Indirect parameters related to vaccine efficacy along the FMD-vaccine production process

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ARGENTINA OIE FMD-STATUS

Outbreaks in neighbor countries

Free with vaccination

Free without vaccination

(PATAGONIA)

FMD IN ARGENTINA

STRENGTHS

- Argentina is a regional leader in FMD control, research and epidemiology
- Local vaccine producers with high quality products (GMP compliance, ISO 9001 certification)
- Efficient controls by the regulatory authorities
- SENASA (regulatory authority)is an OIE reference Lab.
- Networked distribution and application of vaccines controlled by SENASA

COMMERCIALLY AVAILABLE VACCINES IN ARGENTINA

National producers - Excellent quality

- ✓ COMPOSITION:
  - 01 Campos
  - A/Argentina/ 2001
  - A24 Cruzeiro
  - C3 Indaial
  - Oil adjuvant

About 120.000.000 doses applied per year

FMD VACCINE-EFFICACY CONTROL TOOLS

EFFICACY

Ability to produce a desired amount of a desired effect

- Do not induce infection or any undesired effect
- Protect from challenge against the strains included in the vaccine
- Do not elicit antibodies to non-capsid proteins (NCP)

CONTROLLING FMD VACCINES

I	EPIDEMIOLOGICAL STUDIES	• MATCHING OF VACCINE AND FIELD STRAINS • MAbs/SEQUENCING • VN/CROSS PROTECTION
II	IN PROCESS CONTROL	• CELL AND VIRUS BANKS. IDENTITY AND PURITY OF VACCINE STRAINS (MAbs) • QUANTIFICATION OF 140S • FREE OF NCP (rAg + MAbs)
III	FINAL PRODUCT	• SAFETY • ALL SEROTYPES PRESENT • POTENCY: INDIRECT (lp ELISA) • POTENCY PPG (ALEATORY) • FREE OF NCP
IV	EFFECTIVITY OF VACCINATION	• HERD IMMUNITY (slp ELISA) • VIRAL CIRCULATION (3ABC ELISA)

