Handy Helper

Application Note #01

Coping with plate debris in colony counting

The analysis of agar plates for colonies in microbiology is frequently made challenging by the presence of debris and other unwanted material, either embedded within or on the surface of the agar. For manual counting, depending upon the level of experience of the individual involved, this can cause problems in terms of counting errors and loss of valuable laboratory time. In a busy laboratory environment, such activities can waste many hours of otherwise profitable working time during each day.

One practical recommendation is the use of 'filter' stomacher bags when preparing samples for inoculation. These bags strain the unwanted debris out of the sample suspension, without reducing the final count reported. The success of such is restricted by sample type, being ideally suited to certain food sample types. Also, some laboratories feel that for samples giving a total count above a set baseline, the debris particles themselves should also be counted as colony forming units. This subject is open to debate, but the norm for the majority of laboratories is to exclude such from the total plate count.

Since automated colony counting systems can offer huge advantages in terms of timesaving and accuracy of counting, the last thing that a busy laboratory would want to do is to carry out a manual count on every plate tested.

This problem of unwanted debris on plates has therefore been addressed using varying automated approaches in the last few decades. With the more traditional types of automated colony counting systems where there is no exclusion of debris, this can cause errors since such features are counted in addition to the colonies themselves. In cases such as this, where the user needs to rely on cumbersome manual counting software mechanisms, such systems are unwieldy and often take more than using a standard lightbox.

Since its foundation in 1985, Synbiosis, the microbiology division of the Synoptics group, has developed dedicated solutions for the automation of colony counting. Using advanced systems that employ the latest technology available, the user can count virtually any type of agar plate in a fraction of the time it would take to do so with a traditional manual lightbox. Synbiosis has worked very closely with its customers, looking at each counting application in turn, each with its own challenges in terms of automation.

aCOLyte has been designed to fit seamlessly into the busiest of working environments, replacing the traditional lightbox. At its introductory level, Click'n'Count, the operator uses a mouse or wireless pen to manually count to the PC screen, with the counting speed as least as fast as using a traditional lightbox. Over a period of time this speed is actually faster than with a traditional lightbox, due to increased operator comfort and also features such as digital zooming. This allows the operator to look at areas of interest in more detail, allowing very accurate manual counts to be easily made. Even though this is still a manual method, many customers have already reported a dramatic improvement in the ability to choose whether a particular feature is a colony or unwanted debris, due to the live full image display of each plate

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Click'n'Count has the additional benefit of automatically storing all plate results with instant count per ml calculations reported based on the area of the plate read, dilution factor and sample volume. Such data processing would previously have involved manual calculations and entry of final results either to a paper based record system or into a separate PC. With the optional ability to save every plate image with a result, Click'n'Count saves around 50% of laboratory time required for colony counting and also allows operators to instantly produce professional reports.

The more advanced version of aCOLyte, SuperCount, allows the operator to generate an automatic count at the press of a single button. Typical counting speeds of 1 second per plate are achievable for plates with counts in excess of 500 colonies. Through the simple selection of the lighting source and the optional use of a base light diffuser plate, low contrast debris can be screened out without any manual intervention. The slider control Sensitivity feature also allows the operator to detect only colonies based just on contrast. Where debris is of a similar contrast to colonies on the plate, it is recommended that the user perform two counts on a sample, the first when the plate has just been inoculated (prior to incubation) and the second following incubation. A comparison of the two results will show the number of colonies that have grown during incubation.

For laboratories having more severe challenges with plate debris, the ProtoCOL range of products has been developed. These fully integrated systems (ProtoCOL SR and HR) are packed with a wealth of powerful user-selectable software features that can offer the best automatic solution for such applications.

The first method involves a more advanced method for debris counting pre- and post-incubation. In both ProtoCOL SR and HR, this feature automatically subtracts the debris count and displays the final result as a total count per ml.

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An alternative approach is an interactive one, where the mouse is used to draw round larger pieces of debris on the plate image (after incubation). These regions are then automatically eliminated from the count. The area beneath this exclusion region is automatically subtracted from the total plate area when calculating the final count result.

Since debris features are often of different sizes to colonies, the Area Limits feature in ProtoCOL can be used. Either by selecting a suitably sized colony on the displayed image, or entering a known value into the associated field, features beneath (or above) the selected size are automatically screened out. It is possible to apply limits for both the lower and upper size extremes for each count.

A more advanced feature has been introduced recently. This involves the screening out of unwanted features based on a lack of circularity. Since many bacterial colonies approach circularity in gross morphology, a circularity factor can be selected to screen out less circular features. This has been widely used in food microbiology applications, where fibrous debris such as meat fragments often cause counting difficulties.

The final method Synbiosis has employed for screening out unwanted debris has be the introduction of the new simultaneous colour differentiation option for both the ProtoCOL SR and HR systems. This allows the operator to train the system to recognise colonies based on feature colour. Since this is a true colour recognition system, many customers have used this to distinguish between colonies and debris where there is often only a subtle difference in colour. When used in conjunction with chromogenic agars, this feature makes ProtoCOL the most advanced system for automatically coping with debris on agar plates.

For further information on the systems described, please visit our website:
www.synbiosis.com