MEETING REPORT

WHO IB-VPD and ROTAVIRUS Sentinel Global Surveillance Network Meeting:
Global Surveillance, Laboratory and Data Management

16th-21st November 2015

Geneva, Switzerland
## Table of Contents

### Section I: IB-VPD Global Surveillance Meeting and iTAG Meeting
- Background .................................................................................................................. 4
- Meeting Objective ........................................................................................................ 4
- Current Status of Global IB-VPD Surveillance Network ............................................. 4
- Surveillance Highlights from Recommendations ......................................................... 6
- PCV Technical Coordination Project ........................................................................... 7

### Section II: IB-VPD Global Surveillance Meeting and iTAG Meeting
- Overview of laboratory testing and sample referral systems .................................... 8
- Comparative analysis of culture and PCR data from Surveillance database ............... 8
- Rapid Diagnostic Tests in EUR .................................................................................... 9
- Urinalysis reagent strips for CSF chemistry testing .................................................... 10
- CDC/WHO project reviewing use of pneumonia administrative date to show vaccine impact ........ 10
- Results of the use of administrative pneumonia data in two PAHO countries .......... 11
- Early analyses of possible vaccine impact in AFRO .................................................. 11
- Vaccine impact using PCR in South Africa ............................................................... 11

### Section III: IB-VPD Laboratory Meeting
- Background .................................................................................................................. 13
- Meeting Objectives ....................................................................................................... 13
- Session I: QA/QC systems- External Quality Assessment (EQA) ............................... 13
  - 2015 EQA survey ........................................................................................................ 13
  - EQA Trends of laboratory performance .................................................................... 14
- Session I: QA/QC systems- Quality Control (QC) ....................................................... 15
  - Confirmatory testing between RRLs and GRL ......................................................... 15
  - Regional QC between RRLs in AFR (NICD) ............................................................ 16
  - Regional QC between RRL and NLs in WPR (Australia RRL, WPR) ...................... 16
- Session II: Technical update ........................................................................................ 17
  - Implementation of Direct PCR: Pilot test example from RRL AFR (Gambia) .......... 17
  - Robotic DNA Extraction at RRL (Australia RRL, WPR and NICD RRL, AFR) ...... 17
- Session III: Building capacities at national laboratories ............................................. 18
Examples from AMR, EMR, and EUR................................................................. 18
Working with other Surveillance systems: MenAfriNet .................................. 19
Session IV: Improving quality of laboratory data............................................. 19
Guidance for an effective and appropriate presentation of Surveillance data ....... 20

Section IV: ROTAVIRUS Global Surveillance Meeting and iTAG Meeting......... 21
Global overview of WHO ROTAVIRUS Sentinel Surveillance Network ............. 21
WHO Global Rotavirus Surveillance Network: What Analyses Can Be Done with the Data? .......................................................... 22
ROTAVIRUS Sentinel Surveillance: Vaccine Impact in EUR ................................ 23
Use of WHO ROTAVIRUS Surveillance network data in estimating ROTAVIRUS mortality in children <5 years of age, 2000-2013................................................................. 24
TaqMan Array Cards for Rotavirus and other Surveillance and Enteropathogen Detection .......... 25
Norovirus disease burden and vaccine development status.................................. 25
Global Health Security .................................................................................. 26
CDC Collaborations and Synergies .................................................................. 27
Child Health and Mortality Prevention Surveillance (CHAMPS) Network .......... 27

Section V: Rotavirus Laboratory Technical Working Group ............................ 29
Background ................................................................................................. 29
Session I: QA/QC Systems............................................................................ 30
Results from the 2014 EQA and 2015 update................................................ 30
Update on QC Results .................................................................................. 31
Session II: Polio containment and impact on Global Rotavirus Surveillance network .......... 32
Session III: Technical Updates...................................................................... 33
Session IV: Building capacity at National Laboratories .................................. 34
Session V. Gavi presentation .......................................................................... 34

Section VI: Laboratory Special Study for Enteric Pathogens ........................... 36
Background ................................................................................................. 36
Meeting Attendees and Objectives ................................................................ 36
Review of Global Results.............................................................................. 36
Methodology for Analysis of Data .................................................................. 37
Future Directions ......................................................................................... 38
Section I: IB-VPD Global Surveillance Meeting and iTAG Meeting
Day 1: Monday, 14 November 2015

Background
The World Health Organization (WHO)-coordinated Global Invasive Bacterial Vaccine Preventable Disease (IB-VPD) Surveillance network was established from existing Surveillance systems and was standardized across all WHO regions in 2008. The network is coordinated by WHO with Member States and supported by partners. One of the main objectives of the network is to monitor the global burden, etiology, and circulating serotypes/serogroups of IB-VPD, particularly bacterial meningitis disease, and in sentinel sites where possible, pneumonia and sepsis. Another objective of the network is to serve as a platform to monitor vaccine impact and long-term trends in IB-VPD before and after introducing the pneumococcal conjugate vaccine (PCV). Consequently, the network data has reliably contributed to vaccine introduction decisions. The network has been globally representative, particularly focusing on low and middle income countries that have not yet, or have just recently, introduced PCV.

There has been a significant amount of progress since the annual meeting in 2014 where 35 recommendations were made to better understand the data quality and to discuss how to produce more reliable Surveillance data. There has been much progress on prospective linking between clinical and laboratory data as well as zero reporting. While the standard list of variable names has been completed, standard definitions, protocols for Surveillance implementation, and guidelines for data analysis are continuing to be drafted and revised. The web-based data management tool is currently in progress for finalization. Finally, efforts have been made to build upon the IB-VPD network to conduct Surveillance for other vaccine preventable diseases (VPD).

Meeting Objective
To share information and discuss within WHO current status and future directions for WHO-coordinated Global IB-VPD and Rotavirus Sentinel Site Surveillance Networks

Current Status of Global IB-VPD Surveillance Network

Presenters: Adam L. Cohen and Fatima Serhan

The WHO Invasive Bacterial Vaccine Preventable Disease (IB-VPD) Surveillance network collects case-based data on meningitis, pneumonia and sepsis cases in the six WHO Regions (AFR, AMR, EMR, EUR, SEAR, and WPR). Since the strategic review of the network in 2013, great strides have been made in improving the Surveillance network quality, including developing norms and stands for sentinel sites, developing a web-based data management system, analysing the data more regularly, and implementing process and performance indicators to
monitor sentinel sites. The laboratory network has also increased in capacity and quality and now reports case-based serotype and confirmatory testing data. Globally, 68% of this data is linked to the clinical Surveillance database from the sentinel hospitals, with some regions (EUR, SEAR and EMR) linking 90-100% at the country level. The majority of samples received at the Regional Reference Laboratories (RRL) are serotyped or serogrouped and trends can be seen in the data. The majority of positives are typically *Streptococcus pneumoniae* (Spn), but there are also large numbers of *Neisseria meningitidis* (Nm) cases in AFR and EUR in 2013-2014. Recently, the network has been leveraged to begin work on vaccine impact and administrative data studies, as well as a platform for additional VPD Surveillance (namely, typhoid, meningococcal, Japanese Encephalitis, pertussis and enteric pathogens). Overall, the network has been continuously implementing iTAG recommendations and optimizing the scope of the network to ensure sustainability and funding, including re-examining the current and future objectives to determine priority areas.

**Main Discussion Points:**

- There are many *Haemophilus influenzae* (Hi) cases recently being detected, but this is likely because of improving Surveillance for Hi detection.
- India is a very large country in SEAR and globally but does not report in the IB-VPD network. India has multiple Surveillance sites but these are funded by Bill and Melinda Gates Foundation (BMGF) and Johns Hopkins Bloomberg School of Public Health, and they are not included in the IB-VPD network.
- When the Surveillance network increases or changes its capacity, the data can become harder to interpret. The data could be better used for downstream questions like which serotypes should be used for vaccine development, dosing and detection of epidemics, as well as recognizing the importance of integrating across different VPDs.
- Countries that were part of the five-year SURVAC project, a French acronym that stands for Strengthening Surveillance and Response in Central Africa, are concerned about the continuation of Surveillance given that financial support has ended. Perhaps we should monitor these laboratories more closely to assess their performance.

**Action Items:**

- Booster doses for Hi were an issue raised by SAGE and we should focus on a few countries with good data to evaluate.
- Explore how to include large countries such as India in the network.
Surveillance Highlights from Recommendations

Presenter: Adam L. Cohen

Significant progress has been achieved since the 2013 strategic review and 2014 Surveillance meeting. Most of the 75 recommendations made at the two meetings have been met or implemented by the Surveillance network. The only recommendation that has not been discussed or implemented is the need for costing studies. The WHO site assessment tool is not always useful and practical and should be revised with consultation from people who have used the tool. The list of core variables for reporting also needs to be revised to consider the future needs of the network. For example, the variable “vaccine history” is important information for vaccine impact studies, but it is not reliably captured in the WHO Surveillance network. Similarly, information on admission diagnosis and outcome need to be assessed as to whether they need to be mandatorily reported by the sites. The 2015 Surveillance meeting included a large data management component for the first time. This was aimed at helping to enable the data managers to recognize and resolve issues with the data at the source, such as missing core variables or unexpected results.

