Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company’s public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Issues for reporting results

Robert Lannigana, *, James B. Mahonyb

aLondon Health Science Centre and Department of Microbiology and Immunology, University of Western Ontario, London, Ontario, Canada
bDepartment of Pathology & Molecular Medicine, McMaster University and St. Joseph’s Healthcare Hamilton, Ontario, Canada

Keywords: New technologies; Diagnosis; Viral respiratory infections; Reporting practices

1. Introduction

The implementation of new diagnostic tests in clinical laboratories presents many challenges to the lab staff, including how the new test results should be reported out to ordering physicians. This paper deals with issues around reporting RVP test results and provides some general recommendations about how to report results obtained with the xTag RVP. Because multiplex PCR technology is being newly applied for the diagnosis of respiratory viral pathogens there are no published guidelines for reporting results. As such the recommendations made in this paper are of necessity personal opinions of the authors, and only time and experience will determine whether they are correct.

2. What is expected from the laboratory?

In order to position the RVP into the armamentarium of diagnostic tests in the clinical laboratory for the support of programs designed to monitor, diagnose and control respiratory viral infections it is worthwhile to look briefly at what is currently being asked of the laboratory.

Febrile respiratory illnesses due to viruses are a challenge to manage and control for a number of reasons. They are common infections. A wide variety of viruses are involved in these conditions and they all have very similar symptoms and signs at the onset of clinical disease making clinical differentiation unreliable in most situations. Control measures are most effective if they are instituted early. Consequences of respiratory virus infections range from the relatively trivial to possible global pandemic in the case of a new influenza strain; therefore the early, specific detection of the etiological agent either to rule out or rule in a specific pathogen is paramount to instituting appropriate measures for control. The measures that have to be put in place to manage a possible case of avian influenza A or SARS in a human, as opposed to a RSV or parainfluenza virus infection, are substantially different by many orders of magnitude in terms of resources, so the need for accurate, rapid diagnosis either to confirm or exclude a specific viral etiology is crucial. Many of the control measures that need to be put in place are predicated on laboratory results, so confidence in the accuracy of the results is of the utmost importance to laboratorians, clinicians and other health-care workers involved in infection control. These difficulties are compounded when one considers that the initial presentation of the illness usually occurs in the community setting where full-service diagnostic facilities are usually lacking, and if specimens have to be sent to a reference laboratory for diagnosis it is often not feasible to delay the implementation of at least some control measures until definitive results are available. This is a potentially costly and traumatic experience for the community and the individuals involved. Lastly the concerns for the appearance of emerging pathogens, the threat of bioterrorism and the ease with which pathogens, especially respiratory viruses, can be transferred via international travel put additional pressures on the laboratory to provide specific diagnoses in a timely fashion as well as monitor disease activity.

3. Diagnostic tests available

The widespread availability of rapid influenza tests make them attractive for on-site or point-of-care use for community-based hospitals without specialized diagnostic virology labs. Manufacturers and developers of these tests, having recognized the need for such testing, have released many products that are easy-to-use in a general laboratory setting and are capable of detecting influenza and RSV. Tests to detect influenza A and B are perhaps most commonly in use, with respiratory syncytial virus (RSV) detection also being available in many labs that deal with a significant number of pediatric clients. The shortcomings of these rapid EIA tests have been well described, so they will only be covered in generalities here, as follows. Firstly, while the specificity of the tests is generally acceptable, so a positive result in association with the appropriate clinical picture is useful,
the sensitivity of rapid tests that do not amplify the target depends on the amount of the viral antigen present in the clinical sample, and a negative result cannot be used with confidence. Given their low sensitivity, additional testing is required to detect low-level positives missed by rapid EIA tests. Rapid tests have only been developed for influenza and RSV while often the need is to rule in or rule out a wider variety of respiratory viral pathogens not detected by rapid tests. In some virology labs a rapid test is often used as a first step and EIA-negative specimens are tested by DFA and if negative by shell vial culture (SVC) or traditional culture to broaden the spectrum of viruses that can be detected. All this being said these tests, if positive, do provide a head start for more specific control measures to be implemented than if no testing were available.

