
Product characterization of pertussis Whole Cell Vaccine by mass spectrometry

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- Introduction
- Gene and protein regulation during cultivation
- Mass spectrometry
- Antigen composition as measurement of product quality

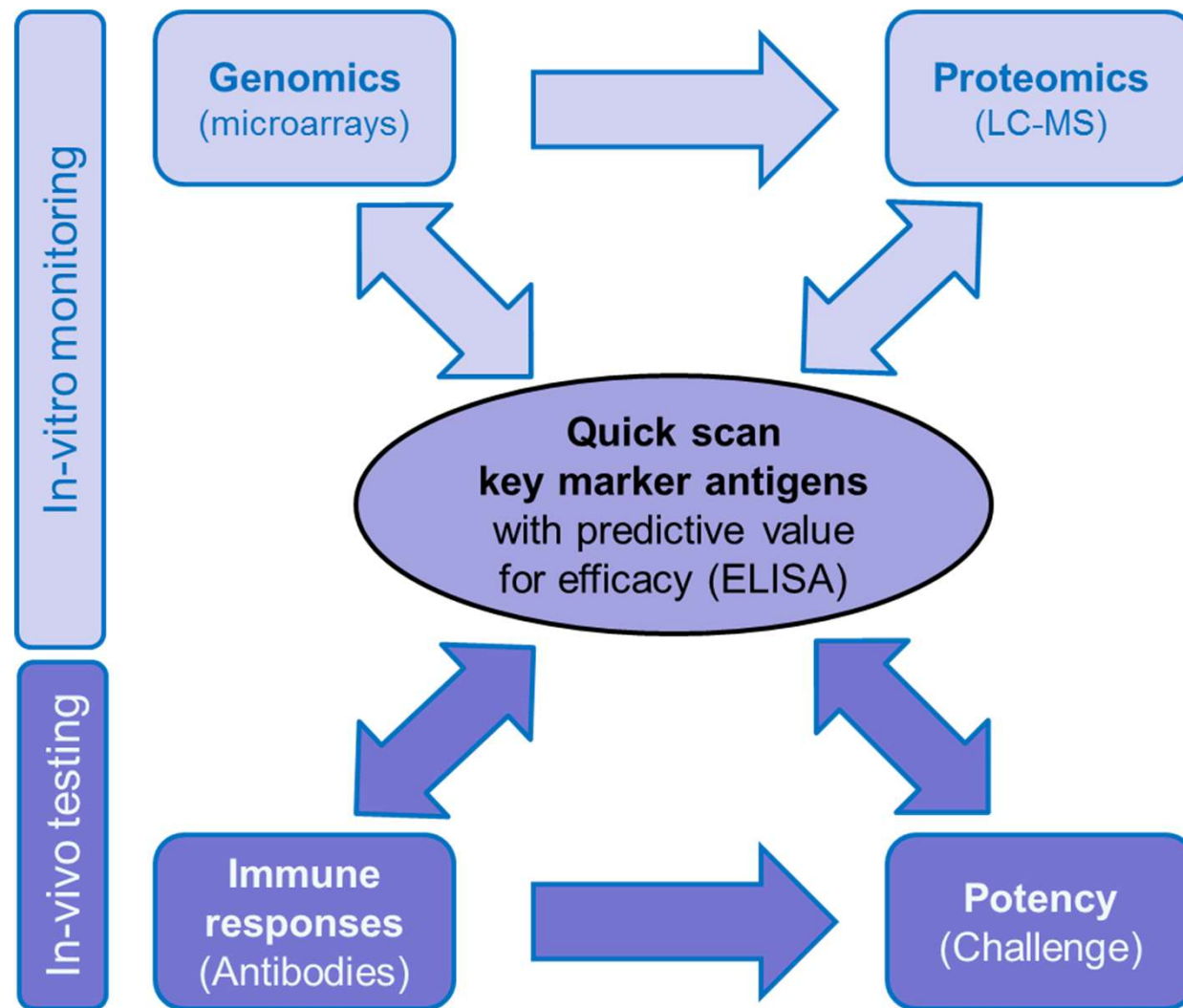
Reasons for characterization of pertussis vaccines

- Resurgence of pertussis in populations well vaccinated with acellular pertussis vaccines triggers development of new pertussis vaccines
- Polio eradication triggers development of hexavalent DPT-Hib-HepB-IPV_{sabin} vaccines necessitating investigation of compatibility of pertussis component
- Potency release tests are not sensitive and reproducible enough and therefore not suitable for this type of research

***B. pertussis* culture**

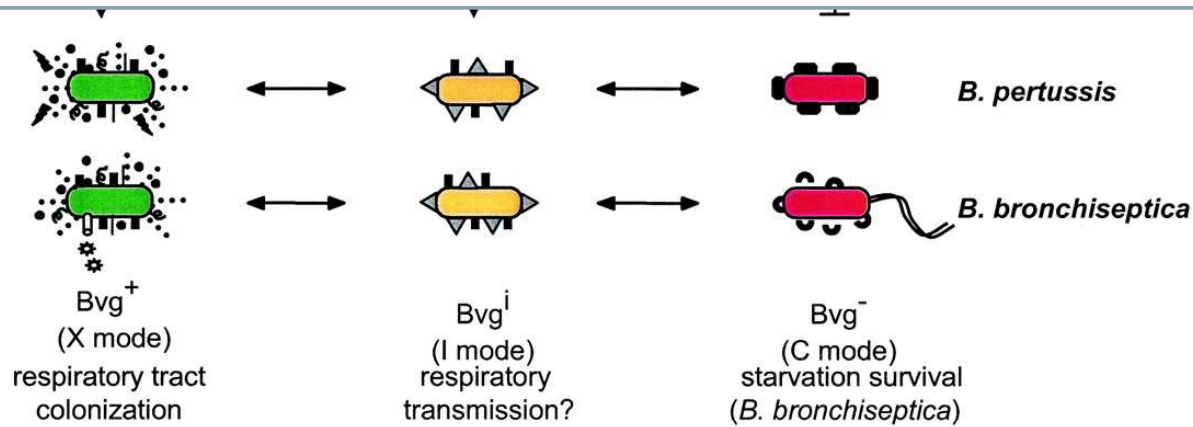
- Start of all pertussis vaccine productions
- Undefined intermediate product
- Product characterization required

