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Combatting Circulating Infectious Diseases in California: Evaluating New Approaches to Surveillance and the Costs of Outbreaks on Public Health Agencies

A dissertation in partial satisfaction of the

requirements for the Doctor of Philosophy

in Environmental Health Sciences

by

John Dave Diaz-Decaro

2018

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2018

ABSTRACT OF THE DISSERTATION

Combatting Circulating Infectious Diseases in California: Evaluating New Approaches to Surveillance and the Costs of Outbreaks on Public Health Agencies

by

John Dave Diaz-Decaro

Doctor of Philosophy in Environmental Health Sciences

University of California, Los Angeles, 2018

Professor Hilary Godwin, Chair

The costs of circulating infectious diseases in California and the need for better approaches to surveillance are major challenges for surveillance laboratories tasked with controlling and preventing the spread of disease. This is particularly true for respiratory pathogens and measles. Although vaccines are widely available for common respiratory tract pathogens as well as measles, outbreaks of both still occur, straining California local and state public health agencies on an annual basis. In the first part of this thesis, I critically evaluate the utility of multiplex molecular diagnostics for public health surveillance of respiratory pathogens and provide recommendations for the development of new assays that better meet the needs of the surveillance community. Next, I present a case study in which multiplex assays were used to study respiratory infections in nursing

homes in California. This case study demonstrates the utility of multiplex assays for studying correlations between environmental contamination and human illness and suggests that this approach could be an effective tool for infection control in healthcare settings. Last, I present a study in which we modeled the epidemiological and economic impact of two recent measles outbreaks in California (the 2014-15 U.S. multi-state and the 2016-17 measles outbreak). The results of this study suggests that, at the county level, population density and the distance from the epicenter of the outbreak are the best predictors of contact counts and costs for these two measles outbreaks. In addition to providing insights into the true costs of prior measles outbreaks, this model also could be used prospectively by local health departments to better manage future outbreaks. Collectively, these studies provide important insights and tools for improving surveillance of respiratory pathogens, more effective controlling respiratory infections in healthcare settings, and decreasing costs of outbreaks.

The dissertation of John Dave Diaz-Decaro is approved.

Robert J. Kim-Farley

Richard J. Jackson

Wendie A. Robbins

Hilary A. Godwin, Committee Chair

University of California, Los Angeles

2018

DEDICATION

To my mom and dad

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LIST OF ABBREVIATIONS

ARI	Acute respiratory tract infection
CA	California
CAP	Community acquired pneumonia
CDC	Centers for Disease Control and Prevention
CDPH	California Department of Public Health
CI	Confidence interval
CPE	Cytopathic effect
CPI-U	Consumer product index for all urban consumers
DFA	Direct fluorescent antibody test
DOR	Diagnostic Odds Ratio
DTaP	Diphtheria, tetanus, and pertussis vaccine
EB	Employee benefits
EIA	Enzyme-linked immunosorbent assay
EV	Enterovirus
FDA	Food and Drug Administration
FN	False Negative
FP	False Positive
FPR	False Positive Rate
FTE	Full-time employee
HIPAC	Healthcare Infection Control Practices Advisory Committee
hMPV	Human metapneumovirus
IgG	Immunoglobulin G

IgM	Immunoglobulin M
ILI	Influenza-like illness
ILINet	U.S. Outpatient Influenza-like Illness Surveillance Network
INF	Influenza
IU	International Units
J	Youden Index
κ	Kappa coefficient
LAC	Los Angeles County
LACPHL	Los Angeles County Public Health Laboratories
LADPH	Los Angeles Department of Public Health
LDT	Laboratory developed test
LR	Likelihood ratio
LRTI	Lower respiratory tract infection
LTC	Long-term care facility
MMR	Measles, mumps and rubella vaccine
NAAT	Nucleic acid amplification test
NCIRD	National Center for Immunization and Respiratory Disease
NGS	Next-generation sequencing
NPS	Nasopharyngeal swab
NNDSS	National Notifiable Disease Surveillance System
NVRESS	National Respiratory and Enteric Virus Surveillance System
PAHO	Pan American Health Organization

PCR	Polymerase chain reaction
PHL	Public health laboratory
PPH	Precision public health
PMI	Precision Medicine Initiative
QTY	Quantity
R_0	Basic reproductive number
ROC	Receiver operator curve
RP	Respiratory panel
RPP	Respiratory pathogens panel
RSV	Respiratory syncytial virus
RTI	Respiratory tract infection
RUO	Research Use Only
RVP	Respiratory viral panel
Sen	Sensitivity
SNF	Skilled nursing home facility
SNS	Strategic National Stockpile
Spe	Specificity
Tdap	Tetanus, diphtheria and pertussis vaccine
TN	True Negative
TP	True Positive
TPR	True Positive Rate
URTI	Upper Respiratory Infection
USD$	United States dollar

VAP	Ventilator associated pneumonia
VRDL	Viral and Rickettsial Disease Laboratory
WGS	Whole-genome sequencing
WHO	World Health Organization
YTD	Year to date

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VITA

EDUCATION

2008…….M.S., Experimental and Molecular Pathology, University of Southern California

2003…….B.S., Biology, Loyola Marymount University

PROFESSIONAL TRAINING & CERTIFICATIONS

2016……PulseNet Laboratory MiSeq Workshop, Centers for Disease Control and Prevention, Atlanta, Georgia

2011……Laboratory Biological Risk Mitigation Training (BSL3), University of California, Los Angeles

HONORS

2017…… Top prize in Laboratory Quality and Efficiency Contest at the Los Angeles County Public Health Laboratories

2012-2013……Association of Schools of Public Health/ Health Resources Services Administration Fellowship

2012-2013……California Wellness Fellowship

2010-2012……California Department of Public Health Laboratory LabAspire Director Program Fellowship

POSTERS AND PRESENTATIONS

- Poster presentation on "Epidemiological Patterns and Clinical Implications of Genotypic Resistance Mechanisms for Carbapenem-Resistant *Enterobacteriaceae* Surveillance Isolates in Los Angeles County" IDWeek 2017 San Diego, CA – October 4-8, 2017
- Guest lecturer for UCLA School of Nursing course, *Fundamentals of Epidemiology* (NURSING 50) "Diagnostic Validity and Screening", 2014-2016.
- Guest lecturer for UCLA Fielding School of Public Health Environmental Health Sciences Departmental seminar (ENV HLT 411). "The Public Health Impact of the Disneyland Measles Outbreak", October 20, 2016.
- Poster presentation on "Prevalence of Respiratory Viruses, including Influenza, Among Nursing Home Residents and High-Touch Room Surfaces" IDWeek 2016 New Orleans, LA – October 26-30, 2016
- Poster presentation on "Epidemiologic Comparison of Laboratory-confirmed Influenza Virus B Yamagata and Victoria Lineage Infections During the 2014-2015 Influenza Season" IDWeek 2015 San Diego, CA – October 7-11, 2015
- Poster presentation on "Global Bio Lab: Enabling Technologies for High-Throughput Screening of Infectious Disease Samples", International Conference on Emerging Infectious Diseases (ICEID), Atlanta, GA, March 11-14, 2012.

CHAPTER 1

Introduction and Overview of the Organization of Thesis

INTRODUCTION

Despite advances in the prevention and surveillance of circulating infectious diseases, such as respiratory tract infections (RTIs) and measles, these diseases continue to pose a substantial burden on public health in the United States. Circulating infectious diseases may be defined as seasonal or endemic communicable disease transmitted through one or several modes of transmission such as direct or indirect (1). Direct transmission occurs when an infectious host comes in direct contact with a susceptible individual. Indirect transmission occurs when the environment acts as a reservoir or vector spreading disease with no direct human-to-human contact. The number of deaths due to selected notifiable infectious diseases in the United States has been increasing in recent years. 1,252,022 deaths occurred in 2000, 1,861,588 deaths in 2010, and 2,082,672 deaths in 2014. This represents an 11.8% increase in 2010 and a 66.34% increase in 2014 since 2000 (2). In the United States, across all age groups age-adjusted death rates for some infectious diseases have decreased but are still more than some chronic conditions (3). Collectively, infectious diseases cost the United States $120 billion in 2014 (4). For comparison, RTIs cost $40 billion annually ($17 billion in direct costs and $22.5 billion in indirect costs) (5, 6). The annual cost of influenza in the United States in direct medical expenses is estimated at $10.4 billion a year with an additional $16.3 billion in lost wages (7). RTIs represent a third of the economic burden due to infectious disease. For measles, despite being declared eliminated from the United States in 2000, outbreaks cost local and state public health agencies ranged from $2.7

million to $5.3 million in 2011 (8). While the cost of measles is relatively low in the U.S. compared to other infectious disease, measles is still a major public health concern as the frequency and size of outbreaks is sporadic and individually very costly.

Respiratory tract infections and measles also place a significant burden upon the health of Californians and on the public health system across the state. The annual economic costs from RTIs are estimated to be $143.3 mil across all 58 California (CA) counties with county-specific costs varying due to demographics (*i.e.*, population size, age structure, and income level) (9, 10). Mortality of RTIs in CA such as influenza accounts for 6 to 14% of all annual deaths in the United States (11). For a common RTI such as the common cold, Californians experience an estimated 78.5 million to 1.5 billion episodes annually (2 to 4 episodes per person) (12). Although mortality is rare among cases measles cases in California (case-fatality rate is 0.2%); 13, 14), the economic burden of measles is a recurring public health concern. Local and state public health agencies spent roughly $141,068 ($10,367 per case) on a measles outbreak in San Diego county where an unvaccinated individual was returning home from an overseas trip (8). Recently, in responding to a measles outbreak originating from a US-bound refugee arriving in Los Angeles International Airport, local response costs ranged from $64,210 to $72,456 (15). The burden of measles and similarly to RTIs in CA has been mostly from controlling and preventing disease in various populations. Thus, heightened surveillance both locally and throughout the state is a priority for public health agencies.

Improved epidemiological tools for surveillance of RTIs and improved understanding of how to control costs of measles can help to mitigate the burden of these diseases. In California, as is the case in many states, Local Health Departments and Public Health Laboratories play a critical role in surveillance of infectious diseases such as RTIs and measles (16, 17). For RTIs, traditional

laboratory methods are the "gold standard" but have been steadily replaced by the commercial release of novel molecular technologies (18). While clinical management of RTIs depend on knowing etiology, respiratory multiplex assays offer rapid detection and characterization addressing many of the limitations of conventional methods. However, while multiplex diagnostic tests have dramatically improved clinical care of RTIs, use of these methods as surveillance tools needs to be further evaluated. Specifically, assessment of the use of multiplexed diagnostic tests as surveillance tools should reflect epidemiological diagnostic measures that are relevant for surveillance purposes. For measles, surveillance initiatives in California have often focused on increasing vaccination rates, yet population based tools that help understand transmission factors and population dynamics are greatly needed as recent costly measles outbreaks have been large and sporadic despite a high vaccination coverage throughout the state. A better understanding of how multiplexed assays perform in surveillance studies on RTIs and a better understanding of what drives costs of measles outbreaks would not only improve communicable disease prevention and control programs but also yield better public health outcomes. While the studies in this thesis focus on Los Angeles and the State of California, they have broad reaching implications for how to improve surveillance of RTIs and reduce costs associated with measles outbreaks in other jurisdictions as well.

RESPIRATORY TRACT INFECTIONS

RTIs are defined as acute respiratory infections affecting either the upper respiratory tract (URTIs) (comprised of the nasal cavity, pharynx and larynx) or lower respiratory tract (LRTIs) (comprised of the trachea, primary bronchi and lungs). (**Figure 1.1**). Symptoms vary depending on etiology, but are often overlapping, which complicates direct diagnosis by clinicians.

Acute disease due to URTIs include the common cold, sinusitis, pharyngitis, epiglottitis and laryngotracheitis. Symptoms vary with type of URTI, patient demographics and medical history, however significant overlap exists. Symptoms for many URTIs include coughing, sneezing, nasal discharge, nasal congestion, runny nose, fever, sore throat and heavy nasal breathing (19). Onset of symptoms typically occurs 1 to 3 days after exposure, with symptoms lasting as much as 3 weeks depending on the type of URTI (20). The most common URTI is the common cold, which is a mild self-limiting disease that annually leads to approximately 1 billion colds in the U.S. (20). Risk factors include close contact with an infected individual with an existing URTI, poor personal hygiene, and overcrowding (especially among children in a group setting or in healthcare facilities), smoking, and a compromised immune status (19, 21). Other risk factors include time of year with most URTIs occurring during the winter months.

Acute disease due to LRTIs include acute bronchitis and bronchiolitis, influenza and pneumonia. Symptoms of less-serious disease include congestion, dry cough, sore throat, low-grade fever and mild headache lasting roughly 2 weeks (22, 23). More serious symptoms include fever, severe cough, rapid breathing, wheezing, chest pain and skin discoloration. As a leading cause of morbidity and mortality in both children and adults, LRTIs have risk factors that are at times disease specific. For bronchitis, risk factors include exposure to cigarette smoke, age, occupational hazards that increase exposure to lung irritants and gastric reflux (24). Influenza risk factors include age, living or working conditions, chronic illnesses, pregnancy and obesity (25). The CDC suggest that flu-related complications are mostly observed in high-risk populations (*i.e.*, children under 5, adults over 65 years of age and older, pregnant women, long-term care facility residents, and American Indians and Alaskan Natives) (26). Risk factors for pneumonia are dependent on the type of pneumonia: community-acquire pneumonia (CAP), hospital-acquired

pneumonia or ventilator-associated pneumonia (VAP) (27-29). Despite the many risk factors, the multi-causal pathways leading to a RTI always begins by first identifying the etiological agents causing disease.

While most URTIs result from viral infection the causative agents for LRTI can be either viral or bacterial (30). The "common cold" is most often associated with viral infections, including rhinoviruses, coronaviruses, parainfluenza, adenoviruses, RSV, and influenza viruses. However, illness diagnosed as a "common cold" can also be bacterial in origin (*e.g. Chlamydia pneumonia, Streptococcus pneumonia, Mycoplasma pneumonia*). Epiglottitis and laryngotracheitis have been associated with infections due to RSV, parainfluenza, and several bacteria, including *Haemophilus influenzae* type b, *Corynebacterium diphtheria, Streptococcus pneumoniae,* and group A streptococci (31). Acute epiglottitis can be a life threatening condition. In recent years, the incidence of this disease has been increasing in adults and decreasing in children (32). Laryngotracheitis, commonly known as croup, is a condition that affects the larynx, trachea and bronchi that is typically associated with a variety of viruses: parainfluenza virus (types 1-4), influenza A and B, rhinovirus, enteroviruses, RSV and measles (in unvaccinated children) (33). While croup is usually due to viral infections, it has occasionally been associated with the bacterium *Mycoplasma pneumoniae* (34). Common causes of acute bronchitis include: influenza A and B, parainfluenza viruses, adenovirus, RSV, rhinovirus, coxsackievirus groups A and B, and echovirus, *C. pneumoniae* and *M. pneumonia* (35, 36). Bronchiolitis is caused by RSV, hMPV, parainfluenza viruses, and adenoviruses (35, 37). Pneumonia is caused by a variety of bacterial and viral respiratory pathogens (38), however influenza is a common cause in both children and adults (39). While viral pneumonia is often associated with community outbreaks of influenza, RSV and parainfluenza (40, 41), the most prevalent causes of viral pneumonia in infants and young

children are RSV and parainfluenza infections (42). Bacterial pneumonia is a frequent complication following a viral RTI. Common etiologies for bacterial pneumonia include *M. pneumoniae*, *Staphylococcus aureus* and gram-negative bacteria (43). Older adults and patients residing in long-term care facilities are at highest risk for bacterial pneumonia (38). While acute respiratory disease may be due to LRTI or URTI, failure to identify viral or bacterial etiology poses a significant clinical risk to patients and population health.

CLINICAL MANAGEMENT OF RESPIRATORY TRACT INFECTIONS

RTIs are a clinical challenge due to the similar symptoms shared by many respiratory viral and bacterial infections. The CDC case definition for influenza-like illness (ILI) includes a fever (temperature of 100°F [≥ 37.8°C] or greater), accompanied by a cough and sore throat without a known cause other than influenza (44). The U.S. Outpatient Influenza-like Illness Surveillance Network (ILINet) is a collaborative effort between the CDC, local and state public health agencies and healthcare providers. With nearly 3000 participating outpatient sites providing data, ILINet collects and trends data of patient visits to heath care providers for ILI. Data shows that on average, during recent flu seasons (2010 to 2015), only 2.6% of all patients visits are because of ILI symptoms (45). State-level data reveals that a majority of ILI's are seldom resolved by initial laboratory testing. Data from World Health Organization (WHO) and National Respiratory and Enteric Virus Surveillance System (NREVSS) collaborating laboratories who screen for influenza A (H1N1pdm09, /H1, /H3) and influenza B, suggests that only a small fraction of all ILI's in California are actually due to either influenza A or B alone (**Figure 1.2).**

Clinical management of RTIs varies with causative agent. Both antivirals and antibiotics are available for treating RTIs but use depends on whether the acute infection is viral or bacterial.

For influenza A and B infections, zanamivir (Relenza ®) and oseltamivir (Tamiflu ®) are widely used antiviral chemoprophylaxis that target neuramindase activity inhibiting progeny viral particle release from an infected cell (46). While zanamivir alleviates symptoms when used early during disease progression, oselatmivir reduces duration of symptoms and viral shedding when used even after 48hrs after onset (47, 48). Recently, oseltamivir and zanamivir resistance has been reported in some circulating influenza A and B strains in very low prevalence (49). For parainfluenza viruses, coronaviruses, rhinoviruses, and enteroviruses many therapeutics are in development, but no specific antivirals are available as respiratory disease associated with these pathogens is often self-limiting (50-52). Similarly, uncomplicated adenoviral infections are often mild and transient with available treatments targeting symptoms rather than mitigating viral infection (53). For RTIs that have a bacterial etiology, many antibiotics are available. For pertussis (commonly referred to as "whooping cough"), current CDC guidelines recommend the following chemoprophyaxes: azithromycin, clarithromycin, erythromycin, and Trimethoprim-sufamethoxasole (54). However, clinicians are advised to consider patient age, tolerability, and cost when choosing a treatment (55). For atypical pneumonia species such as *C. pneumoniae* and *M. pneumonia*, treatment is on a case-by-case basis. However, CDC recommends azithromycin as a first line therapy, but also recommends tetracycline and doxycycline (56). *M. pneumonia* treatment includes macrolides, tetracyclines, and fluoroquinolones (57). In the era of antibiotic stewardship, correct clinical diagnosis of ILI due to influenza, another virus, bacteria or some other respiratory pathogen is critical for patient management.

When available, the best preventative measure against RTIs is vaccination. Immunization against circulating and seasonal influenza strains occurs requires an annual seasonal flu vaccine. An annual seasonal flu vaccine is recommended for anyone 6 months and older. Seasonal flu

vaccines are available as a trivalent type that protects against influenza A/H1N1, influenza A/H3N2, and an influenza B (Yamagata or Victoria strain) virus. Quadrivalent flu vaccines are also available, and provide protection against an additional influenza B virus strain. Due to antigenic drift, influenza viruses are constantly changing, thus the flu vaccine is updated yearly based on recommendations from the CDC. Due to yearly composition changes, the flu shot will not protect against every influenza A and influenza B viral strain. In some years, as little as 10% of those receiving the vaccine were protected against circulating strains (58, 59). However, the effectiveness of the flu shot in individuals and populations is based on the antigenic similarities present in many influenza strains which enables some cross-protection (60). However, in years when there is an antigenic mismatch between the flu shot and circulating strains, overall vaccine effectiveness is reduced but not eliminated. A universal flu vaccine is currently in Phase IIb of clinical trials by Oxford University's Jenner Institute and Vaccitech. The universal flu vaccine targets inner proteins (61). If effective, this universal flu vaccine will provide protection against circulating seasonal influenza strains as well as avian subtypes (62). During past flu seasons (2010 to 2017), the average annual flu vaccination coverage in adults (≥ 18 years of age) has been lower than children (6 months to 17 years of age), 41.7% to 56.5%, respectively (63). Vaccination is also available for other RTIs such as pertussis. Protection against pertussis occurs when receiving either the diphtheria and tetanus toxoids and acellular pertussis vaccine (DTaP) or the tetanus, diphtheria, and acellular pertussis vaccine (Tdap). The DTaP vaccine is administrated as a 5-dose series and is recommended for children and adolescents aged 18 years or younger (64). The Tdap vaccine is recommended for anyone 7 years or older with a booster of tetanus and diphtheria every 10 years (65, 66). Aceullar vaccines have been shown to have high vaccine effectiveness but exhibit waning immunity over time (67). Vaccine effectiveness for DTaP is 80-90% and about 70% for Tdap (68,

69). While preventative treatment in the form of vaccination is preferred over antibiotics and antivirals, in the absence of vaccination and when post-exposure prophylaxis is available, early diagnosis of RTI can greatly improve patient outcomes (70).

PUBLIC HEALTH SURVEILLANCE AND THE CHALLENGE OF RESPIRATORY TRACT INFECTIONS

Surveillance of RTIs provides the evidence for public health decisions and action to mitigate the spread of these diseases (71). For instance, surveillance is used to uncover trends within populations related to prevalence and incidence changes of viruses and bacteria that have the potential to negatively impact human health. Public health surveillance also helps identify the emergence of novel variants and resistant organisms that could lead to increased morbidity and mortality. Additionally, surveillance serves to evaluate effectiveness and improvements in current prevention and control programs and interventions. Based on regional and global trends, the CDC along with collaborating laboratories from around the world provide recommendations each year for selecting the annual candidate strains of seasonal influenza vaccines (72). Similarly, In the U.S., deployment of CDC's Strategic National Stockpile (SNS), which houses medicine and medical supplies to manage diseases such as pandemic influenza and a variety of RTIs is based on surveillance data (73). By recognizing trends, surveillance helps communicable disease programs formulate preparedness and response plans, provide clinical direction for appropriate patient care, and allocate resources during outbreak response. However, a limiting factor in surveillance is having appropriate tools for identifying causes of acute respiratory disease.

Distinguishing between a viral or bacterial RTI is a significant challenge not just for delivering appropriate care but for surveillance purposes as well. Rhinoviruses, coronaviruses,

parainfluenza viruses, adenoviruses, respiratory syncytial viruses, influenza viruses, and a few bacteria (*e.g.*, *Mycoplasma pneumonia* and *Chlamydolphila pneumonia*) clinically present the same ILI symptoms (30) making treatment decisions based on symptoms difficult. For surveillance, failure to characterize definitive causes of disease affects populations by delaying public health decisions and interventions. In cases of a novel outbreak, a delay in distinguishing between a pandemic strain and another pathogen may increase prevalence and incidence leadings to poorer population health outcomes (74). Culture and serology-based assays have been the traditional methods for detecting and characterizing causes of acute respiratory infection. However, while these conventional methods are widely available, delays in turnaround time, poor sensitivity and lack of expertise have limited their usefulness for clinical and surveillance purposes (75). Multiplex respiratory assays address many of these limitations with the added benefit of not only screening for a variety of common RTIs at one time, but also providing qualitative and quantitative data with each test result, which could be used for treatment and screening of disease (76). While respiratory multiplex assays have been a tremendous asset, these methods could significantly improve RTI surveillance.

EPIDEMIOLOGY OF RESPIRATORY TRACT INFECTIONS GLOBALLY AND IN THE UNITED STATES

RTIs are a global public health challenge. As a leading cause of death worldwide across all age groups, total deaths due to RTIs account for more than liver, colon, breast and prostate cancer combined (77). Over the past decade, deaths in children under the age of 5 due to RTIs and RTI-related complications have decreased by 36.8% (95% UI -42.0 to -31.6), however, RTIs are still a leading cause of death under the age of 5 in low income countries and among the top killers in

middle and high income countries (**Table 1.1**) (78). In 2015, 1.8 million deaths due to RTIs and RTI-related complications occurred in children under the age of 5 (79). A decrease in the the burden of RTIs in adults was also observed in adults, as deaths due to RTIs decreased by 3.2% with 2.7 million deaths occurring in 2015. Global trends show that in high-and middle-income countries, the burden due to RTIs in adults has been increasing over time. Risk factors associated with this increase include: malnutrition, population growth, population aging and air pollution (80). For lower income countries such as those in the Sub-Saharan African region, the burden of RTI's is compounded due to mosquito borne diseases, HIV/AIDS, malnutrition and economic instability (81).

In the U.S. a common causative agent for acute respiratory disease is influenza. CDC estimates that on average, 5% to 20% of the American population will contract the flu. Thus, local and state public health agencies can assume that approximately 16 to 64 million Americans will contract the flu annually with certain high-risk populations additionally experiencing flu-related complications (82). **Figure 1.3** depicts the number of weekly flu-related deaths across all ages throughout the United States during past flu seasons. The highest seasonal flu-related deaths (n = 8189) occurred during the 2014-2015 flu season; the lowest occurred during the 2011-12 flu season (n = 738). Collectively, data from previous flu seasons shows a slight shift during the past two flu seasons (2015-16 and 2016-17) with peak mortality observed later in the year suggesting seasonal fluctuations possibly related to social and environmental factors (83). At a state-level, seasonal related-deaths are reported in conjunction with secondary bacterial infections (such as bacterial pneumonia). **Figure 1.4** shows the total number of pneumonia and influenza-related deaths through several flu seasons in California showing peak mortality remaining the same from season to season. In California, 6 to 14% of all annual deaths are due to influenza, which, when coupled

with costs associated with other RTIs, translates to an annual economic burden over $143 mil (84-86). The data presented thus far shows that while a small percentage of people visit healthcare providers due to ILI symptoms and while seasonal flu vaccination coverage has remained relatively the same since 2010, at the local and state level, percentage of deaths due to flu and flu-related complications are a significant economic burden that can plausibly be reduced by increasing adult flu vaccination coverage and visits to healthcare providers. Surveillance data for other RTIs shows additional local burdens due to acute respiratory disease.

In the U.S., the National Respiratory and Enteric Virus Surveillance System (NREVSS) provides laboratory-based surveillance data of select respiratory viruses: respiratory syncytial virus (RSV), adenovirus, parainfluenza viruses, and human metapneumovirus (hMPV) across all regions within the U.S. Data is voluntarily reported by participating U.S. laboratories. For RSV, national trends show a seasonal increase during winter months with a notable peak at the beginning of the year. California shows a similar trend. While adenovirus is both an enteric and respiratory virus depending on genotype, during 2003-2016 the most common adenovirus types (type 3, 2, 1, 4, 7, 14) were all associated with respiratory tract infections (87). Surveillance data on parainfluenza viruses (type-1, -2, and -3) shows year round prevalence with spikes varying on parainfluenza type. During the past year (2017), parainfluenza type-1 peaked during late summer months and remained unchanged until mid-fall. For parainfluenza type-2, peak months occurred during the fall and lasted through early March. Parainfluenza type-3 was found throughout the year but was most prevalent during early spring through summer. While not monitored through NREVSS, parainfluenza type 4 has year round prevalence peaking during the fall during odd-numbered years (88). For hMPV, infections appeared to spike at the end of March and last through early September.

Another source of national surveillance data for common respiratory pathogens is provided by selected U.S. clinical laboratories (89). Over the past two years (2015 - 2017), data has been collected from participating sites whose volume is at least 30 multiplex respiratory tests per week. This data is compiled by private industry whose proprietary technology, the FilmArray Respiratory Panel (RP) will be described below. Surveillance data from clinical sites shows that human rhinovirus/enterovirus is consistently the highest detected respiratory pathogen year-round (low, 10.5% to high, 34.8%). Other viral respiratory pathogens show varying prevalence throughout the year: coronavirus (0.6% to 15%), influenza A (0.2% to 17.9%), RSV (0.8% to 19.7%), and parainfluenza viruses (1.2% to 12.8%). Bacterial respiratory pathogens (*i.e.*, *Bordetella pertussis*, *C. pneumonia*, and *M. pneumonia*) have also been detected at low prevalence throughout the year (0.1% to 2.6%). Prevalence data of viral and bacterial respiratory pathogens from national and private surveillance efforts show a sustained burden of RTIs in the U.S. throughout the year.

DIAGNOSTIC METHODS FOR IDENTIFYING ETIOLOGIES OF RESPIRATORY TRACT INFECTIONS

Traditional Methods

Several standard methods are used in detecting the causative agents of RTIs. Historically, viral and bacterial characterization have included conventional culture methods and serological diagnostic tests. For decades, conventional culture has served as the 'gold standard' for confirming the causative agent of disease. However, a significant drawback of these traditional methods is the limited clinical impact on patient management as they often take as much as 2 weeks (or longer) incubation for interpretable results. For example, influenza viral culture may provide results from 3 to 10 days. For surveillance purposes, a delay in diagnosis of a single day results aides disease

transmission. Rapid culture is possible via shell-vial culture assays, which utilize a monolayer of different cell lines to observe cytopathic effect (CPE). Shell-vial assays while less labor intensive than culture, reduce time-to-result at the expense of reduced sensitivity (90). Serological tests, such as direct fluorescence antibody (DFA) and enzyme immunoassays (EIA), may reduce the time-to-result even further but suffer not only from reduced sensitivity, but also vary in specificity for viral targets and often require technical expertise that is absent in inexperienced laboratories. Some limitations of conventional methods are not inherent to the actual tests, but are due to workflow. Sample transport, collection and processing of samples all can adversely affect pathogen isolation and detection. Despite limitations, conventional methods are far from obsolete as they are a useful tool to validate molecular assays, catch potential new variants that newer tests may not detect, and add important diagnostic and screening information to research and clinical investigation (91).

Molecular Methods: PCR-based Nucleic Acid Amplification Tests **(NAATs)**

A transition from using conventional techniques to molecular based methods for identification of pathogens has been occurring in both clinical and public health laboratories for several decades. The switch to molecular methods has been the product of not only improved technology and lower costs, but also the growing needs of clinical and surveillance labs that are constantly asked to make "active" decisions for delivering timely diagnosis and interventions (92).

One of the most widely used molecular methods is PCR-based nucleic acid amplification tests (NAATs). Nucleic acid amplification by PCR has been the cornerstone of molecular diagnostics (93). PCR assays can be optimized for any pathogen, as long as the sequence is known for a gene that is specific to that pathogen. By offering greater sensitivity and specificity (for nearly all clinically relevant targets), reduced time-to-result (minutes to hours instead of days or weeks),

quantitative data (instead of subjective results) and lower limits of detection (versus variable growth requirements), PCR-based NAATs resolve many of the limitations of conventional methods. When compared to shell vial and conventional culture, real-time PCR for influenza A was significantly more sensitive detecting the virus as much as 7 days after infection (94). Turnaround times were more rapid for PCR (14.8 hrs) than shell vial (49.3 hrs) and conventional culture (199.2 hrs). For some targets, such as *B. pertussis*, PCR-based NAATs have been reported to be nearly twice as sensitive as direct DFA and 6 times more sensitive than culture (95). Real-time PCR based NAATs enable quantification of viral or bacterial titers throughout infection, which allows for disease progression from the onset of disease to recovery to be monitored (91, 96). Limit of detection, which is an important analytical measure of sensitivity, is as low as 1 to 10 copies per target for some PCR-based NAATs (97). In addition, PCR-based NAATs, often require less laboratory expertise than conventional methods (98).

Singleplex NAATs were among the first PCR-based assays to be developed for identifying the causative agents of RTIs. PCR-based singleplex NAATs target a single gene (*e.g.*, the HA gene of influenza A). Singleplex NAATs are optimized to distinguish the target of choice against a backdrop of normal carriage and other potential pathogenic microbes. The first singleplex PCR assay to detect influenza A/H1 was described in 1990 (99). The assay targeted a conserved region of the HA gene segment (100). A year later, a rapid method of isolating viral RNA and synthesis of cDNA was introduced (101). These methods were used to develop singleplex assays for influenza A, influenza B, and influenza C. PCR-based NAATs were viewed as possible alternatives to existing conventional methods (*i.e.*, culture). The first regulatory approved PCR singleplex assay for influenza A was developed by the CDC in 2006 (102). The real-time assay

included two sets of primers and probes targeting the HA gene segment of influenza A/H5 (Asian lineage) making the assay highly specific.

Despite the benefits of singleplex assays over conventional methods, the inability to screen for more than one target is a major limitation for patient management and surveillance. For clinical diagnostics, screening for only one target at a time could severely inhibit healthcare delivery. Similarly, for surveillance purposes, limited screening capacity could mitigate effective disease control and prevention strategies. Additionally, a positive result on a singleplex assay does not rule out the presence of some other pathogen if present.

Unlike singleplex assays, multiplex NAATs screen for more than one target per test run simultaneously amplifying multiple gene sequences in a single reaction (*e.g.*, nucleoprotein gene of human metapnuemovirus and the matrix protein of RSV). The term 'multiplex' is given to any assay that screens for more than one target. Larger multiplex assays identify between 12-20 (103) detecting both viral and bacterial targets. Multiplex NAATs are cost-effective alternatives to singleplex assays benefiting clinical management and disease surveillance capabilities (101). In clinical settings, broad screening improves patient outcomes by rapidly detecting disease in a singular assay instead of several singleplex NAATs. Similarly, for surveillance, broad screening allows epidemiological associations to be revealed sooner rather than waiting on several individual NAATs.

ENHANCING SURVEILLANCE THROUGH THE USE OF MULTIPLEX ASSAYS FOR RESPIRATORY PATHOGENS

Many regulatory-approved major PCR based multiplex platforms are commercially available (**Table 1.2, 1.3**), several of which have point of care potential. In **Chapter 2**, I provide a

critical review of four major multiplex assays for RTIs from an epidemiological perspective: the Luminex NxTAG Respiratory Pathogen Panel (RPP), the BioFire FilmArray Respiratory Panel (RP), the Nanosphere Verigene Respiratory Pathogens Flex Test (RP *Flex*), and the GenMark Respiratory Viral Panel (RVP). Each multiplex platform differs in user complexity, run time, and number (and type) of targets screened. Additionally, while each platform is PCR-based, these instrument systems differ in primer selection, amplification strategy, amplicon detection, and automation (93).

In **Chapter 2**, I review how the multiplex respiratory assays that are FDA approved compare with each other and assess how each meets particular needs of laboratories involved in surveillance of RTIs. These assays differ in turnaround time, target sensitivity and specificity, limit of detection, PCR and chemistry, and cost. In **Chapter 2**, I provide guidance on the use of specific diagnostic performance measures (including ROC space plot, likelihood ratios (LRs), Diagnostic Odds Ratios (DORs), and the Youden Index (*J*)) that can provide insights into which instrument is most appropriate for surveillance of different common RTI analytes. This analysis allows laboratories to identify which multiplex assay would best suit their outbreak response or routine surveillance needs on a per target basis. By identifying which assay performs better for specific targets, communicable disease control programs and public health laboratories can effectively direct public health resources targeting surveillance efforts systematically and precisely.

DIRECT APPLICATION OF RESPIRATORY MULTIPLEX TECHNOLOIGES FOR SURVEILLANCE PURPOSES USING ENVIRONMENTAL SAMPLES

As evidence to support how respiratory multiplex assays can improve surveillance capabilities, I present a case study in **Chapter 3** in which we used the FilmArray RP assay to

conduct surveillance of RTIs in symptomatic nursing home residents and their environment. During the current 2016-17 influenza season, the majority of flu outbreaks in the State of California occurred in long-term care facilities (104). There are currently 1112 licensed long-term care skilled nursing housing as many as 370,000 residents in California, the most in the country (105). The nursing home environment houses a highly susceptible vulnerable population frequently exposed to potential respiratory pathogens from the flow of staff, visitors and other residents (106).

While host transmission of healthcare-associated infections in nursing homes is a well-known concern, our understanding of the role of fomite-mediated transmission in these environments is limited (107). From an infection control perspective, sources of transmission in nursing homes are from both symptomatic residents and any viruses or bacteria on high contact surfaces. The burden of contamination of respiratory viruses and bacteria in the environment is unknown. Thus, the minimum prevalence of disease in the environment is either equal to or greater than the prevalence of disease in residents. In **Chapter 3**, we utilized the minimum prevalence of disease along with likelihood ratios as conditional probabilities to determine the probability of environmental contamination due to shedding from symptomatic residents. This approach involved the application of Bayes' Theorem (108). Overall, our method gives specific environmental shedding probabilities that reveal hygiene and infection control opportunities within the nursing home environment. By using multiplex assays to evaluate the role of the environment in disease transmission, we show how these assays could enhance environmental public health surveillance efforts.

THE RE-EMERGING PUBLIC HEALTH BURDEN OF MEASLES

Like RTIs, measles is another circulating infectious disease that imposes a substantial burden on public health in California. Measles (rubeola) is a highly contagious disease caused by the measles virus (a paramyxovirus). The primary route of transmission is through respiratory secretions (*i.e.*, coughing and sneezing) leading to upper respiratory tract colonization and infection. Clinical diagnosis is based either on laboratory confirmation or by meeting the WHO or CDC clinical case definition, which includes the characteristic maculopapular rash that lasts more than 3 days, accompanied by a temperature of 101º F [38.3º C] with cough, coryza, or conjunctivitis (109, 110). Viral shedding occurs up to 2 days before onset of symptoms and up to 5 days after the appearance of a rash (111). Historical U.S. data shows that roughly 1 out of 4 cases are hospitalized, 1 case out of 1000 develop encephalitis, and death occurs in 1-2 cases per 1000 (112). Once infected, no available treatment resolves measles infection, however providing a dose of measles immune globulin to group at risk for severe disease (e.g. infants under 1-year-old, pregnant women, and immunocompromised persons) can help recovery (113). A single dose of 200000 IU of Vitamin A has also been shown to be associated with reduced mortality for children under 2 years old (114).

