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COMMENTARY

Progress towards Rapid Detection of Measles Vaccine Strains: a Tool To Inform Public Health Interventions

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ABSTRACT Rapid differentiation of vaccine from wild-type strains in suspect measles cases is a valuable epidemiological tool that informs the public health response to this highly infectious disease. Few public health laboratories sequence measles virus-positive specimens to determine genotype, and the vaccine-specific real-time reverse transcriptase PCR (rRT-PCR) assay described by F. Roy et al. (J. Clin. Microbiol. 55: 735–743, 2017, https://doi.org/10.1128/JCM.01879-16) offers a rapid, easily adoptable method to identify measles vaccine strains in suspect cases.

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A highly contagious viral disease that affects only humans, measles causes an acute rash illness that is characterized by high fever, cough, coryza, conjunctivitis, and a generalized maculopapular rash. Complications of measles may be severe in 5% to 25% of cases, especially in infants and populations with high levels of malnutrition, and include otitis media, pneumonia, encephalitis, including rare cases of late-onset encephalitis (subacute sclerosing panencephalitis), and death (1, 2). Measles is a lipid-coated *Morbillivirus* in the family *Paramyxoviridae* and has a 16-kb, nonsegmented, negative-sense, single-stranded RNA genome that encodes 6 structural and 2 nonstructural proteins. The most abundant structural protein is the highly conserved, 525-amino-acid nucleocapsid (N) protein, which encapsulates the genome (3). The N gene mRNA, as the most abundant viral transcript in infected cells, is an effective target for molecular diagnostics (4, 5).

Prior to the introduction of the first measles vaccine in 1963, measles was a common childhood exanthema, resulting in approximately 30 million cases and over 2 million deaths globally each year (6). One half century later, due to the widespread use of a safe, inexpensive, and effective measles-containing vaccine, measles is considered a candidate for global eradication (7). Measles vaccines contain live attenuated virus, and although vaccine recipients may shed virus, transmission from vaccinees to contacts has not been documented (8, 9). Measles antibodies develop in 95% of children over 12 months of age, and mass vaccination efforts since 1980 have resulted in a dramatic decrease in global measles incidence to an estimated 256,000 cases and 134,000 fatalities in 2015 (1, 10). Measles was recently declared eliminated from the Americas and Australia, and many other countries are on track to eliminate the disease by 2020 (10, 11). However, measles remains endemic in some countries, where it continues to cause widespread disease in underimmunized populations and provides reservoirs for importations into countries where measles has been eliminated (1, 12). Due to the highly infectious nature of the virus and the potential for severe disease, when importations or outbreaks occur, an effective public health response is essential.

Expensive and highly labor-intensive, the typical public health response involves patient isolation, laboratory testing, contact tracing, and providing postexposure prophylaxis to high-risk contacts when indicated (13). The vaccine is effective as a postexposure prophylaxis if administered within 72 h of exposure, making it a useful tool for controlling outbreaks. However, approximately 5% of vaccine recipients de-

velop rash and fever 8 to 12 days after vaccination, symptoms that are clinically indistinguishable from those of infections by wild-type measles virus, which can confound an outbreak investigation (14). Knowing that a suspected measles patient was recently vaccinated does not eliminate the concern for a true case; indeed, some may be actual measles cases, as has been seen in previous outbreaks (15–17). The ability to rule out wild-type measles infection in a suspect case puts the brakes on this costly and time-intensive intervention.

Laboratory diagnostics are central to a timely and appropriate public health response to suspect measles cases, and real-time reverse transcriptase PCR (rRT-PCR) assays are a useful and rapid diagnostic tool. The current diagnostic measles rRT-PCR assay (MeV RT-qPCR) used in many public health and reference laboratories detects all genotypes of measles, including the five current vaccine strains (3, 4). While this assay is useful for rapidly diagnosing measles, it cannot differentiate vaccine from wild-type strains. To determine if a suspect case is due to a recent vaccination, the current testing rubric in most reference laboratories involves labor- and time-intensive genetic sequencing.

Genotyping measles virus strains is a critical component of the World Health Organization (WHO) Global Measles and Rubella Laboratory Network (GMRLN), a network of over 700 laboratories that supports surveillance in over 190 countries using standardized protocols (18–21). There are 24 recognized genotypes of measles based on N-450, a 450-nucleotide sequence of the carboxyl terminus of the N gene (20). The curated Measles Nucleotide Surveillance (MeaNS) database serves as a repository of these sequences and provides a mechanism to compare circulating strains (22). Molecular characterization benefits elimination efforts by providing useful information about global strain movement. Through these efforts, the WHO GMRLN has determined that, of the 24 known genotypes, only 6—B3, D4, D8, D9, G3, and H1—have been detected since 2011; there are 11 genotypes that are considered inactive (17, 20). In addition to providing important data to support measles elimination efforts, genotyping also can distinguish vaccine strains from wild-type strains and currently is the most commonly used approach for discrimination. As progress is made toward measles elimination, there is increased interest in rapidly differentiating vaccine from wild-type strains (15, 16). However, with the current recommended Sanger sequencing-based approach, results typically are not available for several days after the initial measles diagnosis, which often is too late to allow early termination of contact tracing efforts in cases of a measles vaccine reaction.