It is well recognized however that culturing for SARS Coronavirus or H5N1 strains of Avian influenza virus is too hazardous for most facilities and requires a BSL3 laboratory. Indeed, culture to detect a wide range of respiratory viruses including Rhinovirus and Metapneumovirus would require several cell types and different temperatures. The sensitivity of even the best culture protocols used by research laboratories is suboptimal and the predictive value of a negative culture is therefore low. Nucleic acid amplification based tests are required therefore to detect the full range of respiratory viruses that culture cannot detect and to increase the predictive value of a negative test.

It is probably fair to say that all labs involved in providing diagnostic services for the detection of respiratory viral infections have been eagerly awaiting a commercially available testing system that is capable of detecting a wide variety of viruses with a high degree of sensitivity and specificity, from a single, easily obtainable clinical specimen, in a clinically useful time frame, without having to culture for potentially dangerous pathogens and without requiring large amounts of additional resources. The RVP test appears to meet several if not all many of the criteria.

4. Issues with respect to reporting of RSV results

In order to highlight issues relating to the reporting of RVP results let us consider some real life experiences with RVP testing in a variety of clinical scenarios and use these experiences to formulate some general recommendations for results reporting. As background to these clinical scenarios our laboratories use the following methods for processing clinical samples for the detection of respiratory viruses. Nasopharyngeal specimens are collected using a flocked swab (Copan or Starplex Starswab Multitrans Collection and Transportation System) inserted into the posterior nasopharynx, which is then placed in the provided viral transport medium. On receipt in the lab the sample is transferred to a biological safety cabinet and vortexed to dislodge any cellular material from the swab; the swab is then discarded and the sample centrifuged. The transport medium is then decanted and saved. Part of the cellular pellet is re-suspended in a small volume of the virus transport medium and used to make smears for DFA (influenza A and B, parainfluenza types 1, 2 and 3, RSV and adenovirus) using a direct fluorescent antibody kit (Diagnostic Hybrids Inc. or Chemicon Int. Light Diagnostics) (this also serves as a check for the sample quality). The decanted medium is then added back to the remaining pellet and the cells are re-suspended and set up for shell vial culture (R-mix shell vials, Diagnostic Hybrids, Athens, Ohio). The remaining sample could then be available for RVP testing (see below for positioning of test). Results from the DFA test are usually reported within 3 hours of receipt in the lab and the shell vial cultures are reported after 16–24 hours, usually the next morning before 11 AM. The viruses detected using EIA, DFA, and SVC are influenza A, B, parainfluenza virus types 1–3, adenovirus, RSV and in some labs human Metapneumovirus since monoclonal antibodies have recently become commercially available. We would not detect influenza H5 strains, SARS, Coronaviruses 229E, OC43, NL63, HKU1, enterovirus or rhinovirus, or subtype influenza.

How to position the RVP test in the clinical laboratory may depend on the needs of the laboratory and its clients. The RVP could be used as a front line test to replace all other tests (EIA, DFA and cultures) or it could be used to test EIA- or DFA-negative specimens. This is discussed elsewhere in this volume and will not be discussed here other than to put the RVP testing in the appropriate context for the following clinical scenarios.

Six scenarios that illustrate various issues with respect to reporting of RVP results are (Table 1):

Scenario 1. A traveler returning from an endemic area for avian influenza H5 and SARS with a clinical picture compatible with an acute viral pneumonia.

Scenario 2. An outbreak in a long-term care facility that was not diagnosed using routine testing.

Scenario 3. Situations in which two viral pathogens were identified.

Scenario 4. Unidentified influenza A detected.

Scenario 5. Retrospective diagnosis of previously undiagnosed viral illness.

Scenario 6. Outbreaks seemingly related to one another in which different etiologies were determined using RVP.

