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Quality Control of polysaccharide Vaccines by the Belgian National Control Lab (OMCL) in the European Batch Release Framework : Activity Report 2014

Scientific Institute of Public Health

Operational Direction « Expertise, Service Provision & Customer Relations »
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List of Abbreviations

BELAC	Belgian Accreditation Body
$CCID_{50}$	Cell Culture Infectious Dose 50 %
DMAB	p-dimethylaminobenzaldehyde
EDQM	European Directory for the Quality of Medicines & Healthcare
EU	European Union
FAMHP	Federal Agency for Medicines and Health Products

Free PS	Free Polysaccharide
GC-FID	Gas-Chromatography-Flame Ionization Detector
Hib	Haemophilus influenza type b
HPLC-PAD	High Performance Liquid Chromatography-Pulsed Amperometric Detector
HPLC-SEC	High Performance Liquid Chromatography-Size Exclusion Chromatography
ICP-AES	Inductively coupled plasma-Atomic Emission Spectroscopy
ISO	International Standardisation Organisation
LAL	Limulus_amoebocyte_lysate
MAA	Marketing Authorisation Application
MJA	Mutual Joint Audit
OCABR	Official Control Authority Batch Release
OMCL	Official Medicine Control Laboratory
OPV	Oral Polio vaccine
Ph.Eur.	European Pharmacopoeia
PS	Polysaccharide
PTS	Proficiency Testing Scheme
UPLC	Ultra Performance Liquid Chromatography
VLP	Virus-like particle
WHO	World Health Organisation
WIV-ISP	Wetenschappelijk Instituut voor volksgezondheid - Institut de Santé Publique

PART 1: GENERAL SECTION

Introduction

The **Biological standardisation** unit of the Scientific Institute of Public Health (WIV-ISP) has a legal activity of quality control for vaccines intended for human use and plasma derived medicinal products. It checks the compliance of each batch of these biological medical products by laboratory analyses prior to their placing on the European market, independently of manufacturers and according to the European Batch release procedure **[1]**. For the international market, batches are released on basis of the protocol review. Testing is not mandatory but may be performed either on a random basis or at the request of local competent authority.

The unit carries out expert activities in these fields, specifically by assessing the "quality" part of registration files, participating in Good Manufacturing Practices (GMP) inspections and the accreditation of blood establishments, as well as participating in various opinion groups and drafting regulatory texts on these matters.
The aim of the service is also to carry out research and development activities in these fields, for normative purposes.

In Belgium, the Federal Agency for Medicines and Health Products **[2]**, founded on the 1st January 2007, is the competent authority responsible for the **quality**, **safety** and **efficacy** of medicines and health products, for granting the marketing authorisation namely to the vaccines manufacturers and for performing the GMP inspections.

The Scientific Institute of Public Health is declared as the Official Medicines Control Laboratory for immunological products in the Royal Decree of 14 December 2006 related to the medicines for human and veterinary use (revision of the Royal Decree of 6 June 1960).

The Biological Standardisation unit performs the OCABR procedure for the human vaccines according the Article 114 of Directive 2001/83/EC as amended by Directive 2004/27/EC.

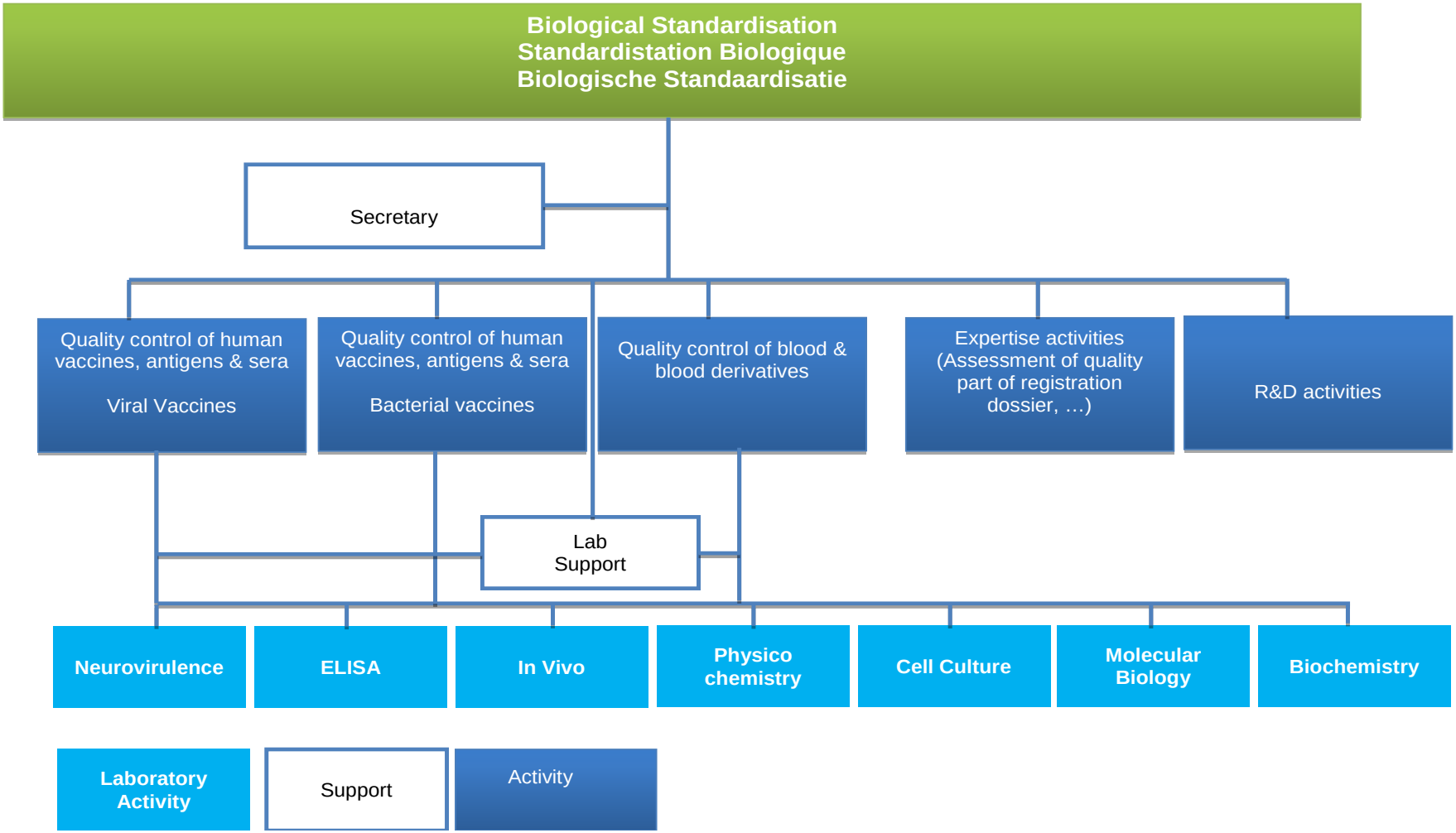
Section A: Organisation of the Competent Authority/OMCL

A.1 General Structure

The Biological Standardisation unit, OMCL for biological products, is part of the Operational Direction “Expertise, Service Provision and Customer Relations” of the Scientific Institute of Public Health.

The structure and the organization of the release activities of the Biological Standardisation unit are shown on the next two pages.


ORGANISATION CHART WIV-ISP
GENERAL DIRECTION
Services of the Executive director
Support services
Managerial posts
Advisor posts
COMMUNICABLE AND INFECTIOUS DISEASES
Food pathogens
Bacterial diseases
Viral diseases
Mycology and aerobiology
Immunology
Platform of animal facility
Laboratory of medical microbiology
Platform BSL3 and Metrology
FOOD, MEDICINES AND CONSUMER SAFETY
Medicines
Toxic and pharmacological residues
Emergent chemical risks
Pesticides
Platform for chromatography and mass spectrometry
PUBLIC HEALTH AND SURVEILLANCE
Toxicology
Healthcare services research
Surveys, lifestyle and chronic diseases
Infectious diseases among the general population
Healthcare associated infections & Antimicrobial resistance (NSIH)
Health and environnement
Cancer Centre
EPI methods for Public health crisis
Public health information system
Special survey
EXPERTISE, SERVICE PROVISION AND CUSTOMER RELATIONS
Biosafety and biotechnology
Biological standardization
Quality of medical laboratories
Platform Biotechnology and molecular biology
Coordination Cell for customer relations and orientation
Dispatching Centre


Biological Standardisation
Standardistation Biologique
Biologische Standaardisatie
Secretary
Quality control of human vaccines, antigens & sera
Viral Vaccines
Quality control of human vaccines, antigens & sera
Bacterial vaccines
Quality control of blood & blood derivatives
Expertise activities (Assessment of quality part of registration dossier, …)
R&D activities
Lab Support
Neurovirulence
ELISA
In Vivo
Physico chemistry
Cell Culture
Molecular Biology
Biochemistry
Laboratory Activity
Support
Activity

A.2 Personnel Matters

Persons listed hereunder have actively participated in xx vaccine testing in 201xx.
Please see also reports to be published from other sections of the unit.

Physicochemical assays, all vaccines
Lorenzo Tesolin, M.Sc, Wim Van Molle, Ph.D. with technical assistance from Saloua El Youssoufi, Dominique Pecher, Rachida Elkhalouki, Ing. Ind. (till March 2014), Laurence Vifquin, Ellen Tuyteleers (from March 2014), Latifa Houbbadi (from May 2014).

Immunochemical assays, all vaccines
Eléonore Dubois, Ph.D., Camille Domicent, M.Sc., Virginie Stygelbout, Ph.D. (from August 2014) with technical assistance from Virginie Misplon (till February 2014), Hanh Van Dang, Sébastien Garcia-Sanchez, Simon Loncke, Véronique Massé, Romain Algoet, Céline Recour (from March 2014), Christophe Vanderstocken (from March 2014).

Biochemical assays, all vaccines
Olivier Carabin, M.Sc, with technical assistance from Fatiha Rahmouni and Sabah Said, Ing. ind.,

Head of Unit Biological Standardisation:
Geneviève Waeterloos, M.Sc. with logistical assistance from Virginie Misplon (from February 2014) and secretarial assistance from Pascale Prévédello.

Section B: Quality Assurance System

The Biological Standardisation unit was first EN 45001 accredited in 1999 and accredited ISO 17025 since 2002. The ISO 17025 accreditation was renewed after a last external audit performed in April 2011 by BELAC, the Belgian Accreditation body **[3]** .

See the accreditation' certificate n° 109-T (valid till 31/01/2015) in annex 1.

List of accredited tests

Assay Code	Sample type	Measured parameter Range of measurement	Description of the assay Equipment
42/III-01/F	Adsorbed Vaccines	Identification & quantification of the HAV antigens content	ELISA Principal equipment colorimeter, washing equipment
42/III-03/F	Adsorbed Vaccines	Identification & determination of the HBsAg antigen content	ELISA Principal equipment colorimeter, washing equipment
42/III-05/F	Bulks of Vaccines	Purity and Identity	PAGE , Coomassie blue and silver staining, and visual evaluation. Principal equipment: PAGE equipment, staining equipment
42/III-09/F	Histological slides - nervous system of monkeys injected with oral poliomyelitis vaccine	Number and lesion scores	Optical Microscopy Principal equipment: Optical microscope
42/III-10/F	Oral Poliomyelitis Vaccines	Number of cultivable live viral particles	Cytopathic effect of virus on cell culture by $CCID_{50}$, neutralisation with specific antiserum – calculation of titre Principal equipment:: laminar flow, incubator and optical microscope

Assay Code	Sample type	Measured parameter Range of measurement	Description of the assay Equipment
42/III/19/F	Rotavirus vaccines	Number of cultivable live viral particles	Cytopathic effect of virus on cell culture by $CCID_{50}$, immunostaining /direct reading – calculation of titre Principal equipment: laminar flow, incubator and optical microscope
42/III-21/F	Measles, Mumps, Rubella, Varicella Vaccines	Number of cultivable live viral particles	Cytopathic effect of virus on cell culture by $CCID_{50}$, neutralisation with specific antiserum – calculation of titre Principal equipment: laminar flow, incubator and optical microscope
42/III-22/F	Varicella Vaccines	Number of cultivable live viral particles	Cytopathic effect of virus on cell culture by $CCID_{50}$, neutralisation with specific antiserum – calculation of titre Principal equipment: laminar flow, incubator and optical microscope
42/III-26/F	Vaccines for human use	pH and visual appearance	pH and visual inspection Principal equipment : pH-meter – Black & White boxes
42/III-28/F	Inactivated Poliomyelitis Vaccines	D-Antigen Content	ELISA Principal equipment colorimeter, washing equipment
42/III-51/F	Vaccines	Determination of 3-O-deacyl-4'Monophoshoryl Lipid A content	GC-FID, Hydrolysis of MPL in fatty acid, derivatization and analysis on a gas chromatograph with flame ionization detector
42/III-52/F	Haemophilus influenzae type b vaccines	Analysis of the total polysaccharides content	HPLC-PAD Dionex, polysaccharide hydrolysis in ribitol-ribose–phosphate units and analysis on anionic column with pulsed amperometric detector (oxydation of carbohydrates).

Assay Code	Sample type	Measured parameter Range of measurement	Description of the assay Equipment
42/III-54/F	Tetravalent Meningococcal Vaccines	Quantification & identity of polysaccharides ACWY	ELISA Principal equipment colorimeter, washing equipment
42/III-57/F	Human Papillomavirus Vaccines	Determination of HPV16 L1 VLP & HPV18 L1 VLP antigen content	ELISA Principal equipment colorimeter, washing equipment
42/III-59/NF	Monovalent pneumococcal conjugated bulks	Determination of total protein content	Lowry colorimetric assay. UV-VIS Spectrophotometer
42/III-60/NF	Monovalent pneumococcal conjugated bulks	Determination of total polysaccharide content	Resorcinol colorimetric assay. UV-VIS Spectrophotometer
42/III-61/F	Monovalent pneumococcal conjugated bulks	Determination of Free-PS4 content	ELISA Principal equipment colorimeter, washing equipment
42/III-62/F	Monovalent pneumococcal conjugated bulks	Determination of Free-PS18C content	ELISA Principal equipment colorimeter, washing equipment

During the last EDQM Mutual Joint Audit performed in March 2009, *in vivo* assays have been included in the scope.
The MJA attestation (EDQM/MJA045) is valid until 12/2014. See Annex 2.
An EDQM mutual joint audit took place in October 2014.

PART 2: TECHNICAL SECTION

Section A: Status of application of Article 114

Article 114 of Directive 2001/83/EC as amended by Directive 2004/27/EC is transposed in the Belgian legislation in the Royal Decree of 14/12/2006 related to the Medicinal Products for Human and Veterinary Use (published the 22/12/2006 in the Belgian official journal).

Article 89 describes the principle of batch release for the immunological products.
In conformity with the EU directive we perform the batch release testing on vaccines batches for the European market. Batch release certificates issued by other OMCLs are accepted.

Section B: Summary of batches tested for OCABR

It is noted that the difference between the number of submitted batches and the number of released batches is due to the fact that the batches which were submitted at the end of one civil year were released the year after.
The report covers the batch release activities during the period January – December 2014.
Lots which do not comply to the approved specifications are rejected by the OMCL and can thus not be put on the market. Those are destroyed by the manufacturer.
Lots which are recalled from the market by the manufacturers are under the supervision of the Belgian Medicines Agency.

In 2014, 60 lots of adsorbed Hib vaccine have been tested and approved. In addition, 49 lots of Hib adsorbed were approved on the protocol review.

Vaccine type	**Submission**	**Release information's**		
Purified meningococcal polysaccharide vaccine, serotypes ACWY		**EU**	**Non-EU**	**Total**
	Submitted	22	26	48
	Tested	22	0	22
	Released	22	26	48
	Rejected/withdrawn	0	0	0

Vaccine type	**Submission**	**Release information's**		
Lyophilised Haemophilus influenzae type b conjugate Vaccine Manufacturer A		**EU**	**Non-EU**	**Total**
	Submitted	48	11	59
	Tested	48	0	48
	Released	48	11	59
	Rejected/withdrawn	0	0	0

Vaccine type	Submission	Release information's		
Haemophilus influenzae type b conjugate vaccine Manufacturer B		EU	Non-EU	Total
	Submitted	22	0	22
	Tested	22	0	22
	Released	22	0	22
	Rejected/withdrawn	0	0	0

WIV-ISP also performs testing and release of pneumococcal monovalent conjugated bulks used for the formulation of a multivalent pneumococcal conjugated vaccine. A certificate of approval is issued by WIV-ISP and transferred to two other OMCL's who are responsible for testing and release of the final container. In 2014 only EU approval certificates where provided. It Is possible that batches that entered in 2013 were tested and released in 2014 and batches that entered in 2014, were tested in 2014 and released in 2015 or tested and released in 2015.

Vaccine type	Status	Release information's		
Pneumococcal Monovalent bulk conjugate		EU	Non-EU	Total
	Submitted	135	0	135
	Tested	152	0	152
	Released	150	0	150
	Rejected	1	0	1

Since 2012, WIV-ISP also performs the release of a conjugated meningococcal polysaccharide vaccine ACWY.

Vaccine type	Status	Release information's		
Conjugated Meningococcal polysaccharide vaccine ACWY		EU	Non-EU	Total
	Submitted	12	27	39
	Tested	11	0	11
	Released	9	27	36
	Rejected	0	0	0

Section C: Technical Details of tests methods applied for OCABR

Batch release of final products is based on the critical evaluation of key parameters of the production and control protocol, and on laboratory testing. The tests performed at WIV-ISP on polysaccharide vaccines (numerous tests are also performed on other vaccine type, see other reports) are listed in the following tables, according to Ph.Eur. Monographs or the Marketing authorisation (MA) when applicable:

Haemophilus influenzae type b conjugate Vaccine	Appearance	Visual inspection (Ph.Eur.)
	pH	pH measurement (Ph.Eur.)
	Endotoxin content	LAL kit by kinetic method (Ph.Eur.)
	Free polysaccharide content	ELISA (MA)
	Molecular size	Size exclusion chromatography (MA)
Pneumococcal monovalent bulk conjugates (Synflorix®)	Protein content	Lowry method (Ph Eur)
	Polysaccharide content	Resorcinol colorimetric method with in house standard and reference (MA)
	Free-polysaccharide content	ELISA (MA)
	Molecular size distribution	HPLC-SEC with dextrane cut-off (MA)
Conjugated Meningococcal polysaccharide vaccine ACWY	O-acetyl content on purified polysaccharide A, C, W and Y bulks	Spectrophotometry (MA)
	Total and free-polysaccharide content on conjugated polysaccharide A bulk	Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES) (MA)
	Total and free-polysaccharide content on conjugated polysaccharide C bulk	DMAB colorimetric method with in house standard and reference (MA)
	Total and free-polysaccharide content on conjugated polysaccharide W and Y bulks	Resorcinol colorimetric method with in house standard and reference (MA)
	Molecular size distribution on conjugated polysaccharide A, C, W and Y bulks	HPLC-SEC with dextrane cut-off (MA)
	Appearance on final lot	Visual inspection (Ph.Eur.)
	Endotoxin content on final lot	LAL kit by kinetic method (Ph.Eur.)
	Total polysaccharide A content on final lot	Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES) (MA)
	Total polysaccharide C+W+Y content on final lot	Ultra Performance Liquid Chromatography (UPLC) with fluorometric detection (MA)
	Total polysaccharide W content on final lot	Ultra Performance Liquid Chromatography (UPLC) with fluorometric detection (MA)
	Total polysaccharide Y content on final lot	Ultra Performance Liquid Chromatography (UPLC) with fluorometric detection (MA)
	Identity on final lot	ELISA (MA)

Section D: Summary of test results

Preliminary remarks

The batch release of final products is based on the critical evaluation of key parameters of the production and control protocol, and on laboratory testing.

On the one hand, the testing verifies the compliance of the key parameters against approved specifications (cf. Marketing authorisation).

On the other hand, consistency limits are set up from a statistical point of view according to yearly historical data of the product. Data from manufacturer and National Control Lab are then evaluated from a consistency point of view.

Out of consistency cases are discussed transparently with the manufacturer and properly justified when needed, ensuring the high quality, safety and efficacy of the final product.

All data have been anonymized.

D.1. Meningococcal polysaccharide vaccine, serotypes ACWY

D.1.1 Product description

This is a vaccine directed against meningococcal invasion (*Neisseria Meningitidis*). Four different polysaccharide serotypes (A,C,W and Y) are formulated directly to obtain the final bulk, followed by filling into final containers and lyophilisation.

D.1.2 Number of batches released

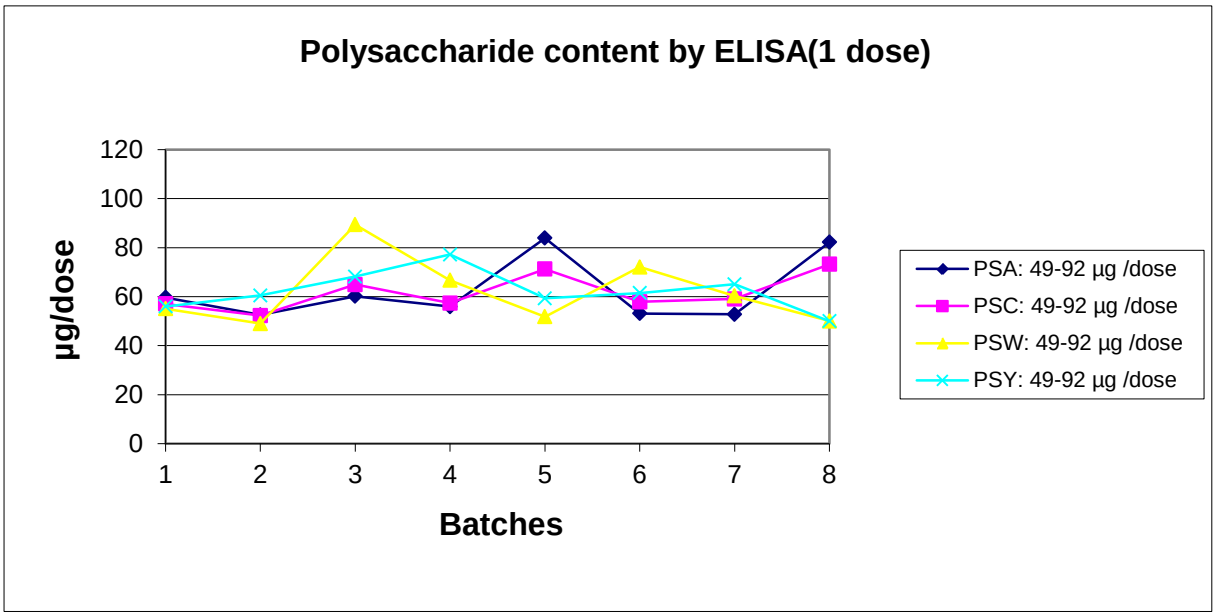
Vaccine type	submission	Release information's		
		EU	Non-EU	Total
Purified meningococcal polysaccharide vaccine, serotypes ACWY	Submitted	22	26	48
	Tested	22	0	22
	Released	22	26	48
	Rejected/withdrawn	0	0	0

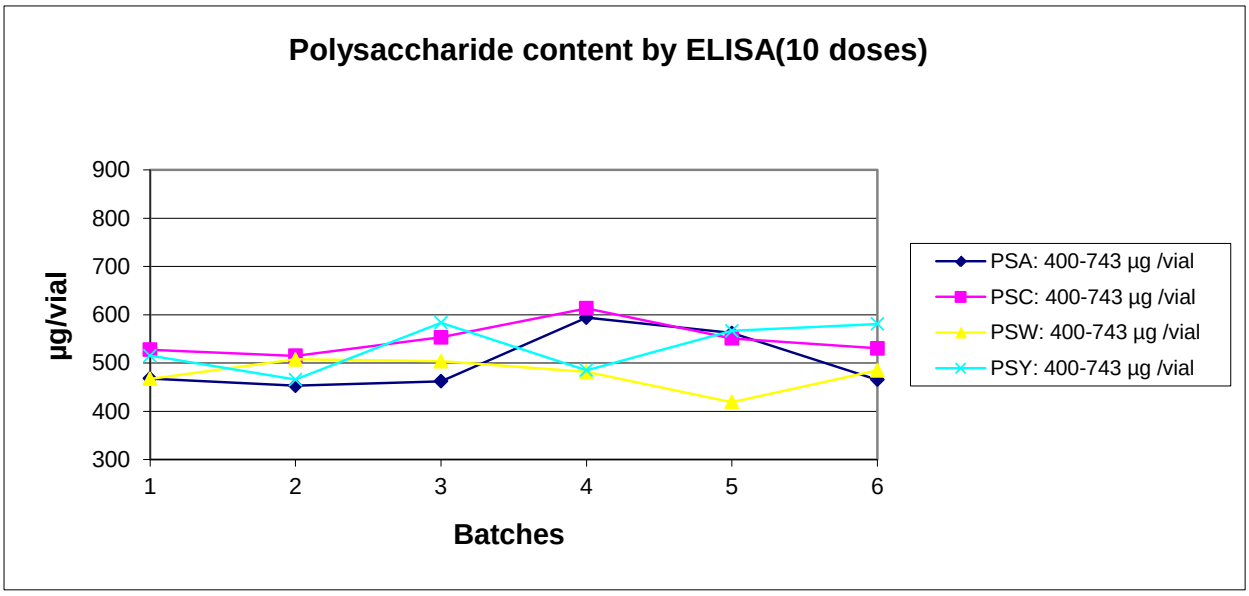
D.1.3 WIV-ISP data

Polysaccharide content

The results remain within specifications. See consistency and graph.

