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)

- World Health Organization (WHO)- HQ, Geneva
- Istituto Superiore di Sanità (ISS, Italy)
- National Institute of Biologicals, Noida, India

Six representatives from three Indian manufacturers were trained

BHARAT BIOTECH

SHANTHA

Zydus Cadila

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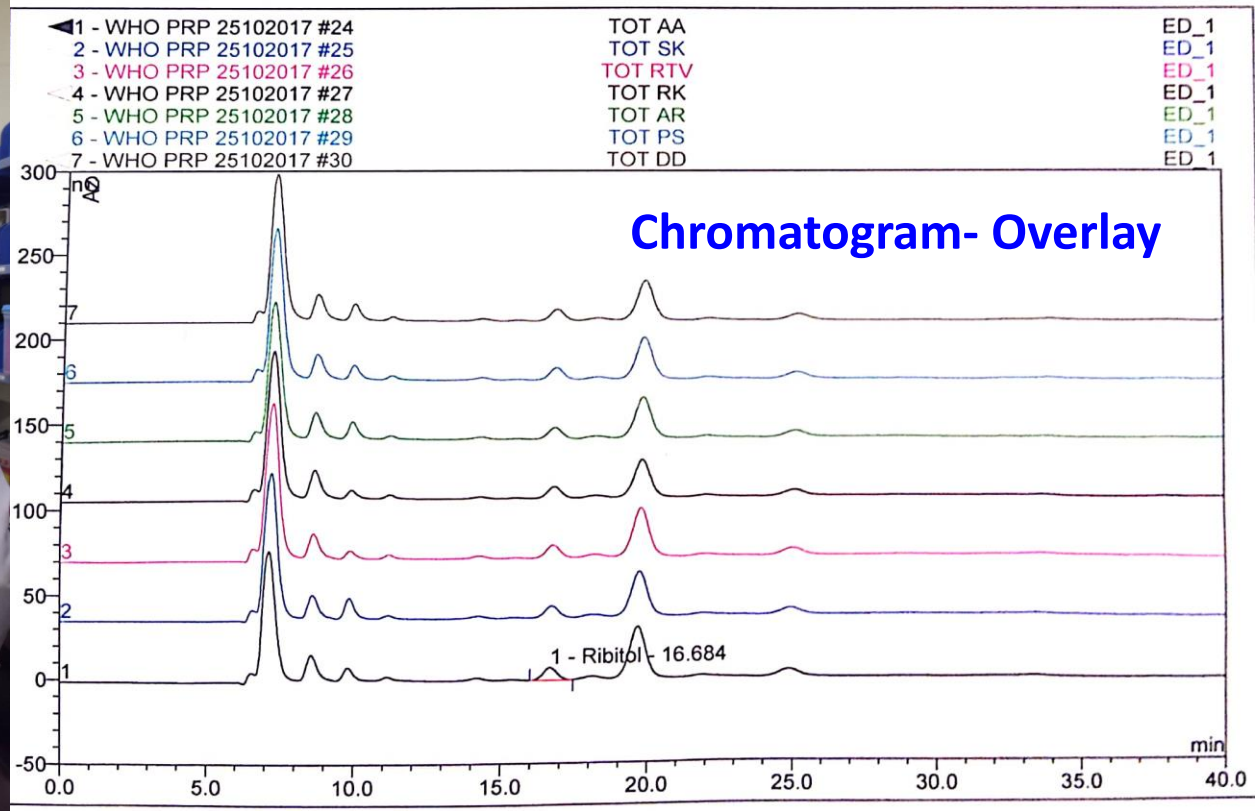
Sample Name:	**TOT AA**	*Injection Volume:*	**100.0**
Vial Number:	**RC5**	*Channel:*	**ED_1**
Sample Type:	**unknown**	*Wavelength:*	**n.a.**
Control Program:	**WHO PRP programme 10012017**	*Bandwidth:*	**n.a.**
Quantif. Method:	**WHO PRP Method 10012017**	*Dilution Factor:*	**1.0000**
Recording Time:	**10/26/2017 7:45**	*Sample Weight:*	**1.0000**
Run Time (min):	**40.00**	*Sample Amount:*	**1.0000**



