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New QC Identity Testing For Acellular Pertussis Combination Vaccines Using LC-MS

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Agenda

- Project Background
  - Current ID testing
  - Project objective
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- Method Development and Validation
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Test Transfer and Implementation

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- Test transfer and implementation in QC
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- Other applications
- Summary and next steps

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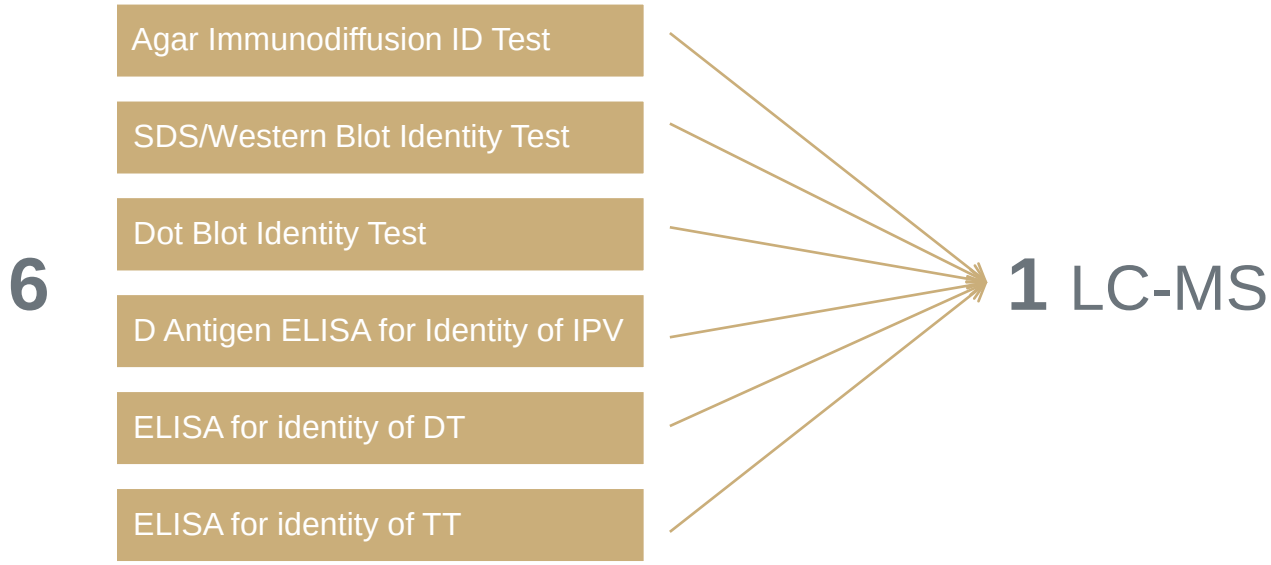
Project Background

Current Identity Testing in QC

- **The identity of acellular pertussis and related combination vaccines is determined at the Final Bulk, Filled Product and/or Labeled Filled Product stages by several QC tests, such as:**
  - Agar Identity for detection of Diphtheria Toxoid (DT) and Tetanus Toxoid (TT)
  - SDS-PAGE/Western Blot Identity Test for detection of Filamentous Haemagglutinin (FHA), Fimbriae (FIM), Pertussis Toxoid (PT), and Pertactin (PRN)
  - Dot Blot Identity Test for detection of high or low dose of Diphtheria Toxoid (DT), high, low or no Filamentous Haemagglutinin (FHA) content, presence or absence of Inactivated Poliomyelitis Vaccine (IPV) Type 1
  - ELISA for Identity of Inactivated Poliomyelitis Vaccine (IPV) Type 1, Type 2, Type 3
  - ELISA for identity of Diphtheria Toxoid (DT)
  - ELISA for identity of Tetanus Toxoid (TT)
- **These tests are labor-intensive, time consuming, and require use of antibodies that are expensive to produce, qualify, and store**
- **Some of these tests generate a high invalidity rate**

Project Objective

- **Develop one LC-MS ID test to replace QC ID tests**
  - Protein antigen identification



