Particle Analytics for Vaccines

particlesellc.com

Kevin Dahl, PhD - 20 years of particle and spectroscopic experience in pharma

Technology Consulting
- Instrumentation, laboratory

Data Consulting
- Reanalysis, interpretation, CMC support

Document Consulting
- Drafting and Review

Method Consulting
- Development, Optimization, Qualification, Validation

Experimental Consulting and Services
- Study design, execution, analysis, interpretation

Training Services
- Selected Instruments

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- ***The reward for work well done is the opportunity to do more.***

- Jonas Salk

Challenges in Vaccine Development

- Discovery
  - Rapid viral evolution – antigenic shift
- Formulation
  - Stabilization/delivery
- Storage and transport
  - Freeze-thaw
- Stability (ICH Q5C)
  - Toxic/immunogenic species
- In-use stability
  - Thaw/recon -> patient
- Release testing
  - Quality (ICH Q6B/Q6A)

Particulate and Spectroscopic Analytical Tools

Regulatory

- Modern vaccines inhabit a gray area between biotherapeutics (DS) and small molecule suspensions (DP)
  - Drug substance covered by ICH Q5A(R1) (safety)
  - Above deal with viral contamination/clearance, concentration, etc.
- What about drug product characteristics as CQAs?
- WHO Expert Committee on Biological Standardization, DRAFT Evaluation of the quality, safety and efficacy of messenger RNA vacc

cons

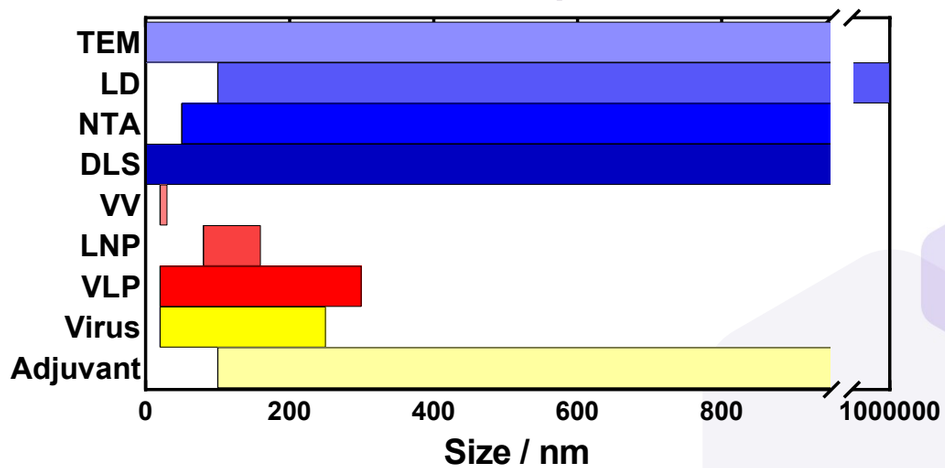
Particle size distribution (purity, consistency, safety)	light scattering such as dynamic or static light scattering; nanoparticle tracking analysis; electron microscopy; size-exclusion chromatography

Vaccines and Analytics

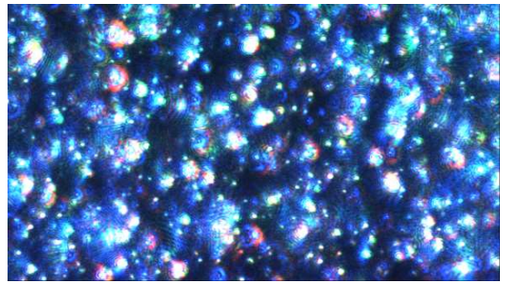
- Vaccine Delivery
  - Live Attenuated or Inactivated Virus - Polio
  - Adjuvanted - Nuvaxovid
  - Lipid Nanoparticle (LNP) – Pfizer/Moderna
  - Viral Vector/AAV – J&J
  - Virus Like Particle (VLP) – Gardasil

PARTICLES!

- Relevant Size Ranges and Common Techniques
  - Cryo-TEM
  - DLS
  - NTA
  - Laser Diffraction (LD)



Nanoparticle Tracking



- Based on video taken of point-scattering from particles
  - Particle movement in suspension described by Brownian motion:

$$D_h = \frac{k_B \boldsymbol{T}}{3\pi \boldsymbol{\eta} D_m}$$

  - Light scattering techniques measure hydrodynamic radius (D_h)

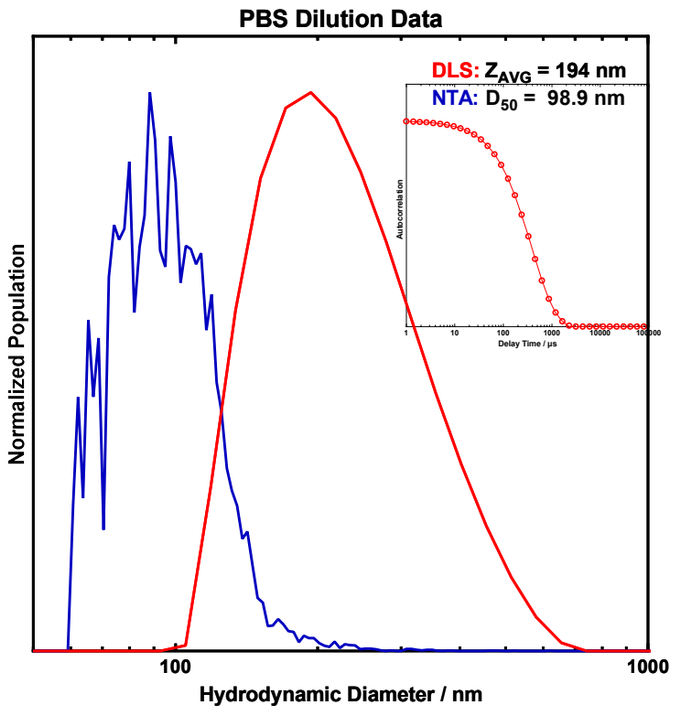
- Strengths
  - Polydisperse (aggregated) samples
  - Single-particle (number-based) technique
  - Less sensitive to oversized material (relative to DLS)
  - Governed by ISO 19430, ASTM E2834-12

Experimental: Vaccine Analytics

- Lyophilized sample with included diluent (1 mL)
  - Modified live virus with adjuvant (proprietary), sub-Q delivery, pH 6-8
  - Reconstitution after aseptic diluent addition, “SHAKE WELL”
- Sample diluted 10^5 in PBS
  - Concentration range match for NTA instrument
- 3-laser NTA system (445/520/635 nm)
  - 350 µl sample stirred in cuvette
  - Practical limit of ~50 nm
  - Fluorescence capable

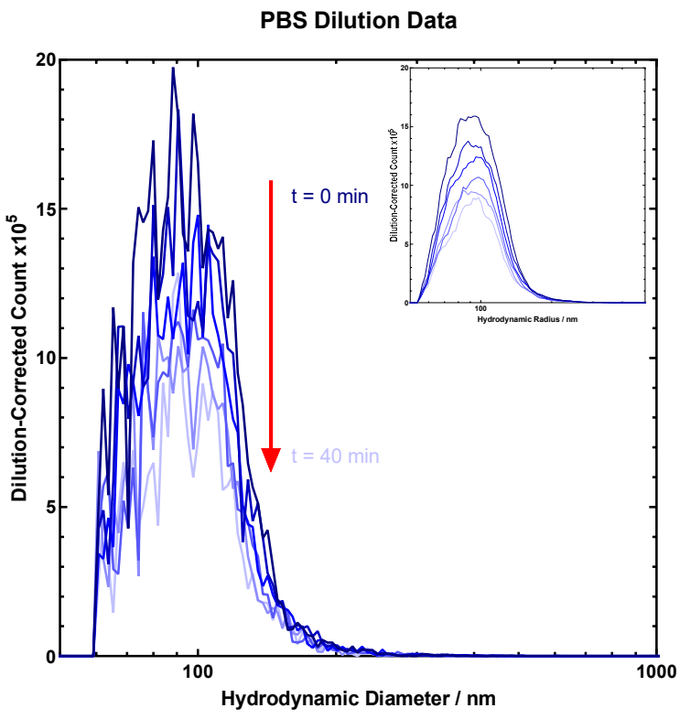
Reference: https://www.durvet.com/product/canine-spectra-6/

How things started:



- DLS results show a broad peak centered at 200 nm
  - Width suggests polydisperse sample – not optimal for DLS
  - Resolving power is ~x3/x5 for two modes
  - Intensity-based distribution – r^6 intensity dependence
- Number-based NTA shows a single mode at 100 nm
  - DLS signal dominated by a small amount of larger debris
    - Reconstitution/dilution?
  - NTA signal characterizes main population in sample

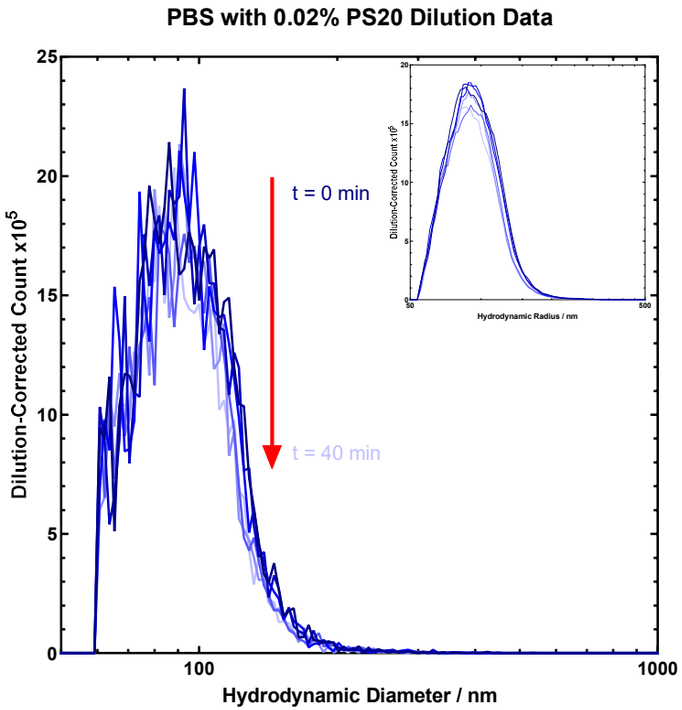
NTA Results: Dilution Only



- 40 min collection time for six replicates
  - 50% decrease in particle concentration after 40 min
    - Access to concentration is critical!
  - D90 of 140 nm (no prominent aggregation observed)
- Change in sample indicates instability
  - Dilution in PBS destabilizes the formulation