	Consistency 2012(mean ±2SD)	Consistency 2013(mean ±2SD)	Consistency 2014(mean ±2SD)
	4 lots	**6 lots**	**8 lots**
PSA 1D	73,0 µg ± 24,8	65.6 µg ± 24.4	62.5 µg ± 26.0
PSC 1D	71,8 µg ± 33,8	64.7 µg ± 23.4	61.6 µg ± 14.8
PSW 1D	63,8 µg± 15,4	63.5 µg ± 17.0	61.8 µg ± 27.6
PSY1D	72,0 µg± 27,8	62.5 µg ± 19.2	62.2µg ± 16.4
	4 lots	**10 lots**	**6 lots**
PSA 10D	532.5 µg ± 51.2	644.3 µg ± 111.4	500.6 µg ± 121.9
PSC 10D	532.5 µg ± 51.2	597.9 µg ± 202.2	548.3µg ± 69.9
PSW 10D	541.4 µg ± 70.8	511.0 µg ± 86.8	477.2 µg ± 64.4
PSY 10D	636.2 µg ± 113.0	539.4 µg ± 86.8	532.6 µg ± 102.4





Endotoxin content:

For the year 2014, results for the endotoxin content are lower than 10 IU/dose except for one batch at 20.8 IU/dose (specification is lower than 48 IU/dose).

D.1.4 Conclusion and remarks

All the results from the manufacturer and WIV-ISP are still compatible with a consistent production ensuring the high quality of the vaccine.
One may observe a discrepancy between the number of lots released on the EU market and the number of lot tested. This come s from the fact that a final fill (lyophilized) can be combined with a different diluent. The final combination package is then released independently.

Data of the manufacturer have been submitted with the production protocol for each lot, reviewed and found consistent compared to previous years.

D.2. Lyophilized Haemophilus influenzae type b conjugate Vaccine, Manufacturer A

D.2.1 Product description

This is a vaccine directed against *Haemophilus influenza type b*. The polysaccharide is conjugated with a protein carrier (Tetanus Toxoid). The monovalent conjugated bulks may be adsorbed on aluminium or directly formulated to obtain the final bulk, followed by filling into final containers and lyophilisation.

D.2.2 Number of batches released

The Hib vaccines in the table hereunder have been released separately. However, Hib vaccines may also be included as a component in combination vaccine. Results in graph may thus show a higher number of batches.

Vaccine type	**submission**	**Release information's**		
Lyophilised Haemophilus influenzae type b conjugate Vaccine		**EU**	**Non-EU**	**Total**
	Submitted	48	11	59
	Tested	48	0	48
	Released	48	11	59
	Rejected/withdrawn	0	0	0

D.2.3 WIV-ISP tests data

On the bulk conjugate:

- identity and molecular size distribution

On the final lot:

- polysaccharide content by HPLC Dionex ™ method
- free polysaccharide content by ELISA or HPLC Dionex™
- description-appearance
- endotoxin content by LAL (Kit)

D.2.3.1. Bulk Conjugate

Molecular Size Distribution : (Specification: >80% of the conjugate PS-TT eluted before Kd 0.2).
The molecular size distribution shows a high consistency of production.

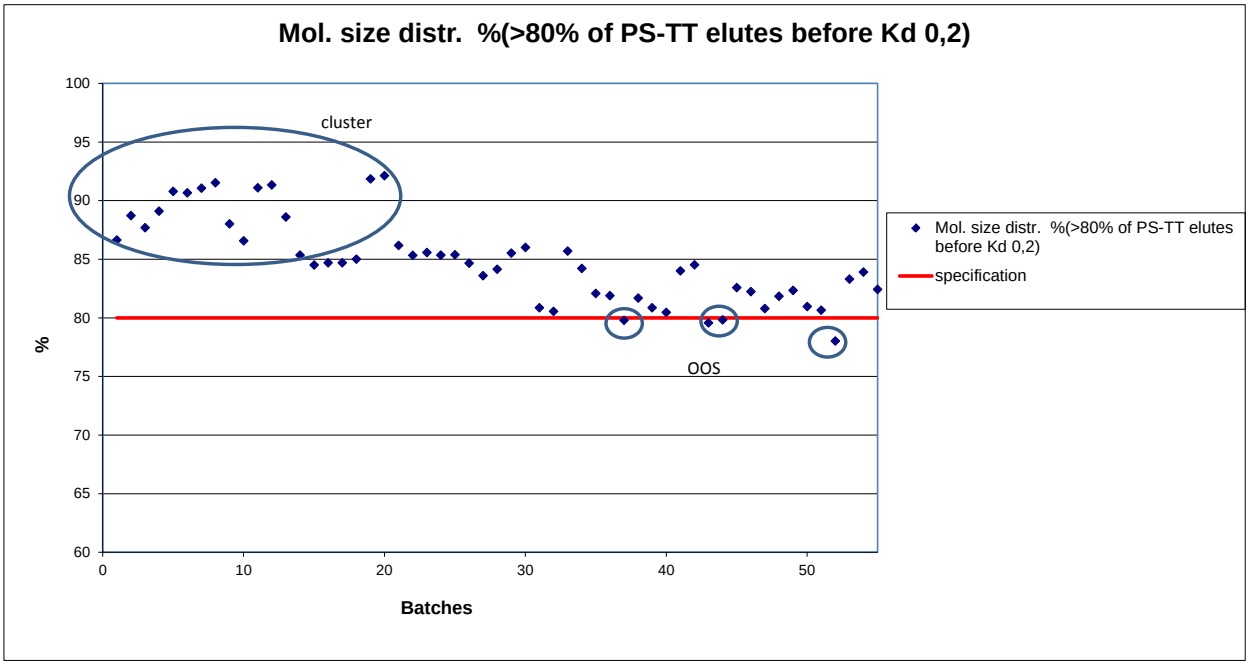
The molecular size distribution shows a high consistency of production.

Years	***Mean***	***Mean - 2*SD***	***Mean + 2*SD***
2012 (N =81)	85.9	82.5	89.3
2013 (N=47)	88.4	85.0	91.8
2014 (N=55)	84.9	77.6	92.2

All WIV-ISP results are below manufacturer's results due to time lag between testing of both labs and the short shelf life of the product itself (6 months). on the graph, one can observe a cluster. those results have be obtained with the old molecular size test (one dextran). Other results have been obtained with the new molecular size (five detrans). It should be mentioned

that the lag between sampling and testing at the OMCL has increased leading to several out of specification batches. Those sample were out of shelf life during testing. Following a thorough investigation at the manufacturer premises (batch record review, raw QC data review, shelf life and formulation dates review), we released all batches. The bulks were formulated during their shelf life and thus were of good quality. We assume that the trend observed in the graph is a combination of factors: new dextrans method show lower results and the increased lag time between sampling and testing.
It is difficult for the company to know the destination of a bulk (EU, Non EU) at the time of bulk production. The short shelf life of the bulk (less than 6 months) is an added constraint in the submission process.

See graph below:

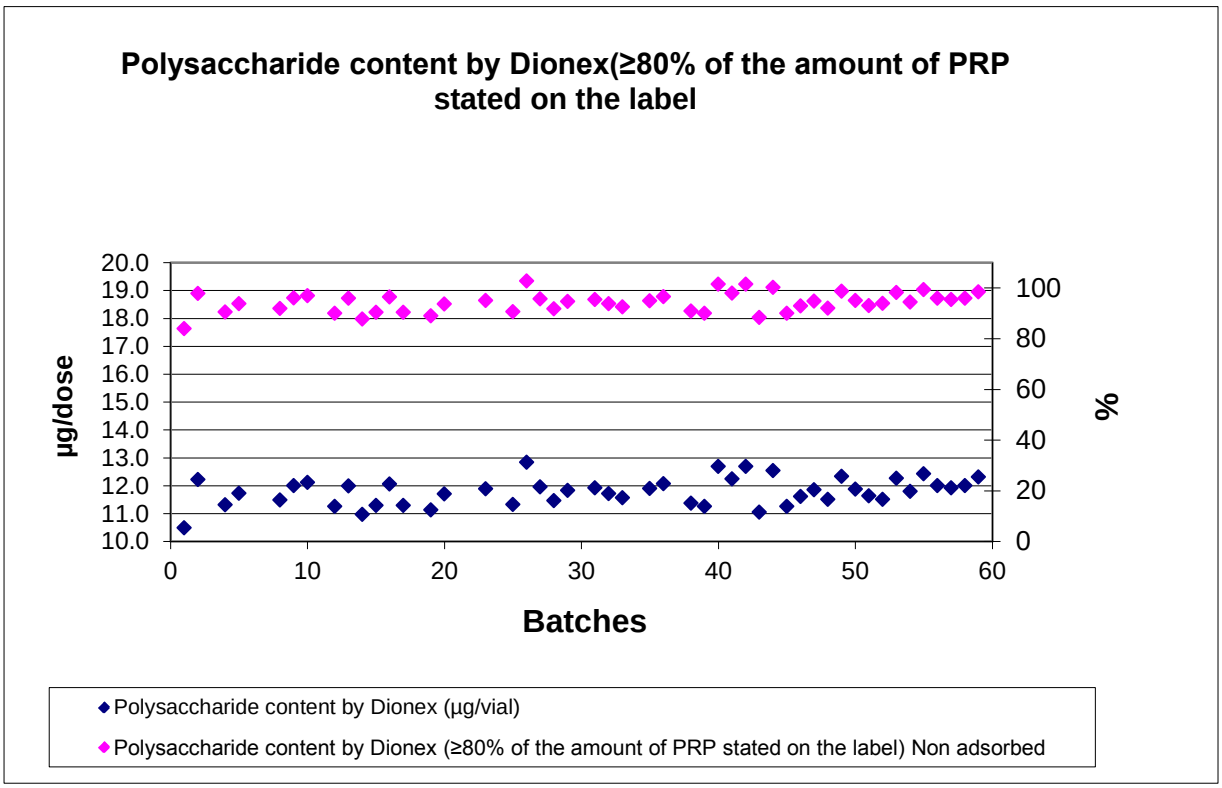


D.2.3.2. Final container

Total polysaccharide content by HPLC with pulsed amperometric detector (refer to section B)

Total polysaccharide non-adsorbed vaccines (10-15µg/dose)
Please note that, in all graphs, all lots are sorted by increasing number. Some 'missing results' may appear. In fact, those lots have not been tested by our lab but released for the international market (Non EU market) based on the production protocol review.
The polysaccharide content shows a high consistency during 2014.

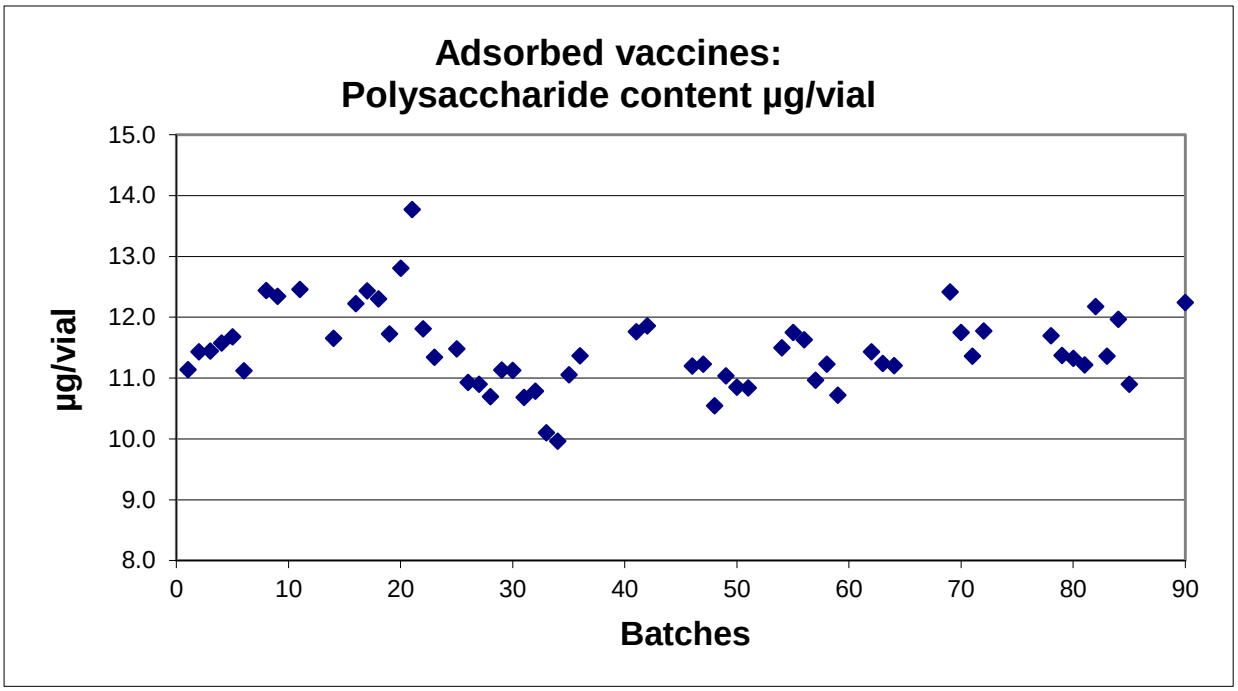
Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N =52)	12.4	11.4	13.4
2013 (N=46)	12.3	10.9	13.7
2014 (N=48)	11.8	10.8	12.8



Total polysaccharide adsorbed vaccines(10-15µg/dose)

Please note that, in all graphs, all lots are sorted by increasing number. Some 'missing results' may appear. In fact, those lots have not been tested by our lab but released for the international market (Non EU market) based on the production protocol review.
The polysaccharide content shows a high consistency during 2014 except for one lot still within specifications.

Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N =59)	12.0	11.0	13.0
2013 (N=89)	12.1	11.1	13.1
2014 (N=60)	11.5	10.1	12.9



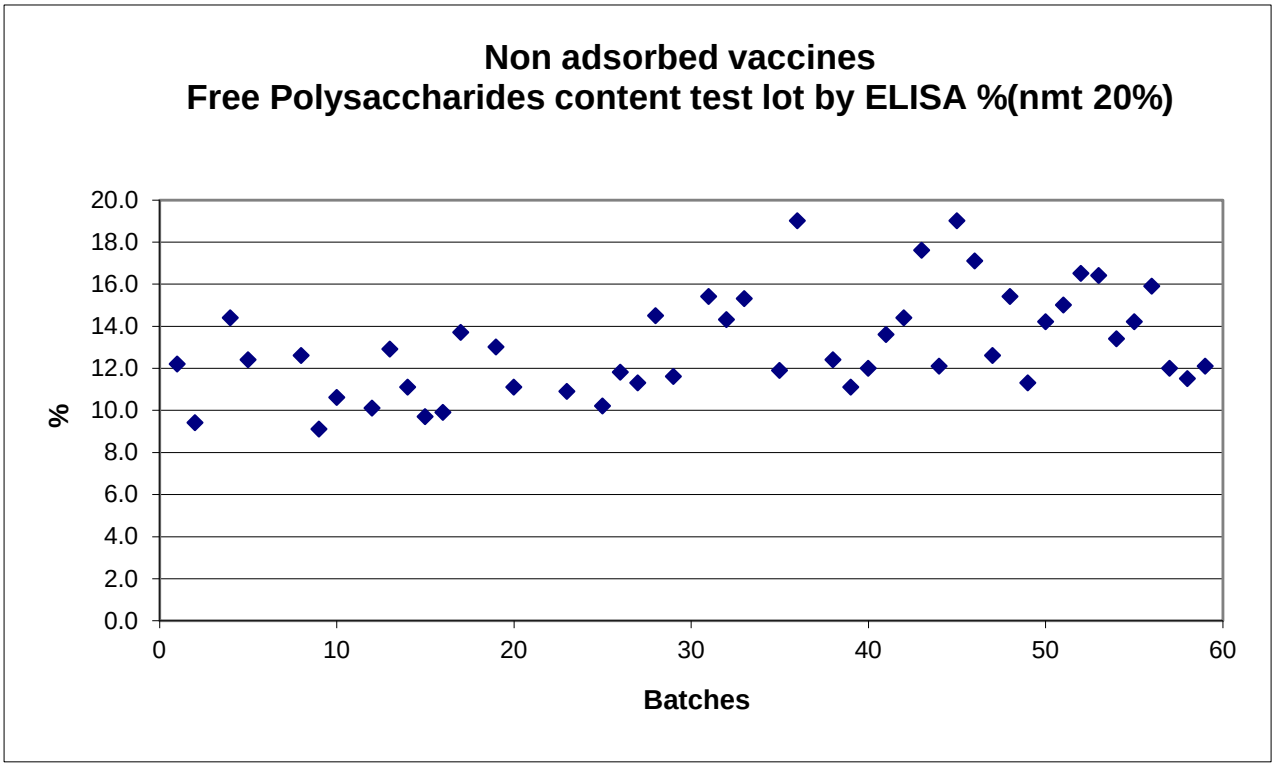
Free polysaccharide content by ELISA (Specification : nmt 20% for non-adsorbed Hib and nmt 20% for adsorbed Hib for use in combination vaccines).

Rem: the specification has been adapted to 20% for adsorbed and non-adsorbed vaccine in 2010.

Free polysaccharide non adsorbed Vaccines (NMT 20%):

Results are higher for WIV-ISP lab in 2 cases due to the high variability of the ELISA test.

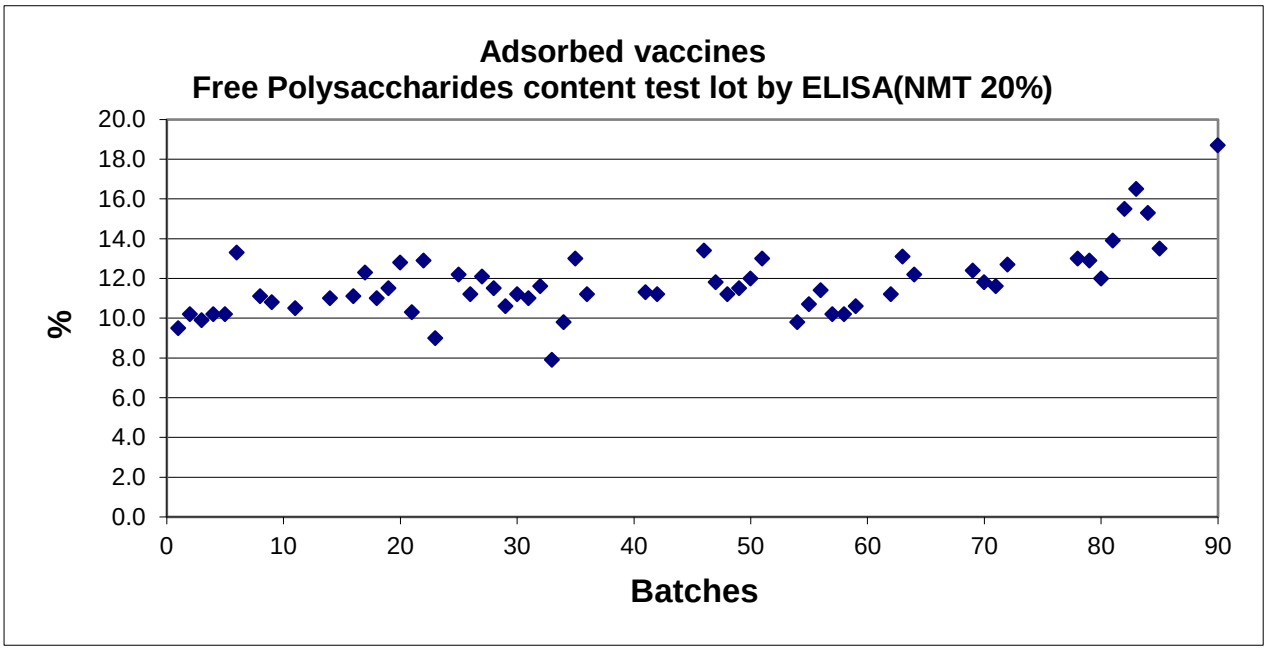
Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N =52)	11.0	7.0	15.0
2013 (46)	12.1	8.3	15.9
2014 (48)	13.1	8.2	18.0



Free polysaccharide adsorbed vaccines(NMT 20%):.

Results are higher for WIV-ISP lab in 3 cases due to the high variability of the ELISA test.

Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N=59)	11.8	8.6	15.0
2013 (N=89)	11.0	7.6	14.4
2014 (N=60)	11.8	8.2	15.4



Endotoxin content by LAL (Specification : <5.00 IU/dose)

Data show that the endotoxin content by LAL test is mostly below 2.5 IU/dose.
The specification is already very low compared to whole cell vaccines, the level of endotoxin of which can reach thousands of IU/dose.

D.2.4 Conclusion

All the results, from the manufacturer as well as from the National Control lab, point towards a highly consistent production ensuring the high quality and safety of the product.
Data of the manufacturer have been submitted with the production protocol for each lot, reviewed and found consistent compared to previous years.

D.3. Lyophilized Haemophilus influenzae type b conjugate vaccine, Manufacturer B

D.3.1 Product description

This is a vaccine directed against *Haemophilus influenza type b*. The polysaccharide is conjugated with a protein carrier (Tetanus Toxoid). The monovalent conjugated bulks are directly formulated to obtain the final bulk, followed by filling into final containers and lyophilisation.

D.3.2 Number of batches released

Vaccine type	Submission	Release information's		
Haemophilus influenzae type b conjugate vaccine		EU	Non-EU	Total
	Submitted	22	0	22
	Tested	22	0	22
	Released	22	0	22
	Rejected/withdrawn	0	0	0

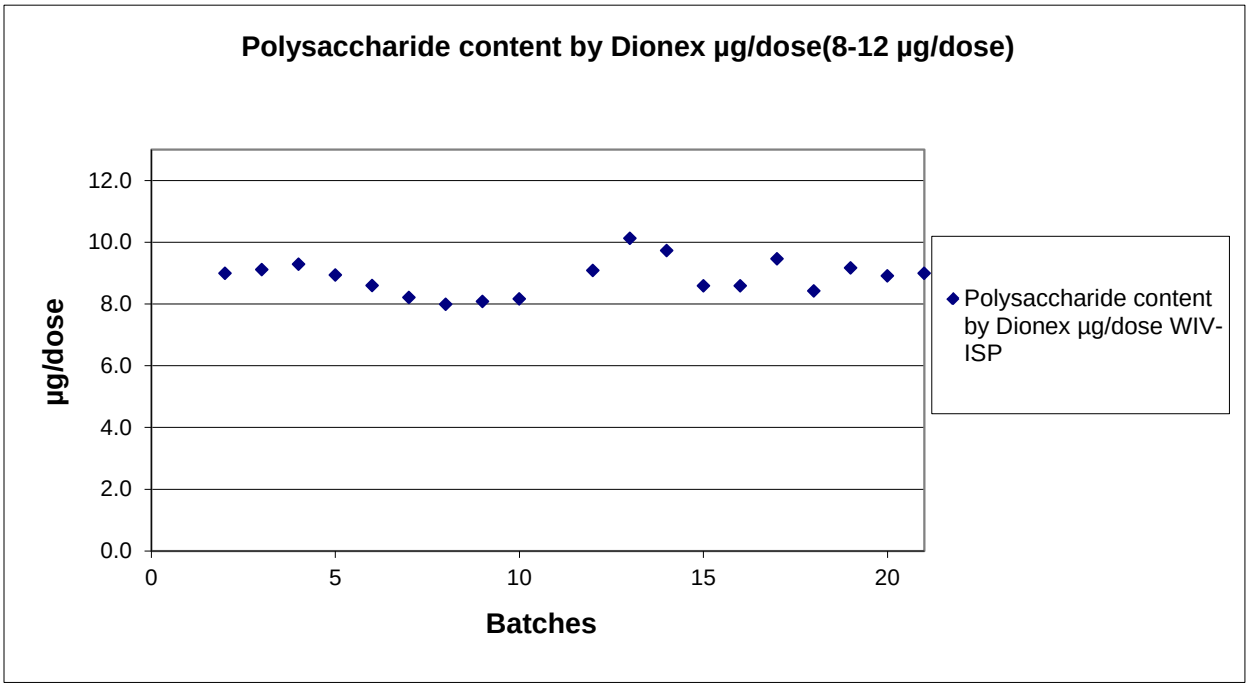
D.3.3 WIV-ISP tests data

All tests performed on final container except molecular size distribution.

Polysaccharide content by HPLC-PAD (Specifications: 8-12µg/ dose)

Results remain within the specifications and shows a high consistency. Note that manufacturer's results have been rounded to unit. WIV-ISP results are below manufacturer's results.

Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N=20)	8.9	8.0	9.8
2013 (N=71)	8.8	8.0	9.6
2014 (N=23)	8.9	7.8	10.0



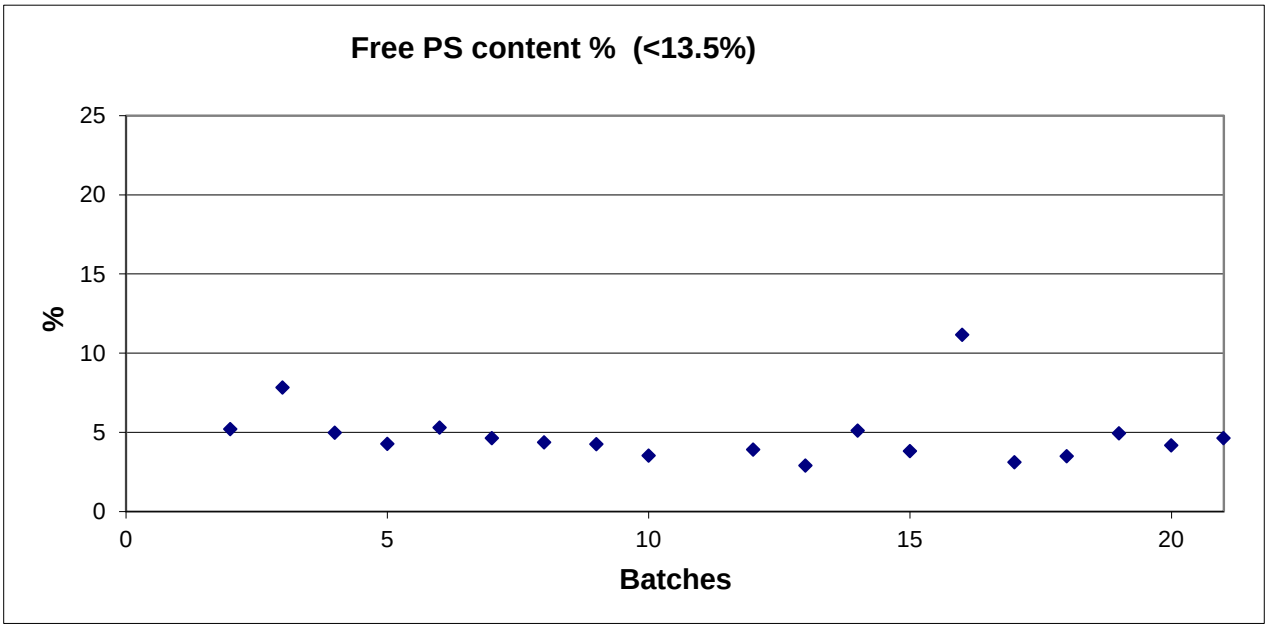
Free polysaccharide (Specification: <13.5%)

See graph below.

Most of the results were below 5%, while the current specification is <13.5%. WIV-ISP results are below manufacturer's results. This is most probably due to the difference in methodology to separate free polysaccharide from conjugated polysaccharide and from the testing itself (HPLC-PAD versus ELISA for WIV-ISP lab). One lot is higher but this is an isolated case.

Please note that in 2012, free polysaccharide content was performed by ELISA. In 2013, we tested the product with a new method (HPLC-pulsed amperometric detection) as well as by ELISA. this is the reason why we have no consistency as such in 2013. In 2014, we tested the vaccine exclusively by HPLC-PAD. This explains the differences observed on consistencies.

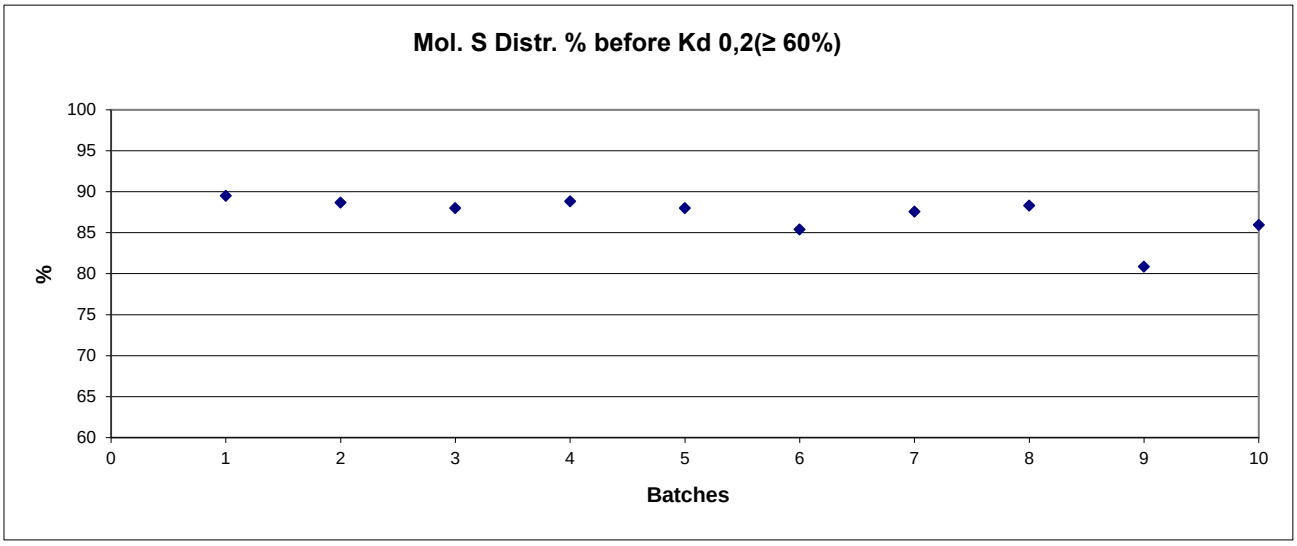
Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N=20)	4.6	3.4	5.8
2013 (N=71)	NA	NA	NA
2014 (N=23)	4.8	1.2	8.4



Molecular size distribution, percentage of conjugate PS-TT eluted before KD 0.2 (Specifications: >60% of the conjugate elutes before Kd 0.2).

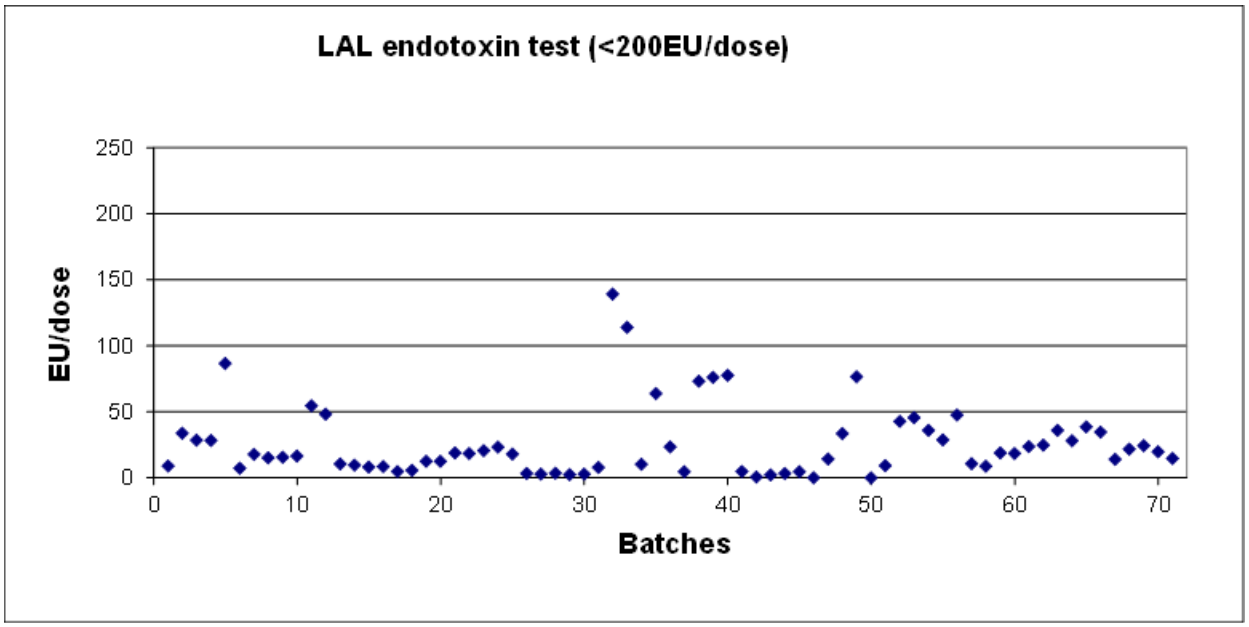
In 2010 the specification was expressed as at least 60 % of polysaccharide elutes before Kd <0,2). The Manufacturer has submitted at that time a variation dossier to express the molecular size with a multiple Angle light scattering equipment : Hydrodynamic radius, Mega Dalton. The variation dossier has demonstrated measurement equivalence. Results are far above the specification.

Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N=9)	89.0	86.3	91.7
2013 (N=19)	90.2	86.8	93.6
2014 (N=10)	87.1	82.1	92.1



LAL endotoxin content LAL (<200 EU/dose).
In 2014, the average of 71 lots is 26 EU/dose ± 54 (2SD).

The manufacturer has submitted variation to introduce a new specification : <200 EU/dose. The purification process has been changed to reduce the endotoxin level. At that moment, the pyrogen test on rabbits was performed. This will be replaced by endotoxin content test. Some lots have a higher endotoxin content but still within specification.



D.3.4. Conclusion

All the results, both from the manufacturer and from WIV-ISP, point towards a consistent production.
Data of the manufacturer have been submitted with the production protocol for each lot, reviewed and found consistent compared to previous years.

D.4. Haemophilus influenzae type b conjugate fraction, part of the fully liquid hexavalent combined vaccine Manufacturer B.

D.4.1 Product description

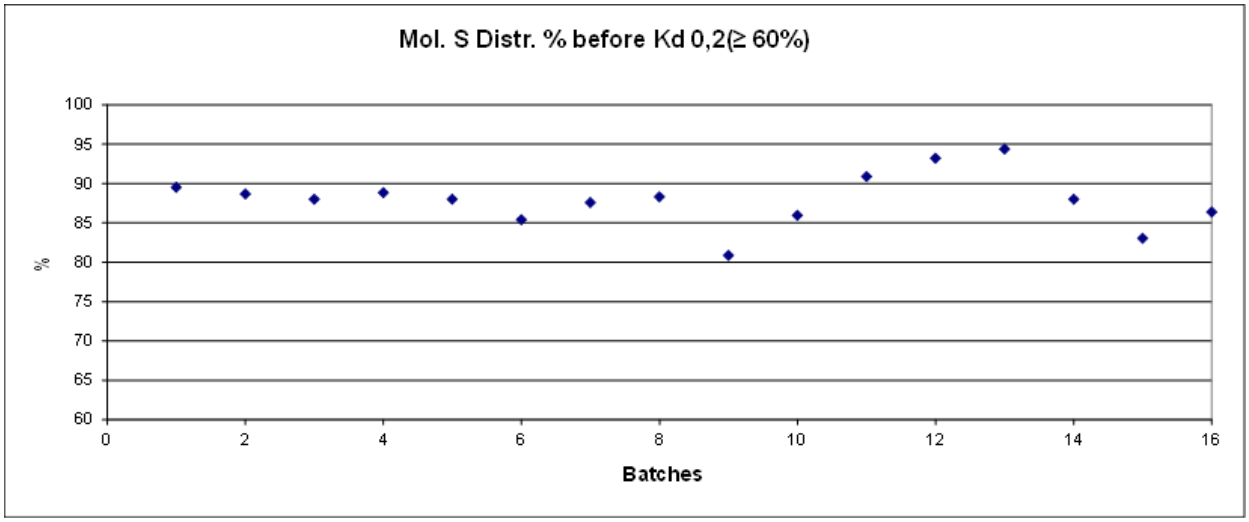
This multivalent vaccine is indicated for primary and booster vaccination of infants and toddlers from six weeks to 24 months of age against diphtheria, tetanus, pertussis, hepatitis B, poliomyelitis and invasive diseases caused by Haemophilus influenzae type b. All antigens are adsorbed on Aluminium hydroxide except the Hib fraction.

D.4.2 Number of released batches

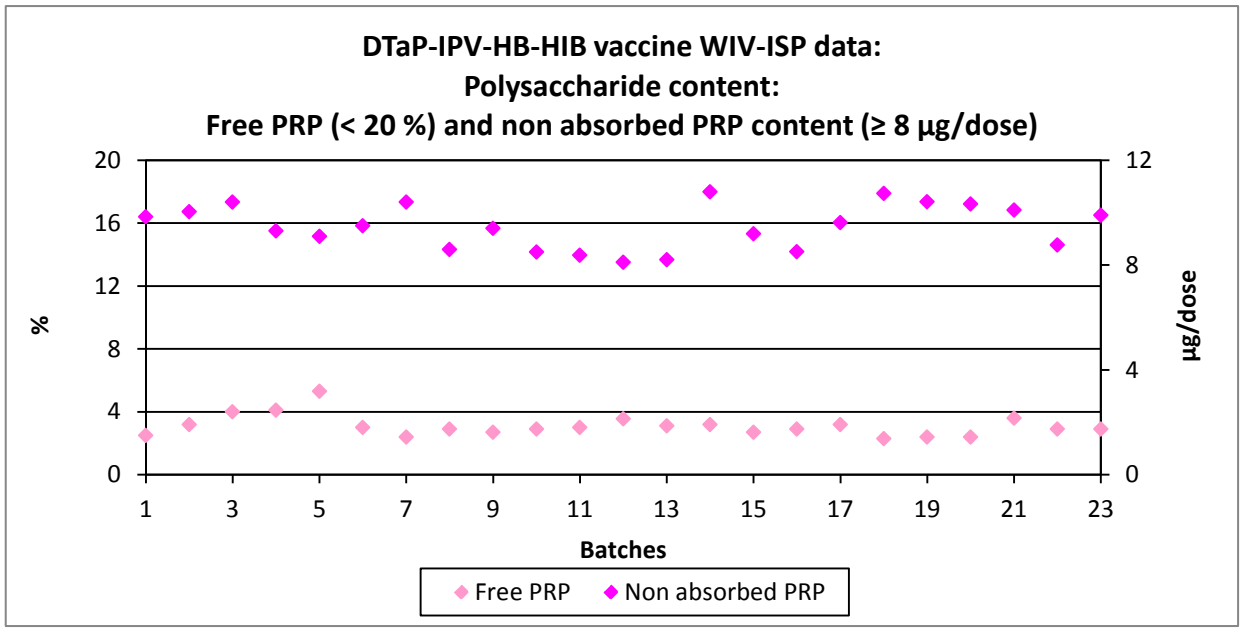
Vaccine type	Status	Release information's		
Adsorbed Diphtheria, Tetanus, acellular Pertussis, hepatitis B inactivated Poliomyelitis and Haemophilus Influenzae type B vaccine		EU	Non-EU	Total
	Submitted	26	0	26
	Tested	26	0	26
	Released	23	0	23
	Rejected/withdrawn	3	0	3

D.4.3 Results

Molecular size distribution on the Hib bulk conjugate alone (not yet formulated with other antigens)



Total non adsorbed polysaccharide content is tested on the final container at WIV-ISP while it is tested on the final bulk by the manufacturer (1 final bulk= 2 to 3 final containers).
In 2014 we detected 2 out of specification lots due to a low polysaccharide content . A presentation was made at the annual meeting 2014. The root cause was a clogging of the filter during formulation of the Hib bulk leading to a low polysaccharide content in the Hexacima final bulk. Those lots were withdrawn from parallel testing by the manufacturer and thus not submitted for release. A third lot was withdrawn for non-compliance regarding the pertussis immunogenicity test.



Depolymerized non adsorbed polysaccharide tested on the final container are also included in the graph. The depolymerized polysaccharide is not conjugated to the protein carrier and thus less immunogenic on the long term.

D.4.4. Conclusion

We have more experience in assessing the quality of this new product. We are now able to assess consistency but one can says it is quite stable for PRP content as well as for depolymerized PRP content. Methods on total PRP and free PRP have to be adapted to better reflect reality as a gap appear between our results and those from the Manufacturer. One can observe the role of an Official Medicine Control Lab in this particular case: 2 lots were detected out of specification by our lab while the results were close to the specification (but still within) at the manufacturer lab. Following a thorough investigation on both sites, the manufacturer decided not to submitted those lots for release. The batches were thus not put on the market.

D.5. Pneumococcal vaccine: monovalent conjugated bulks

D.5.1 Product description

This multivalent vaccine is directed against pneumococcal invasion (*Streptococcus pneumoniae*). The different polysaccharide serotypes are conjugated with a protein carrier. The

monovalent conjugated bulks are adsorbed on aluminium before final formulation into the final container.

D.5.2 Number of released batches

It is possible that batches released in 2013 were tested in 2012, batches tested in 2013 released or to be released in 2014 and batches submitted in 2013, tested and released in 2014.

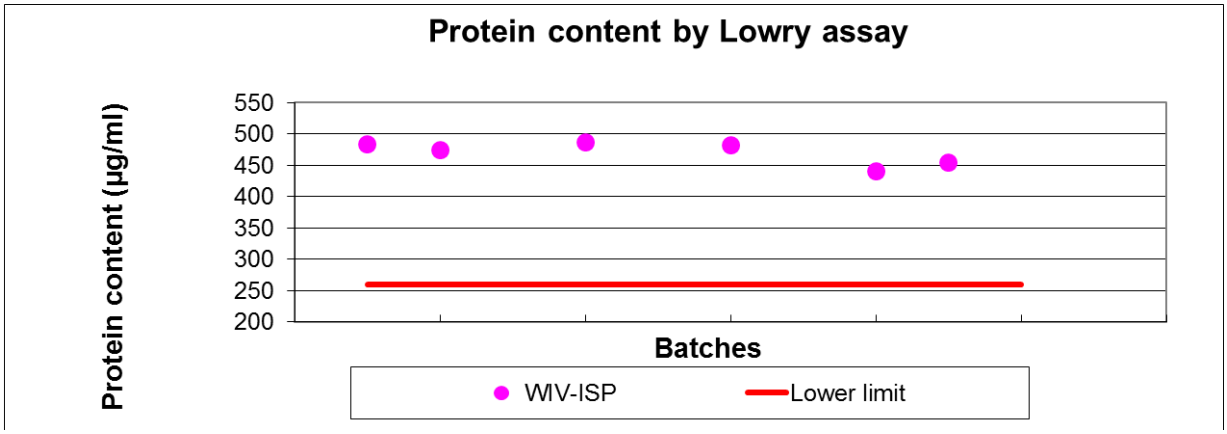
Vaccine type	Status	Release information's		
Pneumococcal Monovalent bulk conjugate		EU	Non-EU	Total
	Submitted	135	0	135
	Tested	152	0	152
	Released	150	0	150
	Rejected	1	0	1

D.5.3 Results

D.5.3.1 PS1-PD

Protein content

It is clear that the results from WIV-ISP are somewhat lower than the company's consistency limits. However, it should be mentioned that the number of batches tested is limited and that many batches were tested in the same experimental session. No OOS data were obtained.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 6)	521	456	586
2013 (N = 9)	508	472	545
2014 (N= 17)	485	451	520

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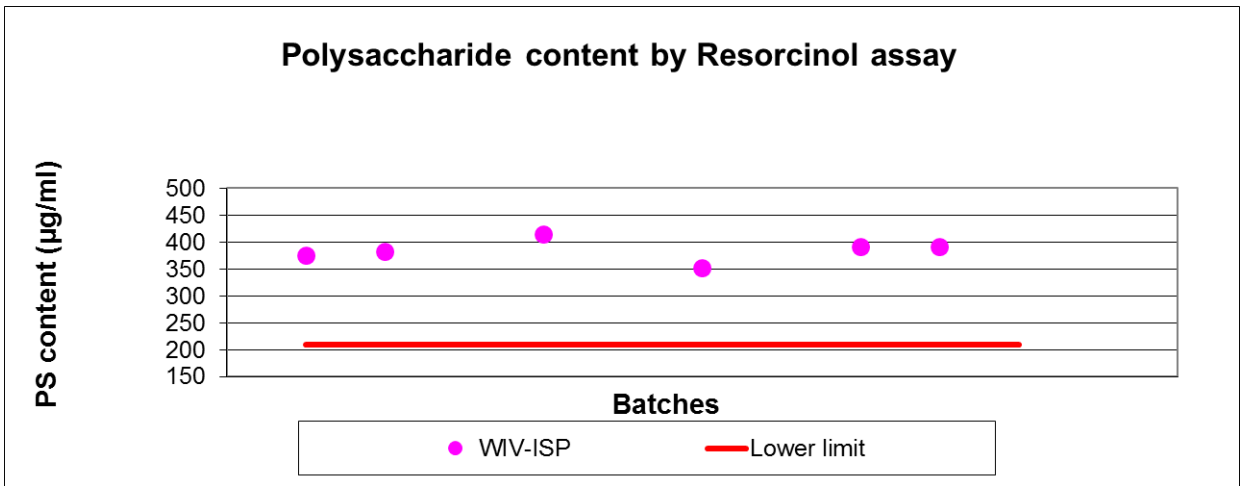
Polysaccharide content

The values of PS content in PS1-PD bulks are well aligned with the manufacturer's consistency.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 6)	376	344	408
2013 (N = 9)	377	358	397
2014 (N = 17)	373	339	406

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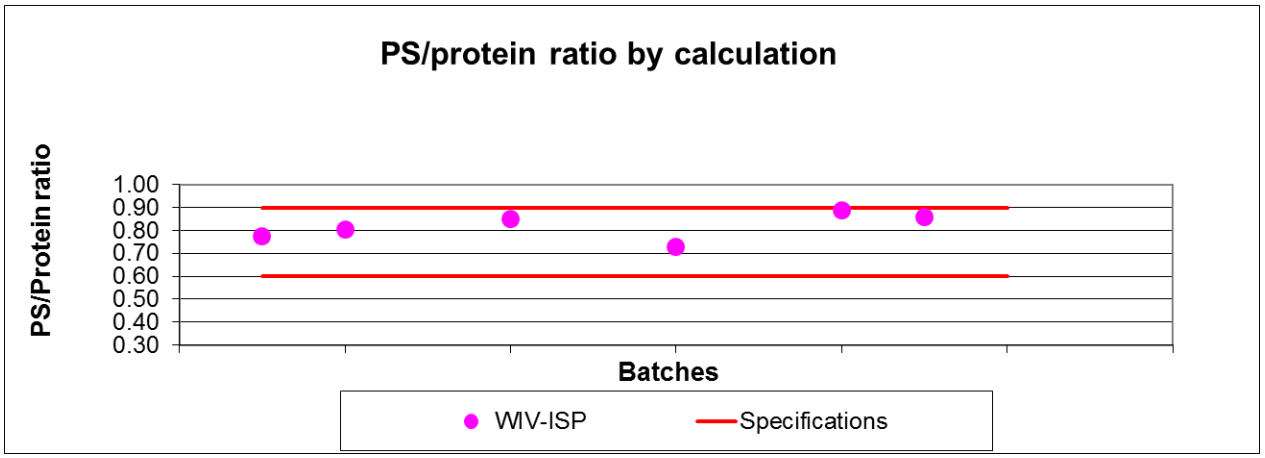
PS/protein ratio

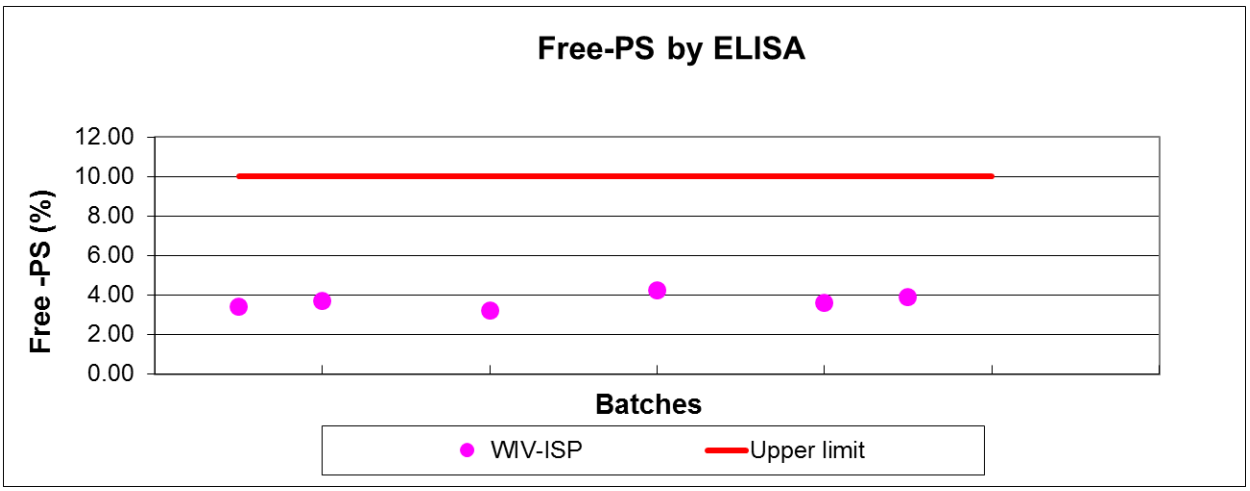
The WIV-ISP results for the ratio are within the consistency limits established by the manufacturer. No OOS data were obtained.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 6)	0.72	0.63	0.82
2013 (N = 9)	0.74	0.67	0.82
2014 (N = 17)	0.78	0.68	0.88

Free-PS

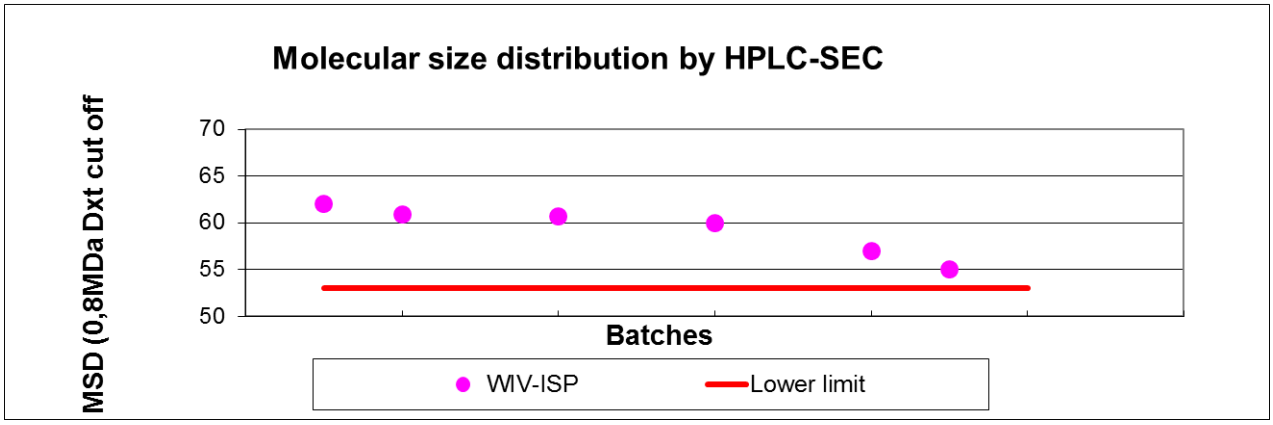
It is observed that the results for free-PS at WIV-ISP very well align with the manufacturer's consistency, even if these limits are very narrow. No OOS results were obtained



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 6)	2.2	1.6	2.7
2013 (N = 9)	2.8	1.5	4.0
2014 (N = 17)	3.1	2.4	3.9

Molecular size distribution

The values obtained by WIV-ISP are lower compared to the consistency limits established by the manufacturer. As testing was performed later at WIV-ISP testing by the applicant, this results are not unexpected. Also, testing at WIV-ISP was done with a new method which gives some lower results. No OOS results were obtained.

