

Multiplexed MMRV chemiluminescent assay for the qualitative determination of IgG antibodies

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Background

Antibody levels to measles, mumps, rubella and varicella-zoster (MMRV) are used to assess an individual's immune status. Multiplexing allows all four specificities to be assayed simultaneously with advantages in terms of time and cost. The Dynex Technologies' Multiplier® with SmartPLEX™ technology fully automates these assays. Antigen coated beads are embedded in the base of a 96 well assay plate and serve as individual targets for IgG binding from the test sample. The assays are based on the standard direct solid phase assay principles with a chemiluminescent endpoint. If IgG antibodies to any of the four analytes are present, a chemiluminescent signal is produced which is proportional to the IgG concentration and is detected by an integrated CCD camera. See Figure 1 for pictures of the SmartPLEX assay plate, endpoint image and the Multiplier instrument.

Results

The results are summarised in the figures below. Precision (Figure 2) and concordance (Figure 3) were found to be comparable to commercially available MMRV ELISA's. ROC analysis gave the following area under the curve results: Measles 0.995, Mumps 0.987, Rubella 0.998 and VZV 0.999.



Figure 1a. Beaded assay plate



Figure 1b. Magnified well

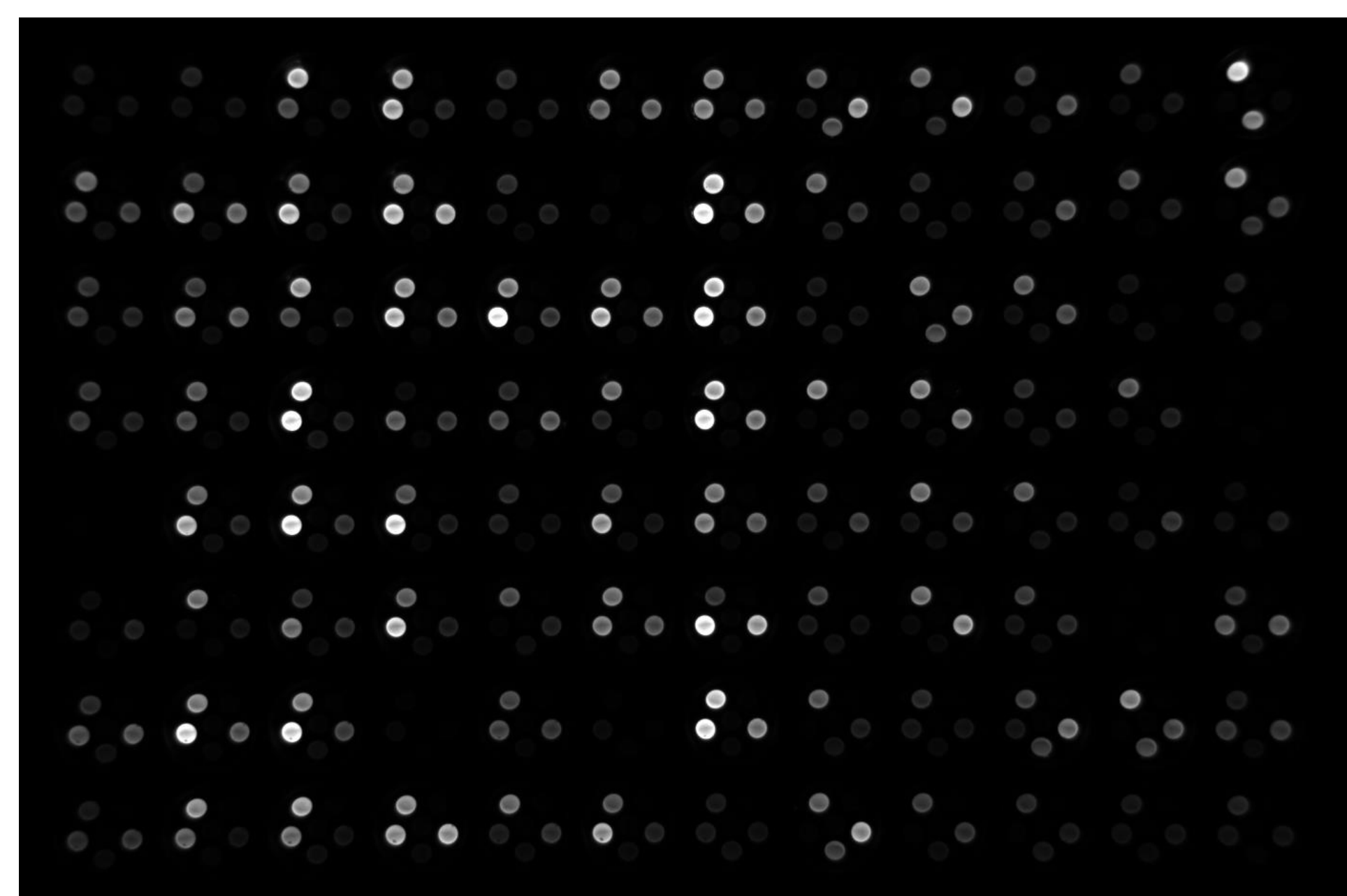


Figure 1c. Typical CCD image of the completed plate