Process and product characterization

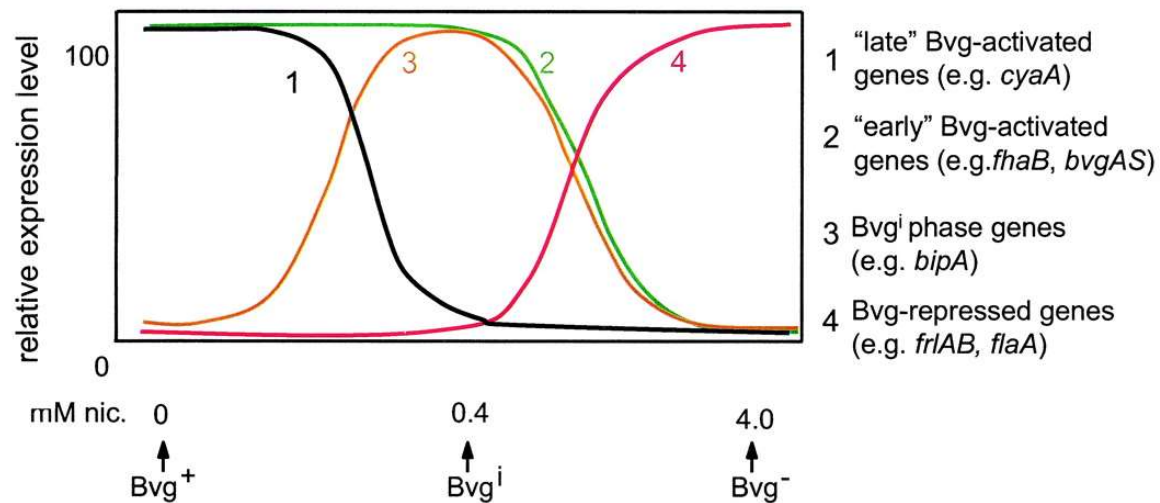


Genomics

Bvg-regulated virulence of *B. pertussis*



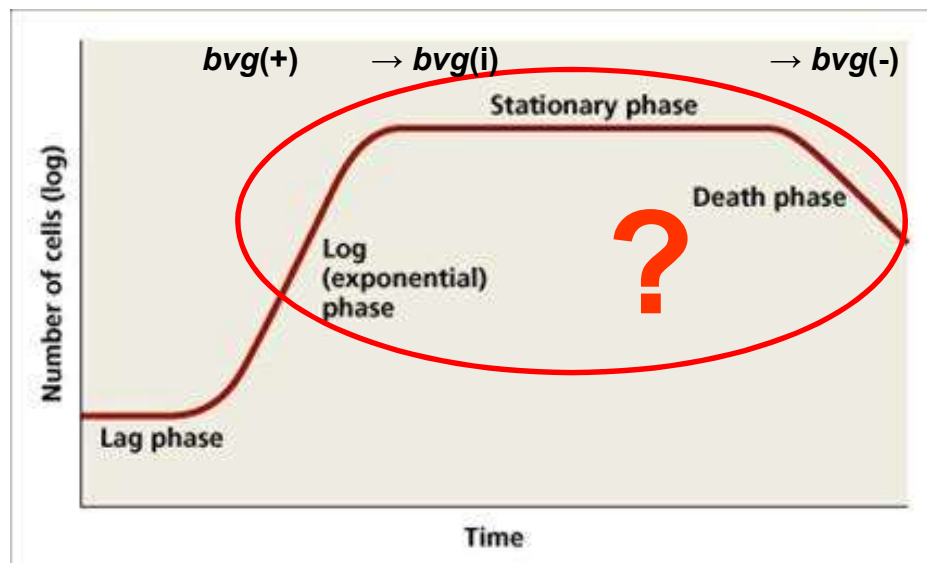
B:



Process knowledge = product quality

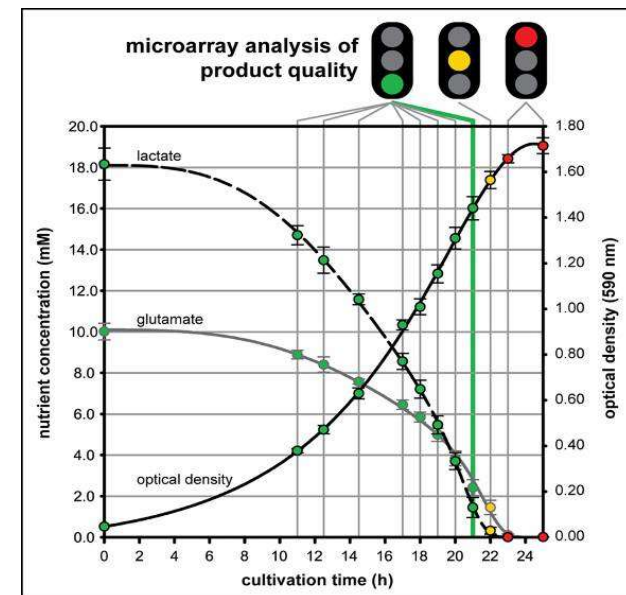
Culture conditions of *B. pertussis* affect the quality of WCV.

- Bvg regulated virulence factors are in general protective
- Expression of virulence factors affected by e.g. nutrient limitations, culture temperature (artificial factors like nicotine or **MgSO₄**)



B. pertussis batch culture

Score based on activation of 56 vag's

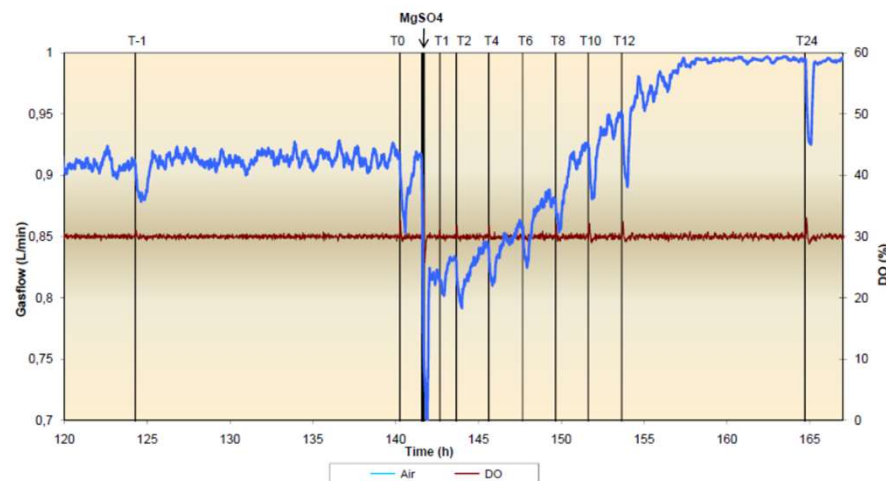


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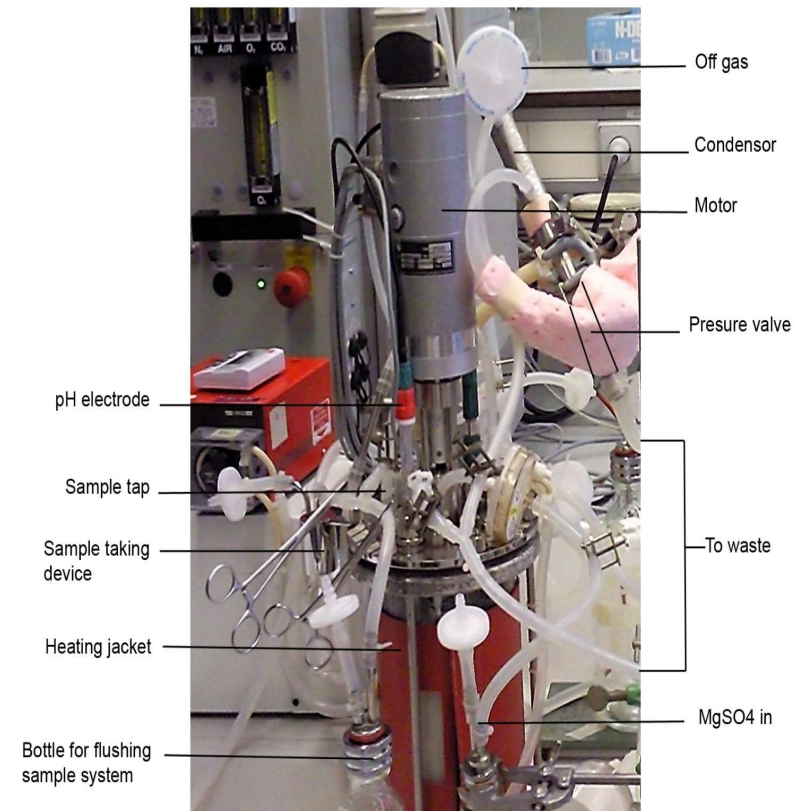
Consistency = process & product knowledge