Main Discussion Points and Action Items

- Key questions: Are data being used at the country level? Sites should use the data in their Gavi applications. Should we consider developing a journal supplement? How can we improve data distribution on the website?
- Non-Gavi eligible sites are not funded but some do continue to report to the WHO IB-VPD network. Their contribution is valuable and we should think about how to support them if we want to continue collecting high quality data from these sites.
- There was a significant emphasis on conducting cost studies and pursuing cost-benefit analyses. Adding other diseases to the existing Surveillance systems may help the Surveillance system be more sustainable and cost-effective.
- Should we continue to drop sites that are non-performers? Not all sites reporting to the network are funded because they were considered as lower performing sites or are in a non-Gavi eligible country. However, the countries getting targeted support performed comparatively worse than the non-supported countries.
- There is a need to be more focused with our efforts, for example including more sentinel Surveillance sites in Asia. We also need to consider how to fund the network more sustainably, such as looking outside of Gavi for funding, persuading countries to contribute funding to the Surveillance, and advocating for its value.
- Local governments should fund national laboratories (NL), not WHO. Some countries are getting money for epidemic meningitis through Global Health Security (GHS). Most Surveillance is extra externally funded. The transition to country ownership should be gradual otherwise the system may fall apart.
PCV Technical Coordination Project

Presenter: Thomas Cherian

There is a need for countries and governments to use the data being generated in the WHO Surveillance networks and to take ownership of the data. The PCV technical coordination project is a BMGF-funded initiative coordinated by WHO and Johns Hopkins Bloomberg School of Public Health. There is a secretariat of partners that will provide technical support and coordination for PCV related projects.

Main Discussion Points:

• WHO Surveillance data for IB-VPD and rotavirus (ROTAVIRUS) should be standing agenda items in NITAG meetings. We need the countries in the Regional Offices to help promote this approach. Surveillance data should also be a required part of Gavi funding applications (HSS).
• Local data should be used to make economic calculations.
• PAHO annual meetings include Surveillance recommendations. The Ministries of Health sign resolutions that have Surveillance mandates. Most PAHO countries now want cost-effectiveness studies, as they have seen the benefit of such studies.
• How can we get countries to invest more in Surveillance? Answers: Countries have to feel ownership and accountability. As an example, this may include purchasing laboratory equipment themselves. Use of “national champions” has been shown to be helpful in some countries.
• Country should link together academic centers of excellence with the Ministries of Health.
Section II: IB-VPD Global Surveillance Meeting and iTAG Meeting
Day 2: Tuesday 17 November 2015

Overview of laboratory testing and sample referral systems
Presenter: Fatima Serhan

The WHO IB-VPD laboratory network exists at both the sentinel hospital and regional level. At the sentinel site, cerebrospinal fluid (CSF) specimens are collected for chemical analysis, microbiological tests, white cell count and appearance. Sentinel sites also perform Gram stain, culture and rapid diagnostic tests including latex agglutination and Binax for laboratory confirmation. CSF samples and isolates from culture are then forwarded to the national or regional reference laboratories (RRLs) where PCR is done for confirmatory and serotyping/serogrouping analysis. In 2014, the iTAG recommended that all CSF samples from suspect meningitis cases should be sent to the national reference laboratory (NRL) or RRL for testing; however, this varies from region to region depending on the following: regional capacities and resources, political challenges, clinical samples agreement between countries, NRL capacities to perform PCR and presence of outbreaks.

Comparative analysis of culture and PCR data from Surveillance database
Presenter: Jillian Murray

PCR is known to have a high sensitivity but it has not been documented in the context of the WHO IB-VPD Surveillance network. The number of additional positive cases confirmed by PCR from those that were culture negative was evaluated in both EUR and AFR. PCR was found to increase the number of confirmed cases detected in both Regions, with a large effect found in sites that have a culture positive rate below 10%. The data from 2014 were also extrapolated for AFR and EUR to determine how many positive cases would have been detected if the 2014 iTAG recommendation to test all suspect meningitis cases was met. It was projected that PCR would have a large impact on increasing the number of positives detected, though it was recognized that the volume of samples to be tested could be a burden to the RRLs. The analysis then projected the number of confirmed cases that would be detected if subsets of suspect meningitis samples were tested. This was done for AFR and EUR as well as the other four regions (AMR, EMR, SEAR and WPR) using data from AFR and EUR. Testing subsets that met probable bacterial meningitis (PBM) criteria by appearance, white cell count, biochemistry results, and combinations of those test results were compared. Testing all CSF specimens by PCR is resource-intensive but ensures all positive cases are detected.
Main Discussion Points:

- Some hesitancy with extrapolating data from different regions because the data are very dynamic.
- Need to consider whether gains in confirmed cases are sufficient to justify increasing the burden on the laboratories doing the testing.
- We need to keep in mind the strategic objective of the Surveillance system, which is to answer questions about pneumococcal disease and learn from the data.
- The low yield in low culture positivity sites is not surprising because each country is different and depends on the reliability of cell counts and laboratory capacity. It would be helpful to look at the negative cases with low WCC that were positive and assess the country Surveillance and laboratory quality since cell counts and biochemistry do not have quality control programs.
- If we need to prioritize testing CSF samples, then we could first test those from countries that have not yet introduced PCV. Could also limit to a proportion of suspect meningitis samples from every country.
- Good quality starts at the country level; can we improve quality of specimens at the country level to improve the yield?
- The Ct values for samples included in this analysis were not assessed. The network recommendations is to use a cut off of 35. Consider going back to the RRL database for all samples that are linked and assess what cut-off was used in the different regions, if data is available.
- WCC may not be reliable in all places for PBM – confirmed cases with low WCC could be in malnourished children who are unable to mount usual immune responses.
- AFR is trying to improve their entire Surveillance system. They are working at the hospitals to have their staff understand their hospital practices and the laboratory system. A large focus is also on implementing the unique patient ID so that samples from the site can be linked to data at the reference laboratory. One big success is the rollout of a new data management tool that will facilitate linking and improvement of data.
- MRC Gambia found that having an onsite data manager was very helpful in improving the quality of data.

Rapid Diagnostic Tests in EUR

Presenter: Dovile Videbaek

The European Region has successfully introduced rapid diagnostic tests into their Surveillance program and in 2014, 81% of all of their suspect meningitis cases were tested with culture, latex agglutination and Binax. Additionally, EUR test approximately 100% of their
suspect meningitis cases with PCR. Using this subset of samples tested by all four diagnostic tests, a comparison of added value was done. Adding latex agglutination and Binax approximately doubled the detection rate for all pathogens (Spn, Hi and Nm). Adding PCR only increased the detection of Spn by a small proportion but doubled the detection of Hi and added about 50% more detection of Nm.

**Urinalysis reagent strips for CSF chemistry testing**  
*Presenter: Linda de Gouveia*

The iTAG recommended investigating urine dipsticks as an alternative for classifying cases as bacterial meningitis. Diagnosis of bacterial meningitis currently uses microscopy, biochemistry and white cell count but reliability of these can vary between regions and it can take time to get the results. The RRL housed at the National Institute of Communicable Diseases in South Africa undertook a study to determine if urinary reagent strips were useful for making semi-quantitative assessment of protein, glucose and leukocyte esterase in CSF samples. They tested 88 CSF samples and determined that it was quick, cheap, simple, and required minimal CSF. Furthermore, no additional equipment was required and the strips can be stored at room temperature. However, interpretation was difficult and contamination of the strips was a possibility since they are normally used with urine. The strips could potentially be used as a rough, rapid clinical diagnostic test.

**Main Discussion Points:**  
- This test was done on stored CSF but the idea is to perform on fresh CSF.  
- Using urine dipsticks may not be accurate but may be better than having no result at all and could be clinically useful in some places because of this.

**CDC/WHO project reviewing use of pneumonia administrative data to show vaccine impact**  
*Presenter: Jennifer Loo Farrar*

Pneumonia is an important end-point for assessing the impact of PCV. Collecting Surveillance data to assess impact of the vaccine can be time and resource intensive and many countries lack adequate baseline data before the vaccine is introduced. Pneumonia administrative data from hospitals has previously been used in middle and high-income settings as a method for assessing impact. However, in low-income settings, it has not yet been done because data quality and Availability can vary. There is on-going work at CDC to try to assess the feasibility of using administrative pneumonia in low-income settings. The overall process of this research is to: 1. Develop an initial list of metrics to assess PCV impact on pneumonia; 2. Conduct a literature search of available data; 3. Review available data; 4. Refine list of metrics
based on literature search; 5. Conduct pilot studies to measure PCV impact using administrative data; and 6. Develop a guidance document based on findings from pilot studies if the method is feasible. CDC has completed the first two steps and has proposed key metrics with a list of 26 published studies available as sources of information.