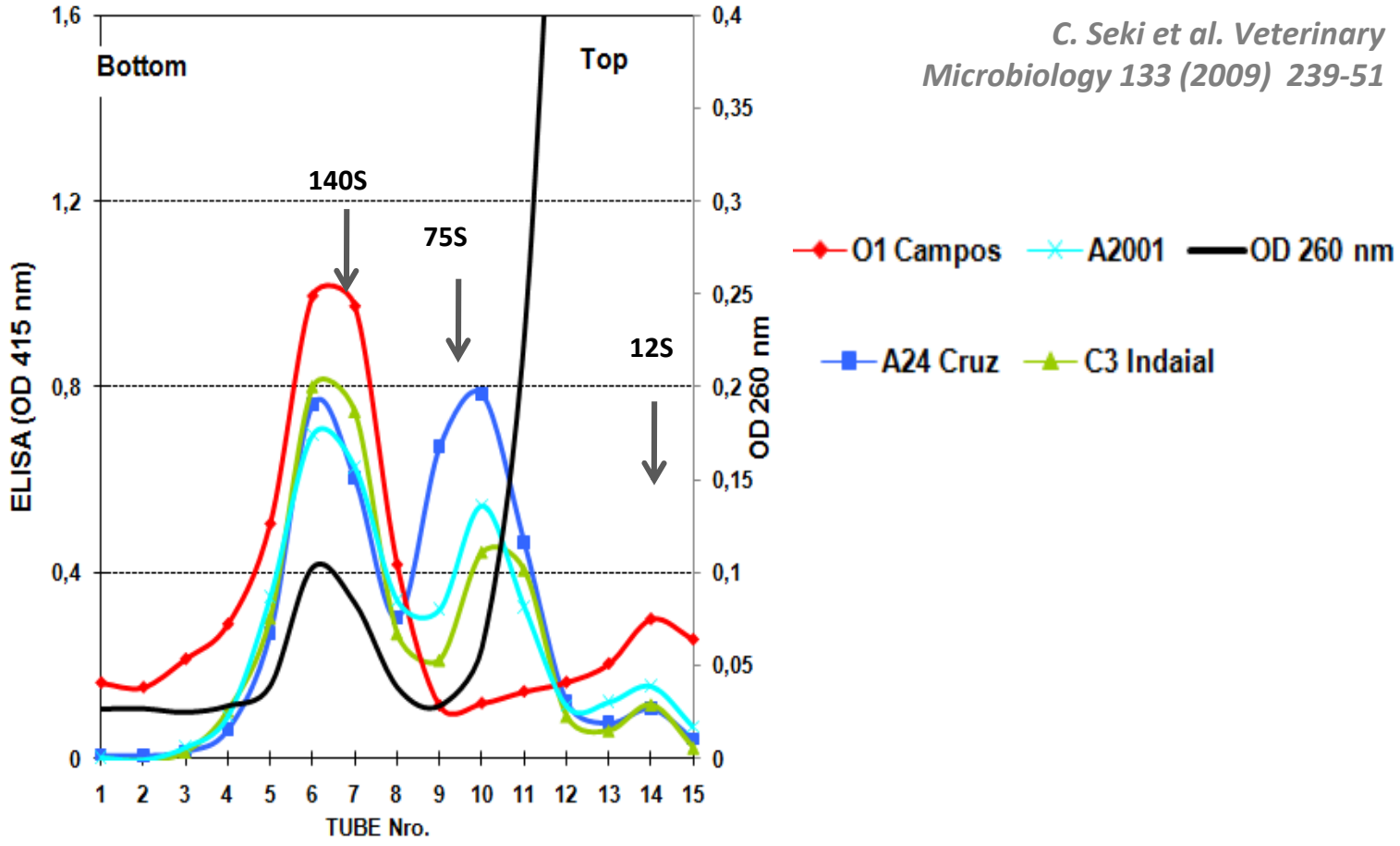
ANTIGEN

Requirements

- **Integrity** - 140S particles, VP1
- **Identity** - of each strain
- **Purity** - Absence of non-capsid proteins able to induce immune responses

ANTIGEN INTEGRITY: 140S particles

CURRENTLY USED: Sucrose gradient ultracentrifugation + serotype-specific ELISA



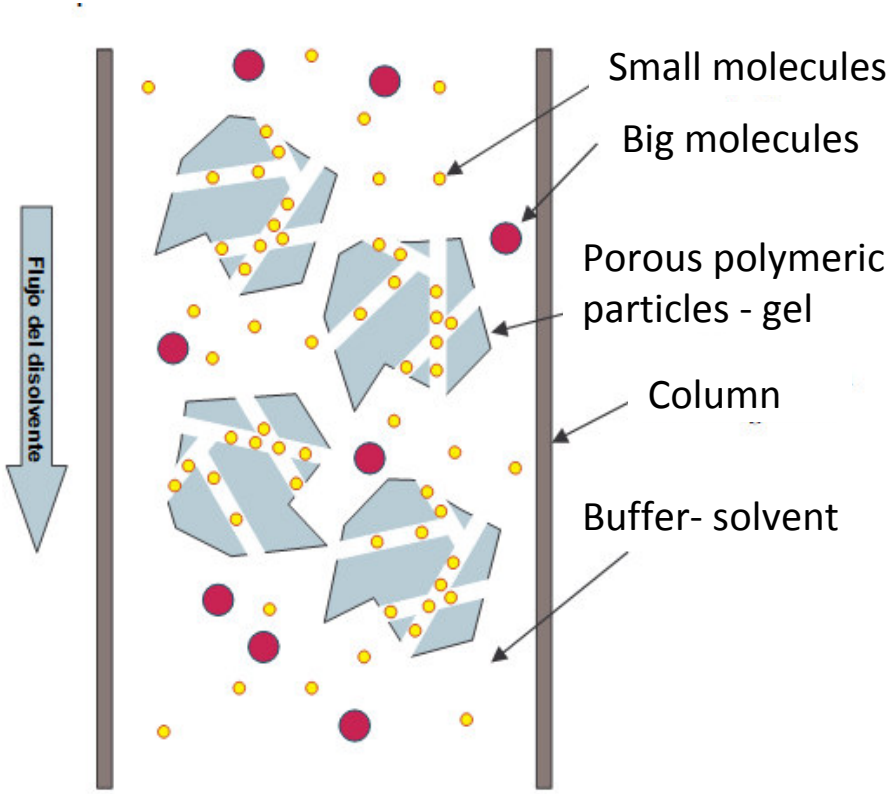
C. Seki et al. Veterinary Microbiology 133 (2009) 239-51

Improved method for quantification of 140S particles: automated

ANTIGEN INTEGRITY: 140S particles

Improved methods for quantification of 140S particles

Separation of whole viral particles by liquid chromatography



Gel filtration is a liquid chromatography technique that separates molecules according to their sizes. It is sometimes called size exclusion or gel permeation chromatography.