Cost Benefit Analysis

	Total Labor Cost ($CAN)	# of Antibody Reagents	# of Test Methods
Current ID Tests	621,504 (4 ID tests)	>18	6
LC-MS ID Test	166,355	0	1
Analysis	455,149 (73%↓)	(100%↓)	(83%↓)

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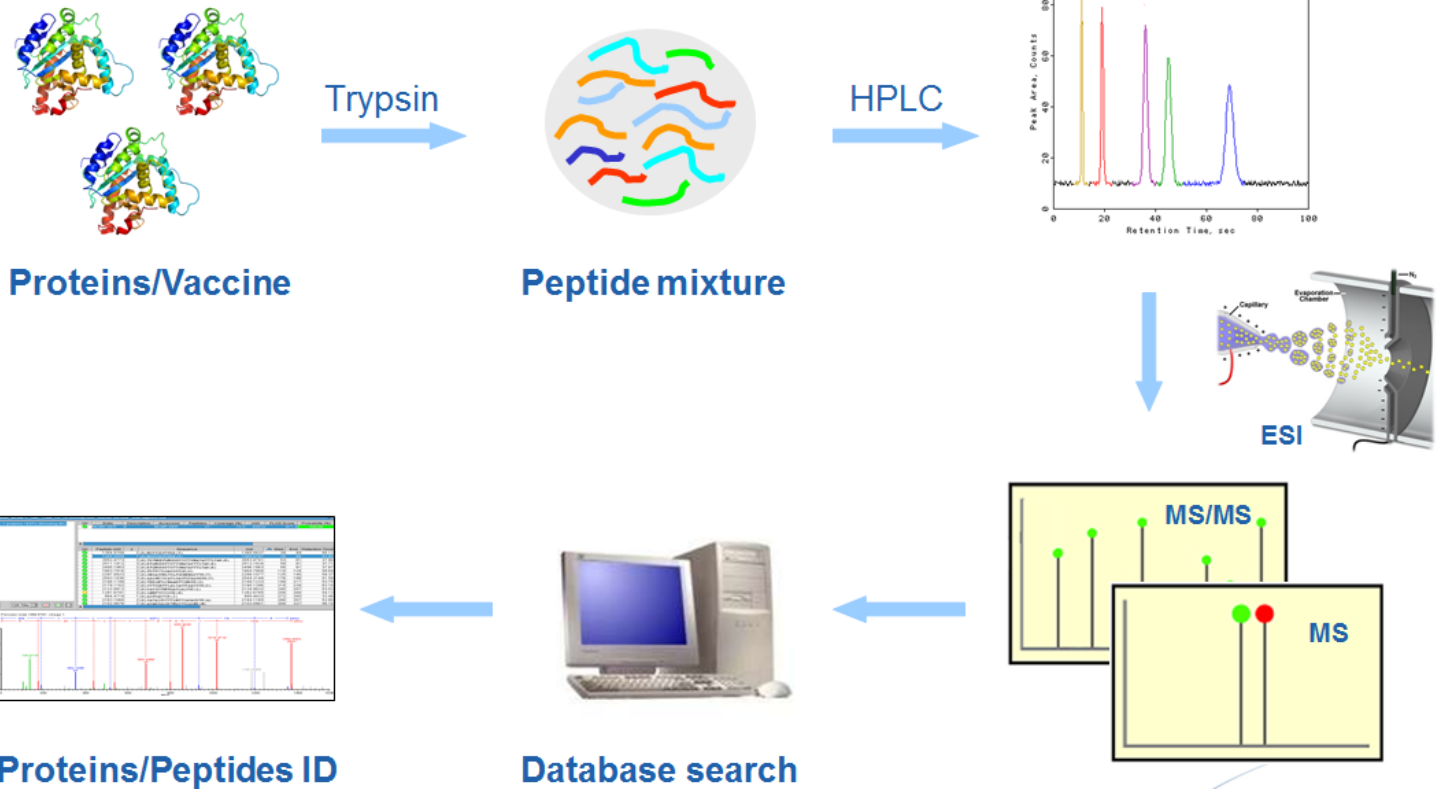
Method Development & Validation

