Rapid Point-of-Care Testing for
Measles Immunity

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ABSTRACT

Measles is a contagious, vaccine-preventable disease that continues to be the leading cause of death in children younger than the age of 5 years. While the introduction of the Measles, Mumps, and Rubella vaccine (MMR) has significantly decreased morbidity and mortality rates worldwide, vaccine coverage is highly variable across global regions. Current diagnostic methods rely on enzyme immunoassays (EIA) to detect IgM or IgG Abs in serum. Commercially available Diamedix Immunosimplicity® Measles IgG test kit has been shown to have 91.1% sensitivity and 93.8% specificity, with a positive predictive value of 88.7% and a negative predictive value of 90.9% on the basis of a PRN titer of 120. There is an increasing need for rapid screening for measles specific immunity in outbreak settings. This study aims to develop a rapid molecular diagnostic assay to detect IgG reactive to three individual measles virus (MeV) proteins.

Measles virus (MeV) genes were subcloned into the pJFT7_nGST vector to generate N-terminal GST fusion proteins. Single MeV cistrons were expressed using in vitro transcription/translation (IVTT) with human cell lysate. Expression of GST-tagged proteins was measured with mouse anti-GST mAb and sheep anti-mouse IgG. Relative light units (RLUs) as luminescence was measured. Antibodies to MeV antigens were measured in 40 serum samples from healthy subjects.

Protein expression of three MeV genes of interest was measured in comparison with vector control and statistical significance was determined using the Student's t-test (p<0.05). N expressed at the highest level with an average RLU value of 3.01 x 10^9 (p<0.001) and all proteins were expressed at least 50% greater than vector control (4.56 x 10^6 RLU). 36/40 serum samples had IgG to N (Ag:GST ratio>1.21), F (Ag:GST ratio>1.92), or H (Ag:GST ratio> 1.23).

These data indicate that the in vitro expression of MeV antigens, N, F, and H, were markedly improved by subcloning into pJFT7_nGST vector to generate N-terminal GST fusion proteins. The expression of single MeV genes N, F and H, are suitable antigens for serologic capture analysis of measles-specific antibodies. These preliminary data can be used to design a more intensive study to explore the possibilities of using these MeV antigens as a diagnostic marker.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>LIST OF FIGURES</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>iii</td>
</tr>
</tbody>
</table>

## CHAPTER

1. INTRODUCTION .......................................................................................................................... 1

2. MATERIALS AND METHODS ........................................................................................................ 7
   2.1 Primer Design, PCR, and DNA Purification ........................................................................ 7
   2.2 Subcloning Into pDONR221 Vector and pJFT7_nGST Vector ........................................... 7
   2.3 Detection of MeV Abs Using Expressed Antigens on the Rapid Antigenic Protein in situ Display (RAPID) ELISA Platform .................................................................................. 7
   2.5 Detection of MeV-specific Abs using Diamedix Immunosimplicity® Measles IgG Test Kit .......................................................................................................................... 8

3. RESULTS .................................................................................................................................... 9
   3.1 Rapid Antigenic Protein in situ Display ELISA for Protein Expression ............................ 9
   3.2 Frequency distribution of serum samples screened using GST control ........................... 10
   3.3 Detection of MeV-specific Abs in Patient Serum using Rapid Antigenic Protein in situ Display ELISA ............................................................................................................ 11
   3.4 Detection of MeV-specific Abs in Patient Serum using Diamedix Immunosimplicity® Measles IgG test kit ................................................................................................. 13

4. DISCUSSION .............................................................................................................................. 15

REFERENCES ............................................................................................................................... 20
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Diagram of Measles Virus Antigenome</td>
<td>1</td>
</tr>
<tr>
<td>2. Protein Expression of N, F, and H antigens</td>
<td>9</td>
</tr>
<tr>
<td>3. Frequency Distribution of Average RLU Values of Serum Samples Screened using GST Control</td>
<td>10</td>
</tr>
<tr>
<td>4. Average GST Ratios for Normal Serum Samples Across the $N$, $F$, and $H$ Antigens</td>
<td>12</td>
</tr>
<tr>
<td>5. EU/ml (ELISA Units/ml) of Serum Samples Screened Using the Diamedix Immunosimplicity® Measles IgG Test Kit</td>
<td>14</td>
</tr>
</tbody>
</table>
1. INTRODUCTION