Sample	Rep	Counts	D50 (nm)	D90 (nm)	Conc. (part/mL)
Spectra 6 - PBS	1	5349	98.9	136.3	1.0E+13
Spectra 6 - PBS	2	4531	99.9	139.2	8.6E+12
Spectra 6 - PBS	3	4067	99.9	140.0	7.8E+12
Spectra 6 - PBS	4	3355	100.6	137.9	6.4E+12
Spectra 6 - PBS	5	3134	99.9	139.5	6.0E+12
Spectra 6 - PBS	6	2730	100.0	142.9	5.2E+12
	Average	**3861**	**99.9**	**139.3**	**7.4E+12**
	%RSD	**25.3**	**0.5**	**1.6**	**25.3**

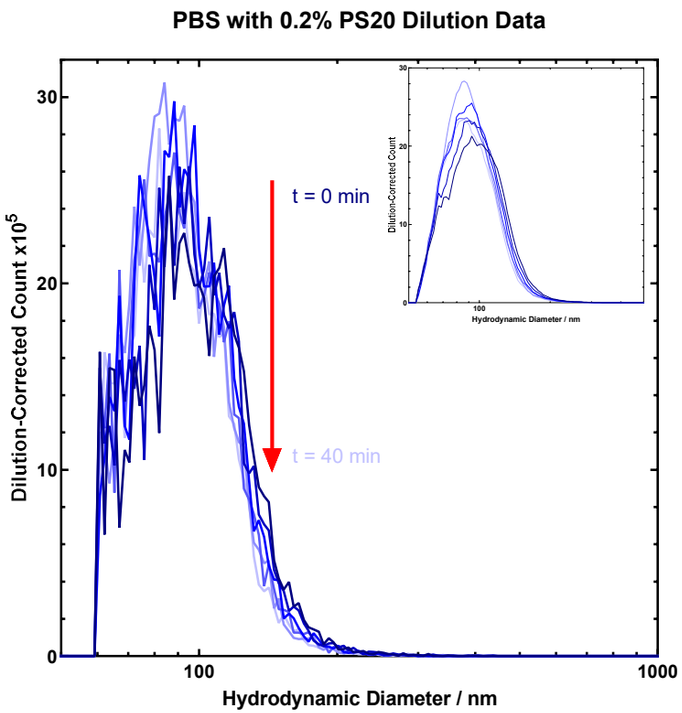
NTA Results: Dilution w/ 0.02% PS20



- Addition of 0.02% PS20 with PBS
  - PS20 should provide surface protection of unstable charge
- 40 min collection time for six replicates
  - ~15% decrease in particle concentration after 40 min
  -

Sample	Rep	Counts	D50 (nm)	D90 (nm)	Conc. (part/mL)
Spectra 6 - PBS+0.02% PS20	1	5981	97.9	134.4	2.3E+13
Spectra 6 - PBS+0.02% PS20	2	5786	97.4	133.6	2.2E+13
Spectra 6 - PBS+0.02% PS20	3	5762	96.7	133.0	2.2E+13
Spectra 6 - PBS+0.02% PS20	4	5123	96.3	131.7	2.0E+13
Spectra 6 - PBS+0.02% PS20	5	5121	94.8	129.8	2.0E+13
Spectra 6 - PBS+0.02% PS20	6	5003	94.5	131.2	1.9E+13
	Average	**5463**	**96.3**	**132.3**	**2.1E+13**
	%RSD	**7.79**	**1.44**	**1.29**	**7.79**

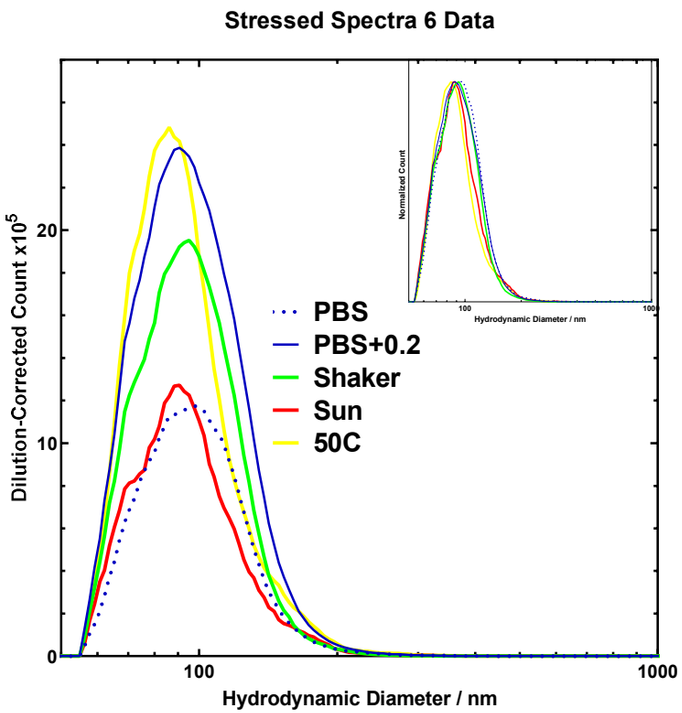
NTA Results: Dilution w/ 0.2% PS20



- Addition of 0.2% PS20 with PBS
- 40 min collection time for six replicates
  - No decrease in particle concentration after 40 min
  - D90 135 nm
  - PS20 reversing “hidden” aggregation?
    - Distribution width shifting and narrowing

Sample	Rep	Counts	D50 (nm)	D90 (nm)	Conc. (part/mL)
Spectra 6 - PBS+0.2% PS20	1	7665	104.1	143.9	2.9E+13
Spectra 6 - PBS+0.2% PS20	2	7852	100.6	140.6	3.0E+13
Spectra 6 - PBS+0.2% PS20	3	8009	97.2	135.2	3.1E+13
Spectra 6 - PBS+0.2% PS20	4	7599	96.0	133.5	2.9E+13
Spectra 6 - PBS+0.2% PS20	5	7904	93.8	129.8	3.0E+13
Spectra 6 - PBS+0.2% PS20	6	7100	94.4	129.6	2.7E+13
	Average	**7688**	**97.7**	**135.4**	**2.9E+13**
	%RSD	**4.24**	**4.07**	**4.28**	**4.24**

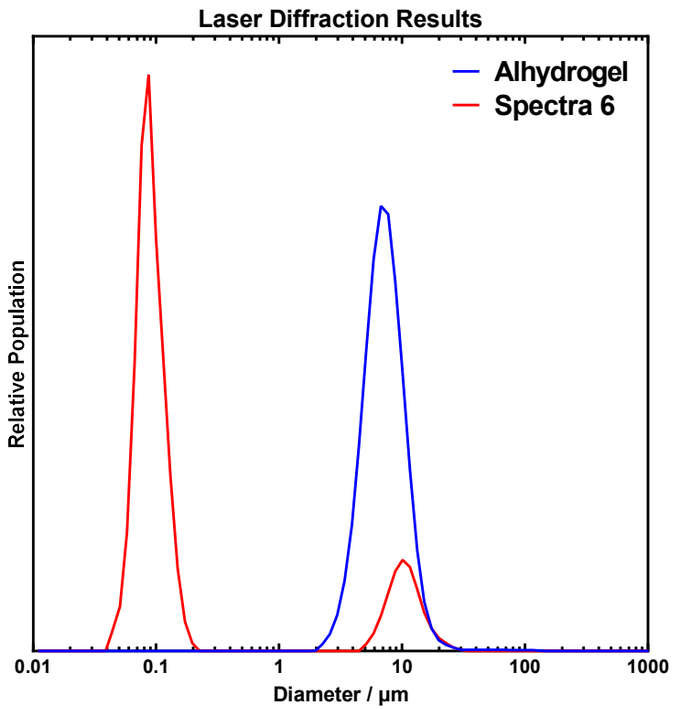
NTA Results: Additional Work



- Three stress conditions tested for 48 hours
  - Shaker plate, direct sunlight, 50 °C
- Sample tolerates shaking
- Sample tolerates elevated temperature well
- Sample does not tolerate direct sunlight (UV)

Sample	Cond.	Counts	D50 (nm)	D90 (nm)	Conc. (part/mL)
Spectra 6 - PBS	None	**3861**	99.9	**139.3**	**7.4E+12**
Spectra 6 - PBS+0.2% PS20	None	7688	97.7	135.4	2.9E+13
Spectra 6 - PBS+0.2% PS20	48 hr. Shake	6693	98.9	133.6	2.6E+13
Spectra 6 - PBS+0.2% PS20	48 hr. Sun	**3648**	**93.6**	**139.6**	**1.4E+13**
Spectra 6 - PBS+0.2% PS20	50C	6828	**91.2**	137.4	2.6E+13

In Addition…



- Laser diffraction records scattering angle vs. intensity of scatter from suspension
  - Widely used for powder/high conc. suspensions
  - Minimum volume ~5 mL
  - Volume-weighted (r^3) distribution, can go below 100 nm
- LD is applicable to PS determination in vaccines
  - Products contain high concentration of particles
- Spectra 6 product shows band at 10 µm in addition to adjuvant band
  - Likely due to incomplete reconstitution
  - Explains poor DLS performance for same product!