The development of a highly effective vaccine for measles was thought to have resulted in elimination of measles in the United States over fifteen years ago. During the pre-vaccine era in the United States, annually roughly 3 to 4 million people were infected, 48000 were hospitalized, 4000 suffered encephalitis, and 400 to 500 died (115). During the 1950s, 95-98% of young adults had serological evidence of a past measles infection (2, 115). Once the first developed and licensed measles vaccine was introduced in 1963 cases declined dramatically. For comparison, in 1960 there were roughly 441703 measles cases in the U.S. and within 7 years after the release of the

vaccine, there were 47351 cases (-89.3%) (116). Once a second dose was recommended in 1989 U.S. measles cases declined even further. A one dose measles, mumps, and rubella (MMR) vaccine is roughly 93% effective; the two-dose series is 97% effective (117). Elimination of measles is achieved when no sustained transmission is observed over the past 12-month period. In theory, an effective vaccine series and high vaccination coverage lead to low levels of susceptibility at a national level. Threshold herd immunity is established when a significant portion of a local population is immunized against a disease. Under a few assumptions, simple threshold herd immunity can be calculated as $1\text{-}1/R_0$ (118). Based on the basic reproductive number (R_0), one measles case can result up to 11-18 secondary cases in a completely susceptible population. The "herd effect" is the risk reduction in susceptible individuals when in close proximity to immune individuals. In the U.S., herd immunity can be achieved if a majority of the population is vaccinated (119). This level was reached almost two decades ago, which led to official elimination of measles being declared in the U.S. in 2000.

GLOBAL, NATIONAL, STATE AND LOCAL BURDEN OF MEASLES

The Measles & Rubella Initiative is the WHO strategic plan to control and eliminate measles in at least 5 WHO regions by 2020, however measles continues to be a burden in many countries (120). Most recent data (2017 YTD) from all six WHO regions show that suspected and confirmed measles cases are found globally with the highest incidence found in the South East Asia and African regions (**Figure 1.5**). The WHO member states with the highest suspected measles cases include: Nigeria, Dominica, and Iran. The highest confirmed measles cases are found in India, Nigeria and Pakistan. Globally, while national efforts continue to help mitigate the spread of measles, the greatest burden has been among children and in low income countries with

weak health systems (121). Children accounted for 89780 global-measles associated deaths in 2016 (122). Recent country-specific data show that more than 50% of deaths were reported in India (123). In Pakistan and Iran, availability of measles morbidity and mortality data is poor due to a weak surveillance structure making assessment difficult (124, 125). In Nigeria, which has a suitable measles-case based surveillance system, social and cultural apprehensions on the use of vaccines have led to large recurring measles outbreaks (126). Endemically sustained measles transmission is found in all but one WHO region: the Americas, which was declared measles-free as of September 2016. However, public health efforts against measles continue even in the Americas region where countries, such as the U.S. continue to see hundreds of measles cases each year.

U.S. measles elimination is a great public heath achievement, however importation of cases and increased susceptibility at the local level contribute to sporadic measles outbreaks in the U.S. Overall, for the past two decades there has been a decreasing number of U.S. measles cases (**Figure 1.6**). However, despite elimination and a decline in cases, since 2010, an average of 198 (range: 55-667) confirmed measles cases have occurred every year (127). Recent trends show that in the post-elimination era, more measles cases in the U.S. have been confirmed in the last 5 years (2009-2014) than previous years (2001-2008) (128). Lack of vaccination coverage may account for the increase in susceptibility, however at a national level vaccination coverage has remained the same in recent years (90.0 to 91.9%) (116). Despite sustained vaccination coverage, measles outbreaks in the U.S. are common. In 2013, the United States experienced 188 confirmed measles cases, 58 of which were from the largest outbreak in the U.S. since 1996 (129). In 2014, incident measles cases tripled from the previous year, with a record-high 644 cases reported from 27 states, recorded from 23 distinct outbreaks, the most during the post-elimination era. Most measles outbreaks in

the U.S. are local and occur in pockets of highly susceptible populations. Thus, the reasons for sporadic measles outbreaks in the U.S. must be addressed as local public health issue rather than a national one.

In recent years, California has recorded several measles outbreaks that show the impact of measles at the local level. In 2008, local and state public health agencies in California spent roughly $141,068 on a measles outbreak in San Diego county where an unvaccinated individual was exposed overseas, contracted measles, and upon returning home exposed 839 people of which 11 became secondary cases (130). Much of the cost of the outbreak was due to clinical care and in investigating contacts. During the summer of 2011, the Los Angeles Department of Public Health (LADPH) was notified of a possible measles cases in a refugee from Burma after an intercontinental flight from Kuala Lumur, Malaysia (131). LADPH and the California Department of Public Health (CDPH) collaborated with nearby states to investigate hundreds of contacts, 3 of which developed measles from exposure to the index case. At the end of 2014, a multi-state measles outbreak began when CDPH confirmed measles in a hospitalized, unvaccinated 11-year-old child. Within weeks, additional measles was confirmed in several California counties, nearby states, Canada and Mexico. Upon investigation, all primary cases shared one notable exposure: all had visited the Disneyland theme park area in Orange County, California (132). The source of the outbreak was likely due to an infectious traveler that had visited the theme park area around December 18-20, 2014. By the end of the outbreak (April 17, 2015) 131 measles cases were confirmed throughout the state, with secondary cases confirmed in household and close contact, community and healthcare setting. Over 20 cases required hospitalization due to measles-related complications. Age distribution among cases: <1-year-old (15/131, 11%), 1-4-year-old (21/131,

15%), 5-19-year-old (24/131, 18%), ≥ 20-year-old (76/131, 56%). Among cases whose immunization could be verified, 70% (57/82) a majority were unvaccinated.

MEASLES MODELING AND COST ANALYSIS OF MEASLES OUTBREAKS IN CALIFORNIA

Recurring measles outbreaks in the U.S. are a major public health concern and a better understanding of the factors that contribute to them would help local health departments contain outbreaks and costs. This year alone, the CDC has reported (as of August 12, 2017) at least 118 cases from several outbreaks in 14 states, including California (133). The reasons for recurring measles cases and outbreaks in California is a complex issue that involves lack of vaccination coverage among population subgroups, social stigmas on the effectiveness of vaccines, and the importation of exposures and disease from other countries where measles is still endemic. However, the underlying drivers of measles transmission during outbreaks also includes interactions between populations (134). Aside from the 2014-15 U.S. multi-state outbreak, the most recent large-scale measles outbreak in California occurred 2016-17 in Los Angeles County in a religious community that resulted in 24 measles cases and thousands of investigated contacts. Our approach to assess the economic burden of recent measles outbreaks in California involves identifying population dynamic factors to estimate the epidemiologically associated contacts. Contacts are estimated through a combination of empirical survey data and regression modelling.

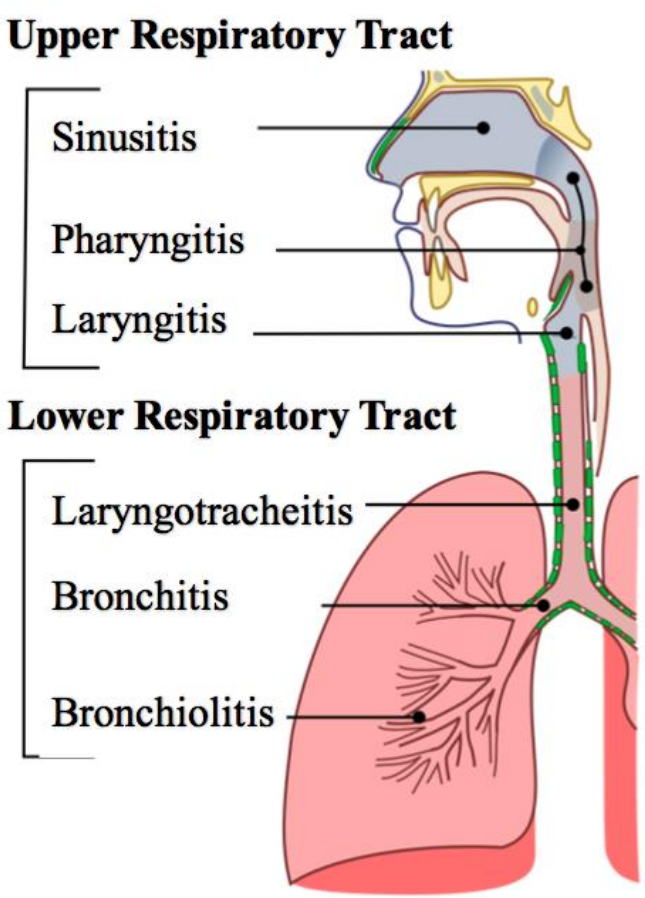
In **Chapter 4**, I present a cost analysis of recent measles outbreaks on local and state health departments in California. The goal of this study was to elucidate the factors that may contribute to measles outbreaks at the local level. Towards this end, we conducted an economic impact analysis of the 2014-15 U.S. multi-state and 2016-17 measles outbreaks on local and state

communicable disease control programs and public health laboratories in California. Based on this analysis, we identify population factors and give recommendations to curb future measles outbreaks in local U.S. populations.

CONCLUSION

The overarching objective fulfilled through this work is to describe how new surveillance tools and costs analyses can be used to reduce the spread and impact of infectious diseases such as RTIs and measles. During outbreaks, public health surveillance also serves as an early warning system mitigating further spread of disease (135). As technology improves, respiratory multiplex assays will become rapider, broader and easier to use and has created an opportunity for surveillance strategies that should be adopted by California surveillance laboratories. In **Chapter 2**, I identify gaps in evaluating current methodologies for public health surveillance for RTIs that are applicable to emerging technologies. In **Chapter 3**, I describe how respiratory multiplex assays can significantly impact public health surveillance in combating circulating infectious diseases in California. Additionally, I demonstrate how FDA-approved respiratory multiplex assays can be used to evaluate concordance between environmental and patient samples for surveillance purposes. In **Chapter 4**, I demonstrate how a cost analysis of measles outbreaks can be used to elucidate important risk factors in outbreak propagation. Additionally, by presenting an epidemiological and economic impact analysis of recent measles outbreaks, I provide not only a method of retrospective count estimation during a measles outbreak event, which alone could be applied to other measles or other vaccine preventable disease outbreak scenarios, but also an approach to estimate communicable disease control programs and public health laboratories response costs during outbreak events. In modeling and economic analysis of measles outbreak,

we shed light on some factors that have contributed to the spread of measles in California, and provide guidance on how strategic investments in education and outreach could be used to reach eradication goals. Taken together, these studies elucidate how enhancing surveillance tools of infectious diseases in local populations such as California can be used to mitigate the overall burden of disease.



Pathogens that infect the Upper Respiratory Tract

- Rhinovirus
- Coronavirus
- Influenza virus
- Parainfluenza virus
- Respiratory Syncytial virus
- *Streptococcus pneumoniae*
- *Mycoplasma pneumoniae*

Pathogens that infect the Lower Respiratory Tract

- Influenza virus
- Parainfluenza virus
- Respiratory Syncytial virus
- Adenovirus
- Human Bocavirus
- *Haemophilus influenzae*
- *Chlamydophila pneumoniae*

Figure 1.1. Upper and lower respiratory tract system and common respiratory infections. At left: Respiratory tract system anatomy depicting conditions specific to the upper and lower respiratory tract. At right: Common viruses that infect the upper and lower respiratory tract. This figure was adapted from a similar figure that is available online from Wikimedia Commons at https://goo.gl/JVFQwg

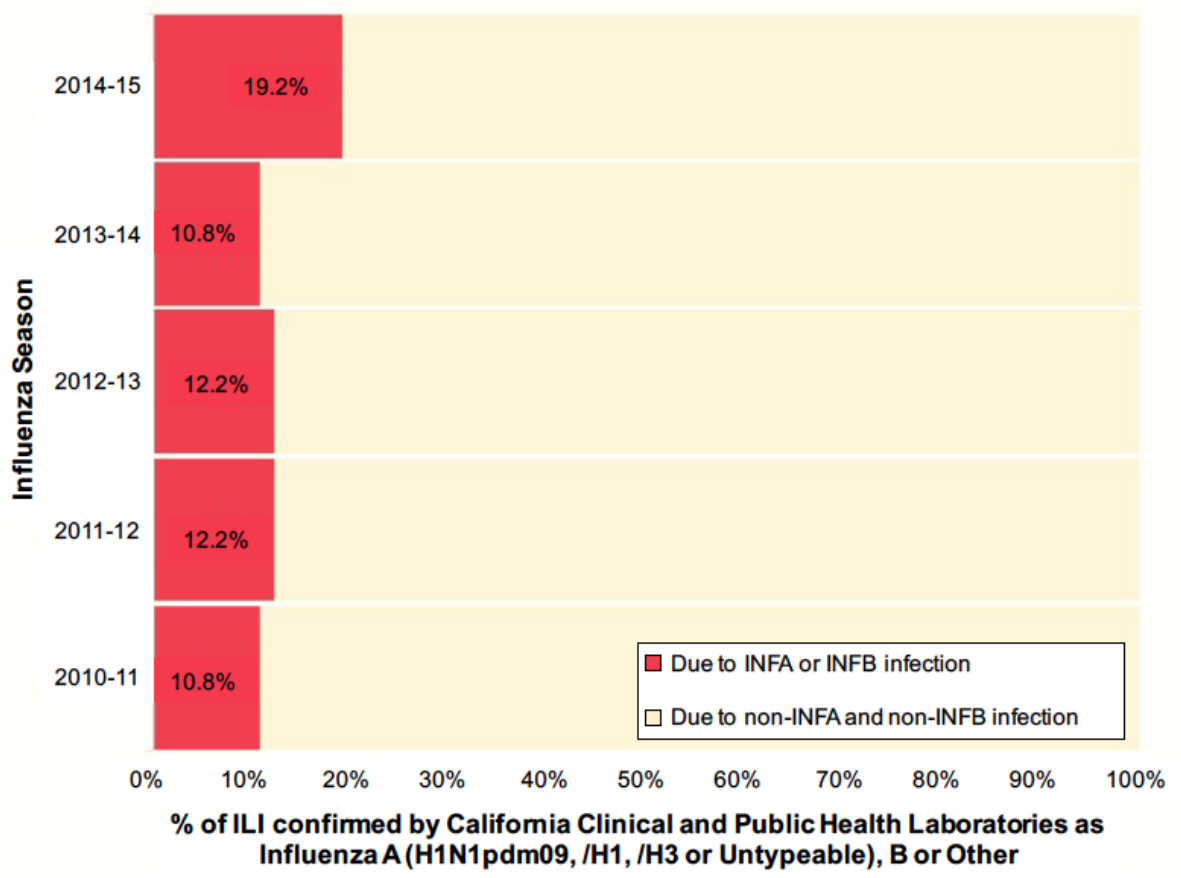


Figure 1.2. **Percentage of influenza-like illness (ILI) laboratory confirmed samples by laboratories designated as World Health Organization (WHO) collaborating and National Respiratory and Enteric Virus Surveillance System (NREVSS) laboratories in California during past influenza seasons.** Samples are screened for influenza A (H1N1pdm09, /H1, /H3 or untypeable) or influenza B. When influenza A and B are not detected, some other respiratory pathogen is assumed to be the causative agent of ILI. However, failure to detect influenza A or influenza B does not completely rule out these targets as limit of detection, storage of samples, time of collection, and excessive freeze-thaw cycles may have affected some samples. Data obtained from Centers for Disease Control and Prevention's national, regional, and state level outpatient illness and viral surveillance (FluView: https://www.cdc.gov/flu/weekly/index.htm).

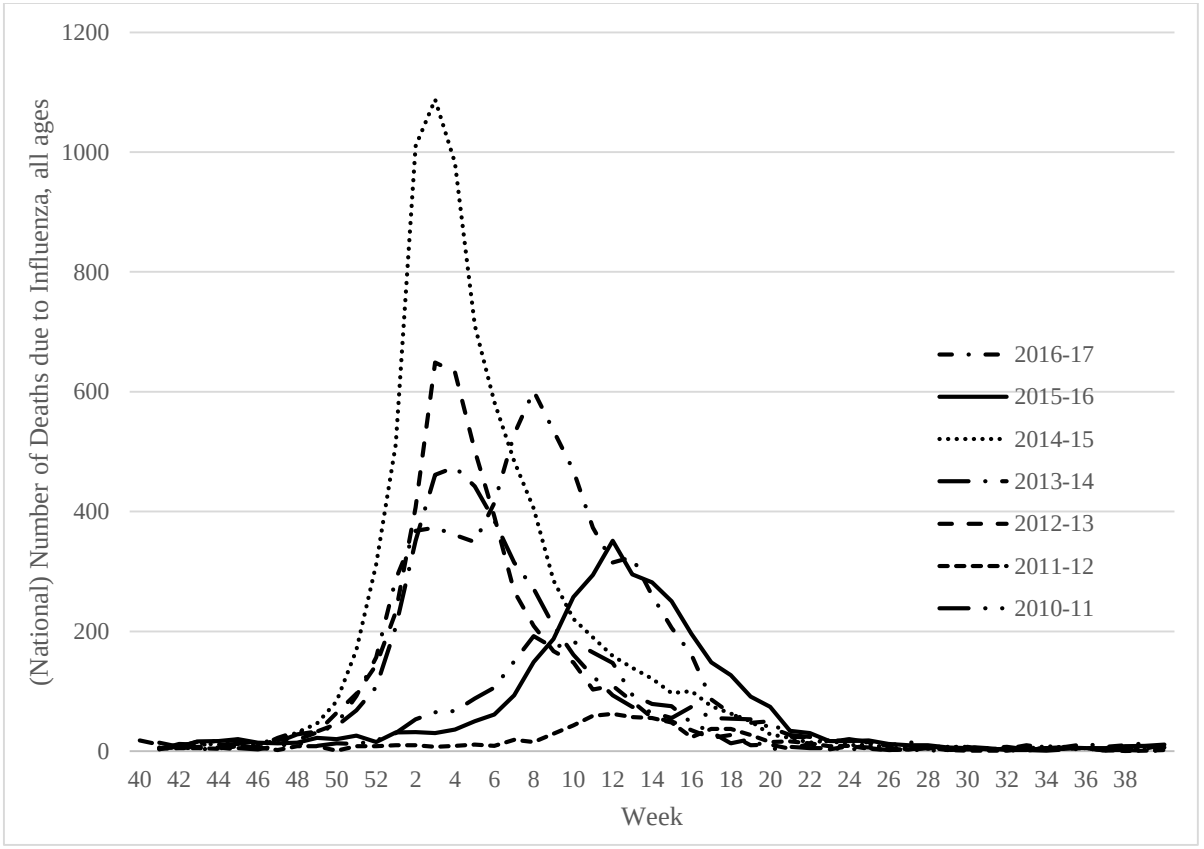


Figure 1.3. Number of flu-related deaths across all age groups throughout the United States during the past seven influenza seasons (2010-11 through 2016-17). Flu season typically begins in October (Week 40) with increasing cases through winter peaking in February (Week 6) slowly declining into the spring months. Data obtained from the Centers Disease Control and Prevention (FluView: https://gis.cdc.gov/grasp/fluview/fluportaldashboard.html)

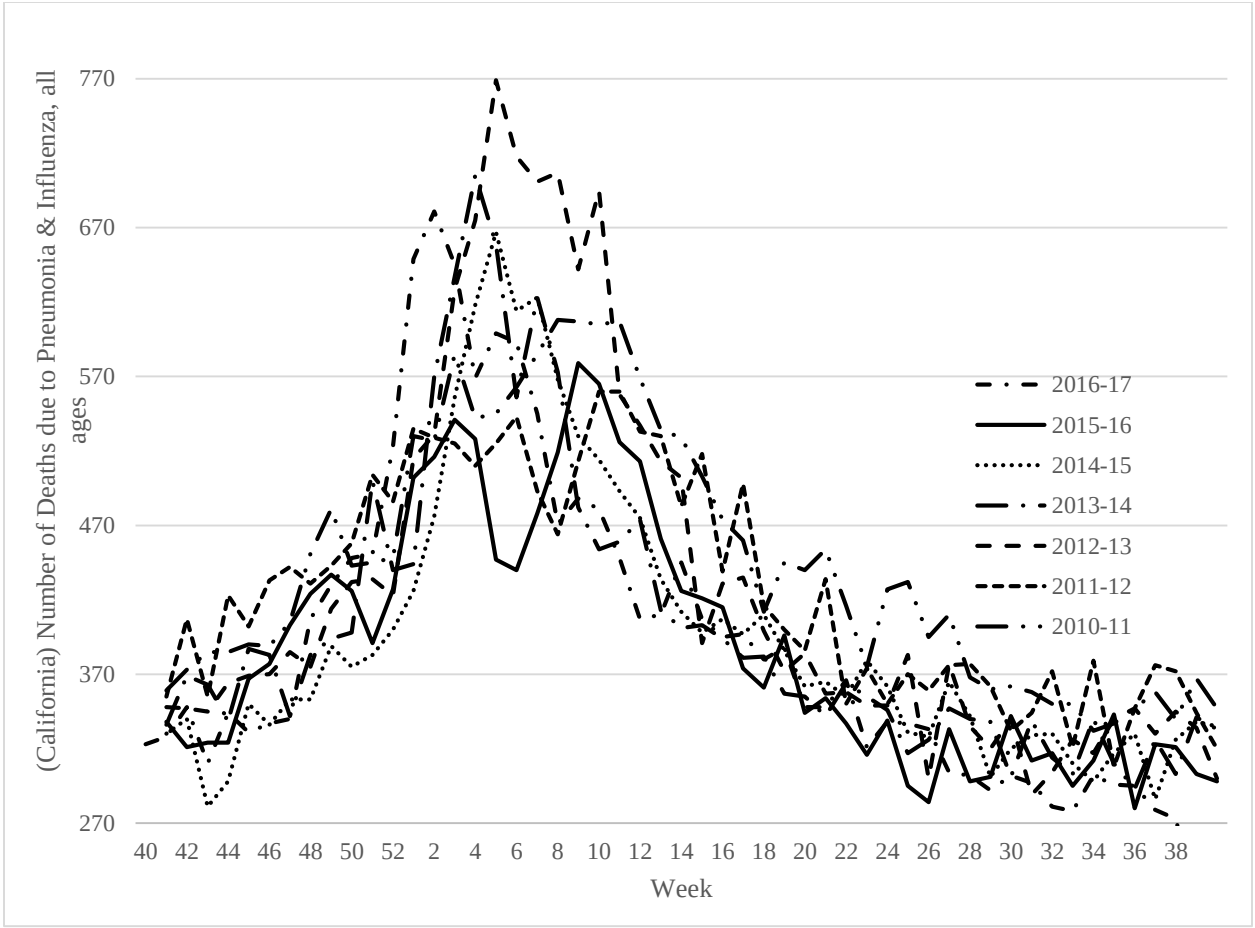


Figure 1.4. Weekly deaths due to pneumonia and influenza across all ages in California for past influenza seasons (2010-11 to 2016-17). Flu season typically begins in October (Week 40) with increasing cases through winter peaking in February (Week 6). Data obtained from the Centers for Disease Control and Prevention. (FluView: https://gis.cdc.gov/grasp/fluview/fluportaldashboard.html)

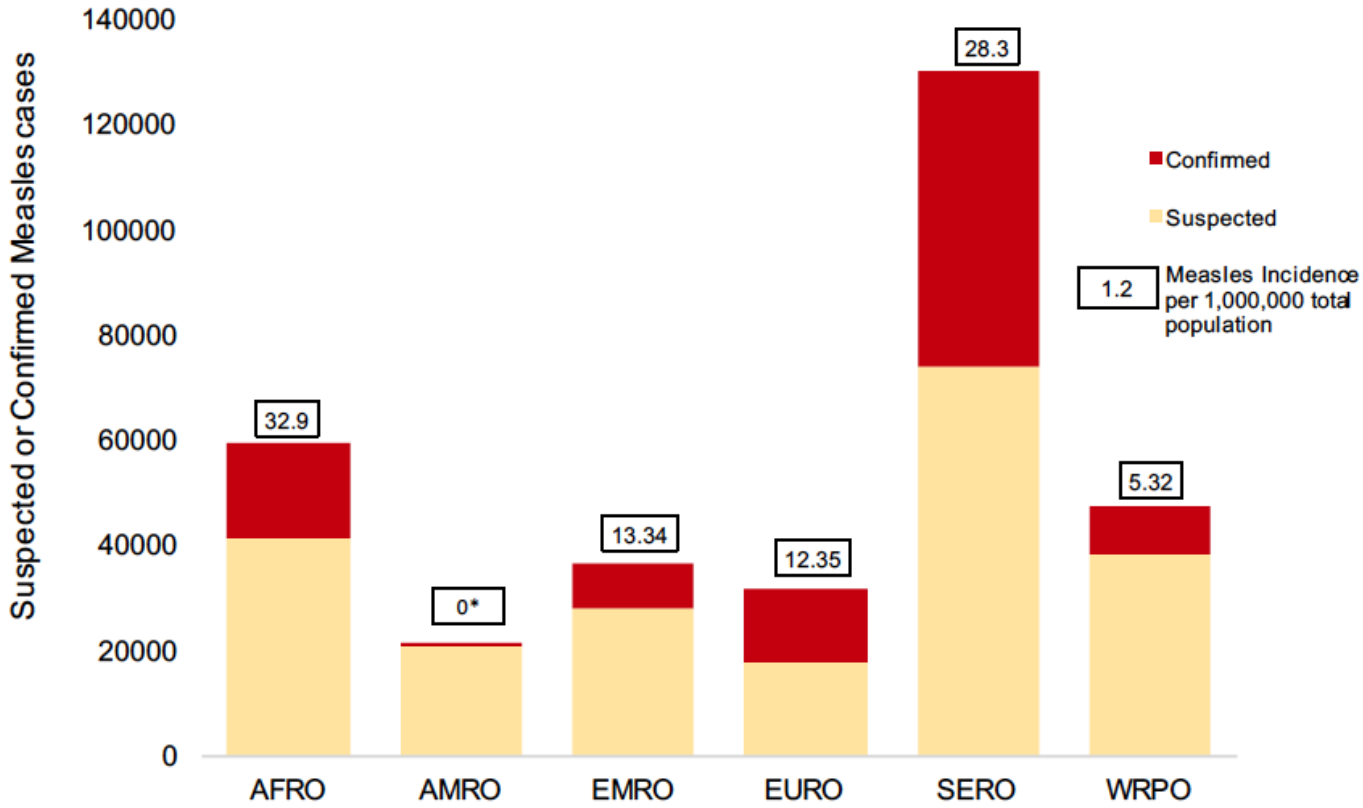


Figure 1.5. **Number of suspected or confirmed measles cases and measles incidence (per 1,000,000 total population) across six World Health Organization (WHO) regions in 2017 (YTD)**. (*) signifies measles elimination in the Americas Region (AMRO) as of September 2016. Data obtained from the World Health Organization (http://www.who.int/immunization/monitoring_surveillance/burden).

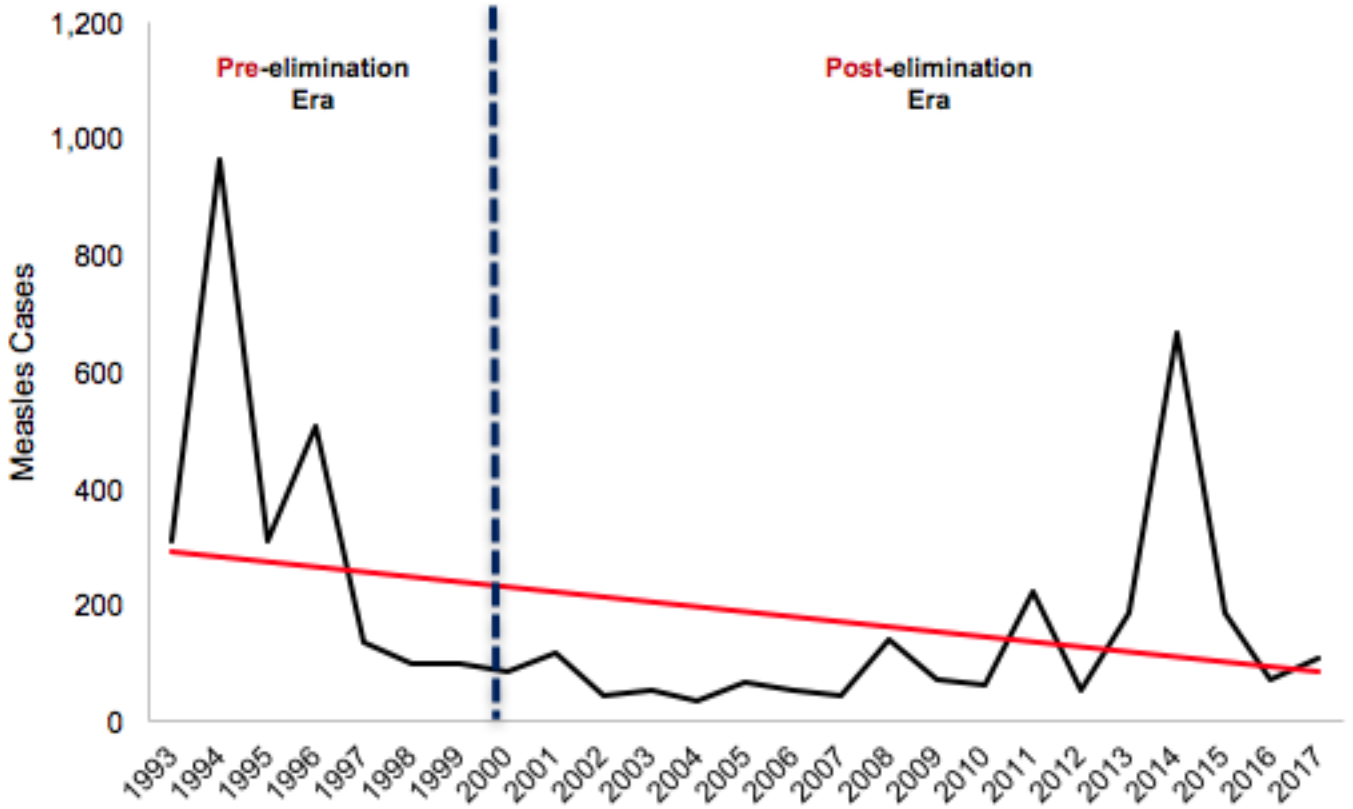


Figure 1.6. **Measles cases in the United States during the pre-elimination and post-elimination era beginning from 1993 through present.** Red line: 24-year trend to date. Dashed blue line: Sustained transmissible measles was declared eliminated from the United States in 2000. Recurring cases in the United States since 2000 due to domestic and international imported cases exposed unvaccinated or undervaccinated pockets of the population. Data obtained from the Centers for Disease Control and Prevention (https://www.statista.com/statistics/186678/new-cases-of-measles-in-the-us-since-1950/)

Table 1.1. The burden of respiratory tract infections (RTIs) in children under 5 and across all ages in middle to high and low income countries. Values shown in **bold** indicates a positive percent change meaning an increase in RTIs. Data obtained from the World Health Organization (http://www.who.int/immunization/monitoring_surveillance/burden).

		Deaths Children younger 5 years	Number per 100,000	Percent change (2005-2015)	Deaths for All Ages	Number per 100,000	Percent change (2005-2015)
Middle to High Income Countries	United States	538.5 (480.9 to 604.8)	2.7 (2.4 to 3.0)	-36.8 (-43.8 to -28.6)	91996.2 (88094.3 to 96175.8)	28.4 (27.2 to 29.7)	**8.0 (3.6 to 12.6)**
	Japan	149.7 (127.1 to 169.7)	2.8 (2.4 to 3.2)	-38.2 (-44.8 to -30.5)	156576.6 (150156.7 to 162966.9)	122 (117.0 to 127.0)	**40.1 (34.3 to 46.0)**
	Germany	42.6 (33.3 to 53.5)	1.3 (1.0 to 1.6)	-28.7 (-44.8 to -7.5)	31582.8 (27970.5 to 35596.7)	37.8 (33.4 to 42.6)	**20.8 (5.0 to 38.9)**
	United Kingdom	151.2 (129.9 to 168.5)	3.8 (3.2 to 4.2)	-19.0 (-27.9 to -9.0)	39930.4 (37967.4 to 41942.9)	62.2 (59.1 to 65.3)	-3.4 (-8.2 to 1.3)
	France	45.5 (33.2 to 60.0)	1.2 (0.8 to 1.5)	-31.9 (-51.0 to -6.7)	25009.3 (21466.4 to 29059.2)	38.3 (32.9 to 44.5)	**20.0 (2.9 to 41.8)**
	Brazil	4677.3 (4125.4 to 5300.3)	31.1 (27.4 to 35.3)	-51.3 (-57.1 to -45.4)	75602.0 (55632.8 to 84415.7)	36.4 (26.8 to 40.6)	**31.6 (19.0 to 43.1)**
	Italy	30.7 (22.9 to 39.1)	1.2 (0.9 to 1.5)	-42.5 (-57.7 to -23.5)	15172.6 (13132.5 to 17635.5)	24.2 (20.9 to 28.1)	**30.9 (11.0 to 54.8)**
	Canada	47.1 (38.1 to 57.0)	2.5 (2.0 to 3.0)	-13.6 (-30.5 to 6.3)	8742.5 (7676.1 to 9963.1)	24.2 (21.2 to 27.6)	**22.8 (6.3 to 41.0)**
Low Income Countries	Central African Republic	3222.2 (2087.7 to 4755.2)	453.1 (293.1 to 668.6)	**9.8 (-31.1 to 79.6)**	7082.6 (4798.9 to 9918.4)	144.4 (97.9 to 202.3)	**16.0 (-18.2 to 63.1)**
	Democratic Republic of Congo	38357.4 (25735.0 to 53739.2)	273.5 (183.5 to 383.2)	-8.2 (-40.7 to 36.1)	72827.3 (53663.3 to 95077.9)	94.1 (69.3 to 122.8)	**8.6 (-16.8 to 45.8)**
	Burundi	5261.2 (3329.3 to 8017.6)	247.0 (156.3 to 376.4)	**0.9 (-35.5 to 31.6)**	9826.4 (6999.3 to 13314.4)	87.4 (62.2 to 118.4)	**9.4 (-21.0 to 49.2)**
	Malawi	8105.2 (5711.7 to 11070.3)	274.5 (193.4 to 379.4)	-10.0 (-37.7 to 24.3)	14233.9 (10768.6 to 18529.2)	82.7 (62.6 to 107.6)	-0.6 (-25.4 to -32.8)
	Mozambique	6557.9 (4615.6 to 9098.8)	135.8 (95.6 to 188.4)	-35.2 (-55.0 to -9.8)	16219.4 (11530.9 to 22061.5)	57.9 (41.2 to 78.8)	-6.8 (-35.8 to 32.6)
	Guinea	7135.2 (5258.5 to 9358.8)	354.9 (261.5 to 465.4)	-20.2 (-42.4 to 7.6)	13571.0 (10473.1 to 17346.0)	107.9 (83.3 to 138.0)	-2.5 (-25.5 to 27.9)
	Eritrea	1996.5 (1331.0 to 2837.9)	240.7 (160.5 to 342.2)	-12.5 (-40.9 to 20.7)	4192.0 (2752.6 to 5955.7)	80.0 (52.5 to 113.6)	**9.4 (-21.3 to 48.5)**
	Madagascar	7804.0 (5361.9 to 11103.9)	209.4 (143.9 to 297.9)	-10.1 (-40.5 to 34.7)	17759.4 (12659.9 to 23847.2)	73.4 (52.3 to 98.6)	**8.9 (-20.7 to 46.2)**
	Global	703917.9 (651385.4 to 763038.7)	104.8 (97.0 to 113.6)	-36.9 (-42.0 to -31.6)	2736714.2 (2500318.4 to 2860842.8)	37.1 (33.9 to 38.8)	-3.2 (-6.9 to 0.4)

Table 1.2. Characteristics and viral analytes covered by widely used FDA-approved respiratory multiplex assays. Assays are categories by whether they have a low (left side of table) or high (right side of table) potential to be used as a point of care test.

		LOW POTENTIAL TO BE USED AS A POINT OF CARE TEST					HIGH POTENTIAL TO BE USED AS A POINT OF CARE TEST				
		Luminex xTAG RVP1	Luminex xTAG RVP FAST	Luminex xTAG RVP FASTv2	Luminex NxTAG RPP	GenMark Dx eSensor RVP	Nanosphere Verigene RV+	Nanosphere Verigene RP *Flex* Test	BioFire FilmArray RP	BioFire FilmArray RP 2	BioFire FilmArray RP EZ
Characteristics	Specimen Type	NPS	NPS	NPS	NPS	NPS	NPS	NPS	NPS	NPS	NPS
	User complexity	High	High	High	High	High	Moderate/ Low	Moderate/Low	Moderate/ Low	Moderate/ Low	CLIA-Waived
	Run Time	8hrs	>5hrs	>5hrs	>5hrs	>4hrs	~2hrs	~2hrs	~1hr	<1hr	~1hr
Viral Analytes	Influenza A	•	•	•	•	•	•	•	•	•	•
	Influenza A (H1)	•	•	•	•	•	•	•	•	•	•
	Influenza (H3)	•	•	•	•	•	•	•	•	•	•
	Influenza (H1N1)pdm09			•	•	•			•	•	•
	Influenza B	•	•	•	•	•	•	•	•	•	•
	Parainfluenza										•
	Parainfluenza 1	•		•		•		•	•	•	
	Parainfluenza 2	•		•		•		•	•	•	
	Parainfluenza 3			•		•		•	•	•	
	Parainfluenza 4	•		•				•	•	•	
	hMPV	•	•	•	•	•		•	•	•	•
	Adenovirus		•	•	•			•	•	•	•
	Adenovirus B/E					•					
	Adenovirus C					•					
	RSV								•	•	•
	RSV A	•			•		•	•			
	RSVB	•			•		•	•			
	Entero-Rhino	•	•	•	•	•		•	•	•	•
	HCoV			•							•
	HCoV HKU1			•	•				•	•	
	HCoV NL63			•	•				•	•	
	HCoV 229E			•	•				•	•	
	HCoV OC43			•	•				•	•	
	HBoV1			•	•						

Table 1.3. Characteristics and bacterial analytes covered by widely used FDA-approved respiratory multiplex assays. Assays are categories by whether they have a low (left side of table) or high (right side of table) potential to be used as a point of care test.

