In Vitro Potency Control for FMD vaccines

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Potency of human vaccines

- **Human vaccines must comply with very strict quality specifications :**
  - **Potency.**
  - **Purity.**
  - **Safety.**
  - **Stability.**

- **Quality attributes of human vaccines are validated during phases I, II and III clinical trials. ➡ Regulatory Authority Approval.**
- **Each batch of human vaccine is released after complying with laboratory quality control assays (*in vitro*).**

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Potency of veterinary vaccines

- Numerous clinical trials in target animals are required for the initial registration of veterinary vaccines with the Regulatory Authority.
- Furthermore, in many cases, the quality attributes of veterinary vaccines are assayed in laboratory animals and/or in target animals for EACH BATCH of vaccine produced.
- Why not applying the same criteria as the human vaccine industry?
  - ➡ In vitro potency and control with validated analytical techniques.
- Worldwide awareness for Animal Welfare: ”3R” movement: Reduce, Refine, Replace.

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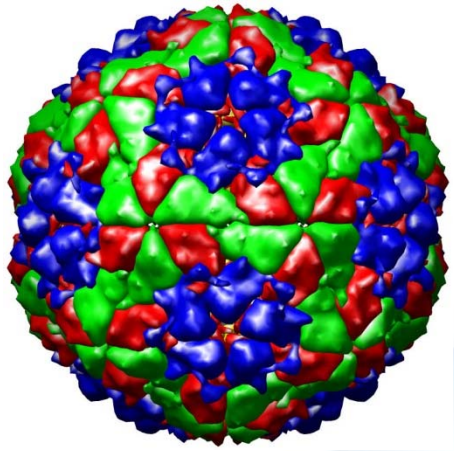
FMD vaccine: correlation of *In Vivo* potency with *In Vitro* parameters

- Parameters measurable *in vitro* that correlates with *in vivo* potency:
  - The antigenic payload of inactivated virus particle per dose of vaccine (Pay and Hingley, 1987).
  - The structural integrity of the viral particle is critical for the vaccine to afford protection (Doel and Chong, 1982).



- The adjuvant used also plays a critical role in the development kinetics, the strength and the duration of the immunity triggered by the vaccine. Therefore, the quality control testing of the final emulsion is critical for the stability and the potency of the vaccine (Aucouturier et al., 2001).

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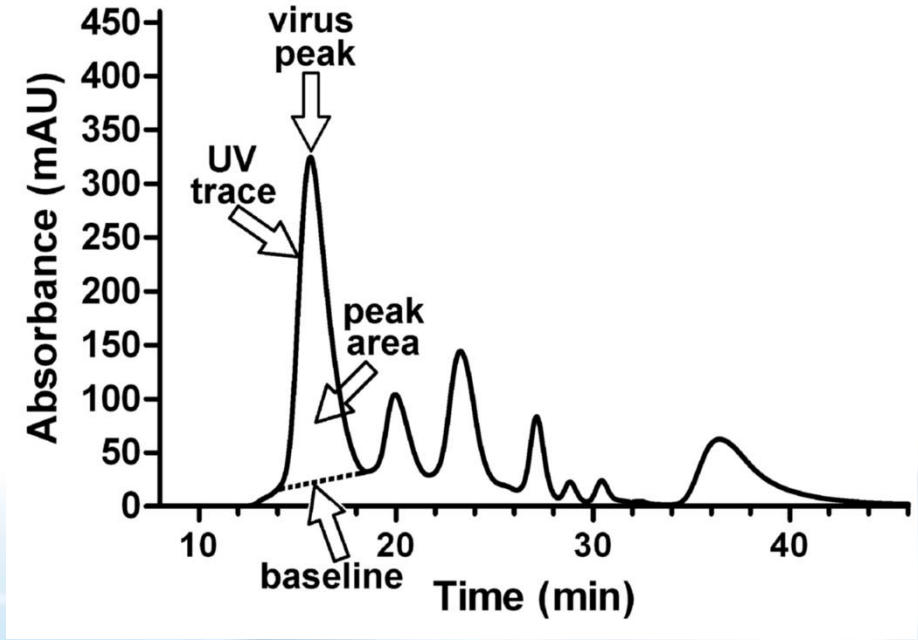
Precise quantification of viral particles by SEC HPLC

- Replacement of sucrose gradient centrifugation “146 S” technique with a robust and precise quantification method by molecular exclusion chromatography (SEC) HPLC:



