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POINT-COUNTERPOINT

Role of Rapid Immunochromatographic Antigen Testing in Diagnosis of Influenza A Virus 2009 H1N1 Infection[▽]

Rapid antigen testing using immunochromatographic devices has become a diagnostic mainstay for detection of influenza virus and respiratory syncytial virus, the two major viruses infecting the respiratory tract. Recent studies have indicated that poor performance in the detection of the novel influenza A virus 2009 H1N1 should preclude their use. A survey of influenza diagnostic methods available on ClinMicroNet and Division C, the two ASM list servers, revealed that, despite this reported poor performance, a majority of the laboratories surveyed intend to continue to offer this testing during the current influenza season. Our two experts have been asked to consider the following question: what is the role of rapid immunochromatographic antigen testing in the laboratory diagnosis of influenza A virus infection during the current 2009 H1N1 pandemic?

POINT

Continuation of testing for influenza virus in the wake of the 2009 H1N1 outbreak by using rapid influenza antigen detection tests (RIDT) is the practice of 84% of community hospital laboratories and of hospitals in several other categories, according to a survey by Selvarangan et al. (7). Although that survey noted a significant decrease in the number of academic institutions performing RIDT, the rate of decrease (30%) appeared to be related to the ability of those laboratories to switch to PCR testing (27% increase). The potential place of RIDT in laboratory diagnosis of influenza A virus infections ranges from a nonexistent role to its use as a frontline, stand-alone method, with variations between those extremes, including its use as a screening test supplemented with methods of greater sensitivity, such as culture or PCR, for implementation when the RIDT result is negative. Taking the position that the use of RIDT for influenza virus infection diagnosis should not be abandoned is based largely on the following three considerations.

Sensitivity of RIDT is an elusive concept. That the performance of RIDT is poor is a generalization that is supported by findings presented in several published reports. However, the range of testing sensitivities reported (11% to 80% [1, 3]) is so vast that it raises the issue of determining what is most important in achieving the best possible sensitivity: is it technical factors in specimen collection or test performance, use of the right kit, testing of the right patients (in the multiple categories of age, stage of illness, and infecting subtype), or some combination of these factors? Each of these deserves the attention of any laboratory considering use of these tests. Pollock et al. (6) recently reported their experience with direct immunofluorescence (DFA) versus PCR for diagnosing 2009 H1N1 infections in a symptomatic group (mean age, 44 years) of individuals whose nasopharyngeal specimens had been collected by trained respiratory therapists. The findings indicated that when very stringent specimen quality criteria were applied, such as specifying ≥60 columnar epithelial cells per sample, the DFA sensitivity approached that of PCR (i.e., 100%). From this, the conclusion can be extrapolated that higher-quality specimens, i.e., epithelial cells from the posterior nasopharynx, are more likely to yield detectable antigen than specimens of lower quality. Additional enhancements of specimen quality are achieved by using flocked swabs (2) and by ensuring the compatibility of viral transport media with the test kit employed, as well as by the obvious precaution of ensuring that each additional step of the RIDT is performed in accordance with the specifications provided in the package insert. Sensitivity may vary according to influenza subtype among kits from different manufacturers (4, 5), and much of the reported experience points to lower sensitivity for 2009 H1N1 than for seasonal H1 and H3 influenza virus. At least one report, however, suggests test sensitivity for 2009 H1N1 similar to that seen with seasonal influenza A virus (5). My own laboratory experience using Directigen EZ Flu A+B (and two other kits on a more limited basis) supports both of the observations mentioned above. With respect to detection of influenza A virus, subsequently subtyped as 2009 H1N1, the sensitivity and specificity of the Directigen EZ Flu A+B test in our hands compared to Luminex xTAG Respiratory Virus Panel (RVP) and CDC PCR assays were 76.6% and 98.7% during the first wave of H1N1 infections in the spring of 2009 (Table 1).

