection, manage patients, and provide timely information to public health policymakers, accurate, timely, and accessible diagnosis of SARS-CoV-2 infection is required (Xiang, et al., 2020), (Morrison, Li, & Loshak, 2020).

In Canada, the initial focus of test development was on molecular methods using real-time reverse transcription-polymerase chain reaction (RT-qPCR) to detect SARS-CoV-2 RNA. While this approach has many advantages, including test performance, opportunities for high throughput of tests, and relatively quick turnaround time upon receipt in the laboratory, these tests can only indicate the presence of viral RNA in patient specimens; it cannot indicate the presence of viable viral particles. While the absence of the viral RNA cannot determine if a person was infected and has since recovered or if the person is infected and the virus was undetectable from the submitted specimen source at that time (Wang, et al., 2020) (Morrison, Li, & Loshak, 2020). Often, RT-qPCR tests are performed on patients who are symptomatic or those who are epidemiologically linked

to COVID-19 cases. This testing strategy likely underestimates the true prevalence of infection in the population by missing those who are asymptomatic or those with very low-levels of symptoms (Khan S. , et al., 2020). Additionally, RT-qPCR methods also face challenges with the accessibility of reagents and equipment, availability of trained personnel, cost, and difficulties with performance (Liu, et al., 2020) (Xu, et al., 2020).

Earlier in the pandemic, it was believed antibody detection might provide a complementary perspective in complement to RT-qPCR testing in the diagnosis of COVID-19 (Xiang, et al., 2020) and possibly serve as an effective tool for COVID-19 screening in close contacts presenting with signs or symptoms but negative SARS-CoV-2 RT-PCR results (Xu, et al., 2020) when paired with clinical and epidemiological data. However, it is more widely understood that serology testing has limited diagnostic capacity for acute infection due to antibodies not being reliably detected until 1-3 weeks post symptom onset and is best utilized to provide population-based information on positivity rates and inform evidence-based decision-making for public health policies and recommendations (Charlton, et al., 2021). Due to these limitations and challenges, serological methodologies are being developed to better quantify the number of people exposed to SARS-CoV-2 from an epidemiological surveillance perspective (Centres for Disease Control and Prevention, 2020) (Morrison, Li, & Loshak, 2020).

Enzyme-linked immunosorbent assays (ELISA) and chemiluminescent microparticle immunoassays (CMIA) each provide semiquantitative *in vitro* measurement of the levels of human antibodies of the immunoglobulin class A (IgA) and G (IgG) against

SARS-CoV-2 in serum. In an effort to provide comprehensive public health and microbiological services during the SARS-CoV-2 global pandemic, clinical assessment of the following three commercial serological tests were performed: Abbott Architect SARS-CoV-2 IgG (A-IgG), EuroImmun Anti-SARS-CoV-2 ELISA IgG (EI-IgG), and EuroImmun Anti-SARS-CoV-2 ELISA IgA (EI-IgA).

Chance favors the prepared mind.

Richard Preston (Preston, 1999)

Our healthcare systems, including screening programs and testing, require continual evaluation, assessment, and development. Sometimes that may mean fine-tuning existing programs or quick and aggressive innovation to address a new, looming threat. Ultimately, once we know better, we can do better; from a technological, clinical, or equity perspective. While this work focuses on methodological and screening perspectives, we must be mindful of the greater context of these factors. Improving access and addressing the social determinants of health benefit us all.

1.5 Study Objectives

1. To investigate the current state of cervical screening in Newfoundland and Labrador through assessment of indicators.
2. To investigate the state of cervical cancer screening from 2002 to 2019 with a focus on low grade abnormalities in Newfoundland and Labrador screening eligible women to understand the changes over time and possible future improvements.
3. To assess diagnostic properties of CINtec PLUS cytology and cobas HPV tests along with genotype 16/18-specific risk threshold among those with a history of LSIL referred to colposcopy to identify those requiring further colposcopy clinic visits and follow-up, and conversely to identify those not at risk, thus ensuring better patient care and resource utilization in a 2-year period.
4. To assess diagnostic indices of CINtec PLUS cytology and cobas HPV test to detect CIN2+ in an LSIL referral population and serve as adjunct tests for triaging LSIL cases referred to colposcopy, thus aiding diagnostic accuracy and treatment and improving overall efficiency in a 2-year period.
5. To assess the performance and utility of three tests for identification of past infection with SARS-CoV-2: Abbott Architect SARS-CoV-2 IgG (A-IgG), EuroImmun Anti-SARS-CoV-2 ELISA IgG (EI-IgG) and EuroImmun Anti-SARS-CoV-2 ELISA IgA (EI-IgA) in a low prevalence setting in 2020 for those with lab-confirmed SARS-CoV-2 infections.

1.6 Involvement of author in thesis

The author was not involved in the conceptualization or implementation of the NL cervical screening initiatives registry that was used for part of this work.

While the testing performed in this work involved clinical specimens (either collected specifically for quality improvement projects or leftover from routine processes) the tests were performed in addition to routine diagnostic work and/or after hours as per research protocols and agreements.

Dr. Sam Ratnam was the principal investigator of the CINtec PLUS LSIL component, which was supported by Roche Diagnostics.

Dr. Lei Jiao was the principal investigator for the SAR-CoV-2 serological assay evaluation component of this work.

The author played a lead role in conceptualizing and conducting the studies presented in this thesis; this includes initial planning and logistics, coordinating contract signing, completing ethics applications and renewals, and obtaining organizational approvals and policy compliance. Author led local coordination including specimen management, database curation and management, and any storage according to ethics approvals and agreements. The author of this thesis was responsible for analysis and manuscript preparation and writing as indicated in the beginning of each manuscript chapter; two as lead author, one as co-lead author, and another as third author. Specific responsibilities are indicated per journal requirements in each chapter. Additionally, author was one of

two trained personnel preparing cervical cytology slides with p16/Ki-67 stains required for CINtec PLUS cytology.

The author was responsible for the statistical analyses in each manuscript and the presentation of the findings from this thesis.

1.7 Organization of thesis

This thesis is divided into eight chapters. Chapter 1 is an overall introduction to the work completed for this thesis. Chapter 2 provides a background to cervical cancer, the role of HPV, and the current state and limitations of cervical cancer screening programs. Additionally, chapter 2 highlights the emergence of SAR-CoV-2 and COVID-19 disease and the impact of diagnostic and screening testing on the pandemic. The research methods employed in the works are included in each manuscript specifically. Chapter 3 through 6 are manuscripts written to include their own Introduction, Methods, Results, and Discussion Sections. Finally, Chapter 7 summarizes the key conclusions, implications, and applications of study results and potential for future research.

Chapter 2 : Background

2.1 Public health screening

Early detection strategies and population-based screening are important public health tools in early detection of disease and population surveillance. While cervical cancer is a long-standing program, requiring review of existing programs and new methodologies, the emergence of the COVID-19 requires an initial evaluation of new test methodologies. This work will examine secondary prevention strategies involving the human papillomavirus (HPV) and subsequent progression to cervical cancer, as well as serological testing involving the SARS-CoV-2 virus, which emerged in 2019, leading to a global pandemic of severe acute respiratory syndrome termed COVID-19 by the WHO (WHO Director-General, 2020).

Various factors impact the morbidity and mortality of diseases on individuals and communities; some are directly medical, many are not. The non-medical factors that affect health outcomes are called the social determinants of health (SDH). SDH are the collective systemic factors that impact health and include economic policies and systems, development agendas, social norms, social policies, and political systems (World Health Organization, 2022).

From a systemic perspective, SDH may have a more significant impact on health outcomes than the healthcare system or lifestyle/individual choices and may account for 30-50% of health outcomes (World Health Organization, 2022). Some examples include income and social protection, education, food insecurity, housing, basic amenities, social inclusion, structural conflict, and access to services. Addressing these factors is critical for improving public health outcomes and reducing disease (World Health Organization, 2022). However, these are more challenging, and in the context of governments and public and private industries, it requires buy-

in, creative approaches, and strong intersectoral relationships. Often this is not where funding and programs are being targeted.

Public health programing is imperative to the health and wellbeing of our populations. Preferably, health leaders and programs should aid to prevent infectious diseases before they begin; this is referred to as “primary prevention” (Gordis, 2009) (Aschengrau & Seage III, 2008) (Shah, 2003). Primary prevention programs can look at immunization programs, addressing risk factors, and strategic programs to address social determinants of health. Much of the work covered here is considered “secondary prevention” programs; this refers to the early detection of infections or diseases, ideally before the start of clinical indications or symptoms, to reduce severity and complications (Gordis, 2009) (Aschengrau & Seage III, 2008) (Shah, 2003). Tertiary prevention refers to reducing the impact of the disease once it has already been diagnosed and includes things like treatment and support (Gordis, 2009) (Aschengrau & Seage III, 2008) (Shah, 2003); this type of programming will not be covered.

To be an appropriate and successful secondary prevention program, screening programs should meet several requirements, including the ability to screen a large proportion of the target population, to provide high quality and caring services, a well-developed and sound referral system to ensure that patients receive follow-up and acceptable treatment (Programme on Cancer Control, Department of Reproductive Health and Research, 2002). In the case of cervical cancer, we see several different program schemes that could fit these criteria and well-established treatments; at the time of this writing, COVID-19 treatment options are not widely available and not affordable in many settings; however, public health screening provides insight into the

prevalence and spread throughout communities and allows for better understanding of the epidemiology of this emerging infectious disease and interventions to decrease spread.

2.2 HPV & Cervical Cancer

2.2.1 Burden

In the early 1980s, Harald zur Hausen and his team discovered HPV in cervical cancers (Durst, Gissmann, Ikenberg, & Zur Hausen, 1983) (Gissmann, Wolnik, Ikenberg, & Koldovsky, 1983). In 2008, zur Hausen won the Nobel Prize in Medicine for discovering human papillomaviruses caused cervical cancer; we now know that nearly all (99.7%) of cervical cancer is caused by HPV progression (Nobel Prize, 2021). The genes from papillomaviruses are incorporated into the host cells' DNA during human cells' normal growth and division phases of human cells (Mcmurray, Nguyen, Westbrook, & Mcance, 2001). Progression of HPV infections has also been implicated in head and neck cancers (Johnson, et al., 2018), anal cancer, penile cancer, and vaginal cancer (Mcmurray, Nguyen, Westbrook, & Mcance, 2001) (Mcmurray, Nguyen, Westbrook, & Mcance, 2001).

The virological process of cell deregulation causes a variety of cancers. Cervical cancer is the most common HPV-associated cancer in women. Globally, cervical cancer remains one of the gravest threats to women's lives and is the second most common cancer; one woman dies of cervical cancer every two minutes (World Health Organization, 2018). In 2000, there were approximately 470,000 new cases and 290,000 deaths

associated with cervical cancer worldwide; 80% of these deaths were in developing countries (Programme on Cancer Control, Department of Reproductive Health and Research, 2002). These numbers have increased to over 600,000 new diagnoses, with over 340,000 deaths in 2020 (Sung, et al., 2021) (Ferlay, et al., 2022). The 5-year survival rate for invasive cervical cancer is 92% with around 44% of people being diagnosed with early-stage cervical cancer; however, if cervical cancer has spread to tissues and/or regional lymph nodes the survival rate drops to 58%. Furthermore, if there is spread to distant parts of the body this rate drops again to 18%, stressing the importance of early detection. (Cancer.net Editorial Board, 2022).

With the availability of vaccinations and screening programs, cervical cancer can be largely prevented (Cohen, Jhingran, Oaknin, & Denny, Cervical Cancer) and possibly eradicated (Canadian Cancer Society, Statistics Canada, Public Health Agency of Canada, Provincial/Territorial Cancer Registries, 2016). Approximately 90% of cervical cancers occur in low-income and middle-income countries that lack organized screening and vaccination programs (Cohen, Jhingran, Oaknin, & Denny, Cervical Cancer).

Nearly 50% of Canadians will develop cancer in their lifetime, which will be responsible for 25% of deaths (Canadian Cancer Society, Statistics Canada, Public Health Agency of Canada, Provincial/Territorial Cancer Registries, 2016). In 2012 there were nearly 3,800 HPV-associated cancers diagnosed in Canada (Canadian Cancer Society, Statistics Canada, Public Health Agency of Canada, Provincial/Territorial Cancer Registries, 2016). Cervical cancer is the second most common type of HPV-associated cancer in

Canada, surpassed by oropharyngeal cancer (Canadian Cancer Society, Statistics Canada, Public Health Agency of Canada, Provincial/Territorial Cancer Registries, 2016). Provincial morbidity and mortality numbers vary, but ultimately there are approximately 1,500 new cases of invasive cervical cancer in Canada and around 300 deaths annually (Canadian Partnership Against Cancer, 2016). This amounts to an overall, age-standardized invasive cervical cancer incidence rate ranging from 8.8 to 12.1 per 100,000 women; Newfoundland and Labrador has the highest rate of 12.1 per 100,000 (Canadian Partnership Against Cancer, 2016).

2.2.2 From HPV to Cervical Cancer

There are over 150 different genotypes of HPV, some causing illness in humans, and some in animals, with 30 infecting the anogenital tract (Hillemanns, Soergel, Hertel, & Jentschke, 2016). Classification of HPV genotypes is based on the nucleotide sequence of the open reading frame (ORF) coding for the capsid protein L1 (Figure 2.2) (Bzhalava, Eklund, & Dillner, 2015). Some of these genotypes are cutaneotropic (1,4,5,8,41,28,60,63, and 65), meaning they are isolated from cutaneous and plantar warts, various lesions, and in some epithelial tumors (Castellsague, 2008). Other genotypes are mucosotropic (6, 11, 13, 44, 55, 16, 31, 33, 35, 52, 58, 67, 18, 39, 45, 59, 68, 70, 26, 51, 69, 30, 53, 56, 66, 32, 42, 34, 64, 73, 54) and have been found in benign and malignant lesions of the human anogenital tract (Castellsague, 2008). There are additional types; however, their association with malignancies is unknown.

Approximately 15 HPV genotypes are oncogenic, meaning that they are cancer-causing. These include types 16, 18[4], 31, 33, 45, and 52; some of the genotypes most frequently associated with cervical cancer (Figure 2.1). When we look at the proportion of these genotypes associated with cancer, we can see from previous studies types 16 and 18 make up approximately 70% of genotypes attributable to cervical cancer (Figure 2.1).

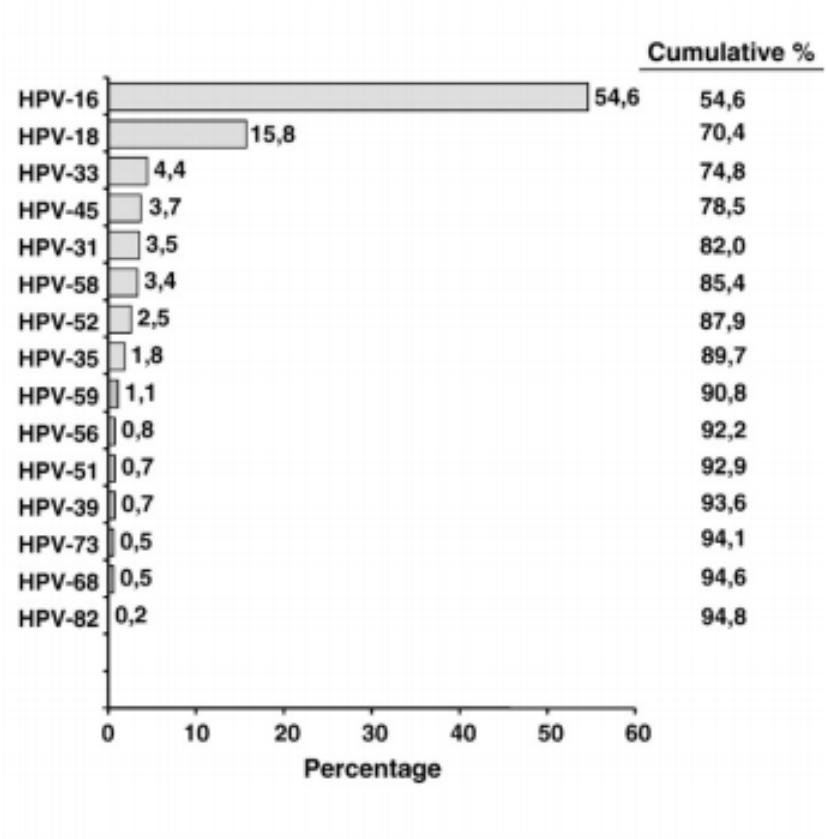


Figure 2.1. Estimated percentage of cases of cervical cancer attributed to the most frequent HPV types. Reproduced with Permission (Castellsague, 2008)

[4] HPV genotype 18 (HPV-18) is the type responsible for Henrietta Lacks "Immortal Cells" which were removed from a cervical biopsy at Johns Hopkins in 1951 (Skloot, 2010)

HPV is a double-stranded DNA virus of approximately 7900 base pairs (Lagstrom, et al., 2019). It is made up of two capsid genes (L1 and L2) and six early proteins (E1, E2, E4-E7), all of which are separated by an upstream regulatory region (URR). The capsid genes and early proteins are responsible for the creation of viral DNA and the assembly of new virus particles once the virus has infected new cells. The URR does not create proteins but contains elements for gene expression regulation, replication of the genome, and viral particle packaging (Figure 2.2) (Munoz, Castellsague, Berrington de Gonzalez, & Gissmann, 2006).

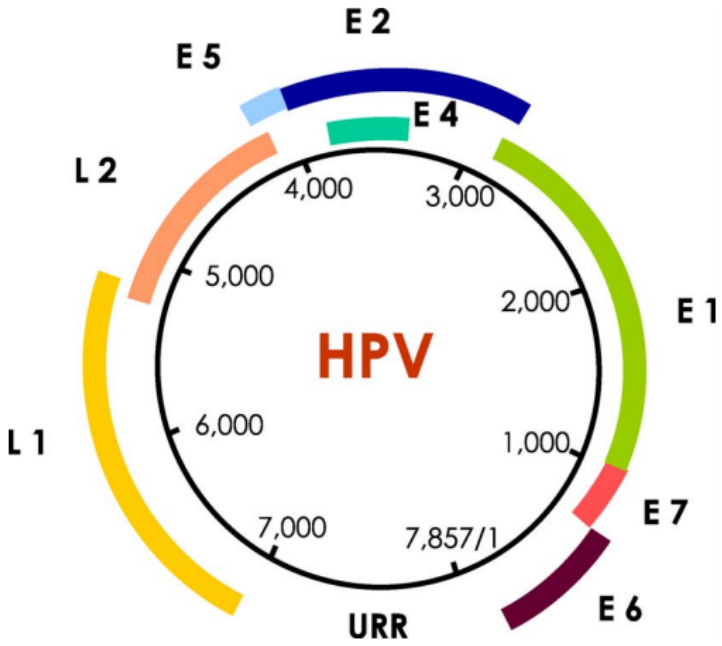
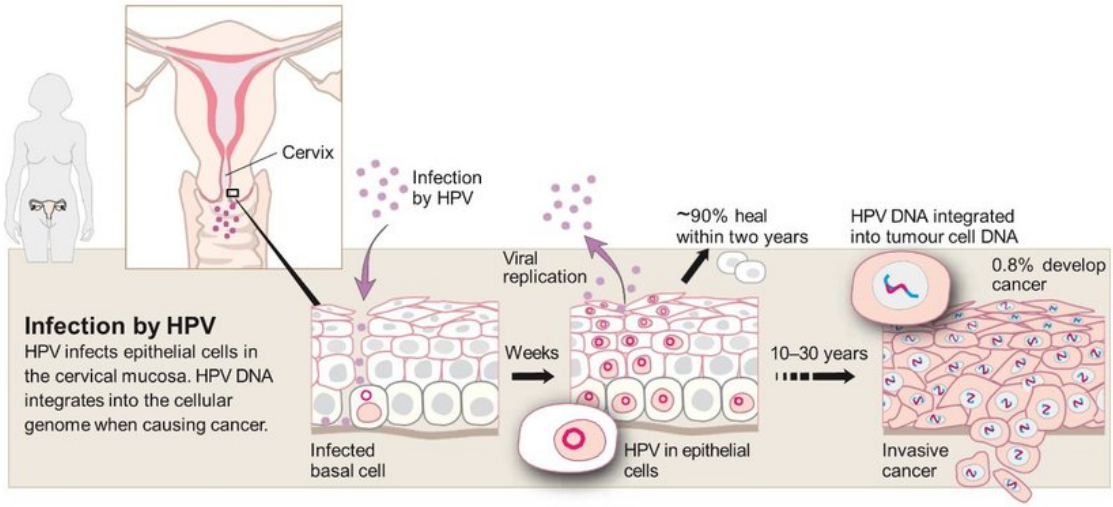


Figure 2.2. Schematic presentation of the HPV genome showing the arrangement of the early E or nonstructural genes, the capsid genes (L1 and L2) and the upstream regulatory region (URR), Reproduced with Permission (Munoz, Castellsague, Berrington de Gonzalez, & Gissmann, 2006)

While the intent of this work is not on the biological mechanisms of the virus pathway, it is essential to understand some of the base biological changes to understand the relationship of HPV and the impact it has on cervical cancer development. Papillomaviruses bind to and enter cells through tiny breaks in the skin and reach the basal layer of the epithelium where they attack the cell machinery of epithelial or mucosal cells and start their cycle. The mechanisms to pathogenesis are well documented (Pfister, 2012). The replication cycle contains two parts: 1) replication of the viral genome, and 2) Pushing the basal cells to the suprabasal compartment (Munoz, Castellsague, Berrington

de Gonzalez, & Gissmann, 2006). As described by Munoz et al, during the replication of the viral genome phase the genome copies to about 100 and maintains this copy level within the infected, replicating, competent cells. In persistent infections, the immune system keeps the viral cycle at this level. E1 and E2 are vital factors in this replication (Munoz, Castellsague, Berrington de Gonzalez, & Gissmann, 2006). The second stage of the replication cycle is when basal cells are pushed to the suprabasal compartment. Once this stage begins, the cells lose their ability to divide and start the terminal differentiation program. HPV can release back into the environment at this stage because of the destruction of epithelial cells. Viral proteins E6 and E7 interact with cellular proteins and induce proliferation, immortalization, and malignant changes; the express of E6 and E7 increases the likelihood of oncogenic progression in HPV-infected cells (Bernard, 2002). The constant activity of E6 and E7 leads to genomic instability, and there is the accumulation of oncogenic mutations; this leads to further loss of cell-growth control and, as such, cancer (Munoz, Castellsague, Berrington de Gonzalez, & Gissmann, 2006). In particular, E5, E6, and E7 also can interfere and actively participate in the down regulation of a host's immune system; an important factor in the control and limiting of HPV infection (Ashrafi & Salman). E7 integration is essential for cancer formation and impacts the risk of oncogenic progressions (Speicher, 2022), (Bernard, 2002).



508

509 *Figure 2.3. Pathogenesis of HPV in cervical cancer, Reproduced under Creative Commons*
510 *License (Non-Commercial-No Derivatives 4.0 International), (copyright: ©The Nobel*
511 *Committee for Physiology or Medicine. Illustrator: Mattias Karlén) (Stark & Zivkovic,*
512 *2018)*

513

514 While we know that HPV is the aetiological agent of cervical cancer, there is a
515 complex interplay between risk factors and HPV infection that contribute to the
516 persistence of infections and the development of pre-cancerous and cancerous lesions.
517 Therefore, HPV is often referred to as an aetiological agent or necessary cause but not a
518 sufficient cause; meaning that other factors are important in the progression from
519 infection to cancer (Munoz, Castellsague, Berrington de Gonzalez, & Gissmann, 2006).
520 These include factors such as (Hillemanns, Soergel, Hertel, & Jentschke, 2016)
521 (Castellsague, 2008):

522 • Early-onset of sexual activity
523 • Multiple sexual partners

- High-risk sexual partners
- History of other sexually transmitted infections
- History of vulvar or vaginal squamous intraepithelial neoplasia (SIN) or cancer
- Immunosuppression (for example, from Human Immunodeficiency Virus [HIV] infection)
- Early age at birth of first child
- Low socioeconomic status (SES) or living in a high poverty country
- Oral contraceptive use
- Having a first degree relative with cervical cancer

HPV is highly contagious and is now considered the most common sexually transmitted infection in most populations (Castellsague, 2008). An estimated 80% of sexually active people are infected with HPV at some point in their lives (Cleveland Clinic, 2018). However, in the majority, the infection is entirely asymptomatic, transient, and cleared within a year or two. Given the likelihood that people are more sexually active when younger, may have more partners, and that HPV is ubiquitous in the general population (Skloot, 2010), these factors contribute to the higher prevalence in younger ages. A higher incidence of abnormal cells can be seen on Pap tests due to HPV infections in younger people. We see a changing trend of decreasing HPV prevalence with age as sexual behaviours, and risk factors decrease with age (Figure 2.4).

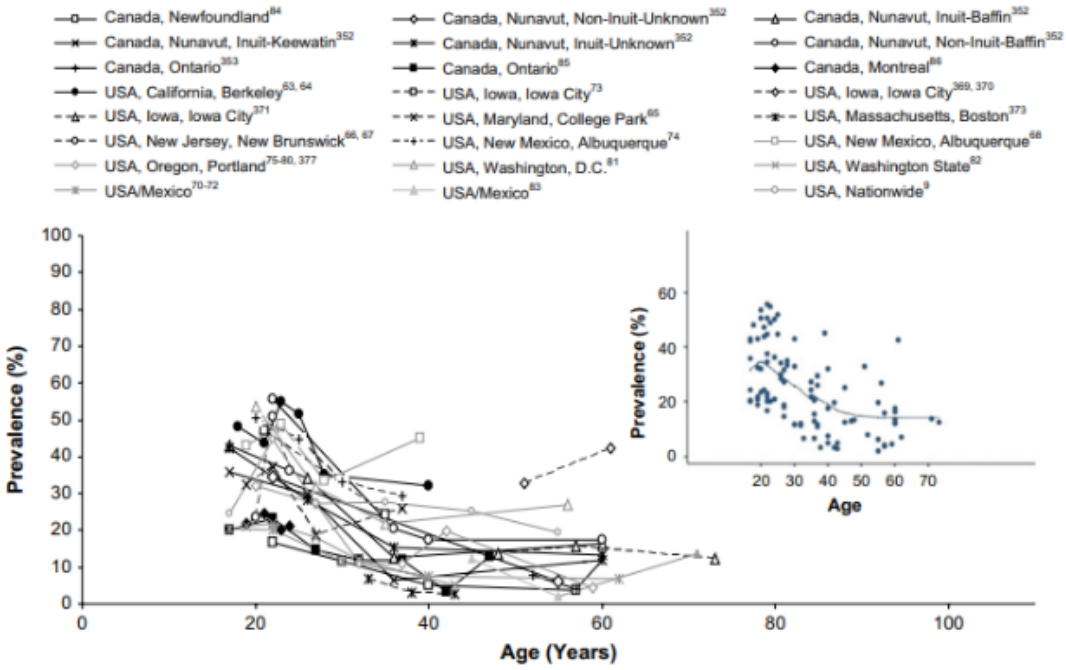


Figure 2.4. HPV prevalence by age in North America among low-risk female populations by country, city, and study, Reproduced with Permission (Smith, Melendy, Rana, & Pimenta, 2008).

The most significant problem with HPV and its association with the natural course of the disease is the persistence of infection (Hillemanns, Soergel, Hertel, & Jentschke, 2016). We know that many of the population are exposed to HPV, many of which can clear infections or experience regression of infection, while some infections lead to host death, some result in latent infections where viral genome remains in cells without detectable activity (Alizon, Murall, & Bravo, 2017). However, a small proportion of patients are unable to clear infections resulting in persistent or chronic infections (Alizon, Murall, &

Bravo, 2017), with people under the age of 30 being more likely to have infections that regress and clear (Canadian Cancer Society, 2022). Therefore, the remaining infections in those >30 years of age are of particular interest for a screening program. The risk of cervical cancer is more significant in this group due to their inability to clear HPV infections, meaning an infection persistence.

There are four major stages of the natural history of cervical cancer:

1. HPV infection, which the majority of women will experience.
2. The inability to recover from HPV or the persistence of infection.
3. Cellular changes caused by this persistence infection (identifiable with cytology).
4. Further progression and involvement of multiple layers of cells to cervical cancer.

We can see a similar pattern when we look at the incidence of cervical cancer with age (Figure 2.5). Although the age at diagnosis peaks in the 30-34 age group, when we compare with Figure 2.4, we see that the HPV prevalence at this same age group is decreasing, reflecting that often persistent HPV infection is the aetiological agent of the development of cervical cancer. The persistence of infection can be caused by age and reduction in immunity to fight off and clear the infection.

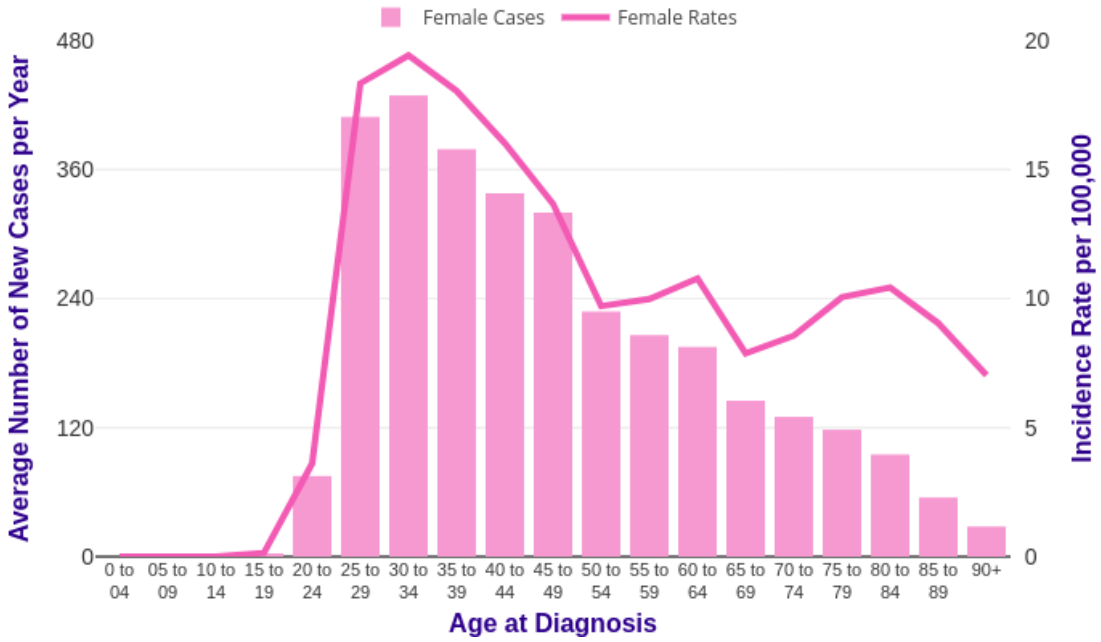


Figure 2.5. Average number of new cases of cervical cancer per year and age-specific incidence rates per 100,000, UK, 2015-2017, Reproduced under Creative Commons (Cancer Research UK, 2020)

2.2.3 Cervical Cancer Screening History

The Pap test, which looks for morphological changes in cervical cells, was introduced in 1949 by Georgios Nikolas Papanicolaou; however, it took many years for the methodology to be accepted widely. In Canada, the early 1970s saw much interest and growing evidence, i.e., the Wilton report (1974) recommended the widespread introduction of the Pap test for cervical cancer screening. It was not until the 1980s before provincial screening programs were implemented; as a consequence of widespread screening a large number of early and late-stage cervical cancers were prevented (Yang, Soulos, Davis, Gross, & Yu, 2018).

The Canadian National Workshop on Screening for Cancer of the Cervix in 1989 led to further development and improvements on existing screening programs; these included reviews of patients being screened too frequently, and some patients not being followed adequately after abnormal cytology results (Health Canada, 1998). Then, in 1995 the Cervical Cancer Prevention Network (CCPN) was created to continue to reduce the morbidity and mortality associated with cervical cancer and its precursors in Canada by facilitating the implementation of organized screening programs (Health Canada, 1998).

Since this time, additional guidance documents and protocols have been released from groups and associations like the Canadian Partnership Against Cancer, the Society of Obstetrics and Gynaecologists of Canada, the Canadian Agency for Drugs and Technologies in Health, and others to continue to make advances in cervical cancer screening programs.

2.2.4 Screening Tests

2.2.4.1 Visual Inspection with Acetic Acid (VIA)

In limited resources settings, more complex or molecular-based methodologies may not be available or possible, as such Visual Inspection with Acetic Acid (VIA) can meet the needs as a screening test. A low-tech alternative, 3-5% acetic acid is used to soak the cervix, followed by non-magnified visual inspection. Reported sensitivities range from 66-

96% with specificities of 64-98% (Programme on Cancer Control, Department of Reproductive Health and Research, 2002).

While there are limitations of VIA such as the potential for over-investigation and over-treatment, its logistic advantages in limited-resource settings make it an elegant and cost-effective solution, particularly in areas with high prevalence. As an example, in a Vietnamese setting, a country with high prevalence, PPV for VIA was 51.2% (Huy, et al., 2018). Additionally, one advantageous element of VIA is providing a test result at the same appointment; given communication infrastructure issues in developing countries, there is a real need for this type of easy and accessible result format. However, in low-socio-economic regions, there are still recommendations for further investigation of abnormal cervical findings (Barut, et al., 2015).

2.2.4.2 Conventional Cytology & Liquid Based Cytology

Pap cytology involves physically examining a cellular smear by specifically trained cytotechnologists and cytopathologists. Conventional cytology involves the direct application of cells from a swab onto a smear. In Liquid Based Cytology (LBC), the cervical swab is placed in a liquid media after collection; media is available from several vendors. For example, in the province of Newfoundland and Labrador, SurePath (BD) collection media is used for LBC (Howlett & Peters, 2015), while in Ontario, ThinPrep (Hologic) is used.

Cytology can be a highly specific test (98-99%), albeit with limited sensitivity (~50%) (Programme on Cancer Control, Department of Reproductive Health and Research, 2002). In the context of these performance indices it is important that in comparison to other methods Pap cytology looks at morphological changes in collected cells and can be subjective; meaning that there can be a risk of false negatives. Its performance in screening programs is improved by repeating the test over defined intervals.

One barrier for cytology, particularly in low-resource settings, is the inability to provide a result immediately to patients, as such infrastructure is required (Programme on Cancer Control, Department of Reproductive Health and Research, 2002).

2.2.4.2.1 Pap cytology nomenclature

The Bethesda System of nomenclature was first developed at a workshop in 1988 to establish terminology to "provide clear-up thresholds for management and decrease interobserver variability" (Nayar & Wilbur). While there have been subsequent updates, the classification system is widely used to report cervical cell abnormalities (Barut, et al., 2015). This includes the categories as stratified below:

Table 2-1. Bethesda System nomenclature categories

Abbreviation	Name	Level of Risk

ASCUS	Atypical Squamous Cells of Undetermined Significance	Lowest
ASC-H	Atypical Squamous Cells cannot exclude HSIL	
LSIL *	Low Grade Squamous Intraepithelial Lesion	
LSIL-H	LSIL cannot exclude HSIL	
HSIL **	High Grade Squamous Intraepithelial Lesion	
AGC	Atypical Glandular Cells	
AIS	Adenocarcinoma in situ	
CIS	Carcinoma in situ	Highest
Adapted from (NL Cervical Cancer Screening Initiatives, 2011)		

646

647 * LSIL is generally CIN1

648 ** HSIL can be CIN 2, CIN2/3, or CIN 3

649 (National Cancer Institute, 2021)

650

651 *2.2.4.3 HPV tests*

652 The presence of HPV DNA may indicate persistent HPV infections; therefore,
653 testing for the virus directly through molecular methodologies may be a better approach
654 for a good screening tool where HPV is the aetiological agent of cervical cancer. Compared
655 to VIA, conventional and LBC cytology, HPV testing is objective; there are no subjective
656 interpretations looking for morphological changes or stains which is a real advantage to
657 improvement of test performance. However, the presence of HPV infection with positive
658 results, meaning HPV has been detected, does not necessarily indicate persistent
659 infections (Arbyn, Roelens, Martin-Hirsch, Leeson, & Wentzensen, 2011).

660 Additionally, HPV tests provided increased sensitivity of at least 30-35% when
661 compared with Pap tests with a specificity loss of 7-10% and HPV tests, in general, have

an incredibly high negative predictive value of 95-98%, for CIN2+ (Bosch, 2007) (Mayrand, et al., 2007). There are various types of HPV tests from several manufacturers with varying performance levels, some detecting HPV DNA (this is the type of tests looked at during this work using the Roche cobas HPV test) and others which detect HPV mRNA. While HPV DNA testing is highly sensitive as it detects the presence of DNA and can detect acute and latent infections, mRNA methodologies may significantly reduce sensitivity because they cannot detect latent infections; however, mRNA tests are more specific in predicting dysplasia (Walker, 2018). Also, some available tests are more advantageous as they can differentiate HPV types. This ability for partial or complete genotyping means there is the potential for risk stratification based on the different levels of risk associated with different genotypes (Figure 2.1). There are no perfect tests and it is critical that jurisdictions evaluate performance indices based on their local prevalence and needs.

There are several barriers for HPV testing in low resource settings, one of which is the inability to provide a result immediately to patients, as such infrastructure is required as well as the extensive and expensive molecular testing instrumentation (Programme on Cancer Control, Department of Reproductive Health and Research, 2002). Additional considerations for implementation in local settings are described in later sections.

