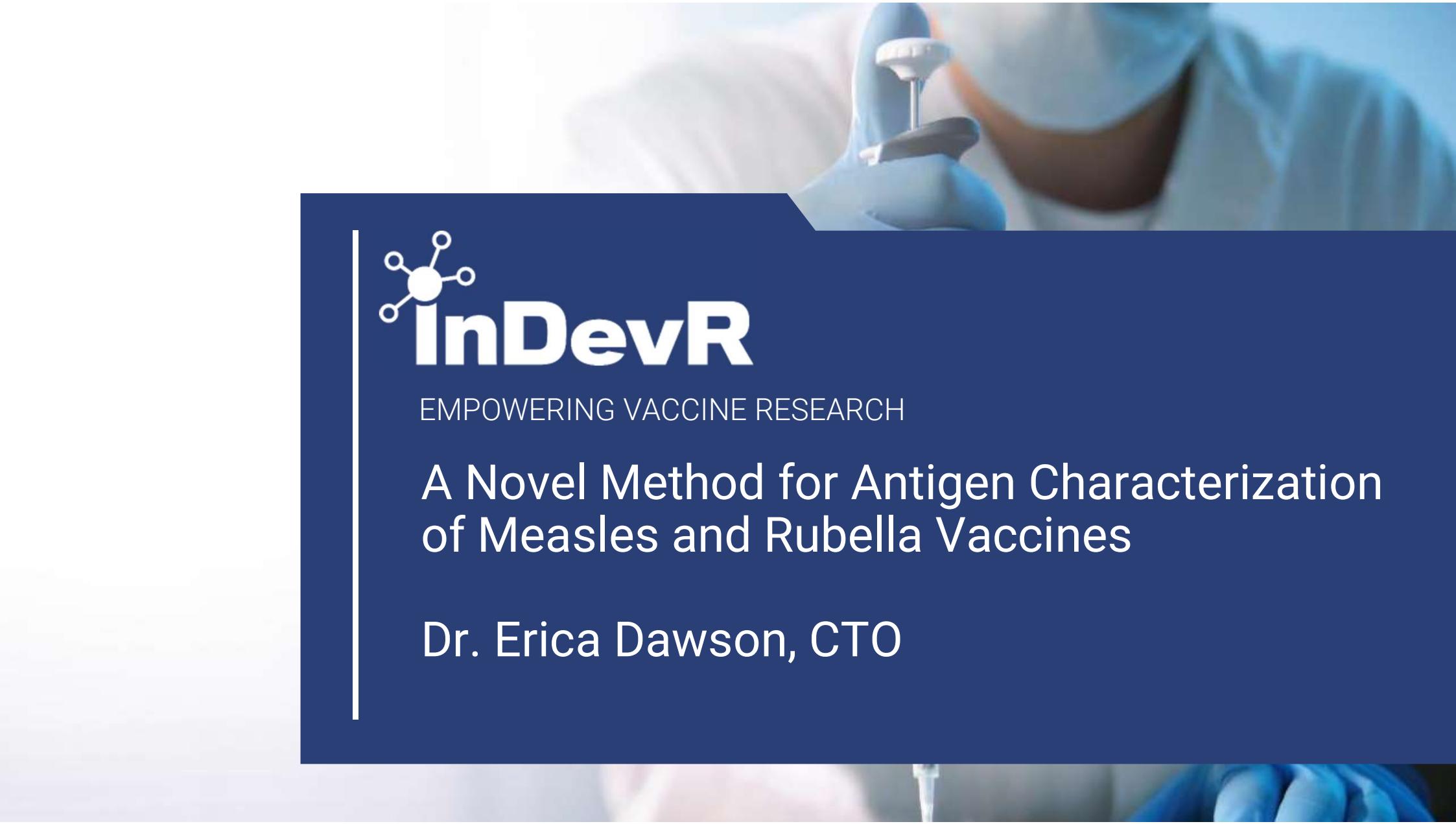


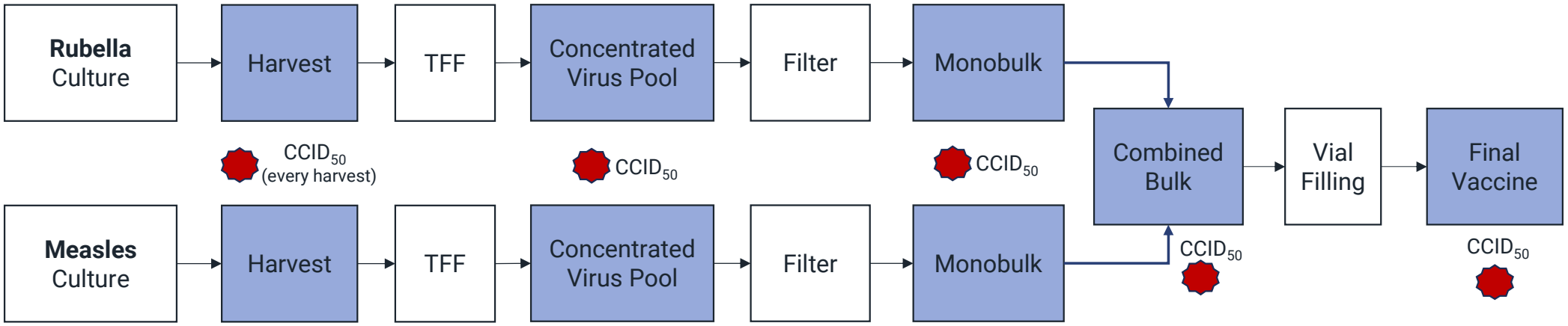
InDevR

EMPOWERING VACCINE RESEARCH

A Novel Method for Antigen Characterization of Measles and Rubella Vaccines

Dr. Erica Dawson, CTO

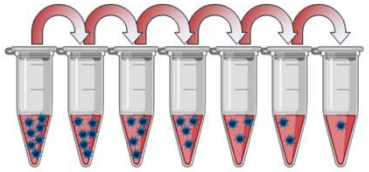
MR Vaccine Manufacturing Overview



$CCID_{50}$ is typically a 10- to 14-day time to result

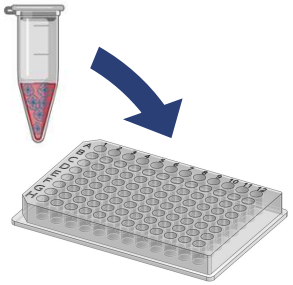
$CCID_{50}$ CV can be 65% (± 0.3 log_{10})

Current $CCID_{50}$ Process for MR Vaccines



Serially Dilute Virus-Containing Sample

Plate 8 replicates of each dilution



Add cells
Measles: Vero cells
Rubella: RK13 cells

10-14 Days in incubator

Evaluate by eye for CPE

	Conc. of virus											
Sample 1	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	+	+	+	+	-	-	-	-	-	-	-	-
B	+	+	+	+	+	-	-	-	-	-	-	-
C	+	+	+	+	-	+	-	-	-	-	-	-
D	+	+	+	-	-	+	-	-	-	-	-	-
E	+	+	+	-	+	-	-	-	-	-	-	-
F	+	+	+	+	+	-	-	-	-	-	-	-
G	+	+	+	+	-	-	-	-	-	-	-	-
H	+	+	+	+	+	-	-	-	-	-	-	-

4.30 $\log_{10}$ $CCID_{50}$/mL

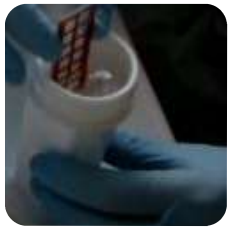
- Manual process with visual inspection
- Method is time-consuming and error prone with ± 0.3 $\log_{10}$ variability possible
- $CCID_{50}$ imprecision can result in expensive lot rejections

VaxArray Process for MR Vaccines

Prepare standard curve and samples

Add samples and standard curve to slide(s)

