Experience With Harmonized Hib Testing Methodology In India

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Hands-on Training on Determination of the Polyribosyl-ribitol-phosphate (PRP) content of the *Haemophilus influenza* type b (Hib) capsular polysaccharide in liquid vaccine presentations by High Performance Anion Exchange Chromatography Pulsed Amperometric Detection (HPAEC-PAD) 23rd -27th October 2017
Hands-on Training on Determination of the Polyribosyl-ribitol-phosphate (PRP) content of the Hib capsular polysaccharide in liquid vaccine presentations by High Performance Anion Exchange Chromatography Pulsed Amperometric Detection (HPAEC-PAD)

Six representatives from three Indian manufacturers were trained:

- World Health Organization (WHO)- HQ, Geneva
- Istituto Superiore di Sanità (ISS, Italy)
- National Institute of Biologicals, Noida, India
Hands-on Training on Determination of the Polyribosyl-ribitol-phosphate (PRP) content of the Hib capsular polysaccharide in liquid vaccine presentations by High Performance Anion Exchange Chromatography Pulsed Amperometric Detection (HPAEC-PAD)
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Chromatogram - Overlay
## WHO - HARMONIZATION OF TEST METHODS

| Polyribosyl-ribitol-phosphate component in *Haemophilus influenzae* type b (Hib) vaccines protects children against invasive Hib infection | Quality of Hib component controlled by determination of total and free saccharide content | Testing of Monovalent or Pentavalent Hib vaccines (liquid formulations) challenging and time intensive | Different protocols used by different manufacturers: challenging for WHO laboratories leading to Out of Specifications (OOS) occurrence |
First Stakeholder’s meeting to include WHO protocol for determination of the PRP content of Hib vaccine by HPAEC-PAD into the Indian Pharmacopoeia

27th October 2017 at National Institute of Biologicals (NIB), Noida, India
Objectives of the meeting

• to harmonize the HPAEC-PAD protocol as different manufacturers are using different protocols for determination of PRP content in Hib vaccine;

• to explore the feasibility of incorporation of WHO PRP protocol in Indian Pharmacopoeia as an alternate method
SALIENT FEATURES OF THE MEETING

• It was proposed that WHO Hib PRP protocol (acid hydrolysis) be included as **Method A** whereas alkaline hydrolysis will serve as **Method B** of Hib monograph in Indian Pharmacopoeia

• **Dr. Christina Von Hunolstein**, Head Bacterial Vaccine unit, ISS, Italy agreed to share the WHO validation protocol with NIB & stakeholders, which can be used as a template for further validation process.

• A validation group was constituted during the meeting comprising of Panacea Biotec, Biological E, Zydus Cadila, Serum Institute of India.

• **Zydus Cadila** was unanimously chosen as **industry group leader**.

Contd.
SALIENT FEATURES OF THE MEETING

• The validation of WHO protocol to be done by Indian manufacturers at their end. NIB to be the nodal point and will co-ordinate the activities between WHO and Indian manufacturers.

• The validation data to be submitted to NIB by the manufacturers for examining. Subsequently NIB to submit the same to Indian Pharmacopoeia Commission for consideration at their end for its incorporation in Indian Pharmacopoeia.

• Indian manufacturers attending the meeting concurred in principle for inclusion of this WHO protocol in Indian Pharmacopoeia Addendum 2020, once the validation studies are completed.

• It was decided that manufacturers should be allowed ample time to switch to WHO protocol for PRP testing using Acid hydrolysis by HPAEC-PAD.
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<thead>
<tr>
<th>S. No.</th>
<th>Name of the Organization</th>
<th>Participants</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>World Health Organisation (WHO)-HQ, Geneva</td>
<td>Dr. Ute Rosskopf</td>
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<tr>
<td>2</td>
<td>Istituto Superiore di Sanità (ISS) Italy</td>
<td>Dr. Christina Von Hunolstein</td>
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<tr>
<td>3</td>
<td>National Institute of Biologicals (NIB), Noida</td>
<td>Dr. Surinder Singh</td>
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<td>4</td>
<td>Central Drugs Standard Control Organization (CDSCO)- HQ, Delhi</td>
<td>Mr. Sanjeev Kumar</td>
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<tr>
<td>5</td>
<td>Indian Pharmacopoeia Commission (IPC), Ghaziabad</td>
<td>Dr. Jaiprakash</td>
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<tr>
<td>6</td>
<td>Biologicals E limited, Hyderabad</td>
<td>Mr. Ramakrishna Chigurambotla</td>
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<td>7</td>
<td>Bharat Biotech, Hyderabad</td>
<td>Dr. Dipankar Das</td>
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<td>8</td>
<td>Shantha Biotech, Hyderabad</td>
<td>Dr. M.R.K Raju</td>
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<td>9</td>
<td>Serum Institute of India, Pune</td>
<td>Dr. Sunil Gairola</td>
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<tr>
<td>10</td>
<td>Panacea Biotec, Baddi</td>
<td>Dr. Sukhjeet Singh</td>
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<tr>
<td>11</td>
<td>Zydus Cadila, Ahmedabad</td>
<td>Dr. Rakesh Sinha</td>
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NIB received the WHO validation protocol from Dr. Mike Ward, Coordinator, Regulatory Systems Strengthening (RSS) Team, Essential Medicines and Health Products (EMP) Department, WHO-Geneva on 15/02/2018.

