WETENSCHAPPELIJK INSTITUUT
VOLKSGEZONDHEID
INSTITUT SCIENTIFIQUE
DE SANTÉ PUBLIQUE

081-TEST / 081-INSP
ISO17025 Accreditation

Quality Control of Polysaccharide Vaccines by the Belgian National Control Lab (OMCL) in the European Batch Release Framework : Activity Report 2013

Scientific Institute of Public Health

Operational Direction « Expertise, Service Provision & Customer Relations »
Biological Standardisation Unit
Rue Juliette Wytsman 14
1050 Brussels – Belgium
www.wiv-isp.be

Biological Standardisation | September 2014 | Brussels, Belgium

Copyright deposit : D/2014/2505/42

Authors

Lorenzo Tesolin, M. Sc.
Phone: +32 2 642 55 43
E-mail: Lorenzo.Tesolin@wiv-isp.be

Wim Van Molle, PhD.
Phone : +32 2 642 56 52
E-mail: Wim.Vanmolle@wiv-isp.be

Contact point

Geneviève Waeterloos, Head of Biological Standardisation Unit
Phone: +32 2 642 53 39
E-mail: Genevieve.Waeterloos@wiv-isp.be

Table of contents

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.

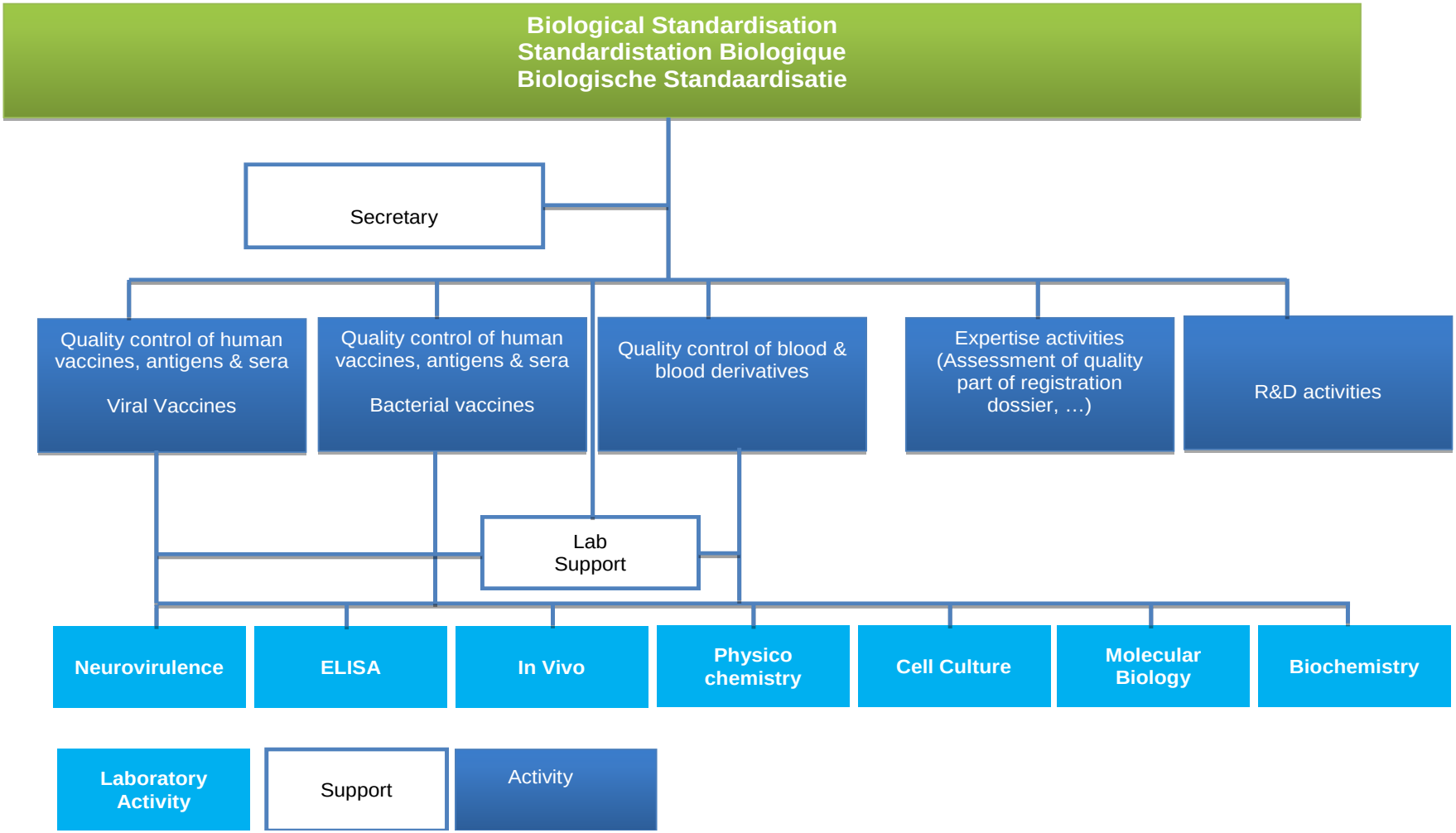
Section A: Organisation of the Competent Authority/OMCL

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 polysaccharide vaccine testing in 2012. Please see also reports to be published from other sections of the unit.

Physicochemical assays, all vaccines
Lorenzo Tesolin, M. Sc, Wim Van Molle, PhD. with technical assistance from: Rita De Brandt (till August 2013), Saloua El Youssoufi, Dominique Pecher, Rachida Elkhalouki, Ing. Ind,, Laurence Vifquin.

Immunochemical assays, all vaccines
Isabelle Hansenne PhD.(till August 2013), Aurélie Bauwin, M.Sc; (till November 2013), Eléonore Dubois, PhD. (from November 2013), Camille Domicent, M.Sc. (from November 2013) with technical assistance Virginie Misplon, Camille Domicent (till October 2013), Hanh Van Dang, Sébastien Garcia-Sanchez, Simon Loncke, Véronique Massé and Romain Algoet.

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 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
42/III-17/F	Vaccines for human use	Osmolality	Measure of freezing temperature based on the osmolality Principal equipment: osmometer

Assay Code	Sample type	Measured parameter Range of measurement	Description of the assay Equipment
42/III-18/F	Haemophilus influenzae b (Hib) Vaccines	Molecular Size Distribution	HPLC by size exclusion with Refractive Index and UV detector Principal equipment: HPLC
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

Assay Code	Sample type	Measured parameter Range of measurement	Description of the assay 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).
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.

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 submitted batches and released batches is due to the fact that the batches which were submitted in 2011, were released in 2012 and that a number of batches submitted at the end of 2012 were released beginning of 2013.
The report covers the batch release activities during the period January – December 2012.
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 2013, 89 lots of adsorbed Hiberix have been tested and approved in combination with a pentavalent vaccine.

Vaccine type	**Submission**	**Release information's**		
Purified meningococcal polysaccharide vaccine, serotypes ACW & ACWY		**EU**	**Non-EU**	**Total**
	Submitted	42	48	90
	Tested	42	0	46
	Released	42	48	90
	Rejected/withdrawn	0	0	0

Vaccine type	**Submission**	**Release information's**		
Lyophilised Haemophilus influenzae type b conjugate Vaccine		**EU**	**Non-EU**	**Total**
	Submitted	46	48	94
	Tested	46	0	46
	Released	46	48	94
	Rejected/withdrawn	0	0	0

Vaccine type	Submission	Release information's		
Haemophilus influenzae type b (Sanofi Pasteur) conjugate vaccine		**EU**	**Non-EU**	**Total**
	Submitted	71	0	71
	Tested	71	0	71
	Released	71	0	71
	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 2013 only EU approval certificates where provided.

Vaccine type	Status	Release information's		
Pneumococcal Monovalent bulk conjugate		**EU**	**Non-EU**	**Total**
	Submitted	77	0	77
	Tested	70	0	70
	Released	73	0	73
	Rejected	0	0	0

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	4	7	11
	Tested	6	0	6
	Released	6	7	13
	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 are listed in the following tables, according to Ph.Eur. Monographs or the Marketing authorisation (MA) when applicable:

	Release test	Brief description
Diphtheria toxoid containing vaccines	Potency on final bulk or final lot	Single or multiple dilution, lethal challenge assay on guinea-pigs vs. in house reference (Ph. Eur.)
	Identity on final lot	Immunodiffusion after desorption
Tetanus toxoid containing vaccines	Potency on final bulk or final lot	Single or multiple dilution, lethal challenge assay on mice vs. in house reference (Ph. Eur.)
	Identity on final lot	Immunodiffusion after desorption
Whole cell Pertussis containing vaccines	Potency on final bulk	Multiple dilution, B.pertussis intracerebral challenge assay (Kendrick test) on mice vs in house reference (Ph.Eur.)
	Specific toxicity on final bulk	Mouse weight gain test (Ph. Eur.)
	Endotoxin content on final lot	LAL test (kit) by kinetic method (Ph.Eur.)
Acellular Pertussis containing vaccines	Potency on final bulk or final lot	Single dilution serological assay on mice vs in house reference. Quantification of serological response by ELISA (MA)
	Residual pertussis toxin on final bulk	Histamine Sensitising Assay (Ph. Eur.)
	Identity on final lot	ELISA (MA) or immunodiffusion (MA)
Purified meningococcal polysaccharide vaccine, serotypes ACW & ACWY	Appearance	Visual inspection (Ph Eur)
	Endotoxin content	LAL test (Kit) (Ph.Eur.)
Bulk conjugate and lyophilised Haemophilus influenzae type b conjugate Vaccine	Polysaccharide content	HPLC-PAD(Dionex™ method)
	Molecular size	Size exclusion chromatography
Haemophilus influenzae type b conjugate Vaccine	Appearance	Visual inspection (Ph.Eur.)
	pH	pH measurement (Ph.Eur.)
	Endotoxin content	LAL test (Kit) (Ph.Eur.)
	Free polysaccharide content	ELISA (MA)
	Molecular size	Size exclusion chromatography
Pneumococcal monovalent bulk conjugates	Protein content	Lowry method (MA)
	Polysaccharide content	Resorcinol colorimetric method with in house standard and reference (MA)
	Free-polysaccharide content	ELISA (MA)
	Molecular size distribution	HPLC-SEC with dextran 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 test (Kit) (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

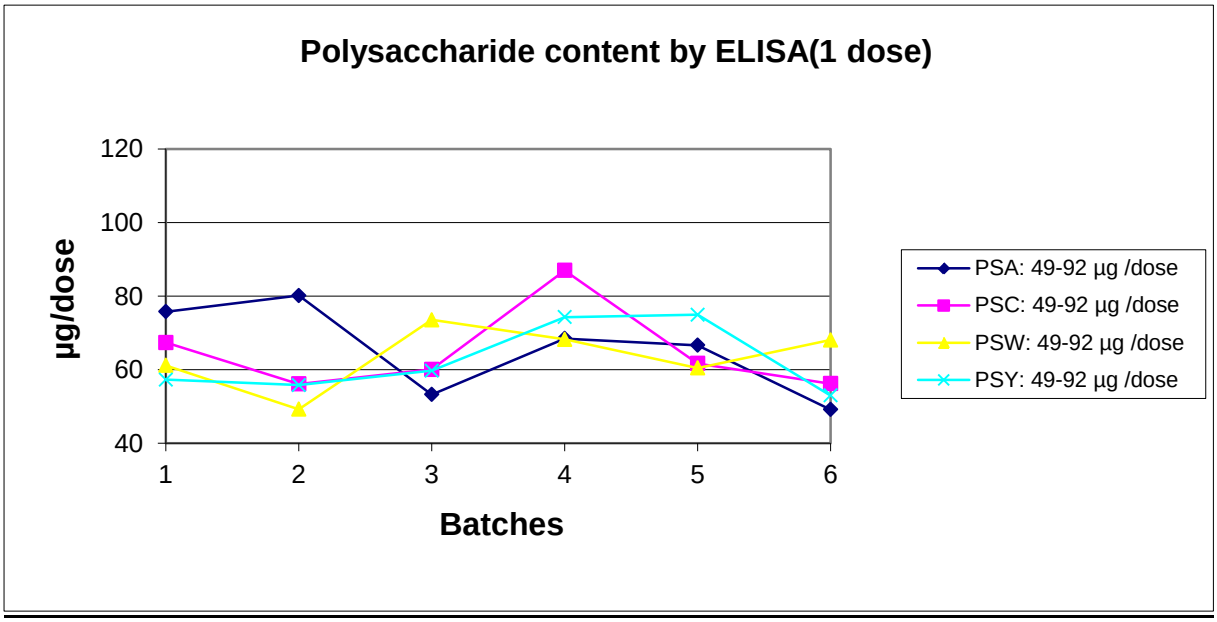
Vaccine type	submission	Release information's		
		EU	Non-EU	Total
Purified meningococcal polysaccharide vaccine, serotypes ACWY	Submitted	42	48	90
	Tested	42	0	46
	Released	42	48	90
	Rejected/withdrawn	0	0	0

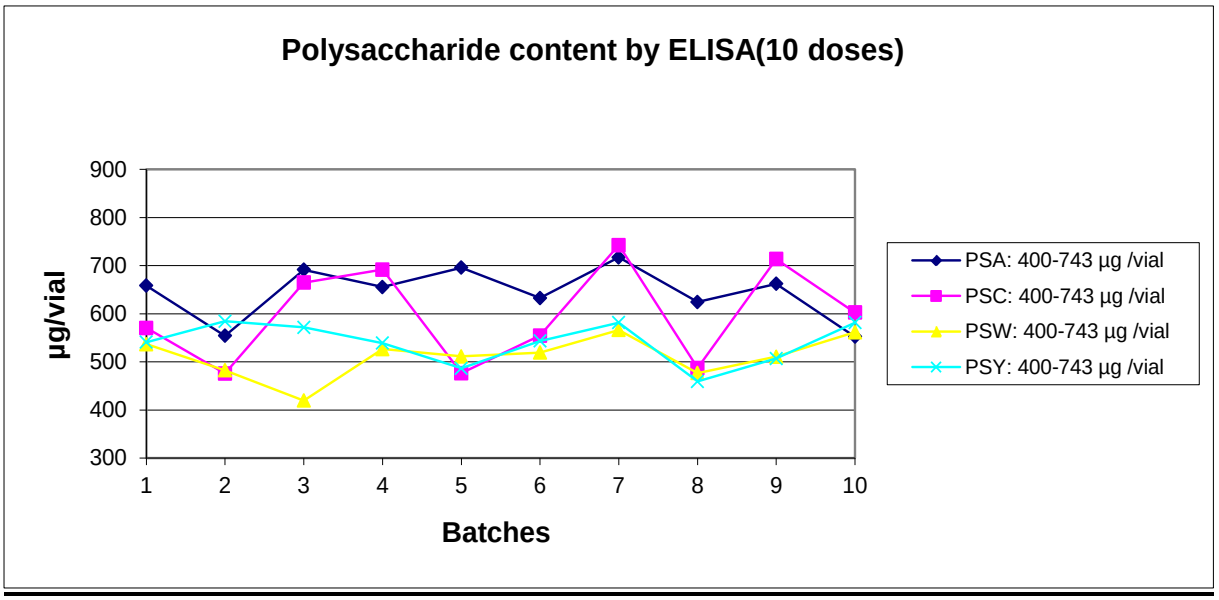
D.1.3 WIV-ISP data

Polysaccharide content

The results remain within specifications. See consistency and graph.

	Consistency 2011(mean ±2SD)	Consistency 2012(mean ±2SD)	Consistency 2013(mean ±2SD)
	4 lots	**4 lots**	**6 lots**
PSA 1D	70,0 µg ± 18.6	73,0 µg ± 24,8	65.6 µg ± 24.4
PSC 1D	67,9 µg ± 16,6	71,8 µg ± 33,8	64.7 µg ± 23.4
PSW 1D	68,8 µg± 13,2	63,8 µg± 15,4	63.5 µg ± 17.0
PSY1D	69,1 µg± 9,0	72,0 µg± 27,8	62.5 µg ± 19.2
	8 lots	**4 lots**	**10 lots**
PSA 10D	547,3 µg ± 63,8	532,5 µg ± 51,2	644.3 µg ± 111.4
PSC 10D	544,3 µg ± 74,4	532,5 µg ± 51,2	597.9 µg ± 202.2
PSW 10D	558,5 µg ± 152,2	541,4 µg ± 70,8	511.0 µg ± 86.8
PSY 10D	565,5 µg ± 66,2	636,2 µg ± 113,0	539.4 µg ± 86.8





Endotoxin content:

For the year 2013, results for the endotoxin content are lower than 10 IU/dose and usually lower than 5 IU/doses (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.

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 vaccines. 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	46	48	94
	Tested	46	0	46
	Released	46	48	94
	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

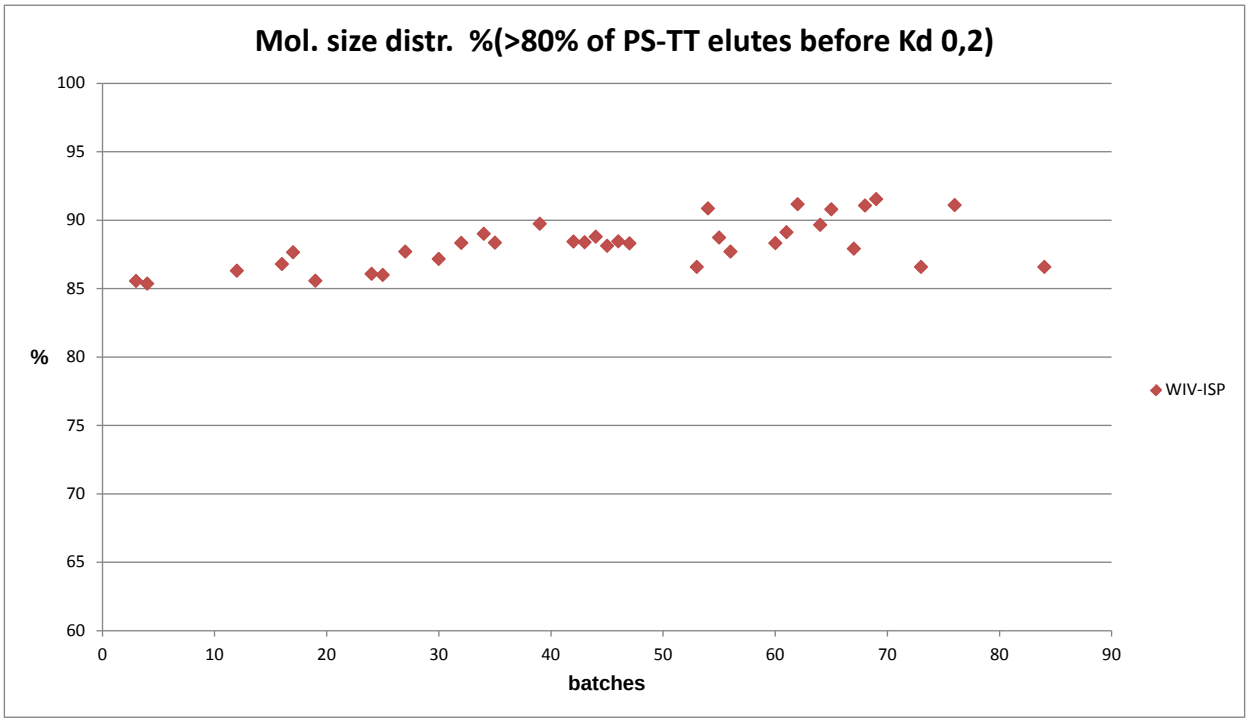
Molecular Size Distribution : (Specification: >80% of the conjugate PS-TT eluted before Kd 0.2).
The molecular size distribution shows a high consistency of production.

The molecular size distribution shows a high consistency of production.

Years	*Mean*	*Mean - 2*SD*	*Mean + 2*SD*
2011 (N = 78)	*87.4*	*84.6*	*90.2*
2012 (N =81)	*85.9*	*82.5*	*89.3*
2013 (N=47)	*88.4*	*85.0*	*91.8*

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).

See graph below:

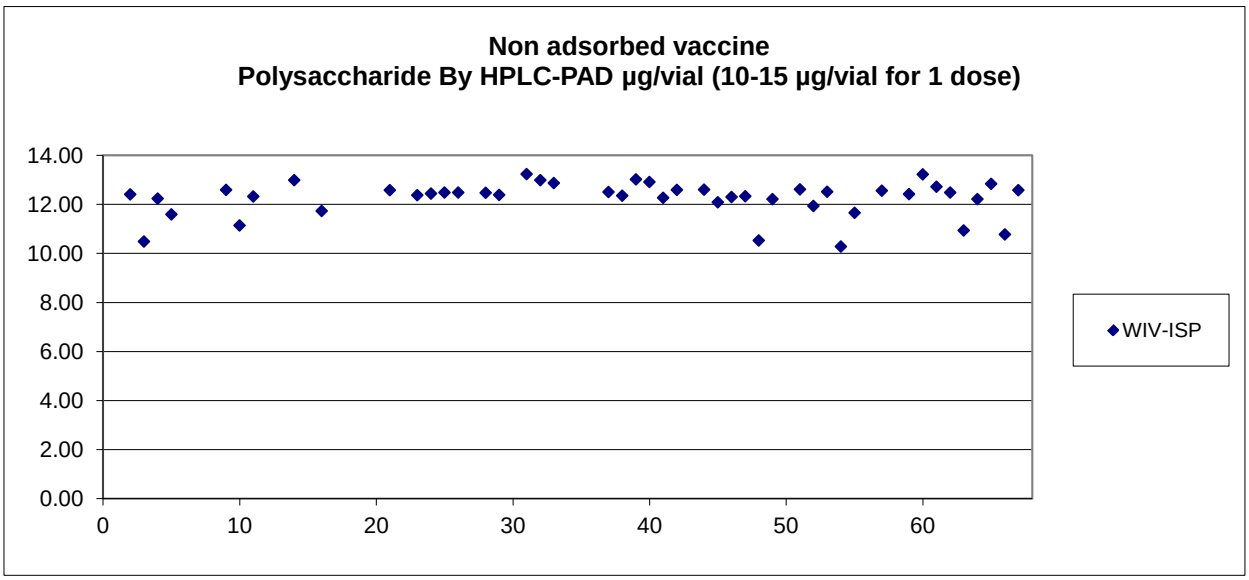

Mol. size distr. %(>80% of PS-TT elutes before Kd 0,2)
100
95
90
85
80
75
70
65
60
%
0
10
20
30
40
50
60
70
80
90
batches
WIV-ISP

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 2013.