Protein Antigen Identification

- **LC-MS identifies protein antigens by detecting antigen specific peptides**



Challenges for Peptide Identification

- **Sample Complexity in Pediacel**
  - Diphtheria & Tetanus (D, TT)
  - Five component acellular Pertussis (FIM, FHA, PT, PRN)
  - Inactivated Poliovirus Types 1, Type 2, and Type 3
  - PRP-T
- **Sequence Similarity of IPV Antigens (% of identical sequence to Type 1) make the identification of IPV type-specific peptides more challenging.**

	VP1	VP2	VP3	VP4
IPV Type 1	100%	100%	100%	100%
IPV Type 2	77.1%	88.6%	87.4%	97.1%
IPV Type 3	75.7%	83.4%	84.9%	92.6%

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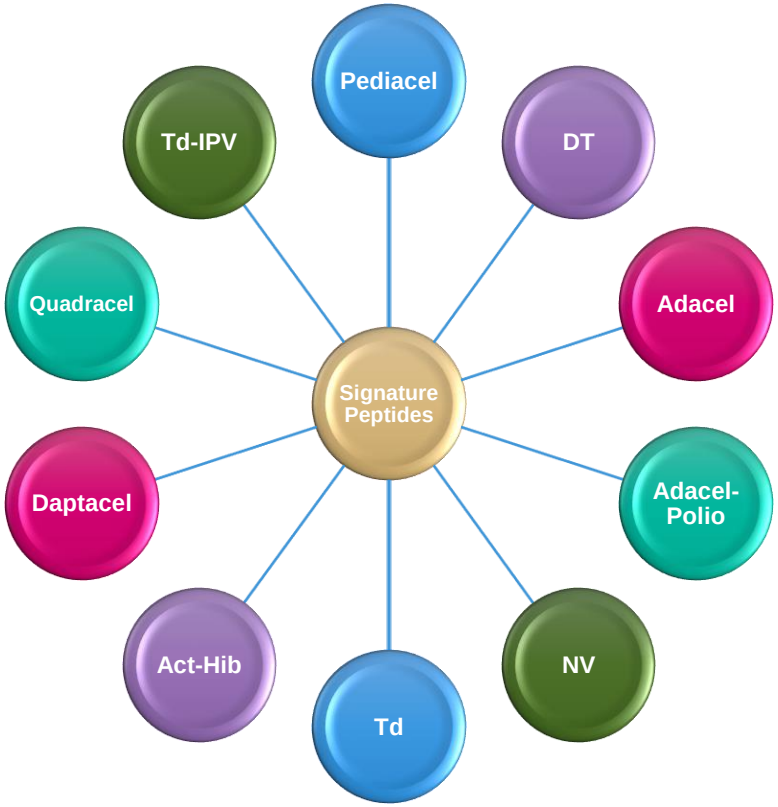
Challenges for Peptide Validation



- Validate peptides across a vaccine panel
- Detected reproducibly throughout vaccine samples at a sufficient level
- Do not contain Cys or Met
- Reasonable size and good fragmentation for MS/MS verification

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Antigen ID Testing Results Using LC-MS

Sample Results

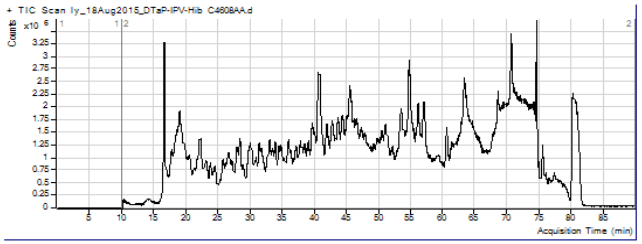
Data Analysis
Batch
D:\MassHunter\Data\GMP\18Aug2015\QuantResults\ly_24Aug2015_Batch18Aug2015.batch.bin

Analysis Time	2015-08-24 10:09	**Analyst**	PASTEUR\i0176191
Report Time	2015-08-24 10:12	**Quant. Method**	LCMS Product ID_v1.quantmethod.xml