2.2.4.4 Immunocytochemical cytology

Biomarkers p16 and Ki-67 are mutually exclusive, naturally occurring proteins within cells; during oncogenesis, cellular changes cause these proteins to be present

683 simultaneously. p16 is a tumor suppressor protein that is overexpressed during the
684 deregulation expression of the E7 oncogene and indicates transforming HPV infections,
685 while Ki67 is a well-known cell proliferation marker (Zhu Y. , et al., 2019). Therefore,
686 immunocytochemical cytology is based on the presence of such biomarkers in changing
687 cells. Since the expression of p16 and Ki-67 is mutually exclusive in normal cells, the co-
688 detection of these proteins simultaneously within the same cervical epithelial cell serves
689 as a specific marker of HPV-mediated oncogenic transformation and predictor of cervical
690 cancer risk. The most common method commercially developed and approved for
691 detecting p16/Ki-67 is CINtec PLUS cytology, where dual specific stains are used to identify
692 the two biomarkers, p16 and Ki-67, both of which are over-expressed in the same
693 transforming cell. These stains are applied to smears of cervical specimens collected in
694 LBC media using special microscopic slides. In the CINtec PLUS test, p16 shows a brown
695 cytoplasmic stain and Ki-67 a red nuclear stain. These stains are evaluated by
696 cytotechnologists and verified by cytopathologists, independent of cytomorphology. For
697 a smear to be classified as positive, at least one cervical epithelial cell must show both the
698 brown cytoplasmic stain and the red nuclear stain; if these stains are not present together,
699 the smear is considered negative. An example of a positive p16/Ki-67 dual-stain smear is
700 shown in Figure 2.6.

701

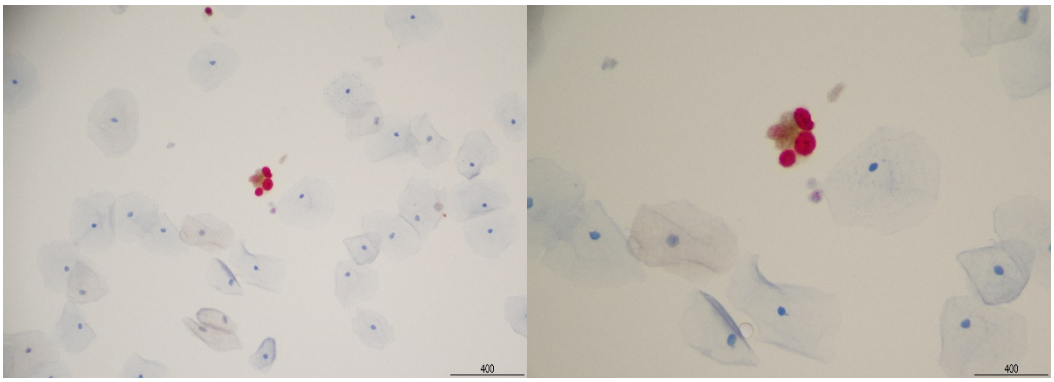


Figure 2.6. Low Power (20x, Left) and High Power (40x, Right) magnifications showing positive dyplastic squamous cells (HSIL) with dual immunostaining. Background shows mature non-dyplastic squamous cells with negative control staining. Photos by Dr. R.Alaghehbandan. Reproduced with Permission.

The CINtec PLUS immunocytological approach is significantly more sensitive than Pap cytology and more specific than HPV testing for detecting CIN2+ where it is actually detecting biomarkers indicative of oncogenic change as opposed to HPV infection with may be simply a transient infection (Ikenberg H. , et al., 2013) (Bergeron C. , et al., 2015) (Killeen J. L., Dye, Grace, & Hiraoka, Improved abnormal pap smear triage using cervical cancer biomarkers, 2014).

Some limitations for use would be the continued reliance on cytotechnologists and cytopathologists, the need for a large staining instrument, and a relatively hands-on process. Additionally, while it seems to be an objective methodology, there can still be differences in inter-observer reliability.

2.2.4.5 Colposcopy

Colposcopy is a more invasive evaluation of a cervix that involves the use of a binocular colposcope and extremely bright lighting to visualize cervical lesions; often, these are performed in scheduled colposcopy clinics, specially organized for colposcopic examination and follow-up. In addition, biopsies and treatment of lesions may occur during colposcopic evaluation. For colposcopy, sensitivity has been reported as 92%, and specificity was 67% (Barut, et al., 2015).

In many jurisdictions, wait times can be lengthy and vary between provinces; however, one set of recommended wait time benchmarks for colposcopy is between 3-8 weeks depending on cytological grade (Wait Time Alliance, 2014), and the procedures are invasive and potentially risky for patients. For example, if a biopsy is performed, patients may experience pain and bleeding for 1-2 days (ACOG, 2021). There is also a considerable psychological impact of recalling patients and referring them for further, more invasive procedures.

2.2.4.5.1 Cervical intraepithelial (CIN) Grades

CIN is pre-invasive lesions as detected by biopsy. As part of the Bethesda system of classification and nomenclature, CIN1 is considered low-grade and relatively begin, and recommendations are to manage conservatively; LSIL cytology falls within this grade. CIN2

is a higher grade than CIN1 and is regarded as the cut-off point to proceed with some sort of treatment as there is an indication of progression; CIN3 is higher again. Both CIN2 and CIN3 are included in HSIL.

Approximately 1.5/1000 women are diagnosed with CIN2/3 annually, with the highest incidence in those between 25-29 years of age (Tainio, et al., 2018). CIN2 or worse (CIN2+) and CIN3 or worse (CIN3+) are typically used in research evaluating the diagnostic performance of tests and include adenocarcinoma in situ (AIS) and other cancers.

2.2.5 Screening Programs

2.2.5.1 Differences in risk and the rationale for triage

Due to the stratified risk of different HPV infections (Figure 2.1), we know that the risk of all HPV infections to develop into cancer is not the same for all genotypes. Ultimately, this principle of stratified oncogenic risk can potentially be used to reduce the number of colposcopy referrals sparing patients not at risk from unnecessary follow-up procedures and ensuring better patient management for those at elevated risk. In addition, by having a triaged or multi-tiered system, we improve the overall performance of our tests and programs as they work in concert.

Historically in a Pap-based screening program, those with ASCUS and worse results would be referred to colposcopy. This is wasteful as most ASCUS cases represent borderline cytologic abnormality and are not really at risk. However, there is a small proportion who may be at risk. For a single ASCUS, the 2-year cumulative risk of CIN3+ is

reported to be 8-9% (Tai, et al., 2018). Therefore, there is a need for follow-up of ASCUS either with repeat cytology or referral to colposcopy or better, risk stratification based on HPV triage. As such, triaging approaches have been evaluated and recommended as part of screening strategy to reduce the unnecessary colposcopy referral in those with ASCUS (Tai, et al., 2018) (Solomon, Schiffman, Tarone, & Group, 2001) (The ASCUS-LSIL Triage Study (ALTS) Group, 2003). Patients >30 years of age with ASCUS Paps receive HPV genotype testing in NL. In this case, although partial genotyping is available, HPV-positive women, regardless of their genotype, are referred to colposcopy, and those who are HPV-negative return to routine Pap screening (Cervical Screening Initiatives, NL, 2016). This triage helps to improve the screening program by better targeting those at risk (Guo, et al., 2019). This question of triage becomes of greater importance in the context of primary HPV screening programs. Many countries have transitioned to HPV primary screening with others to follow in the future, replacing Pap cytology. This change has been challenging to implement due to logistics, up-front costs, and politics. That said, as discussed in Section 2.2.4.3, HPV DNA tests are very sensitive, although a positive test may not indicate persistent infections. Therefore, if we use HPV DNA tests as a primary screening tool and refer all HPV positives to colposcopy, it would be counterintuitive because we know many of those referred will not actually have persistent infections. As such, adding a triage step in an HPV primary screening pathway would make a lot of sense to reduce unnecessary follow-up and systemic costs. One option could be the utilization of a genotyping HPV test

779 to discriminate genotypes 16 and 18 between other HPV genotypes to help identify those
780 at greatest risk (Khan, et al., 2005) (Castle, et al., 2011).

781 Some suggest that Pap cytology could serve as a triage tool in the context of an
782 HPV primary screening program (Wentzensen, Schiffman, Palmer, & Arbyn, 2016). While
783 the HPV test indicates infection, the cellular changes identified in Pap would indicate
784 actual morphological changes; together, you get a complete picture of a patient's
785 infection status. One advantage to this approach is that Pap cytology infrastructure and
786 trained professionals would be readily available.

787 Alternatively, CINtec PLUS (Roche Diagnostics) has emerged as an effective
788 biomarker test for triaging those found to have ASCUS or LSIL in cytology screening (Tjalma
789 W. A., 2017), (Tjalma, Kim, & Vandeweyer, 2017), (Sun, Shen, & Cao, 2019), (Sun M. , Shen,
790 Ren, & al., 2018), (Yu, et al., 2019) and those testing positive for high-risk human
791 papillomavirus (hr-HPV) in HPV primary screening (Clarke, et al., 2019), (Wright T. C., et
792 al., 2015), (Guan, et al., 2012), (Qian, et al., 2018), (Wright Jr, et al., 2017), (Wang, et al.,
793 2017), (Arean-Cuns, et al., 2018), (Gustinucci, et al., 2016), (Bergeron C. , et al., 2015),
794 (Wentzensen, et al., 2015), (Wentzensen, et al., 2019).

795

796 *2.2.5.2 General*

797 The Canadian Task Force on Preventative Health Care recommends routine
798 screening for cervical cancer every two to three years for women 25-69 years of age. As

of 2013, provincially organized cervical cancer screening programs are in every province or territory except Northwest Territories, Nunavut, Yukon, or Quebec (Canadian Partnership Against Cancer, 2018).

"The essential elements for successful cytology screening include:

- Training of the relevant health care professionals, including smear takers, smear readers (cytotechnologist), cytopathologists, colposcopists, and program managers,
- An agreed decision on the priority age group to be screened (initially 35-45),
- Adequately taken and fixed smears,
- Efficient, high-quality laboratory services that should preferably be centralized,
- Quality control of cytology reading,
- A means to rapidly transport smears to the laboratory,
- A mechanism to inform the women screened of the results of the test in an understandable form,
- A mechanism to ensure that women with an abnormal test result attend for management and treatment,
- An accepted definition of an abnormality to be treated, i.e., high-grade lesions,
- A mechanism to follow-up treated women,
- A decision on the frequency of subsequent screens,

- A means to invite women with negative smears for subsequent smears. (Programme on Cancer Control, Department of Reproductive Health and Research, 2002)

Primary cervical cancer screening with cytology is the most common methodology used in the Western world. This technology was first trialled in the 1950s and became widely used in the 1970s and 1980s (Robson-Mainwaring, 2020). There is regular cytology and liquid-based cytology (LBC), and these tests look at cervical specimens and investigate the morphological changes in the cells. Changes in the cells may indicate that there may be precancerous changes taking place or if there is evidence of cancer.

These changes are graded on the Bethesda system (See section 2.2.4.2.1) of nomenclature and are referred according to specific algorithms. For example, any changes from Low-Grade Squamous Intraepithelial Lesions (LSIL) and greater would get referred for colposcopy; anything less would continue to be followed as part of the normal prescribed program.

Colposcopy is a more invasive investigation of the cervix performed by a gynaecologist; biopsy can be taken during this procedure, and the grade of precancer or cancer can be established. Historically, there is a long waitlist for colposcopy referral in many jurisdictions; Newfoundland and Labrador is no different. Also, gyneoncologists are highly paid and specialized physicians. Heavily on this group (possibly in the context of an

HPV primary screening program with no triage) would mean even longer wait times and exorbitant healthcare costs.

The grade Atypical Squamous Cells of Unknown Significance (ASCUS) is a questionable cytology grade, almost like an indeterminate result. As such, these women are followed closely. In NL, the strategy described in following sections, repeat pap and HPV triage for those greater than 30 years of age allows an additional marker to help stratify this in-between group and better establish who needs colposcopy. For example, those who are ASCUS and HPV positive get referred for colposcopy, and those who are ASCUS HPV Negative get additional follow up as part of the general Pap program (Cervical Screening Initiatives, NL, 2016).

Fundamentally, there are some issues with cytology. With regards to sensitivity, it is not the best (just over 50%); however, it is more specific. The performance has traditionally been improved with the repeat of Pap tests over time; thus, the basis for Pap repeat intervals.

Visually inspecting cells is very subjective. Studies have been completed to show that reliability and inter- and intra-observer agreement do falter; this is the case with cytotechnologists and cytopathologists. Also, given the specialized nature of inspection of slides, additional training and experience are required to be a cytotechnologist; typical lab technologists cannot perform the test, unlike HPV tests that can be performed in a standard molecular lab.

Operationally, each Pap slide must be read individually. Where cytology is a pathology procedure, all results require a final sign-off by a cytopathologist, a high-level physician who must be an active member of the result chain and interpretation. Meaning it takes a longer time to release results from Pap cytology.

HPV primary screening involves testing patients using a molecular-based assay, a nucleic acid amplification test (NAAT). In contrast to looking for cellular changes, this test looks directly for the absence or presence of an HPV infection. By determining if a woman has an HPV infection, they can be stratified by risk by several different approaches and algorithms.

One consideration, particularly in HPV testing, is the age at which to start screening. Younger women would most likely test positive for HPV and show some cytological abnormalities; it is not until they become older that the sustained and progressive infection starts to mean something more clinically significant. There is strong evidence that screening younger women less than 25 years of age can cause adverse events like loss of births due to the invasive nature of referring to colposcopy and any potential biopsies, especially when many of these changes and infections are transient and will regress with age. As such, many recommendations for HPV primary screening and cytology are to start at 25 to 30 years of age.

Operational advantages are that HPV primary screening can be performed by a standard laboratory technologist with no specialized, additional training and does not

require a specialized pathologist to sign out results. Also, with molecular methods, specimens can be batched in groups, for example the cobas 4800 platform can include 92 specimens (and 4 controls) and run simultaneously, other platforms may vary. Given the development and shift of laboratory medicine to NAAT, this methodology and basic equipment will be available in centralized locations for the foreseeable future.

As discussed in section 2.2.4.1, one of the biggest challenges of HPV primary screening is the high sensitivity and low specificity of molecular tests, meaning that not all positives will be true positives and potentially swamping a cervical cancer screening program with increased positive tests, overdiagnosis, and overtreatment (Chao, Clark, Carson, & al, 2019). An additional consideration is Positive Predictive Value (PPV), referring to the probability that someone with a positive test result truly has the disease; PPV is dependent on the prevalence of a disease in a population. It is important to remember that HPV infection is quite common, with more than 70% of sexually active Canadians having HPV at some point in their lives (Public Health Agency of Canada, 2020); however, as vaccination programs increase and the cohort of people protected grows HPV population prevalence will need to be considered in screening programs (Chao, Clark, Carson, & al, 2019). One option would be to forward all persons with HPV positive test to colposcopy; however, that would burden the system as there would be more referrals than for a cytology-based approach. The logical approach is to have some sort of triage and additional test to help further “weed out” those at greatest risk. Right now, cytology would be a viable option as it is already an established technique in many areas and has

relatively good specificity. Researchers are also looking at biomarkers and other methodologies like methylation testing for triaging options. These two alternatives would look more at the actual cellular and biological changes to better indicate cancerous changes rather than general non-specific cellular changes like the Bethesda classification system of cytology.

While there are many options, the most-effective strategy for cervical cancer in average-risk women would be Primary HPV with some sort of triage; for example, genotyping, Pap triage, or dual-stain triage (Isidean, et al., 2017). This is in line with high level recommendations (American Cancer Society, 2021) (Ontario Cervical Screening Program (OCSP), 2020) (CADTH, 2019) (Polman, Snijders, Kenter, Berkhof, & Meijer, 2019) (Tota, et al., 2015) (Care, 2013). While available in some jurisdictions and recommended in others, there have been some challenges in implementation such as availability of HPV testing.

A good approach for a screening algorithm for a disease that can be caught early and is treatable is, to begin with a sensitive test and follow up with a more specific test (McNamara & Martin, 2018) which is based on evidence of test accuracy as well as the potential benefits and harms of treatments (World Health Organization, 2013). This is particularly important when highly sensitive screening tests are used to detect target markers in low prevalence populations as the potential for false positives is greater; similar approaches are used in the context of HIV screening (Centres for Disease Control). This type of algorithm makes sense by allowing you to make sure you catch anyone who

could be at risk and then follow up with a more specific supplemental test to increase the overall accuracy. This is a similar approach to what is used in HIV screening and testing, using a general serological screening testing that is highly sensitive and then confirming reactive specimens with a more specific supplemental test such as an immunochromatographic test or a Western blot. Ensuring identification of false-positive screen positives and avoiding false reporting causing severe consequences and undue worry and stress to the patient. With HPV screening, though, it is more to do with the mostly transient infection characterized by the presence of "passenger" viral DNA or mRNA in the target population that does not pose transforming infection, and therefore, the need to identify the small fraction that may be at increased risk through the use of more specific tests. There are psychological and social consequences to telling a patient that they have screened positive in whichever screening model we choose. Health care professionals need to be mindful that we have set up a system that makes the best use of resources and ensures that we are being as correct as possible when we tell patients that they are at risk.

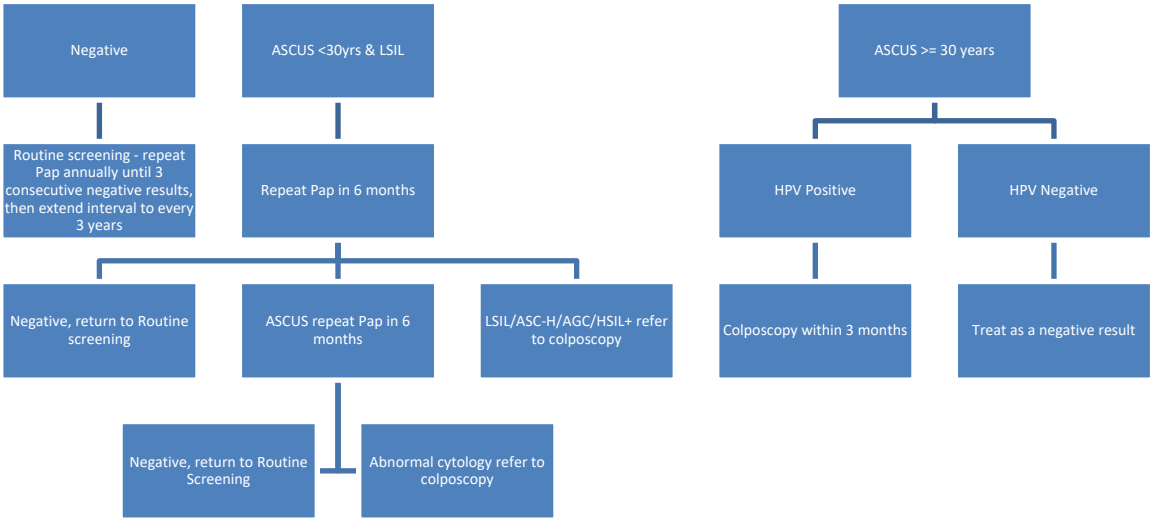
2.2.4.3 Newfoundland and Labrador

Newfoundland and Labrador has historically had a low turn-out for cervical cancer screening programs, with around 40% between 2007 and 2009 (Duke, et al., 2015). This low turn-out can be due to several reasons, including access to primary care physicians, referral delays, providers' attitudes towards screening, limited access to screening, embarrassment, the test being conducted by a males physician, and knowledge of the

benefits of cervical cancer screening (Duke, et al., Effect of vaginal self-sampling on cervical cancer screening rates: a community-based study in Newfoundland, 2015).

The provincial Cervical Screening Initiatives program has done a lot of promotion around the need for screening, retention of patients in screening, and have improved turn out to screening and recall; however, there are limitations in colposcopy wait times and low biopsy rates (Rose, 2016). Based on some national indicators, we see a provincial morbidity rate of approximately 6/100,000 compared to the national rate of 8/100,000. However, NL's mortality rate is approximately 4/100,000 compared to 2/100,000 nationally.

The current provincial screening algorithm is as follows (Figure 2.7):



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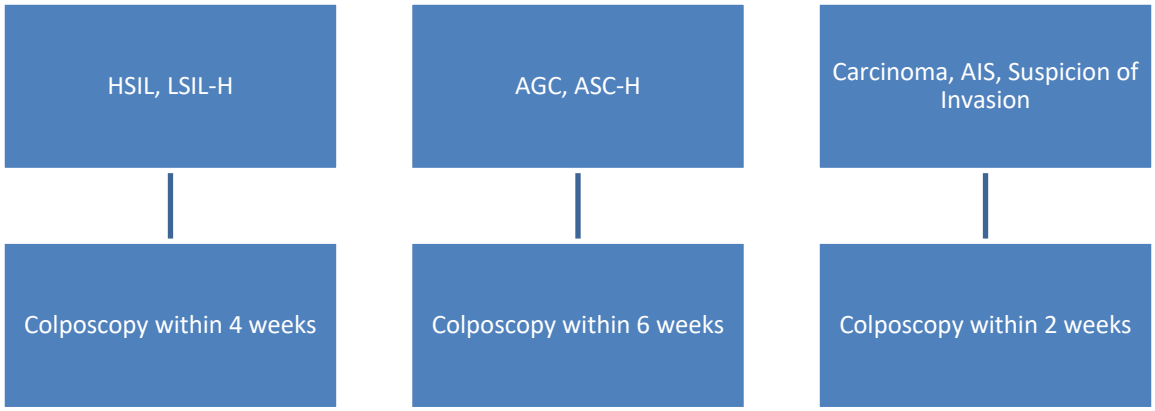


951

952 *Figure 2.7. NL Pap screening algorithm for Women with a Pap test Result of ASCUS <30*
953 *years or LSIL (all ages), Reproduced with Permission, (Cervical Screening Initiatives, NL,*
954 *2016).*

955

Currently in the province, ASCUS-HPV triage is the approach used where those patients with ASCUS results over the age of 30 receive HPV testing. This triage helps identify those women at risk of developing precancerous lesions, reduce colposcopy referral, and avoid unnecessary follow-up for the majority not at risk. In NL, approximately 25% of the ASCUS population test HPV positive; this triage spares 75% from unnecessary colposcopy referral. Of the 25% testing HPV positive, one-third of patients test positive for 16/18, and two-thirds of patients test positive for OHR genotypes. These proportions appear to correlate with the data from the ATHENA trial (Stoler, et al., 2011). As per ATHENA trial, the absolute risk of CIN 2+ is approximately 24% among women positive for 16/18 genotypes and around 9% among "OHR" positive. Thus, in ASCUS-HPV triage, twice as many women test positive for OHR than 16/18 positive but with close to one-third of the CIN 2+ risk. However, per current guidelines in Canada and elsewhere, all those testing HPV positive are deemed at risk and referred for colposcopy (NL Cervical Cancer Screening Initiatives, 2011). There is potential to help better stratify those at greatest risk by taking a closer look at the specific HPV types instead of general HPV positivity. This has yet to be looked at in a NL setting.

From a Canadian perspective, organized screening programs are available in most provinces; as of 2018, Northwest Territories, Nunavut, Yukon or Quebec do not have organized programs, but opportunistic screening may be available (Canadian Partnership Against Cancer, 2018). In general, provinces and territories recommend that cervical cancer screening begin at age 21 or 25, and continue until age 65 to 70 and occur every

977 2-3 years. Each program may vary slightly by means of administration, methodology, and
978 timing.

979 ### 2.2.6 Vaccination programs

980 Vaccination against some HPV strains have been shown to decrease cervical
981 cancer risk drastically (NCI Staff, 2020). From 2008-2014, the proportion of HPV16/18-
982 positive CIN2+ has declined, with the most significant declines observed in vaccinated
983 women (McClung, et al., 2019). Additionally, reductions were observed in those
984 unvaccinated (55.2%-33.3% for vaccinated versus 51.0%-47.3% for unvaccinated),
985 suggesting the positive implications of herd protection (McClung, et al., 2019).

986 The distribution of genotypes (as shown in section 2.2.1) is important to consider,
987 particularly as the research community develops new vaccines for primary protection
988 against infection. The current bivalent vaccine (2vHPV, Ceravix, GlaxoSmithKline) protects
989 against HPV-16 and HPV-18, protecting against approximately 70% of cervical cancers
990 (Meites, et al., 2019). The current quadrivalent vaccine (4vHPV, Gardasil 4, Merck)
991 protects against HPV-16, HPV-18, HPV-6, and HPV-11, so in addition to protecting against
992 cervical cancer, it also protects against the non-oncogenic types HPV-6 and HPV-11
993 causing genital warts. There is a new 9-valent (9vHPV, Gardasil 9, Merck) vaccine which
994 protects against HPV genotypes 6, 11, 16, 18, 31, 33, 45, 52, and 58; this additional
995 protection is valuable in extending protection. The most commonly available vaccines
996 follow either a 2 or 3 dose schedule.

The Canadian National Immunization Strategy has set a goal of 90% of HPV vaccination coverage by 17 years of age for two or more doses of HPV vaccine by the year 2025 (Canadian Partnership Against Cancer, 2018). As of 2017, school-aged HPV vaccination is available for all children in all areas of Canada, and most jurisdictions also have extended eligibility programs which allow for additional groups to receive the vaccine series. Overall, the general immunization coverage for Canada ranges from 57-92%, with NL having the highest immunization update (Canadian Partnership Against Cancer, 2018). Inclusion of males in the HPV vaccination program makes sense from various standpoints, including protection from HPV-associated disease for men (outside the focus of this work) and reduction of transmission to female partners (Shapiro, Perez, & Rosberger, 2016).

Ultimately, given HPV as the aetiological agent of cervical cancer, vaccination programs, in concert with screening programs, provide a comprehensive approach to minimizing disease and catching pre-cancer to prevent a high burden of disease. With the development of HPV vaccination, there are some that may ask if it is necessary to continue to spend money in developing cervical cancer screening programs. Safaeian & Sherman (2013) raise three crucial points why this is the case:

(1) many women fall outside the recommended ages for vaccination,

(2) vaccines would not protect women who are infected before immunization, and

(3) vaccines currently do not protect against all oncogenic HPV genotypes.

In 2007, the implementation of school-aged HPV vaccines in the province also introduced a future factor yet to be realized. The Merck Gardasil® vaccine is the one in current use in Newfoundland and Labrador. This vaccine provides protection from genotypes 6 and 11, the two HPV genotypes attributable to genital warts, and genotypes 16 and 18, the two oncogenic HPV types that contribute to approximately 70% of cervical cancer (Mayrand, et al., 2007). Vaccination will eventually decrease the frequency of cytological abnormalities and lower the cancer risk related to positive screens (Safaeian & Sherman, 2013), yet what about those who have oncogenic HPV types not covered by any current vaccine? The oncogenic HPV types not covered by the quadrivalent widely used vaccine still make up approximately 30% of cervical cancers. The government of NL has mandated the necessity of cost savings across all aspects of the public sector; therefore, it is critical to thoroughly review and critique the existing system and the potential avenues for change.

Ultimately, the combination of high coverage vaccination and effective cervical cancer screening can reduce the burden of cervical cancer to 4 cases /100,000/year; designating cervical cancer as a "rare disease" (de Sanjose & Delany-Moretlwe, 2019). Additional, with the application of HPV vaccination and primary HPV screening, it has been estimated that CIN2/3 and invasive cervical cancer rates are predicted to fall by 40-44% and 42-51%, respectively (Hall, et al., 2018)

2.2.7 Areas for development

Given the 2007 implementation of HPV vaccination in NL, it is essential to shift to HPV primary screening as the young, vaccinated women enter the screening program over time given the expected reduced frequency of screen-identified abnormalities and lower cancer risk for those screening positive (Safaeian & Sherman, 2013). As this becomes a reality, it becomes critical to find a strategy to triage those women who are HPV positive (Isidean, et al., 2017). A CINtec PLUS cytology approach may meet these needs as a more specific test which looks at oncogenic changes.

One aspect that is lacking in NL is the lack of a unified surveillance program to follow the vaccinated cohort of young people. HPV is a concern not only for cervical cancer but also for head and neck, anal, penile, and vaginal cancer (NCI Staff, 2021; Colon-Lopez, Ortiz, & Palefsky, 2010). As a province, it would be valuable to evaluate our vaccination program by following the incidence and prevalence of HPV as indicators. One possible means for this evaluation would be through the Newfoundland and Labrador Centre for Health Information's (NLCHI) HEALTHe NL, an electronic health record (HER), which has data for all patient immunizations administered at community pharmacies, and by Community Health programs within the Regional Health Authorities within the province from 2003 onward as well as any interaction between a patient and a health care provider for services or health assessments (Newfoundland and Labrador Centre for Health Information). It is vitally important to follow these cohorts, and if they develop cancer, establish which HPV genotype is responsible. This genotyping can assist us in better

understanding the natural history of HPV and guide decision-making and policy development as new vaccines become available. In addition, by following these groups, we can truly understand the cost savings and potential reduction in the burden of disease for the young people in our province.

There is room for improvement in our screening programs (Ogilvie, et al., 2017). Worldwide, researchers have been working to improve Pap cytology-based cervical cancer screening programs; this includes strategies like continuing with primary Pap cytology, ASCUS-HPV triage, co-testing, and HPV primary screening (Castle P. , 2015) (Dickenson, et al., 2012) (Mayrand, et al., 2007) (Agoratos, et al., 2015). As new algorithms and technologies emerge it is essential that they be evaluated and assessed from patient safety, quality, and financial perspectives (Sawaya & Huchko, 2017).

Ultimately, risk assessments need to take place for each jurisdiction to balance clinical actions, resources, and well-being for those being screened (Castle, Sideri, Jeronimo, Solomon, & Schiffman, 2007).

We have seen some improvement provincially with the implementation of ASCUS-HPV triage for women over 30 years of age in 2007, which helps to stratify better women who are at less of a risk for cervical cancer and to help reduce the burden on the colposcopy program.

The use of molecular testing in NL allows for simultaneous genotyping, allowing for further risk stratification (i.e., it provides a result HPV Negative, HPV-16, HPV-18, and

HPV Other High Risk). Triage currently uses HPV positive or HPV negative as indicators for colposcopy and yearly follow-up, respectively. We could further reduce colposcopy referral for those testing OHR positive and follow them closely, knowing those testing HPV-16 and HPV-18 positive are the ones at higher risk and require immediate referral to colposcopy.

Ultimately, considerations of new algorithms and methodologies should be considered to improve the identification of patients and more accurately identify those at greatest risk for cervical cancer.

Given the current, dire financial situation in NL, primary HPV screening with cytology triage could easily and effectively be implemented given the capacity of molecular HPV testing, the currently available centralized testing service for HPV at the Public Health Microbiology Laboratory (PHML), and the existing partnership long established between the PHML and the Regional Cytology Laboratories as developed via the ASCUS-HPV triage program. The shift to HPV primary testing, in addition to being better to evaluate the risk of patients, would also be cost-effective (Vigayaraghavan, Efrusy, Mayrand, Santas, & Goggin, 2010) (Cromwell, et al., 2021) and could help to reduce an already backlogged colposcopy referral waitlist in NL.

2.3 SARS-CoV-2 & COVID-19

2.3.1 Connection of SARS-CoV-2 to COVID-19

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the virus that causes coronavirus disease, COVID-19. On February 11, 2020, the name SARS-CoV-2 was selected due to them sharing ~83% genomic similarity to the coronavirus responsible for the 2003 SARS outbreak, and COVID-19 was chosen due to previously developed nomenclature rules developed by international programs (World Health Organization, 2021) (Kaur, et al., 2021).

2.3.2 History & Origins

Humans have experienced pandemics and epidemics of varying severity and impact throughout history. As civilization has grown and developed, pathogens have thrived as smaller populations grew, interacted with other societies, became more urbanized, and relied more on agriculture (Diamond, 1999). For example, the Black Death, 1346-1352 (Diamond, 1999) was responsible for the death of at least 1/3 of medieval Europe (Waltner-Toews, 2007) was caused by *Yersinia pestis,* the bacteria causing the plague. The 1918 Spanish Influenza pandemic had a dramatic impact globally, with an estimated 500 million cases[5] and at least 50 million deaths worldwide (Centers for Disease Control and Prevention, National Center for Immunization and Respiratory Diseases

[5] Approximately 1/3 of the world's population

(NCIRD), 2019), and result in a mortality rate of over 70% in some Labrador communities (Budgell, 2018).

Zoonotic diseases are infectious diseases that have animals as natural reservoirs that are transmitted to humans (World Health Organization, 2020). Zoonoses are a naturally occurring phenomenon and are more common, particularly when humans live in close proximity to animals, thus increasing the risk for infections to jump the species barrier (Waltner-Toews, 2007) (Diamond, 1999). The closer proximity we are to animals, the more we share their infections (Diamond, 1999). Some of the more common animal hosts for zoonotic diseases are fleas, pigs, chickens and other birds, bats, and non-human primates. For example, fleas are responsible for the widespread outbreaks of plague in ancient times (Waltner-Toews, 2007).

While the origins of the SARS-CoV-2 virus are unknown, the World Health Organization (WHO) is leading the investigation; in their report released on March 30, 2021, animal markets or farms supplying animal markets likely played a significant role in the start of the COVID-19 pandemic. For example, the Huanan Seafood market in Wuhan, China, was linked to many of the first COVID-19 cases in December 2019, and 2/3rds of those first cases reported exposure to live or dead animals (Maxmen, 2021). However, since the beginning of the pandemic there have been circulating theories that the origin of SARS-CoV-2 may have started with a leak from a laboratory. In a report released from WHO on June 9, 2022, experts indicated that key pieces of information to establish the cause of the pandemic are still unknown and that the investigative team would remain

open to any evidence. The group even went further to note that there have been past incidents where lab leaks have caused outbreaks, ultimately, no studies have been provided to WHO to assess this hypothesis and there needs to be further investigation (The Associated Press, 2022).

Bats and pangolin viruses have been mentioned as possible jumping points for SARS-CoV-2. In addition to reviewing health records in the province of Hubei for the second half of 2019 to look for any unusual respiratory activity and retrospective testing of 4,500 patients for virus antibodies (Mallapaty, Maxmen, & Callaway, 2021), whole genome sequencing has been performed on over 1000 samples from the Huanan market including pangolins and bats that had similar coronaviruses. At the same time, these viruses were too evolutionarily distant to SARS-CoV-2 but do provide some possible avenues of zoonotic transmission (Maxmen, 2021). Researchers have also demonstrated evidence of SARS-CoV-2 related coronaviruses circulating in bats and pangolins in Southeast Asia (Figure 2.8) and have also detected SARS-CoV-2 neutralizing antibodies in both species (Wacharapluesadee, et al., 2021). Investigations continue to understand the specific origins of SARS-CoV-2 better.

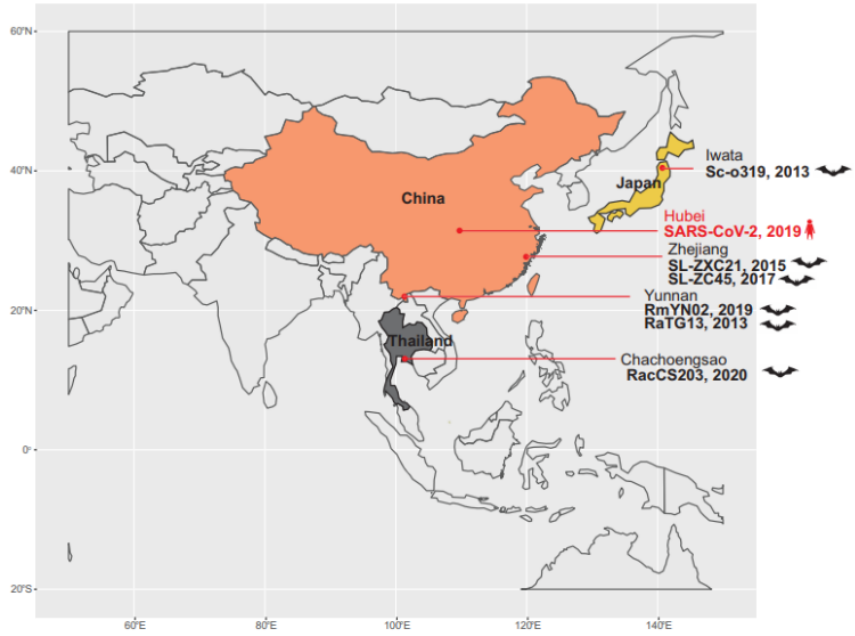


Figure 2.8. Map of Asia illustrating SARS-CoV-2 related coronaviruses detected in the region, Reproduced under Creative Commons Attribution 4.0 International License, (Wacharapluesadee, et al., 2021)

2.3.3 Epidemiology

Since the emergence of SARS-CoV-2 at the end of 2019, there have been over 346 million cases and over 5.5 million deaths worldwide, as of January 23, 2022 (World Health Organization, 2022). To stop the spread of infection, manage patients, and provide timely information to public health policymakers, accurate, timely, and accessible diagnosis of SARS-CoV-2 infection is required (Xiang, et al., 2020) (Morrison, Li, & Loshak, 2020).

