

FROM THE MIND OF THE CHAIR



Hello again and welcome to the summer edition of the PMF division newsletter! I'm looking forward to seeing many of you in San Diego in a few weeks. In this edition, we preview the events, education and research taking place during the 69th AACC Annual Scientific Meeting &

Clinical Lab Expo. Please plan to attend our jointly hosted mixer on Sunday night, following the opening mixer. It will be a great chance to enjoy food and drink while networking with colleagues from the Pediatric and Maternal-Fetal, Industry, Informatics, Clinical Translational Science, and Industry divisions. We will also acknowledge the recipients of our division's awards at this time (read more in this issue). To see the latest science from our areas of interest, check out our poster walk on Wednesday and the sessions we've highlighted on pages 9-10.

In this issue, we reach the end of our ABC's of Laboratory Medicine with letter 'Z', featuring a multi-regional perspectives on Zika virus. Excerpt from the Literature summarizes changes to the sweat chloride reporting recommendations and a clinical case demonstrating the limitations of conventional drug screening cutoffs in pediatrics. I hope that you enjoy this edition of the newsletter and that you will continue to join us in our efforts to advance the practice of pediatric and maternal fetal laboratory medicine. See you in San Diego!

Shannon Haymond, PhD
Chair, AACC PMF Division

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The ABC's of Pediatric Laboratory Medicine:



**Z IS FOR "ZIKA VIRUS TESTING:
FROM BRAZIL TO THE U.S.**

To finish off this round of ABC's of Pediatric Laboratory Medicine we have a special two part article covering the efforts in the clinical laboratories of Brazil and the U.S. to help with the diagnosis of Zika. Thank you for your readership!

Sincerely,

Van Leung-Pineda

PMF Newsletter Editor

1) Zika Virus: a Brazilian overview

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Epidemiology of Zika Virus in Brazil: the beginning of the epidemic

Zika virus (ZIKV) pandemic is alarming although, with the scarcity of literature, the exact details of the disease are not clear. In March 2015, researchers from the Federal University of Bahia confirmed the introduction of Zika virus (ZIKV) in Brazil. Serum samples from 24 exanthematous patients from Camaçari in Bahia were tested and 7 of them presented positive diagnosis to Zika by Real-time PCR (RT-PCR) (1-3). Approximately 440,000-1,300,000 cases of ZIKV in Brazil were reported during the outbreak in 2015. According to data from the

Brazilian Ministry of Health 215,319 probable cases in 2016 were reported, 8 deaths were confirmed in laboratory. In 2017, so far 7,911 probable cases were reported, among which 2,826 (36 %) cases were confirmed. Based on the analysis by geographic region, the Midwest and North region presented the highest incidence of Zika virus infection. Among the Federative Units the highest ZIKV-positive prevalence were observed in state of Tocantins, Roraima and Goiás. Outbreaks suggest that ZIKV is an emerging disease and it might be associated with the increasing numbers of congenital microcephaly cases reported in the country. In 2016, 10,867 cases were reported, of which 3,183 (29,3%) cases remain under investigation and 7,684 (70,7%) cases were investigated and classified, with 2,366 confirmed cases (4).

Laboratory Diagnostics for Zika Virus: Molecular and Serologic Tests

The routine laboratory diagnosis for ZIKV infection is based on the same strategies used for other arboviruses, such as RT-qPCR (Viral RNA Detection) and serologic assays (Antibody-based Detection). Usually, the approach used will depend on the analysis goal, laboratory infrastructure, technical expertise and sampling availability.

The diagnosis of ZIKV infection performed by serological tests can detect ZIKV specific IgM / IgG antibodies after 5-6 days from the onset of symptoms, with increased titers found within 2 weeks. Therefore, these tests are the bottleneck of ZIKV infection diagnosis so far. The high prevalence of dengue seropositivity in Brazilian patients interferes with ZIKV serological tests due to cross-reactivity and it makes a seroprevalence study of ZIKV difficult. A study carried out by a team of researchers from Institute Hermes Pardini aimed to evaluate the performance of commercial Euroimmun ZIKV ELISA test (Euroimmun, Lübeck, Germany) using different panels from Brazilian patients exposed to Dengue (DENV) and Chikungunya (CHIKV) infection. It was possible that positive samples for the dengue virus may also be positive for ZIKV. No cross-reactivity of IgM ZIKV with Chikungunya virus was observed. The IgG

positive samples for DENV and CHIKV may be positive for ZIKV. Despite that, it was not established if this corresponded to a cross reactivity of the test or coinfection in different periods in the same individual in endemic areas. Its positivity should be evaluated in the context of other conditions, such as Epstein Barr virus infections and in malaria infection.