Remove samples and standards, add label

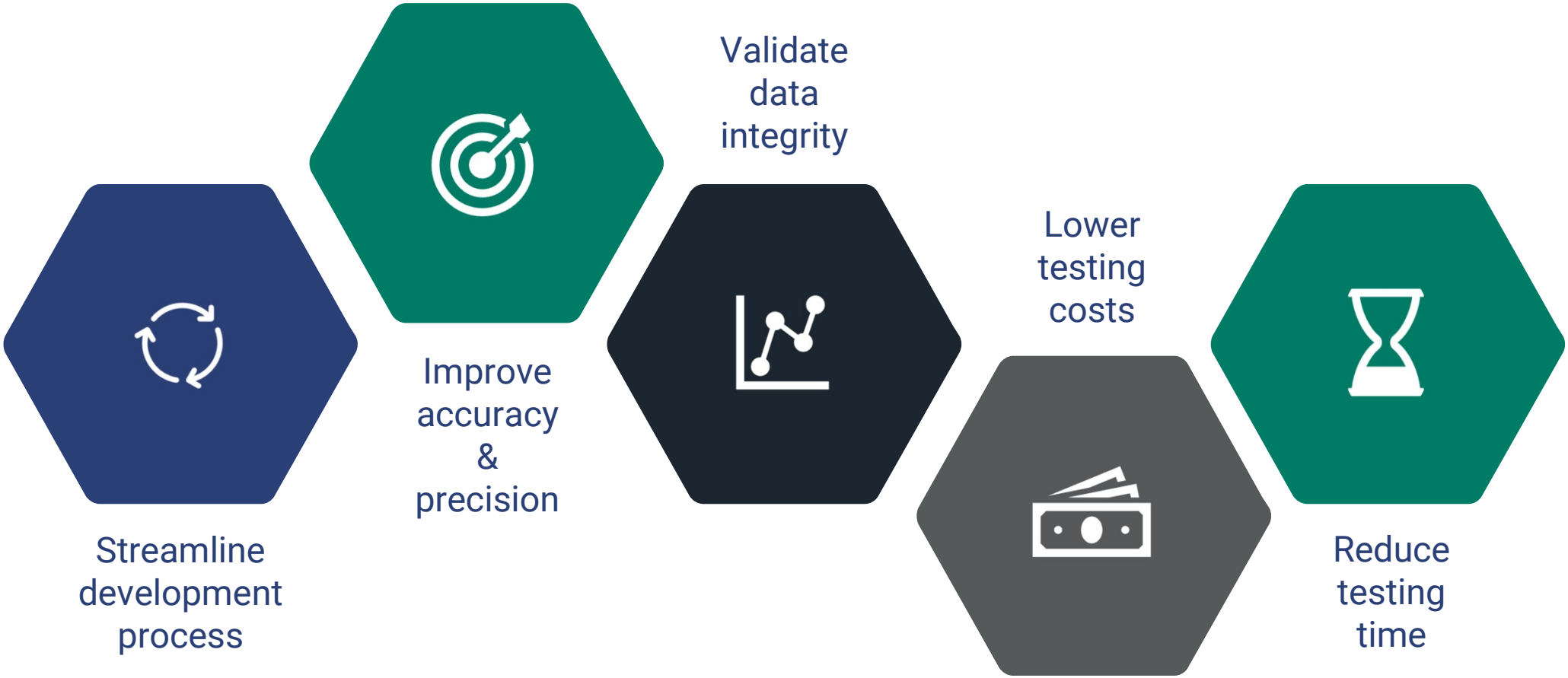


Wash slide and dry

Read on VaxArray Imaging System; Automated data analysis

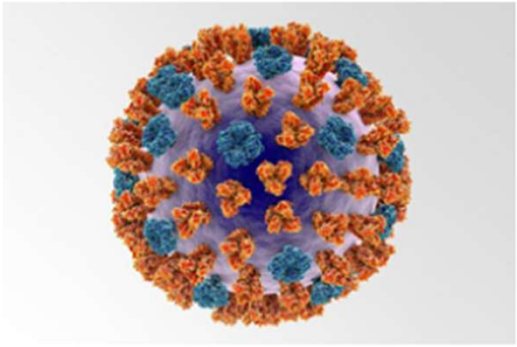
- 5-hour time to result with only 30 minutes hands-on time
- Automated data analysis using accompanying software
- Assay measures antigen concentration, not infectious dose

InDevR's Mission to Empower Vaccine Research



Vaccine Kits Available

Influenza Kits



Simplify and Accelerate Antigen Characterization

Mono and multivalent kits for reliable subtype specific quantification of influenza vaccines

Coronavirus Kits

Quantification of Antigen or Antibody Response for Vaccine Development

Multiplex measurement of antigens or antibodies related to SARS-CoV2

Measles and Rubella Kits

Same Day Antigen Quantitation

Analyze monovalent measles, monovalent rubella, or bivalent vaccine samples

VaxArray Platform

Multiplexed Fluorescence Immunoassay

- Antibody or antigen assays
- Reagent sparing microarray format
- High specificity and stability indicating
- Proven with split virus, subunit vaccines, recombinant proteins, virus-like particles, and serum samples
- Works with purified or crude samples

Platform Includes

- VaxArray Instrument
- Software with CFR Part 11
- IQ/OQ tools

Enabling Global Standardization

Standardized Assay

Globally available

Allows standardization across departments and locations

High Quality

Validated to ICH guidelines

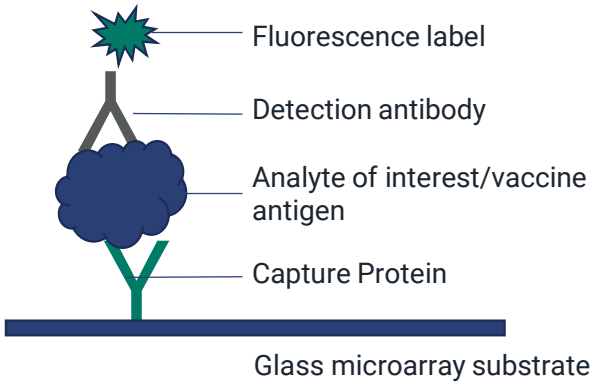
Manufactured under ISO:13485 quality standards

Validation Tools

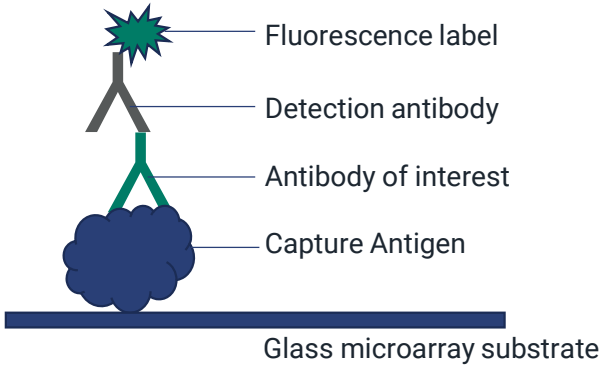
21 CFR Part 11 compatible software

IQOQ tools available

VaxArray Assay Configurations



Antibody Array

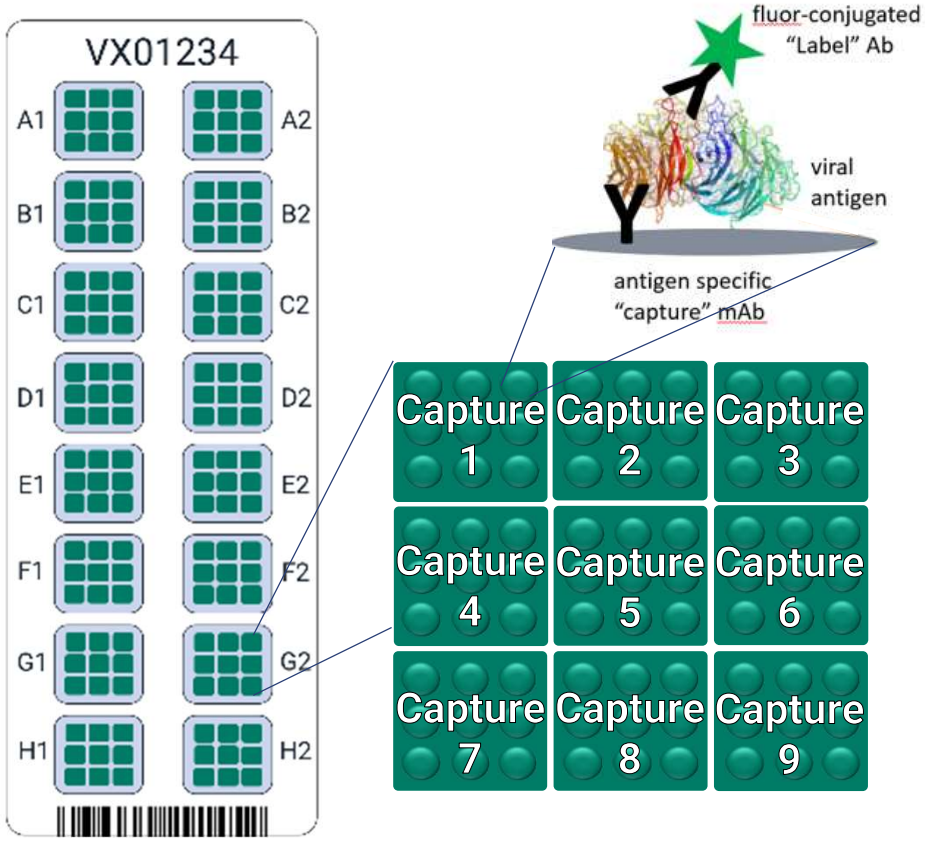


Antigen Array

Fast, Easy, and Versatile

- 30 minutes hands on time
- Same day time to result
- 32 or 64 samples/standards per kit, depending on kit
- Linear range is ng/mL – ug/mL, depending on assay