Steady state culture of *B. pertussis*

- $\text{MgSO}_4 \rightarrow bvg$ down regulation $\rightarrow$ reduction in virulence factors
- **Product characterization**
 - 3 bioreactor runs
 - sampling $\rightarrow T = 0, 2, 6, 12$ and 24 hours
 - Plain inactivated WCV **A, B, C, D** and **E**

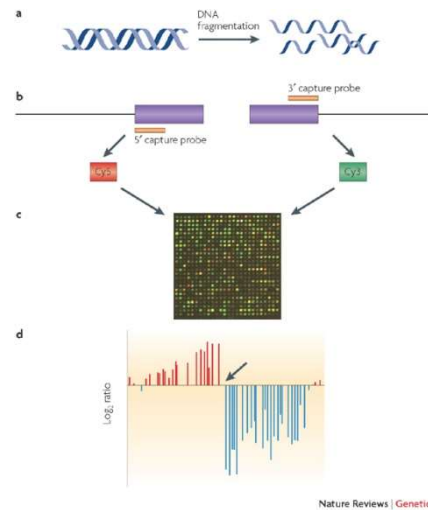


B

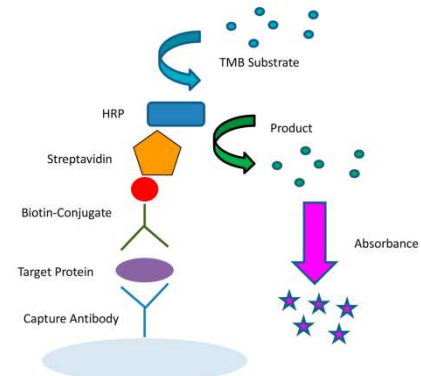


Product characterization

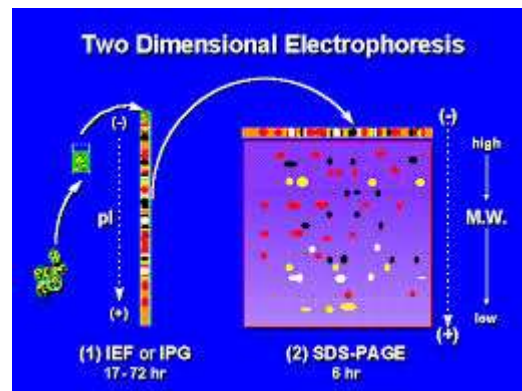
microarray



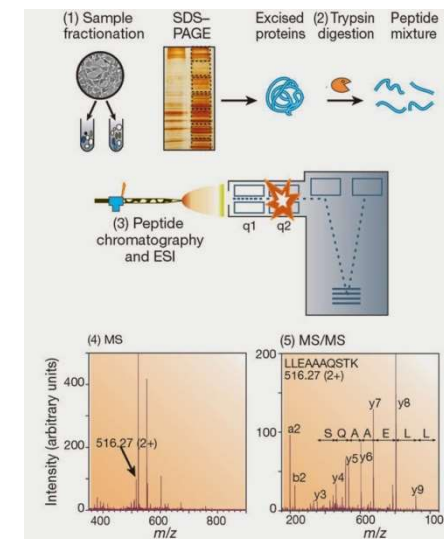
ELISA



2D-electrophoresis



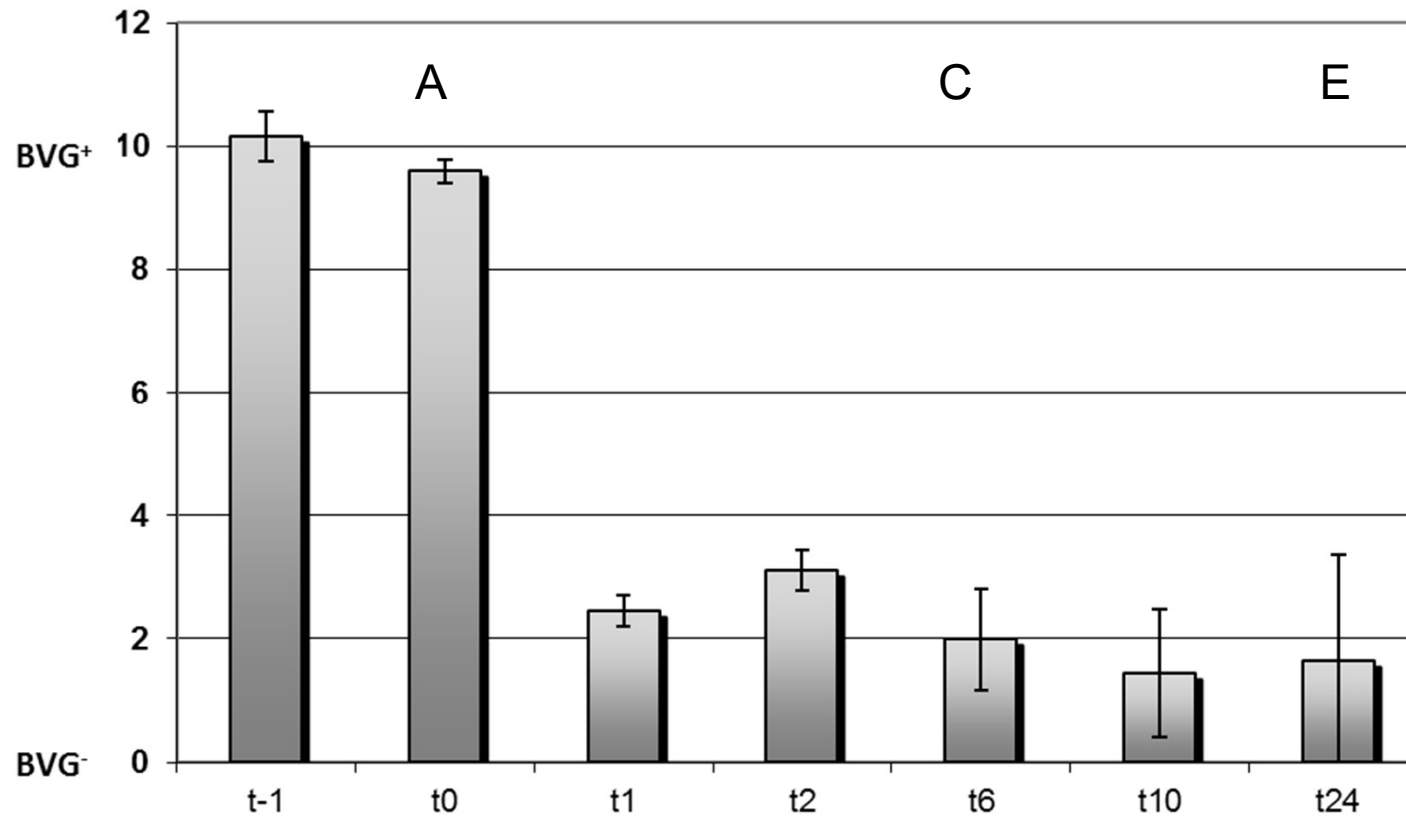
LC-MS



Bernard Metz *et al*, in preparation

Microarrays

Monitoring virulence associated genes