The Measles virus (MeV) is a contagious pathogenic virus that is associated with complications such as encephalitis and pneumonia (1). The measles, mumps, rubella vaccine (MMR vaccine) was introduced over fifty years ago and has led to an overall decline of measles incidence, yet MeV is still a major cause of death in children in developing countries (2). Even with global vaccination rates over 84% (3), there have been numerous outbreaks in recent years (2) due to the contagious nature of MeV. In 2014, there were over 16 deaths every hour due to MeV infections, with a total of 145,000 deaths globally (4). Worldwide, over 21.5 million children were not vaccinated against MeV in 2013 (5). In 2014 alone the United States had 23 MeV outbreaks, and in 2011, 30 countries reported an increase in measles incidence (6). In 2015, a recent measles outbreak in a theme park had spread to over seven different states, encompassing about 113 cases. During the first 6-month period of 2016, there were over 100,000 suspected cases of MeV worldwide, with only about 14,000 of those cases being laboratory confirmed (7).

MeV is a member of the Morbivirus genus in the Paramyxoviridae family (8). It is enveloped, spherical, non-segmented and contains single stranded, negative sense RNA. The MeV antigenome includes eight proteins (Figure 1) with six ORFs encoded by approximately 16,000 nucleotides (9).

Figure 1. Diagram of Measles Virus antigenome. Measles virus contains eight proteins encoded by 16,000 nucleotides (9).

Three MeV genes of interest for this study are: nucleoprotein, fusion protein, and hemagglutinin (N, F, and H). Infection by MeV is initiated on vulnerable cells by the components
present on the viral envelope. Transmembrane proteins, \( H \) and \( F \), are found on the viral envelope (8). The antibodies (Abs) for these transmembrane proteins are able to deactivate the infectious activity of MeV (10). The genomic RNA is surrounded by a nucleocapsid, which forms the ribonucleocapsid (10). \( F \) fuses the viral envelope of MeV with the cellular plasma membrane of the cell to be infected (10). Infected cells express \( H \) and \( F \) on its cell surface. This allows for receptor binding by \( H \) to the surfaces of nearby cells, triggering the fusing activity of the \( F \). Eventually, this results in the formation of giant cells (10). \( N \) aids in packing the MeV genome into a ribonucleoprotein complex (11). MeV is spread through direct contact with respiratory secretions within droplets from infected individuals (12). Droplets that contain MeV can survive in the environment for several hours (12).

Measles outbreaks have been linked to the decrease in vaccine uptake over the years (13). Numbers of MeV cases in the United States has been steadily increasing since 2010, with a peak in the number of cases in 2014 (6) when a large outbreak occurred in an unvaccinated Amish community that included over 383 cases. The pattern of decreasing vaccine uptake can lead to the re-establishment of endemic measles along with an increase in measles mortality rates. The primary reason for increased number of measles cases and transmission of measles is due to failure to vaccinate populations which are susceptible, especially those in Europe. In 2000, the United States had achieved complete elimination of measles due to high vaccination rates (14) (15). But, the potential risk of outbreaks still exist as importation of MeV from foreign countries still occurs (16) and MeV is brought into the United States by unvaccinated travelers. The vaccination rate for the MMR vaccine in 2013 was below 91% in over 17 states (17).

MeV increases susceptibility to other diseases and about 30% of MeV cases have complications (18), including diarrhea, otitis media, pneumonia, encephalitis, and seizures. The best method for prevention of MeV is the measles vaccine, which provides active immunization against MeV (8). This vaccine can be administered to children over the age of 9 months (19). The MMR vaccine is an attenuated strain of MeV taken from the wild type virus. The vaccine is not linked with immunosuppression, but it does provide long-term immunity (10). MeV is still considered endemic worldwide and rapid confirmation of MeV is imperative in outbreak settings.
One dose of the MMR vaccine is 95% effective at preventing MeV, and two doses of MMR vaccine are about 99% effective MeV (20). Between 2-5% do not develop MeV immunity after the first dose of the vaccine (20). The first dose of MMR vaccine is given around 12 months, after loss of passive protection from breast milk, and the second dose is administered at least 28 days after the first dose (21) up until 4-6 years of age. People who have received two doses of the MMR vaccine are considered to have life-long immunity and do not need a booster dose. However, adults may require a booster dose in the event that they do not present evidence of immunity (22). Adults that are traveling to places with high risk of MeV transmission need to ensure that they have had two doses of MMR administered at least 28 days apart. MMR vaccine is effective if administered to patients within the first 72 hours after exposure to MeV (20).