While the situation in Canada has not been as dire as in other countries, there have still been nearly 3 million cases and over 33,000 deaths, as of January 28, 2022

(Government of Canada, 2022), with a relatively even distribution between males and females. Those who are older face particular challenges; 60–79-year old's face higher proportions of disease, particularly when we look at those admitted to ICU, and those 80 years of age and older face higher hospitalization and mortality numbers.

2.3.4 Risk factors for SARS-CoV-2 and COVID-19

There is a variety of risk factors for acquiring SARS-CoV-2. People at most significant risk of exposure to SARS-CoV-2 include those with occupational risks, those living in communal or group settings, and those facing social, economic, or personal barriers limiting access to effective public health measures. People at risk of more severe disease include older people, those who have chronic medical conditions, and those who are immunocompromised (Government of Canada, 2021).

2.3.5 Tests

At the start of the COVID-19 pandemic, the primary focus of SARS-CoV-2 testing in Canada and the province of Newfoundland and Labrador was on the development of molecular methods using real-time reverse transcription-polymerase chain reaction (RT-qPCR) to detect SARS-CoV-2 RNA. At the beginning of the pandemic, national and provincial partners worked quickly to develop new lab-developed tests and evaluate them as they entered the market and achieved Health Canada approval (LeBlanc, et al., Real-

time PCR-based SARS-CoV-2 detection in Canadian laboratories, 2020). While these tests focused on different targets, PCR design and development is outside the scope of this work. It is important to note the important role that high-throughput methods played, meaning that many samples could be batched and processed helping labs maintain capacity during pandemic waves and surges of cases.

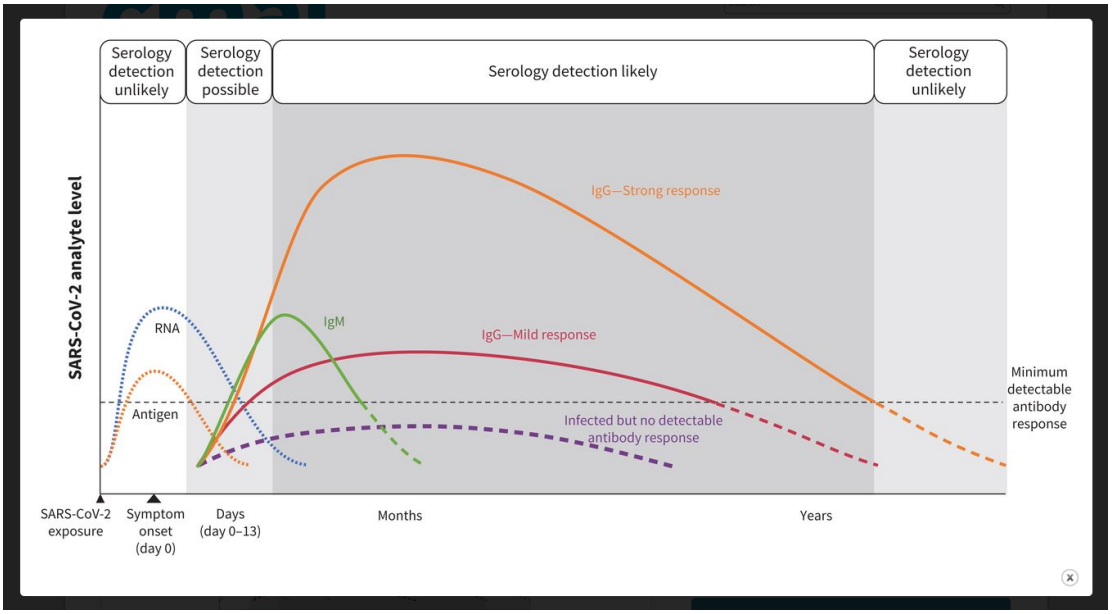
SARS-CoV-2 PCR tests may also indicate a positive test in patients who are asymptomatic in addition to those who have symptomatic disease. The limitation of these molecular tests is that they can only indicate the presence of viral RNA in a specimen; they cannot indicate the presence of viable viral particles. The absence of the viral RNA does not indicate if a person was infected and has since recovered or if the person is infected, but the virus was undetectable from that source at that time (Wang, et al., 2020) (Morrison, Li, & Loshak, 2020). In practice, RT-qPCR tests are performed on those patients who are symptomatic or those who are epidemiologically linked to COVID-19 cases, an approach that likely underestimates the true prevalence of infection in the population (Khan S. , et al., 2020). Additionally, RT-qPCR methods also face challenges with the accessibility of reagents and equipment, availability of trained personnel, cost, and challenges with performance (Liu, et al., 2020) (Xu, et al., 2020).

Due to these limitations, developments in serological testing were undertaken to better quantify the number of individuals exposed to SARS-CoV-2 (Centres for Disease Control and Prevention, 2020) (Morrison, Li, & Loshak, 2020). Additionally, antibody detection may provide a complementary perspective, along with RT-qPCR testing, as an

effective tool for COVID-19 screening in close contacts presenting with signs or symptoms but negative SARS-CoV-2 RT-PCR results (Xu, et al., 2020). However, the clinical utility for serological testing for SARS-CoV-2 is more complicated; it has a limited role in clinical settings because it usually takes a minimum of one to two weeks for patients to develop an antibody response but may help in assessing patients with atypical clinical presentations such as multisystem inflammatory disorder (Van Caeseele, Bailey, Forgie, Dingle, & Krajden, 2020). Its real strength lies in research on natural immunity following exposure or vaccine-induced immunity and population-level epidemiologic approaches (Van Caeseele, Bailey, Forgie, Dingle, & Krajden, 2020).

Enzyme-linked immunosorbent assay (ELISA) and chemiluminescent microparticle immunoassays (CMIA) provide semiquantitative in vitro measurement of the levels of human antibodies of the immunoglobulin class A (IgA) and G (IgG) against SARS-CoV-2 in serum and work under the principals of antibody response to infection (Figure 2.9). In the natural immune response to SARS-CoV-2 infection antibodies, IgM, IgG, and IgA can be detected within 1-3 weeks of infections, with IgM and IgA antibodies declining more rapidly than IgG (Centres for Disease Control and Prevention, 2020). SARS-CoV-2 IgM and IgG have been established as vital in understanding the course and immune response of COVID-19 (Wu, et al., 2020), while less is known about IgA (Centres for Disease Control and Prevention, 2020). There are many different options for serology tests, both traditional and point of care rapid tests, from many vendors. The tests evaluated in this work corresponds to availability and the platforms that were available within the

laboratory; evaluation of different antibodies and their mechanisms are outside the scope of this work. Stein et al. provide a Canadian perspective of the performance of available tests with test performance ranging from 52.1%-89.1% for all time points with lab-based assays (Stein, et al., 2021); additional details can be found in Appendix E.



Note: IgM = immunoglobulin M, IgG = immunoglobulin G

Figure 2.9. Kinetics of antibody response to infection caused by SARS-CoV-2, Reproduced with Permission, (Van Caeseele, Bailey, Forgie, Dingle, & Krajden, 2020)

2.3.6 Areas for development

As we continue to live in the ongoing COVID-19 pandemic, new tests continue to enter the market to meet the needs of the growing demand; these need to be sufficiently vetted to ensure the highest quality and inform appropriate utilization. Not all tests are created equal or perform the same, and some serve purposes better than others; new

evaluations are critical and it is critical that jurisdictions evaluate performance indices based on their local prevalence and needs.

Additionally, partnerships amongst stakeholders like the Canadian Public Health Laboratory Network provide an essential role in consensus-seeking and developing guidelines and best practices. Researchers play a crucial role in providing evidence for these key decision-makers.

The natural life cycle of viruses involves frequent changes through mutations; as such multiple variants have been and are continuing to be identified (Centres for Disease Control, 2021). DNA viruses are relatively stable in comparison to RNA viruses like SARS-CoV-2. Specifically, in some reports SARS-CoV-2 has been accumulating 0.44 substitutions per week and have shown its ability to quickly adapt to new environments and evolve (Amicone, et al., 2022). Variants are currently being tracked as either a notifiable Variant of Concern (VOC), a Variant of Interest (VOI), or a Variant Under Monitoring (VUM). VOCs are the most serious concern due to their increased transmissibility, virulence, and possible decreases in diagnostic, therapeutic, or vaccine efficacy (outbreak.info, 2021); at the time of writing, the main VOCs are called Alpha, Beta, Gamma, Delta, and Omicron per the World Health Organization's VOC nomenclature. As new genomic variants emerge in the global pandemic, surveillance programs and public health testing become even more critical in the early detection of the changing virus. Whole genome sequencing and targeted molecular assays are tools to aid these investigations further.

One area that is emerging considering global vaccination programs for SARS-CoV-2 is the ability to detect immune status using serological assays; this would allow for the assessment of immunity and allow for the estimation of herd immunity (Galipeau, Greig, Liu, & Langlois, 2020). One great example of a large-scale program is the Canadian Blood Services seroprevalence study which undertakes periodic seroprevalence of the antibodies present in donor's blood (COVID-19 Immunity Task Force, 2022). In general, here are certain limitations in some current assays, and to correctly evaluate immunity and vaccine response, serological tests need to have S1-RBD-neutralizing IgG antibody detection as there may be better correlation to immunity due to the utilization of this S1-RBD is aligned with multiple vaccine and is the most common target of vaccine designs (Siemens Healthineers, 2021).

The COVID-19 pandemic is a stark reminder of the importance of infectious disease threats. This event stresses the importance of ongoing infectious disease surveillance and systemic capacity to respond (Christian, et al., 2013). While much progress has been made globally in reducing the overall burden, health disparities and gaps in our systems mean that our populations' health remains vulnerable (McFee, 2013).

Chapter 3 : Paper 1, Improving cervical cancer screening in Newfoundland and Labrador

Unpublished, prepared in manuscript format for potential submission to Canadian Health Policy Journal

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Authors' contributions

LG: Project administration, investigation, study design and methodology, laboratory technical support, resources, data curation and analysis, writing-original draft and writing-review and editing.

Author did not perform testing. All testing was part of routine screening programs in the province of Newfoundland and Labrador.

Author reviewed and approved the final version for submission. Author attests they meet the ICMJE criteria for authorship.

Abstract

Cervical cancer screening is a long-standing program requiring review of existing programs and new methodologies. This study reviewed the Newfoundland and Labrador (NL) cervical screening program data as well as publicly available Canadian data to investigate the state of cervical cancer screening from 2002 to 2019 with a focus on low-grade abnormalities in Newfoundland and Labrador screening eligible women to understand the changes over time and possible future improvements. The findings indicate that while there have been attempts to improve cervical screening participation, high rates of abnormalities, pre-cancerous lesions, and invasive cancers remain troubling. In conclusion, based on the review of local cervical screening programs, there are opportunities for improvement for additional strategies, new strategies, and additional investigation.

3.1 Introduction

HPV is the aetiological agent of cervical cancer; the complex interplay between risk factors and oncogenic HPV infections contribute to the persistence of HPV infections and the development of pre-cancerous and cancerous lesions (Munoz, Castellsague, Berrington de Gonzalez, & Gissmann, 2006). Contributing risk factors include early-onset of sexual activity, multiple sexual partners, high-risk sexual partners, history of other sexually transmitted infections, immunosuppression, oral contraceptive use, having a first degree relative with cervical cancer, and low socioeconomic status (SES) or living in a high

poverty country, to name a few (Hillemanns, Soergel, Hertel, & Jentschke, 2016) (Castellsague, 2008). HPV is highly contagious and is now considered the most common sexually transmitted infection in most populations (Castellsague, 2008). An estimated 80% of sexually active people are infected with HPV at some point in their lives (Cleveland Clinic, 2018).

The Pap test, which looks for morphological changes in the cells of the cervix caused by oncogenic HPV, was introduced in 1949 by Georgios Nikolas Papanicolaou. The Wilton report (1974) recommended the widespread introduction of the Pap test for cervical cancer screening in Canada. However, it did take a while for programming to be implemented across Canada and it was the 1980s before provincial screening programs were implemented in Newfoundland and Labrador. Overall, with this implementation, a 70% reduction in cervical cancer incidence rates was observed (Mayor, 2016).

While new methodologies have been developed, cervical cancer screening with traditional Pap cytology remains the most common methodology used in the developed world. In this system, morphological changes in cervical cells are evaluated, which may indicate pre-cancerous changes taking place or evidence of cancer. Changes are graded on the Bethesda nomenclature system and are referred for additional follow-up according to specific algorithms (Canadian Partnership Against Cancer, 2018). For example, any changes from Low Grade Squamous Intraepithelial Lesions (LSIL) and worse would get referred for colposcopy; anything less would continue to be followed as part of the normal prescribed program.

Colposcopy is a more invasive investigation of the cervix typically performed by a gynecologist; during this procedure biopsy can be taken, and the grade of precancer or cancer can be established. Historically, there is a long waitlist for colposcopy referral in many jurisdictions; the province of Newfoundland and Labrador (NL) is no different. It was noted in a 2013 NL report that the colposcopy wait time for 76% of women (from ages 20-69) was within 12 months (Cervical Screening Initiatives Program, 2013).

ASCUS is a questionable cytology grade; therefore, patients are followed closely. In NL, ASCUS-HPV triage allows for an additional marker to help stratify this in-between group and better establish who needs colposcopy. This triage helps identify those patients at risk of developing pre-cancerous lesions and reduce colposcopy referral while avoiding unnecessary follow-up for the majority that are not at risk. For example, those who are ASCUS and HPV-positive get referred for colposcopy. ASCUS HPV-negative patients get additional follow-up as part of the general Pap program (Cervical Screening Initiatives, NL, 2016).

CIN is pre-invasive lesions as detected by biopsy. As part of the Bethesda system of classification and nomenclature, CIN1 is considered low-grade and relatively begin, and recommendations are to manage conservatively; LSIL cytology falls within this grade. CIN2 is a higher grade than CIN1 and is regarded as the cut-off point to proceed with some sort of treatment as there is an indication of progression; CIN3 is higher again. Both CIN2 and CIN3 are included in HSIL.

Approximately 1.5/1000 women are diagnosed with CIN2/3 annually, with the highest incidence in those between 25-29 years of age (Tainio, et al., 2018). CIN2 or worse (CIN2+) and CIN3 or worse (CIN3+) are typically used in research evaluating the diagnostic performance of tests and include adenocarcinoma in situ (AIS) and other cancers.

In Canada, there are approximately 1,500 new cases of invasive cervical cancer and approximately 300 deaths annually (Canadian Partnership Against Cancer, 2016). In addition, cervical cancer is the second most common type of HPV-associated cancer in Canada, surpassed by oropharyngeal cancer (Canadian Cancer Society, Statistics Canada, Public Health Agency of Canada, Provincial/Territorial Cancer Registries, 2016). However, with the availability of highly efficacious HPV vaccines and HPV vaccination programs playing a vital role in primary prevention strategy combined with secondary screening programs, the disease can be largely prevented (Cohen, Jhingran, Oaknin, & Denny, Cervical Cancer, 2019) and possibly eradicated (Canadian Cancer Society, Statistics Canada, Public Health Agency of Canada, Provincial/Territorial Cancer Registries, 2016).

The national age-standardized invasive cervical cancer incidence rate ranged from 8.8 to 12.1 per 100,000 (Canadian Partnership Against Cancer, 2016), with New Brunswick (NB) having the lowest rate and Newfoundland and Labrador (NL) having the highest rate (Canadian Partnership Against Cancer, 2016). For the age-standardized squamous cell carcinoma rate, NB had the lowest rate of 5.5 per 100,000, and NL had the highest of 8.2 per 100,000 (Canadian Partnership Against Cancer, 2016). In contrast, the age-standardized non-squamous cell carcinoma rate ranged from 2.7 to 4.7 per 100,000, with

British Columbia having the lowest rate and, Manitoba the highest rate (Canadian Partnership Against Cancer, 2016).

Historically, NL has struggled with a low turn-out for cervical cancer screening; this may be due to several reasons, including access to primary care physicians and knowledge of the benefits of cervical cancer screening. The provincial Cervical Screening Initiatives program has focused on health promotion around the need for screening, retention of patients in screening, and have improved turn out to screening and patient recall; however, there are limitations in colposcopy wait times and low biopsy rates (Rose, 2016).

In NL the current cervical screening approach involves routine repeat Pap annual until 3 consecutive negative results, at that time screening is extended to every three years. For those less than 30 years of age with an ASCUS result, Pap is repeated in 6 months with stratification to colposcopy if abnormalities persist. For those 30 and older, ASCUS-HPV triage is used. For all other abnormalities colposcopy referral is required, with varying time frames (Cervical Screening Initiatives, NL, 2016).

Currently in the province, ASCUS-HPV triage is the approach used where those patients with ASCUS results over the age of 30 receive HPV testing. This triage helps identify those women at risk of developing precancerous lesions, reduce colposcopy referral, and avoid unnecessary follow-up for the majority not at risk. In NL, approximately 25% of the ASCUS population test HPV positive; this triage spares 75% from unnecessary colposcopy referral. Of the 25% testing HPV positive, one-third of patients test positive

for 16/18, and two-thirds of patients test positive for OHR genotypes. These proportions appear to correlate with the data from the ATHENA trial (Stoler, et al., 2011). As per ATHENA trial, the absolute risk of CIN 2+ is approximately 24% among women positive for 16/18 genotypes and around 9% among "OHR" positive. Thus, in ASCUS-HPV triage, twice as many women test positive for OHR than 16/18 positive but with close to one-third of the CIN 2+ risk. However, per current guidelines in Canada and elsewhere, all those testing HPV positive are deemed at risk and referred for colposcopy (NL Cervical Cancer Screening Initiatives, 2011). There is potential to help better stratify those at greatest risk by taking a closer look at the specific HPV types instead of general HPV positivity. This has yet to be looked at in a NL setting.

From a Canadian perspective, organized screening programs are available in most provinces; as of 2018, Northwest Territories, Nunavut, Yukon or Quebec do not have organized programs, but opportunistic screening may be available (Canadian Partnership Against Cancer, 2018). In general, provinces and territories recommend that cervical cancer screening begin at age 21 or 25, and continue until age 65 to 70 and occur every 2-3 years. Each program may vary slightly by means of administration, methodology, and timing.

Our initial aims were to conduct a retrospective study to review the cervical screening program within the province of NL from 2002 to 2019 to assess positivity and clinical disease endpoint and to evaluate trends. We reviewed and evaluated trends in publicly available data and a limited data set from the provincial Cervical Screening

Registry. In particular, registry data was obtained for ASCUS and LSIL occurrences and complementary HPV testing. HPV triage was first introduced to the province in 2005, and a change in testing methodology took place in 2014; positivity rates were reviewed and comparison of any available HPV genotype data. However, due to limitations and challenges found during our review, the comprehensive assessment we had hoped for was not possible and elements of a program evaluation were included as a supplement. As such, the objective of this work is to investigate the state of cervical cancer screening from 2002 to 2019 with a focus on low-grade abnormalities in Newfoundland and Labrador screening eligible women to understand the changes over time and possible future improvements.

3.2. Methods

3.2.1. Study protocol

With our initial goals for this study being focused on the NL Cervical Screening Programming specifics, requests were submitted to the Newfoundland and Labrador Centre for Health Information and the Eastern Health regional health authority after receiving ethics approval. This retrospective approach included data from the Newfoundland and Labrador Cervical Screening Initiatives program, including all patients screened from 2002 to 2019 with ASCUS or LSIL pap results. This study was a retrospective review, and as such, all patients were attended to per standard of care. In addition, Pap

data and HPV results, including some genotyping data, from 2002 to 2019 were reviewed and assessed in context with programmatic changes.

To complement the above data, publicly available data from the Canadian Cancer Society, Statistics Canada, Public Health Agency of Canada and the Canadian Partnership Against Cancer were also utilized (Canadian Partnership Against Cancer, 2018) (Canadian Cancer Society, Statistics Canada, Public Health Agency of Canada, Provincial/Territorial Cancer Registries, 2016). These sources were compared and contrasted descriptively.

To better critique and complement the available data, both publicly available and health system data were descriptively reviewed and assessed from a quality improvement perspective using an established program evaluation framework (Centers for Disease Control and Prevention, 1999), where possible.

3.2.2. Ethics

The study was approved by the Newfoundland and Labrador Health Research Ethics Board (HREB) (Appendix F) and Research Proposals Approval Committee (RPAC). Based on HREB criteria and policies, this study was exempt from patient informed consent.

Approvals were also obtained from data custodians at the Provincial Cervical Screening Registry, Eastern Health, the Eastern Health Research and Innovation Team. In addition, we received organizational approval through the Research Proposal Approval Committee (RPAC) for the initial data request.

3.2.3. Study Patient Criteria – Cervical Screening Registry

For registry data, any patients with Pap results with ASCUS and LSIL results and HPV results from 2002 and 2019 were eligible. There were no age limits. Patients must have been eligible for the NL Cervical Screening Initiatives program.

Given the registry is administered outside of this study additional descriptions of the dataset are unavailable (Eastern Health, 2021).

3.2.4 Pap cytology

In NL, cervical specimens are collected in ThinPrep® cytology collection device (BD) for routine cytology per the provincial program. Patients can access Pap cytology through physician offices, women's health clinics, student health clinics, and Planned Parenthood. Cervical specimens are forwarded to regional cytology laboratories in the province for review by cytotechnologists and pathologists sign off. While there are several regional cytology labs in the province, the main site within Eastern Health, St. John's, NL, coordinates the ASCUS triage program in conjunction with the NL PHML and any additional follow-up or triage.

3.2.5. HPV testing

Initially when HPV tirage was introduced in the province in 2005, Hybrid Capture 2 (HC2; Digene, Gaithersburg, MD) was utilized. HC2 is a nucleic acid hybridization assay

with signal amplification using microplate chemiluminescence for the qualitative collective detection of thirteen high-risk HPV genotypes, HPV-16,18,31,33,35,39,45,51,52,56,58,59,68 (Digene, 2002), giving a detected/not detected or positive/negative result.

In 2014, HPV testing changed to the cobas HPV test and was performed on the Roche 4800 automated platform per manufacturer's instructions; this test uses an automated extraction step for HPV and cellular DNA and a PCR amplification step (Roche, 2015). This test simultaneously identifies genotypes 16/18 specifically and 12 Other-high risk types (OHR), HPV-31,33,35,39,45,51,52,56,58,59,66,68, collectively, and reports results as positive for 16 or 18 or both, or positive for 12 OHR or negative for 14 hr-HPV types. Both HPV tests were performed at the PHML in St. John's.

3.2.6. Data analysis

Statistical analysis was performed using SPSS for Windows, versions 23 and 27, Excel, Microsoft Office Professional Plus, 2013, and Social Science Statistics website, 2020. Qualitative variables were studied through different frequencies. Descriptive statistics were prepared for test results, distribution of available cytology grades, and HPV genotypes, as available.

3.2.7 Framework for program evaluation

The contextual framework in which we will evaluate the provincial Cervical Screening Initiatives program is adapted from the Centres for Disease Control "Framework for Program Evaluation in Public Health" (Centers for Disease Control and Prevention, 1999). This can be a comprehensive approach involving standards grouped into four categories: utility, feasibility, propriety, and accuracy. The two primary goals or uses of this evaluation would be 1) to document the level of success in accomplishing screening reach in the province of NL and 2) to assess opportunities for development and risk reduction.

The main indicators that were evaluated included provincial participation, retention, unsatisfactory specimen rate, precancer detection rate, cancer incidence, and screening history of detected invasive cancers.

3.3. Results

The complete data set from the Cervical Screening Registry contained 95,077 test result entries from 2002 to 2019. Of the full data set, 89,594 Pap results were available. This included 45,265 (50.5%) ASCUS results and 44,329 (49.5%) LSIL results, and yearly breakdowns are found in Table 3-1. We can see a varying relationship between the two abnormalities (Figure 3.1). They both demonstrate polynomial trend lines, which are closely inverse of one another. In 2006 and 2016, LSIL and ASCUS exchanged their ranking as the highest proportion. From publicly available data, we can see that ASCUS and LSIL abnormalities do make up the largest percentage of Pap test abnormalities. However, NL has the second-highest at 7.7% combined ASCUS/LSIL percentage of abnormalities only surpassed by New Brunswick (11.8%) (Canadian Cancer Society, Statistics Canada, Public Health Agency of Canada, Provincial/Territorial Cancer Registries, 2016). In comparison, some provinces like Prince Edward Island saw a combined ASCUS/LSIL of 2.6%. Additionally, when we look at the overall percentage of abnormal Pap results while NB has the highest about of abnormal cytology results (~15%), NL had the second highest at ~9%; the remaining provinces ranged from ~4% to 7% (Canadian Cancer Society, Statistics Canada, Public Health Agency of Canada, Provincial/Territorial Cancer Registries, 2016).

From the registry data set, the overall available HPV results were 18,493 with 5,989 (32.4%) positives, 12,501 (67.6%) negatives, and three unknowns, yearly breakdowns are found in Table 3-2. There was an overall HPV test positive rate of 32.4% (Table 3-2). From 2005 to 2013, the HC2 HPV test was in use, overall positivity was 36.6%

(3,983/10,875). From 2014-2019, when the cobas HPV was used, overall HPV positivity was 26.3% (2,006/7,618); there was a significant difference ($p<0.001$, z-score) between these two tests. In Figure 3.2, we can see a gradual trend in decreasing positivity overall, regardless of the test used.

Table 3-3 shows all the available HPV genotyping results from 2012 onward. Per provincial guidelines, only ASCUS cytology should be reflexed with HPV testing. However, upon reviewing registry data, 870 LSILs were triaged with HPV. This is indicative of testing outside of current recommended guidelines in the province. Considering HPV triage testing of both ASCUS and LSIL abnormalities, Table 3-4 shows the differences and variations between the two cytological grades.

In Table 3-3, the HPV genotype distribution is available starting in 2014. Overall, we can see that genotypes 16 and 18 account for a range of 2.70%-11.50%, and the grouping of OHR genotypes accounts for the majority of positive results (25.54% of positive results). Since 2016 there has been an observed decrease in the proportion of positive results containing either genotype 16 or 18 from 11.5% to 5.7% (Table 3-3). One additional element to consider in the available dataset is the inclusion of both ASCUS and LSIL data; based on provincial guidelines, HPV triage is recommended only for ASCUS, not LSIL abnormalities. To better understand the differences between these cytological grades, Table 3-4 highlights the differences in positivity and genotype distribution between ASCUS and LSIL. Notable differences are in the proportion of ASCUS testing positive for HPV (26.90%) versus LSIL (89.10%). If we look at the proportion of those

testing positive, there is a significant difference between ASCUS and LSIL, having 27.2% and 26.5% of all positives (with known genotypes) with 16 and 18 genotypes, respectively.

Nationally the range for age-standardized rates of women 21 to 69 years of age who have had at least one Pap test in 42 months ranges from 62.9% to 73.8%; NL falls at 71.3% (Canadian Cancer Society, Statistics Canada, Public Health Agency of Canada, Provincial/Territorial Cancer Registries, 2016). Looking at the indicator for retention, we see that the percentage of women from 21-66 years of age who has a subsequent Pap test within 42-months of a negative Pap test is relatively consistent across Canada, with the range being 76.7 to 82.0%; NL was at 80.8% retention (Canadian Cancer Society, Statistics Canada, Public Health Agency of Canada, Provincial/Territorial Cancer Registries, 2016).

One of the main goals of a cervical screening program is to detect pre-cancerous lesions and cancers and provide treatment as early as possible. When we look at rates of detection and clinical endpoints, we can see various levels of detection nationally. Those 30-39 years of age NL had the second-highest number of women diagnosed with a pre-cancerous lesion per 1,000 screened from 2011-2013 behind PEI (Canadian Cancer Society, Statistics Canada, Public Health Agency of Canada, Provincial/Territorial Cancer Registries, 2016). The rates for women > 40 years of age were comparable nationally (Canadian Cancer Society, Statistics Canada, Public Health Agency of Canada, Provincial/Territorial Cancer Registries, 2016). As mentioned in the introduction, NL had the highest age-standardized cervical cancer incidence nationally and for both squamous

cell carcinoma and non-squamous cell carcinoma (Canadian Cancer Society, Statistics Canada, Public Health Agency of Canada, Provincial/Territorial Cancer Registries, 2016). When we look at the percentage of invasive cervical cancers diagnosed at Stage 1, there is a range from 46.1 – 81.3%. In NL, 77.3% of invasive cancers are diagnosed at this early stage.

From a programmatic quality perspective, we can see the percentage of unsatisfactory Pap test results ranged from <0.2% to 5%, with NL falling at 0.6%. Additionally, the percentage of patients whole had a length of time from high-grade Pap test result to colposcopy follow-up less than six weeks ranged from 11.7-33.7%.

3.4. Discussion

Some points for consideration within the context of the general cervical screening program including the following changes to local programming in Newfoundland and Labrador (Cervical Screening Initiatives Program, 2013):

- Program launched with partners in Western and Central Regions of the province (2003)
- Expansion to Eastern Rural area (2005)
- Expansion to Labrador Grenfell and Eastern Avalon (2007)
- HPV Triage Reflex officially introduced as standard of care and change from conventional cytology to Liquid Based Cytology (LBC) (2008)
- Provincial Cervical Cytology Registry implemented (2010)

Also, in 2011, changes to screening frequency were adjusted from annual to those who have had no signs of cancer to be screened once every three years (Quinn, 2011). Some of these programmatic changes have led to changes in overall uptake and methodology with the screening rate increasing from 58% of eligible women being screening in 1998 to 76% of all eligible women being screened within the last three years in 2011 (Quinn, 2011).

Based on study data (Table 3-4), approximately 27% of the ASCUS population and nearly 90% of the LSIL population tested positive for HPV. Of this, approximately 12% of ASCUS-HPV positives and about 1% of LSIL-HPV positives had genotypes 16 and 18. HPV genotypes 16/18 account for 70% of cervical cancer (Munoz, et al., 2003) (Khan, et al., 2005) (Guan, et al., 2012) (de Sanjose, et al., 2010). While we are unable to assess the risk of CIN2+ from this study, from the Addressing the Need for Advanced HPV Diagnostics (ATHENA) trial, the absolute risk of CIN 2+ is approximately 24% among those positive for 16/18 genotypes and 9% among those positive for OHR genotypes (Stoler, et al., 2011). Thus, the risk is very different for those with 16/18 genotypes compared to OHR genotypes, and further risk stratification can potentially occur. However, per current guidelines in Canada and elsewhere (Cervical Screening Initiatives, NL, 2016) (Cancer Care Ontario, 2016), all those testing positive for HPV are deemed at risk and referred for colposcopy. There is potential to help better stratify those at greatest risk by taking a closer look at the specific HPV genotypes instead of general HPV positivity and potentially reduce unnecessary colposcopy referrals and associated costs. When we look at Table 3-

4, the differences between those with abnormalities that are HPV positive and those who have either genotype 16 and 18 are apparent, with those with genotypes 16 and 18 making up 27.2% of HPV positive ASCUS patients. In line with this, the remaining 72.8% who are HPV positive, but have genotypes other than 16 and 18 (OHR), are at reduced risk. In this population, triage methodologies may further reduce colposcopy for this group. For example, in the 2002-2019 time period reviewed in this study and estimating colposcopy to be between $84-98 per examination (adjusted inflation) (Benedet, Bertrand, Matisic, & Garner, 2006), if ASCUS-HPV triage only sent 16/18 positive patients to colposcopy directly, over 1000 patients would have been saved from the invasive examination and a possible cost savings of up to $100,000 on one examination alone. These cost estimates are based on a Canadian study which looked at Ontario, Manitoba, Saskatchewan, Alberta, and British Columbia. Additional evaluation with current costing from NL would be beneficial to fully understand the financial implications. While there is no formalized LSIL-HPV triage program in NL, we did see that some LSIL patients did receive the test; additional investigations into the potential utility and possible risks should be assessed for this cytologic grade. If this category of abnormality were triaged, based on the LSIL- HPV positivity of 89.10%, there are possibilities to better target those at increased risk. Even further from a genotyping, 26.5% of LSILs testing positive with available genotyping had genotypes 16 and 18, meaning three-quarters of cases being at reduced risk. Additional studies highlight additional possible options in the context of LSIL triage (Gilbert, et al., 2022) (Ratnam, et al., 2020).

In our data, we see an HPV positivity rate of 26.9% for ASCUS and 89.1% for LSIL; we are very limited in our ability to compare the LSIL positivity widely due to this testing being performed outside of provincial recommendations and, as such, may not be representative of the overall population. When we look at the positivity for the HC2 (36.6%) period versus Roche cobas HPV test (26.3%), this does differ from a large Canadian study to assess the two tests (Cook, et al., 2015). However, this was an assessment of tests used in a general population for primary HPV screening rather than a follow-up test for ASCUS or LSIL cytology, like our study data. Patients already have cytologic abnormalities in a follow-up or triage-based scenario; therefore, we would expect to see more persistent HPV infections than in a general screening population. When we look at similar populations, we see that the HC2 positivity we saw in NL is similar to other observed results (Arbyn, Roelens, Martin-Hirsch, Leeson, & Wentzensen, Use of HC2 to triage women with borderline and mild dyskaryosis in the UK, 2011). NL cobas HPV data did show a lower, albeit similar, positivity to the ATHENA study (Stoler, et al., 2011). To better understand the differences between the two similar tests, their methodologies slightly differ. The HC2 test is a nucleic acid hybridization assay with signal amplification that uses microplate chemiluminescent detection and collectively detects 16, 18, 31, 33, 35, 39, 45, 51,52,56, 58, 59,68 genotypes (Digene, 2002). The cobas HPV test uses amplification of target DNA by PCR and nucleic acid hybridization for the detection of 14 high-risk genotypes in one analysis, genotypes 16 and 18 individually, and genotypes 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 collectively (Roche, 2015) and is more automated than HC2

and can differentiate some genotypes (Wong, Fuller, Pabbaraju, Wong, & Zahariadis, 2012).

The Pan-Canadian Cervical Cancer Screening Network (PCCSN) has a target for 80% of women to have had a Pap test within the last three years (Cancer Care, Eastern Health, 2018). Patients in NL have traditionally had lower cervical cancer screening participation rates; between 2007 and 2009, they were approximately 40% (Duke, et al., Effect of vaginal self-sampling on cervical cancer screening rates: a community-based study in Newfoundland, 2015). However, in the last decade, improvements have been seen. For example, in 2012 and 2013, nearly 80% of women have reported having had a pap test in the previous three years (Cancer Care, Eastern Health, 2018). Additional promotional and compliance work will help meet and achieve the 80% target.

We see a few observations when we compare NL's performance in the Canadian Partnership Against Cancer, Cervical Cancer Screening in Canada, 2011-2013 report. First, we see that NL has age-standardized participation rates within the range of other provinces and, in fact, on the higher end. Additionally, we see that retention is well within the national ranges reported. However, while this retention is positive, nearly 20% of women did not continue to participate in screening (Canadian Cancer Society, Statistics Canada, Public Health Agency of Canada, Provincial/Territorial Cancer Registries, 2016). This is an opportunity for improvement and a group to target for health promotion programming.

The two program quality indicators, unsatisfactory Pap results and time to colposcopy measures, do show NL is performing comparably to other jurisdictions in Canada. However, there is much room for improvement regarding colposcopy wait times to improve access overall better. Based on some standards (i.e., 90% of women with high-grade abnormalities should be seen in colposcopy clinic by four weeks (Canadian Cancer Society, Statistics Canada, Public Health Agency of Canada, Provincial/Territorial Cancer Registries, 2016)), Canada is doing poorly; NL is no different. It is critical to reduce these wait times to lower the risk of adverse outcomes and stress associated with delayed follow-up (Decker, McLachlin, Lotocki, & Group, 2015).

Interestingly, NL seems to have comparably higher rates of abnormalities, pre-cancerous lesions, and cancers from the national perspective. This stresses the importance of participating in screening within the province and improving access to the programs. Often pre-cancerous lesions do not progress into cancer, but treatment and programmatic follow-up are necessary, regardless. However, higher rates of abnormalities and pre-cancerous lesions may also indicate over-calling cytology results meaning that patients are unnecessarily graded higher and referred to colposcopy, adding to the problem of wait time for colposcopy referrals. Alternative methodologies like HPV primary screening may provide an objective approach to reduce this possible over-calling in cytology. Higher rates of cancer in NL stress the importance of including vaccination programs as an arm of any cervical cancer prevention program in concern with screening. One positive measure is NL's percentage of invasive cervical cancers diagnosed at Stage 1

(77.3%), meaning that the existing programs are finding cancers early, this means that treatment may be less aggressive and have better long-term outcomes (Canadian Cancer Society, Statistics Canada, Public Health Agency of Canada, Provincial/Territorial Cancer Registries, 2016). This finding should be promoted extensively in encouraging and promoting the local programs.

Many groups in Canada have come forward to support primary HPV cervical cancer screening programs (Canadian Cancer Society, Statistics Canada, Public Health Agency of Canada, Provincial/Territorial Cancer Registries, 2016) (CADTH, 2019). Additional work has been done in European settings, with varying levels of completeness, to move towards HPV primary screening; however, there is some challenges with implementation such as higher rates of colposcopy referral and higher detection rates of CIN3+ and cervical cancers, highlighting the importance of triage (Maver & Polijak, 2020). Primary HPV screening with cytology triage could efficiently and effectively be implemented in NL given the capacity of molecular testing, the current use of the Health Canada approved primary HPV screening test, and the existing partnership between both the Public Health Microbiology Laboratory and Regional Cytology Laboratory as developed via the HPV ASCUS triage program already long-established. The shift to HPV primary testing, particularly in NL with the higher rates of abnormalities and pre-cancerous lesions than the rest of Canada, in addition to advantages in patient risk evaluation, would also be a cost-effective one (Vigayaraghavan, Efrusy, Mayrand, Santas, & Goggin, 2010; Canadian Cancer Society, Statistics Canada, Public Health Agency of Canada, Provincial/Territorial

Cancer Registries, 2016) and could help to reduce an already backlogged colposcopy referral waitlist in NL.