Results of the use of administrative pneumonia data in two PAHO countries

Presenter: Lucia Oliveria

Results were presented from two countries in Latin America (Chile and Colombia) that have conducted PCV impact analyses using administrative data. The data presented will be made available when published in the peer-reviewed literature.

Main Discussion Points:
- Observational studies have many challenges due to unknown biases. (Jennifer Verani of CDC is leading a review of biases in observational studies.)
- There is an extremely high vaccine efficacy against all mortality in the Chile study. PAHO has sought out assistance from many statisticians to help explain this unlikely finding, but it is still unexplained.
- Suggestions for additional metrics to be included in Jennifer Farrar’s study of pneumonia administrative data: antibiotic use, HIV prevalence, malaria prevention and control programs, and changes in insurance programs. A stable baseline is not often possible, so there were suggestions to use time interrupted series analysis. District and village information were suggested to be required information.

Early analyses of possible vaccine impact in AFRO

Presenter: Jason Mwenda

A presentation of preliminary analyses of data from AFRO and possible PCV impact was given. The data presented will be made available when it has been finalized.

Vaccine impact using PCR in South Africa

Presenter: Anne von Gottberg

PCR is used at NICD in the WHO IB-VPD Surveillance network to serotype and serogroup confirmed cases of meningitis, pneumonia and sepsis. PCR is done on lytA-positive specimens. Isolates are considered to be positive if the PCR cycle threshold (Ct) value is less than 40. While identification of some serotypes can be detected as single serotypes (1, 3, 4, 5, 8, 14, 20, 19A,
23F, and 35B), others are mixed and cannot be identified individually (6A/B, 6C/D, 7A/F, 9A/V/N/L, 10A/B, 12A/B/F, 15A/B/C/F, 18A/B/C, 19B/F, 22A/F, 33A/F/37 and 38/25A/F). The PCR assay can detect 42 serotypes, however some cannot yet be identified. True negatives are considered samples with a Ct value less than 35 and those that are negative for all serotypes in the assay. Samples with a Ct value above 35 have a low probability of detection, and therefore they are not considered a true negative. This cut-off is used in Africa, but this should be adjusted based on the setting because cut-offs will vary with assays. A study was done using data at the NICD to look at the effect of PCV on invasive pneumococcal disease using PCR data. The study found reductions in the proportion of serotypable lytA-positive blood samples from pneumonia cases from 2009 to 2012. However, reductions were found among both vaccine and non-vaccine type disease. The more reliable data came from samples with lytA Ct values less than 35. Better extraction instruments increase the proportion of samples with lower Ct values, which increased their ability to be serotyped. This likely led to an increased detection rate in the laboratory in 2011 and 2012.

Main Discussion Points:

- It was recommended that specimens should only be called negative for vaccine serotypes if the sample is shown to have a significant pneumococcal load. As the lyt Ct value increases, the likelihood of the specimen having pneumococci decreases. South Africa is considering a cut-off Ct value of 34-35.
- The Magnapure 96 extraction machine is good but manual extraction is even better.
- Sites should report the Ct values to WHO HQ to enable better interpretation of data. Sites and ROs should work together on vaccine impact studies because it is important to look at the Ct values when interpreting the PCR results. The serotype assays have different levels of sensitivity, depending on the serotype. It is important to look at the actual Ct value, not just positive and negative results.
- For guidance on choosing which study to do for vaccine impact, a WHO manual (Measuring impact of *Streptococcus pneumoniae* and *Haemophilus influenzae* type b conjugate vaccination, [http://apps.who.int/iris/bitstream/10665/75835/1/WHO_IVB_12.08_eng.pdf](http://apps.who.int/iris/bitstream/10665/75835/1/WHO_IVB_12.08_eng.pdf)) is available; every type of study design has some limitations. The most appropriate design will vary by country.
- It was recommended for AFR to analyse the overall impact of pneumococcal vaccine, not just by select countries.
Section III: IB-VPD Laboratory Meeting
Day 3: Wednesday 18 November 2015

Background

Established in 2011, the laboratory Technical Working Group (TWG) for the WHO-Coordinated Global Invasive Bacterial Vaccine Preventable Disease (IB-VPD) Network has been pivotal in improving the quality of laboratory data collected through the Surveillance network. It has provided guidance for increasing laboratory capacities for the identification and serotyping/serogrouping of Spn, Hi, and Nm. A strategic review of the IB-VPD network was carried out in 2013 with recommendations to strengthen the WHO IB-VPD Surveillance network and these recommendations have since been implemented.

The laboratory TWG convened for the annual meeting in November 2015 to review current regional and laboratory related issues as technical updates and their potential applications in the network were presented. Other objectives of the annual meeting were to provide guidance on Quality Assurance (QA) and Quality Control (QC) systems, building capacities at NLs, and finally, discussing on methods in improving the quality of laboratory data.

Meeting Objectives

- Assess progress of the implementation of QA/QC programmes and review trends of laboratory performance through the EQA
- Review new technical procedures and their potential applications in the laboratory network, including direct PCR for detection and serotyping/serogrouping
- Review current status in different regions on building capacities at national laboratories to support the sentinel site Surveillance at the country level with examples from AMR, EMR and EUR
- Discuss data quality issues and optimize linkages between laboratory and epidemiology data
- Review global analytic data on trends in ST/SG distribution relative to vaccine implementation

Session I: QA/QC systems- External Quality Assessment (EQA)

2015 EQA survey
Presenter: Mary Slack

In 2015, WHO coordinated the global External Quality Assessment (EQA) programme in coordination with the United Kingdom National External Quality Assessment Service (UK NEQAS) at Public Health England (PHE). The EQA for IB-VPD provides proficiency testing panels to assess
the diagnostic capabilities of laboratories in the network, for identification of Spn, Hi, and Nm by culture, microscopy, rapid diagnostic testing, and molecular methods. Antimicrobial susceptibility testing (AMST) was included in the EQA but was not included in the overall scoring. Serotyping/serogrouping capacities were assessed for laboratories with the relevant diagnostic capacities. The panels were also sent to two referee laboratories—the CDC global reference laboratory (GRL) and the Scottish Haemophilus, Legionella, Meningococcus and Pneumococcus Reference Laboratory (SHMPRL), Glasgow, for testing. Preliminary 2015 results from sentinel sites and NLs showed a high level of performance among the laboratories that submitted results. The RRLs and NLs that have PCR capacities received more challenging proficiency testing (PT) panels that included simulated clinical CSF samples and were assessed on methods for strain characterization. Results were returned to UKNEQAS via the online reporting tool.

Challenges of 2015 EQA survey included delays in customs clearance of the infectious disease PT panels and postponing the closing date of the survey. There was an improvement in the participation of laboratories in 2015 compared to 2014. One Gram stain sample and 8 AMST results were excluded from the final scoring because more than 80% of laboratories reported incorrect results. A passing score was ≥90% for the RRLs and ≥75% for the NLs and sentinel site laboratories (SSLs).

The more complex distribution was sent to 9 RRLs, 17 NLs/SSLs and 2 referee laboratories; results were returned by 9 RRLS, 16 NLs/SSLs and 2 referee laboratories. The less complex distribution was sent to 117 NLs/SSLs; results were returned by 93 NLs/SSLs. A total of 9/9 RRLs (100%) achieved overall scores of ≥90% (96-100%). Overall 100/109 (92%) NL/SSLs achieved a passing score of ≥75%.

The L. monocytogenes Gram film and PCR typing of the non-typeable Hi non-culture sample proved problematic for some RRLs, NLs, and SSLs. Samples that proved problematic for some NLs and SSLs were the identification of Streptococcus agalactiae and Hi cultures as well as phenotypic and genotypic typing of Spn. There was also a higher rate of false positive/false negative AST results with all three species of Spn, Hi, and Nm.

**EQA Trends of laboratory performance**

*Presenter: Sapna Manglani*

Regional and global summary analyses were carried out to look at the trends in laboratory performance from the start of the EQA in 2011 up to the most recent survey in 2015. The analysis showed that there has been an improvement at all levels of laboratory performance for the three organisms, in culture identification, microscopy and ST/SG testing. The results of EQA trends of performance analysis highlighted the role of continued technical
assistance, follow-up, training and site visits in the overall improvement of laboratory capacities in the IB-VPD network.

