Rubella Diagnostic Issues in Canada

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With the success of the rubella vaccination program, a goal for the elimination of rubella and congenital rubella syndrome (CRS) by 2010 has been established. To monitor the progress toward elimination, surveillance is critical. The laboratory plays an important role in both diagnostics and surveillance for rubella and CRS. In the elimination phase, there are particular issues and challenges that are important to consider when undertaking rubella diagnostics and surveillance activities. Although immunoglobulin (Ig) M serological testing is the primary diagnostic test used to confirm acute rubella infection, additional tests, such as paired IgG serological testing, molecular detection of rubella virus, and rubella IgG avidity testing need to be considered for confirming cases, depending on the clinical and epidemiologic context of a particular suspected rubella case.

Rubella IgM SEROLOGICAL TESTING

Similar to measles diagnostics, the cornerstone for the laboratory confirmation of rubella cases is IgM serological testing. A rubella IgM-positive result in a single serum sample is confirmatory for an acute rubella infection. However, there are several important issues to consider when interpreting rubella IgM results: test sensitivity, including timing of serum sample collection; test specificity; rubella disease prevalence; and the epidemiologic context.

Rubella immunoglobulin (Ig) M serological testing is the standard method for confirming acute rubella infection. However, additional testing, such as rubella IgG serological testing, and rubella virus detection and/or isolation are also useful for the laboratory confirmation of rubella infection. The epidemiologic context of rubella disease elimination efforts, as well as the laboratory diagnosis of rubella in pregnant women, present challenges, and multiple diagnostic approaches are often required.
an additional 100 dengue-positive serum samples, for a specificity panel of ~583 serum samples. There were no statistically significant differences (z-test) between the sensitivities (95% confidence intervals [CIs]) of the Meddens (76.8% [95% CI, 72.2%–81.4%]), Behring (75.9% [95% CI, 71.3%–80.6%]), Wampole (74.1% [95% CI, 69.3%–78.9%]), and Diamedix (76.1% [95% CI, 71.3%–80.9%]) assays. The timing of sample collection after rash onset is also important to consider because it affects the sensitivity of the assay. Recent studies to characterize and evaluate rubella IgM diagnostic tests show that most patients with acute rubella cases are IgM positive at ≥5 days after rash onset [3]. Therefore, an individual with a suspected rubella case in which serum samples collected <5 days after rash onset initially test IgM negative should have a second serum sample collected >5 days after onset for IgM retesting.

The difficulty of clinically differentiating rubella, measles, and other viral exanthems (including dengue, parvovirus B-19, and human herpesvirus-6 [HHV-6]) has been previously demonstrated [4–7]. In our analysis, there were no statistically significant differences (z-test) between the specificities of Meddens (94.3% [95% CI, 92.4%–96.1%]), Behring (95.2% [95% CI, 93.4%–96.9%]), Wampole (93.3% [95% CI, 91.3%–95.3%]), and Diamedix (92.5% [95% CI, 90.3%–94.6%]). However, it was certainly evident that the Wampole and Diamedix assays had more false-positive rubella IgM results (12 of 100 results and 20 of 100 results, respectively) than did Meddens (4 of 100 results) and Behring (3 of 100 results) for the dengue-positive panel. Although dengue is not an endemic disease in Canada, and thus dengue specificity issues for rubella IgM assays are not a significant issue in Canada, countries where dengue is endemic [8] should be aware of the specificity characteristics of the rubella IgM tests that are being used.

**INTERPRETATION OF RUBELLA IgM SEROLOGICAL TEST RESULTS BASED ON CLINICAL AND EPIDEMIOLOGIC CONTEXT**

In the elimination phase, the clinical and laboratory diagnosis necessary for case-by-case surveillance presents particular challenges as disease prevalence decreases. It has been shown that decreasing prevalence of disease associated with elimination programs results in a decrease in the positive predictive value of IgM serological test results, thus increasing the risk of false-positive results [9]. It is important to understand the sensitivity and specificity characteristics of the kit that is used as well as the epidemiology of other diseases with similar clinical presentation that may occasionally cause false-positive reactions for rubella. In Canada, suspected cases of rubella should also include measles and parvovirus B19 in the differential diagnosis. In some cases in children <2 years of age, HHV-6 (roseola) may also be considered. However, roseola is typically clinically diagnosed with the classic appearance of the maculopapular rash with the disappearance of fever [10]. In reality, the fever-rash illnesses considered in the differential diagnosis, and thus the diagnostic approach, will vary depending on the clinical presentation, the epidemiology (outbreak vs sporadic case), the history of vaccination, and the recent travel history.