The molecular assays for the detection of viral RNA by real-time reverse transcriptase polymerase chain reaction (RT-qPCR) are widely used for virus detection, despite its present limitations due to low viremia in clinical samples. Nevertheless, a well-designed RT-qPCR is highly specific, sensitive and will cross-react with other arbovirus and is a useful tool for detection of ZIKV in virus pandemic areas where viruses including DENV and CHIKV also occur. The reliance on the use of molecular diagnostics to rule out infection requires careful consideration. In 2015 a test for detection of ZIKV in different samples (serum, urine and semen) was implemented at the Hermes Pardini Institute.

In addition, in Brazil, point-of-care-testing methods have obtained the release certificate from ANVISA (National Health Surveillance Agency) such as Zika NS1 Ag Eco Test, Zika IgM/IgG Eco Test (Eco Diagnostica), Rapid Test NS1 Zika (Bahiafarma), Imuno Rapid Zika IgM/IgG (Wama Produtos para laboratório).

Zika Networks in Brazil

Due to the rapid global emergence of ZIKV infections and the rapid increase of microcephaly cases in newborns, the development of specific networks is of great importance for the success of arbovirus control. They could help local, regional and national health authorities in understanding the dynamics of circulation and evolution of infection, which may have repercussions on future epidemics.

According to Mota et al. (5), several research centers in Brazil and other countries joined forces in an attempt to understand the biology of this virus, to develop tools for the treatment of patients and to prevent infection. In São Paulo, a group of 42 laboratories, called the Zika network and coordinated by the Institute of Biomedical Sciences (ICB) at USP, are working

together to better understand the behavior of ZIKV and thus improve the diagnostic methods. Another research network, coordinated by FIOCRUZ Bahia, is involved in the development of a project called ZIBRA (Zika in Brazil Real Time Analysis) which aims to genetically map ZIKV strains collected from several locations in the Northeast region. Another tool to assess the relationship between ZIKV and the host is ZIKV-CBD, developed by FIOCRUZ-Minas.

Several Brazilian institutes are now involved in the development of a vaccine against ZIKV. Bio-Manguinhos/Fiocruz is developing Zika vaccines with different partnerships and platforms by using inactivated, 17 D Yellow Fever/Zika chimeric virus in tissue culture (6). Butantan Institute has been developing an inactivated vaccine in partnership with the US Bio-medical Advanced Research and Development Authority (BarDA). Another study from a collaboration between Harvard University and the University of São Paulo has shown that a single immunization of a plasmid DNA vaccine or a purified inactivated virus vaccine provides complete protection in susceptible mice against challenge with a ZIKV outbreak strain from Northeast Brazil (7). Finally, the University of Pittsburgh in collaboration with the Fiocruz/Aggeu Magalhães Research Centre is now developing a Zika vaccine using a new version of LAMP technology under the sponsorship of the Cura Zika programme (8).

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2) Zika Virus: an overview from the U.S.

Matthew Feldhammer PhD. Department of Pathology and Laboratory Medicine. Emory University

Introduction:

Today, Zika virus is recognized the world over, but it was only 14 months ago that the World Health Organization declared the virus a public health emergency. This action brought the little known Zika virus and its' vector, the *Aedes aegypti* mosquito onto the cover of time magazine (May 16, 2016) and into the lexicon of every household in America. The initial fears of this newly re-discovered threat spread quickly as word of the virus and links with serious birth defects were emerging rapidly from the endemic regions of Brazil that were the most affected. A little over a year later we have learned a great deal about the Zika virus, how it is spread, and the potential effects of vertical transmission on fetal brain development. The response to this

¹Birth defects include brain abnormalities with and without microcephaly, for a complete list please refer to:

epidemic from a public health perspective has shown the ability of governments, private and public health concerns to work together to battle the spread of the virus. Rapid and accurate diagnostics have been developed and deployed under the emergency use authorizations (EUA) mechanism of the Food and Drug Agency throughout the United States. With the growing availability of testing in academic centers, researchers have been able to enroll patients in a variety of clinical trials in attempts to learn as much as possible about the virus. Currently almost a dozen vaccine formulations are in various stages of development and testing.