Recommendations to improve the EQA in 2016:

- WHO to continue the EQA to monitor laboratory performance globally
- Suggestion to include range of Ct values for molecular results
- Penalty for non-answers so that scoring denominators are standard
- A correct result of ST/SG to be considered regardless of the method used

Session I: QA/QC systems- Quality Control (QC)

Confirmatory testing between RRLs and GRL

Presenter: Mahamoudou Ouattara

The purpose of the QC exercise for confirmatory testing between the RRLs and CDC Global Reference Laboratory (GRL) is to strengthen the quality of the data by measuring consensus between laboratories. The QC exercise has been carried out over the last two years. Fifty CSF samples were shipped to GRL from 9 RRLs globally and tested to look at the percentage concordance. Samples were tested for pathogens detection and serotyping/serogrouping. Any discrepancies can be identified and remediated along with the underlying technical issues to ensure a harmonized interpretation of laboratory tests results across the network.

Preliminary results of QC in 2015 showed a high level of concordance between the RRLs and GRL in identification and serotyping/serogrouping of Spn, Hi, and Nm by PCR. GRL received 274 CSF samples from 7 RRLs and 138 isolates from 9 RRLs. Seven out of the nine RRLs were summarized and issues of typical discrepant results were addressed. The average concordance between the RRLs and CDC GRL was 95% for pathogen detection and 97% for ST/SG, which indicated a very high performance for the RRLs and improved level of concordance compared to 2014.

Common reasons for the few discrepancies included transcriptional errors, different testing conditions (direct PCR on CSF vs PCR on DNA, starting volume for extraction, fresh vs stored CSF), different limits of detection, and potential specimen mix-up. Each discrepant result was discussed and addressed by the GRL with the RRLs.

Recommendations to improve the global QC process in 2016:

- Adequate specimen volume, handling and conditioning
- Proper sample selection (i.e. combination of positives, equivocals and negatives/inconclusives with the latter not exceeding 20%)
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Global Meeting 2015

- Problematic and/or difficult specimens can be sent additionally for GRL testing and will not be part of the scored duplicate testing
- Timely referral

Regional QC between RRLs in AFR (NICD)
Presenter: Linda de Gouveia

The process of regional QC between RRLs in AFR was started in 2010, involving the exchange of a batch of isolates between laboratories for identification, serotyping and AMST. It started with 2 participating laboratories and in 2015 there were 4 participating African laboratories: 2 RRL’s and 2 NL’s. Testing was done on spiked samples for PCR and isolates and the consensus that is required between the laboratories is for 3 results per test.

The results of molecular data on CSF samples flagged that NICD had high Ct values due to robotics and methodology. When re-extracted from the original samples using manual Qiagen methods, the results were then comparable. For the isolates testing, the level of concordance between the laboratories was good. AMST was only evaluated for Spn, highlighting a few minor errors.

This QC has been a useful exercise between the RRLs in AFR that contributed sharing of methodologies and troubleshooting issues.

Regional QC between RRL and NLs in WPR (Australia RRL, WPR)
Presenter: Janet Strachan

The RRL in Australia received samples for confirmatory testing for Papua New Guinea, Fiji, and the Philippines while the RRL in South Korea provided QC testing for Mongolia and Cambodia. NLs in these countries have PCR capacities, so the QC exercise included testing of isolates as well as clinical samples or extracted DNA. The lack of concordance of results from NL and RRL highlighted gaps in data reporting, which needs to be taken into account since overall linkage needs improvement. Some challenges that were presented included limited CSF volume to repeat testing and the small number of positive cases.

Recommendations to improve the regional QC for confirmatory testing:

- The laboratories participating in the QC exercise should follow the WHO guidelines and adhere to, including recommendations regarding the following:
  - Composition of the samples to be referred should include positive cases (no more than 20% inconclusive/negatives)
  - Shipping conditions of CSF samples (dry ice) and isolates (e.g., on silica packages)
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Global Meeting 2015

- The regional QC (among RRL and between NL and RRL) is a very useful exercise that enhances laboratory exchange of experience and problem identification/solving; it should be encouraged in all WHO regions.

Session II: Technical update

Implementation of Direct PCR: Pilot test example from RRL AFR (Gambia)

Presenter: Brenda Anna Kwambana-Adams

The rapid real-time PCR (RT-PCR) for detection of Spn, Hi, and Nm in CSF, developed at CDC GRL, was pilot tested in Gambia RRL for AFR. One of the big advantages of this method is that there is no need to extract the DNA. The starting volume of CSF needed per reaction is 2 microlitres, compared to 200 microlitres of CSF needed for DNA extraction to perform the PCR methods used in the network using extracted specimens.

MRC presented the results of the pilot study and found that the Ct values acquired from using the direct PCR were lower than by the traditional indirect method and there was a reduction in inconclusive results. The direct PCR was found to be substantially more cost-effective and required less time to produce results, saving approximately 18 hours/100 samples. Validation at MRC on previously characterized CSF showed concordance between conventional and rapid methods with no evidence of reduced performance with the rapid RT-PCR.

GRL has validated the serotyping of Hi and serogrouping of meningococcus with the direct rapid RT-PCR method. Serotyping of pneumococcus is being optimized and procedures will be shared within the network after validation.

The expansion plan is to validate the direct RT-PCR method in all the regions so it can then be used at NLs as a tool to enhance Surveillance. The NICD RRL for AFR plans to validate it in December 2015 and utilize the method in the year of 2016.

Robotic DNA Extraction at RRL (Australia RRL, WPR and NICD RRL, AFR)

Presenter: Janet Strachan

A study was presented by MDU RRL Australia on the use of robotics for IB-VPD samples, comparing Janus (Parker Elmer) vs Qiacube (Qiagen) machines for robotic DNA and RNA extraction from bacterial cultures and clinical samples. Both systems have the same run time, produce the same purity of DNA, require the same pretreatment and take around two hours to process. The Janus system uses magnetic beads, processes 90 samples, and costs approximately 50% less than the Qiacube.

Automated DNA extraction is usually carried out at the NICD. The EQA and the QC exchange processes highlighted some issues related to variation of the sensitivity depending on
the DNA extraction method used. Few samples fall in the high Ct values range when extracted by the robotic DNA method, compared to lower Ct values when extracted manually. The NICD laboratory receives thousands of samples to be tested from the countries they serve in the Surveillance network. Though the manual DNA extraction processes are more accurate than the robotic methods and provide higher yield of detection and typing, the laboratory uses the robotic DNA extractions to accommodate the countries’ needs in testing all CSF samples from suspect meningitis cases. The NICD laboratory is currently evaluating the direct PCR method developed by CDC GRL for potential implementation, which might allow for higher throughput without compromising sensitivity.

In addition, to improve the classification of probable bacterial meningitis cases, testing was carried out at the NICD on urine strips for measuring the protein and glucose from CSF. Preliminary results from this were presented at the meeting and are included in another section of this report. Based on the results, further testing would be required before the strips can be recommended for use in the IB-VPD Network.

Recommendations on technical issues:
- RRLs to evaluate and/or validate the direct PCR across the network (SOPs and guidance by GRL)
- RRLs to continue evaluating methods critically in the context of their regions as technologies advance and share findings within the network
- PCR data to be interpreted carefully and following the standard procedures recommended in the network (i.e. Ct values cutoff)
- Laboratory data analysis from RRLs should be encouraged at all levels

Session III: Building capacities at national laboratories

Examples from AMR, EMR, and EUR

All WHO regions have a vision to build future national capacities to answer countries’ needs to improve Surveillance and enhance country ownership. As NL capacities improve, Surveillance for VPDs should subsequently improve. It is important for NLs to acquire the right technology; it will reduce some of the workload at RRLs and decrease the burden of sample referral for PCR testing. In EMR, a region with much political conflict, building NL capacities in PCR is crucial in improving the overall Surveillance in the region. Three NLs in EMR have the infrastructure for PCR which may be considered to serve as national referral laboratories. This can further reduce the referring of samples to the RRL.

In EUR, an intensive capacity building occurs in bacteriology. Each country participating in the WHO network is provided with continuous trainings that are tailored depending on its capacity and specific challenges unique to each country.
In AMR, countries have benefited for a long period of time from the assistance of SIREVA (Sistema Regional de Vacunas) laboratory networks to build capacities for diagnosis and serotyping/serogrouping of the IB-VPD pathogens. In addition to the non-molecular methods of serotyping/serogrouping used in AMR countries, the IB-VPD network has provided trainings to build national capacities in PCR.

**Working with other Surveillance systems: MenAfriNet**

To improve coordination of meningitis Surveillance in AFR, the laboratories that perform direct PCR and those working in conjunction with MenAfriNet could possibly initiate discussions with their respective MoHs. In MenAfriNet countries, including Mali, Burkina Faso, and Niger, official meningitis reference laboratories designated by their respective MoHs have the ability in detecting and characterizing Spn, Hi, and Nm. They utilize the CDC laboratory Standard Operating Procedures, which are also widely used by the WHO IB-VPD network. As these capacities already exist systematically in these countries, effort should be made to coordinate Surveillance activities, so they are not done in parallel. When data collection is done in parallel by two different Surveillance systems to detect the same disease, this can undermine data integrity. For instance, specimens can be diverted out of a country for referral, despite the strong and sustainable national Surveillance capacity that already exists. Instead, this capacity should be leveraged and used for the WHO IB-VPD network. With less of a burden in receiving samples per year, RRLs can focus more on performing QC/EQA, identifying any gaps in their Surveillance systems, and supporting training needs. The way forward is to coordinate and work together to protect and use national capacities to generate data necessary for vaccine decisions in the region.