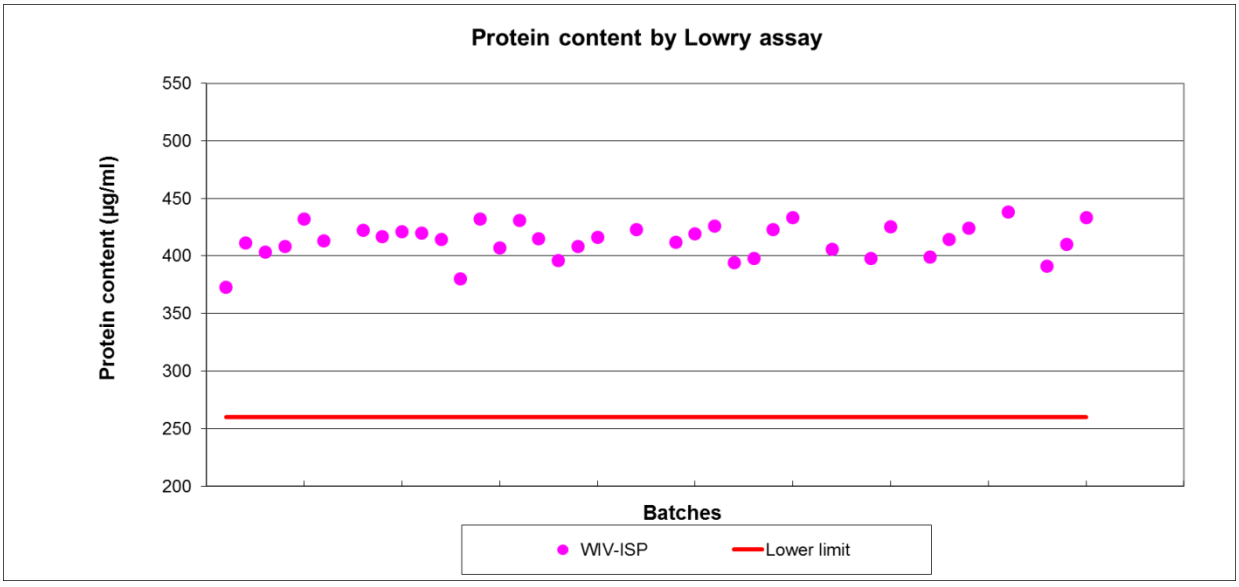


Years	Mean	Mean + 2*SD	Mean - 2*SD
2012 (N = 6)	68	65	70
2013 (N = 9)	66	64	68
2014 (N = 17)	63	59	68

D.5.3.2 PS4-PD

Protein content

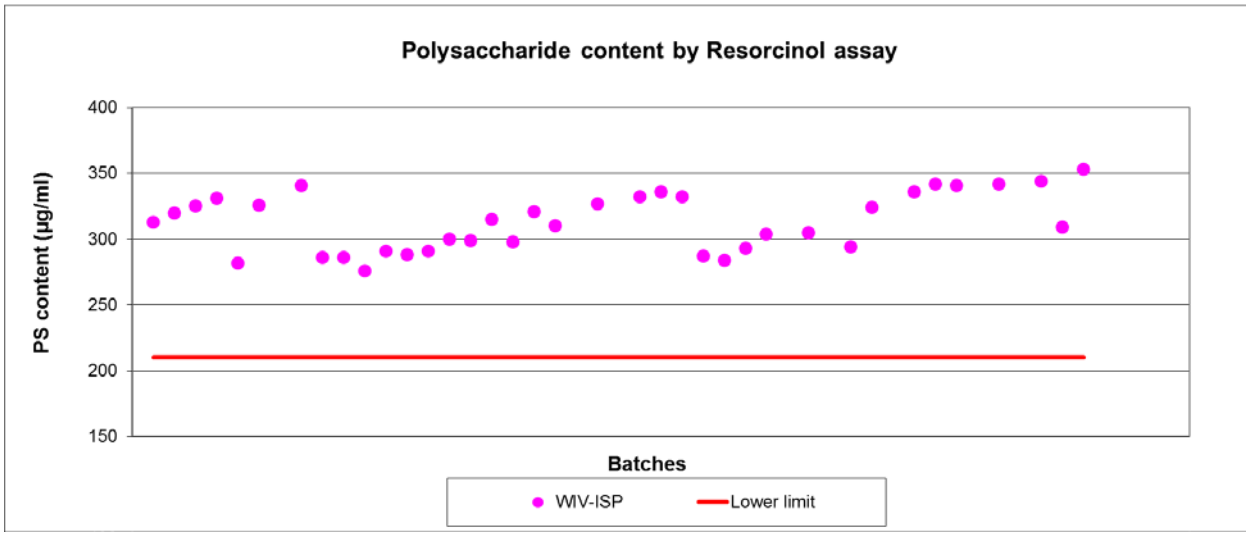
From the graph below, it is clear that some of the results obtained for protein content fall outside the consistency limits set by the manufacturer. Not all bathes produced by the manufacturer are tested by WIV-ISP, so that the WIV-ISP mean is cannot fully be compared with the manufacturer's data. No OOS data were obtained.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 25)	463	396	529
2013 (N = 47)	411	441	472
2014 (N = 74)	442	417	486

Polysaccharide content

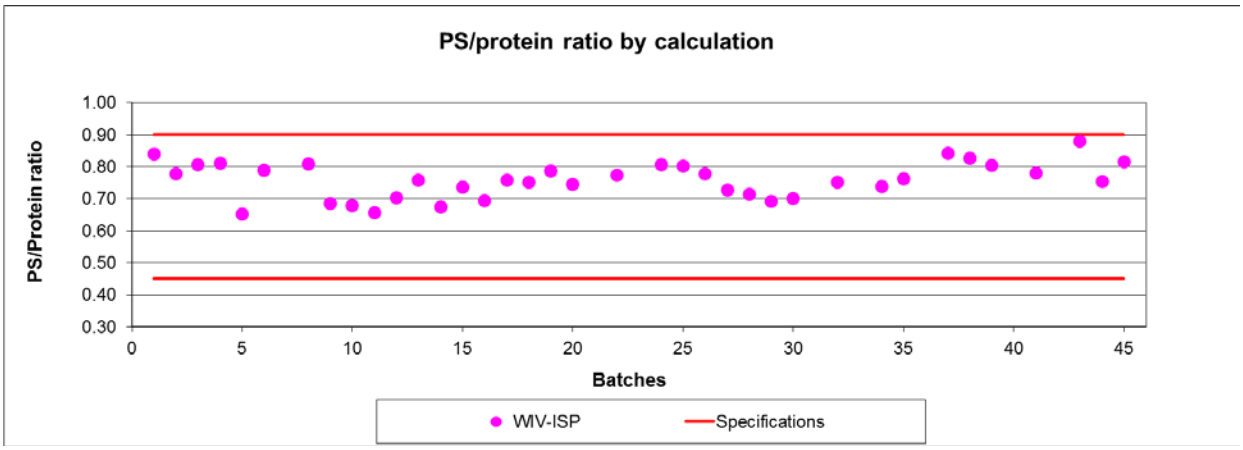
The polysaccharide content obtained at WIV-ISP is very well aligned with the manufacturer's consistency, taking into account the high variability of the assay. No OOS data were obtained.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 25)	313	272	355
2013 (N = 47)	284	306	324
2014 (N = 74)	316	279	352

PS/protein ratio

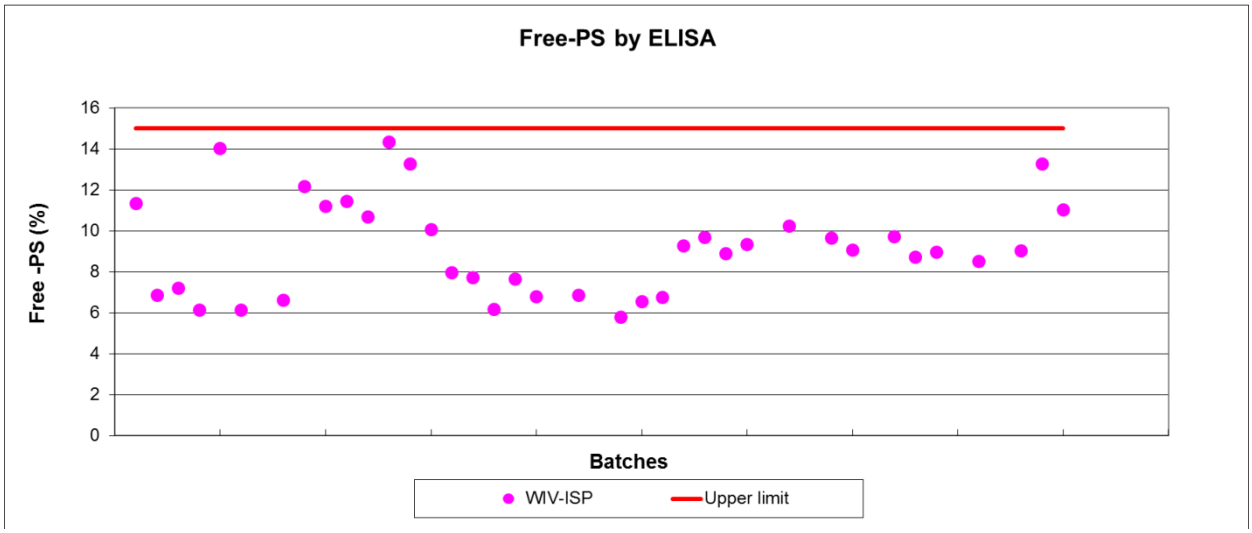
Except from some batches with a higher ratio, nearly all results obtained at WIV-ISP fit into yje consistency established by the manufacturer. It should be kept in mind that for the PS/protein ratio, the result is obtained by calculation using the protein and PS content data.in the 2013 consistency limits.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 25)	0.68	0.59	0.77
2013 (N =47)	0.69	0.64	0.75
2014 (N = 74)	0.72	0.62	0.81

Free-PS

By comparing the WIV-ISP test results with the company's consistency, the overall picture for free-PS is that the levels obtained at WIV-ISP are higher than the levels obtained by the manufacturer. It is reasonable to believe that free-PS is a parameter which can be influenced by the age of the bulk, so that the free-PS will increase over time. Since there was a time delay release testing by the manufacturer and control testing by WIV-ISP, it was not a surprise that free-PS levels were elevated for most of the batches tested. It should also be mentioned that the percentage of free PS is calculated based on two test results, each test with its intrinsic variability. No OOS data were obtained.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 25)	4.2	1.2	7.2
2013 (N = 47)	5.6	3.4	7.8
2014 (N = 74)	7.3	5.2	9.4

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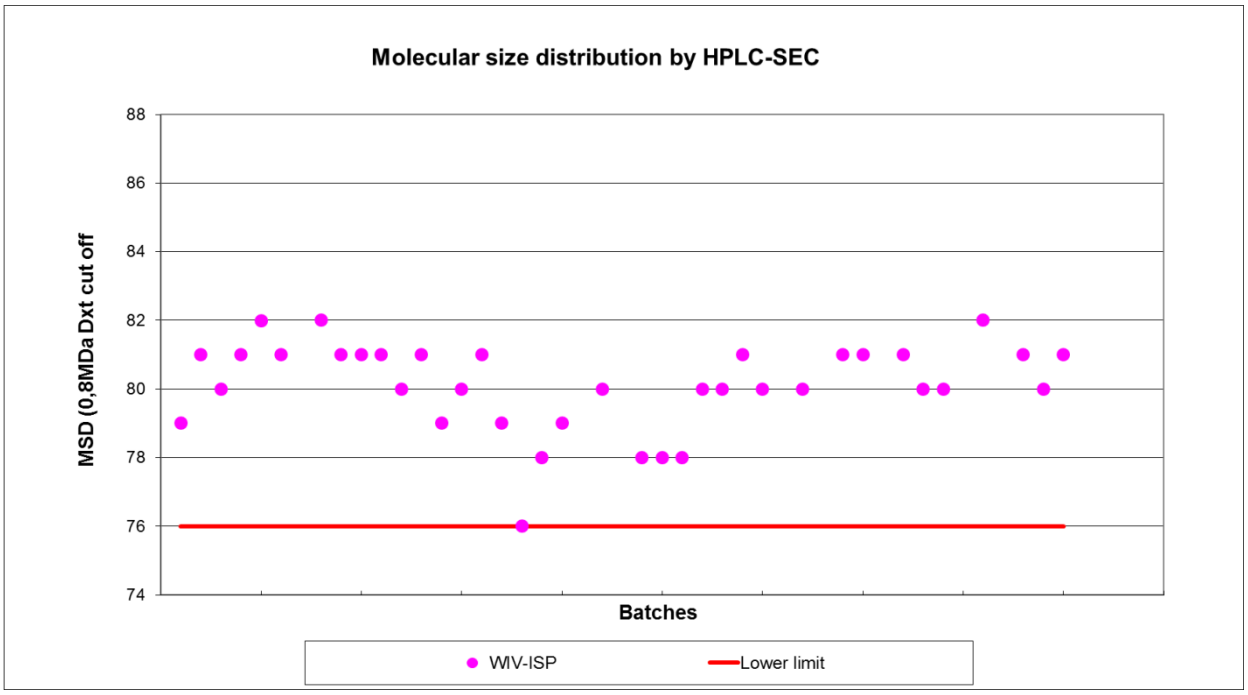
Molecular size distribution

For determination of molecular size distribution by HPLC-SEC, a similar observation can be made as for the free-PS testing. The values obtained at WIV-ISP are different from those obtained by the manufacturer, in this case lower. Molecular size is a characteristic that can also be influenced by the age of the bulk. Since testing was performed later than release testing by the manufacturer, the results are not unexpected. Some results close to the limit were obtained, but no OOS results were generated.

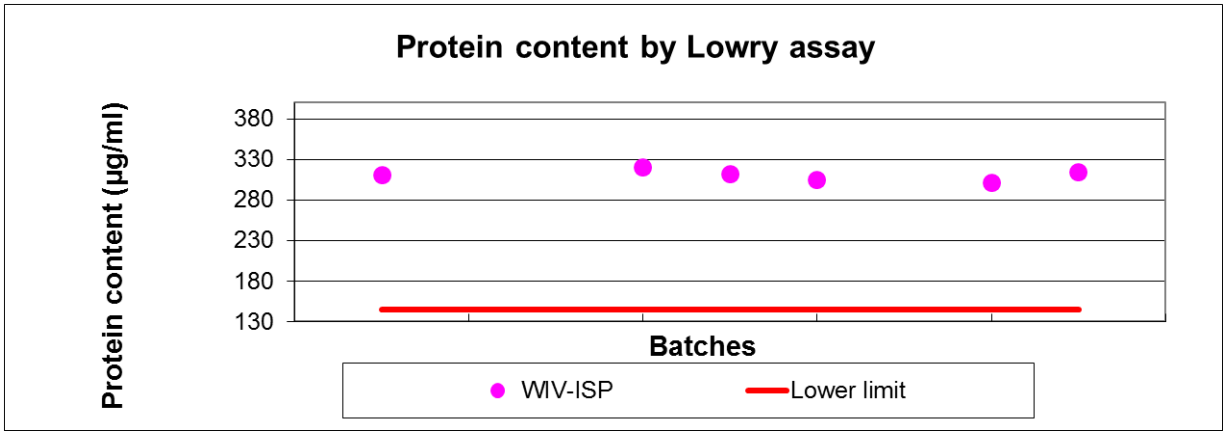


Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 25)	83	80	86
2013 (N = 47)	83	81	85
2014 (N = 74)	83	81	86

D.5.3.3 PS5-PD

Protein content

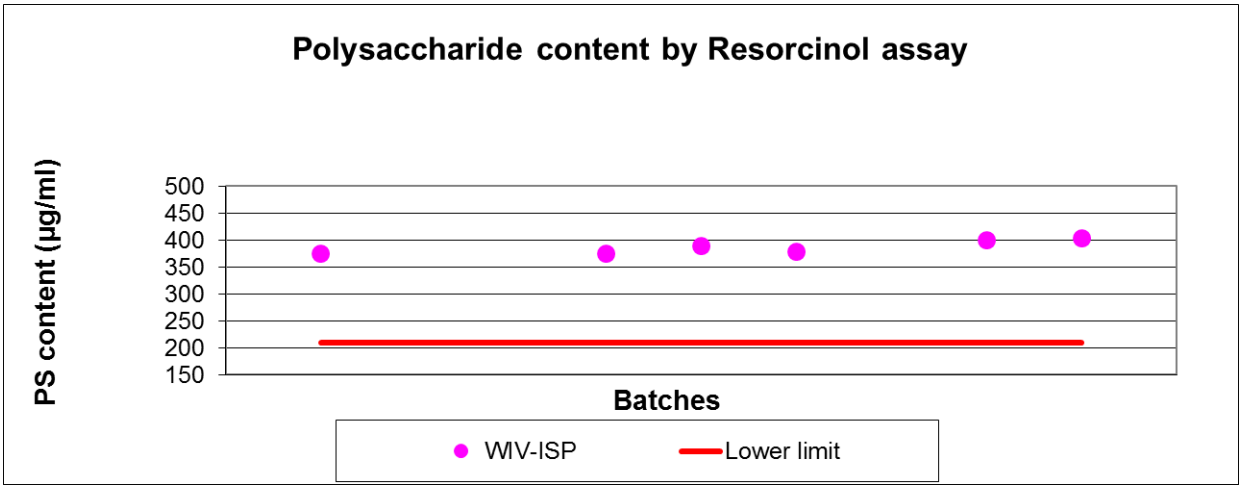
The results obtained by WIV-ISP are somewhat lower than the consistency established by the manufacturer. It should be mentioned that the number of batches tested is limited and that many batches were tested in the same experimental session. Also not all batches manufactured were tested at WIV-ISP. No OOS data were obtained.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 8)	347	320	374
2013 (N = 18)	343	319	367
2014 (N = 21)	329	305	352

Polysaccharide content

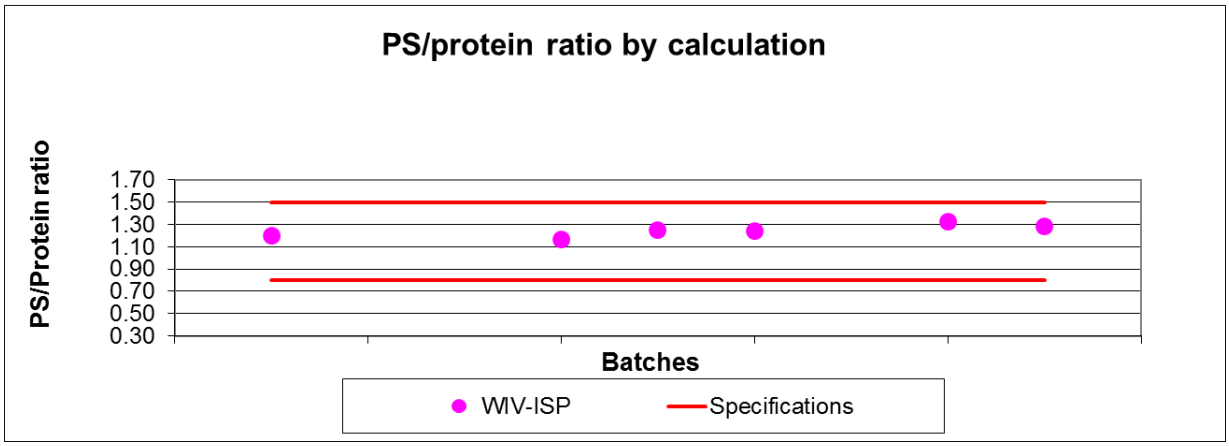
For the determination of the polysaccharide content, the graph shows that the values of PS content in PS5-PD bulks obtained at WIV-ISP very well align with the manufacturer's consistency, even if the limits are very narrow.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 8)	371	352	390
2013 (N = 18)	390	358	421
2014 (N = 21)	383	360	407

PS/protein ratio

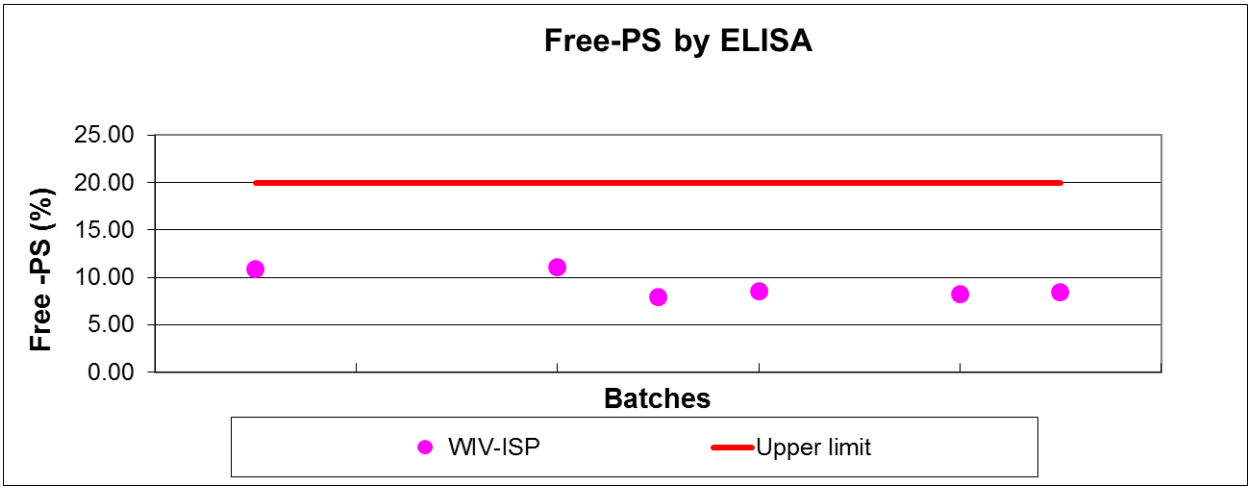
The WIV-ISP results for the ratio are well aligned with the manufacturer's consistency.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 8)	1.07	0.98	1.16
2013 (N = 18)	1.14	1.03	1.25
2014 (N = 21)	1.17	1.08	1.25

Free-PS

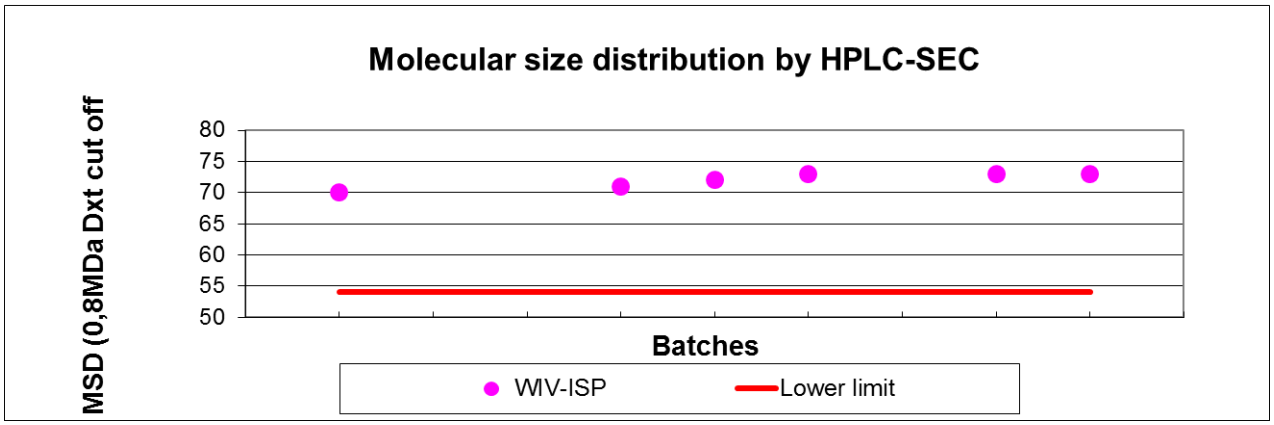
The results demonstrate that there are some differences between WIV-ISP and the manufacture, which are related to the time delay in testing and variability of the method. No OOS results were obtained.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 8)	9.1	7.0	11.2
2013 (N = 18)	11.4	5.6	17.1
2014 (N = 21)	9.8	5.1	14.5

Molecular size distribution

The values obtained by WIV-ISP are for all the batches somewhat lower than the consistency determined by the manufacturer. The fact that testing was performed after release testing by the applicant, these results are not unexpected. The differences observed between testing by the manufacturer and testing at WIV-ISP mostly consists of only several percentages. No OOS results were obtained.