Zika Outcomes in the US:

In contrast, to Central America, South America, and Puerto Rico where there is currently active transmission of the virus, the majority of confirmed Zika cases in the continental United States are travel acquired. The latest statistics (May 3rd, 2017) from the Centers for Disease Control (1) confirms 5,274 cases reported, among which 4,973 (94%) were acquired in travelers returning from endemic areas. The two areas in the United States that had confirmed cases of local transmission were Brownsville, Texas (6 cases) and several small communities in the greater Miami-Dade County region (218 cases). There were an additional 77 cases acquired through other routes: Sexual transmission (46), congenital infection (29), laboratory transmission (1), and unknown person-to-person (1). These figures are a stark contrast to the situation in Puerto Rico where 99% of cases (36,574 total) are due to local transmission. With respect to outcomes, of 1,409 reported infections in pregnant woman, there were 58 instances (4%) of newborns with birth defects¹ and 8 instances (0.6%) of pregnancy loss attributed to Zika infection. Current evidence indicates that the highest risk period for microcephaly is during the first trimester (0.88%-13.2%) and becomes almost zero in the second

<https://www.cdc.gov/zika/geo/pregnancy-outcomes.html>

and third trimester (2). While the majority of attention has focused on the link between Zika and microcephaly there are large numbers of cases of Guillain-Barré syndrome being reported in regions with active Zika transmission. Currently the CDC is reporting 65 patients with Zika linked Guillain-Barré syndrome in the United States and Puerto Rico. Interestingly, new research indicates that previous flavivirus exposure (dengue, chikungunya etc.) can enhance Zika virus pathogenesis (3). We have seen evidence of this in our laboratory specifically with regards to prolonged viremia in patients with previous flavivirus exposures (unpublished observation).

Zika Virus Testing:

Prior to the current Zika epidemic, diagnostic testing was only available through the CDC and a small number of state public health laboratories. Testing is either PCR based in order to identify acute infections or serology based for patients with an exposure outside the acute infection window. In order to respond to the growing need for accurate and rapid diagnostic testing academic hospital laboratories and *in vitro* diagnostic manufacturers worked diligently to develop and validate suitable methods. Testing was and is still available through the CDC and a small number of state public health labs but the turnaround time for some of the tests during peak mosquito season last summer was up to 4-5 weeks in some areas. In order to rapidly respond to the public health emergency the FDA granted an Emergency Use Authorization (EUA) for Zika testing. The initial EUA covered the CDC's PCR Trioplex assay, designed to detect Zika, Dengue, and Chikungunya in acutely infected individuals. Additionally, the EUA covered the CDC's Zika MAC-ELISA for detection of IgM antibodies to Zika virus. Since the initial roll out of the EUA several *in vitro* diagnostics companies as well as reference labs have had their Zika assays approved. Initially, PCR testing was conducted

in serum or plasma for acutely infected patients (7 days post symptom onset) as they were considered to be the most robust matrix platforms. Shortly after the issuance of the EUA newer testing guidelines were issued which drew upon emerging evidence and advocated for the testing of urine in addition to serum or plasma as it could possibly extend the detection window out to 14 days in acute cases (Interim Guidance for Zika Virus Testing of Urine — United States, 2016). More recently several groups have published evidence to suggest that whole blood may allow for the longest detection window ² (4). Similar observations have been reported for West Nile Virus, which like the Zika virus, is a member of the flavivirus genus (5). The authors hypothesize that similar to West Nile, Zika Virus can attach to glycoproteins on the surface of red blood cells, which allows for the prolonged detection of virus after it wanes from the plasma or serum fractions. Under the direction of the NIAID teams from multiple academic centers including our own are participating in studies to investigate the viral reservoirs within the body and how long they persist.

