

The Fifth WHO Global Measles and Rubella Laboratory Network Meeting

26 to 28 September 2007, WHO, Geneva

Final Summary and Recommendations

Global and Regional Update

The measles and rubella laboratory network (LabNet) consists of 679 laboratories serving 164 countries. More than 180,000 serum samples were tested globally for measles IgM in 2006 and more than 89,000 in the first 6 months of 2007. Timeliness and quality of the testing is high with more than 80% of laboratories reporting at least 80% of their results within 7 days. An annual proficiency testing programme has been in place since 2001 when 47 laboratories participated with 96% achieving the pass rate of 90% or better. In 2006, the Region of the Americas' measles and rubella laboratories participated in the WHO proficiency testing programme for the first time. Of the 164 laboratories which received the panel in 2006, 98% passed the measles component and 97% passed the rubella component. The measles and rubella laboratory manual has been completed and is translated into Russian and Spanish and in the process of being translated into French. Two Laboratory oriented WER articles were published over the past 12 months, one covering an update on measles and rubella molecular surveillance and the other with an update on rubella nomenclature.

African Region

Each of the WHO regions has established regional LabNets. The African (AFR) measles LabNet comprises 36 national and 3 regional laboratories and is fully integrated with yellow fever testing, where appropriate. Most laboratories are functioning at a high level of proficiency with only Chad failing to achieve the minimum score. Recently established laboratories in Nigeria, Mozambique, Comoros, and Guinea Bissau will be provided PT panels for first time in 2008. Laboratories in Algeria and Liberia are expected to be integrated into the AFR LabNet in 2008. Training is seen as a priority for the AFR laboratories due to recent establishment of new laboratories, turnover of staff and as new techniques are implemented. The accreditation process is well under way, with 22 laboratories having completed the process. AFRO reported plans for 15 laboratories to undergo onsite reviews and 18 laboratories for desk reviews over the next semester.

The region's main challenges over the past year have been the coordination of the LabNet following the recent African Regional Office decentralization. Measles laboratory supplies will be managed from the Burkina Faso Inter-Country Support Team (IST) office and emergency stocks will be stored at the Harare IST office and distributed upon request. It was recommended that regular communication through conference calls continue on a regular basis with all three AFRO lab coordinators, and partners participating.

Eastern Mediterranean Region

The Eastern Mediterranean region (EMR) has established a measles elimination goal targeted for 2010. All 22 EMR countries have established measles national laboratories. The region saw large outbreaks of measles in 2006 and 2007 in Saudi Arabia, Kuwait, Qatar, Dubai, Egypt, South Sudan, Lebanon, Jordan and Syria, with numbers of serum specimens tested in the first semester of 2007 exceeding that for all of 2006. Twenty

countries passed the most recent proficiency test with only Afghanistan failing to meet the 90% passing score. Seventeen laboratories have been assessed for accreditation and 5 remain to be assessed. A measles and rubella virus isolation and molecular detection training workshop was held in March 2007 with 9 laboratories represented. Following the workshop, Kuwait, Oman, Pakistan and Qatar were able to identify wild type measles virus for the first time. Oman and Tunis regional reference laboratories have been identified as the measles sequencing reference laboratories for the region, though several national laboratories also have the capacity to sequence viruses. Challenges for the EMR LabNet include;

- surveillance problems in Somalia resulting in the laboratory not receiving samples,
- Morocco received fewer than 50 samples,
- staff turnover in Afghanistan, Iraq, and Lebanon has reduced capacity in these laboratories,
- logistics of shipping validation samples from national to regional reference laboratories,
- need to focus more attention on rubella and CRS surveillance

European Region

The European region (EUR) has established measles and rubella elimination targets for 2010. The EUR LabNet comprises 70 laboratories, including 20 sub-national laboratories, three RRLs and one Global Specialized laboratory. Laboratories tested 36,990 IgM samples in 2006, an increase of 3,500 on 2005, mainly due to the increased measles transmission in the region during 2006. The EUR laboratories have shown improved timeliness of reporting for 2006 compared with 2005, (from 81% to 87%). Eighteen countries underwent on-site accreditation assessments in 2006-07, with 13 planned for the next 12 months, 7 laboratories have yet to be assessed and the remainder underwent a desk review. Proficiency was good with only 1 laboratory failing the test in 2007, though 9 laboratories did not participate. Key challenges identified for the EUR LabNet are; enhancing laboratory support for rubella and CRS detection, and transportation of diagnostic samples from the field to the laboratory and QA samples from the national laboratory to the reference laboratory.