**Recommendations for building national capacities:**

- WHO and partners including GRL, RRL, NL and MoH to work together to:
  - Improve NL capacities and overcome regional challenges
  - Harmonize efforts where national capacities exist

**Session IV: Improving quality of laboratory data**

Progress has been made since the WHO and the informal technical advisory group for new vaccines Surveillance have made recommendations to improve linkages between the Surveillance and laboratory data. Effective and regular communication is very important at all levels to achieve 100% linkage. Examples from different regions were shared to show the improvement of their respective data management systems. Enhanced collaborations have been done between the RRL and sentinel sites to track the referred samples, such as by assigning unique patient ID numbers that would allow linking data at all levels of Surveillance.
Guidance for an effective and appropriate presentation of Surveillance data

Presenter: Chris Van Beneden

The session focused on the importance of an appropriate evaluation and presentation of IB-VPD data to support the objectives of the network. The objectives of a data presentation guide are to provide complete, accurate and clear information to stakeholder in a format that allows full and appropriate interpretation of the data to communicate the desired message. Essential elements of effective graphs and tables are to allow trouble-shooting for discrepant laboratory results or low meningitis case counts. Communicating the correct message is very important. The first step in achieving interpretation of data is to identify Surveillance system errors by using performance indicators.

Challenges in IB-VPD Surveillance data presentation include the use of multiple laboratory diagnostics, categorizing and labelling serotyping results correctly, evaluating trends over time amidst changes in Surveillance or introduction of new vaccine into national immunization schedules. Common omissions or mistakes that interfere with effective communication include inconsistent, or no uses of case definitions, where the vaccine-type is unclear or duplicates are included.

The denominator is necessary to put in context that correctly represents the data. The table or graph must describe the data correctly and completely. Details of data are very important and accuracy is critical. When dealing with small numbers, errors or omissions could have greater consequences compared to data with bigger numbers. Incorrect data/bad data may be worse than no data at all.

Recommendations for improving quality of laboratory data:

- There should be continued efforts to improve data quality and linkage of laboratory and clinical data
- To develop a data presentation template at WHO and define the target audience for this document
Global overview of WHO ROTAVIRUS Sentinel Surveillance Network

*Presenters: Adam Cohen & Fatima Serhan*

Now that the rotavirus (ROTAVIRUS) global Surveillance network has met its original objectives, it should now focus on the burden and vaccine impact of rotavirus and pneumococcal vaccines in remaining regions with gaps in data. Long-term changes after vaccine introduction also need to be recognized, such as by monitoring genotype distribution and new vaccine-preventable pathogens. Surveillance is generally present in countries where it is needed and the quality of conducting Surveillance and data from the network have improved. Case-based, linked data are now reported from all six regions, providing a rich source of data for analysis. The Surveillance network has been leveraged as a platform for vaccine impact studies and to monitor additional vaccine-preventable diseases. There is a strong laboratory network consisting of national, regional, and global laboratories that provide data on circulating genotypes. Some of the existing challenges include polio containment, EQA delays, funding, and concerns of sustainability and country ownership.

**Main Discussion Points:**

- External experts have had a strong voice at the global meeting but the country point of view is less reflected. What do the countries want?
- India, South Africa, and Malawi are being excluded from ROTAVIRUS Surveillance network but they have incredible data – why are they always excluded?
- Good to start having people think about publications, but we should consider putting together a bibliography to display or have a repository where all of the network information/data is displayed.
- It will be terrible timing to cut Surveillance funding because the vaccine is just starting to be introduced in some countries and data being published. Surveillance is immensely valuable and it needs to be recognized that an enormous amount of data is coming out of this network.
- Countries in SEAR are keeping a separate list of genotypes in a database. Linkage at country level is not happening, but it is assumed that it is linking at the WHO level. SEAR looks forward in correcting this.
- SEAR only recently got a system for linking data and can link 50% in 2015 which is a good improvement compared to last year.
WHO IB-VPD and ROTA VIRUS Sentinel Site Surveillance Network
Global Meeting 2015

Action items:
- Continue progress in implementing the iTAG recommendations
- Optimize network scope to ensure sustainability and funding
- Use the current Surveillance platform to include additional VPDs; improved criteria to select samples for future testing by Taqman array cards
- GT distribution and monitoring of samples selected for genotyping for future rotavirus vaccine related analysis; involve the laboratories that do generate the GT data in these analysis
- Isolation of unusual strains to be shared with the network: identification of RRL that can assist

WHO Global Rotavirus Surveillance Network: What Analyses Can Be Done with the Data?
Presenter: Catherine Yen

The 2014 case-based data from all regions were reported to WHO HQ and were included in the preliminary analyses if a site reported cases for all 12 months of 2014 and tested ≥100 stool specimens from enrolled cases for rotavirus by enzyme immunoassay (EIA). Basic descriptive epidemiological data were presented including enrollment, testing, rotavirus positivity, rotavirus seasonality, case demographics, and clinical course by region and age group. Among countries that had sufficient data from consistently reporting sites, pre/post-vaccine introduction trends were shown. In 2014, 105 sites from 51 countries reported data on more than 45,000 suspect rotavirus cases. Overall, 31% were positive for rotavirus. A total of 14 countries had introduced the rotavirus vaccine while 22 countries had not. Demonstrating vaccine impact requires a sufficient amount of baseline and post-introduction data, ideally at least 2 years of baseline data with at least 1 year post-introduction data. Assessing vaccine impact requires enhanced technical support to improve surveillance during both phases of pre- and post-vaccine introduction. Basic descriptive epidemiological analyses are still possible to study vaccine impact, yet interpretation must be done with caution by taking into account of previous knowledge and limitations of available data. Furthermore, it is more difficult to evaluate impact of vaccination (e.g., pre/post-rotavirus vaccine introduction trends) in places without enhanced technical support.

Main Discussion Points:
- Including big countries such as India/Nigeria would help monitoring Regional and global trends
- Even if some countries are not reporting to WHO Surveillance, the network may be able to include those countries in global papers as long as they follow a similar surveillance protocol.
• PAHO has over 80 sites but only 2 meet criteria because vaccine introduction reduced diarrhea cases a lot. We may want to reconsider the minimum number of cases that need to be met each year.
• There are considerations for demonstrating seasonality as part of the network, such as variation within countries and whether using the equator to divide countries is valid.
• Seasonality and all-cause diarrhea should be considered when monitoring rotavirus positivity.
• More data analysis with demographic characteristics pre- and post-vaccine introduction.
• The data suggest that boys are sicker than girls; however, in most diarrhea studies, boys are brought in to participate in studies more often than girls.
• Vaccine impact studies are encouraged using data from the network. Publications are highly recommended to reflect all efforts made in the network.
• There was a large number of deaths and this is a very important outcome. In the entire Global Enteric Multicenter Study (GEMS) study, there were only approximately 190 deaths, but in the WHO IB-VPD Surveillance network, there were a lot more deaths that were reported. This network could provide important information about diarrheal deaths.
• Vaccine coverage levels are also very important – there will be differences in coverage in the areas under Surveillance, even in the same country.
• It is also important to look at severity of disease and those that required IV fluid versus not. Based on core variables, we cannot give a clinical severity category, but we can track sites that do report these variables and look at vaccine introduction status and trends of the disease.
• We should look at demographic trends stratified by age group because we might see shifts especially when the disease is more common now in the older age groups.
• We should split graphs of rotavirus positivity by vaccine usage.
• AFR: there has been an improvement in the selection of samples that need to be sent to the RRL. They now have a program that randomly selects from the case-based data for samples to be genotyped.
• We need to look at the data first when deciding on sending samples to the RRL.

**ROTAVIRUS Sentinel Surveillance: Vaccine Impact in EUR**

*Presenter: Danni Daniels*

Rotavirus sentinel Surveillance was conducted in seven countries in EUR in 2015: Armenia, Azerbaijan, Georgia, Moldova, Tajikistan, Ukraine, and Uzbekistan. There were more than 15,000 eligible cases in 2014 and 26% were positive for rotavirus. Trends in disease were shown for all countries, and clear vaccine impact and vaccine effectiveness were shown for
Moldova and Armenia. Early impact data make the case for sustained use of rotavirus vaccine and encourage other countries in the Region to consider vaccine introduction. Continued Surveillance for rotavirus gastroenteritis is important to monitor vaccine uptake and assess medium- and long-term benefits of rotavirus vaccination.