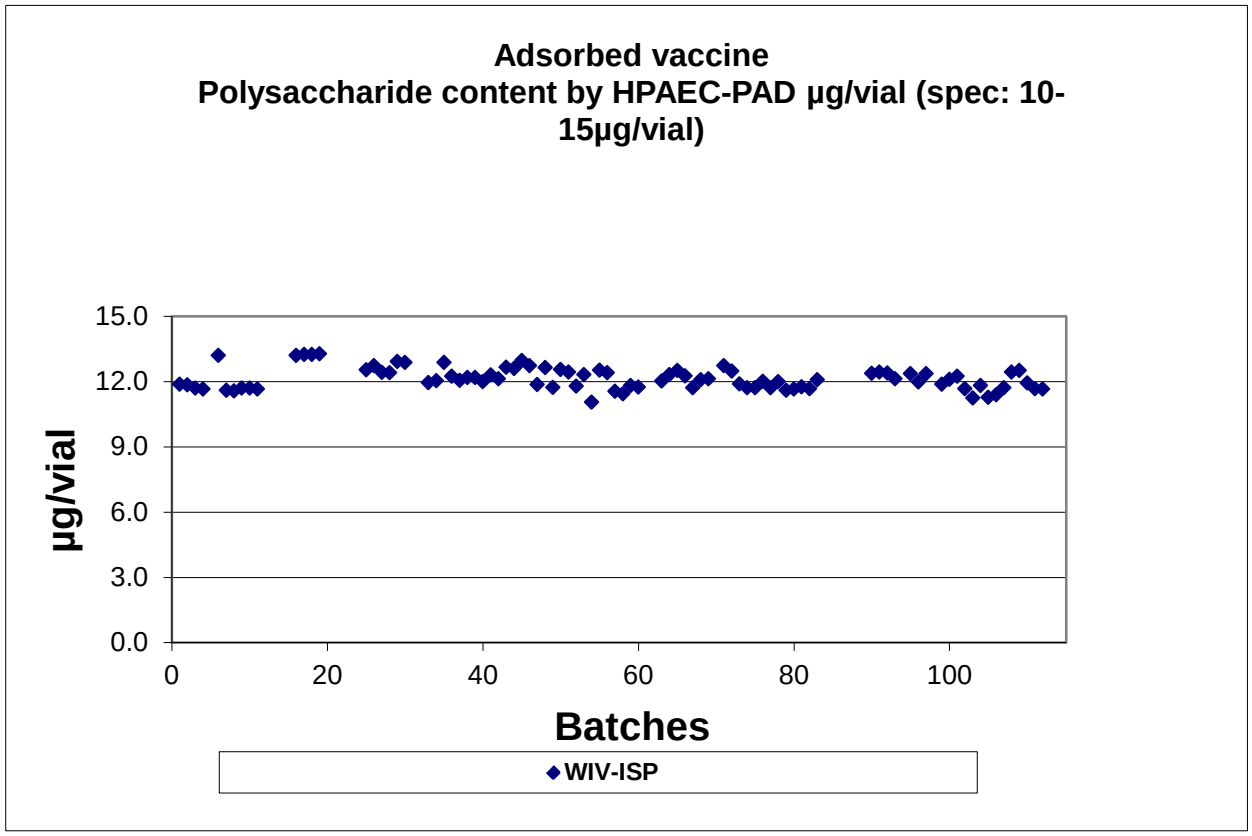
Years	Mean	Mean - 2*SD	Mean + 2*SD
2011 (N = 20)	**12.1**	**10.8**	**13.4**
2012 (N =52)	**12.4**	**11.4**	**13.4**
2013 (N=46)	**12.3**	**10.9**	**13.7**



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

The polysaccharide content shows a high consistency during 2012 except for one lot still within specifications.

Years	Mean	Mean - 2*SD	Mean + 2*SD
2011 (N = 57)	11.6	10.2	13.0
2012 (N =59)	12.0	11.0	13.0
2013 (N=89)	12.1	11.1	13.1



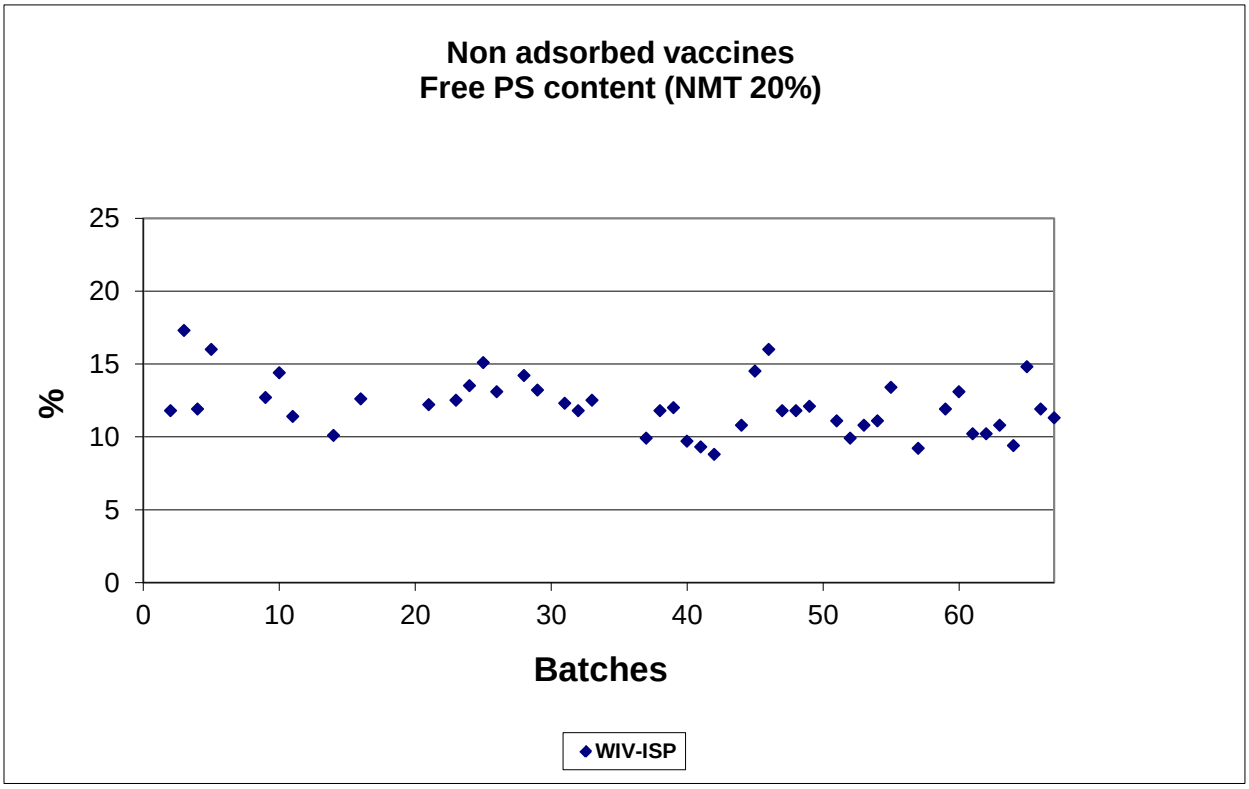
Free polysaccharide content by ELISA (Specification : nmt 20% for non-adsorbed Hib and nmt 20% for adsorbed Hib for use in combination vaccines).

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

Free polysaccharide non adsorbed Vaccines (NMT 20%):

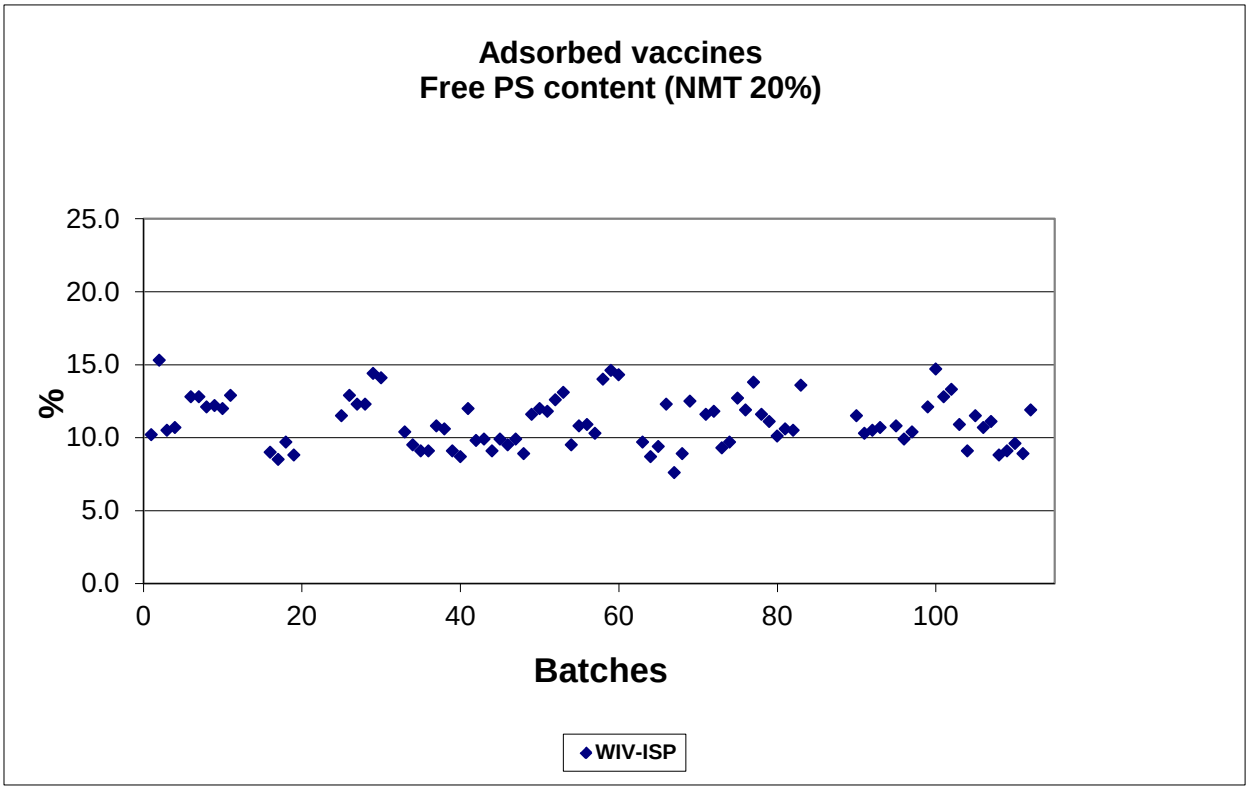
Results are higher for WIV-ISP lab in some cases due to the high variability of the ELISA test.

Years	Mean	Mean - 2*SD	Mean + 2*SD
2011 (N = 20)	9.3	6.7	11.9
2012 (N =52)	11.0	7.0	15.0
2013 (46)	12.1	8.3	15.9



Free polysaccharide adsorbed vaccines(NMT 20%):.

Years	Mean	Mean - 2*SD	Mean + 2*SD
2011 (N=57)	11.4	7.6	15.2
2012 (N=59)	11.8	8.6	15.0
2013 (N=89)	11.0	7.6	14.4



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	71	0	71
	Tested	71	0	71
	Released	71	0	71
	Rejected/withdrawn	0	0	0

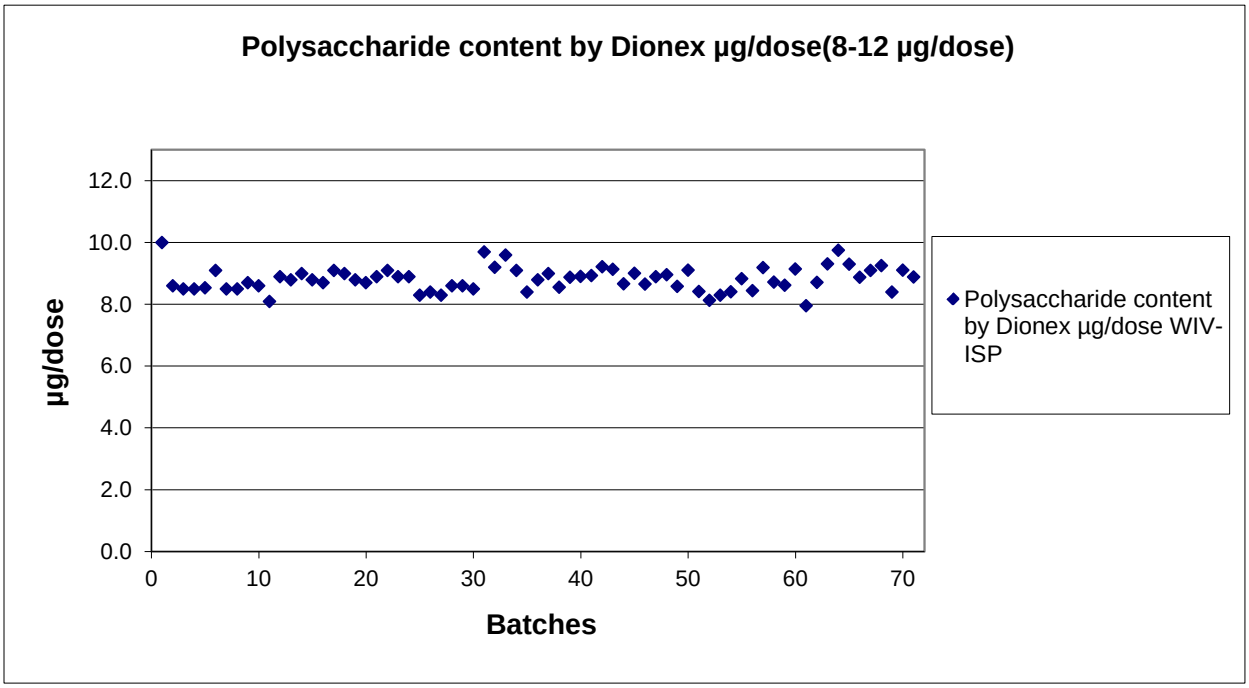
D.3.3 WIV-ISP tests data

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
2011 (N=20)	**9.1**	**8.1**	**10.1**
2012 (N=20)	**8.9**	**8.0**	**9.8**
2013 (N=71)	**8.8**	**8.0**	**9.6**

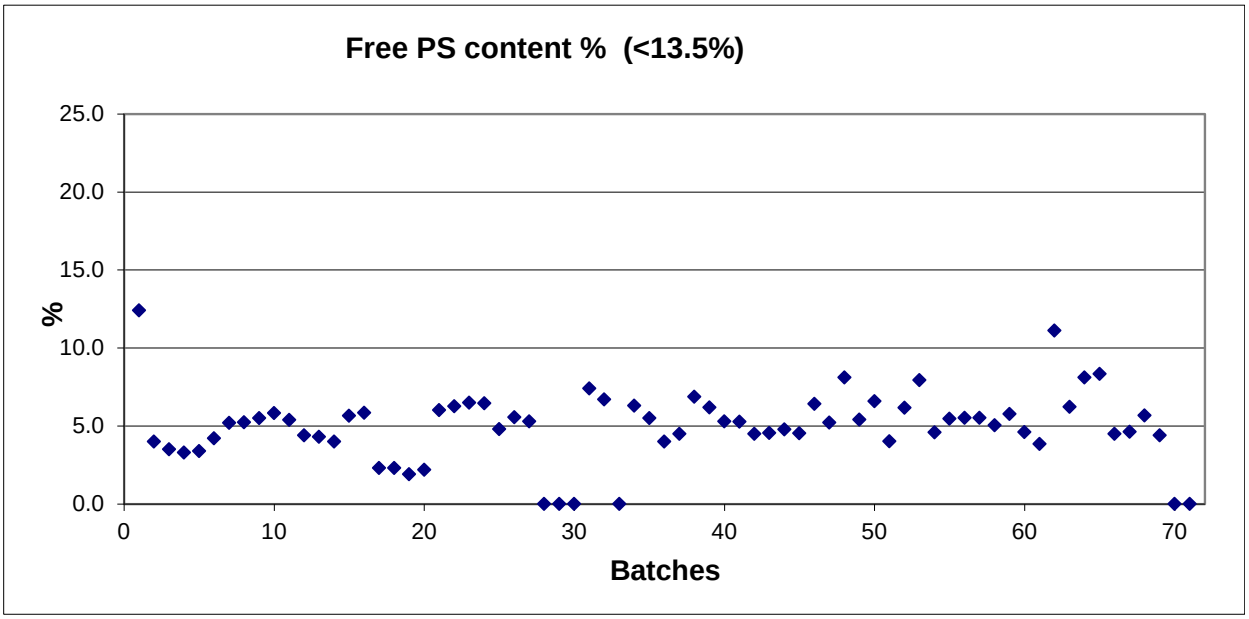


Free polysaccharide content by ELISA (Specification: <13.5%)

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). In 2013, we have introduced the HPLC-PAD for the free PS Testing. Some results are coming from ELISA testing (25 first results) and the rest are coming from HPLC-PAD. In the case of HPLC-PAD, zero results means lower than the detection limit of HPLC-PAD.

Two results are a bit high but still within specification.

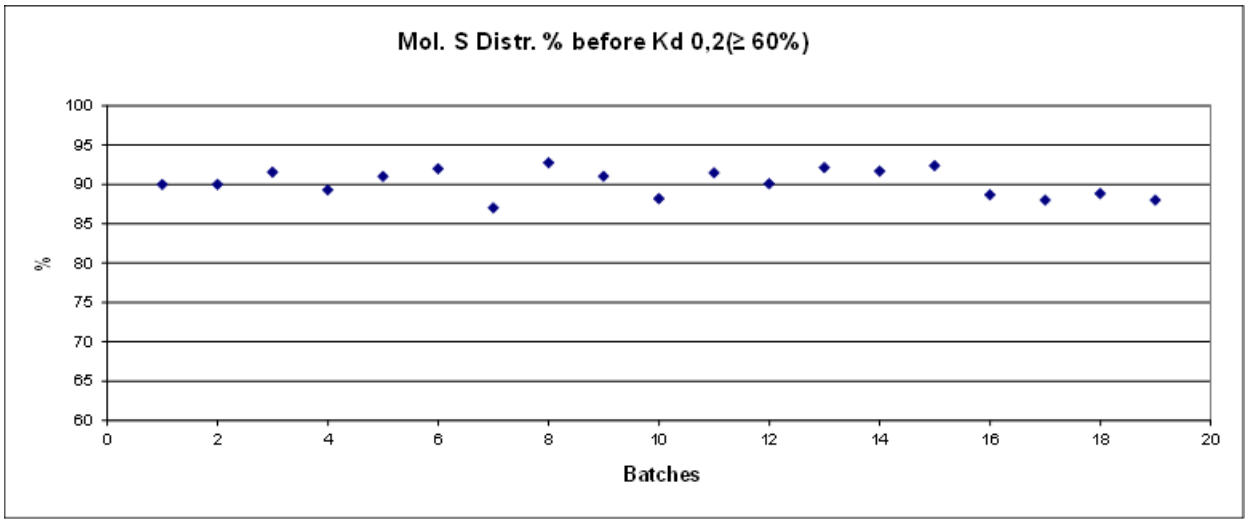
Years	Mean	Mean - 2*SD	Mean + 2*SD
2011 (N=20)	4.3	3.7	4.9
2012 (N=20)	4.6	3.4	5.8
2013 (N=71)	NA	NA	NA



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
2011 (N=11)	89.3	85.7	92.9
2012 (N=9)	89.0	86.3	91.7
2013 (N=19)	90.2	86.8	93.6

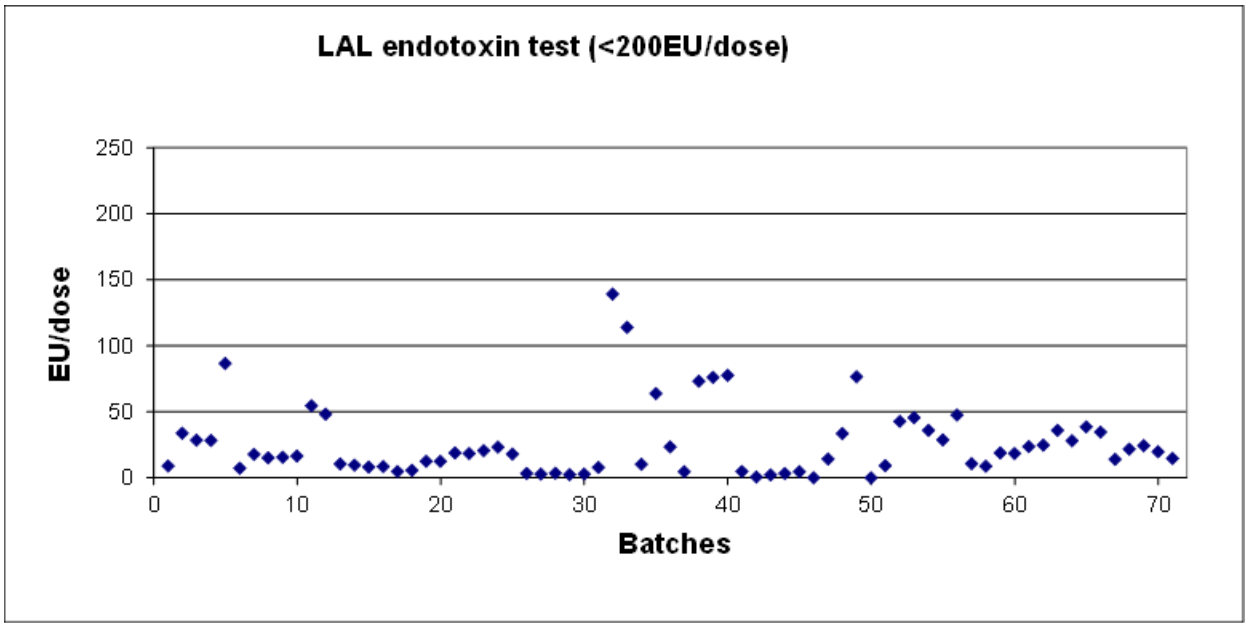


LAL endotoxin content LAL (<200 EU/dose).

All results are within specification.The average of 20 lots is 104 EU/dose ±46 in 2012.