VaxArray Slide Format

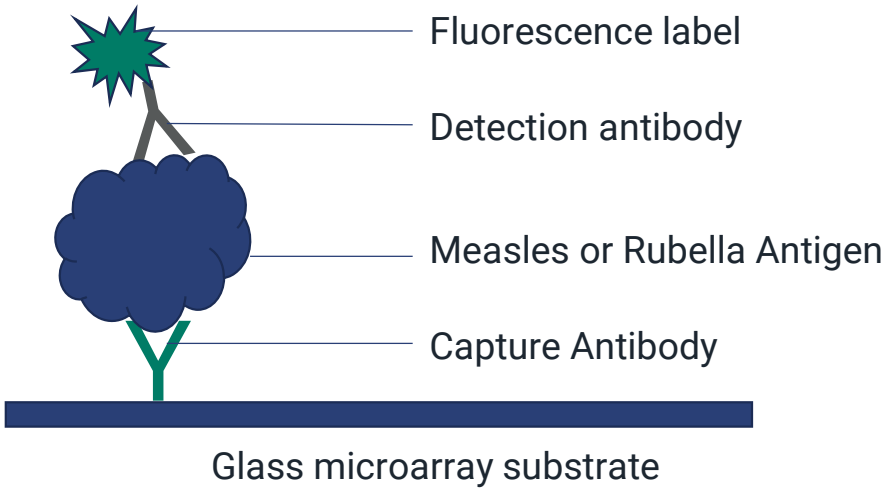


Slide Features

- Slide has an 8 x 2 replicate microarray format
- 16 samples/standards per slide
- Each microarray well contains 1-9 capture arrays
- 9 replicate spots printed of each capture molecule

Measles and Rubella Assay Format

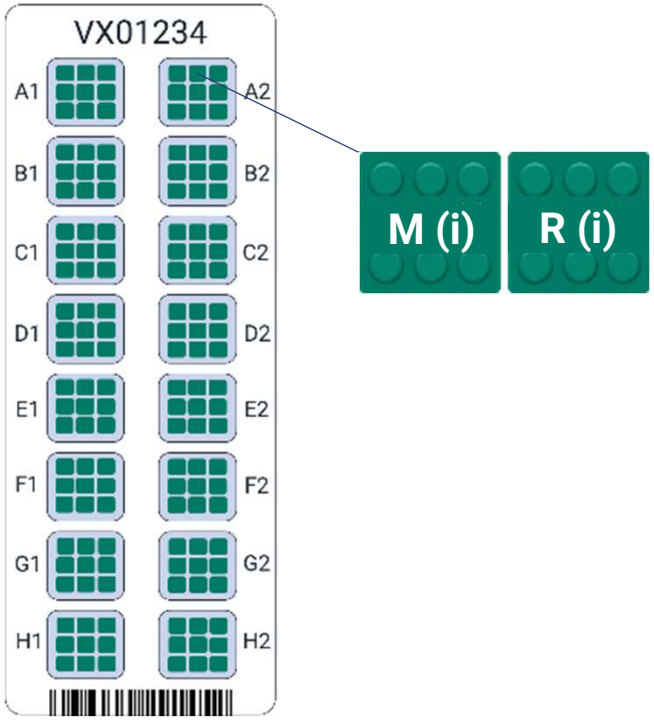
Antibody Array Format



Multiplexed Immunoassay

- Captures both measles and rubella antigens on spatially-separated capture antibodies
- Established VaxArray platform
- Same day result
- Measures total conformational protein
  - Measles NP
  - Rubella E1

VaxArray Measles and Rubella Kit v1.0



MR Kit Features

- 1 capture antibody for each virus
- 9 replicate spots of each antibody within each array
- Analyze monovalent measles, monovalent rubella, or bivalent MR vaccine samples without neutralizing rubella to measure measles
- Quantitation via standard curve using an appropriate user-supplied standard

Measles and Rubella Kit Configuration

Assay Kit

2 VaxArray Measles and Rubella v1.0 Slides
1 bottle 4x MR Lysis Buffer
2 bottle MR Blocking Buffer
2 bottles Wash Buffer 1 Concentrate
2 bottles Wash Buffer 2 Concentrate
6 8-tube strips