Main Discussion Points:

- Reduction in all-cause diarrhea not seen in EU but only rotavirus-associated diarrhea.
- As some countries implemented vaccines, they increased their surveillance so they are capturing more cases. Effect was very visible on rotavirus positives, but the increased number of rotavirus negatives (all-cause) really shows the increased surveillance enrolment.
- Moldova vaccine coverage went from approx. 20% in 2012 up to approx. 60% in 2014
- Armenia vaccine coverage went from approx. 33% in 2012 to 91% in 2014.
- Do countries know what is causing the rotavirus-negative diarrhea? No bacterial data in EUR exist yet to determine the cause of this data.
- EUR shows how rich the data can be when you do a country-level analysis. Comparing across Regions at the global level is also critical, though we do need to be careful about the messages that are being relayed from the data.

Use of WHO ROTA VIRUS Surveillance network data in estimating ROTA VIRUS mortality in children <5 years of age, 2000-2013

Presenter: Jacqueline Tate

Multiple diarrhea and rotavirus mortality estimates have existed over time from different research groups. The objective of this analysis was to update the global rotavirus mortality estimates for children <5 years of age from 2000 to 2013 using the most recent diarrhea mortality estimates. Updated estimates of the proportion of severe diarrhea due to rotavirus were also utilized from literature review and data from the WHO-coordinated Rotavirus Surveillance network. Additionally, the impact of rotavirus vaccine use in early adopter countries was taken into account for the global rotavirus mortality estimates. Trends in the proportion of diarrheal deaths due to rotavirus by region from 2000-2013 were presented. In 2013, there were 216,000 rotavirus deaths. The region with the largest proportion of deaths was from sub-Saharan Africa (56%), followed by southern Asia (32%). India had the largest number of deaths, followed by Nigeria. The Surveillance network will continue to provide timely data on the proportion of severe diarrhea due to rotavirus, and it can help determine the proportion of severe diarrhea that is characterized to be bloody and chronic.
Action items:

- Initiate study in Surveillance sites to explore what proportion of diarrhea hospitalizations are acute, watery, bloody, and persistent.

TaqMan Array Cards for Rotavirus and other Surveillance and Enteropathogen Detection and Rotavirus Genotyping using TAC Assays in SEARO

*Presenters: Eric Houpt (University of Virginia, USA) and Gagandeep Kang (India)*

The TaqMan Array Card (TAC) is a molecular diagnostic technology that can detect more than 20 pathogens and their strains at one time. Compared with culture/ELISA, molecular diagnosis can detect much lower levels of pathogens. This diagnostic technology was used to test specimens from the WHO Global Rotavirus Surveillance Network and retest and reanalyze specimens from GEMS (Global Enteric Multicenter Study). TAC can provide high quality surveillance data that are reproducible between laboratories. It is an open, customizable, and inexpensive molecular diagnostic platform for enteric, respiratory, and invasive infections. In the case of the WHO Global Rotavirus Surveillance Network, TAC has added value to the rotavirus EIA results by identifying high rates of Shigella, ETEC, Cryptosporidium, and norovirus. It can also detect rotavirus in EIA negatives. In SEAR, TAC was employed in India, Nepal, Myanmar, and Sri Lanka. The study found that EIA positive samples had a higher rotavirus load compared to EIA negative samples. There was also good concordance between conventional nested PCR and TAC assays for rotavirus genotyping.

Main Discussion Points:

- Rotavirus was identified by TAC in EIA negative samples, even though rotavirus EIA is a specific test
- The focus of the TAC Array cards in this study should not the rotavirus diagnosis but more to look into other pathogens causing diarrhea
- More careful analysis from the phase I of the study are still needed and WHO should assist in collecting surveillance data on all cases that were tested to help guide the analysis

Norovirus disease burden and vaccine development status

*Presenter: Birgitte Giersing (WHO, IVR/IVB)*

Norovirus is the leading cause of community or outpatient cases of acute gastroenteritis among all ages in the USA, but less is known about the epidemiology of norovirus in low and middle income countries. Norovirus is associated with 18% of diarrheal diseases globally with the most common serotype being GII.4. There are multiple norovirus vaccine candidates under
development, and at least one could be licensed within 5 years. WHO’s Product Development Vaccine Advisory Committee (PD-VAC) advised WHO to explore the possibility of incorporating norovirus Surveillance within the WHO Global Rotavirus Surveillance Network.

**Main Discussion Points:**
- Cross-protection was shown in an in-vitro study because norovirus cannot be cultured.
- PAHO normally does not report deaths for norovirus. Norovirus has replaced rotavirus as the most important pathogen in Brazil (30%).
- Norovirus is recombinant so it is hard to study from its characteristics of evolving quickly.
- Norovirus has not replaced rotavirus in South Africa.
- The two sites in MAL-ED with the most norovirus cases are South Africa and Peru, and both have introduced rotavirus vaccine. The Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development Project (MAL-ED) is a multisite diarrhea etiology study at eight sites in South America, Africa, and Asia.

**Action items:**
- Explore leveraging the WHO Global Rotavirus Surveillance Network to monitor norovirus.

**Global Health Security**

*Presenter: Ben Dahl (CDC)*

A presentation on the US Global Health Initiative was given. The Global Health Security (GHS) initiative is a 1 billion dollar investment made by the US government, the European Union, and other countries to prevent, detect and stop outbreaks. There could be some synergies with Rotavirus and IB-VPD Surveillance because there is an overlap with the epidemiology and laboratory Surveillance and capacity building. GHS is not a US initiative but a global initiative. There is a rotating chair for the committee; the current chair is Finland and the next will be Indonesia. The US commitment to GHS encompasses 17 countries globally.

**Main Discussion Points:**
- Most funding for the WHO-coordinated Global Surveillance Networks is coming from Gavi. GHS is another potential contributor in the future.
- Countries may need to apply independently for GHS funds. In contrast, when WHO applied for Gavi funding, it has been allocated to WHO country offices.
- If GHS funds can be used for health systems strengthening, this would help countries respond to outbreaks.
WHO IB-VPD and ROTAVIRUS Sentinel Site Surveillance Network
Global Meeting 2015

Action items:
- A list of US-supported GHS countries should be distributed in the network, so that Regions can start considering this as a potential synergy. WPR is already going to discuss with Vietnam, which is a US-supported GHS country.

CDC Collaborations and Synergies
Presenter: Umesh Parashar (CDC)

CDC currently has many collaborations with WHO regions for site evaluations and impact evaluations. Much of this work would not have been possible without the surveillance platform at WHO, which serves as the foundation for launching the enhanced evaluations. Most CDC funding for rotavirus surveillance is external from Gavi. Norovirus is an area of work that is more challenging, and CDC would like to remain involved with norovirus surveillance and diarrhea etiology globally. Other areas of work are rotavirus vaccine safety and developing reports and publications with the network data. PAHO has had a good experience working with CDC and found that many of the impact studies and evaluations are necessary to build good capacity in the countries. AFRO has held rotavirus symposia which are good mediums for countries to display their data. The Annual African Rotavirus Symposium will be occurring in Mali in 2016.

Main Discussion Points:
- Supplements are good for getting data from similar countries in one place where people can access it.
- Some of the countries in Asia where they have not yet introduced the vaccine have many years of data – global publications would not preclude countries from publishing.
- There is an opportunity for Regions to invite other Regions to participate in their workshops and share knowledge.

Child Health and Mortality Prevention Surveillance (CHAMPS) Network
Presenter: Duncan Steele (BMGF)

There are three significant challenges when conducting surveillance to determine the cause of death in children <5 years of age globally: incomplete data, poor data quality, and delayed reporting. The primary objective of the CHAMPS Network is to track global causes of childhood death by emphasizing high-mortality areas, focusing on preventable deaths, including neonatal deaths and stillbirths, and prioritizing autopsies as the gold standard. Secondary objectives are to track the incidences of cause-specific, severe disease among children < 5 years of age, including neonates, with an initial focus on severe pneumonia, severe diarrheal disease, and
severe febrile illness. Another objective is to establish a surveillance platform to serve host countries. CHAMPS will employ pathology-based surveillance for causes of child death using MITS (Minimally Invasive Tissue Sampling) and TAC testing.

Main Discussion Points:

- BMGF has had a clear shift in thinking about surveillance; they were unsure of what interventions were important
- There is national and possibly site-specific IRB approval for collecting samples from cases as part of CHAMPS.
- Immunization schedules for rotavirus: BMGF is interested in funding studies to look at rotavirus immunization schedules. PAHO: Mostly just Rotarix, and vaccine is working very well with two doses. PAHO would not think schedule studies are a priority for them.
Section V: Rotavirus Laboratory Technical Working Group

Day 5: Friday 20 November 2015

Background

Following the recommendations made at the WHO global IB-VPD and Rotavirus Surveillance meeting at Geneva in 2011, WHO established a laboratory technical working group (TWG) to give technical guidance needed to strengthen laboratory capacities within the WHO Global Rotavirus Surveillance Network. Other objectives were to improve the quality of diagnostic and genotyping data, standardize laboratory methods/training modules and improve the QA/QC systems.

