VaxArray® Measles and Rubella Assay Correlation to $CCID_{50}$

Overview

The VaxArray Measles and Rubella (MR) assay is a new tool for quantification of measles and rubella antigens in monovalent and bivalent vaccine samples using broadly reactive monoclonal antibodies (mAbs). InDevR prints antibodies reactive to vaccine-relevant measles and rubella strains in an array format on a glass substrate. Readout for this multiplexed immunoassay is based on fluorescent signal from a monoclonal antibody label that binds to the antigen of interest. This rapid measurement offers an advantage throughout the vaccine development cycle over the cumbersome and error prone infectious dose method ($CCID_{50}$).

The cell culture infectious dose 50% ($CCID_{50}$) assay has remained the FDA recommended method to quantify infectious dose at different bioprocess stages from upstream to downstream operation as shown in Figure 1.

VaxArray MR Features

- Same day conformational protein quantitation
- High sensitivity & specificity
- Simultaneous quantification of measles and rubella antigens
- Applicability to all vaccine-relevant strains

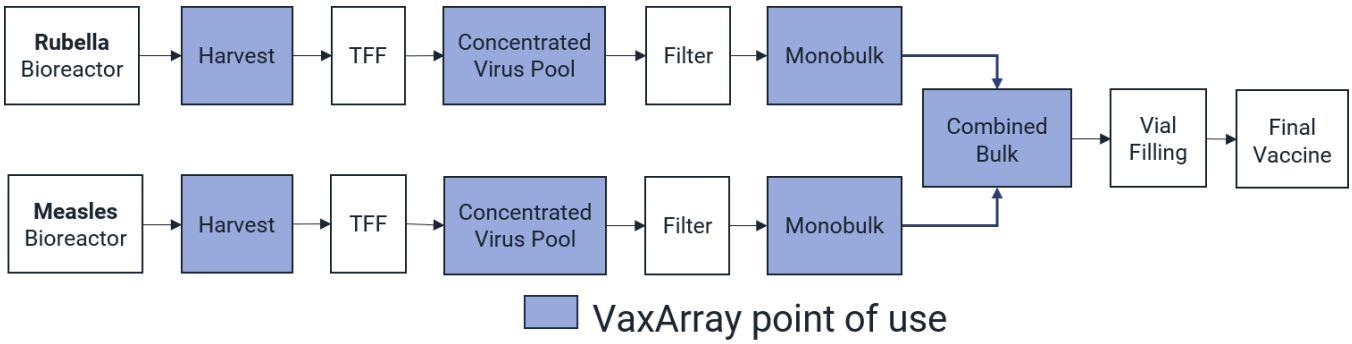

Figure 1. MR Vaccine Manufacturing Process

The $CCID_{50}$ assay is lengthy, time-consuming, subjective, laborious, and imprecise. Cell-culture-based methods typically have a 10 to 14-day turnaround time and suffer from imprecision, which can often result in lot rejection during several bioprocess stages. The VaxArray MR Assay overcomes these drawbacks and helps accelerate vaccine development by producing same-day results while involving only 30 minutes of hands-on time. Although the VaxArray MR assay cannot provide an infectivity measurement, it is an alternative to $CCID_{50}$ for in process samples when rapid antigen tracking is beneficial.

The VaxArray MR assay follows the ICH Q2 (R1) guideline to deliver the required sensitivity, specificity, accuracy, and reproducibility for quantification of measles and rubella antigen(s) in a multiplexed format. This

technical note will demonstrate that the quantification of purified samples measured by the rapid VaxArray MR assay is highly correlated with the titer value measured by $CCID_{50}$.

Purified Samples: Correlation with $CCID_{50}$

Studies were conducted by InDevR, in collaboration with a top biopharmaceutical company, to evaluate the VaxArray MR assay for the quantification of measles strain (CAM-70) and rubella strain (Wistar RA 27/3) in correlation with $CCID_{50}$. Samples from multiple production lots for both strains were collected from various stages of manufacturing. A control monovalent bulk for each strain with a known $CCID_{50}$ value was used as the VaxArray reference standard. Relative fluorescence units (RFU) generated by the MR assay for the samples and the calibrant were used to back-calculate VaxArray-equivalent viral titers (IFU/mL).

For samples containing measles, two monovalent bulk lots and three final vaccine samples (each from a different lot) were used to determine the correlation between the VaxArray MR assay and $CCID_{50}$ as shown in Table 1. The percent difference between the two assays was less than 13% in all cases. The slightly higher variances in the final vaccine could be caused by a combination of factors such as sample degradation or aggregation of CAM-70 measles strain. In addition, the use of a previously characterized final vaccine sample could be a more appropriate calibrant for the analysis of the final vaccine sample.

A similar rubella downstream sample comparison measured the correlation between the VaxArray MR assay and $CCID_{50}$. Fourteen samples were collected, starting from post-tangential flow filtration (TFF) to the product release across seven production lots as illustrated in Table 2 (each lot not represented for every sample type).