Sold Separately

Measles Detection Label
Rubella Detection Label

VaxArray Measles and Rubella Assay Workflow

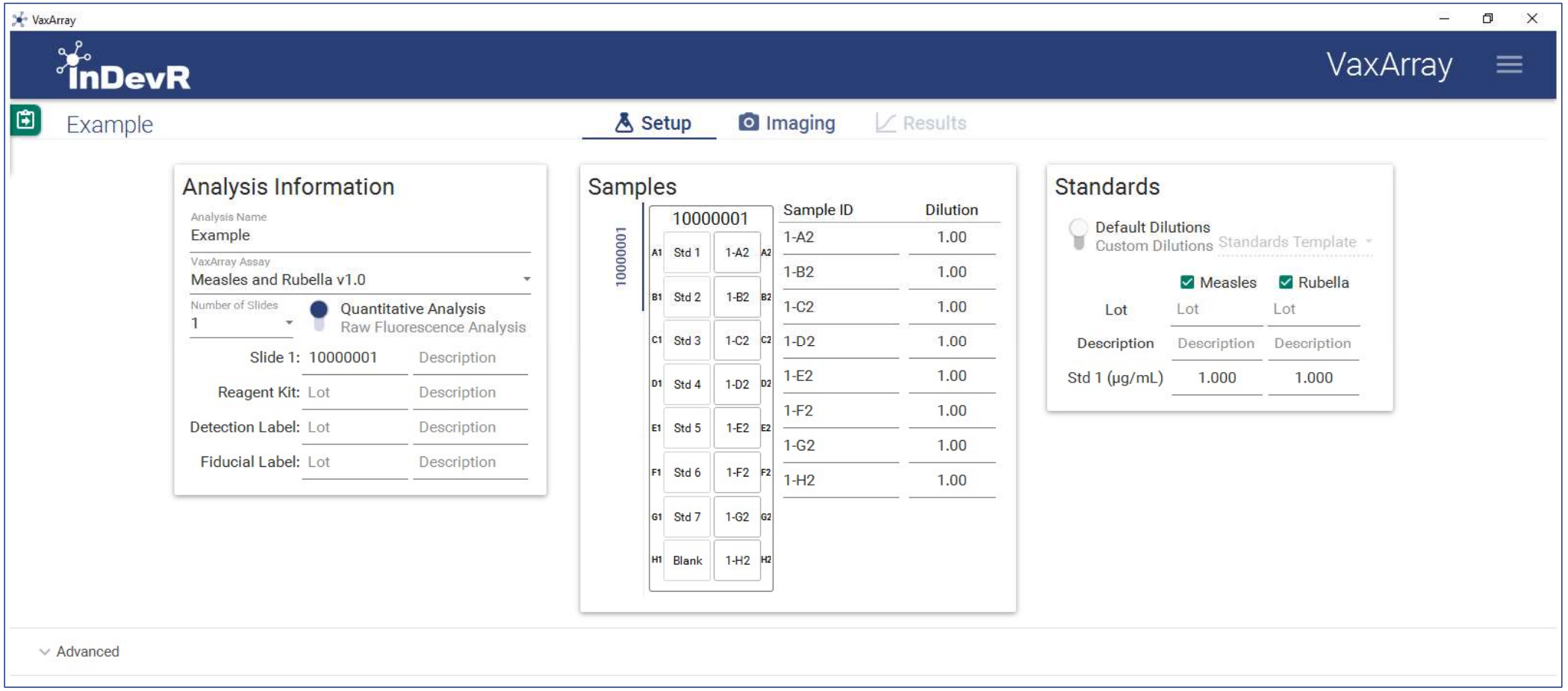
Total time to result is ~ 5 hours

Total hands-on time is ~ 30 minutes

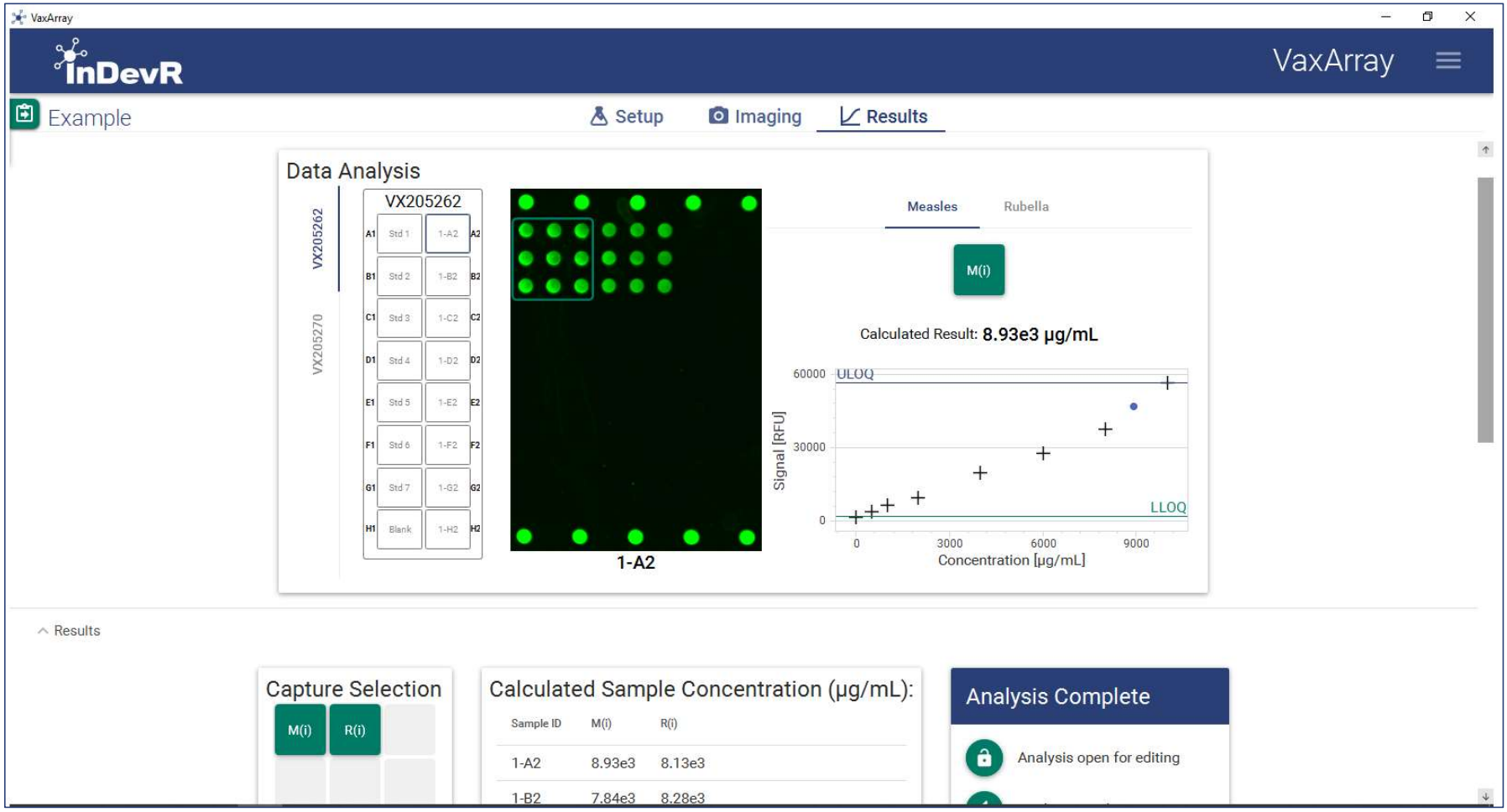
1. Prepare samples and standards
2. Lyse samples for 30 min
3. Combine with MR Blocking Buffer
4. Incubate for 4 hours on shaker

   Note: Overnight incubation provides additional sensitivity if needed
5. Remove excess sample, add detection label, and incubate on shaker 30 min
6. Serial washing steps, then dry slide
7. Imaging is <1 minute per slide

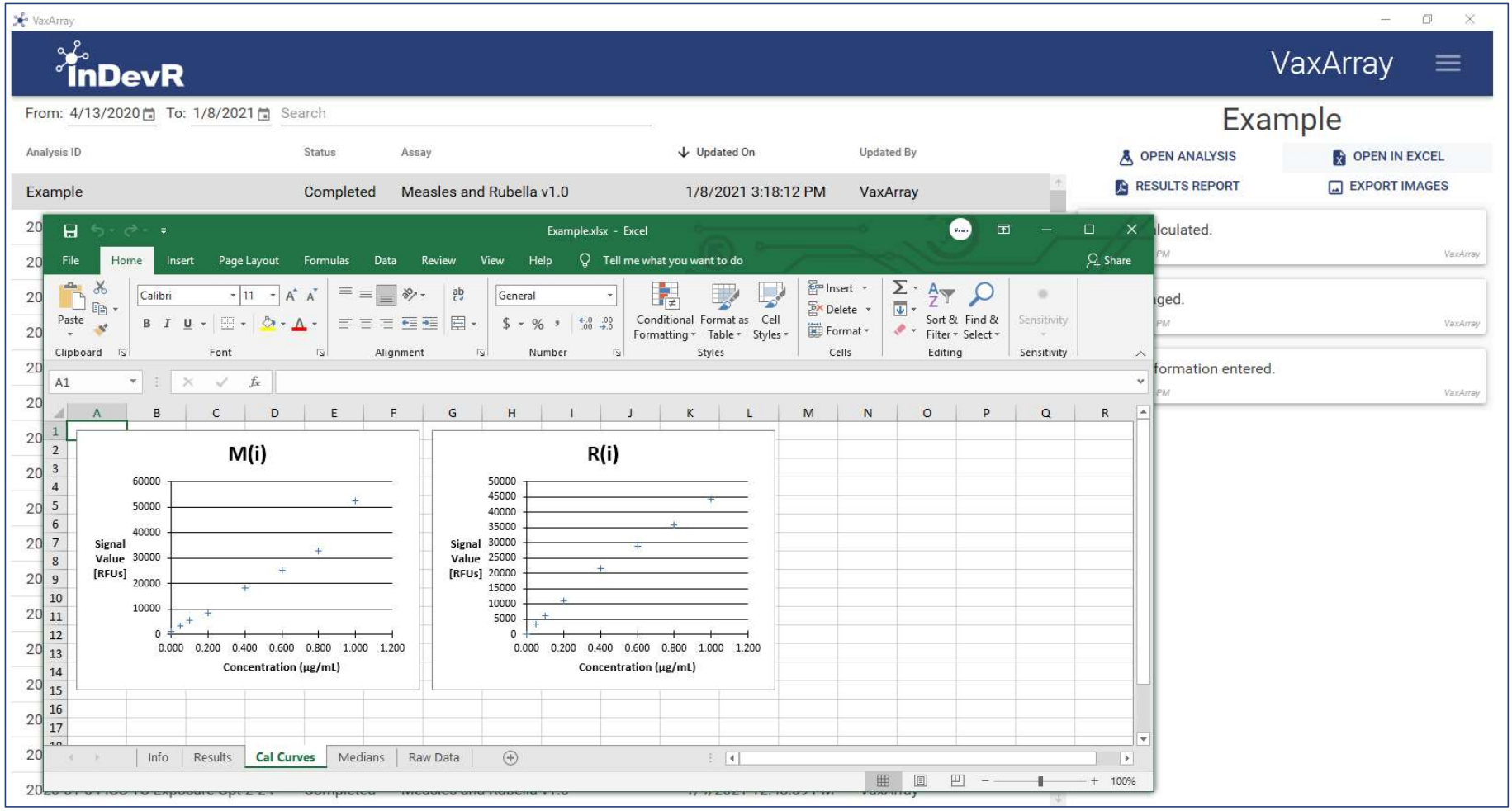
Software Setup is Simple



Software Provides Automated Quantitation



Software Results Exportable to Excel



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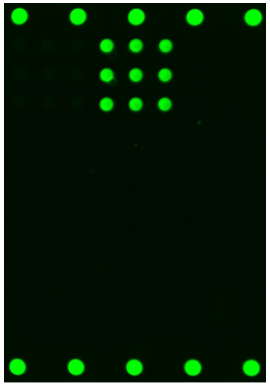
EMPOWERING VACCINE RESEARCH

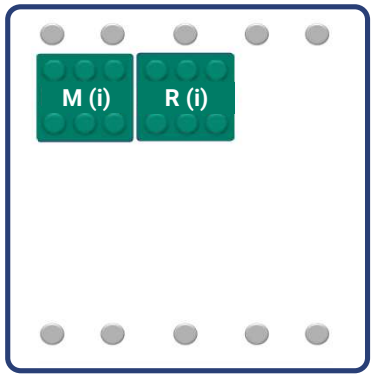
VaxArray MR Assay Performance

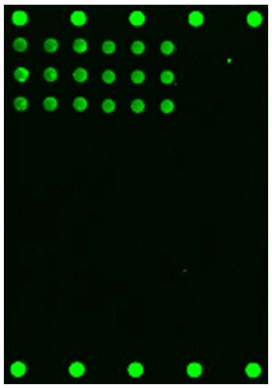
Capture Antibodies are Specific

Measles only



Rubella only





Bivalent sample

- Virus-specific monoclonal antibodies enable analysis of monovalent or bivalent samples using the same kit
- Most testing performed using Measles Edmonston-Schwarz and CAM-70, and Rubella RA 23/7
- Applicable to all M and R strains used in current vaccines
- MMR or MMRV kits also feasible