Another challenge faced when obtaining information about the provincial cervical cancer screening program is the absence of information surrounding screening for vulnerable communities. This is unfortunate and an opportunity for more detailed investigation, particularly in light of the 2021 WHO protocol for cervical cancer screening in under screened populations (Sultanov, et al., 2002). Attempts have been made in this study to be more inclusive in the language surrounding gender, for example, using patients instead of women to be inclusive of all genders with cervices; however, this was a challenge given how data was presented in publicly available reports. As a public health community, we need to work harder to improve these gaps and make sure we serve all communities and evaluate those programs with the same focus as we do others.

The Cervical Screening Registry is one program of the NL Cancer Care Registry as part of Eastern Health's Provincial Cancer Care Program (Eastern Health, 2021). One major challenge with the available data from the Cervical Screening Registry was the absence of histology or biopsy results; only Pap and HPV results were available for assessment. As such, biopsy-confirmed endpoints were unavailable, and test performance values could not be determined. As such, analysis of this data was minimal. Additionally, individual-level data were not available. Therefore, it was impossible to examine any factors associated with HPV positive tests in this piece of work. Also, due to limitations in our data, we cannot compare ASCUS and LSIL with other cytologic abnormalities. Age

distributions and breakdowns are not included because this data was not included in the registry extraction. It was challenging to complete any more extensive interprovincial comparisons for HPV positivity or genotyping due to differences in HPV algorithms across Canada. At the same time, many provinces and territories do have ASCUS triage for those >30 years of age; other jurisdictions use HPV testing for follow-up after treatment. As such, there would be no accurate comparison.

One major limitation in the framework approach to evaluating the program is stakeholder engagement. Therefore, we recommend any further work would involve stakeholders, including clients of the system, such as screening participants, physicians, and colposcopists, to assess qualitative aspects that were unable to be evaluated in this work.

Additional opportunities likely exist to improve the program financially, future work would be beneficial to better support decision makers in consideration of the economic elements of cervical cancer screening, particularly with province specific measures and costings.

4.5. Conclusions

The province of Newfoundland and Labrador has attempted to improve participation in its Cervical Cancer Screening Initiatives programs. It has been successful in some ways including general increases in participation rates, relatively low unsatisfactory rates, and the number of cancers being detected at a low stage, particularly when compared with

the rest of Canada. However, high rates of abnormalities, pre-cancerous lesions, and invasive cancers are troubling. Additional studies may also help to examine the compliance of clinicians to the prescribed standards (ie: HPV tests outside of ASCUS triage and screening more frequently than every three years). Opportunities exist to improve the program clinically through better stratification of patients at risk and systemically through general quality improvements and processes. Additional triaging options or an HPV primary screening algorithm may help with these improvements and outreach to unscreened and under-screened patients. Further work to reduce wait times for colposcopy can further support improvements to the system.

Conflict of interest disclosures

Laura Gilbert is part of a study team that operates under a research grant from Roche Diagnostics (Dr. Sam Ratnam, PI).

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Table 3-1. Available ASCUS and LSIL Pap results from 2002-2019, Newfoundland and Labrador

		Counts		Total
		ASCUS (%)	LSIL (%)	
Year	2002	2698 (67.0%)	1328 (33.0%)	4026
	2003	2152 (61.3%)	1356 (38.7%)	3508
	2004	2109 (60.3%)	1387 (39.7%)	3496
	2005	1967 (53.9%)	1683 (46.1%)	3650
	2006	1709 (47.3%)	1902 (52.7%)	3611
	2007	2239 (42.9%)	2976 (57.1%)	5215
	2008	2056 (42.3%)	2803 (57.7%)	4859
	2009	2369 (45.5%)	2835 (54.5%)	5204
	2010	2633 (40.7%)	3838 (59.3%)	6471
	2011	2598 (41.8%)	3613 (58.2%)	6211
	2012	3272 (48.1%)	3525 (51.9%)	6797
	2013	3006 (47.4%)	3341 (52.6%)	6347
	2014	3174 (48.0%)	3440 (52.0%)	6614
	2015	2992 (49.0%)	3118 (51.0%)	6110
	2016	2718 (54.9%)	2232 (45.1%)	4950
	2017	3160 (58.4%)	2253 (41.6%)	5413
	2018	3043 (62.0%)	1864 (38.0%)	4907
	2019	1370 (62.1%)	835 (37.9%)	2205
Total		45265 (50.5%)	44329 (49.5%)	89594

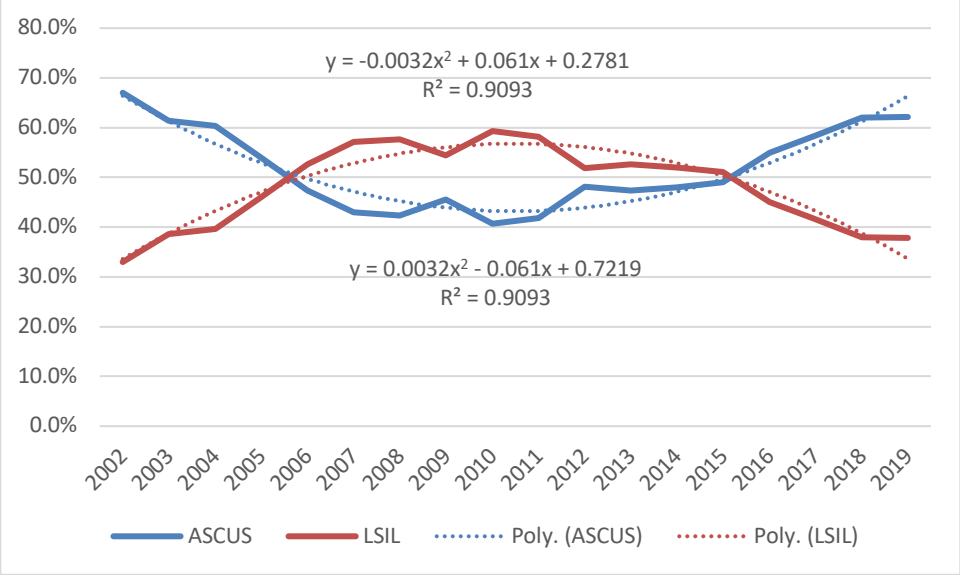

Figure 3.1. Trends in Available ASCUS and LSIL Pap results from 2002-2019, Newfoundland and Labrador

Table 3-2. HPV Results Available for ASCUS and LSIL Pap results from 2002-2019, Newfoundland and Labrador

Count						
		HPV Results for ASCUS & LSIL Pap results				
		Negative	Positive	Unknown	Total	% Positivity
Year	2005	250	182	0	432	42.1%
	2006	1413	802	0	2215	36.2%
	2007	903	593	0	1496	39.6%
	2008	421	374	0	795	47.0%
	2009	429	443	0	872	50.8%
	2010	785	490	0	1275	38.4%
	2011	743	374	0	1117	33.5%
	2012	1065	408	1	1474	27.7%
	2013	881	317	1	1199	26.4%
	2014	1058	312	0	1370	22.8%
	2015	868	325	0	1193	27.2%
	2016	606	330	1	937	35.2%
	2017	1150	402	0	1552	25.9%
	2018	1158	407	0	1565	26.0%
	2019	771	230	0	1001	23.0%
Total		12501	5989	3	18493	32.4%

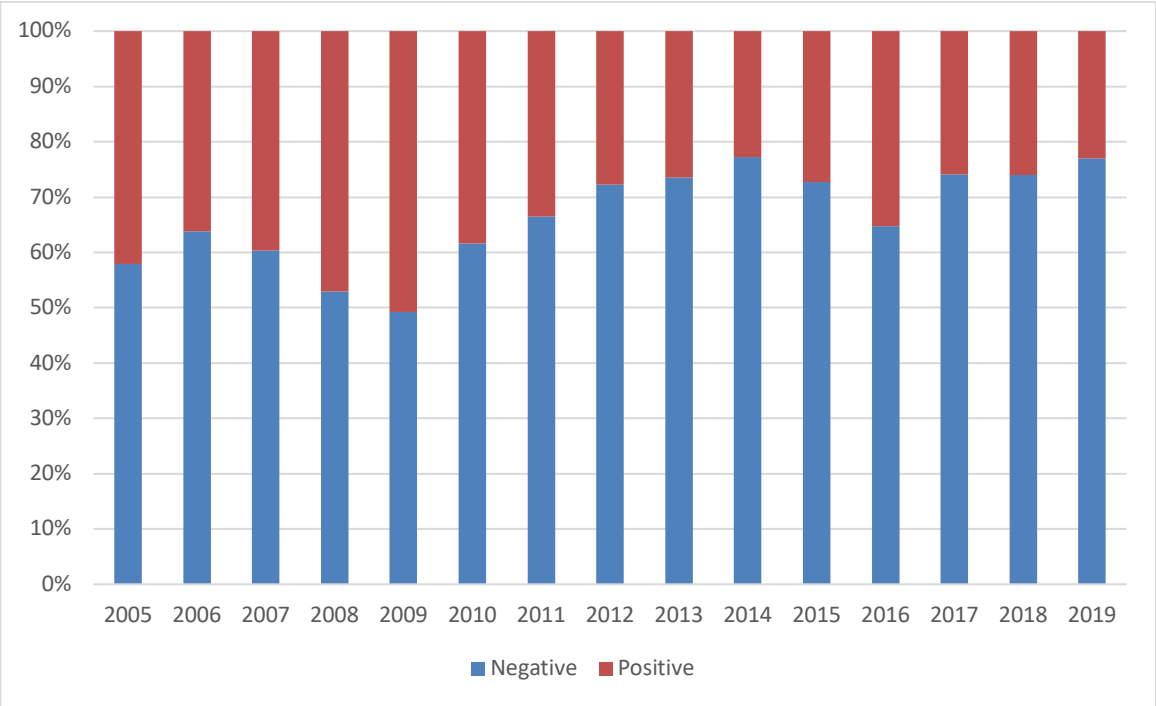

Figure 3.2. Breakdown of HPV positivity for ASCUS and LSIL Pap Results from 2002-2019, Newfoundland and Labrador

Table 3-3. Available HPV Genotypes from 2002-2019, Newfoundland and Labrador

		Negative	HPV 16 Only	HPV 18 Only	HPV OHR Only	HPV 16 & 18	HPV 16 & OHR	HPV 18 & OHR	HPV 16, 18, OHR	Unknown	% with any 16/18	Total
Year	2005	250	0	0	0	0	0	0	0	182	0.0%	432
	2006	1413	0	0	0	0	0	0	0	802	0.0%	2215
	2007	903	0	0	0	0	0	0	0	593	0.0%	1496
	2008	421	0	0	0	0	0	0	0	374	0.0%	795
	2009	429	0	0	0	0	0	0	0	443	0.0%	872
	2010	785	0	0	0	0	0	0	0	490	0.0%	1275
	2011	743	0	0	0	0	0	0	0	373	0.0%	1116
	2012	1065	0	0	0	0	0	0	1	2	0.1%	1068
	2013	881	0	0	0	0	0	0	2	1	0.2%	884
	2014	1058	13	4	90	0	5	7	3	0	2.7%	1180
	2015	868	32	14	238	2	20	15	3	0	7.2%	1192
	2016	606	40	14	221	5	24	21	4	1	11.5%	936
	2017	1150	45	18	286	1	32	16	4	0	7.5%	1552
	2018	1158	39	17	289	0	41	16	5	0	7.5%	1565
	2019	771	22	11	173	0	18	5	1	0	5.7%	1001
Total		12501	191	78	1297	8	140	80	23	3261	3.0%	17579

Table 3-4. Overall HPV Genotype distribution by available Pap grade from 2002-2019, Newfoundland and Labrador

	Pap Test Result		
	ASCUS	LSIL	ASCUS/LSIL
HPV Negative	8852	94	8946
HPV Positive	3260	766	4026
% Positive	26.90%	89.10%	31.00%
HPV 16 Only	147	6	153
HPV 18 Only	55	0	55
HPV OHR Only	1100	25	1125
HPV 16 & 18	7	0	7
HPV 16 & OHR	119	1	120
HPV 18 & OHR	63	1	64
HPV 16, 18, OHR	19	1	20
Unknown	1750	732	2482
Total	12112	860	12972
Total with any 16/18	410	9	419
% with any 16/18	3.40%	1.00%	3.20%

Chapter 4 : Paper 2, CINtec PLUS and cobas HPV testing for triaging Canadian women referred to colposcopy with a history of low-grade squamous intraepithelial lesion: Baseline findings

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SR: Conceptualization, funding acquisition, methodology, supervision, data analysis, writing-original draft, writing- review and editing; DJ: Project administration, supervision, data curation, resources, laboratory technical support, and writing-review and editing; LG: Project administration, investigation, methodology, laboratory technical support, resources, data curation and analysis, and writing- review and editing; RA and MS: Investigation, data analysis, validation, and writing- review and editing; RN: Investigation, data curation, laboratory technical support, and writing-review and editing; AEM: Study co-ordination and technical support; PW: data analysis, writing-review and editing; MR, DS, LE and GZ: Resources, and writing-review and editing; MC: Resources, methodology, supervision, and writing-review and editing.

Abstract

Objective and methods: CINtec PLUS and cobas HPV tests were assessed for triaging women referred to colposcopy with a history of LSIL cytology. Both tests were performed at baseline using ThinPrep cervical specimens and biopsy confirmed cervical intraepithelial neoplasia grade 2 or worse (CIN2+) served as the clinical endpoint.

Results: In all ages, (19-76 years, n = 600), 44.3% (266/600) tested CINtec PLUS positive vs. 55.2% (331/600) HPV positive (p = 0.000). Based on 224 having biopsies, sensitivity to detect CIN2+ (n = 54) was 81.5% (44/54) for CINtec PLUS vs. 94.4% (51/54) for HPV testing (p = 0.039); specificities were, 52.4% (89/170) vs. 44.1% (75/170), respectively (p = 0.129). In women ≥30 years (n = 386), 41.2% (159/386) tested CINtec PLUS positive vs. 50.8% (196/386) HPV positive (p = 0.008). Based on 135 having biopsies, sensitivity to detect CIN2+ (n = 24) was 95.8% (23/24) for both CINtec PLUS and HPV tests; specificities were, 55.0% (61/111) vs. 50.5% (56/111), respectively (p = 0.503).

Conclusions: For women referred to colposcopy with a history of LSIL cytology, CINtec PLUS or cobas HPV test could serve as a predictor of CIN2+ with high sensitivity, particularly in women ≥30 years. Either test can significantly reduce the number of women requiring further investigations and follow up in colposcopy clinics.

4.1 Introduction

Low-grade squamous intraepithelial lesion (LSIL) is the second most common cytological abnormality found in routine cervical screening. While these lesions regress spontaneously in the majority, a small fraction of women with LSIL cytology have an occult high-grade squamous intraepithelial lesion (HSIL) or will progress to HSIL. Consequently, women found to have LSIL in routine cervical screening are either directly referred to colposcopy or followed cytologically, referring those with persistent cytologic abnormalities to colposcopy (The ASCUS-LSIL Triage Study (ALTS) Group, 2003), (Cancer Care Ontario, 2016). In colposcopy clinics, all referred LSIL cases are typically followed with repeat cytology, colposcopy and biopsies for various length of time. This practice increases both unnecessary cost and intervention in patients with a negligible risk of developing cervical cancer; it also subjects many to negative health effects. An effective LSIL triage strategy would identify those women who need to remain in care at the colposcopy clinic and those who can be safely returned to routine screening. In this connection, we previously conducted a multicentre Canadian study for triaging ASCUS and LSIL referral populations using the ProEx C immunoassay (Beckton and Dickinson), an MCM/TOP2a-based biomarker test (Alaghehbandan, et al., 2013). In this study, ProEx C sensitivity to detect cervical intraepithelial neoplasia grade 2 or worse (CIN 2+) was found to be unacceptably low in the range of 68%-72% and precluded this assay in triage.

The CINtec PLUS assay (Roche Diagnostics) is a dual-stain immunocytochemical test which detects p16 and Ki-67 proteins that are over expressed in cervical cells with

transforming HPV infection. This assay has emerged as an effective biomarker-based adjunct test in cervical screening strategy and is well studied (Tjalma W. A., 2017), (Tjalma, Kim, & Vandeweyer, 2017), (Sun M. , Shen, Ren, & Dong, 2018), (Sun, Shen, & Cao, 2019), (Yu, et al., 2019). p16 is a tumour suppressor gene which regulates cell cycle through a cascade of biochemical events; Ki-67 is a nuclear protein and a marker of cellular proliferation. In normal cells, the expressions of p16 and Ki-67 are mutually exclusive. In persistent transforming infection with high-risk human papillomavirus (hr-HPV), E7 oncogene disrupts the negative feedback control on p16 expression, resulting in loss of cell cycle control and continued cell proliferation which lead to over expression of both p16 and Ki-67. Thus, the co-detection of p16/Ki-67 simultaneously within the same cervical epithelial cell serves as a specific marker of HPV-mediated oncogenic transformation and predictor of cervical cancer risk. CINtec PLUS assay has been shown to be more sensitive than cytology with equal specificity, and more specific than HPV testing with relatively comparable sensitivity for detecting CIN2+ in women with LSIL cytology (Schmidt, Bergeron, Denton, & Ridder, 2011), (Ikenberg H. , et al., 2013), (Bergeron C. , et al., 2015), (White, et al., 2016), (Peeters, Wentzensen, Bergeron, & Arbyn, 2019).

The cobas HPV DNA test (Roche Diagnostics) is a PCR-based qualitative assay for the detection of 14 hr-HPV genotypes (HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) in cervical specimens and has been extensively validated (Wright Jr, et al., 2012), (Cox, et al., 2013), (Wright T. , et al., 2015). This is a high throughput partial

genotyping test which differentiates and specifically identifies genotypes 16/18 individually and detects 12 other high-risk (OHR) types collectively in a single analysis. Genotypes 16/18 are far more carcinogenic than any other HPV types, accounting for 70% of cervical cancers; therefore, women infected with these genotypes are at a significantly increased risk for cervical pre-cancer and cancer (Munoz, et al., 2003), (Khan, et al., 2005), (de Sanjose, et al., 2010). This underscores a genotype-specific risk threshold in cervical screening strategy, and the interim clinical guidance in the USA recommends direct referral to colposcopy for those testing positives for genotypes 16/18 in primary HPV screening (Huh, et al., 2015). In this context, the cobas HPV test also has the potential to serve as an adjunct test for triaging LSIL referral population through genotype 16/18-specific risk threshold and reduce the number of women requiring additional investigations and follow up in colposcopy clinics, and thus could aid in better patient care and resource management.

The objectives were to conduct a prospective study to assess positivity rates of CINtec PLUS and cobas HPV tests along with genotype 16/18-specific risk threshold in women referred to colposcopy with a history of LSIL, and to measure each test's ability as well as their clinical performance to predict CIN2+. This report describes the study and presents the data obtained at baseline.

4.2 Materials and methods

4.2.1. Ontario cervical cancer screening guidelines

In the province of Ontario, Canada, liquid-based Papanicolaou cytology is being used for primary cervical cancer screening. If cytology is normal, triennial screening continues. In terms of managing women with LSIL cytology, either direct referral to colposcopy or repeat cytology at 6-month intervals is recommended; for those having persistent atypical squamous cells of undetermined significance (ASCUS) or worse in repeat cytology, colposcopy is recommended (Cancer Care Ontario, 2016). In colposcopy clinics, all referred patients undergo cytology and colposcopic examination with biopsies of lesions detected, and further follow up clinical pathways depend on specific criteria which can take years for some (Cancer Care Ontario, 2016). Cervical screening strategies utilizing HPV triage vary across Canada, with some provinces offering ASCUS-HPV triage for women $\geq$30 years, and LSIL-HPV triage for those $\geq$50 years through government funded programs (Canadian Partnership Against Cancer, 2018). In Ontario, colposcopy services guidelines incorporate HPV testing into the colposcopy clinical pathways for risk stratification to avoid unnecessary follow up colposcopy visits for HPV-negative women (Cancer Care Ontario, 2016). However, this is not practiced since HPV testing is not offered as part of government funded cervical cancer screening program.

4.2.2. Study protocol

The study was conducted within the Ontario cervical screening guidelines and has been designed as a prospective study. Study population comprised of women with a history of LSIL cytology referred to the colposcopy clinic at the Juravinski Hospital, Hamilton, Canada. All study patients were attended to per standard of care, with cervical specimens collected for cytology, and colposcopy and biopsies performed per routine clinical practice. Cytology was carried out as part of routine patient care per standard practice, and CINtec PLUS and cobas HPV tests were performed once at baseline using the residual cervical specimens for the study purpose. Patients' baseline data were recorded, and the study cohort was passively followed by review of their medical records to monitor clinic visits and obtain further relevant study data. Biopsy confirmed CIN2+ served as the clinical endpoint. CINtec PLUS and HPV testing results obtained at baseline together with that of biopsy were recorded as primary study outcomes. CINtec PLUS and HPV positivity rates that would correspond to the proportions requiring further colposcopy clinic visits and follow up were determined. The test results obtained at baseline were correlated with the clinical endpoint observed either at baseline or during follow up to ascertain the clinical performance of the two tests. The study has been designed to follow the cohort for a minimum of one year with a provision to follow them up to three years. This report deals with the results of CINtec PLUS and HPV tests and that of biopsy obtained at baseline.

4.2.3. Ethics

The study was approved by the Hamilton Integrated Research Ethics Board (HiREB) and Newfoundland and Labrador Health Research Ethics Board (HREB) (Appendix G). All women were informed verbally and in writing about the study, use of their residual cervical specimens for CINtec PLUS and HPV testing, and the need to periodically review their medical records to obtain relevant study data during follow up. Those consenting to participate were enrolled in the study with written informed consent.

4.2.4. Patient enrolment

Women with a history of LSIL cytology who had not received treatment were eligible. Enrolment criteria included: 1) Women who had LSIL cytology in routine primary screening and who were directly referred to colposcopy; 2) those who were found to have LSIL cytology initially in routine primary screening and who upon repeat cytology found to have persistent ASCUS or LSIL and referred to colposcopy; and 3) those who were diagnosed as having LSIL cytology among women being followed in the colposcopy clinic. In all instances, enrolment was limited to women with a pre-enrolment history of LSIL. There were no age limits, and pregnant women and women without a cervix were excluded. Eligible patients were enrolled consecutively from November 2017 through February 2019.

4.2.5 Study specimens

Cervical specimens were collected in ThinPrep PreservCyt® cytology collection device (Hologic Inc) for routine cytology at enrolment. The residual cervical specimens in the collection vials were stored at ambient temperature and used in the study as follows: the vials were batched on a weekly basis and slides were prepared for CINtec PLUS testing at the cytology laboratory, St. Joseph's Healthcare Hamilton. A 1 mL aliquot of specimen was pipetted into a tube for cobas HPV testing. Slides and tubes were shipped weekly to the Eastern Health Public Health and Microbiology Laboratory, St. John's for CINtec PLUS and cobas HPV assays. These tests were carried out as described below no later than 6 weeks post collection.

4.2.6. CINtec PLUS assay

An experienced cytotechnologist prepared smears for CINtec PLUS on ThinPrep processor (T5000, Hologic Inc) using special ThinPrep slides (Hologic, Inc). The slides were fixed in ≥ 95% reagent grade ethanol and air dried. These were stained using CINtec PLUS assay kit within 48 hrs and processed on BenchMark ULTRA system (Roche Diagnostics) by trained personnel per manufacturer's instructions.

With the CINtec PLUS assay, the p16 protein appears as a brown cytoplasmic stain and Ki-67 as a red nuclear stain independent of cytomorphology. The CINtec PLUS slides were initially evaluated independently by one of two experienced cytotechnologists who were trained to read these slides. Smears were determined to be positive if at least one

cervical epithelial cell showed both a brownish cytoplasmic immunostaining for p16 and a red nuclear immunostaining for Ki-67 regardless of cellular morphology. If the dual staining was not observed, the smear was considered negative. Smears were deemed unsatisfactory if they did not contain an adequate number of cells (>4 cells per field with a minimum of 10 fields with a 40x objective). Smears were screened systematically with a 10x objective and cells showing the dual staining was confirmed with a 40x objective. All slides were independently reviewed by a study pathologist trained to read CINtec PLUS slides, and the results recorded using the same criteria. Discrepant slides were either internally reviewed by another reader and reconciled or adjudicated independently by an external expert.

4.2.7. cobas HPV test

cobas HPV test was performed on the Roche 4800 automated platform. Testing was performed per manufacturer's instructions by trained personnel. Results were reported as positive for genotypes 16 and or 18, and/or 12 OHR types, or negative for 14 hr-HPV types.

4.2.8. Cervical biopsy

Biopsies were performed by colposcopists per standard clinical practice. Three sections of each biopsy sample were processed with hematoxylin and eosin (H&E) staining per routine practice. p16 immunostaining (CINtec® Histology kit, Roche Diagnostics) was performed on biopsies per manufacturer's instructions as part of the study protocol to

provide supporting diagnostic evidence. p16 results were corroborated with H&E biopsy interpretation. Biopsies were read by staff pathologists at the originating colposcopy clinic site per standard practice. All biopsy slides together with p16 stained slides were independently reviewed by two study pathologists. Discrepant biopsy results were independently adjudicated by a third pathologist, if needed.

4.2.9. Results management

CINtec PLUS and HPV tests were conducted independently, and cytotechnologists and the study pathologists were blinded to these test results as well as cytology and biopsy results obtained at baseline. Colposcopy clinicians did not have access to CINtec PLUS or HPV results at the time of initial patient evaluation. HPV results were provided subsequently to clinicians to aid in patient management as the benefit of HPV testing in cervical screening strategies is well recognized in routine clinical practice. As CINtec PLUS testing was considered experimental, these results were not released.

4.2.10. Data analysis

Statistical analysis was performed using SPSS for Windows, version 23, Excel, Microsoft Office Professional Plus, 2013, and Social Science Statistics website, 2020. Qualitative variables such as test results and cytology grades were studied through review of frequencies. Descriptive statistics such as counts and proportions were prepared for the data collected at baseline for test positivity rates, distribution of cytology grades and HPV genotypes. Study data were analyzed for all ages, <30 years of age and those ≥30 years (Murphy J. , et al., Cervical Screening: Guideline Recommendations, 2011), using contingency tables to determine test positivity rates, and the diagnostic sensitivity and specificity of CINtec PLUS and HPV testing by biopsy confirmed clinical endpoint. McNemar's test was used on paired nominal data and a two-tailed z score was used to compare proportions. P-values less than 0.05 were considered statistically significant.

4.3 Results

4.3.1. Study population

A total of 610 patients meeting the study criteria were enrolled in the study. Of these, 10 were excluded due to insufficient or no cervical specimen for CINtec PLUS and/or HPV testing, or invalid CINtec PLUS or HPV test results, leaving 600 patients with evaluable results (Figure 4.1). Age ranged from 19 to 76 years (median, 33.5), with 386 (64.3%) ≥30 years of age (median, 43).

4.3.2. Positivity rates of CINtec PLUS and HPV tests

Table 4-1 shows in all ages, CINtec PLUS was positive in 266 (44.3%) vs. 331(55.2%) testing HPV positive ($p<0.001$, two-tailed z test). Among the 331 HPV positives, genotypes 16/18 were detected in 93 (28.1%). In women ≥30 years, CINtec PLUS was positive in 159 (41.2%) vs. 196 (50.8%) testing HPV positive ($p=0.008$, two-tailed z test). Among the 196 HPV positives, genotypes 16/18 were detected in 57 (29.1%). There was a significant difference in both CINtec PLUS and HPV positivity rates in women <30 years of age and those ≥30 years (Table 4-1). The % agreement between CINtec PLUS and HPV tests was similar (range, 70.7%-70.8%; kappa, 0.416-0.423) in all ages and the two age groups (Data not shown).

4.3.3 Association of CINtec PLUS and HPV results with biopsy and diagnostic indices

Of the 600 patients in all ages, 232 (38.7%) underwent biopsy per routine clinical practice, and evaluable results were available for 224 (37.3%). Among the 224, biopsy confirmed CIN2+ was diagnosed in 54 (24.1%), including 19 CIN3, with the remaining 170 diagnosed as having ≤CIN1 (Table 4-2). In women ≥30 years, biopsy results were available for 135, and 24 (17.8%) had CIN2+, including 9 CIN3. All CIN2+ biopsy diagnoses were substantiated by a positive p16 result.

Table 4-2 shows of the 54 CIN2+ in all ages, CINtec PLUS was positive in 44 for a sensitivity of 81.5%, vs. 51 testing positives by HPV test for a sensitivity of 94.4% (p = 0.039; Table 4-3, two-tailed z test). Of the 19 CIN3, CINtec PLUS was positive in 18 for a sensitivity of 94.7%, and HPV test was positive in all 19 (Table 4-2). Specificity of CINtec PLUS to detect CIN2+ was 52.4% (89/170) vs. 44.1% (75/170) for HPV testing (p=0.129; Tables 4-2 and 4-3, two-tailed z test). Among women ≥30 years, of the 24 CIN2+, 23 tested positive by both CINtec PLUS and HPV tests for a sensitivity of 95.8% (Table 4-3), with all 9 CIN3 cases testing positive by both tests (Table 4-2); specificity to detect CIN2+ was 55.0% (61/111) for CINtec PLUS vs. 50.5% (56/111) for HPV test (p=0.503: Tables 4-2 and 4-3, two-tailed z test). Distribution of CINtec PLUS and HPV results correlated with biopsy findings are summarized in Figure 4.1.

Table 4-4 shows the comparison of paired results of CINtec PLUS and HPV tests with biopsy results for all ages. Analyses of this data by McNemar test indicated no

significant difference between the two tests. Additional data analysis based on age groups, <30 years and ≥30 years, is shown in Table 4-7 and Table 4-8, respectively, indicating a significant difference in CIN2+ detection between the two tests only for women < 30 years.

4.3.4. Association of HPV genotypes 16/18 with biopsy and diagnostic indices

Table 4-5 shows association of HPV genotypes 16/18 with biopsy results. In women of all ages, among the 51 of the 54 CIN2+ testing HPV positive, genotypes 16/18 were detected in 25 (49.0%), testing for genotypes 16/18 was 46.3% sensitive. Among the 19 CIN3 testing HPV positive, genotypes 16/18 were detected in 11 (57.9%; data not shown). In women <30 years of age, of the 28 CIN2+ testing HPV positive, genotypes 16/18 were detected in 11 (39.3%), testing for genotypes 16/18 was 36.7% sensitive. In women ≥30 years, of the 23 CIN2+ testing HPV positive, genotypes 16/18 were detected in 14 (60.9%), testing for genotypes 16/18 was 58.3% sensitive. There were no significant differences in sensitivities between all ages and the two age groups and between the two age groups. Specificity of testing for genotypes 16/18 was 84.7% in all ages, 86.4% in those <30 years, and 83.8% in women ≥30 years. In all genotypes 16/18 positive cases, type 16 was predominant as a single type in most cases, and in a few it was detected in combination with type 18 or OHR types.

4.3.5. Association of CINtec PLUS and HPV results with cytology

Cytology was performed as part of routine patient care at enrolment, with CINtec PLUS and HPV testing carried out using the residual cervical specimens. The above

cytology results were unknown when patients were enrolled and retrieved from patient medical records to assess the association of CINtec PLUS and HPV test results. The time interval between the index referral LSIL cytology immediately prior to enrolment and cytology performed in the colposcopy clinic at initial referral visit ranged from <1 month to ≥18 months with a median of 7 months (average, 7.9 months). Although the index referral cytology was LSIL in all patients enrolled, cytology performed at the time of initial colposcopy clinic visit showed heterogeneous cytological grades as expected (Table 4-6). In all ages, LSILs appeared to have regressed in 48.9% (291/595) to ASCUS or negative cytology and progressed to HSIL in 8.6% (51/595), with only 41.5% (247/595) still having LSIL at the time of CINtec PLUS and HPV testing. The cytologic lesion regression and progression rates were similar at 50.1% (193/381) and 6.6% (25/381), respectively, in women aged ≥30 years (Data not shown). The positivity rates of both CINtec PLUS and HPV tests uniformly decreased with decreasing cytologic lesion severity (Table 4-6), and this was similar in those ≥30 years (Data not shown). There were significant differences in the positivity rates between CINtec PLUS and HPV tests for LSIL, ASCUS, and overall.

4.4 Discussion

An effective strategy is needed for triaging women referred to colposcopy with a history of LSIL since only a small proportion is at risk for cervical pre-cancer and cancer. The premise of our study was that both CINtec PLUS assay and cobas HPV test with genotype 16/18-specific risk threshold have the potential to identify those at increased risk requiring further investigations and follow up in colposcopy clinics and safely return those not at immediate risk to routine screening. In this context, we also assessed the clinical performance of CINtec PLUS and cobas HPV tests to detect CIN2+.

Although LSIL is a common cytologic finding in cervical screening, it accounts for only 10-20% of CIN2+ (Cuzick, et al., 2013). Regardless, women with LSIL cytology in primary screening are considered at high enough risk for referral to colposcopy, and the ALTS study concluded LSIL cytology is best managed by colposcopy initially (The ASCUS-LSIL Triage Study (ALTS) Group, 2003). It should be noted that in the US, a risk for CIN3+ greater than 5.2% is considered the threshold for colposcopy referral whereas in Europe it is greater than 10% (Castle, Sideri, Jeronimo, Solomon, & Schiffman, 2007), (Arbyn, Roelens, Martin-Hirsch, Leeson, & Wentzensen, Use of HC2 to triage women with borderline and mild dyskaryosis in the UK, 2011). Our baseline study data showed a CIN2+ prevalence of 9% (54/600) in all ages in a routine colposcopy referral setting; it was lower at 6.2% (24/386) in women aged $\geq$30 years. This emphasizes the importance of an efficient triage to identify the small fraction of women at increased risk among the LSIL referral population. On the other hand, it also raises question of following all women with a history

of LSIL cytology with further investigations in colposcopy clinics even though the risk is limited to only a few.

The baseline results from our ongoing study provided some insight into the positivity rates and relative performance of CINtec PLUS and cobas HPV tests for triaging Canadian women referred to colposcopy with a history of LSIL cytology. Based on the test positivity rates, CINtec PLUS identified 44.3% would be at increased risk, vs. 55.2% by HPV test in women of all ages. In women ≥30 years, these figures were not significantly lower at 41.2% vs. 50.8%, respectively (Table 4-1). This implies, in all ages, cutting the size of the LSIL referral population requiring further investigations and follow up in colposcopy clinics slightly over one half by CINtec PLUS assay, and slightly under one half by HPV test. Also, regardless of the test, the proportion requiring further investigations would be lower in women ≥30 years than those <30. In this regard, we note as a strength of our study that our data provide evidence for the benefit of incorporating LSIL-HPV triage for risk threshold and to reduce unnecessary follow up colposcopy visits for HPV-negative women as recommended in the Ontario colposcopy services guidelines (Cancer Care Ontario, 2016).

The reported sensitivity and specificity of CINtec PLUS in detecting CIN2+ among those with ASCUS or LSIL cytology varies in different studies and populations (Tjalma W. A., 2017), (Sun M. , Shen, Ren, & Dong, 2018), (Sun, Shen, & Cao, 2019), (Ikenberg H. , et al., 2013), (Bergeron C. , et al., 2015), (White, et al., 2016), (Peeters, Wentzensen, Bergeron, & Arbyn, 2019), and our observations were comparable to the range of

published figures, in that CINtec PLUS showed a significantly lower sensitivity and non-significantly higher specificity in comparison to HPV testing in women of all ages. CIN2+ sensitivity was 81.5 % for CINtec PLUS vs. 94.4% for HPV test in women of all ages, and these were 70.0% and 93.3%, respectively, in women <30 years (Table 4-3). But, both tests showed identical CIN2+ sensitivity of 95.8% in women ≥30 years. Further, the sensitivities were in the range of 95-100% for both tests in detecting CIN3 in all ages and in those ≥30 years (Table 4-2). This indicates CINtec PLUS could be more reliably used for triaging women ≥30 years than <30 years, whereas HPV testing could be equally reliable in women of all ages, regardless of age groups, referred to colposcopy with a history of LSIL cytology. Further analyses of paired CINtec PLUS and HPV results in women of all ages by McNemar test showed no difference between the two (Table 4-4), indicating that both tests could serve as a predictor of CIN2+ with high sensitivity while conferring a significant reduction in the number of women requiring further colposcopy clinic visits. Since CINtec PLUS sensitivity was found to be lower in women <30 years, this may be of concern if considering CINtec PLUS in LSIL triage for this age group. However, CIN2+ is known to be mostly regressive (Tainio K. , et al., 2018), and CINtec PLUS-negative results may in fact be reflective of regressing lesions, and therefore, clinically more relevant than the higher HPV positivity which in many cases likely represents transient infection. Regardless, the reduced CIN2+ sensitivity of CINtec PLUS should be considered if using this test for LSIL triage in women <30 years. Given the option between Pap cytology and CINtec PLUS for LSIL triage of this age group, the latter would still be a better choice as CINtec PLUS is

more sensitive than cytology (Tjalma W. A., 2017), (Ikenberg H. , et al., 2013). Nevertheless, a further follow up would be warranted for those testing CINtec PLUS negative to ensure CIN2+ is not missed. Alternatively, HPV triage could be an option for this age group, and if using a partial genotyping test such as the cobas HPV assay, there is an added advantage of providing a secondary triage result with genotypes 16/18 information for risk stratification.