Vaccine Development:

Currently the NIAID is pursuing several different vaccine development strategies in order to counter the spread of Zika. These vaccines in large part have been based on the successful vaccine strategies approved for other flaviviruses including Japanese encephalitis and West Nile viruses. A thorough review of the numerous vaccine candidates being pursued is beyond the scope of this article, but briefly, the overall strategies currently either in development or clinical trials include: 1) a NIAID developed DNA based vaccine currently in phase 2 clinical trials in endemic regions throughout South America. 2) A purified and inactivated version of the virus developed by Walter Reed and currently in phase 1 clinical trials. 3) A live-attenuated version of the virus which is scheduled to begin testing at Johns Hopkins

²For the latest guidelines and testing algorithms please refer to the CDC's Zika testing website

<https://www.cdc.gov/zika/hc-providers/testing-for-zikavirus.html>

later this year. 4) Several investigational mRNA based platforms are in various stages of development and/or phase 1 trials and have shown very promising pre-clinical results in animal models (6). As this summer's mosquito season approaches laboratories have already begun to see a renewed increase for Zika testing which only further highlights the growing need to continue to invest in Zika research, vaccines and diagnostics.

protection by a single low-dose nucleoside-modified mRNA vaccination. *Nature* 543, 248-251

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Excerpts from the Literature



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Is This Drug Screen Really Negative? Special Investigation of Drug Screening in Pediatrics

Amy L. Pyle-Eilola, David J. Thornton, Hannah L. Hays, and Marcel J. Casavant. *JALM* Jan 2017, 1 (4) 437-440.

The CDC Morbidity and Mortality Report August 2016, indicates that the incidence of neonatal abstinence syndrome increased drastically between 1999 and 2013 from a high of 3.6 per 1,000 births (Vermont) in 1999 to 33.4 per 1,000 births (West Virginia) in 2013 [1]! Now in 2017, NAS remains a significant concern as well as the increased incidence of young children visiting emergency departments due to intentional and unintentional prescription and illicit drug ingestion.

A case study shared in *The Journal of Applied Laboratory Medicine* January 2017 by Pyle-Eilola et al., highlights the significant risk of using conventional cutoff concentrations in urine drug screens for pediatric patients [2]. The case describes a drug-induced hypoxemia event in three-month old male where preliminary drug screen by conventional qualitative immunoassay was negative (raw result 161 ng/mL, cutoff 300 ng/mL) yet

confirmatory testing was positive for morphine and heroin.

In conjunction with CDC data, this case study underscores the collective need to have methods in place that allow clinicians to readily and confidently detect drug exposure in pediatric patients. Additionally, state laws and the federal Comprehensive Addiction and Recovery Act of 2016 require practitioners to report exposures for appropriate intervention by child protective services.

The authors provide a number of references which support lowering cutoffs for pediatric patients to improve clinical outcomes. At the advice of the authors, we decided to do a retrospective analysis of pediatric drug screens performed across our institution in children 0-12 years of age. Specifically, we looked for instances where the immunoassay raw value was just below the cutoff concentration in each of our 9 immunoassays that we routinely use. Correlation of the lab data with the patient clinical history demonstrated a number of events where we felt the negatively reported immunoassay result was a false negative. Currently, we are discussing alternative approaches with our neonatologists and pediatric ED teams to mitigate these false negatives from happening in the future.

A few suggestions in the manuscript offer ideas on how to approach alternative cutoffs in pediatric patients. Establishing an “indeterminate” range that is between the manufacturer cutoff and a precise point on the validated AMR of the assay may be a great approach as this result could alert a physician that confirmation testing is suggested or it could be readily built into LIS logic to reflex those specimens to confirmation testing.

Clearly, false negatives are as unacceptable as false positives in children. Physicians order drug screens as clinically indicated and conventional screen cutoffs are not likely sensitive enough to correlate with clinical presentation in this demographic. The authors should be applauded for drawing attention to

this matter and for making suggestions on how to improve practice.