Product quality: **good**

poor

Monitoring key marker antigens

Selection of key marker antigens

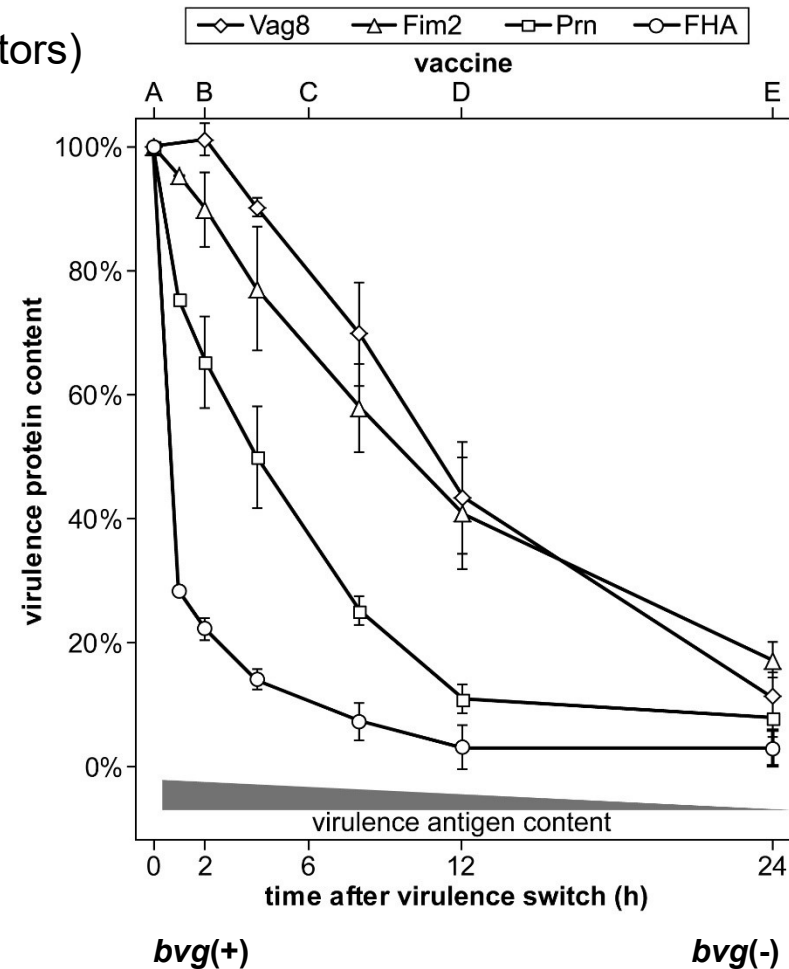
- known protective antigens (generally virulence factors)
 - Ptx (excreted → hardly present in WCV)
 - FHA**
 - Prn** (splitted off → poorly present in WCV)
 - Fimbriae**
 - Vag8** (virulence associated gene 8)

ELISA (quick scan)

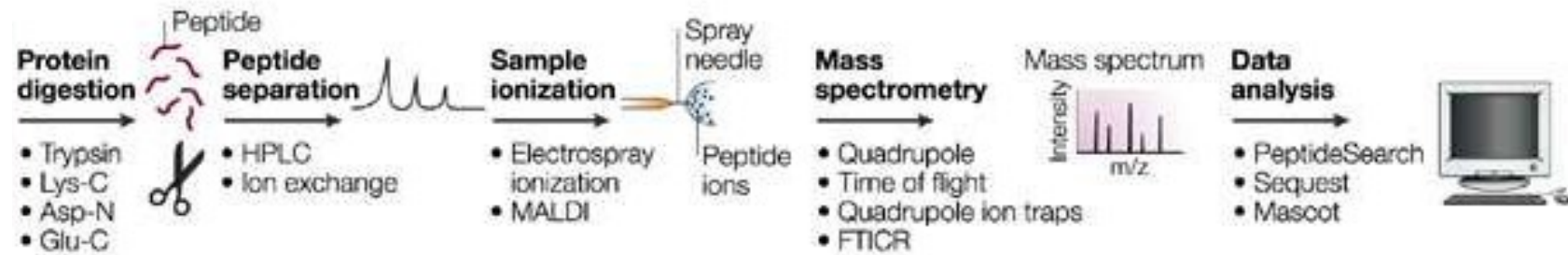
- Coating serial dilutions of whole cell products
- Detection of specific antigens with MoAb's
- Goat anti Mouse IgG-HRP

Is this the right selection?

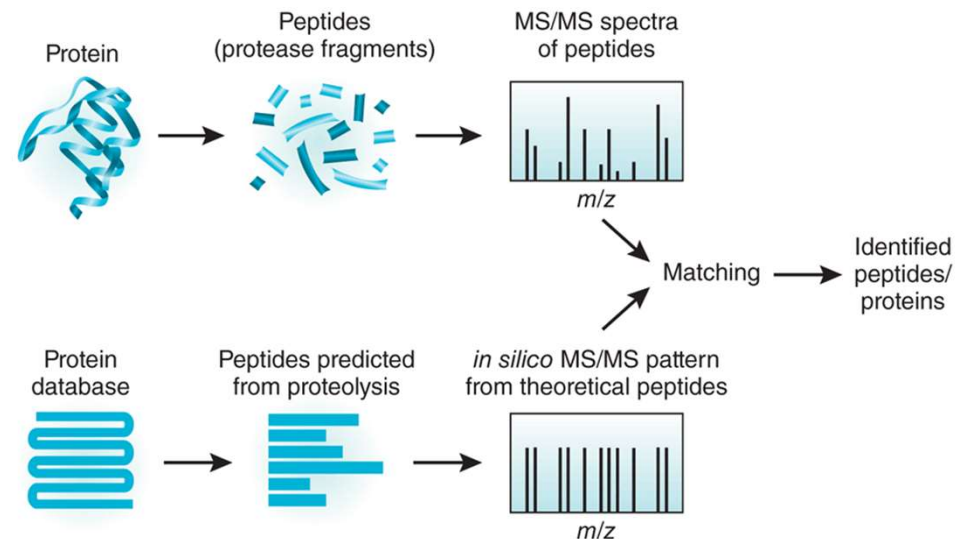
- Gene activation
- Protein production
- Immunoprofiling