MeV vaccine has a failure rate of 2 to 10%, leading to the implementation of the second dose policy (23). Seropositivity has been shown to be 95%, 74%, and 100% for MeV, mumps, and rubella, respectively, for 183 initial seronegative vaccinees 15 years after the second administration of MMR vaccine (24). Geometric mean level of Abs to MeV showed a decline from 1917 to 957 to 729 mIU/mL during the 15-year study, while the mean decay rate for MeV was 3.5%. It should be noted that gender does not affect seropositivity (24). Data has shown that lack of second dose of MMR can lead to waning of immunity from vaccination among young adults (25). As such, implementation of vaccination campaigns to susceptible populations of those between the ages of 15-29, has been suggested as a catch-up method (25).

Side effects of the MMR vaccine may include mild problems such as fever and rash, moderate problems including seizure and temporary low platelet count, and severe problems including serious allergic reactions, deafness, and permanent brain damage (26). However, the MMR vaccine is much safer than actually being infected with MeV and most people who receive the MMR vaccine rarely have any of the serious problems mentioned before (26).

People who are most at risk are those who have defects in cell mediated immunity (12) and are immunocompromised. Unvaccinated children have the highest risk of MeV. Populations that suffer from malnutrition and lack adequate healthcare have MeV cases with mortality rates up to 10% (4). MeV is still common in underdeveloped and developing countries, such as parts of
Asia and Africa. More than 95% of MeV-related deaths occur in countries that have low per capita incomes (4). Thus, it is important to monitor immunization coverage of individuals who are travelling or immigrating to the US and for those who are travelling out of the US (16).

Many developing countries do not have access to state-of-the-art medical devices to be able to accurately and rapidly screen people in outbreak settings (27). It is important to address the absence of proper medical technology that the majority of the world’s population lacks. Many of these people have access to poorly resourced healthcare facilities (28) with little to no supporting clinical laboratory infrastructure. The development of cheap, easy-to-use, and rapid diagnostic assays to test for infectious diseases is paramount to effectively diagnosing and treating infected people in low-resource areas. Furthermore, such devices can be used in large public settings to rapidly determine immunity.

Controlling the spread of infectious diseases, such as MeV, in public places such as colleges and theme parks is very challenging due to the sheer amount of people that aggregate together. Furthermore, such populations of people vary drastically in gender, race, and immunization history (29). In college settings, students live, attend, and spend time together in close proximity, along with also being very mobile. Many people travel abroad and can acquire infectious diseases to bring back, if they lack immunizations (29) or are immunocompromised.

Such outbreaks cause significant financial cost (29) to not only the public health agencies, but also to the families and medical systems. An evaluation of containment costs during the 2008 MeV outbreak in San Diego showed that a strong outbreak response with 95% two dose vaccine coverage and absence of vaccine failure resulted in a net public sector cost of $10,376 per case, while direct medical charges totaled $1,347 per case (30). Young children who could not be vaccinated had to be quarantined, costing families an average of $775 per child (30). With such a high cost associated with containing and eliminating MeV in an outbreak situation, it is more worthwhile and cost effective to actively determine immunity amongst populations rather than attempting to rapidly vaccinate everyone.

For example, outbreaks in college campuses cause significant disruption to young adults’ lives as it impedes their studies and livelihoods. A large MeV outbreak at Washington State in 1995
cost the Department of Health and the institution over $400,000 (31). Previous studies have shown that US colleges and universities that required prematriculation immunization requirements (PIRs) reported less MeV cases and outbreaks compared to colleges and universities that did not require PIRs (32). In this study, the fourteen schools that reported MeV outbreaks did not have any state regulations or require any PIRs for students. Less than 40% of Washington colleges and universities require PIRs and more than 60% of these universities allow exemptions for medical, religious, and/or philosophical reasons (31). Less than 60% of US colleges retain immunization information for students, which means that there could be a potentially under-vaccinated population susceptible to MeV (31) residing in close proximity. Self-reporting of immunizations are not very reliable and can lead to under-reporting of vaccinations (33), making rapid determination of immunity even more important in not only outbreak settings, but also in large public institutions.

