As Published in CLI December 2003

A novel method for automating zone measurement of SRD assays

By Simon Johns

Single radial immunodiffusion (SRD) is a simple yet powerful technique that is routinely used in many clinical laboratories for a wide variety of analyses. Despite its simplicity, the technique as currently practiced suffers from a major drawback due to the fact that in general, the measurement of the reaction zones generated by the assay is performed manually. This is a task that is time-consuming and error-prone. To overcome these problems, a novel method of automating inhibition zone measurement has been developed. The new system has been extensively tested and compared with the standard, manual method. We report here the performance of the new method in the assay of the potency of influenza vaccines.

The principle behind single radial immunodiffusion (SRD) relies on the incorporation of an antibody specific for the analytes being measured into an agar gel as the gel is being poured. Once the agar is set, wells are cut into the agar and different concentrations of the antigen to be measured are added to the wells. As the antigen diffuses into the gel it reacts to form a ring of precipitate around the well (Figure1). This precipitate is formed at a specific, optimal, ratio of antibody to antigen. Since the concentration of the antibody in the agar is constant, the only variable that determines where the optimal ratio of antibody to antigen occurs is the original concentration of the antigen in the well. The diameter of the precipitate ring is proportional to the logarithm of the concentration of antigen. By running several standards, a calibration curve can be constructed, from which the concentration of an unknown sample can be calculated. SRD assays are used to quantify all classes of immunoglobulin, as well as various complement components. and can be used to test the potency of viral vaccines such as the influenza vaccine.

Many laboratories use either a ruler or calibrated viewer to estimate manually the diameter of the SRD reaction zones. Using these methods it can take anything up to 1.5 hours to read an SRD plate consisting of 16 reaction zones. In addition, since the results have to be keyed into a computer for statistical analysis, both methods are time-consuming and labour-intensive.

Automating an SRD assay - A case study

To improve productivity in assessing the serological response of 'flu vaccines, it was decided to switch from using a manual calibrated reader to a specially-designed system, consisting of the ProtoCOL automated zone reader and colony counter (Synbiosis, Cambridge, UK) integrated with a CCD camera on a macrostand over a light box (Figure 2). The system can read an entire SRD plate, measure reaction zones and transcribe the results into an Excel file for reporting in less than 10 minutes, which includes any manual confirmatory checks made for zones automatically highlighted as being non-circular. The system can thus save many hours of highly repetitive work. However, since changing part of a quality control process can have far reaching implications when testing 'flu vaccines, it was necessary to ensure that the automated method introduced was completely validated and correlated with the existing manual method.

A study was therefore undertaken to compare the two methods before introducing automation as part of standard operating procedures.

The SRD assay was carried out according to standard techniques. After incubation the gel was dried in an incubator at 35°C, and stained with Coomassie blue (3% w/v) to visualise the reaction zones.

Measuring reaction zones using a manual method

The SRD plate was placed on a light box attached to a calibrated viewer with an assay data recorder. This viewer was used to manually estimate the size of the reaction zones in

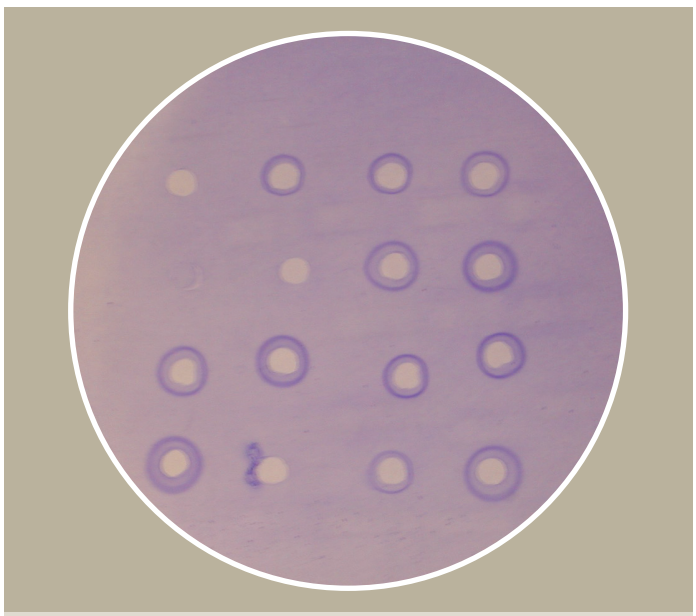

Figure 1. A standard -16 well SRD plate stained with Coomassie blue showing reaction zones around each well, which represent vaccine potency.

two directions at 90° to each other. The print-out from the calibrated viewer was then re-keyed into an in-house programme, which analysed the statistical validity of the test and calculated the HA concentration of the vaccine by comparison with the standard curve of the control antigen.

Measuring reaction zones using an automated method

The SRD plate was placed on the ProtoCOL system's integrated light box where the system's CCD camera captured the plate image and displayed it on screen. A template of eight circles built into the system software was placed over the on-screen image of the SRD plate. The circle templates were then adjusted by mouse clicks to fit around the zone images. Using a single click the software automatically measured the diameter of each zone and transferred the data into an Excel spreadsheet. It also flagged areas of dispute such as fuzzy edges, so this could be measured manually with the on-screen ruler if necessary. The data produced was transferred directly into a statistical analysis programme without the need to re-enter it.

Comparison of reaction zone measurement methods

An SRD assay of three vaccines and one standard was analysed by measuring the reaction zones for each vaccine. One technician measured the assay six times at weekly intervals using both the manual calibrated viewer and the automated method. It was also read six times by a second technician using only the automated system.

Figure 2. Synbiosis ProtoCOL system for automating SRD assays

Vaccine	Technician 1 using manual calibrated viewer method(% CV)	Technician 1 using automated ProtoCOL method(% CV)	Technician 2 using automated ProtoCOL method(% CV)
513/9	1.1	0.7	0.5
757479A	2.4	1.1	0.3
757479B	1.9	0.9	0.5

Table 1. CV analysis of vaccine potency measurements

Vaccine	Technician 1 using manual calibrated viewer method(% CV)	Technician 1 using automated ProtoCOL method(% CV)	Technician 2 using automated ProtoCOL method(% CV)
513/9	1.2	1.2	0.7
757479A	1.3	1.2	0.6
757479B	0.9	0.9	0.5

Table 2. CV analysis of assay precision calculations

Results

The zone sizes obtained from both methods were input into a standard statistical slope-ratio model that produced estimated potencies for the three batches of vaccines. The measured potency data were compared with the value provided for that batch by the manufacturer of the vaccine and enabled an estimate of the precision of the assay to be established. The data were analysed statistically and coefficients of variance (CV) were calculated. Results are shown in Tables 1 and 2.

Discussion

The automated method produced very reproducible results as can be seen from the low CVs. The value of approximately 1% for a coefficient of variation is well within the acceptable limits set by many accreditation services. There was very little difference in the potency results generated by the different technicians (maximum difference of 0.16µg/0.5ml). This was much smaller than the maximum difference seen between the results from the same technician using the manual system and the new automated method(0.53µg/0.5ml). Typically, the confidence interval set by many accreditation services on an estimated potency is ± 1.5µg/0.5ml, so clearly the difference between technicians using ProtoCOL for these assays is acceptable.

Conclusions

The data from this case study demonstrate that automation produces more reproducible readings than a manual calibrated viewer method. In addition, the system offers the benefit of being much quicker, being able to read 16 wells from an SRD plate and provide an electronic report in 10 minutes, compared to around 1.5 hours using a manual method. The ProtoCOL system provides an excellent method of automating reaction zone reading, which should prove invaluable in many laboratories.

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