HPV genotypes 16/18 dominate in high grade lesions accounting for 70% of cervical cancer world-wide (Munoz, et al., 2003), (Khan, et al., 2005), (Guan, et al., 2012). Therefore, testing for these two genotypes has been proposed as an additional tool to allow for more fine-tuned patient management (Arbyn, et al., 2017). This is now technologically supported by several currently available next generation HPV testing platforms such as the cobas HPV test which offer high throughput one-step partial genotyping for 16/18, thus providing immediate access to this information. Based on a meta-analysis of 24 studies involving more than 5000 women with LSIL, Arbyn et al. reported the average risk for CIN3+ to be 19% in genotypes 16/18-positive women compared to 5% in hr-HPV-positive but genotypes 16/18-negative women (Arbyn, et al., 2017). Further, the pre-test probability of CIN3+ was 8.6% whereas the post-test probabilities after triage were 10.6% in hr-HPV positive women, 19.3% in genotypes 16/18-positive women and 3.8% in genotypes 16/18-negative women. This analysis also showed testing for genotypes 16/18 was substantially more specific but less sensitive than testing for hr-HPV in detecting CIN2+. This is relevant when considering genotypes 16/18-

specific threshold for further investigations and follow up of LSIL populations. In our study, among those testing HPV positive, genotypes 16/18 were detected in 28.1% (93/331) in all ages, and 29.1% (57/196) in women ≥30 years. If genotype 16/18-specific risk threshold were to be used for further follow up in colposcopy clinics, based on the above percentages, the proportion requiring additional investigations would be cut down by more than two-thirds among those testing HPV positive. This approach would have detected 49% (25/51) of CIN2+ among those testing HPV positive in all ages, with a sensitivity of 46.3%, and 60.9% (14/23) of CIN2+ among those testing HPV positive in woman ≥30 years, with a sensitivity of 58.3% (Table 4-5). The above sensitivity rates were similar to the pooled genotype 16/18-specific CIN2+ sensitivity of 55.5% (Arbyn, et al., 2017). Our specificity rates of 84.7% and 83.8%, respectively, for these two age groups (Table 4-5), were also like the 76.3% reported in the meta-analysis. It is important, however, to point out while testing for genotypes 16/18 increases efficiency, it is significantly less sensitive than testing for hr-HPV as shown in our study (Tables 4-3 and 4-5) and consistent with the data reported in the meta-analysis (Arbyn, et al., 2017). Whether continued colposcopy follow up or cytological follow up in primary care is considered for hr-HPV-positive, but genotypes 16/18-negative women would depend on local decision thresholds, this can be derived from pre- and post-test probability plots (Arbyn, et al., 2017). Regardless, the above observation takes cue from the US interim clinical guidance that recommends immediate colposcopy referral for those testing positive for genotypes 16/18 in primary HPV screening, and reflex cytology for those

positive for OHR HPV types (Huh, et al., 2015). Although the average risk for CIN2+ is significantly lower in OHR HPV positive women, continued follow up of such cases is warranted to ensure detection of any additional CIN2+ cases that would otherwise be missed by genotype 16/18-based risk stratification if this option is considered. Ongoing studies evaluating triage strategies for HPV positive women should provide further guidance in this regard (Huh, et al., 2015).

Both CINtec PLUS and cobas HPV tests showed a close and consistent correlation with cytological grades found at enrolment. Cytology was performed in the colposcopy clinic an average of 7.9 months after the index referral LSIL cytology, and during this interval LSILs regressed in 48.9% and progressed to HSIL in 8.6% in all ages (Table 4-6). These rates were consistent with a regression of 41.9% and progression of 7% reported after an average of 2-month interval in the ALTS trial of ASCUS population (Solomon, Schiffman, Tarone, & Group, 2001), and 56.4% and 7.8%, respectively, found in a Norwegian study of ASCUS/LSIL populations after a median of 7 months (Trope, et al., 2012). The above observation is particularly important in considering the usefulness of HPV testing for triaging women referred to colposcopy with a history of LSIL cytology as lesion regression would directly influence HPV positivity rates. Since most LSILs regress spontaneously, a large proportion of women with LSIL cytology referred to colposcopy no longer have LSIL by the time they are seen in colposcopy clinics as observed in our study, and this reduces the overall HPV positivity rates, consequently making HPV testing cost-effective in this setting. It is important to note our study was conducted in a routine

colposcopy clinic in a practical setting. Our study showed overall HPV positivity rates of 55.2% in all ages and 50.8% in women ≥30 years (Table 4-1). These were similar to an overall HPV positivity rate of 50.6% found in the ALTS-ASCUS trial which led to an ASCUS-HPV triage recommendation (Solomon, Schiffman, Tarone, & Group, 2001), but substantially lower than the pooled HPV positivity rate of 76% reported in populations with concurrent LSIL (Arbyn, et al., 2009). We could also surmise that the reduced HPV positivity in our study population as described above was the reason for the failure to demonstrate a significantly higher specificity of CINtec PLUS than HPV test that has been shown in many studies (Tjalma W. A., 2017), (Sun M. , Shen, Ren, & Dong, 2018), (Ikenberg H. , et al., 2013), (Bergeron C. , et al., 2015), (Peeters, Wentzensen, Bergeron, & Arbyn, 2019). It is apparent the reduced HPV positivity conferred increased specificity, thus narrowing the difference in specificity rates between CINtec PLUS and HPV tests in our study population albeit showing a non-significantly higher specificity for CINtec PLUS compared to HPV test. Regardless, based our study, it may be concluded that HPV testing, especially with partial genotyping, could be effective for triaging women with a history of LSIL cytology referred to colposcopy in routine clinical settings.

HPV primary screening and ASCUS-HPV triage are recommended for women ≥30 years in many countries. As part of routine cervical screening guidelines, ASCUS-HPV triage was implemented over a decade ago for women ≥30 years in Newfoundland and Labrador. Our records based on cobas HPV testing show an average HPV positivity rate of 30% in this population, thus helping to reduce the number of women requiring immediate

colposcopy referral by about 70%; the reduction could be as high as 85% if using genotype 16/18-specific risk threshold for referral (Gilbert, et al., 2022). Further CINtec PLUS has been approved by Health Canada as an adjunct test for risk stratification to colposcopy referral. These, together with a poor efficacy of ProEx C immunoassay for ASCUS and LSIL triage that we found in our previous study (Alaghehbandan, et al., 2013), provided the basis and impetus to our present study.

Our study was conducted in a routine colposcopy referral setting with a study cohort representative of Canadian LSIL referral populations without any intervention for the purpose of the study, especially regarding the time interval between referral LSIL cytology and initial colposcopy clinic visit. One limitation of this work is that the study population is only from one colposcopy clinic at a regional referral centre, meaning the population may not represent all Canadian populations and colposcopy clinics. The average interval of about 8 months observed in our study is likely representative of colposcopy waiting time in Canadian settings and may be reflective of prevailing colposcopy backlogs and workload. This underscores the importance of using effective triage strategies to reduce unnecessary accumulation of referral women in colposcopy clinics who are not at immediate risk. Our report is preliminary based on baseline findings and a reduced CIN2+ biopsy-confirmed sample size because not all patients in the study underwent biopsy for verification of disease outcome since biopsy was obtained from only those found to have colposcopy-detected lesions per routine clinical practice. Plotting risks for pre-cancer on pre- and post-test probability plots should shed more light in

assessing the efficacy of triage tests including testing for genotypes 16/18 as indicated by Arbyn et al (Arbyn, et al., 2017). This study is ongoing with follow up via medical records review which may provide further data on predictive values of CINtec PLUS and cobas HPV tests for triaging women referred to colposcopy with a history of LSIL cytology.

5.5 Conclusion

Based on our results, it may be concluded that either CINtec PLUS or cobas HPV test could serve as a predictor of CIN2+ with an equally high sensitivity in women ≥30 years referred to colposcopy with a history of LSIL cytology. In women <30 years, CINtec PLUS showed lower sensitivity than HPV test, and this needs to be considered in weighing post-test pre-cancer risk if this test is used for LSIL triage. CINtec PLUS or cobas HPV test can significantly reduce the number of women requiring further investigations and follow up in colposcopy clinics.

5.6 Additional Note

The findings of this study were also presented at CACMID 2019. Appendices A and B are the submitted abstract and poster which was presented, respectively.

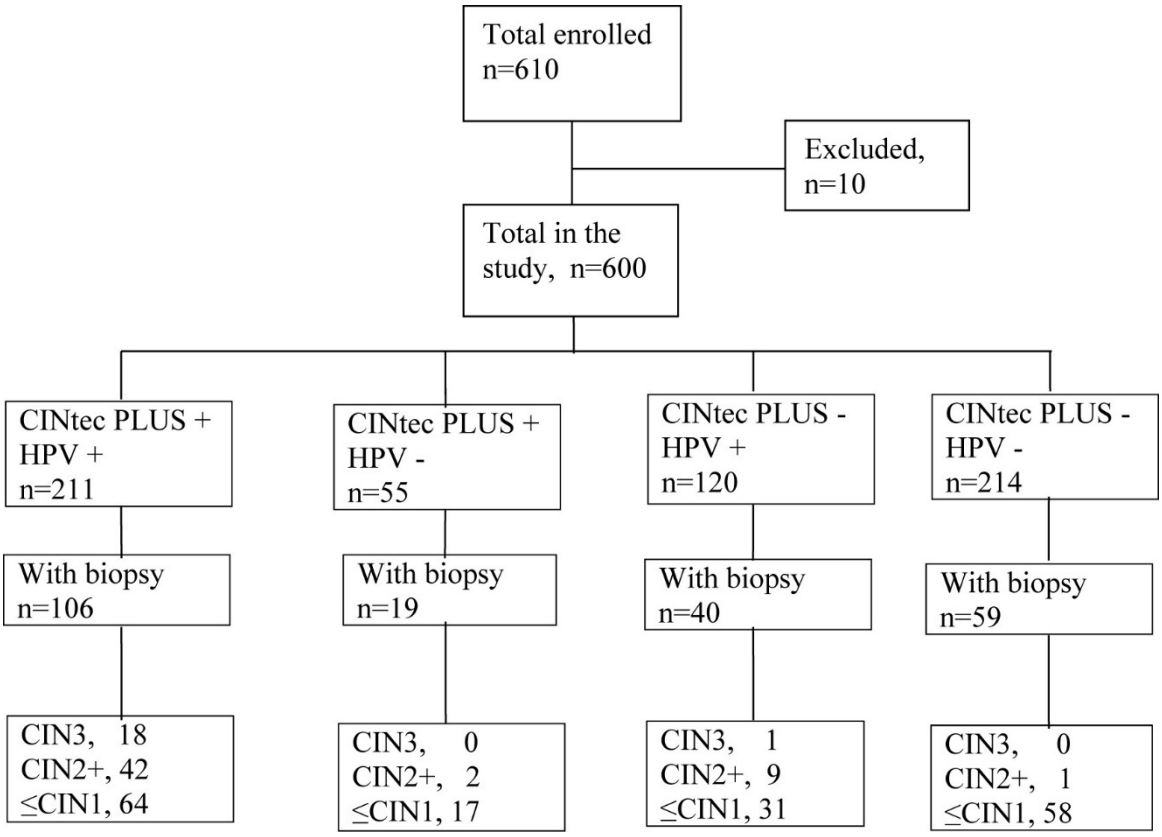

Figure 4.1. Distribution of CINtec PLUS and HPV results and biopsy outcome in ages (18-76 years)

Table 4-1. CINtec PLUS and HPV results by age groups

Test	Result	All ages, n=600	<30 years, n=214	≥30 years, n=386	
CINtec PLUS	Positive	266 (44.3%) [a]	107 (50.0%) [b,*]	159 (41.2%) [c,*]	* p=0.038, CINtec Plus positivity compared between women <30 and ≥30 years, two-tailed z test
	Negative	334 (55.7%)	107 (50.0%)	227 (58.8%)	
HPV	Positive	331 (55.2%) [a]	135 (63.1%) [b,**]	196 (50.8%) [c,**]	** p=0.004, HPV positivity compared between women <30 and ≥30 years, two-tailed z test
	Negative	269 (44.8%)	79 (36.9%)	190 (49.2%)	
p value		[a] p value <0.001, CINtec PLUS positivity compared with HPV positivity in all ages, two-tailed z test	[b] p value=0.006, CINtec PLUS positivity compared with HPV positivity in women <30 years, two-tailed z test	[c] p value= 0.008, CINtec PLUS positivity compared with HPV positivity in women ≥30 years, two-tailed z test	

2357 *Table 4-2. Association of CINtec PLUS and HPV results with biopsy by age groups*

Test	Result	Biopsy result								
		All ages, n=224			<30 years, n=89			≥30 years, n=135		
		≤CIN1, n=170	CIN2+, n=54	CIN3, n=19	≤CIN1, n=59	CIN2+, n=30	CIN3, n=10	≤CIN1, n=111	CIN2+, n=24	CIN3, n=9
CINtec PLUS	Positive	81	44	18	31	21	9	50	23	9
	Negative	89	10	1	28	9	1	61	1	0
HPV	Positive	95	51	19	40	28	10	55	23	9
	Negative	75	3	0	19	2	0	56	1	0

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Table 4-3. Diagnostic indices of CINtec PLUS and HPV tests for detection of CIN2+

Diagnostic index	CINtec PLUS test			HPV test			p value*, All ages
	All ages	<30 years	≥30 years	All ages	<30 years	≥30 years	
Sensitivity % (95% CI)	81.5 (76.4-86.6)	70.0 (60.5-79.5)	95.8 (92.5-99.2)	94.4 (91.4-97.4)	93.3 (88.2-98.5)	95.8 (92.5-99.2)	p=0.039, CINtec Plus sensitivity compared with HPV sensitivity
Specificity % (95% CI)	52.4 (45.8-58.9)	47.5 (37.1-57.8)	55.0 (46.6-63.3)	44.1 (37.6-50.6)	32.2 (22.5-51.4)	50.5 (42.0-58.9)	p=0.129, CINtec Plus specificity compared with HPV specificity
PPV % (95% CI)	35.2 (28.9-41.5)	40.4 (30.2-50.6)	31.5 (23.7-39.3)	34.9 (28.7-41.2)	41.2 (31.0-51.4)	29.5 (21.8-37.2	p=0.960, CINtec Plus PPV compared with HPV PPV
NPV % (95% CI)	89.9 (86.0-93.8)	75.7 (66.8-84.6)	98.4 (96.3-100.0)	96.2 (93.6-98.7)	90.5 (84.4-96.6)	98.2 (96.0-100.0)	p=0.114, CINtec Plus NPV compared with HPV NPV

CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value

* two-tailed z test

Table 4-4. Comparison of CINtec PLUS and HPV tests by biopsy result: All ages (n=224)

		HPV test result					
		≤ CIN1, n=170			CIN2+, n=54		
		Positive	Negative	Total	Positive	Negative	Total
CINtec PLUS result	Positive	64	17	81	42	2	44
	Negative	31	58	89	9	1	10
Total		95	75	170	51	3	54
McNemar		p = 0.059			p = 0.065		

Table 4-5. Association of HPV genotypes 16/18 with biopsy and diagnostic indices

		Biopsy result					
		All ages, n=224		<30 years, n=89		≥30 years, n=135	
		≤CIN1, n=170	CIN2+, n=54	≤CIN1, n=59	CIN2+, n=30	≤CIN1, n=111	CIN2+, n=24
HPV 16/18	Positive	26	25	8	11	18	14
	Negative	144	29 [a]	51	19	93	10
HPV 16/18 genotyping [b]	Sensitivity	25/54 = 46.3%		11/30 = 36.7%		14/24 = 58.3%	
	Specificity	144/170 = 84.7%		51/59 = 86.4%		93/111 = 83.8%	
	p value*, sensitivity: 0.395, All ages compared to <30 years 0.327, All ages compared to ≥ 30 years 0.112, <30 years compared to ≥ 30 years						

[a] Among 29 testing genotype 16/18 negative, 26 tested positive for 12 other high-risk (OHR) genotypes, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.

[b] Sensitivity/specificity based on CIN2+ detection

* two-tailed z test

*Table 4-6. Cytology status at enrolment and association with CINtec PLUS and HPV results: All ages, n=595**

Cytology		CINtec PLUS result		HPV result	
Status	Number of cases (%)	Positive (%)	Negative (%)	Positive (%)	Negative (%)
HSIL	51 (8.6)	45 (88.2)	6 (11.8)	47 (92.2)	4 (7.8)
ASC-H	6 (1.0)	5 (83.3)	1 (16.7)	4 (66.7)	2 (33.3)
LSIL	247 (41.5)	148 (59.9)	99 (40.1)	184 (74.5)	63 (25.5)
ASCUS	101 (17.0)	27 (26.7)	74 (73.3)	43 (42.6)	58 (57.4)
Negative	190 (31.9)	39 (20.5)	151 (79.5)	50 (26.3)	140 (73.7)
Total	595	264 (44.4)	331 (55.6)	328 (55.1)	267 (44.9)

* There were 5 cases with unsatisfactory cytology and excluded from the total of 600 study patients

HSIL, High-grade squamous intraepithelial lesion; ASC-H, Atypical squamous cells of undetermined significance-cannot exclude HSIL; LSIL, Low-grade squamous intraepithelial lesion; ASCUS, Atypical squamous cells of undetermined significance

Table 4-7. Comparison of CINtec PLUS and HPV tests by biopsy result: Women <30 years of age (n=89)

		HPV test result					
		≤ CIN1, n=59			CIN2+, n=30		
		Positive	Negative	Total	Positive	Negative	Total
CINtec PLUS result	Positive	26	5	31	20	1	21
	Negative	14	14	28	8	1	9
Total		40	19	59	28	2	30
McNemar		p = 0.064			p = 0.039		

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2396 *Table 4-8. Comparison of CINtec PLUS and HPV tests by biopsy result: Women ⩾30 years*
2397 *of age (n=135)*

		HPV test result					
		≤ CIN1, n=111			CIN2+, n=24		
		Positive	Negative	Total	Positive	Negative	Total
CINtec PLUS Result	Positive	38	12	50	22	1	23
	Negative	17	44	61	1	0	1
Total		55	56	111	23	1	24
McNemar		p = 0.458			p = 1.000		

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Chapter 5 : Paper 3, Comparison of CINtec PLUS Cytology and cobas HPV Test for Triaging Canadian Patients with LSIL Cytology Referred to Colposcopy: A Two-Year Prospective Study

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Abstract

Objectives & methods: CINtec PLUS and cobas HPV tests were compared for triaging patients referred to colposcopy with a history of LSIL cytology in a 2-year prospective study. Cervical specimens were tested once at enrollment, and test positivity rates determined. Test performance was ascertained with cervical intraepithelial neoplasia grade 2 or worse (CIN2+) and CIN3 or worse (CIN3+) serving as clinical endpoints.

Results: In all ages, (19-76 years, n= 598), 44.3% tested CINtec PLUS positive vs. 55.4% HPV positive (p< 0.001). To detect CIN2+ (n= 99), CINtec PLUS was 81.8% sensitive vs. 93.9% for HPV testing (p= 0.009); genotype 16/18-specific sensitivity was 46.5%. Specificity was 52.9% vs. 36.6%, respectively (p< 0.001). In all ages, to detect CIN3+ (n= 44), sensitivity was 93.2% for both tests; genotype 16/18-specific sensitivity was 52.3%. Specificity was 48.4% for CINtec PLUS vs. 31.1% for HPV testing (p< 0.001). In patients < 30 years, CINtec was 91.7% sensitive vs 95.8% for HPV testing (p= 0.549).

Conclusions: CINtec PLUS or cobas HPV test could serve as a predictor of CIN3+ with high sensitivity in patients referred to colposcopy with a history of LSIL regardless of age while significantly reducing the number of LSIL referral patients requiring further investigations and follow-up in colposcopy clinics.

5.1 Introduction

While some countries have successfully transitioned to human papillomavirus (HPV) primary cervical cancer screening, Papanicolaou cytology remains the mode of primary screening in many jurisdictions, including Canada, for a variety of reasons. In cytology-based cervical cancer screening, a large number of patients are diagnosed as having borderline or low-grade abnormal cytology who are managed at considerable costs, while a small fraction is at risk. It could be beneficial to consider currently available triage options, especially in managing patients referred to colposcopy with a history of low-grade squamous intraepithelial lesion (LSIL), in routine colposcopy clinical practice.

LSIL accounts for a large proportion of abnormal cytology in routine screening but it regresses in the majority of cases. However, a small fraction have high grade squamous intraepithelial lesions (HSIL) or could be at risk of progression to HSIL and cervical cancer. Due to this risk, those found to have LSIL in routine screening are either referred to colposcopy directly or managed cytologically, with those having persistent abnormalities being referred to colposcopy (Cancer Care Ontario, 2016), (Wright Jr, et al., 2007), (Massad, et al., 2013). In colposcopy clinics, LSIL cases are typically followed with cytology, colposcopy, and biopsy as indicated, for an extended period. With the majority being not at risk, this is excessive and unnecessary for most patients and associated with considerable negative health effects due to distress over prolonged period, increased anxiety at every clinic visit, unnecessary invasive procedures and overtreatment etc.

leading to poorer quality of life (Cuzick, et al., 2013) (Tjalma, Kim, & Vandeweyer, 2017). An effective triage of LSIL referral patients can identify those at increased risk who need to remain under care and return those not at immediate risk to routine screening (Cuzick, et al., 2013), (Ronco, et al., 2007), thus eliminating potential negative health effects and reducing systemic costs.

The CINtec PLUS cytology (Roche Diagnostics) has emerged as an effective biomarker-based adjunct test for triaging patients having atypical squamous cells of undetermined significance (ASCUS) or LSIL in cytology screening (Tjalma W. A., 2017), (Tjalma, Kim, & Vandeweyer, 2017), (Sun M. , Shen, Ren, & Dong, 2018), (Sun, Shen, & Cao, 2019), (Yu, et al., 2019), (Bergeron C. , et al., 2015) and those testing positive for high-risk human papillomavirus (hr-HPV) in HPV primary screening (Clarke, et al., 2019), (Guan, et al., 2012), (Wright Jr, et al., 2017), (Wentzensen, et al., 2019). CINtec PLUS is a dual-stain immunocytochemical test which detects p16 and Ki-67 proteins that are over expressed in cervical cells with transforming HPV infection. As the expression of p16 and Ki-67 is mutually exclusive in normal cells, the co-detection of these proteins simultaneously within the same cervical epithelial cell serves as a specific marker of HPV-mediated oncogenic transformation and predictor of cervical cancer risk (Tjalma W. A., 2017), (Tjalma, Kim, & Vandeweyer, 2017), (Sun, Shen, & Cao, 2019), (Sun M. , Shen, Ren, & Dong, 2018), (Yu, et al., 2019), (Ratnam, et al., 2020). CINtec PLUS has been shown to be more sensitive than cytology with equal specificity, and more specific than HPV testing with relatively comparable sensitivity for detecting cervical intraepithelial neoplasia grade

2 or worse (CIN2+) in LSILs (Clarke, et al., 2019), (Schmidt, Bergeron, Denton, & Ridder, 2011), (Ikenberg H. , et al., 2013), (White, et al., 2016), (Peeters, Wentzensen, Bergeron, & Arbyn, 2019). While the clinical applications of CINtec PLUS in LSIL triage have been assessed in several studies in Europe and elsewhere (Bergeron C. , et al., 2015), (Ikenberg H. , et al., 2013), (Possati-Resende J. , et al., 2015), (Wentzensen, Schiffman, Palmer, & Arbyn, 2016), there has been limited evaluation of this method to serve as an adjunct test in LSIL triage in North American settings (El-Zein, et al., 2020).

The ALTS LSIL study precluded LSIL-HPV triage as 83% of LSIL cases tested hr-HPV positive (ASCUS-LSIL Triage Study (ALTS) Group, 2003). However, this study was conducted in patients mostly <30 years. There is evidence that LSIL-HPV triage could be effective in those ≥35 years (Cuzick, et al., 2013), (Ronco, et al., 2007). The cobas HPV DNA test (Roche Diagnostics) is a PCR-based qualitative partial genotyping test, identifying genotypes 16/18 specifically and 12 other high-risk (OHR) types collectively in a single analysis, and has been recommended for genotype 16/18-specific risk threshold in HPV primary screening (Huh, et al., 2015). In this respect, the cobas HPV test also has the potential to serve as an adjunct test for triaging LSIL referral populations within colposcopy clinics, and this could reduce the number of patients requiring additional investigations and follow-up in colposcopy clinics, and thus aid in better patient care and resource management.

We conducted a study to assess positivity rates of CINtec PLUS and cobas HPV tests along with genotype 16/18-specific risk threshold among those referred to colposcopy

with a history of LSIL, to identify those at increased risk and thus potentially reduce the proportion requiring further colposcopy clinic visits and follow-up, and prospectively determined clinical efficacy of the two tests to detect CIN2+. The initial study data were obtained at baseline, and the study cohort remaining under care in the colposcopy clinic was followed up to 2 years to ascertain disease outcome. We previously communicated our baseline findings (Ratnam, et al., 2020), and in this manuscript, we present the complete data obtained in the study.

5.2 Materials and methods

5.2.1. Ontario cervical cancer screening guidelines

In the province of Ontario, Canada, liquid-based Papanicolaou cytology is being used for primary cervical cancer screening. In this system, if cytology is normal, triennial screening continues. For those with LSIL cytology, either direct referral to colposcopy or repeat cytology at 6-month intervals is recommended; for those having persistent atypical squamous cells of undetermined significance (ASCUS) or worse in repeat cytology, colposcopy is recommended (Cancer Care Ontario, 2016). In colposcopy clinics, all referred patients undergo cytology and colposcopic examination with biopsies of any lesions detected, and further follow-up clinical pathways depend on specific criteria as previously described (Ratnam, et al., 2020).

5.2.2 Study design and protocol

The study was designed to assess CINtec PLUS cytology and HPV test positivity at baseline (enrollment) to identify the proportion potentially at increased risk, and therefore, requiring continued follow-up in the colposcopy clinic, and conversely, the proportion that could be returned to routine screening, thus improving overall clinical and systemic efficiency. In relation to this, it was also designed to determine clinical efficacy of CINtec PLUS and HPV tests to detect CIN2+. This was assessed at baseline, and prospectively during a 2-year follow-up of patients who remained under care in the colposcopy clinic.

The study was conducted within the Ontario cervical screening guidelines. The study population comprised of patients with a history of LSIL cytology referred to the colposcopy clinic at the Juravinski Hospital, Hamilton, Canada. All study patients were attended to per standard of care, with cervical specimens collected for cytology, and colposcopy and biopsies performed per routine clinical practice. Cytology was carried out as part of routine patient care, and CINtec PLUS and cobas HPV tests were performed once at enrollment using the residual cervical specimens for the study purpose. Patients' baseline data were recorded, and the study cohort remaining under care in the colposcopy clinic was followed up to 2 years to determine disease outcome. Biopsy confirmed CIN2+ served as the clinical endpoint. CINtec PLUS and HPV testing results obtained at baseline together with that of biopsies performed either at baseline or anytime during the follow-up were recorded as primary study outcomes. CINtec PLUS and HPV positivity rates that would correspond to the proportions requiring further colposcopy clinic visits and follow-up were determined. CINtec PLUS and HPV results obtained at baseline were correlated with biopsy confirmed CIN2+ to ascertain the clinical performance of the tests.

5.2.3. Ethics

The study was approved by the Hamilton Integrated Research Ethics Board (HiREB) and Newfoundland and Labrador Health Research Ethics Board (HREB) (Appendix G). All participants were informed verbally and in writing about the study, use of their residual cervical specimens for CINtec PLUS and HPV testing, and the need to periodically review

their medical records during follow-up. Those consenting to participate were enrolled with written informed consent.

5.2.4. Patient enrolment criteria

Patients with a history of LSIL cytology who had not received treatment were eligible. Enrolment criteria included: 1) Patients who had LSIL cytology in routine primary screening and who were directly referred to colposcopy; 2) those who were found to have LSIL cytology initially in routine primary screening and who upon repeat cytology found to have persistent ASCUS or LSIL and referred to colposcopy; and 3) those who were diagnosed as having LSIL among patients being followed in the colposcopy clinic. There were no age limits. Pregnant persons and those without a cervix were excluded. Eligible patients were enrolled consecutively from November 2017 through February 2019.

5.2.5 Study specimens

Cervical specimens were collected into ThinPrep PreservCyt® (Hologic Inc) cytology medium using their standard collection device for routine cytology at enrolment. Slides were prepared for CINtec PLUS testing using residual cervical specimens at the cytology laboratory, St. Joseph's Healthcare Hamilton. The slides and aliquots of cervical specimens were shipped to the Public Health and Microbiology Laboratory, St. John's for CINtec PLUS and cobas HPV tests. These tests were carried out as described below no later than 6 weeks post collection.

5.2.6. CINtec PLUS Cytology

Slides were prepared on a ThinPrep processor (T5000, Hologic Inc) using special ThinPrep slides (Hologic, Inc) and stained using CINtec PLUS test kits within 48 hrs and processed on BenchMark ULTRA system (Roche Diagnostics) per manufacturer's instructions.

The CINtec PLUS slides were initially evaluated independently by one of two experienced cytotechnologists who were trained to read these slides. Smears were determined to be positive if at least one cervical epithelial cell showed both a brownish cytoplasmic immunostaining for p16 and a red nuclear immunostaining for Ki-67 regardless of cellular morphology. If the dual staining was not observed, the smear was considered negative. Smears were deemed unsatisfactory if they did not contain an adequate number of cells (>4 cells per field with a minimum of 10 fields with a 40x objective). All slides were independently reviewed by a study pathologist trained to read CINtec PLUS slides, and the results recorded using the same criteria. Discrepant slides were either internally reviewed by another reader and reconciled or adjudicated independently by an external expert.

5.2.7. cobas HPV test

The cobas HPV test was performed on the Roche 4800 automated platform per manufacturer's instructions. Results were reported as positive for genotypes 16 and/or 18, and/or 12 OHR types, or negative for 14 hr-HPV types, per standard practice.

5.2.8. Cervical biopsy

Biopsies were performed by colposcopists per standard clinical practice. Three sections of each biopsy sample were processed with hematoxylin and eosin (H&E) staining per routine practice. p16 immunostaining (CINtec® Histology kit, Roche Diagnostics) was performed as part of the study protocol to provide supporting diagnostic evidence. Biopsies were read by staff pathologists at the originating colposcopy clinic site per standard practice. All biopsy slides together with p16 stained slides were independently reviewed by two study pathologists. Discrepant biopsy results were independently adjudicated by a third pathologist, if needed.

5.2.9. Results management

CINtec PLUS and HPV tests were conducted independently. Cytotechnologists and the study pathologists were blinded to test results as well as cytology and biopsy results obtained at baseline. Colposcopy clinicians did not have access to CINtec PLUS or HPV results at the time of initial patient evaluation. Only HPV results were provided subsequently to clinicians to aid in patient management.

5.2.10. Data analysis

Statistical analysis was performed using SPSS for Windows, versions 23 and 27, Excel, Microsoft Office Professional Plus, 2013, MedCalc, 2021, and Social Science Statistics website, 2020 initially at baseline, as previously described (Ratnam, et al., 2020), and after two-years of prospective follow-up. Qualitative variables such as test results,

cytology grades, and HPV genotypes, were studied through different frequencies. Descriptive statistics were prepared for test results, distribution of cytology grades and HPV genotypes. Study data were analyzed using contingency tables to determine test positivity rates, and the diagnostic indices of CINtec PLUS and HPV testing. Receiver operating characteristic (ROC) analyses were performed for CINtec PLUS and HPV testing in detecting CIN2+. The area under the curve was calculated for each test as an alternative single indicator of test performance. All tests were two-tailed, and $p < 0.05$ was considered statistically significant.

5.3 Results

5.3.1 Study population

A total of 610 patients meeting the study criteria were enrolled in the study. Of these, 12 were excluded due to insufficient or no cervical specimen for CINtec PLUS and/or HPV testing, or invalid CINtec PLUS or HPV test results, leaving 598 patients in the study with evaluable results (Figure 5.1). Age ranged from 19 to 76 years (median, 33.0), with 384 (64.2%) ≥30 years of age (median, 43.0). (In our baseline paper (Ratnam, et al., 2020), the total number of patients with evaluable results was reported as 600. Upon final review, 2 patients were found to not to have met study inclusion criteria and were removed from our final analysis, resulting in 598 patients in the study. When reviewing dates and biopsy results, two cases that were reported in our baseline work were reclassified. This reduced the number of patients with biopsy from 224 as reported to 222. These changes had no impact on the conclusions drawn in the baseline paper.

Although the index referral cytology was LSIL in all patients enrolled per study criterion, cytology performed at the time of enrollment showed a heterogeneous cytological grade as expected. The time interval between the index referral LSIL cytology immediately prior to the colposcopy clinic visit and cytology performed in the colposcopy clinic at the time of enrollment ranged from <1 month to ≥18 months with a median of 7 months. During this interval, LSILs regressed in 48.9% and progressed in 9.6% with only 41.5% still having LSIL. The above cytology status of the study population was unknown at the time of patient enrollment. Cytology categories of the study population correlated

with CINtec PLUS and HPV results at the time of enrollment were previously described in our baseline paper (Ratnam, et al., 2020).

Of the 598 patients in the study, per standard practice, biopsies were only performed when clinically indicated. As such, there were 222 evaluable cervical biopsy results available at baseline, and among them 54 (24.3%) were diagnosed as CIN2+. The baseline data obtained with the 54 CIN2+ cases were previously described (Ratnam, et al., 2020). Having reached the clinical endpoint of the study, the 54 CIN2+ cases were not followed any further, leaving 544 patients for prospective follow-up. Of the 544, 264 patients were mostly discharged following a negative HPV test per Ontario cervical cancer guidelines and some were lost to follow-up, leaving 280 patients remaining under care in the colposcopy clinic, representing the follow-up cohort. These were followed for a median of 202 days (36 days to 736 days). Among the 280, 148 patients underwent biopsy during the follow-up as part of routine patient care per standard practice, and of them, 45 (30.4%) were diagnosed as CIN2+. This yielded a total of 99 (27.8%) CIN2+ cases among the 598 patients enrolled in the study, including 42 CIN3 cases and 2 cases of adenocarcinoma in situ, collectively referred to as CIN3+ for analysis purposes. Distribution of the total study population with CINtec PLUS and HPV test results and biopsy outcome of clinical assessment at baseline, along with biopsy outcome during follow-up clinical assessment are schematically shown in Figure 5.1.

5.3.2 CINtec PLUS and HPV test results

Table 5-1 shows CINtec PLUS and cobas HPV results for the total population of 598 for all ages, and those <30 years of age and ≥30 years. In all ages, CINtec PLUS was positive in 265 (44.3%) vs. 331 (55.4%) testing HPV positive (p<0.001, two-tailed z test). Among the 331 HPV positives, genotypes 16/18 were detected in 93 (28.1%). In those ≥30 years, CINtec PLUS was positive in 158 (41.1%) vs. 196 (51.0%) testing HPV positive (p=0.006, two-tailed z test). Among the 196 HPV positives, genotypes 16/18 were detected in 57 (29.1%). There were significant differences in both CINtec PLUS and HPV positivity rates between those <30 years of age and ≥30 years (Table 5-1).

5.3.3 Performance of CINtec PLUS and HPV tests to detect CIN2+ and CIN3+

Of the 598 patients in all ages, a total of 356 (59.5%) had evaluable biopsy results. Among the 356, as indicated above, biopsy confirmed CIN2+ was diagnosed in a total of 99 (27.8%) patients, comprising of 55 CIN2 and 44 CIN3+. All CIN2+ biopsy diagnoses were substantiated by a positive p16 immunostain result.

Table 5-2 illustrates the performance of CINtec PLUS in comparison with HPV testing in detecting 99 CIN2+. In all ages, CINtec PLUS was positive in 81 for a sensitivity of 81.8% while HPV was positive in 93 for a sensitivity of 93.9% (p=0.009, two-tailed z test). Specificity was 52.9% vs. 36.6%, respectively (p<0.001, two-tailed z test). In patients <30 years, CINtec PLUS sensitivity for detection of CIN2+ was 76.0% vs 94.0% for HPV testing (p=0.012, two-tailed z test). For detection of 55 CIN2, in all ages, CINtec PLUS

sensitivity was 72.7% (40/55) vs. 94.5% (52/55) for HPV testing (p=0.002, two-tailed z test), and in patients <30 years, these figures were 61.5% (16/26) and 92.3% (24/26), respectively (p=0.009, data not shown, two-tailed z test).

Table 5-3 shows the performance of CINtec PLUS compared with HPV testing in detecting 44 CIN3+. In all ages, both CINtec PLUS and HPV tests were positive in 41 of these for an identical sensitivity of 93.2%. Specificity was 48.4% vs. 31.1%, respectively (p<0.001, two-tailed z test). Among patients <30 years, CINtec PLUS sensitivity was similar to that of in all ages at 91.7%. Negative predictive values (NPVs) were >96.0% in all age groups for both tests.

