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Dilemmas and Pitfalls in Rubella Laboratory Diagnostics in Low Prevalence or Elimination Settings

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Opinion statement

The changing epidemiology of rubella in populations with either low prevalence levels, or where rubella elimination has been achieved, has challenged laboratory diagnostics. This review will discuss the challenges in identification of acute rubella and outline appropriate confirmatory testing, particularly for cases with low pretest probability. Implementation of pre-analytical screening processes can mitigate follow-up testing required to confirm false positive antibody results, and laboratories should consider not accepting requests for testing that do not have appropriate travel or clinical history. The role of laboratory surveillance will be examined, and challenges in determining protective immunity to rubella infection in light of waning rubella antibody levels in vaccinated populations will be explored. Special populations including prenatal women and the prevention of congenital rubella syndrome will be discussed in the context of low prevalence populations.

Introduction

Viral properties

Rubella is a small positive sense enveloped RNA virus. Entry into host cells is thought to involve the envelope glycoproteins E2 and/or E1, which facilitate entry via clathrin-mediated endocytosis [1, 2], but the specific cellular receptor has not been yet identified. Rubella is

the only member in the genus Rubivirus of the Togaviridae family, and shares genetic similarity to the alphaviruses, which includes mosquito-borne viruses such as the Sindbis and Chikungunya virus. Sequence analysis of rubella viruses shows circulating strains differ by up to 8–10 % in their nucleotide sequence [3, 4], and are separated into two distinct clades (1 and 2) [3, 5], comprised of 12 genotypes (1B, 1C, 1D, 1E, 1F, 1G, 1H, 1I, 1J, 2A, 2B, and 2C) and one provisional genotype (1a) [6]. As some genotypes are geographically restricted, clade and genotype identification can help identify whether a strain is endemically acquired or imported. For example, clade 2 is not currently circulating in North or South America, therefore identification of this clade in the Americas would indicate a case of non-endemic origin [6].

Rubella disease

Rubella typically causes a mild childhood illness with fever, rash, and lymphadenopathy in the postauricular nodes. Transient polyarthralgia appearing 16–20 days after exposure to the virus, and lasting 3–5 days in duration, can also be observed following acute rubella infections [7]. The characteristic rubelliform rash is distinguished by fine erythematous distinct macules that start on the face and later spread to the trunk. Communicability of the virus begins 5–7 days before the start of symptoms, and can last 3–7 days after symptom resolution. Therefore, home isolation is recommended for 1 week after the disappearance of rash [7, 8]. While severe complications have been noted following acute rubella infection, including encephalitis and thrombocytopenia, cases are extremely rare.

The primary concern with rubella infection is when it is acquired during pregnancy. Particularly, fetal infection during the first 16 weeks of gestation can result in congenital rubella syndrome (CRS) development. CRS is associated with severe long-term sequelae including micropthalmia, chorioretinitis, deafness, limb aplasia, and cognitive impairments such as microcephaly [9, 10]. In endemic countries, CRS continues to be reported at high levels. Worldwide, approximately 100,000 cases are estimated per year [11]. Some of these fetal symptoms mirror what have been identified in the current Zika outbreak in Brazil and South America, making identification of CRS increasingly difficult. Worldwide vaccination has resulted in a significant decrease in the incidence of acute rubella and CRS cases [6]. As a result of vaccination programs, the WHO declared rubella and CRS eliminated from the Americas on April 2015 [12•]. Member states of the Pan American Health Organization (PAHO) are required to document elimination by maintaining a high-quality case-based surveillance system, and report all imported CRS and rubella cases, including genotype, to the PAHO and the WHO [13, 14].

Vaccination and immunity screening

Prevention of CRS is the primary goal of prenatal programs worldwide. Targeted vaccination to prepubescent girls was shown to decrease the incidence of CRS; however, it was not until the implementation of universal childhood vaccination programs (for both genders) that a significant reduction in the incidence of acute rubella and CRS cases was observed. Meta-analysis of rubella seroprevalence showed maternally acquired immunity was lost in infants by 9 months, and infants were susceptible to infection from 9 months until vaccination [15]. The WHO therefore recommends childhood vaccination with MMR at 9 months in areas with active measles and rubella transmission, which can be delayed until 12 months in areas of low prevalence [16]. A minimum vaccine coverage rate for rubella is approximately 91 % based on an Ro between 6 and 7 (from England, Wales, and West Germany between 1960 and 1970 [17]), and a vaccine failure rate of 5 % (1-(1/Ro) = 1-(1/7) = 0.857 + 0.05 = 90.7 %). Current coverage rates and vaccination programs are outlined in Table 1.