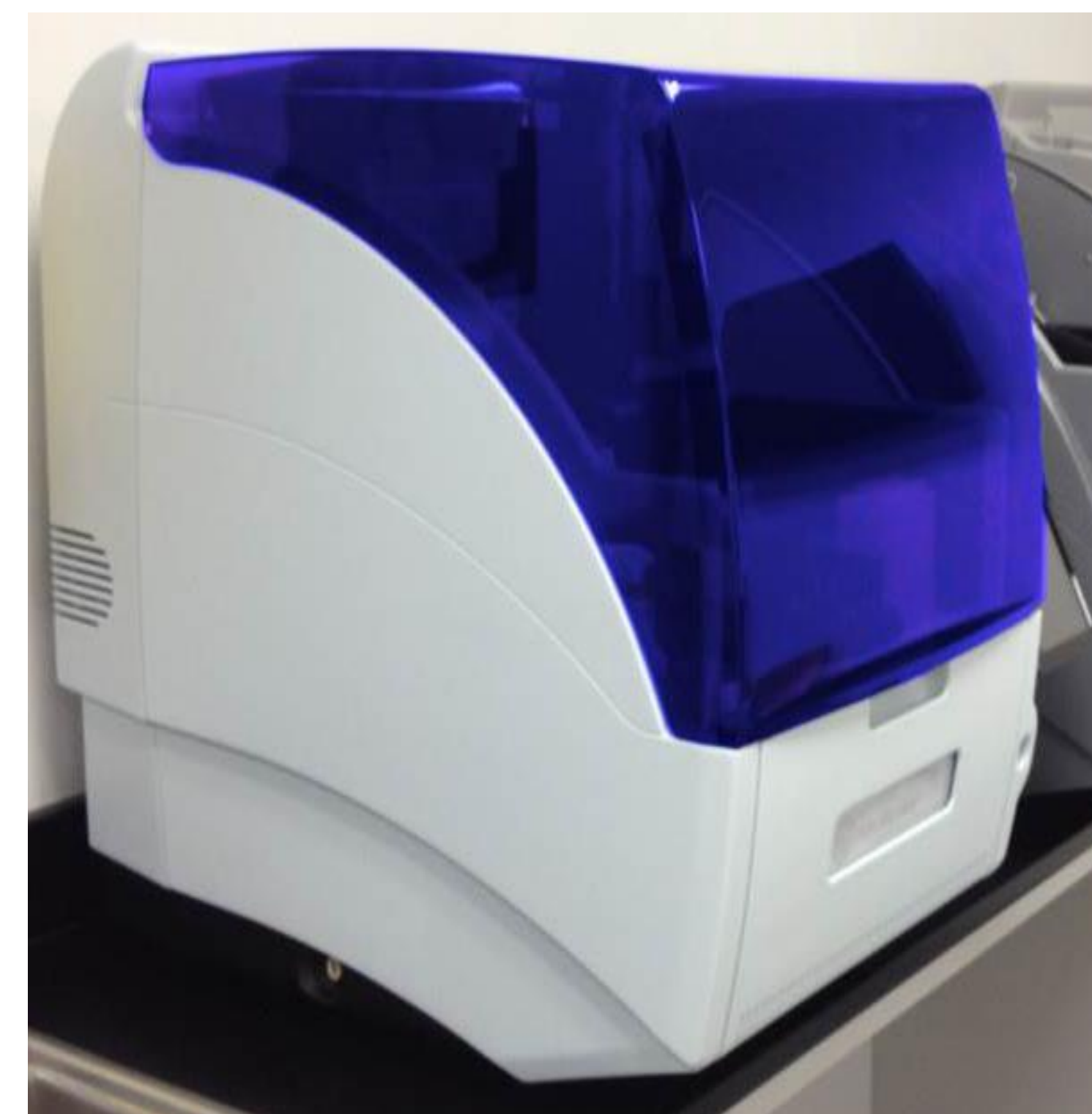


Figure 1d. Multiplier Instrument

Methods

In this study the precision and concordance of the assay have been assessed. An index value was calculated relative to the MMRV single point calibrator, enabling results to be scored as negative, equivocal or positive.

Precision: Precision was assessed by running 14 serum samples with mixed antibody levels, 6x across an assay plate. They were assayed on three Multiplier systems, on three separate days to estimate the within-run, between-run, between-day and between-Multiplier precision coefficients of variation (%CV), Figure 2.

Concordance: Concordance was determined on approximately 930 serum samples collected for MMRV testing. Sample results were compared to those from corresponding individual ELISA assays (510k cleared ELISA assays for Measles, Mumps, Rubella and VZV). Analyse-It software was used to run ROC analysis in order to set the cut-off for MMV and confirm for Rubella, in the latter case the cut-off is defined by the International reference RUBI-1-94. This data was subjected to 2x2 analysis with a Wilson confidence interval set to 0.95%. The concordance is summarised in Figure 3. Samples were analysed in two ways: Indeterminate samples as positive in one case and indeterminate samples as negative in a second case, to give the percent positive agreement (PPA) and percent negative agreement (PNA) respectively.