South East Asian Region

The South East Asian region has established a measles goal of 90% mortality reduction by 2010. Case-based surveillance with laboratory confirmation is encouraged as countries implement catch-up campaigns. All countries have established measles national laboratories with 4 in India to cover southern states and another 5 planned for 2007-2008 to cover the central and northern states implementing case based surveillance. A further 2 laboratories are planned for the Indonesian islands of Sumatra and Sulawesi. Timor Leste's laboratory has yet to establish routine testing. An RRL is well established in Bangkok and two additional RRLs in India are in the establishment phase, one for serum sample validation (Chennai) and one for molecular surveillance (Pune). Data collection has improved in the past 12 months with aggregated laboratory data now published monthly in the SEARO surveillance bulletin.

Western Pacific Region

The Western Pacific region (WPR) has recently appointed a new laboratory coordinator, Dr Youngmee Jee, to replace Dr Kojima. The LabNet comprises 16 National laboratories, 3 regional reference laboratories and 1 global specialized laboratory. The

Hong Kong SAR National Laboratories was re-designated as a reference laboratory in 2007 to provide additional capacity for sample validation and molecular surveillance. In addition China has established a network of 31 provincial and 331 prefecture laboratories which follow a standardized testing and reporting structure and a strong QA programme. In 2006, more than 74,000 serum samples were tested for measles IgM in the region, 70,000 of which were in China. However, reporting of measles data to the regional office is not optimal, with several key reporting entities (Australia, China, China Macau SAR and Japan) yet to establish regular routine reporting. Measles outbreaks were reported in Japan (D5) and Republic of Korea (H1) in 2007 and multiple importations were detected in Australia and Hong Kong during the past 12 months. All WPR national laboratories passed the global proficiency test in 2006 and all except 3 national laboratories have had an accreditation assessment. The remainder are planned to be assessed in the next 12 months. Training workshops were held in the China CDC laboratory for ELISA and virus culture techniques for Provincial and prefecture laboratories. Measles testing in PNG was re-established with the use of dried blood samples (DBS). DBS are also used in Cambodia and are planned for introduction into Laos and the Pacific Island countries. Plans for future development of the WPR network include: identifying budgetary resources for supporting national laboratories; facilitating exchange of measles and rubella genetic information within the LabNet; and implementing training workshops. A measles and rubella laboratory meeting will be convened in 2008, and will likely be combined with polio and JE LabNet meeting and in conjunction with the WPR TAG in June.

Region of the Americas

The Region of the Americas' (AMR) LabNet is made up of 148 laboratories of which 124 are sub-national, 21 national, 2 regional reference and 1 global specialized. More than 36,000 samples were tested in 2006 and 20,000 in the first semester of 2007. The global measles and rubella proficiency testing panel was introduced for the first time in the region in 2006 and all national laboratories which tested the panel passed with 100% score. One laboratory had yet to test the panel.

AMR is currently focusing on improving molecular surveillance. Though there are still many gaps in virus surveillance, numbers of samples collected for virus detection have risen from fewer than 100 in 2005 to 370 by July 2007. Currently 20 national and even some sub-national laboratories have the capacity to perform virus detection. To further strengthen laboratory capacity, a virus detection molecular workshop was held in Mexico in July 2007, for Central American countries, and a meeting for all sequencing laboratories is planned for early 2008. Countries have also been requested to transport their archived rubella positive serum samples to the region's sequencing laboratories in an attempt to amplify virus RNA directly from the serum for establishing a genetic baseline for rubella.

Challenges facing the region include;

- developing a strategy for differentiating true from false positive IgM samples,
- addressing the cost of shipping samples for virus detection or QA purposes,
- strengthening the integration of laboratory and epidemiological surveillance, and
- implementing a new laboratory reporting form.

Status of Alternative testing

Dried blood (DBS) and oral fluid (OF) samples continue to be seen as attractive alternatives to the "gold standard" serum sample for routine surveillance of measles and rubella, under specific circumstances. Evidence has been collected over the past two years, filling some of the knowledge gaps regarding temperature stability for IgM and RNA and the sensitivity of rubella IgM detection. Evidence was reviewed at the 2nd Alternative Sampling Meeting in June 2007 and the findings were presented at the Global LabNet meeting. In summary;

