Genotyping and its utility in verification for both measles and rubella elimination

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Webinar

Measles and rubella genotyping

Moderators

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Introduction, goals and roles

• Strengthening and maintaining global laboratory capacity

Verification of elimination and genotyping

• Methods and nomenclature

Examples of genotyping and how it is used for verification

• Capacity of genotyping in network and expectations
• Lessons learned
• Enhancing resolution
Framework for Verifying Elimination of Measles and Rubella

Criteria for verifying elimination

Documentation of the interruption of endemic measles, or rubella virus transmission for a period of at least 36 months from the last known endemic case

The presence of a high-quality surveillance system that is sensitive and specific enough to detect imported and import-related cases

Genotyping evidence that supports the interruption of endemic transmission

Indicators of the quality of field and laboratory surveillance

- Timeliness of reporting
- Reporting rate of discarded non-measles non-rubella cases
- Representativeness of reporting
- Laboratory confirmation
- Viral detection
- Adequacy of investigation
- Timeliness of specimen transport
- Timeliness of reporting laboratory results

http://www.who.int/wer/2013/wer8809.pdf
A detailed description of the epidemiology of measles and rubella since the introduction of measles and rubella vaccine in the national immunization programme

Population immunity presented as a birth cohort analysis with the addition of evidence related to any marginalized and migrant groups

Quality of epidemiological and laboratory surveillance systems for measles and rubella

Sustainability of the national immunization programme including resources for mass campaigns, where appropriate, in order to sustain elimination

Genotyping evidence that measles and rubella virus transmission is interrupted
Role of GMRLN in verification

Global Measles and Rubella Laboratory Network (est. 2000)

Surveillance
• Classifying cases
• Provide evidence for verification of elimination
• Monitor laboratory performance in meeting quality indicators

Accreditation
• All countries with an elimination goal must have access to a measles-rubella laboratory accredited by GMRLN, with ongoing quality control

Molecular surveillance
• Documenting pre-elimination genotypes
• Monitoring genetic characteristics of circulating strains
• Verifying absence of endemic transmission

Population immunity
• Supporting studies on population immunity/serosurveys
Molecular Epidemiology

Measles and rubella virus

Monitor virus strain distribution to characterize outbreaks and to identify transmission pathways

Provide evidence for interruption of endemic virus circulation

Molecular surveillance should be conducted during all phases of measles and rubella control

Indicator for quality of laboratory surveillance
Primers Amplify 104 Copies of RNA Template

- Primers MeV214 and MeV216 are designed to amplify a 634 nucleotide region coding for the 3' terminus of the nucleoprotein (N) gene in a conventional RT-PCR reaction.

- 11 different genotypes tested

- MV 214 and 216 are also used in sequencing reactions
Target sequence for measles genotyping

MeV216 (nt 1105-1124)

1105  TGG AGC TAT GCC ATG GGA GTA GGA GTG GAA CTT GAA AAC TCC ATG GGA 1152
1153  GGT TTG AAC TTT GGC CGA TCT TAC TTT GAT CCA GCA TAT TTT AGA TTA 1200

↓ start of N-450 window

1201  GGG CAA GAG ATG GTA AGG AGG TCA GCT GCA AAG GTC AGT TCC AGA TTA 1248
1249  GCA TCT GAA CTC GGT ATC ACT GCC GAG GAT GCA AGG CTT GTC TCA GAG 1296
1297  ATT GCA ATG CAT ACT ACT GAC GAC AAG ATC ACT AGA GCG GTC ATT GGA CCC 1344
1344  AGA CAA GCC CAA GCA TCA TTT CTA CAC GCT GAT CAA AGT GAG AAT CAG 1392
1394  CTA CCG AGA TGT GGG GGC AAG GAA GAT AGG AGG GTC AAA CAG AGT CCA 1440
1441  GGA GAA GCC AGG GAG ASC TAC AGA GAA ACC GGG CCC ASC AGC AGA GCA AGT 1488
1489  GAT GGC AGA GCT GCA GAG ATC AGC GCC ACC ATA GAC ATT GAC 1536
1537  ACT GCA TGG GAG TCC AGC CAA GAT CCG CAG GAC AGT CGA AGG TCA GCT 1584
1585  GAG CCC CCTT AGG CTT CAA GCC ATG GCA ATC TCG GAA GAA CAA 1632
1633  GCC TCA GAC ACG GAC ACC CCT ATA STG TAC AAT GAC AGA AAT CCT CTA 1680