Not all influenza-like illnesses are attributable to influenza virus infections. This concept applies to the issue of whether or not to perform any testing at all when the only method available is RIDT. While a clinical diagnosis of influenza without testing may be appropriate for those presenting with mild symptoms and for those who are

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TABLE 1. Comparison of EZ Flu A+B RIDT and PCR results of tests by both methods for influenza A virus in 1,599 samples from pediatric patients of the Children's Medical Center, Dallas, Texas, between 27 April and 31 July 2009[a]

Assay and result	No. of patient samples with indicated result	
	RIDT+	RIDT−
PCR+	154[c]	47[b,c]
PCR−	2[c]	1,396[b]

[a] All results that were positive for influenza A virus by either RIDT or Luminex were subjected to testing by the CDC typing assay.
[b] Data represent the results of testing by the Luminex xTAG RVP assay.
[c] Data represent the results of testing by the CDC 2009 H1N1 typing assay.

not at high risk for complications, greater diagnostic specificity can be achieved by performing an RIDT. Moreover, for those for whom testing is indicated, RIDT can quickly confirm influenza A virus infections. A portion of the negative results would still be positive for influenza A virus by a more sensitive method, but a large portion would also likely contain other respiratory viruses, as experienced in the first wave of 2009 H1N1 infections last spring. The use of RIDT in conjunction with a multiplex assay was an effective approach during this time to manage the high volume of tests and extensive cocirculation of viruses. Overall, 24% of 8,470 patient samples that were tested over the course of 14 weeks contained additional viruses, including rhinoviruses or enteroviruses (1,010 samples), metapneumovirus (365), parainfluenza viruses (297), adenovirus (76), respiratory syncytial viruses (23), influenza B virus (13), and influenza A H3 virus (7).

Faster is better, and tests of greater sensitivity are not readily available in the majority of laboratories. As clinical microbiologists, we must carefully weigh the benefits of complex assays that yield the most accurate and comprehensive results against the pragmatic viewpoint of the majority of clinicians who place considerable importance on obtaining a result while a patient is waiting in an emergency department or a clinic setting so that clinical management plans can immediately be made. Since the specificity is good, especially during periods of moderate to high prevalence of disease, the use of RIDT seems justifiable to satisfy rapid testing needs. In the absence of such an option, the results of the Selvarangan survey would seem to imply that other methods available in nonacademic or nonreference clinical laboratories are practically nonexistent. Only 20% of those laboratories, in contrast to two-thirds of academic centers, have implemented a molecular testing protocol subsequent to the emergence of 2009 H1N1, and none reported offering PCR for deployment in the settings mentioned above.

Conclusions. Achievable sensitivity of >70% for detection of 2009 H1N1 by the use of RIDT is possible. RIDT kits are not interchangeable with respect to sensitivity. Of the kits currently marketed in the United States, at least one (and possibly others) can meet the threshold of >70% sensitivity, provided specimen adequacy is assured. Given the likelihood of long-term circulation of the 2009 H1N1 virus in the human population, well-controlled studies to ascertain kit-specific performance characteristics for detection of this virus compared to seasonal influenza viruses would provide valuable information. Individual laboratories should consider periodically monitoring specimen adequacy, both when verification of the performance characteristics of the RIDT kit chosen for use in their own patient population is performed and when a major shift in virus subtype occurs. As next-generation RIDT kits become available, the expectations of laboratorians with respect to new products from manufacturers must be for improved performance characteristics that are demonstrated in the identification of novel and continuously circulating subtypes of influenza viruses, with claims of performance improvements limited to those kits that provide the most reliable results. In the meantime, there continues to be a role for RIDT, because, in addition to meeting rapid turnaround time needs, those tests can be employed in a cost-effective manner in conjunction with methods of greater sensitivity for backup or for use in multiplex testing formats.