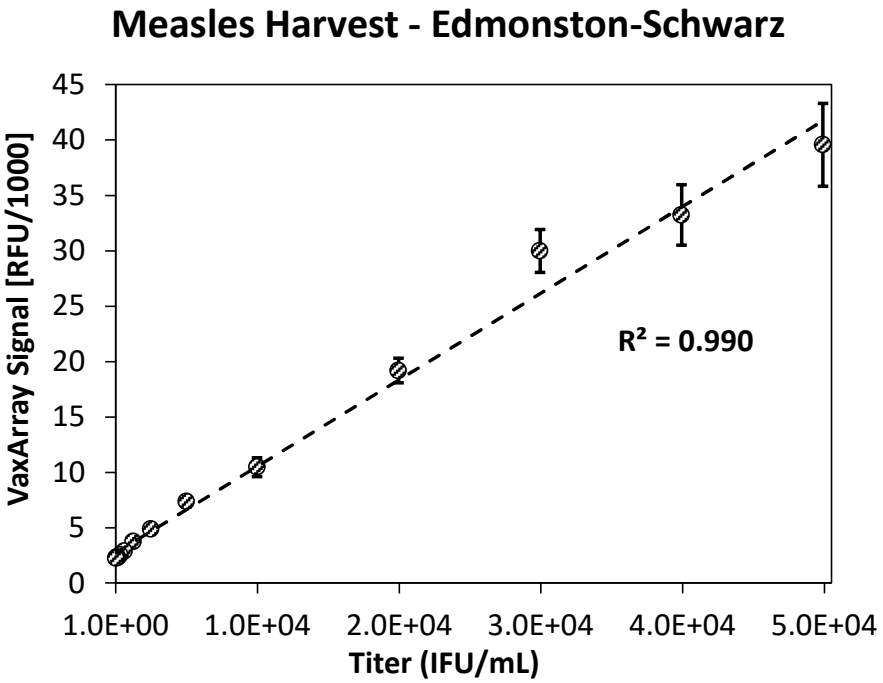
Limits of Quantification and Linear Range

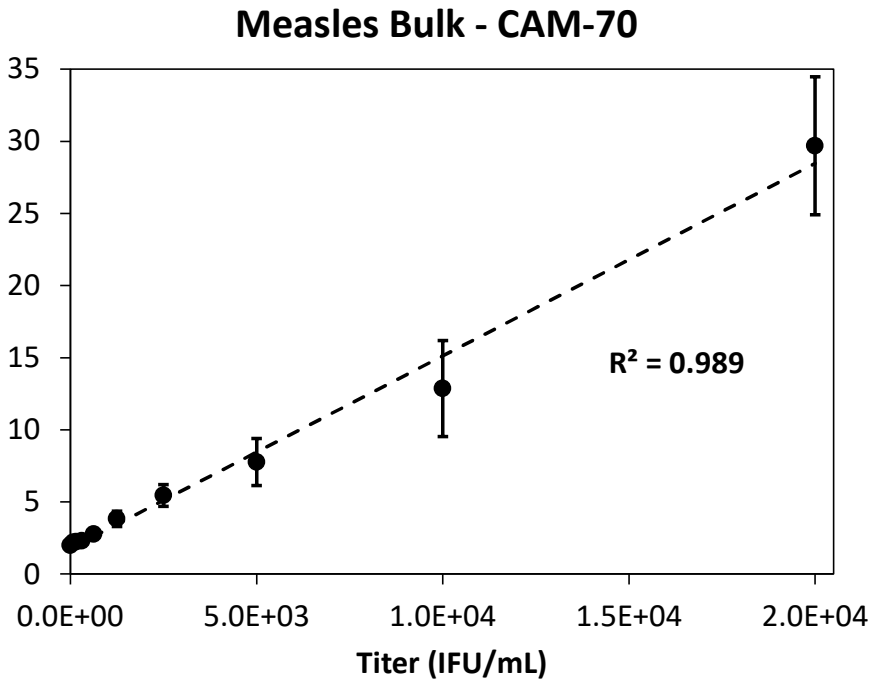
Strain	LLOQ (IFU/mL)	ULOQ (IFU/mL)	Linear Dynamic Range
Measles CAM-70	8.0×10^2 ($\pm$ 6% RSD)	$>1.0 \times 10^5$	≥ 125
Measles Edmonston-Schwarz	3.5×10^2 ($\pm$ 16% RSD)	$>5.0 \times 10^4$	≥ 143
Rubella RA 27/3	3.6×10^2 ($\pm$ 5% RSD)	$>1.55 \times 10^4$	≥ 43