Members of the TWG include WHO global and regional laboratory coordinators and selected technical experts in the field of rotavirus surveillance from the WHO Global Rotavirus Surveillance Network. The previous in-person meeting was held in November 2014 in Rio de Janeiro, which reviewed various topics including rotavirus genotype distribution data from 2013 collected by the WHO surveillance network, leverage of the rotavirus laboratory network for diagnosis of other enteric pathogens pilot studies, and QA/QC systems. This section of the report summarizes the main discussions and recommendations of the rotavirus laboratory meeting where the TWG members of rotavirus laboratory surveillance met in Geneva on the 19 and 20th of November 2015.

Meeting objectives

- Assess progress of the implementation of QA/QC programmes
- Discuss the polio containment in the rotavirus laboratory network context
- Review additional and optimal use of laboratory procedures and building national laboratory capacities for genotyping and strain characterisation
- Review the results of the two-year pilot study funded by the BMGF grant to leverage the WHO rotavirus surveillance network for diagnosing additional enteric pathogens
Session I: QA/QC Systems

Results from the 2014 EQA and 2015 update

Presenter: Mike Bowen (GRL, CDC)

In the 2014 EQA for the Global Rotavirus Surveillance Network, 117 laboratory participated in performing the EIA, while 49 did both EIA and genotyping. Of these, 115/117 laboratories passed the EQA for the EIA and 47/49 for the genotyping. Issues that were identified included incorrect calculation of cut-offs and having controls that were out of range for the EIA.

Of the originally distributed 10 samples, one was removed following the referee laboratory testing. There are challenges in preparing large numbers of panels of lyophilized, non-infectious rotavirus positive and negative samples. Although it was not feasible this year, the referee laboratories will be asked to test the panel prior to distribution in the future. If issues are identified by the referee laboratories, the problematic sample(s) will be removed from the panel prior to distribution.

It was noted that accepting partially correct (some but not all strains identified in mixed samples) results increased the proportion of laboratories providing correct genotypes. The discussion identified that primers and protocols differ by region and some regions have challenges with strains such as G12s which require use of multiple primer sets, and thus, some regions have chosen to eliminate G12 primers and work with sequences of first round products. Availability of primers and protocols for NL and RRL are essential, and the GRL should be contacted in case they are needed.

The WHO ROs have to inform the RRLs on NL results in their Region, so that any identified issues can be worked on by the RRL and the ROs as quickly as possible.

The 2015 EQA exercise started on 26 August 2015 and is expected to end by January 2016. At the time of this presentation, panels were planned to be distributed to approximately 140 laboratories, and results were received from 24 laboratories including the referee laboratories.

Recommendations:

- Continue the exercise globally; complete 2015 survey, and contact referee laboratories for agreement on scoring
- If feasible, panels should be sent to referee laboratories in advance so any potential problematic samples(s) can be excluded before distribution
- Present referee data separately as part of QC of the proficiency testing panels
- RRL should be communicated with the NL and SSL results, so they can implement corrective actions in the laboratories that they are supporting
Update on QC Results

Presenters: Mike Bowen (GRL, CDC) and Nicola Page (NICD)

Following the 2014 exercise of confirmatory testing at the GRL, the 2015 QC included testing of GRL and several RRLs. The GRL received a maximum of 50 samples each from EMR and EUR RRLs. GRL has completed the EMR RRL testing with 100% concordance. AMR RRL (Fiocruz) shipping was being arranged by CDC at the time of this presentation.

NICD, South Africa received samples from Medunsa RRL in South Africa, while the RRL in Ghana had yet to send samples at the time of this meeting. While there was generally a high concordance, there were some issues with mis-priming. The RRLs of WPR and SEAR exchanged 50 samples each, and there was generally a high concordance with some mis-priming, which was resolved on sequencing. WPR also received samples from China and Republic of Korea. There was 98% concordance with Republic of Korea, while China testing was not yet complete.

The mis-priming issues were with specific strains from specific regions with bands being seen for G1G2 for G1 strains, G3s being seen as G3G8, failure to detect G12s, incorrect assignation of mixed P-types (Africa) and G4 strains coming up as G12s (SEAR testing for WPR samples).

The Ghana and Medunsa RRLs in AFR and the Australia RRL in WPR reported on QC testing for NLs in their region and reported high levels of performance in most sites, with NLs improving over time. Site visits and trainings were provided where issues were identified.

Keeping in mind of the several problems identified with primers and with mis-priming for specific strains in different Regions, the discussion focused on the need for a review of current procedures and primers. In order to ensure that the most appropriate primers are used in different Regions, a global review of genotype distribution with data available within and outside the WHO surveillance network is necessary. There appears to be a clear issue with G12 strains circulating at various levels in different parts of the world where the rotavirus vaccine has and has not been introduced. Thus, understanding the drivers of these patterns will be useful. A comprehensive review would provide a baseline for understanding strain distribution over time. It will also establish a base from which future comparisons of circulating strains could be carried out once vaccines have been used for several years in many parts of the world.

In addition, the use of monoclonal antibodies to define the antigenic variations of strains of similar genotypes will be valuable, particularly to study strain distribution after vaccine introduction in Regions.

Recommendations:

- Need a deep review of the data that have been generated. The usage of primers in different laboratories/Regions should also be reviewed.
Regional and bi-Regional QC exercises are very useful and will be continued in the future

WHO plans to convene for a meeting to look closely into the technical issues that arise from the QC exercise

Session II: Polio containment and impact on Global Rotavirus Surveillance network

Presenter: Gloria Rey Benito

An overview of the polio eradication endgame strategic plan, the Global Action Plan III (GAP III), and the regional experience of PAHO were presented. With the trivalent Oral Poliovirus Vaccine (OPV) being replaced by the bivalent OPV following the declaration of the eradication of poliovirus type 2 in April 2016, all laboratories holding wild poliovirus and Sabin type 2 poliovirus are expected to destroy or contain any infectious or potentially infectious material by July 2016. All laboratories not designated as poliovirus essential facilities will be asked not to store any samples (stool or respiratory specimens) that may be potentially infectious and to destroy any existing samples. Retention of such samples will be permitted in only designated poliovirus essential facilities and will require appropriate levels of biorisk management with the defined primary, secondary and tertiary safeguards, and with the requirement of periodic inspections and reporting. In the Americas, there is a regional poliovirus containment plan and other regions are currently developing or have developed their plans.

The need for continuing to work on important pathogens such as rotavirus and the value of the network to inform current and future VPDs were highlighted. The difficulties of implementing GAP III within the short time frame were discussed. It was emphasized that for WHO and the Global Polio Eradication Initiative, outreach, communication, and advocacy are all needed for this global effort.

The implications of GAP III for the network were discussed. Considering the Global Rotavirus Surveillance Network, there are three levels that need to be addressed: The Surveillance sites, the NLs and the RRLs. At the Surveillance sites, no long-term storage of samples should take place, so samples should be sent for testing as early as possible at the NLs. The NLs should test rapidly and send samples within a defined time frame to the RRLs. While some NLs may be designated as essential facilities, the majority will not be identified as essential facilities. The NLs should be encouraged, where appropriate, to destroy samples when testing is completed or ship them to RRLs or designated containment facilities. Given the nature of the work of the RRLs, all RRLs should be considered for essential facility status, and WHO HQ should consider how best to facilitate this with WHO polio staff. Given the current guidance, Phase IIb of polio containment might be feasible in a limited number of laboratories, but Phase III will not be feasible, and therefore, discussions need to be held with appropriate stakeholders in the polio field and those engaged in other forms of Surveillance. They should urgently
consider whether guidance can be developed for facilities that will not be directly handling known poliovirus positive materials.

A consultant was designated to reinforce communication, facilitation and support of WHO HQ Surveillance staff, the RRLs and of the countries where the RRLs are located. They engaged in discussions and devised the best way forward to comply with the current revised version of the GAPIII that was considered acceptable by all participants. Support for this activity may be possible from the BMGF.

**Recommendations:**
- Develop a communication plan to disseminate the most appropriate information with the polio containment team
- Designate a focal person to keep the communication ongoing and assist in implementation of GAPIII from the rotavirus laboratory network
- Determine what is reasonably achievable within the timeline
- Guidelines to be revised

**Session III: Technical Updates**

*Presenter: Mike Bowen (GRL, CDC)*

The GRL has developed a new RT-PCR G and P genotyping assay, which has been tested with 3 different RT-PCR (Qiagen, Mytaq, Superscript III) kits, using mostly all freshly designed primers. The new assay works well on samples from American samples, decreasing untypeable samples.

