A 16-year review of seroprevalence studies on measles and rubella

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Serosurveillance definition

‘Monitoring the immune response’

- Predominantly IgG, but some assays measure mixed Ig response
- Not possible to distinguish between response to immunization or infection
Why use serosurveillance?

- Validate vaccine coverage estimates
- Generate population susceptibility profiles
- Identify at-risk populations and immunity gaps
- Monitor programmatic functions
- Support political/administrative functions
Documenting the Impact of Hepatitis B Immunization: best practices for conducting a serosurvey
What is serosurveillance?

- Functional antibody - CF, SBA, neutralization
- Component recognition - EIA, RIA, chemiluminescence
- Acceptable level of false negatives
- Acceptable level of false positives
- Acceptable level of indeterminates
- Absolute requirements of the test
- Retest/ repeat
- External confirmation/ validation
- Trained staff - number and level
- Facilities/ equipment
- Cost per test
- Manual or automated
- Validation/ calculation
- Tests available
- Sample volume
- Resource requirements
- Time requirements
- Statistical confidence
- Resource availability
- Biased towards groups of greatest interest
- Clear exclusion criteria
- Communication of criteria to staff
- Convenience samples
- Easiest to sample are easiest to immunize
- Capacity to use available packages
- Appropriateness of methods
- Does it make sense?
- Confidence of interpretation
- Sample size

Laboratory test

Serosurvey?

Target Population

- Whole population
- Geographically/ culturally defined group
- Specific age group/ at-risk population
- Historical or current
- Predictive
- Real-time monitoring
- Exposure/ immunization probability
- Known waning immunity
- Compliance
- Political aspects
- Known confounders
- Gender equality

Samples

- Targeted collection or convenience samples
- Sample bias
- Cost of collection
- Type and volume
- Heel/ finger-prick or syringe/ vacutainer
- Oral fluids/ other non-blood
- Immediate sample treatment
- Serum separation
- Bloodspot drying
- Storage temperature
- Storage/ shipment time
- Biosafety
- Sample storage and shipment
- Sample data collection and recording
- Standard epidemiological data
- Vaccination status
- Clinical/ presentation data
Target population considerations

- Whole population
  - Exposure/ immunization probability
  - Known waning immunity

- Geographically/ culturally defined group
  - Compliance
  - Political aspects
  - Known confounders
  - Gender equality

- Specific age group/ at-risk population
  - Predictive
  - Real time monitoring

- Historical or current
Sample considerations

Targeted collection or convenience samples
  - Sample bias
  - Cost of collection

Type and volume
  - Heel/ finger-prick or syringe/ vacutainer
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Immediate sample treatment
  - Serum separation
  - Bloodspot drying
  - Storage temperature
  - Storage/ shipment time
  - Biosafety

Sample storage and shipment
  - Standard epidemiological data
  - Vaccination status
  - Clinical/ presentation data

Sample data collection and recording
Laboratory test considerations

Tests available
- 'Functional antibody' - CF, SBA, neutralization
- Component recognition - EIA, RIA, chemiluminescence
- Available controls/standards

Sensitivity and specificity
- Acceptable level of false negatives
- Acceptable level of false positives
- Acceptable level of indeterminates

Sample volume
- Absolute requirements of the test
- Retest/repeat
- External confirmation/validation

Resource requirements
- Trained staff - number and level
- Facilities/equipment
- Cost per test

Time requirements
- Manual or automated
- Validation/calculation
Results analysis considerations

Sample size
- Statistical confidence
- Resource availability

Selection criteria
- Biased towards groups of greatest interest
- Clear exclusion criteria
- Communication of criteria to staff

Avoiding selection bias
- Convenience samples
- Easiest to sample are easiest to immunize

Statistical package
- Capacity to use available packages
- Appropriateness of methods

Confidence of interpretation
- Does it make sense?
AIM

To conduct a literature review from January 1998 and June 2014

Analyse information in terms of

- Target populations
- Samples
- Laboratory tests
- Results
Method

Pubmed search using key words:
- measles
- rubella, combined with
- serosurvey
- seroprevalence
- immunity and
- population immunity

Expanded using “related articles” links
Results

- 97 articles retrieved
  - 68 relating to measles
  - 58 relating to rubella
  - 30 relating to both measles and rubella
<table>
<thead>
<tr>
<th>Year</th>
<th>Measles publications [reference]</th>
<th>Rubella publications [Reference]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>1 [7]</td>
<td>2 [7, 8]</td>
</tr>
<tr>
<td>2000</td>
<td>1 [9]</td>
<td>2 [9, 10]</td>
</tr>
<tr>
<td>2004</td>
<td>2 [18, 19]</td>
<td>0</td>
</tr>
<tr>
<td>2006</td>
<td>6 [22-27]</td>
<td>6 [22, 24-28]</td>
</tr>
<tr>
<td>2007</td>
<td>3 [29-31]</td>
<td>2 [29, 32]</td>
</tr>
<tr>
<td>2008</td>
<td>7 [33-39]</td>
<td>3 [38, 40, 41]</td>
</tr>
<tr>
<td>2009</td>
<td>2 [42, 43]</td>
<td>4 [43-46]</td>
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<tr>
<td>2010</td>
<td>2 [47, 48]</td>
<td>1 [48]</td>
</tr>
<tr>
<td>2012</td>
<td>7 [57-63]</td>
<td>6 [59, 62-66]</td>
</tr>
<tr>
<td>2013</td>
<td>14 [67-80]</td>
<td>10 [67, 69, 72, 74, 76, 81-85]</td>
</tr>
<tr>
<td>2014</td>
<td>8 [86-93]</td>
<td>11 [86-88, 91, 92, 94-99]</td>
</tr>
</tbody>
</table>

