replication [24]. Mutations in this domain have been shown to reduce its conformational stability at a high temperature [29]. The ts phenotype-determining mutation, Y1042H, found in the KRT vaccine strain rendered the protease domain-containing peptide, $NSP_{994-1301}$, unstable at a high temperature. In contrast, the N1126T and A1277V mutations found in the reversion mutant, TO-336.rev, rendered the TO-336.vac-derived $NSP_{994-1301}$ thermostable. Thus, the present data suggest that reduced stability of the conformation of the protease domain of P150 at a high temperature is a cause of the ts phenotype of some rubella vaccine strains. RuV with any mutations that have similar effects on the protease domain may exhibit a ts phenotype. It is of interest that other vaccine strains also possessed unique mutations in the protease domain.

The most important properties of vaccines are their safety and efficacy. For attenuated live vaccines, avirulence is critical for safety. Therefore, understanding of the molecular bases of the attenuation is crucial for quality control of vaccines. However, no reliable animal models for analyzing RuV virulence have been established. Humans are the only natural host for RuV, and it exhibits poor infection and replication in experimentally infected animals. Nonetheless, infections with clinical isolates of RuV induce considerable levels of humoral immune responses in animals, and the lack of these responses in the majority (>80%) of infected guinea pigs has been used as an *in vivo* marker of licensed rubella vaccines in Japan [6]. This phenotype is documented in the MRBP [8,9]. Although the low potency to induce antibody responses may be correlated with the attenuated phenotype of vaccine strains, no scientific evidence has been provided. A marker test that checks the *in vivo* marker phenotype of vaccine strains has been performed to verify the constancy of the vaccine quality, but not the avirulence [8,9]. It is difficult to determine the safety or avirulence of vaccines using cell culture systems. However, it is generally accepted that a ts phenotype, which can be analyzed in cultured cells, may play a role in virus attenuation [31–37]. Mutations in various genes can cause the ts phenotypes of viruses [33–35,38–43]. Since the body temperatures of guinea pigs and rabbits range from 37.5 to 39.5 °C, the inability of rubella vaccine viruses to elicit humoral immune responses in these animals may be partly and reasonably explained by the ts phenotype [5]. Surprisingly, however, Matsuba.GMK3 with a partial ts phenotype was highly potent in eliciting humoral immune responses in animals. On the other hand, the reversion mutant, TO-336.rev, was able to replicate better than Matsuba.GMK3, but was still unable to elicit these responses. These data demonstrate that a high growth capacity at a high temperature is not necessarily critical for eliciting humoral immune responses in animals. In the view of the care and use of laboratory animals, it is desirable to replace the marker test by a test involving cultured cells. However, our data show that a test for the ts phenotype using cultured cells cannot be a substitute for the marker test using animals. The present data showed that a phenotypic reversion of the virus, by which TO-336.vac became able to grow at a high temperature, was insufficient to elicit humoral immune responses. These data suggest that TO-336.vac has one or more mutations that specifically abolish the potency to elicit these immune responses in animals. It is of interest that TO-336.vac has mutations in the E1 surface glycoprotein, because it is known to be involved in cell entry and induction of neutralizing and HI antibodies. Functional or antigenic changes to this surface glycoprotein may play a role in determining the potency of viruses to elicit humoral immune responses. Analyses of these mutations are in progress in our laboratory.

In summary, the entire nucleotide sequences of all the Japanese rubella vaccines became available with the data obtained in the present study. Nucleotide sequence analyses of progenitor RuV strains and their resulting vaccines revealed mutations that were introduced into the genomes of TO-336.vac and Matsuura.vac during their passages in laboratories. Among these, the N1159S mutation in the protease domain of P150 seems to affect the thermal stability of the protein. The data further suggested that a reduction in the thermal stability of the protease domain is a cause of the ts phenotype of some rubella vaccines. Finally, our data showed that the ability of RuV to grow at a high temperature was not necessarily correlated with the potency to elicit humoral immune responses in animals. These findings indicate that the molecular mechanisms underlying the inability of vaccines to elicit humoral responses in animals are more complicated than the hitherto considered mechanism involving the ts phenotype as the major cause.

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SUPPLEMENT ARTICLE

Status of Global Virologic Surveillance for Rubella Viruses

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The suspected measles case definition captures rubella cases. Therefore, measles surveillance will be improved in the course of the control and eventual elimination of rubella transmission. One aspect of rubella control, virologic surveillance, is reviewed here. A systematic nomenclature for rubella viruses (RVs) based on 13 genotypes has been established and is updated when warranted by increases in information about RVs. From 2005 through 2010, the genotypes of RVs most frequently reported were 1E, 1G, and 2B, and genotypes 1a, 1B, 1C, 1h, 1j, and 2C were less frequently reported. Virologic surveillance can support rubella control and elimination. Synopses of rubella virologic surveillance in various countries, regions, and globally are given, including characterization of viruses from imported cases in a country that has eliminated rubella and studies of endemic viruses circulating in countries without rubella control objectives. Current challenges are discussed.

Measles and rubella are similar rash illnesses that may be difficult to differentiate clinically [1]. Measles control can be impeded when the incidence of rubella results in a substantial number of suspected measles cases that are ultimately classified as rubella cases [2]. Since major rubella epidemics in unvaccinated populations can be separated by 6–9 years, the annual impact of rubella cases on measles surveillance is difficult to predict (Figure 1) [2]. For example, in the country of Georgia, rubella annual incidence rates showed peaks in 1983, 1985, 1988, 1997, and 2004. The 2004 outbreak coincided with a measles outbreak; during this outbreak, 53% of suspected measles case patients tested positive for immunoglobulin M (IgM) to measles virus and 12% tested positive for IgM to rubella virus (RV). Some surveillance programs in support of measles elimination have integrated measles and rubella surveillance, with the final classification of suspected cases determined largely by laboratory results [3, 4]; such integration is cost-effective [5].

After measles elimination, a rubella control or elimination program, including rubella surveillance, can be

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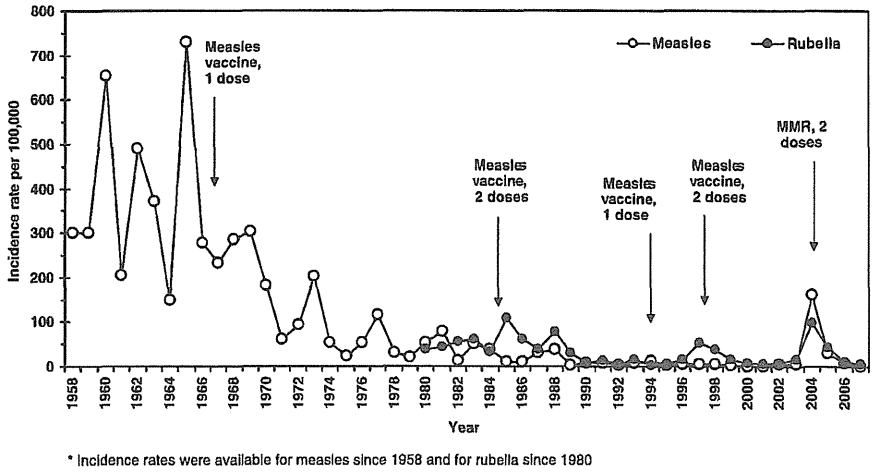

Figure 1. Measles and rubella incidence rates, Georgia, 1958–2007. Reprinted with permission from [2]. MMR, measles-mumps-rubella vaccine.

a catalyst for maintaining measles elimination [6]. This catalytic effect can happen when combined measles and rubella vaccines are used and when high-quality laboratory-based surveillance for rubella, which tests rubella-negative cases for measles, is used [7, 8]. There are rubella and congenital rubella syndrome (CRS) elimination goals and accelerated rubella control programs in many countries (see below).