5.3.4 Performance of CINtec PLUS and HPV tests to detect incident CIN2+ during follow-up

Of the 45 incident CIN2+ detected among 148 having biopsy during the follow-up, 20 were <30 years and 25 were ≥30 years (range, 22-69; median, 30), and included 20 CIN2 and 25 CIN3+. Of the 45 CIN2+, biopsy diagnostic dates were available for 44, and for these cases, the time intervals from enrollment to detection of CIN2+ ranged from 63 to 736 days with an average of 241 days and a median of 199 days (Figure 5.2). In all ages, for detecting CIN2+, CINtec PLUS was 82.2% (37/45) sensitive vs. 93.3% (42/45) for HPV testing (p=0.107, two-tailed z test). Specificities were 47.6% (49/103) vs. 21.4% (22/103), respectively (p<0.001, two-tailed z test). CINtec PLUS showed a positive predictive value (PPV) of 40.7% (37/91) vs. 34.2% (42/123) for HPV testing (p=0.327, two-tailed z test). In

patients <30 years, CINtec PLUS sensitivity was similar to that of all ages at 85.0% (17/20) vs. 95.0% (19/20) for HPV test (p = 0.294, two-tailed z test). NPVs were ≥86% in all age groups for both tests, except 81.3% for CINtec PLUS in those <30 years. Among the 25 CIN3+ in all ages, CINtec PLUS was 92.0% (23/25) sensitive vs. 88.0% (22/25) for HPV testing (p=0.638, two-tailed z test).

5.3.5 ROC analysis

Figure 5.3 shows the results of ROC analysis comparing the overall performance characteristics of CINtec PLUS with HPV testing in detecting CIN2+. For patients in all ages, ROC results showed the areas under the curve for CINtec PLUS and HPV were similar at 0.725 and 0.731 (p>0.05). Further ROC analyses performed for those <30 and ≥30 years of age showed similar results. The area under curve was slightly higher for CINtec PLUS among those ≥30 years, although it was not statistically significant in detecting CIN2+ when compared to HPV.

5.3.6 Genotype specific risk threshold to detect CIN2+ and CIN3+

Table 5-4 shows HPV genotypes 16/18-specific results in comparison with hr-HPV testing to detect CIN2+ and CIN3+. In all ages, to detect CIN2+, genotype 16/18-specific testing was 46.5% sensitive vs 93.9% for hr-HPV testing; for CIN3+, these figures were 52.3% and 93.2%, respectively. As previously reported (Ratnam, et al., 2020), in all

2753 genotype 16/18 positive cases, type 16 was predominant as a single type in most cases,

2754 and in a few it was detected in combination with type 18 or OHR types.

5.4 Discussion

Our study showed an overall CIN2+ prevalence of 16.6% (99/598) in all ages in a routine colposcopy referral setting; it was lower at 12.8% (49/384) in patients aged ≥30 years. CIN3+ prevalence was 7.5% (44/598) and 5.2% (20/384), respectively. These figures are within the ranges reported among LSIL referral population in other studies (Cuzick, et al., 2013), (Solomon, Schiffman, Tarone, & Group, 2001), and underscore the importance of effective triage to identify the small fraction of LSIL referral patients at increased risk, and also raises the question of following all such patients in colposcopy clinics for an extended period.

Our CINtec PLUS positivity rate of 44.3% in LSIL was similar to other studies (Bergeron C. , et al., 2015), (White, et al., 2016), while the HPV positivity rate of 55.4% was significantly lower than those reported in concurrent LSIL (Arbyn, et al., 2017), (Arbyn, et al., 2009), (Table 5-1). This could be attributed to lesion regression in a large proportion of the LSIL referrals by the time they are seen in the colposcopy clinic, leading to lower HPV prevalence and test positivity rate, thus making HPV testing more effective in this setting as described previously (Ratnam, et al., 2020). Based on the above positivity rates, in all ages, CINtec PLUS would reduce the LSIL referral population requiring further investigations and follow-up in a colposcopy clinic by 55.7% (333/598) vs 44.6% (267/598) for HPV testing; these proportions would be higher in those ≥30 years of age. The above difference between the two tests is significant ($p<0.001$, two-tailed z test), and the higher reduction rate of CINtec PLUS is due to its higher specificity. The above data demonstrate

the potential for incorporating CINtec PLUS or HPV triage for patients referred to colposcopy with a history of LSIL to aid in better patient care and overall efficiency.

For CIN2+ detection, in all ages, CINtec PLUS showed a significantly lower sensitivity of 81.8% vs. 93.9% for HPV testing (Table 5-2). CINtec PLUS sensitivity was further dropped to 76.0% in patients <30 years, while remaining higher at 87.8% in those ≥30 years. Further analysis for those <30 years showed the reduced sensitivity of CINtec PLUS was more associated with CIN2 detection (61.5%) than CIN2+ (76.0%). However, when considering that CIN2 is known to be mostly regressive, and the fact that CINtec PLUS is more predictive of transforming HPV infection, it is likely that CINtec PLUS results are more meaningful and of greater clinical relevance and potential utility than an HPV DNA test. It was further substantiated by the observation that, in all ages, CINtec PLUS showed identical sensitivity of 93.2% as the HPV test for detecting CIN3+ (Table 5-3), a more definitive predictor of underlying cancer risk. It is also important to note that in patients <30 and ≥30 years, CINtec PLUS CIN3+ sensitivities were similar to that of HPV at 91.7% and 95.0%, respectively, indicating its clinical utility and value in detecting more severe malignancies regardless of age. Moreover, for the 45 incident CIN2+ cases observed during follow-up, both tests showed similar sensitivity for the total population, without a decrease in CINtec PLUS sensitivity in those <30 years, with similar PPVs. The significantly higher specificity of CINtec PLUS than HPV test was consistently observed, and in this respect, we note that CINtec PLUS is currently approved for triaging those testing positive in HPV primary screening (Slater, 2020). The lower sensitivity, albeit with

the caveats noted, and higher specificity of CINtec PLUS compared to HPV test we observed is consistent with a screening triage study recently reported in a Canadian population (El-Zein, et al., 2020) and many other studies (Schmidt, Bergeron, Denton, & Ridder, 2011), (Possati-Resende J. , et al., 2015), (Prigenzi, Heinke, Salim, & de Azevedo Fochi, 2018), (Zhu Y. , et al., 2019).

The use of CINtec PLUS in LSIL triage especially for patients <30 years of age may be of concern considering its lower sensitivity and NPVs in comparison to all ages in detecting CIN2 and CIN2+. Given the option between cytology and CINtec PLUS for LSIL triage of this age group, the latter would still be a better choice as CINtec PLUS is more sensitive than cytology (Tjalma W. A., 2017), (Ikenberg H. , et al., 2013). Additionally, CINtec PLUS performance being equal to that of HPV testing for detection of CIN3+ must be considered as noted above. Regardless, further follow-up would be warranted for those testing CINtec PLUS negative in LSIL triage to ensure CIN2+ is not missed.

Although there are differences in the overall sensitivities and specificities of CINtec PLUS and HPV reported in other studies (Sun, Shen, & Cao, 2019), (El-Zein, et al., 2020), (Zhu Y. , et al., 2019), (McMenamin, Mckenna, & McDowell, 2018) it is important to note that our study showed both tests having an identical high level of sensitivity to detect CIN3+, a better clinical predictor of risk and progression to assess test performance, and as such, both can potentially be used for LSIL triage in all age groups (Yu, et al., 2019), (Ikenberg H. , et al., 2013), (Possati-Resende J. , et al., 2015), (Das, et al., 2018). We also observed high levels of NPV for both CINtec PLUS and HPV tests in all age groups to detect

CIN3+ as apparent in identical other studies (Cuzick, et al., 2013), (Prigenzi, Heinke, Salim, & de Azevedo Fochi, 2018), (McMenamin, Mckenna, & McDowell, 2018), (Ren, et al., 2019) providing evidence that high grade lesions would be mostly detected in a clinical setting, and reassurance for use of either test for LSIL triage. In this respect we note that the ROC analyses showed no differences in the performance of CINtec PLUS and HPV tests for detection of CIN2+.

We assessed the application of HPV genotypes 16/18-specific threshold in LSIL triage as these genotypes account for approximately 70% of cervical cancer (Munoz, et al., 2003), (Khan, et al., 2005). Our 16/18 positive proportion of 28% among LSILs is similar to those reported in other studies (El-Zein, et al., 2020), (McMenamin, Mckenna, & McDowell, 2018). If this threshold is used, it would mean reducing the number of LSIL referral cases requiring additional follow-up by more than two-thirds. While this will greatly improve efficiency, it is significantly less sensitive for detecting both CIN2+ and CIN3+ than hr-HPV testing as observed in our study (Table 5-4) and reported by others (Arbyn, et al., 2017), (Cox, et al., 2013). Due to this short-coming, if this risk threshold is used in LSIL triage, it would warrant closer follow-up of those testing genotypes 16/18 negative within colposcopy clinics to ensure CIN2+ cases are not missed. As concluded in a meta-analysis, genotypes 16/18-specific risk threshold may be more useful in HPV primary screening than in LSIL triage (Arbyn, et al., 2017).

In this study, we evaluated the application of CINtec PLUS and HPV tests for triaging LSIL referral patients since their risk stratification and management remain of

clinical and programmatic importance in settings where cytology-based cervical screening is used. Overall, triaging LSIL referral populations could help to reduce the number of patients requiring further colposcopy clinic visits and additional investigations, thus decreasing burden on colposcopy clinics and eliminating potential health effects, consequently aiding in better patient care and resources management (Peeters, Wentzensen, Bergeron, & Arbyn, 2019). One aspect that can be difficult to quantify is the reduction in patients' anxiety and peace of mind by not having to continue colposcopy clinic visits for extended periods (Tjalma, Kim, & Vandeweyer, 2017). While not the focus of this work, reductions in the number of patients requiring further clinic visits would also lead to system efficiency by reducing waitlists and wait times for those who truly need colposcopy. Although it may be difficult to quantify the reduction in systems costs due to regional and programmatic differences (Tjalma, Kim, & Vandeweyer, 2017), there are general system cost efficiencies to be found (Tjalma, Kim, & Vandeweyer, 2017) (Arbyn, et al., 2009). Cost-effectiveness modelling with robust economics methodologies will provide important perspectives when assessing patient management strategies as health systems evolve (Wentzensen, Schiffman, Palmer, & Arbyn, 2016).

Our study was carried out in a real-world colposcopy clinic setting without any intervention for study purpose. This shed some light on the lesion regression rate during the interval between referral LSIL cytology and colposcopy, and this has implications in risk stratification and follow-up, and could also impact the outcome of triage tests. Our data on the delay from referral LSIL to colposcopy may be generalizable in Canadian

settings, but this may vary in other jurisdictions. One of the limitations of the study was that only a proportion underwent biopsy as clinically indicated and this prevented ascertaining biopsy-based disease outcome in the total study population. Regardless, in addition to 54 CIN2+ detected at baseline, there were 45 more CIN2+ diagnosed during follow-up. However, the length of our follow-up period was limited; extended follow-up may provide further insight into disease outcome and the PPV of both the CINtec PLUS and HPV tests in LSIL triage (Clarke, et al., 2019). Also, a larger sample size of CIN2+ would be warranted to substantiate our observations.

5.5 Conclusion

Either CINtec PLUS cytology or the cobas HPV test could serve as a predictor of CIN3+, with high sensitivity and NPV in patients referred to colposcopy with a history of LSIL cytology regardless of age. However, the reduced sensitivity of CINtec PLUS for detection of CIN2+ in general, and CIN 2 in particular, especially in patients <30 years, needs to be considered in risk assessments if choosing LSIL-CINtec PLUS triage pathways. Nevertheless, CINtec PLUS was consistently more specific than the cobas HPV test. Our study data provide a basis to improve patient care and efficiency by significantly reducing the number of LSIL referral patients requiring further investigations and follow-up in colposcopy clinics through CINtec PLUS or cobas HPV triage.

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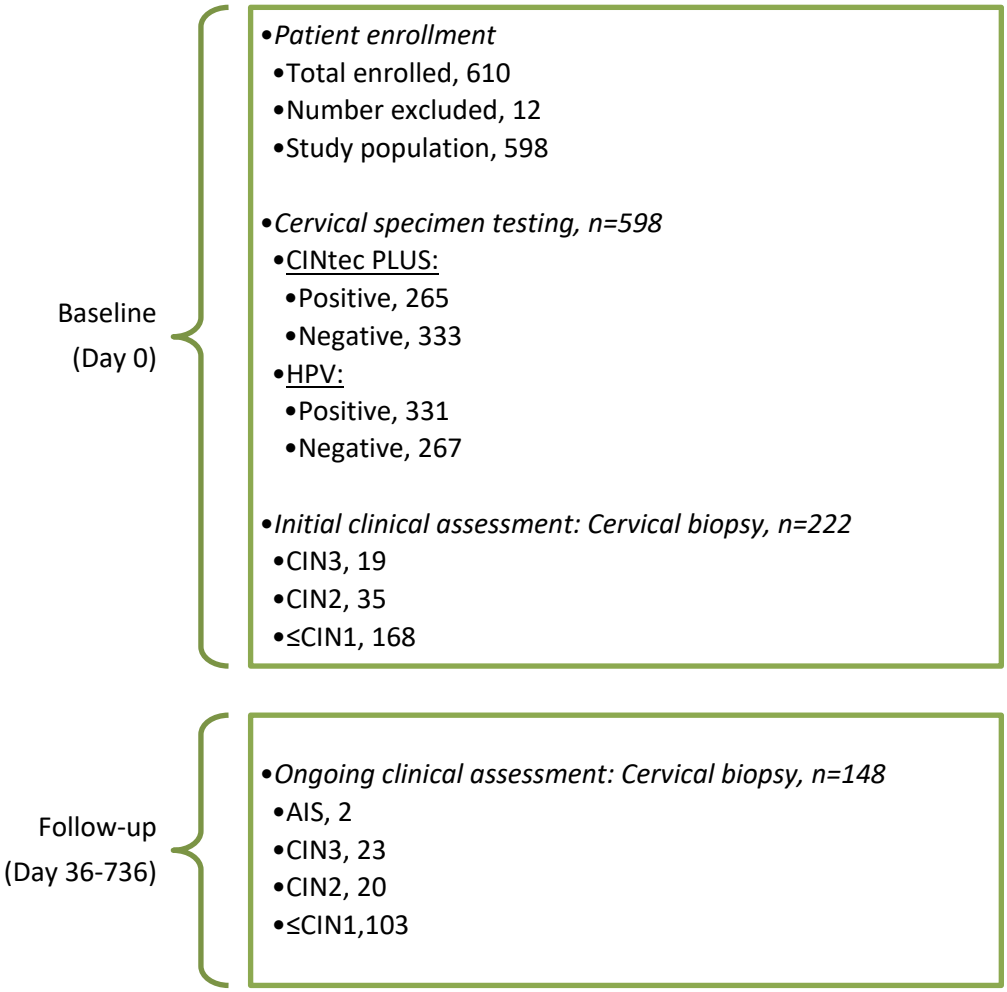
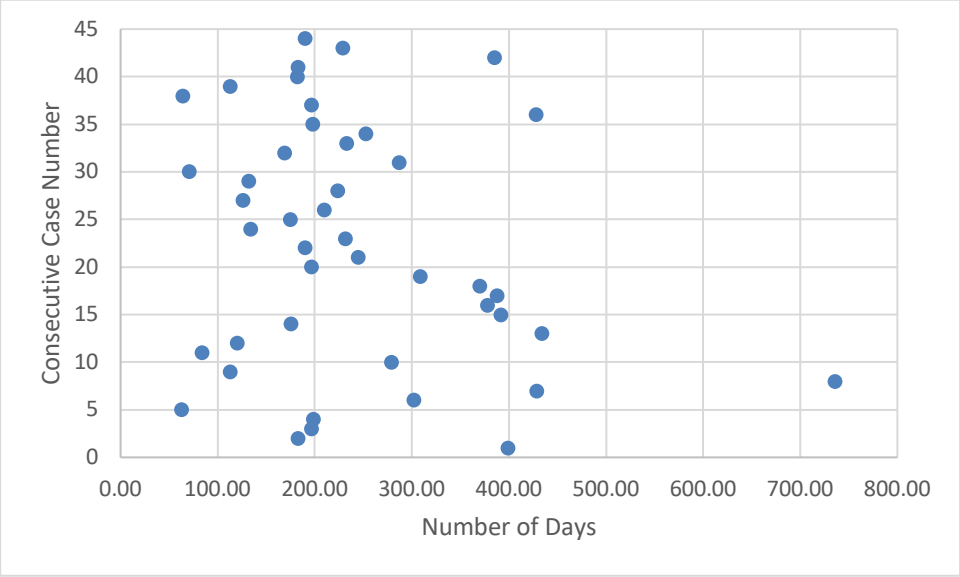


Figure 5.1. Study scheme: patient enrollment, CINtec PLUS and HPV test results and biopsy outcome of clinical assessments at baseline and during follow-up.

AIS, Adenocarcinoma in situ; CIN3, Cervical intraepithelial neoplasia grade 3; CIN2, CIN grade 2; ≤CIN 1, CIN grade 1 or negative.



*45 CIN2+ detected during follow-up; date of biopsy unavailable for one.

*Figure 5.2. Temporal distribution of CIN2+ detected during follow-up (n=44)**

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2911 *Table 5-1. CINtec PLUS and cobas HPV test results by age groups*

Test	Result	All ages, n=598	<30 years, n=214	≥30 years, n=384	
CINtec PLUS	Positive	265 (44.3%) [a]	107 (50.0%) [b*]	158 (41.1%) [c*]	* p=0.037, CINtec Plus positivity compared between women <30 and ≥30 years, two-tailed z test
	Negative	333 (55.7%)	107 (50.0%)	226 (58.9%)	
HPV	Positive	331 (55.4%) [a]	135 (63.1%) [b**]	196 (51.0%) [c**]	** p=0.005, HPV positivity compared between women <30 and ≥30 years, two-tailed z test
	Negative	267 (44.6%)	79 (36.9%)	188 (49.0%)	
p value		[a] p value <0.001, CINtec PLUS positivity compared with HPV positivity in all ages, two-tailed z test	[b] p value=0.006, CINtec PLUS positivity compared with HPV positivity in women <30 years, two-tailed z test	[c] p value= 0.006, CINtec PLUS positivity compared with HPV positivity in women ≥30 years, two-tailed z test	

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Table 5-2. Diagnostic Indices of CINtec PLUS and cobas HPV tests for detecting CIN2+

Test	Sensitivity			Specificity			Positive predictive value			Negative predictive value		
	n/N	% (95%CI)	P*	n/N	% (95%CI)	p	n/N	% (95%CI)	p	n/N	% (95%CI)	P
All ages (n=356)												
CINtec PLUS	81/99	81.8 (72.8-88.9)	0.009	136/257	52.9 (46.6-59.2)	<0.001	81/202	40.1 (36.3-44.0)	0.407	136/154	88.3 (83.0-92.1)	0.131
HPV	93/99	93.9 (87.3-97.7)		94/257	36.6 (30.7-42.8)		93/256	36.3 (33.9-38.8)		94/100	94.0 (87.7-97.2)	
<30 years of age (n=128)												
CINtec PLUS	38/50	76.0 (61.8-86.9)	0.012	40/78	51.3 (39.7-62.8)	0.023	38/76	50.0 (43.2-56.9)	0.741	40/52	76.9 (66.1-85.1)	0.159
HPV	47/50	94.0 (83.5-98.8)		26/78	33.3 (23.1-44.9)		47/99	47.5 (43.2-51.8)		26/29	89.7 (73.5-96.5)	
≥30 years of age (n=228)												
CINtec PLUS	43/49	87.8 (75.2-95.4)	0.294	96/179	53.6 (46.0-61.1)	0.003	43/126	34.1 (30.0-38.5)	0.384	96/102	94.1 (88.2-97.2)	0.063
HPV	46/49	93.9 (83.1-98.7)		68/179	38.0 (30.6-45.5)		46/157	29.3 (26.6-32.2)		68/71	95.8 (88.2-98.6)	

Based on 356 patients in all ages with biopsy.

CIN2+, cervical intraepithelial neoplasia grade 2 or worse.

* two-tailed z test

Table 5-3. Diagnostic Indices of CINtec PLUS and cobas HPV tests for detecting CIN3+

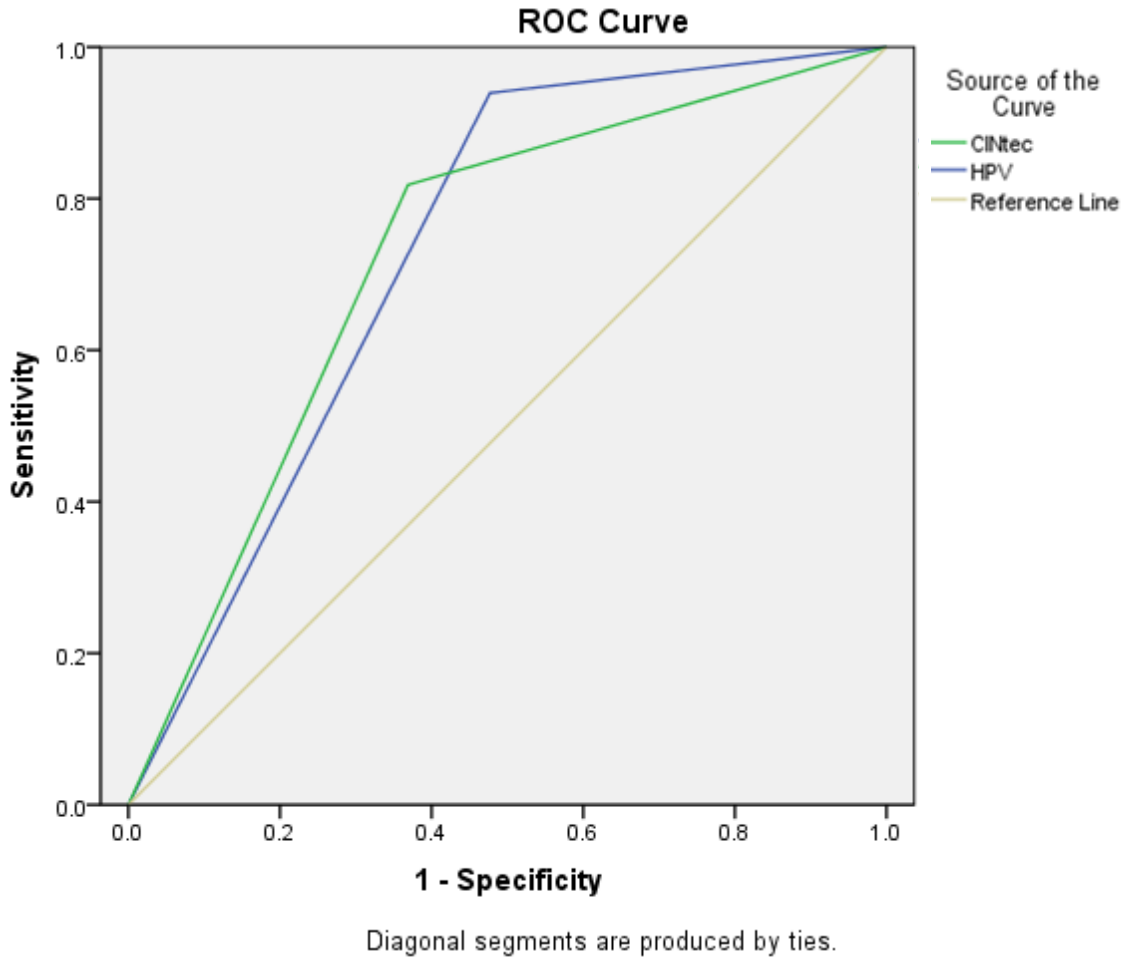
	Sensitivity			Specificity			Positive predictive value			Negative predictive value		
	n/N	% (95%CI)	P*	n/N	% (95%CI)	p	n/N	% (95%CI)	p	n/N	% (95%CI)	p
All ages (n=356)												
CINtec PLUS	41/44	93.2 (81.3-98.6)	1.000	151/312	48.4 (42.7-54.1)	<0.001	41/202	20.3 (18.2-22.6)	0.234	151/154	98.1 (94.4-99.3)	0.589
HPV	41/44	93.2 (81.3-98.6)		97/312	31.1 (26.0-36.6)		41/256	16.0 (14.6-17.5)		97/100	97.0 (91.5-99.0)	
<30 years of age (n=128)												
CINtec PLUS	22/24	91.7 (73.0-99.0)	0.549	50/104	48.1 (38.2-58.1)	0.002	22/76	29.0 (24.6-33.7)	0.390	50/52	96.2 (86.7-99.0)	0.928
HPV	23/24	95.8 (78.9-99.9)		28/104	26.9 (18.7-36.5)		23/99	23.2 (20.8-25.9)		28/29	96.6 (80.0-99.5)	
≥30 years of age (n=228)												
CINtec PLUS	19/20	95.0 (75.1-99.9)	0.549	101/208	48.6 (41.6-55.6)	0.001	19/126	15.1 (13.1-17.3)	0.368	101/102	99.0 (93.7-99.9)	0.363
HPV	18/20	90.0 (68.3-98.8)		69/208	33.2 (26.8-40.0)		18/157	11.5 (9.8-13.4)		69/71	97.2 (90.1-99.2)	

Based on 356 patients in all ages with biopsy.

CIN3+, cervical intraepithelial neoplasia grade 3 or worse (includes 2 cases of adenocarcinoma in situ).

* two-tailed z test

Figure 5.3. ROC curve for CINtec PLUS compared to HPV in detected CIN2+ in all ages (n=598)



Area Under the Curve

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Limit	Upper Limit
CINtec	0.725	0.026	.000	0.673	0.776
HPV	0.731	0.023	.000	0.686	0.777

The test result variable(s): CINtec, HPV has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

[a] Under the nonparametric assumption

[b] Null hypothesis: true area = 0.5

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Table 5-4. HPV genotypes 16/18-specific testing compared with hr-HPV testing to detect CIN2+ and CIN3+

Test	CIN2+						CIN3+					
	Sensitivity			Specificity			Sensitivity			Specificity		
	n/N	%	p*	n/N	%	p	n/N	%	p	n/N	%	p
All ages (n=356)												
HPV16/18	46/99	46.5%	<0.001	225/257	87.5%	<0.001	23/44	52.3%	<0.001	257/312	82.4%	<0.001
hr-HPV	93/99	93.9%		94/257	36.6%		41/44	93.2%		97/312	31.1%	
<30 years of age (n=128)												
HPV16/18	20/50	40.0%	<0.001	67/78	85.9%	<0.001	13/24	54.2%	<0.001	86/104	82.7%	<0.001
hr-HPV	47/50	94.0%		26/78	33.3%		23/24	95.8%		28/104	26.9%	
≥30 years of age (n=228)												
HPV16/18	26/49	53.1%	<0.001	158/179	88.3%	<0.001	10/20	50.0%	0.006	171/208	82.2%	<0.001
hr-HPV	46/49	93.9%		68/179	38.0%		18/20	90.0%		69/208	33.2%	

Based on 356 patients in all ages with biopsy.

CIN2+, cervical intraepithelial neoplasia grade 2 or worse; CIN3+, cervical intraepithelial neoplasia grade 3 or worse (includes 2 cases of Adenocarcinoma in situ)

* two-tailed z test

Chapter 6 : Paper 4, Serological evaluation of human antibodies of the immunoglobulin class A and G against SARS-CoV-2 in serum collected in Newfoundland and Labrador

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HDK & SH: Writing – review & editing, Validation, Supervision

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Abstract

The ability to detect antibodies to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is currently under investigation with various performance characteristics and indications for use. In this article, we analyzed the ability of the Abbott SARS-CoV-2 immunoglobulin class G (IgG), EuroImmun SARS-CoV-2 enzyme-linked immunosorbent assay (ELISA) IgG, and EuroImmun SARS-CoV-2 ELISA immunoglobulin class A (IgA) kits to detect evidence of previous infection with SARS-CoV-2. We tested 49 known coronavirus disease-19 (COVID-19) patients and 111 prepandemic stored serology specimens. This resulted in a sensitivity of 95.9%, 100.0%, and 91.3% and a specificity of 98.2%, 98.2%, and 90.8% respectively, using manufacturer recommended cutoffs after inconclusive results (one for EuroImmun IgG and five for EuroImmun IgA) being excluded in the final statistical analyses. Cross-reactivity of hepatitis C virus seropositive specimens was observed resulting in false positives ($p < 0.05$). If a two-tiered algorithmic approach was applied, that is, testing with Abbott SARS-CoV-2 assay followed by EuroImmun SARS-CoV-2 IgG, 100% specificity and sensitivity could be obtained after six inconclusive results were excluded from data set before statistical analyses. Performance characteristics presented demonstrate the superior performance of IgG class antibodies for investigating previous infections. In addition, utilizing a second antibody test for supplementary testing may significantly enhance performance, particularly in lower prevalence settings.

6.1 Introduction

Since the emergence of SARS-CoV-2, the virus responsible for COVID-19, at the end of 2019, there have been approximately 39 million cases and nearly 1.1 million deaths worldwide, as of 16 October, 2020 (World Health Organization, 2020). In order to stop the spread of infection, manage patients, and provide timely information to public health policy makers, accurate, timely and accessible diagnosis of SARS-CoV-2 infection is required (Morrison, Li, & Loshak, 2020), (Xiang, et al., 2020).

At this time, the primary focus of SARS-CoV-2 testing in Canada, and in the province of Newfoundland and Labrador, has been on molecular methods using real-time reverse transcription polymerase chain reaction (RT-qPCR) to detect SARS-CoV-2 RNA. The limitation of these molecular tests is that they can only indicate the presence of viral RNA in the particular sample; it cannot indicate the presence of viable viral particles. While the absence of the viral RNA cannot determine if a person was infected and has since recovered or if the person is infected but the virus was undetectable from that source at that time (Morrison, Li, & Loshak, 2020), (Wang, Xu, & Gao, 2020). Often, RT-qPCR tests are performed on those patients who are symptomatic or those who are epidemiologically linked to COVID-19 cases; an approach that likely underestimates the true prevalence of infection in the population (Khan S. , et al., 2020). Additionally, RT-qPCR methods also face challenges with accessibility of reagents and equipment, availability of trained personnel, cost, and challenges with performance (Liu, et al., 2020), (Xu, et al., 2020).

Due to these limitations, developments in serological testing are underway to better quantify the number of cases of COVID-19 (Centres for Disease Control and Prevention, 2020), (Morrison, Li, & Loshak, 2020). Additionally, antibody detection may provide a complementary perspective, along with RT-qPCR testing, in the diagnosis of COVID-19 (Xiang, et al., 2020), (Xu, et al., 2020). At this time, it has yet to be established whether there is a long-term immune response against SARS-CoV-2 (Liu T. , et al., 2020) or protection against re-infection (Morrison, Li, & Loshak, 2020).

Enzyme-linked immunosorbent assay (ELISA) and chemiluminescent microparticle immunoassay (CMIA) each provide semiquantitative *in vitro* measurement of the levels of human antibodies of the immunoglobulin class A (IgA) and G (IgG) against SARS-CoV-2 in serum. In order to evaluate the serological tests' ability to detect previous infection with SARS-CoV-2, serum from laboratory-confirmed COVID-19 cases were used as the known positive specimen group and stored historic pre-pandemic serum specimens served as a negative comparator group.

In an effort to provide comprehensive public health and microbiological services during the SARS-CoV-2 global pandemic, assessment was performed for three tests: A-IgG, EI-IgG and EI-IgA. These assays were selected as our laboratory has Abbott Architect and EuroImmun platforms for the standard catalogue of public health serological tests. The objective of the study is to assess the performance and utility of three tests for identification of past infection with SARS-CoV-2: A-IgG, EI-IgG, EI-IgA in a low prevalence setting in 2020 for those with lab-confirmed SARS-CoV-2 infections.

6.2 Materials and methods

7.2.1 Ethics

This research was performed in consultation with the Health Research Ethics Board (HREB) St. John's, NL and was deemed to be a quality assurance project (Appendix H). However, given that additional sampling of patients was needed, a written consent process was implemented to obtain serological specimens from confirmed, previously infected COVID-19 patients.

6.2.2 Sample size

As a quality improvement initiative within the Division of Laboratory in Eastern Health, standard sample size per validation and verification standard operating procedures and policies were used and signed off by clinical leads and laboratory director.

The eligible study population for negative comparators included any patient who had serology specimens submitted to the NLPHL for routine testing within the study period. For SARS-CoV-2 confirmed RT-qPCR positive patients the eligible study population were any patients who had nasopharyngeal specimens submitted for diagnostic testing at NLPHL.

In order to assess the new serological tests, 111 negative comparators, pre-pandemic specimens were withdrawn from local archives. These were selected in two ways: 50 specimens were selected in a random manner and 61 specimens were selected that were known to be positive for various non-SARS-CoV-2 reactive antibodies. The specimens that made up the randomly selected negative control group were matched to the same time period in the previous year, March-June 2019. Samples were selected at random from the laboratory, long-term storage repository in identified ultra-freezer boxes. Through partnership with the local Medical Officer of Health, confirmed RT-qPCR positive patients were identified with strong epidemiological links, and 49 specimens were collected to serve as our known positive specimen group.

6.2.3 Specimen Collection

For specimen collection from COVID-19 patients confirmed by RT-qPCR and quantified as previously published (LeBlanc, et al., Real-time PCR-based SARS-CoV-2 detection in Canadian laboratories, 2020), front line clinicians obtained written consent at local acute care facilities, collected 2 blood samples using serum separator tubes, and forwarded to the PHML. Patient name and healthcare number were provided to match clinical history based on institutional policies; this includes confirming identify, clinical history, and confirmation of epidemiological links with the ministry of health and clinical teams.

6.2.4 SARS-CoV-2 RT-qPCR Viral Load Testing

Molecular testing was performed at the PHML using previously published primers and probes by Corman et al. for E gene (Corman, et al., 2020), (Public Health & Microbiology Laboratory, 2020). A quantitative standard of *in-vitro* transcribed RNA (it-RNA) was developed by performing *in-vitro* transcription using TrancriptAid T7 High Yield Transcription kit (Thermo Scientific), on an amplified gBlock (Integrated DNA Technologies). The it-RNA was extracted using MasterPure Complete DNA and RNA Purification kit (Lucigen) to remove all contaminates including template DNA. The it-RNA was then quantitated with QuantiFlour RNA System (Promega) to determine the copies/µL. All RT-qPCR patient samples were extracted on either the QiaSymphony (Qiagen) or MagNA Pure Compact (Roche), extracting an initial volume of 200 µL and eluting to either 60 µL or 50 µL respectively, all runs included a negative process control. RT-qPCR was performed with using Luna Universal Probe One-Step RT-qPCR Kit (New England Biolabs Inc.) on the Lightcycler 480 II (Roche). A master mix was created with 400 nM of each primer and 200 nM of the probe, with 15 µL added to 5 µL of sample extract. One-Step RT-qPCR was performed with 15 minutes at 50^{o}C, then 1 minute at 95^{o}C, 45 cycles of two step qPCR with 95^{o}C for 5 second and 30 seconds at 58^{o}C. A quantitated positive control at 60 copies per reaction was included on all runs with a stored standard curve applied to each positive specimen.

6.2.5 EuroImmun (EI) Anti-SARS-CoV-2 (IgG) and (IgA)

The EI 2606-9601 G (IgG) is an ELISA that provides semiquantitative *in vitro* detection of IgG human antibodies against the S1 domain of the spike protein including the immunologically relevant receptor binding domain (RBD) of SARS-CoV-2 in serum or plasma (EuroImmun, 2020) while EI 2606-9601 A (IgA) is an ELISA that provides semiquantitative *in vitro* determination of IgA human antibodies against S1 domain of the spike protein of SARS-CoV-2 in serum or plasma (EuroImmun, 2020). Health Canada has approved EI-IgG assay but has not yet approved the EI-IgA. For every group of tests performed, calibrator, positive, and negative control are run and must fall within the limits stated for the relevant test kit lot, as per manufacturer's directions.

6.2.6 Abbott (A) SARS-CoV-2 IgG Assay

The SARS-CoV-2 IgG assay is a CMIA which provides the qualitative detection of IgG antibodies to SARS-CoV-2 in serum and plasma; IgG antibodies to the nucleocapsid proteins are also detected in this methodology (Abbott Diagnostics, 2020). This is a Health Canada approved test and, additionally, a comprehensive evaluation has been performed by Public Health England (Public Health England, 2020). For all tests performed, calibrations, positive and negative controls were run per manufacturer's directions and fell within in the limits stated as per manufacturer directions.

6.2.7 Non-SARS-CoV-2 Serology Assays

For the 61 selected specimens that were known to be positive for various non-SARS-CoV-2 reactive antibodies, all serological testing was performed at the PHML using the Abbott Architect i2000 and i1000 systems and the EuroImmun Analyzer I to detect antibodies. For the purposes of this study, Architect i1000SR is used for the detection of human cytomegalovirus (HCMV) IgG. Architect i2000SR is used for the qualitative detection of hepatitis C virus (HCV) IgG/IgM, HCMV IgM, rubella virus IgG, Epstein-Barr virus (EBV) Epstein-Barr (EBV) Nuclear Antigen (EBNA) IgG, EBV Viral Capsid Antigen (VCA) IgM, hepatitis A virus IgM, human immunodeficiency virus 1 (HIV-1) IgG and Syphilis *Treponema pallidum* (TP) IgG/IgM. The EuroImmun Analyzer I is a platform for semi-quantitative detection of varicella zoster virus (VZV) IgG and herpes simplex virus 1/2 (HSV-1/2) IgG.