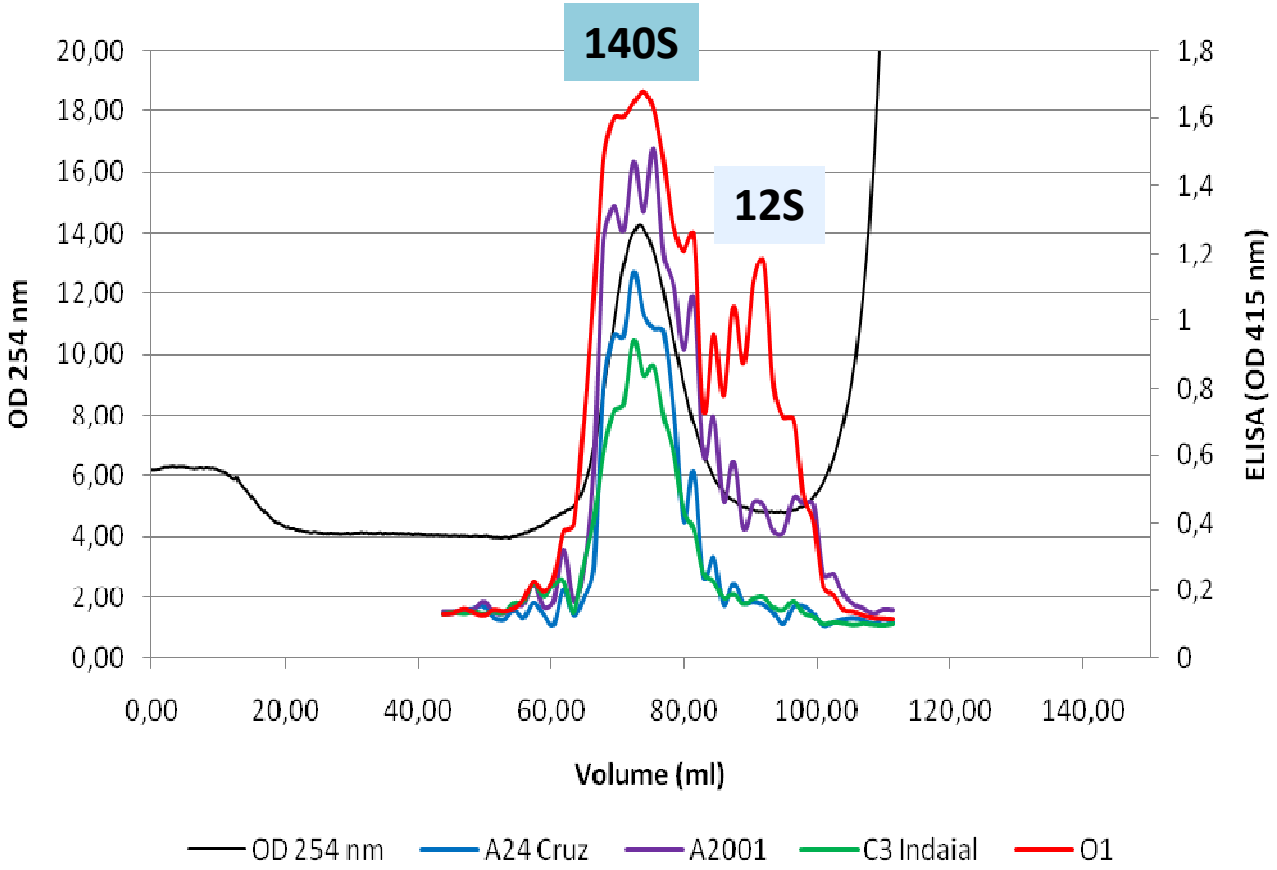
Separation of whole viral particles by liquid chromatography

Tetravalent vaccine

Treatment with DNAse

Sephacryl S400

Reactivity against MAbs



Aqueous phase extracted from a tetravalent argentine commercial vaccine, analyzed with strain specific MAbs ELISA.

- ✓All strains could be purified in the same elution volume.
- ✓ The graph shows that all the strains are present in the vaccine as 140S particles

Separation of whole viral particles by liquid chromatography vs sucrose gradient ultracentrifugation

