



## Rapid Quality Control of Viral Vaccines *A Practical Application of the ProtoCOL system*

### Introduction

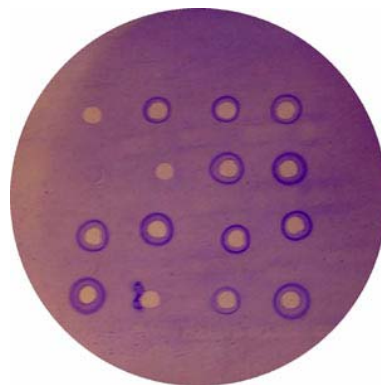
Internationally, a large number of adults and children are routinely vaccinated to protect them against a range of diseases. Many of these vaccines consist of an inactivated strain of a virus that causes the disease. If the concentration of the viral vaccine is incorrect then the protection may be insufficient, which could prove fatal among some of the more vulnerable groups inoculated. In the case of influenza vaccines, the tests for vaccine concentration must also be performed quickly because there is little time available to produce and test the vaccine before the next influenza season begins.

To verify the quality of influenza virus vaccines, most pharmaceutical companies and independent national standards agencies use the Single Radial Immunodiffusion (SRD) assay. The influenza vaccine assay determines the antigenic response a vaccine provokes by measuring the haemagglutinin (HA) concentration of antigens by reaction with specific sheep antiserum in an agarose gel. The size of the circular reaction zone produced is directly proportional to the HA concentration, therefore measurement of reaction zones provides an indirect measure of the potency of the vaccine.

### Measuring Reaction Zones - the Manual Method

Generally to measure SRD reaction zones an SRD plate (Figure 1) is placed on a light box and the circular zones are sized in two directions at 90° to each other using either a ruler or a calibrated viewer. The results are then keyed manually into a computer programme for statistical analysis to determine the validity of the test and to calculate the HA concentration of the vaccine by comparison with the standard curve of a control antigen.

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



/More....

Using this manual method, it is estimated that reading a standard SRD assay containing plates of 36 reaction zones takes approximately 1.5 hours. In addition, keying in and checking the results can take an additional 20-30 minutes. This is not only extremely time consuming but is also a very repetitive and labour intensive task for a scientist to perform.

### Measuring Reaction Zones - the Automated Method

Synbiosis offer the solution to this manual measurement problem by automating the process using a specially designed system. This consists of a Synbiosis ProtoCOL automated zone reader and colony counter integrated with a CCD camera on a macrostand over a light box (See Figure 2). This system can read an entire SRD plate, measure reaction zones and transcribe the results into an Excel format in minutes and therefore offers the opportunity to save hours of highly monotonous work.

Figure 2: Synbiosis ProtoCOL system used for automating SRD assays.



To read an SRD plate rapidly, it is placed on the ProtoCOL system's integrated light box. The ProtoCOL's CCD camera captures the plate image and shows it on the PC's screen. The ProtoCOL software has a template of eight circles that can be placed over the on-screen image of the SRD plate (Figure 3). The circle templates are placed around each reaction zone and are adjusted by simple mouse clicks to fit around the zone image. Using a single click the software automatically measures the diameter of each zone and transfers the data into an Excel spreadsheet.

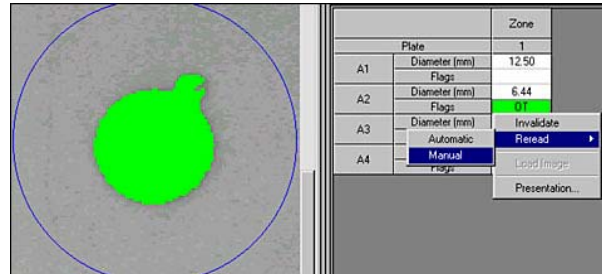
Figure 3: On screen image of an SRD plate showing how the template in the ProtoCOL system's software can be used to measure reaction zones.

A1	Diameter (mm)	10.87	8.92
	Flags		T
A2	Diameter (mm)	10.87	8.92
	Flags		T
A3	Diameter (mm)	10.87	8.92
	Flags		T
A4	Diameter (mm)	8.92	9.39
	Flags		T
A5	Diameter (mm)	8.92	9.39
	Flags		T
A6	Diameter (mm)	8.92	9.39
	Flags		T
A7	Diameter (mm)	8.92	9.39
	Flags		T
A8	Diameter (mm)	8.92	9.39
	Flags		T
A9	Diameter (mm)	8.92	9.39
	Flags		T
A10	Diameter (mm)	8.92	9.39
	Flags		T
A11	Diameter (mm)	8.92	9.39
	Flags		T

/More...

The ProtoCOL's software can also flag any areas of dispute such as fuzzy edges, so that operators can measure this manually with the on-screen ruler, if necessary. The data produced can be transferred directly into virtually any statistical analysis programme without the need to re-key it.

*Figure 4: On screen image of an SRD plate showing how the software alerts users to reaction zones which require manual adjustment.*



### Validating the ProtoCOL System for Zone Measurement

An independent study was performed to compare the ProtoCOL system with a manual calibrated viewer method for measuring reaction zones on SRD assays. The zone sizes obtained from both methods were input into a standard statistical slope-ratio model to produce the estimated potencies for three flu vaccines. The potency figures from each method were then compared.

The results of this study show Synbiosis's ProtoCOL system produces very consistent coefficients of variation (CVs) of approximately one percent for repeated estimates of both potency and precision and the agreement between operators is also excellent. For all parameters tested, the difference between the two methods is well within normally acceptable limits.

### Conclusions

Synbiosis's ProtoCOL offers the benefits of producing rapid results, since it can read SRD plates in normal assays in a few minutes compared to around 1.5 hours using a manual method. Also the system presents these results in a flexible Excel format so they can be exported directly into virtually any statistical programme without the need to re-key them. This not only saves time but also prevents potential keying errors and therefore enhances accuracy. The considerable time saved, will ensure that the ever-increasing number of vaccine doses are tested and released without delay.

In addition, the independent data from the comparative study confirms that the ProtoCOL system produces highly consistent SRD plate readings that are more reproducible than those generated by a manual method. All of these factors make the Synbiosis ProtoCOL an invaluable tool for any pharmaceutical company or quality control organisation that wants to speed up reaction zone reading and the commercial release of influenza vaccines.