Totals 68 58
Purpose of Studies

- **Susceptibility studies** — looking for susceptible populations and immunity gaps;

- **Antibody persistence studies** — looking for evidence of declining antibody levels

- **Vaccination coverage studies** — conducted in association with immunization activities and attempting to provide biological ‘validation’ of vaccine coverage estimates

- **Vaccine immunogenicity studies** — investigating the immunological impact of changing vaccine formulations or schedules in target populations.
Countries surveyed

- Measles surveys in 37 countries
- 12 countries with more than one survey
  - 10 studies in China
  - 5 studies in USA
- Rubella surveys in 36 countries
- 11 countries with multiple surveys
  - 6 studies in Turkey
  - 5 studies in Saudi Arabia
Countries categorised into UN Statistics Division

<table>
<thead>
<tr>
<th>Country status</th>
<th>Measles publications</th>
<th>Rubella publications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Developed</td>
<td>29 (43%)</td>
<td>21 (36%)</td>
</tr>
<tr>
<td>Developing</td>
<td>26 (38%)</td>
<td>29 (50%)</td>
</tr>
<tr>
<td>Least developed</td>
<td>13 (19%)</td>
<td>8 (14%)</td>
</tr>
<tr>
<td>Totals</td>
<td>68</td>
<td>58</td>
</tr>
</tbody>
</table>
## Distribution of Ages of Populations Studied

<table>
<thead>
<tr>
<th>Year</th>
<th>Age grouping</th>
<th>Measles publications</th>
<th>Rubella publications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants</td>
<td>1-12 months</td>
<td>6 (9%)</td>
<td>3 (5%)</td>
</tr>
<tr>
<td>Children</td>
<td>1-12 years</td>
<td>6 (9%)</td>
<td>6 (10%)</td>
</tr>
<tr>
<td>Infants and children</td>
<td>-</td>
<td>7 (10%)</td>
<td>3 (5%)</td>
</tr>
<tr>
<td>Infants to adolescents</td>
<td>-</td>
<td>4 (6%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Children and adolescents</td>
<td>-</td>
<td>1 (1%)</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>Adolescents only</td>
<td>13-17 years</td>
<td>2 (3%)</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>Children to adults</td>
<td>-</td>
<td>7 (10%)</td>
<td>5 (9%)</td>
</tr>
<tr>
<td>Adolescents and adults</td>
<td>-</td>
<td>2 (3%)</td>
<td>15 (26%)</td>
</tr>
<tr>
<td>Adults only</td>
<td>&gt;18 years</td>
<td>13 (19%)</td>
<td>14 (24%)</td>
</tr>
<tr>
<td>Infants to adults</td>
<td>-</td>
<td>20 (29%)</td>
<td>7 (12%)</td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td>68</td>
<td>58</td>
</tr>
</tbody>
</table>
Distribution of Ages of Populations Studied

- 50% of all published rubella surveys targeted either adults alone or adults and adolescents compared with only 22% of measles surveys.
- Rubella studies were often to determine the immunological status of pregnant women whose age generally ranged between 15 – 45 years.
- None of the 97 studies reviewed had only male participants.
### Study sample size

<table>
<thead>
<tr>
<th>Sample size</th>
<th>Measles publications</th>
<th>Rubella publications</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;200</td>
<td>7 (10%)</td>
<td>5 (9%)</td>
</tr>
<tr>
<td>200-499</td>
<td>14 (21%)</td>
<td>14 (24%)</td>
</tr>
<tr>
<td>500-999</td>
<td>11 (16%)</td>
<td>11 (19%)</td>
</tr>
<tr>
<td>1000-2000</td>
<td>17 (25%)</td>
<td>9 (16%)</td>
</tr>
<tr>
<td>&gt;2000</td>
<td>19 (28%)</td>
<td>19 (33%)</td>
</tr>
<tr>
<td>Totals</td>
<td>68</td>
<td>58</td>
</tr>
</tbody>
</table>

Only 17 of the 68 (25%) measles studies and 17 of the 58 (29%) rubella studies reported evidence of power calculations having been performed as part of the study design.
Methods of obtaining specimens