In 2013, 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. We can observe the impact of the process changes on the endotoxin level when comparing consistencies. 2 lots are above 100 EU/dose 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

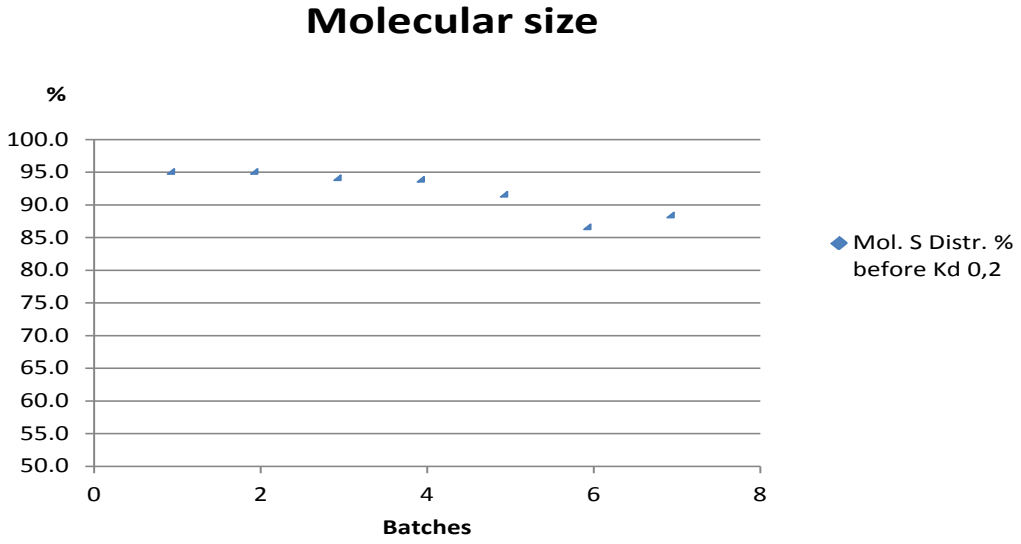
This multivalent vaccine is indicated for primary and booster vaccination of infants and toddlers from six weeks to 24 months of age against diphtheria, tetanus, pertussis, hepatitis B, poliomyelitis and invasive diseases caused by Haemophilus influenzae type b. All antigens are adsorbed on Aluminium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

D.4.2 Number of released batches

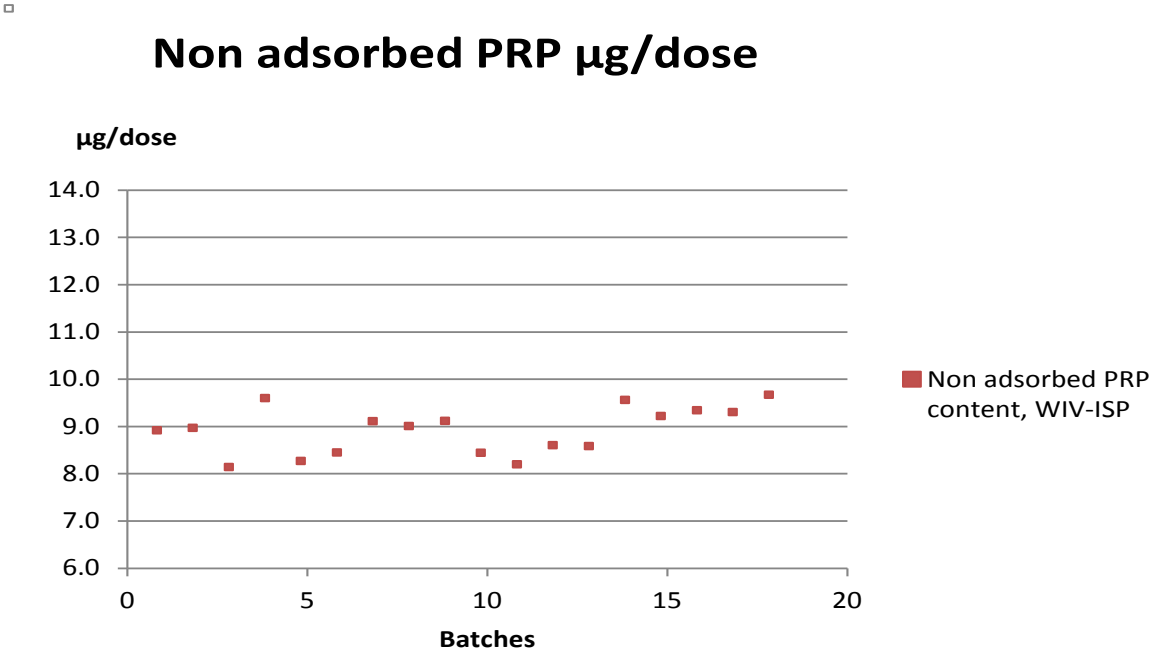
Vaccine type	Status	Release information's		
		EU	Non-EU	Total
Adsorbed Diphtheria, Tetanus, acellular Pertussis, hepatitis B inactivated Poliomyelitis and Haemophilus Influenzae type B vaccine	Submitted	18	0	18
	Tested	18	0	18
	Released	18	0	18
	Rejected	0	0	0

D.4.3 Results

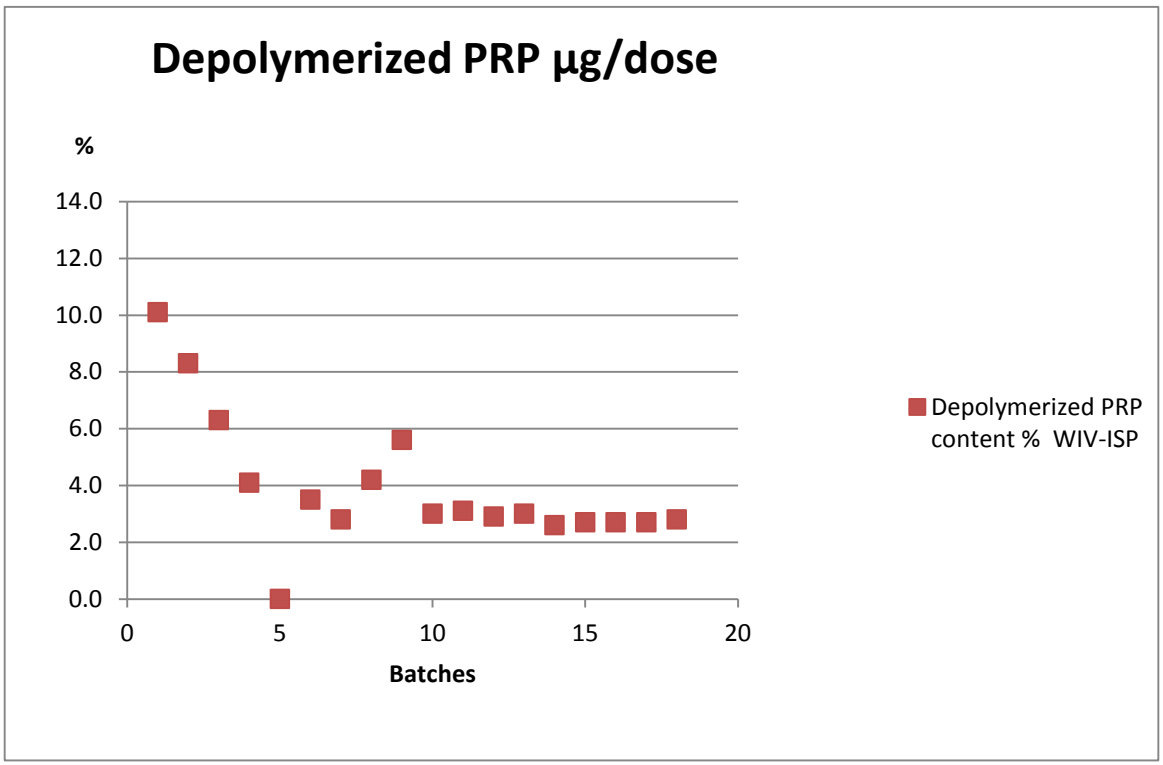
Molecular size distribution on the Hib bulk conjugate alone (not yet formulated with other antigens)



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).



Depolymerized non adsorbed polysaccharide tested on the final container. A trend appeared and can be explained by the new implemented method performed by different technicians. It stabilized after 5-6 batches (zero result means lower than the Limit of Quantitation)



D.4.4. Conclusion

The number of batches is small to assess consistency but one can says it is quite stable for 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.

D.5. Pneumococcal vaccine: monovalent conjugated bulks

D.5.1 Product description

This multivalent vaccine is directed against pneumococcal invasion (S*treptococcus 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.

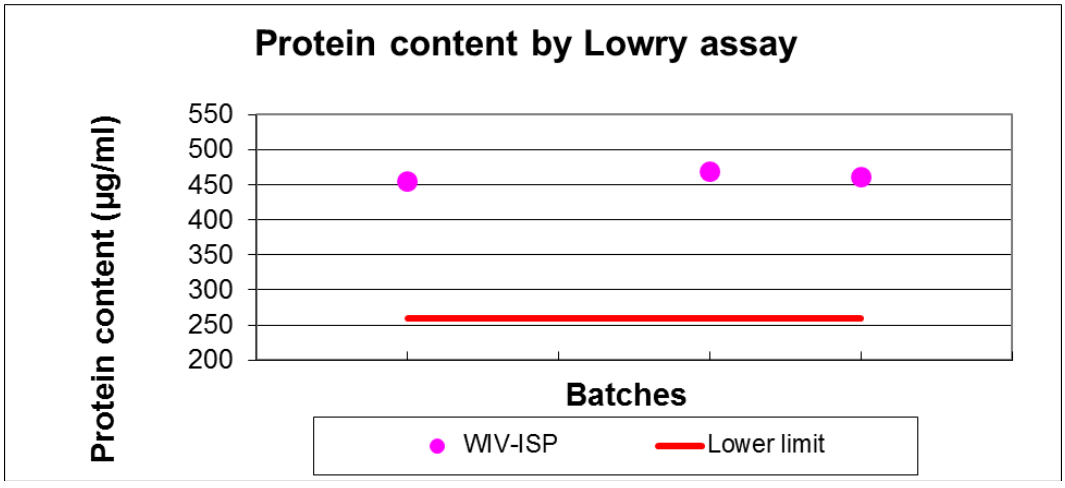
Vaccine type	Status	Release information's		
Pneumococcal Monovalent bulk conjugate		EU	Non-EU	Total
	Submitted	77	0	77
	Tested	70	0	70
	Released	73	0	73
	Rejected	0	0	0

D.5.3 Results

D.5.3.1 PS1-PD

Protein content

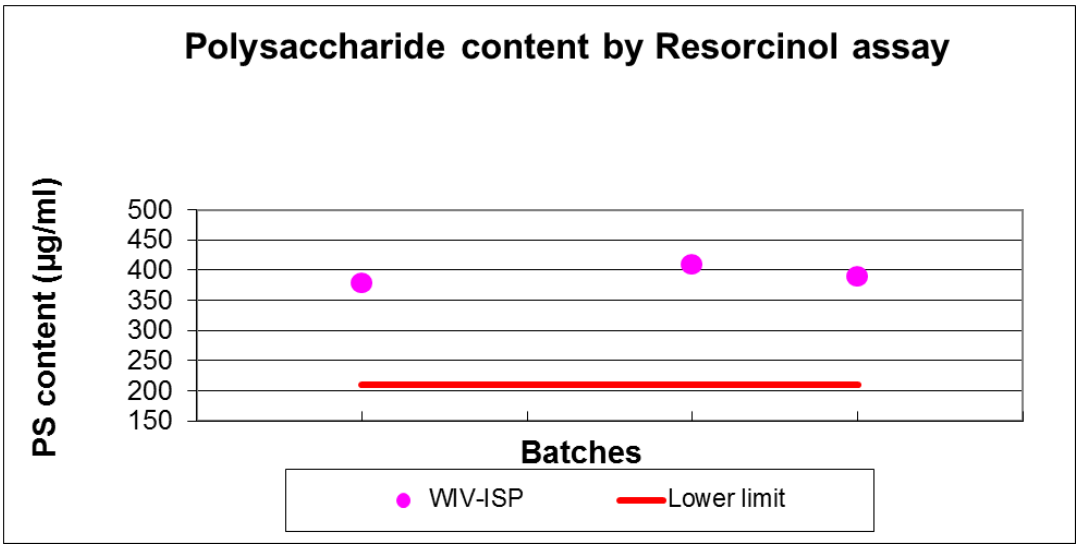
In the following graph, it is clear that the results from WIV-ISP are lower than the consistency limits of the manufacturer. However, it should be mentioned that the number of batches tested is limited and that many batches were tested in the same experimental session. No OOS data were obtained.



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

Polysaccharide content

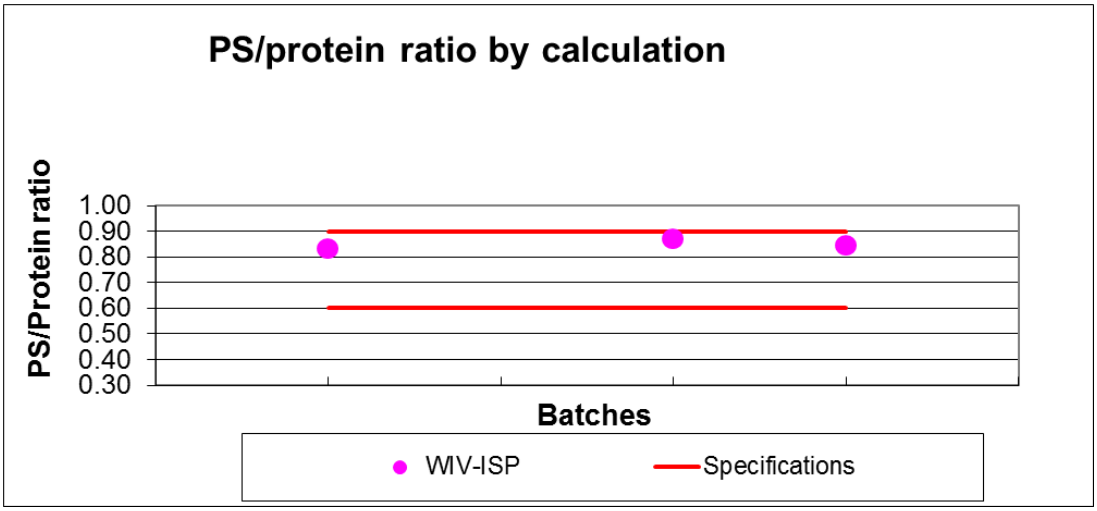
For the determination of the polysaccharide content, the graph below shows that the values of PS content in PS1-PD bulks are well aligned with the between the manufacturer's consistency.



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

PS/protein ratio

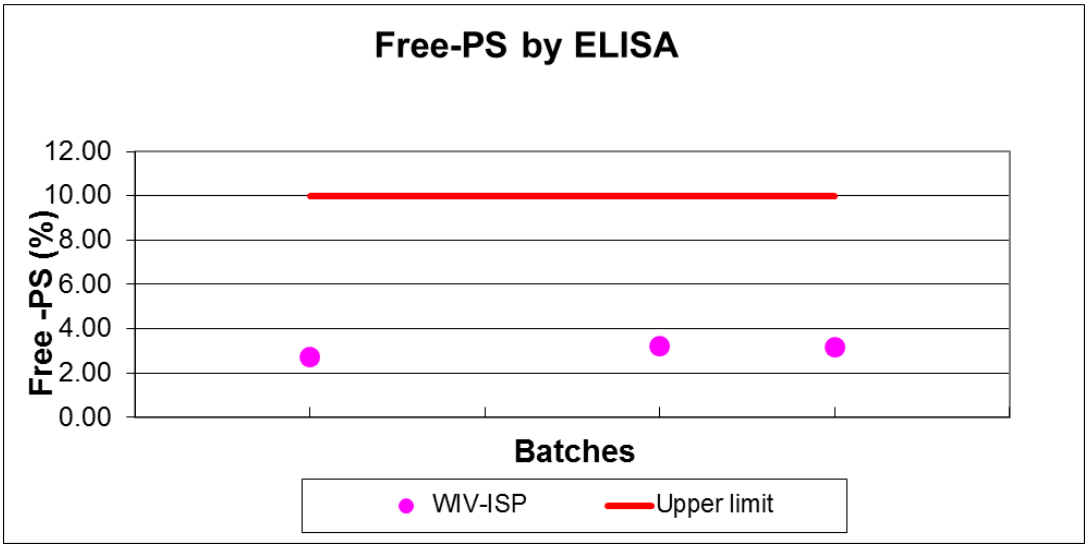
The WIV-ISP results for the ratio are at the upper limit of the consistency. Since the WIV-ISP data for PS or those batches are somewhat higher and those for protein content somewhat lower, it is not surprising that the results for the ratio are also higher. No OOS data were obtained.



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

Free-PS

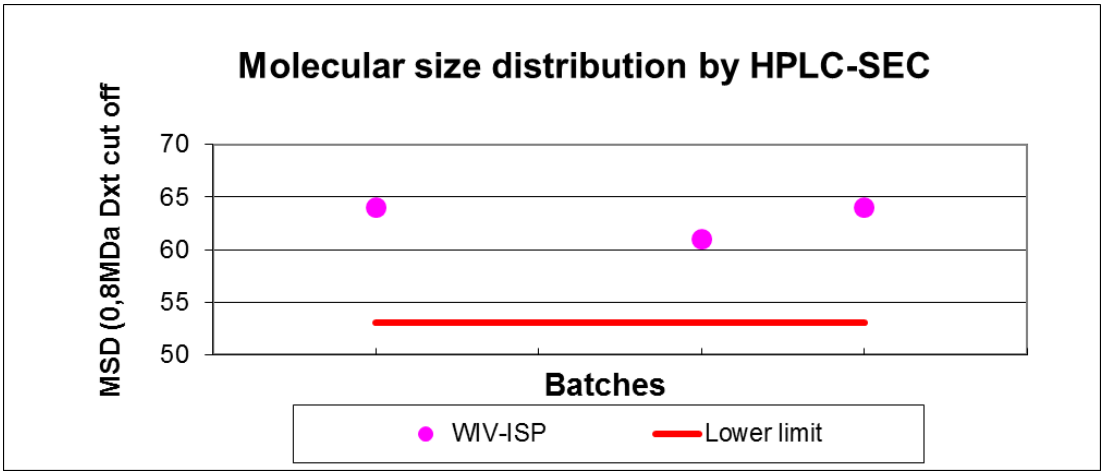
The results in the following graph demonstrate that the results for free-PS at WIV-ISP very well fit into the manufacturer's consistency. No OOS results were obtained.



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

Molecular size distribution

The values obtained by WIV-ISP are for all the batches lower than the consistency limits. .As the molecular size distribution results is dependant from the age of the bulk and testing was performed later at WIV-ISP testing by the applicant, this results are not unexpected. No OOS results were obtained.

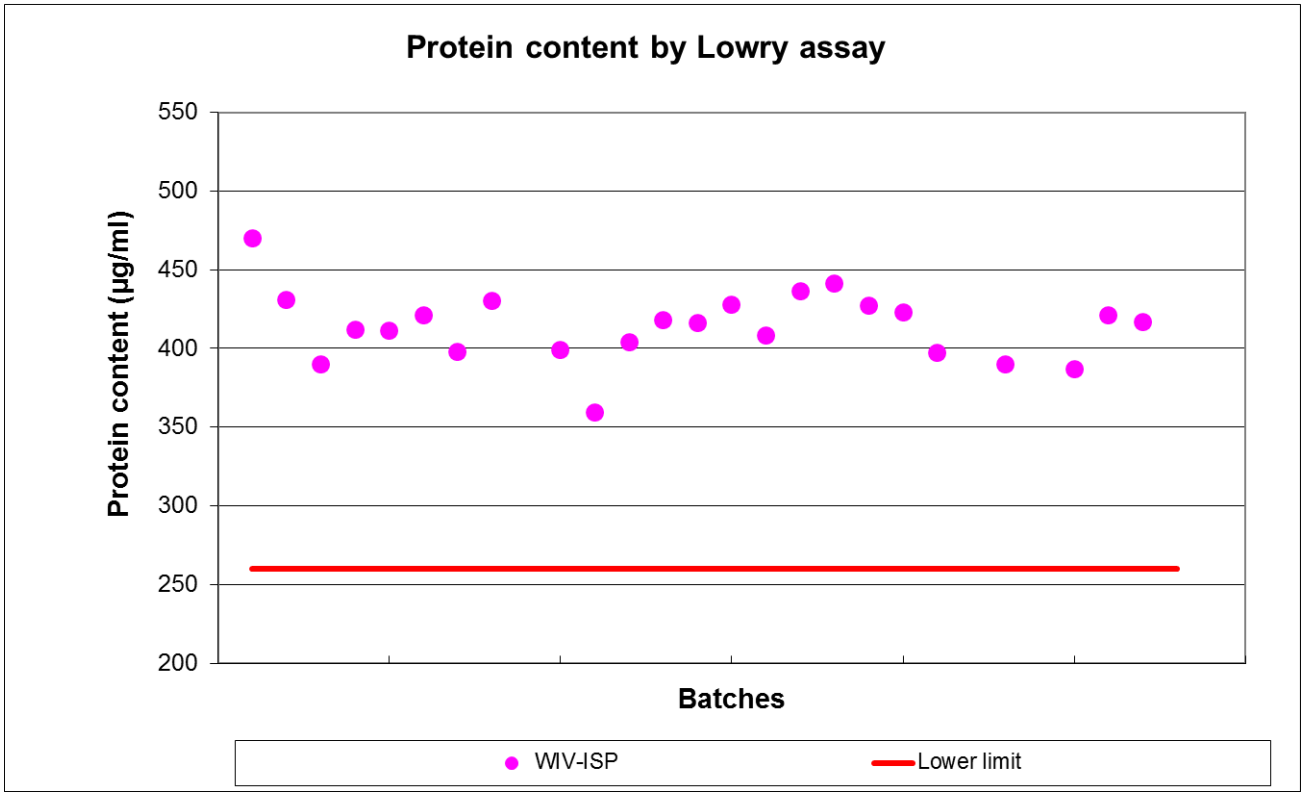


Years	Mean	Mean + 2*SD	Mean - 2*SD
2012 (N = 6)	68	65	70
2013 (N = 9)	66	64	68

D.5.3.2 PS4-PD

Protein content

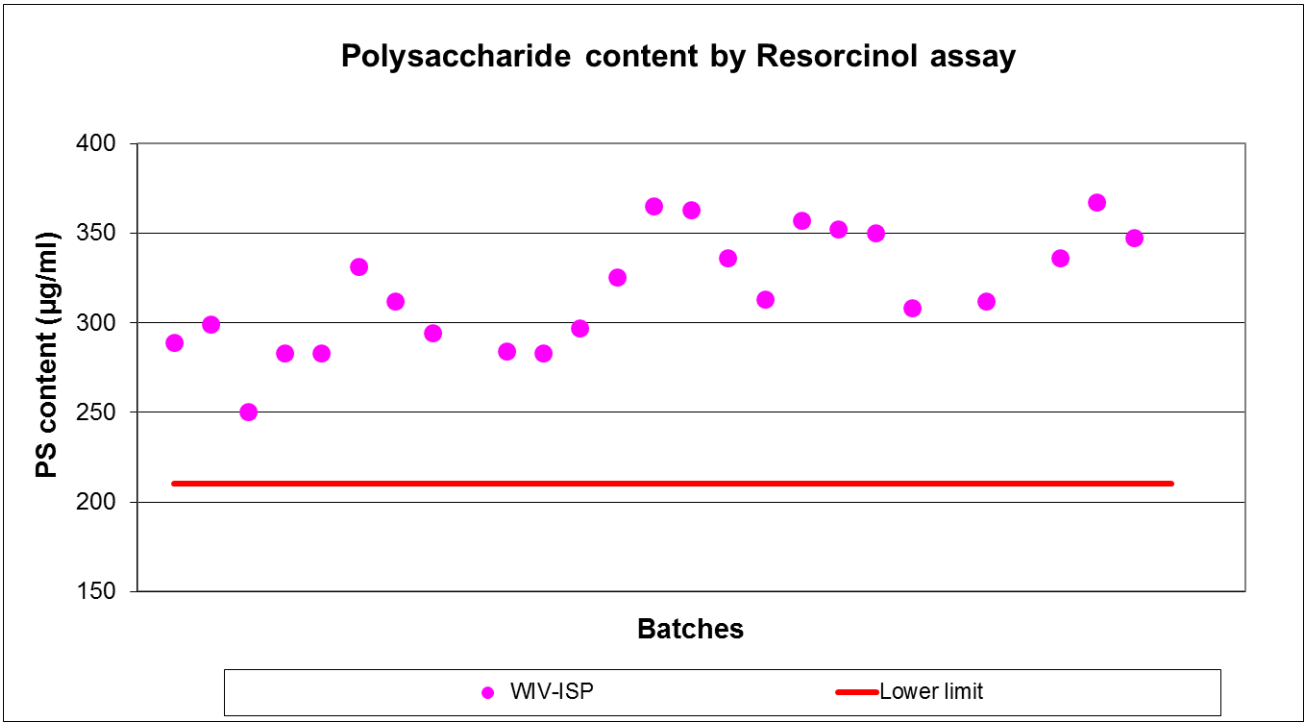
From the graph below, it is clear that the results obtained for protein content fall at the lower limit of the consistency, but no OOS data were obtained.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2011 (N = 24)	489	442	535
2012 (N = 25)	463	396	529
2013 (N = 47)	441	411	472