Data Acquisition

Acq Time	2015-08-21 04:58	**Data File**	ly_18Aug2015_DTaP-IPV-Hib G4608AA.d
Sample Position	P1-C9	**Sample Name**	ly_17Aug2015_DTaP-IPV-Hib G4608AA
Inj. Vol. (µL)	20	**Acq Method File**	APT Product ID_Acq_protein.m
Acq.Operator	PASTEUR\i0176191		

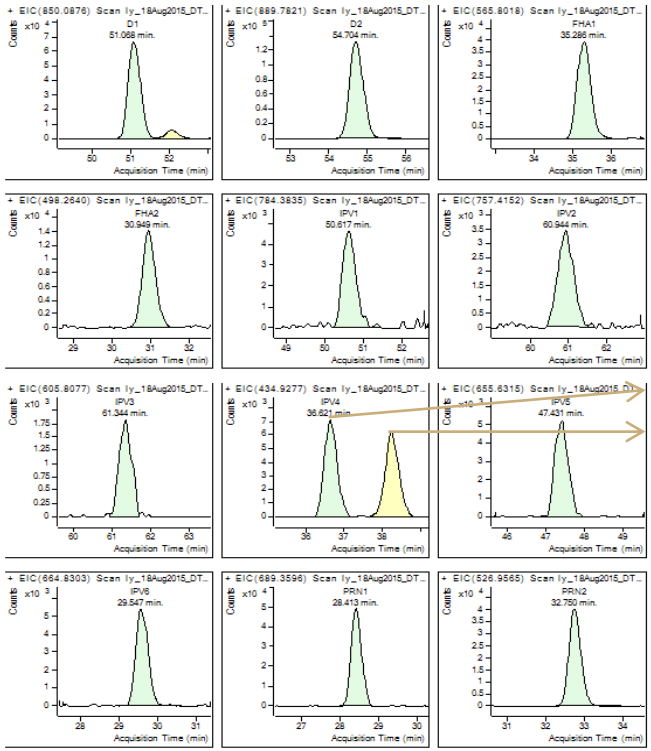
Sample Chromatogram



Sample Results

Peptide	m/z	RT	Area	MI	Height
D1	850.0876	51.068	1527992	☐	66439
D2	889.7821	54.704	3385799	☐	131516
FHA1	565.8018	35.286	1002426	☐	39460
FHA2	498.2640	30.949	349242	☐	14059
IPV1	784.3835	50.617	118143	☐	4650
IPV2	757.4152	60.944	100974	☐	3411
IPV3	605.8077	61.344	41639	☐	1815
IPV4	434.9277	36.621	169677	☐	7101
IPV5	655.6315	47.431	129222	☐	5271
IPV6	664.8303	29.547	123932	☐	5423
PRN1	689.3596	28.413	975945	☐	49091
PRN2	526.9565	32.750	910489	☐	40428
PT1	551.3355	45.512	9220699	☐	350873
PT2	569.2809	30.815	1922001	☐	80803
TT1	578.6391	50.033	384654	☐	15134
TT2	868.4254	40.674	1456927	☐	56622
FIM1	359.7291	29.631	117804	☐	4376