Table 1. Measles Monovalent Bulk and Final vaccine

Sample Type	Monovalent Bulk		Final Vaccine		
Manufacturing Lot	**A**	**B**	**C**	**D**	**E**
VaxArray (log_{10} IFU/mL)	6.83 ±0.06	6.69 ±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
Percent Difference	2.3%	3.0%	7.5%	12.6%	8.1%

Table 2: Rubella downstream sample comparison

Sample Type	$CCID_{50}$ (log_{10} IFU/mL)	VaxArray (log_{10} IFU/mL)	Difference	
			log_{10} IFU/mL	%
TFF Flush	5.43	5.50	0.07	1.3%
Concentrated Virus Pool	6.11	6.06	-0.05	0.8%
	6.24	6.29	0.05	0.8%
	6.24	6.26	0.02	0.3%
	6.07	5.98	-0.09	1.5%
	6.30	6.29	-0.01	0.2%
	5.95	6.30	0.35	5.9%
	6.38	6.11	-0.27	4.8%
Monovalent Bulk	6.13	6.03	-0.10	2.4%
	6.11	6.27	0.16	2.6%
	6.15	6.15	0.00	0%
	5.76	6.12	0.36	6.3%
	6.11	6.21	0.10	1.6%
Vaccine	4.02	4.22	0.20	4.9%

The results showed outstanding accuracy, with less than 7% difference between the VaxArray assay and the $CCID_{50}$ value. As shown in Figure 2, linear regression resulted in an R^2 of 0.92, indicating high goodness of fit.

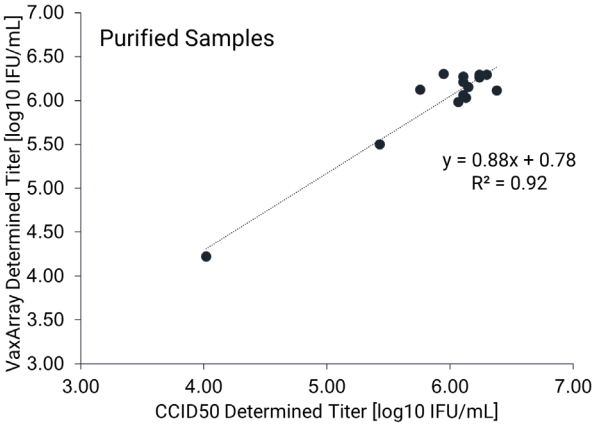

Figure 2. Rubella correlation between VaxArray MR Assay and $CCID_{50}$ for purified samples

A suitability assessment was conducted to examine for analytical specificity and accurate quantification in bivalent analysis using monovalent bulk materials as shown in Figure 3.

The similar linear regressions for monovalent and bivalent analyses indicate that the presence of one virus does not interfere with the ability to measure the second virus. Representative fluorescence images in Figure 4 show the virus-specific monovalent and bivalent response, highlighting the multiplexing capability of the assay.

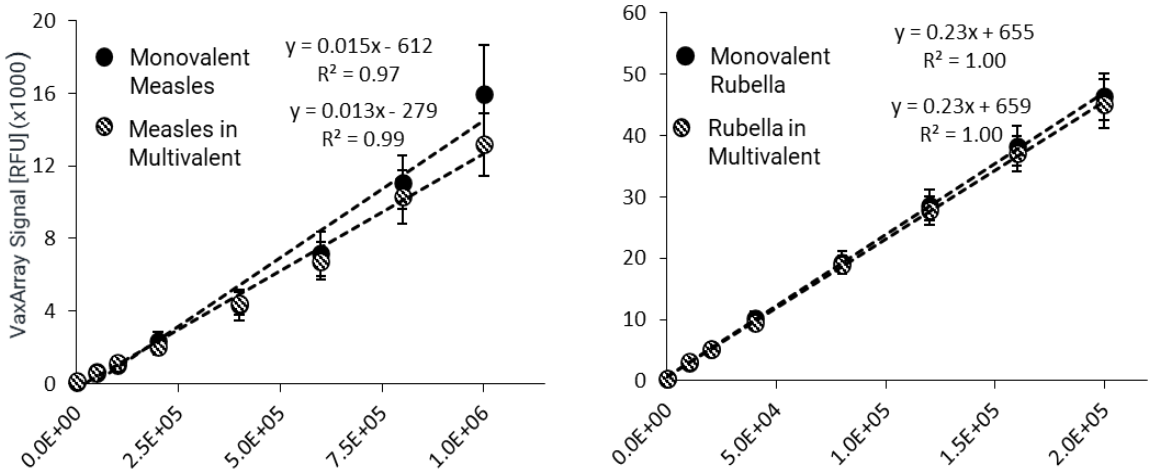

Figure 3. Assay response for monovalent and bivalent (left) measles and (right) rubella samples

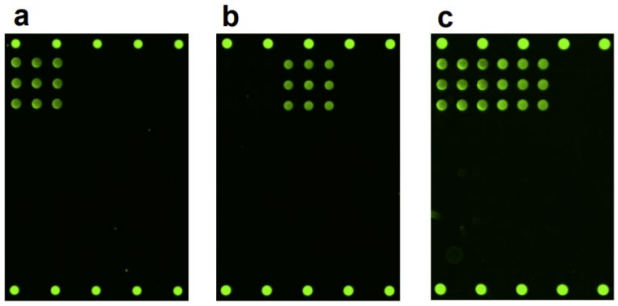

Figure 4. The VaxArray images captured monovalent measles detection (a), monovalent rubella detection (b), and bivalent measles and rubella detection (c)

Summary

The current analytical methods to produce MR vaccines have many bottlenecks which lead to slow and inefficient vaccine development. The current methods also emanate excessive cost due to manufacturing lot rejection. Since the VaxArray Measles and Rubella Assay shows strong correlation with $CCID_{50}$, it offers vaccine developers and manufacturers a much faster, more accurate, and more quantifiable way to assess bioprocess MR vaccine production.

Table 3: Assay Summary Comparison

Metric	$CCID_{50}$	VaxArray	Improvement
Sample Requirements	100 µL per plate	~10 µL	10x
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	Up to 65% RSD	< 15% RSD	3 - 4x improvement
Accuracy	50 – 200% of expected	80 - 120% of expected	Significant improvement

References

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