- The sensitivity and specificity of detecting measles and rubella IgM in DBS and OF is almost equivalent to serum although slightly lower sensitivity for rubella IgM in OF.
- RNA and IgM in DBS are temperature stable when fully dried and can be shipped at ambient temperature
- OF is sufficiently stable at 37 °C and 42 °C for up to 7 days to allow shipping at ambient temperature, when necessary
- The sensitivity for detecting measles and rubella RNA in OF samples ranges from 80-90% in the first week after rash onset and up to 50% 3-4 weeks after rash onset
- Combination testing for IgM and RNA can increase sensitivity of rubella detection in first 4-5 days after rash onset
- There are only marginal cost differences between serum, OF and DBS collection and testing procedures, however both OF and DBS require devices to be supplied to the health centres
- DBS has the potential to reduce transportation costs as it is exempt under IATA dangerous goods requirements
- OF is non invasive and easy to collect
- Training for the collection of DBS is critical to ensure adequate samples are collected
- Data related to the detection of rubella IgM in OF using the commercial Microlmmune assay is limited to two outbreaks. Further field evaluation would be helpful
- A commercially available rubella IgG assay is not yet available for OF
- Supplies of DBS and Oral fluid collection devices are not available at all collection centres
- The use of OF samples is currently limited to measles, rubella and mumps IgM detection. DBS has been validated with a wide range of differential assays but the range is more limited than for serum samples.
- It is not recommended to use OF for the detection of rubella IgM/IgG in pregnant women due to slightly lower IgM sensitivity compared with serum and as there is no rubella IgG assay available for OF.
- Transportation of DBS has not reduced costs in all countries undergoing the field trial in Africa. In DRC the DBS are sent by air due to challenges in transporting by other means in the country, though a reverse cold chain is not utilized. In one country, field staff were reluctant to forgo their transport reimbursement for hand carrying serum samples to the laboratory.

The alternative sampling methods working group recommended developing a summary of the dynamics of alternative sampling procedures in graphic form to help simplify

interpretation of the data. A standardized protocol and a spreadsheet summarizing the characteristics comparing DBS and OF with serum has also been developed.

Evidence was provided of OF sampling being used in the European region for measles and rubella surveillance. Almost all samples for measles and rubella surveillance in the UK are OF and approximately 25% of all samples collected in Western Europe were OF samples and the Netherlands successfully identified monitored a rubella outbreak and subsequent CRS cases using OF samples.

Other countries experiences of alternative sampling included; Brazil considering using oral fluid samples for measles and rubella surveillance at the sub-national level, Luxemburg drying down virus onto filter paper for shipping purposes, and PNG using dried blood filter paper for surveillance.

Data management

An update of the collection and reporting of global measles data showed considerable progress in regional reporting with all 6 regions now reporting data compared with only 3 regions in 2006. Currently 157 countries (81%) report monthly data to HQ, by district and month of onset, but these countries only represent 58% of the global population due to some large countries yet to start reporting (India and China). Data is being reported from both surveillance and laboratory data sources though the two are not always reconciled and there are still discrepancies between annual and monthly datasets.

The WHO measles genotype database has expanded from 1,200 viruses identified from 23 countries in 4 WHO regions 12 months ago to currently more than 2,500 measles virus identified from 59 countries in all regions. Some sequencing laboratories have yet to share their genotype data with the LabNet, however, the last 12 months has seen a number of sequencing laboratories willingly sharing their sequence information on a real time basis, to allow rapid confirmation of whether outbreaks are due imported or indigenous sources of virus. However, only 11% of the viruses which have been submitted to the database have also been submitted to GenBank. Submission of genetic information to GenBank is recommended to allow broader access to this data.

The rubella database currently has 106 viruses submitted from 17 countries in 4 WHO regions, reflecting the more limited rubella molecular surveillance occurring globally. However this is a considerable increase from the 5 viruses submitted to the database at the time of the last meeting 12 months ago.

Some of the issues discussed in relation to improving the WHO database included: making the access to SharePoint site more user friendly; submission of virus sequences to GenBank; sequencing of the recommended window; challenge of curating the database as it continues to increase in size; only representative viruses from outbreaks need to be submitted to the WHO database and GenBank; how to best present genotype data to the programme and the global genotype map needs to be updated on a regular basis. Laboratories need to be reminded that submission of sequences to the WHO genotype database or to GenBank does not constitute prior publication

Developments in measles sequence databases being developed by the Strain banks at HPA and CDC were reported.

CDC reported the development of a measles, mumps, rubella, and herpes virus database containing sequence and related epidemiologic information. Viruses are being sourced from CDC, WHO Collaborating Laboratories and GenBank. The database stores sequence data in fasta format and sequence-related information is being saved in a MS Access database. All sequences included in the CDC database have been validated, converted into a fasta format and submitted to the WHO database. GenBank measles sequences have also been downloaded, validated and assigned genotype and converted to fasta format if necessary. Currently the CDC database has 1588 sequences in the database made up of 214 sequences from CDC sources and 1374 sequences from GenBank.

CDCs is developing a BLAST search function, either at CDC or locally, for real time searching and is investigating an interactive mapping tool such as Google Earth. The database will facilitate monthly reporting to WHO and plan to make the Access epidemiological information database available to participating laboratories.

