POLIOVIRUS 1 SEROPREVALENCE IN NOVA SCOTIA, CANADA

by

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بسم الله الرحمن الرحيم

(bism Allah alruhmin alrahim)

For my Mom,
my Dad,
my Husband and Sons.

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ABSTRACT

Thanks to the efforts of the Global Polio Eradication Initiative, the world has never been closer to eradicating polio as it is today. Without complete eradication from the remaining strongholds, all countries remain at risk. It is the responsibility of polio-free countries to ensure vaccination rates are kept high enough to maintain levels of immunity among the population that prevent reintroduction of poliovirus. Since Nova Scotia has been exclusively using IPV for prevention and control of polio from the time it was licensed in 1955, immunity among adults and seniors may be declining as a result of waning immunity. The risk of virus reintroduction can be assessed by determining the the seroprevalence of neutralizing antibodies in the population. We established and validated the poliovirus standardized microneutralization (MN) assay and developed an immunoadsorption technique to generate poliovirus non-immune serum to use as a negative control. Using the MN assay, we examined the prevalence and levels of neutralizing antibodies against poliovirus 1 (PV1) in Nova Scotia by testing residual sera from three age groups (10 – 29, 30 – 49, and 50 – 64 years old). Although we hypothesized that older adults would have lower levels of antibodies due to waning immunity, seroprevalence rates and geometric mean titers were found to be higher for these age groups. Overall PV1 seroprevalence in Nova Scotia is above the herd immunity threshold range required for protection from poliomyelitis. Seroprevalence rates in the younger age group can be increased by enhancing vaccine uptake and schedule completion. We demonstrated the importance and value of seroepidemiological surveys, and have shown that they provide a more accurate determination of population-based protection than vaccination coverage rates alone. Based on our findings, there is no imminent risk to Nova Scotia from PV1.

LIST OF ABBREVIATIONS USED

°C	degrees Celsius
IU/mL	international units per millilitre
mL	Milliliter
mg	Milligram
µg	Microgram
µL	Microliter
AFP	acute flaccid paralysis
ATCC	American Type Culture Collection
aVDPV	ambiguous vaccine-derived poliovirus
bOPV	bivalent oral polio vaccine
BSC	biosafety cabinet
CDC	Centers for Disease Control and Prevention
CI	confidence interval
CL	containment level
CNICS	Childhood National Immunization Coverage Survey
CNS	central nervous system
CO_2	carbon dioxide
CPE	cytopathic effect
cVDPV	circulating vaccine-derived poliovirus
DBS	dried blood spot
DEA	Diethanolamine
DHA	District Health Authority
DMP	dimethyl pimelimidate dihydrochloride
DMSO	dimethyl sulfoxide
EDTA	ethylenediaminetetraacetic acid
eIPV	enhanced inactivated polio vaccine
ELISA	enzyme-linked immunosorbent assay
EtOH	Ethanol
Fab	antigen-binding fragment of immunoglobulins

FBS	fetal bovine serum
Fc	crystallizable fragment of immunoglobulins
GMT	geometric mean titers
GPEI	Global Polio Eradication Initiative
HEp-2 cells	human epithelial type 2 cells
Hib	*Haemophilus influenzae* type B
H_2O_2	hydrogen peroxide
IAS	Immunoadsorption
IgG	immunoglobulin G
IgM	immunoglobulin M
IHRS	in-house reference sera
IPV	inactivated polio vaccine
iVDPV	immunodeficiency-related vaccine-derived poliovirus
LLD	lower limit of detection
Log	logarithm
MAb	monoclonal antibodie
MEM	minimum essential medium
MN	Microneutralization
mOPV	monovalent oral polio vaccine
NaCl	sodium chloride
NIBSC	National Institute for Biological Standards and Control
NML	National Microbiology Laboratory
OPV	oral polio vaccine
OR	odds ratio
PBS-T	phosphate buffered saline with 0.1% Tween 20
PEESP	Polio Eradication & Endgame Strategic Plan
PHAC	Public Health Agency of Canada
PPE	personal protective equipment
PPS	post-polio syndrome
PV	Poliovirus
PV1	poliovirus type 1

PV2	poliovirus type 2
PV3	poliovirus type 3
QC	quality control
RRPL	Roy Romanov Provincial Laboratory, Saskatchewan Health Authority
RuV	rubella virus
SAE	serious adverse event
SD	standard deviation
sIgA	secretory immunoglobulin A
STD	standard
$TCID_{50}$	median tissue culture infectious dose
TEA	triethanolamine
TDaP	tetanus, diphtheria, acellular pertussis (TDaP)
Tris-HCl	trisaminomethane hydrochloride
tOPV	trivalent oral polio vaccine
ULD	upper limit of detection
VPD	vaccine-preventable disease
VAPP	vaccine-associated paralytic polio
VDPV	vaccine-derived poliovirus
VP	viral polypeptides
WHA	World Health Assembly
WHO	World Health Organization
WPV	Wild-type piliovirus

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CHAPTER 1 INTRODUCTION

1.1 Poliovirus: The global perspective

Polio, also known as poliomyelitis (or infantile paralysis) is a vaccine-preventable disease (VPD) caused by poliovirus. The World Health Assembly (WHA), the decision-making body of the World Health Organization (WHO), issued a global resolution and committed funds in 1988 to eradicate polio by the year 2000 (1). Using strategies recommended by the WHO, polio eradication efforts were effective and successful in countries that were fully committed to the objective or possessed reliable health infrastructure (2). However, despite the significant decline in paralytic polio cases, global eradication was still a challenge in the year 2000, with just under 3,000 cases reported (3). The Polio Eradication & Endgame Strategic Plan (PEESP) 2013-2018, developed by the Global Polio Eradication Initiative (GPEI), had four main objectives that engage and motivate countries, regardless of their polio disease status, to make polio "*the first disease of humans to be eradicated from the earth in the 21st century*" (4). Despite all efforts, eradication has not yet been achieved. The number of polio cases reported in 2018 was 136, including 33 cases attributed to wild-type poliovirus (WPV) and 103 cases associated with circulating vaccine-derived poliovirus (cVDPV). This represented an increase from 2017, where there were 22 WPD and 96 cVDPV cases, respectively (5, 6). Notably, 69 of the 103 cVDPV cases reported in 2018 were from five non-endemic countries (6). The WHO continues to caution that without complete eradication of polio from the remaining strongholds, all countries of the world remain at risk (7-9).

1.2 Poliovirus in Canada

Canada was declared polio-free by the WHO in 1994, with the last case of indigenous WPV infection recorded in 1977 (10). The first vaccine against poliovirus introduced in Canada in 1955 was the inactivated poliovirus vaccine (IPV), which was developed by Jonas Salk. Next came the oral poliovirus vaccine (OPV) in 1962, which was developed by Albert Sabin (10). Since each vaccine has its advantages and limitations, the decision by province regarding the choice of which vaccine to include in its immunization program was based on the clinical and environmental epidemiology of PV within its borders (11). Since its licensure in 1955, Nova Scotia and Ontario have been exclusively using IPV for their polio vaccination program (apart from a brief shortage period in Ontario during the early 1990s) (10). In other parts of Canada, OPV was initially used for mass immunization, after which all provinces and territories eventually transitioned to using IPV between 1994 and 1997 (10). By 1998, all provinces were using the enhanced potency IPV (eIPV) in a pentavalent pediatric vaccine that included coverage for tetanus, diphtheria, acellular pertussis (TDaP), and *Haemophilus influenzae* type B (Hib) (10). Nova Scotia was one of the first provinces in Canada to start an IPV-only immunization schedule in 1955, and maintain it beyond 1962 (even after OPV was introduced) (10). According to the latest Public Health Agency of Canada (PHAC) Childhood National Immunization Coverage Survey (CNICS) in 2015, the estimated coverage rates for IPV immunization [with 95% confidence interval (CI)] by 2, 7, and 17 years were 91.2% (95% CI 87.0-94.1), 90.2% (95% CI 85.9-93.3), and 87.2% (95% CI 82.2-90.9), respectively (12). Polio coverage is determined at $\geq$ 3 IPV doses by 2 years of ages, and at $\geq$ 4 IPV doses by 7 and 17 years of age (12, 13). Similarly for

Nova Scotia, the most recent coverage rates from 2013 were reported as 92.1 (95% CI 88.2-94.8), 82.8 (95% CI 77.9-86.8), and 81.2 (95% CI 76.2-85.3) for 2, 7, and 17 years old, respectively (13).

Despite Canada's elimination of indigenous WPV, four cases resulting from WPV importation were detected in 1978, 1988, 1993, and 1996 (14). Paralytic presentations were reported in the earlier two cases (14). In addition, since 2005, there have been five imported cases of VDPV reported in Canada (15). One case presented with acute flaccid paralysis upon return to Canada from China, but reported receipt of OPV vaccination during their trip. The other four cases presented with non-paralytic illness. These were inadvertently diagnosed after virologic testing by the PHAC National Microbiology Laboratory (NML) (15).

1.3 Poliovirus infection and disease

Poliomyelitis, or polio, is caused by infection with PV, a member of group C *Enterovirus* genus of the *Picornaviridae* family (16). PV a non-enveloped virus with a positive-sense, single-stranded RNA genome. The 30 nm capsid is composed of up of 60 oligomeric protein units, each assembled from four viral polypeptides (VP1-4) in an icosahedral symmetry. PVs are characterized into serotypes 1, 2, and 3 based on antigenically different capsid proteins, and abbreviated PV1, PV2, and PV3, respectively. All three PV serotypes are highly contagious and infectious. PV1 is the most prevalent serotype, and most frequently associated with severe paralytic disease (17, 18). The WHO declared the global eradication of PV2 in 2015 after confirmed elimination in 1999

(19, 20). Ongoing global efforts include controlling and interrupting the circulation of PV1 and PV3, while safeguarding against the re-introduction of PV2 (4).

While PV infection can be induced in higher primates, humans are the sole natural host of PV and as such, the only known reservoir (21). The virus spreads from person-to-person, mainly *via* fecal-oral route (associated with poor hygiene), although transmission by droplet exposure from infected saliva is possible (22). After exposure, the virus adheres to cell receptors on the mucosal surface of the oropharynx and lower gastrointestinal tract, where it then replicates (22). During this asymptomatic phase of infection, the human host is infectious, and sheds infectious virus particles. By penetrating gastrointestinal mucosa, PV enters the blood stream (causing viremia), and spreads to other tissues. In most cases ($\geq$ 95%), the infection remains asymptomatic, despite continued shedding of PV in stool and its presence in the throat. In approximately 4% of infections, cases present with symptoms of gastroenteritis or influenza-like-illness, such as fever, sore throat, headache, vomiting, and fatigue lasting 1 to 2 weeks. These clinical manifestations are referred to as non-paralytic (or abortive) polio, where the patient recovers without sequelae and with life-long protective immunity. In approximately 1 to 2% of infections, PV settles in the neuromuscular junctions post-viremia, and can be transported to the central nervous system (CNS) through a retrograde axonal transport or directly from the viremia (22). Depending on the location and extent of neuron destruction, paralysis may be spinal (i.e. cord involvement), bulbar (i.e. brain stem involvement), or bulbospinal. The musculoskeletal symptoms of spinal paralytic polio are severe; symptoms include loss of reflexes, spasm and muscle pain,

unilateral or bilateral flaccid paralysis leading to the classical withered appearance of polio-deformed limbs. Bulbar or bulbo-spinal involvement constitutes 5 to 10% of the paralytic cases, and is fatal when the damaged nerve cells are those supplying the respiratory muscles. A recurrence of musculoskeletal symptoms, known as post-polio syndrome (PPS), can present decades from the initial acute paralytic attack. Symptoms include muscle pain, weakness, and atrophy, as well as generalized fatigue. It is speculated that PPS results from PV latency in the CNS, or progressive destruction of the motor neurons (9).

1.4 Poliovirus vaccines

Poliovirus infection has no antiviral cure, and consequently, vaccines are the only tool available for control and eradication of this debilitating disease. Historians have documented polio disease dating back to ancient Egypt. By examining Pharaonic inscriptions depicting individuals suffering from withered limbs, or studying mummies with anomalous unilaterally short legs, these ancient presentations were suggestive of polio (9, 16, 23). The late 18th and early 19th century literature includes multiple reports describing paralytic poliomyelitis, leading to epidemics that escalated throughout North America and Europe from 1910s to 1940s. It was not until 1949, when the virus was eventually cultured, that hopes for cure (or vaccines) became possible (10, 16). Research collaborations between Canada and the United States eventually led to the development and use of two vaccines, IPV and OPV; both vaccines are still in use today (10, 16).

IPV is an injectable formulation that contains 3 WPV strains, Mahoney (type l), MEF-I (type 2) and Saukett (type 3), which have been inactivated using formaldehyde (16). For IPV production, virus culture supernatant is purified using gradient ultracentrifugation, then the virus is re-suspended in inactivation medium, filtered, and formaldehyde is added. The formaldehyde-treated virus suspension undergoes inactivation according to protocol conditions, after which any residual formaldehyde is neutralized before confirming virus inactivation and determining the antigen content (24-26). In 1978, a more immunogenic preparation of the inactivated vaccine was produced, using a human-derived cell lines (10, 27-29). It was referred to as enhanced-potency IPV (eIPV). Over the years, combination vaccines, which include IPV antigens as well as other antigens for TDaP and Hib, were licensed to reduce the number of injections needed to complete immunization schedules and improve compliance (30-32). IPV is not associated with any serious adverse events (SAE); most are limited to injection site reactions. The contribution of IPV to these reactions is no longer assessable, as it is incorporated into combination vaccines. Production of IPV requires culturing of the virus strains in large amounts, a process necessitating rigorous biocontainment practices to prevent any accidental escape of PV before inactivation. This is a particular challenge, as the global demand for IPV is on the rise to replace OPV, and with the strict control to prevent the reintroduction of the eradicated PV2 (4).

OPV is a live-attenuated PV (also known as the Sabin vaccine), and as its name infers, is administered orally. The Sabin PV strains are attenuated by serial passages in non-human *in vivo* or *in vitro* culture systems (16, 33). As WPV strains adapt to non-

human cells, they undergo point mutations that reduce virus replication efficiency and genetic loci involved in neurovirulence. This process results in the Sabin PV strains being attenuated for their pathogenicity (33, 34). After oral administration, the Sabin strains replicate locally and trigger both mucosal and systemic immune responses similar to those following natural infection with WPV strains, but without attacking the CNS. Originally, OPV was a trivalent formulation (tOPV), containing all three Sabin strains to protect against exposure to WPVs PV1, PV2, and PV3. Since eradication of PV2, many countries have switched to a bivalent OPV formulation (bOPV), containing PV1 and PV3 only or even a monovalent OPV formulation (mOPV) with either PV1 or PV3 (4, 35). OPV has made significant contributions in the control and prevention of PV infections worldwide. The vaccine is easy to administer without special training, contributes to herd immunity (as those who are vaccinated shed attenuated virus that can infect and vaccinate contacts), and after a complete schedule, it likely induces life-long protection equivalent to natural infection (9, 36). Although the vaccine has an extremely safe profile, vaccine-associated paralytic polio (VAPP) can occur at a rare estimated incidence of 1 case for every 4 to 6 million doses administered (37, 38). A case of VAPP occurs when a Sabin strain reverts to its virulent form, and causes disease in a susceptible individual. Vaccine-derived poliovirus (VDPV) strains causing VAPP have been isolated from immune deficient individuals, and these are described as immunodeficiency-related VDPV (iVDPV). They are termed circulating VDPV (cVDPV) when they are transmitted among communities with suboptimal vaccine coverage, or ambiguous VDPV (aVDPV) when they are detected in environmental samples or isolated from immunocompetent individuals (16, 39). Person-to-person outbreaks from VDPV strains will continue to be a

risk until usage of OPV has completely ended globally (40). Immunization schedules that begin by administering at least one IPV dose before giving OPV, can circumvent the occurrence of VAPP, due to increased systemic antibodies before exposure to the attenuated strains (41).