	Measles	Mumps	Rubella	VZV
Within-run n=14 (6x)	4.54% (2.8-6.7%)	5.35% (3.8-7.2%)	3.69% (2.9-5.4%)	4.08% (2.7-6.8%)
Between-run n=14 (2x)	4.51% (3.3-7.2%)	5.66% (4.3-10.3%)	4.64% (3.4-7.5%)	4.06% (1.6-5.8%)
Between-day n=14 (3x)	3.56% (1.9-5.2%)	4.01% (1.9-5.2%)	4.76% (3.1-6.2%)	3.15% (1.9-3.9%)
Between Multiplier n=14 (3x)	1.41% (0.3-3.9%)	3.19% (1.1-6.1%)	2.24% (0.8-3.7%)	1.37% (0.3-2.1%)

Figure 2. MMRV assay precision, mean and range of 14 samples

		PPA (95% CI)	PNA (95% CI)
Measles n=929	Pos+Eq	95.3% (93.5-96.6%)	94.2% (90.7-97.0%)
	Neg+Eq	93.3% (91.2-95.0%)	95.4% (92.0-97.4%)
Mumps n=867	Pos+Eq	90.2% (87.7-92.2%)	93.3% (88.5-96.2%)
	Neg+Eq	93.3% (91.1-95.0%)	94.6% (90.8-96.9%)
Rubella N=929	Pos+Eq	93.9% (91.9-95.4%)	99.50% (97.4-99.9%)
	Neg+Eq	93.00% (90.9-94.7%)	100.0% (98.4%-100.0)
VZV N=899	Pos+Eq	98.1% (96.8-98.8%)	97.5% (93.0-99.2%)
	Neg+Eq	97.7% (96.3-98.5%)	99.2% (95.6-99.9%)

Figure 3. MMRV concordance vs. commercial 510k cleared ELISA's

Conclusion

Based on these data, the Multiplier instrument and multiplexed MMRV chemiluminescent assay combination offers comparable performance to existing ELISA assays. This multiplexed, fully automated assay gives reproducible semi-quantitative results for MMRV IgG. It is ideal for batch testing, as it can handle up to 92 test samples per plate, producing 368 results in 2 hrs 30 mins.

Disclaimer: This assay is currently for INVESTIGATIONAL USE and is NOT AVAILABLE FOR COMMERCIAL SALE