Meeting Report

WHO Workshop in Training Performance of Rotavirus Vaccine Potency Testing

NIBSC, Potters Bar, United Kingdom

19-23 March 2007
1. **Background**

Rotavirus vaccines are an urgent need for the control of the rotavirus infection in the world. The introduction of these vaccines, in particular to developing countries, will support the effort in controlling the disease.

The capacity of the national control laboratories (NCLs) in testing the vaccine is a crucial part of the process for vaccine licensure and regulation. Potency testing is critical for assuring the efficacy of the vaccines. The status of the rotavirus vaccine potency testing and control in some countries around the world has been investigated through a Questionnaire sent to the NCLs by WHO. There have been difficulties in performing the testing and thus urgent need for technical support in the subject has been identified and requested by many NCLs. In response to the need and in order to strengthen the capacity of the NCLs in testing the rotavirus vaccines, this Workshop in Training Performance of Rotavirus Vaccine Potency Testing has been organized to provide practical knowledge.

There are several rotavirus vaccines currently licensed. Potency testing methods for two of which were taken as the basis of the training given during the workshop due to the broad licensure of the products in many countries worldwide: 1) RotaRix® - produced by GSK and 2) RotaTeq® - Produced by Merck. The RotaRix® is monovalent in its viral composition while the RotaTeq® is pentavalent in its viral composition. The conventional assays used for the potency estimation of live attenuated virus vaccines, such as measles, mumps, rubella, are usually based on the infection of the vaccine virus in cell culture. Assessment of infection is either by observation of plaque formation generation or cytopathic effect, or indirectly using virus specific serological reagents. The RotaRix® vaccine potency assays are carried out on MA104 cells where the vaccine virus titre is measured in terms of CCID$_{50}$ by immunostaining with fluorescent or peroxidase labelled antibodies. The RotaTeq® vaccine is pentavalent vaccine for which reassortant-specific serological reagents are currently not available. Therefore, the potency of vaccine is determined by a sophisticated molecular method in which each reassortant is quantitatively measured by the Polymerase Chain Reaction (PCR). The specificity of each reassortant of the vaccine is determined by the amplifying primers and probe used in the Quantitative Multivalent PCR based assays.

Assays for both rotavirus vaccines, RotaRix® and RotaTeq®, have been developed and validated at the National Institute for Biological Standards & Control (NIBSC), which is the WHO collaborating center for Biological Standards, for the release of vaccine lots. So this workshop has been hosted at NIBSC.

2. **Participants**

The participants were from NCLs, particularly in developing countries who are licensing or will be licensing rotavirus vaccines and indicated urgent need of technical support in that regard. A list of individuals who took part in the workshop is provided.

3. **Objective of the training**

The key objective of the workshop was to provide hands-on training to NCLs on all aspects of the batch release of RotaRix® and RotaTeq® vaccines, in particular to train the participants in the fundamentals and laboratory performance of rotavirus vaccine potency testing (CCID$_{50}$ and Q-PCR assays); review and evaluation of summary protocol of production and control for lot release of rotavirus vaccines; also to provide a forum for
discussion, trouble-shooting and technical communication with regard to the quality testing of rotavirus vaccines; and to identify the gaps and further needs from the developing country NCLs in standardization of rotavirus vaccine testing field. Training is provided so that the trainees, after returning home, should be able to establish and perform related assays accurately and independently. In order to assess the pre-training knowledge of the trainees in the subject area and to share information on current status of rotavirus vaccine production & quality control in the countries, all trainees were requested to give a short presentation on the first day of the workshop elaborating their previous experiences with vaccine testing including, if any, experience for rotavirus vaccines. Although all the participating labs are doing or will be doing the licensure of rotavirus vaccines, the majority of participants had no previous experience of the performance of potency assays of rotavirus vaccines. Two participants had some experience of the batch release assays of the RotaRix® vaccine. None of the participants had any significant experience of testing of RotaTeq® vaccine. The participants acknowledged the importance and demand of receiving such technical training. The assessment carried out through the participant presentations also revealed that all participants had significant knowledge and experience in cell culture and in vitro virus growth areas. Therefore, the main focus of the workshop was to provide training in the key areas of methodologies that is applied for RotaRix® and RotaTeq® vaccines testing. It was also considered to be essential that participants should be provided with the full knowledge of the processes of the protocol review.