1.5 Poliovirus immunity

IPV and OPV vaccines are the backbone for polio eradication strategies. Although both vaccines protect against PV, their immunogenicity profile is not identical. IPV elicits good systemic immunity that prevents WPV viremia, replication and spread to the nervous system. However, the local intestinal immune response after IPV is suboptimal and inferior to OPV (41, 42). IPV-immunized children have been shown to shed higher virus titers than OPV-immunized children following challenge with Sabin strains (41). The immune response following OPV administration resembles that following natural infection, but without clinical disease. OPV elicits both systemic and intestinal mucosa antibodies. The local intestinal immune responses is mucosal, through production of secretory immunoglobulin A (sIgA) which provides protection upon exposure to WPV infection and hinders viral shedding if infected (41, 43). Both systemic and mucosal immune responses are important for safeguarding against poliomyelitis. Neutralizing antibodies are believed to be predominantly immunoglobulin G (IgG), and a 1:8 neutralizing titer in microneutralization assays is considered to be protective (i.e. the correlate of protection against PV infection) (44-46). A four-fold increase in neutralizing IgG against PV denotes recent infection or vaccination (46-48). Mucosal sIgA antibodies also play a role in PV immunity, but the correlate of protection against virus replication

and shedding is unknown; however, there is an association between quantitative levels of sIgA and the rates of viral shedding (49, 50). Overall, IPV and OPV each have their advantages and disadvantages. A comparison between IPV and OPV vaccines is presented in Table 1.1.

Table 1.1 Comparison between IPV and OPV vaccines

Attributes	OPV	IPV
Administration	Oral	Injectable
Type	Live attenuated	Inactivated
Potency	Low with ≥4 doses	Medium to high with 5 doses
Duration of immunity	Lifelong	Not confirmed
Mechanism of protection (individual level)	Systemic and mucosal	Systemic
Mechanism of protection (population/herd immunity)	Shedding of attenuated strains	Prevents viremia
Shedding of VDPV	Yes	No
VAPP[a]	0.4 to 0.6 per 10^6 doses	None
Combination vaccine availability	No	Yes, in use
Cost per dose [(11)]	≤US$0.20	~US$1.00
Production [(11)]	Safe, low risk	Containment requirements to prevent accidental reintroduction
Year introduced	1961	1955

[a] Vaccine acquired paralytic polio

1.6 Poliovirus serological assays

Serological assays use serum to measure and evaluate antibody-based immune responses following infection or immunization. Serologic assays can either be used as a diagnostic tools for the identification of a new or past infection. They can also be used as a screening tools at a population-based levels to determine past exposure to a pathogen from prior infection or immunization against VPDs. Serological testing at the population level is referred to as serological surveillance or serosurveillance (51).

PV serology assays include immunoglobulin M (IgM), IgA- and IgG-specific enzyme-linked immunosorbent assays (ELISA), but ELISA-based detection of any of these has limitations (52, 53). The presence of PV-specific IgA is short-lived, where IgA ELISA sensitivity drops after the first two months following infection or OPV vaccination (54). The benefits of PV-specific IgM ELISA assays is limited in acute illness due to cross reactivity between PV serotypes, as well as between other members of the *Picornavirus* family (53, 55, 56). Both in-house and commercially available IgG ELISA for PV are also rarely used and are not endorsed by the WHO (57, 58). These are not used as a measure for PV immunity, as they do not measure neutralizing antibodies, and like IgM ELISAs are hampered by cross-reactions with other picornaviruses (57, 58).

The neutralization assay is the WHO's reference assay for determining immunity against PV (53, 59, 60). It is used in polio vaccine studies and for assessing individual and population levels of protection (61-63). A serum-sparing, high-throughput "microneutralization" (MN) version of the assay is now regularly used (53, 62, 64-68).

The MN assay detects and measures functional antibodies that neutralize PV. Neutralization titers are determined using live PV, therefore the assay is conducted under biosafety containment level 2 or higher.

Positive and negative sera are required to control for the performance of any serology assay, whether it is used as a diagnostic tool or for seroepidemiology. Positive controls can be easily identified in vaccinated individuals and verified against commercially available reference standards (69) to establish them as in-house reference sera (IHRS). Identifying negative (PV non-immune) sera, on the other hand, can be difficult in a highly vaccinated population. Given the need for negative control serum, the principals of immunoprecipitation and immunoadsorption (IAS) were explored. Immunoprecipitation is technique that separates and collects a protein of interest by capturing it using a specific antibody (70). IAS is an established therapeutic blood-purification technique that removes pathogenic antibodies to treat immunological or immunoglobulin-mediated autoimmune disease (71-73). This study hypothesized that protein A magnetic beads coupled to PV-specific monoclonal antibodies (MAbs) could be used to bind the target PV, and this immune complex could be used to capture PV and in turn remove PV-specific antibodies from PV-immune sera. The generation of negative sera was essential prior to analyzing MN data used for seroepidemiology.

1.7 Seroepidemiology

Serological surveillance (or serosurveillance) plays an invaluable role in understanding the overall epidemiology of VPDs (51). Serosurveillance complements

immunization policies and public health strategies in multiple ways. It provides ongoing evaluation of vaccination programs while estimating disease burden and directing public health attention to trends of emerging or changing infection patterns. With estimates of immunity at the population level, serosurveillance can identify groups or communities at risk from particular VPDs by identifying the proportion of individuals that are unprotected. Over time, serological surveillance data can be modeled and studied to prompt preventative measures, like vaccination, if needed (51, 74). Despite variations in health care and disease epidemiology across countries, many VPDs require collaborative international serosurveillance (75-77). Serum samples for seroepidemiology can be obtained as residual sera from routine diagnostic testing, or actively collected as part of population-based surveys, or cross-sectional studies with interest in specific groups. Although the former approach is considered straightforward (by not requiring active sample collection), it sometimes lacks valuable individual-level information such as medical and vaccination history. Moreover, careful selection of specimens is needed to avoid biases in the analyses. This limitation can be overcome with accurately designed studies that include formal consenting and prospective data collection, but these require a significant amount of research funding. The choice of approach largely depends on cost and the specific objectives or hypotheses being addressed and evaluated.

1.8 Rationale for PV serosurveillance in Nova Scotia

Despite the complete elimination of polio in Canada (10), there are no data on the PV seroprevalence. The number of individuals at risk is unknown. Another area of uncertainty is whether IPV alone confers lifelong protection against PV (68). In Nova

Scotia, adults and seniors do not get boosted against polio, either by vaccination or natural exposure (given PV is not endemic in Canada). With IPV being exclusively used in NS, there could be waning immunity across different age groups. Without seroepidemiological analyses, this remains to be determined. In Canada, like elsewhere, notions of vaccine hesitancy have plagued compliance with children immunization. For polio, vaccine hesitancy is further amplified by the fact that polio is no longer a visible disease in this country. However, all polio-free countries remain at risk of importation and introduction of PV until it is eradicated globally. The province of Nova Scotia receives the highest number of immigrants to Atlantic Canada. The degree at which a country or province may be at risk from imported polio can be assessed by determining the seroprevalence and seroepidemiology of protective neutralizing titres of anti-PV antibodies in the population. In this study, we are interested in determining what level of PV1 immunity exists among Nova Scotians to determine if there are age groups at risk of infection if exposed to PV. Given the unknown duration of immunity offered by IPV and the potential of silent PV circulation in an IPV-only vaccinated population (54), we aim to collect data to evaluate whether boostering is warranted. For this serosurveillance study, we examined the prevalence and levels of neutralizing antibodies against PV1 among Nova Scotians using a standardized MN assay (53).

1.9 Hypothesis and objectives

1.9.1 Hypothesis

Given the lack of endemic poliovirus, we suspect that antibody titers in an IPV-only vaccinated population may have waned over time leaving some age groups at risk of infection if exposed to wild- or vaccine-type poliovirus.

1.9.2 Objective 1

Validate and establish a PV1-specific MN assay to measure protective titres of PV neutralizing antibodies.

1.9.3 Objective 2

Evaluate IAS as a method to generate negative control sera required for MN assays.

1.9.4 Objective 3

Used the validated and controlled MN assay to establish the seroprevalence of PV1 neutralizing antibodies in residual sera collected from residents of Nova Scotia.

CHAPTER 2 MATERIAL AND METHODS

2.1 Biosafety precautions

PV is a risk group 2 pathogen requiring containment level (CL) 2 practices. In addition to standard CL-2 measures, additional precautions were taken while handling the Sabin PV1 strain. Prior to laboratory use of PV1 for this project, staff members were all vaccinated using PEDIACEL (Sanofi Pasteur Ltd.) to boost their immunity, and ensure they are protected. For manipulation of the virus during experiments, CL-2 personal protective equipment (PPE) were used at all times. Gloves were changed frequently, between each step, regardless of suspected soiling and/or accidental contamination. Disinfectants were used, namely 6% sodium hypochlorite (bleach) and 20% hydrogen peroxide (H_2O_2). Liquid biohazard waste was safely discarded into 6% sodium hypochlorite as a safeguard, taking into account that it will get diluted by the volume of waste. Solid waste was discarded directly onto 6% sodium hypochlorite. After allowing 20 to 30 minutes of contact time with disinfectant in the biosafety cabinet (BSC), all waste was secured and carefully disposed of into leak-proof biohazard waste containers and disposed of as per institutional practices.

To disinfect the BSC, pipettes, and any items used in the BSC while handling the live virus, 20% H_2O_2 was used. It was also preferred over bleach for disinfection of laboratory ware and equipment given its effectiveness against PV1 while not being as corrosive (78-80). It was sprayed and allowed at least 10 minutes contact time before wiping off and then spraying the items again using 70% ethanol (EtOH).

2.2 HEp-2 cells

Human epithelial type 2 (HEp-2) cells (CCL-23) were obtained from the American Type Culture Collection (ATCC) (Manassas, VA, USA) (81). HEp-2 cells were used to amplify Sabin PV1 and for the MN assay. All stocks were stored in liquid nitrogen.

2.2.1 Culturing HEp-2 cells

HEp-2 cells were seeded in T-75 flasks (Corning™, Fisher Scientific, Ottawa, ON, CA) and passaged in T-150 or T-175 tissue culture flasks (Corning™, Fisher Scientific, Ottawa, ON), in culture media. Minimum Essential Medium (MEM) (Thermo Fisher Scientific, Mississauga, ON, CA) was the base of all media used for virus culture. For Hep-2 cell propagation and maintenance culture media, MEM was supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) (Sigma Aldrich, Oakville, ON, CA), 10 Units (U)/mL of penicillin, and 10 µg/mL of streptomycin (Pen Strep) (Thermo Fisher Scientific, Mississauga, ON, CA).

The procedure for HEp-2 cell recovery from the ATCC or in-house made stock vials was identical. One frozen vial stored in liquid nitrogen was rapidly thawed in a 37°C water bath for 60 to80 seconds. Before completely thawing, the vial was wiped down with disinfectant (70% EtOH) and transferred immediately inside a BSC. Without delay, the thawed cells were transferred and re-suspended into 9 mL of pre-warmed culture media and placed into a 15 mL conical Falcon tube. Cells were pelleted by centrifuged at 360 *x g*, at 4°C for 10 min. The supernatant was decanted, the cell pellets were re-suspended in 10 mL of pre-warmed culture media, and then added to 5 mL of

pre-warmed culture media in a T-75 flasks. These seeded flasks were incubated at 37°C in 5% CO_2, and examined daily using an inverted microscope (Leica DM IL, Leica Microsystems, Wetzler, Germany) with 40× magnifications until 80 to 90% cell confluency was attained (approximately 48 to 72h). Once the desired confluency was achieved, cells were split and re-passaged as needed.

For cell splitting, flasks were washed once with cold 0.05% Trypsin-ethylenediaminetetraacetic acid (EDTA) (Thermo Fisher Scientific, Mississauga, ON, CA), then trypsinized again for 8 min at 37°C in 5% CO_2. The separated cells were mixed using gentle pipetting to break any clumps, then re-suspended in culture media in a 50 mL conical tube. Once homogenous, the cell suspension volume was noted and the cells were counted using a hemocytometer. Cells were then pelleted by centrifugation (360 *x g*, at 4°C) for 10 minutes. After centrifugation, the supernatant was decanted and the pelleted cells re-suspended in appropriate media (culture or freezing) to give a targeted cell concentration of 2 x 10^6 cells/mL for cryopreservation, 3 x 10^5 cells/mL for the MN assay, or variable concentrations for further passaging depending on the culture flask volume requirements.

2.2.2 Cryopreservation of HEp-2 cells

For storage, HEp-2 cells were preserved in freezing media [5% Dimethyl sulfoxide (DMSO) (Sigma Aldrich, Oakville, ON, CA) in culture media]. Cryovials were tempered in a pre-frozen cold block, termed a Mr. Frosty (Nalgene, (Thermo Fisher Scientific, Mississauga, ON, CA). Washed and pelleted cells were re-suspended in cold freezing media to achieve a final concentration of 2 x 10^6 cells/mL per vial. The cell vials

were stored at -80°C in the Mr. Frosty for 48 to 72 hours, then transferred to liquid nitrogen for long term cryopreservation.

2.3 Sabin PV1

The reference strain of Sabin PV1 (LS-c, 2ab strain; code: 01/528) was acquired from the National Institute for Biological Standards and Control (NIBSC) (Blanche Lane, Ridge, UK) (82). NIBSC is a WHO Collaborating Centre for Reference and Research on poliomyelitis, and is one of the WHO's seven Global Specialized Polio Laboratories. Sabin PV1 was used in both the MN assay and the PV1-antibody immunoadsorption (IAS) experiments.

2.3.1 Sabin PV1 propagation

Sabin PV1 was propagated using HEp-2 cell culture. Each Sabin PV1 culture were grown in batches, in three T-150 tissue culture flasks. When confluent monolayers of HEp-2 cells were attained, the first two flasks were inoculated with Sabin PV1, while the third flask served as an uninfected control. Immediately prior to inoculation, the flasks with confluent HEp-2 cells were washed once with 10 mL serum-free MEM (including Pen/Strep antibiotic supplementation), followed by the addition of 3 ml of MEM with 2% FBS. An inoculum of Sabin PV1 adjusted to 3.0×10^5 median tissue culture infectious dose ($TCID_{50}$) in approximately 250 µL media was added to each virus-infected flask, and an equal volume of MEM with 2% FBS was added to the uninfected control flask. All the flasks were incubated at 35°C, 5% CO_2 for 60 min, after which 12 mL of MEM with 2% FBS were added to each of the flasks, incubated again for

24-48 hours, and monitored daily for observation of cytopathic effect (CPE) (Figure 3.4). Once adequate CPE was observed, the culture content of the infected flasks was completely detached using a cell scraper, collected, and pooled into 50 mL conical tubes, and virus was harvested by subjecting the culture material to three freeze/thaw cycles at -80°C and then room temperature. The virus suspension was clarified by centrifugation at 3000 *x g* for 5 min at 4°C, the supernatant then divided into 100-200 µL aliquots stocks, and stored frozen at -80°C pending titration.