		LOW POTENTIAL TO BE USED AS A POINT OF CARE TEST					HIGH POTENTIAL TO BE USED AS A POINT OF CARE TEST				
		Luminex xTAG RVP1	Luminex xTAG RVP FAST	Luminex xTAG RVP FASTv2	Luminex NxTAG RPP	GenMark Dx eSensor RVP	Nanosphere Verigene RV+	Nanosphere Verigene RP *Flex* Test	BioFire FilmArray RP	BioFire FilmArray RP 2	BioFire FilmArray RP EZ
Characteristics	**Approved Specimen Type**	NPS	NPS	NPS	NPS	NPS	NPS	NPS	NPS	NPS	NPS
	User complexity	High	High	High	High	High	Moderate/ Low	Moderate/Low	Moderate/ Low	Moderate/ Low	CLIA-Waived
	Run Time	8hrs	>5hrs	>5hrs	>5hrs	>4hrs	~2hrs	~2hrs	~1hr	<1hr	~1hr
Bacterial Analytes	*Bordetella* Group (*B. pertussis, B. parapertussis, B. bronchiseptica*)							•			
	B. pertussis							•	•	•	•
	B. parapertussis									•	
	B. holmesii							•			
	Chlamydophila pneumoniae				•				•	•	•
	Mycoplasma pneumonia				•				•	•	•
	Legionella pneumophila				•						

REFERENCES

1. Cortez MH, Weitz JS. Distinguishing between indirect and direct modes of transmission using epidemiological time series. *Am Nat* 2013 Feb; 181(2):E43-54
2. U.S. Department of Health and Human Services. Health, United States, 2016 with chartbook on long-term trends in health. 2016.
3. CDC, National Center for Health Statistics. Table 017: Age-adjusted rates for selected causes of death by sex, race, and Hispanic origin: United States, selected years 1950-2015. Retrieved from: https://www.cdc.gov/nchs/hus/contents2016.htm#infectious. Accessed January 16, 2017.
4. Research America. Infectious diseases. 15.2.315. Retrieved from: http://www.researchamerica.org/sites/default/files/InfectiousDiseaseFactSheet_0.pdf . Accessed January 16, 2017.
5. Fendrick AM, Monto AS, Nightengale B, *et al.* The economic burden of non-infuenza related viral respiratory tract infection in the United States. *Arch Intern Med* 2003 Feb 24;163(4):487-94
6. Dixon RE. Economic costs of respiratory tract infections in the United States. *Am J Med* 1985 Jun 28;78(6B):45-51
7. CDC Foundation. Business challenges. Retrieved from: https://www.cdcfoundation.org/businesspulse/flu-prevention-infographic Accessed January 16, 2017.
8. Ortega-Sancez IR, Vijayaraghavan M, Barskey AE, *et al.* The economic burden of sixteen measles outbreaks on United States public health departments in 2011. *Vaccine* 2014 Mar 5;32(11):1311-7.

9. Mao L, Yang Y, Qiu Y, et al. Annual economic impacts of seasonal influenza on US counties: spatial heterogeneity and patterns. Int J Health Georg 2012 May 17;11:16.
10. Sugerman DE, Barskey AE, Delea MG, et al. Measles outbreak in a highly vaccinated population, San Diego, 2008: role of the intentionally undervaccinated. Pediatrics 2010 Apr; 125(4):747-55.
11. Centers for Disease Control and Prevention. Pneumonia and influenza mortality surveillance from the National Center for Health Statistics Mortality Surveillance System. Retrieved from: https://gis.cdc.gov/grasp/fluview/mortality.html Accessed October 9, 2017.
12. About the common cold (online). Retrieved from: https://www1.villanova.edu/villanova/studentlife/health/promotion/goto/resources/illnesses.html Accessed January 16, 2018.
13. Szabo, L. (2015, July). Measles kills first patient in 12 years. *USA Today* (online). Retrieved from: https://www.usatoday.com/story/news/2015/07/02/measles-death-washington-state/29624385/ Accessed October 10, 2017.
14. Centers for Disease Control. Epidemiology and Prevention of Vaccine-Preventable Diseases. Retrieved from: https://www.cdc.gov/vaccines/pubs/pinkbook/meas.html#modalIdString_CDCTable_0 Accessed October 10, 2017.
15. Coleman MS, Burke HM, Welstead BL, et al. Cost analysis of measles in refugees arriving at Los Angeles International Airport from Malaysia. *Hum Vaccin Immunother* 2017 May;13(5):1084-1090.
16. Thacker SB, Qualters JR, Lee LM. Public health surveillance in the United States: evolution and challenges. *MMWR Supplements* July 27, 2012 61(03);3-9.

17. Dowdle WR. The future of the public health laboratory. *Annu Rev Publ Health* 1993 14:649-64.
18. Langley G, Besser J, Iwamoto M, *et al*. Effects of culture-independent diagnostic tests on future emerging infections program surveillance. *Emerg Infect Dis* 2015 Sep;21(9):1582-88.
19. Baletine JR, Nabili SN, Shiel WC. Upper Respiratory Tract Infection. Retrieved from: https://www.medicinenet.com/upper_respiratory_infection/article.htm. Accessed November 20, 2017.
20. Mossad SB, Upper Respiratory Tract Infections. August 2013. Retrieved from: http://www.clevelandclinicmeded.com/medicalpubs/diseasemanagement/infectious-disease/upper-respiratory-tract-infection/. Accessed November 20, 2017.
21. John Hopkins Medicine. Upper respiratory infections. Retrieved from: https://www.hopkinsmedicine.org/healthlibrary/conditions/pediatrics/upper_respiratory_infection_uri_or_common_cold_90,P02966. Accessed November 20, 2017.
22. Temple Lung Cancer. Lower respiratory tract infections (LRTI) symptoms. Retrieved from: https://lung.templehealth.org/patient-care/conditions-we-treat/lower-respiratory-tract-infections/lower-respiratory-tract-infections-lrti-symptoms. Accessed November 20, 2017.
23. European Lung White Book. Acute lower respiratory infections. Retrieved from: https://www.erswhitebook.org/chapters/acute-lower-respiratory-infections/. Accessed November 20, 2017.
24. Mayo Clinic. Bronchitis. Retrieved from: https://www.mayoclinic.org/diseases-conditions/bronchitis/symptoms-causes/syc-20355566. Accessed November 20, 2017.
25. Mayo Clinic. Influenza (flu). Retrieved from: https://www.mayoclinic.org/diseases-conditions/flu/symptoms-causes/syc-20351719. Accessed November 19, 2017.

26. Centers for Disease Control and Prevention (CDC), National Center for Immunization and Respiratory Diseases (NCIRD). People at high risk of developing flu-related complications. August 25, 2016. Retrieved from: https://www.cdc.gov/flu/about/disease/high_risk.htm. Accessed November 19, 2017.
27. Torres A, Peetermans WE, Viegi G, *et al.* Risk factors for community-acquired pneumonia in adults in Europe: a literature review. *Thorax* 2013 Nov;68(11):1057-65.
28. Lynch JP 3rd. Hospital-acquired pneumonia: risk factors, microbiology, and treatment. *Chest* 2001 Feb;119(2 Suppl):373S-384S.
29. Bonten MJ, Kollef MH, Hall JB. Risk factors for ventilator-associated pneumonia: from epidemiology to patient management. Clin Infect Dis. 2004 Apr 15;38(8):1141-9.
30. Dasaraju PV, Liu C. 1996. Chapter 93 Infections of the Respiratory System In *Medical Microbiology*, 4th ed. Galveston (TX): University of Texas Medical Branch at Galveston.
31. Gompf, SG. Epiglottitis. February 08, 2017. Retrieved from: https://emedicine.medscape.com/article/763612-overview?pa=m%2F6cUAlUiUsdSbSDhvSiw9ZyPb1o9Htowl90k%2F9O1mwtYJr4eHKYMItsOuqjqbSe3VTCoOeXbjkt6yTmgC%2BNJwfqwaG3lIao9g53Y4UE8Q%3D#a4. Accessed November 18, 2017.
32. Ryan M, Hunt M, Snowberger T. A changing pattern of epiglottitis. *Clin Pediatr* (Phila). 1992 Sep;31(9):532-535.
33. National Health Service (NHS). Croup. July, 31, 2017. Retrieved from: https://www.nhs.uk/conditions/croup/causes/. Accessed November 18, 2017.
34. Worrall G. Croup. *Can Fam Physician* 2008 Apr;54(4):573-574.

35. Chamberlin NR. Respiratory airway infections. August 21, 2014. Retrieved from: https://www.atsu.edu/faculty/chamberlain/website/lectures/lecture/reairin2.htm. Accessed November 17, 2017.
36. Carolan, PL. Pediatric bronchitis. December 19, 2016. Retrieved from: https://emedicine.medscape.com/article/1001332-overview#a4". Accessed November 16, 2017.
37. NHS. Bronchiolitis. October 9, 2015. Retrieved from: https://www.nhs.uk/conditions/bronchiolitis/. Accessed on November 17, 2017.
38. Henig O, Kaye KS. Bacterial pneumonia in older adults. *Infect Dis Clin North Am* 2017 Dec;31(4):689-713.
39. Fraser CS, Jha A, Openshaw PJ. Vaccines in the prevention of viral pneumonia. *Clin Chest Med* 2017 Mar;38(1):155-169.
40. Chien JW, Johnson JL. Viral pneumonias. *Postgrad Med* Jun 30, 2015;107:41-52.
41. World Health Organization (WHO). 2016. Diseases of the respiratory system (J00-J99) In International statistical classification of diseases and related health problems, 10th revision (ICD-10), 2016.
42. Greenberg SB. Viral pneumonia. *Infect Dis Clin North Am* 1991 Sep;5(3):603-21.
43. Jung HS, Kang BJ, Ra SW, *et al*. Elucidation of bacterial pneumonia-causing pathogens in patients with respiratory viral infection. *Tuberc Respir Dis* (Seoul) 2017 Oct;80(4):358-367.
44. CDC, NCIRD. Overview of influenza surveillance in the United States. October 13, 2017. Retrieved from: https://www.cdc.gov/flu/weekly/overview.htm. Accessed November 15, 2017.

45. CDC, National Respiratory and Enteric Virus Surveillance (NREVSS). Week ending of November 11, 2017. Retrieved from: https://gis.cdc.gov/grasp/fluview/fluportaldashboard.html. Accessed on November 15, 2017.
46. Winquist, AG, Fukuda K, Bridges CB, Cox NJ. Neuraminidase inhibitors for treatment of influenza A and B infections. *MMWR* December 17, 1999 48(RR14);1-9.
47. Hayden FG, Osterhaus AD, Treanor JJ, et al. Efficacy and safety of the neuraminidase inhibitor zanamivir in the treatment of influenza virus infections. GG167 Influenza Study Group. *N Engl J Med* 1997 Sep 25;337(13):874-80.
48. Fry AM, Goswami D, Nahar K, et al. Efficacy of oseltamivir treatment started within 5 days of symptoms onset to reduce influenza illness duration and virus shedding in an urban setting in Bangladesh: a randomized placebo-controlled trial. *Lancet Infect Dis* 2014 Feb;14(2):109-18.
49. CDC, NCIRD. Antiviral drug resistance among influenza viruses. November 3, 2016. Retrieved from: https://www.cdc.gov/flu/professionals/antivirals/antiviral-drug-resistance.htm. Accessed November 10, 2017.
50. CDC, NCIRD. Coronavirus prevention and treatment. November 9, 2017. Retrieved from: https://www.cdc.gov/coronavirus/about/prevention.html. Accessed November 15, 2017.
51. Bailly B, Dirr L, El-Deeb IM, et al. A dual drug regimen synergistically blocks human parainfluenza virus infection. *Sci Rep* 2016 Apr 7;6:24138.
52. Rotbart HA. Antiviral therapy for enteroviruses and rhinoviruses. *Antivir Chem Chemother* 2000 Jul;11(4):261-71.

53. CDC, NCIRD. Adenoviruses prevention and treatment. May 23, 2017. Retrieved from: https://www.cdc.gov/adenovirus/about/prevention-treatment.html. Accessed November 15, 2017.
54. CDC. Pertussis (whooping cough). August 7, 2017. Retrieved from: https://www.cdc.gov/pertussis/clinical/treatment.html. Accessed November 14, 2017.
55. Tiwari T, Murphy TV, Moran J. Recommended antimicrobial agents for the treatment and postexposure prophylaxis of pertussis, 2005 CDC guidelines. *MMWR* December 9, 2005, 54(RR14);1-16.
56. CDC, NCIRD. *Chlamydia pneumoniae* infection. September 26, 2016. Retrieved from: https://www.cdc.gov/pneumonia/atypical/cpneumoniae/hcp/treatment.html. Accessed November 15, 2017.
57. CDC, NCIRD. *Mycoplasma pneumoniae* infection. March 15, 2016. Retrieved from: https://www.cdc.gov/pneumonia/atypical/mycoplasma/hcp/antibiotic-treatment-resistance.html. Accessed November 15, 2017.
58. Belongia EA, Kieke BA, Donahue JG, *et al*. Effectiveness of inactivated influenza vaccines varied substantially with antigenic match from the 2004-2005 season to the 2006-2007 season. *J Infect Dis* 2009 Jan 15;1992(2):159-67.
59. CDC, NCIRD. Influenza (flu): seasonal influenza vaccine effectiveness, 2005-2017. August 24, 2017. Retrieved from: https://www.cdc.gov/flu/professionals/vaccination/effectiveness-studies.htm. Accessed: November 15, 2017.
60. Ichinohe T, Tamura S, Kawaguchi A, *et al*. Cross-protection against H5N1 influenza virus infection is afforded by intranasal inoculation with seasonal trivalent inactivated influenza vaccine. *J Infect Dis* Nov 1;196(9):1313-20.

61. Lillie PJ, Berthoud TK, Powell TJ. Preliminary assessment of the efficacy of a T-cell-based influenza vaccine, MVA-NP+M1, in humans. *Clin Infect Dis* 2012 Jul;55(1):19-25.
62. The Jenner Institute. Human influenza vaccine programme. 2017 Retrieved from: http://www.jenner.ac.uk/human-flu. Accessed November 15, 2017.
63. CDC, NCIRD. Influenza (flu): Flu vaccination coverage, United States, 2016-17 influenza season. September 28, 2017 Retrieved from: https://www.cdc.gov/flu/fluvaxview/coverage-1617estimates.htm. Accessed November 18, 2017.
64. CDC. Recommended immunization schedule for children and adolescents aged 18 years or younger, United States, 2017. July 17, 2017.
65. CDC. Recommended immunization schedule for adults aged 19 years or older, United States, 2017. July 17, 2017.
66. CDC, National Center for Immunization and Respiratory Diseases (NCIRD). Vaccines and preventable diseases, diphtheria, tetanus, and pertussis vaccine recommendations. November 22, 2016. Retrieved from: https://www.cdc.gov/vaccines/vpd/dtap-tdap-td/hcp/recommendations.html. Accessed November 18, 2017.
67. Schwartz KL, Kwong JC, Deeks SL, *et al.* Effectiveness of pertussis vaccination and duration of immunity. *CMAJ* 2016 Nov 1;188(16):E399-E406.
68. CDC, NCIRD. Pertussis (whooping cough). August 7, 2017. Retrieved from: https://www.cdc.gov/pertussis/about/faqs.html. Accessed November 17, 2017.
69. Koepke R, Eickhoff JC, Ayele RA, *et al*. Estimating the effectiveness of tetanus-diphtheria-aceullular pertussis vaccine (Tdap) for preventing pertussis: evidence of rapidly waning immunity and difference in effectiveness by Tdap brand. J Infect Dis 2014 Sep 15:210(6):942-53.

70. Rappo U, Schuetz AN, Jenkins SG, *et al.* Impact of early detection of respiratory viruses by multiplex PCR assay on clinical outcomes in adults patients. *J Clin Microbiol* 2016 Aug;54(8):2096-103.

71. Nsubuga P, White ME, Thacker SB, *et al.* Public health surveillance: a tool for targeting and monitoring interventions. In *Disease Control Priorities in Developing Countries.* 2nd edition. (2006) New York: Oxford University Press 997-1015.

72. CDC. Overview of influenza surveillance in the United States. (September 18, 2017). Retrieved from: https://www.cdc.gov/flu/weekly/overview.htm.

73. U.S. Department of Health and Human Services. Strategic national stockpile (SNS). September 29, 2017. Retrieved from: https://chemm.nlm.nih.gov/sns.htm. Accessed November 12, 2017.

74. Schnepf N, Resche-Rigon M, Chaillon A, *et al.* High burden of non-influenza viruses in influenza-like illness in the early weeks of H1N1v epidemic in France. *PLoS One* 2011;6(8):e23514.

75. Leland DS, Ginocchio CC. Role of cell culture for virus detection in the age of technology. *Clin Microbiol Rev* 2007 Jan;20(1):49-78

76. Edwards MC, Gibbs RA. Multiplex PCR: advantages, development, and applications. *PCR Methods Appl* 1994 Feb;3(4):S65-75.

77. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet.* 2015;385:117-71.

78. WHO Media Centre. The top 10 leading causes of death by country income group (2012). Retrieved from: http://www.who.int/mediacentre/factsheets/fs310/en/index1.html. Accessed March 28, 2016

79. WHO. Global Health Observatory data repository: acute lower respiratory infections. February 2, 2010. Retrieved from: http://apps.who.int/gho/data/view.main.CM1002015WORLD-CH9?lang=en Accessed on January 17, 2018.

80. Global Burden of Disease 2015 Collaborators. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory tract infections in 195 countries: a systematic analysis for the global burden of disease study 2015. *Lancet Infect Dis* 2017 Nov;17(11):1133-1161.

81. Gessner BD, Shindo N, Briand S. Seasonal influenza epidemiology in sub-Saharan Africa: a systematic review. *Lancet Infect Dis* 2011 Mar; 11(3):223-35.

82. CDC. Seasonal Influenza Q & A. What is seasonal (flu)? September 18, 2015. Accessed April 30, 2016

83. Lofgren E, Fefferman NH, Naumov YN, *et al.* Influenza seasonality: underlying causes and modeling theories. J Virol 2007 Jun;81(11):5429-36.

84. CDC. Pneumonia and influenza mortality surveillance from the National Center for Health Statistics Mortality Surveillance System. Accessed October 9, 2017. Retrieved from: https://gis.cdc.gov/grasp/fluview/mortality.html

85. Mao L, Yang Y, Qiu Y, *et al.* Annual economic impacts of seasonal influenza on US counties: spatial heterogeneity and patterns. *Int J Health Georg* 2012 May 17;11:16.

86. Sugerman DE, Barskey AE, Delea MG, *et al.* Measles outbreak in a highly vaccinated population, San Diego, 2008: role of the intentionally undervaccinated. *Pediatrics* 2010 Apr; 125(4):747-55.

87. Binder AM, Biggs HM, Haynes AK, *et al.* Human adenovirus surveillance- United States, 2003-2016. MMWR October 6, 2017 66(39);1039-1042.

88. Frost HM, Robinson CC, Dominguez SR. Epidemiology and clinical presentation of parainfluenza type 4 in children: a 3-year comparative study to parainfluenza types 1-3. *J Infect Dis* 2014 Mar 1;209(5):695-702.

89. Meyers L, Ginocchio CC, Faucett AN, *et al.* Automated collection of pathogen-specific diagnostic data for real-time syndromic epidemiological studies. (preprint).

90. Gavin PJ, Thomson Jr. RB. Review of Rapid Diagnostic Tests for Influenza. *Clin Appl Immun Rev.* (2004) 4(3):151-172.

91. Hodinka RL, Kaiser L. Point-Counterpoint: Is the Era of Viral Culture Over in the Clinical Microbiology Laboratory? *J Clin. Microbiol.* January 2013, 51(1), 2-8.

92. Thompson C, Cullum N, McCaughan D, *et al.* Nurses, information use, and clinical decision making: the real world potential for evidence based decisions in nursing. *Evid Based Nurs* 2004;7-68-72

93. Buchan BW, Ledeboer NA. Emerging technologies for the clinical microbiology laboratory. Clin Microbiol Rev 2014 Oct;27(4):783-822.

94. Zitterkopf NL, Leekha S, Espy MJ, *et al.* Relevance of influenza A virus detection by PCR, shell vial assay, and tube cell culture to rapid reporting procedures. *J Clin Microbiol* 2006 Sep;44(9):3366-3367.

95. Loeffelholz MJ, Thompson CJ, Long KS, *et al*. Comparison of PCR, culture, and direct fluorescent-antibody testing for detection of *Bordetella pertussis*. *J Clin Microbiol* 1999 Sep;37(9):2872-2876.

96. Lee DH, Kim JI, Lee JW, *et al.* Quantitative measurement of influenza virus replication using consecutive bronchoalveolar lavage in the lower respiratory tract of a ferret model. *J Vet Sci* 2014 Sep; 15(3):439-442.

97. Ieven M. Currently used nucleic acid amplification tests for the detection of viruses and atypicals in acute respiratory infections. *J Clin Virol* 2007 Dec;40(4):259-76.

98. Leland DS, Ginocchio CC. Role of cell culture for virus detection in the age of technology. *Clin Microbiol Rev* 2007 Jan; 20(1):49-78.

99. Bressoud A, Whitcomb J, Pourzand C, *et al.* Rapid detection of influenza virus H1 by the polymerase chain reaction. *Biochem Bioph Res Co*. 167;2:425-430.

100. Zhang WD, Evans DH. Detection and identification of human influenza viruses by the polymerase chain reaction. *J Virol Methods*. 1991 Jun;33(1-2):165-89.

101. Elnifro EM, Ashshi AM, Cooper RJ, *et al.* Multiplex PCR: optimization and application in diagnostic virology. *Clin Micrbiol Rev* 2000 Oct;13(4):559-570.

102. FDA. 510(k) Denovo decision summary, k060159.

103. Schreckenberger PC, McAdam AJ. Point-counterpoint: large multiplex PCR panels should be first-line tests for detection of respiratory and intestinal pathogens. *J Clin Microbiol* 2015 Oct;53(10):3110-5.

104. Epson, E. (Producer). February 15, 2017. *Influenza prevention and outbreak control in long-term care facilities*.

105. California Association of Health Facilities. Facts and statistics: long-term care providers. (October, 2016). Retrieved from: http://www.cahf.org/Resources/Media-Center/Facts-and-Statistics.

106. Montoya A, Mody L. Common infections in nursing homes: a review of current issues and challenges. *Aging Health* 2011 Dec;7(6):889-899.

107. Boone SA, Gerba CP. Significance of Fomites in the spread of respiratory and enteric viral disease. *Appl Environ Microbiol* 2007, 73(6):1687.

108. Lopez Puga J, Krzywinski M, Altman N. Points of significance: Bayesian statistics. *Nat Methods* 2015 May;12(5):377-8.

109. WHO. Measles, disease surveillance. Retrieved from: http://www.emro.who.int/health-topics/measles/disease-surveillance.html. Accessed November 13, 2017.

110. CDC. National notifiable diseases surveillance system (NNDSS), measles/rubeola, 2013 case definition. Retrieved from: https://wwwn.cdc.gov/nndss/conditions/measles/case-definition/2013/. Accessed November 13, 2017.

111. Marcdante K, Kliegman. February 25, 2014. Chapter 97: Infections characterized by fever and rash In *Nelson essentials of pediatrics e-book.* (available online) Elseiver Health Sciences. p 329.

112. CDC. Measles data and statistics. April 27, 2016. Retrieved from: https://www.cdc.gov/measles/downloads/measlesdataandstatsslideset.pdf. Accessed November 15, 2017.

113. Los Angeles County Public Health Department (LACPHD). Measles immune globulin factsheet. April 2014. Retrieved from: http://publichealth.lacounty.gov/ip/providers/Alert/Measles_Immune_Globulin_20140401.pdf. Accessed November 14, 2017.

114. D'Souza RM, D'Souza R. Vitamin A for treating measles in children. Cochrane Database Syst Rev 2001;(2):CD001479.

115. CDC, NCIRD. Measles (rubeola). March 3, 2017 Retrieved from: https://www.cdc.gov/measles/about/history.html. Accessed November 14, 2017.

116. Hinman AR, Orenstein WA, Bloch AB, *et al*. Impact of measles in the United States. *Rev Infect Dis* 1983 May-Jun;5(3):439-44.

117. CDC. Measles vaccination. February 13, 2017. Retrieved from: https://www.cdc.gov/measles/vaccination.html. Accessed November 18, 2017.

118. Fine P, Eames K, Heymann DL, et al. "Herd immunity": a rough guide. *Clin Infect Dis* 2011 Apr 1;52(7):911-6.

119. Ventola CL. Immunization in the United States: recommendations, barriers, and measures to improve compliance. *PT* 2016 Jul;41(7):426-436.

120. WHO. Global measles and rubella strategic plan: 2012-2020. 2012.

121. WHO. Measles, immunization. February 6, 2014. Retrieved from: http://www.who.int/immunization/topics/measles/en/. Accessed November 12, 2017.

122. WHO. Measles, fact sheet. October 2017. Retrieved from: http://www.who.int/mediacentre/factsheets/fs286/en/. Accessed November 12, 2017.

123. Shrivastava SR, Shrivastava PS, Ramasamy J. Measles in India: challenges and recent developments. *Infect Ecol Epidemiol* 2015;5.

124. Niazi AK, Sadaf R. Measles epidemic in Pakistan: in search of solutions. *Ann Med Health Sci Res* 2014 Jan-Feb;4(1):1-2.

125. Moghadam M, Afsarkazerooni P, Ebrahimi M, *et al*. Measles outbreak in south of Iran, where vaccine coverage was high: a case-series study. *Iran J Public Health* 2014 Mar;43(3):375-380.

126. Salako AA, Sholeye OO. Control of measles in Nigeria: a critical review of the literature. *Br J Med Med Res* 2015 5(2):160-168.

127. CDC, NCRID. Measles cases and outbreaks. November 13, 2017 Retrieved from: https://www.cdc.gov/measles/cases-outbreaks.html. Accessed November 15, 2017.

128. Fiebelkorn AP, Redd SB, Gastanaduy PA, *et al.* A comparison of post-elimination measles epidemiology in the United States, 2009-2014 versus 2001-2008. *J Pediatric Infect Dis Soc* 2017 Mar 1; 6(1):40-48.

129. Lebo E, Wallace G, Redd S, *et al.* Measles- United States, January 1- August 24, 2013. *MMWR* September 13, 2013 62(36);741-743.

130. Sugerman DE, Barskey AE, Delea MG, *et al.* Measles outbreak in a highly vaccinated population, San Diego, 2008: the role of the intentionally undervaccinated. *Pediatrics* 2010 Apr;125(4):747-55.

131. Kodagoda D, El Amin AN, Nguyen V, *et al.* Measles outbreak associated with an arriving refugee- Los Angeles County, California, August-September 2011. *MMWR* June 1, 2012 61(21);385-389.

132. Zipprich J, Winter K, Hacker J, *et al.* Measles outbreak- California, December 2014-February 2015. *MMWR* February 20, 2015 64(06);153-154.

133. CDC. Measles outbreaks and cases. Retrieved from: https://www.cdc.gov/measles/cases-outbreaks.html Accessed August 12, 2017.

134. Finkenstadt B, Keeling M, Grenfell B. Patterns of density dependence in measles dynamics. *Proc Biol Sci* 1998 May 7;265(1398):753-762.

135. World Health Organization. Public health surveillance. (2017) Retrieved from: http://www.who.int/topics/public_health_surveillance/en/

CHAPTER 2

Critical evaluation of FDA-approved respiratory multiplex assays for epidemiological surveillance.

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ABSTRACT

Introduction: Clinical management and identification of respiratory diseases has become more rapid and increasingly specific due to widespread use of PCR multiplex technologies. Although significantly improving clinical diagnosis, multiplexed PCR assays could have a greater impact on local and global disease surveillance. We wish to propose methods of evaluating respiratory multiplex assays to maximize diagnostic yields specifically for surveillance efforts.

Areas Covered: Here, we review multiplexed assays and critically assess what barriers have limited these assays for disease surveillance and how these barriers might be addressed. We focus specifically on the case study of using multiplexed assays for surveillance of respiratory pathogens.

Expert Commentary: Current commercially-available respiratory multiplex PCR assays are widely used for clinical diagnosis; however, specific barriers have limited their use for surveillance. Key barriers include differences in testing phase requirements and diagnostic performance evaluation. In this work we clarify phase testing requirements and introduce unique

diagnostic performance measures that simplify the use of these assays on a per target basis for disease surveillance. Additionally, we recommend that future efforts focus on developing performance standards for next-generation sequencing platforms.

INTRODUCTION

Multiplexed diagnostic assays have the potential to revolutionize disease surveillance, however, these technologies have not had as great of impact on control and prevention efforts as compared to healthcare utilities because surveillance needs are fundamentally different from clinical diagnostic priorities. Whereas diagnostic assays are used to identify disease etiology and appropriate treatment for infected individuals, disease surveillance is conducted at a population level to identify trends and disease burden. Despite the substantial benefits (*e.g.,* high efficiency, rapid turn-around-time, high through-put, screening for a multitude of targets) of multiplexed assays to enhance surveillance efforts, no comprehensive review has assessed whether multiplex assays are actually meeting surveillance needs or whether future studies should focus on specific laboratory needs when performing surveillance and outbreak testing. Here, we review what the barriers are to using multiplexed assays for respiratory tract infections (RTIs) surveillance. By focusing on the limitations of current respiratory multiplex assays for surveillance, we are able to highlight how new multiplexed assays could be designed to better meet the needs of laboratories conducting surveillance.

Currently, RTIs are among the top five leading causes of death worldwide across all age groups accounting for 4.2 million deaths annually; total deaths from RTI's account for more than liver, colon, breast and prostate cancer combined (1). RTIs are a cause of significant morbidity, leading to loss of productivity and directly impacting national markets. For instance, seasonal

influenza affects 16 to 64 million American each year (2), and can cost the United States close to a billion dollars in particularly bad years (3). Hallmark symptoms of RTIs are due to a wide range of clinically indistinguishable respiratory pathogens. Not identifying etiological causes of respiratory disease can lead to poor antibiotic stewardship that can pose problems for the patient, at risk populations, and the healthcare system as a whole. Sensitivity (Sen) is defined as the ability of a diagnostic test to correctly identify individuals with disease. When clinicians base treatment decisions on symptoms alone Sen can be as low as 36%, resulting in only a small percentage of patients receiving appropriate care (4). Given the cost, public health burden, and diversity of pathogens that cause similar symptoms, RTIs were among the first group of pathogens tested in a multiplex format (5).

Respiratory multiplex testing algorithms enhance treatment of influenza in children with acute respiratory tract infections and have shown to aide surveillance efforts (6). There are four major FDA-approved respiratory multiplex assays: Luminex NxTAG Respiratory Pathogen Panel (RPP), Nanosphere Verigene Respiratory Panel (RP) *Flex*, BioFire Film Array Respiratory Panel (RP), and eSensor Respiratory Viral Panel (RVP). Because RTI treatment and clinical management depends on the etiological agent, the ability to rapidly characterize respiratory pathogens is extremely important. Multiplex methods are particularly valuable in the case of RTIs, allowing clinicians to simultaneously test for a wide range of possible pathogens, as opposed of relying on numerous individuals tests. Correct diagnosis of RTIs is also critical to antibiotic stewardship, since roughly 1 in 3 antibiotics prescribed are unnecessary and most of these unneeded prescriptions are for respiratory infections (7). Another challenge is diagnosing RTI's across geographic regions where respiratory disease prevalence may be seasonal or region specific. A broad spectrum assay screening a variety of targets, would identify changes in seasonal trends

significantly impacting regional clinical decisions throughout the year. RSV infections, for example, while seasonal, affect different parts of the United States at different times of the year with outbreaks varying within regions and between communities (8). Multiplex methods also help resolve genotype variation. Recognizing genotypic differences helps in diagnosis by determining the course of clinical treatments. For instance, adamantane-resistant A/H3N2 strains have circulated globally for the past decade and oseltamivir-resistant seasonal A/H1N1 have circulated globally since 2007 (9). During the 2016-2017 influenza season, the predominant strain was A/H3N2, compared to a year earlier when H1N1pdm09 was prevalent. These trends call to attention of the need to not only identify, but also characterize respiratory pathogens if possible to circumvent ineffective treatments. The benefits of multiplex assays have resulted in an increasing number of hospital and clinical laboratories adopting these methods strictly for clinical use (10). Multiplex technologies can help aide clinical decisions by simultaneously screening for a variety of respiratory pathogens often detecting specific genetic differences increasing the value of these methods for diagnostic purposes. For surveillance, limited studies have shown the epidemiological use of these technologies on control and prevention but when applied, respiratory multiplex assays tremendously benefit public health. For example, despite not being included in the first generation Luminex xTAG RVP (released in 2008), post-analytical results could still be used as a rule-out method for 2009 H1N1pdm09 strain (11). Similarly, during the 2014 EV-D68 outbreak, both the eSensor RVP and the FilmArray RP, were used as reliable diagnostic tests in surveillance algorithms to distinguish between Rhinovirus and Enterovirus-D68 (12, 13).

Multiplex assays have the potential to transform surveillance efforts of RTIs by supporting control and prevention activities. For instance, during the 2009 H1N1 pandemic, epidemiological surveillance was just as important as the narrow focus of patient care that typical in most diagnostic

labs (14). Through the use of multiplex methods, public health laboratories (PHLs) helped confirm susceptible H1N1pdm cases by identifying transmission patterns and outbreak clusters in affected populations. Multiplex methods can also strengthen surveillance activities by allowing PHLs to meet the surge capacity needs of surrounding health jurisdictions and to provide baseline trends that helps resource planning. By screening acute respiratory infections against a variety of pathogens, and rapidly providing results, multiplex assays allow the public health community to recommend appropriate clinical interventions and hence shorten the duration of an outbreak within the populations served for a given community. Despite these potential advantages to the surveillance community, multiplexed PCR-assays have been more slowly adopted by surveillance labs than by their clinical counterparts. Currently, a minimum of only 13 state public health laboratories have fully implemented respiratory multiplex methods for surveillance use (15). This begs the question of whether there are substantive reasons related to the use, design or commercialization of these tests that has slowed their uptake by labs focused on surveillance.

CURRENT FDA-APPROVED MULTIPLEXED ASSAYS FOR RESPIRATORY TRACT INFECTIONS

In many clinical laboratories, the use of culture and serology has been greatly reduced (if not altogether replaced) due to the availability of nucleic acid amplification tests (NAATs). NAATs dramatically reduce turn-around time allowing rapid response for control and prevention and by confirming suspect cases in hours instead of days and weeks. NAATs have also shown greater sensitivity above conventional methods for all public health relevant targets. NAATs are sufficient for making clinical decisions and unlike traditional methods remove subjective interpretation that can differ from one technician to the next. The epidemiological use of NAATs

such as RT-PCR allows understanding of possible transmission in a population, but also important gives information for defining an appropriate case definition. Despite the widespread use of NAATs, only a few major FDA-approved multiplex methods are available for the detection of respiratory pathogens.

Luminex NxTAG Respiratory Pathogen Panel

In 2008, the Luminex Corporation (Austin, Texas) unveiled the xTAG RVPv1, a high complexity high through-put bead-based qualitative PCR assay capable of detecting a variety of respiratory pathogens. The xTAG RVPv1 assay was the first FDA-approved broad respiratory panel and is extensively used in clinical and PHLs. Utilizing a bead hybridization fluid array, the most current version of the panel is the Luminex NxTAG RPP that can screen 20 total targets from NPS: RSV type A and B, influenza A variants, influenza A H1 and H3, influenza B, parainfluenza 1, 2, 3, and 4, human metapneumovirus (hMPV), adenovirus, rhinovrus/enterovirus, coronavirus type HKU1, NL63, 229E, OC43, human bocavirus (hBoV), and bacterial targets, *C. pneumoniae* and *M. pneumoniae*. Results of up to 96 samples can be obtained in a single working day (3hrs post-extraction). Operator time is anywhere between 20 to 25 minutes. Samples are added directly to pre-plated lyophilized wells on a 96 well plate. As a closed tube-system, the NxTAG RPP requires PCR and hyrbridization prior to adding the plate to Luminex's MAGPIX® instrument. The Luminex MagPix instrument functions as a fluorescent imager (instead of a flow cytometer of the original RVPv1 assay). Calls are qualitative based on mean fluorescent intensity (MFI).