Polysaccharide content

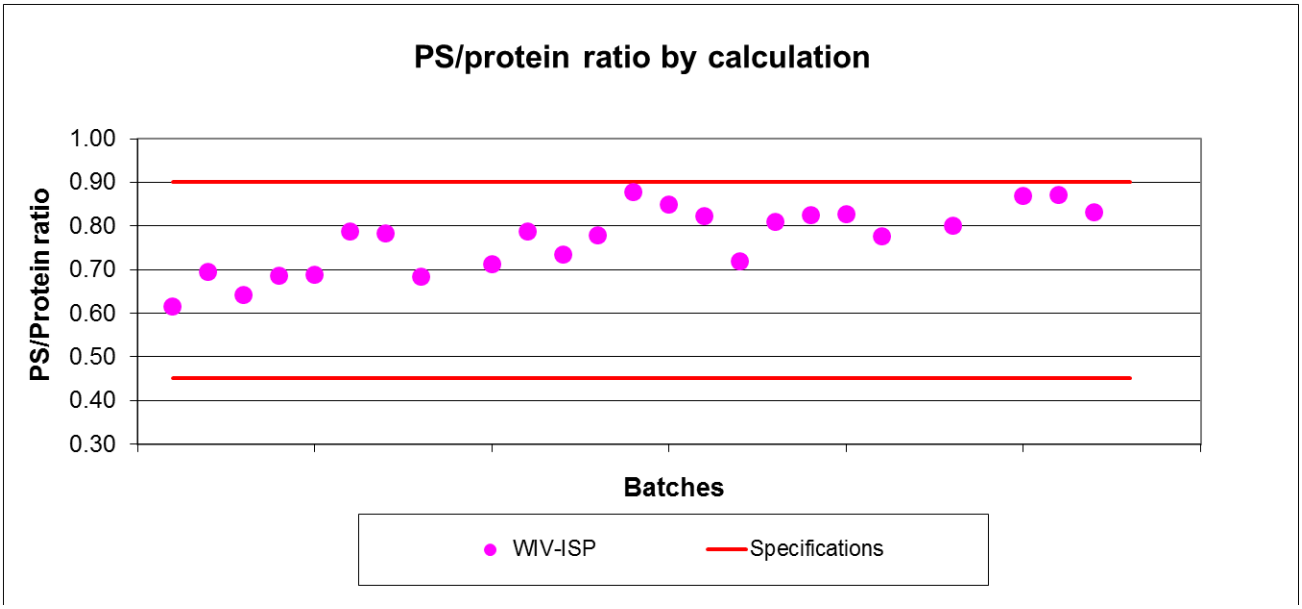
The polysaccharide content is also determined at WIV-ISP. From the graph below, it can be concluded that there is some more variability for the results obtained at WIV-ISP as compared to the consistency limits provided by the manufacturer. It should be taken into account that the resorcinol assay is a highly variable assay. No OOS data were obtained.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2011 (N = 24)	320	295	345
2012 (N = 25)	313	272	355
2013 (N = 47)	306	284	324

PS/protein ratio

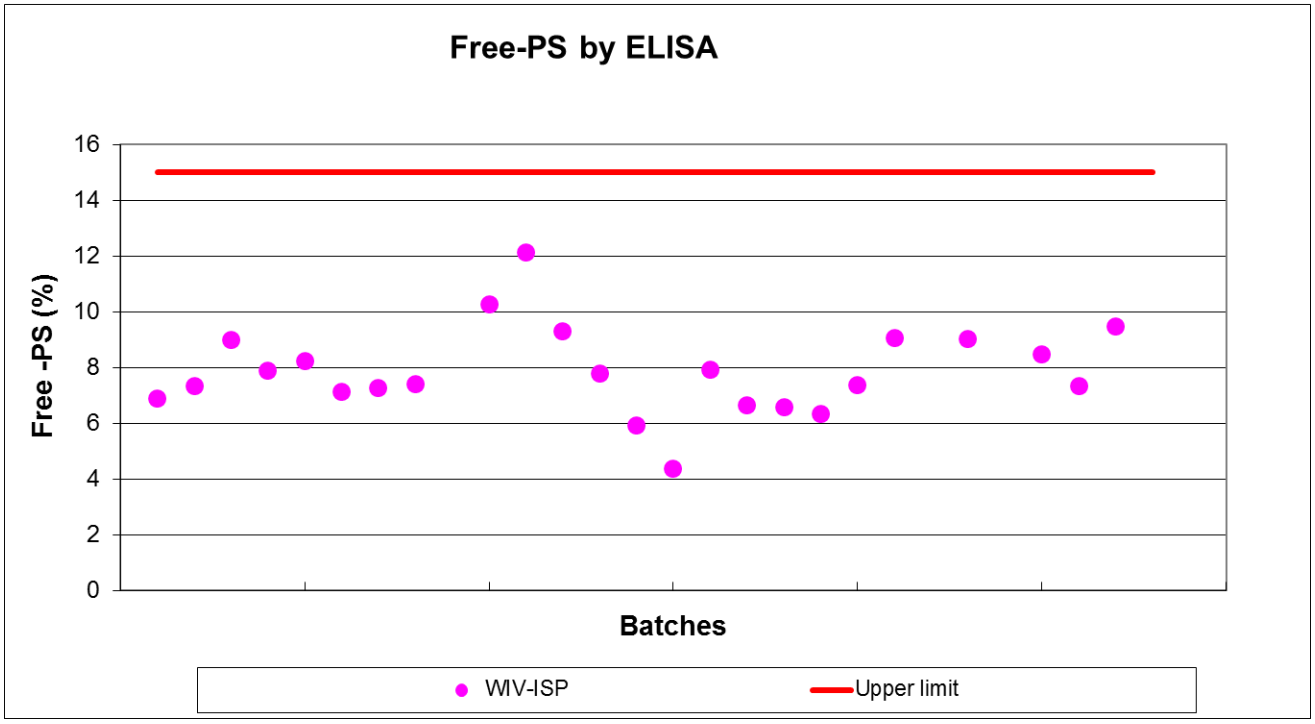
The graph below shows that the values at WIV-ISP are towards the upper consistency limit and more variable. The result is a ratio of PS content by protein content, and since PS content was higher at WIV-ISP and protein content lower, it is logical that the ratio is higher. No OOS data were obtained.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2011 (N = 24)	0.66	0.59	0.73
2012 (N = 25)	0.68	0.59	0.77
2013 (N =47)	0.69	0.64	0.75

Free-PS

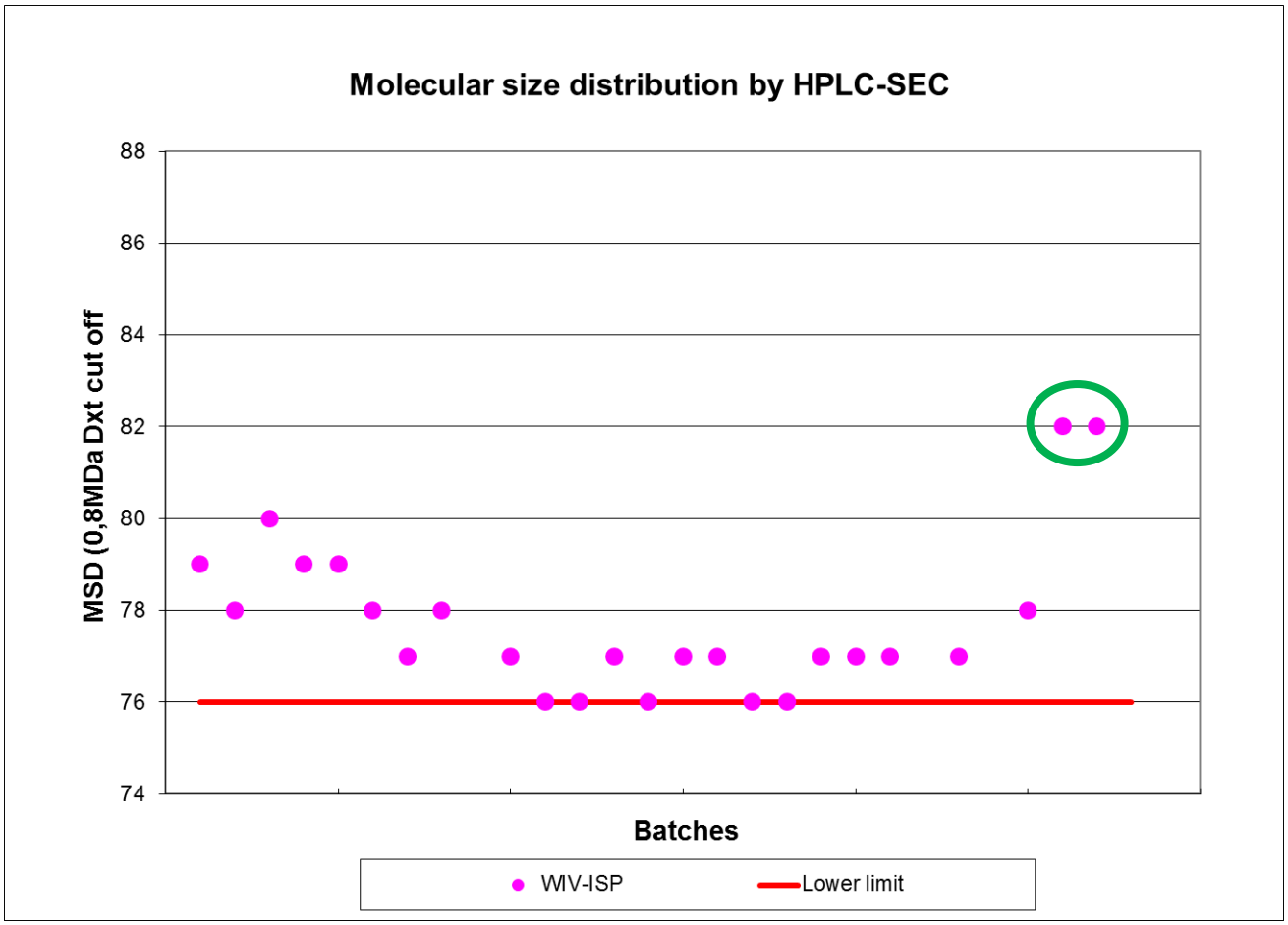
By comparing the WIV-ISP test results with the manufacturer's consistency, the overall picture for free-PS is that the levels obtained at WIV-ISP are higher than the levels obtained by the manufacturer. It is reasonable to believe that free-PS is a parameter which can be influenced by the age of the 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
2011 (N = 24)	4.0	2.6	5.4
2012 (N = 25)	4.2	1.2	7.2
2013 (N = 47)	5.6	3.4	7.8

Molecular size distribution

Since the MSD result is dependant of the age of the bulk some delay in testing might explain differences. 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. The last two results (green circle) were obtained with the new validated 5 dextran method (at WIV-ISP only), were a better correlation can be found between the manufacturer and WIV-ISP.

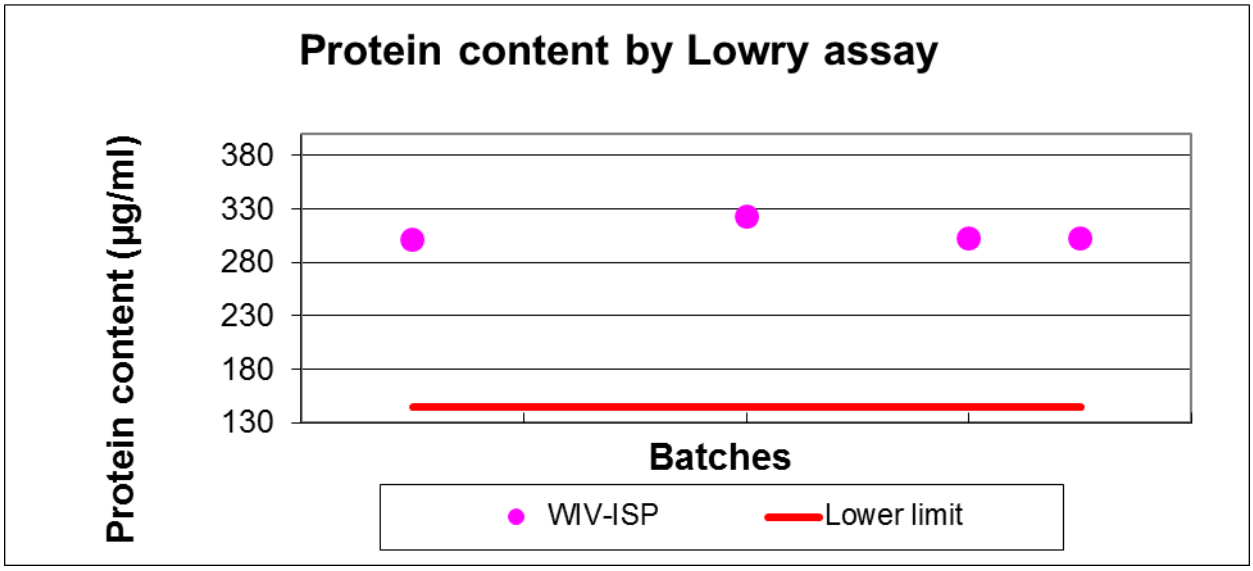


Years	Mean	Mean - 2*SD	Mean + 2*SD
2011 (N = 24)	85	84	87
2012 (N = 25)	83	80	86
2013 (N = 47)	83	81	85

D.5.3.3 PS5-PD

Protein content

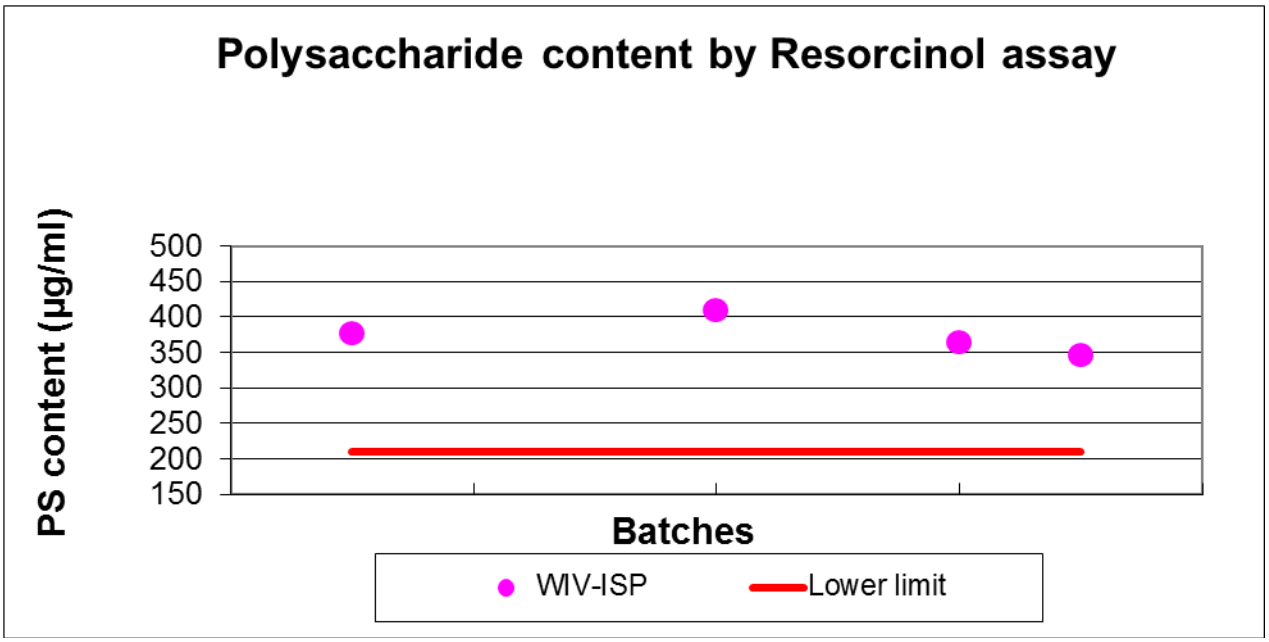
In the following graph, it is clear that the results of WIV-ISP are lower than the consistency provided by the manufacturer. It should be mentioned that the number of batches tested is limited and that many batches were tested in the same experimental session. No OOS data were obtained.



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

Polysaccharide content

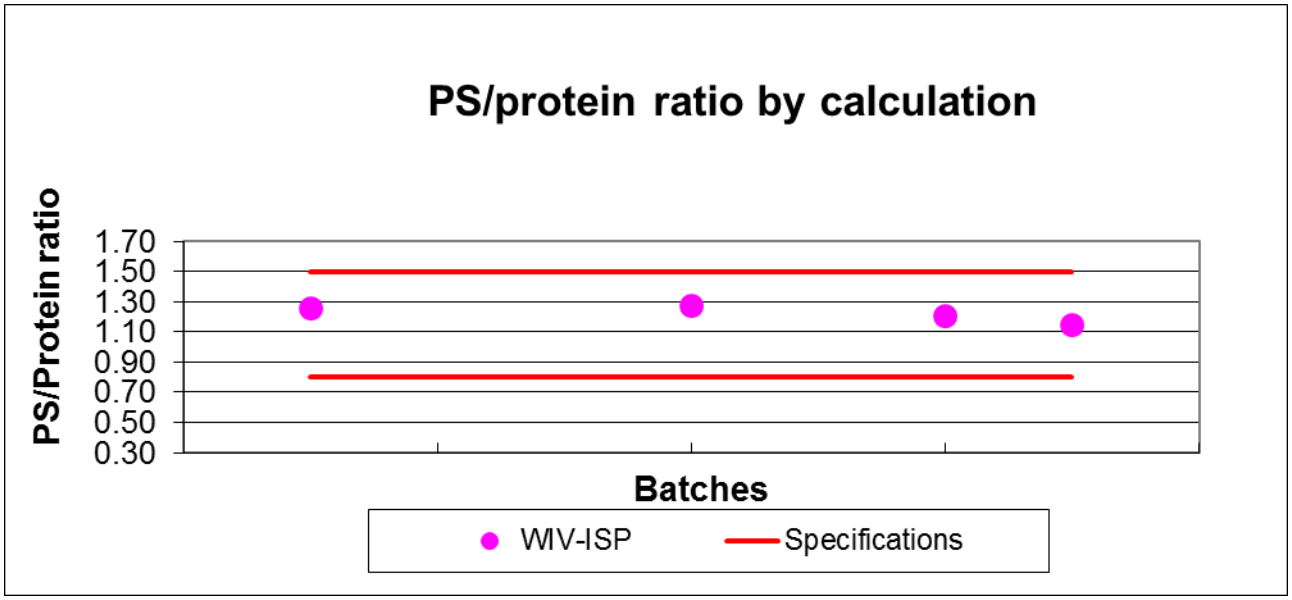
For the polysaccharide content, the graph below demonstrates that the values of PS content in PS5-PD bulks are well aligned with the manufacture's consistency. No OOS data were obtained.



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

PS/protein ratio

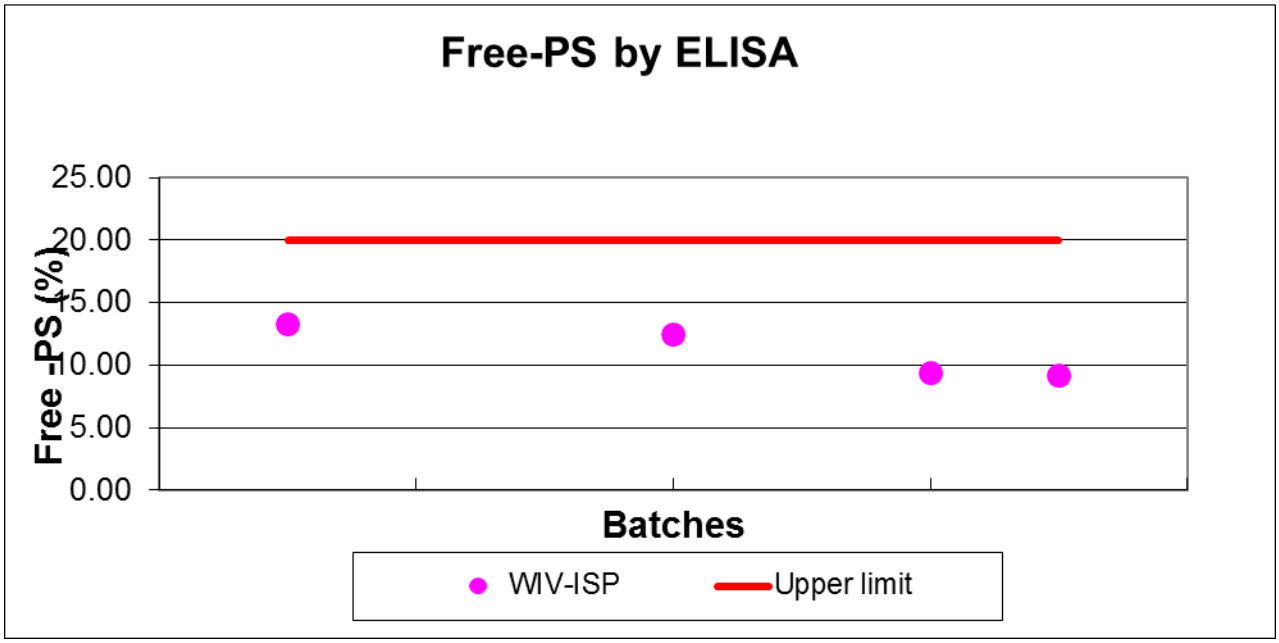
The WIV-ISP results for the ratio fit very well into the manufacturer's consistency limits. No OOS data were obtained.