Laboratory diagnosis of rubella is needed to confirm those rubella cases that are investigated in the course of measles surveillance due to the recommended use of a measles case definition that also captures rubella cases. The diagnostic assays and the clinical interpretations of laboratory results are sufficiently different for measles and rubella that laboratories must have specific experience in both diseases [9]. Two important techniques used in laboratory support for rubella diagnosis are the detection of rubella IgM and the detection of RV RNA by conventional and real-time reverse-transcription polymerase chain reaction assays [10]. Molecular epidemiology, defined here as virologic surveillance that allows the differentiation of circulating RVs, can be used to monitor transmission pathways and to identify interruption of endemic virus transmission [9]. The current status of virologic surveillance for circulating RVs worldwide is summarized here.

NOMENCLATURE

Rubella virus, the sole member of the *Rubivirus* genus in the Togaviridae family, is a positive-polarity RNA virus with a genome of 9,762 nucleotides. Five protein products are encoded by the genome: 2 non-structural proteins (P90 and P150) and 3 virion proteins (the capsid and 2 envelope glycoproteins, E2 and E1). A systematic nomenclature for RVs is necessary for effective virologic surveillance. The World Health Organization (WHO) Measles and Rubella Laboratory Network has recommended the collection of RV genotype data to support control and elimination programs globally [9]. Although rubella is a serologically monotypic virus, sequence analysis of the E1 glycoprotein revealed that distinct genetic variants of RVs exist [11]. In 2005, a systematic nomenclature was adopted by the WHO and has been described elsewhere [12–14]. Briefly, genetic characterization has identified 2 clades that differ by 8%–10% at the nucleotide level. Clade 1 is divided into 10 genotypes (1a, 1B, 1C, 1D, 1E, 1F, 1G, 1h, 1i, and 1j), of which 6 are recognized and 4 are provisional (designated by lowercase letters). Clade 2 contains 3 genotypes (2A, 2B, and 2C). All recognized genotypes must be represented by at least 2 well-characterized reference viruses for which the 3,192-nucleotide structural protein open reading frame (SP-ORF) has been sequenced. A 739-nucleotide region (nucleotides 8,731–9,469) within the SP-ORF was designated as the minimum sequence window necessary for assigning genotypes by comparison with the reference virus sequences. Figure 2 shows phylogenetic trees produced using 32 reference virus sequences. Accepted or proposed reference virus sequences are now available for all 13 genotypes. Genotype 1a virus sequences, currently represented by vaccine and laboratory viruses from the 1960s, do not cluster as a single group and should probably be further subdivided. Bootstrap values are robust for all genotypes except for 1D, using the SP-ORF sequences. The bootstrap values are less robust for some genotypes by means of the 739-nucleotide sequence window. The criterion for a valid analysis using 739-nucleotide sequences is the proper clustering of reference viruses, not bootstrap values [12].

OVERALL STATUS OF VIROLOGIC SURVEILLANCE FOR RVs

Global Distribution of RVs

Four of the 13 rubella virus genotypes were not reported from January 2005 through May 2010. Genotype 2A viruses have not been isolated since 1980, except for several isolates of the Chinese 2A vaccine strain, BRDII, from vaccine-associated

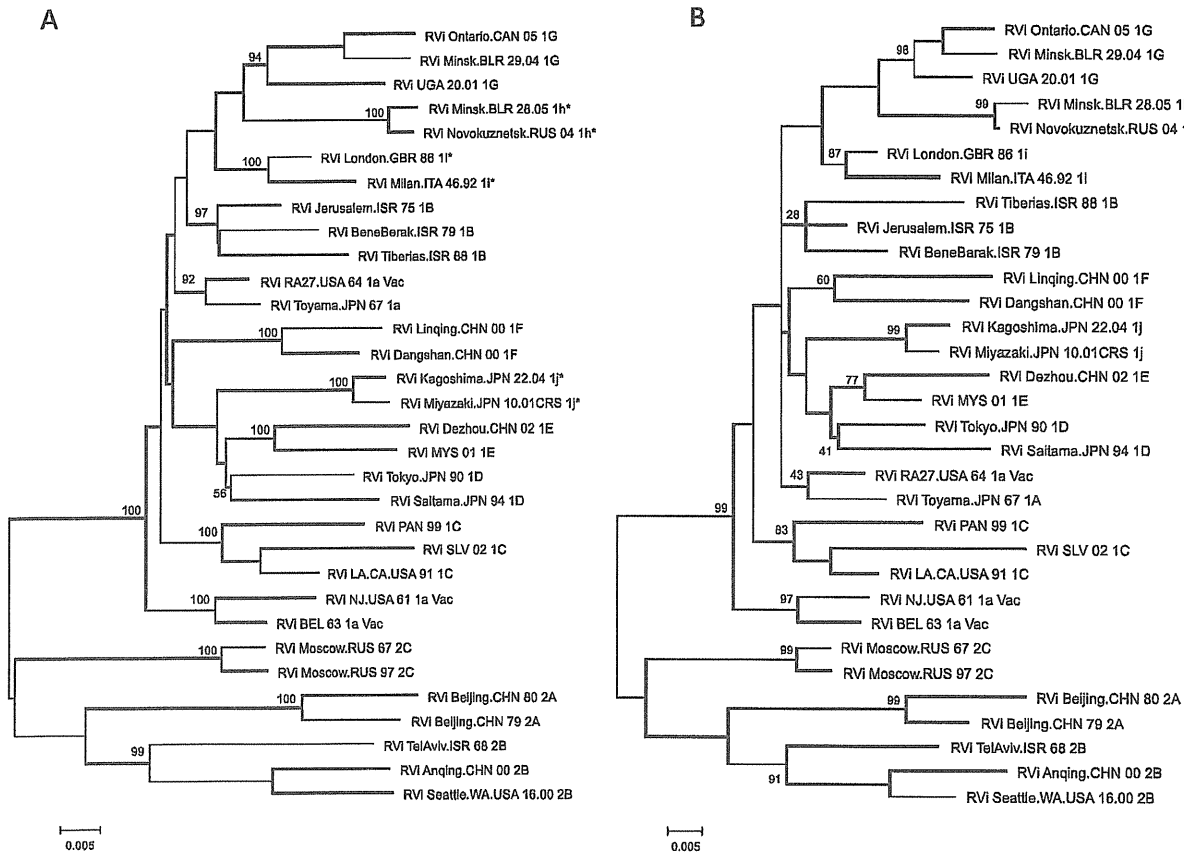

Figure 2. Phylogenetic analysis of 32 rubella reference virus sequences. Phylogenetic analyses were conducted using MEGA4 Software (version 4.0) [15]. The evolutionary history was inferred using the neighbor-joining method [16]. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown next to the branches for each genotype. The branch lengths are in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Tamura 3-parameter method [17] and are in units of the number of base substitutions per site. *A*, Phylogenetic tree based on the rubella structural protein coding region (3,192 nucleotides in length). Proposed reference virus sequences are marked with asterisks (E. Abernathy, unpublished data). *B*, Phylogenetic tree based on the 739-nucleotide region recommended by the World Health Organization for genotyping of rubella viruses.

cases. Viruses of genotypes 1i and 1F were last found in Italy (1994) and China (2002), respectively [18, 19]. Genotype 1D has not been detected since 1996. In 2007, 1j was determined to be a provisional genotype [13]. Some viruses that are now classified as 1j were considered to belong to genotype 1D before genotype 1j viruses were defined (see Figure 2). Therefore, care should be taken when working with virus sequences identified as genotype 1D before 2007. Reclassification of additional viruses by subdivision of some current genotypes (eg, genotypes 1E, 1G, and 2B) will likely be necessary to enhance the utility of RV molecular epidemiology.