Summary

- Vaccines are analytically ‘different’
  - Biologic product necessitates specific testing
  - Particles are the product, testing with new technologies
- Incorporation of particle techniques
  - Directly measure CQA of Drug Product
  - Useful in formdev, stability, transport, and in-use studies
  - Can be readily worked into product release workflow
- Kevin Dahl, PhD: kd@particlesellc.com

A-TEEM for Vaccines

Vaccines Are Challenging Samples

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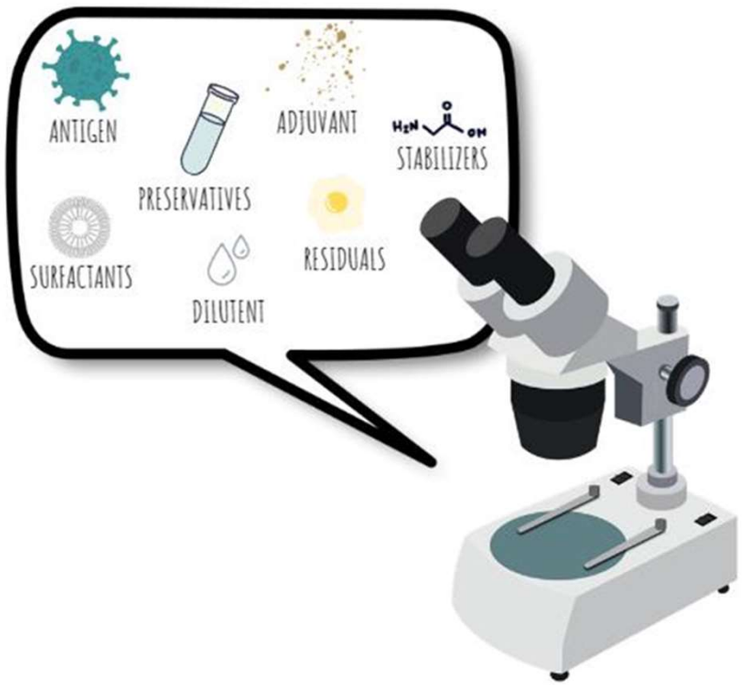
Vaccines - Characterization Challenges



Content and Format of Chemistry, Manufacturing and Controls Information and Establishment Description Information for a Vaccine or Related Product

2. Specifications and Methods

A description of all test methods selected to assure the identity, purity, strength and/or potency, as well as the lot-to-lot consistency of the finished product and the specifications used for the drug product should be submitted. Certificates of analysis and analytical results for at least three consecutive batches should be provided.

3. Validation Results

The results of studies validating the specificity, sensitivity, and variability of each method used for release testing should be provided. Where applicable this should include descriptions of reference standards and their validation. For analytical methods in compendial sources, the appropriate citations should be provided.

Up to 70% of manufacturing process is QC

- **Need same information - but faster**

and comparing various PAT methods and instruments. In-line monitoring and RTR, for example, would enable a substantial reduction in product release time (1–2 days compared to weeks–months). They would also improve the quality, efficiency and supply of the product through enhanced in-line monitoring, and indirect and multivariate sensors along with multivariate analysis and predictive modeling.

Vaccines Roadmap

NIMBL Manufacturing USA BioPhorum

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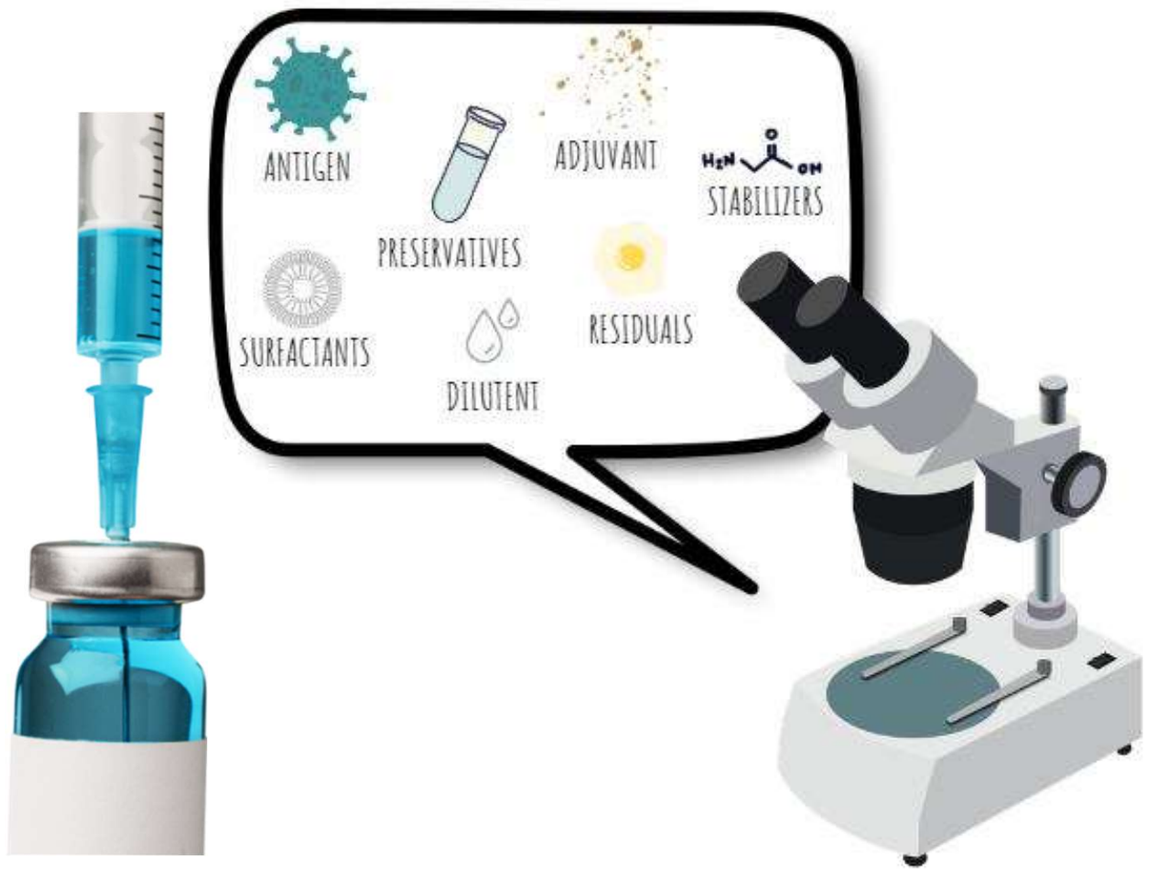
Vaccines – Struggle for Vibrational Spectroscopy

Vibration Spectroscopy – go-to option for **rapid analysis**

- Struggle with vaccine samples



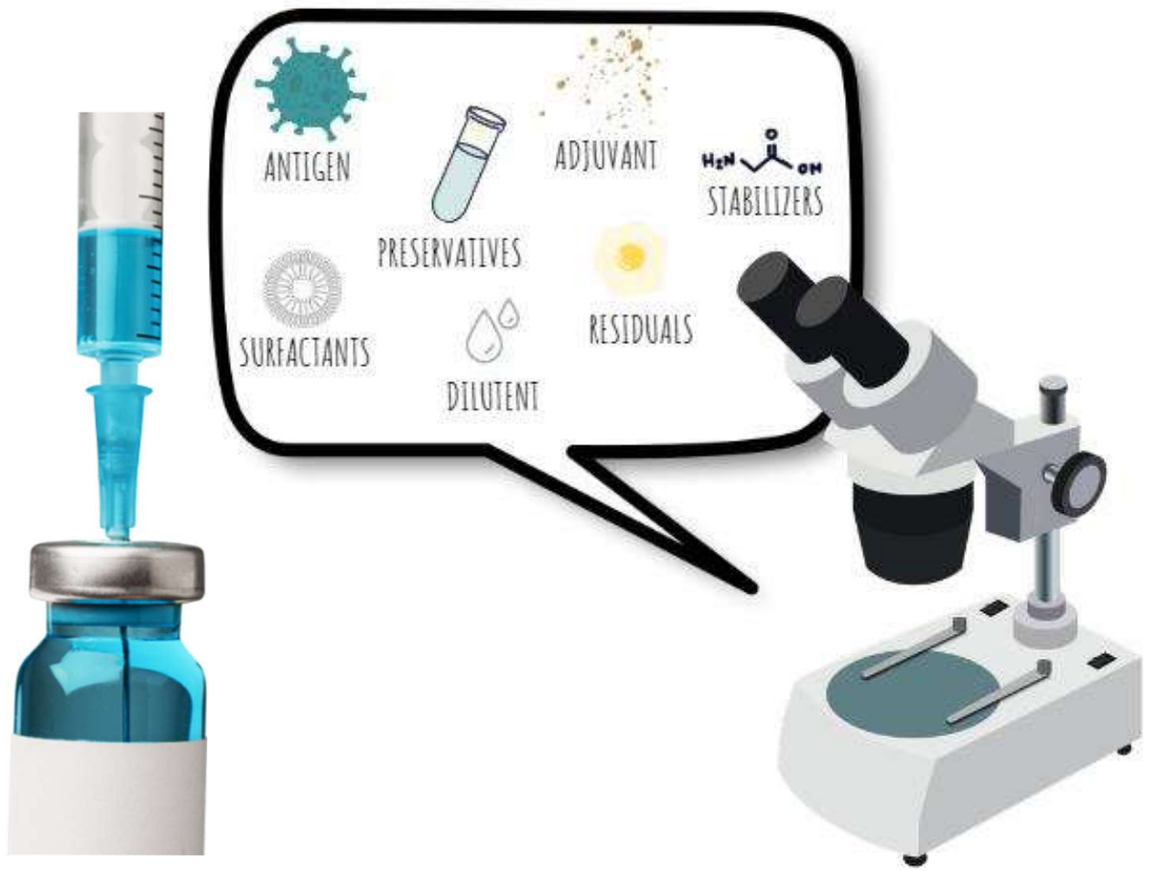
Shingrix - lyophilized powder	Amount	Role	Concentration
Glycoprotein E			0.1 mg/ml
Sucrose			40 mg/ml
Polysorbate 80	0.8 mg	Excipient	
Sodium Dihydrogen Phosphate	0.16 mg	Excipient	
Dipotassium phosphate	0.116 mg	Excipient	
Water-based diluent			

Challenge for Raman

Challenge for FTIR/NIR

Vaccines – Ideal for Fluorescence

Fluorescence is sensitive to protein, while being insensitive to pure water and sugar. Could that work?