Testing for acute rubella and congenital rubella syndrome

Who should be tested?

Detection of rubella-specific IgM is recommended by the WHO [13] and the US CDC [32] as the frontline diagnostic test for surveillance and diagnosis of suspected rubella cases. The WHO requests each participating country to submit the total number of IgM tests performed to evaluate the quality of their surveillance program. The number of IgM tests should exceed 2/100,000 population [33]. IgM is the first class of antibodies produced following a rubella

Table 1. Select vaccination programs for rubella and relative rates of congenital rubella syndrome (CRS)

Institution or country	Target population	Vaccine coverage	Cases of CRS	Reference
USA	Universal childhood immunization (2 doses: 12 months and 4–6 years), since 1998	91.5 % with ≥1 dose for those 19-35 months of age	4 Cases (between 2005 and 2011)	[18, 19]
Canada	Universal childhood immunization (2 doses: 12 months and 4–6 years), since 1996	89.2 % with ≥1 dose by 2 years of age 94.5 % with ≥1 dose by 7 years of age	3 Cases (between 2005 and 2014)	[20, 21]
Australia	Universal childhood immunization MMRV (2 doses: 12 months and 18 months), since July 2013, MMR vaccination since 1993	>80 % with ≥1 dose by 2 years of age	0.3 per 100,000 between 2003 and 2014	[22–24]
Germany	Universal childhood vaccination MMRV (2 doses: 11–14 months and 15-23 months), since 2009	>90 % across all birth cohorts	1.1 per 1,000,000 in 2015	[25–27]
UK	Universal childhood vaccination (2 doses: 12-13 months and 3-4 years of age), vaccine implemented 1988	91.5 % with ≥1 dose by 2 years of age 95.2 % with ≥1 dose by 5 years of age	1 case in 2014	[28, 29]
China	Universal childhood immunization (2 doses: 8 months and 18–24 months of age), vaccine implemented 2005	70 % with ≥1 dose by 9 years of age	9.32 per 100,000 (167 cases) in 2011 0.87 and 0.59 per 100,000 in 2014 and 15 respectively	[30, 31]

infection and are detectable in virtually 100 % of cases 4–28 days after rash onset [34], and may persist for several months thereafter [35]. IgM antibodies are neutralizing [36–38], and their rapid production is an effective device to limit the spread of the virus and reduce viremia.

Detection of IgM is a very sensitive and rapid way to confirm a suspected rubella case, but its specificity is not absolute. Although the best commercial enzyme immunoassays (EIA) kits for rubella IgM have excellent performance, with a specificity of around 95 % [39–42], false positive results are always possible. In low prevalence or elimination settings, the positive predictive value of the rubella IgM assay drops virtually to zero. In these scenarios, all IgM positive results are likely to be false positives in the absence of a definite exposure history [43]. In the Americas, where rubella and CRS were declared eliminated in April 2015, detection of rubella IgM antibodies is indicated in cases with probable exposure to rubella, or with history of travel to endemic areas. In these settings, confirmation of an IgM positive result by direct detection of the rubella virus from nasopharyngeal or throat swabs, urine, and/or serum is recommended (see molecular diagnostics section below).

Immunization with rubella-containing vaccines produces a rubella-specific IgM response, which is not linked to rubella infection. It is not possible to distinguish between vaccine-induced and naturally induced

rubella antibodies and therefore serology testing for rubella within 6–8 weeks after rubella immunization is problematic.

False positive rubella IgM

False positive rubella IgM results can occur following infection with other viruses, namely parvovirus B19, Epstein-Barr virus, cytomegalovirus, and measles, which all can produce cross-reactive IgM [44–48]. The presence of rheumatoid factor (an anti-human immunoglobulin autoantibody most commonly of the IgM class) is also a common source of interference in IgM assays [49–51]. Rheumatoid factor is found in 10 % of the population, even without disease symptoms [49], and it can be induced by a number of bacterial and viral infections [51, 52]. Indirect EIA IgM kits are well validated [39, 41, 53] and are most commonly used in WHO-accredited laboratories [54••]; however, for maximum specificity, they require a pretreatment step to remove rheumatoid factor and other IgG antibodies that may interfere with the assay. IgM capture EIA kits are also available for rubella IgM testing [39, 40]. They are based on a different principle, which minimizes interference with some non-specific IgMs and specific IgG and may decrease the level of false positive results [41]. It is good practice to repeat equivocal or contradictory rubella IgM results using EIA kits based on different methodologies [50].