2.3.2 Sabin PV1 stock titration

For titration of Sabin PV1, a single aliquot of stock virus was thawed, and 10-fold serial dilutions were performed in MEM with 2% FBS spanning 10^{-1} to 10^{-9}. In parallel, a suspension containing 3 x 10^5 cells/mL of HEp-2 cells in MEM with 10% FBS was prepared. The virus dilutions were added sequentially to wells of columns one to ten in 96-well sterile cell culture plates (Corning Costar, Fisher Scientific, Ottawa, ON, CA), as illustrated in Figure 2.1. No virus dilutions were added to wells from columns 11 and 12, which were used as controls. In the first two rows of wells, 100 µL the first dilution (10^{-2}) was added, followed by the next dilution (10^{-3}) in the next two rows, and this process was repeated until the remaining dilutions (10^{-4} to 10^{-9}) were completed (Figure 2.1). For cell controls, 100 µL virus-free media (MEM with 2% FBS) was added (columns 11 and 12). Then, 100 µL of HEp-2 cell suspension were added to all wells to achieve a final concentration 1.5 x 10^5 cells/mL, and the plates incubated for 5 days at 35°C, 5% CO_2.

After confirming absence of contamination in all the cell control wells, the wells for each virus dilution were examined to count the number of wells per dilution showing CPE. Virus titer was calculated using the following method/s: 1) - Log_{10} $TCID_{50}$/mL = -1

- [(total CPE % / 100) - 0.5] x $\log_{10}$ (correction factor), the Spearman-Kärber method (83, 84) and 2) Log_{10} TCID_{50}/mL = -[(total # of CPE wells/# of wells per dilution) + 0.5] x log dilution factor, a simplified version of the Spearman-Kärber method (85).

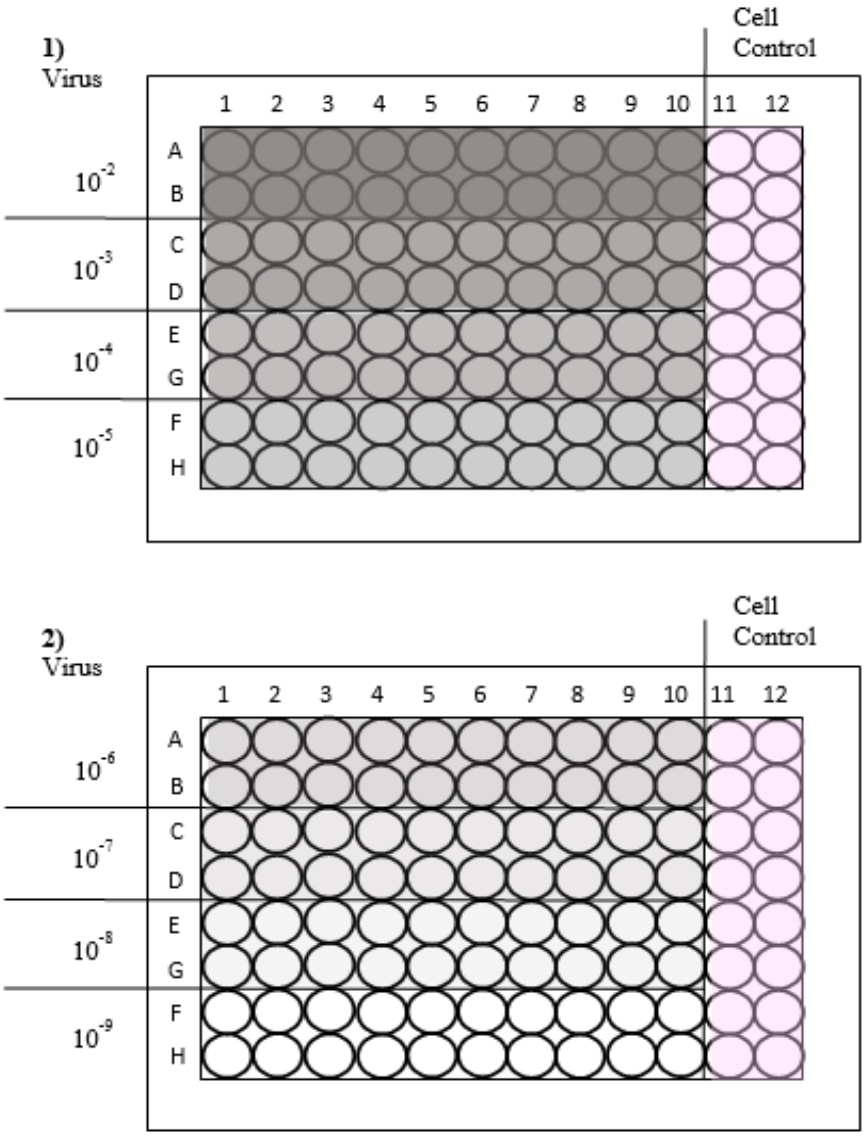

Figure 2.1 Layout of Sabin PV1 titration plates 1 and 2. Virus dilutions 10^{-2} to 10^{-9} were added sequentially to two rows each, from columns 1 – 10; cell control were columns 11 and 12.

2.4 MN assay for PV1

2.4.1 MN assay - Overview

The MN assay used in this project was based on the Centers for Disease Control and Prevention's (CDC) validated "micro", serum-sparing version of the original neutralization assay (53). The specifics of this standardized MN assay was published in 2016 by Weldon *et al* (53). Assay procedures, including Sabin PV1 and HEp-2 cell growth, were only adapted to accommodate local biosafety requirements, scale of testing, and experiments required.

Each experiment using the PV1 MN assay requires a week to complete. Briefly, the steps included serum dilution, plate staining, reading, and analysis of the data; however, prior to serum addition, the virus had to be cultured and titered, and HEp-2 cells needed to be grown to desired confluency.

2.4.2 MN - Experiment

HEp-2 cells were cultured 24 to 72 hours ahead of the MN experiment to ensure confluent flasks on the day of the experiment. Serum samples and controls were pre-organized according to a 96-well plate map to match their location in the deep-well dilution microplate and track their location on the assay plates. A complete experiment tested 96 sera in 24 assay plates, plus one virus back titration plate, and one cell control plate. The day of the experiment was marked as the day the virus was added to the assay plates.

2.4.2.1 MN - Heat inactivation of serum

A 40 µL aliquot of each serum sample was loaded into the deep-well microplate; the plate was sealed and the serum samples were heat-inactivated in a water bath at 56°C for 30 min. The heat-inactivated serum was stored at 4°C in the covered microplate pending addition to the assay plates. Before addition of serum samples, the MN assay plates were prepared by adding 25 µL MEM with 2 % FBS to each well of the serum plates and the virus back titration plate, and 50 µL MEM with 2 % FBS to each well of the cell control plates. The heat-inactivated serum samples in the deep-well plate were diluted 1:4 with 120 µL of MEM with 2 % FBS and 25 µL of each diluted serum sample was transferred in triplicate to row A of the assay plates for a 1:8 dilution of the serum (Figure 2.2). Unless the experiment was completed on the same day, the assay plates were stacked, wrapped in plastic wrap and stored overnight at 4°C, for a maximum of 24 hours.

2.4.2.2 MN - Serum processing

Starting at row A of the MN assay plates, using a multichannel pipette, serum samples were serially diluted from 1:8 to 1:1024 (Figure 2.2); 25 µL of diluted sample were discarded from row H for a final volume of 25 µL in all the wells. Stock PV1 was diluted in MEM with 2 % FBS for a working virus titer of 100 $TCID_{50}$/25 µL (4 x 10^3 $TCID_{50}$/mL). Starting at row H (the highest dilution of serum) using a multichannel repeater pipette, 25 µL of working virus suspension was added to the diluted serum samples in the MN assay plates. For the virus back titration plate, 3 ten-fold dilutions were made from the 100 $TCID_{50}$/25 µL, for 10, 1, and 0.1 $TCID_{50}$/25 µL virus dilutions.

Each virus dilution was added to two full rows (25 µL/well), starting with 100 $TCID_{50}$ in rows A and B, then C and D, E and F, and G and H for the subsequent dilutions (Figure 2.2).

All the plates were wrapped in plastic wrap and incubated at 35°C in 5 % CO_2 for 3 hours to allow any neutralizing antibodies to bind to virus. During this neutralizing step, a fresh HEp-2 cell suspension at 3 x 10^5 cells/mL in MEM with 10% FBS was prepared. After the 3-hour incubation, 25 µL of cells were added to every well of every plate; lastly, the plates were wrapped in plastic wrap and incubated at 35°C, 5 % CO_2 for 5 days.

Following the five days incubation, all media in the assay plates was aspirated and discarded, and the plates were stained by adding 50 µL 0.05% (w/v) crystal violet (Sigma Aldrich, Oakville, ON, CA) [prepared in 0.5% Tween 20 (Fisher Scientific, Ottawa, ON, CA) and 24% EtOH], and incubating at room temperature for at least 45 minutes. The dye was then aspirated and the plates washed 4 times using a Vaccu-Pette/96 (Sigma Aldrich, Oakville, ON, CA), filling the wells with 250 to 300 µL of distilled water per wash. The plates were left in the BSC to dry overnight.

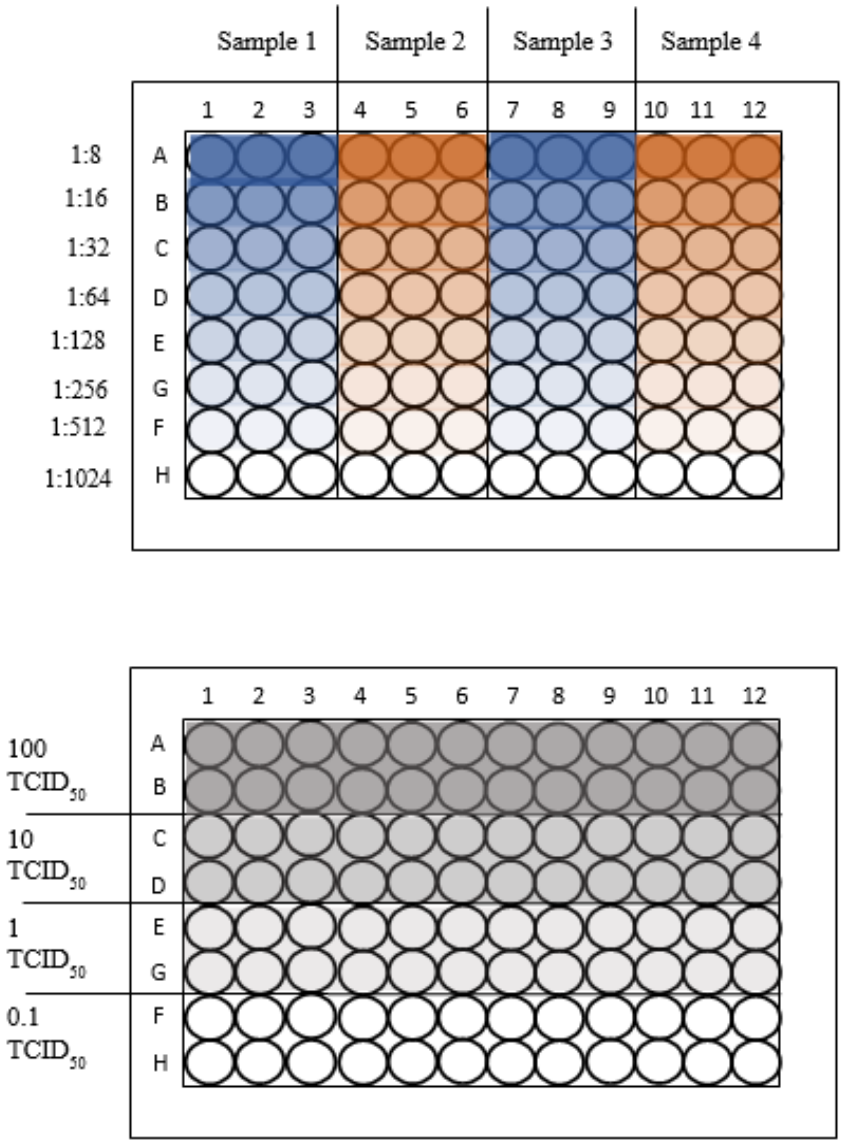

Figure 2.2 Layout of MN assay plates.
Top plate shows samples loaded in triplicate. Bottom plate shows loading of virus dilution 100 – 0.1 $TCID_{50}$ in back titration plate.

2.4.2.3 MN - Quality control analysis

Quality control (QC) for each MN experiment was verified before extracting raw data and calculating PV1 neutralization titers. The cell control plate was visually assessed for the presence of CPE in any of its wells. A cell control plate showing any CPE would disqualify the experiment due to suspicion of cross-contamination or unexplained cell death. For a control plate to be valid, the requirement was absence of CPE.

Next, the working titer of the virus suspension used in the experiment was confirmed using the back titration plate. Virus titer was calculated by counting the number of wells with CPE in the virus back titration plate "***S***", and using the following formula:

$$\boldsymbol{Log}\mathbf{TCID_{50}} = \boldsymbol{S - 0.5}$$

The targeted virus titer was 2.0 log base 10 (2.0 $\log_{10}$), corresponding to 100 $TCID_{50}$. For a back titration plate to be valid, the calculated virus titer had to fall between 1.5 and 2.5 $\log_{10}$, corresponding to 32 to 320 $TCID_{50}$, respectively. The virus titers calculated from the back titration plates were monitored over time for each MN experiment.

Lastly, for QC, control sera were included in all MN experiments, and their titers were monitored over time. The control sera comprised in-house reference sera (IHRS) established by repeated measurement of their PV1 neutralizing antibody titers, the WHO's 3rd international anti-poliovirus reference standard (69), and a commercial human serum deficient of immunoglobulins (Human Serum Minus IgG, IgA, and IgM, Sigma Aldrich, Oakville, ON) (86). For a control serum to be valid, the standard

deviation (SD) for its inter-assay neutralization titer over time could not exceed ±1.0 log base 2 (log_2).

2.4.2.4 MN - Calculating neutralization titers

The number of wells positive for neutralization, shown by absence of CPE and stained purple (Figure 3.4), were summed for each triplicate test serum, and the neutralization titers calculated using the following formula:

Neutralization titer = (no. of positive wells / 3*) + 2.5

(* where this is the number of test serum replicates)

The upper limit of detection (ULD) and lower limit of detection (LLD) neutralization titers are 10.5 and 2.5 respectively, where a titer of 2.5 was considered negative.
The reciprocal titers were calculated through exponentiation of the base 2 (the dilution factor), by the neutralization titer, as follows:

Reciprocal titer = $1:2^{\text{neutralization titer}}$

The cutoff for positivity was defined as reciprocal titers of anti-PV1 neutralizing antibodies that were ≥1:8. As per the ULD of the assay, 1:1448 was the highest reported reciprocal titer.

2.5 MN assay validation

Prior to testing any clinical specimens for evaluation of the seroprevalence of PV1 antibodies in Nova Scotia, the MN assay was validated for accuracy with the help of the CDC laboratory (53). Using 52 de-identified serum samples, the results of the in-house MN assay were compared with those of the CDC. The 52 samples included a collection

of IHRS and a group of residual sera from the Roy Romanov Provincial Laboratory (RRPL), Saskatchewan Health Authority. The serum from the RRPL were among their repository of anti-polio positive and negative samples; they were shared with us for validation purposes only. All the samples are de-identified and not linked to any identifying or confidential information.