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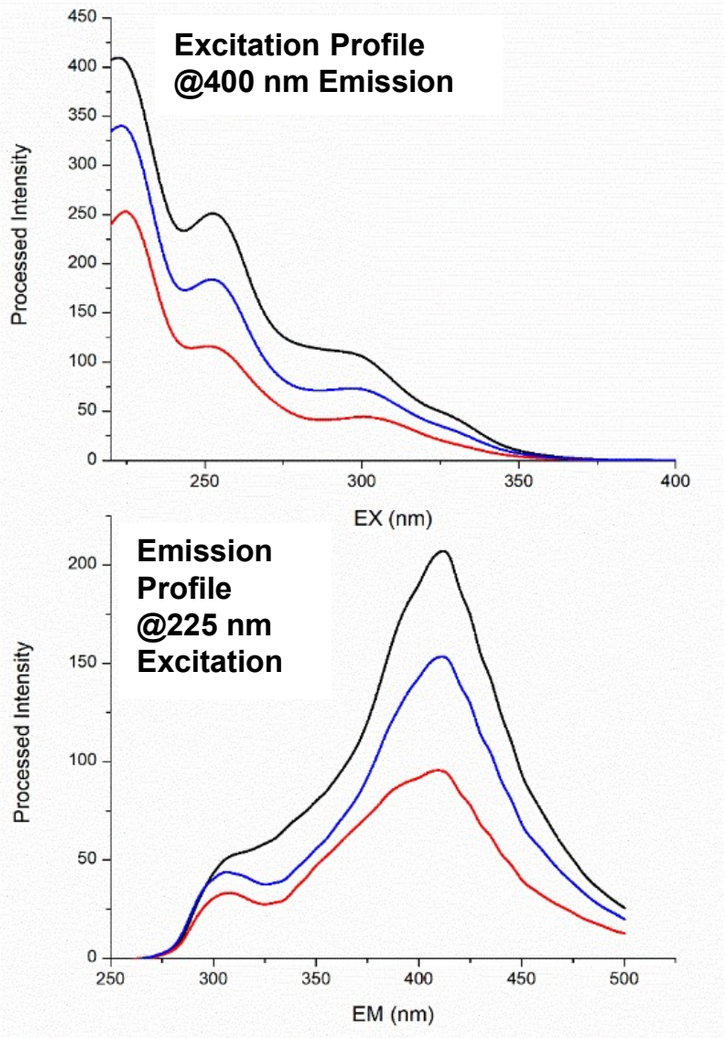
Polysorbate 80	0.8 mg	Excipient	
Sodium Dihydrogen Phosphate	0.16 mg	Excipient	
Dipotassium phosphate	0.116 mg	Excipient	
Water-based diluent			

No problem for fluorescence

No problem for fluorescence

What about Fluorescence Spectroscopy?

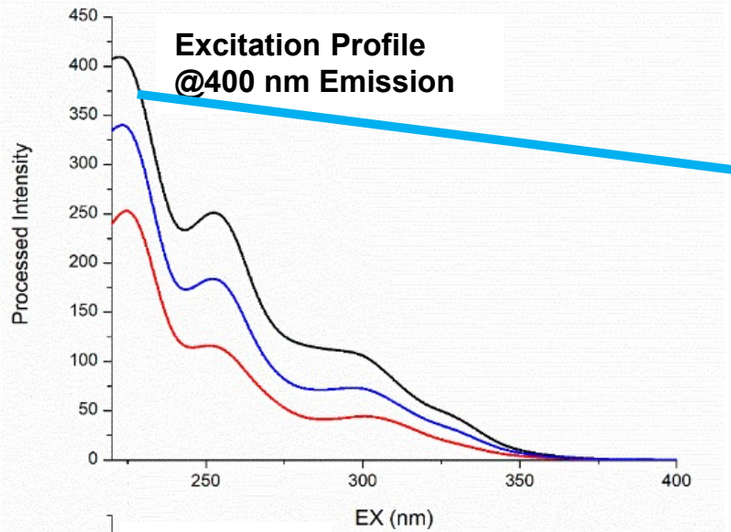


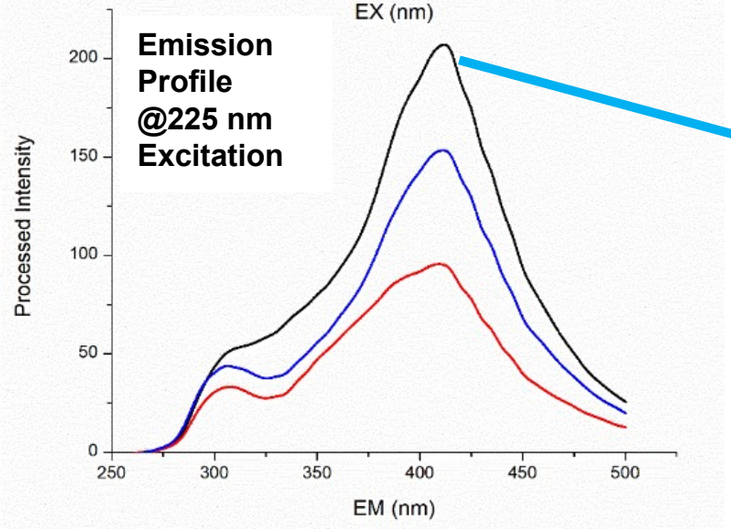
Limited information content…

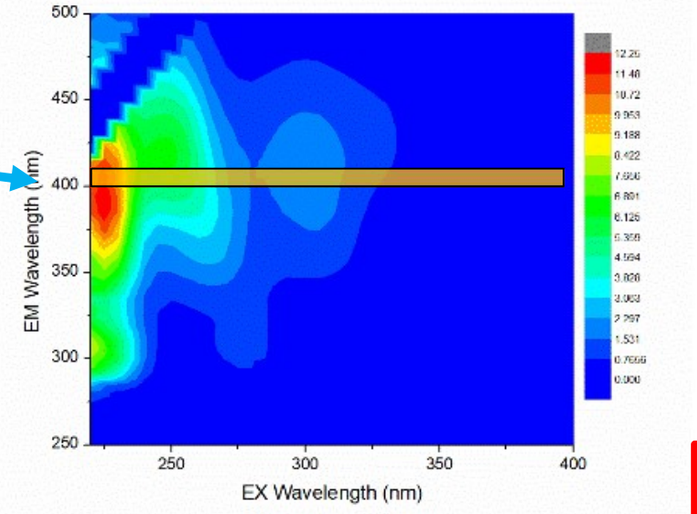
IF

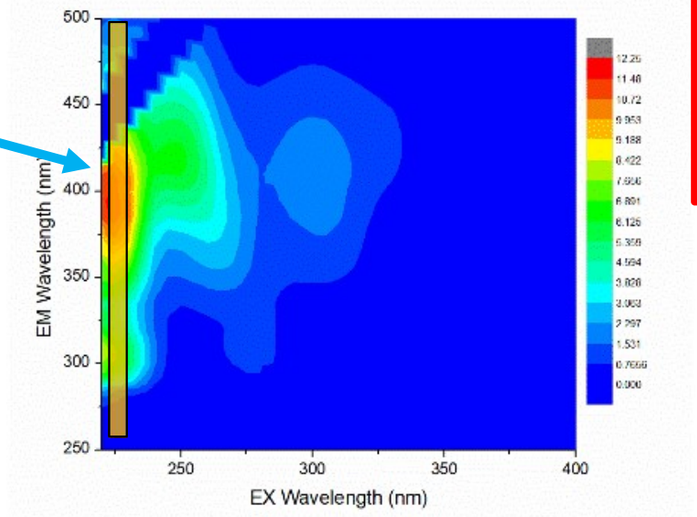
Just excitation or just emission

Add More Dimensions! Emission for Every Excitation









Fluorescence Excitation Emission Matrix (EEM)

Too Slow.
Concentration Effects (IFE).
Too Much Information?

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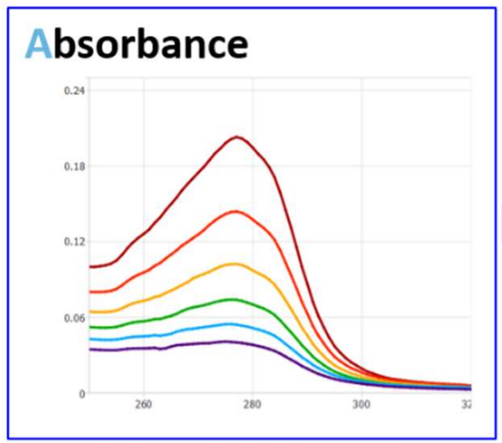
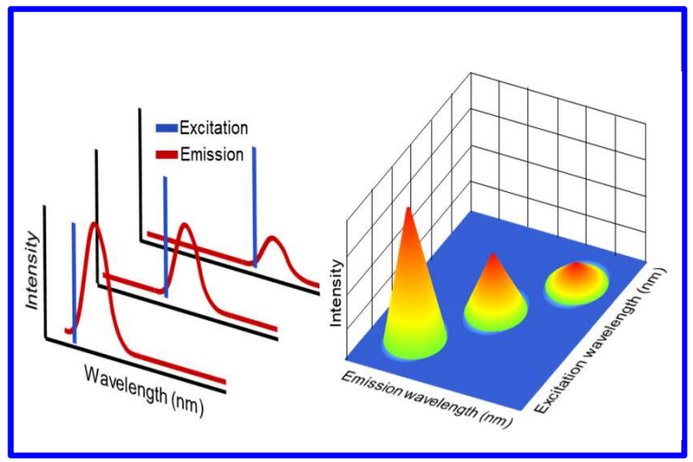
A-TEEM* – Clear Molecular Fingerprint

Data Collection: Same Sample @ Same Time

CCD collects emission trace in an instant





- CCD Detector Collects full EEM in 60 sec ~~Too Slow.~~
- 2-in-1 UV/Vis + EEM for robust IFE correction ~~Concentration Effects (IFE).~~
- Multivariate analysis ~~Too Much Information?~~

***A**bsorbance – **T**ransmission **E**xcitation **E**mission **M**atrix

(12) **United States Patent**
Gilmore et al.