Nanosphere Verigene Respiratory Panel Flex

The Verigene system (now manufactured by Luminex) a moderate complexity, customizable throughput assay that detects a variety of pathogens not found in other major FDA-approved respiratory multiplex panel such as a *Bordetella* spp group. The Verigene platform consists of a reader and a separate processing unit capable of sample-to-results in about 2 hours with minimal hands-on time and is fully automated. On-board extraction is followed by PCR amplification and subsequent elution of nucleic acids into a test cartridge for hybridization. Targets are detected by utilizing gold nanoparticle-conjugated probes that hybridize virus specific amplicons on a microarray. The signal from the hybridized probes are amplified by a silver staining process. Light scatter is measured on gold nanoparticle probes bound specifically to target-containing spots on the microarray. Qualitative analysis is then done and reported by the Verigene Reader. The most recent panel offered on the Verigene system is the Respiratory Pathogens *Flex* (RP *Flex*), which received FDA approval in 2015. The panel screens against both viruses and bacteria highlighting the *Bordetella* spp bacterial group. The RP *Flex* is the first multiplex respiratory test to allow customization by user's reporting preference allowing a 'pay for what you need' option. Five reporting blocks that may be released to users. Block 1: adenovirus and hMPV, Block 2: influenza A, A/H1, A/H3, and influenza B, Block 3: parainfluenza 1-4 and rhinovirus, Block 4: RSV A and B, and Block 5: *B. pertussis*, *B. holmesii* and *B. parapertussis/B.bronchiseptica*. While on-board PCR multiplexing is performed for all targets, only those results pre-selected are paid for and reported. Endemically circulating viruses may be targeted in specific populations while budgeting for targets of interest. For example, Block 1 and Block 4 may be of great interest for laboratories serving senior or pediatric populations as outbreaks in RSV and adenovirus are common in these sub-populations (16, 17). Additionally,

during flu season, Block 2 would give a great profile on circulating influenza viruses. For diagnostic laboratories, surveillance and population-based trend data are highly valued and therefore the flex option would mostly likely be bypassed in favor of a fixed report for all targets.

eSensor Respiratory Viral Panel (RVP)

GenMark Diagnostics, Inc. (Carlsbad, California) received FDA approval for the eSensor Respiratory Virus Panel (RVP), a high complexity high-throughput assay in 2012. The assay runs on the GenMark's XT-8 instrument modular system which can integrate from one to three analyzers processing up to 8 samples each. The underlying technology combines microfluidics and electrochemical detection to characterize 14 viral respiratory pathogens. The initial workflow begins with 60 minutes of prep time and includes: pre-extraction, reagent prep, amplification, and a single-strand specific exonuclease reaction step. The target DNA is mixed and hybridized with specific target signal probes that each contain a ferrocene label. The mixture is placed into an eSensor loading cartridge then analyzed by the XT-8 system. Within the cartridge exists a microfluidic chamber with pre-assembled capture probes bound on gold electrodes. After the target DNA/signal probe complex runs through the cartridge chambers a binding step occurs by capture probes giving off a positive signal when voltage is carried through each electrode. The latter part of the workflow is roughly 3.5 hours. The respiratory viral panel itself contains several Influenza targets, RSV subtypes, parainfluenza types, and hMPV. Additionally, the panel only reports Rhinovirus instead of a combined rhinovirus/enterovirus result however, cross-reactivity with Enterovirus D68 (EV-D68) has been reported (18). The panel also is the only multiplex panel to specifically detect adenovirus types (B/E and C).

FilmArray Respiratory Panel (FilmArray RP)

The FilmArray RP is an FDA-approved (2011) low-throughput, moderate/low complexity, multiplex nested PCR assay automated from extraction to result. The assay is able to identify and characterize 17 viral as well as 3 bacterial pathogens manufactured by BioFire Diagnostics (Salt Lake City, Utah). The 17 viral targets include: adenovirus, coronavirus HKU1, NL63, 229E and OC43, hMPV, rhinovrus/enterovirus, influenza A, influenza A subtype H1, H3 and (H1N1)pdm09, influenza B, parainfluenza 1,2,3 and 4, and RSV. The 3 bacterial targets include: *B. pertussis*, *Chlamydophila pneumonia*, and *Mycoplasma pneumonia*. A major benefit of the FilmArray RP is the minimal hands-on operator time (2-4 minutes/sample). The system includes onboard automated nucleic acid extraction. NPS samples are injected into a reagent pouch, which house freeze-dried regents for sample preparation, purification, and nested multiplex PCR. Endpoint melting curve analysis confirms or rejects a call. The time-to-result is about 1 hour. A drawback of the FilmArray RP system is that only one sample can be processed at a time. Thus, in a single 8hr workday, a total of 6 to 8 samples may be processed. The FilmArray 2.0 System received FDA clearance in early 2015. The updated model could include up to 8 modular units sequentially linked to one PC allowing higher throughput. In early 2016, Biofire Diagnostics (now a subsidiary of BioMerieux) received FDA clearance for an expanded instrument system, the FilmArray Torch. The newer platform is comprised of scalable modules that may include up to a 12-module system capable of screening up to ~90 samples per an 8hr work-day. The newer platform follows the same technology and allows the processing of the same RP assay.

ANALYTICAL REQUIREMENT DIFFERENCES FOR SURVEILLANCE AND CLINICAL DIAGNOSIS

The analytical requirements for surveillance of respiratory pathogens differ from those of clinical diagnosis (**Table 2.1**). In the clinical setting, rapid identification is a top priority so that the appropriate treatments can be identified and delivered. By contrast, for surveillance purposes, definitive characterization is critical to resolving etiologies of outbreaks, understanding epidemiological trends and aiding disease control and prevention. Here, we review the testing requirement differences for the three phases (pre-analytical, analytical, and post-analytical) of clinical and surveillance testing.

Pre-analytical and Analytical Requirements

The primary differences between the pre-analytical testing requirements for disease surveillance and clinical diagnosis begins during specimen collection. Because, healthcare facilities only collect specimens from individuals exhibiting RTI symptoms, the likelihood of identifying the source of infection is high (19). This selective specimen collection by clinical labs minimizes unnecessary testing, which in turn reduces cost and use of medical services. For laboratories performing surveillance-based testing, specimens are collected from both symptomatic and asymptomatic individuals. Comparing the test results for symptomatic and healthy individuals provide an evidence base for epidemiological associations. Critically, active surveillance studies have reported that as many as 44% of viral respiratory infections may go unreported due to lack of symptoms (20). Thus, monitoring asymptomatic individuals can potentially mitigate further spread of infection by detecting asymptomatic carriers of respiratory disease.

Another difference between clinical and surveillance testing occurs during related to specimen processing. All four of the current major FDA-approved multiplex assays (FilmArray RP, Nanosphere RP *Flex*, Luminex NxTAG RVP, GenMark eSensor RVP) have only received FDA approval for the testing of nasopharyngeal specimens (NPS) only. Despite several proof-of-principal studies (21-24) that have shown comparable diagnostic sensitivity (Sen) (defined as the ability of a diagnostic test to correctly identify individuals with disease) and specificity (Spe) (defined as the ability of a diagnostic test to correctly identify individuals without disease) when using other samples types (*e.g.*, sputum, bronchoalveolar lavage fluids, bronchial washes, anterior nares swabs, throat swabs and tracheal aspirates), processing of other specimen types and collection methods requires additional independent studies by the testing laboratory especially if results are used to make a clinical decision. Independent studies include analytical validation studies of lab-developed tests (LDTs) that establish comparable precision, accuracy, reference range and reportable range. Laboratories are also required to assess additional assay performance characteristics (*e.g.*, analytical specificity and analytical sensitivity and linearity) (25). For surveillance testing, similar independent studies are required especially when results are reported to healthcare agencies for clinical purposes. However, because surveillance testing is used to establish disease trends and reveal etiology, a variety of sample types are often collected and processed since microbial load is unknown and may be higher in one type than another (26). To report these results, surveillance labs are also required to perform additional independent validation studies or report results as Research Use Only (RUO) or Investigational Use Only (IUO). The required additional validation of other sample types likely limits the adoption of multiplexed assays for surveillance purpose.

Finally, there are differences between the number of samples typically processed for clinical diagnostic and surveillance work. Because effective clinical care depends on rapid turn-around time, clinical labs are more likely to process samples individually or in smaller batches. When identifying the source of an outbreak, rapid results are critical for control and prevention also requiring small batch testing. However, for routine surveillance whose aim is to trend population data, samples can be tested in large batches to minimize costs. However, large batch testing is often not possible for some multiplex assays due to the inherent design by manufacturers. For instance, both the FilmArray RP and the Nanosphere RP *Flex* can only process one sample at a time, while the Luminex NxTAG RPP and the eSensor RVP can process multiple samples at one time. It should be noted that if needed, both the FilmArray RP and the Nanosphere RP *Flex* allow for modular expansion to test more than one sample at a time but even then there are limitations to the number of samples that can be processed at one time. Thus, the need for surveillance labs to perform large batch testing has not been met which likely has slowed the adoption of respiratory multiplex assays by surveillance labs.

Post-Analytical Requirements

There are also differences between the requirements for tests from the clinical and surveillance standpoint at the post-analytical phase. The original intended use of the major FDA-approved respiratory multiplex assays was to provide rapid results to aide clinical management (27-30). As a result, since the commercial release of these assays, studies have mostly reported the post-analytical use of respiratory multiplex results in clinical settings (6, 31). Post-analytical interpretation of results varies depending on how the results from multiplex testing are used. For example, in the clinical environment, and in some instances during surveillance testing, results

from multiplex testing are used for healthcare decisions. However, for the majority of surveillance testing, results are most often used to trend population data, identify the causative agent of an outbreak and confirm cases.

Critical Attributes of Multiplex testing based on Analytical Requirements

Diagnostic Validity for Clinical Testing

Differences during the pre- and post-analytical phases of testing for clinical and surveillance purpose define the critical attributes needed in multiplex assays when used for clinical diagnostic testing or surveillance (**Table 2.1**). A major critical attribute is diagnostic validity. While the basic measures of diagnostic validity for clinical diagnostic and surveillance labs are the same, they are used and prioritized in different ways. These basic diagnostic measures are Sen (sensitivity or true positive rate, TP) and Spe (specificity or true negative rate, TN). Sen, sometimes referred to as the True Positive Rate, is often expressed as True Positives (TP) and False Negatives (FN) given by the following:

$$\frac{TP}{TP + FN}$$

(Eq. 2.1)

Spe, sometimes referred to as the True Negative Rate, is often expressed in terms of True Negatives (TN) and False Positives (FP) given by the following:

$$\frac{TN}{TN + FP}$$

(Eq. 2.2)

Despite the universal use of Sen and Spe in assessing assay performance, these measures are not without limitations (32). Sen and Spe are summarized population parameters used to confirm the

presence or absence of disease. Conceptually, Sen and Spe are not appropriate on a case by case basis (as disease progression differs from one patient to another). More critically, Sen and Spe are independent of disease prevalence, which changes how an assay may perform (33-34). Thus, appropriate clinical-decision making using these population parameters requires more parametric inputs to aid healthcare decisions (35). The interpretation of Sen and Spe in the clinical area is incomplete requiring more scrutiny and explanation for patient management (36).

A better measure of assay performance for clinical diagnostic purposes is the likelihood ratio (LR) particularly when used to describe tests having more than just a dichotomous (positive/negative) result (32). LRs are defined as the ratio between the probability of the presence or absence of disease in a patient to the probability of the same result in a patient with or without disease. LRs provide direction and strength of a test result in ruling-in and ruling-out disease (36, 37). For clinicians, LRs answer the frequent question: Given a test result, how likely is it that a patient may truly have disease? In practice, LRs are presented as positive likelihood ratios (LR+) or negative likelihood ratios (LR-). LR+, is the ratio of the probability of a positive test when disease is present to the probability of the same test result when disease is absent and may be expressed as Sen and Spe:

$$LR+ = \frac{Sen}{1 - Spe}$$

(Eq. 2.3)

Conversely, LR-, the probability of a negative test result given the disease is present to the probability of the same test result when disease is absent is presented as:

$$LR- = \frac{1 - Sen}{Spe}$$

(Eq. 2.4)

Using prior evidence to calculate the probability that disease is actually present allows a much more stable and clinically applicable diagnostic interpretation (39), with application to surveillance. Bayes' Rule or Bayes' Theorem describes the probability of an outcome based on prior knowledge. For clinical diagnosis, prior knowledge includes prevalence of disease and diagnostic validity of the assay used to screen for disease. In assessing the clinical utility of an assay, Bayes' Theorem allows the post-test probability of disease to be calculated from the pre-test (*i.e.,* prevalence of disease) and conditional probabilities (inherent to the diagnostic test calculate post-test used, LRs). Graphically, the relationship between these input probabilities can be shown via a Fagan nomogram, a graphical scaling tool designed to help clinicians determine the probability of a patient actually having disease based on the value of LR+ for a test and prevalence of disease in the population (40). For clinically relevant respiratory pathogens such as Influenza A/H1, H3, Influenza B and *B. pertussis*, Fagan nomograms can be used by healthcare professionals comparatively to reveal which assay best suites clinical care needs. Prevalence in a Fagan nomogram analysis can range from 0 to 100% and be applied to any population.

Use of Fagan nomogram analysis in calculating post-test probabilities (the probability of positive test given prevalence and the LR+) for each of the major FDA-approved multiplex respiratory assays is summarized in **Table 2.2**. The LR+ shown in **Table 2.2** is calculated based on the Sen and Spe reported in the FDA 510(k) summaries for each of these assays. The prevalence of influenza A subtypes and Influenza B shown here reflects yearly seasonal Influenza trends where roughly 5-20% of the U.S. population get the flu each year (41). This calculation uses data from a study conducted in a general clinical laboratory supporting inpatient and emergency room services where respiratory samples were initially screened for RSV and 2% of the sample population tested positive for *B. pertussis* (42). A positive result for influenza A/H1 on the

Luminex NxTAG RPP has a post-test probability of 98.1% (*i.e.*, that the individual who tested positive on the assay is actually infected with influenza) (**Table 2**). The BioFire FilmArray RP performs better in detecting Influenza A/H1, H3 and B than all other multiplex platforms listed. However, the Nanosphere RP *Flex*, performs better than the FilmArray RP when detecting *B. pertussis*. Calculating post-test probabilities for different assays given specific parameters for specific respiratory pathogens can increase clinical effectiveness by identifying which assay performs better per target in a given population through a quantifiable value that helps clinicians provide the correct treatment.

Diagnostic Validity for Surveillance Testing

While comparison of post-test probabilities potentially holds much value for clinical diagnoses and helps in understanding the critical attributes for multiplex assays, for surveillance purposes, diagnostic odds ratio (DOR) tend to be more useful. DOR can be calculated as:

$$\frac{LR+}{LR-}$$

(Eq. 2.5)

or equivalently:

$$\frac{(Sen)(Spe)}{1 - Sen - Spe + (Sen)(Spe)}$$

(Eq. 2.6)

DOR is interpreted as the ratio of the odds of a positive test in the presence of disease to the odds of positive test in the absence of disease. The higher the DOR the better discriminatory power of a test (43). DOR differentiates between those in the population with disease and those without

disease based on the likelihood of a positive test result or equivalently calculated from the Sen and Spe for a specific assay. As a measure, the DOR shows the overall strength and epidemiological importance of association of between test result and disease (43, 44). For instance, a larger DOR would describe a very strong association and conversely, a low DOR would describe a weaker association. A higher DOR translates to better discriminatory test performance to identify patients with disease (44). The reported Sen and Spe from the 510(k) FDA approval summary reports of the major FDA-approved respiratory multiplex assays are summarized in **Table 2.3**, along with the DOR for each assay and for all targets available per assay. The DORs for all of the FDA-approved multiplex respiratory assays are high, which dictates that each of these assays has good discriminatory power. The strength of association for each of the assays varies depending on the target (*i.e.*, pathogen of interest). As a result, which assay is the "best" depends on the target being studied. The eSensor RVP shows greater diagnostic performance than other multiplex assays (*i.e.*, the FilmArray RP and the Luminex NxTAG RPP) when confirming outbreak cases of adenovirus (specifically types B/C and E), common in the United States as recently as 2013 (45). By contrast, the FilmArray RP performs better than all other comparable assays for all influenza types (A/H1, A/H3, A/H1-2009, and B). The Nanosphere RP *Flex* performs better than all other comparable assays for *Bordetella* bacterial targets (*i.e.*, *B. pertussis, B. parapertussis/B. bronchiseptica, B. holmesii*). In summary, the best assay among targets that overlap across all assays is the FilmArray RP. However, not all targets overlap as many assays have a variety of respiratory pathogens targeted by the panel, thus the best performing assay needs to be evaluated at a per target basis.

COMPARISON OF EXISTING MULTIPLEXED ASSAYS FOR SURVEILLANCE OF RESPIRATORY PATHOGENS

A relatively small number of studies have directly compared the diagnostic performance between the major FDA approved multiplex respiratory assays currently available and have mostly concluded similar diagnostic performance among all these assays (**Table 2.4**). These studies have used Cohen's Kappa, κ (46) for comparing diagnostic performance. Popwitch et al. presented the most complete comparison of more than two FDA-approved multiplex respiratory assays (47). The study included an analytical comparison of the Biofire FilmArray RP, GenMark eSensor RVP, Luminex xTAGv1 and Luminex RVP FAST using retrospective samples collected in general clinical setting screened by each panel. The eSensor RVP reported the highest Sen (100%) for nearly all targets (rhinovirus/enterovirus, 90.7% the lone exception). Low Sen for adenovirus was a weakness for most assays: FilmArray RP, 57.1%, xTAG RVPv1, 74.3%, xTAG RVP FAST, 82.9%, eSensor RVP, 100%. Detection of Influenza B was also difficult: xTAG RVP FAST, 45.5%, FilmArray RP, 77.3%, xTAG RVPv1, 95.5%, eSensor RVP, 100%. When assessing age-specific assay performance only the eSensor RVP failed to show any statistically significant difference across age groups. The xTAG RVP FAST and FilmArray RP showed higher Sen for adults ($\geq$18) than children ($\leq$5) and teens (<18). Other comparative studies have similarly evaluated diagnostic performance based on Sen and Spe comparing the FDA-approved multiplex respiratory tests to culture, serology and LDTs. The consensus shows that for all comparable targets, Sen and Spe are superior to traditional methods and relatively the same across each multiplex assay with few target exceptions (see **Table 2.4**).

ROC curves provide an alternative approach for evaluating diagnostic performance providing better insight and guidance in differentiating the usefulness of respiratory multiplex

assays for either clinical or surveillance use. ROC curves are simple visualizations that allow comparison of classifiers (*i.e.,* tests) by plotting the True Positive Rate (TPR= Sen) against the False Positive Rate (FPR= 1-Spe) (48). For ARIs, FPs represent a burden to healthcare systems especially if the infection is in vulnerable sub-populations or in individuals where co-infection exacerbate an already existing condition. For surveillance, FPs represent cases of misallocated resources, where efforts are required to treat a non-existing condition in hopes of mitigating the further spread of disease in subpopulations. If comparing routine clinical care to routine surveillance, FPs are a much greater burden to the healthcare industry especially if the test is either expensive or if follow-up is invasive or expensive. Within ROC space, a point (FPR, TPR) on the left hand side denotes a classifier that exhibits better performance than random chance (**Figure 2.1**). Better performing classifiers are always preferred. The discriminatory line separates "liberal" and "conservative" classifiers. Any point in the liberal space represents a classifier that will likely identify all the TP despite a weak signal and at the expense of having a high FPR. Conversely, any point in the conservative space will identify all the TPs in the presence of strong a strong signal at the expense of a low FPR. During an outbreak from a novel respiratory variant (*e.g.,* influenza, coronavirus (49)), a high FPR is acceptable especially if we are assured that by identifying all the TPs, appropriate control and prevention could mitigate the spread of infection. Additionally, because milder forms of RTI go unreported (50) and not every respiratory infection is detected (for many reasons) true prevalence and incidence is difficult to estimate. A high FPR respiratory multiplex test could potentially minimize the error of not knowing true prevalence estimates by including false positives in the total number of positives observed. While may not be ideal for correcting true prevalence estimates, a high FPR assay could help in approaching the true burden of disease. Finally, from the ROC space plot analysis, clinical care benefits from the use of

conservative diagnostic tests but for routine surveillance, liberal diagnostic tests would be more effective. Using ROC space provides a valuable method for comparing the major FDA-approved respiratory multiplex assays for either clinical diagnostic and surveillance use.

Upon closer inspection of the ROC space plot (**Figure 2.2**), important similarities and differences arise between the FilmArray RP, Nanosphere RP *Flex*, Luminex NxTAG RPP and the eSensor RVP. While all of these assays are better performers than random chance, a slight majority of target calls lie on the conservative side of discrimination suggesting that when considering assays as whole performers, few false positives will be reported as most calls will correctly identify positive infection. Every assay has calls throughout the ROC space suggesting that despite an overall liberal or conservative classification for any assay, these assays could still be used for either patient care or surveillance. The ROC space plot also reveals that the most conservative multiplex assay is the Luminex NxTAG RPP which suggests the assay would be most beneficial over other assays when used for clinical diagnostic purposes. The most liberal test is the eSensor RVP, which would serve well for those performing surveillance work. From the ROC space plot, we can also make per target assay specific recommendations. The FilmArray RP has the lowest FPR across all targets except when liberal calling rhinovirus/enterovirus, RSV and CoHKU1 and conservative calling adenovirus infections. Thus, in an outbreak of rhinovirus/enterovirus, RSV or CoHKU1, the FilmArray RP would perform much better than other assays. The Nanosphere RP *Flex* also has a low FPR, but unlike the FilmArray RP, hardly makes any liberal calls except for RSV B suggesting a greater clinical application than others. For highly relevant clinical and public health targets (*e.g.*, Influenza A, A/H1/ A/H3, A/H1-2009 and influenza B) the FilmArray RP is nearly perfect by detecting relatively few false positives when correctly identifying positive infection. For pandemic potential targets such as coronaviruses, the FilmArray RP is again an excellent

diagnostic test that has both a higher Sen and lower FPR comparable to all other assays with the sole exception of CoNL63, detected slightly better on the Luminex NxTAG RPP. Only two assays screen for *Bordetella* spp: the FilmArray RP and the Nanoshpere RP *Flex*. Among these two assays, the Nanosphere RP *Flex* is a near perfect assay by correctly calling positive *Bordetella* spp infections. The worst performing targets across all assays were conservative calls for parainfluenza 2 (Luminex NxTAG RPP), parainfluenza 4 (Luminex NxTAG RPP) and *B. pertussis* (FilmArray RP). The use of ROC plots to assess graphically the diagnostic value for respiratory multiplex assays adds tremendous value where each target could be evaluated independently of other targets and in comparison to the same target across each multiplex assay.

Another useful measure often presented in conjunction with ROC curves is the Youden Index (*J*) (51). *J* is a statistical summary measure that (similar to DOR) incorporates both Sen and Spe. *J* is interpreted as diagnostic test maximum effectiveness representing the optimal cutoff threshold where the maximum difference between TPR and FPR exists (52). Essentially, *J* is a point (Sen, 1-Spe) in ROC space that defines where a diagnostic test will be able to correctly identify the most positives and the fewest false positives. *J* has a value between 0 and 1. *J* graphically represents the farthest point in ROC space away from random performance. *J* is expressed as:

$$J = \text{Sen}+\text{Spe}-1. \qquad \text{(Eq. 2.7)}$$

Unlike other measures, *J* is not dependent on disease prevalence but does suffer from assuming a dichotomous test result which may not always be applicable depending on the assay (53). For instance, characterizing influenza A strains on the FilmArray RP gives one of three results, “Detected”, “Not Detected”, and “Equviocal”. Similarly, the Luminex xTAG RVP provides

"Equivocal' results for some targets (54). For evaluating respiratory multiplex assays, *J* can provide a simple measure of diagnostic effectiveness for either clinical or surveillance use. Upon calculating *J* for each of the respiratory multiplex assays, a heatmap (**Figure 2.3**) can be plotted that allows a visual comparison of diagnostic performance. The lighter the color the closer $J = 1$ meaning the better performing assay for that specific target. Grey boxes indicate targets not screened for in the respective assays. When used in conjunction with ROC space plots, the Youden Index provides yet another valuable measure of diagnostic accuracy.

LIMITATIONS OF CURRENT MULTIPLEXED ASSAYS FOR SURVEILLANCE OF RESPIRATORY PATHOGENS

Despite the tremendous benefits and widespread use of respiratory multiplex technologies revealed through the use of several diagnostic accuracy measures, these complex assays are not without limitations. One of the important aspects in designing PCR based assays is primer selection. All of the FDA-approved multiplex respiratory technologies presented contain primer pools and probes corresponding to common upper respiratory targets. Optimized primers yield reliable qualitative results for all the targets in each individual panel. However, a universal challenge for all manufactures is selecting appropriate genomic target sequences to optimize hybridization and thereby minimizing unwanted nonspecific interactions which could lead to diagnostic errors (*i.e.,* FP and FN). Two targets have challenged manufacturers: adenovirus and rhinovirus/enterovirus. Early assay releases of the Luminex xTAG RVP and the BioFire FilmArray RP reported poor adenovirus detection and resulted in reduced sensitivity when presented in clinical and contrived samples (21, 55, 56). Current versions of the FilmArray RP and the xTAG RVP now include updated primers aligned against the complete coding sequences of all adenovirus

serotypes. The updated FilmArray RP panel has shown diagnostic improvement over the previous version (57). eSensor RVP is the only multiplex assay capable of detecting subgroups B/E and C. For surveillance, assay choice depends on surveillance population needs. The increasing DOR order of the major FDA-approved respiratory multiplex panels as designed: Nanosphere RP *Flex*, FilmArray RP and Luminex NxTAG RPP.

Another problematic targets for respiratory multiplex testing are rhinoviruses and enteroviruses. Due to genetic homology, these pathogens lack resolution when detected on either the Luminex NxTAG RVP or FilmArray RP and as a result, when detected are simply reported as either rhinovirus or rhinovirus/enterovirus without differentiation. The primer similarity of rhinoviruses and enteroviruses increases the risk of cross-amplification and interference during multiplex testing. Per the product insert, Nanosphere RP *Flex* has observed cross-reactivity of rhinovirus/enterovirus primers with Human poliovirus 2 and 3, coxsackievirus A24 and EV-D68. When samples are called 'positive' for rhinovirus/enterovirus, laboratories should reflex to either culture or sequencing to further resolve identification. Detecting and follow-up testing for rhinovirus/enterovirus samples has become an important concern due to a nationwide outbreak of EV-D68 across the United States (58). Symptoms included hallmark cold symptoms (fever, runny nose, muscle aches) as well as difficulty breathing. A recent study has since described the association of EV-D68 infection and acute flaccid myelitis (related to acute flaccid paralysis) (59) highlighting the importance of good resolution between rhinovirus and enterovirus for all respiratory multiplex assays. A similar primer cross-reactivity issue is present for coronavirus OC43 and HKU1 viruses in the FilmArray RP assay. Another limitation of respiratory multiplex assays is that precise quantification of infection cannot be determined as all of the assays presented

are FDA-approved qualitative test unable to determine viral or bacterial load, which (if available) may be helpful during surveillance in identifying an appropriate case definition.

CONCLUSION

The current landscape of respiratory multiplex testing includes a variety of technologies that have revolutionized clinical practice, however, due to specific barriers these technologies could impact surveillance efforts even more. Despite numerous studies showing real world application, full adoption of these methods for routine surveillance testing and outbreak response is limited in large part due to how these technologies are evaluated. When compared to culture and serology, all of the FDA-approved assays presented in this report provide equivalent Sen and Spe, measures that have traditionally been markers for diagnostic performance. However, better measures of diagnostic performance such as LRs, DORs, and the Youden Index make assay use and result interpretation clearer and much more effective. These measures allow comparisons on a per-target basis, providing a much more specific use of these technologies for either surveillance or clinical diagnosis. The next few years will see the evolution of respiratory multiplex technologies in more rapid, more efficient, easier to use (and easier to interpret) panels capable of screening a broader range of pathogens. The challenge bestowed on laboratories is evaluating whether newer technologies will meet clinical and public health demand.

FIVE-YEAR VIEW

Within five years, while multiplex respiratory assays become more rapid, more comprehensive and more automated, next-generation sequencing (NGS) will begin to dominate the diagnostic market due to the tremendous benefits of this technology. Innovation has defined

laboratory diagnostics for detecting respiratory pathogens. What began with culture and serology was later improved upon by the introduction of PCR-based assays to now the wide adoption of multiplex platforms and assays. The identification of respiratory pathogens has progressively become more specific and precise. NGS is the latest advancement that may change not just clinical practice but also public health. NGS is a powerful high-throughput tool used for a variety of different healthcare and non-healthcare applications (60, 61). As a diagnostic method, multiplex NGS has been shown to confirmed viral infections as detected by a PCR-based multiplex assay (62). Similarly, Graf et al., showed the reliability of metagenomics (a specific NGS-type of analysis) as a comparable method to the eSensor RVP not just in agreement but also in detecting additional pathogens (63). For surveillance of respiratory pathogens, NGS provides detailed genomic information that aides in identifying resistance, virulence and sequences changes over time (64-69). One of the biggest challenge of applying NGS towards surveillance and clinical care is the need of FDA regulatory standards and methods. NGS presents a unique regulatory challenge that requires evaluation not just of the clinical performance of the method itself, but also of the bioinformatic pipelines used for detection, characterization, and phylogenetic relationships.

KEY ISSUES

- RTIs are among the top 5 leading causes of death worldwide across all age groups accounting for 4.2 million deaths annually; total deaths that account for more than liver, colon, breast and prostate cancer combined
- The public health system includes state and local public health and clinical laboratories whose responsibility in outbreak response is managing and tracking disease.

- Advances in multiplex PCR instrument systems have replaced most traditional methods for detecting respiratory pathogens
- Within the past decade, several multiplex platforms such as the Luminex NxTAG RPP, Verigene RP *Flex*, eSensor RVP and the FilmArray RP have acquired FDA approval for use in public health and clinical laboratories. FDA-approved respiratory multiplex assays have successfully demonstrated public health benefits in a variety of patient populations.
- From a clinical standpoint, respiratory multiplex assays should be evaluated not just through Sen and Spe measures, but through LRs especially through Fagan nomogram analysis that allows incorporating prevalence of disease and assay performance into a post-test probability on an individual basis.
- From a public health surveillance perspective, respiratory multiplex assays should be compared against epidemiologically relevant and interpretable diagnostic measures such as the DOR.
- ROC space also provides a great tool in comparing diagnostic assays on a per target basis
- Surveillance needs for future respiratory multiplex technologies include broader panels and optimizing multiplex assay performance
- The future of laboratories performing diagnostic and surveillance testing is directed toward adopting NGS technologies and in establishing regulatory guideline.

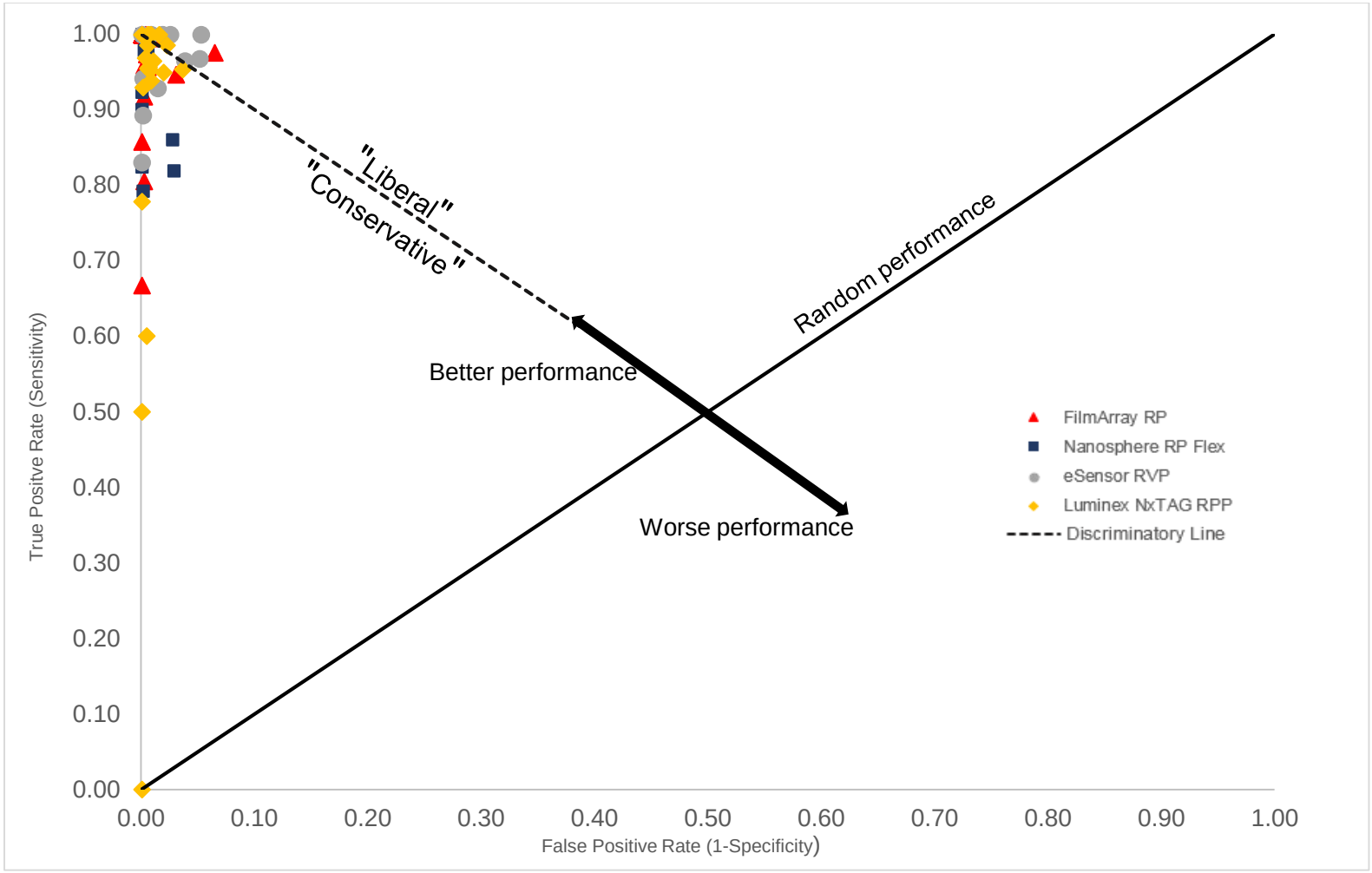

Figure 2.1. ROC Space of major FDA-approved respiratory panels showing "Liberal" and "Conservative" calls from discrimination. Better performing assays lie on the left of random chance; worse performing assays lie on the right. Outlier at (0,0) is for *C. pneumo* sample tested by Luminex NxTAG RPP during clinical prospective studies; sample was discrepant with follow-up clinical site testing unknown.

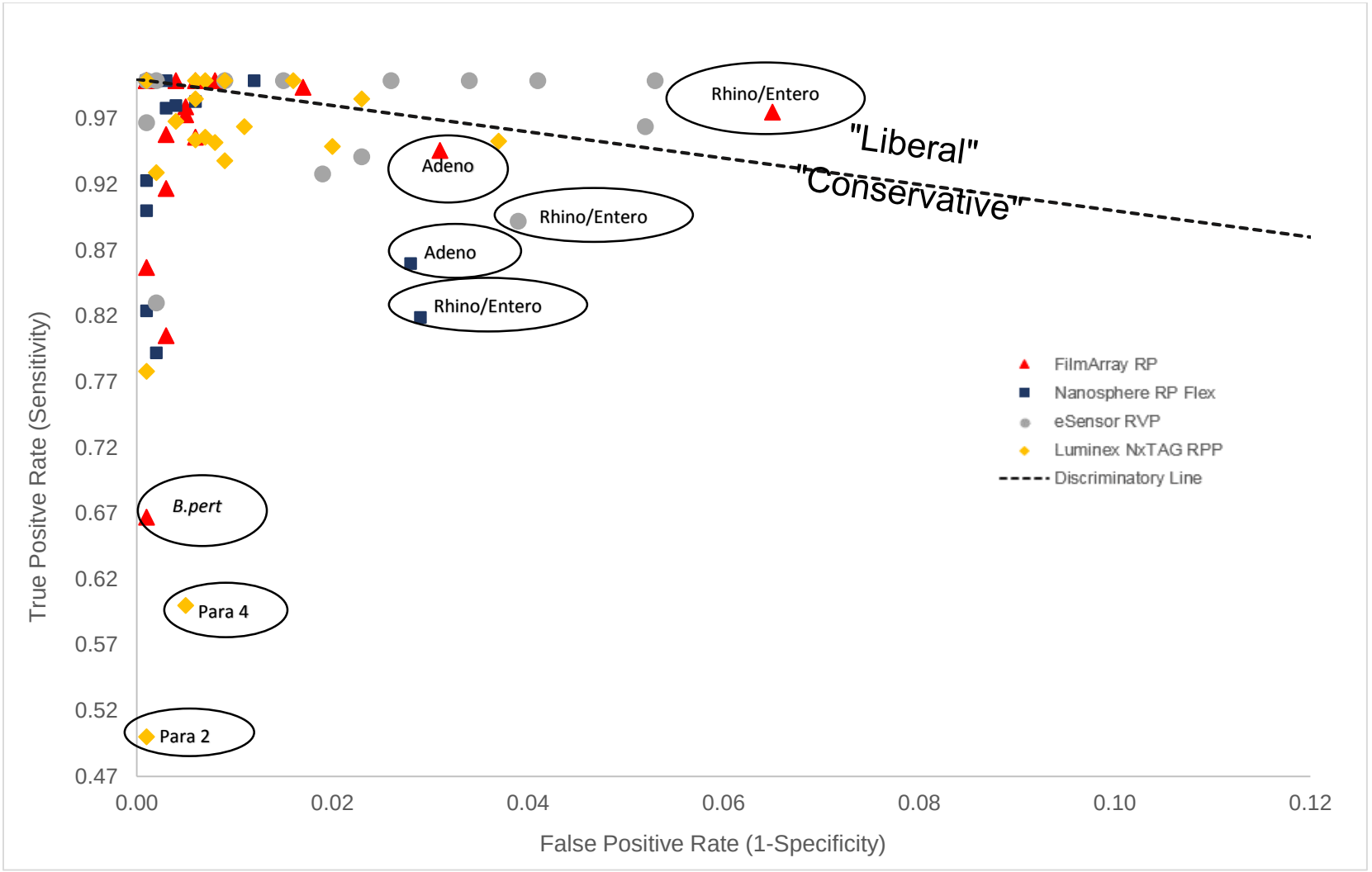

Figure 2.2. Figure 2.1 with *x* and *y*-axis adjusted (minus *C. pneumo* for Luminex NxTAG RPP) to view targets details in ROC Space. Outliers circled and identified.