False positive rubella IgM results are particularly troublesome during pregnancy, because they may lead to the decision to abort the fetus. Rubella infection in the first 12 weeks of pregnancy carries a 90 % risk of congenital abnormalities that constitute congenital rubella syndrome (CRS). The most common source for false IgM positive results in pregnancy is the erroneous request of rubella IgM during, as part of routine prenatal testing, or as part of the diagnostic work-up for a flu-like illness with or without rash [43, 55]. Pregnant women should therefore not be tested for rubella IgM unless there is a history of contact with a laboratory-confirmed rubella case, or history of travel to endemic areas during pregnancy. Clinical and reference laboratories in countries of very low rubella incidence should remind physicians of the futility of ordering a rubella IgM test without credible history of exposure.

Confirmation of positive rubella IgM results

Once a positive rubella IgM result has been obtained, further testing to confirm or exclude rubella may be necessary. Nasopharyngeal and urine samples should be tested for viral detection by PCR; however, beyond 7 days after rash onset the sensitivity of detection of rubella virus drops dramatically. Therefore, in the absence of appropriate travel history, or appearance of a rash, a negative rubella PCR cannot exclude a recent infection. IgG seroconversion, or a fourfold increase of IgG titers, can also be used to reliably confirm recent rubella infection in the presence of rubella IgM antibodies. Acute (within 7 days post-rash) and convalescent (at least 20 days after rash onset) samples for IgG are required.

An informative and specific laboratory test is the measurement of IgG avidity, i.e., the strength with which IgG antibodies bind to their specific target. During primary infection, IgG antibodies undergo a process of maturation, during which B cell clones producing antibodies of higher affinity for their

specific target are selected [56–58]. As a result, IgG in serum bind more tightly even in the presence of denaturing agents such us urea or diethylamine. Avidity is calculated as a function of percentage of IgG bound in the presence or absence of a denaturing agent, which increases from "low" to "high" within 2 months following rubella infection. Thus, a low avidity rubella IgG combined with a positive rubella IgM, strongly suggests rubella infection occurred within the past 2 months. Conversely, high avidity results indicate a more remote infection, or an anamnestic IgG response (if there was a rise in IgG titers). Unfortunately, since IgM can sometimes persist past the time of IgG maturation [59], a high avidity result later in pregnancy cannot exclude rubella infection occurred before conception.

In summary, confirmation of a positive IgM result requires extensive additional testing which can be prevented with appropriate pre-analytical processing of samples, specifically, by canceling all rubella IgM requests without appropriate history of illness or exposure, such as those requested as part of a routine serological screening of a pregnant woman. More information and references can be found in the WHO "Manual for the laboratory diagnosis of measles and rubella infection" second edition [54••]. The third edition is in preparation.

Molecular detection of rubella virus

Detection of rubella virus in suspected cases can be used to confirm a case of rubella and can resolve any uncertainty that may remain after serological testing. Detection of the rubella virus is performed using molecular methods, especially real-time RT-PCR, for which several well-validated assays are available [60–62]. Culture isolation and direct immunofluorescence are no longer in general use, being supplanted by the faster and more sensitive molecular tests.

While the molecular detection and the interpretation of results are rarely problematic for the modern clinical microbiology laboratory, timing of specimen collection is crucial to ensure the sensitivity of detection [55]. Nasopharyngeal (NP) swabs or urine are the specimens of choice, and should be collected as soon as possible after rash onset and, for optimal sensitivity, no later than 5 days [54••]. Additionally, rubella virus can be detected in serum, but with lower sensitivity compared to NP swabs or urine specimens. Like all PCR-based methods, a negative result does not exclude the presence of a recent infection, especially if a rash is not detected, and the date of exposure is not known.

Vaccine strains of rubella virus can be detected in urine or NP swabs up to 28 days after immunization [63], and this should be considered when interpreting positive RT-PCR results in recently vaccinated individuals. Genotyping can be used to differentiate between a wild-type infection and a vaccine-derived PCR positive, and is performed by RT-PCR amplification and sequencing of the WHO standardized 739 nucleotide region of the E1 envelope protein gene [64]. PAHO requests all member states to genotype isolates and submit the sequence of all sporadic cases or outbreaks [13] to the WHO worldwide database (RubeNS). Sequence comparisons against the RubeNS can help track imported infections and document elimination.