References:

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Excerpts from the Literature



Brenda Suh-Lailam, PhD, DABCC, FACB, Assistant Director, Clinical Chemistry and Mass Spectrometry, Ann & Robert H. Lurie Children’s Hospital of Chicago. Assistant Professor of Pathology, Northwestern University Feinberg School of Medicine, Chicago, IL, USA

Diagnosis of Cystic Fibrosis: Consensus Guidelines from the Cystic Fibrosis Foundation

Farrell PM, White TB, Ren CL, Hempstead SE, Accurso F, Derichs N, Howenstine M, McColley SA, Rock M, Rosenfeld M, Sermet-Gaudelus I, Southern KW, Marshall BC, Sosnay PR. *J Pediatr*. 2017 Feb;181S:S4-S15.

Cystic fibrosis (CF) is the most common lethal genetic disease within the Caucasian population, affecting approximately 1 in 4000 newborns in the United States. CF is a

progressive, multisystem disorder that causes persistent lung infections and limits the patient's ability to breathe. CF is caused by mutations in the gene for the CF transmembrane conductance regulator (CFTR), which encodes an ion channel protein. To date, over 2000 mutations have been linked to CF.

Newborn screening (NBS) for cystic fibrosis was not universally done in the US until recently in 2010 when it was implemented by all 50 states plus the District of Columbia. This, in addition to laboratory advances and international collection of CF clinical data, has led to a significant increase in the amount of phenotypic and genotypic information on CF which has greatly enhanced the interpretation of CF status in many patients. With the growth of information on CF, the goal of this publication was to revise the 2008 CF Foundation diagnostic guidelines, harmonizing terminology and diagnostic criteria with the European CF Society (ECFS). One of the things the international committee convened by the CF Foundation did was to take a critical look at the reference intervals for sweat chloride which is the diagnostic test for CF and made changes based on the available new data. Laboratorians should be aware of the new consensus guidelines as these affect laboratory reporting of test results.

Some of the main take-aways for laboratory professionals are shown on the following table (summary from the publication and laboratory sweat chloride procedures):

2008 Guidelines	New Consensus Guidelines
<ul style="list-style-type: none"> The term "borderline" is used to describe sweat chloride results in the range 30-59 (for ≤ 6 months old) and 40-59 (for 	<ul style="list-style-type: none"> The term "intermediate" is used to describe sweat chloride results in the range 30-59 mmol/L for all ages

>6 months old) mmol/L	
<ul style="list-style-type: none"> Different reference intervals for ages ≥ 6 months: Sweat chloride: < 40 mmol/L was normal threshold for ages ≥ 6 months (exceptions occur) <p>a. Neonates (≤ 6 months old)</p> <ul style="list-style-type: none"> Normal: 0-29 mmol/L Borderline: 30-59 mmol/L Consistent with Cystic Fibrosis: >60 mmol/L <p>b. >6 months old</p> <ul style="list-style-type: none"> Normal: 0-39 mmol/L Borderline: 40-59 mmol/L Consistent with Cystic Fibrosis: >60 mmol/L 	<ul style="list-style-type: none"> Same reference intervals for all ages: Sweat chloride: < 30 mmol/L is normal threshold for all ages (exceptions occur) All ages Normal (Cystic Fibrosis unlikely): 0-29 mmol/L Intermediate: 30-59 mmol/L Indicative of Cystic Fibrosis: >60 mmol/L
<ul style="list-style-type: none"> CFTR mutations: Used ACMG/ACOG panel of 23 mutations 	<ul style="list-style-type: none"> CFTR mutations: use CFTR2 mutation list, with guidelines given for mutations not included in CFTR2
<ul style="list-style-type: none"> Presumptive diagnosis of CF not addressed 	<ul style="list-style-type: none"> Presumptive diagnosis of CF: can be made (positive NBS and 2 CF mutations or signs and symptoms of CF; or meconium

	ileus) and treatment started; diagnosis must be confirmed with a sweat test
<ul style="list-style-type: none"> Genetic analysis: recommended if not part of NBS 	<ul style="list-style-type: none"> Genetic analysis: recommended in addition to that done during NBS

2017 AACC Annual Scientific Meeting and Clinical Lab Expo: PMF Sessions of Interest and Meeting Highlights

JULY 30-AUGUST 3, 2017 IN SAN DIEGO, CALIFORNIA

Sunday, July 30th

Opening Plenary:

Jennifer Doudna, PhD

CRISPR Biology, Technology & Ethics: The Future of Genome Engineering. 11001.