Values obtained from rubella and measles bulks and harvest samples

Lowest allowable concentration in vaccine is 2×10^3 IFU/mL

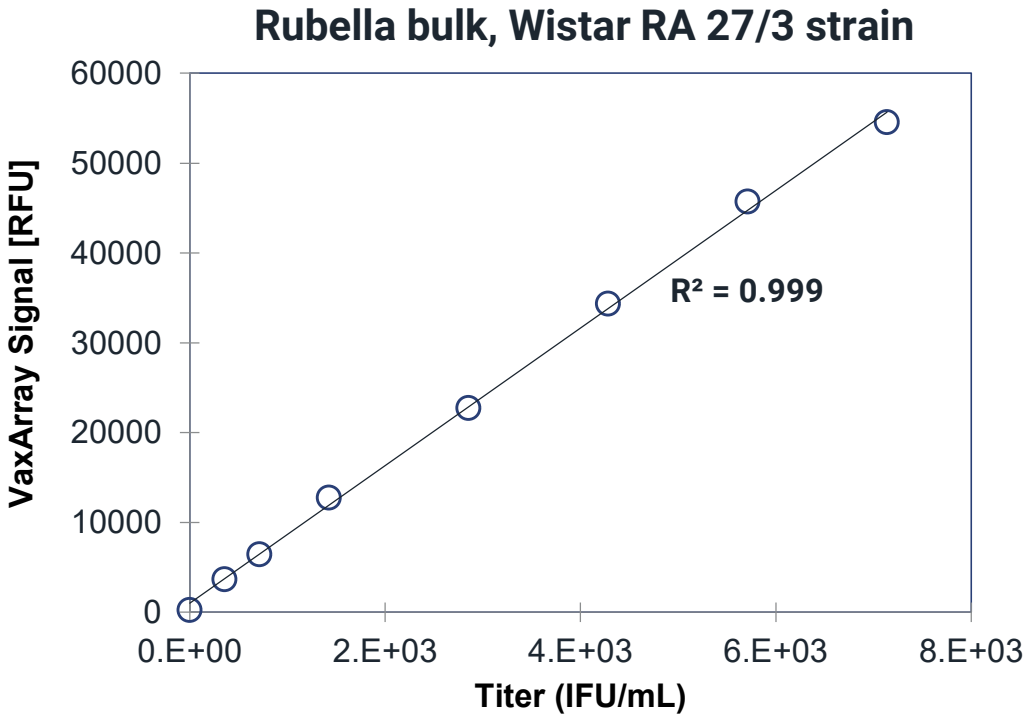
Measles Linearity





Excellent linearity with dilution for measles

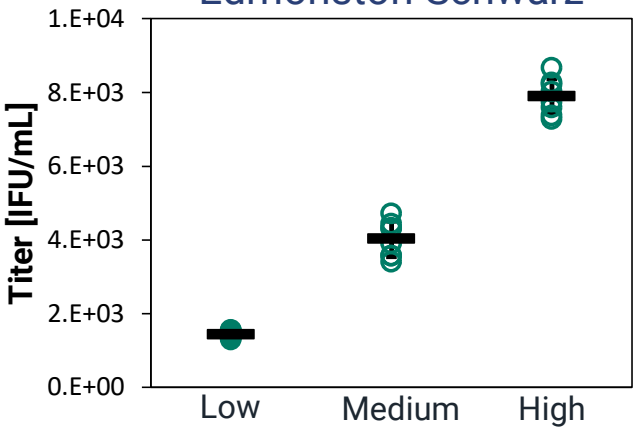
Rubella Linearity



Standard curve shows excellent linearity for rubella as well

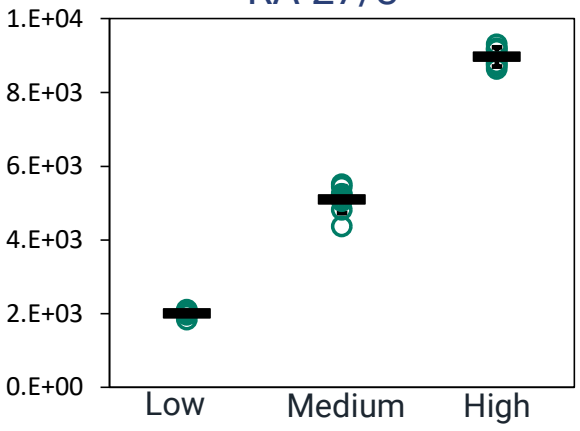
Assay Demonstrates High Precision

Measles Harvest
Edmonston-Schwarz



Sample	%CV
High	**6.1**
Medium	**11.9**
Low	**7.0**

Rubella Bulk
RA 27/3

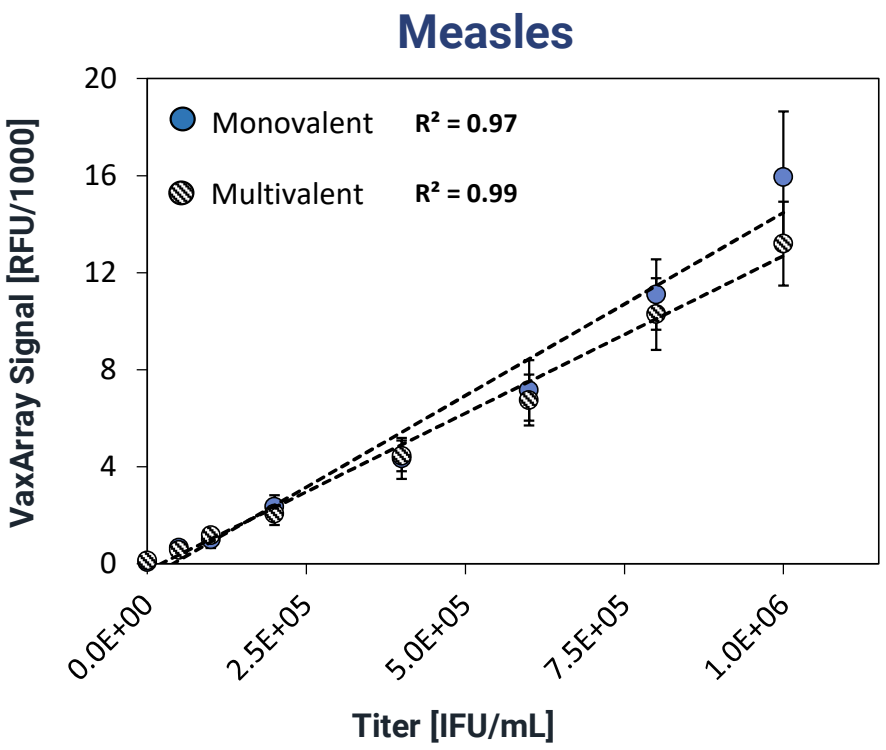


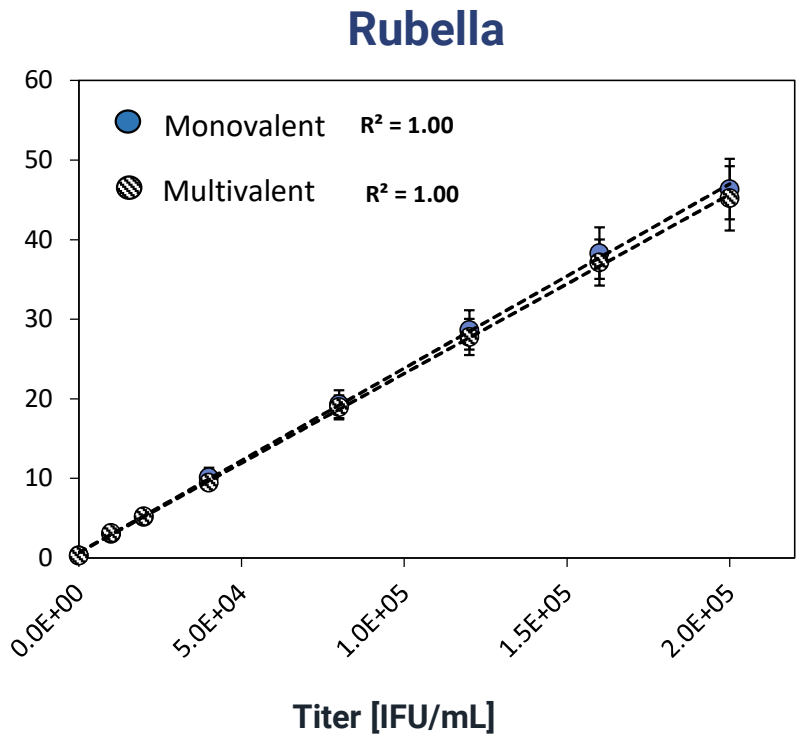
Sample	%CV
High	**2.9**
Medium	**7.2**
Low	**4.0**

- 8 replicates of three samples analyzed: high, medium, and low concentration
- 1 user, 1 day
- Samples analyzed against a 7-point standard curve
- Solid bar is 8-replicate average
- Error bars are standard deviation of average (8 replicates)

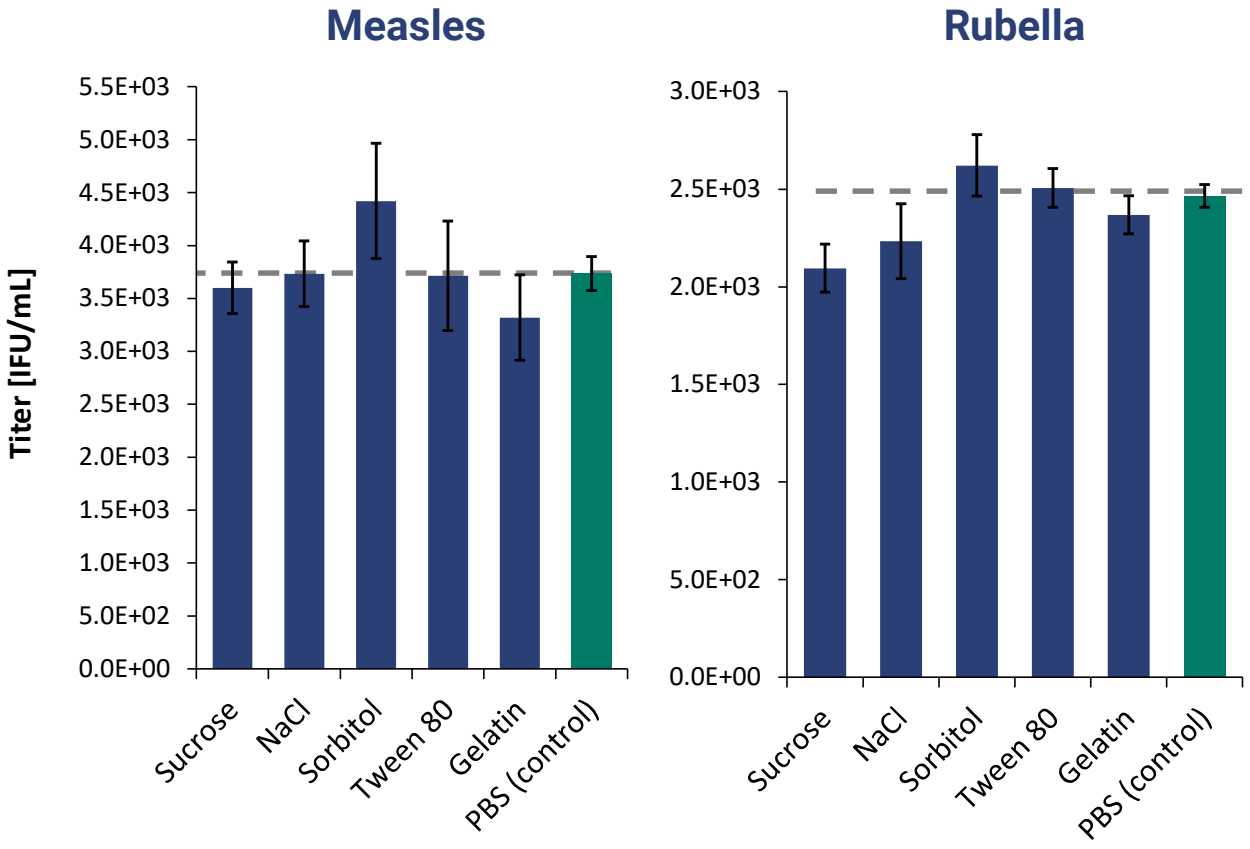
Monovalent or Bivalent Analysis

Response curves for each are not significantly affected in the presence of the other virus