The ***Wilcoxon rank sum test*** and ***Spearman's rank correlation test*** were used to compare anti-PV1 neutralization titers during validation. The null hypothesis for the Wilcoxon test is "no difference" between results by both laboratories. As such, the requirement was no statistical significance for acceptability of the validation. The null hypothesis for the Spearman test is "correlation" between results by both laboratories, and as such, the requirement was a significant positive correlation for acceptability of the validation.

2.6 Generating negative serum using Immunoadsorption (IAS)

In addition to positive control sera, there was a need for non-immune (negative control) serum to establish and validate performance of the PV1 MN assay. The ability to find negative sera in the highly vaccinated Nova Scotian population is difficult. Attempts to obtain negative sera from different sources were unsuccessful. Alternatively, it was decided to attempt to generate negative serum in-house using IAS. IAS is a blood-purification technique that removes pathogenic antibodies as part of the treatment of immunological or immunoglobulin-mediated autoimmune disease (71-73). IAS columns were made up of protein A magnetic beads coupled to the crystalizable fragment (Fc) of virus-specific MAbs. The antigen-binding fragment (Fab) of MAbs then captured the

target virus, and in turn the virus binds virus-specifc antibodies in serum. Following magnetic immobilization, the serum can be removed, reducing the concentration of virus-specific antibody immobilized by the bead/MAb/virus complex.

In this study, IAS was used to remove of PV1-specific antibodies in immune serum samples, and anti-RuV titers were used as a negative analyte control. A parallel series of experiments were performed to remove anti-RuV antibodies from immune sera to determine if the IAS methodology could be reproducible for other VPDs. In the anti-RuV IAS, anti-PV1 titres were used as the negative analyte control. Both experiments were prepared separately to avoid MAbs, virus or serum cross-contamination.

2.6.1 IAS - Preparation of MAbs

IAS columns used protein A magnetic beads, monoclonal antibodies (MAbs) with strong binding affinity to protein A, and a concentrated virus suspensions. For IAS removal of anti-PV1 antibodies, the MAb used was anti-poliovirus 1 mouse monoclonal antibody (HYB 295-15, BioPorto Diagnostics A/S, Hellerup, Denmark). For IAS removal of anti-RuV antibodies, anti-rubella mouse monoclonal antibody (MAB925, EMD Millipore, Burlington, MA, USA) was used. Both MAbs were used at a concentration of 1 mg/mL.

2.6.2 IAS - Preparation of protein A magnetic beads and MAbs coupling

Protein A conjugated magnetic beads (SureBeads, Bio-Rad Laboratories, Hercules, CA, USA) were the affinity matrix of choice for IAS given their ability to capture the Fc portion of MAbs and their intrinsic property of being immobilized using a

magnetic rack. Such immobilization simplifies antibody capture steps and serum recovery. This method also reduces serum dilution, by minimizing the dead volume.

A vial of protein A magnetic beads was thoroughly re-suspended, and 100 µL (1 mg) of beads were transferred to 1.5 mL Eppendorf tubes. A magnetic rack was used to immobilize the beads, and the storage buffer was removed. Then, the beads were washed 3 times with 1 mL phosphate buffered saline with 0.1% Tween 20 (PBS-T), with magnetic immobilization and resuspension at each step. For a final concentration of 10 µg of MAbs per bead tube, 10 µL of a 1 mg/mL solution of MAbs were added to 190 µL of PBS in each tube. After removing the third PBS-T wash, the beads were re-suspended in the MAbs, and incubated for a mimimum of one hour, with slow mixing using a tube rotator at 4 to 6 RPM for gentle agitation. Following incubation, the MAb-coupled beads were ready for chemical cross-linking.

First, the MAb-coupled beads were washed twice with 1 ml of 0.2M sodium borate pH 9.0, the binding buffer. For cross-linking, the MAb-coupled beads were re-suspended in 25mM dimethyl pimelimidate dihydrochloride (DMP) in fresh 0.2M Na borate and the reaction mixture was incubated for 30 minutes at room temperature. Then, 400 µL of 0.2M triethanolamine (TEA) were added to the DMP, and incubated for an additional 5 minutes at RT. The tube was magnetized to remove the DMP/TEA cross-linking buffer. The process of cross-linking with DMP and TEA was repeated 2 more times, before quenching the beads with 1 ml of 50M diethanolamine (DEA) for 5 minutes at room temperature. The tube was magnetized to remove the reagent, and quenching was repeated one more time. Finally, the beads were washed 3 times with PBS-T as described earlier, and stored in the third wash at 4°C pending viral capture.

2.6.3 IAS - Virus preparation and viral capture

The viruses used for IAS were undiluted Sabin PV1 stock or the M33 strain of RuV (ATCC VR-315™). RuV was cultured in-house and the suspension was used undiluted. Briefly, the RuV stock was propagated on 85-90% confluent monolayers of Vero cells (ATCC VR-81) in MEM with 10% FBS were prepared in three T-150 culture flasks at 37°C in 5% CO_2, two flasks for the virus culture and one flask to serve as a negative, uninfected control. On the day of virus inoculation, the cells were washed with 10 ml of serum-free MEM. Then, 3 ml of MEM with 5% FBS and Pen/Strep (10 U/mL of Pen, and 10 µg/mL of Strep) antibiotic supplementation were added to each flask. While keeping the frozen vial of RuV on ice, 250 µL of warm serum-free MEM media were dispensed and mixed with the virus material, then immediately all the thawed liquid was collected and inoculated in to the culture flasks. For the uninfected flask, 250 µL of serum free media were added. All the flasks were incubated at 35°C, 5% CO_2 for 1 hour, after which 12 mL of MEM with 5% were added to each flask. The flasks were incubated at 35°C, 5% CO_2 for up to 7 days until CPE was observed. On day 3, 5 mL of media were substituted from each flask with fresh MEM with 5%. Upon observation of rubella CPE, the layer of virus/cell debris was scraped and collected in 50 mL conical tubes, and frozen at -80°C. After 3 freeze/thaw cycles, the culture material was centrifuged at 1000 *x g* for 10 minutes at 4°C to clarify the harvested virus. The virus suspension was frozen undiluted at -80°C in multiple aliquots for single time use.

To facilitate viral capture by the MAbs-coupled beads, high titer, undiluted virus suspensions were prepared in trisaminomethane hydrochloride (Tris-HCl) 50mM pH 8.0, sodium chloride (NaCl) 150mM, and EDTA 2mM. For viral capture, 500 µL of virus

suspension were added to the coupled beads and incubated overnight at 4°C with slow rotation. The following day, unbound virus was safely removed and discarded. The bead complex (bead/MAb/virus) was washed 3 times with PBS-T, and stored in the third wash at 4°C until used for IAS of immune serum.

2.6.4 IAS - Processing of immune serum

Each tube of bead complex was magnetized to remove the PBS-T storage buffer. Then, 500 μL of immune sera was added and incubated overnight at 4˚C with slow rotation. After magnetic immobilization to remove the serum, the IAS incubation was repeated using a fresh bead complex up to 7 times. A fraction of serum was taken from step aliquots to measure anti-PV1 and anti-RuV titers.

Antibody titres to PV1 were determined using the MN assay. Neutralization titers were characterized as low (≤5.5), moderate (>5.5 and ≤8.8), and high (>8.8). Serum aliquots were also screened using the Architect Rubella IgG assay (Abbott Diagnostics) by the Microbiology Division, Departmental of Pathology and Laboratory Medicine, Nova Scotia Health Authority (NSHA) (87). The Architect Rubella IgG titers are interpreted as follows: <8.0 IU/mL is negative (non-immune); 8.0 to 15.0 IU/mL is considered in the grey zone; >15 IU/mL is positive (immune).

2.7 Seroprevalence study samples

Serum samples used for determination of the seroprevalence of PV1 antibodies were previous collected as part of a project lead by Dr. Todd Hatchette for the serosurveillance of zoonotic infections among residents of Nova Scotia, entitled:

"***Emerging Zoonotic Infections in Nova Scotia: How Many Humans Have Been Infected***?" (88). This collection consists of 1,850 anonymized residual sera originally submitted for diagnostic testing between May 1 and August 30, 2012 at regional Nova Scotia District Health Authority (DHA) laboratories across the province. These residual sera were originally submitted for routine diagnostic testing as part of routine or prenatal screening of healthy individuals. Although a convenience sample collection, it is geographically representative of the whole province. Sera are stratified by age, gender, DHA and Nova Scotia Provincial Authority Management Zones, also known as health zones (Figure 2.3) (89).

The original sample collection was proportionate to the Nova Scotia population, and grouped in 10-year age bands for subjects 10 – 59 years of age, and one 5-year age band for subjects 60 – 64 years of age (88). For the purpose of this research focus on PV, the age bands were widened and samples stratified to 10 – 29, 30 – 49, and 50 – 64 years, while maintaining representation of the population in Nova Scotia by conserving original sampling proportions across DHA, gender, and 10-year/5-year age groups. The age bands were chosen to allow comparison of PV titers between different age groups, and representation of Nova Scotia to allow comparison between different parts of Nova Scotia.

It was important to ensure that the sample size tested would allow the detection of statistically significant differences (p-value <0.05) between PV1 seroprevalence rates among age groups. Based on various literature reports of PV1 seroprevalence (62, 63, 90), as well as polio vaccination coverage rates Nova Scotia and Canada (12, 13), it was

determined that 215 samples per age group are required to detect any difference with a power of 80% and a confidence level of 95% (alpha level 0.05).

2.8 Statistical analysis

Statistical analysis was carried out using R software (R Foundation for Statistical Computing, Vienna, Austria) (91), and *p*-values <0.05 were considered statistically significant. Student's one-sample *t* test was used to evaluate virus and control sera titers over time. Validation and proficiency for conducting the MN assay were assessed using the Wilcoxon rank sum test and Spearman's rank correlation test.

Study population descriptive analysis was performed using gender and median age for age groups by zone. Seroprevalence proportions and 95% confidence intervals (CI) were calculated for age groups and zones. One-way ANOVA was used to compare geometric mean titers (GMT) and 95% CI between age groups and zones. Multivariate logistic regression was used to identify factors associated with seroprevalence.

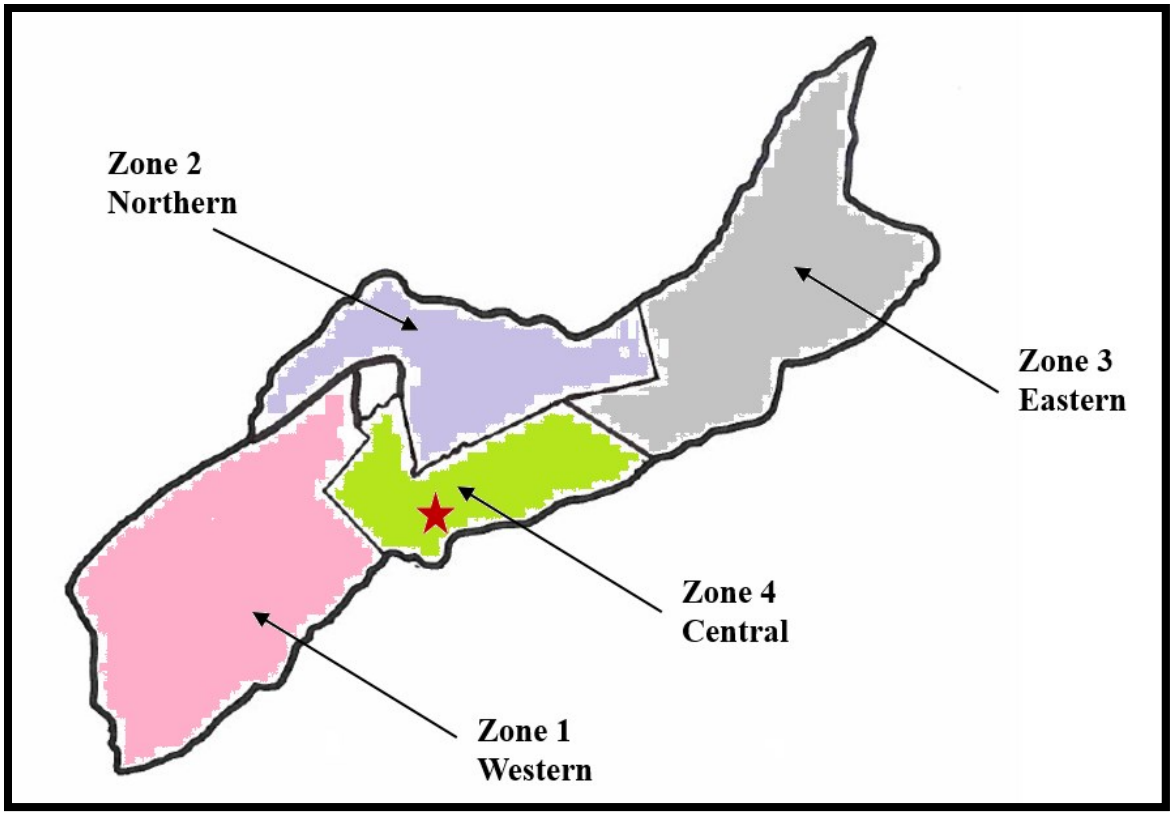

Figure 2.3 Nova Scotia Health Authority. Health zones in Nova Scotia identified by name and number.

CHAPTER 3 RESULTS

3.1 Sabin PV1 titration and back titration

Two batches of Sabin PV1 were cultured and used throughout this project. Based on culture date, they were identified as Sabin PV1 Lot: 20170116 and Lot: 20180217. Both virus batches were monitored over time. Each time a lot was diluted to 100 $TCID_{50}$/25 μL (working titer) for use in a MN experiment, data from the back titration plate was accrued for quality purposes. With each use over time, both batches were within allowable virus titer range of the MN assay (Table 3.1, Figure 3.1).

Table 3.1 Mean titer of Sabin PV1 lots 20170116 and 20180217

Sabin PV1 Lot	n	$TCID_{50}$ [1] Mean (95% CI)	Log10 [2] Mean (95% CI)
20170116	14	55.1 (51.3, 58.9)	1.7 (1.7, 1.8)
20180217	15	61.8 (59.4, 64.2)	1.8 (1.8, 1.8)

[1] Must be between 32 and 320 $TCID_{50}$; [2] Must be between 1.5 and 2.5 Log_{10}.