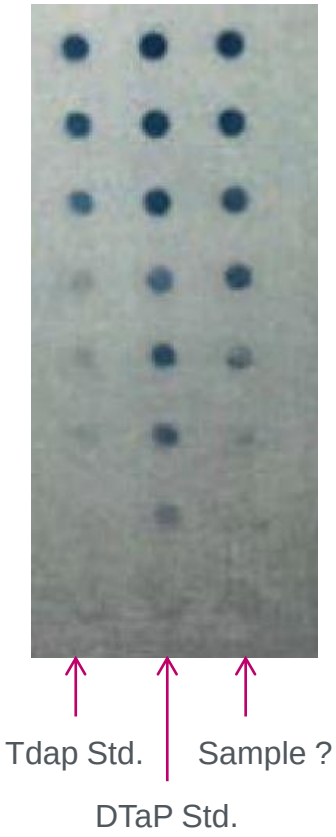
Sample Results

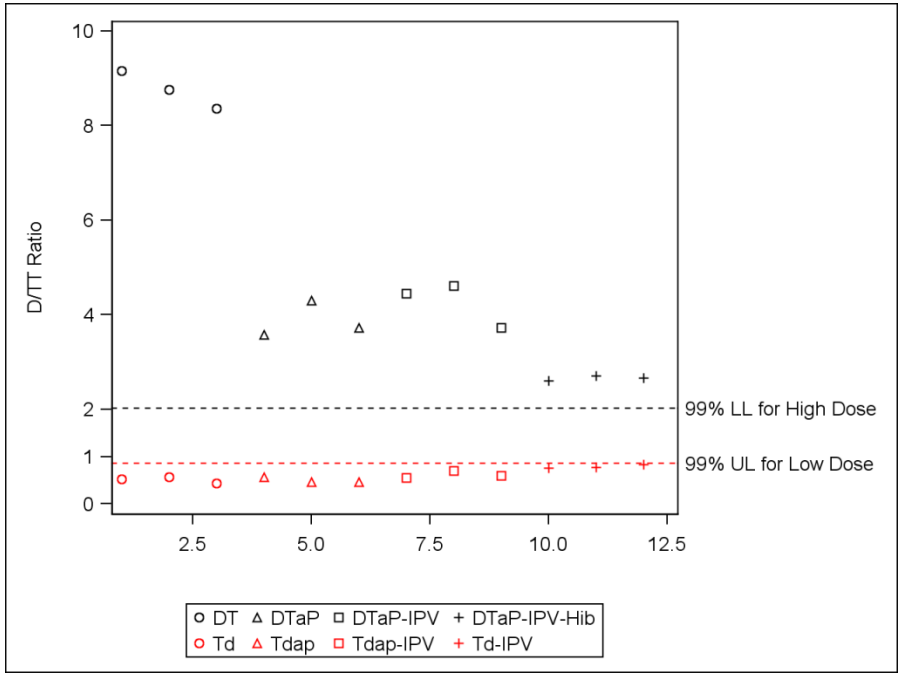


FTEP**I** KDVLIK
FTEP**L**KDVLIK

Dosage Differentiation Using LC-MS ID Method

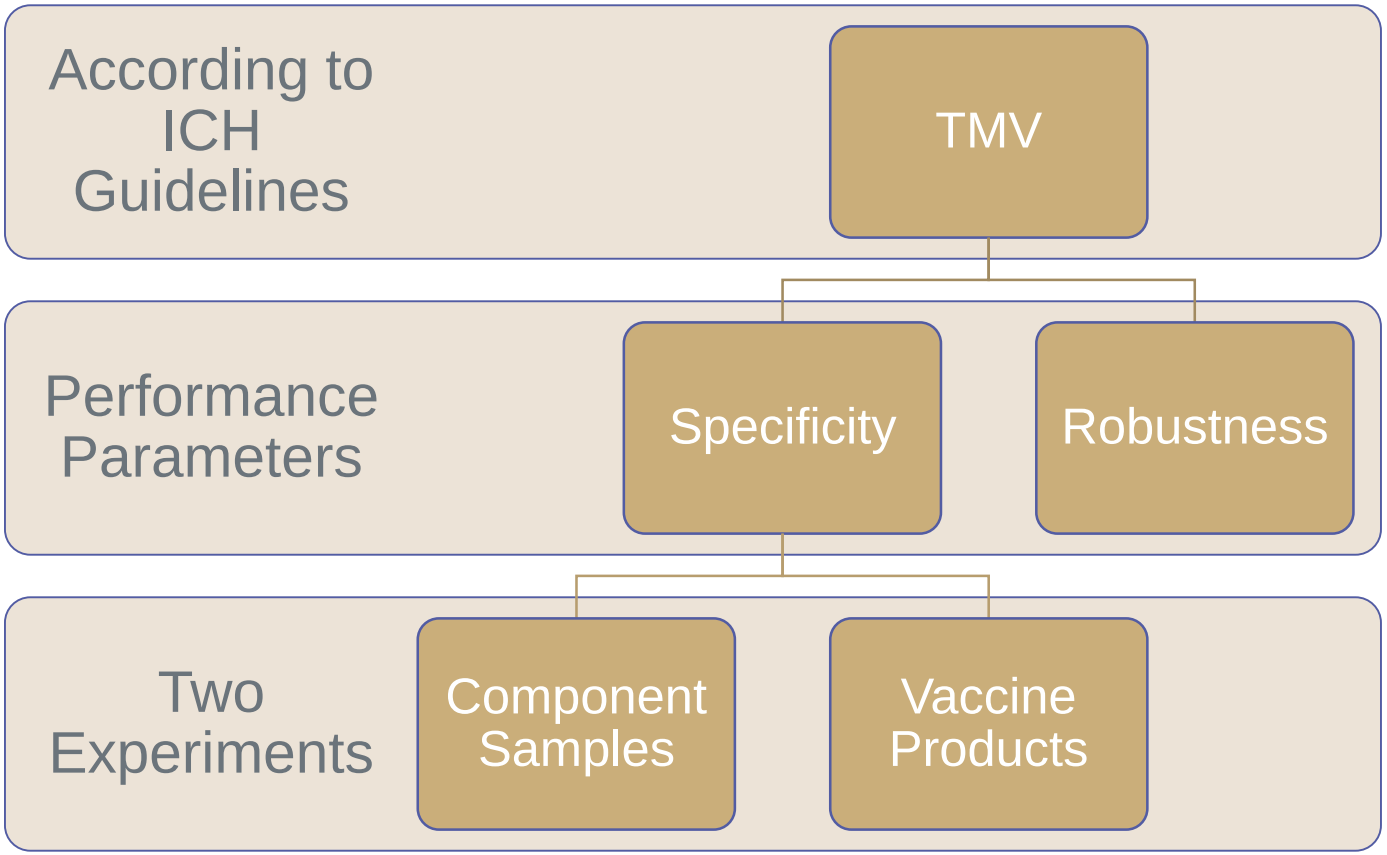
- **Successfully developed a simple and reliable method for dosage differentiation.**