Performs Well with Interfering Substances

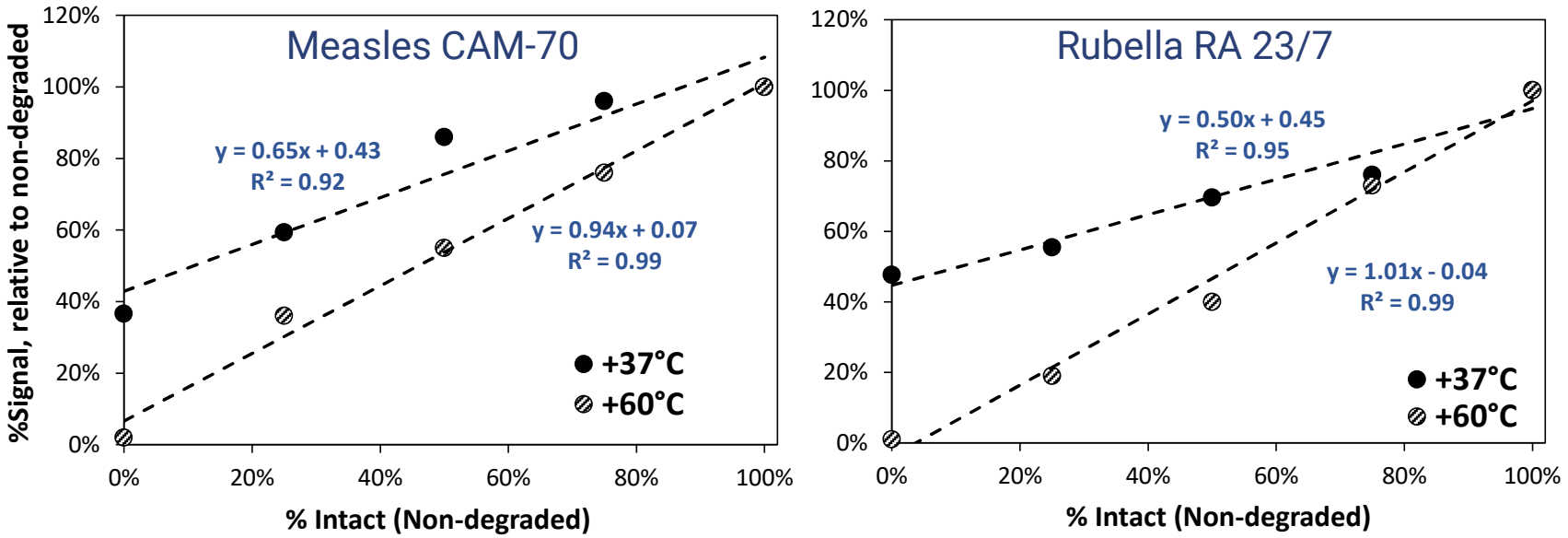


Demonstrates good accuracy, with all producing < 18% difference from control

Substance	Testing Conc
Sucrose	0.38%
NaCl	0.90%
Sorbitol	5.0%
Tween 80	0.125%
Gelatin	1.6%

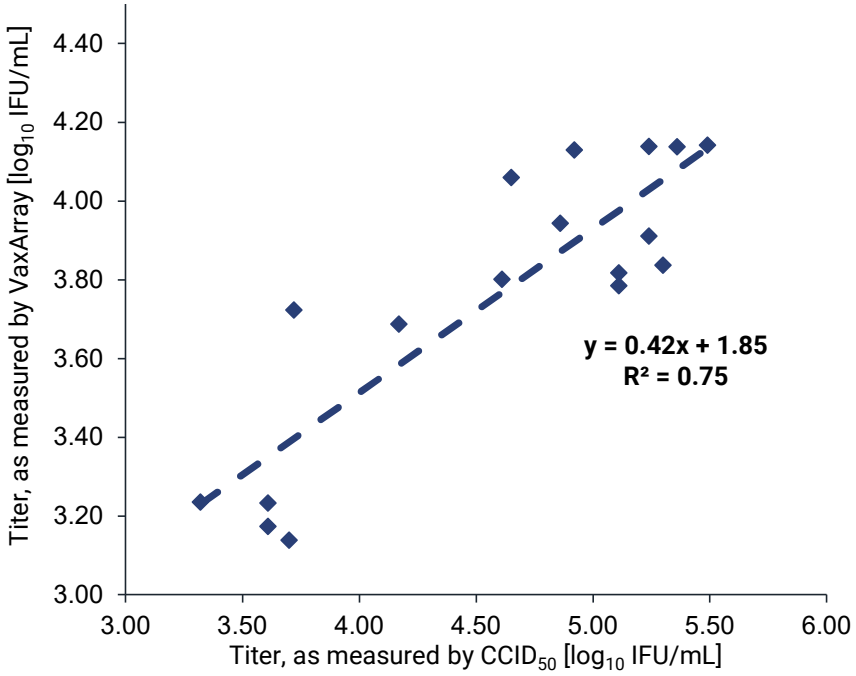
Thermal Degradation

Untreated vs. heat-treated at 60C for 48 hrs and 37C for 24 hrs



- **60C data:** VaxArray MR assay is measuring only the intact protein in the sample (slope=1)
- **37C data:** did not show complete loss of VaxArray signal, but $CCID_{50}$ (not shown) showed loss of infectivity; 37C likely does not fully denature the epitopes probed by the VaxArray antibodies

Rubella Harvest Samples Correlation to $CCID_{50}$



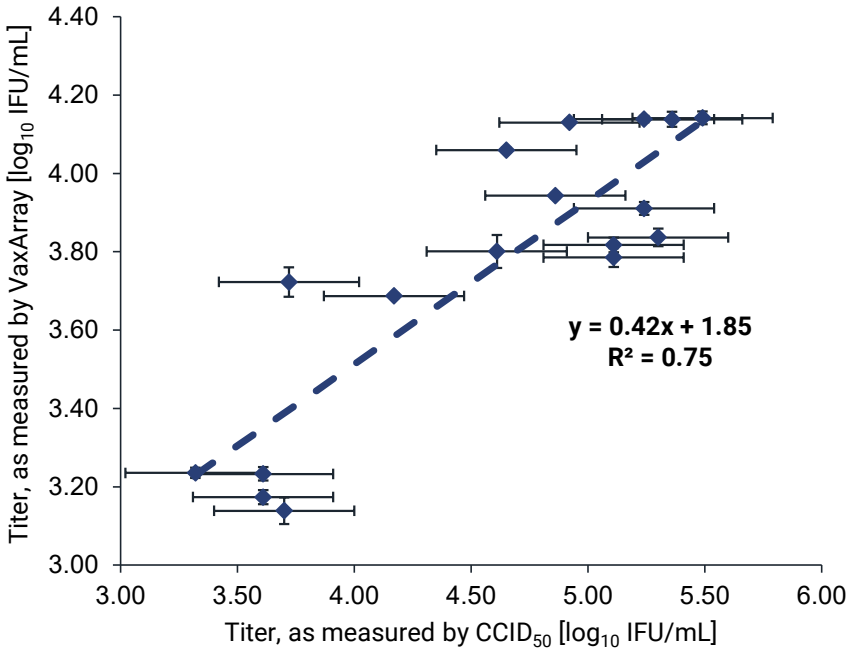
Experimental Design

- 17 in-process rubella samples analyzed by $CCID_{50}$ and VaxArray
- Varying harvest days of 2-20 days post infection and production lot
- Different lot of vaccine monobulk used as VaxArray calibrant

Results

VaxArray generally correlated to $CCID_{50}$ (BUT NOT EQUIVALENT)

Rubella Harvest Samples Correlation to $CCID_{50}$

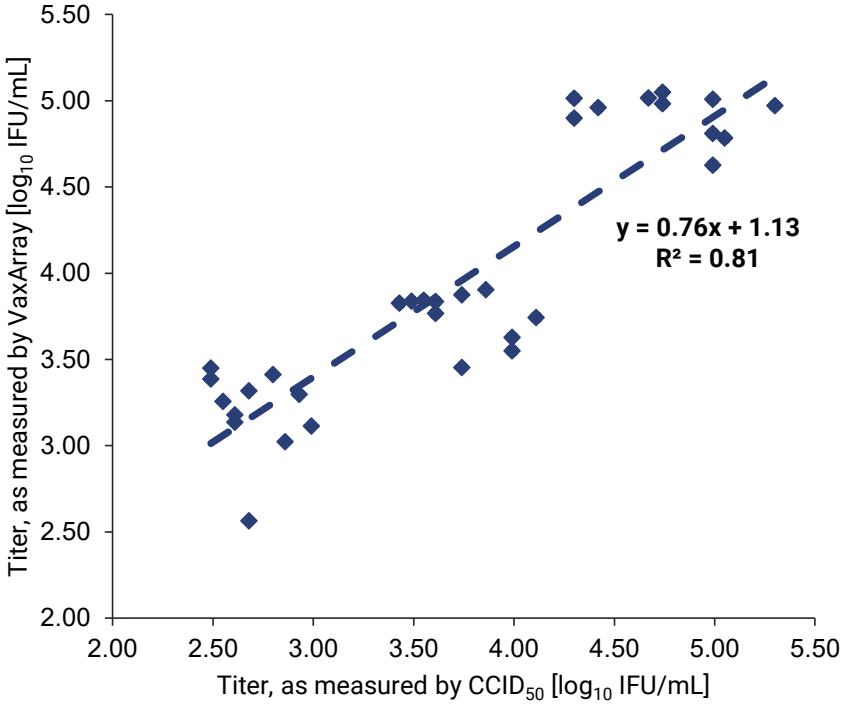


Same data as previous slide with added error bars

Results

- $CCID_{50}$ error bars shown in x direction are assumed typical error of ± 0.3 log_{10}
- VaxArray error bars in y direction (± 1 std dev of mean, n=4) are tiny in comparison