The gold standard method to test for MeV immunity is the plaque neutralization test (PRNT), which measures functional neutralizing Abs (34). This assay is quantitative and requires a serum dilution less than 1:120 that reduces the number of plaques by 50% (PRNT titre) in order to have a level of Ab protection against MeV (34). PRNT detects all isotypes of Abs directed against MeV. PRNT can take up to seven days to complete and it is an assay that is sometimes difficult to standardize. In comparison, MeV IgG ELISA’s are very easy and rapid to perform (34), but only detect IgG specific for MeV.

Current diagnostic methods of detecting MeV Abs include indirect MeV commercial immunoglobulin M (IgM) capture assays and immunoglobulin G (IgG) capture assays. IgM Abs are produced in the event of exposure to a disease, such as MeV. IgM serology is the standard for laboratory diagnosis of MeV and commercial IgM immunoassay (EIA) kits are used for determination of presence of IgM Abs. IgG Abs are produced after long term immunity is developed and is an indicator of past exposure to disease. Commercial IgG EIA kits are used for determination of immunity through detection of IgG Abs.

Four commercial IgM capture assays (Behring, Clark, Gull, and PanBio) were evaluated to determine their ability to detect MeV-specific IgM Abs. 308 serum samples from patients involved in a MeV outbreak and 454 serum samples from healthy subjects without MeV were
screened. Comparison of these four assays determined that they have detection rates between 57% to 80% and sensitivities that range from 82.8% to 88.6% (2). Most indirect MeV commercial IgM assays measure reactivity against the N antigen as Abs to N predominate and appear early during infections. The N antigen is a major target of the T-cell response that is needed for viral clearance (35). These assays also have routine false-negative and false-positive results (2), which poses a problem when trying to rapidly test multiple MeV cases to detect the presence of MeV.

Various commercial IgG ELISA kits are available and can measure reactivity against the N antigen or cell culture grown native virus antigens. Two such assays (Microimmune and Dade Behring), one using recombinant N antigen and the other using cell culture grown native virus antigens, were evaluated for MeV immunity testing (34). Data showed that both ELISA’s has similar performance characteristics and provided false negative results in 10% of serum samples (34). Both ELISA’s showed a sensitivity of 89.6% with a specificity of 100%.

A commercially available Diamedix Immunosimplicity® Measles IgG test kit (Diamedix Corporation, Miami, Fl) has been shown to have 91.1% sensitivity and 93.8% specificity (36) at a PRN cut off of 120. This Diamedix enzyme immunoassay (EIA) test detects Abs to MeV antigen, a purified extract of Vero cells infected with Edmonston strain of MeV (37). Previous studies have shown that this EIA test has very low sensitivity in detecting MeV Ab at low levels, making it unreliable for MeV vaccine-related studies (36). This EIA kit has been shown to have a positive predictive value of 99.5% and a negative predictive value of 45.5% on the basis of a PRN titer of 8, the lowest detectable Ab level by the plaque reduction neutralization test, and have a positive predictive value of 88.7% and a negative predictive value of 90.9% on the basis of a PRN titer of 120, indicating the possibility of false positive results (36). For the purposes of this study, the Diamedix Immunosimplicity® Measles IgG test kit was used to screen the same serum samples as was used for the RAPID ELISA.

Rapid antigenic protein in situ display ELISA (RAPID ELISA) will be utilized for protein expression and serological analysis. This alternative method for detecting Abs in sera across all MeV antigens will allow for a more sensitive high-throughput serologic screening (38) using cDNA
fused to GST-tags which will be expressed in situ using in vitro transcription/translation (IVTT) with human cell lysate. High sensitivity and reproducibility are the most important advantages associated with the RAPID ELISA assay (38). RAPID ELISAs are also cost-effective; a 96-well plate costs $59.11, with each well costing $0.62. In contrast, enzyme-linked immunosorbent assays (ELISAs) are limited because these assays require the use of known antigens and a priori protein purification (38).