↓ end of N-450 window

1681  GAC TAG GTG CGA GAG GCC GAG GGC CAG AAC AAC ATC CGC CTA CCC TCC 1728
1729  ATC ATT GTT ATA AAA AA
Groups are defined by phylogeny of the last 450 nt of N protein coding gene.
Sequences submitted to MeaNS and RubeNS


Number of sequences

Measles
Rubella

0 50 100 150 200 250 300 350 400 450
4000 3500 3000 2500 2000 1500 1000 500 0

05/12/2017 | Measles and Rubella Virus Genotyping
Global Distribution of Measles Genotypes: 2010-2015

Size of circle is proportional to the number of specimens with a genotype result.

- **B3**
- **D8**
- **D3**
- **D9**
- **D4**
- **G3**
- **D6**
- **H1**

Countries with genotype data available

Not applicable
Distribution of measles genotypes (last 12 months)

Data Source: MeaNS database (Genotypes) and IVB Database (Incidence) as of 2017-11-10 and covering the period 2016-10-01 to 2017-09-30 - Pie charts proportional to the number of sequenced viruses
Rubella virus genotyping

Genomic RNA

Non-structural proteins (NSP)
- P150
- P90

Structural proteins (SP)
- C
- E2
- E1

E1 coding region

Molecular window (739-nt)
- 8258
- 8731
- 9469
- 9700

5’ Cap

polyA 3’

1 2 M
Rubella Virus Clades and Genotypes

Groups are defined by phylogeny of the last 739 nt window of E1 gene

• Rubella Virus Clades and Genotypes
  3 Measles sequence diversity
Groups are defined by phylogeny of the last 739 nt window of E1 gene

- RVi/Minsk.BLR/29.04/[1G]
- RVi/Ontario.CAN/0.05/[1G]
- RVi/Kampala.UGA/20.01/[1G]
- RVi/Minsk.BLR/28.05/2[1H]
- RVi/Ryazan.RUS/09.08/[1H]
- RVi/Bene Berak.ISR/0.79/[1B]
- RVi/Tiberius.ISR/0.88/[1B]
- RVi/Jerusalem.ISR/0.75/[1B]
- RVi/Miyazaki.JPN/10.01/[1J]
- RVi/Kagoshima.JPN/22.04/[1J]
- RVi/Shandong.CHN/0.02/[1E]
- RVi/Kuala Lumpur.MYS/0.01/[1E]
- RVi/Pennsylvania.USA/0.64/[1a]
- RVi/Toyama.JPN/0.67/[1a]
- RVi/Brussels.BEL/0.63/[1a]
- RVi/New Jersey.USA/0.61/[1a]
- RVi/Anhui.CHN/0.00/2[2B]
- RVi/Washington.USA/16.00/[2B]
- RVi/Tel Aviv.ISR/0.68/[2B]
- RVi/Beijing.CHN/0.79/[2A]
- RVi/Beijing.CHN/0.80/[2A]

substitutions per site over 739 nucleotides

1 Clades

2
Global Distribution of Rubella Genotypes: 2010-2015
Distribution of rubella genotypes (last 12 months)

Data Source: RubNS database (Genotypes) and IVB Database (Incidence) as of 2017-11-10 and covering the period 2016-10-01 to 2017-09-30 - Pie charts proportional to the number of sequenced viruses.
Monitoring Laboratory Performance

Timely and complete reporting

- Diagnostic and genotyping data

Accreditation

- Ensure WHO performance indicators are met
- Timely reporting of lab data, incl genotyping

Quality control

- Confirmatory testing by supervisory laboratory

Quality assurance

- Proficiency testing serologic and **molecular**
Molecular EQA program

FTA cards; CDC and INSTAND e.V.
Conclusions

Network laboratories with existing molecular infrastructure (RT-qPCR, sequencing) trained for measles and rubella molecular testing

- Infrastructure mostly developed through GPLN, influenza programme, or GHSA

Workshops have been repeatedly conducted in all WHO Regions

Molecular external quality assurance programme established to closely monitor the performance of labs

- Including ability to analyse and upload quality sequences to MeaNS and RubeNS