Automating Color Colony Counting

by Simon Johns

To check the safety of foods, microbiologists routinely liquefy and plate samples onto agar plates. They then examine the food for potentially pathogenic bacteria such as *Salmonella*, *E. coli* 0157, *Bacillus cereus*, and *Listeria monocytogenes*, as well as anaerobes such as *Campylobacter*. On the basis of the level of potentially harmful bacteria in the sample, decisions are made as to whether to market the product.

In the case of testing therapeutics, counting bacterial colonies can provide information on different aspects of a drug. For example, with a vaccine for a bacterial infection such as meningitis, counting the number of colonies that survive after treatment with a new vaccine can indicate the effectiveness of the therapy. Additionally, enumerating the number of microbes isolated from finished pharmaceutical products or the water used to produce them can help production managers assess if the sterility levels of the product are acceptable for consumers.

To perform efficient colony counts, microbiologists often work with colored bacterial colonies because they are often easier to visualize. For example, some colonies can be very similar in color to the agar on which they are plated; therefore, coloring makes them not only visible but also easier to count. Another advantage to working with colored bacterial colonies is that specific bacteria in a culture of mixed bacterial colonies stand out from the others, simplifying identification and enumeration.

Colonies can be colored by using chromogenic media or by adding staining agents following the incubation of sample plates. However, manually counting a colored colony requires that the color vision of the microbiologist performing the count be perfect, which is not always the case. Also, the color vision of each microbiologist needs to be uniform, which is impossible because the human eye and brain see and interpret colors differently. When analyzing colonies in a mixed population, for example, manual counting can be very time consuming and tedious, making it prone to error, especially when different microbiologists are assessing the same plate types.

To overcome these issues, automated colony counters have been developed that can capture, display, and simultaneously count different color colonies from one plate image. The ProtoCOL SR (*Figure 1*) and ProtoCOL HR (Synbiosis, Cambridge, U.K.) consist of a PC linked to a fully enclosed cabinet with a built-in, high-resolution camera and LED lighting above and below the plate holder. The camera, which is integrated to powerful image analysis software, can detect colonies 0.2 mm in diam (ProtoCOL SR) and colonies as small as 0.1 mm diam (ProtoCOL HR). The systems can simultaneously capture, differentiate, and display an unlimited number of different color colonies on one plate image.

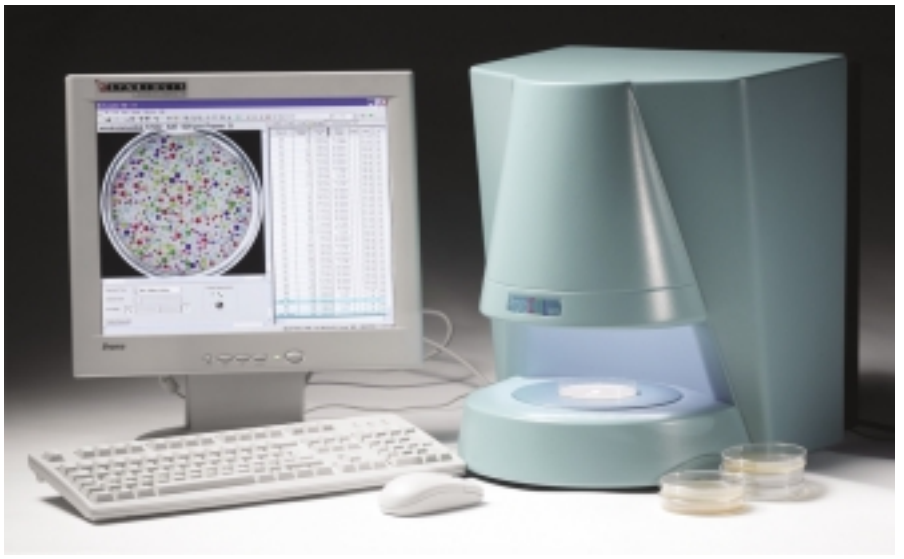

Figure 1 ProtoCOL SR automated colony counter.

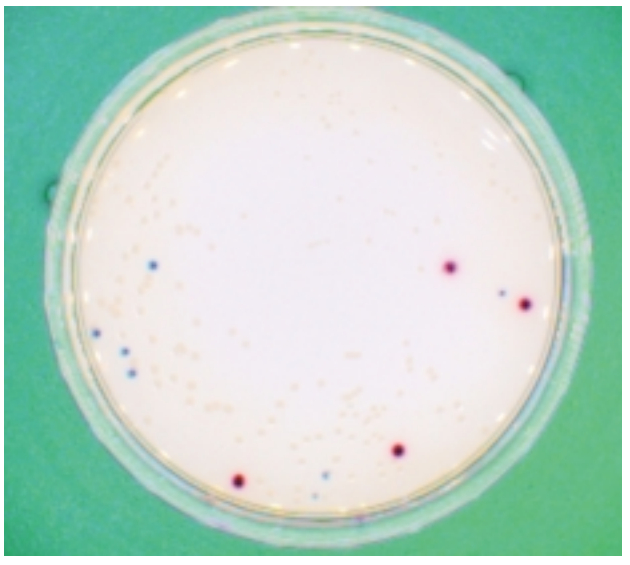

Figure 2 COLI ID plates showing E. coli *(red) and coliforms (blue).*

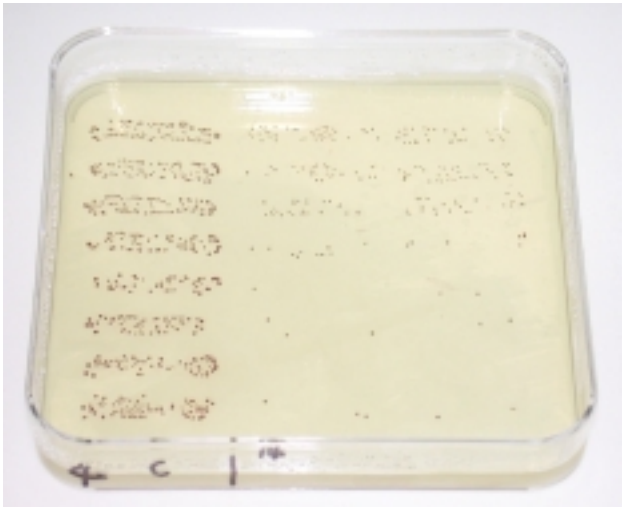

Figure 3 Pneumococci on Todd-Hewitt agar plates with yeast extract and an agar overlay containing antibiotics and TCC.