(10) **Patent No.:** **US 8,901,513 B2**
(45) **Date of Patent:** **Dec. 2, 2014**

A-TEEM Method on Aqualog- UV/Vis and Fluorescence

Aqualog with autosampler – preferred configuration for A-TEEM in Biopharma

A-TEEM on Aqualog

FAST-01 – Batch Sampling Accessory
For unattended, Multi-sample Measurements

Eliminates “blur”

- Clear molecular fingerprint
- Low limit of detection
- Sensitive and specific
- Rapid and inexpensive

A-TEEM – Meets USP Specifications

⟨857⟩ ULTRAVIOLET-VISIBLE SPECTROSCOPY

- Photometric accuracy
- Absorbance and Fluorescence Excitation Wavelength Accuracy
- Stray Light
- Emission Wavelength Accuracy
- Emission Spectral Shape Accuracy
- Water Raman Band Accuracy
- Water Raman Band Signal to Noise (SNR)
- Water Raman Band Area Scattering Units (RSU)
- Quinine Sulfate Units (QSU)

⟨853⟩ FLUORESCENCE SPECTROSCOPY

Measuring Absorbance and Excitation Wavelength Accuracy and Excitation Spectral Correction

Measuring Water Raman Sensitivity and Wavelength Calibration

Measuring Photometric Accuracy and Linearity

Measuring Stray Light

Spectral Correction of Fluorescence Emission

Installation Operation Qualification (IQ/OQ)

Tackling Quantitative Analysis – Beyond LOD

Technique	Sensitive	Selective	Repeatable	Reproducible	Comments	LOD (PPM)
A-TEEM (2-in-1) Fluorescence	↑	↑	↑	↑	UV/Vis & Fluorescence, Quantitative across broad concentration range	<0.001
3D/EEM Fluorescence	↑	↑	●	●	Poor analytical quantification, molecular fingerprint is concentration dependent	<0.1
Raman	●	●	↑	↑	Struggles with low concentrations	25-150
FTIR	●	●	↑	↑	Water interferes with molecular fingerprint	100-2000
UV/VIS	●	↓	↑	↑	Low selectivity Low information content	0.3
NIR	↓	↓	↑	↑	Struggles with low concentrations Low selectivity	0.1-1

A-TEEM for AAVs

AAV – rapid determination of empty/full ratio

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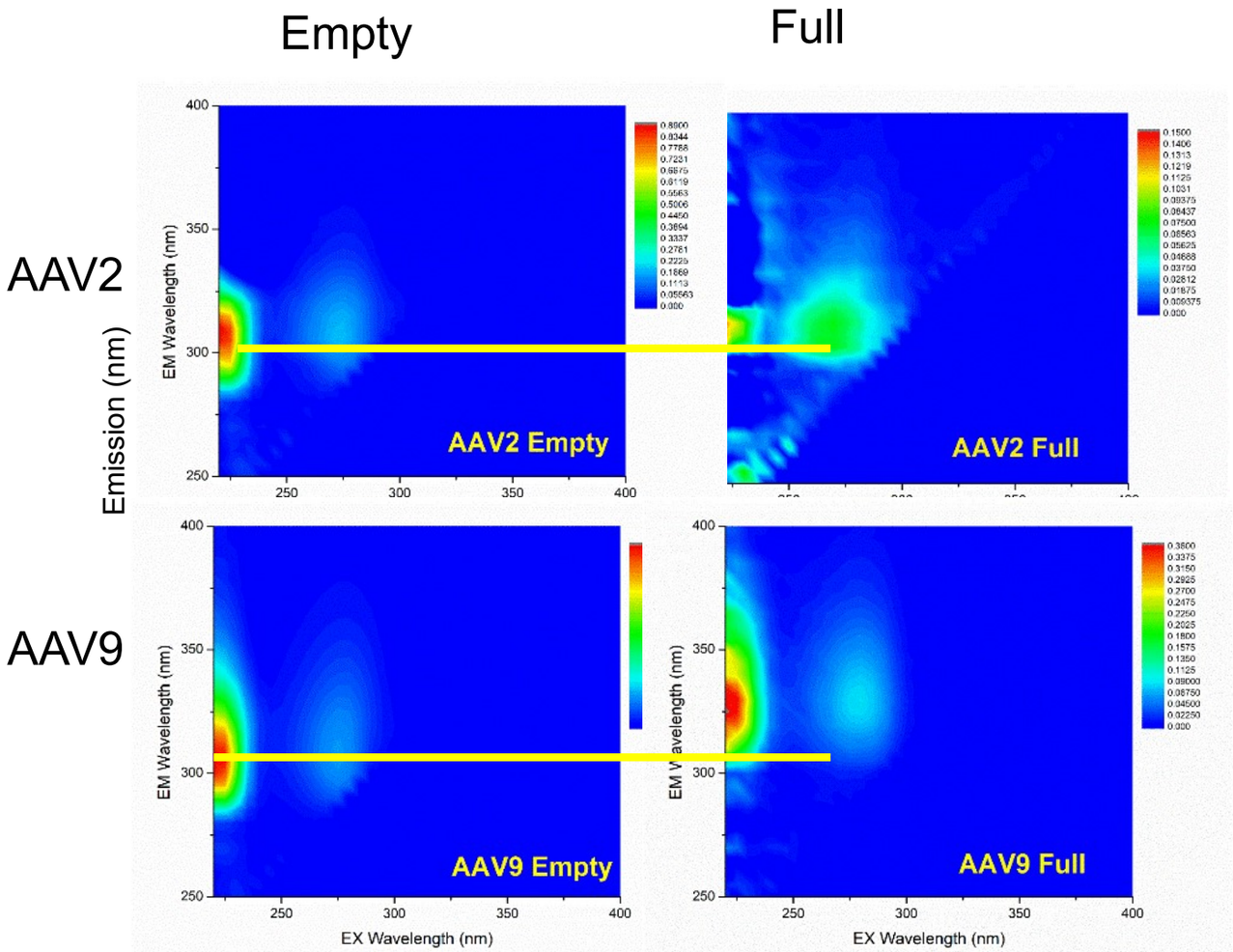
AAV Capsid Reference Samples

Serotype differentiation – AAV2 vs AAV9
Empty/Full ratio determination – Reference Samples

Serotype	Part Number	Physical Particle Count / mL	% Empty	Empty or Full
AAV2	RS-AAV2-ET	1.27×10^{12}	99.5%	Empty
AAV9	RS-AAV9-ET	1.76×10^{12}	93.1%	
		Vector Genome Count / mL	**% Full**	
AAV2	RS-AAV2-FL	1.82×10^{11}	71.2%	Full
AAV9	RS-AAV9-FL	3.86×10^{11}	82.3%	

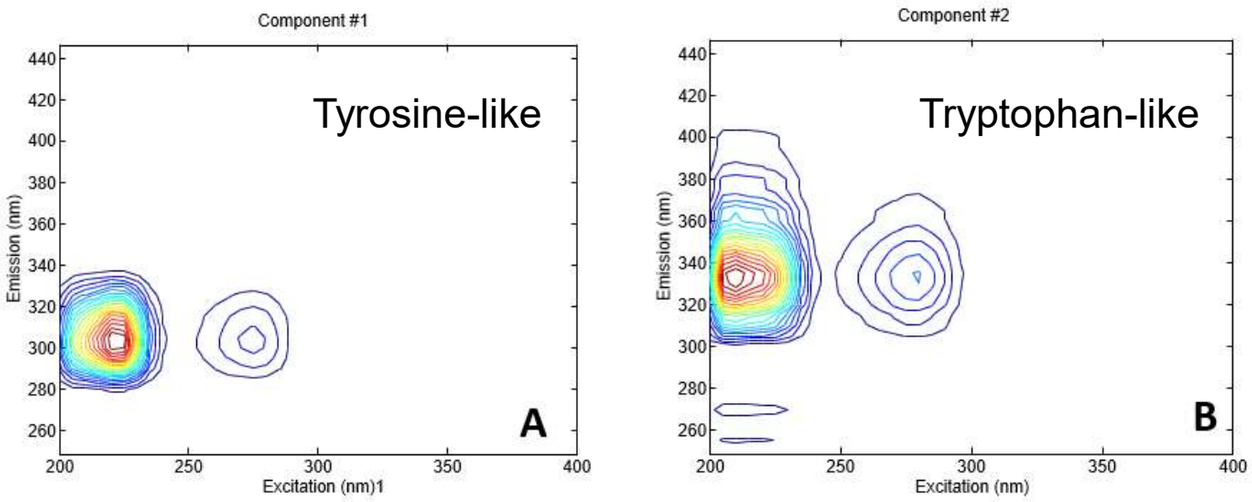
Samples preparation - 100X dilution with PBS

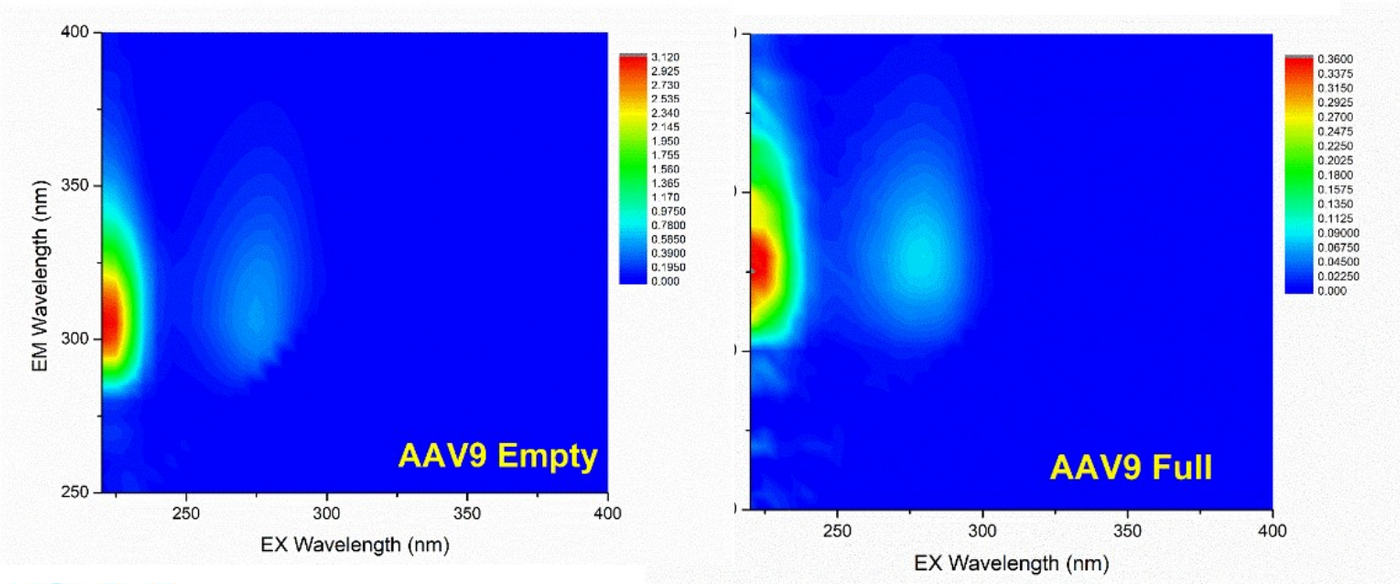
A-TEEM Fingerprints – empty and full AAV capsids