M.A. Spitteler et al. / Vaccine 29 (2011) 7182–7187

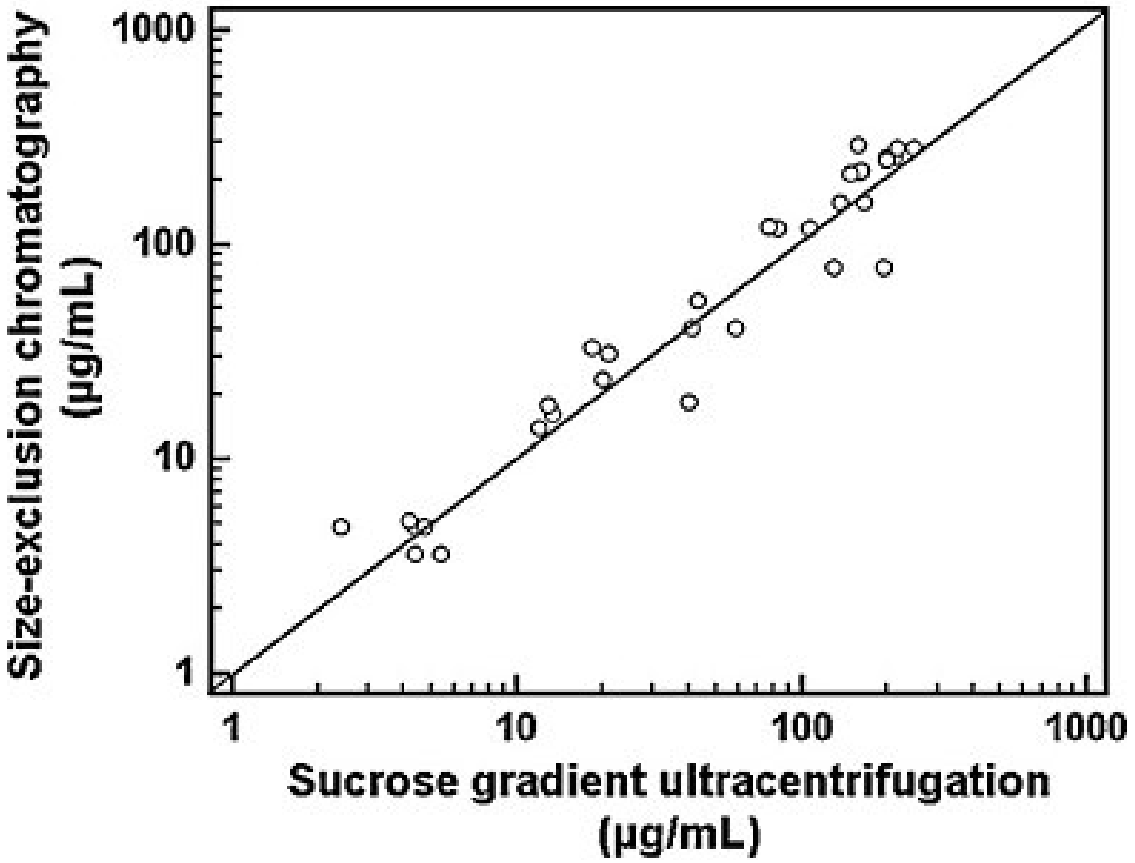


Fig. 6. Concordance analysis. A parity plot was constructed with results obtained from size exclusion chromatography (*Y* axis) and sucrose gradient centrifugation (*X* axis), each symbol represents results corresponding to analysis of process samples containing either FMDV O1 Campos, A24 Cruzeiro, A Argentina 2001 or C3Indaial or

VACCINE PURITY REQUIREMENT

NCP FREE VACCINES

VACCINES MUST BE CONTROLLED TO GUANTANTEE THEIR INCAPACITY TO INDUCE ANTIBODIES TO NCP (3ABC)

VACCINATION - DIVA TESTING

Discriminate infected from vaccinated animals

INFECTION

✓ *NCP ELISA (+)*
✓ *CP ELISA (+)*

Antibodies against capsid and non capsid proteins

VACCINATION

✓ *NCP ELISA (-)*
✓ *CP ELISA (+)*

Antibodies against capsid proteins

FMD vaccine purity assessment

- **Serology after repeated vaccination** ***Available now***
  - *Naïve* cattle are vaccinated 3 times using double dose of vaccine
  - Serology is performed at regular intervals (up to 90 dpv)

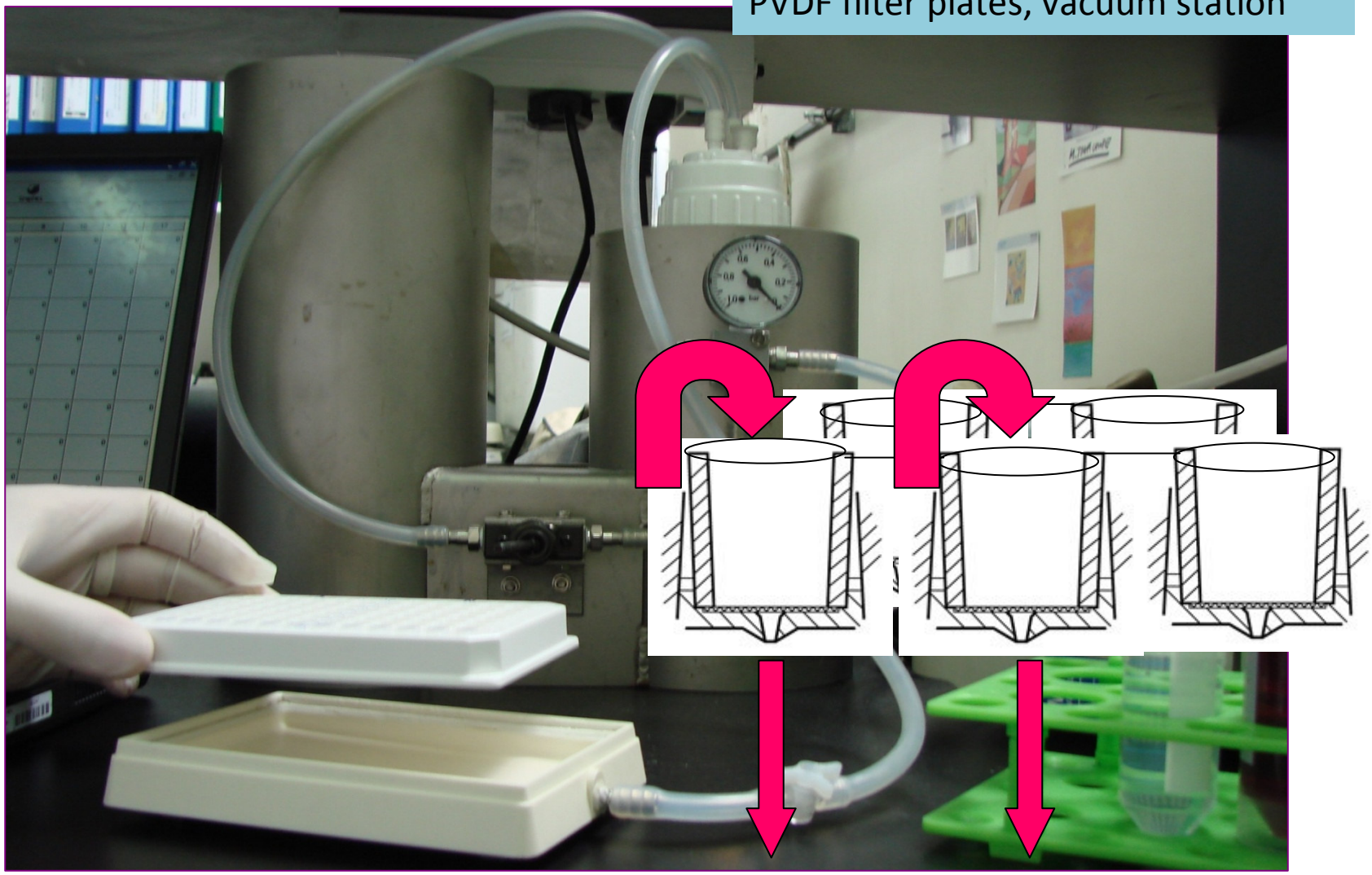
- **Lowest amount of 3ABC to induce antibodies after 3 vaccinations:** about 10 ng (from rec 3ABC vaccination studies)