Applications

Identifying potentially harmful bacteria in food

The ProtoCOL SR automated colony counter is being used at a major food testing center in the U.K. to automatically count a wide range of different-colored bacteria and yeast cells on spiral, pour, and spread plates. One of its main uses is to differentiate and count the numbers of potentially harmful red *E. coli* colonies from a background of blue coliform colonies on selective chromogenic COLI ID plates (bioMérieux, Marcy L'Etoile, France) (*Figure 2*). By eye, it can be difficult to differentiate between the blue of the coliforms and the red of the *E. coli*. Since these bacteria are isolated from meat and shellfish destined for consumers, the ability to use the colony counter to quickly and precisely determine the numbers of potentially pathogenic bacteria means that contaminated food can be prevented from going to market. This ultimately protects the public from the potential of food poisoning, which in some cases can be fatal.

Testing the effectiveness of vaccines

Scientists in the Department of Pathology at the University of Alabama at Birmingham (UAB) (Tuscaloosa, AL) use the ProtoCOL system to save time in the evaluation of new pneumococcal vaccines. The system is being used as part of an improved opsonophagocytic killing assay (OPKA) to automatically count surviving antibiotic-resistant pneumococci on Todd-Hewitt agar plates with yeast extract and an agar overlay containing antibiotics and 2,3,5-

triphenyl tetrazolium chloride (TCC). The pneumococci plated are those that survived the opsonizing effect of antibodies induced with different pneumococcal vaccines.

Manual colony counting for the OPKA is tedious and time consuming. However, the low contrast between the medium and colonies means that it is difficult to distinguish between the two. The TCC colors pneumococcal colonies red (*Figure 3*), and the colony counter with its enhanced color contrast is able to detect and easily count these red colonies

Using a ProtoCOL, researchers at UAB have found that they can instantly count thousands of colonies with ease. This allows them to routinely plate bacteria from 24 reaction wells onto a single square Petri dish, reducing the number of plates to a manageable amount and ensuring that colony counting is no longer the rate-limiting step in their OPKA work.

Since the colony counter produces live, color, on-screen images that can be saved with a time-and-date stamp for GLP compliance, images can be referred to later or printed out for reports or presentations. This feature is important to researchers testing therapeutics because it provides secure records that are compliant with the information required by external regulatory auditors such as the Medicines Control Agency (MCA) and the Food and Drug Administration (FDA).

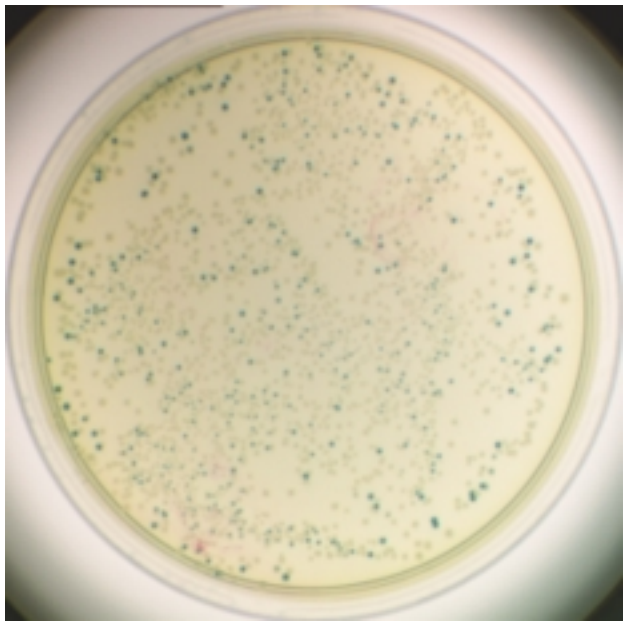

Figure 4 Blue (nonrecombinant) and white (recombinant) E. coli *colonies derived from* LacZ*-based selection vectors on tryptone soya agar.*

Discussion

In addition to differentiating red colonies from a cream background and distinguishing and simultaneously counting blue and red colonies in the examples above, the ProtoCOL SR and HR systems can be used for a number of other applications, for instance, to speed up genetic studies by counting recombinant clones. This is achieved by simultaneously differentiating and automatically counting blue (nonrecombinant) and white (recombinant) *E. coli* colonies derived from *LacZ*-based selection vectors plated on the same surface (*Figure 4*). Since the system can count up to 1000 colonies in less than 2 sec while automatically correcting for background variations, it enables researchers to quickly make important decisions as to whether to discard plates or continue with further studies of their clones.

Conclusion

Coloring bacterial colonies facilitates automated colony counting by increasing the contrast between bacterial colonies and agar plates, allowing the ProtoCOL HR and SR systems to detect bacterial colonies more easily. This is demonstrated by the above examples, which show the range of colors and bacteria that can be visualized and counted. Due to their GLP compliance, the systems can reliably improve the accuracy and productivity of food testing and a range of therapeutic applications.

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