HPA developed a web based measles sequence database as part of an EU time limited funded project (ELSM) in 2002. The funding for the project expired in 2006 and the database had 1,949 patient details and 505 sequences submitted during this 4 year period.

HPA, in collaboration with EURO, have developed plans to expand concept of the ELSM database to include one which has the capacity to store measles sequences/trace files, facilitate genotyping and allow comparison/phylogeny with viruses in other countries. The quality of all submitted sequences will be checked and tools provided to search for identical or similar Measles sequences and/or to genotype measles strains. The database will feature the capability to automatically report to EURO, and the ability to upload to GenBank and the WHO genotype database. Access to the database will be open to LabNet and academia but will require prior registration and an agreement on the use of any data. The database currently contains ELSM and HPA data and it is planned to upload all GenBank measles data. The timetable for launching is December 2007 and it should be ready to receive trace files by March 2008.

Molecular epidemiology

Measles

Sequencing laboratories in each of the WHO regions updated recent measles and rubella molecular epidemiological surveillance data. There has been a big improvement in the collection of measles molecular epidemiological data in the past 12 months as more laboratories develop sequencing capacity and as an awareness of the programmatic worth of sequence information improves. In the past 12 months, 948 measles viruses identified in 2006 were submitted to the WHO measles genotype database.

The African regional molecular data revealed that B2, B3 and D4 genotypes continue to predominate in the region. B2 was detected in outbreaks in Angola, DRC, Kenya, Uganda and South Africa, and strains show up to 2.4% diversity. B3.1 was found uniformly throughout the region with Chad having identical sequences to Kenya. D4 is also found over much of AFR with up to 10.9% diversity detected. One D4 strain was found throughout Botswana, NW South Africa, Zambia and Zimbabwe between 2005 and 2006. Most of the AFR data is collected from sequencing IgM positive serum sent to

the south African RRL for QA purposes but also obtained through collection of urine samples and throat swabs from measles outbreaks. Clinical data is limited or lacking for many of the virological samples however, making full analysis difficult.

The European region reported a large diversity of measles viruses detected in the past 12 months. Three genotypes (D6, D4 and B3) predominated during 2005-2006. Multiple importations of genotypes D4 and B3 were detected from Africa and Asia and each showed considerable diversity. The D4 genotype, first identified during the large outbreak in Romania in 2004, continued to be found in Romania in 2007. The same D4 was also detected during sporadic outbreaks involving mostly Roma or Sinti populations in the Balkans, Germany, Italy, Portugal and Spain. Circulation of the European endemic genotype, D6, has yet to be interrupted and was found widely throughout Europe following spread from the Ukraine outbreak. Sporadic detection of other genotypes (B2, D5, D8, D9, G3 and H1) reflects multiple importations from other parts of the world. The absence of genotypes C2 and D7 as well as earlier variants of genotype D6 suggests that several chains of transmission may have been interrupted. The UK experienced outbreaks of B3 and D4 which were found mostly in under-immunized highly mobile populations. Sustained measles outbreaks were detected in Italy (D4), Switzerland (D5) and Germany (D5, D8). Measles cases or outbreaks in most of Europe were due to continued transmission of endemic measles virus strains and prolonged circulation of imported strains.

In the Eastern Mediterranean region there has been a steady improvement in molecular surveillance over the past 3 years. Oman (D5), Qatar (D4) and Kuwait identified measles viruses for the first time in the past 12 months following a virus detection workshop in March. However, collecting samples for virus detection is still a low priority for many countries in the region. Genotype D4 predominates in the region and is found in contiguous countries from Pakistan to Egypt. B3 has been found in Somalia, Sudan and Libya in the past year.

In the Western Pacific region, Japan reported recurrent measles outbreaks occurring in 1998, 2001, 2006 and 2007. Measles strains isolated in 1990-1999 and 2000-2003 were classified mainly as D5 Palau.BLA/93 like, but strains identified in 2007 were classified as belonging in the Bangkok.THA/93/1 cluster. Measles strains isolated during the past 2 years in Japan were closely related to D5 virus strains detected in Thailand, Canada, USA and UK. The Japanese 2007 D5 outbreak resulted in 2525 cases from "pediatric" sentinel sites being detected, (986 in the 0-4 year age group, 740 in 10-14 year group) and 805 cases were confirmed in adults.

In China, the measles LabNet is very active and has made a large contribution to the early diagnosis of measles outbreaks in the country. Genotype H1 is widely distributed throughout the country and approximately 100,000 measles cases were detected in 2006. Nineteen provinces reported 356 viruses isolated in the 12 months September 2006 to August 2007. Multiple transmission pathways were detected, but genetic diversity appeared to decrease compared to the previous 12 month period. The DPR Korea outbreak in early 2007 was confirmed as due to an H1 virus, which was shown to be very similar to a Henan isolate identified in 2007.