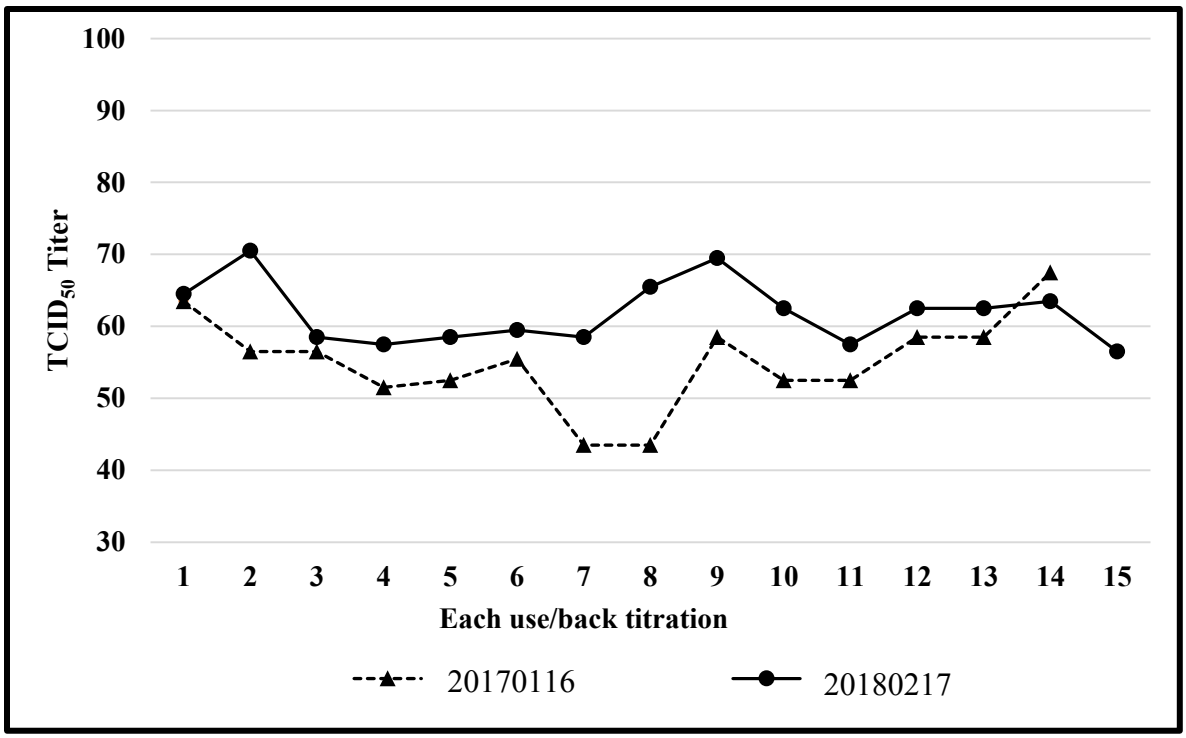

Figure 3.1 With each use over time, the $TCID_{50}$ titers for the two lots of Sabin PV1 fell within the qualification range of 32 and 320 $TCID_{50}$. The y-axis shows the $TCID_{50}$ titer, and the x-axis shows the sequence number of the back titration.

3.2 MN - Validation

The validation serum set (n=52) was tested by our in-house method and compared to results obtained by the CDC reference laboratory in Atlanta, GA. In addition to comparing neutralization titers for the entire set (n=52), a subgroup of samples with a neutralization titer ≤4.8 (n=17) by either laboratory were analyzed separately to focus on performance at the lower end of detection for the assay. There was no significant difference (*p*-values > 0.05) between the neutralization titers reported by the two laboratories, for both overall and low titer samples (Table 3.2). Furthermore, the paired neutralization titer results showed a significant degree of correlation (Table 3.3, Figure 3.2). Collectively, this data confirmed the accuracy of the MN assay for use in testing the study samples.

Table 3.2 The Wilcoxon rank sum test is not significant confirming no difference between the in-house neutralization titers when compared to those reported by the Centers for Disease Control and Prevention (CDC).

Validation samples	n	Difference estimate (95% CI)	*p*-value
Subgroup at lower end of the assay*	17	-0.67 (-1.30, 6.87e-05)	0.1014
All	52	0.30 (-0.67, 1.70)	0.5508

* Neutralization titers ≤4.8 by either laboratory.

Table 3.3 The Spearman's rank correlation coefficient confirms strong correlation between the in-house neutralization titers when compared to those reported by the Centers for Disease Control and Prevention (CDC).

Validation samples	n	Spearman's *rho*	*p*-value
Subset at lower end of the assay	17	0.871	5.237^{-6}
All	52	0.964	$< 2.2^{-16}$

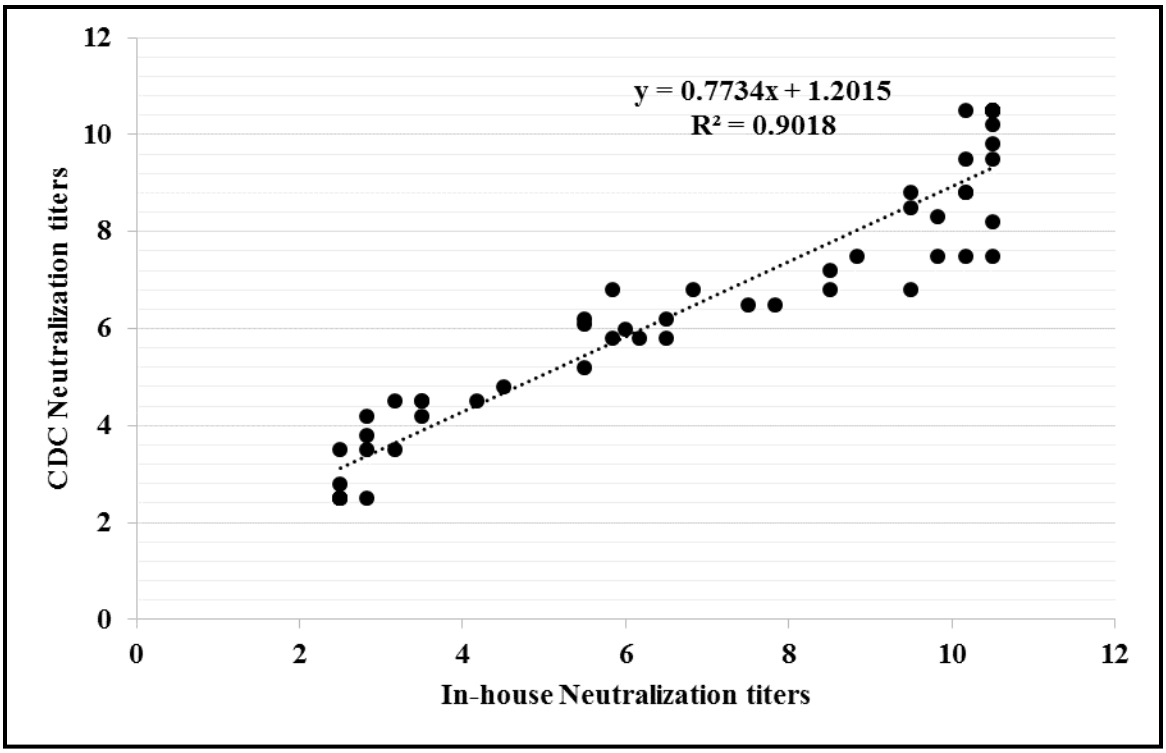

Figure 3.2 Strong positive correlation between titers of the validation panel sera tested in-house and at the Centers for Disease Control and Prevention (CDC). The y-axis represents the neutralization titer results by the CDC, and the x-axis represents the in-house neutralization titer results.

3.3 MN - Monitoring positive and negative control sera

Six main control sera were used throughout the MN experiments, and their titers were monitored over time. Of note, these six control sera were included among the 52 validation panel sera. The control sera included 4 IHRS (3 anti-PV1 positives and 1 anti-PV1 negative), the WHO's 3rd international anti-poliovirus standard (69), and the commercial negative. As shown in Table 3.4 and Figure 3.3, the performance of the control sera over time fell within the allowable qualification parameter set at neutralization titers SD over time not exceeding ± 1.0 $\log_2$. The two negative sera at the assay's LLD (IHRS-3 and commercial negative) and the one high positive serum at the ULD (IHRS-2) did not show any deviation over time, whereas the other three positive control sera (IHRS-1, IHRS-4, and WHO standard) showed some variation as verified by their 95% CI and SD (Table 3.5).

Table 3.4 The control sera used with the microneutralization assay all fell within the qualification requirement of neutralization titer standard deviation ±1.0 log_2 over time.

Control	n	Neutralization titer [1] Mean (95% CI)	Neutralization titer SD [2]	Reciprocal titer [3] Mean (95% CI)
IHRS-1	18	6.1 (5.8, 6.4)	0.6	73 (59, 87)
IHRS-2	19	10.5 (10.5, 10.5)	0.0	1448 (1448, 1448)
IHRS-3	12	2.5 (2.5, 2.5)	0.0	6 (6, 6)
IHRS-4	10	6.6 (6.3, 6.8)	0.4	98 (80, 115)
WHO STD	10	8.5 (8.1, 8.8)	0.5	370 (288, 452)
Commercial Negative	14	2.5 (2.5, 2.5)	0.0	6 (6, 6)

IHRS = In-house reference serum; WHO STD = WHO standard serum; [1]Calculated by dividing the number of neutralized wells by 3 and then adding 2.5; [2]SD = standard deviation, should be within ±1.0 log_2; [3]Calculated using $2^{\text{Neutralization titer}}$

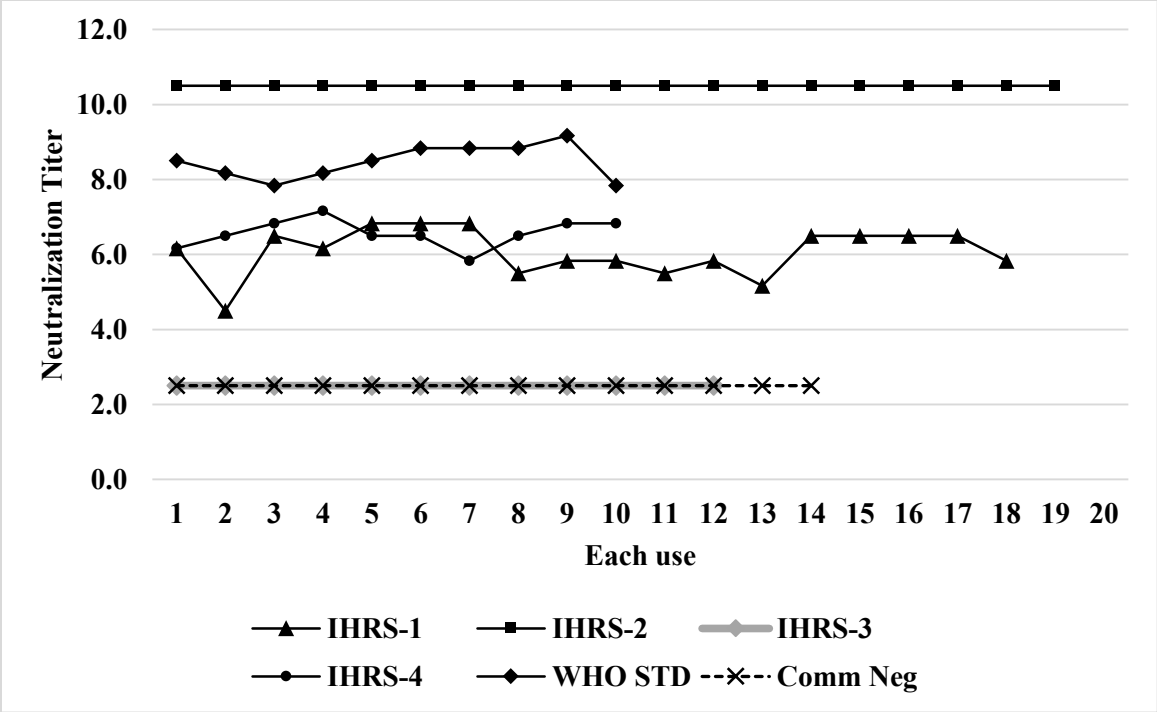

Figure 3.3 Neutralization titers of the six control sera used with the microneutralization assay fell within the qualification requirement of neutralization titer standard deviation ±1.0 log_2 over time, even when their titers varied.
The y-axis shows the calculated neutralization titer, and the x-axis shows the sequence number of the control use. IHRS = In-house reference serum; WHO STD = WHO standard serum; Comm Neg = commercial negative serum.

3.4 PV1 seroprevalence and geometric mean titers in Nova Scotia

A total of 648 anonymized sera were selected and tested using microneutralization to measure individual anti-PV1 titers and determine the GMT of antibodies to PV1 among different age strata and health zones across Nova Scotia.

The sera were selected randomly, but preserved the weighted distribution adopted in the original 1,850 sample collection (88) from the DHAs representing Nova Scotia. As the numbers per age group per DHA were too small for analysis by DHA, all analysis was performed using the distribution of samples by age and Nova Scotia health zones 1 to 4 (Table 3.5). The three age bands, 10 – 29, 30 – 49, and 50 – 64 years were equally represented (Table 3.5).

Males and females were equally distributed among the zones, both overall and by age group (Table 3.6). The median age per age band per zone demonstrated similar age distribution in the various groups, with the exception of the 10 – 29 years cohort who were slightly older in zone 4 compared to the other zones. Seroprevalence rates were similar among males and females (Table 3.7).

Table 3.5 Sample breakdown by Age, Nova Scotia DHA, and Zone

Zone No. (name)	DHA#	Age group (in years)			Total # for DHA	Total # for Zone
		10 - 29	30 - 49	50 - 64		
1 (Western)	1	20	21	27	68	232
	2	23	23	25	71	
	3	31	30	32	93	
2 (Northern)	4	14	14	13	41	84
	5	6	5	7	18	
	6	8	8	9	25	
3 (Eastern)	7	8	8	9	25	97
	8	25	21	26	72	
4 (Central)	9	81	86	68	235	235
Total		216	216	216	648	648

Table 3.6 Demographics of the study population

	Zone 1	Zone 2	Zone 3	Zone 4	Total
	n = 232	n = 84	n = 97	n = 235	N = 648
Gender					
Male (%)	49.6	50.0	48.5	50.2	49.7
Age group distribution (n)					
10 - 29 years	74	28	33	81	216
30 - 49 years	74	27	29	86	216
50 - 64 years	84	29	35	68	216
Median age					
10 - 29 years	19	18.5	21	29	20
30 - 49 years	41	39	43	39	39.5
50 - 64 years	60	60	60	59	59.5

Table 3.7 Similar seroprevalence of anti-PV1 antibodies among males and females in Nova Scotia.

	Males	Females	*p*-value
	n (% positive)		
All ages	290 (90.1)	288 (88.3)	0.527
10 - 29 years	91 (85.8)	84 (76.4)	0.084
30 - 49 years	102 (95.3)	101 (92.7)	0.569
50 - 64 years	97 (89.0)	103 (96.3)	0.066

The seroprevalence of antibodies within each age group that were above the proposed threshold for protection ≥1:8 for polio was then determined (47, 48). Seroprevalence was assessed by health zone, by age group, or overall (Figure 3.5). Overall, the seroprevalence rate in Nova Scotia was 89.2% (Figure 3.5). When comparing health zones (all ages combined in each zone), the seroprevalence rates were not significantly different ($p = 0.597$). However, provine-wide, the percentage of individuals in the youngest age group that had a titer of ≥1:8 was significantly lower than the older age groups ($p < 0.001$). While not significant, the lowest seroprevalence, 75.0%, was observed in the youngest age group (10 to 29 years) in Zone 1, while the highest rate was 97.7% in the middle age group (30 to 49 years) in Zone 4 (Figure 3.5). Focusing on the age groups and by health zones in the province, the lowest seroprevalence rate was always among the youngest age group (10 to 29 year olds), while the other two age groups (30 to 49 and 50 to 64 years) were both higher than the youngest age group in all areas, without any pattern between them (Figure 3.5). Zone 1 was the only zone for which all the seroprevalence rates, all ages combined and by age group, fell below 90.0%.