The geographic distribution of the 9 active genotypes during the observation period is shown in Table 1 (GenBank; WHO database; D. Featherstone, personal communication). In some cases there are insufficient data to classify these viruses as either endemic or imported. Of these 9 genotypes, 6 (1a, 1B, 1C, 1h, 1j, and 2C) were reported sporadically or in geographically restricted regions. For example, viruses of genotype 1h were detected only in Russia and neighboring countries during the observation period [20], and genotype 2C was reported only from Perm, Russia (GenBank). The decline and likely elimination of viruses of genotype 1C from the Americas has been monitored by virologic surveillance, and no virus of this genotype has been reported for almost 5 years (Table 1) [14].

Viruses of the remaining three genotypes, 1E, 1G, and 2B, had a wide geographic distribution and were frequently found. Genotype 1E viruses have been identified in 1 Middle Eastern country, 7 European countries, 3 Southeast Asian countries, 3 African countries, and 4 Western Pacific countries, and are the dominant genotype in China [19]. Viruses of genotype 1G were found in 5 European countries and 7 African countries. Viruses of genotype 2B were reported from 2 Middle Eastern countries, 5 European countries, 4 Southeast Asian countries, 4 South and

Table 1. Global Distribution of Reported Rubella Virus Genotypes, 2005–2010

Genotype, country	Year or years
1a	
Cambodia	2009
Japan	2008
Kazakhstan	2006
1B	
South Africa	2007, 2008
1C	
Chile	2005
Peru	2005
1E	
Belarus	2005, 2006
China	2005, 2006, 2007, 2008,[a] 2009, 2010[a]
China HK SAR	2008, 2009, 2010
France	2005[a]
Kazakhstan	2006
Laos	2009
Malaysia	2005[b]
Mongolia	2010
Poland	2007, 2008[b]
Russia	2005, 2006, 2007, 2008, 2010
South Africa	2008
Sri Lanka	2008
Sudan	2005
Thailand	2005, 2009
Tunisia	2008
Ukraine	2007
United Kingdom	2008
United States	2008[c]
Vietnam	2007[b]
Yemen	2008
1G	
Algeria	2007
Belarus	2005
Ghana	2005, 2008
Côte d'Ivoire	2005,[b] 2008
Kenya	2005, 2010[b]
Libya	2009
Netherlands	2005[a]
Russia	2005,[b] 2006, 2008
Sudan	2005
Uganda	2007[b]
Ukraine	2009
United Kingdom	2007
1h	
Belarus	2005, 2006
Kazakhstan	2008[a]
Kyrgystan	2009
Russia	2005, 2006, 2007, 2008, 2009, 2010
1j	
Brazil	2005[c]
Philippines	2010[a]

Table 1. (Continued)

Genotype, country	Year or years
Spain	2005[c]
United Kingdom	2006[c]
United States	2010[c]
2B	
Argentina	2008
Bangladesh	2009
Bosnia and Herzegovina	2009, 2010
Brazil	2006, 2007, 2008, 2009
Chile	2007
China	2008
China HK SAR	2008, 2009
Dubai	2009[b]
Egypt	2007[b]
France	2009
India	2005, 2007, 2008,[b] 2010[b]
Italy	2008[b]
Japan	2007[c]
Kazakhstan	2008[bc]
Mexico	2008[b]
Nepal	2008, 2009, 2010
Russia	2009[c]
South Africa	2007, 2008
Spain	2009
Sri Lanka	2008
Sudan	2006
Ukraine	2010
United Kingdom	2006, 2007, 2008, 2010
United States	2007,[c] 2009,[c] 2010[c]
Vietnam	2006,[b] 2009
Yemen	2008
2C	
Russia	2005

NOTE. Genotypes 1D, 1F, 1i, and 2A were inactive during this period. Country (or countries) and year (or years) of report are indicated for each genotype. HK SAR, Hong Kong Special Administrative Region.

[a] Found endemically and as an export.

[b] Exported virus; importation countries are shown in Table 2.

[c] Probable import, but links are unknown.

Central American countries, 3 African countries, and 2 Western Pacific countries.

Limited Number of RVs From Virologic Surveillance and Systematic Reporting of Sequences

There are a total of 647 virus entries in the WHO rubella virus genotype database for viruses found from 1966 through June 2010, and only 534 of these viruses have been sequenced through the 739-nucleotide window required for genotyping. GenBank contains rubella sequences, but these sequences are not evaluated for accuracy and updated when new information becomes available (eg, new genotype assignments) and often lack epidemiologic linkages. In addition, no dedicated database of rubella

sequences is currently available; the WHO genotype database currently does not provide sequence information apart from linkages to GenBank accession numbers, when the submitter provides them. Furthermore, there are still many countries that lack sufficient baseline data on circulating viruses, and there are only a few countries where RVs have been sampled over time [18–21].

CURRENT STATUS OF VIROLOGIC SURVEILLANCE FOR RVs IN THE WHO REGIONS

The WHO Region of the Americas

The member states of the Pan American Health Organization are working to complete the goal of elimination of rubella and CRS [22]. Using routine immunization, supplemental immunization activities (SIAs), and an integrated surveillance system, endemic rubella and CRS cases in the Americas are now at an all-time low [23, 24]. One notable recent achievement was work to eliminate rubella and CRS from Brazil by the administration of ~70 million doses of rubella-containing vaccine, which targeted women and men aged 20–39 years in all states and adolescents aged 12–19 years in 5 of 27 states [25].

Virologic surveillance has been important to elimination efforts in the Americas. Country-specific data from virologic surveillance in Canada and the United States supported the documentation of elimination of endemic rubella in these 2 countries [14]. The available information on RVs of genotypes 1C, 1E, 1G, 1B, 1j, and 2B found in the Americas from 1997 through 2007 has been recently summarized [14]. Viruses of genotype 1C (first identified in about 1983) have previously been found circulating only in the Americas, have not been found anywhere in the world since 2005, and are thus likely eliminated [24]. Prior to 2006, all genotype 2B viruses identified in the Americas were associated with importation events. In 2006, a virus or viruses of genotype 2B caused a large epidemic, predominantly in Brazil and Chile, which lasted for >1 year and thus met the criterion for endemic circulation [26, 27]. However, good virologic surveillance efforts after 2006 provided evidence for the decline and likely elimination of endemic viruses of genotype 2B in the Americas [24].

As for the other rubella virus genotypes previously found in the Americas, viruses of genotypes 1E and 1G are distributed widely in the world and viruses of genotype 1B have been found occasionally throughout the world from 1972 through 2008. Viruses of genotypes 1B and 1G were found in the Americas (mostly in Brazil) over a 7-year time span, indicating that these viruses were likely endemic. Viruses of genotype 1E may also have been endemic, but they were found only from 1997 through 1999, which would also be compatible with multiple importations. Furthermore, the period 1997–1999 was before major rubella control activities in most countries in the Americas, so if genotype 1E was endemic, its disappearance after 2 years would be surprising. Viruses of genotype 1j have only been found in the Americas as either known or likely imports [42, M. M. Siqueira and E. Abernathy, unpublished data].

The WHO European Region

The WHO European Region (EUR) has established a rubella elimination and CRS prevention goal by 2015 [5]. Among the different countries in this region, there are considerable differences in strategies and approaches to rubella control and rubella and CRS surveillance. In addition to different vaccination schedules with different kinds of rubella-containing vaccines and implementation of SIAs [28], many different reporting systems are currently in place. In some countries, reporting is mandatory for both rubella and rubella in pregnancy/congenital rubella infection (CRI)/CRS cases; in other countries, it is mandatory only for rubella cases or only for rubella in pregnancy/CRI/CRS cases. Some countries rely on voluntary laboratory-based reporting systems or operate supplementary sentinel systems [29]. In addition, there are discrepancies related to whether clinical cases or only laboratory-confirmed cases are reported, further complicating comparisons of reported cases between countries [29–31].