Empty	λex (nm)	λem (nm)	Comments
AAV2	223	310	Tyr-like profile
	275		
AAV9	223		
	275		
λex (nm)	**λem (nm)**	**Comments**	**Comments**
AAV2	223	312	Quench, slight λ shift
	274		
AAV9	223	330	Quench, large λ shift to Trp-like in polar
	277		

Multivariate Modeling – PARAFAC Analysis





Empty	λex (nm)	λem (nm)	Comments
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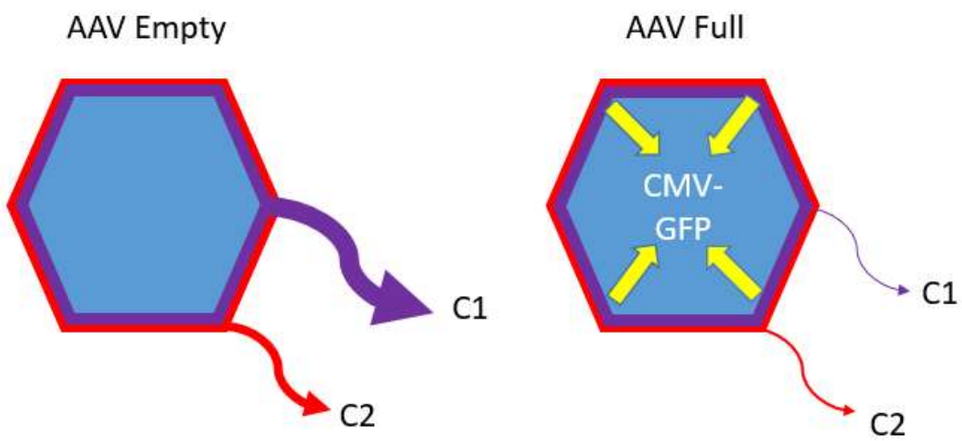
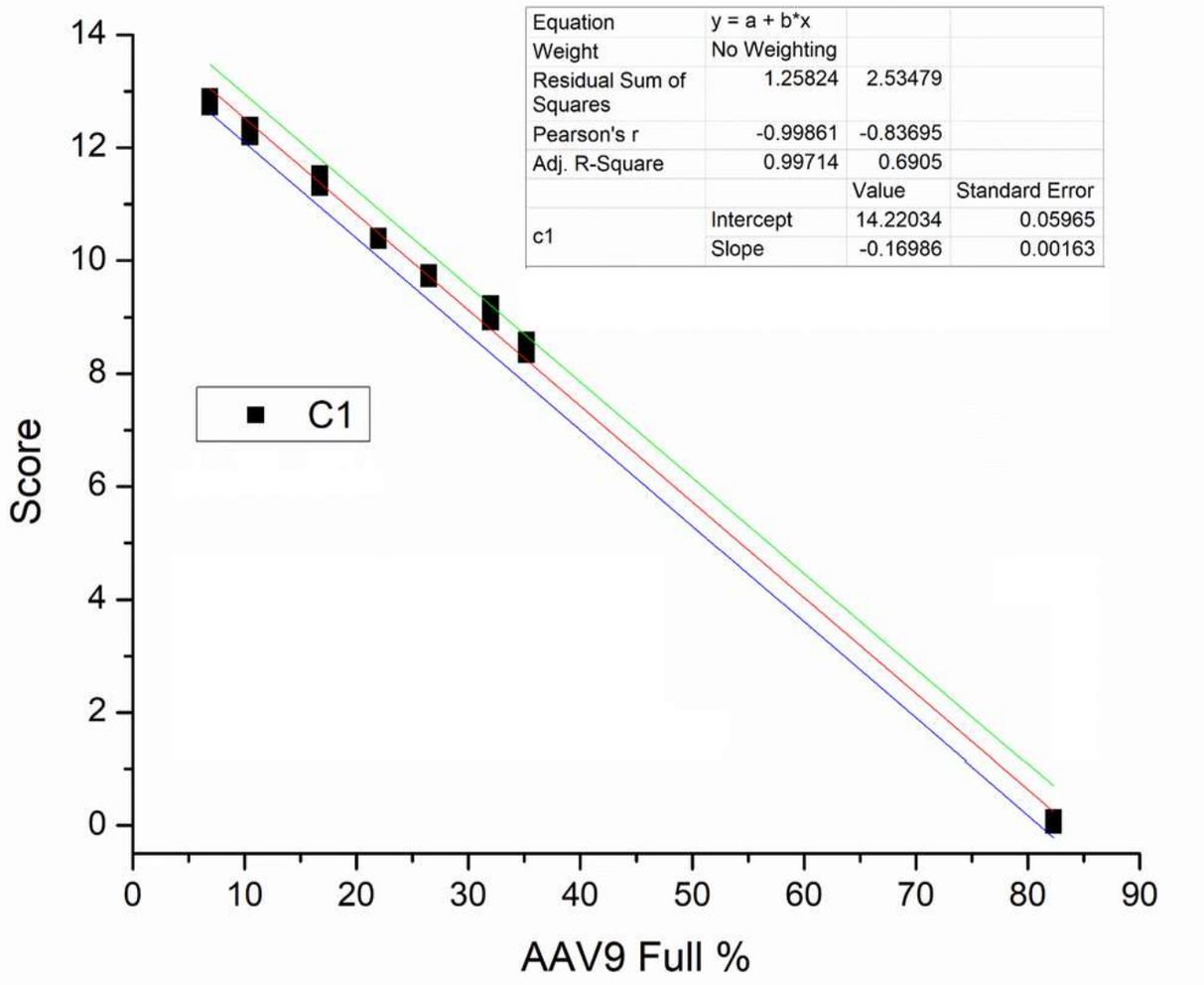
λex (nm)	**λem (nm)**	**Comments**	**Comments**
AAV2	223	312	Quench, slight λ shift
	274		
AAV9	223	330	Quench, large λ shift to Trp-like in polar
	277		

Quantitative Modeling – Quantification of “Full”

- Component 1 (Tyr) fluorescence is quenched (linearly) by the presence of DNA payload.
- PARAFAC component scores – plotted against “full” metric provided by AAV manufacture





Serotype Differentiation – XGBoost MVA Tool

Samples

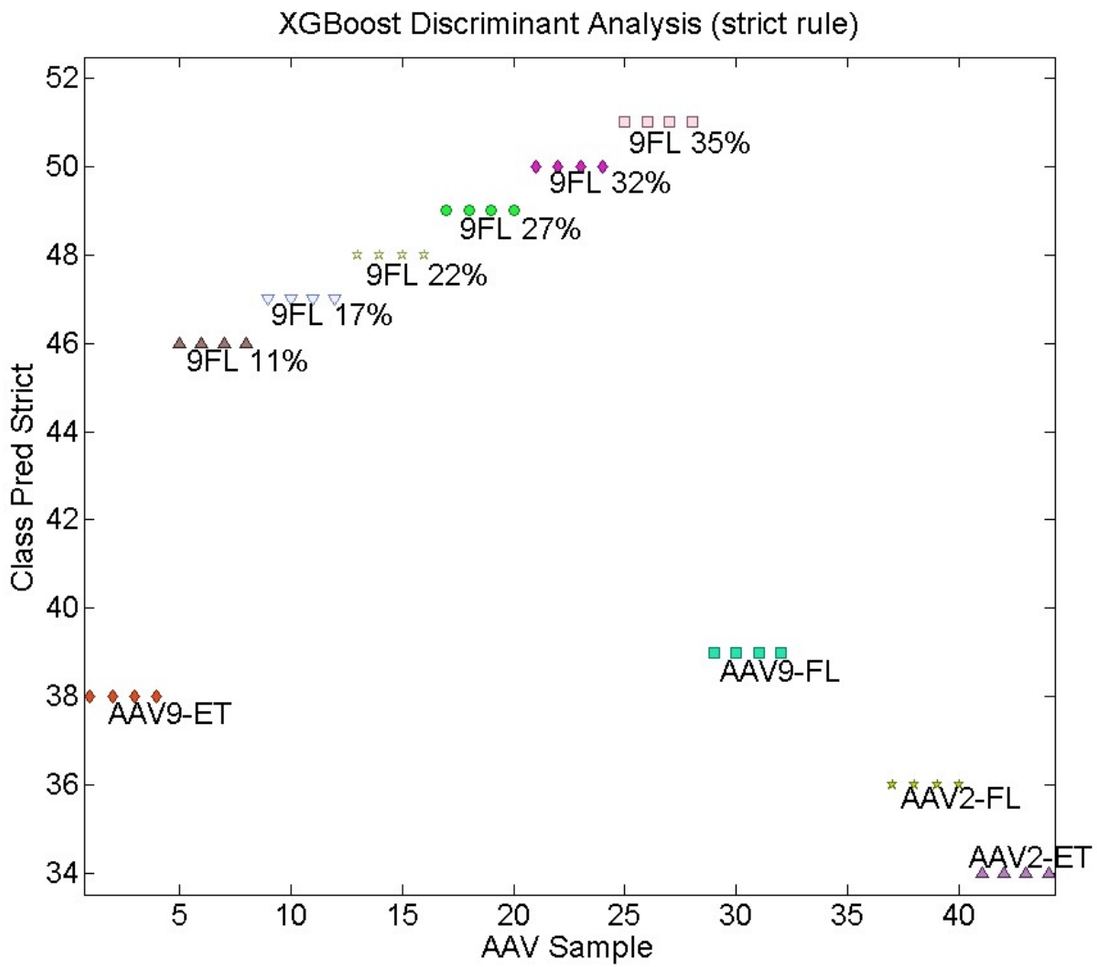
- AAV9 and AAV2 FL (loaded) and ET (empty)
- Titration series of AAV9 Empty and Full

Goal

- Assess capacity to resolve concentrations

Result

- All samples correctly identified and grouped



A-TEEM for Rapid AAV Empty/Full Ratio Determination

- AAV2 vs AAV9 serotypes are differentiated by A-TEEM
- Empty/Full Ratio can be determined by A-TEEM
- Discrimination of loaded AAV's is possible with a precision of better than 3%

A-TEEM for Vaccine Characterization

Repeatable? and Reproducible?