In the Region of the Americas, measles incidence remains at very low levels (187 cases in 2006 and 141 in 2007). However, surveillance activities have identified imported

cases in USA, Canada, Brazil and Venezuela some of which have resulted in small to moderate sized chains of transmission. A B3 measles outbreak in Venezuela began after an importation from Europe in 2006 and continued to circulate for more than 12 months and resulted in 111 cases. In 2007, measles cases were imported into the USA from Europe and Asia, but outbreaks were small and self contained. Canada had a similar picture to the USA, with cases or small outbreaks having epidemiological and/or virological links to Asia, Europe and the Middle East. An outbreak of 57 measles cases in rural Bahia, Brazil, could not be epidemiologically linked to an importation. However, the D4 sequence was identical, (over 450 nts) to a virus identified in Europe in 2006 and was not closely related to any previous Brazil D4 viruses.

From the South East Asian region, India reported the results of measles molecular surveillance from 17 sub-national centres, which was coordinated by NIV, Pune. More than 32 outbreaks were investigated over much of India during a 2 year period and 146 virus sequences were completed. Measles virus genotypes D4, D7 and D8 were detected in the past 3 years and showed considerable genetic diversity. The Bangkok sequencing laboratory reported recent (2006-07) D5 viruses identified in Thailand and Myanmar, D9 viruses in Java, and D4 and D8 in India.

Rubella

Global molecular surveillance for rubella has developed more slowly than measles, reflecting both that the virus is more difficult to isolate than measles and that only 2 WHO regions, the Americas and EUR, have established rubella regional elimination goals. As of September 2007, only 106 rubella viruses had been submitted to the WHO rubella genotype database, compared with 2530 for measles, although 481 rubella viruses can be found on GenBank. However, in the past two years regional surveillance activities have improved, new rubella virus identification techniques introduced and virus identification training workshops held in EMR and the Region of the Americas. These combined activities should enhance the capability of the LabNet to increase the sensitivity of detecting rubella viruses in the future.

In EUR, 35 countries reported rubella cases in 2006, 7 of which reported more than 1,000. In 2006, 6 countries also provided sequence information to the WHO genotype database. Of the 14 EUR countries with known rubella genotype information, 4 genotypes predominate, 1E, 1G, 1h and 1i, however genotypes 1D, 2B and 2C have also been detected. Russia (Perm) recently submitted a series of 2C viruses to GenBank, the most recent from 2005.

Japan has had annual rubella outbreaks with approximately 125 cases per week at the peak. A large outbreak in 2004 resulted in 4239 rubella cases and 10 CRS cases being detected. A new two dose MR vaccine schedule was introduced in 2006, the first dose to be given at 12-24 months and the second at 5-6 years, before school entrance. Eight rubella genotypes have been detected in Japan since 1966, and a possible novel genotype, which circulated between 1987-1991 and only recently recovered from the freezer. A 2B genotype virus was detected from a CRS case in 2007.

China has experienced large periodic rubella outbreaks every 7-8 years, the last in 2001-02. A national case-based reporting system has yet to be established but an internet direct reporting system was established in 2005. Serosurveillance studies held in Beijing and Chongqing cities indicated that 18% of women of child-bearing-age are at risk of rubella infection. Thirteen provinces identified 178 rubella viruses during 18

month period 2006-July 2007. Genotype 1E was the predominant genotype detected, with 2B also found in Sichuan.

Recent rubella virus genotypes identified in the Americas include:
Canada; 2007, 2B (imported from Egypt)
USA; 2007, 1G (imported from Uganda) and 2B (Unknown source)
Brazil; 2005, 1D; 2006-07, 2B
Chile; 2007, 2B (endemic)

The 4th Global LabNet meeting in 2006 reported the following information on rubella virus genotypes.

- Some genotypes are geographically restricted (e.g. 1C, 1D)
- Some genotypes are widely distributed (e.g. 1E, 1g)
- Genotype 1E which was first observed in 1997, is now widely distributed
- Africa, Eastern Europe, the Middle East, Southeast Asia and the Americas have major gaps in molecular surveillance.

Since the 4th Global LabNet meeting, new information on rubella genotypes has become clear.

- A clade 2 virus has now been found to be endemic in the Americas (genotype 2B).
- The wild-type rubella virus nomenclature update in the June 2007 WER article addressed the subgroups in the former provisional genotype 1g (now 1G, 1h, and 1i) and a new genotype from Japan reported at the 4th global meeting (now provisional genotype 1j).
- Some genotypes (other than provisional genotype 1g) exhibit “sub-genotypic” clusters (e.g. 1E).