Monday, July 31st

Plenary Session:

Teresa Woodruff, PhD

Oncofertility: From Bench to Bedside to Babies. 12001.

Brown Bag Sessions:

Critically High Ammonia in an Adolescent: Review of a Clinical Case Study from the Clinical Chemistry Journal. 42103 & 52203.

Anti-Mullerian Hormone (AMH): An Emerging Biomarker for the Assessment of Reproductive Function. 42113 & 52213.

Sensitive Estrone and Estradiol Quantitation in the Clinical Lab: Why, When and How? 42116 & 52216.

Updates in Pediatric Lipid Testing. 42121 & 52221

Short Coursers:

Anti-Mullerian Hormone from the Laboratory Perspective. 72414

Tuesday, August 1st

Plenary Session:

Jay Shendure, MD, PhD

Beyond Sequencing: New Frontiers in Genomics. 13001.

Brown Bag Sessions:

Laboratory Assessment of Pediatric Metabolic Syndrome. 43108 & 53208.

Small Blood Samples, Challenges for the Lab. 43122 & 53222.

Short Coursers:

Multi-Marker Testing Strategies in Women's Health. 73102.

Wednesday, August 2nd

Brown Bag Session:

Thyroid Function and Laboratory Assessment of Thyroid Disease. 44101 & 54201.

Innovative Applications in the Work-up of Primary Aldosteronism: Assays and Diagnostic Management Teams. 44102 & 54202.

Afternoon Symposia : Developed with the Pediatric Maternal Fetal Medicine Division

Current Practices and New Innovations in Newborn Screening. 34215.

Newborn screening is a fast-changing field requiring screening programs, clinical labs, birthing centers and clinicians to stay informed. At the AACC meeting in San Diego this summer, the PMF division along with CLSI and CDC will sponsor a Wednesday afternoon symposium that addresses a number of challenges from sample collection to patient treatment. These challenges will be discussed in light of CLSI guidance documents (Dr Ron Whitley), CDC's national quality management efforts (Dr Carla Cuthbert), global interpretive tools (Dr Piero Rinaldo), the lab's role in follow-up of screen positive results (Dr Uttam Garg), and current patient management initiatives (Dr Jennifer Gannon). Despite these challenges, with innovative technologies and better treatment options, the newborn screening system will continue to grow and improve patient outcomes.

Please Join Us!

Event: Pediatric and Maternal-Fetal, Industry, Informatics, Clinical Translational Science, and Industry Divisions Joint Mixer

Date: Sunday, July 30, 2017

Time: 7:30pm-9:00pm

Location: Marriott Marquis San Diego Marina Hotel (Balboa Room)

Relax and enjoy food and drinks in the company of your PMF colleagues!

Event: Pediatric and Maternal-Fetal Poster Walk. Dr. Mark Kellogg will guide through highlights in PMF research

Date: Wednesday, August 2, 2017

Time: 12:30pm-1:30pm

Location: San Diego Convention Center

PMF Division Awardees

Please help us congratulate the winners of this year's PMF Division Awards. The awards will be presented during the Pediatric and Maternal-Fetal, Industry, Informatics, Clinical Translational Science, and Industry Divisions Joint Mixer on **Sunday July 30, 2017 from 7:30pm-9:00pm at the Marriott Marquis San Diego Marina Hotel (Balboa Room).**

Best Abstract by a Student or Young Investigator:

- **Camila Santos Nobre and Ticiane Henriques Santa Rita**, Laboratorio Sabin, Brasilia, DF, Brasil
- Title: Non-invasive fetal sex determination using cell-free fetal DNA isolated from maternal capillary blood obtained by fingertip puncture: The elimination of exogenous male DNA from the collection site is crucial

Best Abstract:

- **Paris Dolph**, Laboratories Northwest/Multicare Health System, Tacoma, WA, USA
- Title: Evaluating neonatal minimum volumes with Abbott Architect c8000 and Sysmex XN

Outstanding Contributions to Pediatric Materna-Fetal Laboratory Medicine:



Piero Rinaldo, MD, PhD, Professor of Laboratory Medicine and Pathology and T. Denny Sanford Professor of Pediatrics, Mayo Clinic, Rochester, Minnesota, USA

2017 PMF Division Executive Board:

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