If ratio ≥1 → High dose (15 Lf D)
If ratio <1 → Low dose (2 Lf D)

Test Method Validation (TMV)



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Specificity for Antigen Components

Antigen Component	# of Lot	D peptides	TT peptides	FHA peptides	FIM peptide	PT peptides	PRN peptides	IPV Type 1 peptide	IPV Type 2 peptide	IPV Type 3 peptide
D	3	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
TT	3	Neg	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Neg
FHA	3	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg	Neg
FIM	3	Neg	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg
PT	3	Neg	Neg	Neg	Neg	Pos	Neg	Neg	Neg	Neg
PRN	3	Neg	Neg	Neg	Neg	Neg	Pos	Neg	Neg	Neg
vIPV Type 1	3	Neg	Neg	Neg	Neg	Neg	Neg	Pos	Neg	Neg
vIPV Type 2	3	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Pos	Neg
vIPV Type 3	3	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Pos

- LC-MS method was able to identify all the antigen specific peptides in the antigen components. No detection of antigen specific peptides was observed in the antigen components where the corresponding antigen is absent.

Specificity Assessment for Products

Product Sample	# of Lot	D peptides	TT peptides	FHA peptides	FIM peptide	PT peptides	PRN peptides	IPV Type 1 peptide	IPV Type 2 peptide	IPV Type 3 peptide	PRP
DT	3	High Dose	Pos	N/Ap	N/Ap	N/Ap	Neg	N/Ap	N/Ap	N/Ap	N/Ap
Td	3	Low Dose	Pos	N/Ap	N/Ap	N/Ap	Neg	Neg	Neg	Neg	N/Ap
Td-IPV	3	Low Dose	Pos	N/Ap	N/Ap	N/Ap	Neg	Pos	Pos	Pos	N/Ap
Tdap	3	Low Dose	Pos	Pos	Pos	Pos	Pos	Neg	Neg	Neg	N/Ap
DTaP	3	High Dose	Pos	Pos	Pos	Pos	Pos	Neg	Neg	Neg	N/Ap
Tdap-IPV	3	Low Dose	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	N/Ap
DTaP-IPV	3	High Dose	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg
DTaP-IPV-Hib	3	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos

- LC-MS method is able to identify all acellular pertussis combination vaccines and each product can be differentiated from other similar vaccine products produced at Sanofi Pasteur Limited.