The quality and sensitivity of surveillance differs between countries, but compared with the total number of reported cases, the number of laboratory-confirmed cases and/or cases with known epidemiological links or importation status still seems low (WHO database; D. Featherstone, personal communication) [30]. The majority of rubella cases seem to occur among unvaccinated individuals, and there are still many cases reported among individuals ≥20 years old [29–31].

From 2007 through 2009, WHO received aggregated case numbers from 49 of the 53 EUR states [32], but data on virus genotypes were available from only 7 countries (WHO database; D. Featherstone, personal communication). Both WHO and EUVAC.NET data from this period indicate that the bulk of cases were reported from only few countries and that several countries reported few or no cases from 2007 through 2009 [29–32]. Some countries in the region may have achieved rubella elimination, whereas others are still far from realizing this goal.

Little or no baseline information on endemic rubella virus genotypes is available from most EUR countries. Sufficient information on whether a case is endemic or imported may not be collected, and thus it is sometimes difficult to classify viruses as endemic or imported. From 2007 through 2009, reports of 88 RV genotypes (31 of genotype 1h, 24 of genotype 1E, 21 of genotype 2B, and 12 of genotype 1G) from 7 different countries in the EUR (Russia, United Kingdom, Kazakhstan, Bosnia and Herzegovina, Poland, Ukraine, and Kyrgyzstan) were submitted to the WHO genotype database, most of them from Russia (n = 51) and from the United Kingdom (n = 20) (WHO database; D. Featherstone, personal communication). RV sequence information for ~300 sequences from 18 EUR countries is

available on GenBank. For several years, viruses of genotypes 1E, 1h, and 1G were among the most widespread in the EUR (WHO database; D. Featherstone, personal communication) [20, 21, 33, 34], whereas in the United Kingdom, many cases of genotype 2B were registered (WHO database; D. Featherstone, personal communication) [35]. The latter genotype has lately been identified more often in other EUR countries (eg, Bosnia and Herzegovina, Kazakhstan, Russia, and France) both from sporadic cases and from outbreaks (WHO database; D. Featherstone, personal communication) [21, 36].

The WHO Eastern Mediterranean, Western Pacific, African, and South-East Asian Regions

Overall, countries in the Eastern Mediterranean Region (EMR), Western Pacific Region (WPR), African Region (AFR), and South-East Asian Region (SEAR) of WHO have significantly improved virologic RV surveillance in recent years. There are considerable differences in the extent of rubella control and elimination programs among the countries within these regions. The WPR has an accelerated rubella control and CRS prevention objective [37]. The genotype of at least 1 RV has been determined from many countries in the AFR, EMR, SEAR, and WPR (Table 1). Most of these countries are working to establish genetic baseline data prior to embarking on significant rubella control and elimination efforts.

The strong virologic surveillance required in countries where rubella is controlled has often provided the genotypes of viruses from countries without strong rubella virologic surveillance. Such surveillance of imported cases has enabled the identification of the genotype of viruses in the country of exposure (Table 2) [WHO database; D. Featherstone, personal communication, 38–40]. This information has proved valuable in the exporting regions and at the global level. Thorough investigation of imported cases is important, since infections can occur in airports, in airplanes, or in transit centers [40].

SPECIFIC EXAMPLES FROM CURRENT VIROLOGIC SURVEILLANCE

Virologic Surveillance in a Country That Has Eliminated Endemic RVs and CRS

In the United States, the Centers for Disease Control and Prevention reported in 2005 the results of an expert review panel that considered the status of rubella and CRS elimination in the United States [41]. Based on a review of the history of the rubella vaccination program in the United States, epidemiology, molecular epidemiology, seroprevalence, vaccine coverage, and adequacy of surveillance, the panel concluded that RV was no longer endemic in the United States [42]. A recent summary of all available data provided evidence that elimination of endemic rubella has been maintained. The data in support of maintenance of elimination included a virologic surveillance profile of rubella and CRS that showed a pattern of viral genotypes consistent with viruses originating outside the United States [43].

Table 2. Reported Export and Import Pairs of Rubella Virus, 2005–2010

Exporting country, importing country	Genotype	Year
China		
United States	1E	2008
United Kingdom	1E	2010
Dubai		
Canada	2B	2009
Egypt		
Canada	2B	2007
France		
United Kingdom	1E	2005
India		
United States	2B	2008, 2010
Italy		
United Kingdom	2B	2008
Côte d'Ivoire		
United States	1G	2005
Kazakhstan		
Russia	1h	2008
United Kingdom	2B	2008
Kenya		
United States	1G	2010
Malaysia		
United States	1E	2005
Mexico		
United States	2B	2008
Netherlands		
Canada[a]	1G	2005
Philippines		
China HK SAR	1j	2010
Poland		
United Kingdom	1E	2008
Russia		
United Kingdom	1G	2005
Uganda		
United States	1G	2007
Vietnam		
China	2B	2006
China (Province of Taiwan)	1E	2007

NOTE. The criteria for establishment of epidemiological links may not be consistent between pairs. HK SAR, Hong Kong Special Administrative Region.

[a] This importation led to a large outbreak in Canada (see text for further discussion).

Since rubella and CRS have been eliminated from the United States, identified cases receive intense investigation, including, when possible, determinations of the genotypes of the viruses. From 2007 through 2010, the genotypes of viruses from 14 cases found in the United States were determined to be 2B (9 cases), 1E (2 cases), 1G (2 cases), and 1j (1 case). Viral RNA was

detected in a specimen from 1 additional imported case, but insufficient RNA was available to determine a genotype. Genotypes of 7 of these viruses were consistent with the epidemiologic data about the country of exposure to rubella (India, China, Uganda, Kenya, and likely Mexico), and 7 cases did not have epidemiologic data indicating a country of exposure. Although good sequence information from these 7 viruses was available (5 genotype 2B viruses, 1 genotype 1E virus, and 1 genotype 1j virus), there is insufficient baseline data on RVs of these genotypes in the world to allow assignment of country of exposure on the basis of sequence information alone. As expected, documented cases of CRS occurring in the United States as the result of exposures in other countries have been found after 2005 [38].

Use of Virologic Surveillance in Regions Seeking to Certify Rubella Elimination

In countries or regions where rubella and CRS control has been achieved, molecular epidemiological data can help to support the process of certification of rubella and CRS elimination, especially if comprehensive baseline data are available for comparison. As described above, the Americas have adopted a rubella elimination goal, and virologic surveillance data will certainly contribute to certification of elimination. Tracking the elimination of viruses of genotypes 1C and 2B as well as virologic investigations of imported cases (Table 2) will likely be key contributions to certification [44]. The EUR of WHO is working toward a similar use of virologic surveillance data.

Use of Virologic Surveillance in a Country With Many Importations

Frequent importations of RVs pose challenges for some countries that are trying to eliminate rubella. Molecular epidemiological data obtained in such settings can help to distinguish between endemic and imported viruses. In the United Kingdom, for instance, most rubella cases during the past years were caused by genotype 2B viruses. Importations of viruses of genotypes 1E (eg, from Romania, France, Poland, and China), 1G (eg, from Africa and Russia), 1j (from the Philippines), and 2B (eg, from Italy and maybe Kazakhstan) were documented during the past 8 years, mostly to London [40].