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

Free-PS

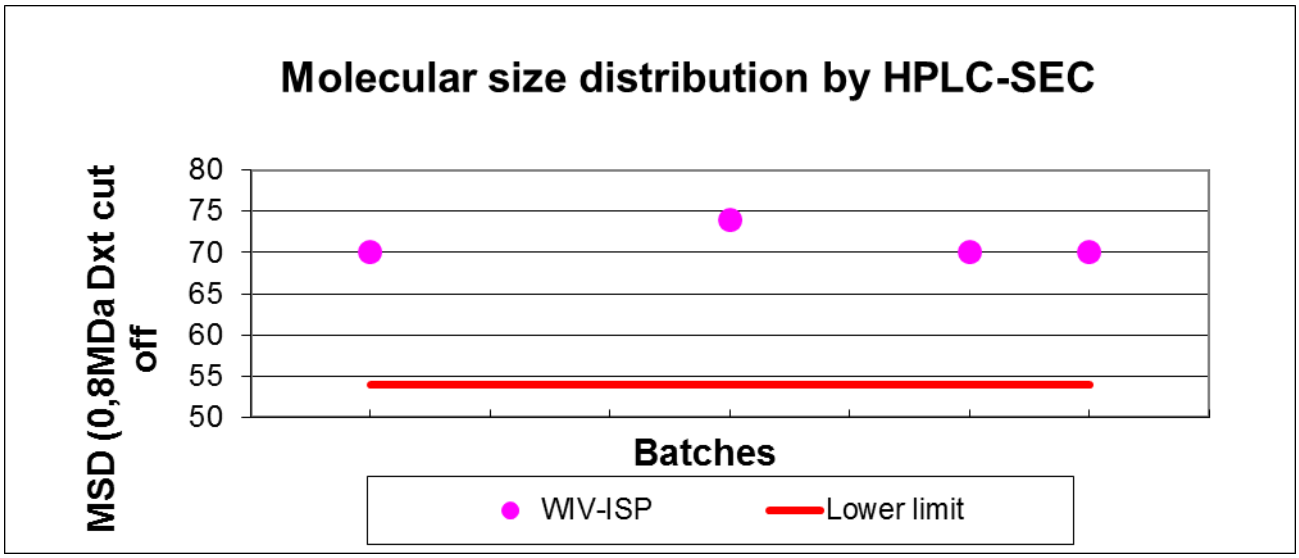
The results in the following graph demonstrate that the WIV-ISP results are corresponding to the consistency obtained by the manufacturer. No OOS results were obtained.



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

Molecular size distribution

The values obtained by WIV-ISP are for all the batches towards the lower consistency limit. The fact that testing was performed after release testing by the applicant makes that 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.

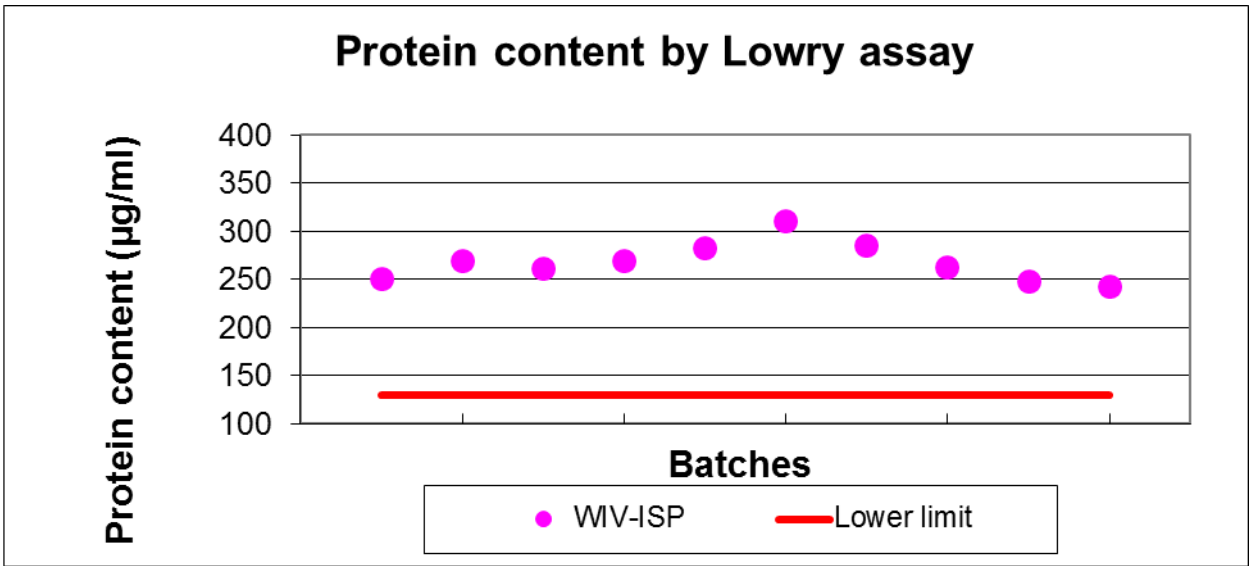


Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 8)	73	74	76
2013 (N = 18)	75	73	78

D.5.3.4 PS6B-PD

Protein content

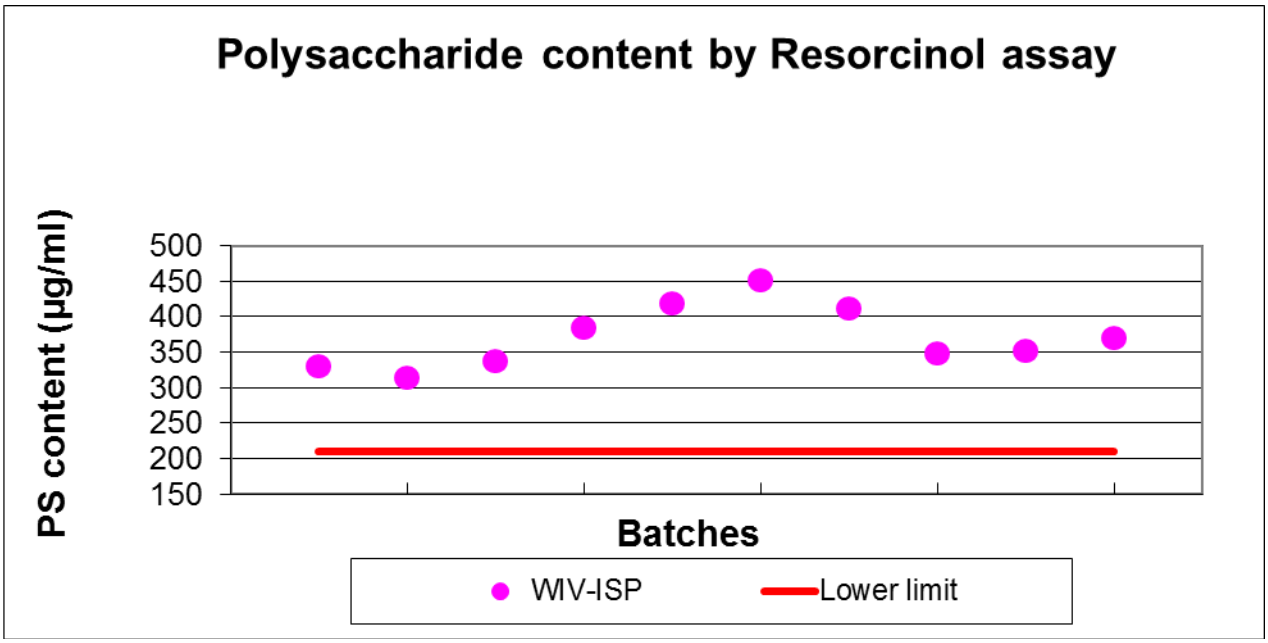
The results obtained by WIV-ISP are very well aligned with the manufacturer's consistency.



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

Polysaccharide content

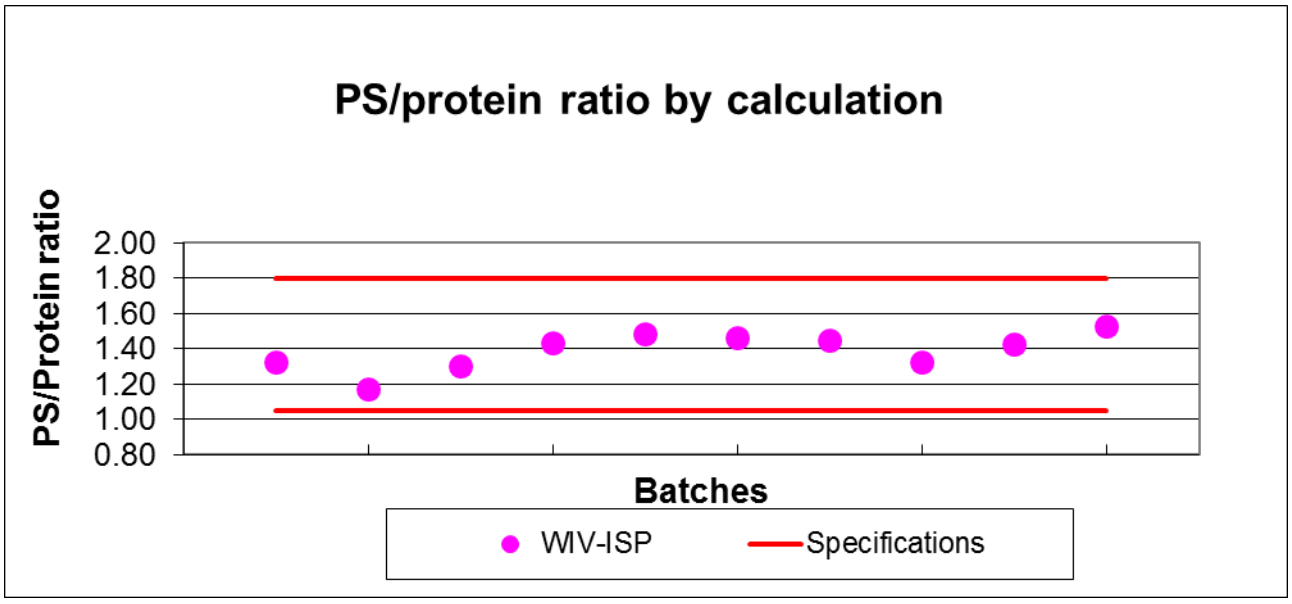
The results obtained at WIV-ISP are towards the upper consistency limit. The assay is variable and the results obtained by the manufacturer are based on a mean of two determinations, while at the OMCL's only one determination per batch sis performed. No OOS data were generated.



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

PS/protein ratio

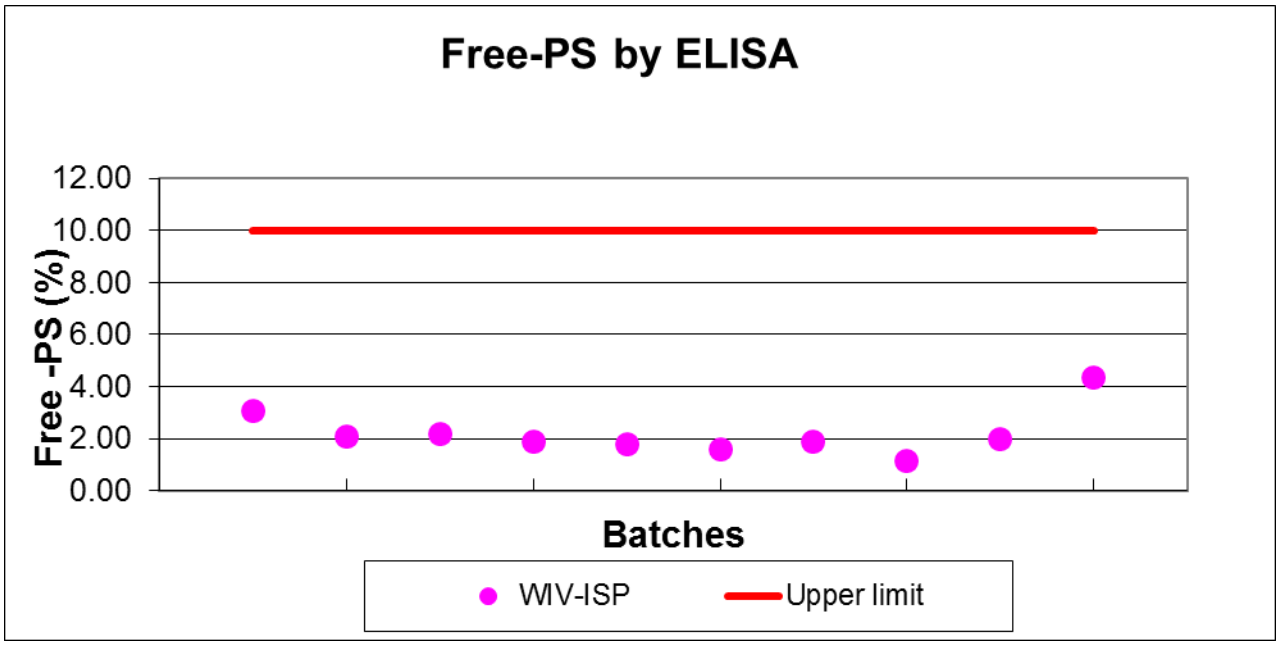
The results obtained by WIV-ISP fit into the manufacturer's consistency.



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

Free-PS

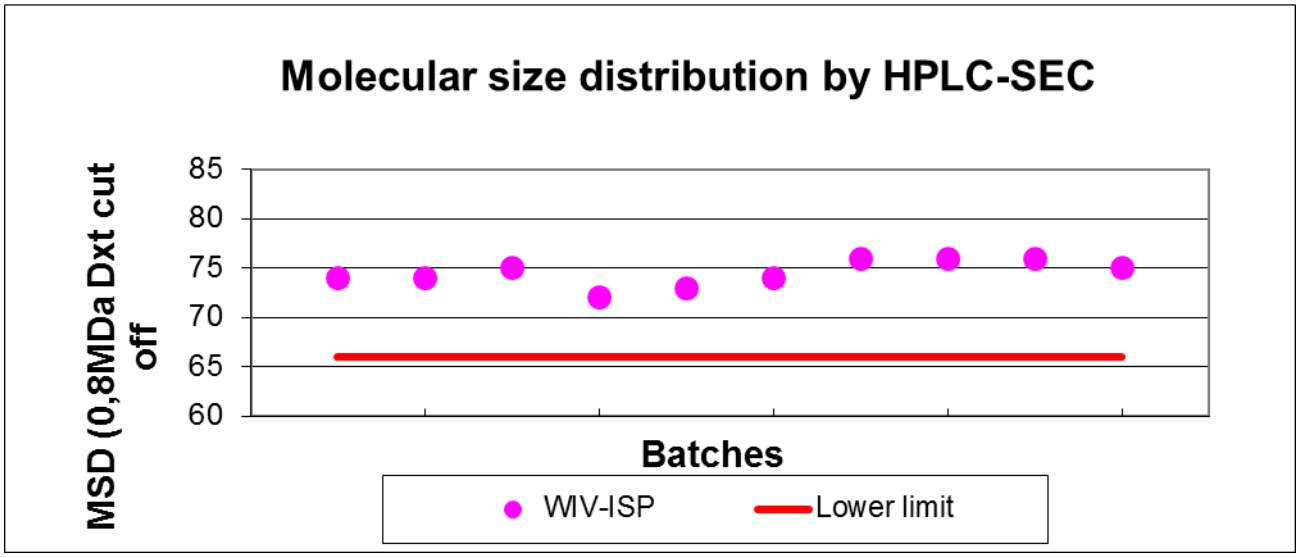
Most of the results obtained by WIV-ISP are within the consistency limits and far below the upper specification limit. The increases values are due to delay in testing and since free-PS increases with the age of the bulk, the results are not abnormal.



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

Molecular size distribution

The results obtained at WIV-ISP all meet the specifications and are somewhat towards the lower consistency limits. The delay in testing is probably the cause for the lower results.

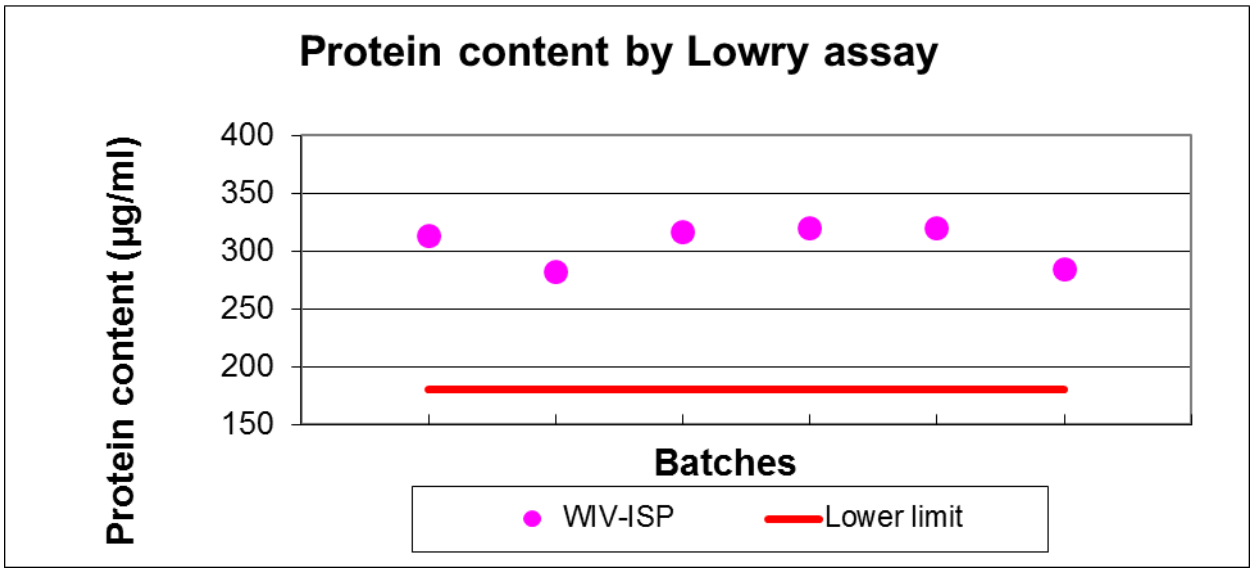


Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 17)	77	74	79
2013 (N = 3)	76	75	77

D.5.3.5 PS7F-PD

Protein content

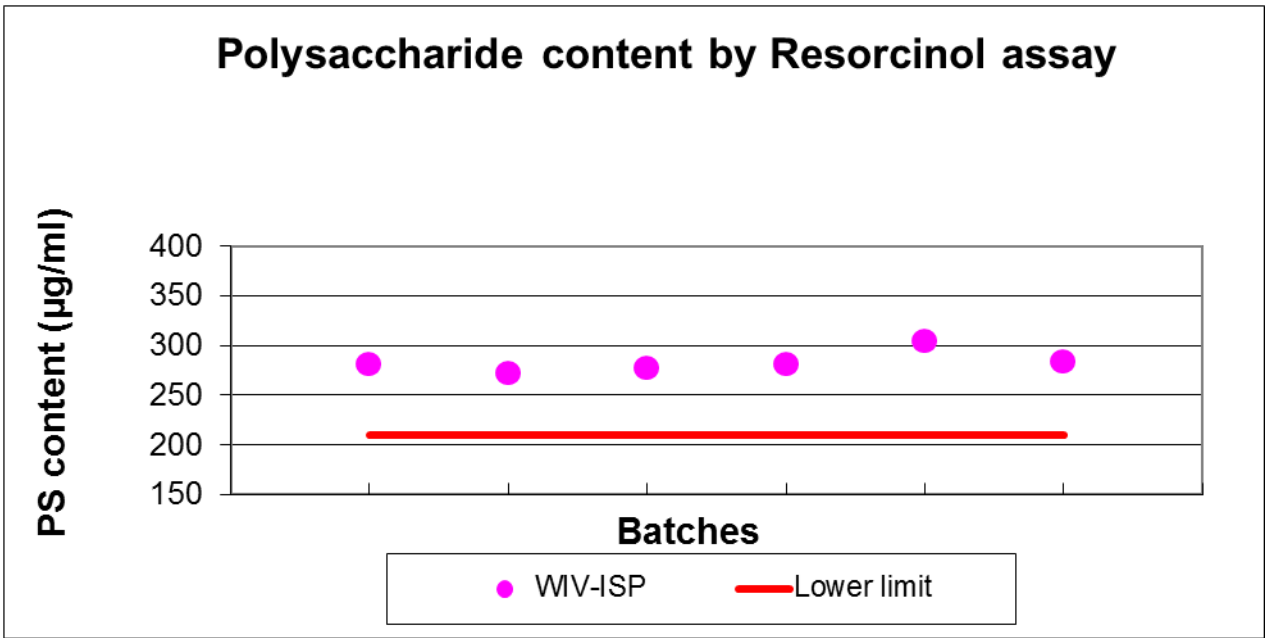
The results obtained at WIV-ISP are within the specifications and are very well aligned with the manufacturer's consistency.



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

Polysaccharide content

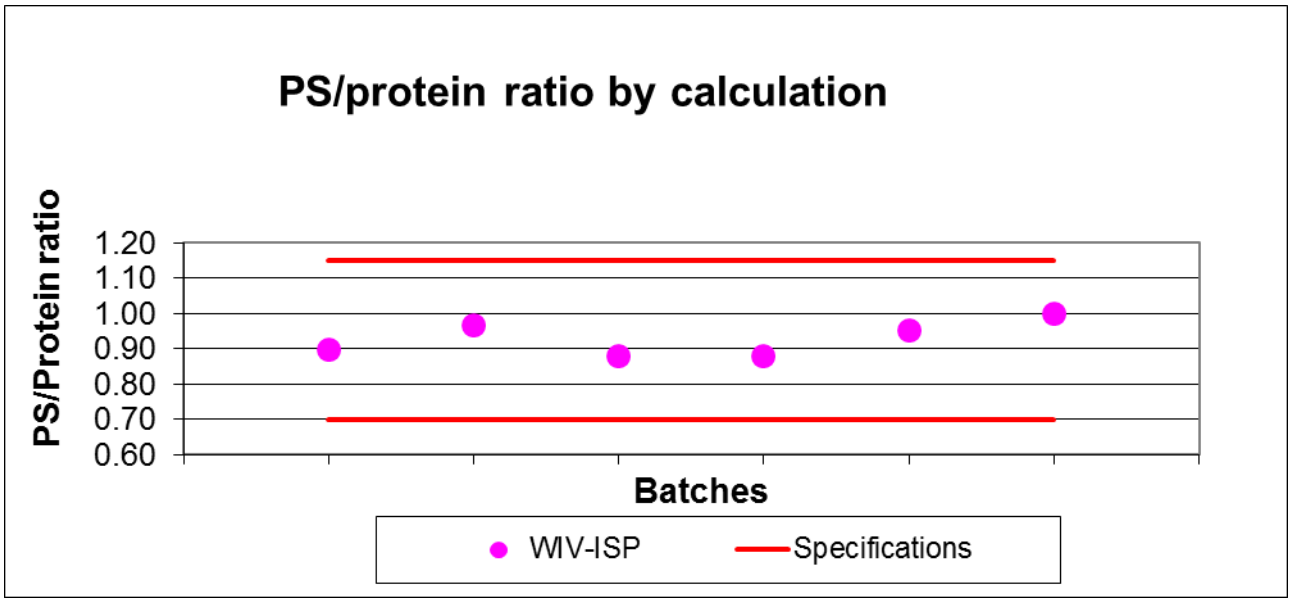
For the determination of the polysaccharide content, the graph below shows that the values of PS content in PS7F-PD bulks are well aligned with the manufacture's consistency.