The confirmation of rubella outbreak–related cases is relatively straightforward in that an IgM-positive result or an epidemiologic link to a confirmed rubella case is confirmatory. However, the confirmation of sporadic cases presents more of a challenge. In sporadic cases in which there is recent travel to an area with known or potential rubella activity and clinical symptoms compatible with rubella or measles, parallel or sequential testing for measles and rubella IgM is a reasonable approach. A positive result for rubella IgM antibody would then be diagnostic for an acute rubella infection. However, if there is no known travel history and no epidemiologic link to a confirmed rubella case as well as no known rubella activity in the geographic area, then the predictive value of rubella IgM serological testing is low, and thus there is a significant risk of a false-positive IgM result. In such situations in Canada, additional laboratory testing (such as paired IgG serological testing and/or virus isolation and detection) is required for confirming the rubella infection. Repeat testing using alternative IgM assays is typically not considered to be appropriate; determining which kit gave the “correct” result in the case of discrepant results is not possible, because most Canadian laboratories use kits that have comparable sensitivity and specificity.

**RUBELLA IgG SEROLOGICAL TESTING**

The detection of rubella-specific IgG is indicative of an exposure to rubella virus either from wild-type virus infection or from vaccination. Rubella IgG testing is used for diagnosis of acute rubella infection by paired IgG serological testing and by IgG avidity testing.

Rubella IgG can be used for diagnosis of acute rubella infections by testing paired acute and convalescent phase serum samples. Qualitative testing showing a negative acute phase sample and a positive convalescent phase sample would indicate a seroconversion and would be confirmatory for an acute rubella infection. In the case of a positive IgG result in an acute phase sample, a convalescent phase serum sample should be collected at least 10–14 days after the first sample, and the relative titers between the paired serum samples need to be determined. A >4-fold increase in the relative titer between the acute and convalescent phase serum samples is considered to be significant and indicative of an acute infection. Although the commercial IgG enzyme immunoassays give a quantitative value (optical density [OD] or IU/mL) with a single dilution sample, it is important to do an end-point dilution series to determine the relative titers. As an example, a particular IgG kit gave a 2.7 OD reading for an acute phase serum sample, whereas the
RUBELLA VIRUS DETECTION

In addition to serological testing, rubella virus isolation or virus detection by reverse-transcription polymerase chain reaction (RT-PCR) is useful for the confirmation of rubella infection [15]. Rubella virus isolation is not used frequently for confirmatory purposes but is very useful for subsequent genotyping and molecular epidemiologic purposes: tracking transmission pathways, linking outbreaks and cases, and documenting the elimination of a rubella virus strain from a geographic region [2, 16]. The genotyping of rubella virus has been standardized by the World Health Organization [16]. In Canada, RT-PCR has become a very useful supplementary test for confirming measles, mumps, and rubella infections, in particular for sporadic cases in which the interpretation of IgM serological testing results can prove challenging. The ideal samples for rubella isolation and detection are nasopharyngeal or throat specimens collected as soon as possible after the onset of symptoms (<5 days after rash onset). The challenge that we see in Canada for sporadic cases is ensuring that specimens for rubella virus isolation and detection are collected early in the course of disease, at the same time as the collection of the serum sample for IgM serological testing.