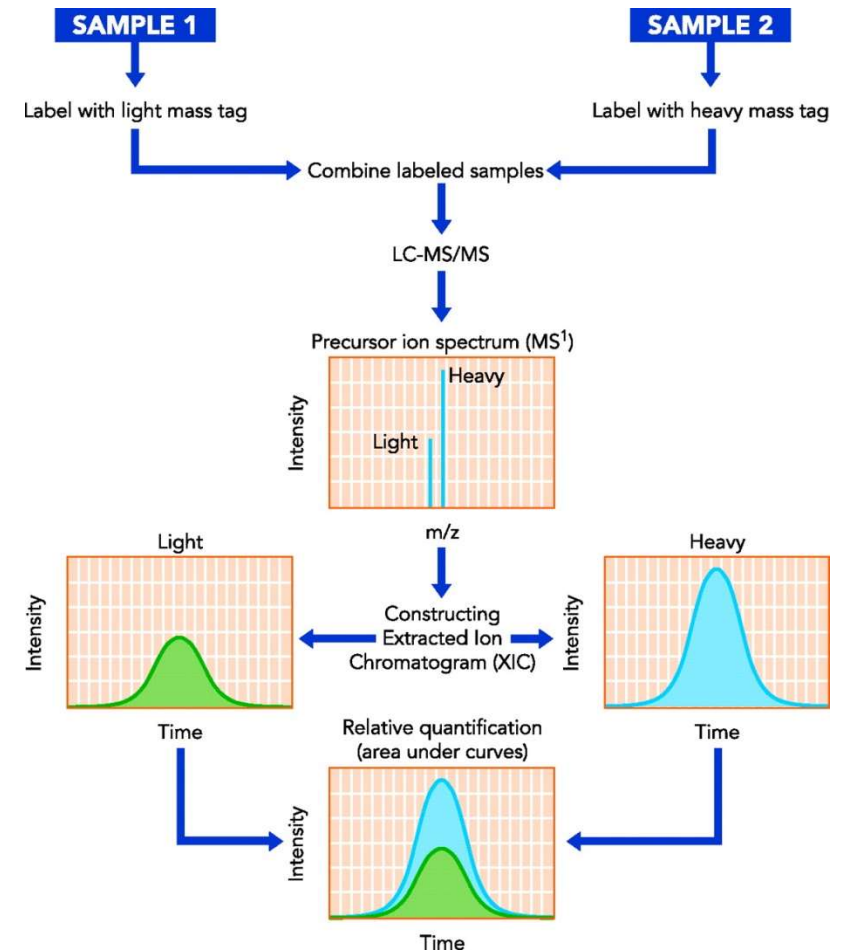
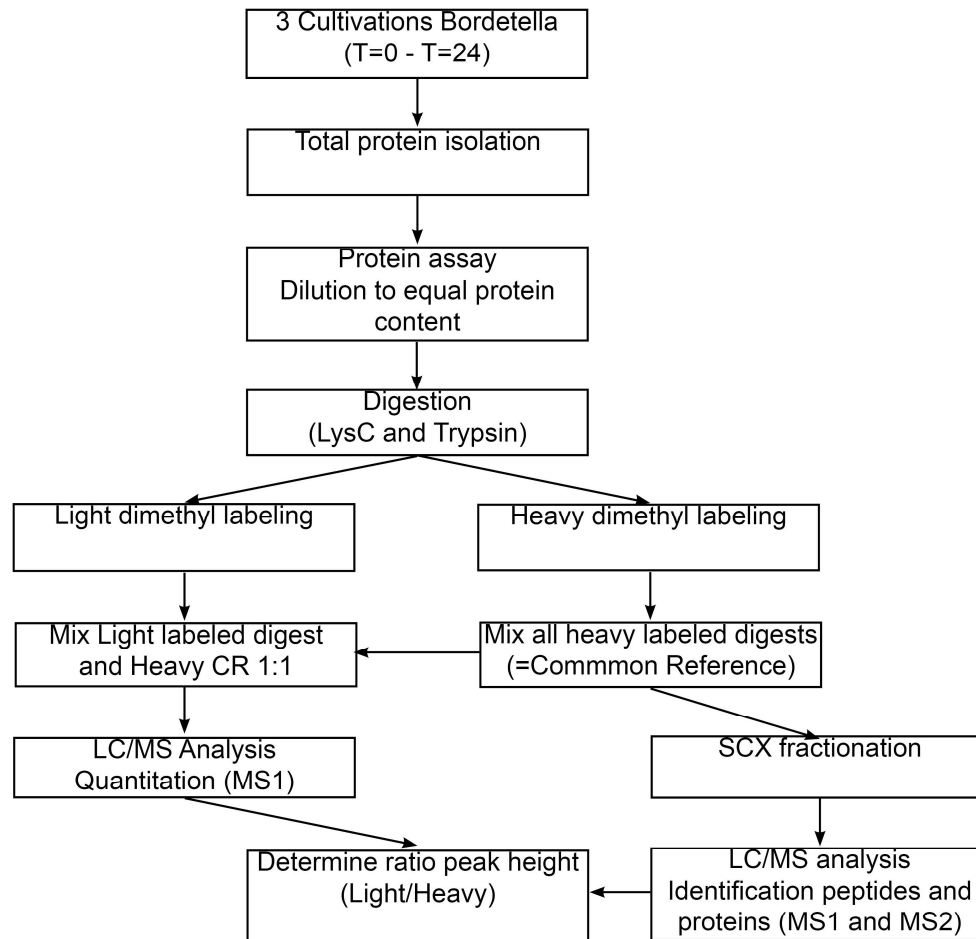
Identification of proteins by LC-MS