6.2.8 Statistical Analysis

Statistical analysis was performed using Excel, Microsoft Office Professional Plus, 2013 and MedCalc Diagnostic Test Evaluation Calculator. Contingency tables were used to determine test positivity rates and the diagnostic sensitivity and specificity of each serological test. Clinical or diagnostic sensitivity and specificity refers to the determination of people with a given disease or diagnosis what were identified by the test being evaluated as positive (Saah, 1997). Confidence intervals for sensitivity and specificity are "exact" Clopper-Pearson confidence intervals. Differences in positive predictive values

and negatives predictive values were also calculated based on varying levels of prevalence for comparison of test performance. The t-test was used to determine the significance in our cross-reactivity analysis by comparing the A-IgG values for the HCV seropositive specimens and the HCV seronegative specimens. Precision and analytical sensitivity and specificity were also assessed to determine intra-operator reliability, relative lower limit of detection (LLOD), and possible cross-reactivity, respectively. Analytical sensitivity refers to the smallest amount of substance in a sample that can be correctly detected by an assay (Saah, 1997).

6.3 Results

For this study we had 160 patient specimens in total; 49 of which were SARS-CoV-2 positive RT-qPCR confirmed cases and 111 were historic serological specimens collected prior to the emergence of SARS-CoV-2. Of the 49 COVID-19 cases, 24 (49.0%) were female and 25 (51.0%) were male; the average age was 55 years with a minimum of 18 and maximum of 81 years (median 57 years of age). Of the 111 historic serological specimens, 72 (64.9%) were female and 39 (35.1%) were male. The average age for these historic specimens was 44 years of age with a minimum of 11 and a maximum of 83 years (median 42 years of age). Viral load at time of diagnosis was found to be an average of $2.0x10^6$ ($1.3x10^3$ to $6.5x10^8$) copies/mL at 5 (-5 to 14) days post symptom onset, with two patients being asymptomatic. Patients diagnosed prior to symptoms onset were known contacts of other symptomatic cases and are represented with negative days to onset. Figure 6.1 and Figure 6.2 illustrates days from symptom onset in comparison to viral load and A-IgG index values, EI-IgG and EI-IgA ratio values, respectively. Figure 6.1 results are in line with the literature and help to demonstrate the importance of early testing for SARS-CoV-2, as viral shedding decreases over time (Salvatore, et al., 2021). For the positive SARS-CoV-2 RT-qPCR confirmed cases, there was a median of 60 days from symptom onset to collection of serum specimen (min = 38, max = 67). For the 111 negative sample set, a panel of 61 specimens with previous non-SARS-CoV-2 antibody positivity was selected. These included 10 HCMV IgG, 9 HCV IgG/IgM, 5 HCMV IgM, 5 rubella virus IgG, 5 VZV IgG, 3 EBV EBNA IgG, 2 EBV VCA IgM, 3 hepatitis A virus IgM, 4 HSV-1/2 IgG, 5 HIV-1 IgG, 4

Syphilis TP IgG/IgM, 6 samples positive for various hepatitis B viral markers. Precision assessments of the assays were performed with positive and negative controls performed 10 times within one day and on 10 different days, both intra-run and inter-run coefficients of variation were <10% for all assays.

The sensitivity of all three assays was assessed with specimens from positive SARS-CoV-2 RT-qPCR cases and analytically with a dilution panel of pooled positive samples. The positive clinical specimens demonstrated sensitivities *of A-IgG, EI-IgG and EI-IgA were* 95.9%, 100.0% and 91.3%, respectively as presented in Table 6-1 when calculations were performed by not including the inconclusive results. Further calculations were performed with alternative interpretations of the inconclusive results (shown in Table 6-1). The two samples that were falsely negative for A-IgG produced index values of 0.99 and 1.28, demonstrating a lower titer of antibody that did not meet the manufacturer established cut-off of 1.4. The EI-IgG assay produced one sample that was inconclusive, which was the same sample the produced the lower false negative using the A-IgG assay. The EI-IgA assay produced four false negative results that did not always correlate with the IgG titer of either assay or days from symptom onset to serum collection. Analytical sensitivity, also referred to as LLOD was performed by pooling known positives then diluting them as per Table 6-2, and this is an average relative determination. The limit of detection was found at the 1:16 dilution for A-IgG assay and 1:4 for both the EuroImmun assays. Overall, clinical sensitivity was found to be slightly higher for the EI-IgG assay, with the A-IgG assay being more analytically sensitive.

The specificity of all three assays was measured by testing 111 pre-pandemic clinical specimens composed of two groups, 50 random samples and 61 samples known to be positive for non-SARS-CoV-2 reactive antibodies. For the A-IgG assay, there were two false positive results on two HCV IgG/IgM positive samples. Additionally, the next highest index value, 0.49, of the negative sample set, was also from an HCV positive patient. The EI-IgG assay produced one false positive result against HCMV IgM and one false positive result against a sample with anti-EBV/VZV IgG antibodies. The EI-IgA assay was positive for 10 different patients including samples from both randomly pulled samples and specimens positive for various non-SARS-CoV-2 reactive viral antibodies. The A-IgG and the EI-IgG assay were not falsely positive on the same samples. Overall, the A-IgG, EI-IgG and EI-IgA assays produce clinical specificity of 98.2%, 98.2%, and 90.8% respectively.

6.4 Discussion

Detection of anti-SARS-CoV-2 antibodies will influence medical, public health, and societal decisions in the coming months and years as result of the COVID-19 pandemic. Presented here is an initial evaluation of two classes of antibodies, from two different commercial providers, EuroImmun and Abbott. The assays detect separate antigenic targets with potential different implications on clinical performance (Liu, et al., 2020), and potential protective implications. Our results present slightly lower performance than the package insert for Abbott Architect assay and slightly better than the EuroImmun assays (Abbott Diagnostics, 2020), (EuroImmun, 2020), (EuroImmun, 2020). IgG antibody test showed superior sensitivity and specificity over IgA antibody test in our study. IgM antibody test for SARS-CoV-2 was not evaluated in this study, IgM (Long, Liu, Deng, & al., 2020) tests by themselves are less sensitive and specific than IgG results; however, may improve diagnostic sensitivity slightly when performed in tandem with IgG testing. Additionally, the performance of the Abbott IgG assay in our setting showed a higher sensitivity and a lower specificity than a comprehensive evaluation of over 1000 specimens conducted by the Clinical Service Unit at Public Health England Colindale (Public Health England, 2020). Compared to a USA study (n=1020) we found similar specificity, albeit slightly inferior sensitivity, for the performance of the A-IgG assay (Bryan, et al., 2020) (Morrison, Li, & Loshak, 2020), demonstrating consistency with the manufacturer reported performance. Ultimately, there were no significant differences

(Table 6-1) between both of the IgG assays which was also found in work completed in the USA (Titus, 2020).

Our sample subset likely impacted performance factors for two main reasons. Firstly, our known positive samples were collected from mildly symptomatic patients that were managed in the community via the Communicable Disease Control team. There were two discordant results from our positive sample set for IgG antibodies. One sample collected 41 days post-symptom onset was negative on A-IgG and inconclusive on EI-IgG; and the other sample, which was collected 62 days post-symptom onset, was negative on the A-IgG and positive on the EI-IgG. As demonstrated in Table 7-3, there was no significant correlation between days of symptom onset to collection of serum and antibody index/ratio to suggest waning of antibodies during the time period tested. This information is in contrast to a study in Wuhan, China that found 10% of patients lost SARS-CoV-2 antibodies within weeks of infection (Liu T. , et al., 2020), albeit different serology tests were used in this case. However, it has been previously shown that more severe disease produces a stronger antibody response, and thus our mild disease cohort may result in a lower sensitivity (Zhao Jr, et al., 2020). Secondly, our negative sample subset was made up of two categories of randomized retrospective samples and a cross-reactivity panel of specimens with known antibody status for various non-SARS-CoV-2 pathogens. All discordant IgG and 8 of 11 of the IgA samples from presumed negative samples were from patients with known non-SARS-CoV-2 reactive viral antibodies.

Given the ubiquitous nature of coronaviruses in general, there is a rightful concern of cross-reactivity in the development of serological testing methodologies (Khan S. , et al., 2020). In the package insert, EuroImmun reported cross-reactions to other human pathogenic coronaviruses; however, no other viruses were mentioned (EuroImmun, 2020), (EuroImmun, 2020). However, in the Abbott IgG documentation, it states that there is no-cross reactivity observed in a non-COVID-19 population for coronaviruses and only one case out of 181 (0.6%) showed cross-reactivity with HCMV IgG (Abbott Diagnostics, 2020). As this was an anticipated challenge for investigating these new tests we selected a cross-reactivity panel of specimens with known immune status for various pathogens. One limitation in our setting is that we do not test for non-SARS-CoV-2 human pathogenic coronaviruses by serology and chose to focus on other pathogens. Overall, anti-SARS-CoV-2 antibodies may cross-react with certain pathogens or under some medical conditions. It was interesting to note that in other studies, and in the manufacturer information, this type of cross-reactivity was not identified (Matushek, et al., 2020) (EuroImmun, 2020). That being said, in Matushek et al. (Matushek, et al., 2020) work they focused on other respiratory coronavirus cross-activity; something not investigated or evaluable in our work given the availability of tests and a consideration for future work. Seropositive HCV samples were found to cross react to the A-IgG assay in this validation significantly ($p<0.05$) in comparison to the non-HCV-infected persons. Additional studies are required to fully evaluate the potential issues with cross-reactivity and the impact it may have on the seroprevalence investigations for SARS-CoV-2.

Given these limitations, current recommendations for lower prevalence area like Newfoundland and Labrador are to proceed with two-tiered testing (Centres for Disease Control and Prevention, 2020). In our Table 6-1, sensitivity and specificity is shown for a two-tiered testing algorithm, where EI-IgG was applied as a supplementary test to all A-IgG results of index value ≥ 0.7. Tests were still interpreted per manufacturer instructions however, if EI-IgG was indicated per testing algorithm and produced different qualitative results from A-IgG this was deemed to be inconclusive. This cut-off was developed by observing the lowest index value from a known COVID-19 case, 0.99 and the highest index value from a true negative result in our negative sample set, 0.49, applying the average rounded to the nearest tenth. In Newfoundland and Labrador, the confirmed case prevalence by RT-qPCR is approximately 0.05%, while some studies have suggested that actual prevalence rates may be under-represented by RT-qPCR by as much as 55-fold (Bendavid, et al., 2020). In Table 6-4, Positive Predictive Values (PPV) and Negative Predictive Values (NPV) are calculated based on both the confirmed prevalence and the largest estimate from published literature. Given the low prevalence in our province, even small drops in specificity can make very significant changes in the PPV. Additionally, in Figure 6.3, we compared variances in prevalence and the impact on the PPV values for A-IgG, EI-IgG, and a two-tiered model. In both A-IgG and EI-IgG, a PPV of 95.0% is not reached until the overall prevalence is approximately 25.0%. Based on the low-prevalence in our province, a two-tiered model would maximize PPV and performance.

No additional clinical information, such as antibodies to seasonal coronavirus, respiratory distress, oxygen saturation, or radiological findings were investigated in our work. This is a potential limitation and additional clinical perspective may provide some insight into the clinical progression and manifestation of disease in patients as included in other works (Kohmer, Westhaus, Ruhl, Ciesek, & Rabenau, 2020) (Xiang, et al., 2020).

Serological specimens from COVID-19 cases were collected on average 52.7 days from the RT-qPCR results**.** Ideally, blood is collected at different phases of infection as clinically feasible to help understand the dynamic of the antibody production (Xiang, et al., 2020) and the differing performance of the assays based on time after diagnosis (Kohmer, Westhaus, Ruhl, Ciesek, & Rabenau, 2020); at this time we were unable to obtain specimens corresponding to disease severity. This is a limitation and certainly an opportunity for further future investigation.

When we analyzed viral load at the time of initial diagnosis, it did not correlate with antibody response. However, viral load negatively correlated with days from symptom onset; this added assurance to our symptom onset date which in mild disease can be ambiguous. This reduction of viral load over the length of illness is in line with discussions regarding specimen suitability (Alberta Health Services: COVID-19 Scientific Advisory Group, 2020), (Long, Liu, Deng, & al., 2020), (Wang, Xu, & Gao, 2020).

In our study population, age positively correlated with strength of antibody response. This may be due to more severe disease in older population; however,

additional investigation is necessary. Previous work in this area has shown a correlation with severity and antibody response (Zhao Jr, et al., 2020).

6.5 Conclusion

Serological testing may serve a critical role in truly quantifying the number of COVID-19 cases. The EI-IgG and A-IgG assays may also serve as an effective tool in helping detect past SARS-CoV-2 infection, independently or in concert with other diagnostic modalities. However, given the high cross-reactivity found in EI-IgA there may be limited utility in the use of this immunoglobulin class for detection of previous infection. Cross-reactivity in HCV seropositive specimens was a unique observation which resulted in false positives. Additional studies would add to the strength of this evidence and help to better establish the relationship between severity of illness and antibody response.

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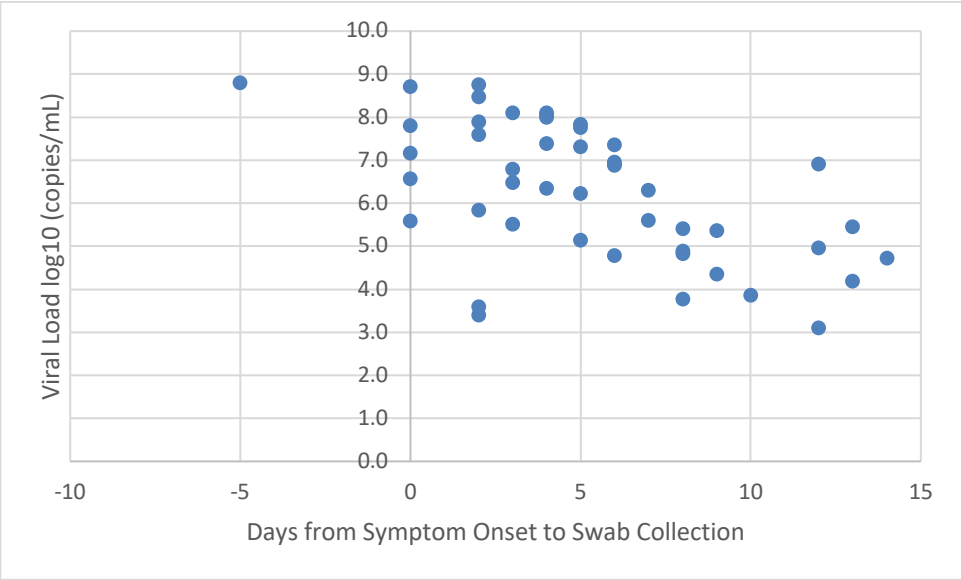
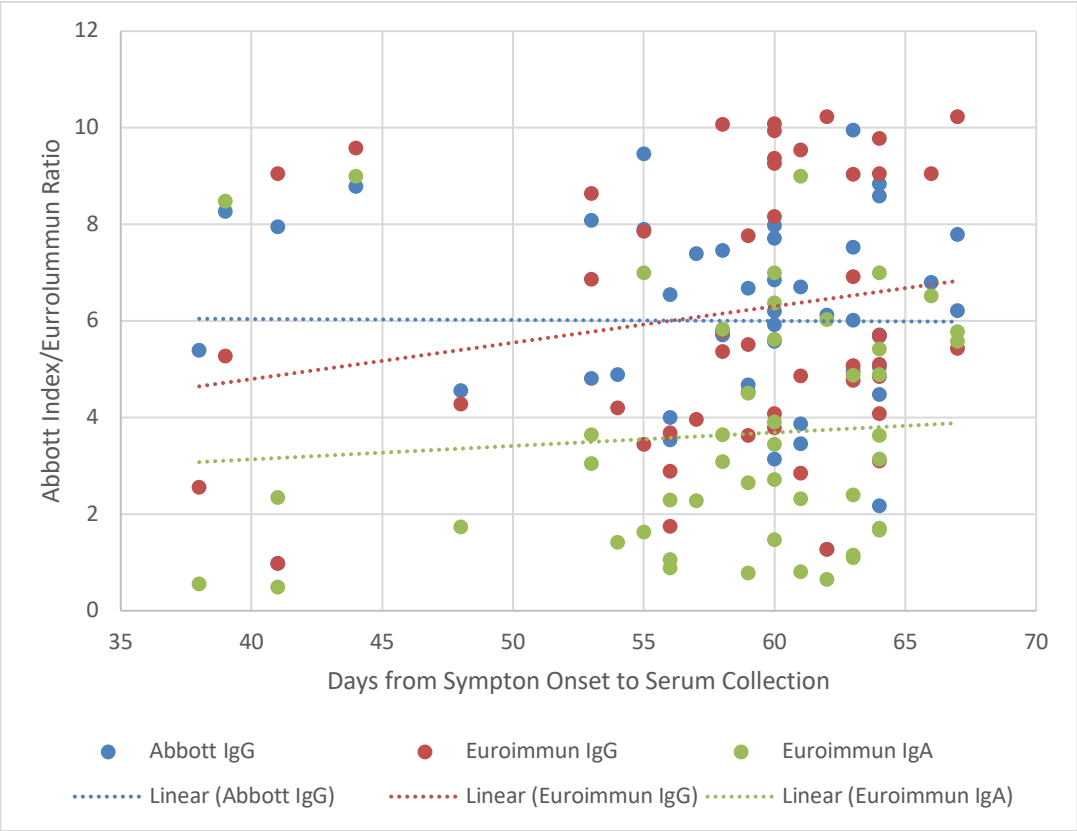
Conflict of Interest

The authors have no conflict of interest to declare.



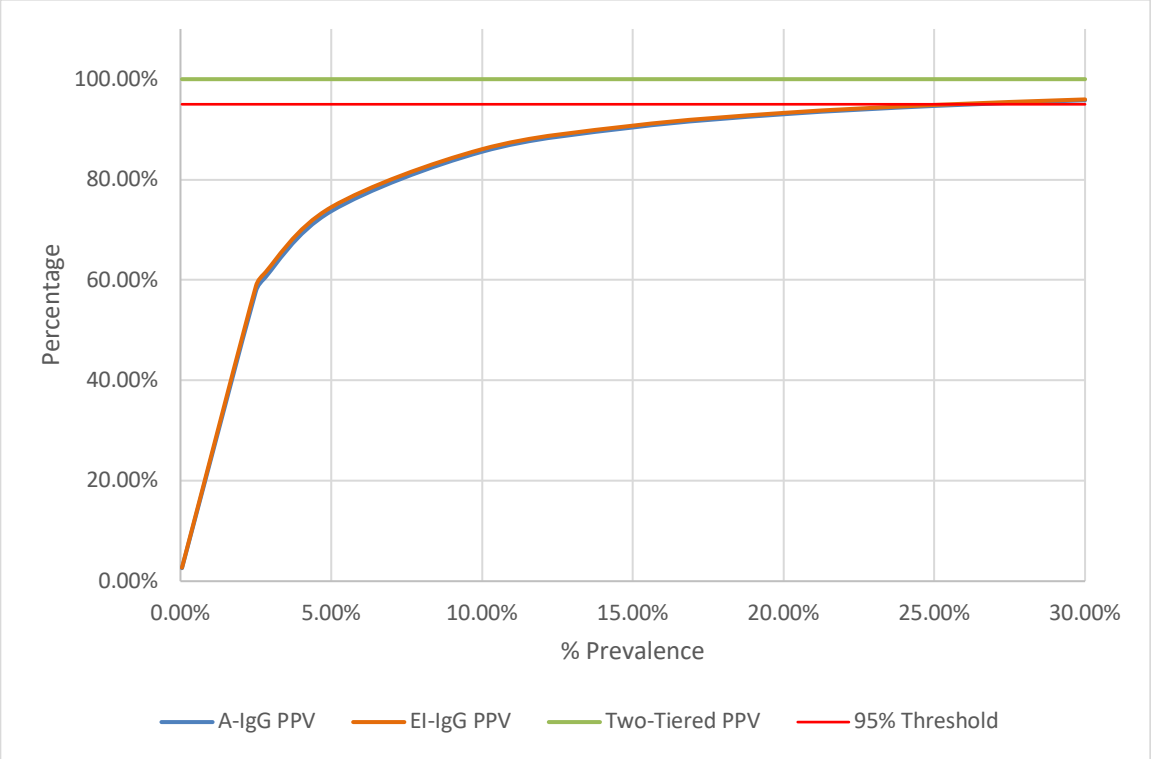
*2 Positive COVID-19 specimens did not have date of symptom onset.

Figure 6.1. Days from Onset vs Viral Load (n=47)



*2 Positive COVID-19 specimens did not have date of symptom onset.

Figure 6.2. Days from Onset vs Abbott IgG Index values and EuroImmun IgG and IgA Ratio Values (n=47)*



*A-IgG and EI-IgG PPV lines overlap in many places and may be difficult to discern.

*Figure 6.3. Impact of prevalence on Positive Predictive Value (PPV) for IgG Tests, Independently and in a Two-Tiered Model**

3371 *Table 6-1. Diagnostic performance of EuroImmun IgG/IgA and Abbott IgG*

3372

		+(PCR Confirmed)	-(Pre-Pandemic)	Total
Abbott IgG	+	47	2	49
	-	2	109	111
	+/-*	0	0	0
	Total	49	111	160

Sensitivity	95.9 (86.0-99.5)[#]	Specificity	98.2 (93.6-99.8)[#]

EuroImmun IgG	+	48	2	50
	-	0	109	109
	+/-*	1	0	1
	Total	49	111	160

Sensitivity	100.0 (92.6-100.0)[#]	Specificity	98.2 (93.6-99.8)[#]
Sensitivity	100.0 (92.8-100.0)[##]	Specificity	98.2 (93.6-99.8)[##]
Sensitivity	98.0 (89.1-100.0)[###]	Specificity	98.2 (93.6-99.8)[###]

EuroImmun IgA	+	42	10	52
	-	4	99	103
	+/-*	3	2	5
	Total	49	111	160

Sensitivity	91.3 (79.2-97.6)[#]	Specificity	90.8 (83.8-95.5)[#]
Sensitivity	91.8 (80.4-97.7)[##]	Specificity	89.2 (81.9-94.3)[##]
Sensitivity	85.7 (72.8-94.1)[###]	Specificity	91.0 (84.1-95.6)[###]

Two Tier IgG	Confirmed Positive**	47	0	47
	Confirmed Negative ***	0	109	109
	Inconclusive	2	2	4
	Total	49	111	160

Sensitivity	100.0 (92.5-100.0)[#]	Specificity	100.0 (96.7-100.0)[#]
Sensitivity	100.0 (92.8-100.0)[##]	Specificity	98.2 (93.6-99.8)[##]
Sensitivity	95.9 (86.0-99.5)[###]	Specificity	100.0 (96.7-100.0)[###]
*Inconclusive, Not included in calculations			
** Confirmed Positive = Positive by both Tests			
*** Confirmed Negative = Negative by both Tests			
# Calculated with inconclusives not counted ## Calculated with inconclusives counted as positive results ### Counted with inconclusives counted as negative results			

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Table 6-2. Analytical Sensitivity with Limits of Detection (LOD)

Analytic Sensitivity	EuroImmun		Abbott
	IgA	IgG	IgG
1:1 dilution	6.27 Pos	6.76 Pos	8.38 Pos
1:4 dilution	2.53 Pos	3.30 Pos	5.14 Pos
1:16 dilution	0.87 Ind	1.00 Ind	1.83 Pos
1:64 dilution	0.52 Neg	0.38 Neg	0.40 Neg
1:256 dilution	0.40 Neg	0.19 Neg	0.10 Neg
1:1024 dilution	0.62 Neg	0.26 Neg	0.04 Neg

Table 6-3. Pearson R Relationships between Age, Study Time Frames, PCR Viral Loads, A-IgG, EI-IgG, and EI-IgA, (N=49)

Comparison	Pearson R
Age vs PCR Viral Load	0.23
Age vs Abbott IgG	0.33
Age vs EI IgG	0.33
Age vs EI IgA	0.20
Onset to Swab Collection vs PCR Viral Load	-0.57
Onset to Serum Collection vs Abbott IgG	-0.01
Onset to Serum Collection vs EI IgG	0.20
Onset to Serum Collection vs EI IgA	0.08
PCR Viral Load vs Abbott IgG	-0.04
PCR Viral Load vs EI IgG	-0.04
PCR Viral Load vs EI IgA	-0.04

Table 6-4. Impact of prevalence on Positive Predictive Value (PPV) and Negative Predictive Value (NPV) for IgG Tests, Independently and in a Two-Tiered Model (With the gold-standard being used for comparison being Positives: PCR positive COVID-19 cases and Negatives: Pre-pandemic samples)

	A-IgG		EI-IgG		Two-Tiered	
Prevalence	0.05%	2.75%	0.05%	2.75%	0.05%	2.75%
PPV	2.6%	60.1%	2.7%	61.1%	100.0%	100.0%
NPV	100.0%	99.9%	100.0%	100.0%	100.0%	100.0%

6.6 Additional Note

The findings of this study were also presented at CACMID 2021. Appendices C and D are the submitted abstract and poster which was presented, respectively.

The findings of this work regarding SARS-CoV-2 have also been part of national initiatives for testing development through partnerships with the Canadian Public Health Laboratory Network (CPHLN). As such, authors contributed to an additional manuscript with Federal Territorial and Provincial (FTP) partners highlighting the over significance and summary from a national perspective. This paper is included as Appendix E.

Chapter 7 : Conclusions

7.1 Potential improvements to cervical screening programs

The province of Newfoundland and Labrador has attempted to improve participation in its Cervical Cancer Screening Initiatives programs. It has been successful in some ways including general increases in participation rates with increases in the participation rate from 58% in 1998 to 76% in 2011 (Quinn, 2011), relatively low unsatisfactory rates, and the number of cancers being detected at a low stage, particularly when compared with the rest of Canada. However, high rates of abnormalities, pre-cancerous lesions, and invasive cancers are troubling. Additional studies may also help to examine the compliance of clinicians to the prescribed standards (ie: HPV tests outside of ASCUS triage and screening more frequently than every three years). Opportunities exist to improve the program clinically through better stratification of patients at risk and systemically through general quality improvements and processes. Additional triaging options or an HPV primary screening algorithm may help with these improvements and outreach to unscreened and under-screened patients. Further work to reduce wait times for colposcopy can further support improvements to the system.

Additionally, there is an opportunity to look at the LSIL population for better risk stratification. Finally, there is an opportunity for either CINtec PLUS or the cobas HPV test to serve as predictors of CIN3+, with high sensitivity and NPV in patients referred to colposcopy with a history of LSIL cytology. Our study data provides a basis to improve

patient care and efficiency by significantly reducing the number of LSIL referral patients requiring further investigations and follow-up in colposcopy clinics through CINtec PLUS or cobas HPV triage.

7.2 Applications of SARS-CoV-2 serology

Serological testing may serve a critical role in truly quantifying the number of COVID-19 cases from a population perspective. At the same time, initial considerations were given to clinical applications, further investigations have demonstrated the more appropriate utilization in the context of population and screening. In the case of populations with lower disease prevalence, two-tiered methodologies would provide improved test performance.

8.3 Future research and considerations moving forward

Upon review of the available data from the NL Cervical Cancer Registry, it is incredibly apparent that histology and biopsy data must be included within this dataset; this was a considerable limitation and challenge when assessing and reviewing available data. Due to this limitation, diagnostic indices were unable to be examined, and limited information regarding clinical endpoints and disease outcomes can be obtained.

With the abundance of recommendations for the utilization of HPV primary testing from national and international groups, programmatic reviews should take place in NL to best evaluate opportunities for improvement and potential systemic challenges. Additional data and programmatic information should be obtained and reviewed to comprehend the implications and any financial impact fully. Additionally, within the context of a province with a high HPV vaccine uptake (the NL public vaccination program began in 2007 (Cervical Screening Initiatives Program, 2013)), considerations should be taken, and risk assessments should take place to understand the implications of any screening approach better.

For COVID-19 screening programs, more work needs to be done to fully understand the impact that vaccination programs and different vaccines have on the (Cervical Screening Initiatives Program, 2013) immunological response in the context of serology testing. Also, with the emergence of VOCs and VOIs, additional reviews need to take place to assess any differences in the immune response and antibody development.

One element that I could not explore in this work is the importance of social determinants and anthropological factors on infections and inequalities. Qualitative variables to better understand the impact on access in cervical cancer screening programs and the SARS-CoV-2 pandemic is critical to understand the intricacies of challenges fully and to work towards better public health systems.

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Appendices

Appendix A: CACMID 2019 Abstract

HPV Testing for Triaging Women with Low Grade Squamous Intraepithelial Lesion (LSIL) Cytology in Cervical Cancer Screening: Preliminary Findings from a Canadian Study.

Laura Gilbert[1,2], Sam Ratnam[2,3,4], Dan Jang[4], Reza Alaghehbandan[5], Miranda Schell[6], Rob Needle[1,2], Arnav Wadhawan[4], Dustin Costescu[6], George Zahariadis[1,2], Max Chernesky[4]

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Background: Cervical cancer screening relies on Pap cytology to detect precancerous lesions. A majority of women with abnormal cytology have either atypical squamous cells of undetermined significance (ASCUS) or low-grade squamous intraepithelial lesion (LSIL), and in most, these are not predictive of cancer risk. However, all such cases are followed with repeat cytology or colposcopy because some may have an underlying high-grade disease. ASCUS-HPV triage is recommended to better identify those at increased risk. In this regard, LSIL-HPV triage may also be helpful. We assessed the usefulness of LSIL-HPV triage as part of an ongoing study investigating the application of CINtec PLUS (Roche), a dual-stain biomarker test, in LSIL triage.

Methods: LSIL cases seen at the colposcopy clinic, Juravinski Hospital, Hamilton were prospectively enrolled with informed consent. Cervical specimens were collected at enrolment in ThinPrep for routine Pap. The remnant from the ThinPrep vials was used for HPV testing utilizing cobas 4800 assay (Roche). Biopsy confirmed cervical intraepithelial neoplasia grade 2 or worse (≥CIN2) served as the clinical endpoint.

Results: Preliminary analysis was based on 347 patients (target, n=600). Ages ranged from 19-76 (median 33), with 204 (58.8%) ≥30 years of age. Of the 347, 188 (54.2%) tested HPV+, and 159 (45.8%) HPV-. There were 34 cases of ≥CIN2, and 33 had tested HPV+ (sensitivity, 97.1%). Of 313 without ≥CIN2, 158 tested HPV- (specificity, 50.5%; negative predictive value, 158/159 = 99.4%). Among the 188 testing HPV+, most were positive for high-risk oncongenic types other than 16 and 18 regardless of biopsy result.

Conclusions: LSIL-HPV triage may have the potential to safely relegate half of women to routine screening with a very high negative predictive value, while maintaining superb sensitivity to detect ≥CIN2. Longitudinal studies could provide additional clinical data to assess the long term negative predictive value of LSIL-HPV triage.

Appendix B: CACMID 2019 Poster

HPV Testing for Triaging Women with Low Grade Squamous Intraepithelial Lesion (LSIL) Cytology in Cervical Cancer Screening

Preliminary Findings from a Canadian Study

Laura Gilbert[1,2], Sam Ratnam[2,3,4], Dan Jang[4], Reza Alaghehbandan[5], Miranda Schell[6], Rob Needle[4,2], Arnav Wadhawan[4], Dustin Costescu[6], George Zahariadis[4,2], Max Chernesky[4]

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Background

In routine Pap cytology screening, a majority of women with abnormal cytology have either atypical squamous cells of undetermined significance (ASCUS) or low-grade squamous intraepithelial lesion (LSIL). In most, these are not predictive of cervical cancer risk.

HPV triage is recommended to better identify those at increased risk among ASCUS Pap. ASCUS-HPV triage allows for focusing on the smaller proportion of women likely to be at risk, and avoiding unnecessary colposcopy and follow-up for the majority not at risk. As for LSIL, Canadian guidelines recommend that LSIL cases are either referred to colposcopy directly or managed with cytology. In this regard, LSIL-HPV triage may also be helpful.

We assessed the usefulness of LSIL-HPV triage as part of an ongoing study investigating the application of CINtec PLUS (Roche), a dual-stain biomarker test, in LSIL triage.

Methods

LSIL cases seen at the colposcopy clinic, Juravinski Cancer Clinic, Hamilton were prospectively enrolled with informed consent. Cervical specimens were collected at enrolment in ThinPrep for routine Pap. The remnant from the ThinPrep vials was used for HPV testing utilizing cobas 4800 assay (Roche). Biopsy confirmed cervical intraepithelial neoplasia grade 2 or worse (≥CIN2) served as the clinical endpoint.

Results

Preliminary analysis was based on 347 patients (target, n=600). Ages ranged from 19-76 (median 33), with 204 (58.8%) ≥30 years of age. Of the 347, 188 (54.2%) tested HPV positive, and 159 (45.8%) HPV negative. Among those testing positive, a much smaller proportion tested positive for types 16 and 18 compared with those testing positive for 12 other high risk (OHR) types. Figure 1 shows HPV results by age group and genotype distribution. There were 34 cases of ≥CIN2. HPV testing showed a sensitivity of 97.1% and specificity of 50.5% with a negative predictive value of 99.4% (Table 1). Figure 2 shows HPV genotype distribution by CIN grade.

Figure 1. HPV Results by Age Group and Genotype

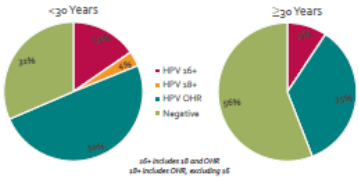
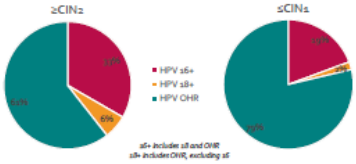


Table 1. HPV Test Performance

		≥CIN2	<CIN1	Total
HPV	+	33	155	188
	-	1	158	159
Total		34	343	347

Sensitivity	33/34=97.1%	PPV	33/188=17.6%
Specificity	158/313=50.5%	NPV	158/159= 99.4%

Figure 2. HPV Genotype Distribution by CIN Grade



Discussion

Our preliminary study showed only about one half of LSIL cases, ≥30 years of age, tested positive for HPV, thus requiring colposcopy and further follow-up. The other half testing HPV negative is not at risk, and therefore, could be safely returned to routine screening, hence avoiding unnecessary follow-up and testing. Additionally, the proportion requiring immediate colposcopy could be further reduced based on 16/18 risk stratification.

The benefit of LSIL-HPV triage is dependent on the screening population and age (1), and our results are similar to those reported for women ≥35 years of age in other populations (2,3). In this respect, LSIL-HPV triage for a Canadian population, ≥30 years, could be as effective as ASCUS-HPV triage.

As the study reaches completion, the ability of LSIL-HPV triage can be better evaluated. Additionally, the utility of CINtec PLUS to act as an adjunct test would also be evaluated with complete study data. These will be valuable in the context of primary HPV screening as well as cytology screening.

Conclusions

- LSIL-HPV triage of women ≥30 years may have the potential to help focus on those at risk and safely relegate the rest to routine screening with a very high negative predictive value, while maintaining superb sensitivity to detect ≥CIN2.
- Longitudinal studies could provide additional clinical data to assess the long term negative predictive value of LSIL-HPV triage.

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We would like to thank our support from Roche Diagnostics, as part of the Canadian LSIL CINtec Research Study, staff of the colposcopy clinic at Juravinski Cancer Centre, Hamilton, Anne Ecobichon-Morris, Cytology Laboratory, St. Joseph's Healthcare, Hamilton, and Thomas Barrett and Ken Melvin, Eastern Health Cytology Laboratory, St. John's.