4. Key areas of training

The training was provided in the following key areas:

4.1 In the monovalent vaccine (RotaRix®) area

- Cell culture and infection
- Cell staining (immuno-fluorescent and immuno-peroxidase staining)
- Reading of micro plates and calculation of CCID$_{50}$ values
- Calculation of results
- Protocol review for RotaRix® vaccine

4.2 In the pentavalent vaccine (RotaTeq®) area

- Cell culture and infection
- Setting up quantitative PCR plates
- Performance of QPCR amplifications in ABI Prism 7000 sequence detection system
- Importation of data from ABI Prism 7000 sequence detection system to a PC for further analysis
- Data analysis by a software based on the parallel line analysis
- Estimation of virus titre of each reasserting relative to the pentavalent vaccine standard
- Protocol review for RotaTeq® vaccine

5. Trouble-shooting sessions

At the end of each day sufficient time was allocated to discuss trouble-shooting of the
methodology applied during the day. A frank and open discussion was the key element of these sessions. Some of the issues discussed in these sessions are reported below:

Related to RotaRix® vaccine:

- MA-104 cells - their maintenance, usage and passage level records
- Optimisation of trypsin concentration for RotaRix® vaccine assay
- Assay design with relation to dilutions and number of replicates per dilution
- Scoring strategy - positive confirmation of at least two foci in the assay cut-off well
- Microplate antibody staining- hybridisation, washing and plate reading related issues

Related to RotaTeq® vaccine:

- Probes and primers sequences and supply of associated oligonucleotides.
- Importance of the accurate pipetting during the cell infection and optical plate formation.
- Permissible number of freeze-thaw cycles to which the TaqMan assay reagents and infection plate could be safely subjected without compromising the assay results.
- Production of master-mix stocks and their role in achieving consistency between assay results.
- Role of assay design in terms of incorporation of dilution and replicate numbers per assay to achieve statistically valid results.
- Impact of the assay outliers in obtaining the statistically valid results, ramification of the outliers.

6. Protocol review sessions
It was envisaged by the workshop trainers that some laboratories will probably release rotavirus vaccines based on the review of the manufacturer’s product release protocol alone rather than by batch-release testing. Therefore, sufficient time was allocated for these sessions to discuss protocol review.

6.1 Protocol review of RotaRix® vaccine
This session covered the principles of Protocol Review and how this might apply to the review of batches of the monovalent rotavirus vaccines. The trainees were provided with a scenario in which a new manufacturer was submitting the protocol for a monovalent rotavirus vaccine to the NCL. They were asked to review this protocol in the light of the exiting specifications. They were also provided with the WHO guidelines and the relevant Pharmacopoeial monographs.

The training session went well and the trainees were able to identify most of the “problems” that had been introduced into the protocols on purpose. All trainees had a grasp of the principles of Protocol Review and felt the session provided good indications for what to look for in monovalent rotavirus vaccine protocols.
6.2 Protocol review of RotaTeq® vaccine
The trainees were made aware of the key principle of the protocol review which essentially is to review and record the information that a manufacture supplies with its product, in this case RotaTeq® vaccine. For the training purpose participants were given a copy of the Release Protocol originally supplied by Merck to NIBSC in connection with a release of a batch of RotaTeq® vaccine. Additionally, the participants were also given a pre-drafted template form to record information which is essential to monitor before a vaccine batch could be recommended for release. The trainees were made aware that protocol review and laboratory testing of vaccine are two independent tasks and the successful completion of one must not be viewed as the successful completion of the other.

After the training the trainees were given some mock product release protocols to review independently. Some of these protocols had some errors in them (introduced purposely) while the others were identical to the original release protocol produced by the company. Most trainees were able to spot the errors correctly, leading to the conclusion that participants had well understood the concept of the protocol review procedures and are competent to form them independently.