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

PS/protein ratio

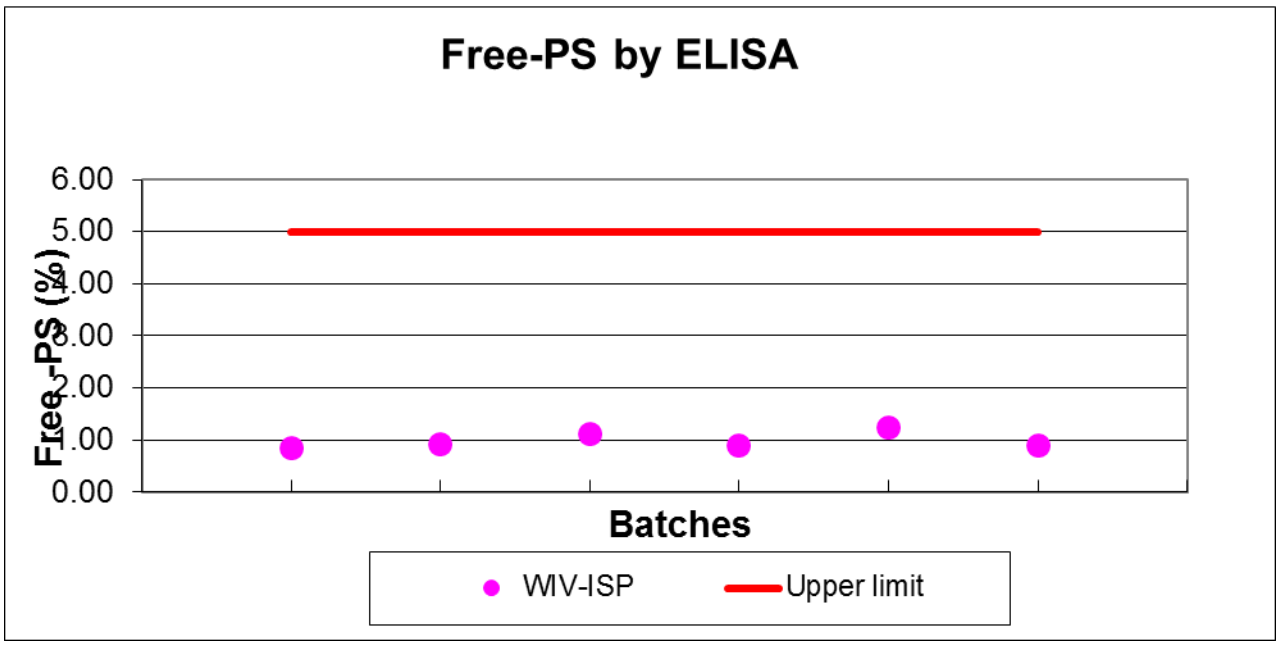
The WIV-ISP results for the ratio are comparable with those obtained by the manufacturer. No OOS data were obtained.



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

Free-PS

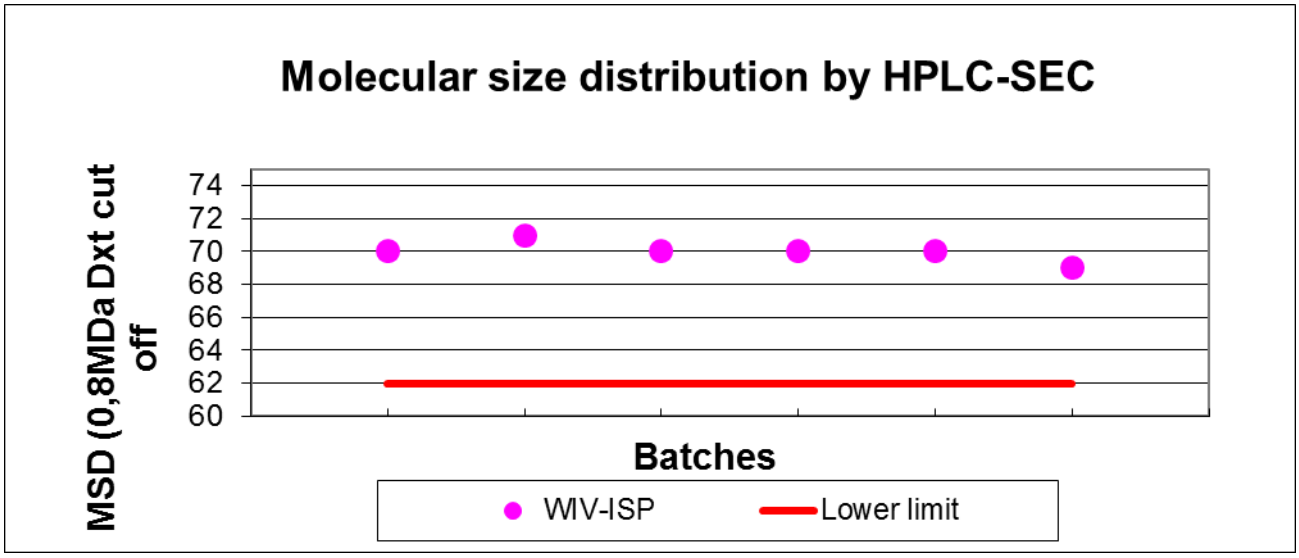
The results in the following graph demonstrate that the results for free-PS at WIV-ISP fit within the manufacturer's consistency. No OOS results were obtained.



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

Molecular size distribution

The values obtained by WIV-ISP all met the specifications and are well aligned with the manufacturer's consistency.

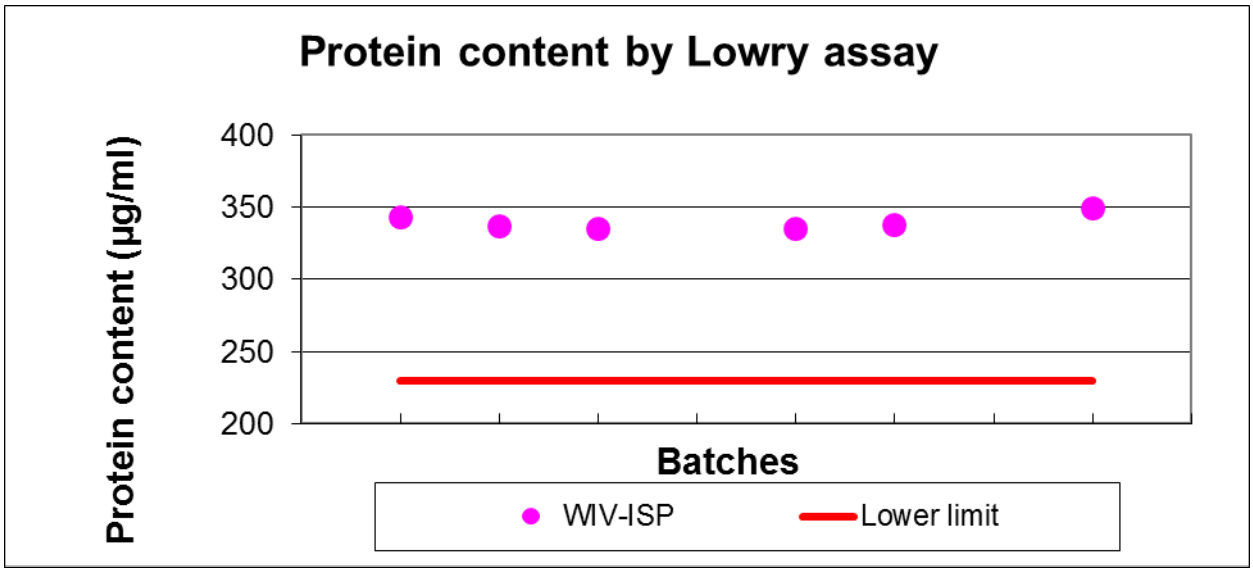


Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 2)	72	70	73
2013 (N = 12)	70	69	71

D.5.3.6 PS9V-PD

Protein content

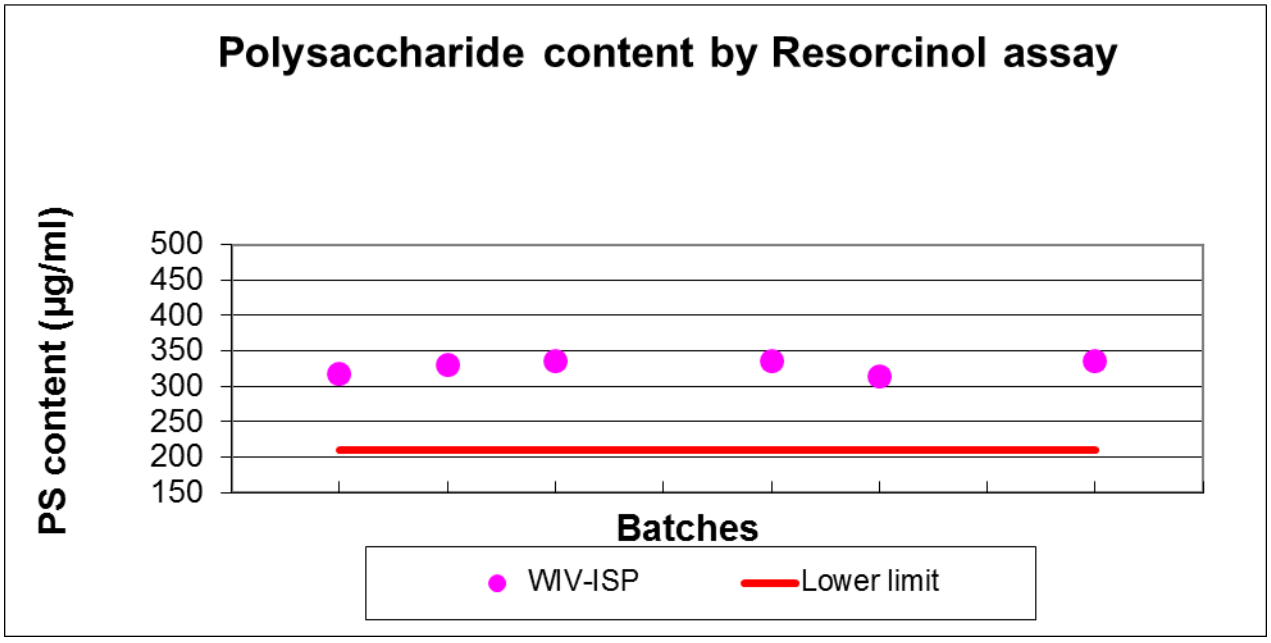
It is clear that the results of WIV-ISP are towards the lower consistency limit, but the number of batches tested is limited and no OOS data were obtained.



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

Polysaccharide content

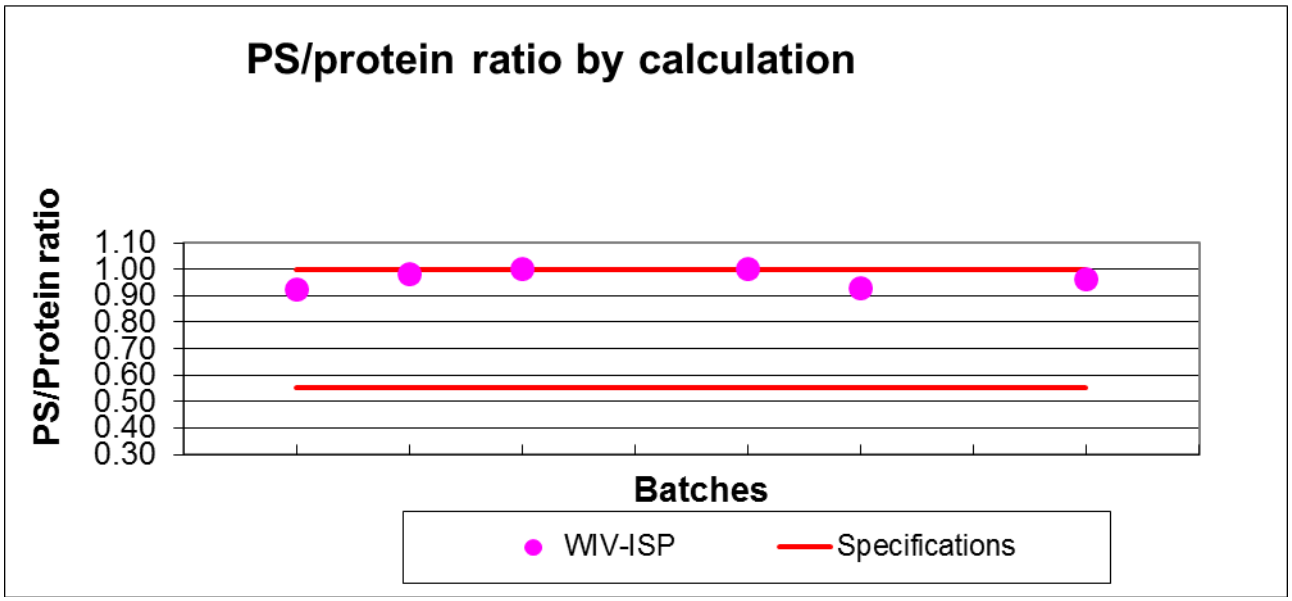
For the determination of the polysaccharide content, the graph below shows that the values of PS content in PS9V-PD bulks are well aligned with the manufacturer's consistency. No OOS data were obtained.



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

PS/protein ratio

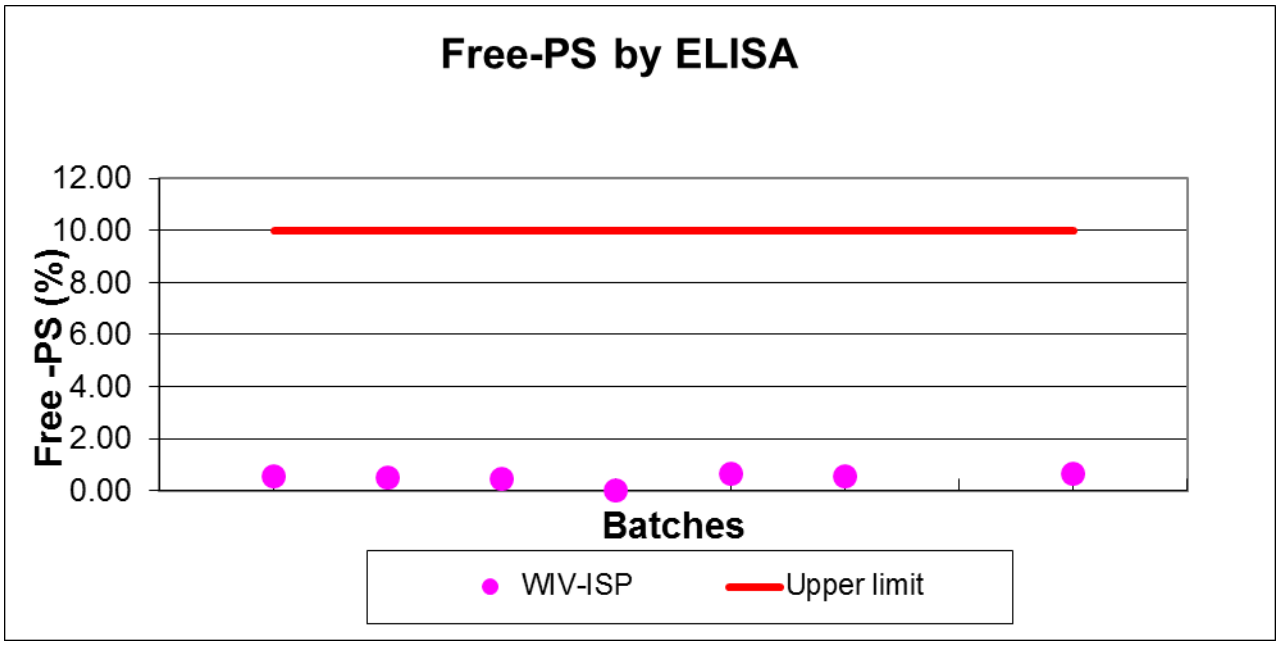
The WIV-ISP results for the ratio are higher and very close to the upper limit. This is due to the fact that polysaccharide values are higher and protein values are lower at WIV-ISP. No OOS data were obtained.



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

Free-PS

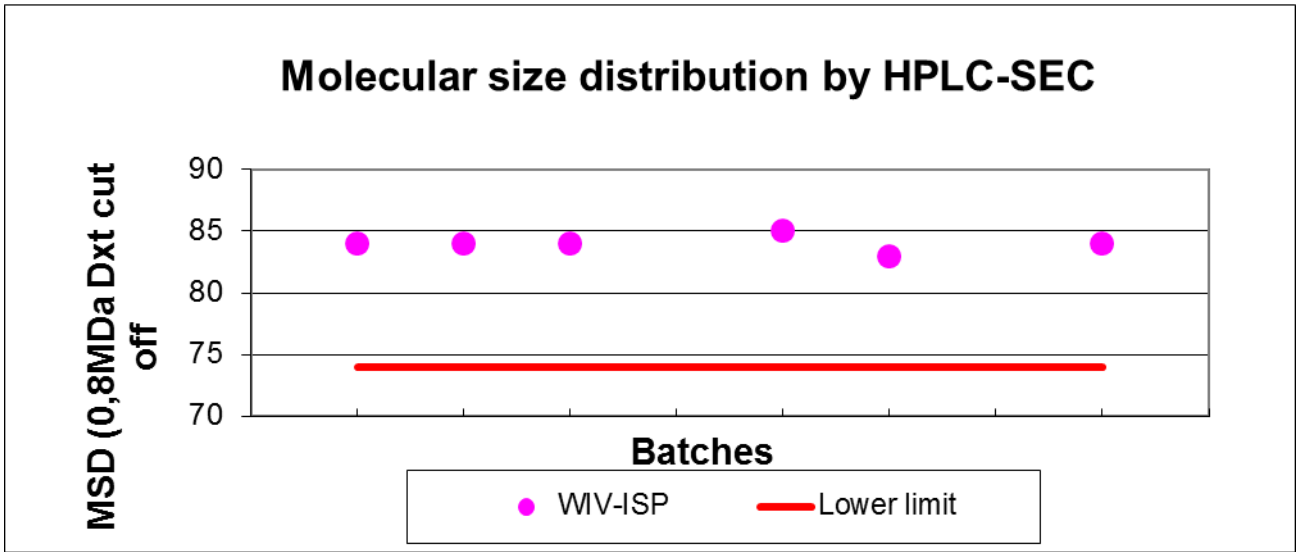
The results in the following graph demonstrate that the results for free-PS at WIV-ISP fit very well into the manufacturer's consistency. No results were obtained.



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

Molecular size distribution

The values obtained by WIV-ISP are highly comparable to the values obtained by the manufacturer. No OOS results were obtained.