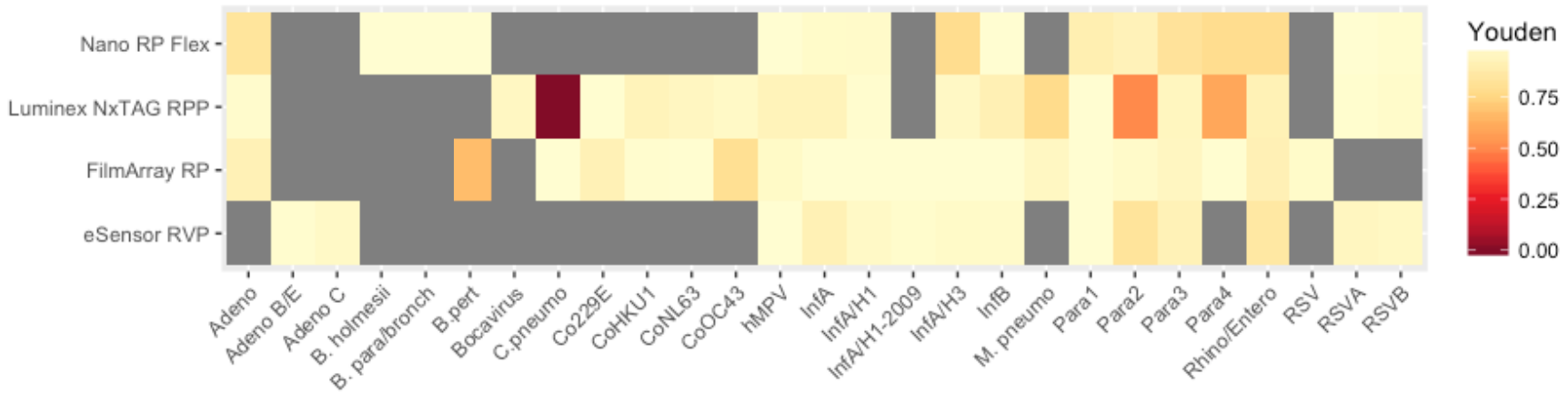

Figure 2.3. Heatmap illustrating Youden Index (*J*) for all FDA-approved respiratory multiplex assay targets. Boxes in gray denote not detected targets. No 95% CI calculated per the assumption that some of the index values calculated are close to 0 or 1. Per the FDA decision summary, only one *C. pneumo* target for the Luminex NxTAG RPP was tested during clinical prospective studies with discrepant site testing unknown.

Table 2.1. Analytical Requirements for Multiplex Diagnostic Assays for Diagnosis and Surveillance of Respiratory Pathogens

	Clinical Diagnosis of Respiratory Pathogens		Surveillance of Respiratory Pathogens & Outbreak Control	
	Characteristics	**Critical Attributes for Assays**	**Characteristics**	**Critical Attributes for Assays**
Pre-analytical	Samples processed individually or in small batches; number of samples varies depending on size of laboratory and prevalence of disease.	Small labs (or labs in low prevalence areas) require low-cost individuals tests; larger labs (or labs in high prevalence areas) may do better with moderate to high-throughput assays.	Periodically, large numbers of samples are screened to determine etiology and origin of outbreak.	Potential to process large number of samples rapidly at minimal cost to support surge testing.
	Typically collect specimens from individuals with clinical symptoms, who are more likely to have a high viral or bacterial load.	Per sample cost, low turnaround time and ability to differentiate between viral and bacterial respiratory pathogens (*e.g.*, viruses versus bacterial) more important than sensitivity or specificity.	Because syndromic surveillance is performed for identification of causative agents and possible risk factors, labs often test asymptomatic individuals in addition to symptomatic ones.	Critical that assays are both sensitive and specific.
	Likely to have standard specimen type with minimal variation for a given pathogen.	FDA-approval is required for standardization in clinical decision-making; closed assay systems (*i.e.*, not modifiable) are ideal preventing operator error and contamination.	Because remnant samples may be used and collected specimen types may vary, sample matrices are highly variable, which may limit interpretation on FDA-approved assays.	FDA-approval is required for clinical decision-making; however, laboratories tailor and validate assays for new sample types and emerging pathogens depending on need.
Post-analytical	Treatment and patient care decisions are based on results.	Need to accurately distinguish between pathogens which have similar symptoms but different treatments.	Results used both for individual clinical management *and* for understanding population-level trends.	Need to accurately distinguish between pathogens which have similar symptoms but different treatments for clinical care AND need phylogenetic information to identify epidemiological trends/identify sources of outbreaks.

Table 2.2. Comparison of positive likelihood ratios and post-test probabilities for high-prevalence pathogens for commercially-available and FDA-approved respiratory panels based on Fagan Nomogram Analysis of data reported by manufacturers to the FDA as part of the approval processes for the assay. A positive likelihood ratio **(**LR+) greater than 10 is considered significantly large to rule in or rule out disease. A larger value for the post-test probability indicates that the test has a higher probability of being accurate (*i.e.*, the probability of the individual patient having the disease if a positive assay result is obtained is greater). In general, assays with higher post-test probabilities are better for clinical diagnostic purposes.

	Assay	Prevalence in Population	Positive Likelihood Ratio (LR+)	Post-Test Probability
Influenza A/H1	FilmArray RP	10%	999*	99.1%*
	eSensor RVP		967*	99.1%*
	Luminex Nx TAG RPP		111.11	92.5%
	Nanosphere RP *Flex*		326	97.3%
Influenza A/H3	FilmArray RP	5%	999*	98.1%*
	eSensor RVP		38.46	66.9%
	Luminex Nx TAG RPP		42.82	69.3%
	Nanosphere RP *Flex*		499.5	96.3%
Influenza B	FilmArray RP	5%	999*	98.1%*
	eSensor RVP		48.84	72.0%
	Luminex Nx TAG RPP		136.57	87.8%
	Nanosphere RP *Flex*		245	92.8%
Bordetella pertussis	FilmArray RP	2%	667	93.2%
	eSensor RVP		n.r.**	n.r.**
	Luminex Nx TAG RPP		n.r.**	n.r.**
	Nanosphere RP *Flex*		999*	98.1%*

*In cases where the specificity was reported as 100%, we substituted a value of 99.9% so that the positive likelihood ratio could be calculated using the standard formula.
***B. pertussis* is not a target in the panel

Table 2.3 Comparison of sensitivity (Sen), specificity (Spe) and diagnostic odds ratio (DOR, with 95%CI) of FDA-approved respiratory panels, based on data reported by manufacturers to the FDA as part of the approval processes for the assays.

	FilmArray RP				Nanosphere RP *Flex*				eSensor RVP				Luminex NxTAG RPP			
	Sen	Spe	DOR	95% CI	Sen	Spe	DOR	95% CI	Sen	Spe	DOR	95% CI	Sen	Spe	DOR	95% CI
Adeno	95%	97%	547.6	192-1561.3*	86%	97%	213.2	2.8-469.9*	n.d.	n.d.	n.d.	n.d.	100%	98%	61438.5	52533.3-71853.3*
Adeno B/E	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	100%	99%	110001	46915.4-257915.7*	n.d.	n.d.	n.d.	n.d.
Adeno C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	100%	97%	28383.6	11911.8-67631.5*	n.d.	n.d.	n.d.	n.d.
CoHKU1	100%	99%	123876	49003-313149.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	93%	100%	6529.2	533.9-79836.2*
CoNL63	100%	99%	165501	82619-331528.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	95%	99%	3435.8	1469.6-8032.4*
Co229E	92%	100%	3671.7	395.8-34059.4*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	100%	99%	165501	129533.6-211455.4*
CoOC43	81%	100%	1371.9	426.1-4417.5*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	97%	100%	7532.3	798.6-71039.91*
hMPV	97%	100%	7171.4	1496.1-34374.1*	100%	100%	332001	5.7-778167.9*	100%	100%	498501	121426.9-2046525*	94%	99%	1665.9	1183.1-2345.6*
Rhino/Entero	98%	94%	561	295.3-1065.6*	82%	97%	151.5	3.7-219.2*	89%	96%	203.5	46.3-895.1*	95%	96%	527.7	442.2-629.8*
InfA	100%	100%	998001	295.3-1065.6*	98%	99%	9579.4	1.4-74460.2*	96%	95%	488.2	190.7-1249.8*	95%	98%	911.8	745.3-1115.5
InfA/H1	100%	100%	998001	319033.3-3121950*	98%	100%	14773.7	1.3-125258.6*	97%	100%	29273.7	3983.8-215106.9*	100%	99%	110001	90760.01-133321.1*
InfA/H3	100%	100%	998001	374104-2662377*	100%	100%	498501	4.6-1530067*	100%	97%	37424.1	23683.6-59161.3*	99%	98%	2789.4	1374.7-5659.9*
InfA/H1-2009	100%	100%	998001	790354.4-1260202*	n.d.	n.d.	n.d.	n.d.	100%	99%	65601	36662.8-117380.4*	n.d.	n.d.	n.d.	n.d.
InfB	100%	100%	998001	22630.6-4394843*	98%	100%	12201	1.4-9445.6*	93%	98%	665.5	239.3-1850.4*	96%	99%	3082.2	1603.4-5924.7*
Para1	100%	100%	998001	126362.2-7882150*	100%	100%	8991	1.8-55995.1*	100%	100%	998001	111447.8-8936976*	100%	100%	998001	94900.4-10495281*
Para2	98%	100%	9277.2	1136.6-75719*	100%	100%	11975.0	1.1-123475.6*	83%	100%	2436.3	189.0-31397.8*	50%	100%	999	2.78-357781.9*
Para3	96%	99%	3599.5	765.4-16927.5*	82%	100%	4677.1	1.9-25206.3*	94%	98%	677.5	226.6-2025.2*	95%	99%	2459.3	279.6-21630.8*
Para4	100%	100%	248751	92525.8-668754.6*	79%	100%	1900.0	2.2-7629.9*	n.d.	n.d.	n.d.	n.d.	60%	100%	298.5	48.7-1828.4*
RSV	99%	98%	9579.4	1287.9-71249.8*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
RSVA	n.d.	n.d.	n.d.	n.d.	100%	100%	998001	4.4-3408002*	100%	95%	17850.1	12343.6-25812.9*	100%	99%	141715.3	120945.7-166051.6*
RSVB	n.d.	n.d.	n.d.	n.d.	100%	99%	82251	4.6-221854.5*	100%	96%	23366.9	14391.4-37939.9*	99%	99%	10878.8	5528-7-21405.9*
Bocavirus	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	96%	99%	2407.6	289.3-20032.8*
B.pert	67%	100%	2001	90.2-44392.2*	100%	100%	998001	3.3-4702441*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B. para/bronch	n.d.	n.d.	n.d.	720.4-49759.4*	100%	100%	998001	1.5-11010489*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B. holmesii	n.d.	n.d.	n.d.	n.d.	100%	100%	998001	2.2-7088148*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C.pneumo	100%	100%	998001	116527.2-8547411	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	–	100%	0	n.a.
M.pneumo	96%	100%	7580.4	851.6-67473.8*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	78%	100%	3501	372.4-32916.5*

DOR: Diagnostic Odds Ratio calculated as LR+/LR- per (44) FilmArray RP: InfluA/H1 Sen not reported. Luminex xTAP RPP: *C.pneumo* site testing results for discrepant specimens were available or reported.
n.d. : target is not included as a target in the panel
- : As reported in the FDA-decision summary, Sen was reported as 0%; thus, DOR was not able to be calculated.
95% CI: Calculated as LN(DOR) ± 1.96 (√(1/TP + 1/TN + 1/FP + 1/FN)),*P $\leq$ 0.05
NOTE: When reported, instead of using Sen and Spe at 100%, we used 99.9% in our calculations to prevent a DOR = undefined.

Table 2.4. List of all direct comparative studies of the major FDA-approved multiplex respiratory assays.

Study	Comparison	Cohen's Kappa, κ (95% CI)	Interpretation of κ[61, 74]
Lee *et al.* (2017) [70]	Luminex NxTAG RPP: Luminex xTAG RVPv2	0.85 (0.757-0.932)	Strong
Chen *et al.* (2016) [71]	Luminex NxTAG RPP: FilmArray RP	0.92 (0.90-0.94)	Almost Perfect
Hwang *et al.* (2014) [72]	xTAG RVP: Nanosphere RV+	0.908*	Almost Perfect
Popowitch, *et al*. (2013) [47]	FilmArray RP: eSensor RVP: Luminex xTAG RVPv1: Luminex xTAG RVP	Not reported	n/a
Babady *et al.* (2012) [23]	Luminex xTAG RVP: FimArray RP	0.685**	Moderate
Rand *et al.* (2011) [69]	FilmArray RP: Luminex xTAG RVP	0.91 (0.85-0.97)	Almost Perfect
Pabbaraju *et al.* (2011) [73]	Luminex xTAG RVP: Luminex xTAG RVP FAST	0.548-1.00***	Weak to Almost Perfect

*study only included comparison of RSV, influenza A and B

**no 95% CI reported

***calculated κ for all targets individually. Low end kappa coefficient is for influenza B only.

REFERENCES

1. CDC. Chronic Diseases: The Leading Causes of Death and Disability in the United States. Retrieved from: http://www.cdc.gov/chronicdisease/overview/. Accessed February 29, 2016.
2. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*. 2015;385:117-71.
3. Mao L, Yang Y, Qiu Y, *et al.* Annual economic impacts of seasonal influenza on US counties: Spatial heterogeneity and patterns. *Int J Health Geogr*. 2012 11:16.
4. McCulloh RJ, Andrea S, Reinert S, *et al.* Potential Utility of Multiplex Amplification Respiratory Viral Panel Testing in the Management of Acute Respiratory Infection in Children: A Retrospective Analysis. *J Pediatric Infect Dis Soc* 2014 Jun;3(2):146-53.
5. Schreckenberger PC, McAdam AJ. Point-counterpoint: Large Multiplex PCR panels should be first-line tests for detection of respiratory and intestinal pathogens. *J Clin Microbiol* 2015 Oct; 53(10): 3110-3115.
6. Subramony A, Zachariah P, Krones A, *et al*. Impact of Multiplex Polymerase Chain Reaction Testing for Respiratory Pathogens on Healthcare Resource Utilization for Pediatric Inpatients. *J Pediatr* 2016 Jun;173:196-201.
7. Fleming-Dutra KE, Hersh AL, Shapiro DJ. Prevalence of Inappropriate Antibiotic Prescriptions Among US Ambulatory Care Visits, 2010-2011. *JAMA* 2016 May 3;315(17):1864-73.
8. CDC. RSV Census Regional Trends (2017). Retrieved from: https://www.cdc.gov/surveillance/nrevss/rsv/region.html. Accessed February 4, 2016.

9. Hayden FG, de Jong MD. Emerging Influenza Antiviral Resistance Threats. *J Infect Dis* 2011 Jan 1;203(1):6-10.
10. Su S, Fry AM, Kirley PD. Survey of influenza and other respiratory viruses diagnostic testing in US hospitals, 2012-2013. *Influenza Other Respir Viruses* 2016 Mar; 10(2):86-90.
11. Ginocchio CC, St George K. Likelihood that an unsubtypeable influenza A virus result obtained with the Luminex xTAG respiratory virus panel is indicative of infection with novel A/H1N1 (swine-like) influenza virus. *J Clin Microbiol*. 2009 Jul;47 (7):2347-8.
12. McAllister SC, Schleiss MR, Arbefeville S, *et al*. Epidemic 2014 Enterovirus D68 Cross-Reacts with Human Rhinovirus on a Respiratory Molecular Diagnostic Platform. PLoS One 2015;10(3):e0118529.
13. Shibib DR, Matushek SM, Beavis KG, *et al.* BioFire FilmArray Respiratory Panel for Detection of Enterovirus D68. *J Clin Microbiol* 2016 Feb;54(2):457-9.
14. Nowak JA, Kaul KL. The Role of Community Molecular Diagnostics Laboratories in the H1N1 Pandemic. *J Mol Diagn* 2009 Sep;11(5):369-370.
15. APHL. Use of Commercial Respiratory Virus Panels at State Public Health Laboratories. December 1, 2015.
16. National Foundation for Infectious Diseases. Respiratory Syncytial Virus in Older Adults: A Hidden Annual Epidemic (2016).
17. Ghebremedhin B. Human adenovirus: Viral pathogen with increasing importance. *Eur J Microbiol Immunol (Bp)* 2014 Mar; 4(1):26-33.
18. McAllister SC, Schleiss MR, Arbefeville S, *et al.* Epidemic 2014 enterovirus D68 cross-reacts with human rhinovirus on a respiratory molecular diagnostic platform. *PLoS One* 2015 Mar 23;10(3):e0118529.

19. Jansen RR, Wieringa J, Koekkoek SM, *et al*. Frequent Detection of Respiratory Viruses without Symptoms: Toward Defining Clinically Relevant Cutoff Values. *J Clin Microbiol* 2011 July;49(7):2631-2636.
20. Lau LL, Cowling BJ, Fang VJ, *et al.* Viral shedding and clinical illness in naturally acquired influenza virus infections. *J Infect Dis* 2010 May 15;201(10):1509-1516.
21. Ruggiero P, McMillen T, Tang YW, *et al.* Evaluation of the BioFire FilmArray respiratory panel and the GenMark eSensor respiratory viral panel on lower respiratory tract specimens. *J Clin Microbiol* 2014 Jan;52(1):288-90.
22. Byington CL, Ampofo K, Stockmann C, *et al*. Community Surveillance of Respiratory Viruses Among Families in the Utah Better Identification of Germs-Longitudinal Viral Epidemiology (BIG-LoVE) Study. *Clin Infect Dis* 2015 Oct 15;61(8):1217-1224.
23. Babady NE, Mead P, Stiles J, *et al*. Comparison of the Luminex xTAG RVP Fast Assay and the Idaho Technology FilmArray RP Assay for Detection of Respiratory Viruses in Pediatric Patients at a Cancer Hospital. *J Clin Microbiol* 2012 Jul;50(7):2282-8.
24. Song Cho G, Moon BY, Lee BJ, *et al.* High Rates of Detection of Respiratory Viruses in the Nasal Washes and Muscosae of Patients with Chronic Rhinosinusitis. *J Clin Microbiol* 2013 Mar;51(3):979-984.
25. Halling KC, Schrijver I, Persons DL. Test verification and validation for molecular diagnostic assays. *Arch Pathol Lab Med* 2012 Jan;136(1):11-3.
26. Lieberman D, Shleyfer E, Castel H, *et al.* Nasopharyngeal versus oropharyngeal sampling for isolation of potential respiratory pathogens in adults. *J Clin Microbiol* 2006 Feb; 44(2):525-528.

27. Mahony J, Chong S, Merante F, *et al.* Development of a respiratory virus panel test for detection of twenty human respiratory viruses by use of multiplex PCR and a fluid microbead-based assay. *J Clin Microbiol* 2007 Sep;45(9):2965-70.
28. Poritz MA, Blaschke AJ, Byington CL, *et al*. FilmArray, an automated nested multiplex PCR system for multi-pathogen detection: development and application to respiratory tract infection. *PLoS One* 2011;6(10):e26047
29. Pierce VM, Hodinka RL. Comparison of the GenMark Diagnostics eSensor respiratory viral panel to real-time PCR for detection of respiratory viruses in children. *J Clin Microbiol* 2012 Nov;50(11):3458-65
30. Jannetto PJ, Buchan BW, Vaughan KA. Real-Time Detection of Influenza A, Influenza B, and Respiratory Syncytial Virus A and B in Respiratory Specimens by Use of Nanoparticle Probes. *J Clin Microbiol* 2010 Nov;48(11):3997-4002.
31. Marchand-Austin A, Farrell DJ, Jamieson FB, *et al.* Respiratory Infection in Institutions during Early Stages of Pandemic (H1N1) 2009, Canada. *Emerg Infect Dis* 2009 Dec;15(12):2001-2003
32. Parikh R, Parikh S, Arun E, *et al*. Likelihood ratios: Clinical application in day-to-day practice. *Indian J Ophthalmol* 2009 May-Jun;57(3):217-221.
33. Brenner H, Gefeller O. Variation of sensitivity, specificity, likelihood ratios and predictive values with disease prevalence. *Stat Med* 1997 May 15;16(9):981-91
34. Moons KG, van Es GA, Deckers JW. Limitations of sensitivity, specificity, likelihood ratio, and bayes' theorem in assessing diagnostic probabilities: a clinical example. *Epidemiology* 1997 Jan;8(1):12-7.

35. Idrovo AJ. Three Criteria for Ecological Fallacy. Environ Health Perspect 2011 Aug;119(8):a332.
36. Akobeng AK. Understanding diagnostic tests 1: sensitivity, specificity and predictive values. *Acta Paediatr* 2007 Mar;96(3):338-41.
37. Gallagher EJ. The problem with sensitivity and specificity. *Ann Emerg Med* 2003;42:298-303.
38. Grimes DA, Schulz KF. Refining clinical diagnosis with likelihood ratios. Lancet 2005 Apr 23-29;365(9469):1500-5.
39. Simon D, Boring III JR (1990). Chapter 6 Sensitivity, Specificity, and Predictive Value. In *Clinical Methods: The History, Physical, and Laboratory Examinations*. 3rd edition (Online). Butterworths, Boston, MA, USA.
40. Fagan TJ. Nomogram for Bayes theorem. (Letter). *N Engl J Med* 1975 July31;293(5):257.
41. CDC Foundation. Flu Prevention. Retrieved from: http://www.cdcfoundation.org/businesspulse/flu-prevention-infographic. Accessed April 30, 2016.
42. Walsh P, Overmeyer C, Kimmel L, *et al*. Prevalence of *Bordetella pertussis* and *Bordetella parapertussis* in Samples Submitted for RSV Screening. *West J Emerg Med* 2008 Aug;9(3):135-140.
43. Simundic AM. Measures of Diagnostic Accuracy: Basic Definitions. EJIFCC 2009 Jan;19(4):203-211Ridderhof JC, Moulton AD, Ned RM, et al. The Laboratory Efficiencies Initiative: Partnership for Building a Sustainable National Public Health Laboratory System. *Public Health Rep* 2013 Sep-Oct;128(Suppl 2): 20-33.
44. Glas AS, Lijmer JG, Prins MH, *et al*. The diagnostic odds ratio: a single indicator of test performance. *J Clin Epidemiol* 2003 Nov;56(11):1129-35.

45. Scott MK, Chommanard C, Lu X, *et al.* Human Adenovirus Associated with Severe Respiratory Infection, Oregon, USA, 2013-2014. *Emerg Infect Dis* 2016 Jun;22(6):1044-1051.
46. McHugh, ML. Interrater reliability: the kappa statistic. *Biochem Med* (Zagreb) 2012 Oct;22(3):276-282.
47. Popowitch EB, O'Neill SS, Miller MB. Comparison of the BioFire FilmArray RP, GenMark eSensor RVP, Luminex xTAG RVPv1, and Luminex xTAG RVP fast multiplex assays for detection of respiratory viruses. *J Clin Microbiol*. 2013 May;51 (5):1528-33.
48. Fawcett T. An Introduction to ROC analysis. Pattern Recogn Lett 27(2006) 861-874.
49. Coleman CM, Frieman MB. Coronaviruses: important emerging human pathogens. *J Virol* 2014 May;88(10):5209-12.
50. WHO. Morbidity rate for children aged 0-4 years due to acute respiratory illness. In Children's Environmental Health Indicators. Retrieved from: http://www.who.int/ceh/indicators/ARImorbidity0_4.pdf?ua=1. Accessed on May 5, 2016.
51. Youden WJ. Index for rating diagnostic tests. *Cancer* 1950 Jan;3(1):32-5.
52. Ruopp MD, Perkins NJ, Whitcomb BW, *et al.* Youden Index and Optimal Cut-Point Estimated from Observations Affected by a Lower Limit of Detection. *Biom J* 2008 Jun;50(3):419-430.
53. Zhou XH, McClish DK, Obuchowski NA (2009). Combined Measures of Sensitivity and Specificity. In *Statistical Methods in Diagnostic Medicine*. John Wiley & Sons. New York, New York, USA (23-24).
54. Rand KH, Rampersaud H, Houchk HJ. Comparison of Two Multiplex Methods for Detection of Respiratory Viruses: FilmArray RP and xTAG RVP. *J Clin Microbiol* 2011 Jul;49(7):2449-2453.

55. Esposito S, Scala A, Bianchini S, *et al*. Identification of Human Adenovirus in Respiratory Samples with Luminex Respiratory Virus Panel Fast V2 Assay and Real-Time Polymerase Chain Reaction. *Int J Mol Scie*. 2016 Feb 26;17(3):297.

56. Faden H, Wynn RJ, Campagna L, *et al.* Outbreak of adenovirus type 30 in a neonatal intensive care unit. *J Pediatr*. 2005 Apr. 146 (4):523-7.

57. Song E, Wang H, Salamon D, *et al.* Performance Characteristics of FilmArray Respiratory Panel v1.7 for Detection of Adenovirus in a Large Cohort of Pediatric Nasopharyngeal Samples: One Test May Not Fit All. *J Clin Microbiol.* 2016 Jun;54(6):1479-86.

58. CDC. Enterovirus D68 (2016). Retrieved from: https://www.cdc.gov/non-polio-enterovirus/about/ev-d68.html. Accessed on: May 9, 2016.

59. Aliabadi N, Messacar K, Pastula DM, *et al*. Enterovirus D68 Infection in Children with Acute Flaccid Myelitis, Colorado, USA, 2014. *Emerg Infect Dis* 2016 Aug;22(8):1387-94.

60. Struelens MJ, Brisse S. From molecular to genomic epidemiology: transforming surveillance and control of infectious diseases. *Euro Surveill.* 2013;18(4):20386

61. Koser CU, Ellington MJ, Cartwright EJ, *et al.* Routine use of microbial whole genome sequencing in diagnostic and public health microbiology. *PLoS Pathog*. 2012;8(8):e1002824

62. Zoll J, Rahamat-Langendoen J, Ahout I, *et al.* Direct multiplexed whole genome sequencing of respiratory tract samples reveals full viral genomic information. *J Clin Microbiol*. 2015 May 66:6-11.

63. Graf EH, Simmon KE, Tardif KD, *et al*. Unbiased Detection of Respiratory Virus by Use of RNA Sequencing-Based Metagenomics: a Systematic Comparison to a Commercial PCR Panel. *J Clin Microbiol* 2016 Apr;54(4):1000-7.

64. McGinnis J, Laplante J, Shudt M, *et al*. Next generation sequencing for whole genome analysis and surveillance of influenza A viruses. *J Clin Virol* 2016 Jun;79:44-50.

65. Koser CU, Ellington MJ, Cartwright EJ, *et al.* Routine use of microbial whole genome sequencing in diagnostic and public health microbiology. *PLoS Pathog*. 2012;8(8):e1002824

66. Dallman TJ, Byrne L, Launders N. *et al*. The utility and public health implications of PCR and whole genome sequencing for the detection and investigation of an outbreak of Shig toxin-producing *Escherichia coli* serogroup O26:H11. *Epidemiol Infect*. 2015 Jun;143(8):1672-80.

67. Carriço JA, Sabat AJ, Friedrich AW, *et al.* Bioinformatics in bacterial molecular epidemiology and public health: databases, tools and the next-generation sequencing revolution. *Euro Surveill*. 2013;18 (4).

68. Koser CU, Ellington MJ, Peacock SJ. Whole genome sequencing to control antimicrobial resistance. *Trends Genet.* 2014 Sep; 30(9):401-407.

69. Aanensen DM, Feil EJ, Holden MT, *et al.* Whole-Genome Sequencing for Routine Pathogen Surveillance in Public Health: a Population Snapshot of Invasive *Staphylococcus aureus* in Europe. *MBio*. 2016; May 5;7(3).

70. Lee CK, Lee HK, Ng CWS, *et al.* Comparison of Luminex NxTAG Respiratory Pathogen Panel and xTAG Respiratory Viral Panel FAST Version 2 for the Detection of Respiratory Viruses. *Ann Lab Med* 2017 May;37(3):267-271.

71. Chen JH, Lam HY, Yip CC, *et al.* Clinical Evaluation of the New High-Throughput Luminex NxTAG Respiratory Pathogen Panel Assay for Multiplex Respiratory Pathogen Detection. *J Clin Micrbiol* 2016 Jul;54(7):1820-5.

72. Hwang SM, Lim MS, Han M, *et al*. Comparison of xTAG respiratory virus panel and Verigene Respiratory Virus Plus for detecting influenza virus and respiratory syncytial virus. *J Clin Lab Anal* 2015 Mar;29(2):116-21.
73. Pabbaraju K, Tokaryk KL, Wong S, *et al*. Comparison of the Luminex xTAG Respiratory Viral Panel with In-House Nucleic Acid Amplification Tests for Diagnosis of Respiratory Virus Infections. *J Clin Microbiol* 2008 Sep;46(9):3056-3062.
74. Sim J, Wright CC. The kappa statistic in reliability studies: use, interpretation, and sample size requirements. *Phys Ther* 2005 Mar;85(3):257-68.

CHAPTER 3

Bayesian evidence of environmental contamination from acute respiratory infection in long-term care facilities

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ABSTRACT

Skilled nursing home facilities (SNFs) house a vulnerable population frequently exposed to respiratory pathogens. Our study aims to gain a better understanding of the transmission of nursing home-acquired viral respiratory infections in non-epidemic settings. Symptomatic surveillance was performed in 3 SNFs for residents exhibiting acute respiratory symptoms. Environmental surveillance of 5 high touch areas was performed to assess possible transmission. All resident and environmental samples were screened using a commercial multiplex PCR platform. Bayesian methods were used to evaluate environmental contamination. Among nursing home residents with respiratory symptoms, 19% had a detectable viral pathogen (parainfluenza-3, rhinovirus/enterovirus, RSV, or influenza B). Environmental contamination was found in 20% of total room surface swabs of symptomatic residents. Environmental and resident results were all concordant. Target period prevalence among symptomatic residents ranged from 5.5 to 13.3% depending on target. Bayesian analysis quantifies the probability of environmental shedding due

to parainfluenza-3 as 96.1% and due to rhinovirus/enterovirus as 62.5%. Our findings confirm that non-epidemic viral infections are common among SNF residents exhibiting acute respiratory symptoms and that environmental contamination may facilitate further spread with considerable epidemiological implications. Findings further emphasize the importance of environmental infection control for viral respiratory pathogens in long-term care facilities.

INTRODUCTION

Long-term care facilities present a unique public health problem: a highly susceptible population in a crowded institutional setting constantly exposed to respiratory pathogens from the flow of visitors, personnel and other residents. Nursing home acquired infections cost the U.S. healthcare system roughly $673 million to $2 billion annually, and are a significant concern in long-term care populations where prevalence of co-morbid disease is high (1). Nearly half (49%) of long-term care populations are arthritic and over a quarter are suffering from other chronic ailments (2). Among acute morbidities, influenza, upper respiratory tract infections and nursing home acquired pneumonia have presented a challenging and prevalent public health concern (3-8). Vaccines are available, but even for the most seasonal respiratory tract infection (*i.e,*. influenza) vaccine efficacy is <70% (9). And even in highly vaccinated nursing home populations, influenza outbreaks still occur leading to substantial morbidity and mortality (10). Outbreaks in long-term care facilities have been caused by a variety of respiratory pathogens including influenza B, coronavirus, parainfluenza, and *Bordetella pertussis* (11-13).

Previous data have suggested that the physical environment plays a prominent role in respiratory disease transmission. Influenza A H1N1 has been shown to survive on common surfaces for up to 17 days, remaining infectious for at least a week (14, 15). Other respiratory

pathogens such as coronavirus 229E remain infectious for at least 5 days on a variety of materials including ceramics, rubber, and glass (16). Despite appropriate hygiene and prevention control, residual pathogenic microbial contamination persists in healthcare environments (17). Data on fomite contamination of respiratory pathogens in endemic (*i.e.*, non-outbreak) settings is limited. Respiratory viral contamination is a particular concern given that the environmental burden of respiratory pathogens may facilitate transmission, exacerbate existing health conditions, and be a potential source of outbreaks. This study is part of a larger collaboration known as PROTECT Project, a pilot investigation to study decolonization of nursing home residents against healthcare-associated infection. The intent of this sub-study is to assess baseline epidemiology and report surveillance results of respiratory pathogens from residents and the environments of three skilled nursing home-facilities (SNF) in Southern California. The probability of viral shedding in SNFs due to symptomatic residents is estimated by applying Bayes' Theorem providing evidence on the importance of infection control in long-term care facilities.

METHODS

Specimen Collection

Between May 2015 and July 2015 infection control nurses at each participating site identified patient residents with clinical symptoms suggestive of influenza-like illness (ILI), *i.e.*, fever, congestion, rhinorrhea, cough (with or without sputum production), shortness of breath, or other pulmonary complaints (pleurisy, wheezing). Symptomatic patients underwent nasal and environmental swabbing using a viral collection system involving flocked tipped swabs (one per nostril or two per surface) placed in M4 viral transport media. For residents with suspected ILI, nursing staff returned 3 days later to swab five common high-risk exposure objects (bed side

table/bed rail, call button/remote/phones, door knobs (room and bathroom combined), light switch, bathroom handles (toilet flush handle and sink handles combined) using the above described viral collection system. Two-hundred sixty environmental samples were collected. All samples were transported on cold pack, immediately frozen and stored frozen at -70ºC prior to testing. All specimens were processed and tested at the Los Angeles County Public Health Laboratories.

Respiratory Multiplex Testing

All resident and environmental swabs were processed using the FilmArray RP v.1 (BioFire Diagnostics, Salt Lake City, Utah) an FDA-approved multiplex nested PCR-based respiratory assay capable of detecting 17 viral targets (adenovirus, coronavirus (HKU1, NL63, 229E and OC43), human metapnuemovirus, rhinovrus/enterovirus, influenza A, influenza A subtypes H1, H3 and (H1N1)pdm09, influenza B, parainfluenza types 1, 2, 3 and 4, and respiratory syncytial virus) and 3 bacterial targets (*Bordetella pertussis*, *Chlamydophila pneumonia*, and *Mycoplasma pneumonia*). Prospective and retrospective studies have shown the reliable diagnostic performance of the FilmArray RP in detecting a variety of respiratory pathogens in vulnerable populations (18-20). A complete overview of our surveillance and multiplex testing algorithm is found in **Figure 3.1**.

Application of Bayes' Theorem in Environmental Surveillance

Fagan nomograms are clinical graphical Bayesian tools that determines disease probability conditional on the probabilities of input parameters (21). The input parameters include 1) a prior (pre-test) probability defined as the disease prevalence in the population and 2) the diagnostic performance measures of the test being used to determine the presence of disease. Input parameters

determine the posterior (post-test) probability of disease providing a much more confident result interpretation. While the use of Fagan nomograms has been limited to clinical practice the underlying probabilistic mechanics of Bayes' Theorem have had a wide range of applications (22-24). Because we are using a clinical diagnostic test for environmental surveillance purposes, the pre-test probability cannot be defined to just disease prevalence within SNF populations. From an infection control perspective, the potential for transmission of respiratory pathogens in the environment is due to symptomatic individuals, but also fomites in the general environment (25, 26). Therefore, total prevalence of transmissible pathogens within SNFs includes disease prevalence in residents and also the unknown disease prevalence in the general environment. Thus, application of the Fagan nomogram is only applicable by including Bayes' pre-test probability as minimum disease prevalence, which we obtained from symptomatic surveillance efforts. Logically, the disease prevalence in our SNF environments is either equal to or greater than the minimum disease prevalence found among residents.

The second Bayesian parameter included in our Fagan nomogram is the positive and negative likelihood ratio calculated from the diagnostic measures (sensitivity and specificity) reported in the FDA-approved summary report of the BioFire FilmArray RP. Both Positive (LR+) and Negative Likelihood Ratios (LR-) were calculated directly from sensitivity and specificity: LR+ = Sensitivity/1-Specificity, LR- = 1-Sensitivity/Specificity. By standard methods, sensitivity is also called the true positive rate, and 1-specificity is called the false positive rate (27).

Using the above Bayesian parameters of minimum disease prevalence and the diagnostic performance measures of the FilmArray RP, the post-test probability can then be interpreted as the minimum probability of environmental contamination of high contact surfaces due to either shedding from an environmental source or a symptomatic resident.

All statistical analyses and data visualizations were done in R Studio (Version 1.0.143).

Institutional Review Board approval was obtained from the Los Angeles County Public Health Department, University of California, Los Angeles and the University of California, Irvine.

RESULTS

Demographics

The demographics including comorbidities of all patients at risk are listed in **Table 3.1**. The source population for this study included facilities whose mean annual admission was 762 patients and 47033 resident days. The mean length of stay among patients at all SNFs was approximately two months. A majority of residents were white females between the ages of 65-85, and a third of the total population was >85 years of age. Nearly all residents were admitted from local hospitals, and many had existing comorbidities at the time of the study including diabetes, fecal incontinence, and a wound and/or rash (**Table 3.1**).

Symptomatic Surveillance

Fifty-two residents were identified as symptomatic for acute respiratory illness. Ten of 52 residents (19%) had a detectable viral pathogen: parainfluenza type 3 (n=4), rhinovirus/enterovirus (n=4), RSV (n=1), and influenza B (n=1). (**Figure 3.2**) All positive results were from two SNFs with no FilmArray RP targets detected from a third SNF. Parainfluenza-3 (13.3%) and rhino/enterovirus (10.0%) were the most common targets detected. Additional targets identified

include influenza B, RSV and rhinovirus/enterovirus (5.5% each). Total period prevalence is stratified per facility and per target in **Table 3.2**.