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COUNTERPOINT

For more than 20 years, rapid influenza antigen diagnostic tests (RIDTs) have been the mainstay of influenza testing, due to the ease of use, the rapid turnaround time, and the limited number of hospital laboratories that offer comprehensive viral diagnostics. RIDT performance character-

istics were established through controlled clinical trial studies for U.S. Food and Drug Administration (FDA) clearance. To obtain optimal performance data, RIDT trial studies are designed so that only patients with defined symptoms (generally, a minimum of two to three symptoms) who have been symptomatic for less than 5 days are selected, to ensure that virus is being shed at reasonably high and detectable titers. The predominance of pediatric samples, especially from children less than 5 years of age, enhances RIDT performance, as children shed higher titers of virus (5, 9) for longer time periods than do adults (9). Low levels of virus shedding are particularly problematic in diagnostic testing for geriatric patients, a group frequently tested using RIDTs when influenza outbreaks in chronic care facilities are suspected. Sample types used in trials (nasopharyngeal swabs, washes, and/or aspirates) are restricted to specific specimen types, collected, transported, stored, and tested under tightly controlled conditions, within specific time frames. Imperfect "gold standards" for these evaluations are direct immunofluorescence (DFA) and/or viral culture, although it is well-documented that nucleic acid amplification tests (NAAT) are more sensitive for detection of influenza viruses (1, 4, 7, 8, 10).

Keeping this in mind, RIDT users must be cautious in assuming that manufacturer performance claims match RIDT performance in routine clinical settings, including Clinical Laboratory Improvement Amendment-certified laboratories, outpatient clinics, emergency departments, or physician offices. In contrast to controlled trials, most laboratories do not have clinical information and test all specimens submitted. Testing includes samples collected beyond the time frame for virus detection and from the "worried well," as was done frequently for outpatients during the influenza A virus 2009 H1N1 pandemic. Sample collection is problematic, as many practitioners are not appropriately trained to collect nasopharyngeal specimens and patients are reluctant to do so, resulting in submission of suboptimal nasal or throat swabs. Uncontrolled sample transport and storage can affect test results. Finally, testing is performed in a variety of clinical settings by diverse personnel with various levels of technical skills, with or without appropriate training and control procedures, as Clinical Laboratory Improvement Amendment requirements have been waived for many RIDTs.

Antigenic shift or drift, which occurred during the 2007 to 2008 influenza season, or the emergence of a new strain, as seen with the 2009 H1N1 pandemic, can compromise RIDT performance. During the 2007 to 2008 season, after complaints that the RIDT (BinaxNOW Influenza A&B; Binax, Inc., Scarborough, MA) that we use in the North Shore-Long Island Jewish Health System was performing poorly, we compared BinaxNOW to the 3M Rapid Detection A+B test (3MA+B; 3M Medical Diagnostics, St. Paul, MN), DFA (Diagnostic Hybrids [DHI], Athens, OH), and R-Mix culture (DHI) (6). Sensitivities of BinaxNOW and 3MA+B for influenza A detection were 48.9% and 72.3%, respectively, and for influenza B detection were 36% and 88%, respectively. This was a substantial decrease in BinaxNOW sensitivity from our initial evaluation (for influenza A virus, 75%; for influenza B virus, 72%) performed years previously. This decrease in BinaxNOW sensitivity may have been related to an antigenic change in the influenza viruses that season. RIDTs are not regularly modified by manufacturers to accommodate antigenic changes that may reduce RIDT sensitivity. Most testing sites are not able to reevaluate RIDT performance yearly and may continue to use RIDTs with suboptimal performance.

The 2009 (H1N1) pandemic provided an enormous opportunity to evaluate RIDT performance under real-life conditions. Studies demonstrated that RIDTs detected 2009 (H1N1) (1, 3, 4, 7, 8, 10) but that sensitivities ranged widely (10% to 70%) (1, 4, 7, 8, 10). In a large analysis of RIDT performance that included 11 hospital laboratories and a core facility that serves a large number of outreach clients, sensitivities for influenza A virus detection versus DFA/R-Mix culture results were 9.6% for BinaxNOW and 40% for 3MA+B (7). Overall sensitivity was 17.8% (for both RIDTs combined) compared to NAAT (xTag Respiratory Virus Panel [RVP]; Luminex Molecular Diagnostics, Toronto, Canada) (7). Studies with a more limited sample number, performed by the Centers for Disease Control and Prevention (CDC) (1) and by Vasoo et al. (10), determined that the sensitivities of RIDTs compared to NAATs were 38.3% and 40%, respectively, for BinaxNOW, 46.7% and 49%, respectively, for the EZ Flu A+B test (BD, Franklin Lakes, NJ), and 53.3% and 69%, respectively, for the Quickview Influenza test (Quidel, San Diego, CA).