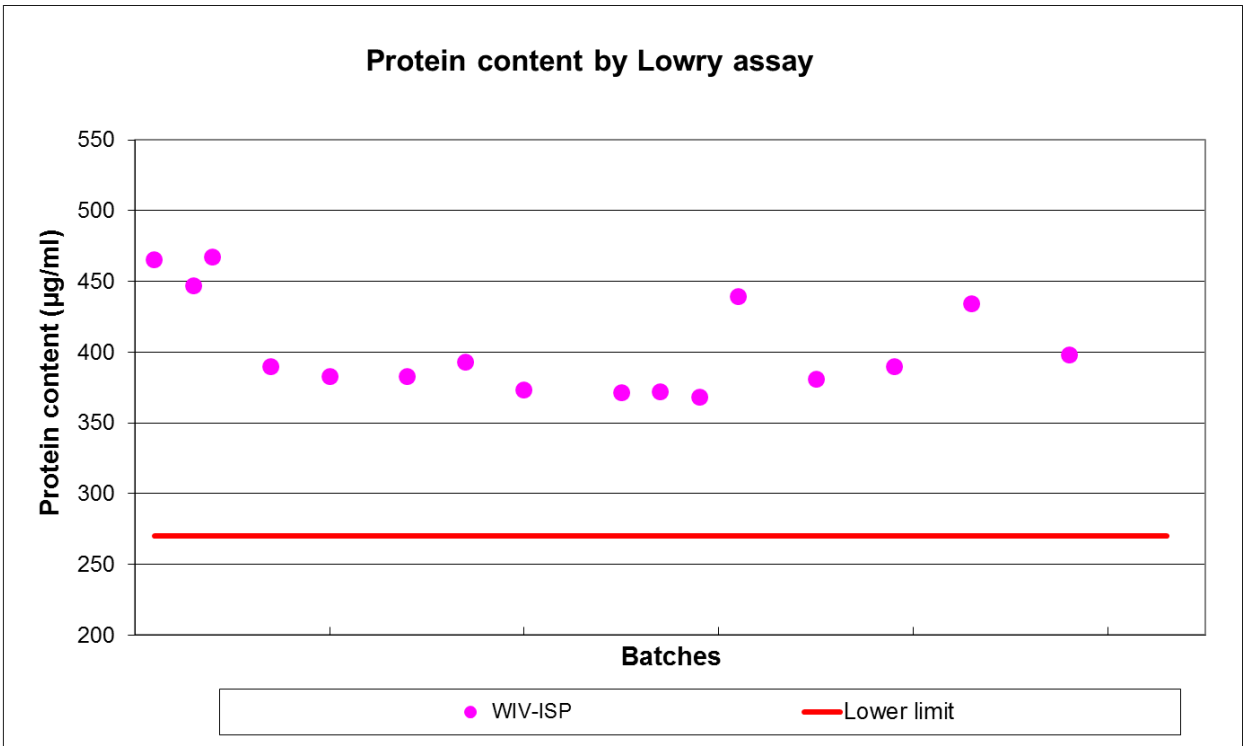


Years	Mean	Mean - 2*SD	Mean + 2*SD
2012 (N = 19)	86	84	88
2013 (N = 14)	85	83	86

D.5.3.7 PS18C-PD

Protein content

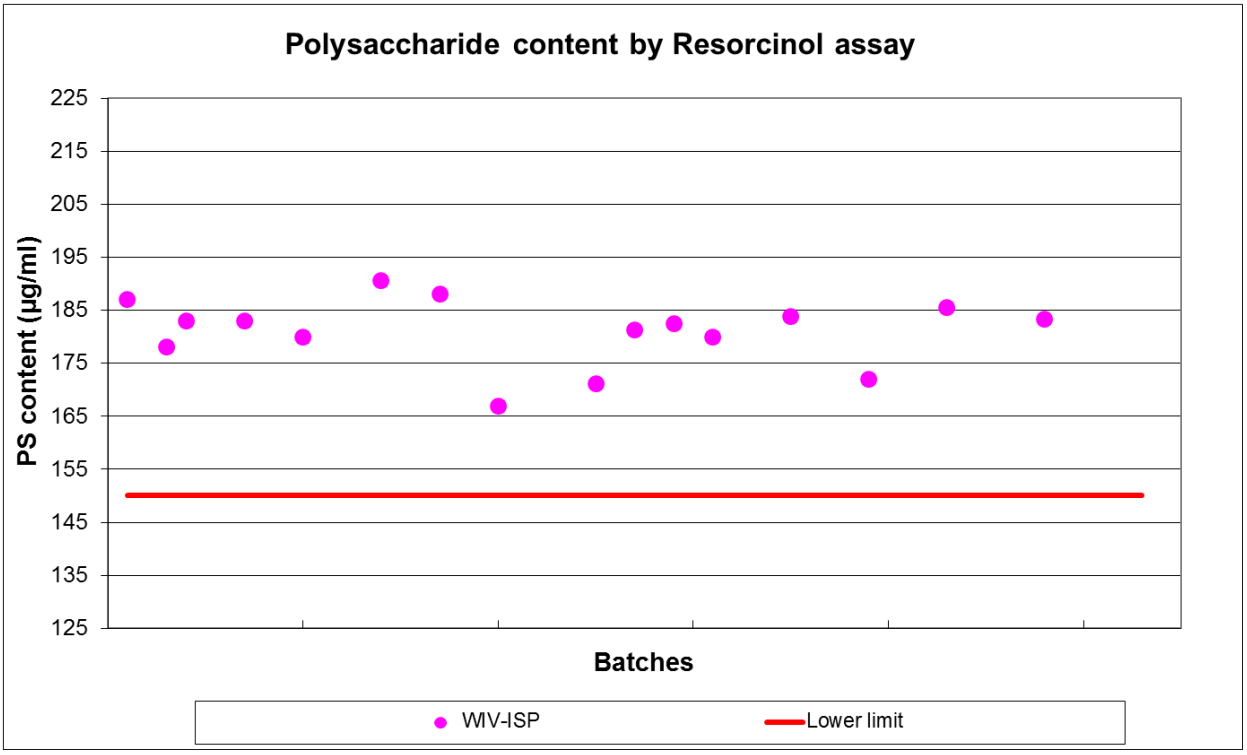
In the following graph, it is clear that the results of WIV-ISP are somewhat towards the lower consistency limit. It should be mentioned that the number of batches tested is limited and that many batches were tested in the same experimental session. No OOS data were obtained.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2011 (N = 21)	446	411	481
2012 (N = 45)	447	357	536
2013 (N = 79)	462	370	554

Polysaccharide content

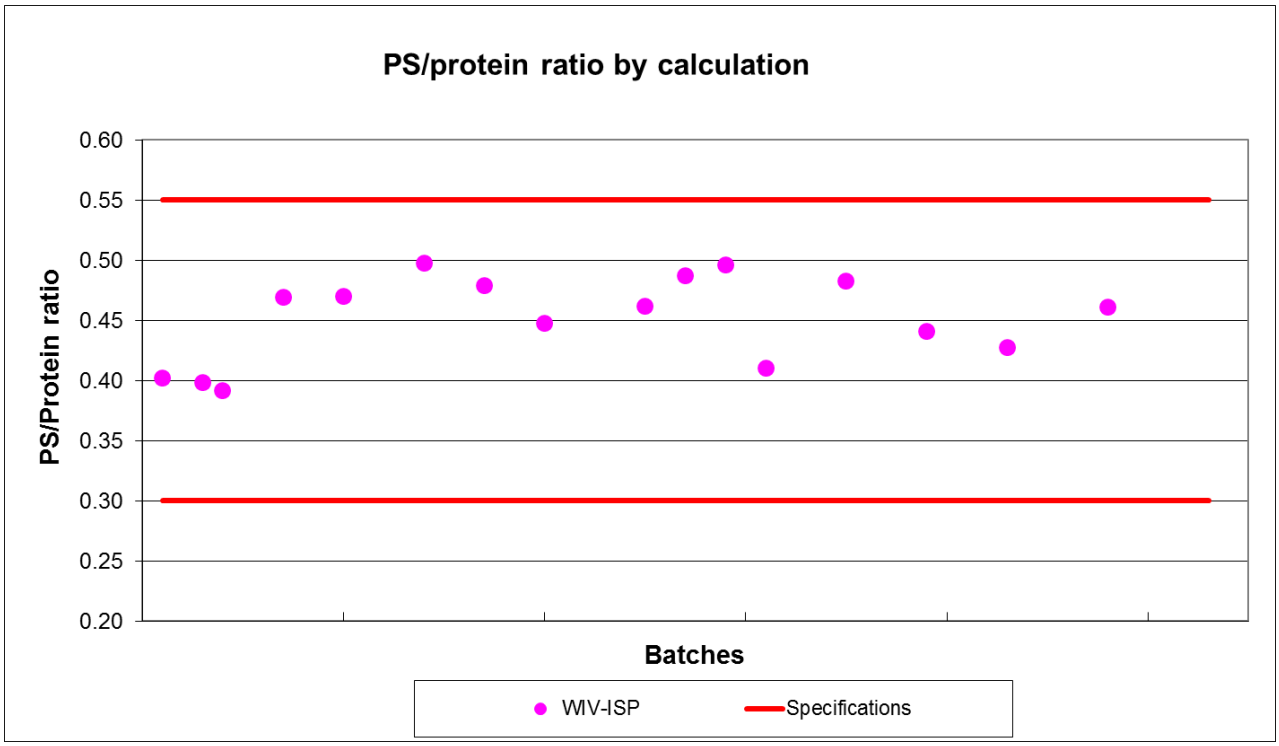
For the determination of the polysaccharide content, the graph below shows that the values of PS content in PS18C-TT bulks are well aligned between WIV-ISP and the manufacturer's consistency. No OOS data were obtained.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2011 (N = 21)	185	163	207
2012 (N = 45)	180	164	197
2013 (N = 79)	181	156	207

PS/protein ratio

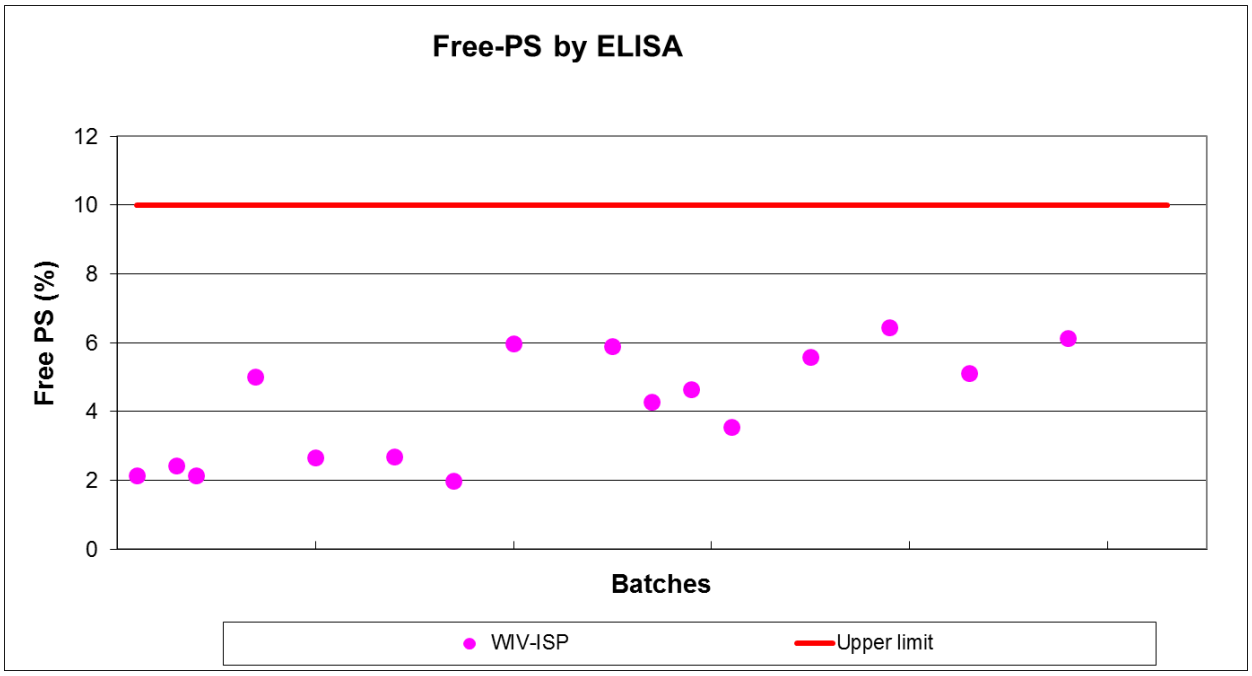
The WIV-ISP results for the ratio are toward the upper consistency limit. Since the WIV-ISP data for PS or those batches are somewhat higher and those for protein content somewhat lower, it is not surprising that the results for the ratio are also higher. No OOS data were obtained.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2011 (N = 21)	0.45	0.32	0.49
2012 (N = 45)	0.41	0.33	0.48
2013 (N = 79)	0.39	0.34	0.45

Free-PS

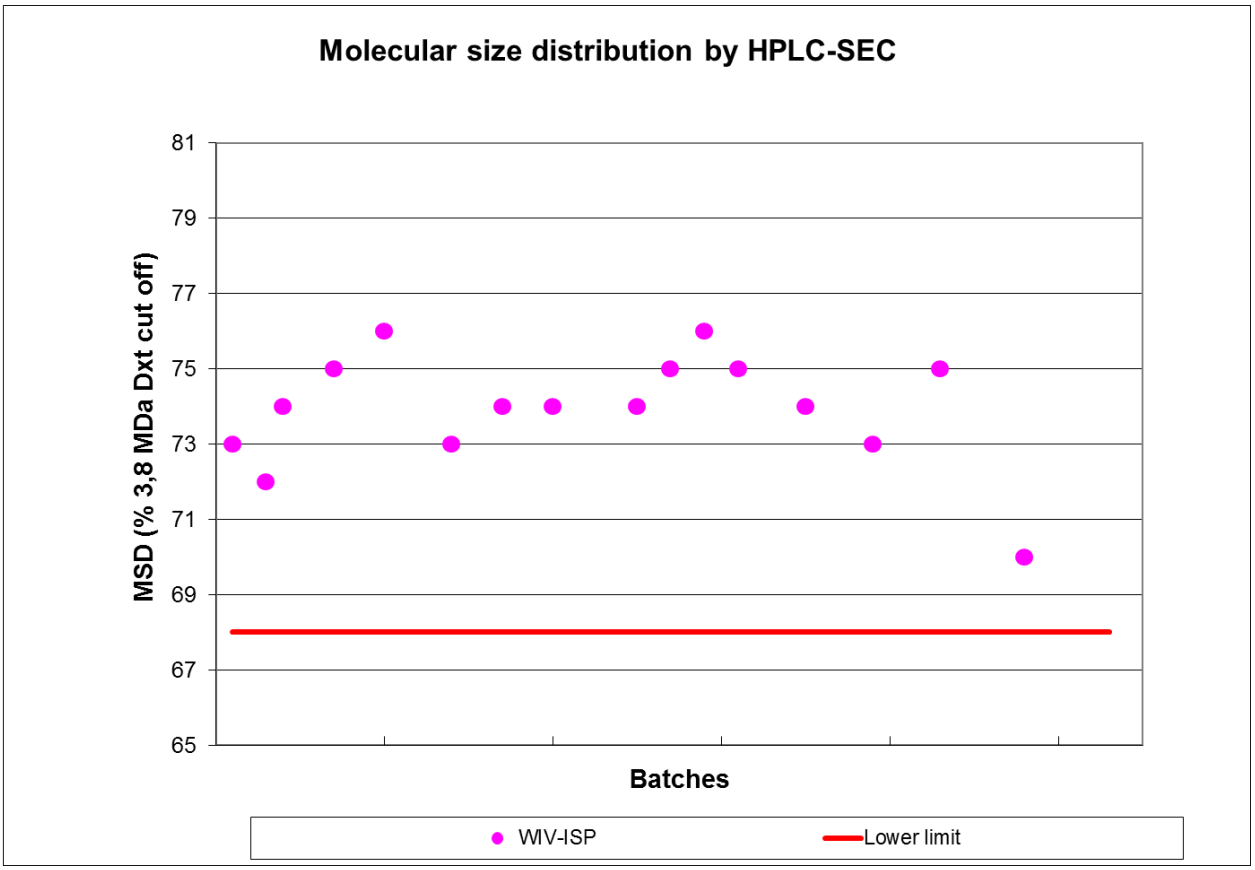
The results in the following graph demonstrate that the results for free-PS at WIV-ISP are towards the upper consistency limit. This is due to the delay between testing by the manufacturer and testing by WIV-ISP. Free-PS can be considered as a stability indicating factor that increases in function of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



Years	Mean	Mean - 2*SD	Mean + 2*SD
2011 (N = 21)	2.4	1.8	3.0
2012 (N = 45)	2.6	1.8	3.5
2013 (N = 79)	3.2	1.3	5.0

Molecular size distribution

The values obtained by WIV-ISP fit very well into the manufacturer's consistency. No OOS results were obtained.



Years	Mean	Mean - 2*SD	Mean + 2*SD
2011 (N = 21)	74	71	77
2012 (N = 45)	74	70	78
2013 (N = 79)	74	69	78

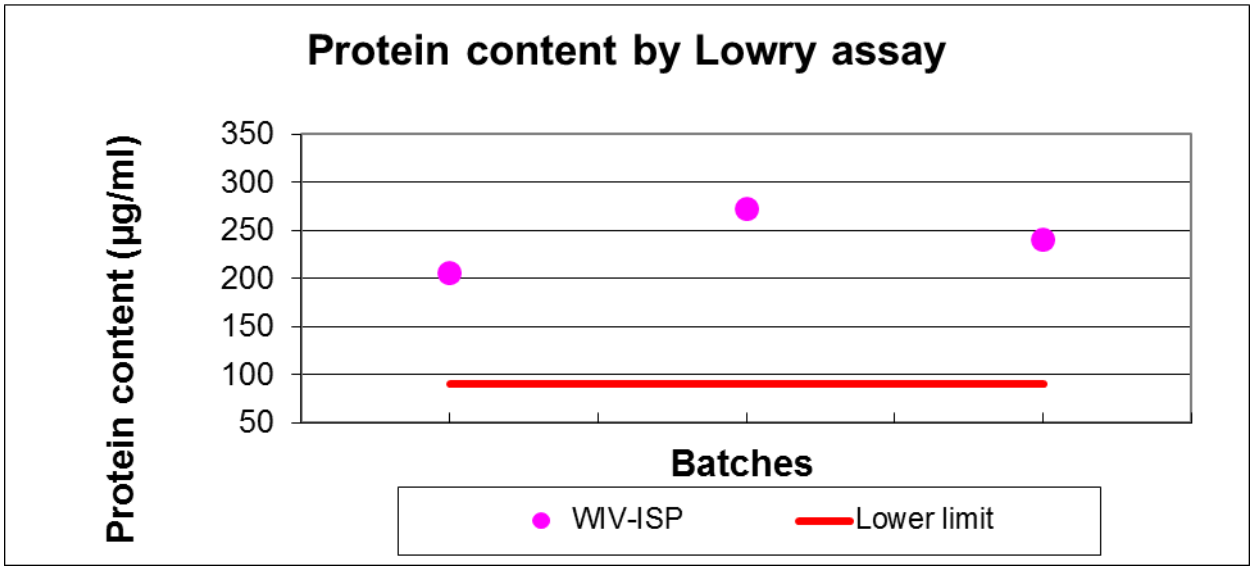
D.5.3.8 PS19F-DT

Only one batch of PS19F-DT was released and the results for protein content, polysaccharide content, PS/protein ratio, free-PS and molecular size distribution met the specifications.

D.5.3.9 PS23F-PD

Protein content

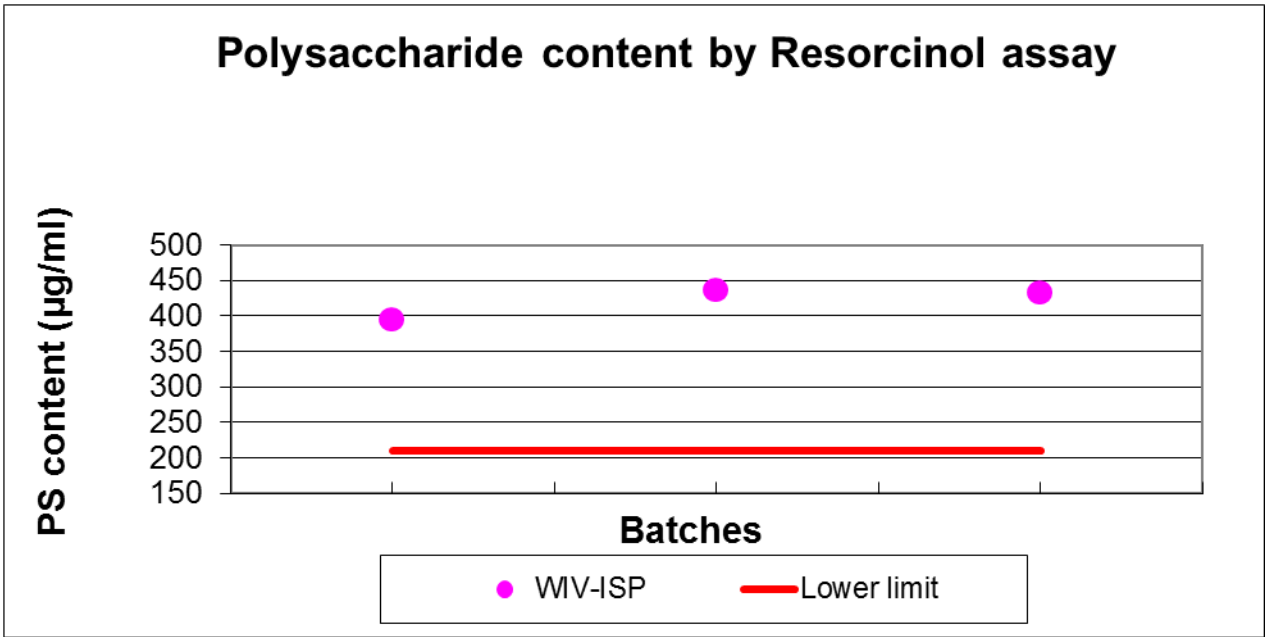
In the following graph, it is clear that the results of WIV-ISP are more or less within the manufacturer's consistency, but a very limited number of batches have been tested. No OOS data were obtained.



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

Polysaccharide content

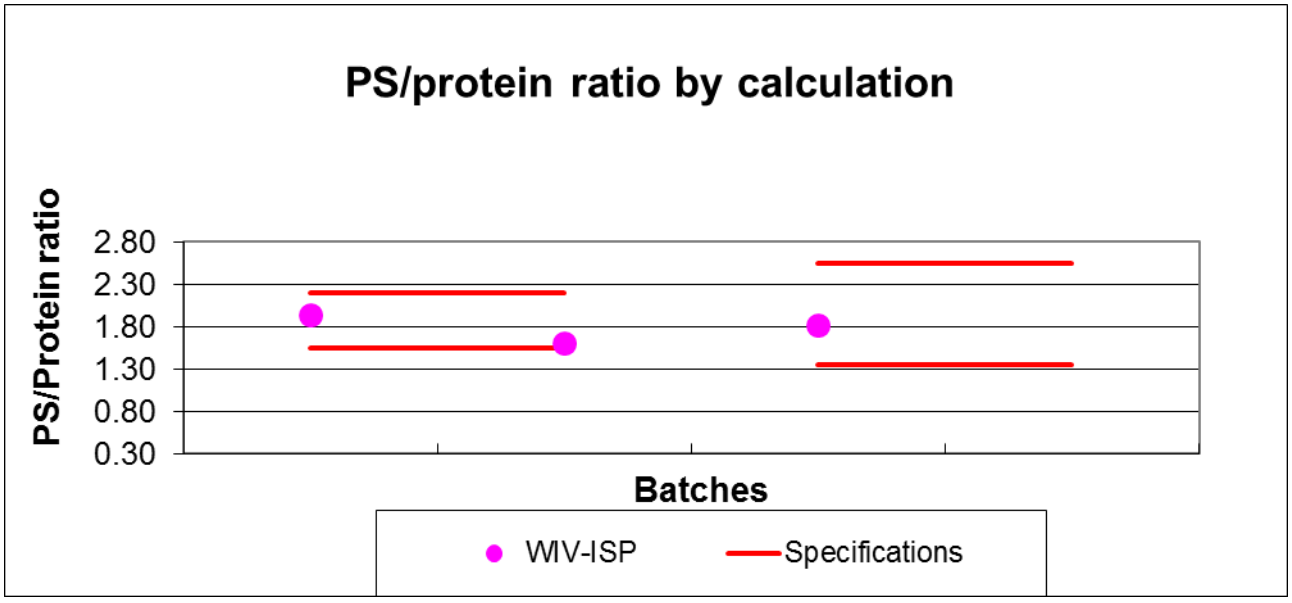
For the determination of the polysaccharide content, the graph below shows that the values of PS content in PS23F-PD bulks fit very well into the manufacturer's consistency. No OOS data were obtained.