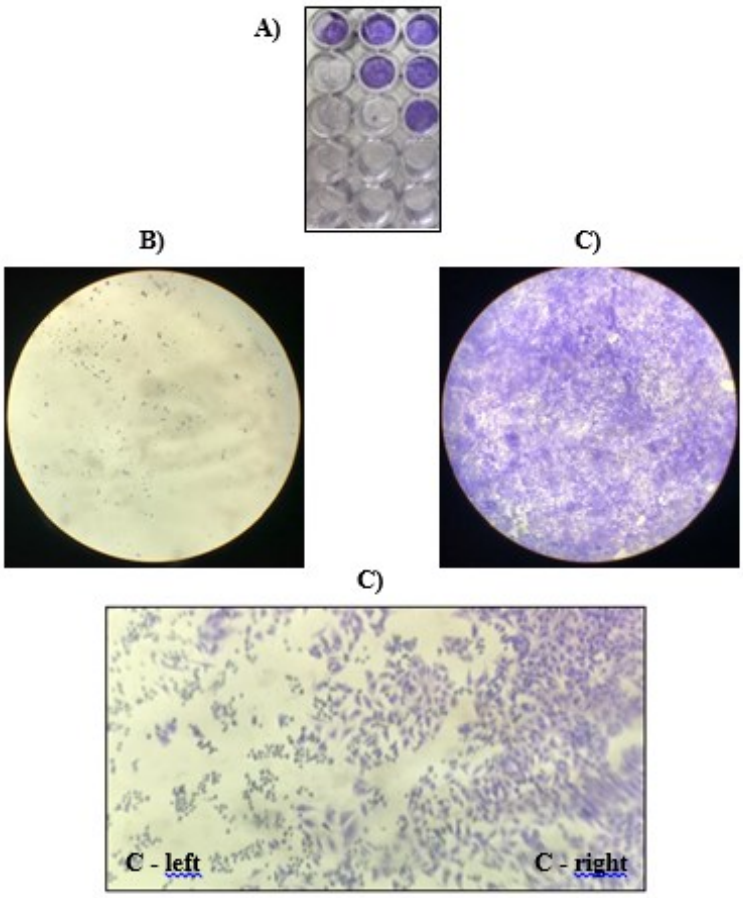

Figure 3.4 Appearance of cytopathic effect (CPE) on HEp-2 cells.
A) Purple wells show stained cells (no CPE), transparent wells show CPE; B) the appearance of a well with CPE under the microscope; C) the appearance of a well with no CPE under the microscope; C) a combination of CPE (left) and no CPE (right) under the microscope.

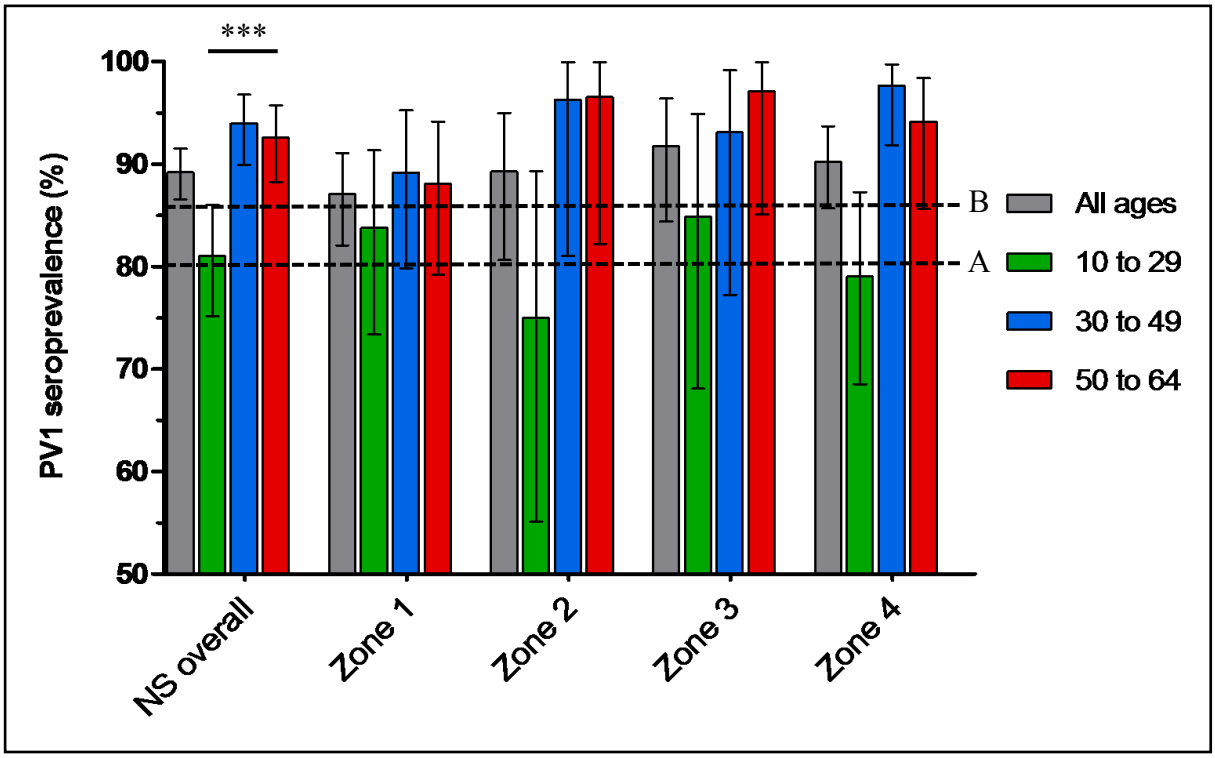

Figure 3.5 Seroprevalence of anti-PV1 in NS shows no apparent risk to PV1. Seroprevalence of anti-PV1 antibodies in the youngest age group was significantly lower than the older age groups (***p<0.001); there was no significant difference (p=0.597) between seroprevalence rates among all ages of each health zone (grey bars under each zone).
Dotted lines A, at 80%, and B at 86%, are the threshold range for herd immunity against poliovirus; NS = Nova Scotia.

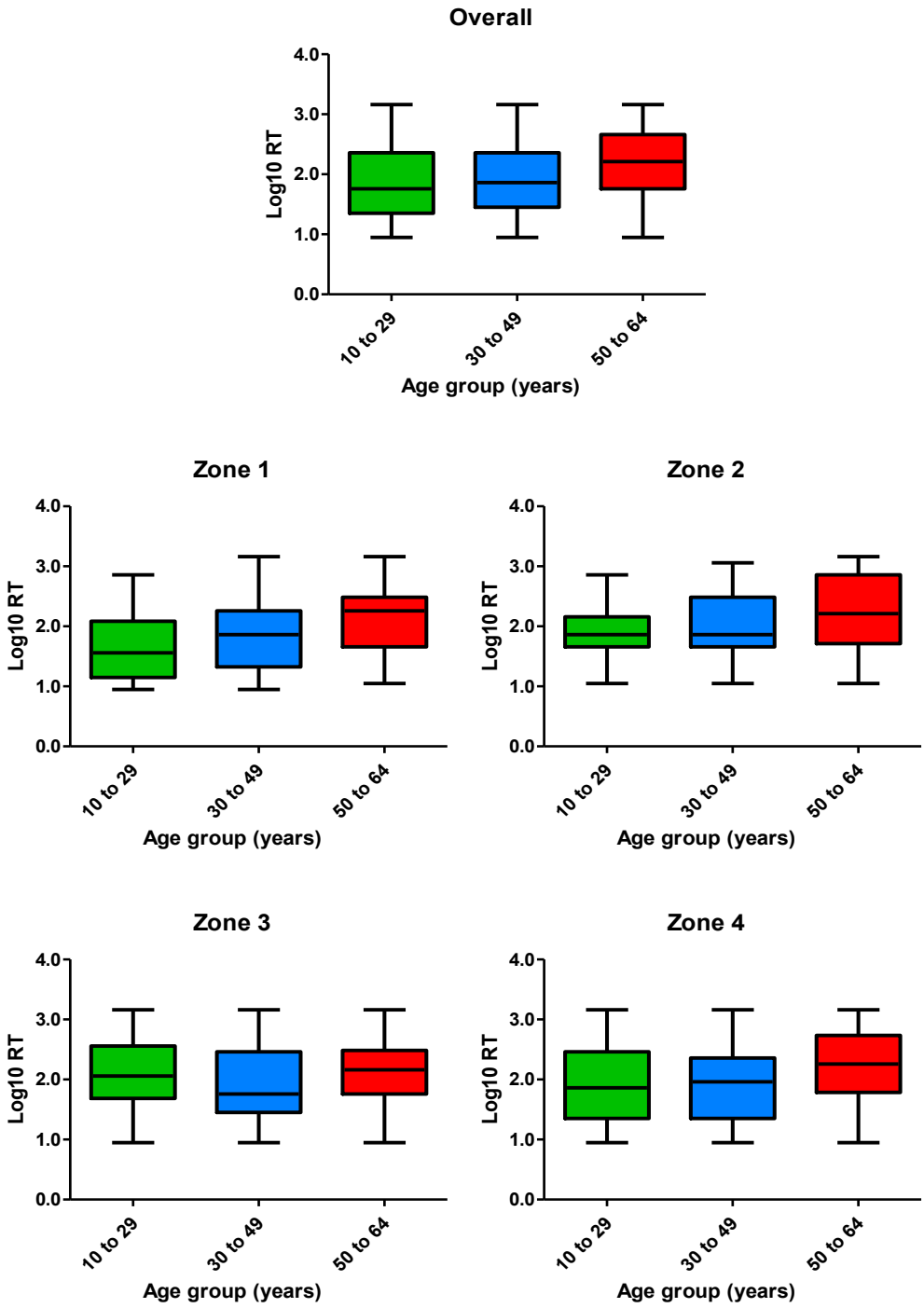

Figure 3.6. Distribution of the seroprotective log_{10} reciprocal titers (RT) of anti-PV1 antibodies among different age groups, province-wide and by healthcare zone in Nova Scotia.

While not significant, the older age group had higher anti-PV1 titers than the other two age groups in Nova Scotia and in each health zone, with the median always falling within the upper half of the titer spectrum measured by the MN assay (Figure 3.6). In zones 1 and 2, upper quartile titers of the younger age group did not reach the assay's ULD. Zones 1 and 4 had a similar age group pattern as the province overall. In general, titers among the younger age group were skewed towards the assay LLD, titers among the older age group were skewed towards the ULD, and the middle age group titers varied between being similar to either the younger or older age group (Figure 3.6).

The GMTs for the three age groups and the four zones were also assessed (Figure 3.7-A). The GMTs of the three age groups were significantly different (p-value <0.001), due to the GMT of older age being much higher than the other age groups. The GMTs between zones were also significantly different as shown in Figure 3.7-B (p-value <0.05). However, when the data was further analyzed for the 578 seropositive samples, there was nearly equal distribution of reciprocal titers between age groups (Figure 3.8). Only a few outliers contributed to the increase in GMT for older age groups.

The multivariate logistic regression for seroprevalence (Table 3.8) showed that the middle and older age groups (30 to 49 and 50 – 64 years) in Nova Scotia are more likely to be seropositive against PV1, compared to the younger 10 – 29 years of age cohort. The odds of being seropositive are at least three times higher in the age groups 30 to 49 and 50 – 64 years, when compared to the 10 – 29 years or age. There was no significant difference in the likelihood of being seropositive across the four health zones (Table 3.8).

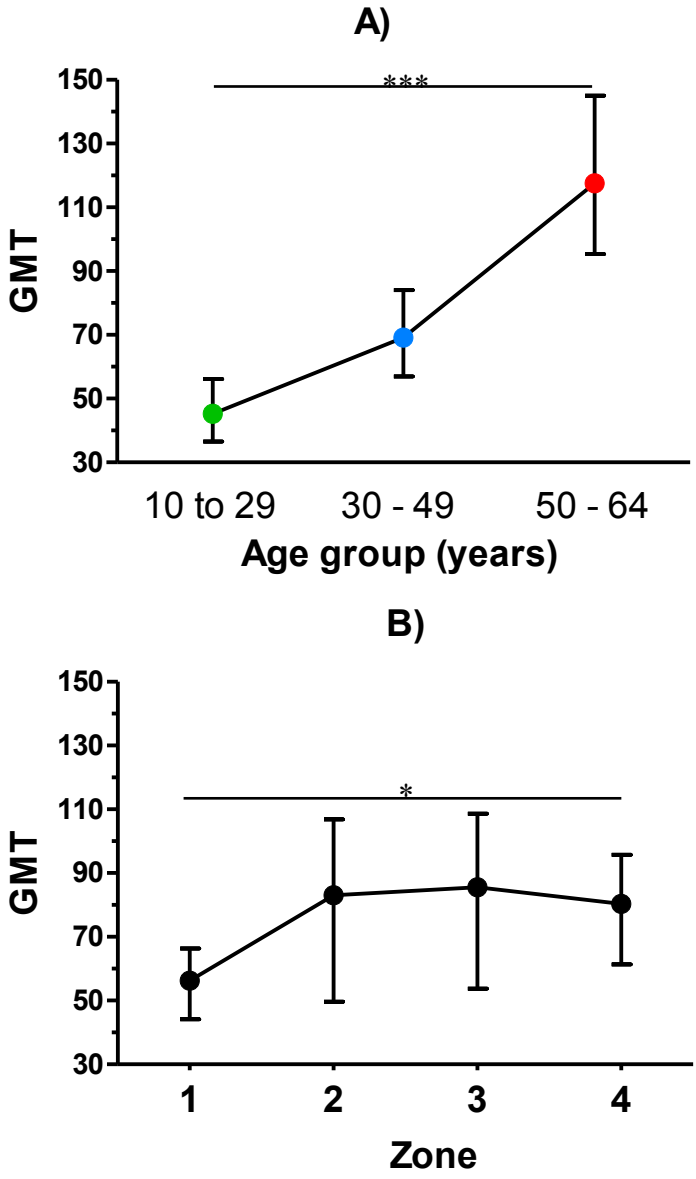

Figure 3.7 Geometric mean titers (GMT) are significantly different between age groups (*p<0.001) and health zones (*p<0.05).**
The horizontal lines indicate that the GMTs of all age groups (A), or all zones (B) were compared using the test statistic.

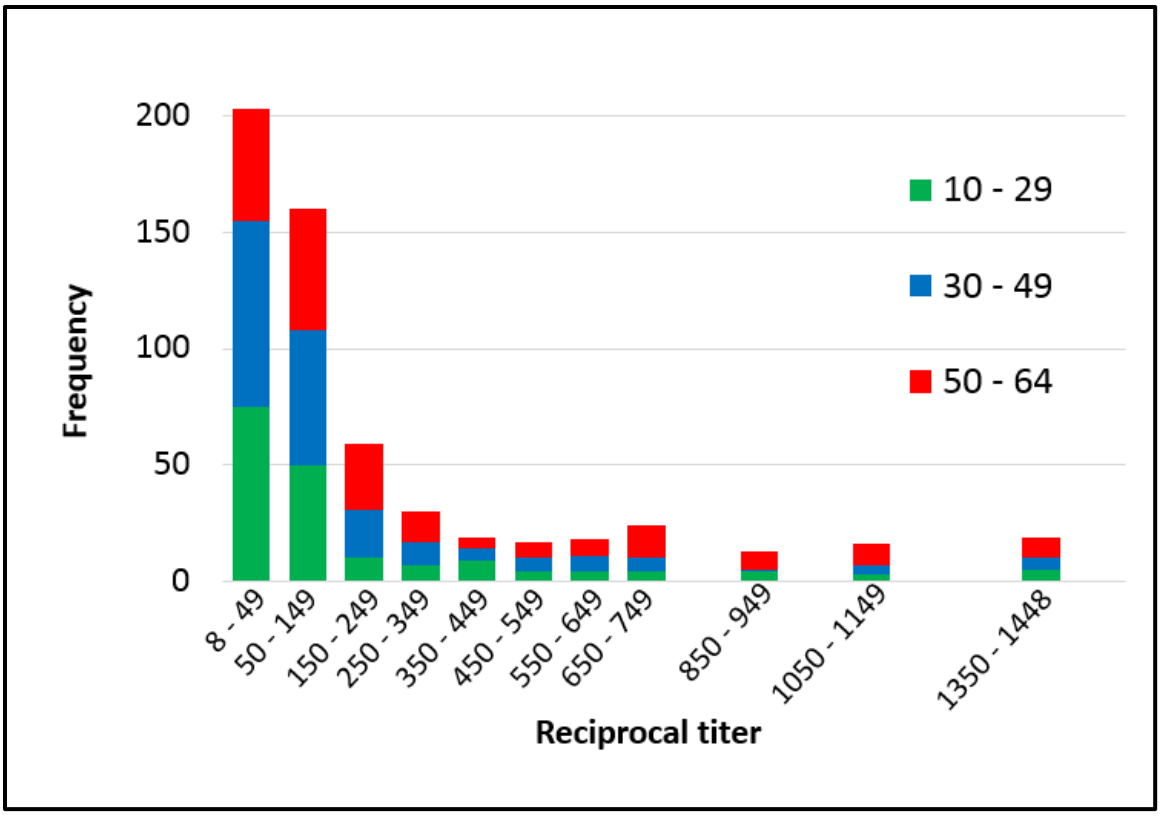

Figure 3.8. Frequency of reciprocal titers among seroprotected individuals was equally distributed amongst age groups.