Collection of Baseline Virologic Data in Countries With Endemic Rubella

Some countries are collecting rubella baseline data. In China, for instance, rubella vaccination was included in the national immunization program only in 2007, and large-scale investigation of RVs collected from 1979 through 2007 provided valuable information from the prevaccination era [4]. Five different genotypes (1a, 1E, 1F, 2A, and 2B), some of which cocirculated, were documented in China (eg, 1a and 2A from 1979 through 1984, 1F and 2B from 1999 through 2000, and 1F and 1E from 2001 through 2007). In Anhui province, genotype 1F and 2B viruses were likely replaced by 1E viruses in connection with an epidemic in 2001. From 2001 through 2007, genotype 1E viruses were clearly predominant in the 17 of 31 provinces surveyed in China, indicating that a similar genotype shift likely took place in other provinces [19]. Additional examples of countries with recent baseline data collections include Italy [18], Russia [34, 45], Japan [46], Belarus [20], and France [21].

Use of Virologic Surveillance to Document the Spread of Rubella Outbreaks

Molecular epidemiological data can be used to document importation followed by an outbreak or sustained circulation in the new location. In 2004–2005, a rubella outbreak was reported in the Netherlands, which spread to Canada, where it caused sustained transmission for a prolonged period [33]. In total, 387 cases were reported in the Netherlands and 309 cases were reported in Canada; 97% of cases in both countries were among unvaccinated people belonging to orthodox Protestant communities. RV isolates obtained in the Netherlands and in Canada both belonged to genotype 1G and were closely related. These data were consistent with the spread of rubella from the Netherlands to Canada despite the lack of a known contact between cases in the Netherlands and the first cases in Canada [33].

SUMMARY AND CHALLENGES

Virologic surveillance has contributed significantly to major control and elimination programs for polio and measles [47, 48]. The suspected measles case definition also captures rubella cases. Therefore, measles surveillance will be improved in the course of the control and eventual elimination of rubella transmission. An important part of rubella surveillance is virologic surveillance for RVs.

Data from virologic surveillance for RVs contributed to the program to document the elimination of rubella and CRS from the United States and has already contributed to major rubella control programs in the Americas and Europe [42]. However, the lack of comprehensive rubella surveillance in other parts of the world, the paucity of RVs that have been sequenced, and the lack of an established sequence database for RVs limits the utility of data from virologic surveillance for RVs. It has not been possible to test the utility of virologic surveillance data in a number of situations, including tracking of the source of imports with sequence data alone (eg, some viruses imported into the United States and United Kingdom) and identification of the source of major outbreaks with sequence data alone (eg, the 2006 epidemic in Brazil). The current lack of understanding of the dynamics of RV circulation in unvaccinated populations is due to limited virologic surveillance over time [19]. Furthermore, a better understanding of the molecular evolution of RVs over time should allow rigorous assignment of RVs to genetic groups and determinations of other key epidemiologic

information (eg, duration of endemicity of specific RVs in a given location), as has been done for other viruses [49].

There have been significant efforts in virologic surveillance in countries that have eliminated rubella, in countries that seek to eliminate measles and rubella, and in some countries with no rubella control goals that are seeking to eliminate measles. However, considerable efforts to strengthen virological surveillance of rubella are needed to fully exploit data of this type in support of elimination efforts.

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Expansion of the Global Measles and Rubella Laboratory Network 2005–09

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Enhancing measles surveillance with integration of epidemiologic and laboratory information is one of the key strategies for accelerated measles control and elimination. The World Health Organization (WHO) Global Measles and Rubella Laboratory Network (LabNet) has been developed since 2000 to currently include 690 laboratories serving 183 countries. The LabNet testing strategy follows well-validated, standardized procedures for confirming suspected cases and for monitoring measles and rubella virus transmission patterns. The strength of the LabNet is a strong quality assurance program that monitors the performance of all laboratories through annual proficiency testing and continuous assessment. In the 5-year period 2005–2009, the results of >1 million measles immunoglobulin M (IgM) tests have been reported by the LabNet and, in addition, sequence information on >7000 measles and 600 rubella viruses has been shared. Progress with the development of the LabNet during 2005–2009 is discussed.

Progress with measles control since the year 2000 has been dramatic. It is estimated that >4.5 million measles deaths have been prevented as of 2008 through implementation of the strategies developed by WHO and the United Nations Children's Fund [1]. Four World Health Organization (WHO) regions (Americas [AMR], Eastern Mediterranean [EMR], European [EUR] and Western Pacific [WPR]) have established elimination goals, and the WHO African Region (AFR) has developed strategies leading to an elimination goal that they term "pre-elimination." The Southeast Asian Region (SEAR) has a mortality reduction goal.

Enhancing measles surveillance with the integration of epidemiologic and laboratory information is one of the key strategies for accelerated measles control and elimination, and the WHO Measles and Rubella Laboratory Network (LabNet) has been established to provide timely and accurate data for the surveillance of measles and rubella.

FUNCTION OF THE GLOBAL MEASLES AND RUBELLA LABORATORY NETWORK

The LabNet plays a critical role in confirming suspected cases of measles through the detection of specific immunoglobulin M (IgM) and testing measles-negative cases for evidence of rubella infection. In addition, identification and characterization of measles and

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rubella viruses provide information about the origin of outbreaks, whether endemic or imported, which is required for monitoring progress toward elimination.

As disease prevalence falls, medical personnel become less experienced in recognizing clinical measles and rubella, thus increasing the importance of laboratory methods to distinguish measles and rubella from other diseases with similar clinical presentations. The strategy of testing measles-negative cases for rubella was developed primarily to enhance the measles surveillance system in the WHO Region of the Americas. This strategy revealed previously unrecognized widespread circulation of rubella virus in many countries in the region. Furthermore, various studies indicated that the disease burden of congenital rubella syndrome was significant in the Americas and underscored significant morbidity from rubella and congenital rubella syndrome in the Western hemisphere [2–4]. After measles elimination was accomplished in the AMR in 2002, a rubella elimination program was established using the same strategies, including laboratory-based surveillance.

As of July 2010, the LabNet consists of 690 laboratories, all of which follow a standardized set of testing protocols and reporting procedures with a strong focus on quality assurance. The structure of the LabNet was based on the successful polio laboratory network [5] and was established in 4 tiers of laboratories: national level (n = 162), regional reference (n = 19), global specialized (n = 3), and subnational level (n = 506) (Figure 1).

The cornerstones of the LabNet are the national-level laboratories, which provide timely and accurate case confirmation of suspected measles and/or rubella cases using standard enzyme-linked immunosorbent assay (ELISA)-based IgM detection from serum samples collected at first contact with the health system. These laboratories are closely linked with the national disease control programs and often are responsible for surveillance of other exanthematous diseases as well, including dengue, parvovirus B19, and human herpesvirus type 6. Differential testing for other exanthematous disease can improve the interpretation of measles and rubella test results, especially in low-incidence settings. By June 2010, 160 of the 193 member states of WHO had established national laboratories with a further 24, mostly countries with small populations, served by another country's laboratory. Some large countries or those with logistical challenges in transporting samples have established laboratories at the subnational level to facilitate the timely testing and reporting of results. For example, China has established 362 laboratories at the first (n=31) and second (n=331) administrative levels (provincial and prefecture, respectively) [6]. In 2009, the Chinese laboratory network tested more than 86,000 serum samples for measles and rubella IgM antibodies following the same testing strategies and comprehensive quality assurance and accreditation assessments as the remainder of the global LabNet.