Filtration-Assisted Chemi-Luminometric Immunoassay "FAL-ELISA"

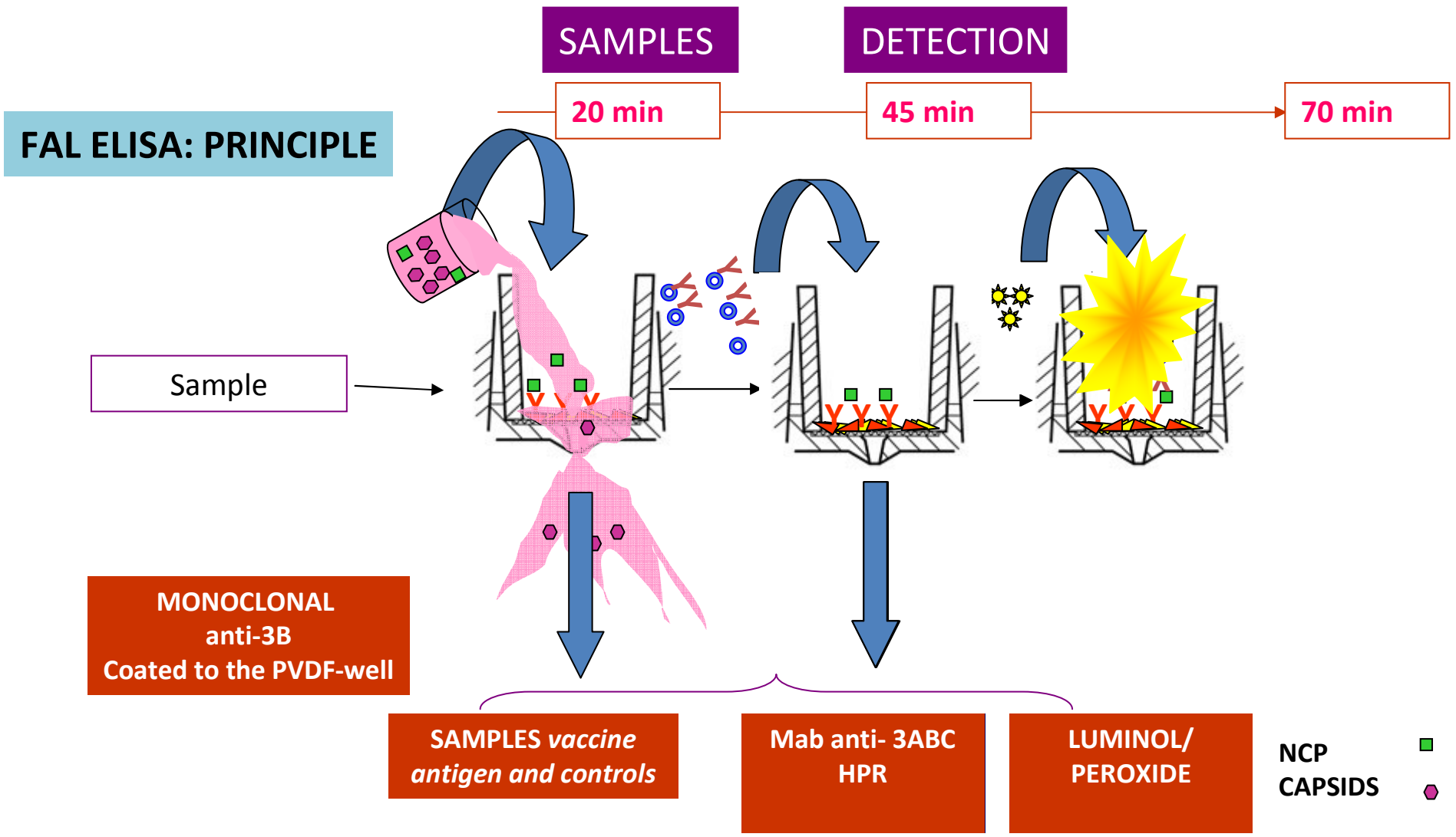
Limitations on the development		SOLUTION
Need of high sensitivity	Data from r3ABC immunization: <42 ng/dose (one dose) <10.2ng/ dose (three doses)	
Volume of the sample	VARIABLE – Unknown concentration factor to yield detectable NCP	
Sample composition	VARIABLE and mostly unknown, with possible interferents for immune interactions and chemical reactions	