Currently methods for genotype analysis are not specified, only that proper genotypic groups must be found for reference viruses. Analysis with multiple programs is desirable.

CDC described promising results with looking at improving the sensitivity for genotyping rubella viruses directly from clinical samples. The method uses 2 overlapping primers for the 739 nt recommended window and was sensitive down to about 10 copy numbers in the small number of samples evaluated.

Quality Assurance

VIDRL presented a review of the proficiency testing (PT) programme for the past 12 months. From 163 laboratories which returned results in the time for the meeting, 86% and 96% scored 100% for measles and rubella respectively, and 98% and 97% scored 90% or above. One problem arising from Panel 00607 was that a small number of samples were contaminated with bacteria which degraded the IgM. However, the individual results from laboratories that encountered problems with these samples were discounted. The new 00705 panel for 2007/08 has been filtered through 0.22 µm membrane filter and a subset held at room temperature for several weeks to confirm sterility and IgM stability before shipping to laboratories.

It was reported that a small number of laboratories used an assay to test the PT which was different to that used for their routine testing. One of these laboratories also stated that they did not need to send validation sera to the RRL as they had passed their PT.

Promising data was reported from HPA and CDC with evaluating serum samples dried onto filter paper for shipping purposes. Though the number of samples tested was limited, a very close correlation between the ODs of both dried and liquid serum was found by both laboratories, even with different drying and extraction procedures. HPA showed dried serum had a mean drop in OD of ~2% per day at 37 °C though there was some variability with individual samples. As most regions reported challenges in transporting samples from the national to the regional laboratory for validation purposes, the dried serum technique appeared to offer a possible solution and further field evaluation of the techniques was considered appropriate.

China's measles and rubella LabNet constitutes almost half the number of laboratories in the Global LabNet. More than 60,000 samples were tested in 2006 and 794/858 outbreaks were confirmed as measles and 73/84 outbreaks confirmed as rubella. The quality assurance programme in China parallels that of the rest of the LabNet, with proficiency testing, sample validation, timeliness indicators and accreditation. As part of the quality strengthening in China, hands-on training workshops were held in 2006 and 2007 for provincial laboratories and prefecture laboratory training organized at the provincial level. Annual laboratory coordination meetings are held for provincial laboratory heads. China CDC successfully participates in the Global PT programme and coordinates a similar programme for all 31 provincial laboratories. In 2006, all provincial laboratories except three achieved 100% and only Qinghai and Tibet did not reach the minimum score (90%). More than 2400 measles and rubella serum samples were sent by the provincial laboratories to the China CDC for confirmation in 2006, and all achieved $\geq 90\%$, and most 100%. The accreditation programme is carried out with support from specialized laboratories and WHO laboratory specialists. The RRL in China CDC is assessed annually and about 6-12 provincial laboratories are assessed each year on a rotational basis. Most provincial laboratories in China use the Virion Serion assay and 3 locally made kits (Shenzhen Haitai, Kerenda and Bell) are also used, however there were some lot to lot variability problems reported for the local assays.

Measles Serosurveillance

A report was presented on a pilot project evaluating different surveillance sensitivities in the Russian Federation (RF). Measles incidence in the RF has been declining steadily since 1999, and in 2006 the national average was 0.8/100,000, although 8 territories had an incidence of $>1/100,000$ and 1 $>10/100,000$. To determine whether cases were being missed through the routine measles surveillance programme, 11 territories were surveyed at different levels of intensity, from 1/100,000 to 7/100,000 over a 3 year period. It was found that measles cases were found under the enhanced surveillance protocol which would have been missed under the earlier surveillance scheme. Most of the missed cases would have been diagnosed as; rubella, pseudo-tuberculosis, allergic rash, infectious erythema or "other" and not measles and would therefore not have been tested in the laboratory. Most of the measles cases were in older age groups, with a mean of 23 years. The RF has subsequently selected a target of 2/100,000 to help capture some of the measles cases previously missed.

A seroprevalence study was also carried out as part of the pilot project. The Behring IgG assay was used to determine antibody levels in the age groups: 9-10 years, 15-17 years, and 23-25 years. From 12-24% of all the age groups were found to be IgG negative. However, this did not appear to reflect low immunity as measles transmission in these populations was very restricted, even following confirmed virus introduction. Further studies are under way to evaluate a subset of the Russian Behring ELISA IgG results with a PRNT assay at KTL, Finland.