Two or three regional reference laboratories have been selected in each WHO region. The regional reference laboratories are regional centers of excellence that support the capacity building of the national-level laboratories under their

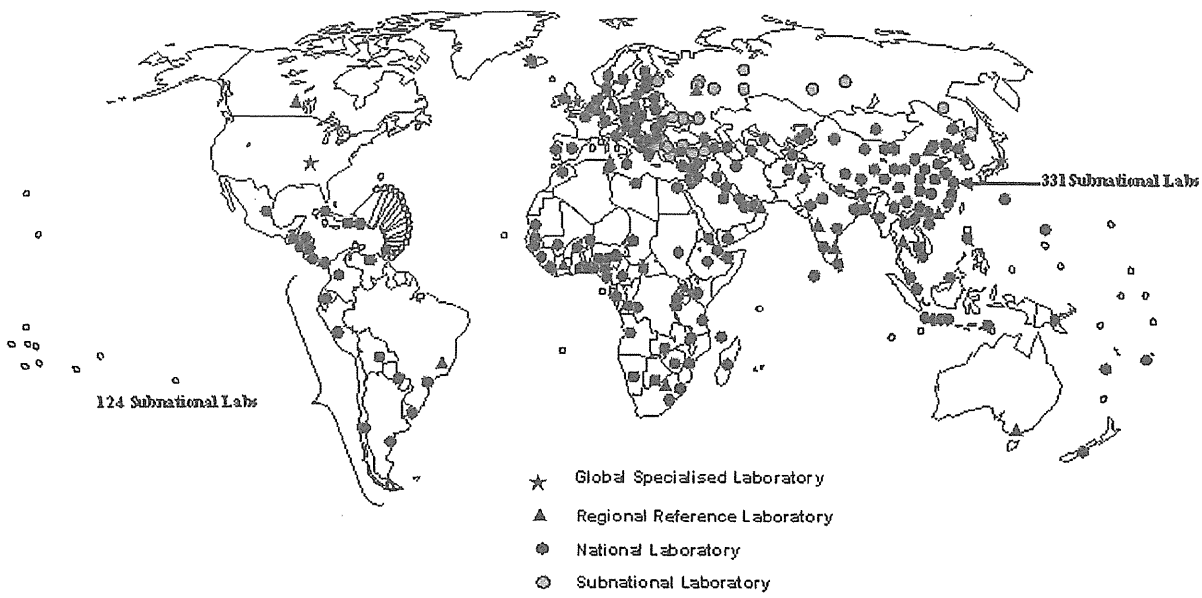

Figure 1. Location of laboratories belonging to the Measles and Rubella LabNet.

responsibility and provide confirmatory testing of a proportion of the samples tested by the national-level laboratories. They also support genetic characterization of circulating wild-type viruses to help monitor viral transmission pathways and progress toward measles elimination. Global specialized laboratories are global centers of excellence that have been established in Japan, the United Kingdom, and the United States. The global specialized laboratories contribute to the standardization of procedures and protocols, development and validation of new techniques, and global capacity building through focused training, and some provide financial support.

LABORATORY TESTING STRATEGIES

The standard LabNet test for measles and rubella confirmation is the detection of IgM in serum, oral fluid, or eluates of dried blood spots collected from suspected cases at their first contact with a health facility [7]. For molecular testing, preferred samples are throat swabs or oral fluid for direct virus detection of viral RNA by reverse-transcription polymerase chain reaction assay. Throat swab samples are also used to isolate measles virus in cell culture. Urine samples and peripheral blood mononuclear cells can also be used for virus culture, although in practice these have proven to be more difficult to collect, to transport, and to process successfully. The LabNet has introduced the Vero/hSLAM cell line as the standard for virus isolation, provided by Dr Yanagi [8]. This cell line was demonstrated to be sensitive for both wild-type measles and rubella virus culture [9](unpublished data from LabNet) and has proven to be easily handled in the LabNet.

ELISA-based IgM detection in serum was established as an accurate, simple, and rapid procedure to confirm cases of suspected measles or rubella. Various commercial assays were comprehensively validated [10], and assays with high sensitivity and specificity are currently used by most national laboratories in the LabNet. National and subnational laboratories in some countries use locally produced commercial assays. Locally produced assays in the United States, the Netherlands, Japan [11, 12], China, and the Russian Federation have been evaluated (unpublished data from LabNet), some of which have shown sensitivity and specificity comparable with the commercial assays used by most laboratories in the LabNet.

A serum sample collected at first contact with a health facility is considered the gold standard for measles IgM ELISA detection, because sensitivity has been reported to be 77% within 72 hours after rash onset and 100% at 4 days [13]. Oral fluid and dried blood samples have been used for enhancing the diagnosis of numerous diseases [14–20] and have been considered for use in the LabNet. The LabNet has comprehensively evaluated oral fluid and dried blood sampling techniques, using IgM detection for measles and rubella diagnosis and viral RNA detection and sequencing for molecular surveillance, determining their temperature stability outside the cold chain, and assessing their ease of use [21, 22]. In 2010, five African countries (Benin, Cote d'Ivoire, Kenya, Malawi, and Zimbabwe) began evaluating oral fluid sampling techniques as a mechanism to enhance surveillance through use of a single sample for both case confirmation (IgM) and molecular surveillance (RNA). If results from the trial are encouraging, this technique will be considered for introduction to a larger number of countries in the region. Several countries within the WHO Southeast Asian Region are also considering trying the use of oral fluid samples to enhance surveillance.

QUALITY ASSURANCE AND SURVEILLANCE INDICATORS

Quality assurance is a major strength of the LabNet and includes confirmatory testing of national and subnational laboratory results, a global proficiency testing program, implementation of in-house quality control measures, and an accreditation program. The global proficiency testing program for the LabNet began in 2000, and initially 38 laboratories were involved [23]. The proficiency testing serum panels consist of 20 samples from patients with recent measles, rubella, or other exanthema and are produced and analyzed by the Victorian Infectious Diseases Reference Laboratory in Melbourne, Australia, one of the LabNet's regional reference laboratories for the Western Pacific Region. In 2009, there were 220 laboratories participating in the proficiency testing program, including all national, regional reference, and global specialized laboratories, the 31 Chinese provincial laboratories, and all subnational laboratories in the Russian Federation, Turkey, and Ukraine. For measles, 98% of laboratories achieved a passing score ($\geq$90%), with 84% of laboratories achieving 100%. For rubella, the scores were 99% and 94%, respectively.

Because the proficiency testing program provides a snapshot of the accuracy of IgM testing, a continual assessment of a laboratory's testing accuracy was established through a confirmatory testing program. This program requires national and subnational laboratories to forward annually a representative sample (approximately 10% of all samples) to the designated regional reference laboratory for repeat testing with an expected concordance of $\geq$90%. The WHO accreditation program monitors performance of laboratories on a continual basis, but the assessment is expected to be completed annually. Seven performance criteria are assessed: reporting timeliness of case confirmation results, reporting timeliness of genetic characterization data, accuracy of results compared with the reference laboratory, implementation of specified quality control procedures, a passing score in the annual proficiency test, a minimum number of 50 tests performed per year, and a passing score on a comprehensive onsite review of the laboratory's work practices. National laboratories and reference laboratories have similar

criteria, but the expected passing scores are higher for the reference laboratories.

The function of the accreditation program is not only to monitor the quality of the LabNet but also, and most importantly, to identify any capacity building that is needed and to troubleshoot any problems that may have arisen. A laboratory that achieves less than the passing score on any one of the performance criteria will work with the WHO regional laboratory coordinator to identify areas in which improvement is needed and to develop and to implement a workplan to reach the full accreditation benchmarks as soon as possible. Quality assurance of serologic testing is comprehensive, and plans have been developed for a similar program to ensure the accuracy of molecular techniques and the sensitivity of virus culture.