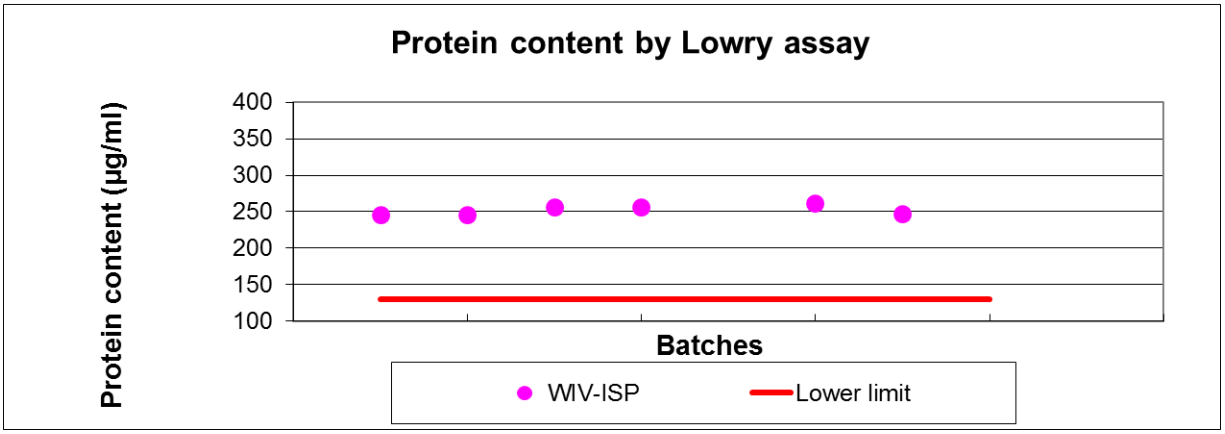


Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 8)	73	74	76
2013 (N = 18)	75	73	78
2014 (N = 21)	76	72	79

D.5.3.4 PS6B-PD

Protein content

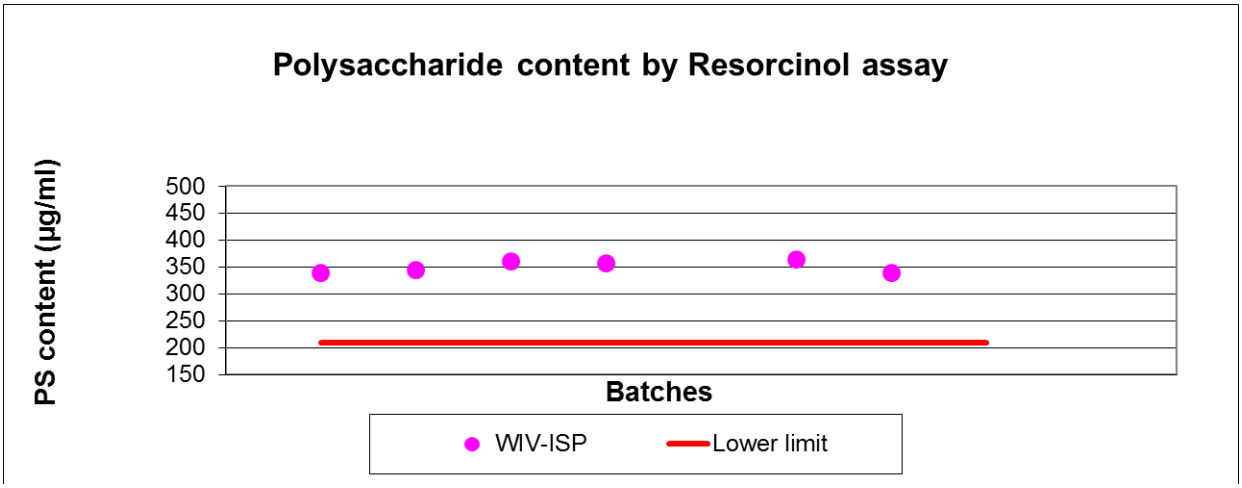
The results obtained by WIV-ISP are very well aligned with the manufacturer's consistency.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N =17)	273	250	303
2013 (N = 3)	253	242	264
2014 (N = 15)	263	235	290

Polysaccharide content

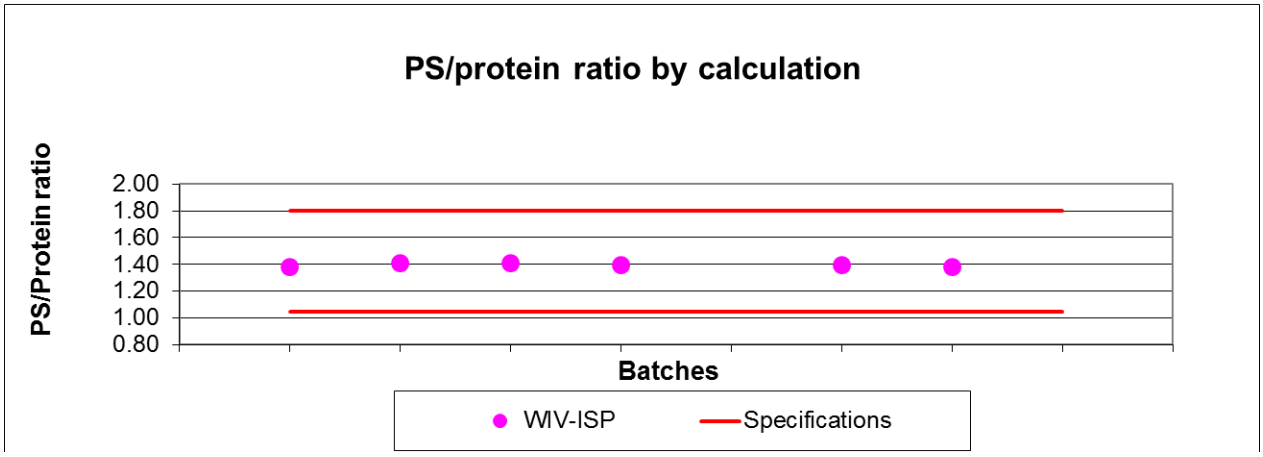
The results obtained at WIV-ISP fall within the consistency established by the manufacturer. No OOS data were generated.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 17)	378	343	412
2013 (N = 3)	387	366	409
2014 (N = 15)	365	333	397

PS/protein ratio

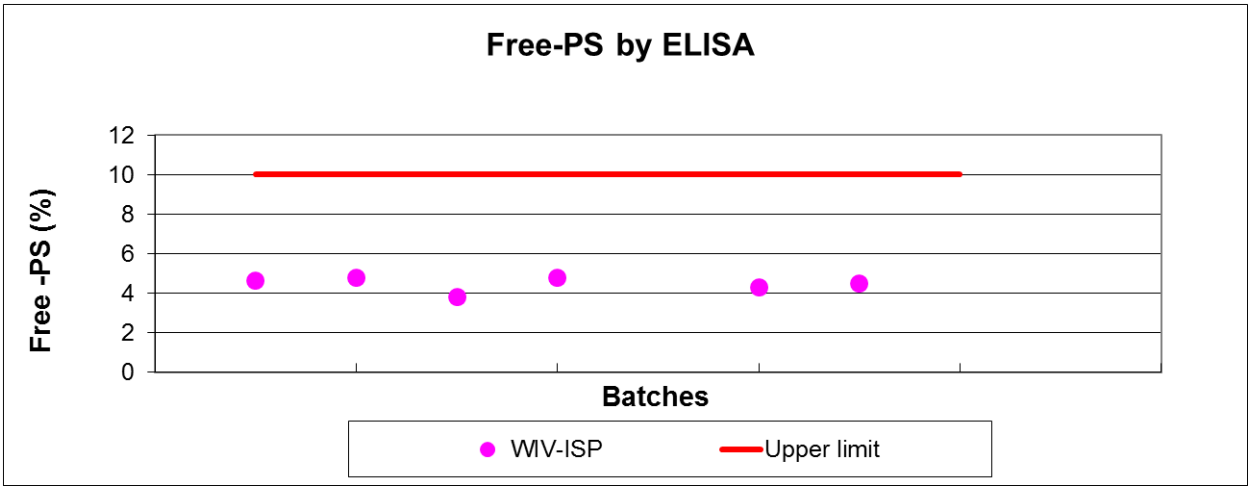
The results obtained by the manufacturer and obtained by WIV-ISP are highly comparable.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 17)	1.37	1.22	1.52
2013 (N = 3)	1.53	1.46	1.61
2014 (N = 15)	1.39	1.26	1.53

Free-PS

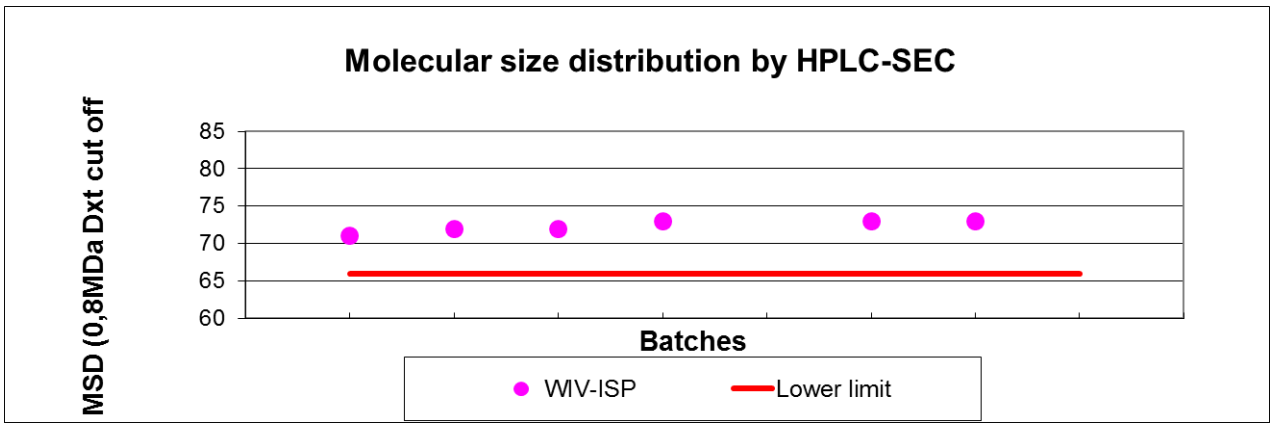
The results obtained by WIV-ISP fall outside the consistency of the manufacturer, but are still far below the upper specification limit. The time difference in testing explains the increased free-PS level, which is considered as a stability indicating factor.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 17)	1.8	0.9	2.8
2013 (N = 3)	1.8	1.1	2.5
2014 (N = 15)	2.1	1.5	2.6

Molecular size distribution

The results obtained at WIV-ISP are very well aligned with the manufacturer's conssitency, even if the limits are very tight.

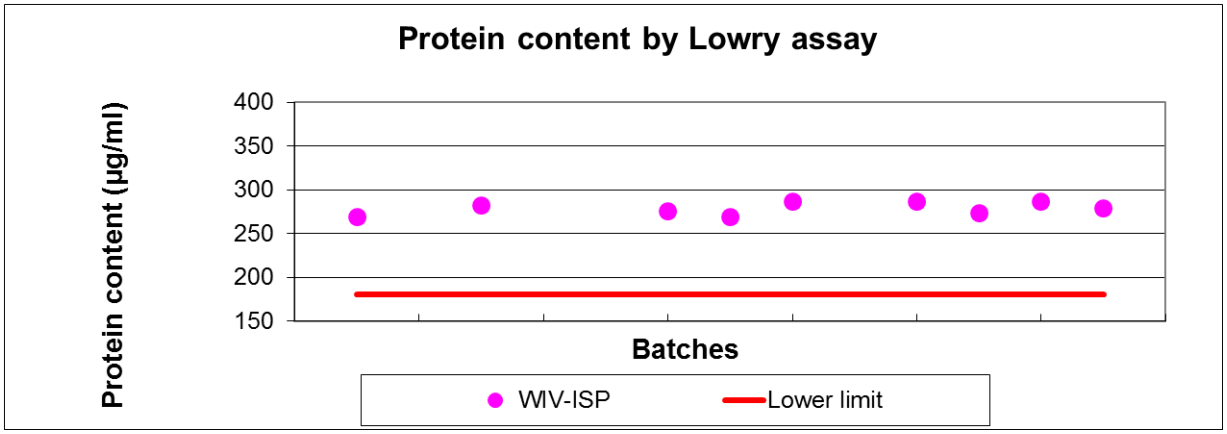


Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 17)	77	74	79
2013 (N = 3)	76	75	77
2014 (N = 15)	75	74	76

D.5.3.5 PS7F-PD

Protein content

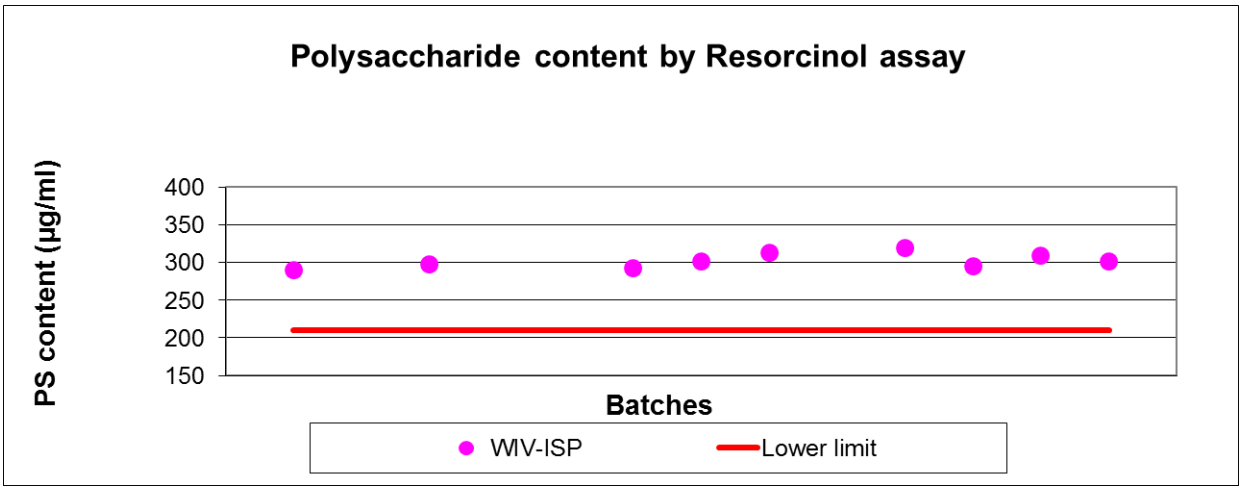
The results obtained at WIV-ISP are somewhat outside the consistency limits established by the manufacturer. The number of batches tested is limited.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 2)	317	315	318
2013 (N = 12)	293	263	323
2014 (N = 15)	298	282	315

Polysaccharide content

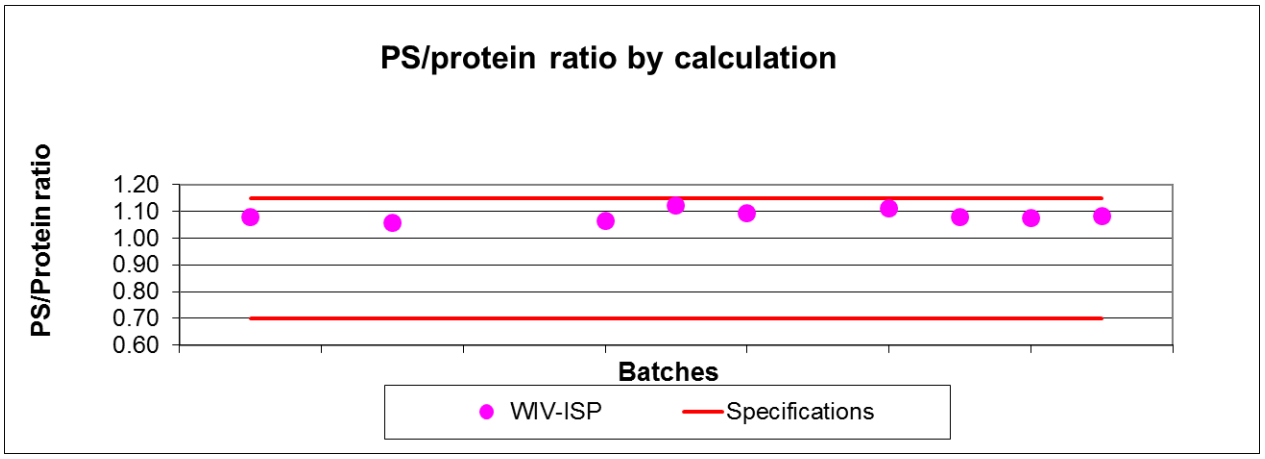
For the determination of the polysaccharide content, it is clear that the values of PS content in PS7F-PD bulks are towards the higher border of the consistency limits. All data are within the specifications.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 2)	286	278	294
2013 (N = 12)	259	242	275
2014 (N = 15)	278	240	317

PS/protein ratio

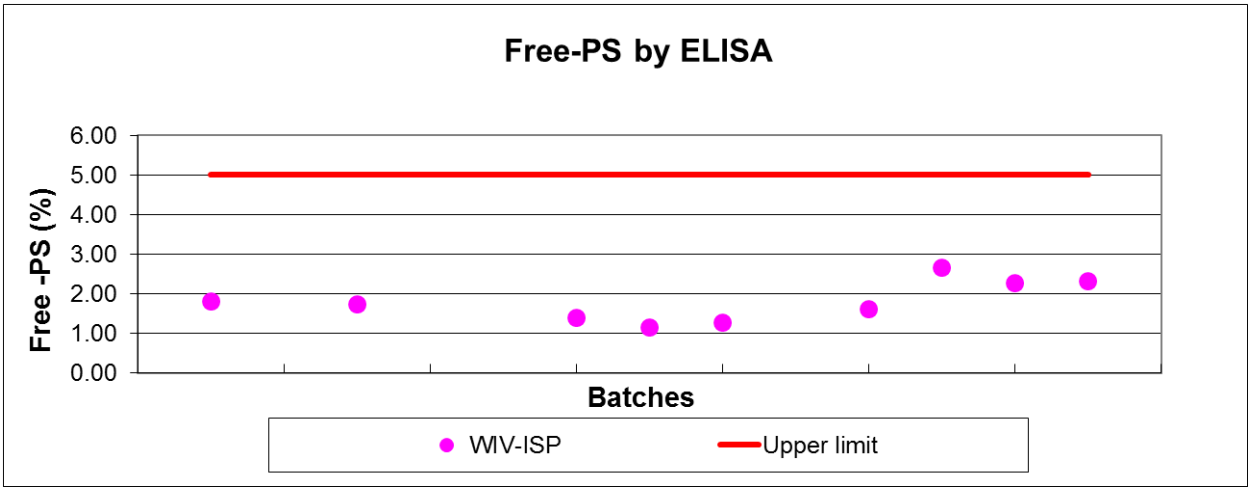
The WIV-ISP results for the ratio are higher due to the higher values for PS content. No OOS data were obtained.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 2)	0.90	0.87	0.93
2013 (N = 12)	0.89	0.83	0.94
2014 (N = 15)	0.93	0.81	1.05

Free-PS

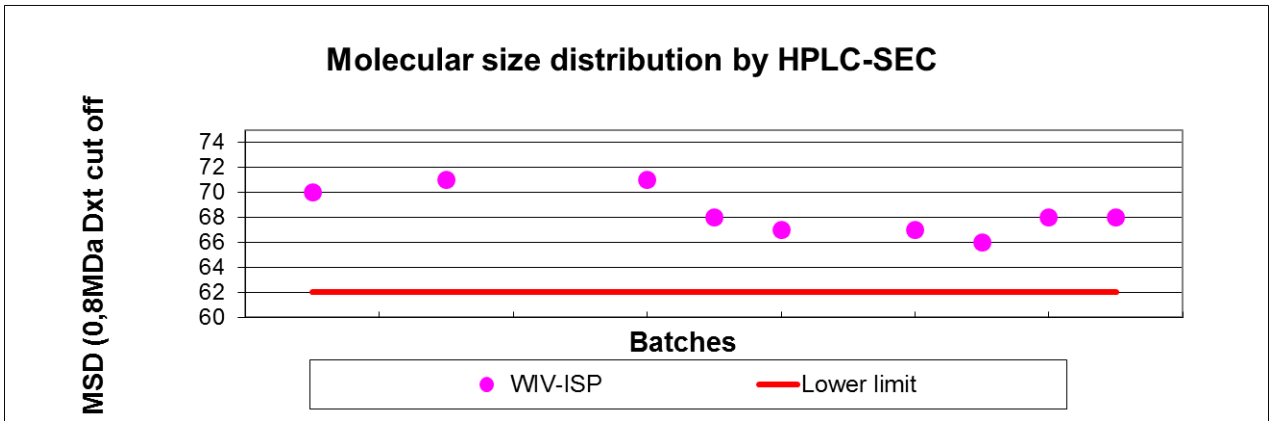
The results for free-PS at WIV-ISP are towards the upper border of the manufacturer's consistency limits. This is due to a greater delay between testing by the manufacturer and testing by WIV-ISP. Free-PS can be considered as a stability indicating factor that increases in function of bulk age. No OOS results were obtained.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 2)	0.5	0.2	0.8
2013 (N = 12)	0.9	0.2	1.6
2014 (N = 15)	1.3	0.6	2.1

Molecular size distribution

The values obtained by WIV-ISP nicely fit the consistency established by the manufacturer.

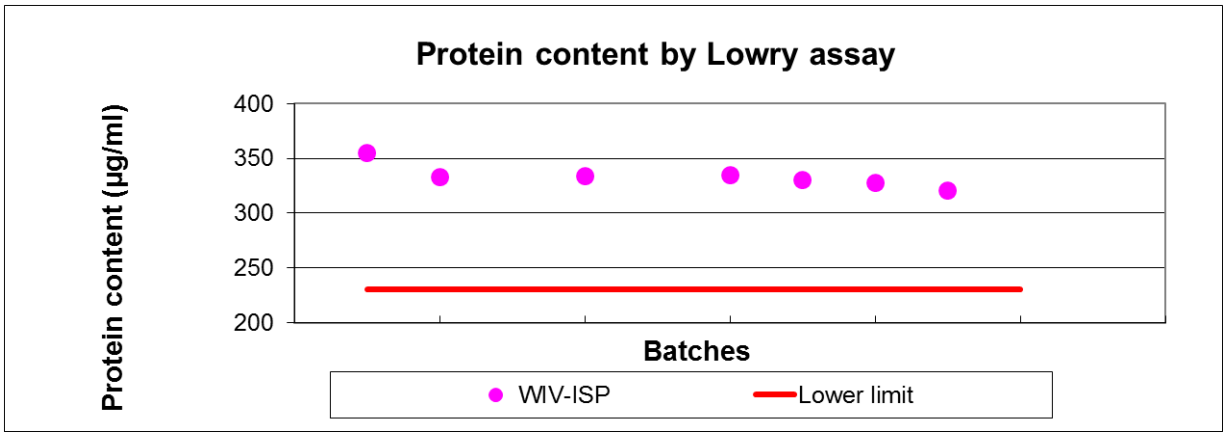


Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 2)	72	70	73
2013 (N = 12)	70	69	71
2014 (N = 15)	70	67	72

D.5.3.6 PS9V-PD

Protein content

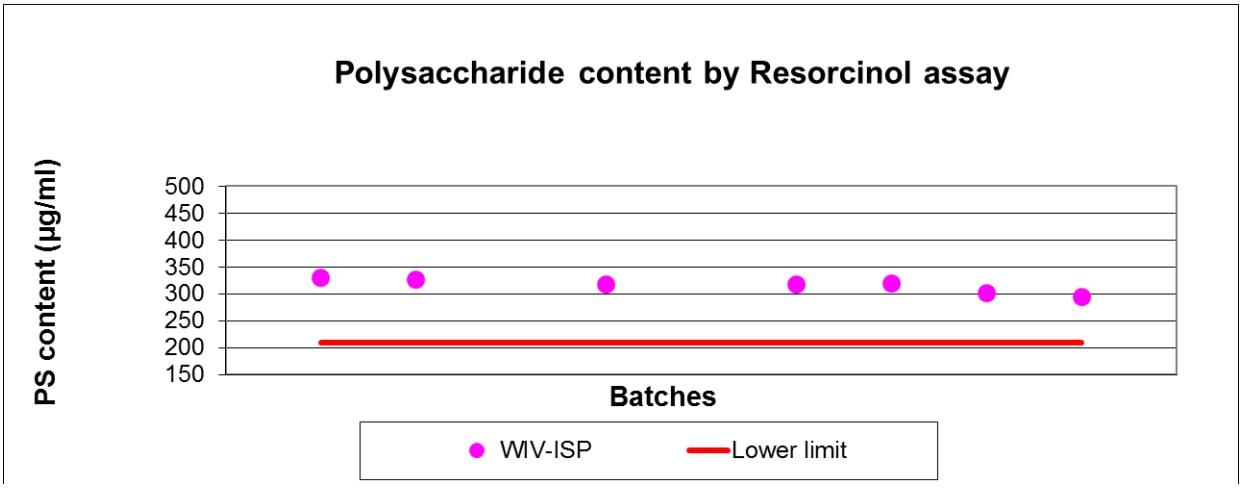
The results of WIV-ISP are within the manufacturer's consistency. No OOS data were obtained.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 19)	369	330	408
2013 (N = 14)	370	354	386
2014 (N = 19)	354	330	378