Environmental Surveillance and Bayes' Theorem

Two hundred sixty environmental surfaces were swabbed during environmental surveillance. Among residents with detectable viral infection, environmental contamination of the same pathogen was found in 20% (2/10) of high-contact surfaces tested (*i.e.*, bedrail, doorknobs). The FilmArray RP confirmed the presence of parainfluenza type 3 and rhinovirus/enterovirus in the environment. All positive viral environmental specimens were concordant with confirmed resident results. No bacterial respiratory pathogens were detected among any resident or environmental samples.

Using disease prevalence from symptomatic surveillance as the minimum pre-test probability and the LRs calculated from the reported BioFire FilmArray RP target-specific sensitivity and specificity, true environmental contamination probability due to shedding is 96% for parainfluenza 3 and 63% for rhinovirus/enterovirus. A Fagan nomogram for parainfluenza 3 using the appropriate parameters is found in **Figure 3.3**. Of note, each parameter is used as a linear scale bisected to yield the minimum post-test probability. Prevalence and LR- can also be used to give the minimum post-test probability of a negative result, meaning < 1% probability of parainfuenza 3 environmental shedding upon a positive diagnostic result. Results from the complete Bayesian analysis is found in **Table 3.3**.

For our Bayesian analysis, minimum pre-test probability is limited to only the disease prevalence as determined from our symptomatic surveillance. However, if known, including environmental disease prevalence would allow greater approximation of the post-test probability

of environmental contamination due to shedding within the SNF environment. To assess how increased disease prevalence would affect environmental contamination due to shedding, we extend our Bayesian analysis by simulating how post-test probability would be affected by altering parainfluenza 3 and rhinovirus/enterovirus pre-test probabilities (*i.e.*, disease prevalence). As illustrated in **Figure 3.4**, viral detection is saturated, marked by an exponential decay relationship between environmental contamination due to shedding and increased true disease prevalence, which occurs at higher prevalence for either viral target. **Figure 3.4** suggests that evidence of environmental transmission is more likely to occur when disease prevalence is high but also limited by the detection method.

DISCUSSION

Over the next few decades, older populations will continue to grow at an accelerated rate increasing the demand for long-term care facilities and creating new public health challenges in managing respiratory health. As of 2012, the long-term care facility workforce functioned in hospices, adult day service centers, home health agencies, assisted living communities and nursing homes totaling 58600 workers for the 4 million Americans in long-term care facilities (1 long-care service worker for every ~67 patient residents) (28, 29). While nursing homes comprise nearly a third of all long term care facilities and are expected to increase, appropriate surveillance of respiratory tract infections among the elderly should be an important public health priority as most of this population is highly susceptible to respiratory outbreaks (30).

The viral pathogens found among residents and in the environment in this study have all been previously implicated in previous outbreaks affecting vulnerable populations in healthcare settings (10, 31). From symptomatic surveillance we confirmed the presence of parainfluenza 3,

rhinovirus/enterovirus, RSV and influenza B infection in non-epidemic, i.e., endemic settings. A parainfluenza type 3 outbreak was in an adult hematology unit contributed to nosocomial transmission occurred over a 5-month span (32). The source of the outbreak was a chronically infected resident that had been placed in isolation, suggesting an environmental component of transmission in the outbreak. In the present study, all four parainfluenza type 3 specimens found during surveillance were detected in the same SNF population (SNF1) within a one-week period, plausibly suggesting intra-facility transmission. However, confirmation of intra-facility transmission could be resolved only by whole genome sequencing to compare viral genetic profiles and phylogeny. Evidence of parainfluenza type 3 environmental contamination due to shedding was observed as a symptomatic resident's call button/TV remote positively detected the virus. What role, if any, this shedding had on parainfluenza type 3 transmission to other residents is unclear; however, application of Bayes' Theorem reveals that environmental shedding of parainfluenza 3 due to this resident is highly probable.

The significance of the environmental shedding reported in our study increases upon considering that sustained transmission is plausible in a semi-closed population such as a nursing home environment (33). This strengthens the use of prevalence of infection as a pre-test probability performed during our Bayesian analysis. We additionally found evidence of rhinovirus/enterovirus shedding in the environment of the same SNF population (SNF1) on resident door knobs. Once again, using a Fagan nomogram helps interpretation: given a 10% prevalence of rhinovirus/enterovirus in this subpopulation and given 97.5% true positive rate and 6.5% false positive rate, the probability of shedding in the environment by rhinovirus/enterovirus from a symptomatic individual is 62.5% suggesting the possible presence of either a different pathogen as the aetiological cause of infection or minimal viral shedding in the environment. RSV, influenza

B, and rhino/enterovirus were all detected from resident samples collected on the same day from one SNF, however no co-infection was detected. The co-circulation and burden from multiple respiratory viruses among vulnerable populations is unknown but evidence suggests respiratory tract infections may be compounded by the synergistic effect of multiple pathogens (34).

Based on our clinical and environmental surveillance, clear epidemiological facility differences were present. While SNF 1 had the most viral targets confirmed by multiplex testing, SNF 2 had greater viral diversity. Additionally, SNF 3 had no targets detected at all. Our observations may reflect differences in sanitation and hygiene control, but also may be due to unequal foot traffic flow at each facility. High traffic flow would allow greater opportunity for resident exposure, colonization and infection; and conversely, low traffic flow would minimize the opportunity of the same. Overall, our study did not coincide with the wintertime seasonality of most viral respiratory infections, which explains why no targets were confirmed in SNF 3, but additionally provides evidence that our reported disease prevalence for each detected viral pathogen is likely an underestimate.

The confirmed presence and absence of respiratory pathogens in our study supports some national trends. Based on Western United States Census Region RSV 2015 data gathered from the National Respiratory and Enteric Virus Surveillance System (NREVSS), the RSV clinical sample identified in this study coincided with a period of low viral isolation among the general US population (35). NREVSS data suggests a high percent positive rate for antigen detection of parainfluenza 3 in the US compared to RSV at the same time (36). While influenza A was not found in any clinical and environmental sample, influenza B infection was confirmed, supporting national and regional-level outpatient surveillance from the 2014-2015 flu season, where influenza B was the prevalent circulating influenza strain (37). Additionally, influenza B strains are often

most prevalent at the end of most influenza seasons, which coincides to when our study was conducted (*i.e.*, late spring, early summer). Seasonal trends of other respiratory pathogens may help explain the low detection rates reported in our study.

Our results provide evidence supporting a recent report revealing major gaps in the knowledge, practice and policy in infection control of environmental surfaces in healthcare settings (38). In our study, environmental contamination was highly likely shed from symptomatic residents. Fagan nomograms are methods of Bayesian analysis incorporating conditional probabilities for evaluating environmental contamination and person-to-person transmission, but only if a baseline prevalence is known. Our data suggests environmental contamination is site specific with possible viral shedding only found in SNF 1. This evidence suggests minor differences in the adherence to environmental hygiene practices within and between facilities. Recommendations for basic and environmental infection control practice in health-care facilities have been created by the CDC and the Healthcare Infection Control Practices Advisory Committee (39). Per recommendations, high-level disinfectants on noncritical areas or environmental surfaces is not required in long term care facilities, however a more frequent cleaning schedule of high-contact surfaces is suggested.

The main limitation of our study is related to respiratory viral detection. While some residents were confirmed, a majority of residents had no detectable respiratory infection. Results do not exclude the presence of other respiratory pathogens not included in the FilmArray RP. Despite the high sensitivity of the FilmArray RP, detection may also have been hindered by low microbial load or equally likely, routine facility-specific cleaning procedures preventing the capture of pathogens altogether. Another significant issue is that the FilmArray RP is only FDA approved for processing nasopharyngeal swab (NPS) specimens; however, due to resident co-

morbidities, a non-invasive sample collection method (*i.e.*, nares swabs) was preferred. However, nares swab specimen processing on the FilmArray RP is effective for detecting respiratory pathogen (40). Additionally, the time of collection may have not coincided with high viral titres. For instance, sampling outside of the general incubation of parainfluenza infections (two to four days) or environmental shedding (three to ten days) may have affected detection (41). Additionally, the FilmArray RP is FDA-cleared only for *in vitro* diagnostic use with no clear application for environmental testing. However, environmental swab testing and processing has been recommended as a method to prevent environmental contamination during routine clinical testing (42).

Our results suggest that heightened surveillance among vulnerable populations in a crowded institutional setting may help identify residents with transmissible respiratory infections, thereby enhance prevention efforts. Ideally, year round surveillance activities especially during influenza season would provide a clearer picture on the role of the environment on respiratory pathogen transmission. Additionally, evidence for inter-facility circulation of respiratory viruses may be under reported and may be amenable to intervention. For long-term care facility staff, heightened awareness about the potential for viral respiratory pathogen spread is necessary as well as reinforcement of standard infection control practices of ILI patients. To our knowledge this is the first report to use the BioFire FilmArray RP for environmental monitoring for respiratory pathogens, and also the first report to use this technology for testing samples on a strictly older population in a LTC setting.

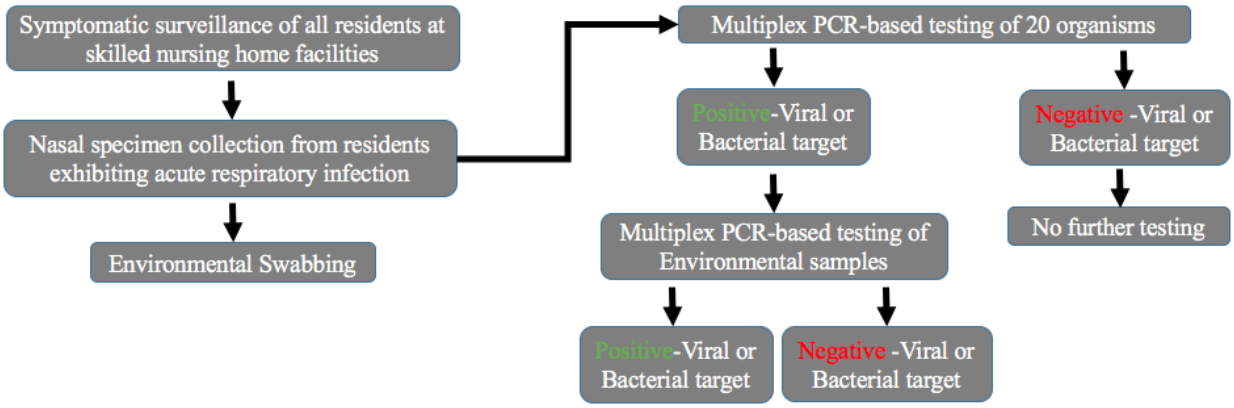

Figure 3.1. Surveillance and testing algorithm for symptomatic resident and environmental multiplex testing.

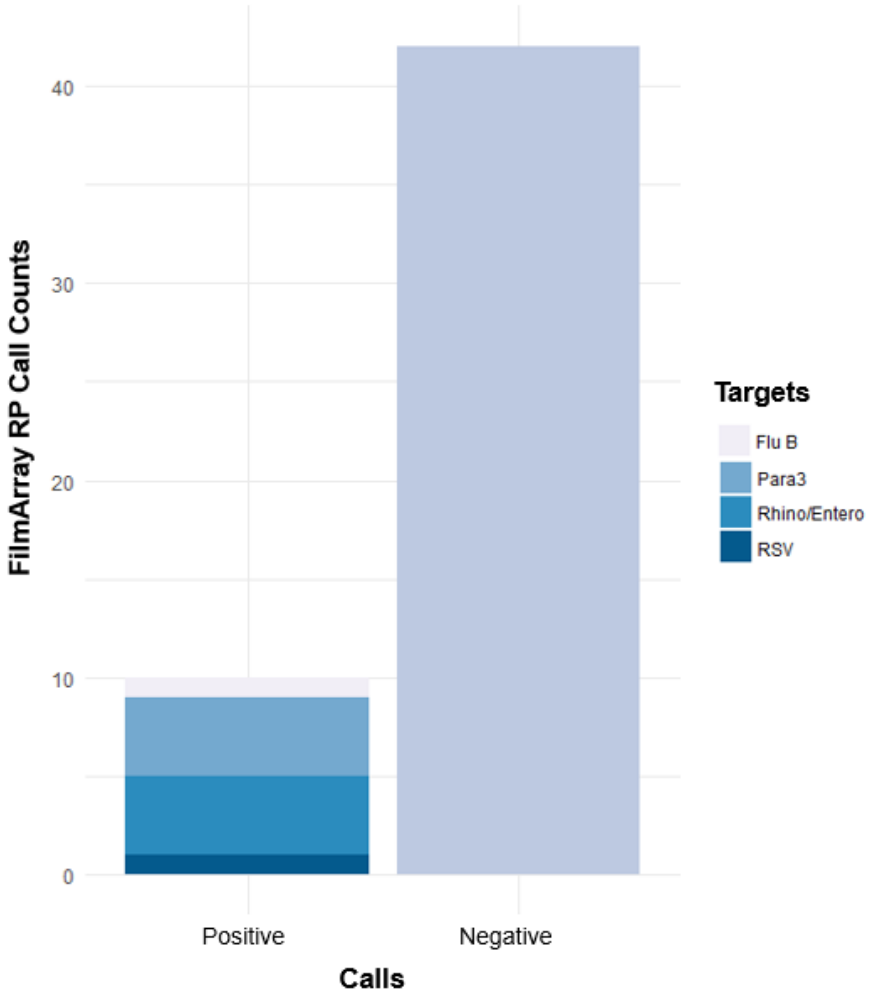

Figure 3.2. Summary of symptomatic surveillance based on BioFire FilmArray RP positive and negative call counts separated by target. 10 out of 52 (19.2%) symptomatic residents had a detectable viral infection.

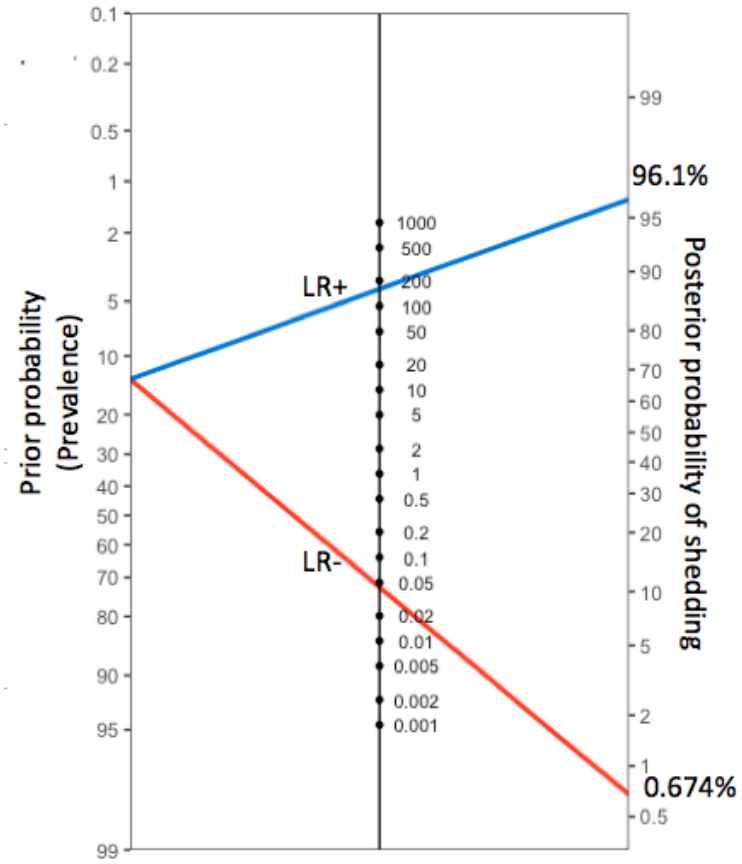

Figure 3.3. Parainfluenza-3 Fagan nomogram of incorporating disease prevalence from symptomatic surveillance in SNF1 and LRs calculated from the diagnostic measures of the FilmArray RP to yield the post-test probability of shedding in a high contact surface.

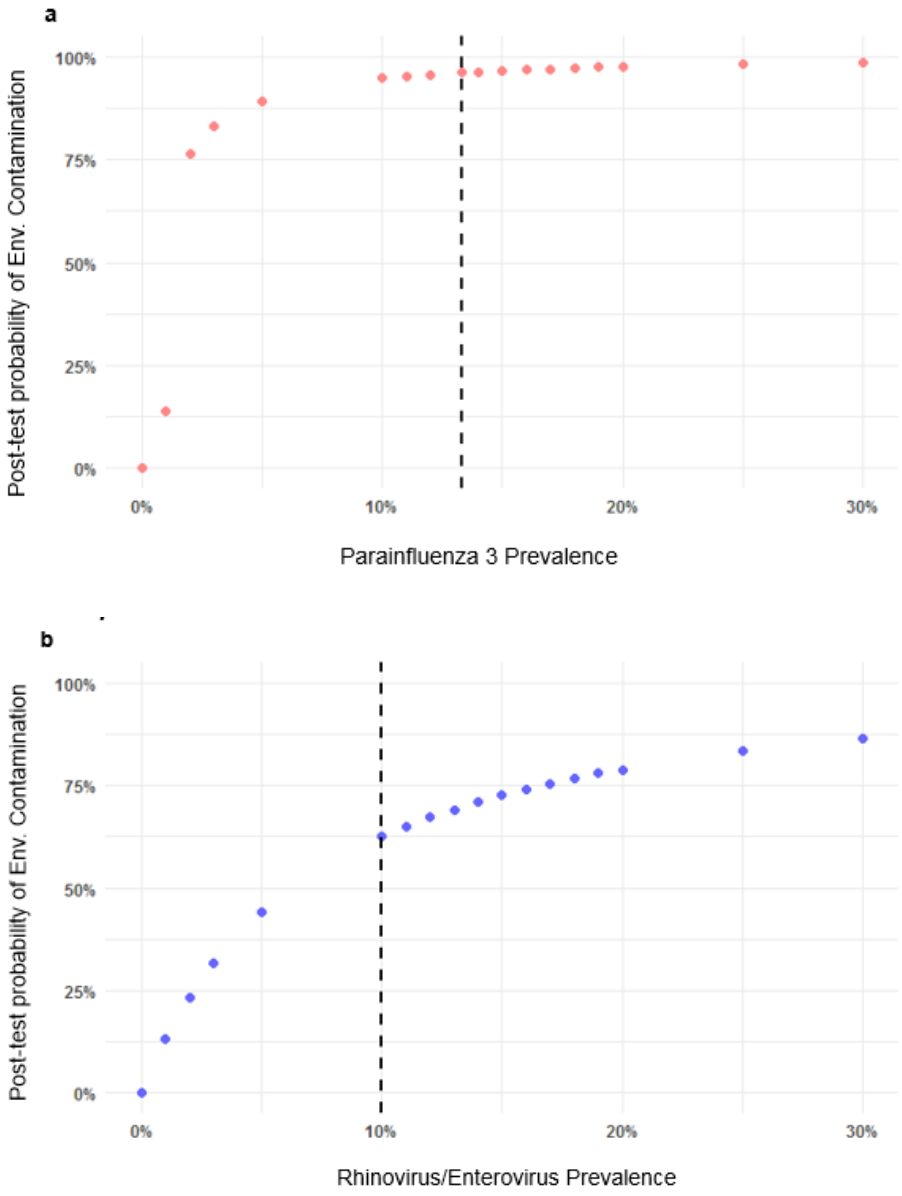

Figure 3.4. Post-test probability of environmental contamination with increase prevalence of respiratory disease. Dashed line indicates viral prevalence as found during symptomatic surveillance (parainfluenza 3:13.3%, rhinovirus/enterovirus:10.0%)

Table 3.1. Skilled Nursing Home Facility-level characteristics.

Facility-level Variable	SNF1	SNF2	SNF3
Annual Volume		N	
Admissions	562	832	892
Resident Days	32,638	49,928	58,532
Mean Length of Stay (Days)	58	60	66
Demographics and Insurance		%	
Age			
<65	18	38	12
65-85	46	42	50
85+	36	20	38
Male	34	46	41
Race			
White	92	91	79
Black	2	3	10
Asian/Pacific Islander	6	6	11
Hispanic Ethnicity	25	27	9
<High School Education	32	19	11
Medicare Insured	38	20	18
Admitted from Hospital	95	94	92
Illness and Comorbidities			
Mechanical Ventilation	0	0	0.2
End Stage Renal Disease	7	8	9
Diabetes	40	42	34
Wounds or rash	83	68	90
Fecal Incontinence	35	35	34

Table 3.2. Epidemiological summary of symptomatic surveillance showing prevalence and overall burden from confirmed targets found in SNF 1 and SNF 2.

	Positive target detected	Prevalence (%)	Estimated number of annual cases*
SNF1	**7**	**23.3**	**28**
Para 3	4	13.3	16
Rhino/Entero	3	10.0	12
SNF2	**3**	**16.6**	**12**
Flu B	1	5.5	4
Rhino/Entero	1	5.5	4
RSV	1	5.5	4

*assuming period prevalence is sustained during a 12-month period

Table 3.3. Application Bayes' Theorem to calculate minimum Post-test probability of environmental contamination from confirmed targets detected during environmental surveillance

	Diagnostic performance of the BioFire FilmArray RP*					
Confirmed target from environmental surveillance	**Sen**	**Spe**	**LR+**	**LR-**	**Prior Probability: Minimum prevalence in the environment****	**Post-test probability: Minimum probability of environmental contamination*****
Parainfluenza 3	0.956	0.994	159.33	0.0443	13.3%	96.1%
Rhinovirus/Enterovirus	0.975	0.935	15	0.0267	10.0%	62.5%

*Sensitivity (Sen); Specificity (Spe); Positive Likelihood Ratio (LR+); Negative Likelihood Ratio (LR-)

**Total prevalence would have included prevalence of respiratory pathogens on surfaces and inanimate objects within SNFs however this is unknown.

***Minimum probability of environmental contamination given a positive test result on the BioFire FilmArray RP and a minimum prevalence as observed from our symptomatic surveillance

REFERENCES

1. Strausbaugh LJ, Joseph CL. The burden of infection in long-term care. *Infect Control Hosp Epidemiol* 2000 Oct; 21 (10): 674-679.

2. CDC. Difficulty in physical functioning, ages 18+: US, 1997-2014, National Health Interview Survey. Available at: http://205.207.175.93/HDI/TableViewer/tableView.aspx?ReportId=641. Accessed: June 1, 2016.

3. CDC. Outbreak of pneumococcal pneumonia among unvaccinated residents of a nursing home—New Jersey, April 2001. *MMWR* 2001; 50(33): 707-710.

4. Mill K, Graham, Winslow BT, Springer, KL. Treatment of nursing home-acquired pneumonia. *Am Fam Physician* 2009; 79 (11): 976-982.

5. Monto AS, Rotthoff J, Teich E, *et al.* Detection and control of influenza outbreaks in well-vaccinated nursing home populations. *Clin Infect Dis* 2004 August; 39(4): 459-464.

6. Quinn C, Demirjian A, Watkins LF, *et al.* Legionnaires' disease outbreak at a long-term care facility caused by a cooling tower using an automated disinfection system--Ohio, 2013. *J Environ Health* 2015; 78(5): 8-13.

7. Harris TG, Sullivan MJ, Proops D. Delay in diagnosis leading to nosocomial transmission of tuberculosis at a New York City health care facility. *Am J Infect Control* 2013 Feb; 41 (2): 155-160.

8. Dowell SF, Anderson LJ, Gary HE Jr, *et al.* Respiratory syncytial virus is an important cause of community-acquired lower respiratory infection among hospitalized adults. *J Infect Dis* 1996 Sep; 174(3): 456-462.

9. Osterholm MT, Kelley NS, Sommer A, *et al*. Efficacy and effectiveness of influenza vaccines: a systematic review and meta-analysis. *Lancet Infect Dis* 2012 Jan; 12(1): 36-44.

10. Morens DM, Rash VM. Lessons from a nursing home outbreak of influenza A. *Infect Control Hosp Epidemiol* 1995 May; 16 (5): 275-280.

11. Nicolle LE, Garibaldi R, Strausbaugh LJ. Infections and antibiotic resistance in nursing homes. *Clin Microbiol Rev* 1996 Jan; 9(1): 1–17.

12. Falsey AR, Dallal GE, Formica MA *et al.* Long-term care facilities: a cornucopia of viral pathogens. *J Am Geriatr Soc* 2008 Jul; 56(7): 1281-1285.

13. Addiss DG, Davis JP, Meade BD, *et al.* A pertussis outbreak in a Wisconsin nursing home. *J Infect Dis* 1991 Oct; 164(4): 704-710.

14. Perry KA, Coulliette AD, Rose LJ *et al.* Persistence of influenza A (H1N1) virus on stainless steel surfaces. *Appl Environ Microbiol* 2016 May 16; 82 (11): 3239-3245.

15. Thomas Y, Vogel G, Wunderli W, *et al.* Survival of influenza virus on banknotes. *Appl Environ Microbiol* 2008 May;74(10): 3002-3007.

16. Warnes SL, Little ZR, Keevil CW. Human coronavirus 229E remains infectious on common touch surface materials. *mBio* 2015; 10: 6(6): e01697-15.

17. Tuladhar E, Hazeleger WC, Koopmans M, *et al.* Residual viral and bacterial contamination of surfaces after cleanings and disinfection. *Appl Environ Microbiol* 2012; 78 (21): 7769-7775.

18. U.S. Food and Drug Administration. 510(k) Summary, Idaho Technology Inc. FilmArray RP Test System. K103175.

19. Pierce VM, Elkan M, Leet M, *et al.* Comparison of the Idaho Technology FilmArray system to real-time PCR for detection of respiratory pathogens in children. *J Clin Micrbiol* 2012 Feb; 50(2): 364-371

20. Hammond SP, Gagne LS, Stock SR, *et al*. Respiratory virus detection in immunocompromised patients with FilmArray respiratory panel compared to conventional methods. *J Clin Microbiol* 2012; 50(10): 3216-3221.

21. Fagan TJ. Letter: Nomogram for Bayes theorem. *N Engl J Med* 1975 Jul 31; 293(5): 257.

22. Hall GH. The clinical application of Bayes' theorem. *Lancet* 1967 Sep 9; 2(7515): 555-7.

23. Gronewold AD, Vallero DA (2010). Application of Bayes' theorem for predicting environment damage. In *AccessScience*. McGraw-Hill Education (online).

24. Rubin DB. Some applications of Bayesian statistics to educational data. *Journal of the Royal Statistical Society. Series D (The Statistician)* Mar-Jun, 1983; 32(1/2): 55-68.

25. Pica N, Bouvier NM. Environmental factors affecting the transmission of respiratory viruses. *Curr Opin Virol* 2012 Feb; 2(1); 90-95.

26. Goins WP, Talbot HK, Talbot TR. Health care-acquired viral respiratory diseases. *Infect Dis Clin North Am* 2011 Mar; 25(1): 227-244.

27. Ting KM (2011). Sensitivity and specificity. In *Encyclopedia of Machine Learning*. Springer Science + Business Media LLC.

28. CDC. U.S. Department of Health and Human Services. Long-term care services in the United States: 2013 Overview. *Vital and Health Statistics Series* 2013; 3.

29. CDC. Nursing homes and assisted living (long-term care facilities [LTCs]) (2017). Available at: https://www.cdc.gov/longtermcare/index.html. Accessed August 29, 2017.

30. Gaspard P, *et al*. [Article in French] Respiratory tract infections in institutions for elderly people: the GROG Geronto-Alsace, strategy of surveillance and alert. [Abstract] *Medecine Et Maladies Infectieuses* 2007; 37 Suppl; 3: S215-222.

31. Libow LS, Neufield RR, Olson E, *et al.* Sequential outbreak of influenza A and B in a nursing home: efficacy of vaccine and amantadine. *J Am Geriatr Soc* 1996; 44 (10): 1153-1157.

32. Jalal H, Bibby DF, Bennett J, *et al*. Molecular investigations of an outbreak of parainfluenza virus type 3 and respiratory syncytial virus infections in a hematology unit. *J Clin Microbiol* 2007 June; 45 (6): 1690-1696.

33. Juthani-Mehta M, Quagliarello VJ. Infectious diseases in the nursing home setting: challenges and opportunities for clinical investigation. *Clin Infect Dis* 2010 Oct 15; 51 (8): 931-936.

34. Hebert-Dufresne L, Althouse BM. Complex dynamics of synergistic coinfections on realistically clustered networks. *Proc Natl Acad Sci USA* 2015 Aug 18; 112 (33): 10551-10556.

35. CDC. National Respiratory and Enteric Virus Surveillance System. Western census region RSV data 2015. Updated: April 15, 2015.

36. CDC. National Respiratory and Enteric Virus Surveillance System. US Data parainfluenza 3 data 2015. Updated: April 15, 2015.

37. CDC. FluView. Accessed March 25, 2016.

38. Quinn MM, Henneberger PK, National Institute for Occupational Safety and Health, *et al.* Cleaning and disinfecting environmental surfaces in health care: toward an integrated framework for infection and occupational illness prevention. *Am J Infect Control* 2015 May 1; 43 (5): 424-434.

39. CDC and HIPAC. Guidelines for environmental infection control in health-care facilities—June 6 2003. *MMWR* 2003; 52(RR10) ;1-42.

40. Blaschke AJ, Allison MA, Meyers L, *et al.* Non-invasive sample collection for respiratory virus testing by multiplex PCR. *J Clin Virol* 2011 Nov; 52 (3): 210-214.

41. Public Health Agency of Canada. Human Parainfluenza Virus Fact Sheet. Available at: www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/parainfluenza-eng.php. Accessed: June 8, 2016.

42. BioFire Diagnostics LLC. Contamination prevention and decontamination, technical Note. FLM1-PRT-0230-01.

CHAPTER 4

Modeling the economic impact of recent measles outbreaks to public health infrastructures in California

(This chapter has been prepared for submission as a manuscript to *Vaccine*.)

ABSTRACT

Introduction: Despite being eliminated from the United States since 2000, measles remains a great public health concern with significant economic impact on local and state public health departments. Here, we provide a cost analysis of two recent measles outbreaks in Southern California: the 2014-15 multi-state and 2016-17 measles outbreaks. We focus on the estimated cost to local and state communicable disease control programs and public health laboratories (PHLs).

Methods: To capture empirical data about local outbreak response, we sent surveys to all members of the California Association of Communicable Disease Controllers and all members of the California Association of Public Health Laboratory Directors. We additionally sent surveys to the California Department of Public Health to capture state outbreak response. Empirical data included costs (in 2017 USD$) to communicable disease control programs due to contact investigations, isolation, and personnel costs; and to PHLs due to measles testing and personnel costs. Empirical data from the 2014-15 U.S. multi-state measles outbreak was used to model estimated epidemiologically-linked measles contact counts at the local level in California. Our model is then

applied to estimate the public health epidemiologic and laboratory response to the 2014-15 U.S. multi-state measles outbreak and the 2016-17 measles outbreak. We additionally include estimated hospitalization costs to capture overall economic burden from a public health perspective.

Results: We estimate that during the 2014-15 multi-state outbreak there were 10,702 epidemiologically-linked contacts costing CA local and state public health departments $3,743,000 - $4,085,000 ($349-$381 per contact, $29,000 - $31,000 per case). In contrast, the 2016-17 measles outbreak cost $338,000 ($318 per contact, $14,000 per case) from the estimated 1063 contacts. The difference in cost between both suggests that measles control and prevention has improved over time across all public health jurisdictions. These costs are comparable to other recent measles outbreaks.

Discussion: Our study provides an economic case study to the need of global eradication of measles. Further, we present a robust and stable dynamic contact count model that could be used retrospectively or prospectively for future measles or other vaccine-preventable disease outbreaks both in and outside California.

INTRODUCTION

Despite the international and domestic successes against measles, cases and sporadic outbreaks still occur even in 'eliminated' areas. In early Fall 2016, the Pan American Health Organization/World Health Organization (PAHO/WHO) declared the Americas as the first global region to have eliminated sustained endemic measles transmission (1). Measles elimination in the Americas was facilitated by several factors including, the availability of an effective vaccine, state

and local vaccination requirements for daycare and school entry, heightened surveillance systems, and the concerted use of better diagnostic tools across local public health infrastructures. Despite achieving measles elimination in 2000, the United States has experienced on average 196 annual confirmed measles cases since 2010. While the cause of measles in the U.S. is due to case importation from countries where sustained measles transmission continues (2), the underlying susceptible population in the US that results primarily from personal beliefs allows for and contributes to measles transmission. Measles will continue until further elimination occurs abroad and until higher vaccination rates are observed domestically. However, until measles elimination and vaccination campaigns are successful worldwide, the U.S. is expected to bear some social and economic burden of measles.

Measles resurgence in the U.S. is a recurring economic issue for public health infrastructure. In 2004, the Iowa state public health laboratory and the state public health departments spent $142,000 in containment and investigation from one exposed U.S. college student returning from abroad (3). In 2008, an unvaccinated 7-year-old boy was exposed and infected while on vacation overseas with his family, who upon returning home to San Diego resulted in measles exposure of 839 persons and 11 additional cases (all unvaccinated children). In response to the outbreak, local and state agencies spent an estimated $124,000 ($10,000 per case) in control, outbreak and healthcare response (4). Again in the same year, an infectious Swiss traveler arrived in the U.S. and exposed thousands of individuals, costing local health care facilities $799,000 ($57,000 per case). In 2011, a relatively small outbreak of 9 cases began when an unvaccinated 15-year-old refugee traveling from Malaysia to Los Angeles, CA exposed other refugees and unvaccinated American passengers. The index case propagated contact investigations in several states with estimated costs to state health departments ranging from $621 (Oklahoma)

to $35,115 (California). These costs included labor, laboratory testing, treatment and hospitalization (5). In early 2014, an economic analysis of 16 outbreaks across the U.S. found that the cost per contact investigated was $298, with a cost range of $12,000-$30,000 per measles case (6).

In late 2014, a multi-state measles outbreak began when 42 individuals were exposed to measles from an unspecified source while attending the Disney theme parks in California (7). This exposure sparked a multi-state outbreak that affected several nearby states: Arizona, Utah, Nevada, Washington, Colorado, and Oregon. Additional cases were found outside of the U.S. in Mexico and Canada. Sustained transmission of the outbreak lasted for 120 days, with the outbreak being declared officially over on April 17th, 2015. Within California a total of 131 cases were confirmed in 14 counties across the state. While the highest attack rate was among 20-29 year olds, the highest incidence was among the very young (<1-year-olds). Among the 82 cases for which immunization status could be verified 70% were unvaccinated. Thirty-one secondary cases were confirmed among households or close contacts (23.6%), 14 among community settings (10.6%), and 44 among unknown epidemiologically linked sources (33.5%).

In early December 2016, measles began circulating in two Jewish day schools and within an Orthodox Jewish community in Los Angeles County (LAC). Eventually, 24 cases were identified within Los Angeles (n = 22), Ventura (n = 1) and Santa Barbara (n = 1) counties. Reports confirmed that at least 16 out of the first 18 cases identified were unvaccinated (8). Reports also estimated that over 2000 individuals may have been exposed, with 10% of them being unvaccinated (9). Among the suspected cases confirmed for measles at the LAC Public Health Laboratory (LACPHL), a majority were under the age of 20 (data not published).

The aim of our study is to assess the economic burden of the 2014-15 U.S. multi-state measles outbreak. We are specifically interested in evaluating the cost to public health infrastructure in the state of California, addressing the economic impact to local and state public health laboratories, as well as to local and state communicable disease control programs involved in response and control. We further evaluate the economic burden of the more recent measles outbreak in Southern California that occurred late 2016 through early 2017.

METHODS

A combination of empirical data and modeling was used to estimate the total cost of the 2014-15 U.S. multi-state and the 2016-17 measles outbreaks in California. Our economic perspective focused on estimating the local and state public health response cost across all California counties that both had communicable disease departments that investigated contacts and local PHLs that conducted measles testing. We additionally added estimated hospitalization costs to assess overall economic burden from a public health perspective. We limited our analysis to only those counties that had both public health departments and public health laboratories as the activities involved in investigating contacts and confirming measles cases are formally tasked to these agencies.

Survey Data

To capture empirical costs of the 2014 multi-state measles outbreak, we developed questionnaires tailored to gather data from both the laboratory and epidemiological staff at local health departments in CA. Surveys were sent to all members of the California Association of Public Health Laboratory Directors to capture public health laboratory response costs, and to all members

of the California Association of Communicable Disease Controllers across the 61 local health jurisdictions in California to capture the public health epidemiologic response costs. The questionnaires were sent to all counties and health jurisdictions, regardless of whether a case or contact was ever identified in them, because investigations and laboratory testing of potential measles cases or exposures lead to costs even in the absence of confirmed cases or contacts. Both questionnaires asked respondents to provide information regarding direct costs associated with the outbreak response. Direct costs include personnel salaries, fringe benefits, and supplies, shipping and courier costs, additional staffing and stand-by time.

To capture state laboratory data from both outbreaks, we sent out abbreviated surveys to the California Department of Public Health (CDPH) which included laboratory questions for the Viral and Rickettsial Disease Laboratory (VRDL). These abbreviated surveys were meant to capture staff and laboratory specific data corresponding to outbreak response from the state public health perspective. (A more detailed explanation of the methods used to gather these data and the questionnaires themselves are provided in **Appendix A**.)

Imputation of Number of Contacts

Based on survey results, contacts and response costs were only available from a subset of counties. Thus, using observed data and other epidemiologically-linked parameters, a Poisson regression model was used to estimate contacts (μ) for every county whose response included communicable disease control and public health laboratory testing information. Contacts are considered epidemiologically-linked to cases and resulted in investigation or contact tracing. The covariate selection for our regression model is based on previously identified causally associated parameters of measles transmission or outbreak propagation (10-15). Our model is fitted with the

following predictors: confirmed measles cases within each county (x_1), county distance from the outbreak epicenter (x_2), and population density per county (x_3).