It is therefore of paramount importance to obtain a timely NPS or urine specimen for molecular analysis in every suspected case or rubella.

Recommendations

- A positive rubella IgM result is strongly indicative of a recent rubella infection in high endemicity areas following probable exposure, or in low incidence/elimination areas with a history of travel to endemic rubella areas or exposure to a laboratory confirmed case.
- In very low incidence settings, screening of pregnant women for rubella IgM without history of exposure should be avoided, since virtually all positive results will be false positives.
- In these cases, positive IgM results should be confirmed with alternative testing methods including IgM retesting, rubella virus detection by RT-PCR, IgG seroconversion, and IgG avidity testing.
- Appropriate pre-analytical consideration is needed for rubella IgM, as laboratory testing may not be able to confirm or exclude a positive or equivocal rubella IgM result in the absence of a clear exposure history.
- Molecular testing (RT-PCR) will confirm a suspected case of rubella, but not exclude it, if precise history of exposure or rash is not available.

Acute rubella and CRS surveillance

The WHO recommends rubella surveillance in all countries that have implemented rubella-containing vaccination for their populations [13]. As of 2015, 703 laboratories involved in the detection of rubella and CRS in 199 countries participated in the WHO Global Measles and Rubella Laboratory Network (GMRLN) [65]. Countries that are close to, or have achieved, elimination are recommended to monitor for all cases of febrile and rash based illnesses, while countries that have active circulation of rubella should monitor for cases of congenital infection.

Rubella elimination was achieved in 2004 in the USA and 2005 in Canada, respectively. Rubella and CRS were officially declared eliminated from the whole region of the Americas in April 2015 [12•]. To maintain elimination, PAHO has requested each country to collect and report on three key indicators that must be fulfilled: (1) maintenance of high vaccine coverage, including hard to reach populations (where coverage rates of approximately 91 % are required to maintain herd immunity), (2) maintenance and strengthening of surveillance systems to detect all acute rubella and CRS cases, and (3) to promote and support rapid response to outbreaks [14].

Nationwide surveillance for acute and congenital rubella in Canada is primarily an active system, where acute and CRS cases are submitted to the Public Health Agency of Canada by each province and territory on a weekly basis. Epidemiological data including patient demographic information, vaccination status, and travel history are linked with laboratory

Table 2. Surveillance systems for acute rubella and CRS cases

Surveillance system	Location	Active or passive?	Cases	Publically available data sources
Canadian Measles/ Rubella Surveillance System (CMRSS)	Canada	Active/passive*, since 1998	Acute and CRS	[66]
MARS (measles and rubella surveillance)	Canada (BC, Alberta, Newfoundland, and Labrador)	Active, since 2011	Acute and CRS	[66]
Centers for Disease Control (CDC)	USA	Active/passive*, since 1966	Acute and CRS	[67, 68]
European Center for Disease Prevention and Control (ECDC)	28 member states (within the European Union (EU) and European Economic Area (EEA))	Active/passive*, since 2012	Acute and CRS	[69]
Australian Paediatric Surveillance Unit (APSU)	Australia	Active/passive*, since 2004	Acute and CRS	[70]
National Epidemiological Surveillance of Infectious Diseases (NESID)	Japan	Active/passive*, since 1999	Acute and CRS	[71, 72]
World Health Organization (WHO)	Global Measles and Rubella Laboratory Network (GMRLN); includes SEAR, AMR, EUR, WPR, EMR, and AFR	Active/passive*, implementation dates vary by region	Acute and CRS and febrile illness for countries who have not achieved elimination	[73]

*Active/Passive systems require data to be supplied to public health agencies via legislation etc., but rely on a manual (i.e. not automatic) process of submission

data from the National Microbiology Laboratory when specimens are submitted for further work-up. Likewise, the USA legislates that all acute and CRS cases be reported to their respective state public health laboratories, and other international organizations regularly publish acute rubella and CRS cases (see Table 2 for outline of different surveillance methods); however, like Canada, these systems rely on accurate and timely reporting from healthcare workers, schools, and laboratories, among others.