Polysaccharide content

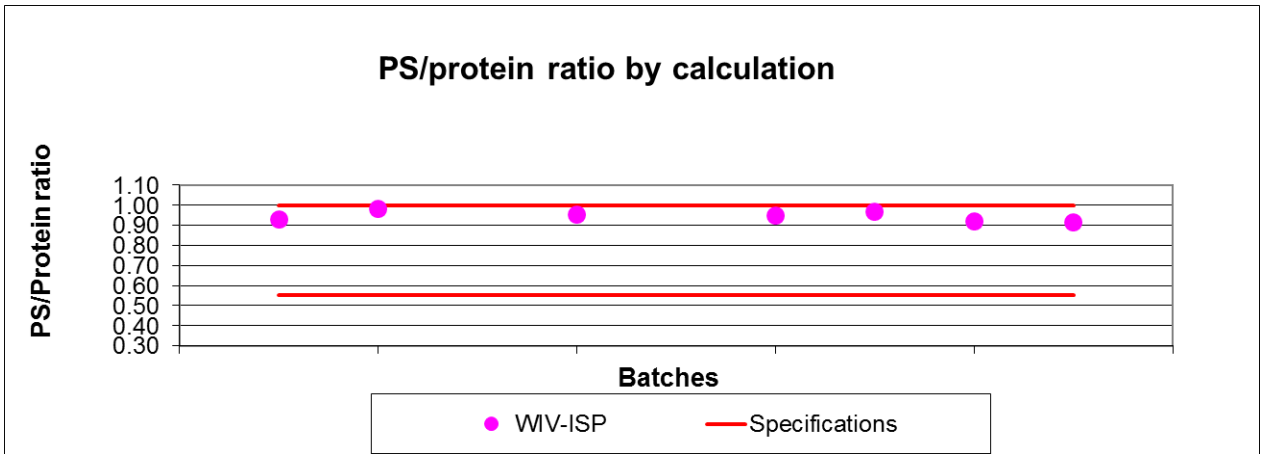
For the determination of the polysaccharide content, the values of PS content in PS9V-PD bulks are towards the higher border of the consistency limits established by the manufacturer. No OOS data were obtained.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 19)	291	262	319
2013 (N = 14)	297	261	333
2014 (N = 19)	288	255	321

PS/protein ratio

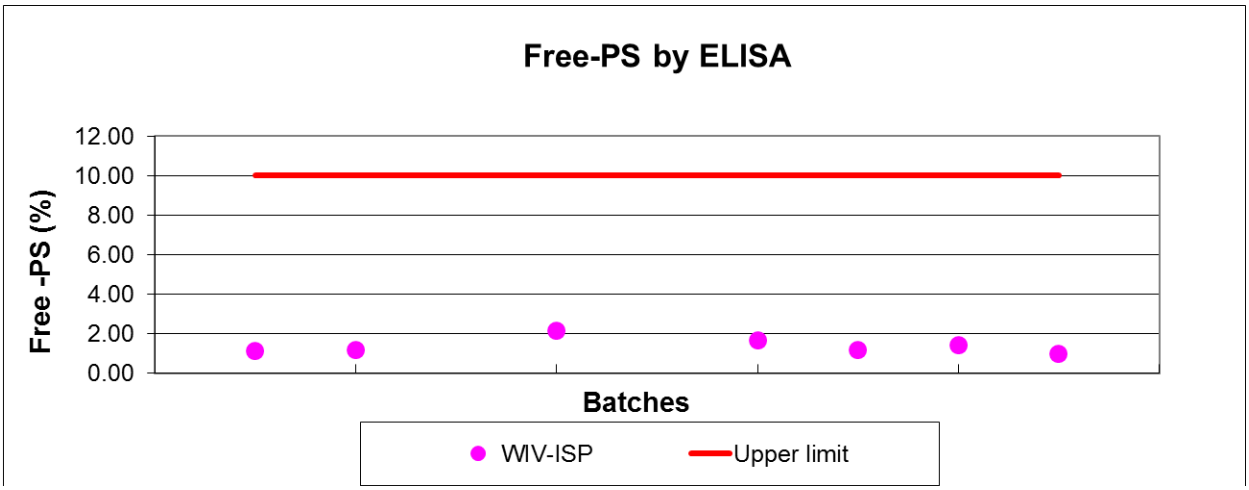
The WIV-ISP results for the ratio are higher and very close to the upper limit. This is due to the fact that polysaccharide values are higher and protein values are lower at WIV-ISP. No OOS data were obtained. The results are above the upper border of the manufacturer's consistency.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 19)	0.79	0.69	0.89
2013 (N = 14)	0.81	0.71	0.90
2014 (N = 19)	0.81	0.72	0.91

Free-PS

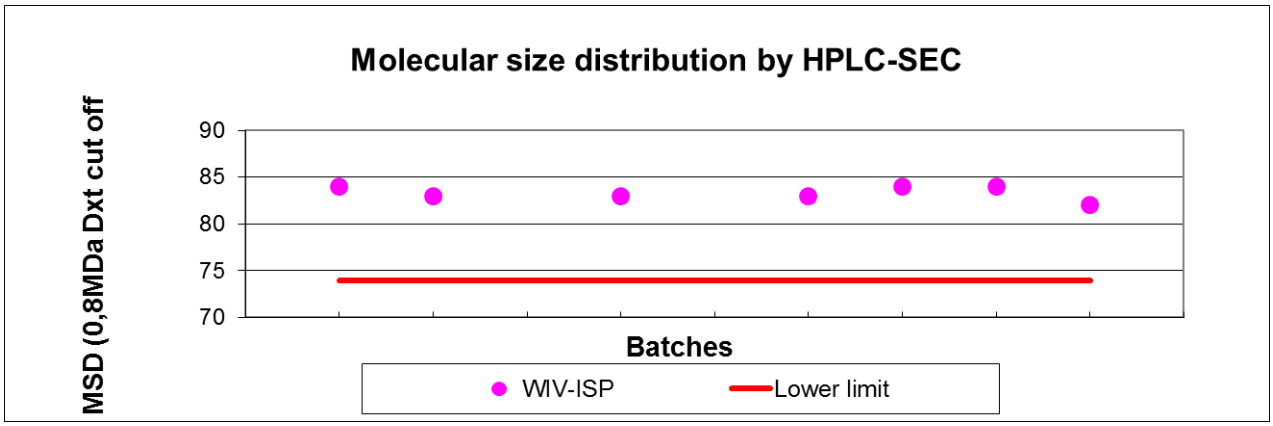
The results obtained at WIV-ISP are higher due to later testing. No OOS results were obtained.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 19)	0.4	0.1	0.8
2013 (N = 14)	0.4	0.2	0.7
2014 (N = 19)	0.6	0.3	0.8

Molecular size distribution

The values obtained by WIV-ISP are within the manufacturer's consistency. No OOS results were obtained.

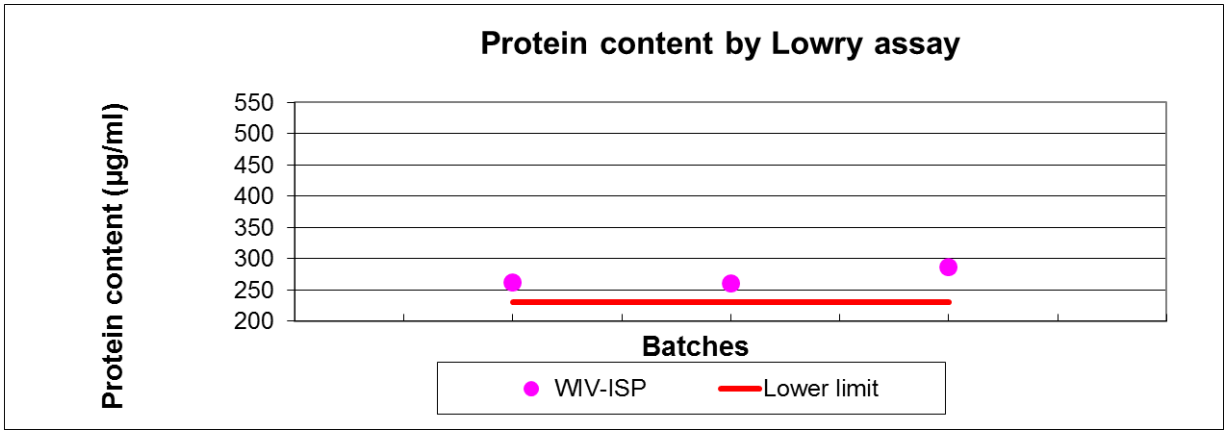


Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 19)	86	84	88
2013 (N = 14)	85	83	86
2014 (N = 19)	85	82	86

D.5.3.7 PS14-PD

Protein content

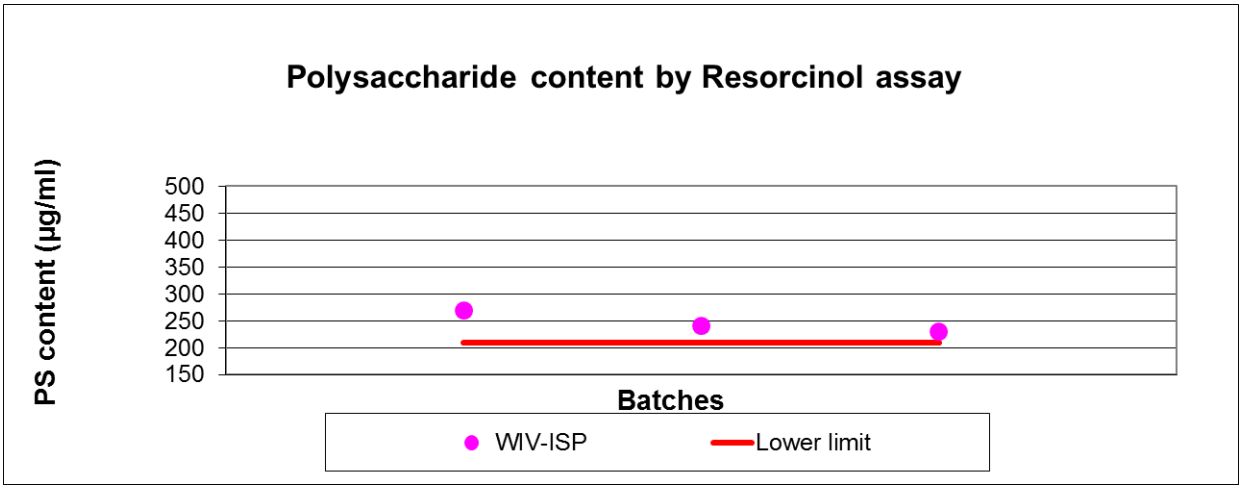
The results of WIV-ISP are comparable with the manufacturer's consistency. No OOS data were obtained.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 6)	308	289	327
2013 (N = 10)	288	271	305
2014 (N = 38)	289	260	317

Polysaccharide content

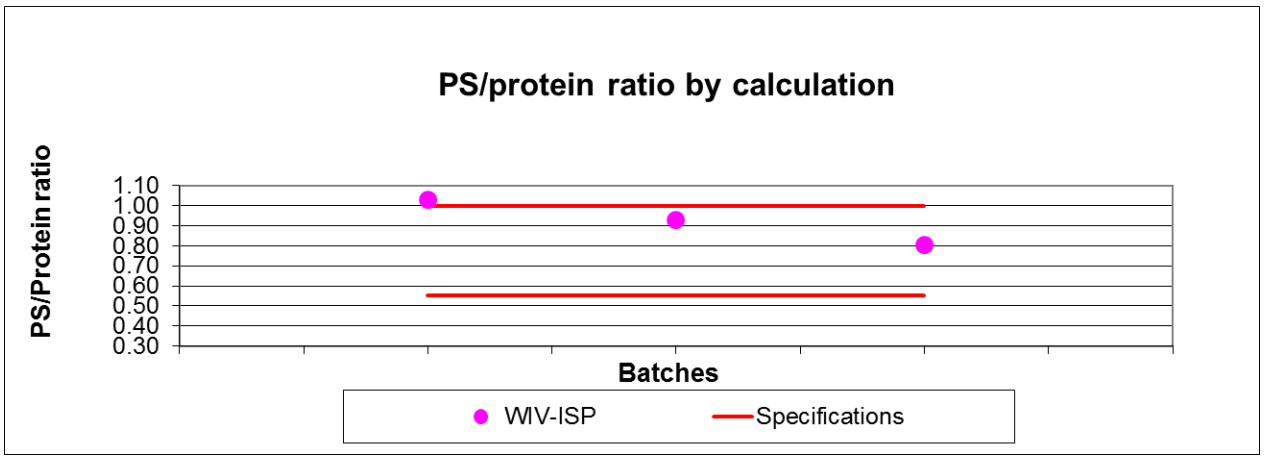
For the determination of the polysaccharide content, the graph below shows that the values of PS content in PS14-PD bulks are well aligned with the consistency established by the manufacturer.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 6)	241	224	259
2013 (N = 10)	235	222	247
2014 (N = 38)	237	223	251

PS/protein ratio

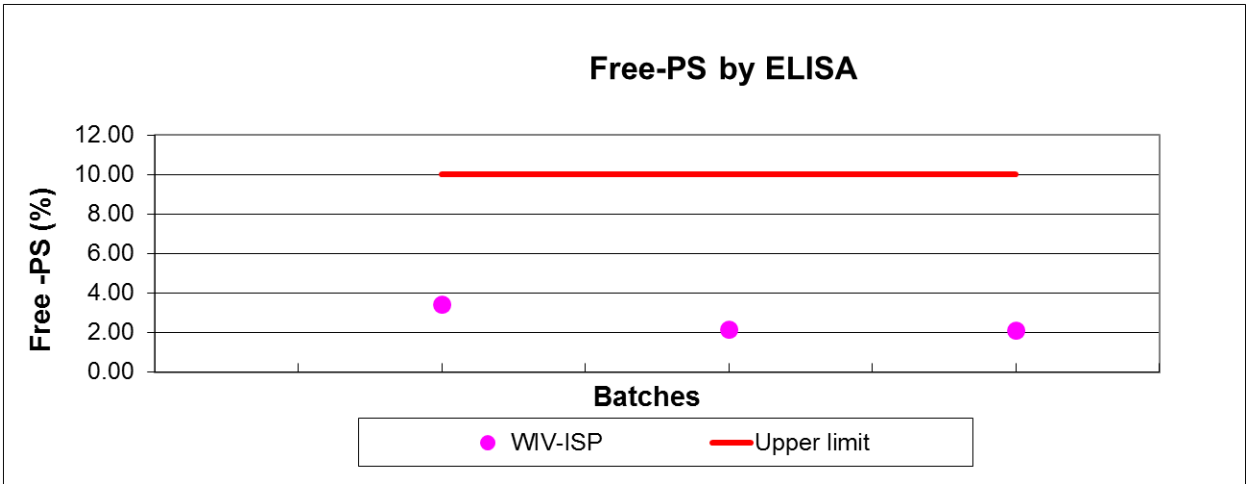
The WIV-ISP results for the ratio are higher and very close to the upper limit, with even one OOS result. The ratio is determined by dividing the result of two tests. The variability for the result for PS content and protein content was within the acceptance limit and the OOS result is due to assay variability. The batch was released based on the result of the manufacturer which was within the specifications.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 6)	0.79	0.70	0.87
2013 (N = 10)	0.82	0.75	0.88
2014 (N = 38)	0.82	0.74	0.91

Free-PS

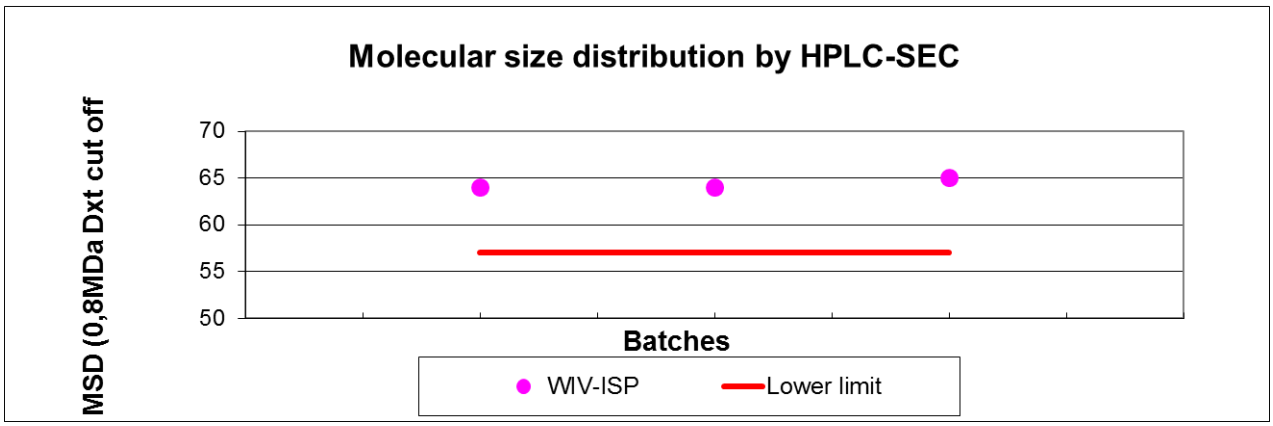
The results for free-PS at WIV-ISP and the result of the manufacture are very comparable. No OOS results were obtained.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 6)	3.1	2.1	4.1
2013 (N = 10)	2.8	2.1	3.5
2014 (N = 38)	3.3	2.0	4.6

Molecular size distribution

The values obtained by WIV-ISP are highly comparable to the values obtained by the manufacturer. No OOS results were obtained. All the results for molecular size distribution are within the manufacturer's consistency.

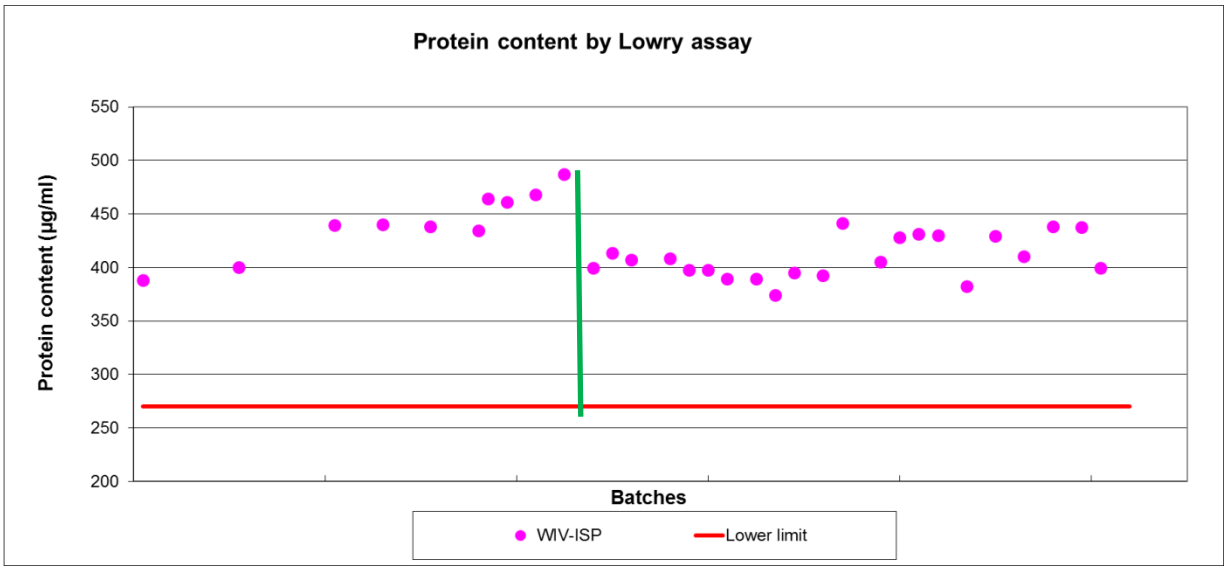


Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 6)	66	63	68
2013 (N = 10)	65	64	66
2014 (N = 38)	65	62	68

D.5.3.8 PS18C-PD

Protein content

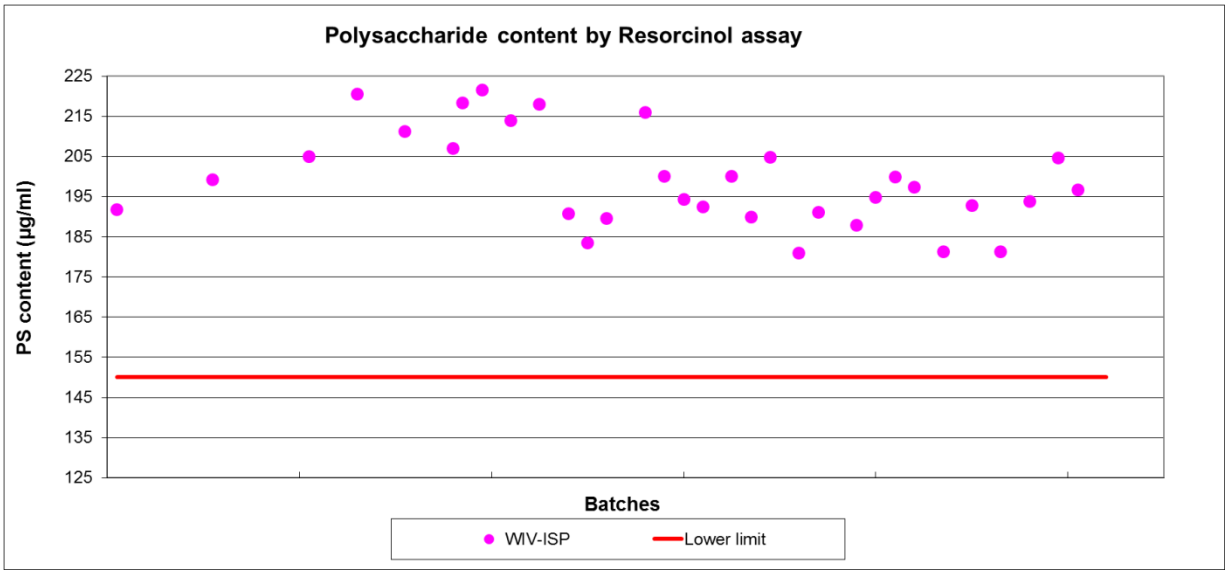
It is clear that the results of WIV-ISP are somewhat divergent from the manufacturer's consistency. The apparent drop in protein content, observed by WIV-ISP testing, is linked to the site of production (green line). At the additional site, the protein content is lower, but still within the specifications.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 45)	447	357	536
2013 (N = 79)	462	370	554
2014 (N = 84)	489	410	567

Polysaccharide content

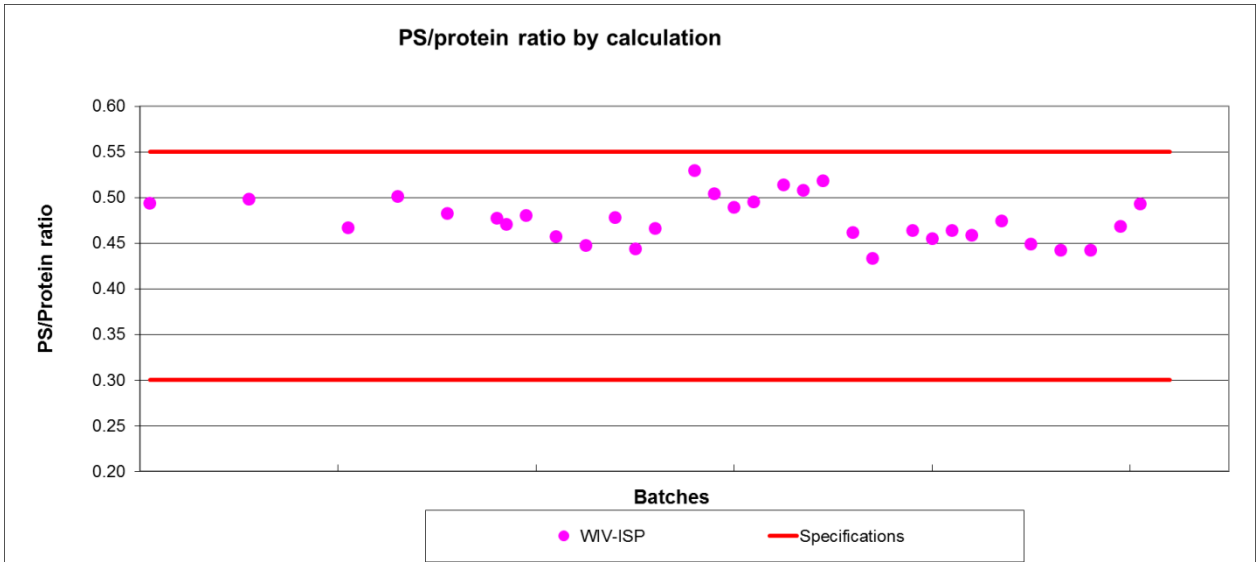
For the determination of the polysaccharide content, the graph shows that the values of PS content in PS18C-TT bulks are higher at WIV-ISP, in comparison with the manufacturer's consistency. No OOS data were obtained.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 45)	180	164	197
2013 (N = 79)	181	156	207
2014 (N = 84)	190	161	219