HPA and RIVM reported their experiences with seroepidemiological studies in the UK and the Netherlands. A UK 1994 study confirmed measles susceptibility of up to 12-13% in the under 16 years of age population, with 2-14 year olds having the lowest percentage of detectible antibody. A school age campaign targeting the susceptible age groups significantly reduced the number of notified and confirmed cases in the following years. The Netherlands reported several population studies where PRNT and ELISA data have been correlated.

Published data shows 100% sensitivity of Behring IgG with PRNT and specificity of 90%, and a 2004 HPA correlation showed R^2 values of 0.36. It was suggested that in countries where most measles antibody is derived from vaccination, some ELISA assays may have insufficient sensitivity.

It is recognized that the ELISA and PRNT measure different antibodies and will not provide a 100% correlation all of the time. The PRNT can provide an accurate measure of protection, but inter-assay variation can exceed 2 fold. Use of the International standard serum is critical to minimize assay variability and to standardize results. The latest WHO standard (3rd International Standard) has recently been approved for use with PRNT assays but its use for ELISA is still pending.

Recommendations
5th Global Measles and rubella Laboratory Network Meeting
26-28 Sept 2007

1. Given the increased demand on the laboratory network with the addition of JE (and other new viral VPDs), additional support for the Laboratory Coordinators should be considered for SE Asian, W Pacific Regions and the Region of the Americas. **Action:** WHO/HQ, Regional Offices and Partners. **Timeline:** Ongoing,
2. Despite the progress in documenting the diagnostic performance of alternative sampling methods (DBS and OF), there has been limited uptake of these techniques in the programme. The recommendations from the recent alternative sampling methods meeting should be summarized and disseminated to programme managers and presented at upcoming Regional Immunization TAG Meetings and Inter-country Measles and Rubella Meetings to encourage appropriate use of these methods. **Action:** WHO/HQ, Regional Offices. **Timeline:** Ongoing,
3. The concept of developing a restricted access genotype/sequence database for measles and rubella should be pursued. Some of the characteristics any new database should contain are:
 - a. The core-variables of the current WHO genotype database
 - b. Regular and timely reporting requirements
 - c. Automatic cross-linking with other LabNet genotype/sequence databases and GenBank
 - d. Sequences submitted should meet defined QC proceduresGuidelines for submitting and performing QC on sequences added to the database(s) will be developed. **Action:** All LabNet sequencing laboratories. **Timeline:** Database development ongoing, QC guidelines, 2nd quarter 2008.
4. Only approximately 10% of the entries submitted to the WHO LabNet genome database currently have a GenBank accession number. It is strongly recommended that laboratories submit their sequence information to GenBank in a timely manner and that these submissions are annotated appropriately. Submission of viruses to GenBank should be updated at least quarterly. **Action:** All LabNet sequencing laboratories. **Timeline:** Ongoing
5. Laboratories are required to submit representative genotype information about circulating measles and rubella strains to the WHO genotype database, preferably on a real-time basis, but at least monthly, including negative reporting. The reporting will be to either the WHO genotype SharePoint database or by email to global and regional laboratory Coordinators. A modification of the current strain naming protocol to reflect those viruses known to be directly imported will be developed. **Action:** GSLs, RRLs and all LabNet sequencing laboratories. **Timeline:** Ongoing
6. The rubella genotype database should be maintained using the WHO recommended minimum sequencing window of 739 nt. Data on potential new windows should be evaluated as it accumulates. Genotype data for rubella viruses determined from a window smaller than the recommended minimum should still be submitted to the WHO database, however, the naming of the

genotype of these sequences should clearly identify the specific window used until the full window has been assessed. **Action:** All LabNet sequencing laboratories **Timeline:** Ongoing