As previously mentioned, each predictor ($x_j = x_1 \ldots x_3$) in our model has a plausible association in contributing to the spread of measles outbreaks and potentially contributes to determining the number of contacts during the outbreaks involved in this study. The number of confirmed cases (x_1) within each county was known based on CDPH measles surveillance during the 2014-15 multi-state measles outbreak. The distance between counties (x_2) was calculated in geographical space instead of transit infrastructure space (*i.e.*, distance measured by streets and highways) to account for non-linear effects of distance on the number of contacts, x_2 is defined as (distance in miles/100)^2. Finally, county population densities calculated from American Community Survey data census estimates from 2014 and 2016 and expressed as x_3. The estimation of μ in each county took the form:

$$log(\mu) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3, \quad \text{(Eq. 4.1.)}$$

where β_0 has a constant value regardless of county and $\beta_i = \beta_1 \ldots \beta_n$ are the regression coefficient values.

Cost Analysis

A standardized approach was taken to calculate laboratory testing costs by assuming that PCR was the preferred method of measles testing in each PHL when appropriate. A more detailed explanation of the method used is presented in **Appendix A**. Serology was also performed, but costs were negligible to overall economic impact. We verified whether PCR was performed in

each PHL by either direct survey response, follow-up or by accessing online publicly available test requisitions from each laboratory. When available, we applied real test costs for either serology or PCR. In instances when measles testing was not performed at all, we assumed that state laboratory send-outs were conducted at a courier cost of $42.50 per sample (figure based on mean internal LAC PHL courier send-out data). In order to include salary information in test cost we used a public service website created by the Nevada Policy Research Institute, a non-profit think tank that provides the titles, salaries and fringe benefits information for all individual CA county and state workers by title and classification. When available, we verified microbiologist (or equivalent) salaries within each CA county against county specific class specifications and arrived at a mean microbiologist salary per hour for each county during 2014 and 2016, measles PCR assay hands-on time (3 hrs.) to give full test cost of confirming measles in each county inclusive of personnel salary in performing measles testing.

Because public health epidemiologic response costs were not reported by every county, we calculated them for each county by multiplying the estimated number of contacts, μ (obtained from our regression model) by the public health epidemiologic response cost per contact ($250, which was calculated from data provided by the Los Angeles Department of Public Health (LADPH)). This estimate included direct expenses from several internal subdivisions within Los Angeles County involved in the public health epidemiologic response divided by the total contacts investigated within LAC.

We acknowledge that samples from every suspected contact will not necessarily undergo laboratory testing. Thus, using the LAC PHL data from the 2014-15 multi-state measles outbreak, we estimated that roughly 1 of every 15 suspected contacts investigated were tested for measles. This rate estimate (15.14) was applied to μ at the county level to estimate how many approximated

investigated contacts underwent laboratory testing in each CA PHL. Once known, the number of contacts that underwent laboratory testing was multiplied by the cost of measles PCR reagents only ($25.60). In summary, the total measles laboratory cost per county is either a direct send-out ($42.50 per laboratory specimen) or includes the PCR test cost for all μ factored through a rate estimate (15.14) and by the cost to run the test inclusive of the average CA microbiologist salary (plus benefits) at the time of the outbreak (Eq. 4.2).

$$\left(\frac{\exp^{\mu}}{15.14}\right) * \ \$25.60\ (PCR\ test\ cost) +$$

$$2014\ or\ 2016\ CA\ microbiologist\ salary\ per\ hour * PCR\ test\ hands\ on\ time$$

(Eq. 4.2)

To capture hospitalization response associated with measles complications during the 2014-15 multi-state outbreak, we added hospitalization costs for 21 confirmed measles cases that required hospitalization. Based on previous reports, mean hospitalization cost per measles case was estimated to be $25,000 (2017 US$) (16).

2016-17 Measles Outbreak

Our regression model and standardization approach of laboratory costs was further applied to estimate the public health epidemiologic and PHL cost of μ and the overall cost of the 2016-17 measles outbreak. We updated our input data to reflect 2016 conditions, such as population density data and 2016 CA microbiologist salaries.

Validation and Sensitivity Analysis

The Poisson model was validated internally by assessing parametric relationships via a correlation matrix and externally by comparing the expected μ to the observed μ. Finally, a local sensitivity analysis and perturbation-based sensitivity analysis were performed to evaluate the robustness and stability of our model and overall approach.

Modeling, statistics and GIS

All modeling and statistical analysis was performed using the statistical software package R (version 3.4.1). A map of California with overlaid μ for each county was created using ArcGIS Online (2017).

RESULTS

Survey Data

Survey results for the 2014-15 multi-state measles outbreak provided the observed costs of the public health epidemiologic and PHL response from each county, when available **(Figure 4.1)**. The public health epidemiologic survey provided observed outbreak counts. Overall, while there was a 34.2% response rate among all counties, public health epidemiologic programs comprised a majority (>70%) of survey respondents. Based on survey responses, the outbreak response required a variety of staff including public health nurses (20%), communicable disease controllers (18%), infection control staff (13%), epidemiology analysts (13%), administrative staff (13%), medical epidemiologists (9%), students and interns (7%), and other (7%). Our survey also revealed that for exposures that occurred in healthcare settings, public health epidemiologic response was often aided by in-house hospital epidemiologists. Among public health epidemiologic respondents,

self-isolation was recommended in at least five counties and one city health jurisdiction (Marin, Alameda, San Joaquin, Nevada, San Luis Obispo and the City of Berkeley). Communicable disease control within communities included supplying MMR vaccinations and post-exposure prophylaxis treatments (*e.g.,* immunoglobulin). At least two counties and one health jurisdiction activated the Incident Command Structure (Fresno, Alameda, and the City of Berkeley) for public health epidemiologic response.

Survey results from the state laboratories revealed number of tests performed and method of testing. During the 2014-15 multi-state outbreak, VRDL tested 1850 samples with PCR bulking a majority of the testing. Additional testing included serological immunoassays and next-generation sequencing. During the 2016-17 outbreak, CA state laboratories reported 106 total samples tested with a majority undergoing next-generation sequencing. PCR and serology were also performed. Culture was not performed during either outbreak response.

Poisson modeling

Our regression modeling of the 2014-15 multi-state outbreak, predicts that epidemiologically-linked contacts would be spatially distributed throughout the state (**Figure 4.2**). Contacts are predicted to be clustered in counties directly adjacent to Orange County and within 170 miles of the Disney theme parks area. Additionally, contacts were predicted throughout central California and in smaller closely adjacent counties near the northern part of the state. Contacts are expected as far south as San Diego County (87.5 miles) and as far north as Humboldt County (703.0 miles). The total number of epidemiologically-linked measles contacts is predicted from the model is 10,591.

When applying our regression model to the 2016-17 measles outbreak, our regression analysis predicted 1,063 epidemiologically-linked contacts in Los Angeles County alone. Additionally, despite confirmed cases found in both Santa Barbara and Ventura counties, our model predicts zero epidemiological linked contacts in these areas. Our model also predicted no linked contacts in the adjacent counties near LAC (*i.e.*, San Diego, Orange, Riverside or San Bernardino counties).

Cost calculations

We estimate that total public health epidemiologic and PHL response cost across all counties from 10,591 expected contacts and 131 confirmed cases during the 2014-15 multi-state measles outbreak was $2,757,000 (**Figure 4.3)**. An alternative estimate can be derived by substituting contact counts, public health epidemiologic and PHL costs for every county where survey data was reported, using regression predictions only for counties with missing data; the total investigated counts during the 2014-15 Multi-state outbreak when combining both expected and observed data then become 10,702 with a total public health epidemiologic and PHL response cost of $3,059,000 (**Table 4.1**). When hospitalization costs ($531,000) for 21 individuals and state costs ($126,000 - $469,000) are included, the total cost for the 2014-15 multi-state measles outbreak to local and state public health infrastructure comes to $3,743,000 - $4,085,000.

When applying our regression model to the epidemiological and PHL response for 2016-17 measles outbreak, we estimate that the LAC public health department spent $279,000. When including the state response ($60,000), the total cost of the outbreak to local and state public health infrastructure becomes $338,000.

Validation and Sensitivity Analysis

To assess the validity of our regression model to predict accurate counts of suspected measles contacts, we compared expected μ to observed μ. There is a strong positive correlative relationship ($R^2 = 0.945$) between expected and observed μ that is highly significant ($p > 0.0001$) providing evidence that our model does well in predicting μ **(Figure 4.4**).

To assess the overall robustness of our model, we performed a one-way sensitivity analysis, where we varied parameter β_i by twice the standard error for each coefficient in the regression model. We then applied μ to our standardization approach for obtaining public health epidemiologic and PHL costs, comparing the new estimates to the baseline cost of $2,757,000 from our Poisson model. Results of this analysis are shown in **Table 4.2**. The cost range shows minimal differences from baseline: the lowest cost ($2.47 million) is 3.17% lower than baseline, while the highest cost ($3.27 million) is 18.66% higher than baseline. These results suggest that our predicted cost estimates are robust to uncertainty.

To conduct a broader assessment of the stability of our cost analysis, we performed a perturbation-based sensitivity analysis, in which small random perturbations were introduced simultaneously for each parameter β_i and the constant, β_0 for 45 iterations. We then applied μ to our overall approach and compared the resulting estimates to the baseline cost estimate, $2,756,808. **Figure 4.5** shows the results, in which there is minimal variation across all iterations, once again suggesting that our predicted cost estimates are robust to measurement and numerical error.

DISCUSSION

We present a functional model and overall approach to determining measles contact counts retrospectively and assessing the economic impact of recent measles outbreaks to California's public health infrastructure. For the 2014-15 U.S. multi-state measles outbreak, 10,702 epidemiologically-linked contacts cost local and state public health agencies $3,743,000 - $4,085,000 ($349-$381 per contact, $29,000 - $31,000 per case) in outbreak control, contact investigations, laboratory testing, and hospitalizations. During the 2016-17 measles outbreak, California spent $338,000 ($318 per contact and $14,000 per case) from the expected 1063 contacts. Combining costs from both outbreaks, the total economic burden is $4,081,000 to $4,424,000 throughout the state with an average $110,000 to $120,000 spent at the county-level. Overall, our estimated outbreak costs combine contact investigations, recommended isolation, laboratory screening of measles, hospitalization from cases experiencing complications from measles infection.

Aside from shedding light on the economic impact of measles outbreaks in California, our model confirms several real-world epidemiological observations form Orange and San Francisco counties. During the 2014-15 outbreak, more epidemiologically-linked measles contacts should be expected in Orange County than in surrounding counties. The higher count would be due to the greater number of confirmed measles cases (n = 35), a more population dense area, and the outbreak epicenter occurring within the Disney theme parks area, located in Orange County. This evidence suggests a higher measles burden in Orange county. However, survey results revealed more counts in Los Angeles County (n = 2180) than Orange County (n = 1955). By predicting more counts in Los Angeles County (n = 2022) than Orange County (n = 1969), our model agrees with this epidemiological observation, which may also be due to immunization disparities between

counties. The threshold count at which this observation is reversed (*i.e.*, more contacts in Orange than Los Angeles) is when we simulate either just one additional case in Orange County (n = 36) or one less case in Los Angeles (n = 28) (data not shown). Additionally, during the 2014-15 outbreak, it was observed that although San Francisco County was the most population dense county in the state, the surrounding counties of Alameda, Marin and San Mateo all had confirmed measles cases, and the immunization status among public and private schools was < 90%, however, CA state health department did not confirm any measles cases in San Francisco County. By predicting no contacts within this county, our model agrees with this observation. In using our model to simulate 100 measles cases in San Francisco county and maintaining the outbreak epicenter in Orange County while keeping population density the same, we assessed if the absence of expected counts is an artifact or source of error within our model. We estimated that there were over 500 contacts in San Francisco county. These observations are likely due to underlying correlation dynamics among predictors. These observations support the ability of our model to possibly forecast measles contact trends.

By describing measles contact trends, a further application of our model may be to help in outbreak response prospectively. Our methods could be used by communicable disease and control programs to predict the severity of a measles outbreak, to plan accordingly in allocating appropriate resources at a county-level, and to project the economic impact at the local and state levels. For PHLs, estimating contact count would allow laboratories to anticipate workload in screening suspected measles cases, which would allow appropriate planning for any surge capacity testing as well as anticipating possible laboratory costs. Additionally, our model may be applicable for evaluating the epidemiological impact of other vaccine preventable diseases such as mumps or *Bordetella pertussis*, both of which have had notable outbreaks in recent years (17, 18). However,

our model may need adjustment to account for various transmission factors for these other infectious diseases.

Our study reveals the need for specific local and state guidelines for assessing outbreak costs and planning preparedness. Local public health agencies tasked with assessing the economic impact of a measles outbreak should make every effort to capture all response costs. Our surveys used to identify outbreak costs were created to capture most direct and indirect costs. However, due to our low response rate our estimates did not include costs associated with personnel over-time, immunoglobulin prophylaxis, vaccines and mileage. These expenditures could represent significant costs to local and state public health departments (ref). We recommend that these costs be strongly emphasized in surveys as important determinants of local outbreak costs. Our study reveals the highly unpredictable costs of measles outbreaks at the local level. To lessen local costs, state public health agencies should have a policy in place that allows a state fiscal reserve to be maintained, which could be dispersed to local health departments rapidly in outbreak events for communicable control and prevention activities and laboratory testing. Rapid funding for at-risk counties and health jurisdictions would mitigate outbreak costs in local health departments.

We acknowledge several limitations in our study that we encourage should be corrected for future measles outbreak economic analyses. Our surveys were designed to gather epidemiological and laboratory-based data from public health departments and laboratories but do not account for other response costs not reported by counties (*e.g.*, outreach, education, additional laboratory testing, mileage, immunoglobulin prophylaxis, and personnel overtime). These additional costs mean that the costs reported should be viewed as underestimates/lower bounds on possible costs. Another concern regarding our surveys, which could not be anticipated were concurrent events that occurred while our surveys were deployed. Our surveys were released at

the end of April 2015 with data collection occurring over several months, which at the time, coincided when Zika began to draw attention of most public health agencies throughout CA. Thus, retroactive interference may be present when we captured empirical data. The baseline cost per contact from Los Angeles County used in our economic analyses is based on the payroll code used to document time spent on measles response. (A more detailed explanation of how this analysis was performed is provided in **Appendix A**). It is likely that staff could have forgotten to document the appropriate time worked; however, it is unknown how this bias could have impacted our results. Poisson, quasi-Poisson and negative binomial modeling are used to estimate count data with the latter two directly correcting for over dispersion of input data. Blumberg *et al.* presented a negative binomial model to determine the effective reproductive number from the 2014-15 Multi-state Outbreak during the first-generation measles cases, which was 0.69 using a dispersion parameter of 0.27 (21). The dispersion parameter, *k*, allows for modeling the heterogeneity within datasets. However, when fitted to our observed data, while both the quasi-Poisson and negative binomial models have similar covariate standard errors, these methods have greater standard errors than the classic Poisson model we used for our economic analysis (data not shown). A similar observation was reported showing that negative binomial models created too much over dispersion in some data leading to overestimation of outbreak counts that when compared to other modeling methods, did not match observed data (22). We find that when our model is presented without standard error adjustment, we do not grossly overestimate or underestimate the expected counts in relation what was captured by our surveys and observed data. By accepting over dispersion within our model, we acknowledge that this may be a source of error in our cost estimates but it is unlikely due to the strong correlation between the observed and expected contact counts and because of the validity of our model. Our analysis of laboratory data assumes uniform testing across all CA PHLs.

However, despite the presence of recommended testing algorithms, we find measles testing greatly varies across PHLs dependent staff, availability of lab equipment and funding. While every effort was made to include true costs of testing in every CA PHL, it is unknown how many samples were tested in every PHL. We further do not know how many duplicate or discordant tests were performed and we further do not differentiate the obvious duplicate testing by both local and state. We also do not account for possible measles genotyping that may have been performed at county public health laboratories. Thus, we acknowledge our laboratory testing assumptions may bias our economic analyses.

Despite measles elimination in the US, measles outbreaks continue to occur, and as our study points out, responding to these outbreaks is costly. As previous economic studies have reported, measles outbreaks require concerted efforts across public health agencies and until measles elimination goals are reached in endemic areas around the world or until vaccination rates increase across all social groups domestically, measles outbreaks will continue. As of this writing, the Centers for Disease Control and Prevention (CDC) reports 117 measles cases recorded from 13 states (California, Florida, Kansas, Maine, Maryland, Michigan, Minnesota, Nebraska, New Jersey, New York, Pennsylvania, Utah and Washington) (23). Without change or even a slight decline in national MMR vaccination rates, public health sector costs will continue to accrue because there are pockets in our population where this is a substantial drop in vaccination rates leading to localized risk, even though the overall average rate only dips slightly (24).

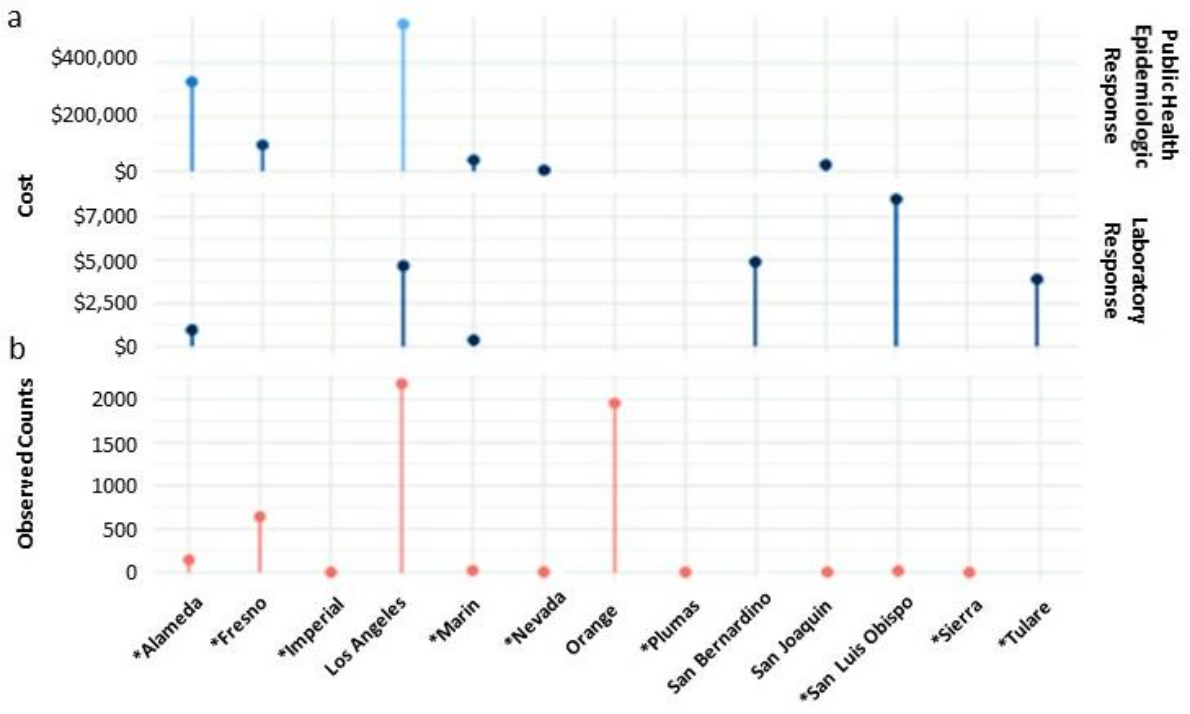

Figure 4.1. Laboratory and Communicable Disease Control survey and follow-up results from 2014-2015 U.S. Multi-state measles outbreak. a) Costs associated from outbreak control and laboratory response. Not shown is City of Berkley reported $25,792.88 in communicable disease response expenses. Costs adjusted to CPI-U (2017 US$). b) Denotes observed susceptible contacts investigated by Disease Control which include CA counties with and without (*) confirmed cases.

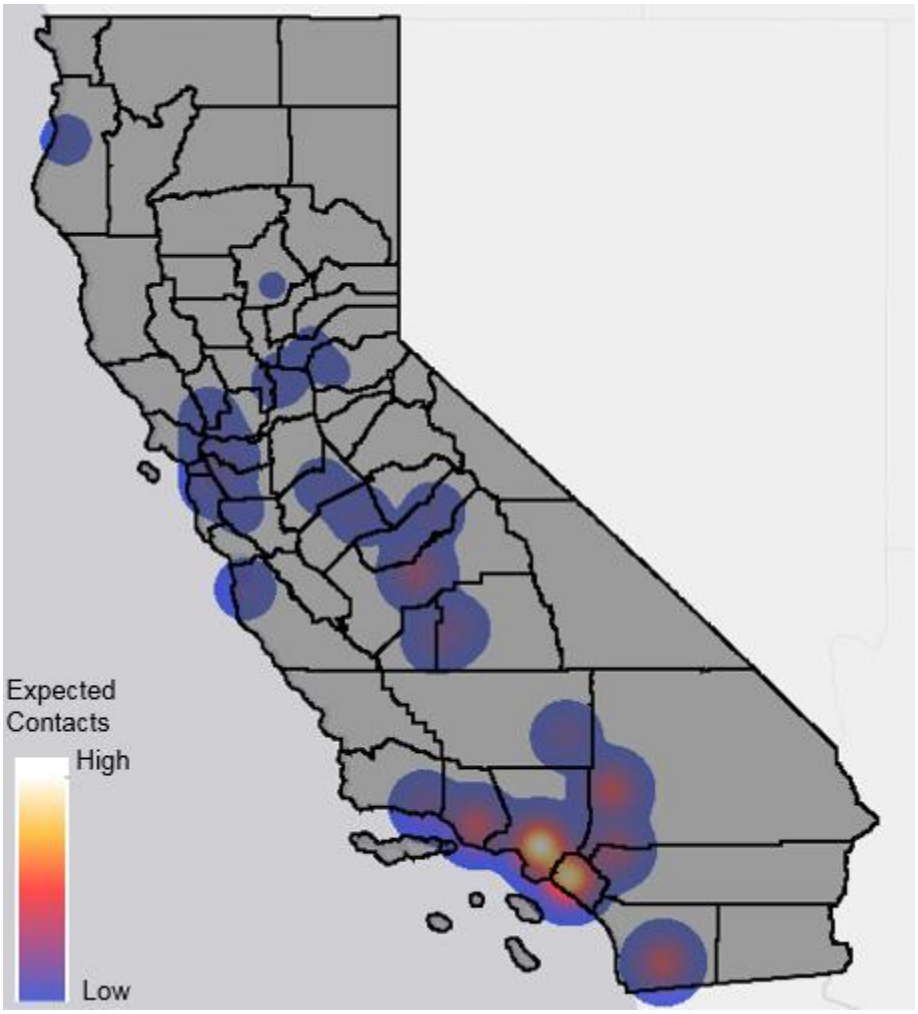

Figure 4.2. **Expected measles contacts in California counties during the 2014-15 U.S. multi-state measles outbreak.** Determined from statistical modeling using covariates x_1: confirmed cases, x_2: distance from outbreak epicenter, and x_3: CA county population density

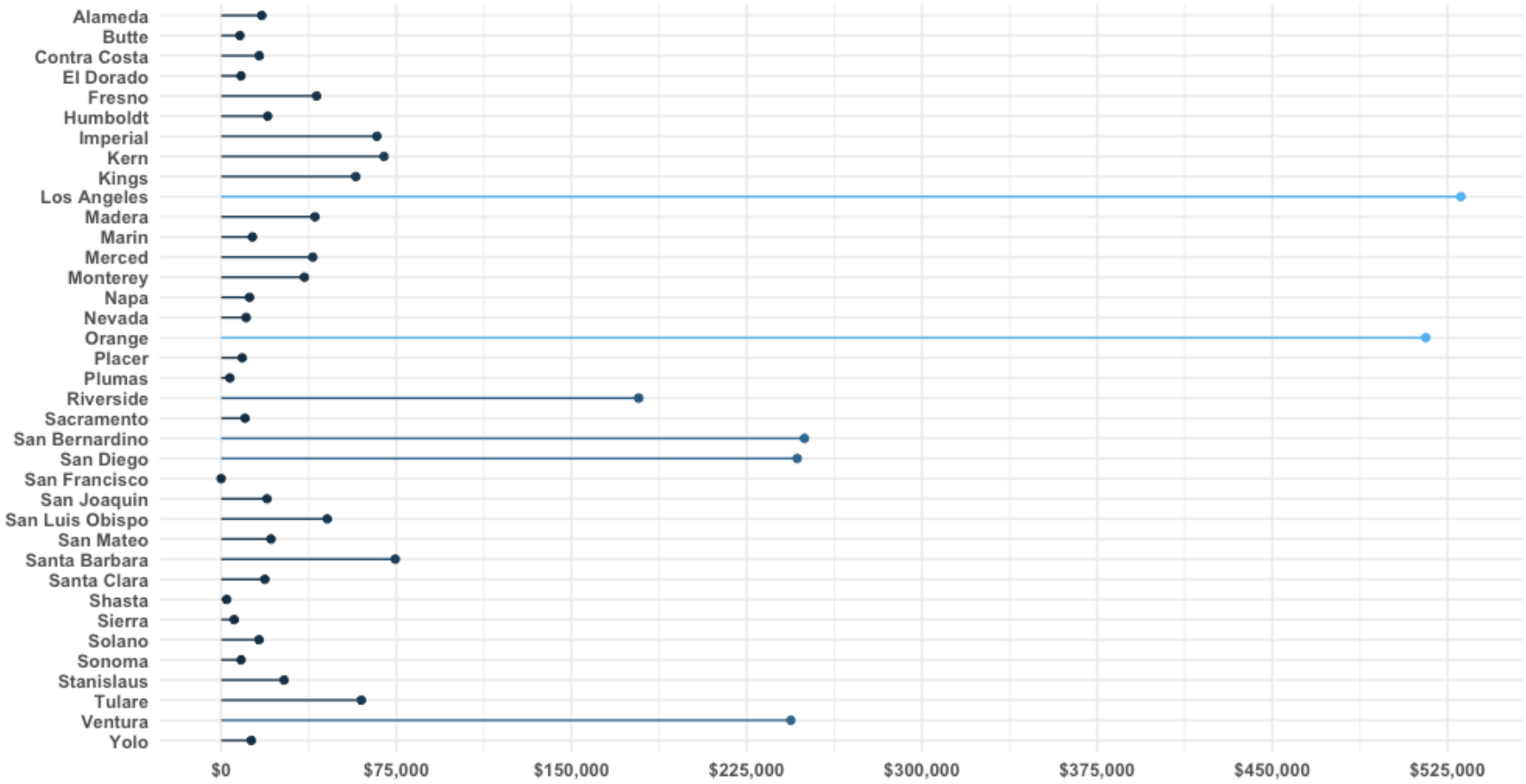

Figure 4.3. **Communicable control programs and public health laboratories estimated total costs from modeled expected epidemiologically-linked measles contacts (n=10,591) and confirmed cases (n=131) in CA counties**. Hospitalization costs are not included.

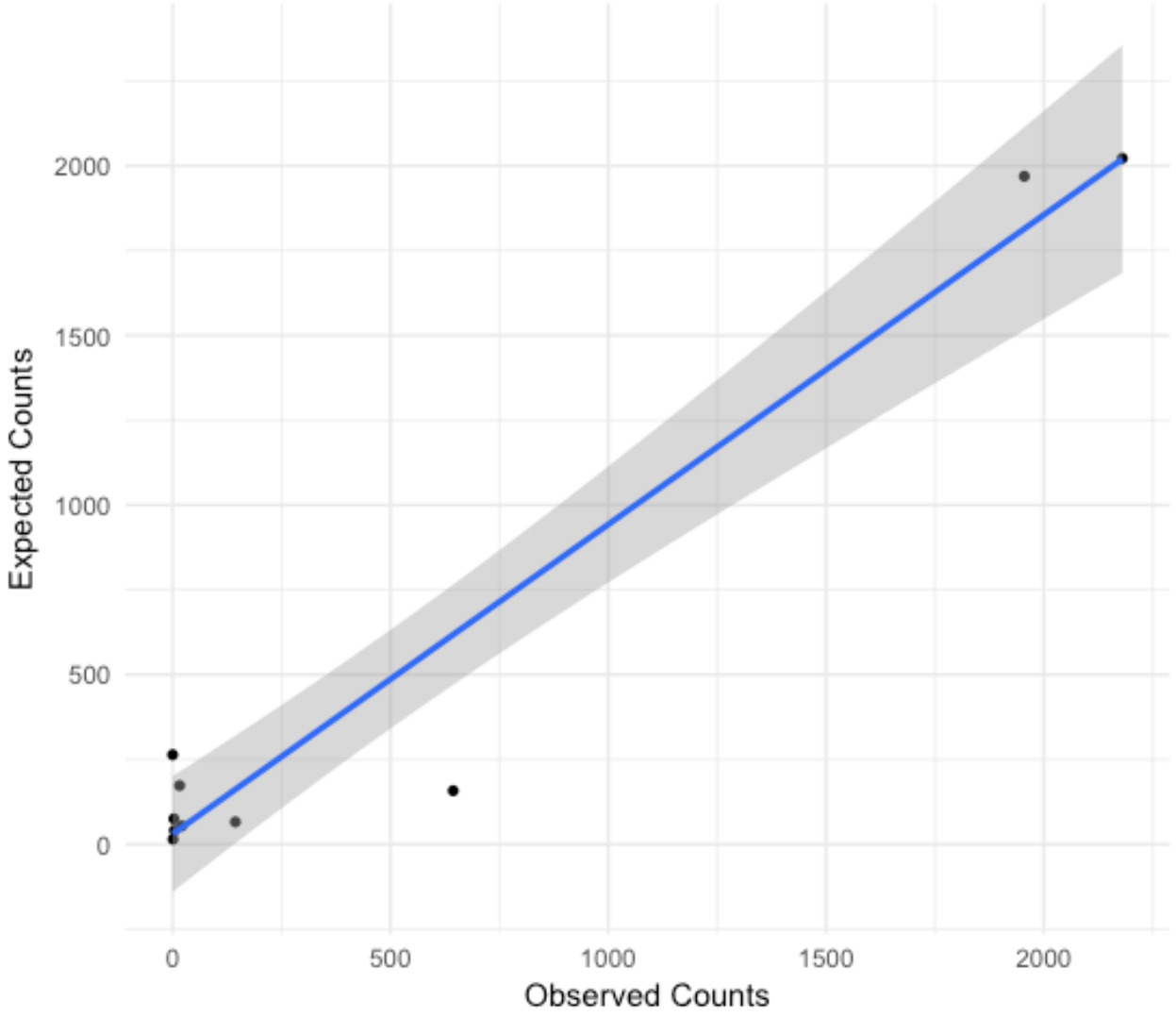

Figure 4.4. Poisson model validation of expected counts versus observed counts (from our survey) with 95% CI. R^2 = 0.945, p <0.0001.

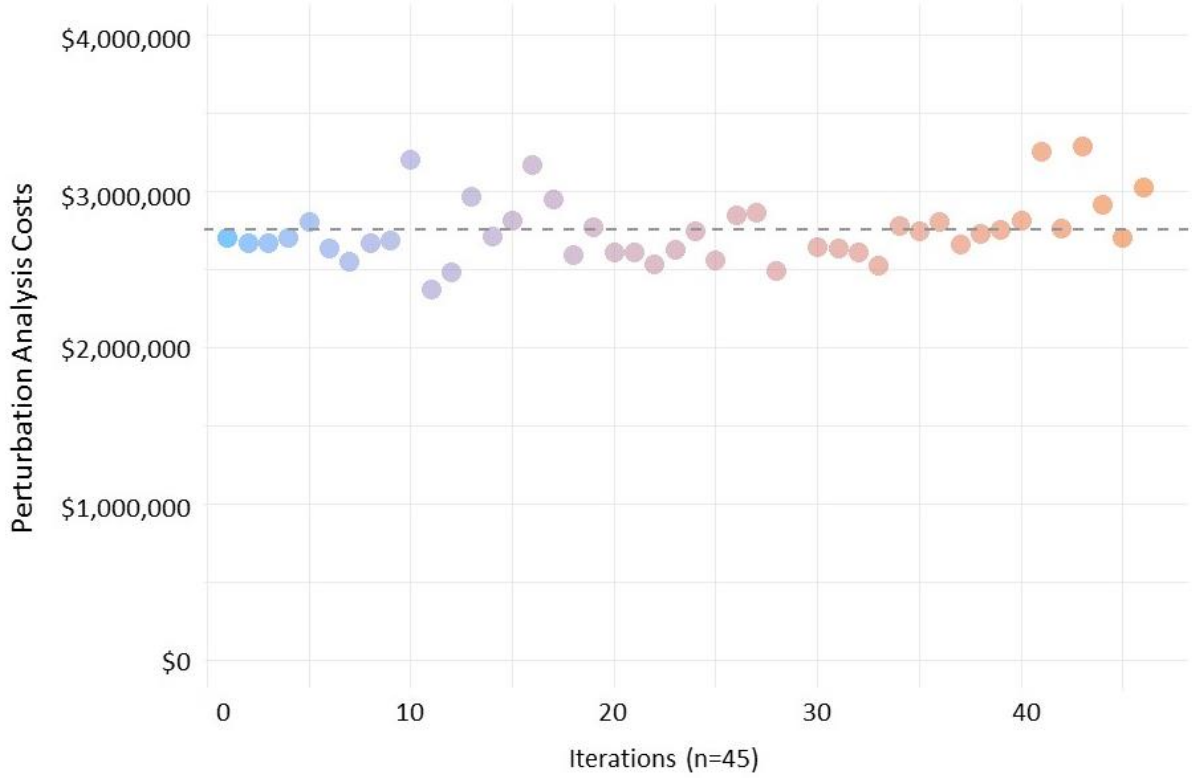

Figure 4.5. Perturbation-based Sensitivity Analysis with 45 iterations. Each iteration introduces small random perturbations simultaneously applied to all covariates and intercept. Dashed line represents the expected cost from our model and standardization approach: $2,756,808.

Table 4.1. Communicable disease response and Laboratory response costs from expected and observed data stratified by county. All costs CPI adjusted (2017 US$).

California County	*Communicable Disease Response*				*Laboratory Response*
Alameda		$330,116			$985
Butte		$7,706			$356
Contra Costa		$15,585			$723
El Dorado		$8,401			$98
Fresno		$96,930			$5,427
Humboldt		$19,202			$733
Imperial		$0			$0
Kern		$68,890			$804
Kings		$56,953			$665
Los Angeles		$543,865			$4,851
Madera		$39,699			$463
Merced		$37,672			$1,551
Monterey		$34,233			$1,403
Napa/Solano/Yolo/Marin*	$41,469	$12,133	$14,776	$12,921	$1,455
Nevada		$1,000			$46
Orange		$488,887			$23,069
Placer		$8,927			$104
Plumas		$250			$3
Riverside		$171,525			$7,221
Sacramento		$9,780			$435
San Bernardino		$239,927			$5,093
San Diego		$234,653			$11,932
San Francisco		$12			$-
San Joaquin		$23,920			$40
San Luis Obispo		$4,001			$8,884
San Mateo		$20,175			$1,168
Santa Barbara		$73,659			$860
Santa Clara		$17,618			$1,109
Shasta		$2,328			$27
Sierra		$0			$0
Sonoma		$8,110			$447
Stanislaus		$26,622			$311
Tulare		$59,356			$4,056
Ventura		$232,932			$10,931
TOTAL		**$2,964,235**			**$95,248**

*Napa-Solano-Yolo-Marin counties have individual public health departments but share the same PHL

Table 4.2. **One-way Sensitivity Analysis with percent difference from baseline cost.**
Baseline costs includes only communicable disease and laboratory expenses only.