The received report was prepared by Dr. Christina and L. Ralli, the National Centre for the Control and Evaluation of Medicines at the Istituto Superiore di Sanità, Italy.

NIB forwarded the WHO validation protocol to all the stakeholders for their comments and queries on the protocol on 19/02/2018.

NIB compiled all the queries received from the stakeholders and forwarded to Dr. Christina for her reply on 11/05/2018.

After receipt of reply from Dr. Christina on 21/05/2018 it was decided to organise second stakeholder’s meeting to finalize the roadmap.
Second Stakeholder’s meeting to include WHO protocol for determination of the PRP content of Hib vaccine by HPAEC- PAD into the Indian Pharmacopoeia

• 23rd May 2018 at National Institute of Biologicals (NIB), Noida, India
Objectives of the meeting

• to optimize the protocol for final validation studies
• to have a consensus among the stakeholders for final inclusion of WHO protocol in Indian Pharmacopoeia Addendum 2020.
Decision taken and Plan of Action

The protocol be taken up for liquid formulated Pentavalent vaccine only.

Regarding freeze dried and hexavalent formulation decision will be taken up in future separately.

Validation study to be conducted by the manufacturers will include:

- Preparation of Validation protocol by individual stakeholder and the same to be submitted to Zydus Cadila (Group leader) who in turn will finalize the protocol for the study.
- LOD and LOQ to be included in the validation protocol.

Use of different kind of Ion Chromatography system equipment i.e. ICS 3000, ICS 5000

For spiking studies, higher as well as lower concentration should be considered.

HPAEC- PAD (ICS 5000+)
Both Ribitol and PRP to be used as standard and can be used as a spiking agent in vaccine samples.

Ribitol Reference Standard used in the protocol received from WHO, was procured from M/s. Sigma. However, Manufacturers may use Ribitol available with other suppliers in addition to M/s Sigma.

For Acid hydrolysis Heating Block, Hot Air Oven and Water Bath can be used.

Each manufacturer will test their own product using this WHO method.
It was agreed that stakeholders will use the methods as mentioned against their names.

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<tr>
<th>S. No.</th>
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<th>Method</th>
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<tbody>
<tr>
<td>1.</td>
<td>Manufacturer 1</td>
<td>Gravity flow and explore the possibility of Vacuum Pump</td>
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<tr>
<td>2.</td>
<td>Manufacturer 2</td>
<td>Gravity flow</td>
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<tr>
<td>3.</td>
<td>Manufacturer 3</td>
<td>Gravity flow and Vacuum Pump</td>
</tr>
<tr>
<td>4.</td>
<td>Manufacturer 4</td>
<td>Gravity flow and Vacuum Pump</td>
</tr>
<tr>
<td>5.</td>
<td>Manufacturer 5</td>
<td>Gravity flow</td>
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Free PRP results can be obtained with two methods: Gravity flow and Vacuum Pump.
Manufacturers need to validate the sample hold time and same can be included as a part of robustness studies e.g. 12, 18, 24, 30 and 36 Hrs. respectively.

SPE C4 cartridge to be used in the protocol may be procured from either M/s Vydaic or any other supplier, if available.

Optimization studies to understand the binding capacity of the SPE C4 cartridge should be kept in the validation protocol.
Timelines finalized for different activities

- Till 23/06/2018: Stakeholders to submit draft protocol to Zydus Cadila (Group Leader)
- Till 07/07/2018: Group Leader to prepare and circulate final validation protocol
- Till 31/07/2018: Optimization time required for Manufacturers to initiate the validation study
- 01/08/2018 to 30/03/2019: Time required for Validation Study
- 31/03/2019: Final Submission of document to NIB
- Till 30/04/2019: Verification at NIB
**Current Status**

**Time required for Validation Study** 01/08/2018 – 30/03/2019 **extended to 30/06/2019** as requested by the manufacturers.

All stakeholders yet to submit their validation data to NIB.

After verification of validation data, NIB in turn will submit the data by **31/07/2019** to Indian Pharmacopoeia Commission for its incorporation into Indian Pharmacopoeia Addendum 2020.
Thank you