2. MATERIALS AND METHODS

2.1 Primer Design, PCR, and DNA Purification

Primers specific for N, F, and H were designed using attB sites. Genes were amplified from measles plasmid, pB(+)MeVvac2(ATU)P, through PCR and run through a 1% agarose gel. DNA collected from the PCR reactions was purified using a QIAquick® PCR Purification Kit (Qiagen Sample and Assay Technologies, Hiden, Germany).

2.2 Subcloning Into pDONR221 Vector and pJFT7_nGST Vector

PCR product was recombined with the Gateway pDONR 221 entry vector through a BP reaction to generate an entry clone. The entry clone was recombined with the Gateway-compatible pJFT7_nGST expression vector, obtained from the DNASU Plasmid Repository at the Biodesign Institute at Arizona State University, through LR reactions to generate N-terminal GST fusion proteins.

2.3 Detection of MeV Abs Using Expressed Antigens on the Rapid Antigenic Protein in situ Display (RAPID) ELISA Platform

Wells in a 96-well plate were coated with anti-GST antibody and blocked with 5% milk PBST 0.2%. GST-tagged proteins were expressed from cDNA using in vitro transcription/translation (IVTT) with Hela reagents and human cell lysate. GST-tagged proteins were captured onto the anti-GST, washed, and incubated with Mouse anti-GST primary antibody diluted to 1:3000. Wells were washed and incubated with Mouse anti-GST primary antibody
diluted to 1:3000 and then with sheep anti mouse IgG secondary antibody diluted to 1:6250.
SuperSignal ELISA Pico Chemiluminescent Substrate (Thermo Scientific, Rockford, Illinois) was used for detection (39) and luminesce was detected using Glomax 96 Microplate Luminometer (Promega, WI).

Serum samples from healthy subjects were screened for MeV-specific Abs and to determine frequency of seropositivity using the RAPID ELISA system.

After expression, GST-tagged proteins were captured onto the anti-GST coated wells, washed, and incubated with serum samples diluted to 1:3000. Wells were subsequently washed and incubated with sheep anti mouse IgG secondary antibody diluted to 1:6250. SuperSignal ELISA Pico Chemiluminescent Substrate (Thermo Scientific, Rockford, Illinois) was used for detection (39) and luminesce was detected using Glomax 96 Microplate Luminometer (Promega, WI).

2.5 Detection of MeV-specific Abs using Diamedix Immunosimplicity® Measles IgG Test Kit

Serum Ab detection using the Diamedix Immunosimplicity® Measles IgG test kit was performed as described in the package insert (37). A 1:101 dilution of serum samples was prepared using the Sample Diluent provided in the kit. Serum samples, along with 100 μl of provided Calibrator and provided Controls were added to their respective wells in the provided 96-well plate. Plate was incubated at room temperature for 30 min and then washed with Wash Solution 3 times. 100 μl of provided Conjugate was added to all of the wells and then plate was incubated at room temperature for 30 min and washed as before. 100 μl of Substrate Solution was added to all wells and plate was again incubated at room temperature for 30 min. 100 μl of Stop Solution was added and absorbance was detected and quantified using Glomax 96 Microplate Luminometer (Promega, WI).
3. RESULTS

3.1 Rapid Antigenic Protein in situ Display ELISA for Protein Expression

Three full-length proteins (N, F, and H) fused to n-terminal GST protein were tested for protein expression. All three antigens were strongly expressed, captured and displayed, as detected with an anti-GST antibody. Proteins were expressed, with RLU values ranging between 3.01 x 10⁹ and 1.20 x 10⁹ (Figure 2). N had an average RLU value of 3.01 x 10⁹ (p<0.001) while F had an average RLU value of 1.24 x 10⁹ (p<0.001). H had average RLU value of 1.20 x 10⁹ (p<0.001). Average RLU value for the negative control was 4.56 x 10⁶ RLU, indicating that N, F and H expressed at least 50% greater than the control. There was over an 8-fold improvement in expression levels using N-terminal GST fusion proteins.