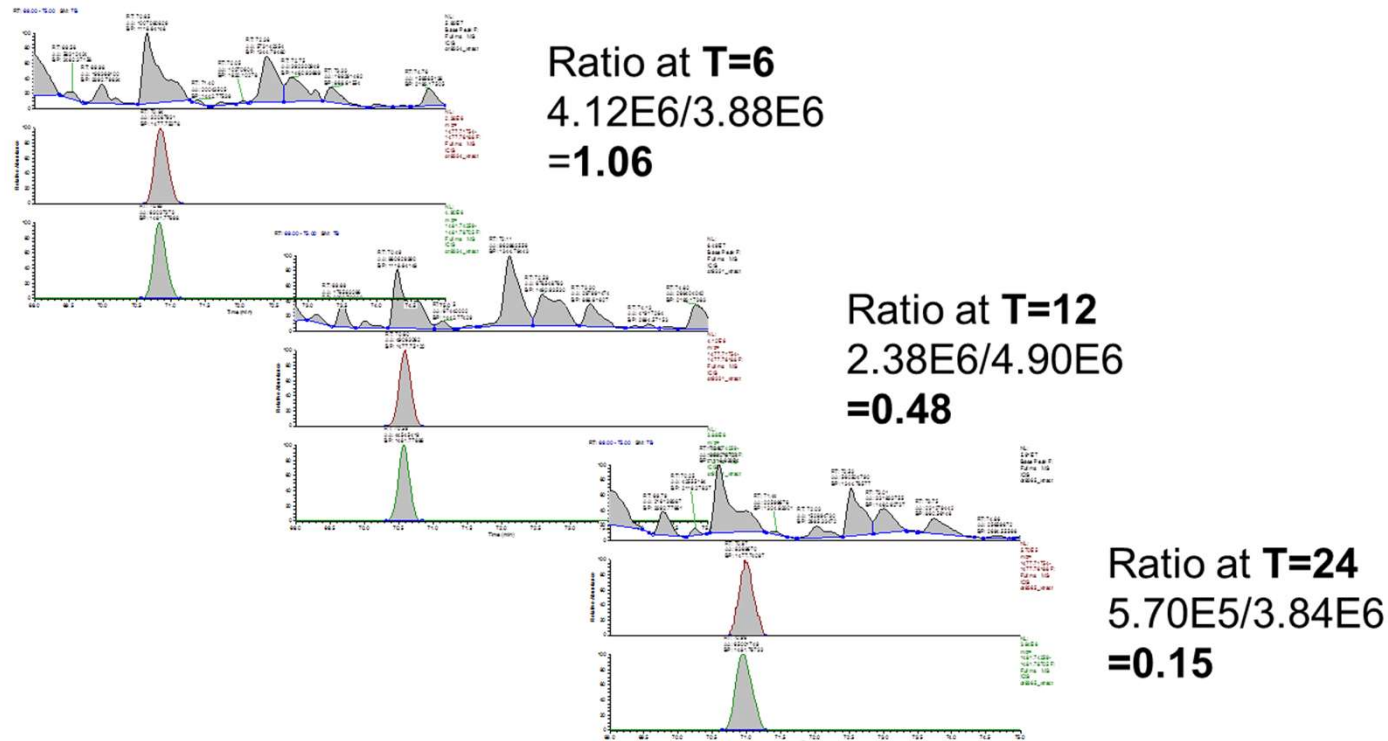
Nature Reviews | Molecular Cell Biology



Quantitation of proteins by LC-MS



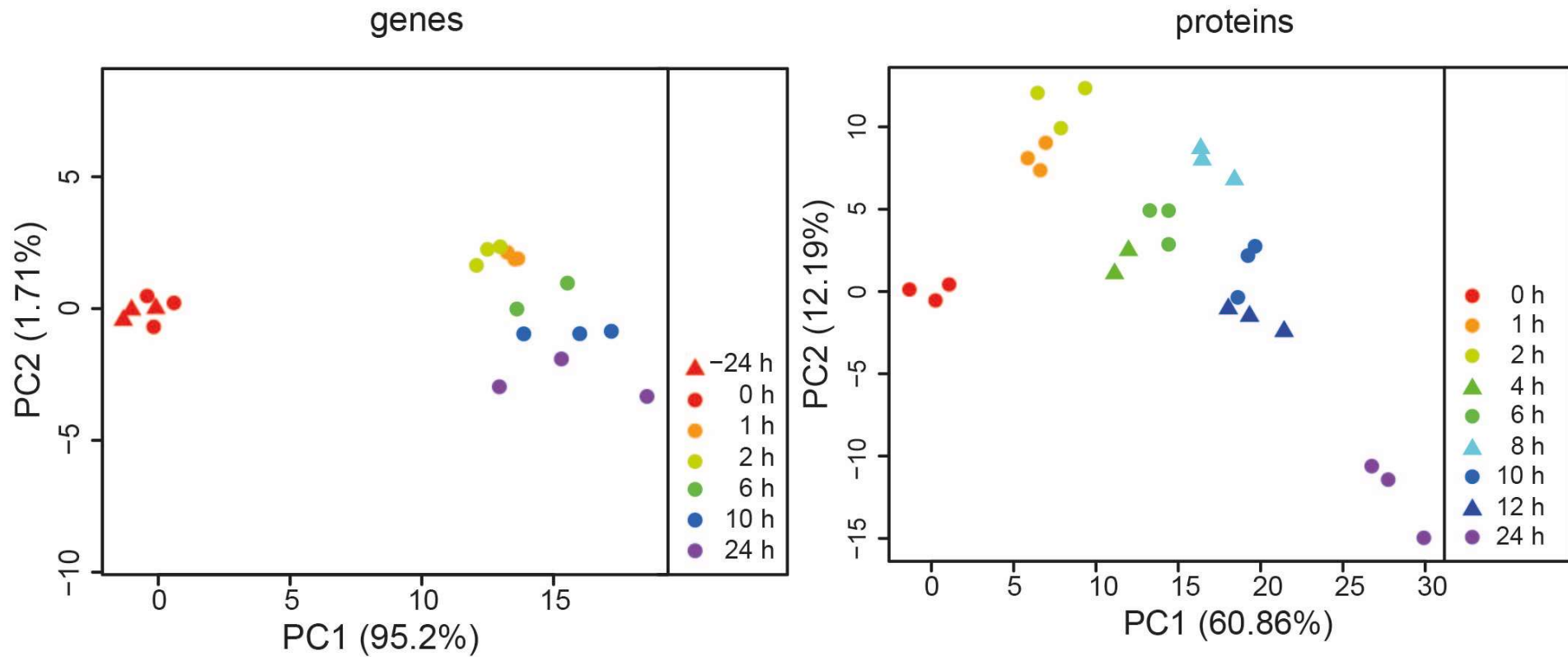
Quantitation of peptides and proteins



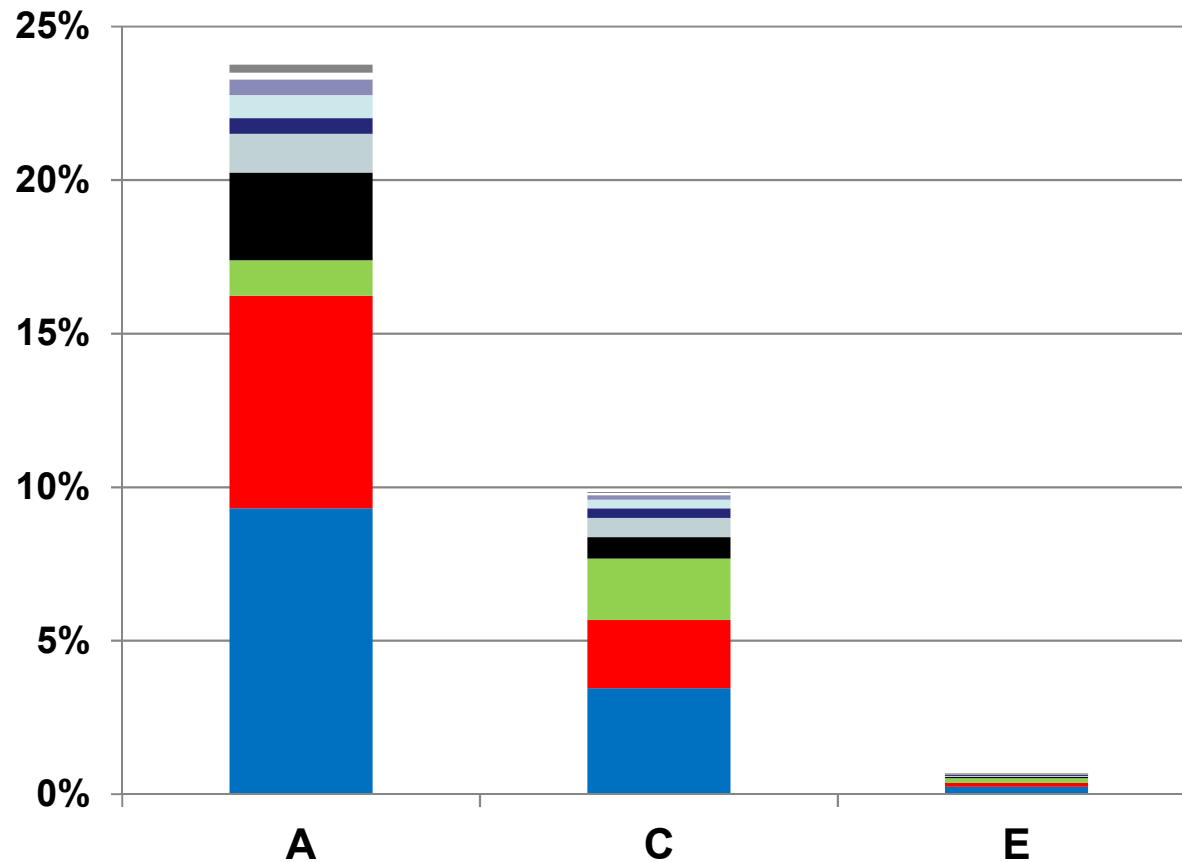
Up and down regulation
of protein expression