Coefficient Name	Baseline Value	Baseline Cost	Coefficient Range in Sensitivity Analysis	Cost Range in Sensitivity Analysis	% Difference from Baseline Cost
Pop. Density	-0.000400	$ 2,756,808	(-0.00048, -0.000319)	($2.47 mil, $3.12 mil)	(-3.17, 3.67)
Cases	0.0926172	$ 2,756,808	(0.0826, 0.103)	($2.36 mil, $3.27 mil)	(-14.25,18.66)
$(Distance/100)^2$	-0.097036	$ 2,756,808	(-0.0861, -0.108)	($2.67 mil, $2.86 mil)	(-10.22, 13.55)

REFERENCES

1. PAHO and WHO (28 September 2016). Region of the Americas is declared free of measles. Retrieved from http://www.paho.org.
2. WHO. Reported measles cases and incidence rates by WHO Member States 2013, 2014 as of 11 February 2015. Retrieved from http://www.who.int/en/.
3. Dayan GH, Ortega-Sanchez IR, LeBaron, *et al.* The cost of containing one case of measles: the economic impact on the public health infrastructure-Iowa, 2004. *Pediatrics* 2005 Jul;116(1):e1-4.
4. Sugerman DE, Barskey AE, Delea MG, *et al.* Measles outbreak in a highly vaccinated population, San Diego, 2008: role of the intentionally undervaccinated. *Pediatrics* 2010 Apr;125(4):747-55
5. Coleman MS, Burke HM, Welstead BL, *et al*. Cost analysis of measles in refugees arriving at Los Angeles International Airport from Malaysia. *Hum Vaccin Immunother*. 2017 May;13(5):1084-1090.
6. Ortega-Sanchez IR, Vijayaraghavan M, Barskey AE, *et al*. The economic burden of sixteen measles outbreaks on United States public health departments in 2011. *Vaccine* 2014 Mar 5:32(11):1311-7.
7. CDPH. California measles surveillance update, April 17, 2015. Retrieved from https://archive.cdph.ca.gov/HealthInfo/discond/Documents/Measles_update_4-17-2015_public.pdf
8. Arom, E. L.A. Orthodox community hit by measles outbreak. Jan 25, 2017 *Jewish Journal*

9. KPCC 89.3 LA County measles outbreak said to spread among Jewish Day Schools Rebecca Plevin. January 23, 2017. http://www.scpr.org/news/2017/01/23/68384/l-a-county-measles-outbreak-said-to-spread-among-j/
10. Hu H, Nigmatulina K, Eckhoff P. The scaling of contact rates with population density for the infectious disease models. *Math Biosci* 2013 Aug;244(2):125-34
11. Tarwater PM, Martin CF. Effects of population density on the spread of disease. *Complexity* July/August 6(6):29-36.
12. Greenwood PE, Gordillo LF. (2009) Stochastic Epidemic Modeling. In *Mathematical and Statistical Estimation Approaches in Epidemiology*. London New York. Springer Science + Business Media B.V. p31-52.
13. Katriel G, Stone L. Pandemic dynamics and the breakdown of herd immunity. *PLoS One* 2010 Mar 15;5(3):e9565.
14. Britton T, House T, Lloyd A, *et al.* Five challenges for stochastic epidemic modes involving involving global transmission. *Epidemics* 2015 Mar;10:54-7.
15. Riley S. Large-scale spatial transmission models of infectious disease. *Science* 2007 Jun1:316(5829).
16. Whitney CG, Fangjun Z, Singelton J, *et al*. Benefits from Immunization During the Vaccines for Children Program Era – United States, 1994-2013. *MMWR* April 15, 2014 63(16);352-355.
17. CDC. (2016, June 1). Mumps Cases and Outbreaks. Retrieved from https://www.cdc.gov/mumps.
18. CDC. (2017, August 7). Pertussis (Whooping Cough): Surveillance and Reported. Retrieved from https://www.cdc.gov/pertussis.

19. Blume S. Anti-vaccination movements and their interpretations. *Soc Sci Med* 2006 Feb;62(3):628-42.
20. SB-277. (2015, June 30). Public health: vaccinations. Retrieved from: https://leginfo.legislature.ca.gov.
21. Blumberg S, Worden L, Enanoria W, *et al.* Assessing Measles Transmission in the United States Following a Large Outbreak in California. *PLoS Curr* 2015 May 7;7.
22. Bishai D, Johns B, Lefevre A, *et al.* Cost effectiveness of measles eradication. WHO Final Report. August 12, 2010.
23. CDC. (2017, August 23). Measles (Rubeola) Measles Cases and Outbreaks. Retrieved from: https://www.cdc.gov/measles.
24. Lo NC, Hoetz PJ. Public Health and Economic Consequences of Vaccine Hesitancy for Measles in the United States. *JAMA Pediatr.* 2017 July 24. Epub ahead of print.
25. CDPH VRDL March 2017. Measles Laboratory Testing Guidance. Retrieved from https://archive.cdph.ca.gov.
26. Hummel KB, Lowe L, Bellini WJ, *et al.* Development of quantitative gene-specific real-time RT-PCR assays for the detection of measles virus in clinical specimens. *J Virol Methods* 2006 Mar; 132(1-2):166-73.

CHAPTER 5

Overarching Conclusions and Recommendations for Future Studies

INTRODUCTION

New approaches in local and state surveillance methods and technologies are required to address the high burden of circulating infectious diseases. Surveillance is a systems effort conducted by a broad array of public health agencies, a key component of which is the local public health laboratory (PHL). PHLs are tasked with laboratory testing to confirm or rule out infection and identifying outbreaks (1). While PHLs have historically relied on culture and serology, molecular methods, such as respiratory multiplex assays have the capacity to greatly mitigate morbidity and mortality due to RTIs by detecting infection early during the course of disease. For surveillance, early detection allows early treatment limiting the further spread of disease. Additionally, multiplex methods meet one of the long term goals of Healthy People 2020 of improving surveillance of infectious diseases (2). However, before these assays enhance surveillance, a key challenge is in how these assays are evaluated for diagnostic performance. Traditionally, diagnostic assays have relied on the use of Sensitivity and Specificity to assess performance, however these assays are often evaluated without recommendations for surveillance purposes. Another challenge is in how the same multiplex assays perform in different populations given variable prevalence of disease. A final challenge for surveillance is in assessing economic impact to evaluate existing disease prevention and control strategies and policies.

To address these challenges, we present useful diagnostic clinical and epidemiological measures that compare and contrast multiplex respiratory assays on a per target basis regardless of assay categorizing the strength of each assay for surveillance in **Chapter 2**. In **Chapter 3**, we

describe how these measures have statistical inference application for environmental surveillance allowing control and prevention resources to be targeted to specific populations in need. In **Chapter 4**, we present a modeling approach for determining the number of epidemiologically-linked contacts during a measles outbreak, which we then used to estimate costs to local and state communicable control and laboratory agencies. Although the work in **Chapters 3** and **4** focuses on case studies in California, the results from this work have broad implications for improving surveillance of RTIs and reducing costs of measles outbreaks in other jurisdictions. Collectively, these studies provide tools that can be used strengthen control and prevention efforts in local and state public health laboratories and agencies.

IMPROVING SURVEILLANCE USING MULTIPLEX RESPIRATORY ASSAYS

Classification of respiratory multiplex assays according to their utility for surveillance can help drive improved performance of these assays. *In vitro* diagnostic assays can be classified by different criteria. For example, the federal criteria set by the FDA categorizes *in vitro* diagnostic assays by complexity: waived, moderate or high complexity (3). By evaluating diagnostic performance through the use of ROC space plots (**Chapter 2**) respiratory multiplex assays can be further classified as either "liberal" or "conservative" assays based on true positive and false positive rates. We describe how these measures are extended to specific analytes as well. This additional classification scheme could serve to identify the best performing assay for surveillance and clinical needs. For manufacturers, this data could be used to market existing diagnostic assays for either surveillance or clinical use or both. Classifying multiplex assays by ROC space plots strengthens the role of communicable disease control programs and public health laboratories by identifying specific assays that should be used for surveillance of select targets.

In **Chapter 2**, we present other diagnostic performance measures, beyond just ROC space plots, that should be considered when evaluating respiratory multiplex assays for surveillance. In comparison studies, Sensitivity (Sen) and Specificity (Spe) are often used to evaluate assay performance (4, 5). While these measures are often touted as having universal application unaffected by disease prevalence, meta-analysis studies have shown the contrary (6). We recommend that the additional diagnostic measures described in **Chapter 2** (*i.e.*, LRs, Diagnostic Odds Ratios and Youden Index) be regularly used for comparison of respiratory multiplex assays to evaluate performance for surveillance purposes. Because disease prevalence may be included in the interpretation of these measures, they provide a powerful tool in comparing and contrasting respiratory multiplex assay performance in different populations. For example, we describe the use of nomograms in a vulnerable population using LRs and disease prevalence (**Chapter 3**), which together provide a robust evaluation of the performance of respiratory multiplex assays in specific populations.

Highlighting diagnostic performance differences between multiplex assays will help manufacturers identify multiplex targets that need improvement. One of the challenges for manufacturers in releasing multiplex assays and subsequently obtaining regulatory approval to market these assays, is addressing the tradeoff between detecting specific viruses and bacteria and minimizing the cross-reactivity with other targets. For instance, low detection of adenovirus respiratory tract infections in immunocompromised patients using the BioFire FilmArray RP has been a concern based on real-world performance, but has since improved with the release of an updated version of the assay (7). To address poor performing targets, the ROC space plots described in **Chapter 2** can provide guidance for manufacturers to identify problematic targets

during assay development. Using the diagnostic performance measures discussed in **Chapter 2**, targets with a low true positive rate or a high false positive rate could be identified and improved prior to release. Additionally, manufacturers could utilize ROC space plots to develop assays specific for surveillance purposes by improving liberal target calls or similarly, develop assays for specific for clinical diagnosis by improving conservative target calls. By developing of surveillance-specific assays separate from clinical-specific assays, manufacturers could target separate diagnostic markets broadening their assay portfolio all the while increasing revenue. There is potential for manufacturers in using and applying additional diagnostic performance measures during assay development and upon assay release.

Respiratory multiplex assays can fulfill critical public health initiatives. Respiratory multiplex assays also have the potential to contribute to the development of Precision Public Health (PPH), an important new public health initiative. PPH is a budding framework that began in 2015 with the introduction of the Precision Medicine Initiative (PMI) under the Obama Administration (8, 9). PMI has the intention of improving health care management through an individualized approach to disease. At the core of PMI is the realization that health status is an integrated relationship between genetic, behavioral and environmental influences that is unique to every patient (10). Similarly, PPH depends on the assumption that disease and disease transmission are not random but reflect population differences due to several characteristics unique to populations. The goal of PPH is to identify populations differences that drive the distribution of disease and disease transmission. A key component of this framework is the efficient use of data to guide interventions at the population level (11), which can be achieved by using "precise" disease tracking to identify disease trends. Respiratory multiplex technologies can fulfill PPH by

providing surveillance programs by precisely identifying etiological characteristics of disease in populations. And because current multiplex assays can be directly linked to laboratory information systems, reporting data can be quickly analyzed and added to existing databases that collectively help in local surveillance efforts.

APPLICATION OF STATISTICAL INFERENCES FOR SURVEILLANCE USING RESPIRATORY MULTIPLEX ASSAYS

Multivariate nomograms for respiratory tract infections surveillance. The work described in Chapters 2 and 3 highlights how multivariate nomograms might offer an improved approach for determining how useful a particular assay is for surveillance purposes. The traditional Fagan nomogram, which is typically used by clinicians to determine which assay has the greatest utility for *clinical diagnosis* of a particular disease, consists of two predictive scales: the prevalence of disease (pre-test probability) and the diagnostic performance of the assay being used. These scales are used to arrive at a post-test probability that is weighted with the values and assumptions of the predictor scales (12). However, within the field of nomography there is also a multivariate approach (13-15). A multivariate nomogram for RTI surveillance could incorporate prevalence of disease, different diagnostic performance measures, and other population characteristics to predict disease burden in a specific population. However, two points should be considered. First, local public health agencies differ in terms of what multiplex respiratory assays are available and how they are used. Seldom do public health laboratories have multiple resources to identify the same pathogen. Thus, likelihood ratios (LRs) would vary across laboratories dependent on what respiratory multiplex assays are available. Additionally, population differences (*e.g.*, demographics, co-morbidities, environmental) will increase or decrease the severity and spread of

disease in a population. In light of these variations, multivariate nomograms could be used to assess population risk in delaying response to an outbreak. Ultimately, this approach would allow decision makers to evaluate control needs based on specific population needs.

Precise population Bayesian analysis. Local public health efforts to control and prevent disease can be improved by population-specific statistical inferences used in Fagan nomograms. Fagan nomograms are visual representations of Bayes' Theorem in which prior and conditional probabilities are used to estimate a posterior probability (12). The application of Bayes' Theorem that we employed in **Chapter 3** uses a population approach instead of the traditional clinical use of Fagan nomograms. Based on extensive literature search, we are the first to use Fagan nomograms in a population specific approach. In calculating the post-test probability of environmental shedding due to a symptomatic resident, we use a baseline prevalence from the same population and not from any other source. This approach in including probabilities from only those populations from which prior and conditional probabilities are calculated minimizes bias or error that may be introduced when using probabilities from other populations. This allows a precise interpretation of disease burden probabilities specific to individual populations, which helps local public health efforts (*i.e.,* control and prevention, public health laboratory algorithms and clinical diagnoses).

THE NEXT DIAGNOSTIC FRONTIER FOR DISEASE SURVEILLANCE

In reviewing and testing the capabilities of multiplexed assays, one cannot help wondering whether it is worth spending time on optimizing this technology for surveillance of infectious disease given how rapidly whole genome sequencing (WGS), particularly with the advent of next

generation sequencing methods, has supplanted multiplexed PCR in the research realm. However, even though WGS has eclipsed multiplexed PCR as a tool in basic research, WGS still poses significant challenges when it comes to routine disease surveillance. Whole genome sequencing has the advantage of providing comprehensive genomic information which can be used to identify genetic variations from a reference strain or de novo assembly of possible new variants. As a result, WGS has many advantages over multiplexed PCR methods for identification, typing, resistance detection, and virulence profiling (16). The utility of WGS for surveillance of influenza and *B. pertussis* has been demonstrated (17, 18). Several next generation sequencing (NGS) platforms are commercially available (*e.g*., HiSeq and Miseq (Illumina), Ion Torrent (Thermo Fisher), Sequel (Pacific Biosciences), 454 GS Junior (Roche), MiniION and PromethION (Oxford Nanopore) that can be used for this type of surveillance work. These platforms differ in sequencing chemistry, data accuracy, application, read length, ease of use, run time, throughput and price. Comparative studies showing differences in analytical sensitivity (19) and accuracy based on single nucleotide polymorphisms (20) can be used to evaluate diagnostic performance. However, due to the vast amounts of data generated, NGS platforms present a considerable regulatory challenge (21). A framework and appropriate performance measures are needed to standardize comparisons allowing these newer technologies to be used for public health surveillance.

As clinical and public health laboratories adopt NGS platforms, for public health surveillance managing NGS data is a present concern. NGS technologies are capable of interfacing directly with laboratory information systems (LIMS), however each sequencing run holds considerable data that is impossible to manage with existing LIMS systems. For example, Illumina's HiSeq-4000 system generates roughly 1300-1500 GB/run using 2 x 150 bp reads (22). Additionally, bioinformatic pipelines are required to tease out, analyze and interpret information

from NGS data. However, for many laboratories the computational expertise necessary to execute pipelines is lacking (23). Future research efforts in public health surveillance should focus on big data analytics and data management.

LOCAL AND STATE EPIDEMIOLOGICAL AND ECONOMIC BURDEN OF RECENT CALIFORNIA MEALSES OUTBREAKS

Epidemiological assessment of outbreaks. The model presented in **Chapter 4** potentially benefits communicable disease programs for controlling and preventing future measles outbreaks as well as other vaccine preventable diseases. This model was formulated and optimized to include several dynamic parameters: confirmed cases, population density and epicenter distance. Our model differs from previous economic measles outbreak models which only included confirmed cases, contacts investigated and duration of outbreak (24). Our model allows for numerating counts of epidemiological-linked measles contacts at a local level. By estimating which counties have greater contact counts, our model reveals local populations where disease burden is greatest helping communicable disease programs allocate resources to counties that have the greatest need. Additionally, while our model bases population density on 2014 census data and the epicenter of the 2014-15 U.S. Multi-state measles outbreak being Orange County, simulations that exchange and substitute these parameters specific to other outbreaks would be plausible. For instance, when applying our model to the 2016-17 measles outbreak, our analysis substitutes population densities to reflect 2016 census estimates as well as setting the outbreak epicenter in Los Angeles County rather than Orange County (**Chapter 4**). Our model may be used for future measles outbreak scenarios to estimate the epidemiological burden of disease at a local level. Likewise, the model may also be useful for estimating the epidemiological burden of other vaccine preventable diseases

that have recently sparked outbreaks across the U.S., such as mumps, *Bordetella pertussis* and bacterial meningitis (25-27).

Economic evaluation of public health laboratory outbreak response. Previous economic analysis studies of past measles outbreaks have given little attention to public health laboratory response costs (24, 28). In **Chapter 4**, we highlight the importance of accounting for public health laboratory costs by reporting the combined public health laboratory costs for the 2014-15 multi-state and the 2016-17 measles outbreaks (~$107,000). These costs, while not as great as the combined local communicable disease control costs (~3.2 mil) are still significant especially for smaller local public health agencies. Our method of calculating local laboratory costs focuses on capturing specific local data such as salary information and type of diagnostic testing performed. The importance of these gathering this local data allows a much more focused cost analysis at the local level. We recommend that future outbreak analysis avoid generalizing laboratory response costs to fully reveal the impact of local outbreak response.

CONCLUSION

The future of surveillance will be marked by the introduction of novel methods and technologies. The challenge for control and prevention efforts in the next decade will be to utilize new strategies for detecting respiratory pathogens as multiplex diagnostic platforms become broader and much more rapid. A primary challenge within public health will be to ensure that these platforms are useful not only for clinical point of care but also for surveillance purposes. NGS systems are powerful diagnostic tools that have only been used to a limited extent thus far for routine public health surveillance efforts, but with established regulatory standards and the

development of bioinformatics pipelines, NGS has the potential to replace most PCR-based methods for surveillance. Additionally, novel epidemiological and economic models are needed for other vaccine preventable diseases to better understand transmission patterns, identify high-risk groups and provide further proof of the effectiveness of vaccines. Novel technologies will drive the future of surveillance.

REFERENCES

1. Association of Public Health Laboratories (2014). The core functions of public health laboratories. Retrieved from: https://www.aphl.org/aboutaphl/publications/documents/aphlcorefunctionsandcapabilities_2014.pdf
2. U.S. Department of Health and Human Services. Immunization and Infectious Diseases. Retrieved from: https://www.healthypeople.gov/2020/topics-objectives/topic/immunization-and-infectious-diseases. Accessed on November 25, 2017.
3. California Department of Public Health. (September 12, 2016). Influenza vaccination among healthcare personnel in California hospitals for the 2015-16 influenza season. Retrieved from: https://www.cdph.ca.gov/Programs/CHCQ/HAI/CDPH%20Document%20Library/HCP_InfluenzaVaccReport2015_2016rev091216ADA.pdf
4. California Department of Public Health. 2014-2015 Immunization status of kindergarten students, California. Retrieved from: https://www.cdph.ca.gov.
5. U.S. Food & Drug Administration. CLIA categorizations. (April 16, 2014). Retrieved from: https://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/IVDRegulatoryAssistance/ucm393229.html.
6. Popowitch EB, O'Neill SS, Miller BB. Comparison of the Biofire FilmArray RP, Genmark eSensor RVP, Luminex xTAG RVPv1, and Luminex xTAG RVP fast multiplex assays for detection of respiratory viruses. *J Clin Microbiol* 2013 May;51(5):1528-1533.
7. Rand KH, Rampersaud H, Houck HJ. Comparison of two multiplex methods for detection of respiratory viruses: FilmArray RP and xTAG RVP. *J Clin Microbiol* 2011 Jul;49(7):2449-2453.

8. Leeflang MMG, Rutjes AWS, Reitsma JB, *et al.* Variation of a test's sensitivity and specificity with disease prevalence. *CMAJ* 2013 Aug6:185(11):E537-E544.
9. Doern CD, Lacey D, Huang R, *et al*. Evaluation and implementation of FilmArray version 1.7 for improved detection of adenovirus respiratory tract infection. *J Clin Microbiol* 2013 Dec; 51(12):4036-4039.
10. Holst L. The Precision Medicine Initiative: data-driven treatments as unique as your own body. (January 30, 2015) Retrieved from: https://obamawhitehouse.archives.gov/blog/2015/01/30/precision-medicine-initiative-data-driven-treatments-unique-your-own-body.
11. Dowell SF, Blazes D, Desmond-Hellmann S. Comment: Four steps to precision public health. *Nature* 540:7632; 189-191.
12. Fagan TJ. Letter: Nomogram for Bayes theorem. *N Engl J Med* 1975 Jul 31;293(5):257.
13. Moons KG, van ES GA, Deckers JW, *et al*. Limitations of sensitivity, specificity, likelihood ratio, and bayes' theorem in assessing diagnostic probabilities: a clinical example. *Epidemiology* 1997 Jan; 8(1):12-17.
14. Chen JJ, Pugach J, Patel M, *et al.* The renal length nomogram: a multivariable approach. *J Urol* 2002 Nov; 168(5):2149-2152.
15. Stephenson AJ, Nguyen CT, Kattan MW. Nomograms in the Management of Prostate Cancer. In *Recent Advances in Prostate Cancer: Basic Science Discoveries and Clinical Advances.* (2011) Singapore: World Scientific.
16. Kwong JC, McCallum N, Sintchenko V, *et al.* Whole genome sequencing in clinical and public health microbiology. *Pathology* 2015 Apr; 47(3):199-210.

17. McGinnis J, Laplante J, Shudt M, *et al*. Next generation sequencing for whole genome analysis and surveillance of influenza A viruses. *J Clin Virol* 2016 Jun;79-44-50.
18. Marchand-Austin A, Tsang RSW, Guthrie JL, *et al.* Short-read whole-genome sequencing for laboratory-based surveillance of *Bordetella pertussis*. *J Clin Microbiol* 2017 May; 55(5):1446-1453.
19. Frey KG, Herrera-Galeano JE, Redden CL, *et al*. Comparison of three next-generation sequencing platforms for metagenomics sequencing and identification of pathogens in blood. *BMC Genomics* 2014 Feb 4; 15:96.
20. Quail MA, Smith M, Coupland P, *et al.* A tale of three next generation sequencing platforms: comparison of Ion Torrent, Pacific BioSciences and Illumina MiSeq sequencers. *BMC Genomics* 2012 Jul 24;13:341.
21. Department of Health and Human Services, Food and Drug Administration. December 2014) Optimizing FDA's regulatory oversight of next generation sequencing diagnostic tests-preliminary discussion paper. Retrieved from: https://www.fda.gov/downloads/MedicalDevices/NewsEvents/WorkshopsConferences/UCM427869.pdf.
22. Illumina, Inc. (2018). HiSeq 3000/HiSeq 4000 System quality and performance. Retrieved from: https://www.illumina.com/systems/sequencing-platforms/hiseq-3000-4000/specifications.html. Accessed on January 3, 2018.
23. Oakeson KF, Wagner JM, Mendenhall M, *et al.* Bioinformatic analyses of whole-genome sequence data in a public health laboratory. *Emerg Infect Dis.* 2017 Sep;23(9):1441-1445.

24. Ortega-Sanchez IR, Vijayaraghavan M, Barskey AE, *et al.* The economic burden of sixteen measles outbreaks on United States public health departments in 2011. *Vaccine.* 2014 Mar 5;32(11):1311-1317.
25. CDC. Mumps cases and outbreaks. (September 20, 2017) Retrieved from: https://www.cdc.gov/mumps/outbreaks.html.
26. CDC. Pertussis surveillance reports. (August 7, 2017) Retrieved from: https://www.cdc.gov/pertussis/surv-reporting.html.
27. CDC. Meningococcal outbreaks. (May 4, 2017) Retrieved from: https://www.cdc.gov/meningococcal/outbreaks/index.html.
28. Sugerman DE, Barskey AE, Delea MG, *et al*. Measles outbreak in a highly vaccinated population, San Diego, 2008: role of the intentionally undervaccinated. *Pediatrics* 2010 Apr; 125(4):747-55.

APPENDIX A

Supporting Information for Chapter 4

DETAILED METHODS AND QUESTIONNAIRES USED TO GATHER DATA FROM LOCAL HEALTH DEPARTMENTS IN CALIFORNIA ON MEASLES OUTBREAK COSTS

The public health epidemiologic and PHL questionnaires were made electronically available accessible via web link for 18 months beginning in April 2015. Aggressive follow-up was conducted among non-respondents. For the disease controller questionnaire, we asked about the number of personnel, wages, hours spent per full time employee, fringe benefits, overhead costs and when reported the cost of post-exposure prophylaxis. We additionally asked disease controllers to disclose how many contacts were investigated in their county and if any case was recommended for self-isolation along with the appropriate cost. For both disease controller and laboratory questionnaires we asked if, as a result of the outbreak, there was any delay in other projects, surveillance or disease control efforts. For the laboratory questionnaire, respondents were asked about number of personnel, wages, hours spent per full-time employee (FTE), fringe benefits, overhead rate, courier cost and the general cost of each laboratory test used for confirming a susceptible case or exposure (*i.e.*, PCR, IgG EIA, IgM IFA, etc.). The abbreviated surveys sent to CDPH asked questions regarding outbreak response time including number of weeks spent, average hours per week, overtime as well as salary information. We assume a 30% benefit rate to account for additional costs associated with salary. state laboratory questions asked cost and quantity of the following diagnostic tests: PCR, IgG EIA, IgG IFA, IgM EIA, IgM IFA, culture and genotyping. We also asked questions regarding send-outs and shipping costs.

Economic Impact of the U.S. Multi-state Measles Outbreak 2014-2015 in California

Survey Informed Consent

Purpose: This survey is part of an independent research study conducted by the Los Angeles County Public Health Laboratory and UCLA Fielding School of Public Health. The purpose of this study is to understand the economic impact of the 2014-2015 U.S. Multi-state Measles Outbreak in public health jurisdictions across California. This survey will take approximately 20-30 minutes.

Security Information: All information collected in this survey will be kept in a secure manner. Your IP address will NOT be retained.

Decision to quit at any time: Participation is voluntary; you are free to withdraw from this survey at any time. If at any point you do not want to continue, you can simply leave the website. If you do not click on the "submit" button at the end of the survey, your answers and participation will not be recorded.

How the findings will be used: Your responses will be pivotal in understanding the economic burden of this outbreak to the California public health response infrastructure. Data will be shared with the California Department of Public Health and the Centers for Disease Control and Prevention.

Agreement: By clicking the "I AGREE TO PARTICIPATE" button and beginning the survey, you acknowledge that you have read this information and agree to participate in this survey with the knowledge that you are free to withdraw your participation at any time without penalty.

* Thank you for your participation in our survey, we appreciate your time and feedback.

- ◯ I AGREE TO PARTICIPATE IN THIS SURVEY
- ◯ I DO NOT WISH TO PARTICIPATE IN THIS SURVEY

Economic Impact of the U.S. Multi-state Measles Outbreak 2014-2015 in California

Survey Responder information

* Responder Information

What is your name?	
What is your title?	
What is your health jurisdiction? (eg Los Angeles County, San Mateo County, etc)	
What is your department?	

Economic Impact of the U.S. Multi-state Measles Outbreak 2014-2015 in California

Responder Identifier

* Please identify the title that most closely matches your position:

- ◯ Lab Director
- ◯ Lab Manager
- ◯ Communicable Disease Controller
- ◯ Staff Epidemiologist

Economic Impact of the U.S. Multi-state Measles Outbreak 2014-2015 in California

End survey

Thank you for your response. Please comment as to why you chose not to participate.

Comment

Economic Impact of the U.S. Multi-state Measles Outbreak 2014-2015 in California

End Survey

Thank you for your response. Please feel free to leave any comments.

Comment

* May we contact you for additional information if needed?

- ◯ Yes, please contact me
- ◯ No

If yes, please leave your phone number and email:

Economic Impact of the U.S. Multi-state Measles Outbreak 2014-2015 in California

Laboratory Survey (page 1 of 8)

Please answer the following in regards to the U.S. Multi-state Measles outbreak that occurred from December 2014 to April 2015.

How many full-time equivalent (FTE) **laboratory workers** were involved in responding to the outbreak?

Approximately how many total **regular work hours** were spent (do not include overtime, but do include any time spent by the **individuals included in the number above** in responding to the outbreak: laboratory testing and investigation, laboratory meetings, phone calls, webinars)?

Approximately how many **over-time hours** were spent by laboratory staff?

What was the total personnel cost? (ie include **only** base salaries)

What is your county Employee Benefits (EB) rate? (eg 28%)

Economic Impact of the U.S. Multi-state Measles Outbreak 2014-2015 in California

Laboratory Survey (page 2 of 8)

Were there any additional costs incurred due to laboratory response? (such as increased security, shippers, courier cost, instrument purchase, reagent purchase, additional staffing/temp staff, or stand-by time)

- ◯ Yes
- ◯ No
- ◯ Unsure

If answered yes to the previous question, what were the additional incured costs?

Increased security:	
Shippers cost:Stand by time cost:	
Courier cost:	
Purchase instruments cost:	
Overall additional Measles reagents cost:	
Part-time/ extra help staff cost:	
Stand by time cost:	
Other cost (please define):	

Economic Impact of the U.S. Multi-state Measles Outbreak 2014-2015 in California

Laboratory Survey (3 of 8)

Please give your general overhead rate (ie indirect costs) for your department.

How many of **each** of the following methods to test/confirm measles specimens were performed during this outbreak?

PCR	
Serology, IgG, EIA	
Serology, IgG, IFA	
Serology, IgM, IFA	
Serology, IgM, EIA	
Culture	
Other test	

Economic Impact of the U.S. Multi-state Measles Outbreak 2014-2015 in California

Laboratory Testing Cost per Sample (page 4 of 8)

Below, please estimate the per-unit cost of testing for each assay. NOTE: Include only the price of the reagent and test, not lab labor costs.
***Please type "N/A" if Not Applicable**

What was the laboratory testing cost for PCR? (includes price of reagents and tests used)

What was the laboratory testing cost for Serology, **IgG EIA**? (includes price of reagents and tests used)

What was the laboratory testing cost for Serology, **IgG IFA**? (includes price of reagents and tests used)

What was the laboratory testing cost for Serology, **IgM IFA**? (includes price of reagents and tests used)

What was the laboratory testing cost for Serology, **IgM EIA**? (includes price of reagents and tests used)

What was the laboratory testing cost for **Culture**? (includes price of reagents and tests used)

Economic Impact of the U.S. Multi-state Measles Outbreak 2014-2015 in California

Laboratory Survey (page 5 of 8)

Were there any discordant testing results? (Public Health lab versus State lab results)

- ◯ Yes
- ◯ No

If yes, please indicate how many

What was the overall shipping cost per sample? (cost of FedEx, GSO, etc. only and not shippers)

What was the overall courier cost per sample pick up if provided to clinical sites?

Economic Impact of the U.S. Multi-state Measles Outbreak 2014-2015 in California

Laboratory Survey (6 of 8)

As a result of the outbreak, was there any delay in other laboratory projects?

- ◯ Yes
- ◯ No

If yes, please specify what project(s) or testing were delayed

Due to the outbreak, was Incident Command System (ICS) mode activated in the laboratory or health department?

- ◯ Yes
- ◯ No
- ◯ Unsure

Economic Impact of the U.S. Multi-state Measles Outbreak 2014-2015 in California

Laboratory Survey (page 7 of 8)

In your opinion, what would have helped improve your laboratory's response to the outbreak?

- [ ] More staff
- [ ] Additional testing methods available for diagnostic workflow efficiencies
- [ ] More communication with epidemiologists
- [ ] Real-time dashboard results notifications
- [ ] Our response would not have benefited from any of the above

Other (please specify)

Economic Impact of the U.S. Multi-state Measles Outbreak 2014-2015 in California

Laboratory Survey (8 of 8)

In your opinion, please briefly comment as to some of the challenges that were experienced by your laboratory?

In your opinion, please briefly comment as to some of the lessons learned.

Economic Impact of the U.S. Multi-state Measles Outbreak 2014-2015 in California

Public Health Department Epidemiologist Survey (1 of 6)

Please answer the following in regards to the U.S. Multi-state Measles outbreak that occurred from December 2014 to April 2015.

How many full-time equivalent (FTE) workers **in your department** were involved in responding to the outbreak?

Approximately how many **total regular work hours by the individuals included in the number above** were spent in contact tracing/investigation? (do not include over-time)

Approximately how many **total regular work hours** were spent in analysis of epidemiological data for this outbreak? (do not include over-time)

Approximately how many **over-time** hours were spent in both contact tracing/investigation and analysis?

What was the total personnel cost? (ie include**only** base salaries)

What is your county Employee Benefits (EB) rate? (eg 28%)

Economic Impact of the U.S. Multi-state Measles Outbreak 2014-2015 in California

Public Health Department Epidemiologist Survey (2 of 6)

How many of the following staff type were involved in the outbreak response?

Communicable Disease Controller	
Medical Epidemiologist	
Infection Control Staff	
Epidemiology Analyst	
Public Health Nurse	
Clerical/Admin Support	
Student Worker/Intern	
Other epidemiologist staff	

How many contacts were investigated within your county?

How many confirmed cases occurred within your county?

Due to the outbreak, was Incident Command Structure (ICS) mode activated in your department or program?

- ◯ Yes
- ◯ No
- ◯ Unsure

Economic Impact of the U.S. Multi-state Measles Outbreak 2014-2015 in California

Public Health Department Epidemiologist Survey (3 of 6)

For exposures occurring in a hospital health care setting did you receive hospital epidemiologist involved assistance?

- ◯ Yes
- ◯ No

If yes, please list the hospitals involved:

Was any case recommended for self-isolation?

- ◯ Yes
- ◯ No

How many cases were recommended for self-isolation?

Economic Impact of the U.S. Multi-state Measles Outbreak 2014-2015 in California

Public Health Department Epidemiologist Survey (4 of 6)

Were there any overhead costs? (such as increased security, shippers, courier costs, additional staffing/temp staff and stand-by time)

- ◯ Yes
- ◯ No

If yes, please list

If applicable, how many immunoglobulin (IG) post-exposure prophylaxis treatments were given during the measles outbreak?

If applicable, what were the department costs in providing immunoglobulin to the**public**?

If applicable, how many measles vaccinations were given to the**public**?

If applicable, what were the department costs in providing measles vaccination to the**public**?

Economic Impact of the U.S. Multi-state Measles Outbreak 2014-2015 in California

Public Health Department Epidemiologist Survey (5 of 6)

What is the **adult** MMR vaccination coverage for your county (≥19 years old)?

What is the **childhood** MMR/MMRV vaccination coverage for your county (≤18 years old)

As a result of the outbreak, was there any delay in surveillance projects or disease control efforts?

- ◯ Yes
- ◯ No

If yes, please specify what project(s) or surveillance were/was delayed

Economic Impact of the U.S. Multi-state Measles Outbreak 2014-2015 in California

Public Health Department Epidemiologist Survey (6 of 6)

In your opinion, please briefly comment as to some of the challenges that were experienced by your department during the outbreak?

In your opinion, please briefly comment as to some of the lessons learned.

Abbreviated LABORATORY Survey

Economic Impact of the U.S. Multi-state Measles Outbreak 2014-2015 in California

Q1. What is your name: _____

Q2. What is your title: _____

Q3. What is your department: _____

Q4. What is your health jurisdiction/county: _____

Staff Questions:

Q5. How many of each of the following staff type were involved in the outbreak response?

	QUANT.	**Approx hrs spent on response/day***
Microbiologist	_____	_____
Laboratory Assistant	_____	_____
Clerical/Admin Support	_____	_____
Manager	_____	_____
Student Worker/ Intern	_____	_____
Other (please specify) _____	_____	_____
Other (please specify) _____	_____	_____

*Outbreak lasted from Dec '14- Apr '15.

Q6. What is your county Employee Benefits (EB) rate? (eg 28%): _____

Q7. Please give your general overhead rate (ie indirect costs) for your department: _____

Laboratory Testing Questions:

Q8. How many of each of the follow methods to test/confirm measles specimens were performed during this outbreak?

	QUANT.	**COST/test***
PCR	_____	$_____
Serology, IgG, EIA	_____	$_____
Serology, IgG, IFA	_____	$_____
Serology, IgM, IFA	_____	$_____
Serology, IgM, EIA	_____	$_____
Culture	_____	$_____
Other (please specify) _____		$_____
Sendouts**	_____	

*Include only the price of the reagent and test, not lab labor costs

**How many samples were sent out to the State laboratory or other laboratory for confirmatory or add'tl testing

Q9. How many confirmed cases occurred within your county/jurisdiction? _____

Q10. Please specify and list any miscellaneous costs: _____

CDPH State Measles Follow-up Survey

Disney Outbreak Questions

Occupation By-Category*	QTY*	Avg Salary (without benefits)**	Approximate Range of Days Involved in Response
Microbiologist	8		
Laboratory Assistant	4		
Clerical/Admin Support	2		
Manager	5		
Student Worker/Intern	2		
Lab-Epi Liaison	4		
LIMS Support	2		
Other (please specify):_____			
Other (please specify):_____			
Other (please specify):_____			

*These categories and quantities of each are from previous survey responses from VRDL (2015)
**if Salary plus benefits are available please provide instead with an "*" to signify salary and benefit quantity

Total Number of measles contacts in the state:_____	

Jewish Orthodox Measles

Total Measles Cases	
Total Measles Contacts	
Outbreak Duration (MMDDYY-MMDDYY)	

General Comments:

DETAILED METHODS FOR ANALYSIS OF LOS ANGELES COUNTY DATA FOR MEASLES OUTBREAK

During the beginning of the outbreak, all staff of the Los Angeles Department of Public Health was allowed to input a special payroll code in their timesheets to account for any time spent in responding to the 2014 measles outbreak. Payroll and finance data was used as a baseline to approximate the cost per contact in Los Angeles county ($250). In total, our baseline cost per contact included direct (salary and fringe benefits) and indirect expenses (operation and maintenance costs for facilities and equipment) from several internal subdivisions. Costs associated with personnel over-time, immunoglobulin prophylaxis, and mileage were not included. A summary of expenses as defined by timesheet and purchasing codes was requested from our Finance Management Department within the Los Angeles Public Health Department. From this summary expense we subtracted all costs associated with the Los Angeles County Public Health Laboratories which gave us a total cost of the outbreak within LAC as $545,000, or $250 per contact among the 2180 contacts (confirmed by our Immunization Program) within our county.

DETAILED METHODS FOR STANDARDIZATION OF PUBLIC HEALTH LABORATORY DATA FOR MEASLES OUTBREAK

Based on national and the state guidelines, it is recommended that when receiving suspected measles samples, local public health labs should perform measles IgG/IgM serological testing and PCR (where available) depending on specimen type. However, based on survey results it was clear that some CA PHLs do not perform measles testing and instead send out specimens to the state laboratory for testing and confirmation (25). While serology testing is performed for determining exposure. the preferred method for confirming an acute measles case is PCR (26). Additionally,

discordant or follow-up testing is also performed depending on initial laboratory results. This testing algorithm presents a considerable challenge in assessing the overall laboratory response to any outbreak that affects more than one county. Thus, a standardized approach was taken as described in the text.