FAL ELISA PLATFORM

PVDF filter plates, vacuum station



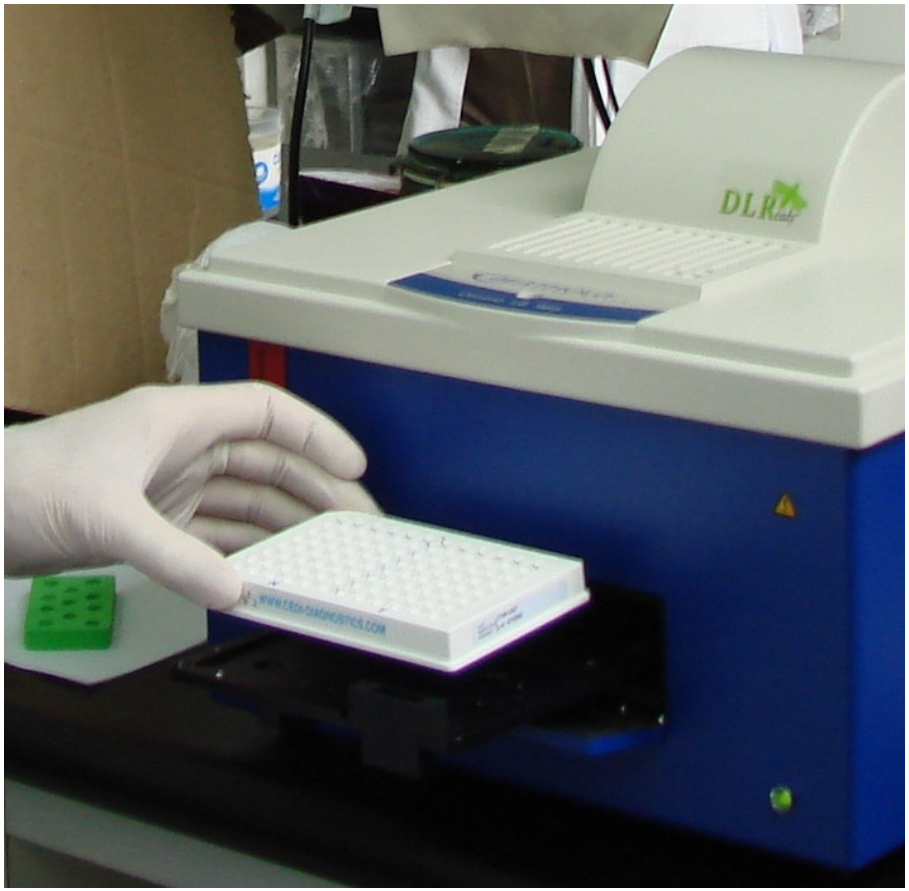
FAL ELISA STEP by STEP

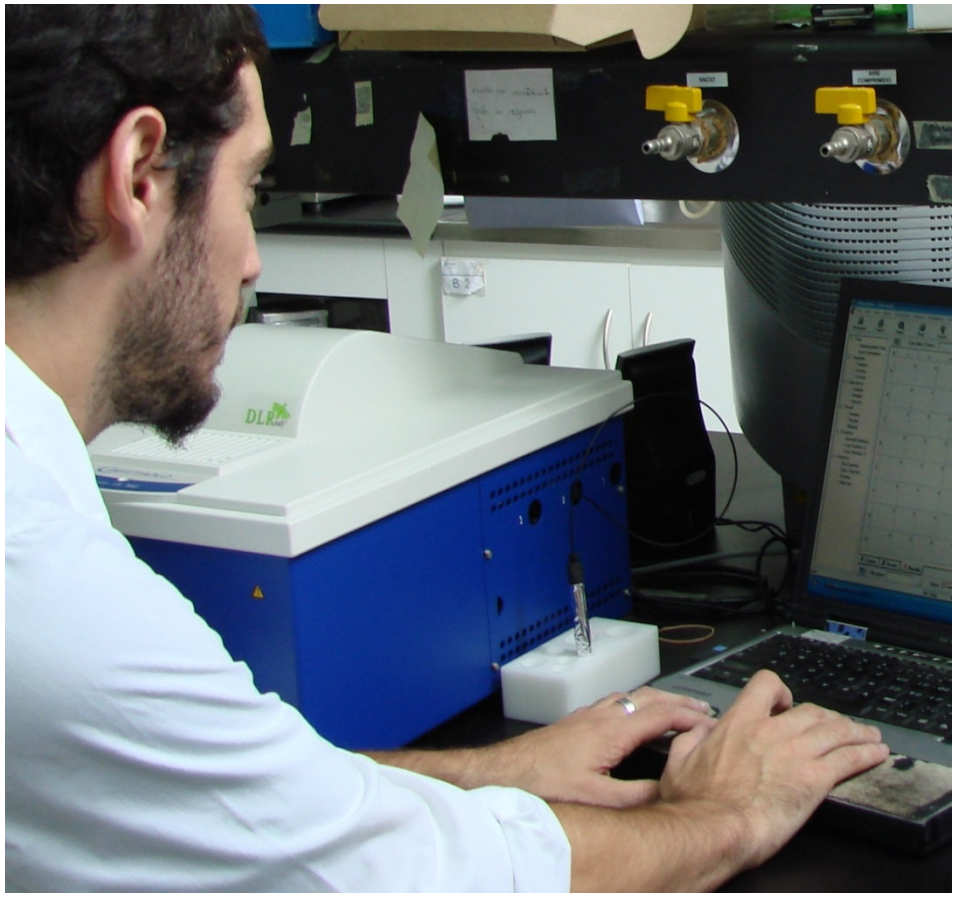


ROOM TEMPERATURE

FAL ELISA PLATFORM

Photoluminometer





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Development of an in process control filtration-assisted chemiluminometric immunoassay to quantify foot and mouth disease virus (FMDV) non-capsid proteins in vaccine-antigen batches