Categorised into four groups; random, convenience, total and other/not stated

<table>
<thead>
<tr>
<th>Collection methods</th>
<th>Measles publications</th>
<th>Rubella publications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Random</td>
<td>30 (44%)</td>
<td>22 (38%)</td>
</tr>
<tr>
<td>Convenience</td>
<td>25 (37%)</td>
<td>27 (47%)</td>
</tr>
<tr>
<td>Total</td>
<td>8 (12%)</td>
<td>7 (12%)</td>
</tr>
<tr>
<td>Other/Not stated</td>
<td>5 (7%)</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>Totals</td>
<td>68</td>
<td>58</td>
</tr>
</tbody>
</table>
Specimen testing

- 55 of the 68 (81%) measles studies used only serum samples.
- All specimens were stored frozen below -20°C Celsius.
- Two measles studies reported using oral fluid exclusively.
- Alternative sample type in addition to serum, including breast milk, oral fluid and blood derived from the umbilical cord, earlobe or finger prick.
### Assay Formats Used

<table>
<thead>
<tr>
<th>Laboratory test</th>
<th>Measles publications</th>
<th>Rubella publications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutralization assay</td>
<td>7 (10%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>MTP EIAs</td>
<td>52 (76%)</td>
<td>40 (69%)</td>
</tr>
<tr>
<td>Siemens Enzygnost</td>
<td>21 (31%)</td>
<td>14 (24%)</td>
</tr>
<tr>
<td>Virion/Serion</td>
<td>9 (13%)</td>
<td>2 (3.5%)</td>
</tr>
<tr>
<td>Other commercial MTP EIAs</td>
<td>3 (4%)</td>
<td>22 (38%)</td>
</tr>
<tr>
<td>MTP EIA not identified</td>
<td>19 (28%)</td>
<td>2 (3.5%)</td>
</tr>
<tr>
<td>Automated immunoassay</td>
<td>1 (1%)</td>
<td>12 (21%)</td>
</tr>
<tr>
<td>Abbott AxSYM</td>
<td></td>
<td>5 (9%)</td>
</tr>
<tr>
<td>bioMerieux VIDAS</td>
<td>1 (1%)</td>
<td>4 (7%)</td>
</tr>
<tr>
<td>Other commercial automated immunoassays</td>
<td></td>
<td>3 (5%)</td>
</tr>
<tr>
<td>Bioplex assay</td>
<td>1 (1%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Haemagglutination inhibition assay</td>
<td>4 (6%)</td>
<td>0</td>
</tr>
<tr>
<td>MTP EIA and neutralisation</td>
<td>2 (3%)</td>
<td>0</td>
</tr>
<tr>
<td>Assay not described</td>
<td>1 (1%)</td>
<td>3 (5%)</td>
</tr>
<tr>
<td>MTP EIA and gel haemolysis</td>
<td>0</td>
<td>1 (2%)</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>68</strong></td>
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</tr>
</tbody>
</table>

MTP EIA: Microtitre plate enzyme immunoassays
Measles EIA

- 53 of 68 studies used EIAs or AIs
- 21 (40%) used the Enzygnost® Anti-Measles Virus/IgG
- 9 (17%) used Serion Measles IgG
- 20 measles studies used assays from a total of 19 different commercial sources
- 3 assays were unspecified
Rubella EIAs

- 40 rubella studies used MTP EIAs
- 14/40 (35%) used Enzygnost® Anti-Rubella Virus/IgG
- 5 used ETI-RUBEK-G Plus
- Remaining 21 studies used commercial MTP EIAs from 13 different manufacturers
- 5 used the AxSYM Rubella IgG assay
- 4 used the VIDAS Rub IgG
Of 68 measles studies
- 39 (57%) reported in mIU/mL
- 22 (43%) reported qualitative results
- 2 studies reported the results as titres
- 5 did not specify

About half (32 of 68; 47%) applied a range for equivocal results

Quantitative immune/non-immune cut-offs ranged from 63 to 350 mIU/mL
Use of International Standard

- 13 measles studies and 6 rubella studies used international standard to calibrate non-commercial assays
- 2nd WHO international standard for measles only recommended for Enzygnost EIA
- 3rd WHO international standard for measles (current) is not recommended to be used to calibrate MTP EIAs
Reporting of Rubella Results

- 44 of 58 (76%) studies reported results in IU/mL
- 22 (38%) including an equivocal range
- Equivocal ranges generally were
  - 5 to 10 IU/mL (N=5),
  - 10 to 15 IU/mL (N=4),
  - 5 to 15 IU/mL (N=2),
- 18 of the 58 (31%) studies used 10 IU/mL as the cut-off
- A further 10 studies (17%) used a cut-off between 4 and 20 IU/mL
Major Findings

- Various samples types were used in seroprevalance studies
- Functional assays such as PNT for measles and PNT or HAI for rubella are considered the most appropriate test
- EIAs known to be ~10% less sensitive that PNT
- Wide range of non-functional assays used
- Random use of cut-offs, equivocal ranges
- Measles international standard is used inappropriately to calibrate MTP EIAs
Conclusion

Results from seroprevalance studies cannot necessarily be compared due to lack of standardisation.

Suggest use of functional assays calibrated using international standard.

Introduce a standard non-functional assay (MTP EIA or AI) with standard sample type, cut-off and equivocal range.
Acknowledgement

Dr Mick Mulders
World Health Organization
Global VPD Laboratory Networks