



# Handy Helper

Application Note #09

## Using ProtoCOL for rapid assessment of the OPKA assay

### Introduction

*Streptococcus pneumoniae* is a major cause of pneumonia in young children and the elderly. New vaccines against this bacterium are required because conventional antibiotics are becoming less effective due to the increasing numbers of multi drug-resistant *S. pneumoniae*. To evaluate novel pneumococcal vaccines, an enzyme-linked immunosorbent assay (ELISA) is commonly used alongside a modified *in vitro* opsonophagocytic-killing assay (OPKA). The ELISA method allows antibody quantitation but cannot distinguish between functional and non-functional antibodies. The OPKA is useful as an additional test for measuring antibody function and is a good surrogate assay for immune protection. Patient blood samples are taken before and after vaccination, serially diluted and tested by OPKA and plated out onto Todd-Hewitt agar plates with yeast extract. An agar overlay containing antibiotics and 2, 3, 5-triphenyl tetrazolium chloride dye is added and the resulting red bacterial colonies are counted the next day to determine the dilution of patient serum that kills 50% of bacteria compared to the control (no serum). This method evaluates the killing function of the antibody induced following vaccination. Multiplexing is possible by use of antibiotic resistant strains of *S. pneumoniae*.

### Evaluating results of the OPKA assay

In a typical clinical trial of a new pneumococcal vaccine, anything up to 11,000 colonies can be generated from every patient at each bleed and therefore samples can take days to process manually. Since colony enumeration provides the data on which vaccine efficacy is based, it is essential to obtain the most precise colony counts possible. But manual methods of enumerating the OPKA assay require microbiologists to use a light box and pen and then key the results manually into a computer. This is not only time consuming but can lead to reading and transcription errors, especially when counting such large numbers of *S. pneumoniae* colonies, many of which can be less than 1 mm in diameter. Additionally, because this method does not produce any digital images of the plate alongside the colony count an independent audit cannot be carried out by regulatory authorities, which is compulsory for the approval of new vaccines.

### The Automated Alternative

To overcome the difficulties associated with manually counting colonies on large OPKA assay plates, Synbiosis provides a ProtoCOL, automated colony counter, (Figure 1) with a stand and platform containing a high resolution camera integrated to a computer. The system can evaluate the colonies on large OPKA plates because its software automatically compensates for different agar thickness and artefacts such as bubbles or debris that can occur during overlaying and can even enumerate touching colonies and colonies of different sizes.

The ProtoCOL produces results from OPKA assays which are compliant with GCP (Good Clinical Practice) guidelines so can be presented to regulatory authorities such as the European Medicines Control Agency (EMA) and Food and Drug Administration (FDA). For example, the colony count results are automatically transferred into a table for storage in a secure database. The database is password protected and so ensures that batches of results cannot be deleted. Additionally, count editing is recorded with a coded flag next to the revised result. Every detail of the sample including pictures of the OPKA plates, system configuration, member of staff that read the plate, date and time are recorded in a professional report.

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Figure 1: ProtoCOL colony counting system for OPKA assays



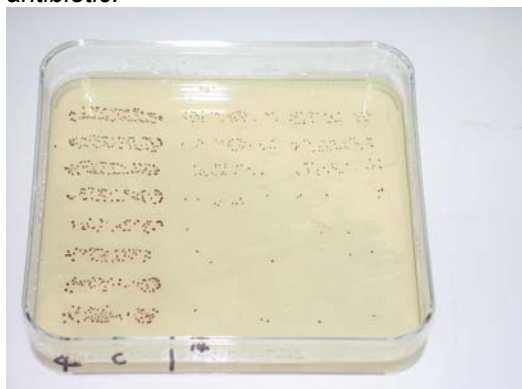
#### Application of ProtoCOL in Clinical Trials of Pneumococcal Vaccines

The ProtoCOL is being successfully used for assessing the results of OPKA assays at a number of prestigious UK and US research institutes. These include the Institute of Child Health (ICH) in the UK and the University of Alabama at Birmingham, USA (the authors of the modified OPKA assay).

At the Immunobiology Unit of the ICH, scientists are running clinical trials in which children are vaccinated with new types of pneumococcal vaccines. They have been using the ProtoCOL daily since 2006 to assess 20 plates from the OPKA assay of approximately 45,000 *S. pneumoniae* colonies. They have found that the system can distinguish between close colonies and even visualise very small colonies with a diameter of less than 0.2mm (Figure 2). This is enabling scientists at the ICH to accurately generate results the same day the colonies appear, whereas processing 45,000 colonies manually would take days to perform.

Note: The children will not contain any *S. pneumoniae* as these are not contained in the vaccine. The bacteria are only added to the serum in the assay. See first paragraph for what results mean.

Figure 2: *S. pneumoniae* on a Todd- Hewitt agar–yeast extract plate with an overlay containing TTC and antibiotic.



#### Conclusions

With the increase in antibiotic resistance of *S. pneumoniae*, the production of new pneumococcal vaccines is critical and is an area where automated colony counting could help generate clinical data more rapidly. However, accurately enumerating colonies from an OPKA assay is a task very few automated systems can perform successfully, yet the use of the ProtoCOL colony counter in clinical trials at the ICH for example, shows the system offers such high resolution imaging that it can generate precise, GCP compliant results. Therefore, the system is highly applicable for any institute or pharmaceutical company, which needs automated colony counts from the OPKA assay, making using the ProtoCOL an excellent method for rapidly evaluating new pneumococcal vaccines.