In all six scenarios issues arose regarding how to report the results, and from these occurrences some recommendations can be made. In scenario 1, where a traveler returned from a SARS and H5N1 endemic area, the issue was primarily to rule out H5 avian influenza and SARS. We initially reported that neither of these agents had been detected. This prompted a flurry of calls to the laboratory asking if any other viruses had been found. The first recommendation would be that the report clearly indicate all the viruses that are tested for and the results for each virus indicated as “detected” or “not detected”.

In scenario 2 we had a situation in which our routine testing failed to identify the cause of an outbreak in
a long-term care facility. Subsequent to our negative results the samples were sent through the RVP which identified the causative agent which in this case was human metapneumovirus (hMPV) that would not normally have been detected on routine testing. In this situation we ended up sending amended reports to the charts of those patients in which metapneumovirus was detected indicating that “on further investigation human metapneumovirus was detected”. We also informed the infection control service directly that we had identified a causative agent even though hMPV was not in fact a reportable disease in our jurisdiction. The second recommendation then is that even if results are available after a previously reported negative and that the outbreak has been successfully managed, the amended report listing all the respiratory viruses detected or not detected should become part of the clinical record so that retrospective studies looking at the epidemiology of suspected respiratory viral outbreaks will have complete records to correlate with the clinical presentation.

In scenario 3 two viral pathogens were isolated from a single sample, one being an enterovirus/rhinovirus. The attending physician had one or two responses. Either he or she called the lab to discuss the possible interpretation of the results or they assumed that the enterovirus/rhinovirus was insignificant and ignored it. This second response may be because of how we were using the RVP test at the time as it was essentially used as a backup to our routine testing so the clinician already had a diagnosis in hand. If the RVP became the front line test the question of the significance of two pathogens would likely occur more often. The third recommendation then is that when more than one pathogen is detected they should all be reported and become part of the clinical record. The report should not say “two respiratory viruses detected” but rather should list all the viruses tested for and whether they were detected or not detected. One of the surprising results in using the RVP is that samples containing more than one pathogen were much more common than previously supposed. In our experience with the RVP test between 5 and 8% of positives are dual infections with two or more viruses present (Mahony et al., 2007). A careful review of the literature indicates that dual infections detected by a combination of DFA, culture and NAAT have been reported at frequencies as high as 30–50% (Chiu et al., 2005; Kuypers et al., 2007; van der Hoek et al., 2007). It is not possible to interpret the significance of dual infections at this time; this will have to be evaluated in appropriate clinical studies.

In scenario 4, an untyped influenza A virus is detected by a signal on the pan-reactive Influenza A matrix bead in the absence of a signal on the H1 or H3 subtyping beads. The RVP assay was developed with this scenario in mind to identify new subtypes such as H5N1 or H7N2 circulating in the human population. The matrix gene PCR target is highly conserved so that all influenza A subtypes will be detected (Mahony et al., 2007). In developing the test it was noted that that matrix PCR was more sensitive than the H1 or H3 PCR so that occasionally an H1N1or H3N2 may be detected by the matrix bead in the absence of a signal on the typing bead. In this scenario the influenza strain should be sent to a reference laboratory for subtyping.

In scenario 5 the RVP was used retrospectively to investigate the etiology of previously undiagnosed cases, sometimes from months earlier. We felt it prudent to phone the attending physician prior to sending the report so they would fully understand why the result was now being reported. We left it to the discretion of the attending physician to inform the patient rather than the lab informing the patient directly. We did this for two reasons. First the physician ordered the test, not the patient, so the physician remains the laboratory client, and secondly we were not
aware of the outcome of the case whereas the treating physician was. Again if the RVP is adopted by the lab and becomes the primary test for respiratory viral infections this scenario would not occur in future, though labs doing a look back study may be faced with this scenario. However it is not unlikely that in the future other new technologies will become available that may be used in a similar fashion for retrospective diagnosis so the recommendation would still be to contact the attending physician prior to submitting a report to the clinical record.