PS/protein ratio

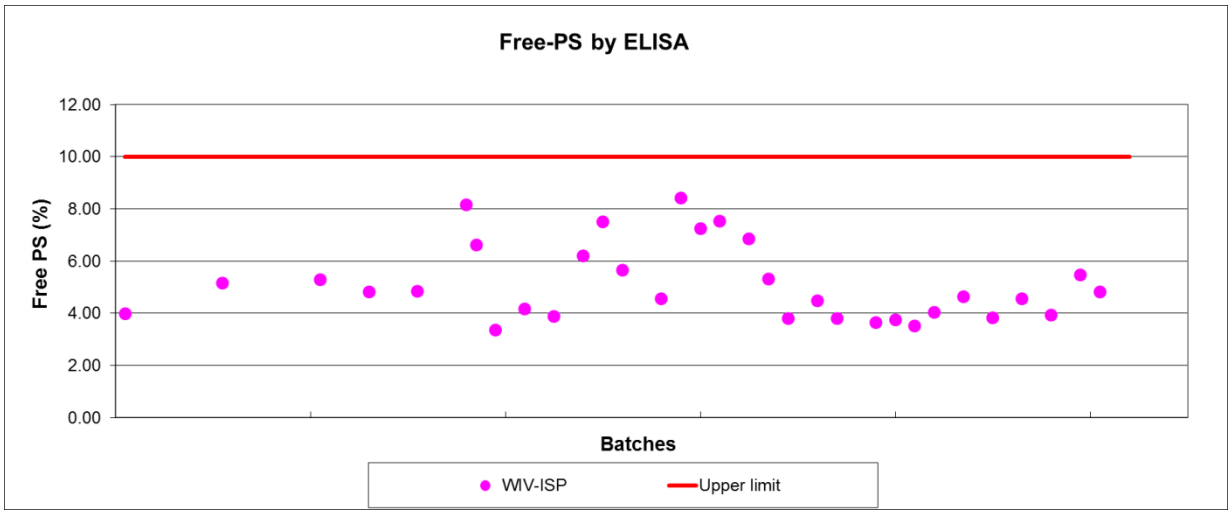
The WIV-ISP results for the ratio are higher than those obtained by the manufacturer, because of higher PS content values and lower protein content values. No OOS data were obtained.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 45)	0.41	0.33	0.48
2013 (N = 79)	0.39	0.34	0.45
2014 (N = 84)	0.39	0.30	0.49

Free-PS

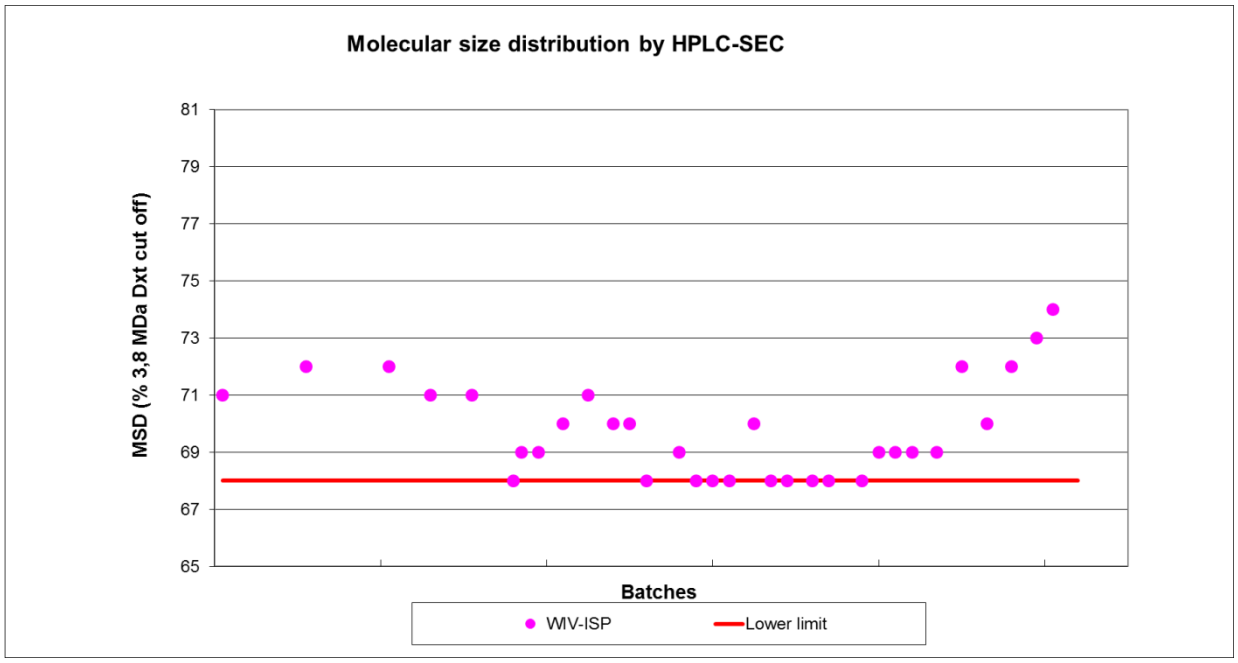
The graph shows that the results for free-PS at WIV-ISP are outside the manufacturer's consistency. This is due to the delay between testing by the manufacturer and testing by WIV-ISP. Free-PS can be considered as a stability indicating factor that increases in function of bulk age. No OOS results were obtained.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 45)	2.6	1.8	3.5
2013 (N = 79)	3.2	1.3	5.0
2014 (N = 84)	3.0	2.0	4.0

Molecular size distribution

The values obtained by WIV-ISP are for most of the batches somewhat lower than the consistency established by the manufacturer. The fact that testing was performed after release testing by the applicant, this results are not unexpected and are fully supported by the stability data of the manufacturer. The values obtained at WIV-ISP close to the specifications were due to some technical problems with the HPLC column. No OOS results were obtained.

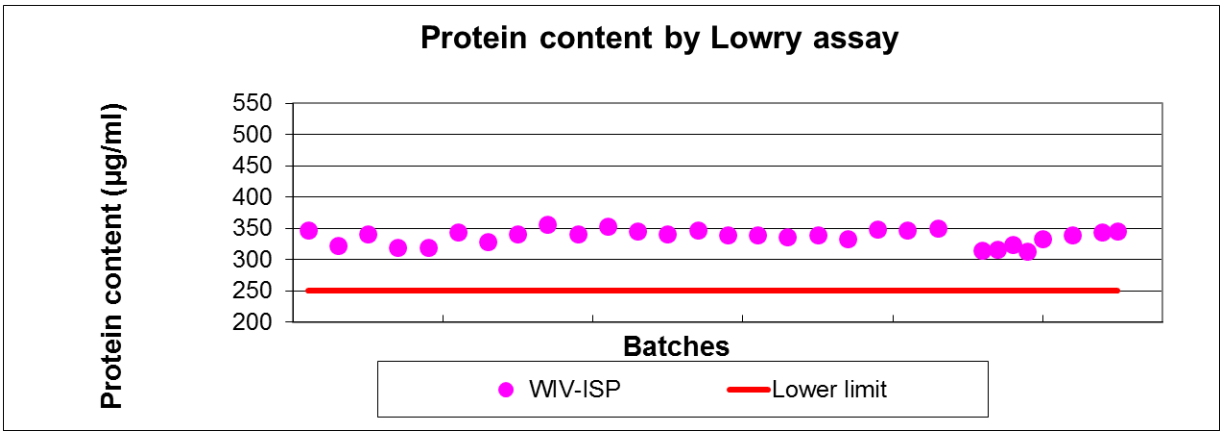


Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 45)	74	70	78
2013 (N = 79)	74	69	78
2014 (N = 84)	75	71	78

D.5.3.9 PS19F-DT

Protein content

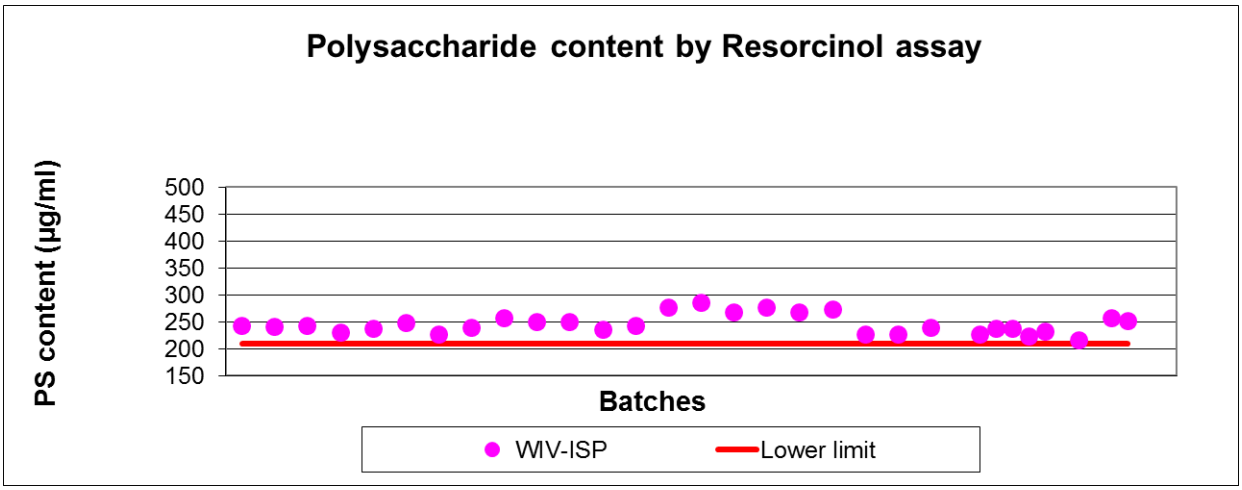
There is a good alignment between the data generated by WIV-ISP and the consistency established by the manufacturer. No OOS data were obtained.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 16)	363	334	392
2013 (N = 51)	349	301	396
2014 (N = 95)	356	304	407

Polysaccharide content

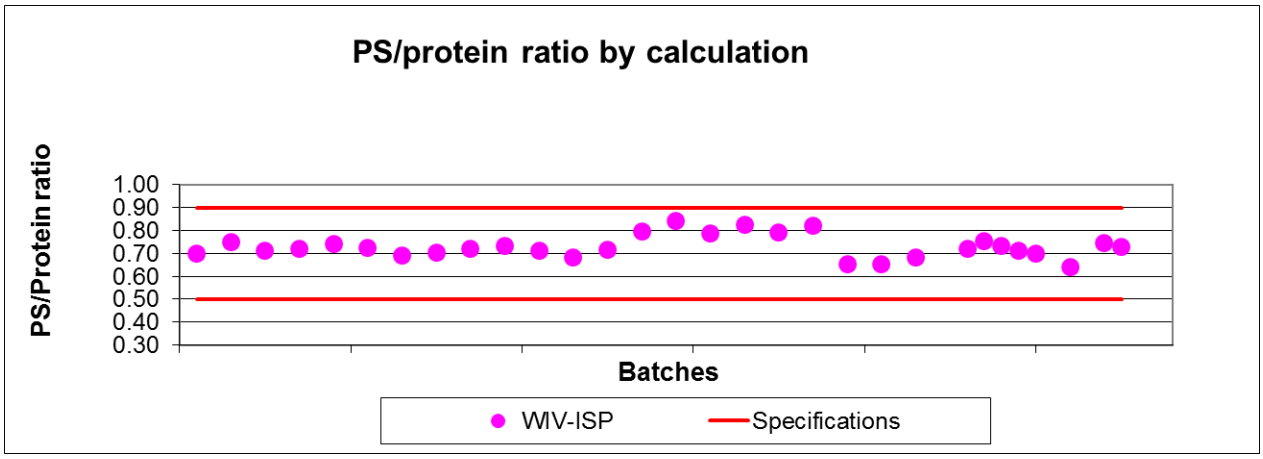
For the determination of the polysaccharide content, the graph below shows that there is a good alignment between the manufacturer's consistency and the WIV-ISP results. No OOS data were obtained.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 16)	267	249	285
2013 (N = 51)	246	220	272
2014 (N = 95)	256	226	287

PS/protein ratio

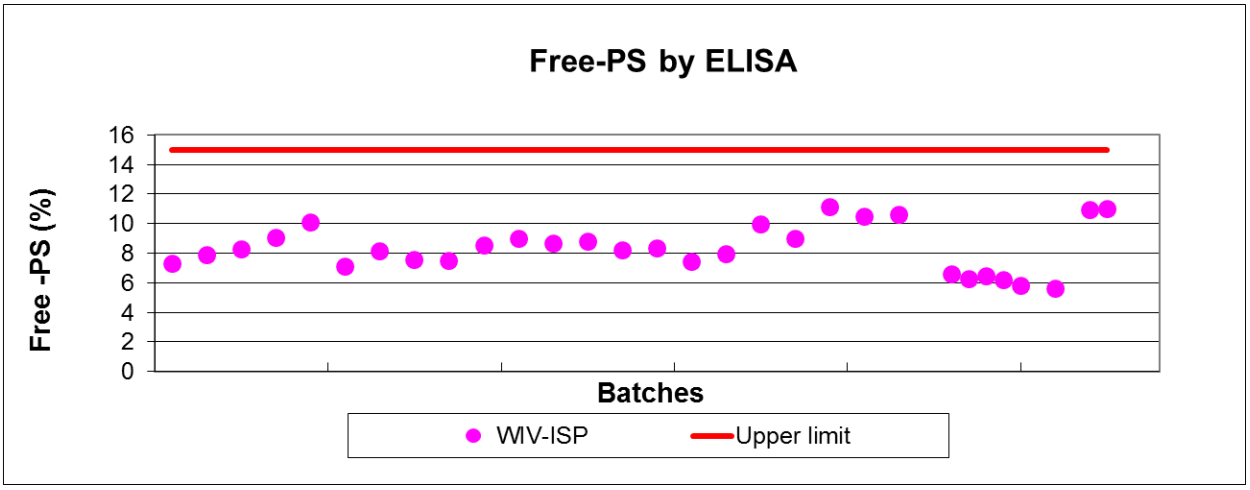
The WIV-ISP results are highly comparable to those obtained by the manufacturer.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 16)	0.74	0.64	0.84
2013 (N = 51)	0.71	0.63	0.78
2014 (N = 95)	0.72	0.66	0.79

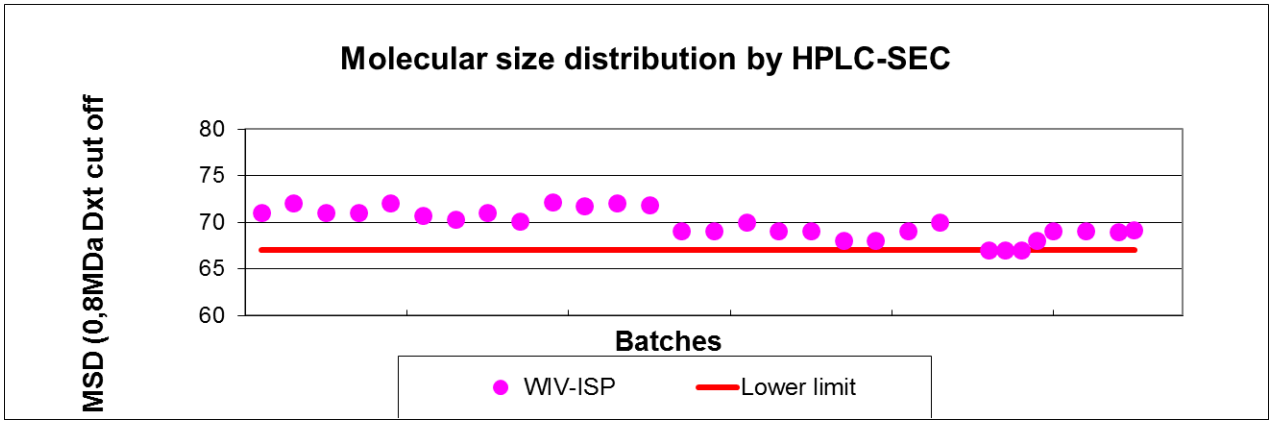
Free-PS

The graph demonstrates that the results for free-PS at WIV-ISP have a shift towards higher values compared to the manufacturer's consistency. This is due to later testing in time, since free-PS increases over time. No OOS results were obtained.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 16)	7.1	3.5	10.6
2013 (N = 51)	5.4	2.3	8.6
2014 (N = 95)	6.8	3.8	9.8

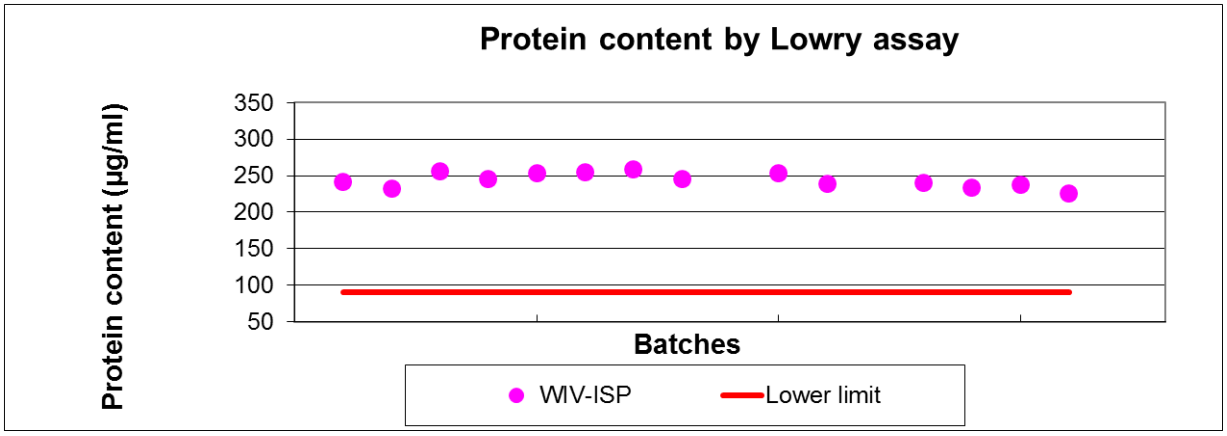
Molecular size distribution
The values obtained by WIV-ISP are lower due to the later testing, since a decrease in MSD is observed over time. No OOS results were obtained.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 16)	72	70	74
2013 (N = 51)	73	71	75
2014 (N = 95)	73	71	75

D.5.3.10 PS23F-PD

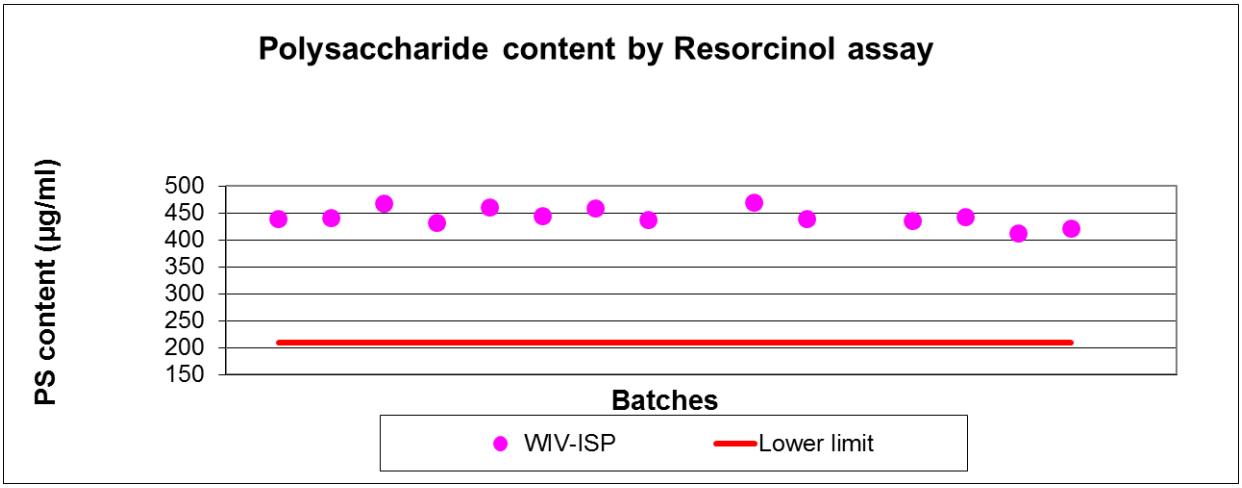
Protein content
The results obtained at WIV-ISP are well aligned fit well into the manufacturer's consistency . No OOS data were obtained.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 12)	265	233	296
2013 (N = 9)	258	246	271
2014 (N = 14)	254	243	265

Polysaccharide content

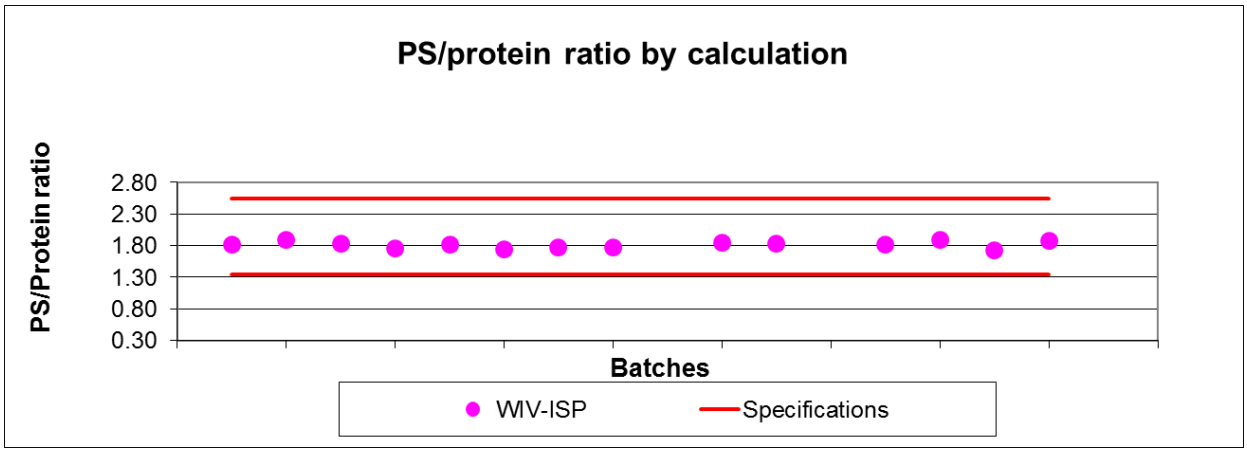
For the determination of the polysaccharide content, the graph shows that the values of PS content in PS23F-PD bulks are well aligned between the manufacturer and WIV-ISP. No OOS data were obtained.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 12)	444	400	487
2013 (N = 9)	433	402	463
2014 (N = 14)	441	410	473

PS/protein ratio

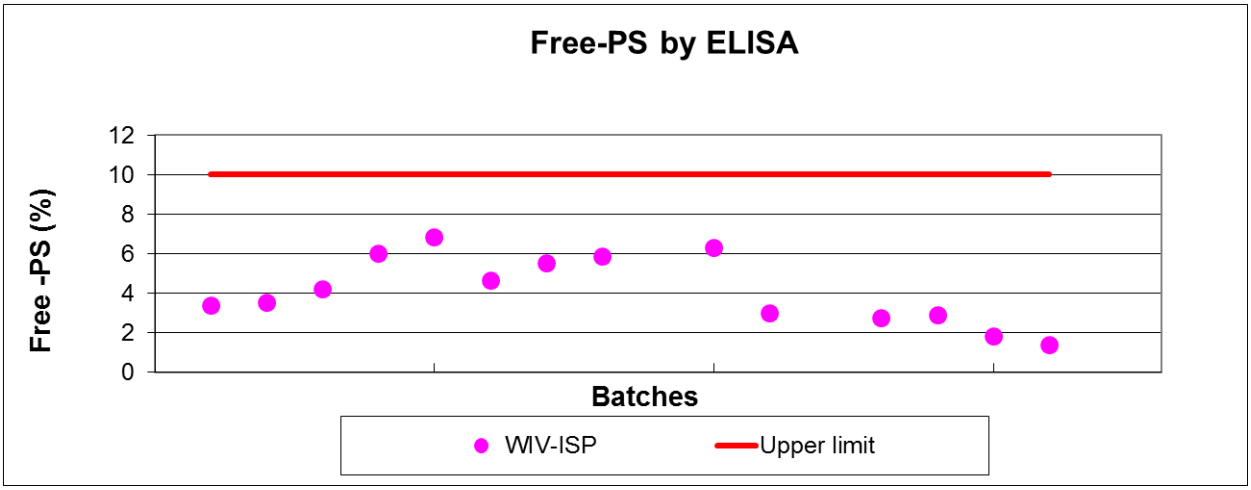
The WIV-ISP results are highly comparable to those obtained by the manufacturer.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 12)	1.68	1.41	1.96
2013 (N = 9)	1.67	1.58	1.77
2014 (N = 14)	1.74	1.62	1.85

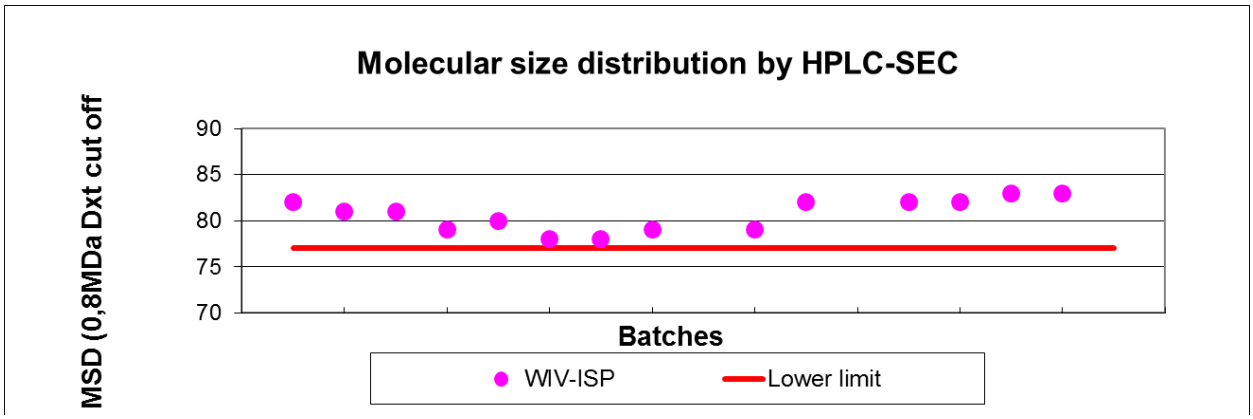
Free-PS

The results for free-PS at WIV-ISP are higher due to later testing. No OOS results were obtained.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 12)	2.3	1.5	3.0
2013 (N = 9)	2.7	2.1	3.2
2014 (N = 14)	2.7	1.5	3.9

Molecular size distribution
The values obtained by WIV-ISP are lower due to later testing. No OOS results were obtained. .



Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 12)	85	81	90
2013 (N =9)	86	84	88
2014 (N = 14)	85	81	90

D.6 Conjugated meningococcal polysaccharide vaccine, serotypes ACWY

D.6.1 Product description

This is a vaccine directed against meningococcal invasion (*Neisseria meningitidis*). Four different polysaccharide serotypes (A,C,W and Y) are separately conjugated with a Tetanus Toxoid protein carrier. The monovalent conjugated bulks are mixed to obtain the final bulk, followed by filling into final containers.

D.6.2 Number of released batches

In 2014, 39 batches were submitted and 11 batches were fully tested. A total of 36 batches (EU and WHO) were released

Vaccine type	Status	Release information's		
		EU	Non-EU	Total
Conjugated Meningococcal polysaccharide vaccine ACWY	Submitted	12	27	39
	Tested	11	0	11
	Released	9	27	36
	Rejected	0	0	0

D.6.3 Results

D.6.3.1 Purified polysaccharides

D.6.3.1.1 PSA

In 2014, 2 different bulks were submitted for testing to WIV-ISP. O-acetyl content was determined by WIV-ISP. While the manufacturer used NMR, WIV-ISP should a spectrophotometric method to determine O-acetyl content. Each test has its own specifications. All the results met the specifications.

D.6.3.1.2 PSC

Only one batch of purified PSC was submitted and tested by WIV-ISP and result for O-acetyl content met the specifications.

D.6.3.1.3 PSW

O-acetyl content was determined both by the manufacturer and by WIV-ISP. While the manufacturer used NMR, WIV-ISP should a spectrophotometric method to determine O-acetyl content. Each test has its own specifications. All test results met the specifications. Two samples were tested by WIV-ISP.

D.6.3.1.4 PSY

O-acetyl content was determined both by the manufacturer and by WIV-ISP. While the manufacturer used NMR, WIV-ISP should a spectrophotometric method to determine O-acetyl content. Each test has its own specifications. All test results met the specifications. Two samples were submitted for testing and one sample was submitted for WHO release.

D.6.3.2. Monovalent conjugated bulks

D.6.3.2.1 PSA(ah)-TT

Two batches of PSA-TT were submitted for testing to WIV-ISP. WIV-ISP performed testing on polysaccharide content, free-PS content and molecular size distribution. All results met the specifications.

D.6.3.2.2 PSC(ah)-TT

One batch of PSC-TT was submitted for testing to WIV-ISP. WIV-ISP performed testing on polysaccharide content, free-PS content and molecular size distribution. All results met the specifications.

D.6.3.2.3 PSW-TT

Two batches of PSW-TT were submitted for testing to WIV-ISP. WIV-ISP performed testing on polysaccharide content, free-PS content and molecular size distribution. All results met the specifications.

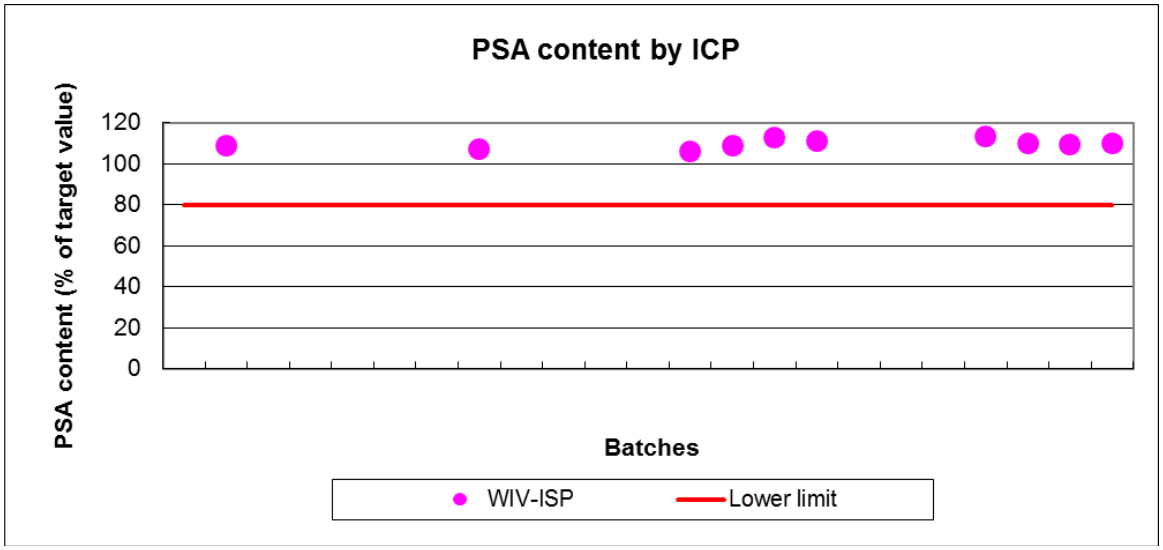
D.6.3.2.4 PSY-TT

One batch of PSY-TT was submitted for testing to WIV-ISP. WIV-ISP performed testing on polysaccharide content, free-PS content and molecular size distribution. All results met the specifications.

D.6.3.3 Final container

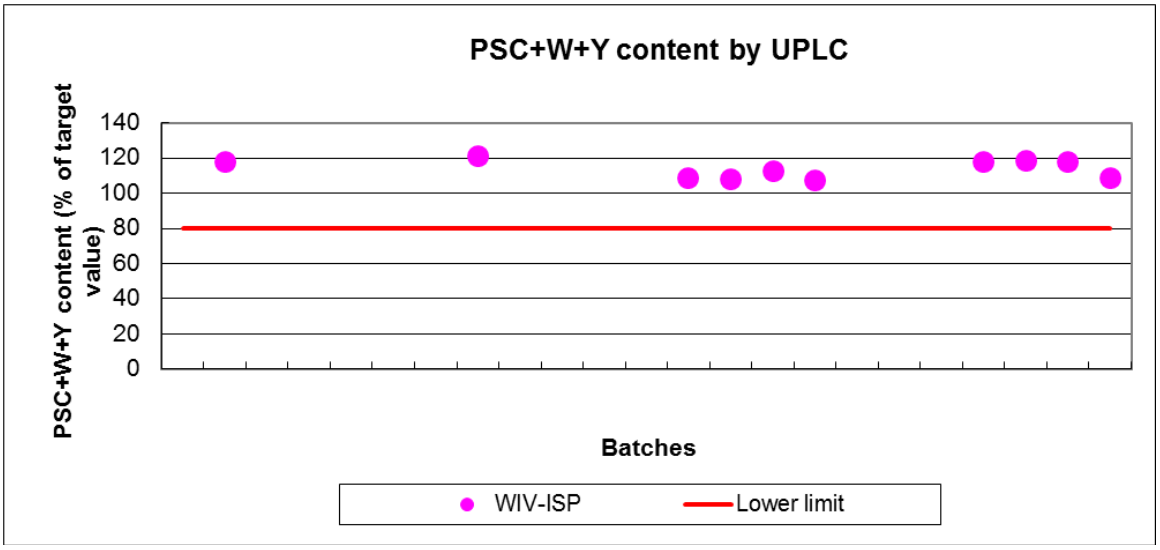
PSA content

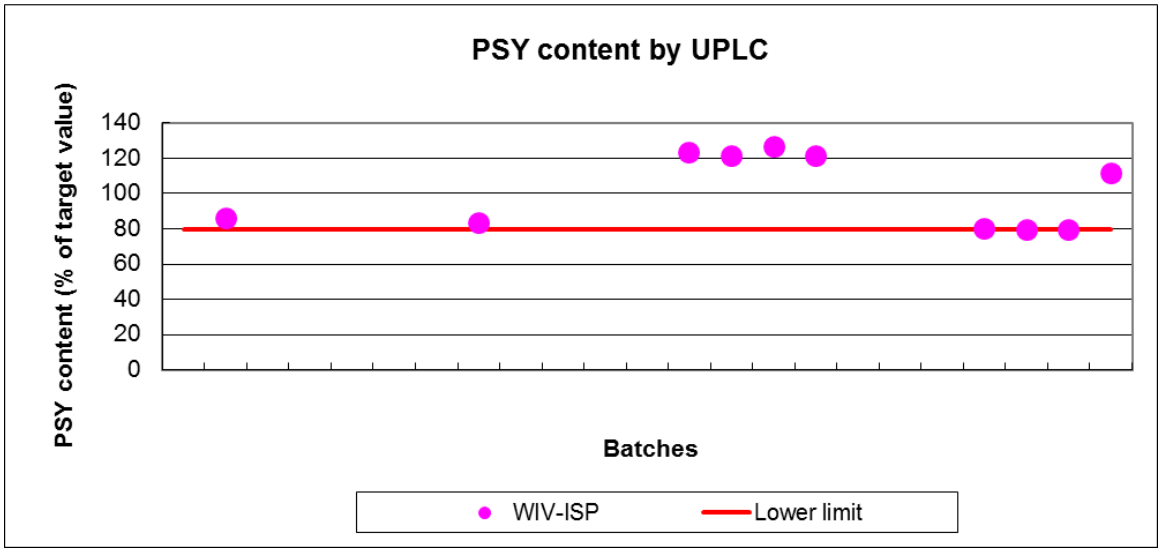
The PSA content in the final container is determined by ICP. The results obtained by WIV-ISP met the specifications. 13 additional batches were submitted for WHO market.

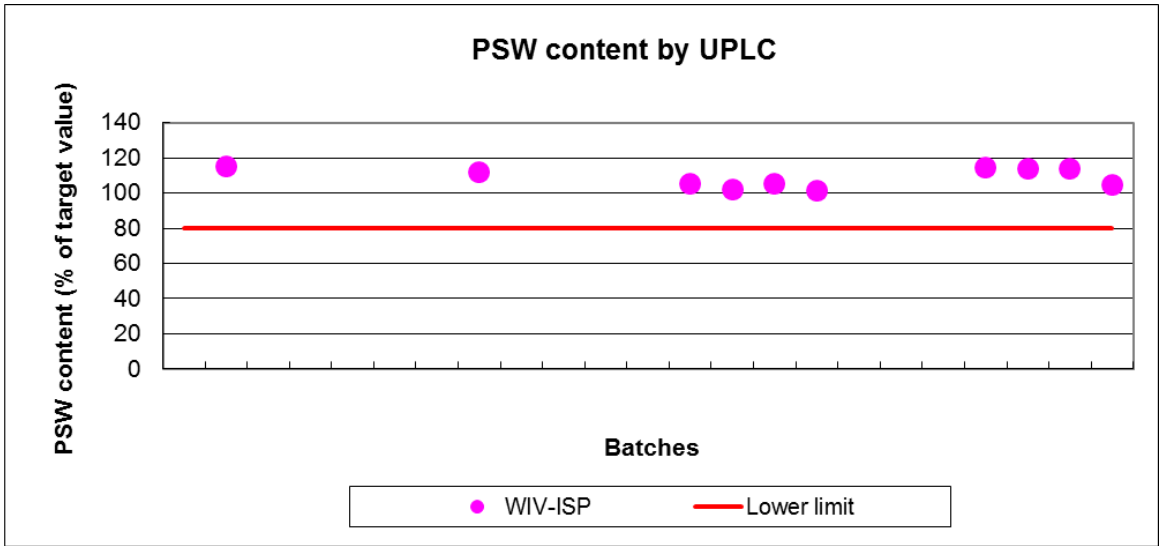


PSC+W+Y content

The total PSC+W+Y content and PSW and PSY content are determined by UPLC. The results from WIV-ISP all meet the specifications.

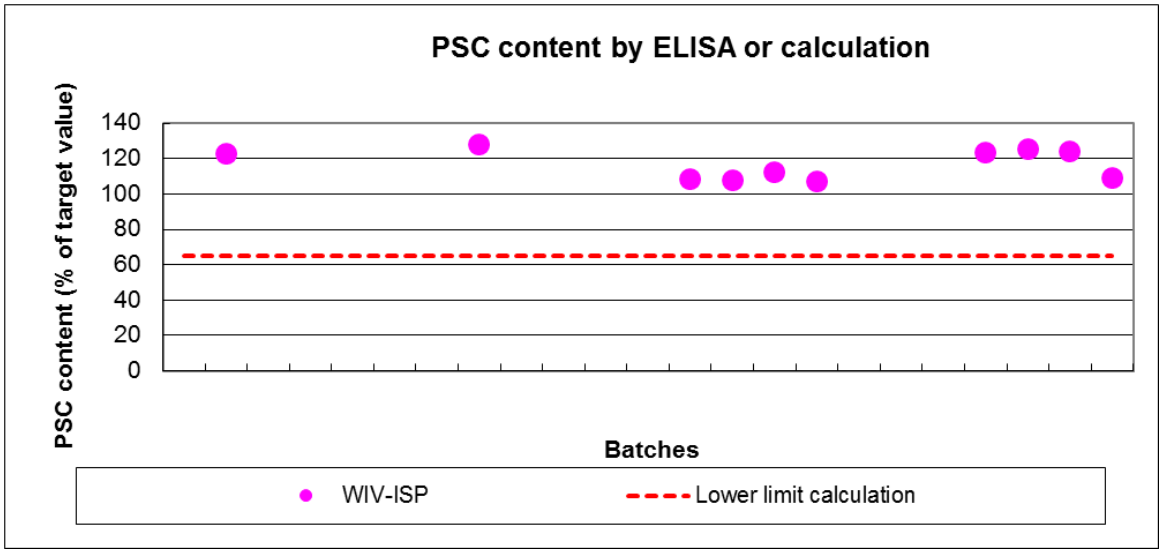






PSC content

The PSC content in the final container is obtained by calculation. All results meet the specifications.


PSC content by ELISA or calculation
PSC content (% of target value)
140
120
100
80
60
40
20
0
Batches
WIV-ISP
Lower limit calculation

D.7 General conclusion

Vaccines are heterogeneous immuno-biological products containing various components such as antigens, adjuvants, excipients and preservatives. Due to this complexity and the intrinsic variability of the vaccine production process, each vaccine batch (lot/serial) of final product is regarded as unique and it is therefore required that quality control is performed on each batch of a vaccine before its release on the market.

Testing is only one component of an operational quality system. One can observe the **important role of an Official Medicine Control Lab** in this particular case: 2 lots were detected out of specification by our lab while the results were close to the specification (but still within) at the manufacturer lab. Following a thorough investigation on both sites, the manufacturer decided not to submitted those lots for release. The batches were thus not put on the market.

On the other hand, the follow up of the consistency in production through the review of in-process characteristics and parameters (and not only the licensed release specifications) measured on the intermediates and the final product represents a major improvement in quality evaluation.

Nowadays it is a well-established philosophy that the quality of a vaccine is not ensured by testing alone, but also built through the strict application of the quality assurance system.
To reinforce the quality evaluation and the overview of the quality product, the Biological Standardisation has participated, in 2014, as product expert to **8 GMP inspections** on site with GMP auditors from the Belgian Inspectorate (FAMHP).

In addition, our quality experts have also been involved in evaluation of the quality part of Marketing Authorization dossiers according to the EMA centralised procedure (**2 licensing dossiers as well as 93 process variation dossiers in 2014** (EU and non EU format))[**4][5]** .

They have also participated to the evaluation of **30 scientific advice dossiers** according to the EMA consultation procedure and FAHMP (Belgian agency) scientific technical advice procedure**[6]**.

This combination of different points of view (Batch release, GMP inspection, review of quality dossier) is quite unique and allows the Scientific Institute of Public Health to be one of the major key players in the field of vaccines within Europe.

Section E: Network Activity

- **The Biological Standardisation Programme (BSP) [7]**

One of the missions of the Biological Standardisation Programme is to elaborate European Pharmacopoeia Reference Standards and working standards for biologicals in order to improve international harmonisation.

To achieve such a goal, the secretariat of the Biological Standardisation, OMCL and HealthCare Department (DBO) of the EDQM coordinates collaborative studies run under the BSP with those planned by the World Health Organization (WHO) and the U.S. Food and Drug Administration (FDA).

The BSP studies may include national labs as well as labs of different European manufacturers in order to provide Biological Reference Preparations (BRP) to the networkn or to standardise a testing method.

The full reports of the concluded collaborative studies are published in 'Pharmeuropa Bio & Scientific Notes' **[8]** .

We have participated in **2 BSP's** in 2014.

- **The Proficiency Testing Scheme (PTS) [9]**

In order to build mutual trust between OMCLs, the concept of Proficiency Testing Scheme has been introduced within the European network of OMCLs.

The Proficiency Testing Scheme is an external assessment of quality control management systems using inter-laboratory comparisons. It is organized by the EDQM to ensure the performance of individual laboratories.

PTS studies are carried out on a regular basis on biological and physico-chemical methods.

This helps to build a homogeneous quality level of analysis between members as well as a mutual recognition within the network.

We have participated to **1 PTS** in 2014.

- **Mutual joint audit (MJA) [10]**

A common approach for developing and implementing Quality Management systems (QMS) in all OMCLs of the Network is an essential step to mutual recognition. This was especially important due to the increasing exchange of results and data (e.g. batch release of biologicals, market surveillance of centrally authorised products) among members.

In order to harmonize the quality management systems of the OMCLs, the mutual joint audit program has been adopted and refers to the ISO/IEC 17025 quality standard.

MJAs of OMCLs are carried out by experts, from the Network. They check that OMCLs quality management systems comply with the requirements laid down in ISO/IEC 17025, in the General European OMCL Network Quality Management Guidelines and in the European Pharmacopoeia. They take place on a routine basis.

Our experts from the Biological Standardisation unit participated in **2 different mutual joint audits** in 2014.

- **Quality Management Guidelines**

They have been drafted by experts from the OMCL Network in order to support laboratories in implementing the ISO/IEC 17025 requirements, while taking into account the specific OMCL environment.

The following new or updated OMCL guidelines revised by our experts were adopted by the network:

1/ Sub-contracting of tests PA/PH/OMCL (14) 18 3R

2/ Evaluation and Reporting of Results PA/PH/OMCL (13) 113 2R

Section F: Batch release at the request of WHO

Among its activities, our Biological Standardisation unit ensures the control and the release of batches of vaccines for human use before their marketing. Within this framework, our unit developed, thanks to its expertise acquired during the two last decades, a close collaboration with the World Health Organisation **[11]**.

Our collaboration is based on the following 3 activities/expertise:

A) The technical expertise for the analysis and the evaluation of new "candidate vaccines" subjected to a pre-qualification procedure or already pre-qualified vaccines. This is a procedure of acceptance of the vaccines by WHO for use at the time of vaccination campaigns of the UNICEF. **[12]**

The TSA contract (Technical Service Agreement) **[13]** lists the type of vaccines and the number of batches which will be sent by WHO as well as the tests to carry out. The samples come from the company who wishes to enter the pre-qualification process or to maintain its pre-qualified status.

WHO sends samples 2 or 3 times a year accompanied by the documentation relative to their production and control by the company.

During this pre-qualification procedure, the experts of the WIV-ISP are invited to evaluate the dossier submitted according to the WHO standards (Product Summary file) and take also part in a GMP quality audit of the manufacturer.

Batches tested in 2014 are listed in the table below

Others: WHO (TSA)	Nr of tested batches		
Vaccines	**2012**	**2013**	**2014**
bOPV (bivalent OPV type 1 & type 3)	**8**	**7**	**0**
Bulk Hib	**1**	**1**	**0**
Hib	**5**	**5**	**0**
DTPw-HepB	**20**	**8**	**4**
DTP	**1**	**0**	**0**

B) The participation in the drafting of recommendations related to quality (production and control), safety and effectiveness of new "candidate vaccines" as well as the revision of existing recommendations. **[14]**

C) Scientific advice in direct connection with problems occurring on the field (e.g. when an out of specification result is detected on a critical test) or research projects (impact of temperature on vaccine activity). **[15]**

This expertise is also valuable during the evaluation of national regulatory authorities of other countries. Indeed, the competent authorities of a country are audited by their peers in order to standardize the dossier evaluation and analysis practices. This allows WHO to guarantee an equivalent quality level of assessment of the pre-qualification procedure, whatever the country of manufacturing. Within this framework, our scientists are invited as technical experts to share their expertise and knowledge of the vaccines.

One expert has participated as a facilitator to 2 training courses for the evaluation of the quality part of vaccine dossiers: Iran and Turkey.

Acknowledgment

We would first like to thank the Manufacturers for the exchange of information on batches and the associated transparency in the European batch release framework

We would like to thank the European Directorate for the Quality of Medicine & Healthcare (EDQM) for giving us the opportunity to take part, as vaccine experts, to PTS & BSP testing and auditing.

We also would like to thank the World Health Organisation (WHO) for the fruitful collaboration in the field of testing and auditing.

Finally, We would like to thank all Biological standardisation teams for their expertise and support during testing, especially Geneviève Waeterloos, Head of the Biological standardisation unit, Eleonore Dubois, Camille Domicent, Virginie Stygelbout and Alexandre Dobly, Mathias Janssen, Fabrice Ribaucour, Olivier Carabin and Koen Brusselmans, senior scientists.

Conflict of Interest Statement

The authors have neither any professional nor personal conflicts of interest.

see also:

http://www.ema.europa.eu/docs/en_GB/document_library/contacts/ltesolin_DI.pdf

http://www.ema.europa.eu/docs/en_GB/document_library/contacts/wmolle_DI.pdf

List of References

[1] http://www.edqm.eu/en/human-biologicals-611.html
[2] http://www.fagg-afmps.be/en/
[3] Belac: http://economie.fgov.be/en/entreprises/life_enterprise/quality_policy/Accreditation/
[4] http://ec.europa.eu/health/files/eudralex/vol-2/a/vol2a_chap7_rev_2008_07_en.pdf
[5]http://www.ema.europa.eu/docs/en_GB/document_library/Regulatory_and_procedural_guideline/2009/10/WC500004089.pdf
[6]**http://www.fagg-afmps.be/en/human_use/medicines/medicines/scientific_technical_advice/applicvation_procedures/**
[7] BSP: http://www.edqm.eu/en/Biological-Standardisation-Programme-mission-60.html
[8] Pharmeuropa : http://www.edqm.eu/en/pharmeuropa-bio-and-scientific-notes-584.html
[9] PTS: http://www.edqm.eu/en/Proficiency-Testing-Scheme-47.html
[10] MJA: http://www.edqm.eu/en/quality-management-19.html
[11]Tesolin L., Le WIV-ISP, partenaire clé de l'OMS dans le contrôle qualité des vaccins. WIV-ISP rapport d'activités 2011-2012 ; numéro de dépôt : D/2013/2505/23 ; Bruxelles, 2013.
[12] http://www.who.int/topics/prequalification/en/
[13] http://www.who.int/biologicals/areas/vaccines/lot_release/en/
[14]http://www.who.int/immunization_standards/vaccine_quality/pq_consultation_2010/en/index.html
[15] Zipursky S. et al, Vaccine, JVAC-D-11-00507R1 (http ://dx.doi.org/10.1016/j. vaccine.2011.06.011)

List of Annexes

Annex 1 : ISO 17025 Belac accreditation certificate
Annex 2 : MJA Attestation

Annex 1 : BELAC certificate

Organisme belge d'Accréditation
Belgische Accreditatieinstelling
Belgische Akkreditierungsstelle
Belgian Accreditation Body

Signatory to EA, ILAC and IAF Multilateral Agreements

Certificat d'Accréditation n° 081-TEST

En application des dispositions de l'arrêté royal du 31 janvier 2006 créant BELAC, le Bureau d'Accréditation atteste que le laboratoire d'essais

SERVICE PUBLIC FEDERAL (SPF)
SANTE PUBLIQUE, SECURITE DE LA CHAINE ALIMENTAIRE
ET ENVIRONNEMENT
INSTITUT SCIENTIFIQUE DE LA SANTE PUBLIQUE
Rue Juliette Wytsman, 14
1050 BRUXELLES - Belgique

possède, conformément aux critères de la norme NBN EN ISO/IEC 17025:2005, la compétence pour effectuer les essais décrits dans l'annexe qui fait partie intégrante du présent certificat. Le respect des conditions d'accréditation fait l'objet de surveillances régulières.

La Présidente du Bureau d'Accréditation BELAC,

Nicole MEURÉE-VANLAETHEM

Date d'émission : **2010-06-22**

Date de validité : **2015-01-31**

La version originale de ce certificat est en néerlandais.

Annex 2: MJA Attestation

edqm
European Directorate for the Quality of Medicines & HealthCare

COUNCIL OF EUROPE

COUNCIL OF EUROPE

European Directorate for the Quality of Medicines & HealthCare

OMCL NETWORK QUALITY MANAGEMENT SYSTEM

ATTESTATION

The EDQM, European Directorate for the Quality of Medicines & HealthCare, hereby declares that

Scientific Institute of Public Health
Juliette Wytsman, 14, B-1050 Brussels, Belgium
Biological Standardisation Section

has been audited in accordance with the EDQM instruction *IS7/02* on the OMCL Network Mutual Joint Audit Scheme.

The above-mentioned OMCL is entitled to declare that it has satisfactorily implemented a Quality Management System in accordance with ISO/IEC 17025.

Detailed information can be found in the Audit Report, which is consigned in document *PA/PH/OMCL-QA (09) 04 DEF* corresponding to the *MJA 01/09*, and in the enclosed Scope of Assessment. The original documents are archived at the Department of Biological Standardisation, OMCL Network & HealthCare (DBO) of the EDQM and the Director of the OMCL has received a certified copy.

Attestation number: EDQM/MJA-045

Strasbourg, 30 June 2011

Valid until: 12/2014

Karl-Heinz Buchheit
Deputy Head of the DBO, EDQM