**Figure 2.** Protein Expression of N, F, and H antigens.
3.2 Frequency distribution of serum samples screened using GST control

93% (40/43) of the serum samples from healthy donors and the positive control and calibrator screened using GST control had average RLU values that fell between +/- 1 SD of the mean for the whole sample set (Figure 3), indicating normal distribution with values that had negligible variation. The z-score is defined as the number of standard deviations each value is from the mean. The mean of the RLU values across all of the samples screened was $2.65 \times 10^8$, while the standard deviation was $1.68 \times 10^8$.

All average RLU values for serum samples screened were below $1 \times 10^9$. Similar patterns of detection have been seen in previous studies using GST control plates/wells as well, indicating that current average RLU values are consistent with normal detection rates. No extreme outlier was detected within this sample set. Samples 35 and 31 had the highest RLU averages of $9.21 \times 10^8$ and $8.61 \times 10^8$, respectively. Sample 32 had the lowest RLU average of $1.12 \times 10^8$. The minimum and maximum RLU averages fall within the range of what has been seen in previous studies.

Figure 3. Frequency Distribution of Average RLU Values of Serum Samples Screened using GST Control
3.3 Detection of MeV-specific Abs in Patient Serum using Rapid Antigenic Protein in situ Display ELISA

N, F, and H were screened across 40 serum samples from healthy subjects and the GST ratios were calculated. N, in general, had higher GST ratios, as compared to F and H. It was assumed that majority of sera was positive for MeV-specific Abs, as MMR vaccinations are required. Cutoff values for N, F, and H, were calculated as the mean +3 standard deviations of the GST ratios as observed among the samples screened. Cutoff values for N, F, and H were 1.21, 1.92, and 1.23 (mean+3SD of negative control), respectively. Based on this criterion, 36 of the 40 positive serum samples had GST ratios above the cutoff values, indicating significant detection of MeV-specific Abs.

The calibrator, positive control, and negative control from the Diamedix kit were also screened for comparison purposes. The calibrator and positive control had had GST ratios above the cutoff values mentioned above for all three antigens, while the negative control had GST ratios well below the cutoff values for all three antigens.
Figure 4. Average GST Ratios for Serum Samples Screened Across N, F, and H Antigens.
3.4 Detection of MeV-specific Abs in Patient Serum using Diamedix Immunosimplicity® Measles IgG test kit

ELISA UNITS/ml (EU/ml), standardized using provided references which includes the calibrator, were calculated in order to determine the potency of immunologically active substance in the serum samples that were screened. EU/ml was determined as reactivity in this assay for the particular antigen and is expressed per unit volume equal to one milliliter. EU/ml for each serum sample was calculated by using the following formula: \[\frac{(EU/ml \text{ of Calibrator}) \times \text{Absorbance of Sample}}{(EU/ml \text{ of Calibrator})}\]. Reference ranges were provided in the package insert, and any sample with an EU/ml above 20.0 was considered to be reactive (positive) for anti-MeV IgG and presumed to have immunity against MeV. Any sample with an EU/ml below 15.0 was considered to be nonreactive (negative) for anti-MeV IgG and presumed to be non-immune to MeV. The magnitude of the measured result, above the cut-off, is not indicative of the total amount of antibody present. The magnitude of the reported IgG level cannot be correlated to an endpoint titer.

The Diamedix test kit presented results that indicated that samples 1-40 were reactive (positive) and had MeV-specific Abs present; indicating presumed immunity to MeV. These samples had EU/ml that were above the cut-off value of 20.0 EU/mL, and ranged between 46.58 EU/ml and 126.75 EU/mL. The calibrator had an EU/ml of 100, while the positive control had an EU/ml of 76.42. The negative control was shown to be nonreactive, or negative, for MeV-specific Abs, with an EU/ml of 5.608.
Figure 5. EU/ml (ELISA Units/ml) of Serum Samples Screened Using the Diamedix Immunosimplicity® Measles IgG Test Kit.
4. DISCUSSION

The highly contagious nature of MeV makes rapid diagnostic analysis crucial during outbreaks (18). Before the MMR vaccine was implemented globally, MeV caused over 2.6 million deaths every year (4). People who are most at risk are unvaccinated children, pregnant women, and non-immune people (4). 2014 had over 20 outbreaks of MeV cases, constituting about 89% of all reported cases (6). Due to the contagious nature of MV and its ability to live for up to two hours in an area where an infected person has coughed or sneezed, it is imperative that there are measures put in place in order to ensure the safety of the general public. A person infected with MeV can infect 90% of the people they come into contact with if those people are not vaccinated (15).