	T=0	T=6	T=12	T=24
Pertactin (P14283)	1.69	1.06	0.48	0.15
Putative periplasmic substrate binding protein (Q7VWX9)	0.11	1.03	1.82	2.47

Gene and protein expression



% virulence proteins in WCV

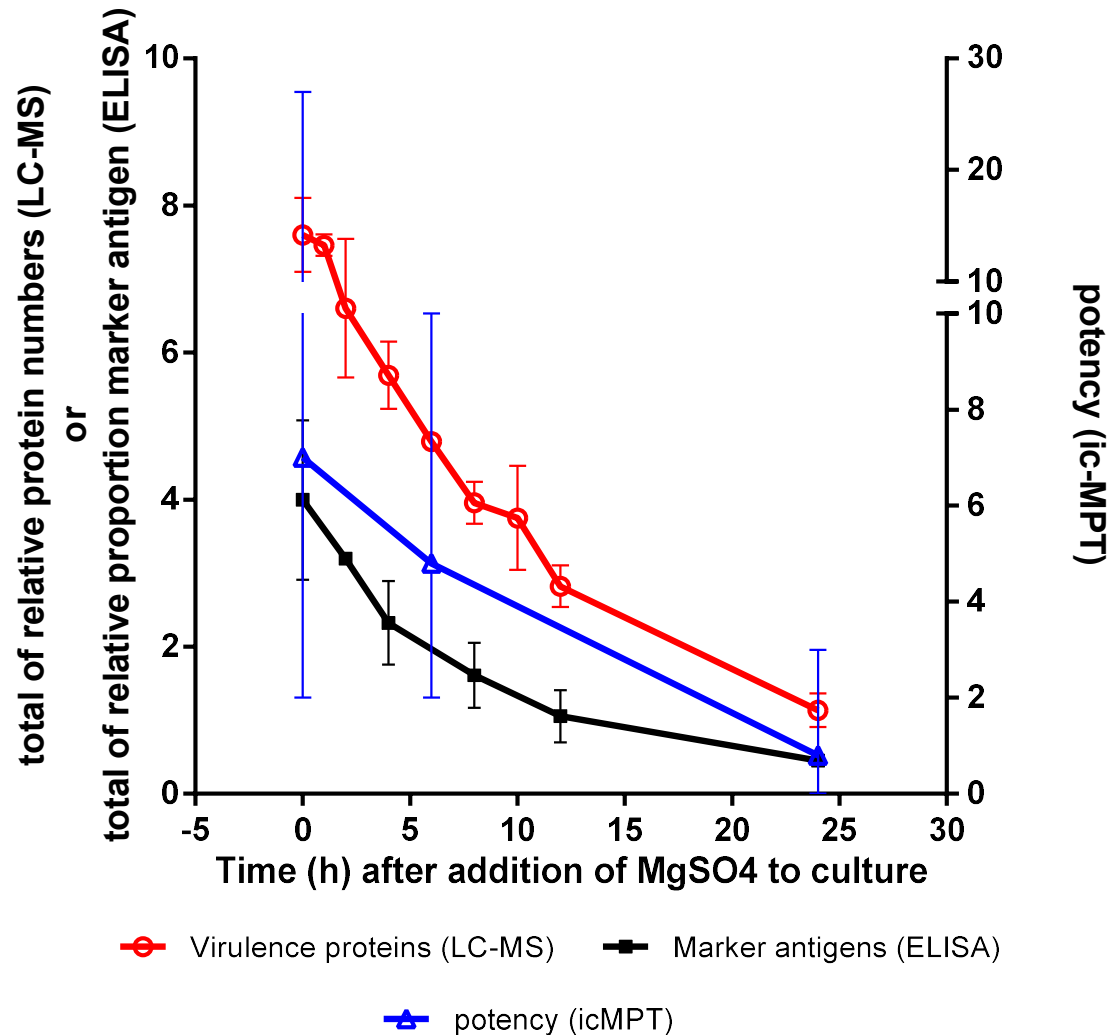


Vaccine A
good quality (bvg+)

Vaccine C:
Intermediate quality

Vaccine E
Poor quality (bvg-)

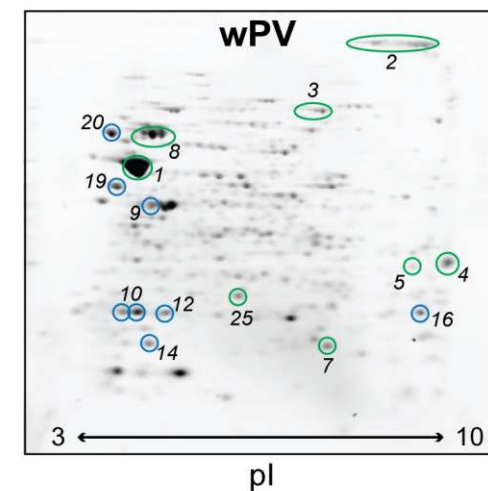
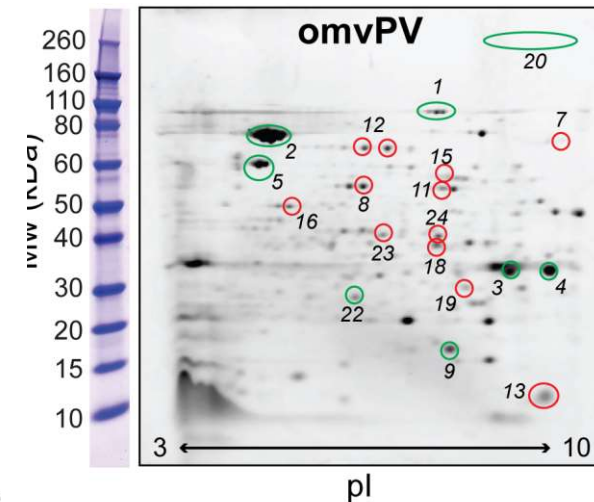
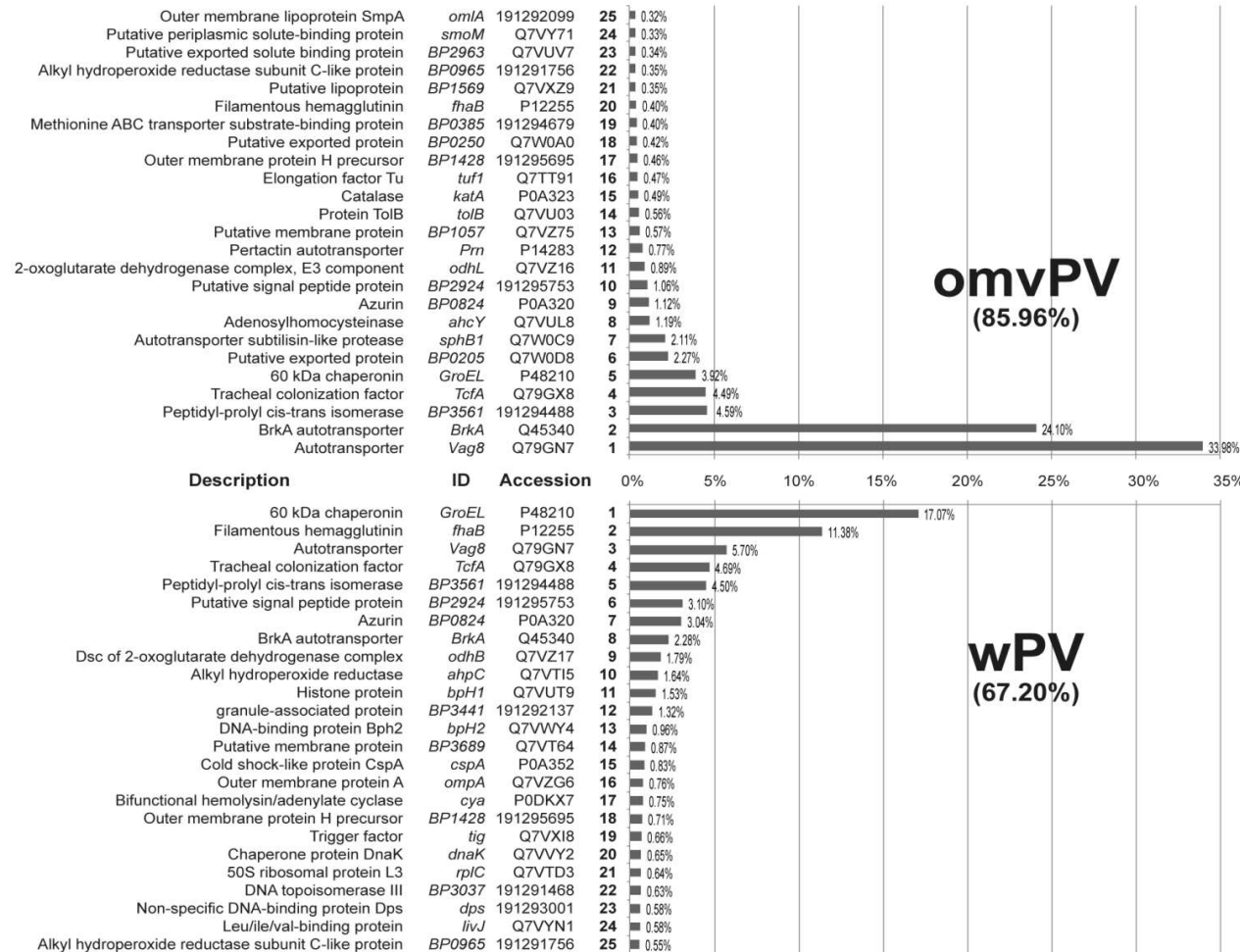
Relation antigen content and protection