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ABSTRACT

In many countries, foot and mouth disease (FMD) is controlled by vaccination and surveillance against non-capsid proteins (NCP); therefore vaccines are required not to induce antibodies against NCP. Vaccine purity is evaluated by repeated inoculation of naïve cattle, an expensive and time consuming protocol that raises several animal welfare concerns. We have developed an in process control filtration-assisted chemiluminometric immunoassay (FAL-ELISA) to detect and quantify NCP in vaccine antigen batches

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FAL-ELISA to quantify FMDV-3ABC : VACCINE ANTIGEN BATCHES

Do vaccine components interfere with the reaction?

"SPIKING" VACCINE ANTIGEN BATCHES with known amounts of 3ABC

- TWO BATCHES:
  - 1X (filtered)
  - PEG concentrate

FAL-ELISA to quantify FMDV-3ABC : VACCINE ANTIGEN BATCHES

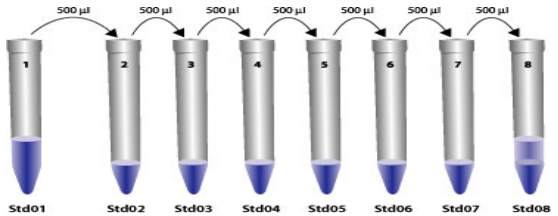
Vaccine components DO NOT interfere with 3ABC detection