Measles Harvest Samples Correlation to $CCID_{50}$



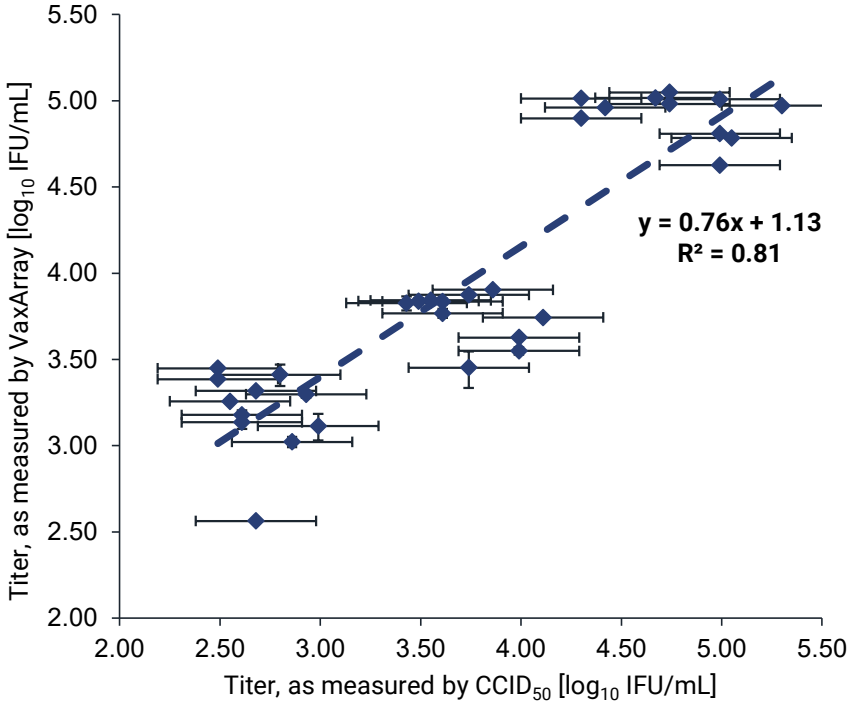
Experimental Design

- 33 in-process measles samples (Edmonston-Schwarz) analyzed by $CCID_{50}$ and VaxArray
- Separate harvest sample (not included in sample set) used as VaxArray calibrant

Results

VaxArray generally correlated to $CCID_{50}$ (BUT NOT EQUIVALENT)

Measles Harvest Samples Correlation to $CCID_{50}$



Same data as previous slide with added error bars

Results

- $CCID_{50}$ error bars shown in x direction are assumed typical error of ± 0.3 log_{10}
- Similar to rubella, VaxArray precision of the replicates is significantly better than $CCID_{50}$

VaxArray Measures Total Conformational Protein

VaxArray measures total conformational protein
and not quantity of infectious virus particles

Ratio of total conformational protein to infectious particles may
vary batch to batch, as a function of harvest time, etc.

Crude harvest samples do not produce equivalent results to $CCID_{50}$
but are generally correlated (R^2 ~ 0.75 – 0.8)

VaxArray vs. $CCID_{50}$ for Purified Rubella

Rubella bulk previously characterized by $CCID_{50}$ used as VaxArray calibrant

	Concentrated Virus Pool					Bulk			Final Vaccine		
Lot	A	B	C	D	E	B	C	F	G	H	I
VaxArray (log_{10} IFU/mL)	6.06 (±0.06)	6.27 (±0.05)	6.26 (±0.04)	5.98 (±0.02)	6.38 (±0.04)	6.15 (±0.02)	6.03 (±0.03)	6.27 (±0.03)	4.31 (±0.18)	4.36 (±0.04)	4.38 (±0.04)
$CCID_{50}$ (log_{10} IFU/mL)	6.11	6.24	6.24	6.07	6.30	6.15	6.13	6.11	4.34	4.26	4.22
Difference (log_{10} IFU/mL)	0.05	0.03	0.02	0.09	0.08	0.00	0.11	0.16	0.03	0.10	0.16

More purified rubella samples produce much closer equivalency to $CCID_{50}$

VaxArray vs. $CCID_{50}$ for Purified Measles

- Measles bulk (CAM-70) characterized by $CCID_{50}$ used as VaxArray calibrant
- Bulk material produced closer equivalency to $CCID_{50}$
- Measles component in vaccine measured ~0.5 log_{10} lower by VaxArray compared to $CCID_{50}$

	Bulk		Final Vaccine		
Lot	A	B	C	D	E
VaxArray (log_{10} IFU/mL)	6.83 (±0.06)	6.83 (±0.05)	5.18 (±0.13)	5.36 (±0.08)	5.28 (±0.08)
$CCID_{50}$ (log_{10} IFU/mL)	6.99	6.90	4.82	4.76	4.88
Difference (log_{10} IFU/mL)	0.16	0.21	0.36	0.60	0.40

- Possible explanations?
  - Measles aggregation in both bulk and vaccine could be affecting VaxArray measurement
  - Measles degradation over time ($CCID_{50}$ values historically assigned by collaborator)
  - Monovalent bulk not an appropriate calibrant for this strain (use previously characterized vaccine?)

VaxArray MR Assay Advantages over $CCID_{50}$

Metric	$CCID_{50}$	VaxArray	Improvement
Sample Requirements	100 µL per plate	~10 µL	10x
Information Content	1 plate per sample; multiple replicates of each dilution required	16 arrays/slide, both viruses simultaneous when needed	Multiplexed—simultaneous analysis in bivalent samples
Hands On Time	4-6 hours	30 minutes	Less time in lab
Time to Result	10-14 days	5 hours	Faster answers
Standardization	Home-brew assay; likely site to site differences	Global product with standardized reagents	Standardization reduces risk
Precision	65% RSD ($\pm$ 0.3 $\log_{10}$ IFU/mL)	< 15% RSD	3-4x improvement
Accuracy	50 – 200% of expected	80 - 120% of expected	Significant improvement

InDevR Services Available

Testing Services

Testing Services

InDevR offers a range of testing services to support vaccine research and development

Custom Testing & Kits

Customize VaxArray Assay to Your Needs

Custom array design under ISO 13485:2016 requirements to meet your project needs

GLP Testing Services

Testing Service Options

Run commercially available kits

Create and run custom kits

SRID confirmation

MUNANA confirmation

Purity Adjusted Total Protein

Antibody Kits Available

- Influenza Seasonal Hemagglutinin
- Influenza Pandemic Hemagglutinin
- Influenza Seasonal Neuraminidase
- Influenza Nucleoprotein
- Influenza Monovalent H1, H3, B/V, B/Y
- Coronavirus Spike Protein
- Measles and Rubella

Antigen Kits Available

- Coronavirus SeroAssay

InDevR Custom Services

Custom Options

Antibody arrays

Antigen arrays

Multiple targets in each test

Print what you want and need

Obtain more results in less time

Applications

- Custom antibody kits for rapid quantification of antigen levels during bioprocess development, monitoring, or potency testing
- Custom antigen kits to measure vaccine response in clinical specimens or for serosurveillance studies
- Replacement of ELISAs to improve time to result and reduce reagent usage

Custom Kit Development Process

Printing Feasibility

- Screen reagents for optimal printing conditions
- Determine specificity and relative sensitivity

Performance Assessment

- Measure LOD, LOQ
- Evaluate linear range
- Test intra-lot precision

Manufacturing Validation

- Test inter-lot precision
- Develop QC protocols
- Create documentation and custom packaging

Examples of Custom VaxArray Assays

- Custom antigen characterization (potency) assay for recombinant influenza vaccine
- Custom influenza serological assay for use in vaccine clinical trials
- Custom COVID-19 antigen characterization (potency) assay for recombinant spike protein-based vaccine
- Custom COVID-19 serological assay for use in vaccine clinical trials

Thank You!

Thank you for your time and attention

We welcome any questions you may have regarding the VaxArray platform—please contact us at:

sales@indevr.com