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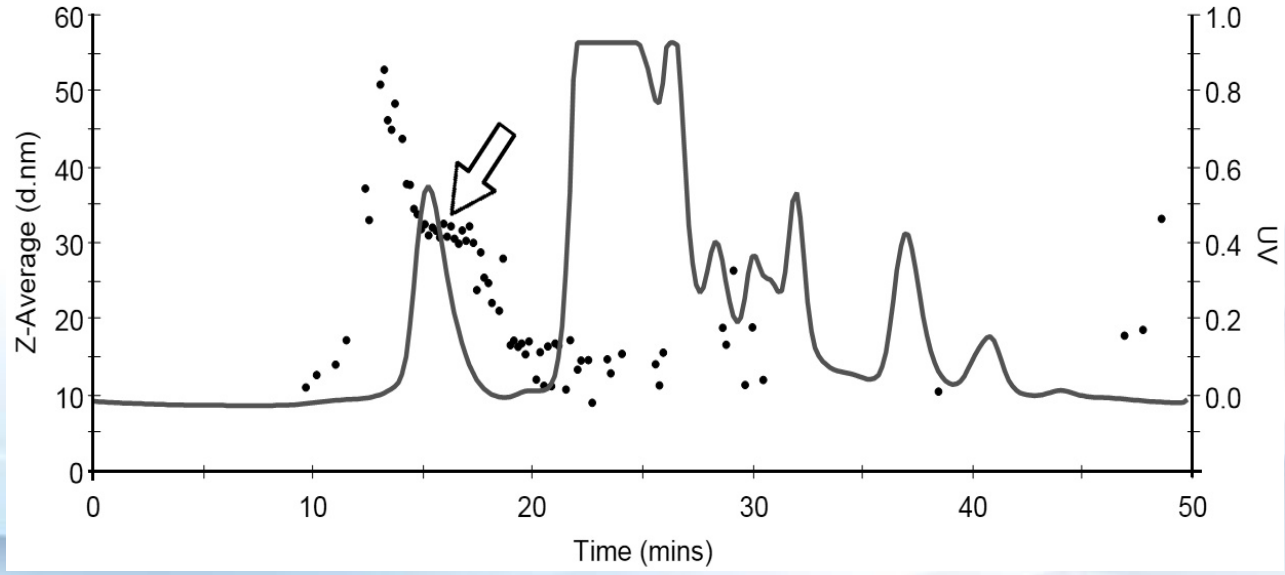
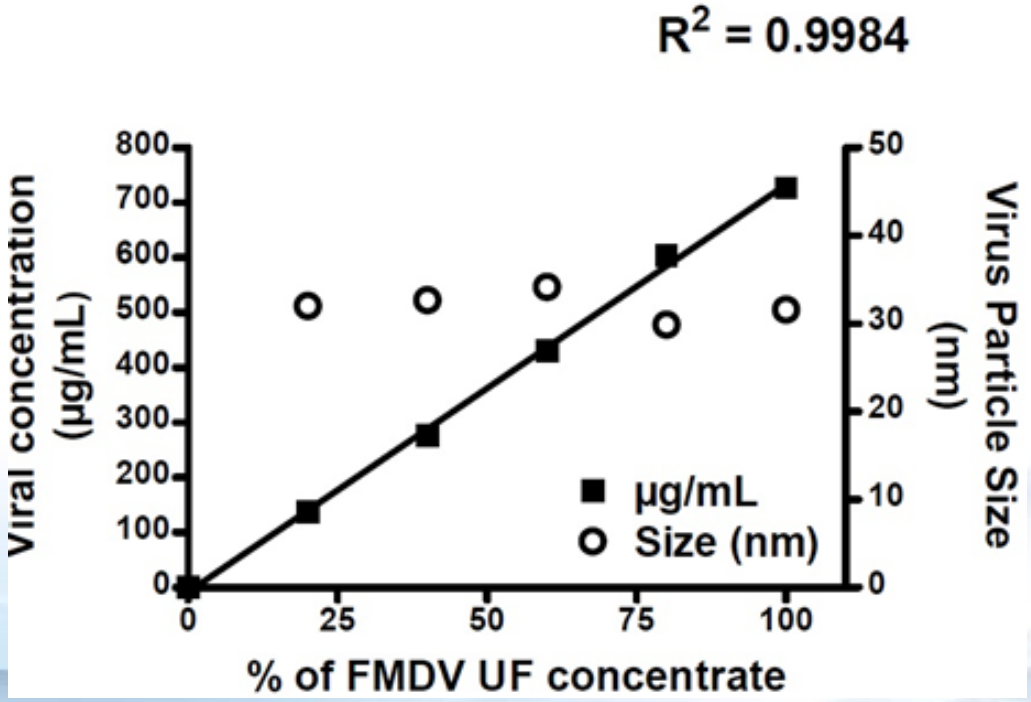
Characterization of size and integrity of virus particles by Dynamic Light Scattering