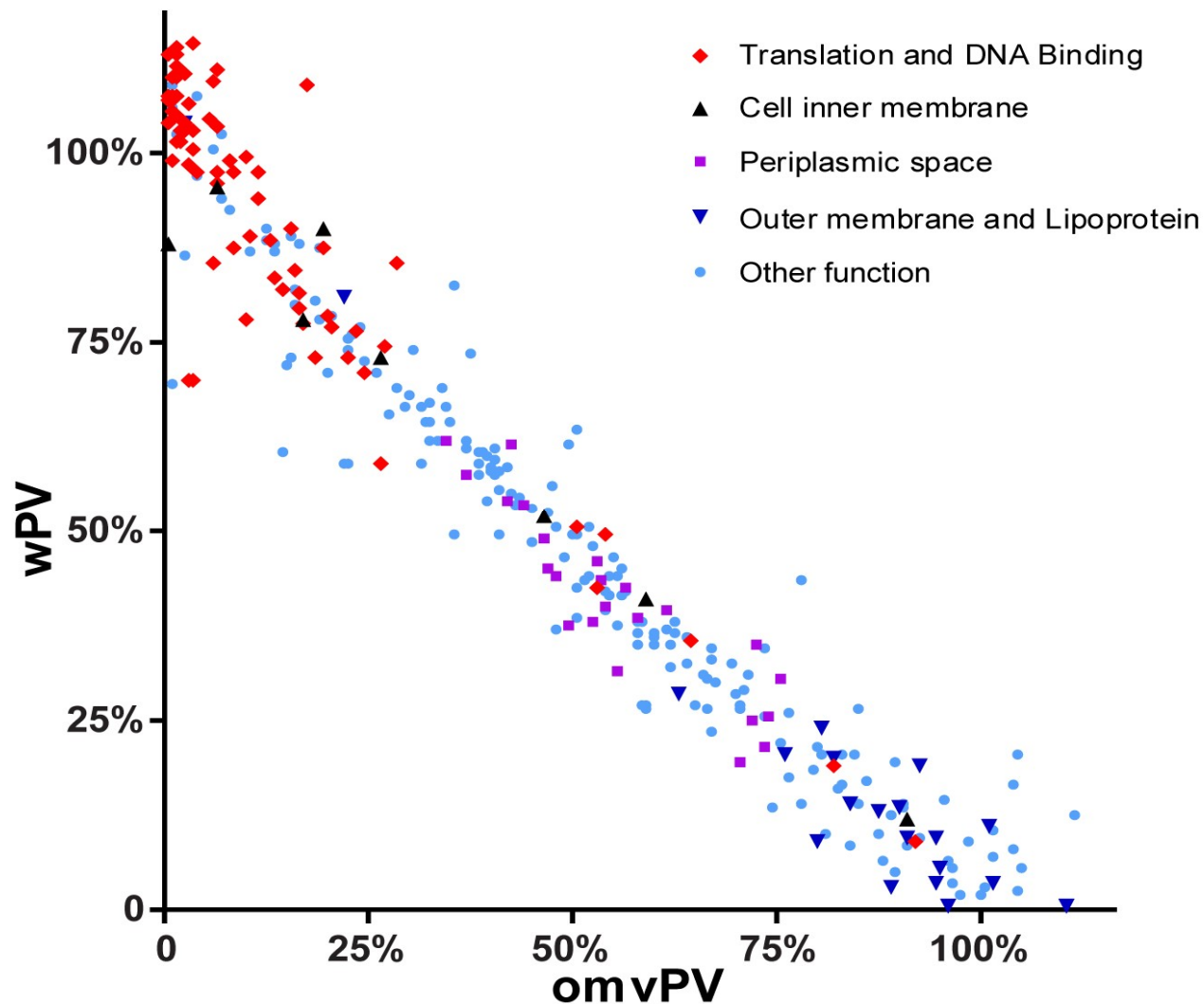
Vaccine protein composition

LC-MS

2DE



Vaccine protein composition



LC-MS

Common reference

Functional analysis

DAVID

GO-terms

omvPV profile:

Enrichment of
outer membrane
proteins

Absence ribosomal
proteins

Proteomics whole cell based vaccines

	WCV	OMVs
Peptides identified	7600	-
Proteins identified	1200	-
Proteins quantified	332	268
Proteins differently expressed	151	-
% virulence factors	≈25%	50-75%
Top 5 of known antigens	groEL, fhaB, vag8, tcfA, brkA	Vag8, brkA, tcfA, groEL, sphB1

Conclusions

Antigenic composition of whole cell based pertussis vaccines is largely determined by production process conditions

Key marker antigens

- expected: Ptx, FHA, Prn, Fimbriae
- determined: FHA, Vag8, BrkA, TcfA

Efficacy of pertussis vaccine seems to be related to the proportion of specific antigens in the product

LC-MS is a powerful tool to characterize undefined vaccines like WCV

More detailed information can be requested by mail:

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