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Test Transfer and Implementation

Regulatory Submissions and Implementation in QC

- **Submissions and Regulatory Approvals**
  - Receiving approval from Regulatory Angecies (FDA, Health Canada, and etc.) for implementing LC-MS identity test for releasing pertussis vaccines
- **Test Transfer**
  - Installation and qualification of LC-MS equipment in QC completed in June 2016
  - 2 QC Analysts and Manager were trained
  - Test Transfer to QC Chemistry for all Acel products completed in Oct 2016
- **Implementation**
  - Tests implemented in QC Chemistry for testing of Pediacel CA (Mar 2018), Quadracel/Pentacel US&CA (May 2018), Repevax CA (June 2018), Td US&CA (Sep 2018), Daptacel US&CA (Sep 2018), DT US (Nov 2018)

Example: Benefits after Implementing LC-MS ID Test for Pediacel in QC

Antigens to be detected in Pediacel	D peptides	TT peptides	FHA peptides	FIM peptide	PT peptides	PRN peptides	IPV Type 1 peptide	IPV Type 2 peptide	IPV Type 3 peptide
LC-MS Test Results	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos

- A single LC-MS test identifies all 9 protein antigens which otherwise would require 6 QC ID tests to confirm the presence of each protein antigens in the vaccine

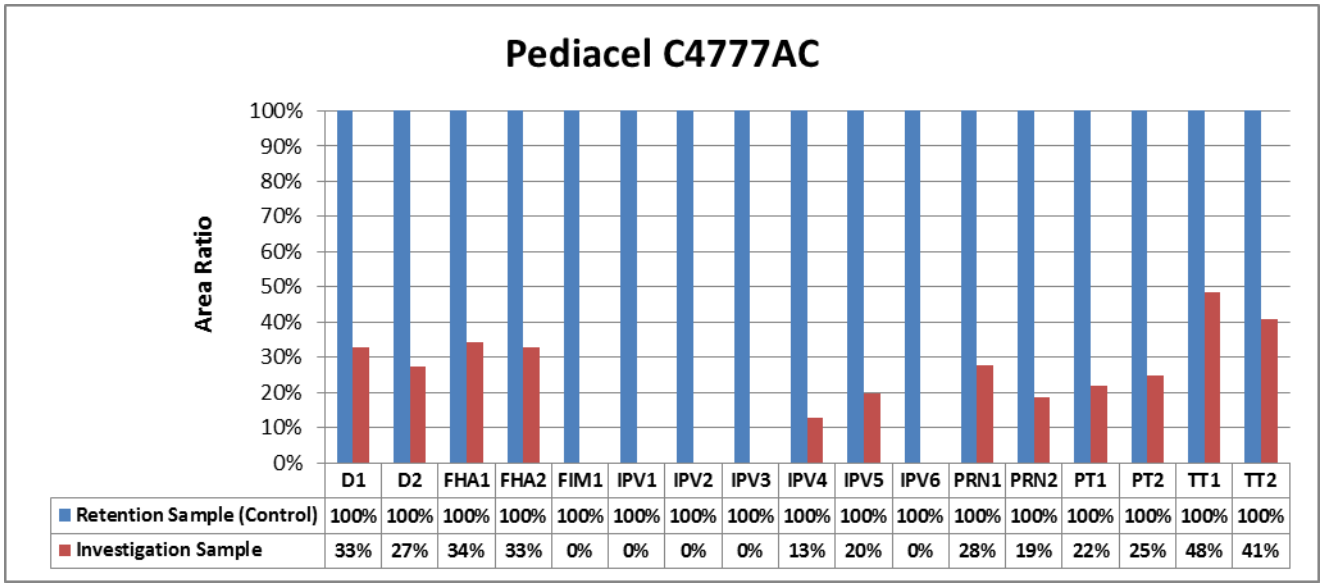
	ID tests required for Pediacel ID testing using old methods
1	Agar Identity of Diphtheria Toxoid and Tetanus Toxoid
2	Dot Blot Identity Test for detection of high or low dose of Diphtheria Toxoid (DT), high, low or no Filamentous Haemagglutinin (FHA)
3	Western Blot Identity Test for detection of FHA, FIM, PT and PRN
4	ELISA for Identity of Inactivated Poliomyelitis Vaccine (IPV) Type 1
5	ELISA for Identity of Inactivated Poliomyelitis Vaccine (IPV) Type 2
6	ELISA for Identity of Inactivated Poliomyelitis Vaccine (IPV) Type 3