Chromatogram- Overlay

No.	Ret.Time min	Peak Name	Height nC	Area nC*min	Rel.Area %	Amount	Type
1	16.68	Ribitol	7.131	3.888	100.00	0.413	BMB
Total:			7.131	3.888	100.00	0.413	

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

Stakeholders in the meeting: 27th October 2017

S. No.	Name of the Organization	Participants
1	World Health Organisation (WHO)-HQ, Geneva	Dr. Ute Rosskopf
2	Istituto Superiore di Sanità (ISS) Italy	Dr. Christina Von Hunolstein
3	National Institute of Biologicals (NIB), Noida	Dr. Surinder Singh
4	Central Drugs Standard Control Organization (CDSCO)- HQ, Delhi	Mr. Sanjeev Kumar
5	Indian Pharmacopoeia Commission (IPC), Ghaziabad	Dr. Jaiprakash
6	Biologicals E limited, Hyderabad	Mr. Ramakrishna Chigurambotla
7	Bharat Biotech, Hyderabad	Dr. Dipankar Das
8	Shantha Biotech, Hyderabad	Dr. M.R.K Raju
9	Serum Institute of India, Pune	Dr. Sunil Gairola
10	Panacea Biotec, Baddi	Dr. Sukhjeet Singh
11	Zydus Cadila, Ahmedabad	Dr. Rakesh Sinha

Progress after 1st stakeholder's meeting

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

HPAEC- PAD (ICS 5000+)

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

Decision taken and Plan of Action

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.

Ribitol from M/s. Sigma

Decision taken and Plan of Action

Gravity flow

Vacuum pump

Free PRP results can be obtained with two methods

It was agreed that stakeholders will use the methods as mentioned against their names

S. No.	Stakeholders	Method
1.	Manufacturer 1	Gravity flow and explore the possibility of Vacuum Pump
2.	Manufacturer 2	Gravity flow
3.	Manufacturer 3	Gravity flow and Vacuum Pump
4.	Manufacturer 4	Gravity flow and Vacuum Pump
5.	Manufacturer 5	Gravity flow

Decision taken and Plan of Action

Pack of SPE C4 cartridge from M/s Vydac

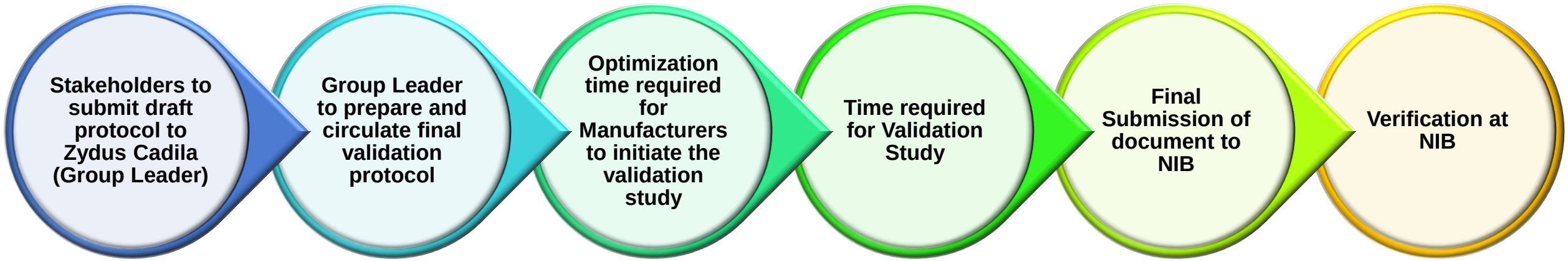
Manufacturers needs to validate the sample hold time and same can be included as a part of robustness studies e.g. 12, 18, 24, 30 and 36 Hrs. respectively.

SPE C4 cartridge to be used in the protocol may be procured from either M/s Vydac 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

Decision taken and Plan of Action

Timelines finalized for different activities



- Till 23/06/2018
- Till 07/07/2018
- Till 31/07/2018
- 01/08/2018 to 30/03/2019
- 31/03/2019
- Till 30/04/2019

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