A number of the surveillance indicators that are used to assess the quality of measles surveillance are dependant on laboratory function. These indicators include the percentage of cases with an adequate sample, the percentage of samples that arrive in the laboratory within 3 days, the percentage of results reported within 7 days, and the percentage of suspected cases discarded by the laboratory. LabNet member laboratories have achieved the target of 80% for most of these indicators; however, in some regions obtaining adequate samples is difficult because of logistic problems or inadequate case investigations, and often timely transportation of samples to the laboratory is difficult to achieve.

LABNET COORDINATION

Coordination of the LabNet is a key facet to a successfully functioning network. Communication among all levels of laboratories is carried out through regular meetings, workshops, and information exchange through telephone and email. Global LabNet meetings have been held annually since 2001, and regional laboratory coordination meetings are held in each of the 6 WHO Regions every 1–3 years. Each of the 6 WHO Regions has a dedicated laboratory coordinator with expertise in virology and experience with managing laboratory networks. These coordinators are positioned in the regional or subregional offices, and one is located in WHO headquarters. Their primary roles are to work with Ministries of Health to assess and to select appropriate laboratories, to expand the capacity and capability of LabNet through standardized training activities, to ensure laboratories are provided with appropriate equipment, to provide standardized supplies, to establish regular reporting structures, to perform accreditation reviews, and to coordinate the resolution of any performance issues. The regional reference and global specialized laboratories play a key role in strengthening capacity through support of training, providing troubleshooting advice, and developing and validating new technologies to improve laboratory-based surveillance.

LABNET INTEGRATION AND CAPACITY BUILDING

The LabNet was built on the experience gained from establishing the polio laboratory network and capitalized on the same infrastructure in a number of countries. In turn, the measles and rubella LabNet has been used to build similar laboratory-based surveillance networks globally. As of June 2010, these include laboratories focusing on yellow fever in West and Central Africa (n=23), Japanese encephalitis in SEAR (n=13) and WPR (n=9) (Figure 1), and human papillomavirus infection (n=10) globally. The rotavirus laboratory network was established in the late 1990s in WPR and SEAR and extended to all WHO regions by 2007, using a similar proficiency testing and data sharing model [24–26]. The pediatric bacterial meningitis laboratory network for *Haemophilis influenzae* type b surveillance, dating from the early 2000s in the African region, used a similar proficiency testing and data sharing program. There is considerable overlap in personnel, laboratories, and institutions used for these different surveillance activities. A number of measles LabNet personnel have also been involved in the enhanced global surveillance for pandemic influenza and in establishing routine surveillance for seasonal influenza. The more recently initiated global disease surveillance programs benefit from the capacity developed in establishing the polio and measles and rubella LabNets, especially through the linkages developed with WHO and specialized laboratories, the provision of standardized equipment and supplies, and the skills developed through the extensive training programs implemented.

The capacity of the Measles and Rubella LabNet has developed rapidly, especially in the past 5 years. The number of samples tested for measles IgM has increased from 119,000 in 2005 to 173,000 in 2009, with most of the increase in the Western Pacific Region. The number of samples from SEAR is expected to increase considerably after India begins case-based surveillance (Figure 2). Approximately 67% of all samples were also tested for rubella IgM, with approximately 400,000 IgM tests being performed in total by the LabNet in 2008. In 2009, there were 193,120 samples received for testing in the LabNet, of which 327,082 tests were performed for measles and/or rubella IgM antibodies (Table 1).

For monitoring and tracking molecular surveillance data, genotype databases for measles and rubella were established at WHO headquarters in 2006, which include genotype and basic epidemiologic data. As of 1 June 2010, 7675 measles viruses from 123 countries have been sequenced and key variables submitted to this database, and details on 600 rubella viruses from 36 countries have been submitted [27, 28]. In 2008, a joint Health Protection Agency and WHO measles sequence database (MeaNS) was also established, which included sequences from 4,200 viruses by 1 June 2010.

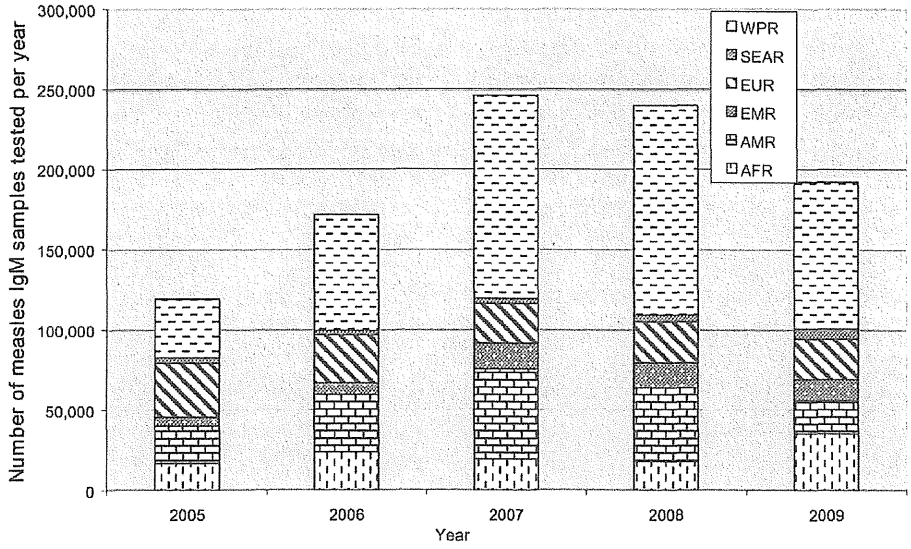

Figure 2. Measles immunoglobulin M (IgM) samples tested per year, by World Health Organization region, 2005–09. Two large countries, India and Pakistan, have yet to establish case-based surveillance. AFR, African Region; AMR, Americas Region; EMR, Eastern Mediterranean Region; EUR, European Region; SEAR, Southeast Asian Region; WPR, Western Pacific Region.

RESOURCES

Resources for the LabNet are provided largely through bilateral support mechanisms. Countries provide the infrastructure costs and cover staff salaries and overheads; and WHO, with the aid of its partners—a key one of which is the US Centers for Disease Control and Prevention—supports training activities and assists laboratories in obtaining optimal levels of equipment and supplies [29]. In some WHO Regions, operational costs may be provided to priority countries in addition to supplies and training.

DISCUSSION

The development of LabNet has progressed rapidly during the past 10 years. The number of laboratories has increased from 60 in 2000 to 690 in 2010 but has likely reached a plateau, because almost all countries have established laboratory-based measles surveillance. However, the complexity of testing will continue to increase as molecular surveillance to determine transmission patterns and to distinguish between imported and endemic measles cases is fully embraced. LabNet testing is critical for adding specificity to surveillance information when incidence

Table 1. Reported Laboratory Workload and Performance of Laboratories Participating in the LabNet by World Health Organization (WHO) Region, 2009

		Measles			Rubella			
WHO Region	No. of serum samples received	No. of samples tested	No. of samples IgM positive	% Positive	No. of samples tested	No. of samples IgM positive	% Positive	Results reported within 7 days,[a] %
AFR	36,558	35,242	10,088	29	29,611	5157	17	59
AMR	20,668	20,415	89	0.4	20,415	18	0.09	84
EMR	13,222	13,222	5950	45	11,824	770	7	95
EUR	24,929	18,665	3314	18	17,660	1094	6	78
SEAR	6561	6128	1103	18	5368	2656	49	70
WPR	91,182	71,426	34,412	48	49,084	12,787	26	>90
Total	**193,120**	**165,098**	**54,956**	**33**	**133,962**	**22,482**	**17**	

NOTE. AFR, African Region; AMR, Americas Region; EMR, Eastern Mediterranean Region; EUR, European v; SEAR, Southeast Asian Region; WPR, Western Pacific Region.