SPIKING VACCINE ANTIGEN BATCHES with known amounts of 3ABC

- 1X (filtered)
- PEG concentrate

VS

SERIAL DILUTIONS OF rec 3ABC



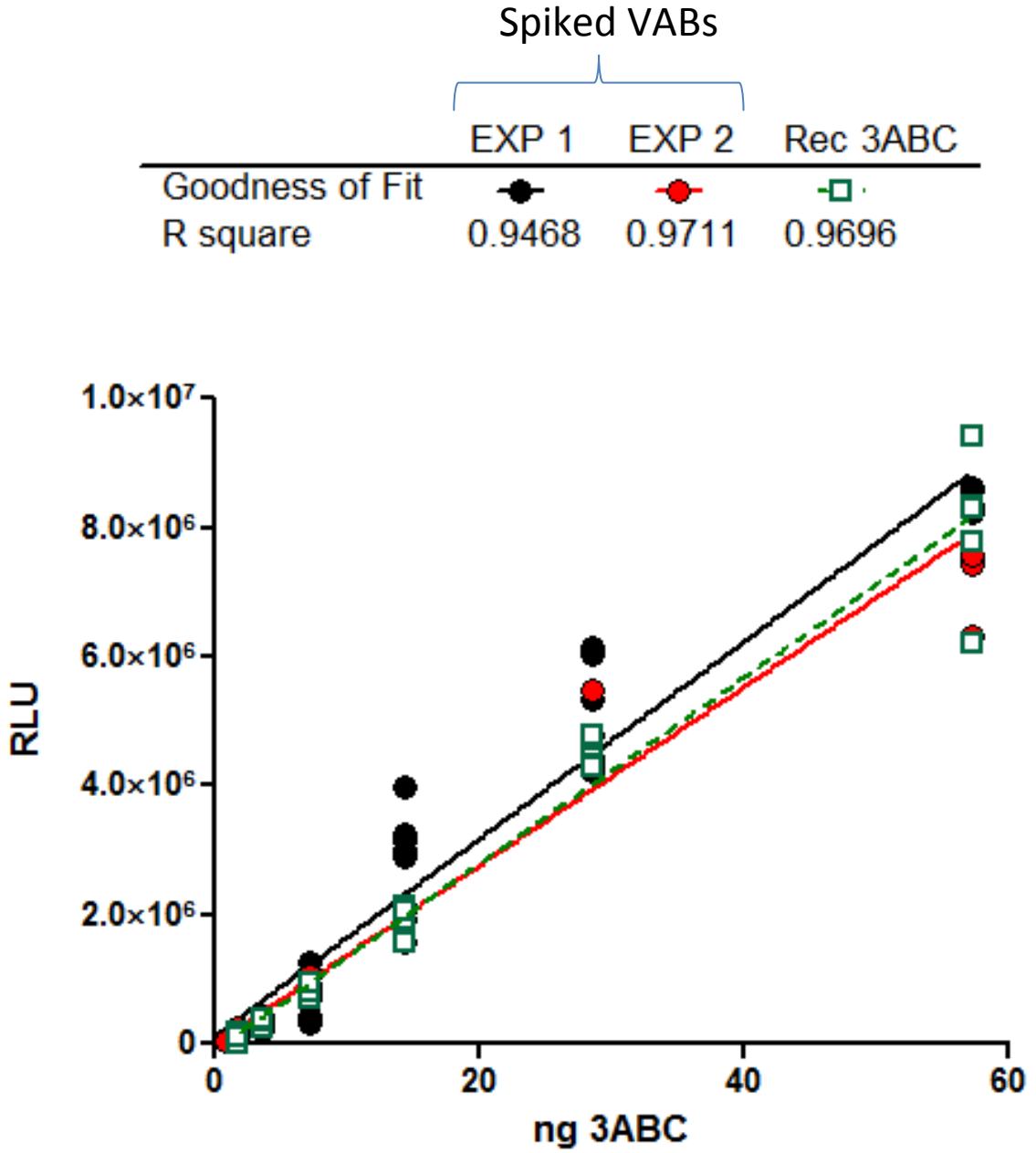
INITIAL SENSITIVITY: 2ng/well

Linear range: 2-30 ng/well

FAL-ELISA applied to VACCINE ANTIGEN BATCHES

THE KIT

Linear detection range
0.89 - 57ng/well



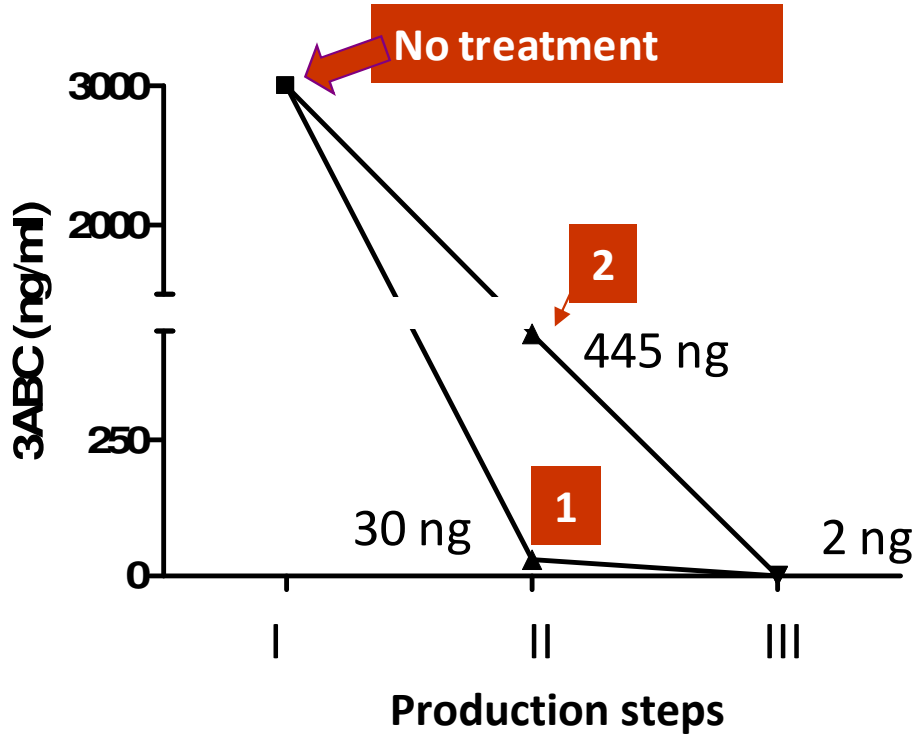
FAL-ELISA applied to improve vaccine production

Application

EXAMPLE: FAL ELISA APPLIED TO FMDV VACCINE ANTIGEN PRODUCTION PROCESS

Production steps

I- PRE-PURIFICATION TREATMENT	Unique
II- PARTIAL PURIFICATION TREATMENT	1 vs. 2
III-FINAL PURIFICATION TREATMENT	Unique



Quantitation of 3ABC in FMD vaccine antigen batches using FAL ELISA

Conclusions

- The test is sensitive enough to detect 3ABC
- The use of filtration allows the concentration of large volumes and also "cleans up" samples, which is particularly important when they are from diverse origin and complex composition (i.e.: industrial antigenic preparations).
- The application of the assay allow producers to optimize their production process
- New efforts are focused in applying the assay to formulated vaccines and to determine the lowest amount of NSP that can elicit specific antibodies in vaccinated cattle

Indirect parameters related to vaccine efficacy along the FMD-vaccine production process

Conclusions

- New methods are being applied based on new technologies to quantify whole particles, their integrity and identity
- These techniques allow to reduce the use of animals in vaccine efficacy testing
- Development of improved technologies to *allow in vitro* indirect vaccine testing is mandatory

FMD research in Argentina

RIIDFA: Network for FMD research and development

INTA

- IMMUNOLOGY
  - Maternal immunity and response to vaccines in calves
  - Onset of the immune response in bovines: vaccination and oro-nasal infection
- New assays to study cross-protection
  - IFN-gamma
  - Isotype-ELISA (IgM, IgG1, IgG2)
  - Avidity ELISA
- Cross-protection experiments in cattle
- Collaboration with companies: product development
- Molecular epidemiology

THANK YOU - GRACIAS