Although RIDT specificities and positive predictive values are high when testing is performed during the influenza season, false positives also occur. Conversely, due to poor sensitivities, negative predictive values can be low (48.5%) (7). Unfortunately, many clinicians are not aware of the poor performance of RIDTs and rely on the results to make clinical decisions. Therefore, professional societies, regulatory agencies, and the CDC recommend that, in the appropriate clinical situations, RIDT-negative samples should be subjected to tests of greater sensitivity, such as DFA, culture, or NAAT (2). Despite a negative RIDT result, a diagnosis of influenza should be considered on the basis of a patient's clinical presentation, and, if indicated, empiric antiviral treatment should be considered.

RIDTs cannot differentiate influenza A virus subtypes (seasonal H1 and H3 or 2009 H1N1). Some FDA-cleared NAATs differentiate seasonal H1 and H3 (Luminex RVP, ProFlu-ST [Gen-Probe Prodesse, Waukesha, WI]) and/or 2009 (H1N1) (ProFlu-ST, Influenza A H1N1 [2009] real-time PCR [Focus Diagnostics, Cypress, CA]). Subtyping may be important for seriously ill hospitalized patients, as >99% of the current seasonal H1 strains are oseltamivir resistant (cdc.gov/flu/). Although little resistance has been reported for

2009 (H1N1), there is the potential that oseltamivir resistance may rise and that subtyping may be necessary to assure appropriate antiviral selection.

With the development of fully automated, easy-to-use molecular systems such as GeneXpert (Cepheid, Sunnyvale, CA), Jaguar (HandyLab, Ann Arbor, MI), and the FilmArray system (Idaho Technology, Salt Lake City, UT), highly sensitive NAATs with subtyping capability will shortly be available for all laboratories, regardless of size and technical expertise. Although the cost may be higher (approximately $35 to $60 per test) than that of RIDTs (approximately $10 to $20), the enhanced performance far outweighs the added expense. Performing one NAAT would be less expensive overall than performing RIDTs with the necessity of subjecting negative samples to additional testing. Time to results (1 to 4 h) would be appropriate for therapeutic and infection control intervention, whereas the additional testing required with negative RIDTs would severely impact the time to results. Studies have demonstrated that rapid positive results for the detection of respiratory viruses can lead to reduced antibiotic use and to shorter duration of antibiotic therapy and length of stay.

As laboratory experts, we need to balance many factors in selecting a diagnostic test, including performance, turnaround time, convenience, cost, and technical expertise required. However, above all, we have an obligation to provide accurate test results. Although under certain situations and for certain patient populations (i.e., in the field of pediatrics), the use of RIDTs may initially be an acceptable practice, we must clearly understand the limitations of RIDTs and provide that information to our clinicians. As sensitive, rapid, and easy-to-use NAATs with subtyping capability become available, we must discourage the use of poorly performing RIDTs.

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SUMMARY

Points of agreement:

- RIDTs are best done in the pediatric population early in the disease course, during periods when disease incidence is high.
- High-quality specimens containing ciliated epithelial cells are essential to the accuracy of this test, so it is important to ensure that the individuals who collect these specimens are adequately trained.
- Because of the comparatively poor sensitivity of RIDTs, confirmatory testing with more-sensitive NAAT tests or culture should be performed on RIDT-negative specimens when a diagnosis of 2009 H1N1 needs to be established.
- RIDTs do not allow subtyping, which is important given the different antiviral susceptibility characteristics of the seasonal versus 2009 H1N1 viruses.

Issues to be resolved:

- How well RIDTs actually work in clinical settings rather than in research settings, especially in times of antigen shift or even drift, is not well established. It is incumbent upon manufacturers to provide this information on a continuing basis, with at least annual updates of RIDT package inserts.
- Only a minority of community hospital laboratories have the capability to perform influenza NAATs of greater accuracy. It is important that future iterations of influenza NAATs should be formatted for broad use, including in the community hospital setting.

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The views expressed in this feature do not necessarily represent the views of the journal or of ASM.