- **Development of a method for the characterization of size and integrity of virus particles by Dynamic Light Scattering (DLS) performed “in line” with the SEC HPLC analysis (FMDV´s size described in literature as between 25 and 35 nm):**



Linearity, Robustness and Range of Detection of SEC HPLC + DLS

- Linear response of SEC HPLC quantification over a broad range of antigen concentration and consistent DLS characterization of virus size at all points of the analysis:



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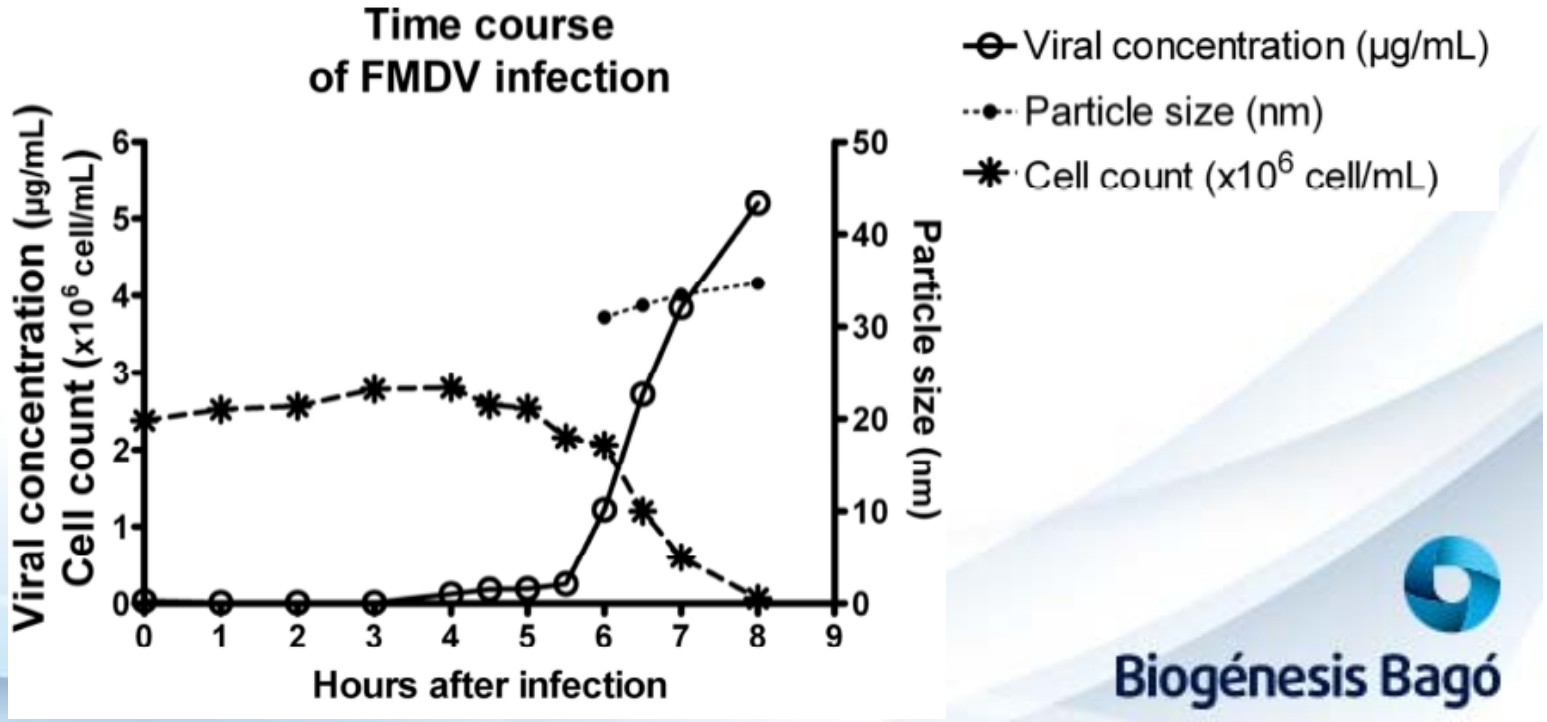
Robustness of the analysis at low viral concentration