A fully active surveillance pilot project was implemented in Canada in 2011, to track real-time laboratory data in three jurisdictions (BC, Alberta and Newfoundland, and Labrador). All patient specimens submitted for rubella or measles testing are reported via the local and provincial laboratories to the PAHO where cases are identified and tracked through a web-based system. This automatic reporting system captures all samples tested for rubella, and offers real-time coordination between laboratory and epidemiology data. Detection of acute rubella and CRS cases is however still dependent on clinical suspicion

and appropriate laboratory testing. A stronger link to epidemiological data and laboratory data is therefore needed globally to fully understand the disease burden of rubella, and to overcome challenges with accurate and timely reporting of results [65].

Testing for protective immunity to rubella

Who should be tested?

Detection of rubella-specific IgG is recommended for specific populations, including prenatal women by the WHO [12•] and the US CDC [14] as a preventative measure for development of fetal CRS. From a public health perspective, serosurveys for rubella IgG is recommended to understand the overall level of protection across populations, which can be used to target vaccine uptake to at risk populations. Particularly for health care workers, use of rubella IgG testing following exposure to a case patient may help to identify risk of infection.

In areas of low prevalence, rubella antibody screening of prenatal women is often included in the routine screening programs. Integrated screening and vaccination programs offer postnatal vaccination to all women with antibodies below the level that is considered to be protective. On April 1, 2016, the UK became the first country to end rubella immunity screening in pregnant women. The UK achieved rubella elimination due to high MMR vaccine coverage (95.2 % coverage by the age of 5 years [29], which is greater than the required 91 % coverage estimated for herd immunity). Because rubella screening identifies women who may not be protected from rubella infection, but vaccine is contraindicated during pregnancy, no preventative action can therefore be taken on the initial screening result. The UK therefore decided that vaccination prior to pregnancy was more advantageous than upfront screening and targeted postnatal vaccination.

In countries that have achieved elimination, possible inaccuracies in IgG screening results have been observed, including waning immunity (discussed in detail below). As countries decide how to handle the changing epidemiology of rubella screening results, screening strategies will likely diversify as countries identify the most effective health care strategies for their populations.

Neutralizing versus total antibody

Neutralizing antibody testing is considered the most reliable indicator of protective immunity, as it can detect antibodies to E1 and E2 rubella envelope proteins [74]. Cell cultures are challenged with live rubella virus in the presence, or absence, of patient serum. The viability of cell monolayers is therefore a direct measure of the ability of serum antibodies to block infection of rubella virus [75], and results can be used to effectively correlate levels of immunity. It is important to note that neutralization assays were used to determine what level of antibody constitutes a protective immune response. The earliest vaccine trials used neutralization testing to correlate with clinical immunity, and these studies were used to develop the currently used cut offs for rubella IgG levels [76].

Due to the variability and high labor cost of the assay, neutralization antibody testing generally fell out of favor as a routine screening test, and

was replaced most recently by enzyme immunoassays (EIA) [74]. The high throughput design and relative low cost of EIAs compared to the neutralization antibody assay facilitated incorporation into the routine clinical laboratory. Many commercial assays are now available for use in the clinical laboratory, and use a range of detection systems (including chemiluminescence, electrochemiluminescence, and immunofluorescence) and rubella antigens (including viral lysates, purified proteins, and recombinant protein). Because the rubella antigens used by manufactures differs, variability in the amount of rubella-specific IgG detected is often observed when identical samples are tested between different platforms [77–81, 82•]. While EIAs detect total levels of rubella-specific IgG, it is not a direct measure of the level of protection those antibodies would provide upon challenge with rubella virus (compared to the neutralization test, not all rubella-specific IgG will provide protection from infection), making interpretation of values close to the cut off difficult [83••].

Rubella IgG cut offs

The Rubella Subcommittee of the National Committee on Clinical Laboratory Standards (NCCLS) first established a cut off of >15 IU/ml rubella antibodies in 1985 [76]. Samples from patients testing >15 IU/ml were considered immune, and those testing <15 IU/ml were considered susceptible to infection. In 1992, this cut off was revised to >10 IU/ml following additional clinical and epidemiological studies [84–87], showing that a secondary immune response was elicited by patients with antibody levels <15 IU/ml following challenge with live-attenuated vaccine, or reinfection [88]. Using an antibody titer as a marker of immunity presents a number of diagnostic challenges: first, the numerical antibody value is known to vary between testing assays [82•], which may result in different interpretations when the same sample is tested on multiple assays. In fact, we have recently shown that a 30 % difference in titer can be seen between assays manufactured by the same company [89]. Secondly, using a quantitative titer, rather than a positive or negative qualitative value, challenges the limits of serology testing. This creates an assay where positive values are within one IU/ml from negative values, and no statistical difference (i.e., a difference of 2 standard deviations) separates negative from positive results. Mathematical modeling of a large prenatal population showed a natural distribution of true negative values, and true positive values, with a difference between the means representing a 126-fold change in IgG levels [90].