MeV genes of interest were successfully subcloned into the Gateway entry vector, pDONR 221, and Gateway expression vector, pANT7_cGST (from previous studies) and pJFT7_nGST. This was confirmed through sequence analysis using Sequencher 5.0 (Gene Codes Corporation, Ann Arbor, Michigan). Subcloned MeV genes of interest were tested for protein expression and three of them successfully expressed at least 50% greater than the control, ensuring that all MeV fusion proteins could be used serologic analysis. N had the strongest expression, while F and H had weaker expression. There was over an 8-fold improvement in expression levels using N-terminal GST fusion proteins, indicating that these fusion proteins were suitable for serum screening. Protein expression levels can vary, but significant protein expression has been seen to range above $1 \times 10^9$ from previous studies.

A programmable ELISA assay, termed RAPID ELISA, was developed using three antigens from the MeV genome for serologic detection of MeV-specific Abs. RAPID ELISAs have been used in previous studies to detect p53-Autoantibodies (AAbs) for use as prognostic biomarkers and have been shown to detect higher frequency of p53-specific AAbs, as compared to published reports (40).

From a small sample set (40 serum samples from healthy donors) that was used to screen N, F and H antigens, it could be seen that most MeV-specific Abs were directed against the N antigen. This data shows that MeV-specific antibodies in sera can be detected at significant
levels, using all three antigens. Additionally, the absence of MeV-specific antibodies can also be detected, as seen with the low GST ratio for the negative control that was provided with the Diamedix test kit. Cutoff values for positive serology was defined as the mean +3 standard deviations of the GST ratio observed among the set of samples screened. Calibrator, positive control, and negative control provided in the Diamedix Immunosimplicity® Measles IgG test kit was also screened using this platform. The negative control, which was non-reactive for MeV IgG Abs, had very low detection (37) with GST ratios well below the cutoff values for all three antigens. The calibrator, which was highly reactive for MeV IgG Abs (37), had high GST ratios above the cutoff values, while the positive control, which was moderately reactive for MeV IgG Abs (37), also had GST ratios above the cutoff values.

Evaluation of the combination of three antigens showed that, of the 40 serum samples that were screened, 36 had detection of IgG to N, F, and/or H, greater than the cutoff values, indicating good signal strength and detection levels. Because this assay uses IVTT with human cell lysate to generate the production of fusion proteins, there can be differences in protein folding and structure. Furthermore, it might be beneficial to excise the transmembrane region of the H and F antigens, as that might be affecting protein folding and inhibiting proper Ab capture. Currently, this assay has not been tested using a large sample of negative controls.

To ensure that GST control had normal distribution with negligible variation across all of the serum samples that were screened, z-scores were calculated and it was determined that 93% (40/43) of the samples had average RLU values that fell between +/-1 SD of the sample mean. As expected, all average RLU values for serum samples screened were below 1x10^9 and similar patterns of detection were seen between this study and previous studies using GST control plates/wells. This data indicates that GST control plates had a range of similar values across all serum samples that were screened and no large outliers were detected.

Samples tested using the Diamedix Immunosimplicity® Measles IgG test kit had EU/ml greater than the cutoff value of 20.0 EU/ml, indicating that all of the samples had detectable levels of MeV-specific Abs, which was expected. Comparisons between the two assays show similar detection patterns, with the exception of four samples which fell below the cutoff values for
all three antigens on the RAPID ELISA platform. Samples 10, 18, 25, and 31 had GST ratios that fell below the cutoff values calculated for each antigen. Consequently, these four samples also had higher average RLU values for GST control, as compared to the other serum samples. GST ratios may have been lower due to high background for these samples. For further studies, it will be necessary to optimize the background signal to lower it and to determine concentration of Abs and serum that will be needed for optimal signal detection.

The Diamedix kit had better detection of MeV-specific Abs in serum, as compared to the RAPID ELISA platform, which may be due to the fact that this kit detects Abs to the whole MeV antigen, which is a purified extract of Vero cells infected with Edmonston strain of MeV (37). It may be beneficial to create custom protein microarrays using Vero cells infected with Edmonston strain of MeV and screen serum samples to see if better detection is achieved, as compared to the three antigen RAPID ELISA platform. Using the entire MeV antigen could prove to overcome some of low detection that was seen with some samples and allow for better Ab capture, as the entirety of the antigen can be spotted onto these arrays. For further studies, it may be extremely advantageous, in terms of moving forward with a point of care assay, to test for MeV immunity using custom protein microarrays spotted with the whole MeV antigen.