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Vaccine Samples – Multicomponent

Vaccine Formulations

SOLO-JEC 5
5-way
SOLO-JEC 5 delivers protection against five common canine infectious diseases.

SOLO-JEC 6
5-way + corona
SOLO-JEC 6 delivers the same protection as SOLO-JEC 5 plus additional protection against coronavirus.

SOLO-JEC 9
5-way + 4-way lepto
SOLO-JEC 9 delivers protection against four types of lepto that are known to infect dogs.

SOLO-JEC 10
5-way + 4-way lepto + corona
SOLO-JEC 10 delivers the same protection as SOLO-JEC 9 plus additional protection against coronavirus

	Basic Program Solo-Jec 5&6		Lepto Included Solo-Jec 9&10	
Disease	Solo-Jec 5	Solo-Jec 6	Solo-Jec 9	Solo-Jec 10
Distemper	●	●	●	●
Hepatitis		●	●	●
Adenovirus 1	●			
Adenovirus 2	●	●	●	●
Coronavirus		●		●
Parainfluenza	●	●	●	●
Parvovirus	●	●	●	●
Lepto (4 types)			●	●
Gentamicin	●	●	●	●
Amphotericin B	●			
Thimerosal		●	●	●
Adjuvant	●	●	●	●

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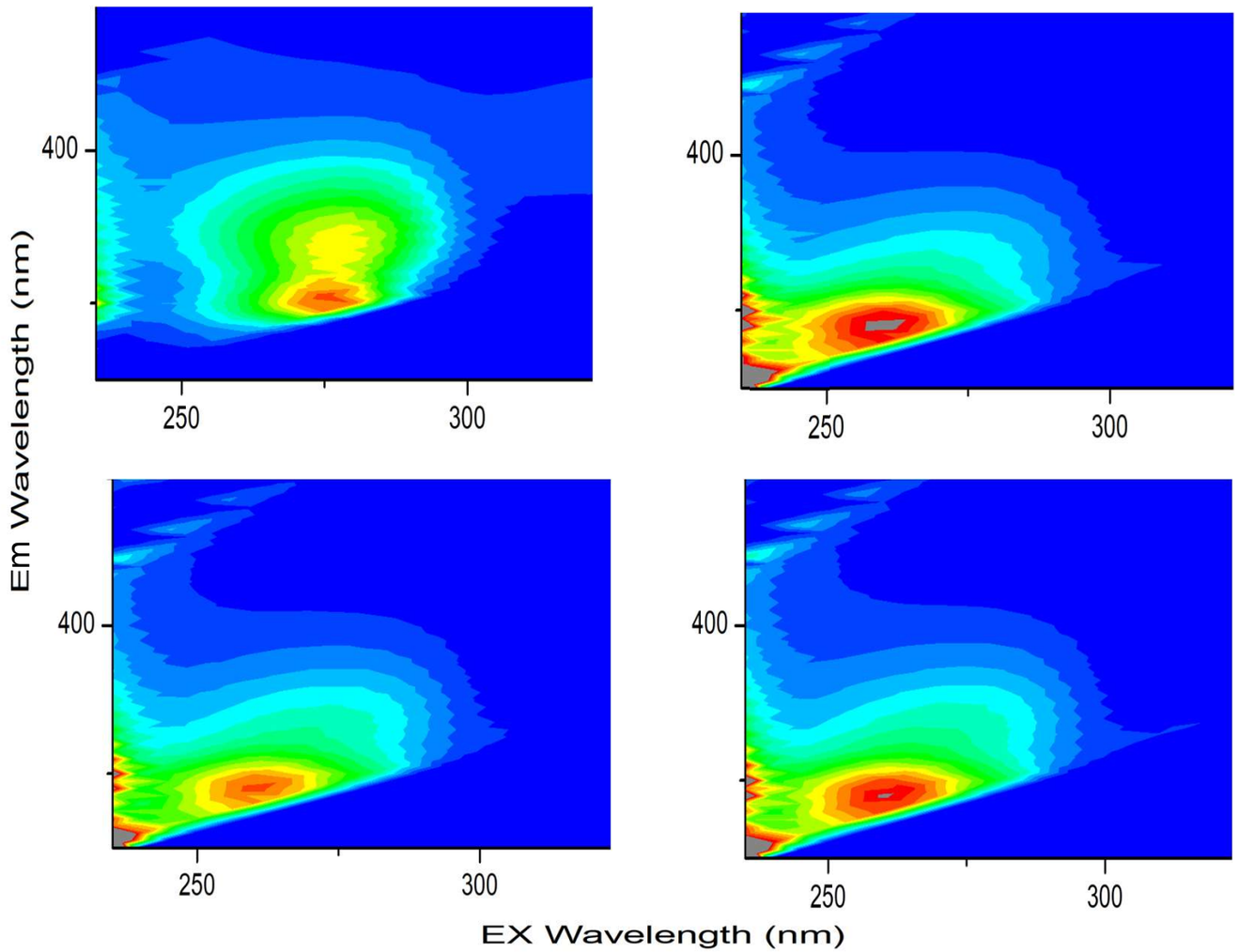
Vaccine Analysis: Repeatable & Reproducible?

Experimental:

1. Vaccines reconstituted per package instructions
2. Diluted with DI water – 60X
3. Measured one sample on two separate days – reproducibility of measurement
4. Measured a second sample - different operator on a different instrument, different day – to test model validation/reproducibility

Corrected A-TEEM Data – Quick Glance

Solo-Jec 5



Solo-Jec 9

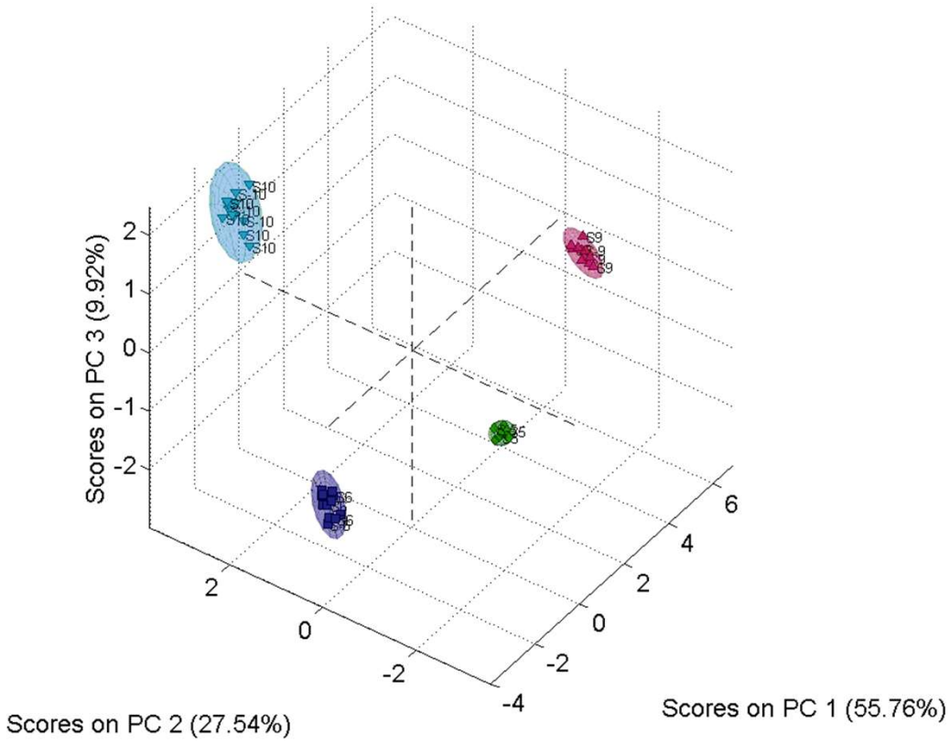
Solo-Jec 6

Solo-Jec 10

Differentiate? Yes

Repeatable? Yes

Multiple measurements of same vaccine tightly clustered
Different vaccine formulations well separated
3 components – 93% of variance



Date of acquisition	Spectra vaccine code labels
November 19	S5, S6, S9, S10
December 1	S-5, S-6, S-9, S-10

Principal Components Analysis
Percent Variance Captured by PCA Model

Principal Component Number	% Variance Captured This PC	% Variance Captured Total
1	55.76	55.76
2	27.54	83.30
3	9.92	93.23

Method Validation – Model Stability

- **New batch of the vaccines - U5, U6, U9, U10**
  - Measured on a different instrument
  - Prepared and measured by different operator
  - Added to the calibration set

- **Samples S-5, S-6, S-9, S-10**
  - Served as validation set

Prediction Probability – Tabular

100% Correct Classification of "unknowns" using model developed previously

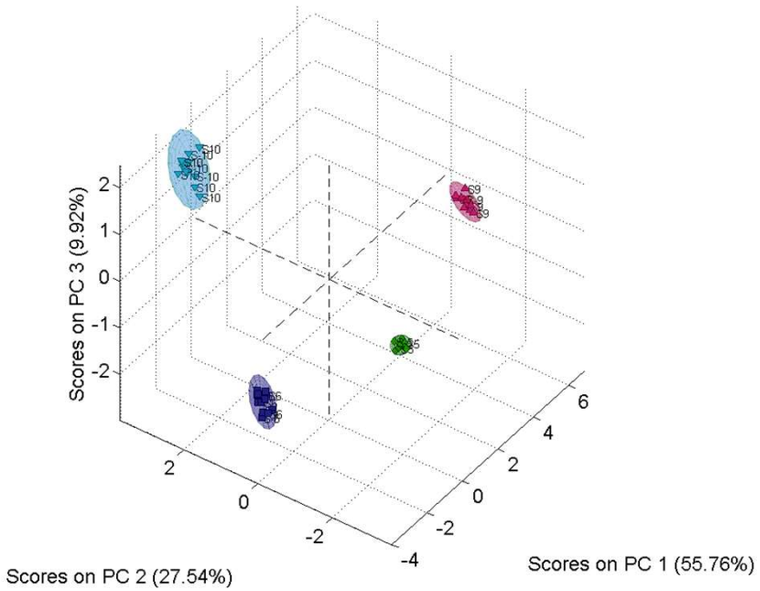
- 5 repeats
- 4 vaccine products

Sample	Class Pred Probability S-5,S5	Class Pred Probability S-6,S6	Class Pred Probability S-9,S9	Class Pred Probability S-10,S10	Misclassified
U5	0.95	0.01	0.01	0.03	0
U5	0.95	0.01	0.01	0.03	0
U5	0.94	0.01	0.01	0.04	0
U5	0.95	0.01	0.01	0.03	0
U5	0.95	0.01	0.01	0.03	0
U6	0.01	0.98	0.01	0.00	0
U6	0.01	0.98	0.01	0.00	0
U6	0.01	0.98	0.01	0.00	0
U6	0.01	0.98	0.01	0.00	0
U6	0.01	0.98	0.01	0.00	0
U9	0.01	0.01	0.94	0.05	0
U9	0.01	0.01	0.95	0.04	0
U9	0.01	0.01	0.94	0.05	0
U9	0.01	0.01	0.94	0.05	0
U9	0.01	0.01	0.94	0.05	0
U10	0.05	0.05	0.05	0.85	0
U10	0.06	0.06	0.06	0.81	0
U10	0.05	0.05	0.05	0.85	0
U10	0.05	0.05	0.05	0.85	0
U10	0.05	0.05	0.05	0.85	0