- **Monitoring the infection kinetics using the combined method of quantification and characterization of size and integrity by SEC HPLC + in-line DLS:**



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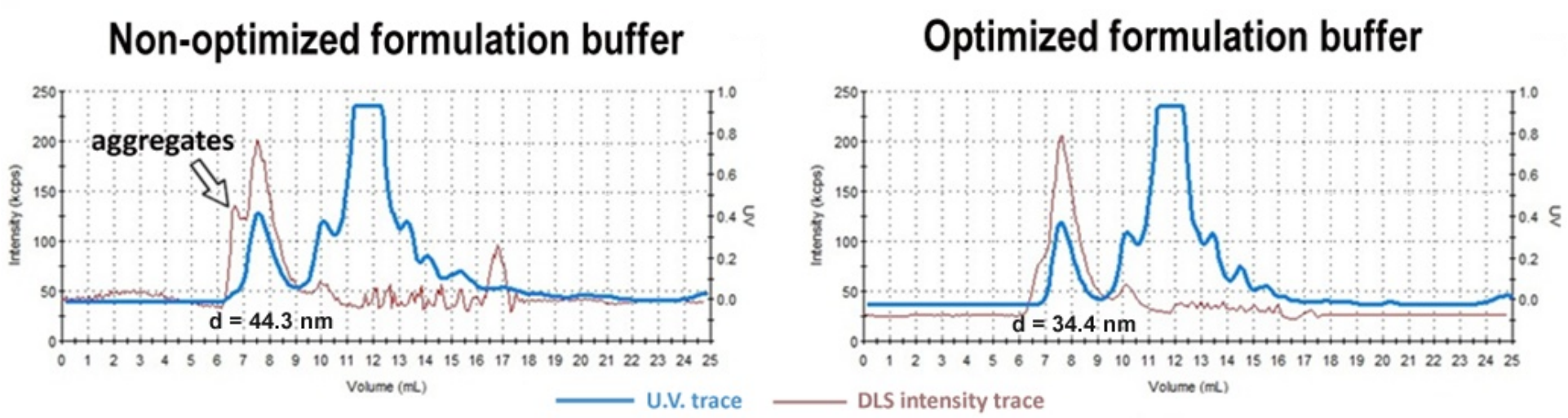
Vaccine optimization with DLS analysis

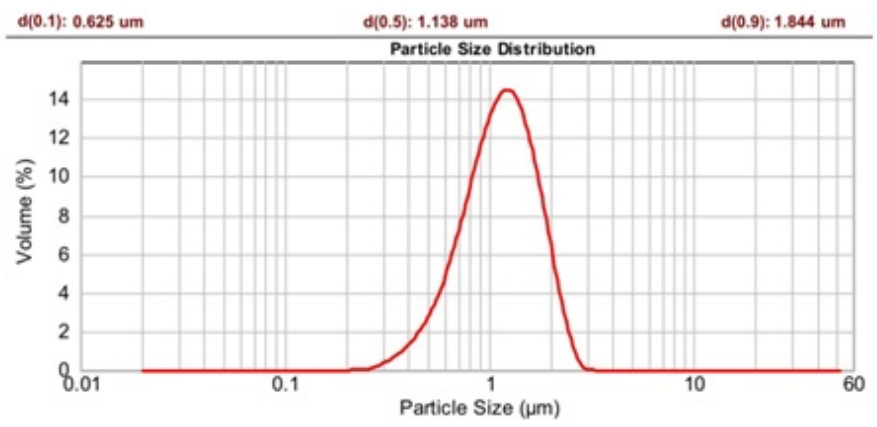
- **Although the SEC HPLC analyses yield the same UV trace for both samples (same virus concentration), the in-line DLS analysis enables the detection of aggregates in vaccine formulated with non-optimized buffer:**