A large weakness of this study is the lack of negative samples used for serologic screening. Sensitivities and specificities could not be determined for this present study as negative cases and controls were not screened due to accessibility issues. For an assay to be clinically useful, it would be necessary to have very high specificity, indicating lower numbers of false positives, and significantly high sensitivity as well. Most IgM MeV assays have reported sensitivities between 83-89% and specificities of 95-100% (41). Current IgG MeV assays have sensitivities that range from 70-88% and specificities of 99-100%, making it necessary for any new IgG assays to have comparable rates in order to be as clinically useful. Limitations associated with using the RAPID ELISA platform for MeV IgG testing include potential false negative results, as indicated by the four samples that had very low detection using this assay. The probability of false positive results cannot be determined at this time.
The RAPID ELISA platform has many strengths, including the ability to screen a large number of clinical samples against a limited number of proteins. Furthermore, antigens are readily expressed as full length N-terminal GST tagged fusion proteins that are captured onto the 96-well plates using anti-tag Abs. This method eliminates the need to amplify sequences by PCR and to prepare quantified plasmid DNA for screening processes as recombinant proteins can be captured in situ using anti-tag Abs. A large advantage of the RAPID ELISA platform is the ease and flexibility of using cDNA as source antigens without having to separately express and purify them. Because of this, antigens can also very readily be printed on custom microarrays for point of care testing. From previous studies, it is already known that the sensitivity of detection for the RAPID ELISA platform is comparable to standard ELISAs.

This current study needs to be expanded to include a larger sample set with negative cases and controls that can be used to accurately determine the sensitivity and specificity of the RAPID ELISA platform. A large limitation to this present study was the fact that only positive serum samples were available, making it harder to determine if the RAPID ELISA platform could also detect the absence of MeV-specific Abs. For further studies, pre- and post-vaccination sera will need to be screened in order to accurately ascertain the validity and strength of using the RAPID ELISA platform for rapid determination of MeV immunity.

Though the RAPID ELISA system was weaker than the standard Diamedix kit, with proper optimization and testing of a larger sample set, this assay can be used for point of care testing. The ability to rapidly detect MeV-specific immunity is extremely important so that individuals that are susceptible and need to be vaccinated/treated can be rapidly identified in outbreak settings. Real time determination of vital information about individuals being tested can significantly increase outbreak containment and elimination procedures (42). Such assays can help facilitate rapid diagnosis in countries where resources are limited, aiding in MeV surveillance and treatment response time. In addition, implementation of such assays in public areas with high traffic, such as amusement parks and airports, can also help rapidly screen the thousands of people traveling from various areas of the world in order to ensure the general public is not being exposed to the virus. The main objective of a point-of-care assay is to generate a rapid result so
that appropriate treatment can be administered, leading to an improved clinical and economic outcome (43).

A rapid point-of-care assays can be used for personal testing, at the scene of an accident/outbreak, in hospitals, and in underdeveloped countries. Such implementation of point-of-care assays can completely revolutionize health care delivery. Having a point-of-care assay can also help with disaster recovery in ensuring emergency preparedness. Furthermore, there is a national laboratory technologist shortage, with a projected need for 13,000 technologists every year (42), making it significantly harder to have and to use large and complex machinery for laboratory diagnosis. Studies have shown that point-of-care devices can reduce hospital stay, improve adherence to treatment, and reduce complications that might arise (43).

In conclusion, this study shows that all of the expressed single MV genes were able to capture IgG antibodies present in 90% of the serum samples tested using the RAPID ELISA platform. Though the RAPID ELISA platform had higher levels of false negative results, as compared to the Diamedix Immunosimplicity® Measles IgG test kit, with proper optimization and calibration, application of this assay using programmable protein arrays (FLEX NAPPA) displaying these antigens for rapid determination of MeV immunity has great potential and applicability in the field, for personal use, and in healthcare facilities.
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