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

PS/protein ratio

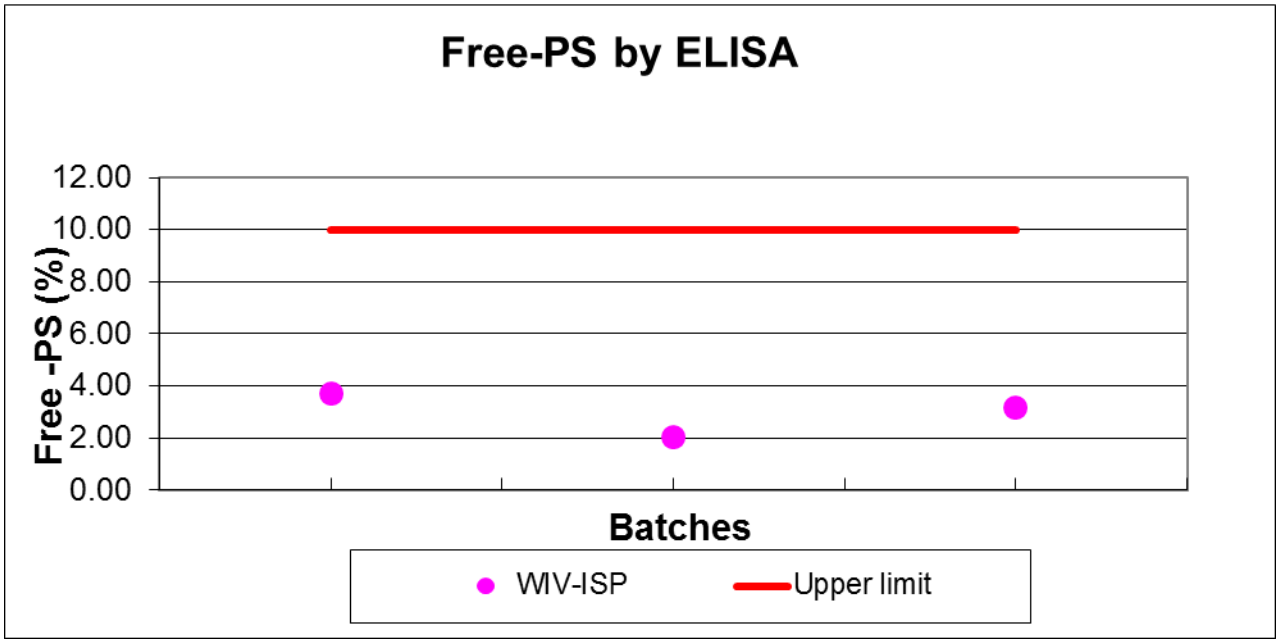
The WIV-ISP results are highly comparable to those obtained by the manufacturer. Between batch two and three, the specifications have been changed by the manufacturer.



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

Free-PS

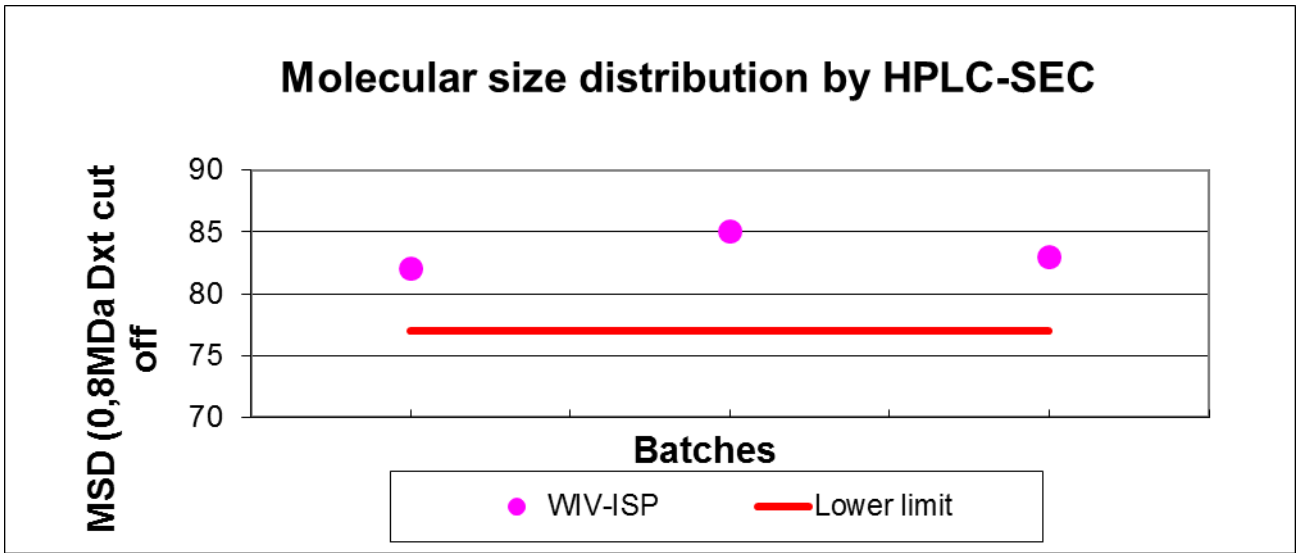
The results in the following graph demonstrate that the results for free-PS at WIV-ISP and the result of the manufacture very well align. No OOS results were obtained.



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

Molecular size distribution

The values obtained by WIV-ISP are towards the lower consistency limit. The delay is testing by the manufacturer and WIV-ISP can influence the reported result since the molecular size distribution is depending on the age of the bulk. 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

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 2013, 4 batches were submitted and 6 batches were fully tested and released

Vaccine type	Status	Release information's		
		EU	Non-EU	Total
Conjugated Meningococcal polysaccharide vaccine ACWY	Submitted	4	7	11
	Tested	6	0	6
	Released	6	7	13
	Rejected	0	0	0

D.6.3 Release specifications and limits

Tests	Manufacturer	WIV-ISP
Purified PSA bulks		
O-acetyl content	≥ 2,5 µmole per mg PS dry weight (by spectrophotometry)	same as manufacturer
	≥ 0.80 mole/ mole PS (by NMR)	Test not performed at WIV-ISP

Tests	Manufacturer	WIV-ISP
Purified PSC bulks		
O-acetyl content	≥ 1,5 µmole per mg PS dry weight (by spectrophotometry)	same as manufacturer
	≥ 0.81 mole/ mole PS (by NMR)	Test not performed at WIV-ISP

Tests	Manufacturer	WIV-ISP
Purified PSW bulks		
O-acetyl content	≥ 0,3 µmole per mg PS dry weight (by spectrophotometry)	same as manufacturer
	≥ 0.49 mole/ mole PS (by NMR)	Test not performed at WIV-ISP

Tests	Manufacturer	WIV-ISP
Purified PSY bulks		
O-acetyl content	≥ 0,9 µmole per mg PS dry weight (by spectrophotometry)	same as manufacturer
	≥ 0.62 mole/ mole PS (by NMR)	Test not performed at WIV-ISP

Tests	Manufacturer	WIV-ISP
Conjugated PSAah-TT bulks		
polysaccharide content	≥ 240 µg/ml	same as manufacturer
free-polysaccharide	≤ 15 %	same as manufacturer
molecular size distribution	≥ 65 % before dextran 0,8Mda/≥ 65 % before cut off 875 KDa	same as manufacturer

Tests	Manufacturer	WIV-ISP
Conjugated PSCah-TT bulks		
polysaccharide content	≥ 300 µg/ml	same as manufacturer
free-polysaccharide	≤ 15 %	same as manufacturer
molecular size distribution	≥ 60 % before dextran 3,8Mda/≥ 60 % before cut off 2900 KDa	same as manufacturer

Tests	Manufacturer	WIV-ISP
Conjugated PSW-TT bulks		
polysaccharide content	≥ 360 µg/ml	same as manufacturer
free-polysaccharide	≤ 15 %	same as manufacturer
molecular size distribution	≥ 50 % before dextran 3,8Mda/≥ 50 % before cut off 2300 KDa	same as manufacturer

Tests	Manufacturer	WIV-ISP
Conjugated PSY-TT bulks		
polysaccharide content	≥ 220 µg/ml	same as manufacturer
free-polysaccharide	≤ 20 % or ≤ 15 % (depending on manufacture date)	≤ 15 %
molecular size distribution	≥ 60 % before dextran 3,8Mda/≥ 60 % before cut off 2600 KDa	same as manufacturer

Tests	Manufacturer	WIV-ISP
Final container		
Appearance	White cake or powder. After reconstitution with diluent: clear and colourless liquid.	same as manufacturer
Identity of all four serotypes	Positive	same as manufacturer
Endotoxin content	≤ 10.00 IU per dose	same as manufacturer
PS A content	≥ 80% of target value	same as manufacturer
PS C + W + Y content	≥ 80% of target value	same as manufacturer
PS Y content	≥ 80% of target value	same as manufacturer
PS W content	≥ 80% of target value	same as manufacturer
PS C content	≥ 70 % of target value (by ELISA)	≥ 65 % of target value (by calculation)

D.6.4 Results

D.6.4.1 Purified polysaccharides

D.6.4.1.1 PSA

In 2013, 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.6.4.1.2 PSC

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

D.6.4.1.3 PSW

No batches of purified PSW were submitted to WIV-ISP for testing.

D.6.4.1.4 PSY

In 2013, 1 bulk was submitted to WIV-ISP for testing. While the manufacturer used NMR, WIV-ISP should a spectrophotometric method to determine O-acetyl content. Each test has its own specifications. The test results met the specifications.

D.6.4.2. Monovalent conjugated bulks

D.6.4.2.1 PSA(ah)-TT

One batch of PSA-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.6.4.2.2 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.6.4.2.3 PSW-TT

No batches of PSW-TT were submitted for testing to WIV-ISP.

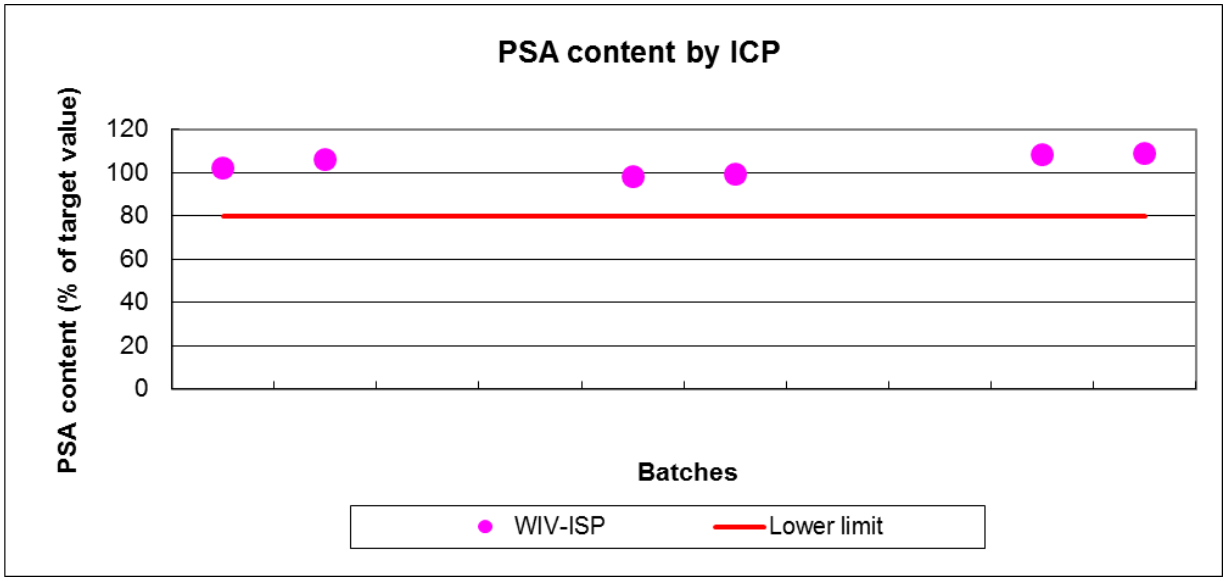
D.6.4.2.4 PSY-TT

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.6.4.3 Final container

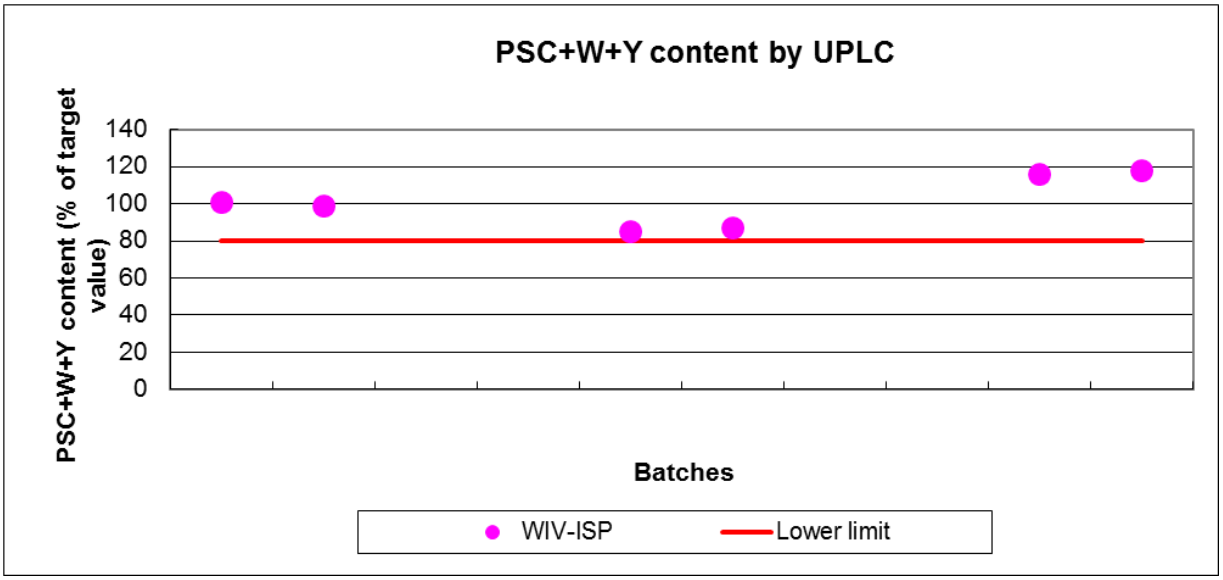
<u>PSA content</u>

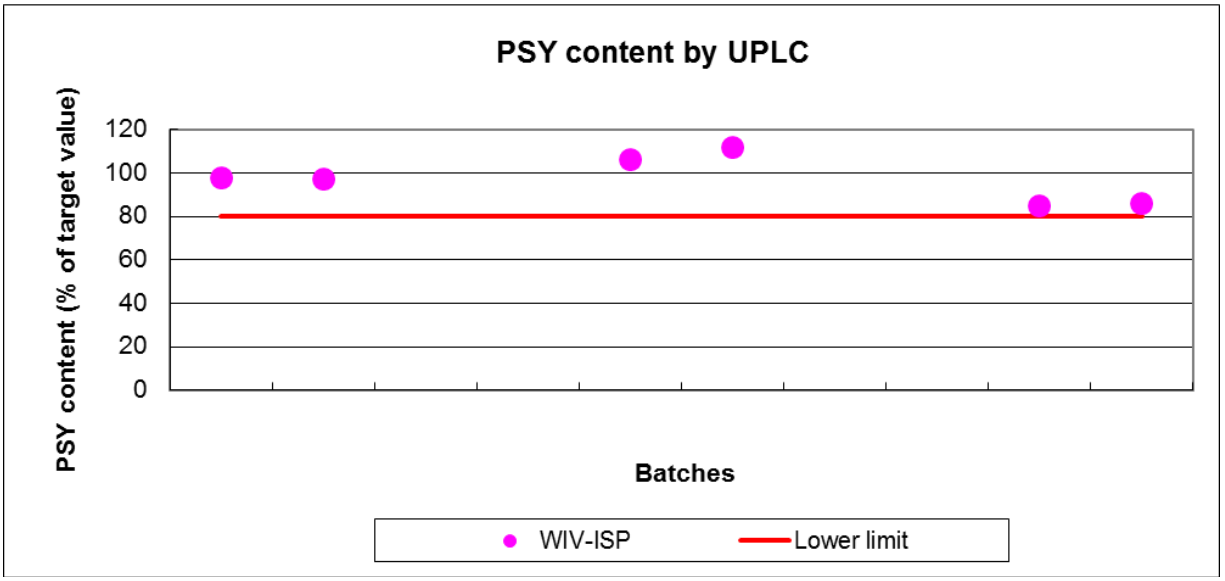
The PSA content in the final container is determined by ICP. The results obtained by WIV-ISP met the specifications. Since the product is new on the market, no consistency data are available yet.

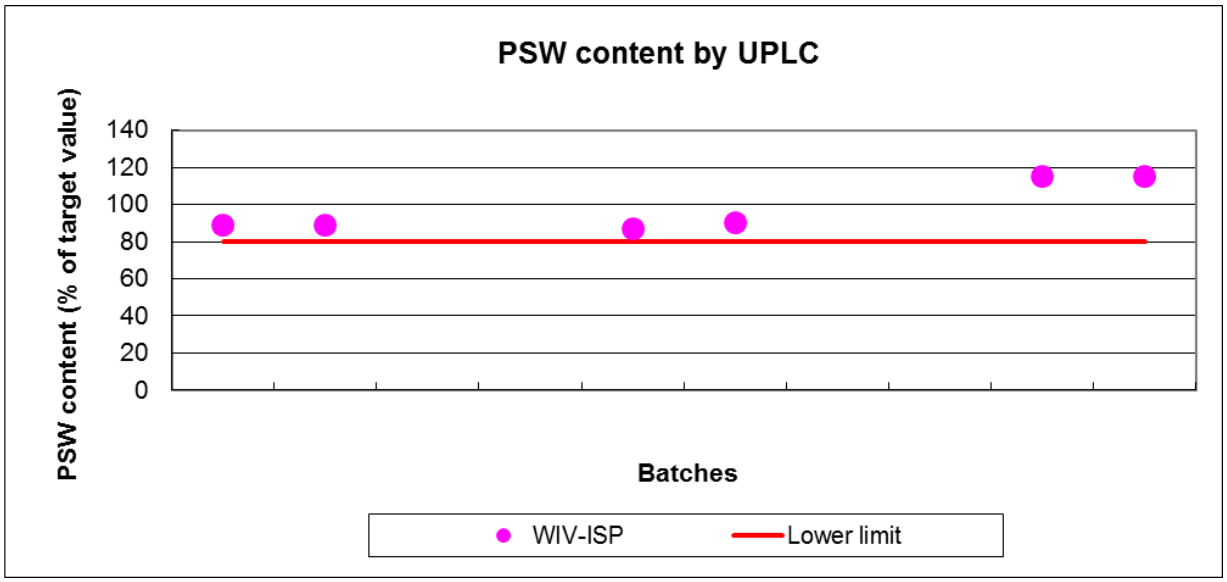


PSC+W+Y content

The total PSC+W+Y content and PSW and PSY content are determined by UPLC. The results from WIV-ISP all meet the specifications. Since the product is new on the market, no consistency data are available yet.

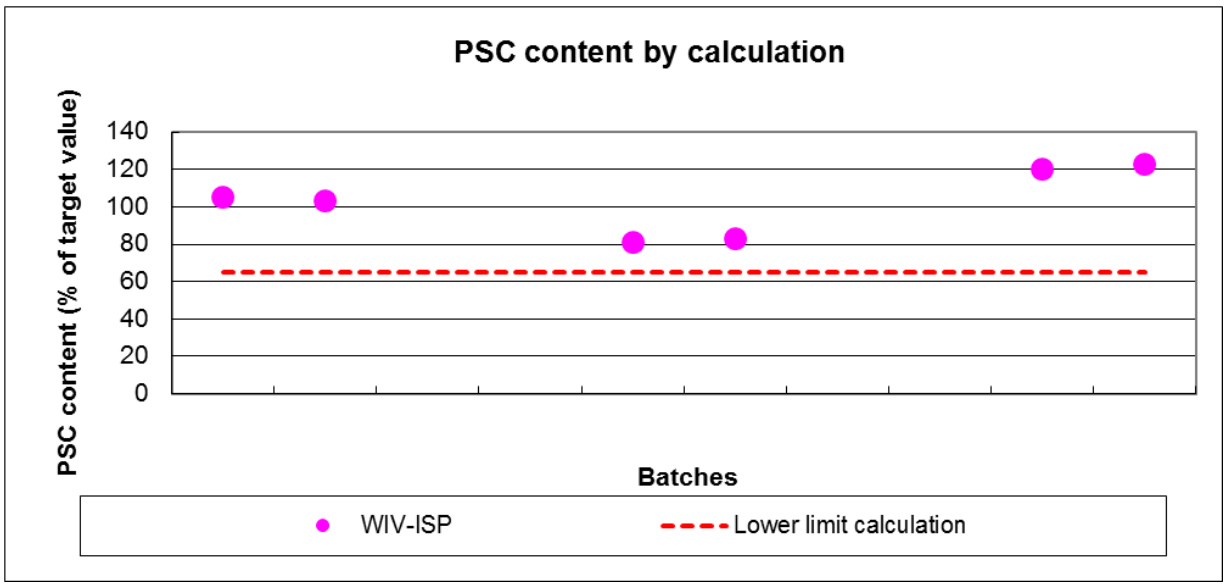






PSC content

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



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, which includes careful validation and maintenance of the manufacturing process.

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 2013, as product expert to **9 GMP inspections** on site with GMP auditors from the Inspectorate (FAMHP).

In addition, our quality experts have also been involved in evaluation of the quality part of dossiers (**3 licensing dossiers as well as 102 process variation dossiers in 2013).** They have also participated to the evaluation of **28 scientific advice dossiers.**

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) [4]**

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 network.

The full reports of the concluded collaborative studies are published in 'Pharmeuropa Bio & Scientific Notes' **[5]** .

We have participated in **3 BSP's** in 2013.

- **The Proficiency Testing Scheme (PTS) [6]**

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 **5 PTS** in 2013.

- **Mutual joint audit (MJA) [7]**

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.

One expert from the Biological Standardisation unit participated in **2 different mutual joint audits** in 2013.

- **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 were adopted by correspondence and presented at the 2013 annual OMCL meeting: Qualification of Balances Annex 8 to the OMCL Network Guideline “Qualification of Equipment” PA/PH/OMCL (12) 77 7R

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 **[8]**.

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. **[9]**

The TSA contract (Technical Service Agreement) **[10]** 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 2013 are listed in the table below

Others: WHO (TSA)	Nr of tested batches
bOPV (bivalent OPV type 1 & type 3)	**8**
Bulk Hib	**1**
Hib	**5**
DTPw-HepB	**20**
DTP	**1**

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. **[11]**

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). **[12]**
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.

In 2013, one of our experts has been involved in a GMP site audit for an Indian manufacturer.
On expert has participated as a facilitator to a training course for the evaluation of the quality part of vaccine dossiers.

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 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] BSP: http://www.edqm.eu/en/Biological-Standardisation-Programme-mission-60.html
[5] Pharmeuropa : http://www.edqm.eu/en/pharmeuropa-bio-and-scientific-notes-584.html
[6] PTS: http://www.edqm.eu/en/Proficiency-Testing-Scheme-47.html
[7] MJA: http://www.edqm.eu/en/quality-management-19.html
[8]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.
[9] http://www.who.int/topics/prequalification/en/
[10] http://www.who.int/biologicals/areas/vaccines/lot_release/en/
[11]http://www.who.int/immunization_standards/vaccine_quality/pq_consultation_2010/en/index.html
[12] 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