Scenario 6 also relates to the management of outbreaks in a long-term care facility and perhaps requires more extended explanation of the actual situation. Two outbreaks occurred on separate but closely associated wards in a long-term care facility. On one ward the outbreak was limited to two individuals, whereas on the other ward five individuals became involved in the outbreak. It was assumed initially, as these outbreaks had occurred at the same time, they were likely related to one another and probably had the same etiology. We were able to determine by direct testing that the outbreak involving two individuals was related to influenza A but the cluster of five patients on the other ward were negative for all pathogens tested for in our routine direct testing. At that time, both outbreaks were assumed to be of common etiology and preparations were being made to treat those individuals infected on both wards and also to prophylax the entire institution against influenza A. We ran all samples from both of these outbreaks through the RVP test and results were available later the same day. We were able to confirm influenza A in the two-patient outbreak, but the five patients who had been negative by our direct testing were all negative for influenza A but had coronavirus NL63 detected by the RVP, thereby establishing different etiologies for the outbreaks. As a result, management was modified to prophylax only those individuals on the ward where influenza A had been identified and simply use cohorting measures to deal with the other ward. Neither of these outbreaks spread any further than the wards concerned. It is often not possible to identify an etiological agent for all respiratory outbreaks and thus, if an etiology is found in any of the patients and the outbreaks are felt to be related, it is simply assumed that all individuals involved in the outbreaks have the same etiology. The RVP test provided a clear differentiation of etiologies and therefore the implementation of more appropriate infection control measures by providing accurate diagnoses. Perhaps as experience grows with the use of the RVP it will be recognized that more than one respiratory virus can circulate simultaneously in an institution. This is quite possible because in Hamilton in January of 2006 the RVP test identified 10 respiratory viruses co-circulating in the community. In this scenario there were no reporting issues but it does demonstrate the value of having RVP available both for management, as in this case, as well as better defining the epidemiology of respiratory viral infections in institutions.

In light of the need for good communication between the laboratory and its clients, physicians using a specific laboratory need to be informed when tests for new analytes or new tests for existing analytes are added to the testing menu. It is good laboratory practice to inform users by official memo about new tests that have been introduced. The memo should include a rationale for the new test and if possible a few facts on the new analytes (respiratory viruses) being tested, the methodology being used, how results will be reported (including any normal values or ranges), any action that may be required relating to infection control or public health, and the name and telephone number of a contact person in the laboratory who can address questions and/or concerns.

5. Summary

In summary we have described various clinical scenarios where we have used the RVP test, demonstrated its added value, and made recommendations about reporting results. As mentioned at the outset, these recommendations are our opinions and perhaps more widespread use of the RVP test will refine these initial suggestions. The recommendations however fall under two general principles that apply to all laboratory test results but are particularly important when introducing new diagnostic methods, namely: close communication by the laboratory with the ordering physician and other health-care workers that will be using the results for patient management, and the inclusion of all results in the patient record to allow for future clinical–pathological correlations to be carried out to refine the understanding of the epidemiology and management of disease states.

Conflict of interest statement

RL: honorarium for services. JBM: Stockholder, honoraria, patent applications (Luminex).

References

Chiu SS, Chan KH, Chu KW, Kwan SW, Guan Y, Poon LL, et al. Human coronavirus NL63 infection and other coronavirus infections in children hospitalized with acute respiratory disease in Hong Kong, China. Clin Infect Dis 2005;40:1721–9.

Kuijpers J, Martin ET, Heugel J, Wright N, Morrow R, Englund JA. Clinical disease in children associated with newly described coronavirus subtypes. Pediatrics 2007;119:e70–6.

Mahony J, Chong S, Merante F, Vaghoubian S, Sinha T, Lisle C, et al. Development of a respiratory virus panel (RVP) test for the detection of twenty human respiratory viruses using multiplex PCR and a fluid microbead-based assay. J Clin Microbiol 2007;45:2965–70.

van der Hoek L, Sure K, Ichorst G, Stang A, Pyrc K, Jebbink MF, et al. Human coronavirus NL63 infection is associated with croup. PLoS Med 2005;2(8):e240. Epub 2005 Aug 23. Republished in: Adv Exp Med Biol 2006;581:485–91.

S50 R. Lannigan, J.B. Mahony / Journal of Clinical Virology 40 Suppl. 1 (2007) S47–S50