→ **1** LC-MS

- Cost savings
  - 73% reduction in labor cost
  - Eliminating the use of more than 18 antibody reagents
  - Reducing cost for the lifecycle management of 1 analytical procedure versus 6 procedures
  - Reducing the invalidity rate to 0

Other Applications of LC-MS ID Test — Counterfeit Investigations

- LC-MS ID testing method were successfully used to test counterfeit samples and the corresponding retention samples to verify that the content within the returned Pediacel products (C4732AB and C4777AC) is not the correct Pediacel vaccine manufactured at Sanofi Pasteur Limited.



	D1	D2	FHA1	FHA2	FIM1	IPV1	IPV2	IPV3	IPV4	IPV5	IPV6	PRN1	PRN2	PT1	PT2	TT1	TT2
Retention Sample (Control)	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
Investigation Sample	33%	27%	34%	33%	0%	0%	0%	0%	13%	20%	0%	28%	19%	22%	25%	48%	41%

Comparison of the relative amount of antigen-specific peptides between the Pediacel C4777AC retention sample (Control) and investigation sample

Summary and Next Steps

- **Successfully developed, validated, and transferred a LC-MS method to QC for replacing 6 ID tests of acellular pertussis combination vaccines**
- **It not only identifies all the protein antigens but also differentiates the products which have the same antigen but different dosage in a single LC-MS experiment**
- **Benefits**
  - Efficiency: One LC-MS identity test replaces 6 QC identity tests affording reduced test cycle times
  - Test Reliability: It has reduced invalidity rates to zero since implementation in March 2017
  - Cost:
    - Save 73% labor cost
    - Eliminate the use of more than 18 antibody reagents associated with high costs
    - Reduce maintenance time and cost since only one instead of 6 procedures require life cycle management for SOP updating, reagent and equipment maintenance, training, re-validation, regulatory submission, etc.
  - Greater customer assurance of data quality due to automation and test reliability

Summary and Next Steps

- **Next Steps**
  - Test transfer to other Sanofi Pasteur sites
    - Feasibility completed in 2017 and received ARC endorsement
    - Implementation of LC-MS ID tests in 2019 for
      - Hexaxim & Axim family to replace identity test by Luminex, Ouchterlony, etc. (VDR, MLE)
      - Shan5 & Shan6 to replace ELISAs and Agglutination (SHA)
      - Fluzone to replace Ouchterlony (SWF)

Acknowledgement

Michael Leach (AS)
Matthew Balmer (AS)
Sue Nelson (AS)
Seeven Vydelingum (AS)
Rob Maharajh (AS)
Lois Yin (AS)
Alex Listigovers (AS)
Bruce Carpick (AS)
Artur Pedyczak (AS)

Olivier Faure (QO QC)
Rosa Nisman (QC Chemistry)
Na Guan (QC Chemistry)
Pilly Chan (QC Chemistry)
Cynthia Ma (QC Chemistry)
Kei He (QC Biostatistics)
Meili Li (QO AE)
Helen Sarantis (QO AE)
Jim Yu (QO AE)

Dave Jaipersad (QO VS)
Tatjana Cvetkovic (QO VS)
Shahneela Usman (QO VS)
Peter Doherty (QO VS)

Maureen Barbalinardo (RA)
Leanne Chinn (RA)
Priya Persaud (RA)

William Zhu (IS)
David McIntyre (IS)
Henry Pan (IS)

Graham Phillips (IO)
Reddy Donga (IO ENG)

THANK YOU

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