Table 3.8 The final multivariate logistic regression model shows that age predicts PV1 seropositivity in Nova Scotia.

	Odds ratio (OR)	95% CI	*p*-value
Age groups (years)			
10 - 29	*Reference*		
30 - 49	3.67	1.96 – 7.37	2.410^{-7}
50 - 64	2.99	1.65 – 5.67	
Health Zones			
Zone 1	*Reference*		
Zone 2	1.27	1.59 – 3.00	
Zone 3	1.73	0.79 – 4.24	0.5064
Zone 4	1.42	0.79 – 2.59	

3.5 Generating negative control serum for MN using IAS

IAS was used to develop anti-PV1 negative serum. Following successful results adsorbing anti-PV1 antibodies, the approach was then further validated by adsorbing anti-rubella antibodies. Generating non-immune serum to two different viruses from paired aliquots of the same sample offered the opportunity to assess the impact of adsorbing specific antibodies to one virus on the titer of antibodies against the other virus.

IAS to remove PV1 antibodies was attempted on serum samples from 7 different individuals after confirming they were anti-PV1 positive. Of these 7 individual sera, 3 were also anti-rubella immunoadsorbed separately. Sample profiles in terms of neat anti-PV1 titer, neat anti-rubella titer, priming polio vaccine, and IAS conducted (anti-PV1 only, or both anti-PV1 and anti-rubella) are shown in Table 3.9. Sample selection for IAS was based on neat titers as well as availability of enough sample to conduct more than one IAS experiment. Samples B, C, and D were subjected to both anti-PV1 and anti-rubella IAS (Table 3.9). The separate and distinctly identified fractions from each of these samples that were either anti-PV1 or anti-rubella immuneadsorbed were also cross tested for the titers of the virus antibodies that were *not* adsorbed.

Table 3.9 Serum samples used for immunoadsorption

	A	B	C	D	E	F	G*
Neat titer							
anti-PV1	6.0	7.3	6.2	6.8	6.8	7.2	9.4
anti-rubella	-	46.1	306.8	76.3	-	-	-
IAS							
PV1 Abs	Y	Y	Y	Y	Y	Y	Y
Rubella Abs	N	Y	Y	Y	N	N	N
Polio vaccine priming	OPV	IPV	IPV	OPV	OPV	IPV	IPV

*Sample G was also IPV boostered; PV1= polio virus 1; IAS= immunoadsorption; Y= yes; N= no; OPV= oral polio vaccine; IPV= inactivated polio vaccine

Although extracorporeal IAS has been used to remove pathogenic antibodies from whole blood, there was no measure or experience around its potential for successfully adsorbing serum antibodies or how many overnight IAS incubations would effectively convert immune serum to non-immune serum for the virus captured in the affinity matrix. Since the affinity matrix was made in 1.5 mL tubes, 500 uLs of serum was considered an appropriate starting volume in relation to the tube capacity to facilitate adequate and constant mixing during incubation with slow rotation. With accumulation of results and experience after each IAS experiment, we increased or decreased the number of IAS incubations based on the rate of antibody titer decline for each sample. We also adjusted how often to collect a fraction of the serum to test and monitor the gradual decline of titer with more IAS incubations. Therefore, the number of titer assessments for each sample varied across the different IAS experiments. With this variation in testing time points per sample, the titers (including the neat titer) were plotted, and log trend lines were added to demonstrate the performance of IAS per sample (Figures 3.9 and 3.10).

Samples A and D, both OPV primed, were the only two samples to become completely anti-PV1 negative (Figure 3.9) with a neutralization titer $\leq$2.8 (negative); sample E, also OPV primed, showed a major decline in titer. Samples B and C, both IPV primed, showed minimal to moderate decline in their anti-PV1 titers. Lastly, Samples F (IPV primed) and G (IPV primed and boostered) lacked any indication of declining titer trend (Figure 3.9). To assess the effect of anti-PV1 IAS on rubella titers, the endpoint serum fractions of samples B, C, and D recovered after completing each anti-PV1 IAS experiment were then tested to assess their rubella titers to determine if they differed from the neat measurements. As shown in Table 3.10, the rubella titers remained stable,

with minimal variation as evident by the SD. The SD of the anti-rubella titers after anti-PV1 IAS were proportional to the original neat titer, the higher the neat titer the bigger the SD (Table 3.10). None of the rubella titers differed to the extent to change the positive/immune interpretation of the sample titer as defined by the ARCHITECT assay (<8.0 IU/mL is negative/non-immune; 8.0-15.0 IU/mL is grey zone; >15 IU/mL is positive/immune).

Anti-rubella IAS, was successfully performed on samples B, C, and D (Figure 3.10). Although sample C had a very high neat titer, it did show notable titer decline, but did not reach the assay grey zone or positivity cutoff (Figure 3.10 – A). Sample B became negative after 7 IAS incubations, and sample D titer declined prominently (Figure 3.10 – B). To assess the effect of anti-rubella IAS on PV1 titers, the endpoint serum fractions of samples B, C, and D recovered after completing each anti-rubella IAS experiment were then tested to assess their PV1 titers to determine if they differed from the neat measurements. The SD of the anti-PV1 titers after anti-rubella IAS fell within the allowable qualification for control sera neutralization titers over time of ± 1.0 $\log_2$ (Table 3.11), thereby indicating unchanged PV1 titers .

Table 3.10 Rubella titers are not affected by anti-PV1 immunoadsorption.

	Rubella virus titer after anti-PV1 IAS			
Sample: neat rubella titer (IU/mL)	**Anti-PV1 IAS #1**	**Anti-PV1 IAS #2**	**Anti-PV1 IAS #3**	**SD**
B: 46.1	46.6	43.6	37.6	*3.6*
C: 306.8	211.3	253.7	199	*42.2*
D: 76.3[1]	67.3	75.6	63	*5.6*

[1]Sample D with became negative anti-PV1 IAS; SD = standard deviation.

Table 3.11 PV1 titers are not affected by anti-rubella immunoadsorption.

	PV1 virus titer after anti-rubella IAS			
Sample: neat anti-PV1 neut. titer	**Anti-rubella IAS #1**	**Anti- rubella IAS #2**	**Anti- rubella IAS #3**	**SD**
B: 7.3	6.2	6.5	8.2	*0.8*
C: 6.2	5.5	6.8	N/A	*0.5*
D: 6.8	5.2	6.5	6.8	*0.7*

[1]Sample B with anti-rubella IAS became negative. SD = standard deviation.

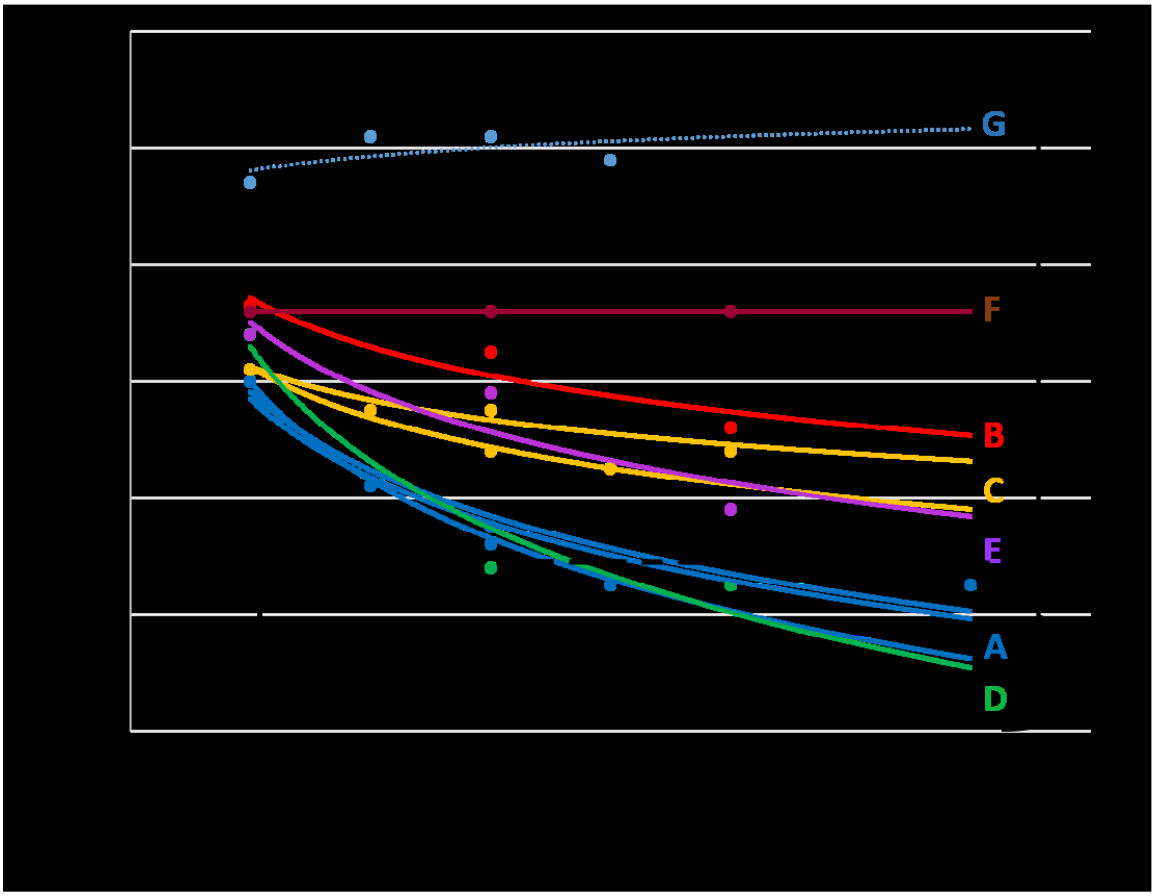

Figure 3.9 Titer declining with anti-PV1 immunoadsorption (IAS).
The change in anti-PV1 titer among 3 oral polio vaccine (OPV) primed samples, A, D, E, and 4 inactivated polio vaccine (IPV) primes samples, B, C, F, and F with IAS incubations.

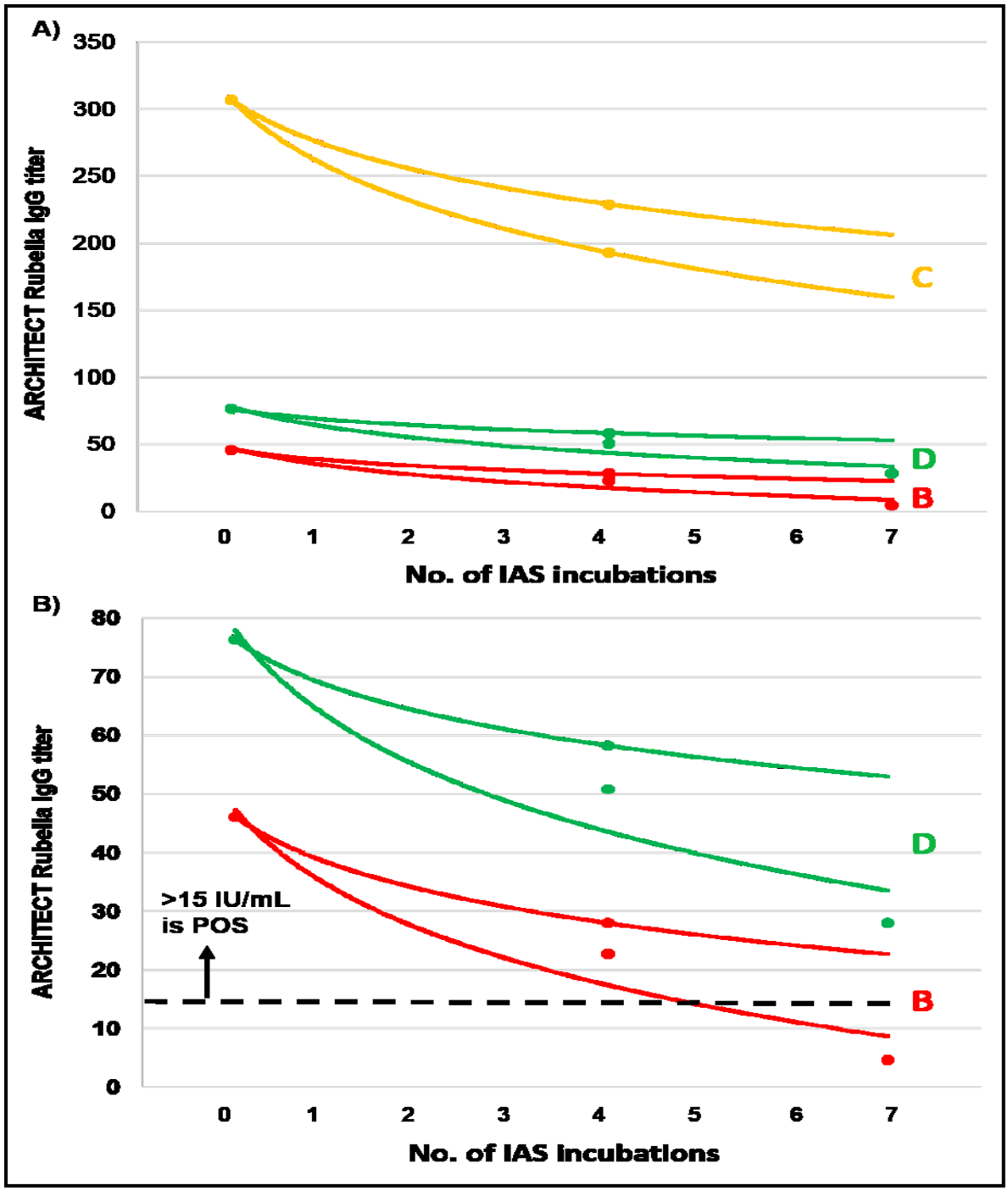

Figure 3.10 Titer declining with anti-Rubella immunoadsorption (IAS).
The change in anti-rubella titer among samples, B, C, and D. A) Presents all 3 samples that were anti-rubella immunoadsorbed; B) focuses on the low-medium neat titer samples, B and D, at the cutoff of the immunoassay.