Roy and colleagues have described a newly designed assay for the rapid detection and identification of measles vaccine strains in clinical specimens (17). Primers and a locked nucleic acid (LNA) probe for this TaqMan rRT-PCR assay (MeVA RT-qPCR) were designed using available genotype A sequences. The LNA probe targets a 23-base sequence that is the same among vaccine strains but has 1 to 5 nucleotide differences from sequences of wild-type strains within the highly conserved amino terminus of the N gene (3). The narrow specificity is intended to eliminate the need for sequencing to confirm a vaccine-related event rather than a wild-type infection. Assay performance was assessed for sensitivity and specificity in three large measles reference laboratories using two PCR platforms that are commonly used in many public health and reference laboratories.

The ability of MeVA RT-qPCR to discriminate between the 5 current vaccine strains and all but 5 of 24 wild-type genotypes was assessed. Casting a broad net, a total of 370 clinical samples, viral isolates, and synthetic RNA were tested that covered a diversity of genotypes, including 13 that are no longer in circulation (20). Under optimized assay conditions, the specificity of the assay was excellent at 99%. Although one wild-type D5 strain was incorrectly identified as a vaccine strain, this genotype has not been detected since 2009; however, while this genotype may no longer be circulating, the nonspecific detection of this wild-type strain highlights one of the challenges in molecular assay design.

While this assay is highly discriminatory for measles vaccine strains, the somewhat lower sensitivity of 94% hampers its potential use as a diagnostic assay. The vaccine-specific assay performed with approximately 10-fold-lower sensitivity than the MeV RT-qPCR assay that is widely used by public health and reference laboratories. A similar result was seen with clinical samples tested in both MeV and MeVA RT-qPCR assays. Thus, some vaccine-related cases may be falsely identified as wild type. As the authors point out, due to the lowered sensitivity, this assay does not yet preclude the subsequent need for sequencing to confirm a MeV RT-qPCR-positive/MeVA RT-qPCR-negative specimen as wild type. A more expanded multicenter evaluation of these two assays against a defined set of clinical samples may help set a maximum threshold cycle (C_T) value above which all MeV RT-qPCR-positive/MeVA RT-qPCR-negative specimens should be sequenced, so that vaccine strain specimens will not be misidentified. One caveat to this continued reliance on sequencing is that when MeV RT-qPCR C_T values are $>$35, genotyping can be challenging due to the low viral load. Similarly, since only specialized laboratories perform N-450 sequencing, the need to ship specimens to a reference laboratory for confirmation will further delay confirmation of identification of a case strain as a vaccine strain or a wild-type strain.

A rapid, real-time RT-PCR approach to discriminating between vaccine and wild-type strains of measles virus as we approach global measles elimination is an attractive alternative to genetic sequencing. Not only would it eliminate the time and expense for additional laboratory testing, but it would also halt the need for extended epidemiological investigations when vaccine strains are identified. One important point to consider, prior to adoption of this targeted TaqMan assay approach to vaccine strain discrimination, however, is whether the assay as designed can adequately distinguish among strains in a more diverse set. Not all strains of a given measles virus genotype are genetically identical, and even within the highly conserved N gene, nucleotide differences can be seen within a circulating genotype in a given outbreak. Although Roy et al. analyzed a large number of samples, the degree of strain diversity within a given genotype was not described. A larger multicenter study using additional measles virus strain repositories would provide the opportunity to better assess strain diversity and thus assay robustness.

The study by Roy et al. also highlighted the importance of thoroughly evaluating a new assay when it is adopted for use: increased nonspecificity was observed when MeVA RT-qPCR was run with a different rRT-PCR kit at one of the laboratories. Since the Invitrogen kit that gave false positives from three different wild-type genotypes is recommended for the MeV RT-qPCR, laboratories that currently use this kit for measles diagnosis may need to change to the MeVA RT-qPCR-recommended Qiagen kit or run the two assays separately. Measles can be a devastating disease, either in its acute form or due to long-term or late sequelae, and to misdiagnose a true case as a vaccine-related event may unwisely put other vulnerable patients at risk.

Although some questions remain, the multicenter study by Roy et al. provides an important step toward rapidly identifying measles vaccine strains in suspect cases, especially in outbreak settings, when suspicion for wild-type strains is high. As countries move toward and achieve measles elimination, it will behoove public health laboratories to rapidly and accurately determine the status of a suspect case—is it a true infectious case, or is it due to a recent vaccination? While the advent of this and other vaccine-specific assays pushes the field in this direction, further evaluation with a greater diversity of circulating strains is warranted.

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