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Appendix C: CACMID 2021 Abstract

Title: Evaluation of human antibodies of the immunoglobulin class A and G against SARS-CoV-2 in serum: A provincial public health laboratory experience

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Word Count Limit: 300

Objective: The initial focus of SARS-CoV-2 testing has been on molecular methods using real-time reverse transcription polymerase chain reaction (RT-qPCR) to detect severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA. This methodology indicates

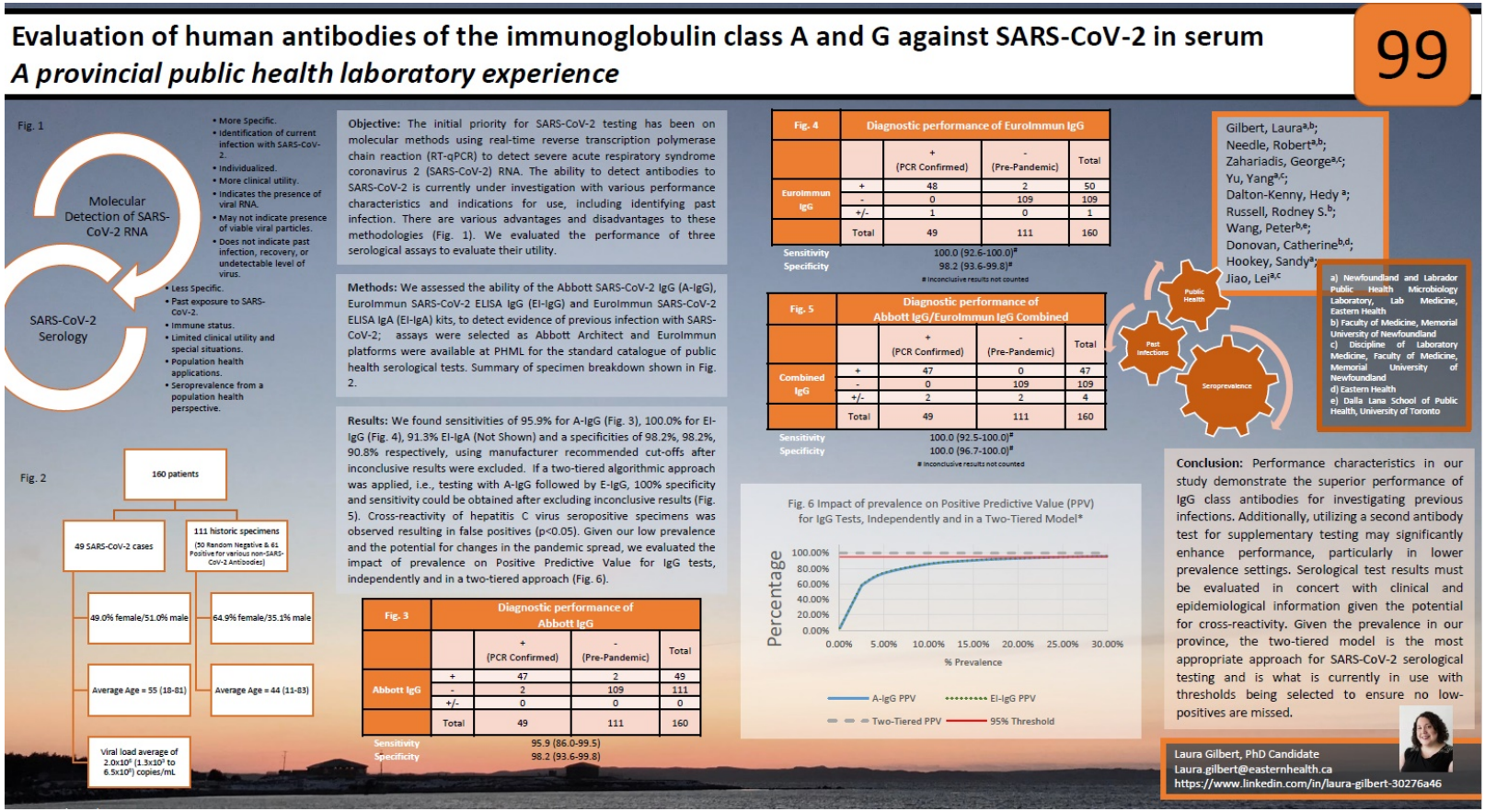
the presence of viral RNA; not necessarily the presence of viable viral particles. Additionally, the absence of viral RNA does not indicate past infection, recovery, or undetectable levels of virus. The ability to detect antibodies to SARS-CoV-2 is currently under investigation with various performance characteristics and indications for use, including identifying past infection. In an effort to provide comprehensive public health and microbiological services, we evaluated the performance of three serological assays to establish their utility.

Methods: We assessed the ability of the Abbott SARS-CoV-2 IgG (A-IgG), EuroImmun SARS-CoV-2 ELISA IgG (EI-IgG) and EuroImmun SARS-CoV-2 ELISA IgA (EI-IgA) kits, to detect evidence of previous infection with SARS-CoV-2. These assays were selected as our laboratory has Abbott Architect and EuroImmun platforms for the standard catalogue of public health serological tests. We tested 49 known Coronavirus disease-19 (COVID-19) patients and 111 pre-pandemic stored serology specimens.

Results: We found *sensitivities of* 95.9% for A-IgG, 100.0% for EI-IgG, 91.3% EI-IgA and a specificities of 98.2%, 98.2%, 90.8% respectively, using manufacturer recommended cut-offs after inconclusive results were excluded. If a two-tiered algorithmic approach was applied, i.e., testing with A-IgG followed by E-IgG, 100% specificity and sensitivity could be obtained after excluding inconclusive results. Cross-reactivity of hepatitis C virus seropositive specimens was observed resulting in false positives ($p<0.05$).

Conclusion: Performance characteristics in our study demonstrate the superior performance of IgG class antibodies for investigating previous infections. Additionally, utilizing a second antibody test for supplementary testing may significantly enhance performance, particularly in lower prevalence settings. Serological test results must be evaluated in concert with clinical and epidemiological information given the potential for cross-reactivity.

Appendix D: CACMID 2021 Poster



Appendix E: National SARS-CoV-2 Manuscript

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Virology

Evaluation of commercial SARS-CoV-2 serological assays in Canadian public health laboratories

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ABSTRACT

The COVID-19 pandemic has led to the influx of immunoassays for the detection of antibodies towards severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) into the global market. The Canadian Public Health Laboratory Network Serology Task Force undertook a nationwide evaluation of twelve laboratory and 6 point-of-care based commercial serological assays for the detection of SARS-CoV-2 antibodies. We determined that there was considerable variability in the performance of individual tests and that an orthogonal testing algorithm should be prioritized to maximize the accuracy and comparability of results across the country. The manual enzyme immunoassays and point-of-care tests evaluated had lower specificity and increased coefficients of variation compared to automated enzyme immunoassays platforms putting into question their utility for large-scale sero-surveillance. Overall, the data presented here provide a comprehensive approach for applying accurate serological assays for longitudinal sero-surveillance and vaccine trials while informing Canadian public health policy.

1. Introduction

On December 31st 2019, Chinese officials confirmed dozens of cases of pneumonia with an unknown cause. By January 7th 2020, the cause of the outbreak was determined to be a novel coronavirus termed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing coronavirus disease (COVID-19). The outbreak of SARS-CoV-2 has evaded containment efforts and has spread worldwide with over 78 million infections and 1.7 million deaths as of December 2020 (Dong et al. 2020). Canada has taken proactive measures to prevent community transmission; however, over 500,000 cases and over 14,000 deaths have been confirmed across the country to date. Long-term care homes have been particularly affected by the SARS-CoV-2

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pandemic with >80% of deaths occurring in those settings (Holroyd-Leduc and Laupacis 2020).

Serological assays have been developed in response to the ever-growing pandemic in the hopes of providing widespread testing, which may determine the magnitude of community transmission (Bonelli et al. 2020; Charlton et al. 2020; Lassaunière et al. 2020; Theel et al. 2020a). However, the development of humoral responses can take anywhere from several days to weeks following infection to develop. Additionally, some studies suggest that up to 40% of asymptomatic infections may become seronegative in the convalescent phase, further complicating the role of serology in the diagnosis of COVID-19 (Long et al. 2020b). The critical window for detection of SARS-CoV-2 infection remains the symptomatic period when antibody testing is, by its nature, insensitive, making PCR based methodologies the preferred diagnostic tool of acute SARS-CoV-2 infection (LeBlanc et al. 2020).

Antibodies to SARS-CoV-2 are believed to target the viral nucleocapsid or spike proteins and spike protein is believed to be the main target of neutralizing antibody responses (Prévost et al. 2020; Long et al. 2020a). Cross-reactivity with other circulating human coronaviruses particularly in the nucleocapsid region may hinder serological assays as a diagnostic tool (He et al. 2004; Khan et al. 2020). In the absence of definitive data on the duration of antibody responses and their utility as a correlate of protection, SARS-CoV-2 serological assays are currently limited to sero-surveillance studies, outbreak cluster analysis, and as an aid in diagnosing rare COVID-19 related disorders such as multi-inflammatory syndrome in children (MIS-C) (Bryant et al. 2020; Theel et al. 2020b).

There are two types of commercial serological platforms/assays currently available, which include laboratory and point-of-care (rapid cassettes) based tests. The laboratory-based assays are further categorized as being implemented on high-through-put chemiluminescent platforms (CLIA) or medium-through-put enzyme immunoassays (EIA). With the rapid development of serological tests and the extensive number of assays available for testing in the North American market, the Canadian Public Health Laboratory Network Serology Task Force conducted a nationwide evaluation of SARS-CoV-2 serological assays in order to better inform serological testing in Canada.

2. Methods

The Canadian Public Health Laboratory Network conducted a nationwide evaluation of SARS-CoV-2 serological assays. Common sample criteria were applied across the study in order to generate comparable data. All specimens analyzed for sensitivity were confirmed positive for SARS-CoV-2 RNA by RT-PCR targeting the nucleocapsid or envelope gene from nasopharyngeal swabs. Patient results were stratified into groups by symptom onset including 0-7, 8-14, >14, or >21 days. Pre-outbreak samples utilized for specificity were collected prior to December 1, 2019 (Canada's first reported case was January 25, 2020) (maximum 240 specimens). Cross-reactivity was evaluated using serum samples from patients who tested positive by PCR for other common respiratory infections including within 6 weeks postsymptom onset: influenza A (n = 25), influenza B (n = 15), respiratory syncytial virus (n = 5), adenovirus (n = 9), rhinovirus (n = 13), and human coronaviruses (n = 30), 229E (n = 1), OC43 (n = 4), HKU1 (n = 5), and NL63 (n = 7). In addition, sera positive for antibodies to syphilis (n = 39), Epstein-Barr virus IgM/IgG (n = 22), parvovirus IgM/IgG (n = 2), cytomegalovirus IgM/IgG (n = 39), human immunodeficiency virus 1 (n = 19), hepatitis A/B/C virus IgM (n = 51), herpes simplex virus (n = 8), varicella zoster virus (n = 9), rubella (n = 12), measles (n = 4), mumps (n = 10), rabies (n = 25) toxoplasma (n = 3) and other autoimmune disorders, such as, rheumatoid arthritis (n = 51) were included in the panel as these specimens often result in cross-reactivity. Also, specimen's positive for anti-nuclear antibody (n = 31) and anti-double stranded DNA (n=18) were included. Serology testing was conducted using the manufactures' instructions for the following CLIA/EIA tests: Architect SARS-CoV-2 IgG (Abbott, Chicago, IL, USA), BioRad SARS-CoV-2 IgM, IgG or Platelia Total (Bio-Rad Laboratories, Hercules, CA, USA), Liaison SARS-CoV-2 IgG (DiaSorin, Saluggia, Italy), EDI Novel Coronavirus COVID-19 IgM or IgG (Epitope Diagnostics Inc., San Diego, CA, USA), Euroimmun SARS-CoV-2 IgA or IgG (EUROIMMUN, Lubeck, Germany), VITROS Anti-SARS-CoV-2 IgG or Total (Ortho-Clinical Diagnostics, Raritan, NJ, USA), Elecsys Anti-SARS-CoV-2 Total (Roche, Basel, Switzerland). EIA based tests were performed using an automated Dynex platform in all laboratories with the exception of a one being performed manually. Several point-of-care rapid cassettes were also evaluated which included: Artron IgM/IgG, Biocan IgM/IgG, BioEasy IgM/IgG, Biolidics IgM/IgG, BTNX IgM/IgG, and NADAL IgM/IgG. Each province provided aggregate data for each test, which allowed analysis of overall sensitivity and specificity across the country. Additionally, there were limited lot numbers available resulting in all laboratories using the same lot number for their respective test. Overall sensitivity and specificity values were calculated by combining aggregate results from each individual public health laboratory. In addition, the coefficient of variation was calculated to measure variability between the reported sensitivity and specificities for the respective laboratories. Sensitivity and specificity analyses were calculated using Graphpad Prism v6. Positive predictive values for individual and combined testing algorithms were calculated as previously described (Bryant et al. 2020).

3. Results

3.1. Laboratory-based SARS-CoV-2 serological assays

Twelve different laboratory-based CLIA/EIA serological tests were evaluated for sensitivity and specificity in order to develop a serological testing algorithm for use by the provinces for sero-surveillance and the diagnosis of rare COVID-19 related disorders. Of the twelve assays evaluated, only four were licensed by the Medical Devices Branch (MDB) of Health Canada at the time of this study for use in Canada including the Abbott, DiaSorin, Roche, and Ortho-Clinical tests (https://www.canada.ca/en/health-canada). All four of these tests are implemented on high-volume instrumentation capable of processing hundreds of specimens per hour. All serological assays irrespective of manufacturer were relatively insensitive when serum samples were collected less than 7 days post-symptom onset [range: 25.0% – 67.9%] (Table 1 and Supplemental Fig. 1). Sensitivity improved considerably for all tests when specimens were assayed >14 days post-symptom onset [range: 55.9% – 96.7%]. The Abbott IgG, DiaSorin IgG, Roche total and Ortho-Clinical total tests achieved sensitivities >14 days postsymptom onset of 90.2%, 85.0%, 86.6%, and 94.0% respectively. While individually the Euroimmun IgA and IgG test achieved 92.8% and 91.2% sensitivity, respectively, combining the two assays improved the sensitivity to 96.7% >14 days postsymptom onset.

Given that specificity is a key metric in establishing positive predictive value (PPV) in low prevalence settings, clinical specificity and cross-reactivity serum panels were compiled by each provincial laboratory including pre-December specimens and specimens known to be reactive for antibodies to non-SARS-CoV-2 respiratory infections across the country. The Abbott, DiaSorin, Roche, and Ortho-Clinical test kits achieved 99.0% to 100.0% specificity, while the manual EIA test kits generally achieved lower specificity [range; 84.7% – 98.6%]. Aggregate test results where more than one laboratory evaluated a particular platform were used to estimate the variability associated with performing these tests on a national scale (Table 2). The Euroimmun IgA test showed the least variation between laboratories when overall sensitivity was considered (7.8% CV) while the BioRad IgM test was the most variable (26.8%). The variation in reported overall specificities

Table 1
Performance characteristics of commercial laboratory serological assays for SARS-CoV-2.

Manufacturer / Assay	Isotype	SARS-CoV-2 PCR-Positive Patients															Negative Samples (Pre Dec 2019)			Cross-Reactivity Samples			# Prov. Labs
		<7 d			7-14 d			>14 d			>21 d			All time points									
		Neg	Pos	Sens.	Neg	Pos	Sens.	Neg	Pos	Sens.	Neg	Pos	Sens.	Neg	Pos	Sens.	Neg	Pos	Spec.	Neg	Pos	Spec.	
Auto (CLIA)																							
Abbott[T]	IgG	69	36	34.3	37	88	70.4	30	274	90.1	22	262	92.3	140	444	76.0	240	2	99.2	453	4	99.1	6
DiaSorin[T]	IgG	84	34	28.8	68	82	54.7	33	186	84.9	58	197	77.3	166	377	69.4	219	3	98.6	395	3	99.2	6
Ortho[T]	Total	62	36	36.7	26	90	77.6	12	187	94.0	7	182	96.3	101	354	77.8	88	0	100.0	286	1	99.7	3
Ortho	IgG	72	24	25.0	46	54	54.0	19	155	89.1	16	152	90.5	140	272	66.0	88	0	100.0	284	3	99.0	3
Roche[T]	Total	57	24	29.6	35	92	72.4	45	292	86.6	33	251	88.4	137	408	74.9	98	0	100.0	271	0	100.0	4
Manual (EIA)																							
BioRad	IgM	32	18	36.0	34	67	66.3	56	71	55.9	38	17	30.9	128	158	55.2	124	1	99.2	216	4	98.2	4
BioRad	IgG	21	24	53.3	20	72	78.3	19	90	82.6	17	38	69.1	62	192	75.6	122	3	97.6	201	15	93.1	4
BioRad	Comb.	21	24	53.3	19	72	79.1	17	92	84.4	15	40	72.7	58	194	77.0	122	3	97.6	201	15	93.1	4
BioRad Platelia	Total	58	43	42.6	25	29	53.7	32	85	72.6	26	72	73.5	115	125	52.1	45	3	93.8	195	4	98.0	2
Epitope Diagnostics	IgM	23	23	50.0	25	63	71.6	22	72	76.6	18	38	67.9	76	160	67.8	100	0	100.0	162	8	95.3	4
Epitope Diagnostics	IgG	22	24	52.2	17	78	82.1	18	89	83.2	16	52	76.5	58	198	77.3	110	2	98.2	209	10	95.4	5
Epitope Diagnostics	Comb.	16	30	65.2	15	73	83.0	10	84	89.4	10	46	82.1	42	194	82.2	98	2	98.0	139	13	91.4	4
Euroimmun	IgA	17	36	67.9	17	86	83.5	11	141	92.8	12	109	90.1	50	308	86.0	162	13	92.6	247	61	80.2	5
Euroimmun	IgG	35	29	45.3	43	73	62.9	18	187	91.2	10	127	92.7	97	338	77.7	173	2	98.9	294	11	96.4	5
Euroimmun	Comb.	17	36	67.9	17	86	83.5	5	147	96.7	4	117	96.7	39	319	89.1	162	13	92.6	268	64	80.7	5

[T]Health Canada Approved, [Neg] Negative, [Pos] Positive, [Sens] Sensitivity, [Spec] Specificity

between laboratories was considerably smaller (<5% CV), with the exception of the Euroimmun and Epitope Diagnostics test kits.

3.2. Point-of-care SARS-CoV-2 serological assays

The Serology Task Force also evaluated six commercially available point-of-care cassettes in order to determine their feasibility for large-scale sero-surveillance in areas where laboratory testing poses logistical challenges. Similar to CLIA/EIA based assays, rapid cassettes were also relatively insensitive with samples collected less than 7 days postsymptom onset (Table 3) ranging from 34.1 – 74.2%. Sensitivity was drastically improved when specimens were assayed >14 days postsymptom onset with the Artron test cassette achieving 95.3% sensitivity while the BioEasy cassette achieved the lowest reported sensitivity of 80.0%. The specificity of the cassettes varied considerably with the BTNX cassette achieving 89.3% overall specificity while the Biocan cassette performed the best with 99.6% overall specificity. The variability (Table 4) in the reported overall sensitivity between laboratories ranged from as high as 30.6% (BioEasy) to as little as 4.6% (Artron v2) with the majority of variability associated with acute specimens. The variation in reported specificities between laboratories ranged from 0.4% (Biocan) to 8.5% (BTNX).

4. Discussion

This study was designed to evaluate the analytical performance of commercial SARS-CoV-2 serological test kits / platforms in clinical laboratories across Canada. In addition, these data represent testing that occurred in multiple provincial public health laboratories from over six provincial jurisdictions making it one of the most comprehensive national data sets to date. Indeed, a total of twelve different CLIA/EIA laboratory based assays as well as six different point-of-care rapid cassettes were evaluated for the detection of SARS-CoV-2 antibodies. Overall, the sensitivity of laboratory-based assays was quite variable less than 7 days post-symptom onset underscoring the limitation of serological testing for clinical diagnosis of acute SARS-CoV-2 infection. While the IgM assays tended to have improved sensitivity during acute infection compared to IgG, and the Euroimmun IgA

Table 2
Variability of commercial laboratory serological assays for SARS-CoV-2.

Manufacturer / Assay	Isotype	SARS-CoV-2 PCR-positive patients										Overall specificity		# Prov. labs
		<7 d		7-14 d		>14 d		>21 d		All time points				
		Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV	
Auto (CLIA)														
Abbott[T]	IgG	41.5	41.3	75.6	18.5	90.8	6.7	94.8	6.2	82.3	15.6	98.9	1.3	6
DiaSorin[T]	IgG	36.6	43.6	57.0	23.4	90.2	11.3	85.2	14.4	71.7	18.1	99.0	1.0	6
Ortho[T]	Total	43.7	55.3	77.0	3.4	94.0	6.4	96.7	3.2	79.3	11.4	99.7	0.4	3
Ortho	IgG	30.3	61.4	53.3	6.6	89.7	13.0	91.7	8.0	68.3	15.7	99.3	1.2	3
Roche[T]	Total	43.0	65.1	77.0	20.8	85.5	10.3	87.0	8.2	78.3	20.4	100.0	0.0	4
Manual (EIA)														
BioRad	IgM	42.0	39.8	68.8	27.6	56.3	31.7	43.8	51.6	56.3	26.8	98.8	1.4	4
BioRad	IgG	59.3	16.9	82.0	12.3	85.0	18.0	78.8	32.1	78.8	16.2	94.1	2.6	4
BioRad	Comb.	59.3	16.9	82.5	11.0	86.3	15.9	73.3	34.3	79.8	14.7	94.1	2.6	4
BioRad Platelia	Total	41.0	58.6	81.0	0.0	90.0	0.0	91.0	0.0	77.5	11.9	97.3	1.0	2
Epitope Diagnostics	IgM	59.8	32.1	77.0	23.5	81.3	16.2	65.3	38.8	74.5	21.5	94.8	6.9	4
Epitope Diagnostics	IgG	58.3	26.1	88.6	13.4	89.0	14.5	80.0	30.6	84.6	16.2	95.1	3.7	5
Epitope Diagnostics	Comb.	74.3	27.3	87.0	11.2	92.0	10.7	78.3	32.8	86.5	12.7	90.6	9.2	4
Euroimmun	IgA	62.8	22.8	84.0	11.2	89.0	12.2	91.0	11.4	85.8	7..75	84.6	7.9	5
Euroimmun	IgG	48.8	23.2	63.8	32.0	88.6	6.7	91.2	6.9	78.6	21.5	97.3	1.6	5
Euroimmun	Comb.	62.8	22.8	84.0	11.2	94.8	6.8	93.0	13.0	89.4	8.4	84.6	6.9	5

[T] Health Canada Approved, [%CV] Coefficient of variation.

Table 3
Performance characteristics of commercial point-of-care (rapid cassette) serological assays for SARS-CoV-2.

Manufacturer / Assay	Isotype	SARS-CoV-2 PCR-Positive Patients															Negative Samples (Pre Dec 2019)			Cross-Reactivity Samples			# Prov. Labs
		<7 d			7-14 d			>14 d			>21 d			All time points									
		Neg	Pos	Sens.	Neg	Pos	Sens.	Neg	Pos	Sens.	Neg	Pos	Sens.	Neg	Pos	Sens.	Neg	Pos	Spec.	Neg	Pos	Spec.	
Artron v2.	IgM/IgG	8	23	74.2	9	51	85.0	10	202	95.3	9	125	93.3	27	276	91.1	35	1	97.2	85	3	96.6	2
Biocan	IgM/IgG	54	28	34.1	29	79	73.1	30	185	86.0	24	120	83.3	113	292	72.1	59	0	100.0	209	1	99.5	3
BioEasy	IgM/IgG	7	8	50.0	8	20	56.0	1	9	80.0	0	1	100.0	16	37	58.0	36	0	100.0	23	2	92.0	2
Biolidics	IgM/IgG	3	7	70.0	4	20	83.3	2	23	92.0	0	10	100.0	9	50	84.7	59	2	96.7	0	0	0.0	2
BTNX	IgM/IgG	47	41	46.6	20	104	83.9	14	222	94.1	7	147	95.5	81	367	81.9	108	1	99.1	177	33	84.3	4
NADAL	IgM/IgG	41	27	39.7	17	61	78.2	13	181	93.3	9	155	97.0	71	269	79.1	45	0	100.0	170	8	95.5	2

[Neg] Negative, [Pos] Positive, [Sens] Sensitivity, [Spec] Specificity

Table 4
Variability of commercial point-of-care (rapid cassette) serological assays for SARS-CoV-2.

Manufacturer / Assay	Isotype	SARS-CoV-2 PCR-Positive Patients										Overall Specificity		# Prov. Labs
		<7 d		7-14 d		>14 d		>21 d		All time points				
		Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV	
Artron v2.	IgM/IgG	74.0	1.9	85.5	17.4	96.0	3.0	93.0	0.0	92.0	4.6	95.5	6.7	2
Biocan	IgM/IgG	44.3	57.6	75.7	19.8	88.3	9.5	83.0	5.1	75.7	19.0	99.8	0.4	3
BioEasy	IgM/IgG	55.0	12.9	78.0	39.9	90.0	15.7	100.0	0.0	74.0	30.6	98.0	2.9	2
Biolidics	IgM/IgG	70.0	20.2	86.5	22.1	95.0	7.4	100.0	0.0	87.5	12.1	98.0	2.9	2
BTNX	IgM/IgG	59.0	39.8	82.8	12.0	93.8	4.1	96.7	3.0	83.8	12.0	92.2	8.5	4
NADAL	IgM/IgG	42.5	25.0	78.0	5.4	93.5	2.3	94.5	3.7	79.5	8.0	96.0	1.9	2

[%CV] Coefficient of variation.

reaching 67.9% sensitivity, these assays also suffered from increased cross-reactivity and generally poorer specificity (Table 2). Early diagnosis <14 days post-symptom onset is a key factor in immediate public health interventions such as patient isolation and contact tracing to limit community transmission. However, the sensitivity of these assays improved with time following the onset of symptoms, specifically, >14 days postsymptom onset. Importantly, all four serological tests licensed by the MDB of Health Canada consistently achieved specificity values exceeding 99% which is a key metric when cross-reactivity can occur with other circulating human coronaviruses in low COVID-19 prevalence settings.

The majority of the rapid cassettes achieved between 90 and 100% specificity; however, in a low prevalence setting they would not achieve the necessary PPV that would be required for implementing large-scale, sero-surveillance testing. Examination of lot-to-lot variability of all COVID serological testing platforms is needed. In addition, our studies of rapid test cassettes made use of serum as opposed to capillary blood which is the preferred specimen for these particular test kits. A recent study demonstrated that various sample sources can have profound effects on serological test results indicated further validation of these platforms is needed (Flower et al. 2020).

Given the current low estimated prevalence in some jurisdictions across Canada a two-tiered orthogonal algorithm should be considered when conducting sero-surveillance studies, or rare diagnostic testing (Skowronski et al. 2020). The PPV of any single test at an estimated prevalence of 1% for example, would be as high as 43.4% for the Abbott IgG CMIA and as low as 11.3% for the BioRad EIA (Fig. 1). In contrast, when combining some of the top-performing assays such as those produced by Abbott and DiaSorin or Abbott and Ortho-Clinical at a prevalence of 1%, PPVs significantly improve to 98.1% and 99.8% respectively.

We recognize that the variability analysis between laboratories was not based on overlapping specimens measured by each respective laboratory. A national panel is currently being constructed in order to measure the variance on a national scale for multiple platforms. However, our data represent a comprehensive sampling of COVID-19 sera from across the country, and while sensitivity varied considerably for specimens collected before 14 days postsymptom

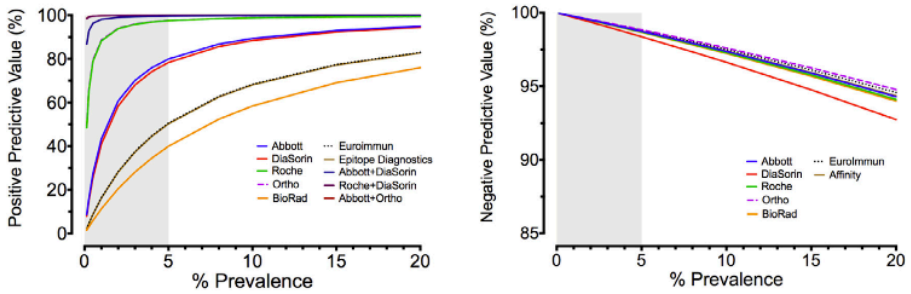

Fig. 1. Performance of individual and combined commercial serological assays for SARS-CoV-2 infection. he positive and negative predicative value of individual and combined assays were plotted based on estimated prevalence rates using the sensitivity and specificity characteristics of each serological test.

onset, sensitivity and reproducibility between laboratories markedly improved >14 days post-symptom onset.

A consideration of utmost importance in implementing serological testing for SARS-CoV-2 infection is that antibodies have yet to be shown to provide immunity to reinfection. In fact, a recent study has reported that antibodies diminish considerably 2-3 months following infection, making the value of reporting individual results contentious in regards to immune status or protection (Long et al. 2020b). The use of serological testing for SARS-CoV-2 may provide some insight into cases where late presentation (2 weeks post-symptom onset) occurs outside the window of detection offered by molecular nasopharyngeal testing. In these cases, documenting seroconversion using an acute and convalescent specimen could be considered. Studies with semi-quantitative serological assays documenting increases in signal between specimens should also be considered as a strategy for identifying recent or recurrent infections. In addition, virus neutralization assays must also be performed to better understand the possible correlates of protection and how their results may align with the serological assays / platforms assessed in this study. Moreover, serological testing may become helpful in understanding the etiology of multi-system inflammatory syndrome in children (MIS-C) (Perez-Toledo et al. 2020; Riollano-Cruz et al., 2021). Finally, a key role for serological testing will be its use in understanding the spread of SARS-CoV-2 infection across the country, informing evidence-based public health policy decisions that affect all aspects of Canadian society and health.

Credit statement

Derek R. Stein: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – Original, Writing –Review & Editing, Supervision, Project administration; Ainsley Gretchen: Methodology, Investigation, Data Curation, Writing – Review & Editing; Laurel Thorlacius: Methodology, Investigation, Writing – Review & Editing; Denise Fudge: Methodology, Investigation, Writing – Review & Editing; Amanda Lang: Conceptualization, Data Curation Methodology, Investigation, Writing – Review & Editing; Inna Sekirov: Conceptualization, Methodology, Investigation, Writing – Review & Editing; Muhammad Morshed: Conceptualization, Methodology, Investigation, Writing – Review & Editing; Paul N. Levett: Conceptualization, Methodology, Investigation, Writing – Review & Editing; Vanessa Tran: Conceptualization, Data Curation Methodology, Investigation, Writing – Review & Editing; Julianne Kus: Jonathan Gubbay: Conceptualization, Methodology, Investigation, Writing – Review & Editing; Vandana Mohan: Conceptualization, Methodology, Investigation, Writing – Review & Editing; Carmen Charlton: Conceptualization, Methodology, Investigation, Writing – Review & Editing; Jamil N. Kanji: Conceptualization, Methodology, Investigation, Writing – Review & Editing; Graham Tipples: Conceptualization, Methodology, Investigation; Bouchra Serhir: Conceptualization, Methodology, Investigation, Writing – Review & Editing; Christian Therrien: Conceptualization, Methodology, Investigation, Writing – Review & Editing; Michel Roger: Conceptualization, Methodology, Investigation, Writing – Review & Editing; Lei Jiao: Conceptualization, Methodology, Investigation, Writing – Review & Editing; George Zahariadis: Conceptualization, Methodology, Investigation, Writing – Review & Editing; Robert Needle: Conceptualization, Methodology, Investigation, Writing – Review & Editing; Laura Gilbert: Conceptualization, Methodology, Investigation, Writing – Review & Editing; Guillaume Desnoyers: Conceptualization, Methodology, Investigation, Writing – Review & Editing; Richard Garceau: Conceptualization, Methodology, Investigation, Writing – Review & Editing; Ihssan Bouhtiauy: Conceptualization, Methodology, Investigation, Writing – Review & Editing; Jean Longtin: Conceptualization, Methodology, Investigation, Writing – Review & Editing; Nadia El-Gabalawy: Writing - Review & Editing, Data Curation; Antonia Dibernardo: Conceptualization, Data Curation, Methodology, Investigation, Writing – Review & Editing; Carla Osiowy: Conceptualization, Methodology, Investigation, Writing – Review & Editing; L. Robbin Lindsay: Conceptualization, Methodology, Investigation, Writing – Review & Editing; Michael Drebot: :Conceptualization, Methodology, Investigation, Writing – Review & Editing.

Declaration of competing interest

The authors declare no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.diagmicrobio.2021.115412.

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4255

245

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4256

246

Appendix F: NL Cervical Screening Ethics Forms

Research Ethics Office
Suite 200, Eastern Trust Building
95 Bonaventure Avenue
St. John's, NL
A1B 2X5

July 14, 2020

Suite 1, 100 Forest Road
St. John's, NL
A1A 3Z9

Dear Ms. Gilbert:

Researcher Portal File # 20200141
Reference # 2019.095

RE: Review of Provincial Cervical Cancer Screening Program ASCUS HPV Triage, 2002-2019

Your application was reviewed by a subcommittee under the direction of the HREB] and the following decision was rendered:

X	Approval
	Approval subject to changes
	Rejection

Ethics approval is granted for one year effective July 14, 2020. This ethics approval will be reported to the board at the next scheduled HREB meeting.

This is to confirm that the HREB reviewed and approved or acknowledged the following documents (as indicated):

- Variable List 2020/06/29 acknowledged
- Proposal 2019/02/14, approved

Please note the following:

- This ethics approval will lapse on July 14, 2021 It is your responsibility to ensure that the Ethics Renewal form is submitted prior to the renewal date.
- This is your ethics approval only. Organizational approval may also be required. It is your responsibility to seek the necessary organizational approvals.
- Modifications of the study are not permitted without prior approval from the HREB. Request for modification to the study must be outlined on the relevant Event Form available on the Researcher Portal website.
- Though this research has received HREB approval, you are responsible for the ethical conduct of this research.
- If you have any questions please contact info@hrea.ca or 709 777 6974.

The HREB operates according to the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS2), ICH Guidance E6: Good Clinical Practice Guidelines (GCP), the Health Research Ethics Authority Act (HREA Act) and applicable laws and regulations.

We wish you every success with your study.

4259

4260

Appendix G: CINtec Ethics Forms

NEWFOUNDLAND AND LABRADOR
HREB
HEALTH RESEARCH ETHICS BOARD

Ethics Office
Suite 200, Eastern Trust Building
95 Bonaventure Avenue
St. John's, NL
A1B 2X5

February 23, 2016

Dr Sam Ratnam
Public Health Laboratory
Dr LA Miller Centre
Suite 1, 100 Forest Road
St. John's, NL
A1A 3Z9

Dear Dr Ratnam:

Reference #15.287

RE: Canadian CINtec-ASCUS/LSIL Study

This will acknowledge receipt of your correspondence.

This correspondence has been reviewed by the Chair under the direction of the Board. ***Full board approval*** of this research study is granted for one year effective **December 10, 2015.**

This is your ethics approval only. Organizational approval may also be required. It is your responsibility to seek the necessary organizational approval from the Regional Health Authority or other organization as appropriate. You can refer to the HREA website for further guidance on organizational approvals.

This is to confirm that the Health Research Ethics Board reviewed and approved or acknowledged the following documents (as indicated):

- Application, approved
- Revised consent form ASCUS-HPV positive cases, dated February 16, 2016, approved
- Revised consent form LSIL cases, dated February 16, 2016, approved
- Proposal approved
- Study Registration Card, approved
- Follow up call/emails, approved
- Budget, approved

MARK THE DATE

This approval will lapse on December 10, 2016. It is your responsibility to ensure that the Ethics Renewal form is forwarded to the HREB office prior to the renewal date; you may not receive a

email: info@hrea.ca Phone: 777-6974 FAX: 777-8776

reminder. The Ethics Renewal form can be downloaded from the HREB website http://www.hrea.ca.

If you do not return the completed Ethics Renewal form prior to date of renewal:

- ***You will no longer have ethics approval***
- *You will be required to stop research activity immediately*
- *You may not be permitted to restart the study until you reapply for and receive approval to undertake the study again*
- *Lapse in ethics approval* ***may result in interruption or termination of funding***

You are solely responsible for providing a copy of this letter, along with your approved HREB application form; **to the Office of Research Services** should your research depend on funding administered through that office.

Modifications of the protocol/consent are not permitted without prior approval from the HREB. **Implementing changes without HREB approval may result in your ethics approval being revoked, meaning your research much stop**. Request for modification to the protocol/consent must be outlined on an amendment form (available on the HREA website) and submitted to the HREB for review.

The Health Research Ethics Board operates according to the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans, the Health Research Ethics Authority Act and applicable laws and regulations.

You are responsible for the ethical conduct of this research, notwithstanding the approval of the HREB.

We wish you every success with your study.

4263

Appendix H: SARS-CoV-2 Serology Ethics Forms

HREA

Research Ethics Office
Suite 200, 95 Bonaventure Avenue
St. John's, NL A1B 2X5

Dear Dr. Jiao,

The Research Ethics Office (REO) has determined that this project of 'Serological evaluation of human antibodies against SARS-CoV-2 in blood collected in Newfoundland and Labrador' is likely to be a quality assurance or quality improvement project, and as such would not require ethics review. If it is the case that this project's intent is for internal use to assess and improve the practices of a strictly designated program, and furthermore that the intent is not to produce generalizable knowledge, then this project does not meet the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2) definition of research and as such is exempt from ethics review.

- Research is defined by the TCPS 2 as: "an undertaking intended to extend knowledge through a disciplined inquiry and/or systematic investigation." (Application of Article 2.1)

However, if the intent is to produce generalizable information beyond a specific program then ethics review will be required. Whether or not a study is program evaluation depends largely on the intent of the researcher and as per your correspondence, "the project's intent is to assess and improve the practice of a strictly designated program, i.e. improve the testing modality of COVID-19 infection at NL." If you have any questions, or would like more information, please do not hesitate to contact the Ethics Officer at ethicsofficer@hrea.ca.

Sincerely,

[redacted]

Ethics Officer
Health Research Ethics Authority
95 Bonaventure Ave, Suite 200
St. John's, NL A1B 2X5
T: 709-777-8115
F: 709-777-8776
E: ethicsofficer@hrea.ca

email: info@hrea.ca Phone: 777-8949 FAX: 777-8776