CHAPTER 4 DISCUSSION

This is the first report on PV1 seroprevalence from Canada, since the country was officially certified as polio-free by the WHO in 1994 (10). The neutralizing titers to PV1 among three age groups (10 to 29, 30 – 49, and 50 - 64 years old) in Nova Scotia were evaluated using the MN assay. This study confirms that serosurveillance is an important tool for assessing population-level immunity against VPDs like polio, and that most Nova Scotians have shown protective immunity to PV1.

The measurement of serum antibodies, or serology, is fundamental for assessing protection against VPDs like polio, where it can be used to define personal or population-based protection (47, 48). Serological surveillance can monitor rates of protective immunity and evaluate vaccination programs, allowing timely intervention to control or minimize risk (92-94). The benefits of serosurveillance are multifold. Seroepidemiology can answer the "where" and "who" in terms of vaccination gaps and can be analysed to model population immunity. Seroepidemiology could inform vaccination policy in terms of recommended schedule, as well as cost and stockpile of doses (95, 96). Although PV resurgence and outbreaks are unlikely in Canada, there is value to knowing our PV seroepidemiology and ensure that we are achieving herd protection rates. Herd protection, or herd immunity, is determined as the threshold of seroprevalence rates within a population that prevent person-to-person transmission, and in turn prevent secondary cases of infection. Not everyone who gets vaccinated, develops a protective serological response due to underlying individual conditions, such as malnutrition, diet, and immune disorders. Consequently, using vaccine coverage rates alone to determine herd immunity levels maybe inaccurate. The use of, seroepidemiological surveys can

provide a more accurate determination of protection. Presently, there are no Canadian data on the seroprevalence of polio, and with potential introduction from infected travelers, it is important to better understand if Nova Scotia is seroprotected, and not rely solely on vaccine coverage rates.

For polio, the estimated herd immunity threshold is 80 – 86%, requiring vaccination coverage of 84 – 90% (97). There have been concerns when polio vaccination rates dropped below 90% (98, 99) or even 95% (100), especially in polio-free countries that do not want PV reintroduction pending global eradication. Herd protection indirectly protects individuals whose titers fall below the correlate of protection. In this study, the overall seroprevalence to PV1 among our representative sample population was 89.2%. Assuming maintained vaccine coverage rates since last reported in 2013, we suggest that residents of Nova Scotia have adequate protection from PV. However it is important to realize the samples tested were collected in 2012, and more recent samples would be required to confirm this.

Although the overall PV1 seroprevalence in Nova Scotia was shown to be above the herd immunity threshold range required for protection from poliomyelitis, the seroprevalence rates were not the same for all age groups. When compared across the three age groups, the seroprevalence in the youngest age group (10 – 29 years) of 81%, was significantly lower than the older age groups 30 – 49 and 50 – 64 years, at 94% and 92.6%, respectively. Looking at smaller geographical clusters of the population, seroprevalence by health zones was 87.1%, 89.3%, 91.8%, and 90.2% for zones 1, 2, 3, and 4, respectively. Within these smaller populations, seroprevalence rates of the younger age group reached 75% in zone 2. The observed pattern of lower seroprevalence among

the younger age group compared to the other groups is an exploratory endpoint requiring verification.

It was hypothesized that older adults would have lower levels of antibodies due to waning immunity, however, seroprevalence rates and GMTs were found to be significantly higher for these age groups. Furthermore, the two older age groups were more likely to be seropositive than the younger age group, with an odds ratio (OR) 3.67 for 30 – 49 years old (95% CI 1.96 – 7.37) and OR 2.99 for 50 – 64 years old (95% CI 1.65 – 5.67). Immunity to PV is acquired after exposure to PV (wild type or attenuated), or vaccination. The introduction and use of polio vaccines in Canada resulted in a remarkable and prompt decline in the incidence and prevalence of PV disease. While most provinces were transitioning from OPV to IPV before a complete shift in 1998, Nova Scotia has been exclusively using IPV for polio vaccination since 1955. Compared to OPV, the long-term duration of humoral immunity after IPV series remains unclear (96). Additionally, with IPV-exclusive vaccination, mucosal protection is negligible such that it does not protect against shedding if the individual is exposed to the virus (43, 101, 102). Nonetheless, like the rest of Canada, virus circulation in Nova Scotia was successfully interrupted and has been controlled for decades.

It is unlikely that the higher seroprevalence identified in the older age groups of this study is the result of natural exposure to WPV or attenuated strains from cVDPV. The last case of indigenous, paralytic WPV in Canada was recorded in 1977 (10, 98). Later, imported paralytic and non-paralytic WPV cases were last reported in 1988 and 1996, respectively (14). Subsequent cases of VDPV in Canada were imported, the last paralytic case in 2009, and the last non-paralytic case in 2012 (15). The live attenuated

polio vaccine was not used in Nova Scotia with the exception of the mass immunization with attenuated strains in Yarmouth County, south of Nova Scotia (103). Interestingly, the rates from Zone 1, where Yarmouth falls, has the lowest seroprevalence rates overall and for each age group (while not significant compared to the others age groups or zones).

Why individuals in these older age groups have a greater chance of being seropositive is not fully clear. Two recent studies from the United States reported high overall seroprevalence rates, >91%, to PV1 among all age groups with IPV-only schedules, provided full 4-dose vaccine schedule was complete (67, 68). For our younger age group, the most recently available Nova Scotia vaccine coverage rates were reported in 2013, one year after our samples were collected where 82.8 (95% CI 77.9, 86.8) had ≥ 4 IPV doses by 7 years old, and 81.2 (95% CI 76.2, 85.3) by 17 years old (13). This study did not have vaccination history or rates of vaccination among individuals in the older age groups. However, these individuals were infants and children at a time when the fears and shocking images of paralytic polio disease were still vivid to their parents and care givers, suggesting a large proportion would likely have received IPV, and moreover, given an IPV booster (10, 104, 105). Scandinavian countries, Sweden, Finland, and Iceland, started an IPV-exclusive schedule shortly after Nova Scotia, after which they eliminated polio cases and prevented PV introduction, with the exception of a wild-type PV3 epidemic in Finland in 1984 (106, 107). A 1987 study from Sweden reported a decline in titers occurs 2-5 years after IPV immunization, and thereafter humoral immunity levels remain mostly stable or shows only slow decline, without stating whether they reached non-protective levels (106). Vaccine recommendations in Canada

include an IPV booster when travelling to PV endemic regions. Although minimal, this practice may also be contributing to the higher immunity levels among the older age groups, especially those from urban parts of Nova Scotia. The high IPV-induced PV1 seroprevalence rates among our older age groups most certainly contribute to the reduction of infection risk and infectiousness from PV1 in Nova Scotia (108). This immune cohort provides indirect herd protection to non-immunized individuals, so long as susceptible pockets are minimal (108). When looking at titers in seropositive individuals in our study, the titres were nearly equally distributed across age groups, suggesting again that no differences could be noted between age groups and waning immunity amongst older age cohorts was not evident.

The latest polio vaccination rates from Nova Scotia for the youngest age group are approaching the lower accepted level required for herd protection (13). If the rate of vaccinated cohorts declines, and the number of vaccinated individuals drops over time, there will be increasingly less contribution to indirect/herd protection, ultimately putting the unvaccinated at risk. Based on this study's findings, there is no imminent risk from PV1 among 10 – 29 years old in Nova Scotia, especially if the seroprevalence rates are the same if measured today. However, it is important to focus efforts on sustaining vaccination coverage, and ensuring that all health zones in Nova Scotia maintain polio vaccination within uptake ranges that provide herd protection. Strategies to improve vaccine coverage are the same for all VPDs (109), but recently they require parallel interventions to address vaccine hesitancy in order to be successful. Interventions include providing clear, scientific-based facts about vaccine to the public, educating children about vaccines in schools, and empowering health care providers (110, 111). Vaccine

hesitancy is a serious and global threat to immunization programs in general and the GPEI specifically, given the paramount role of vaccines for achieving polio eradication (112). Although the impact is unclear in Nova Scotia, vaccine hesitancy is a growing problem in many countries (113). Our data also points to another potential factor, difficulty of access to vaccine clinics or health centers, especially that a large proportion of the Nova Scotia population reside in rural communities (114, 115). Although the difference in seroprevalence rates between health zones is not significant, further investigation into its possible association with access to vaccine can help improve coverage.

While the possibility of PV importation remains until global eradication is accomplished, our data indicates that widespread transmission in Nova Scotia is unlikely. Our results reject the hypothesis of lower protection rates in older residents of Nova Scotia, nevertheless, it was important to assess what the overall and age-specific PV1 seroprevalence rates mean in the context of population dynamics. Nova Scotia receives the highest number of immigrants to the Atlantic region, including migrants from areas of political unrest, especially the last few years (116). The virus can cause asymptomatic infections and shedding that allows for it to be unsuspectingly carried by travelers from PV endemic countries or by new migrants (117). A single case of polio can have serious consequences, especially with susceptible and/or unvaccinated groups among the population (98, 118, 119). In Bulgaria, the 2001 importation of wild type PV1 by a 13-month old child resulted in an outbreak of 42 paralytic cases that required supplemental immunization activities at the local and national levels (120). Among other response efforts, a serology survey of hospitalized children was also conducted to assess

neutralization titers for that susceptible cohort. The survey identified unpredicted gaps of immunity among minorities, and helped in expeditiously vaccinating them (120). Similarly in 2007, Australia had its first case of polio 30 years from the last reported case. This PV1 importation triggered a broad public health response that was reported as a guideline for other countries (121, 122). These examples of PV outbreaks serve as a reminder that a polio outbreak can occur from one confirmed polio case. Currently in Canada, the only surveillance for polio is acute flaccid paralysis (AFP) surveillance (15). The most recent numbers on AFP surveillance by PHAC indicate that the laboratory testing rate falls below the WHO recommendation (15). Not only is there a chance for missing a case of polio infection, but diagnosis after an AFP presentation only identifies cases after virus introduction and active disease, without any alert for gaps in protective immunity within the population (95). Knowing that paralytic polio occurs in 1 – 2% of the infections, and that the basic reproductive number (R_0) from one infectious polio case is 5-7 secondary infections/case, then a single paralytic case may just be the tip of the iceberg for much broader underlying asymptomatic virus activity (22, 97). While AFP surveillance is very important, maintaining and demonstrating high population immunity is essential for staying polio-free and for global eradication. This year, 2019, marks the 31st year since the Global Polio Eradication Initiative or GPEI was launched. According to the GPEI, the global polio figures at the end of 2018 include 3 endemic countries, 6 outbreak countries and 15 key-at-risk countries (123). At least two of these are among the top ten birth countries of recent immigrants to Canada (124). Before the end of February 2019, 6 cases of paralytic wild type PV1 have been reported from two endemic countries, in addition to cVDPV cases and detection of WPV1 in environmental samples in other

countries (123). While the GPEI focuses efforts and funds towards polio-affected countries, it underscores that "*polio-free countries play a critical role in maintaining sensitive surveillance and high population immunity, including thorough strengthened routine immunization services*" (4).

This study, by testing Nova Scotia samples using the MN assay, has provided PV1 seroprevalence and seroepidemiology data that is otherwise unavailable. Given the limitations of ELISA-based serologic methods, the MN assay will remain the reference for measuring PV neutralizing titers. Serological assays require positive and negative controls for validation, standardization and monitoring of assay performance. Driven by the need for an anti-PV1 negative serum samples, IAS was shown to be a promising approach for generating negative sera. Small-scale IAS was successful at adsorbing anti-PV1 or anti-RuV antibodies, demonstrating its applicability for two different viral antibodies. The ability to remove specific viral antibodies from sera is invaluable in generating negative controls to aid in assessing population-based protection against VPDs in highly vaccinated populations where negative specimens may be difficult to find. Although our approach was validated by removing antibodies specific to another highly vaccinated virus, IAS can be likely further refined by optimizing conditions such as beads to serum ratios, temperature, and capture antibody. Moreover, further in depth research is required to study and explain why IAS using Sabin strains is successful at adsorbing anti-PV1 antibodies from OPV-primed and not IPV-primed serum samples. It is possible that IPV primed individuals generate similar antibodies as used for viral capture in IAS. This possibility could be assessed by alternating serum purifications with bead complexes with different MAbs used for viral capture. Regardless of the need to further evaluate

differences between IPV and OPV primed sera, this study showed IAS could effectively convert PV1 immune sera to a negative status, without sample dilution, or affecting other serum properties, including other antibody titers. To our knowledge, this method to generate negative serum has not been previously explored.

This study has some limitations. The most important limitation is the lack of individual level data on immunization and medical history, this limits the data generalizability. While convenience or residual sera are commonly used for seroepidemiology (51), and offer a number of advantages (such as cost savings, relatively easy access to the specimens, and the can be anonymously linked to certain medical records containing demographics or outcome data); however, caution to ensure non-biases when sera are selected. In this study, the residual sera were primarily collected from routine visits of individuals to healthcare settings, which would not account confounding factors such as co-morbidities, conditions of immune compromise, and vaccine history. Individual vaccine history can only be obtained through population-based recruitment and sampling, that are time consuming, costly, and not entirely bias-free (125). Another limitation is the lack of pediatric samples. Seroepidemiology data on infants and children is invaluable for evaluating vaccine programs, and essential for ensuring that this vulnerable age group is protected. Collecting and monitoring data over time can also help determine if seronegative rates in older age groups are due to waning immunity or low vaccine uptake, by comparing rates of current adult seroepidemiology rates against vaccine coverage rates from when they were infants and children. This is particularly important for PV since serology data from that age group can better inform and explain the seronegative portion in older age groups. Determining immunity levels

against only PV1 can be considered a limitation, but given the eradication of PV2, and WPV1 circulating more than PV3, PV1 serology is a reasonable surrogate for protection levels against other serotypes. The residual sera from this study were collected in 2012; a more recent collection would provide more current PV seroepidemiology in Nova Scotia.

To better assess PV1 seroprevalence in Nova Scotia moving forward, testing of pediatric samples would definitely be included. With a broader overall age span, paired-wise comparison of protective rates between age groups could be conducted. While our study focused on Nova Scotia, it has highlighted the importance of promoting and supporting PV serosurveillance in other parts of Canada. Seeking collections of residual sera for MN assay testing may be the only option for accumulation of broader data on PV seroprevalence in Canada. However, population-based samples would be more informative with specific groups of most susceptibility or concern, like school-aged children and immunocompromised patients. Moreover, this design could be used to assess waning immunity, by administering and testing the effect of an IPV booster on older adults that are seronegative.

IPV-only programs are effective when vaccine coverage and schedule completion are optimal, especially given the lack of IPV associated mucosal immunity and ambiguity of its waning immunity. As public health strategies continue to improve vaccine program outreach and coverage, they would benefit from parallel systematic serological surveillance to inform public health decision and recommendations. It is very unlikely that Nova Scotia, or Canada, will use OPV for polio control and prevention. In Canada, the risk of causing cVDPV cases due to OPV outweighs its protective benefit, since there is no evidence of introduction or circulation of WPV. While the continued risk of PV

importation, the Canadian setting warrants high polio vaccine uptake, ideally supplemented with some degree serosurveillance to ensure herd immunity is maintained. In addition to PV, it is important to assess population-based immunity against other VPDs. Supporting the establishment of a Canadian seroepidemiology network is an important goal to reduce the potential resurgence of VPDs like polio, and ensure that we are protected.

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