[a] For the AMR, percentage of results reported within 4 days.

decreases and the predictive value of clinical diagnosis diminishes. In addition, monitoring progress toward elimination through sequence analysis of currently circulating wild-type viruses is needed to determine whether a virus is endemic or imported.

Despite the large improvement in molecular surveillance, gaps in baseline molecular surveillance for measles still exist and no genotype data are available for 72 countries, although 25 of these are from the Region of the Americas, which has already eliminated measles, and 20 are small countries with a very low incidence of measles. Gathering information on baseline rubella virus sequence data is still at an early stage of development, with no data reported from 158 countries.

As countries progress toward elimination of measles, laboratories will be required to provide genotype information from 80% of the chains of transmission. Achieving this target will require close coordination between those conducting case investigations and the LabNet and will increase the number of samples that must be processed and tested by the laboratory. Also, in elimination or pre-elimination settings, rapid confirmation of rashes due to vaccine reactions using molecular techniques will become increasingly important.

The positive predictive value of the procedure of using a single IgM ELISA on serum for confirming suspected measles and rubella cases in the LabNet is high when incidence of disease is high, but concerns have been raised as to whether these testing procedures are appropriate under low-incidence settings, because the positive predictive value decreases significantly [30]. Although the WHO Region of the Americas has successfully documented elimination of both measles and rubella using these testing strategies, the LabNet is investigating procedures that may improve the positive predictive value in low-incidence settings. In addition, specialized serologic testing, such as IgG avidity assays and plaque-reduction neutralization assays, are used in more advanced laboratory settings to aid in the classification of more difficult cases that sometimes arise in low-incidence settings [31, 32].

CHALLENGES

As of 2009, only 12 (Algeria, Comoros, Guinea Bissau, India, Mauritius, Monaco, Morocco, Pakistan, San Marino, Sao Tome and Principe, Seychelles, and Somalia.) of the 193 member states of WHO have yet to implement case-based surveillance with laboratory confirmation; however, 2 are large-population countries: India and Pakistan. However, LabNet laboratories are performing measles outbreak confirmation in all 12 except Mauritius, San Marino, Sao Tome and Principe, and Seychelles. The LabNet has shown that it has the capacity to conduct up to 400,000 IgM tests annually (2008) for measles and rubella, and individual laboratories have responded to an almost 30-fold increase in weekly baseline testing rates. When all countries establish case-based surveillance and meet the minimum surveillance and testing criteria, the annual number of LabNet tests will increase by an estimated 70,000. The LabNet has the capacity to meet this increased throughput; however, finding the resources to procure additional kits and to train and to equip additional laboratories in the countries that have yet to meet the surveillance indicators will require increased advocacy efforts and more country commitment to supporting measles and rubella laboratory-based surveillance.

Despite progress in measles control, large measles outbreaks occurred in 2009 and 2010 in Bulgaria and several African countries. Testing all suspected cases during large outbreaks is not essential if countries follow WHO's recommended guidelines to epidemiologically link suspected cases to laboratory-confirmed cases [33]. However, some countries continue to collect samples from almost all suspected cases during an outbreak, often increasing the laboratory's workload by many-fold. For example, in 2009 South Africa experienced a large outbreak of measles that saw the national laboratory reporting almost 17,000 positive cases during a 12-month period starting in mid-2009 [34]. The laboratory tested approximately 50 serum samples per week before the outbreak but progressed to testing >1400 specimens per week at the peak of the outbreak, within a period of several months. Although the national laboratory performed exceptionally well in managing the heavy workload, it was put under considerable pressure to provide an acceptable turn-around time while at the same time maintaining high-quality results. Laboratories in other countries, including Botswana, Cambodia, Lesotho, Malawi, Philippines, Swaziland, Ukraine, Viet Nam, and Zimbabwe, have experienced a large increase in workload following outbreaks. Although the weekly increase in number of samples tested was less than in South Africa, the impact on increasing the reporting turnaround time has been similar. Procuring sufficient test kits, maintaining quality, and timely reporting of results under these circumstances make a considerable challenge.

Some countries have a considerable number of private laboratories in the health sector that are used for testing suspected measles cases. Many of these laboratories are funded through health insurance and may test only for the specific diseases covered by the reimbursement. It has been reported from the European and Western Pacific regions that the large number of these laboratories in some countries makes it difficult to monitor the quality and accuracy of test procedures and to ensure regular reporting through the public health system. In the Region of the Americas, the United States reported that approximately 25,000 measles IgM tests were performed by private laboratories in 1997 [35]. External monitoring of quality control in commercial laboratories is a challenge, although in some cases, LabNet laboratories provide confirmatory and follow-up testing procedures.

Table 2. Laboratory-Based Training Workshops Held January 2009–June 2010 for Measles, Rubella, Yellow Fever, and Japanese Encephalitis Surveillance

Region	No. of countries	No. of laboratories participating	No. of participants
AFR	24	26	38
EMR	11	12	18
SEAR	11	18	40
WPR	14	46	82
Total	60	102	178

NOTE. AFR, African Region; EMR, Eastern Mediterranean Region; SEAR, Southeast Asian Region; WPR, Western Pacific Region.

As part of the quality assurance program, national and subnational laboratories are required to send a proportion of their IgM samples to their designated reference laboratory for confirmatory testing. The international shipment for these procedures has amounted to a considerable cost burden for some countries and has posed multiple logistic difficulties when samples are transported across national borders. The LabNet has validated the use of drying serum onto filter paper for temperature-stable shipment, thus reducing the cost of shipping under refrigeration temperature. Furthermore, dried serum samples are approved as exempt from dangerous goods requirements and regulations [36].

The LabNet has strengthened the health system by building capacity, strengthening laboratory infrastructure, and developing human resources. In the 18 months from 2009 to June 2010, >170 national and reference laboratory staff were trained to perform IgM ELISA procedures and polymerase chain reaction and sequencing techniques, and to improve data and laboratory management for measles, yellow fever, and Japanese encephalitis surveillance (Table 2). However, as countries strive to reach their measles elimination goals, partnership support for the LabNet must increase as the complexity of testing and number of samples increase.

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SUPPLEMENT ARTICLE

Global Distribution of Measles Genotypes and Measles Molecular Epidemiology

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A critical component of laboratory surveillance for measles is the genetic characterization of circulating wild-type viruses. The World Health Organization (WHO) Measles and Rubella Laboratory Network (LabNet), provides for standardized testing in 183 countries and supports genetic characterization of currently circulating strains of measles viruses. The goal of this report is to describe the lessons learned from nearly 20 years of virologic surveillance for measles, to describe the global databases for measles sequences, and to provide regional updates about measles genotypes detected by recent surveillance activities. Virologic surveillance for measles is now well established in all of the WHO regions, and most countries have conducted at least some baseline surveillance. The WHO Global Genotype Database contains >7000 genotype reports, and the Measles Nucleotide Surveillance (MeaNS) contains >4000 entries. This sequence information has proven to be extremely useful for tracking global transmission patterns and for documenting the interruption of transmission in some countries. The future challenges will be to develop quality control programs for molecular methods and to continue to expand virologic surveillance activities in all regions.

A critical component of laboratory surveillance for measles is the genetic characterization of circulating wild-type viruses to provide support for molecular epidemiologic studies [1, 2]. The World Health Organization (WHO) Measles and Rubella Laboratory Network (LabNet) provides for standardized testing and reporting, with laboratories in 183 countries. The primary function of LabNet is to provide laboratory confirmation of suspected cases of measles and rubella [3, 4]. LabNet also supports genetic characterization of currently circulating strains of measles virus and is responsible for standardization of the nomenclature and laboratory procedures that are used for genetic characterization of wild-type measles and rubella

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