How to interpret waning immunity in populations with universal vaccination

In countries where universal vaccination was adopted greater than 10 years ago, a significant decline in total rubella IgG antibody levels has been observed [81, 90–92]. Particularly in younger age cohorts, a growing proportion have antibody levels below the level considered to be protective [90]. While the cases of acute rubella and CRS have not increased over the same time frame [91, 93, 94] (highlighting the effectiveness of the vaccine program), it is difficult to accurately assess the level of protective immunity in these populations. The reason for the reduction in rubella-specific antibodies in vaccinated individuals, compared to those who acquired immunity via natural infection, is potentially twofold: longevity of antibodies and decreased circulation of wild type virus.

Longevity studies using the tetravalent vaccine have shown 100 % seropositivity for rubella 3 years post-vaccination [95]. The time since vaccination appears to be inversely proportional however, as 12 years after immunization with the trivalent vaccine, 69 % of children were either seronegative, or had low levels of rubella antibody, whereas only 7 % had low or no antibodies 1 month after vaccination [96]. Revaccination of women with no, or low, rubella antibody levels resulted in IgG levels >15 IU/ml in 55.8 % of cases after a mean of 1.1 years since time of vaccination [90], or in 26 % of cases after 5 years [97]. The timing of vaccination in these populations may also play a role in the longevity of rubella IgG levels. As seen in Table 1, the first immunization dose is typically given at 1 year of age; however, in 2005, China adopted an accelerated vaccine schedule with doses at 8 and 18 months. Antibody persistence was shown for the duration of the study (10 months) and participants in the trial group (vaccine at 8 and 18 months) had statistically higher total rubella IgG antibody titers compared to the control group (vaccine at 12 and 22 months); however, both levels were well above the >10 IU/ml cutoff of positivity [98]. As these updated vaccination schedules were introduced in China in 2005, it will be interesting to examine the presence of antibodies as this cohort ages, and whether the longevity of antibody titers is maintained into adulthood.

A compounding factor to the observed decline in antibody levels is the reduction of circulating wild-type virus due to herd immunity. As more people are vaccinated, the number of acute rubella infections decreases (as evidenced in the decreased case rates of rubella and CRS) [90, 91, 93, 94]. Thus, the number of people who would have experienced a boost to their immunity from post-vaccine exposure to the virus has also decreased. In Israel, decreased IgG levels observed in younger age cohorts were attributed to lack of circulating wild-type virus and lower vaccination coverage [99]. Those born in 1977–78 had a seroprevalence of 95.6 % compared to those born in 1988–89 who had 85.7 % seroprevalence for measles, which was shown to correlate with the seroprevalence for rubella [99].

Recommendations

- A positive rubella IgG is a strong indicator of protective immunity, and patients can generally be considered protected from infection when levels are >10 IU/ml.
- A positive rubella IgG result <10 IU/ml suggests that a patient was previously vaccinated or exposed to rubella, but may be susceptible to infection. However in areas with low prevalence/elimination, antibody levels below 10 IU/ml may still be protective, as herd immunity is maintained despite waning antibody levels.
- Prenatal screening programs should consider the incidence of rubella in their population and overall vaccination coverage to determine the best screening strategies.

Conclusions

The changing epidemiology of rubella prevalence has challenged laboratory diagnostics. Low disease prevalence can cause uncertainty with a high number

of false positive results, and waning antibody levels in vaccinated populations make it difficult to determine true protective immunity. Understanding the pretest probability and limiting IgM testing to only cases with a clinical history of rubella-like infection can decrease the number of false positive results, and subsequent follow-up testing. Re-evaluation of when to use rubella IgG as a screen for protective immunity, particularly in populations where rubella has been declared eliminated may decrease patient anxiety and physician uncertainty when results are below the level that is considered protective.

Compliance with Ethical Standards

Conflict of Interest
Dr. Charlton and Dr. Severini declare that they have no conflicts of interest.

Human and Animal Rights and Informed Consent
This article does not contain any studies with human or animal subjects performed by any of the authors.

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