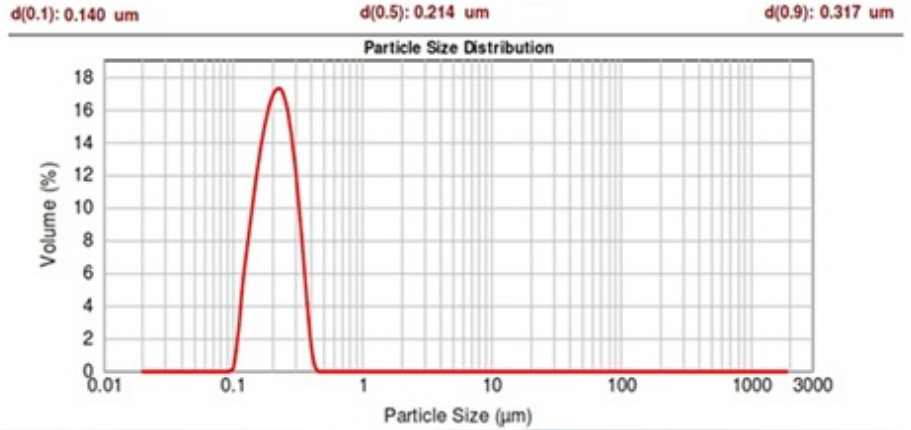
Mie Scattering analysis for emulsion control

- **The stability of emulsion (single W/O or double W/O/W) is critical for potency and stability of the vaccine: the quality of emulsion is characterized by determination of the distribution of the droplet size of dispersed phase in the continuous phase by Mie Scattering.**

Single emulsion, W/O

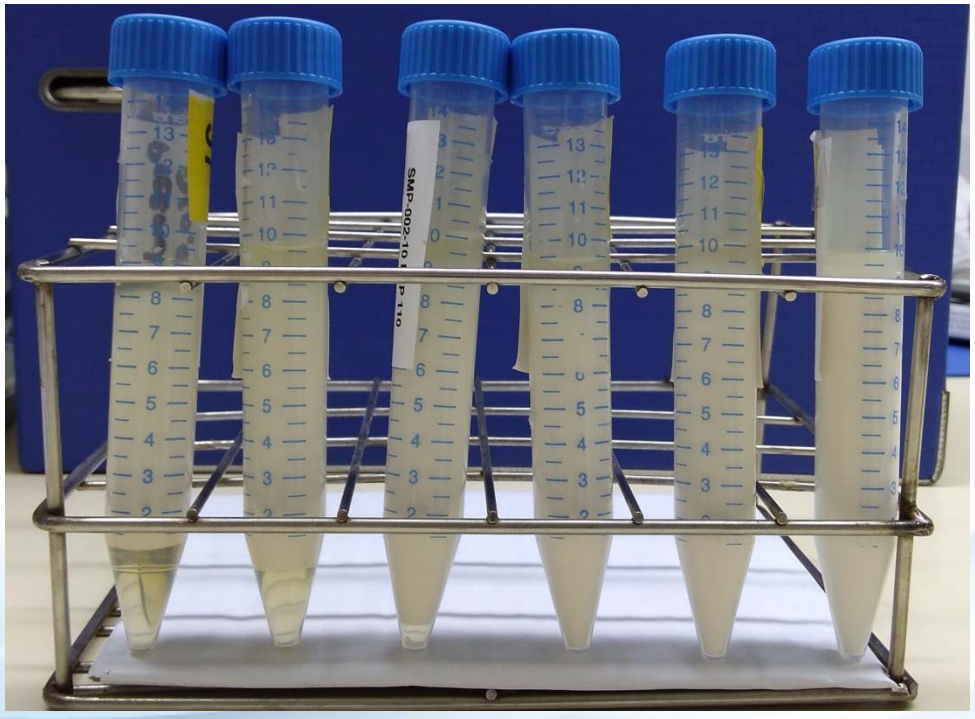


Double emulsion, W/O/W



Mie Scattering analysis as a guarantee of emulsion stability

- **Single W/O emulsion: correlation between decreasing value of droplet size and stability of the emulsion after 30 days at 37°C:**



d(0.9): 6 µm 5 µm 4 µm 3 µm 2 µm 1 µm

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Conclusions

- **Biogénesis-Bagó S.A. has successfully developed, validated and implemented a holistic method to quantify the FMD virus content and characterize the size and integrity of the viral particles. This technique can be applied to vaccine manufacturing intermediates, finished vaccines, vaccine stockpiles and antigens banks. We have also demonstrated that Mie Laser Scattering analysis of the vaccine emulsions can predict and guarantee the stability of the final vaccine.**

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Conclusions

- These combined techniques constitute an *in vitro* potency toolkit which provides a much higher level of confidence in the product quality than in vivo assays on the final product.

- Regulatory agencies and vaccine manufacturers, with the support of the scientific community, should therefore encourage the replacement of the use of test animals (3R) for FMD vaccines potency testing.

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Thanks !

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