7. Discussions on the standardization issues for rotavirus vaccine testing and recommendations

7.1 Rotavirus vaccine standards
The initial discussion focused on suitable reference materials for the cell culture assay of monovalent rotavirus vaccine where NCLs had obtained references from the manufacturer. However it was apparent from the discussion that:
- NCLs found it hard to obtain reference from the manufacturer
- The number/amount provided was always small i.e. which would lead to a constant need to revalidate with each new batch of reference and,
- In most cases there was no protocol and/or little information provided with the reference

None of the NCLs felt they had the facilities or the expertise to produce their own in-house reference preparations.

With respect to the material already obtained from GSK for establishing an International Reference Preparation, NIBSC outlined their plans to more broadly survey NCLs with an interest in rotavirus vaccines to ascertain how they might envisage using a reference that was available from NIBSC. The preferred position from NIBSC’s point of view was to establish a primary reference against which NCLs could calibrate their own in-house references and/or secondary/regional reference preparations. The participants considered that these secondary references may not be available at present or/and in the near future. Presence of working reference preparation from NIBSC would be of help due to little amount of working references supplied by manufacturer.
NIBSC agreed to investigate the possibility of making their own bulks for production of secondary reference preparations and to also look at the suitability of other materials i.e. the Chinese lamb vaccine.

One possibility that found unanimous agreement from the NCLs was the desirability of a WHO proficiency panel for monovalent rotavirus vaccine. It was felt that this would help to harmonize assays between laboratories and would provide laboratories establishing the assay with suitable materials upon which to proactive. WHO and NIBSC agreed to look into the feasibility and costs of establishing such a proficiency panel.

7.2 MA-104 cell bank
Another issue that was raised during the discussion was the availability of qualified MA-104 cells. MA-104 cells that all NCLs had obtained to date had come from the manufacturer. However, it was considered that it would be preferable to have an "International Cell Bank" on which they could draw in the future. Most NCLs have claimed the difficulties when culturing MA-104 cells. All NCLs present indicated they had the facilities to freeze-down and maintain their own "working banks" and so an “International Bank” would act as a master bank.

WHO and NIBSC agreed to look at the feasibility and costs of establishing such a cell bank.

7.3 Standardized assay methodologies
The issue was raised by WHO as to whether a Manual of Methods was needed for the assay of rotavirus vaccines. While it was considered by participants that it would be useful especially for Q-PCR, NIBSC indicated that it did not feel able to undertake the lead role in this matter considering the product-specific issues. NIBSC commented that each laboratory should set up the assays and validate in a proper way.

7.4 Validation guidelines
It was suggested by the participants that if they could be provided with the written guidelines for the validation of a method/assay and its associated reagents. NIBSC’s view on this was more reserved on the ground that validation of an assay or its associated reagents are exclusively dependent on the facilities, resources and methodology available in the laboratory. However, it was suggested based on NIBSC's experience that assays should be repeated in reasonable numbers and if the data generated are statistically robust then the methodology will probably be acceptable.

8. Conclusions
Based on plenary and personal discussions with participants and information provided on the feedback forms collected at the last day of the workshop it can be concluded that:

- The workshop had been successful in achieving its key objectives.
- The participants were given full training for conducting potency assays related to monovalent as well as pentavalent rotavirus vaccines.
- The participants from China and Brazil were more eager to learn the assays
related to pentavalent vaccine as monovalent testing was already being undertaken and they were anticipating the release of RotaTeq® vaccine soon after their return to the home country. Rota Rix® vaccine is currently licensed in Egypt and vaccine samples are received for Batch release purpose.

- NIBSC staff assured all participants that NIBSC would be willing to provide further assistance on all aspects of the methodologies applied during the training, should it be needed in the future. This could easily be achieved by exchanging of emails.
- NIBSC would be willing to run the MA-104 cell bank if some financial resources are committed for it either by WHO or any other donors.
- NIBSC would continue its efforts for the development of reference preparations for rotavirus vaccines. A close contact in this regard will be kept with the WHO staff related to the project area.
- NIBSC would also take an initiative to conduct a proficiency study between various laboratories at an appropriate time.
- NISBC will be willing to host further rotavirus vaccine testing training workshop(s) should that be required by the WHO in the future.
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