STRATEGY FOR RUBELLA LABORATORY CONFIRMATION IN SPORADIC AND OUTBREAK SITUATIONS

It is recognized that measles and rubella surveillance are integrally linked because of the similar clinical presentation of the diseases (fever, maculopapular rash) and the common goal of eliminating endemic measles and rubella from Canada. Figure 1 outlines a general approach to diagnosing rubella that is very similar to the approach for measles diagnosis. This algorithm takes into account the timing of sample collection, whether the case is sporadic or epidemiologically linked (ie, outbreak related), and the use of IgG serological testing and/or virus isolation and detection as supplementary tests to IgM serological testing. However, in practice, the approach taken when investigating a particular case should take into account the clinical and epidemiologic context. For example, if there was recent travel to an area with a known rubella outbreak, then rubella may be more highly suspected than measles. In cases with no travel history and no known local measles or rubella activity, then more common fever-rash illnesses (eg, parvovirus B19 infection) would be more highly suspected.

CONFIRMATION OF RUBELLA INFECTIONS IN PREGNANT WOMEN

This increased possibility of false-positive IgM results is of particular concern when investigating suspected rubella cases in pregnant women, for whom the risk of CRS entails critical care management decisions [11]. The risk of CRS is dependent on when during the pregnancy the rubella infection occurs and on whether it is a primary infection. There is a >80% risk of CRS when a primary rubella infection occurs within the first 12 weeks of pregnancy [17]. The risk decreases sharply after this, with a minimal risk of CRS after 20 weeks gestation [18]. It has been shown that, for asymptomatic reinfection, there was an 8% risk of fetal infection in the first 16 weeks, but malformations were rare. It is therefore critical to perform additional laboratory testing when investigating suspected rubella cases in pregnant women. In such cases, additional laboratory testing could include paired IgG serological testing, rubella virus isolation and detection, and rubella IgG avidity testing. This latter method is able to differentiate primary infection from past infection or...
reinfection and has been shown to be very useful for the investigation of rubella infection in pregnant women [11].

**CRS LABORATORY CONFIRMATION**

CRS cases are very rare in Canada [2]. As described elsewhere [15, 19, 20], congenital rubella syndrome can be confirmed in the laboratory by (1) the detection of rubella-specific IgM in the serum or cord blood of the infant, (2) the detection of persistent rubella IgG in the infant after ~6 months following waning of maternally acquired IgG, or (3) the isolation and detection of rubella virus in urine or respiratory specimens collected during first few months of life.

**SUMMARY**

In summary, it is critical to understand the characteristics of the tests being used and the clinical and epidemiologic context for
Table 1. Relative and Overall Sensitivities and Specificities of Rubella Immunoglobulin M Antibody Tests

<table>
<thead>
<tr>
<th>Assay</th>
<th>Sensitivity, % (95% CI)</th>
<th>Specificity, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meddens</td>
<td>76.8 (72.2–81.4)</td>
<td>94.3 (92.4–96.1)</td>
</tr>
<tr>
<td>Behring</td>
<td>75.9 (71.3–80.6)</td>
<td>95.2 (93.4–96.9)</td>
</tr>
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<td>Wampole</td>
<td>74.1 (69.3–78.9)</td>
<td>93.3 (91.3–95.3)</td>
</tr>
<tr>
<td>Diamedix</td>
<td>76.1 (71.3–80.9)</td>
<td>92.5 (90.3–94.6)</td>
</tr>
</tbody>
</table>

**NOTE.** CI, confidence interval.

* Specificity panel includes dengue, measles, parvovirus B19 infection, human herpesvirus 6 infection, Epstein-Barr virus infection, Mycoplasma infection, and rheumatoid arthritis samples.

b Results determined during previous rubella sensitivity and specificity testing [3].

the proper interpretation of laboratory results for the investigation of suspected cases of rubella and CRS. Sporadic suspected cases of rubella require additional testing (eg, IgG titers or RT-PCR) in addition to IgM-positive serological test results to conclusively confirm rubella cases, whereas outbreak-related cases can be either epidemiologically linked to laboratory confirmed cases or confirmed by IgM serological testing alone. This approach to confirming suspected cases of rubella is similar to the approach for confirming suspected cases of measles. Particular care must be taken when confirming rubella infection in pregnant women, and additional tests, such as IgG avidity testing, are useful. Only through careful consideration of clinical, epidemiological, and laboratory information can the most appropriate public health response and patient management decision be taken.

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**References**