7. Further studies should be encouraged to develop sensitive PCR tests to enable direct sequencing of rubella virus from clinical samples. A time-frame for evaluation and dissemination of the protocols will be developed. **Action:** WHO/HQ, CDC, HPA, RKI and Luxembourg. **Timeline:** Ongoing
8. Any use of sequence data by a second party is expected to follow the protocol of consulting the contributing and/or sequencing laboratory before any publication occurs. All members of the WHO LabNet will be required to indicate agreement with this protocol before gaining access to the genotype database. Access to WHO sequence and genotype databases will also be available to all LabNet Laboratories that perform sequencing and who agree to the above protocol. All laboratories should be aware that submission of genotype information and sequence data to WHO does not constitute prior publication and that laboratories will be able to publish their sequence data as long as they comply with the protocol. **Action:** WHO HQ, all LabNet sequencing laboratories. **Timeline:** Protocol to be circulated for comment by end of November 2007 and implemented by end of December 2007.
9. Sequencing laboratories should report data from their national measles and rubella surveillance programmes, but should strongly encourage other countries, for which they are providing sequence support to report their own data also or obtain permission from them to submit the data on their behalf. Sequencing laboratories can encourage countries to report their own data by providing sequence data in a format compatible with the LabNet database needs. **Action:** All LabNet sequencing laboratories. **Timeline:** Ongoing
10. An Agreement for Transfer of Scientific Materials for exchange of clinical specimens and data should be considered in light of the intellectual property rights concerns raised with the avian influenza surveillance programme. The development of an overall data sharing agreement may need to be considered for laboratories undertaking genotyping, as opposed to numerous MTA's. **Action:** Dr Tashiro to report developments from Influenza programme which may impact on the measles and rubella laboratory Network. **Timeline:** Ongoing
11. Efforts should be made to collect as much epidemiologic and laboratory data as possible from cases of genotype A measles viruses and rubella vaccine viruses to determine whether their origins are related to vaccine strains or to indigenous circulation. (These should be reported in the monthly update to WHO complete with epidemiological data). Viruses identified from cases with a confirmed history of recent vaccination need not be reported to the database. **Action:** GSLs and all sequencing laboratories. **Timeline:** Ongoing
12. Data Management: Laboratories should be encouraged to work with their national surveillance counterparts to reconcile laboratory and surveillance data to allow compatible data submission to the global monitoring of measles and rubella surveillance. **Action:** All laboratories in LabNet and Regional Laboratory Coordinators. **Timeline:** Ongoing

13. The LabNet will keep an updated map and table of the latest genotype information on the public access WHO website. However, this data will only be helpful if genotype and outbreak data is provided to WHO on a timely basis. **Action:** WHO/HQ to formalize format. **Timeline:** Quarterly update, beginning January 2008.
14. Regular conference calls will be set up between Regional Laboratory Coordinators and HQ and key partners, but due to regional time differences these will be staggered. **Action:** WHO/HQ and Regional Laboratory Coordinators **Timeline:** Bimonthly, beginning January 2008.
15. Training opportunities within the LabNet should be provided on a regular basis to account for LabNet staff turnover and the expansion of laboratory activities into viral detection. There is a recognized need for laboratories to be instructed in the development of SOPs and laboratories with expertise in this field will be encouraged to provide support. **Action:** WHO Regional Laboratory Coordinators, GSLs and RRLs. **Timeline:** Ongoing
16. Laboratory Coordinators will review whether the current Accreditation/Evaluation checklist needs updating. Timeliness indicators for sequence/genotype reporting will also be included in the NL checklist to incorporate those NLs which also perform sequencing or send samples to a sequencing laboratory. Any changes required will be implemented for the start of the 2008 accreditation period. **Action:** Laboratory Coordinators and GSL, RRLs. **Timeline:** Comments to HQ by November 30 2007. Implementation, January 2008
17. Considerable strain variation within specific measles genotypes has been noted by the LabNet and it is recommended that these variations continue to be monitored. A decision will be made at the next global meeting on whether these variants need to be documented in a format other than that outlined by the current naming protocols. **Action:** GSLs, sequencing laboratories. **Timeline:** 3rd quarter 2008
18. Serum dried onto filter paper shows considerable promise for facilitating the transportation of serum for validation purposes. However, this technique will need to undergo evaluation under field conditions before widespread use. Pilot studies will be rolled out in 2008 in limited countries. The recommended protocol(s) for the field evaluation will be finalized by the end of November 2007. **Action:** HPA, CDC, WHO/HQ. **Timeline:** Protocol, 30 November 2007. Roll out 1st quarter 2008
19. It is recognized that the use of a serum standard is critical for validating measles sero-epidemiological studies. Laboratories that choose to use PRNT assays to validate their ELISA IgG sero-epidemiological studies should use the recommended protocol finalized by the PRNT working group, currently available on the HPA website, and to be published shortly in Vaccine. The new 3rd International Standard is now available for validating PRNT assays. **Action:** LabNet and WHO/HQ and ROs. **Timeline:** Ongoing

20. The draft HQ surveillance guidelines, which outline the recommended frequency of sampling measles and rubella viruses from chains of transmission and during outbreaks, will be circulated to LabNet when finalized. It is recommended that representative strains of currently circulating virus are deposited in the one of the two strain banks at CDC and HPA. **Action:** WHO/HQ, Strain Banks, LabNet. **Timeline:** Guidelines circulated, 1st quarter 2008. Strains deposited, ongoing.
21. Given the challenges with diagnosing CRS cases, a testing and reporting algorithm based on current diagnostic technologies will be developed and shared with the LabNet. **Action:** GSLs, WHO/HQ **Timeline:** 1st quarter 2008