All RRLs agreed to evaluate up to 100 samples for genotyping during 2016 if the kits, reagents, and protocols were provided. If validated across regions and appropriately costed, this might be a suitable replacement for current procedures and should then be included in a revised Rotavirus Laboratory Procedures Manual. In addition, the current CDC red box primers should continue to be available to all laboratories that need them.

The idea of a central repository for sequence data was discussed and it was agreed to communicate with EuroRotaNet that has such a resource before making further decisions. This could potentially be done at a parallel meeting to discuss sequence data and primer selections at the International Rotavirus Symposium in Melbourne in September 2016, or possibly at the WHO Rotavirus Meeting late March 2016.

There was a discussion in 2014 on culturing of type and reassortant rotavirus strains. Several RRLs agreed that they would make the cultured rotavirus available and attempt to adapt unusual or reassortant strains to culture, recognising that adaptation to culture happens only in a proportion of wild-type strains from stool. The involvement of the University of Liverpool would also be helpful in the future.
Recommendations:
- RRLs to validate the RT-PCR using 100 samples across the network before updating the laboratory manual
- Ensure that old primers will still be available (WHO to discuss with CDC GRL)
- Share the SOPs on virus isolation and culture of strains and clarify objectives before identification of laboratories that can help
- A small working group meeting is needed to review the primers, sequences, strains and GT distribution
- Collate sequence data from Regions in a common database

Session IV: Building capacity at National Laboratories
Presenter: Jason Mwenda (AFRO)

Progress on capacity building at the NLs in AFR were presented. A request from the MOH was followed by a WHO assessment and report. In AFR, through other grant mechanisms, 4 NLs were trained in genotyping. There are additional laboratories that are in need of training, but it appeared that after external funding had ceased, only Mauritius and Mozambique would have domestic resources to continue the work. The need for QC between the NLs and RRLs was emphasized.

Recommendations:
- Define potential scope for engagement and opportunities in laboratory capacity
- Emphasize QC systems to ensure good quality data that are continuously collected through the network
- Highlight the importance of the role of the RRLs to implement QC with NL

Session V. Gavi presentation
Presenter: Hope Johnson

Gavi has been revisiting its scope in Surveillance. A panel of experts helped Gavi consider what needs to be done in the coming years to improve the quality of surveillance, the use of performance indicators, and the use of data. Gavi is considering how non-financial and financial levers can be used to improve delivery, coverage, and equity by strengthening basic country surveillance and regional networks of surveillance sites with enhanced capacity where additional studies can be done. Priorities are based on which countries have the furthest to go, and special attention is given to countries that are applying or expected to apply for health systems strengthening (HSS) grants. Over a 5 year period, Gavi is proposing 7% of its total
spending on data which is an equivalent to US$ 500 million over 5 years that will include funding for HSS. Although the approach is still at a high level, defining minimum standards of immunisation surveillance and key questions must be addressed for decision making in vaccines.
Section VI: Laboratory Special Study for Enteric Pathogens (closed session by BMGF grant to leverage Global Rotavirus Laboratory Network)

Day 6: Saturday 21 November 2015

Background

A pilot project to leverage the Global Rotavirus Surveillance Network was initiated in 2013 with the University of Virginia (UVA). The purpose of the project was to explore the feasibility of using available samples and sampling strategies in order to detect additional pathogens using TAC, particularly in the samples that were not tested positive for rotavirus in the enzyme immunoassay.

The UVA investigators presented their experience in using TAC at GEMS and other sites in 2013 as the process of trainings and testing plans were also described. Preliminary data of year 1 from the testing done in SEAR and WPR were presented. The data emphasized the need for samples of good quality from the network, but most importantly, the data showed that, as expected by multiplexed testing of high sensitivity, multiple potential pathogens can be identified in both rotavirus positive and negative samples from children with gastroenteritis. Subsequently, training and testing activities are being continued for the remaining laboratories that plan to utilize TAC.

Meeting Attendees and Objectives

The meeting was attended by WHO RO laboratory focal persons from AFR, AMR, SEAR, and WPR, WHO HQ, GRL/CDC representatives, 6 RRL representatives, and the UVA staff involved in the pilot TAC project. The objectives were introduced briefly by Duncan Steele to review past experiences, available results, and future plans.

Review of Global Results

The transfer of equipment, training and testing were described by Darwin Operario. While SEAR and WPR laboratories already had equipment available, appropriate equipment were procured and placed at NICD, South Africa, Fiocruz, Brazil and Noguchi Institute, Ghana. Training was conducted and sample testing was initiated at all five sites.

Overall, 771 EIA negative and 563 EIA positive samples were tested from several countries in each of the RRLs. The sample selection criteria varied in terms of numbers and distribution by country and the period of collection was either 2013 or 2014 depending on the site and time of testing. The results demonstrated high internal validity and reproducibility.
WHO IB-VPD and ROTA VIRUS Sentinel Site Surveillance Network
Global Meeting 2015

The results for rotavirus EIA positive samples were comparable by TAC and EIA. Overall, there was 88% accuracy for rotavirus detection, but TAC detected slightly more rotavirus in the EIA positive samples, although this varied by site. The results for the EIA negative samples showed a high level of positivity for rotavirus across all sites, with much higher levels in SEAR and AFR. TAC was able to accurately genotype 86-90% of samples that had been conventionally genotyped.

In both the rotavirus EIA positive and negative samples, high levels of multiple pathogens were detected. The increased detection was particularly high for five pathogens, including rotavirus (mainly contributed by SEAR and AFR), EIEC/Shigella (highest in AMR and AFR, but high in all regions), cryptosporidium (mainly in AFR and WPR), norovirus genogroup II (in all regions, particularly SEAR), and adenovirus 40/41 (in all regions, particularly SEAR).

The discussion focused on issues of typing, timing of sample collection, and sample selection. The regional offices and laboratories reported back on their experience in working with the assays and agreed that in terms of laboratory performance, although the extraction process was laborious, the assay was easy to implement and report results. The necessity in providing feedback to countries and acknowledgement of all participants' efforts were emphasized.

Methodology for Analysis of Data

James Platts-Mills spoke on the methodology of GEMS, subsequent studies, and the ways in which estimates of pathogen specific burdens of disease can be derived in the absence of controls. Analysis of the GEMS data using a conditional logistic regression model, with pathogen quantity and status, permitted association of quantity of individual pathogens with moderate or high likelihood of diarrhea. This could be further analysed for acute watery diarrhea and dysentery. Additional data on choice of samples, clinical data on dysentery, timing, and use of vaccines could help improve the estimates of disease burden.

The discussion highlighted the importance of such analysis for the Global Rotavirus Surveillance Network. The use of cut-offs could potentially eliminate the use of controls, but if the goal is to understand the cause of hospitalized diarrhea, then dysentery cases would need to be included. While the network might consider expanding to a broader Surveillance of the causes of diarrhea and burden of disease, issues of value, size and funding would need to be further explored. It was discussed that the work itself would be done at the RRLs, with sites contributing randomly selected samples. The discussion considered the value of a second pilot before moving to a larger scale by testing with controls and evaluating cut-offs.
Future Directions

Eric Houpt presented the current phases of the project and emphasized the need for sample quality and careful extraction methods. Further analysis is required for samples that tested positive by TAC but negative by EIA, and all sites agreed to retest such samples by EIA. While 8% of samples with low CT values (<15 cycles) were negative by EIA but 14.5% of EIA positive samples while TAC positive, were in amounts that would not be considered rotavirus-associated diarrhea amounts. It was decided that these will be explored further by UVA by obtaining data from RRLs.

Publication of the data was discussed where data should be linked and coordinated by UVA, WHO HQ and ROs. An additional 250 cards are available and the most appropriate use of the cards requires further discussion.

Conducting phase 2 requires consideration on whether additional laboratories need to be equipped and where. The goals of the stakeholders (WHO, GAVI and BMGF) for current and future surveillance, and potentially vaccine testing and impact monitoring should be prioritized in preparation for phase 2. Suggestions for expansion included EUR and AMR laboratories as well as China and the Republic of Korea that would require resources. Unrepresented areas such as India and Democratic Republic of Congo were also considered.

Several decisions are necessary before conducting phase 2, but phase 2 TAC and the Global Rotavirus Surveillance Network need to be considered separately, so that the countries participating in the Global Rotavirus Surveillance Network are not impacted as phase 2 TAC is implemented in a limited number of sites in different regions. Questions were raised whether the TAC could be modified depending on what might be regionally important, such as by taking into account of newly emerging noroviruses and the possibility of simplifying extraction methods for TAC.

Recommendations:

- Complete and finalise phase I data (including retesting EIA negatives, sharing raw data, OD values and linking case based data)
- A phase II pilot is needed and discussion among stakeholders is required to discuss scope and strategy
- EMR and EUR are priority Regions for phase II expansion
- WHO Surveillance and laboratory coordinators should look into case-based surveillance to understand more epidemiological characteristics of samples that were used for the pilot study
- Synergize with the WHO antibiotic treatment of diarrhea and pneumonia project