Extreme Gradient Boost – Discriminant Analysis

A-TEEM for Vaccine Characterization

1. Rapid – minutes not hours
2. Differentiate similar, multi-component vaccines
3. Repeatable & Reproducible
4. Validatable



Date of acquisition	Spectra vaccine code labels
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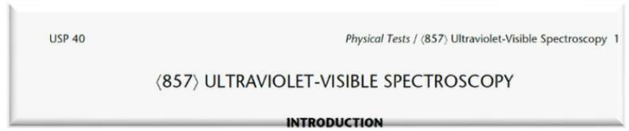
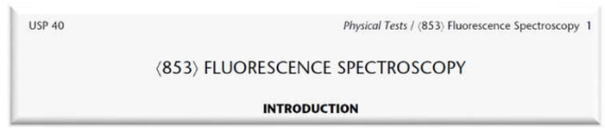

USP 40 Physical Tests / ⟨857⟩ Ultraviolet-Visible Spectroscopy 1

⟨857⟩ ULTRAVIOLET-VISIBLE SPECTROSCOPY

INTRODUCTION


USP 40 Physical Tests / ⟨853⟩ Fluorescence Spectroscopy 1

⟨853⟩ FLUORESCENCE SPECTROSCOPY

INTRODUCTION

Differentiate ✚ Reproducibly ✚ Validatable

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A-TEEM – High Sensitivity Tool for Vaccines

Application Examples

- AAV – serotype differentiation
- AAV – rapid empty/full ratio determination
- Vaccines – differentiate similar, multicomponent vaccines, QC

Compared to NIR/FTIR/Raman

- Lower Limits of Detection
- Exquisite discrimination
- Excellent quantification

Compared to EEMs

- Speed of acquisition
- Robust IFE correction – true A-TEEM Fingerprint
- 2-in-1 modality, adds UV/Vis modality to data set

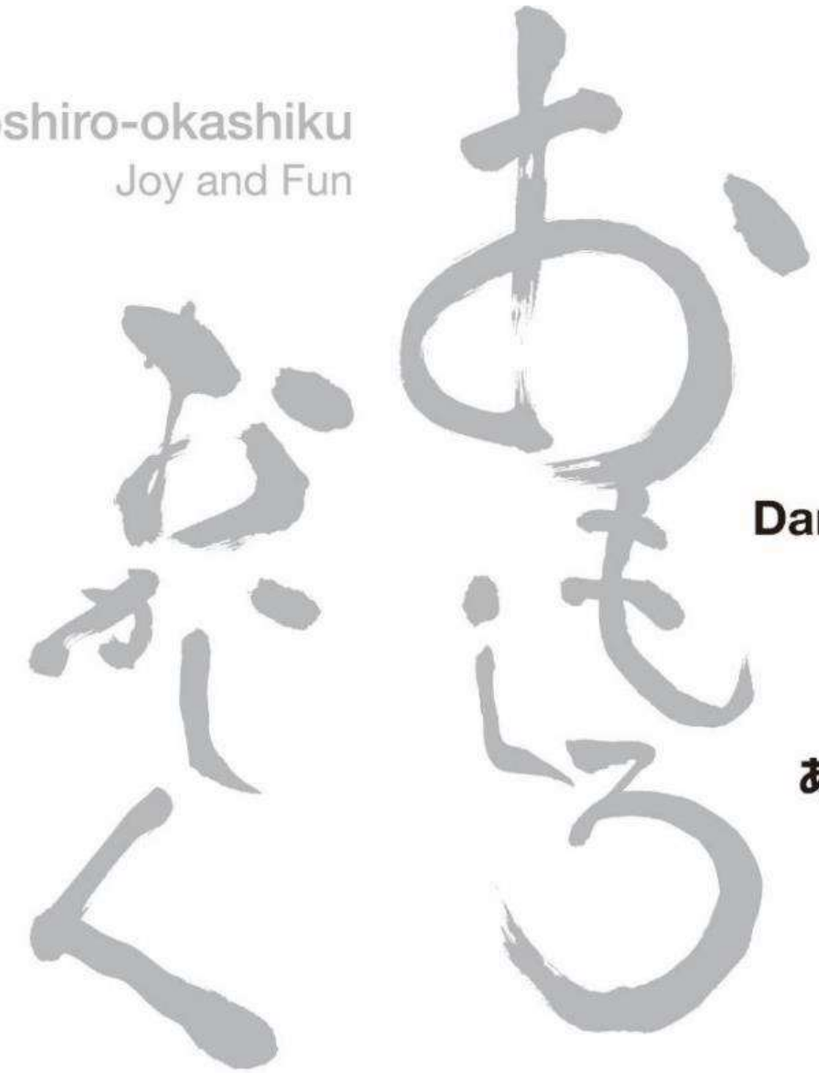
Acknowledgements

Adam Gilmore, A-TEEM Product Manager, HORIBA

Karoly Csatorday, HORIBA, *retired*

Omoshiro-okashiku

Joy and Fun



Terima kasih

Gracias

谢谢

Danke

Σας ευχαριστώ πάρα πολύ

Tack ska du ha

ধন্যবাদ

Grazie

THANK YOU

شُكْرًا

ขอบคุณครับ

Obrigado

Большое спасибо

ありがとうございました

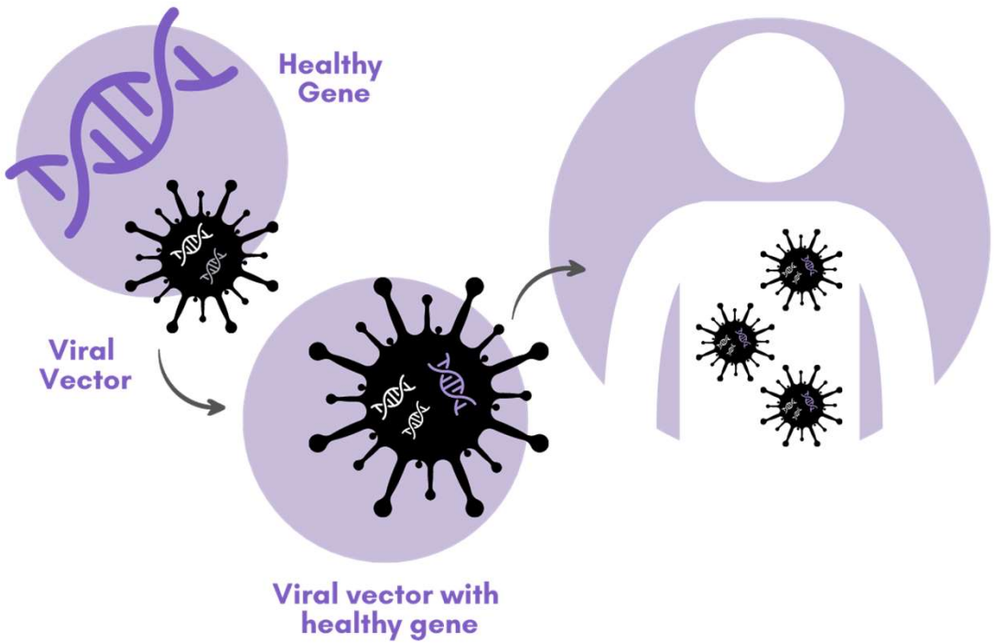
Cảm ơn

Merci

감사합니다

Dziękuję

Viral Vectors – Characterization Challenges



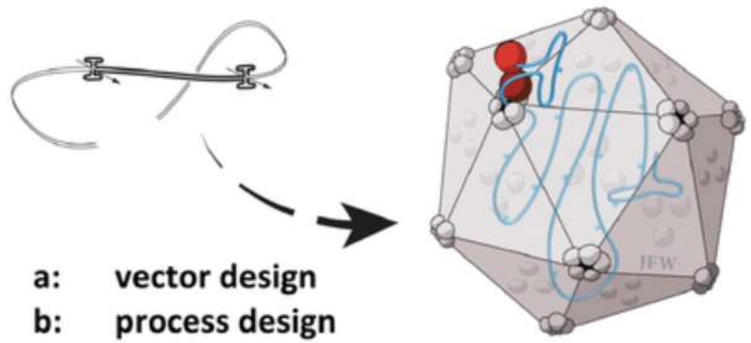
Human Gene Therapy Products Incorporating Human Genome Editing

Draft Guidance for Industry

Abstract

This article describes quality control testing and characterization of adeno-associated virus (AAV) gene therapy vectors for clinical product development. It explores the relationship between AAV vector design and manufacturing process design with product critical quality attributes required for safety and durable efficacious expression, with emphasis on vector immunogenicity.

rAAV purity, potency and safety = *f* (a, b, c)



a: vector design
b: process design
c: process control

Wright, J.F. (2021), Quality Control Testing, Characterization and Critical Quality Attributes of Adeno-Associated Virus Vectors Used for Human Gene Therapy. Biotechnol. J., 16: 2000022. https://doi.org/10.1002/biot.202000022