An investigation into analyst interpretation of results

Rachael Smith
LGC Standards, Proficiency Testing, Bury, United Kingdom

Introduction

The ability to identify and enumerate colonies on an agar plate is an essential skill for microbiologists. However, most proficiency testing schemes involve testing a sample from start to finish, including resuscitation, dilution, plating, culturing and counting steps. When the microbiologist obtains an incorrect result, it is not always easy to know where the error happened. The QMIS 103 paper exercise removes the majority of these testing variables, allowing analysts to be compared based on their interpretation, counting and calculation skills alone. Participants were provided with a photograph of colonies on a plate and informed of the volume and dilution plated, and were asked to count and calculate the level of microorganism in the original sample. Some examples are illustrated below:

Figure 1 shows yeast and mould growth on a 10^{-2} dilution plate from 100µl aliquot of a 10g food sample on Rose Bengal Chloramphenicol agar. The assigned value was 36,497 cfu/g with a range of 35,600 cfu/g to 38,000 cfu/g and a standard deviation of 0.01 log10. Some of the mould colonies were growing at the edges of the plate and were therefore missed by some participants. This shows that counting technique alone can have a significant effect on the final result depending upon what the analyst chooses to enumerate. For example, very small colonies, colonies obscured by other colonies, or colonies growing close to the edges of the petri-dish may affect the final count. Enumeration when mould is present is always challenging due to the spreading colonies.

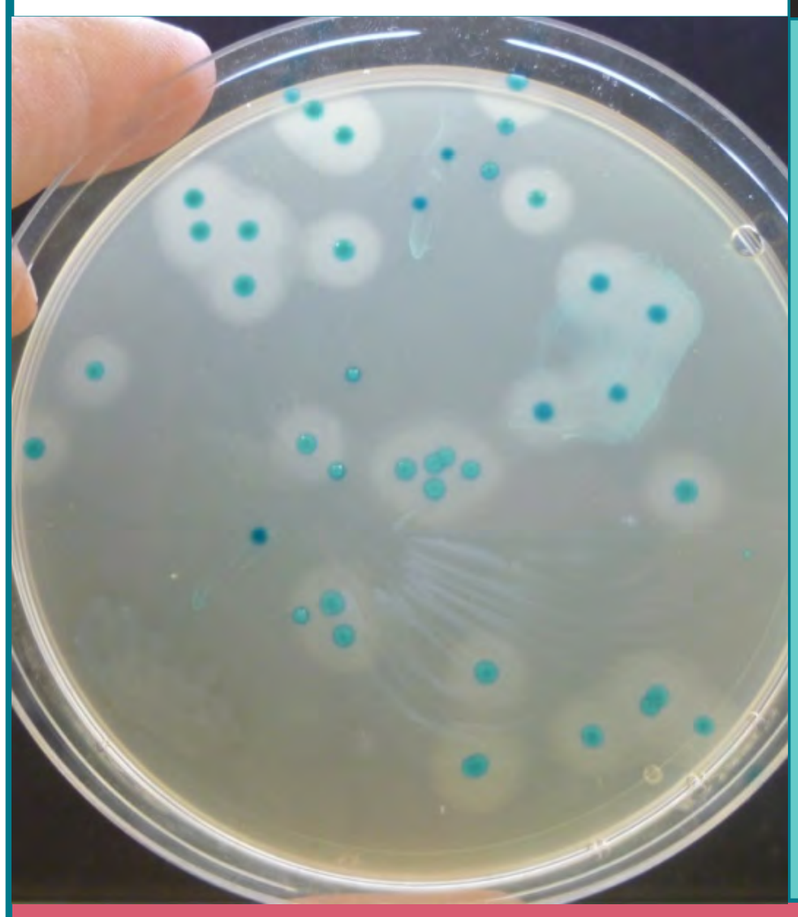

Figure 2 shows the microbiological growth obtained on the 10^{-2} dilution plate from 100µl aliquot of a 10g food sample on chromogenic Listeria agar. Participants were asked to enumerate both Listeria species and Listeria monocytogenes. On Listeria chromogenic agar, typical Listeria monocytogenes colonies appear as entire turquoise or blue/green colonies with a visible halo or zone of precipitation. Other Listeria species grow without this zone surrounding the colonies. Difficulties can arise when colonies without zones grow close to those with zones, which can make differentiating between presumptive Listeria monocytogenes and Listeria species problematic. A small number of participants, when enumerating the Listeria species colonies visible, did not include those with halos (presumptive Listeria monocytogenes colonies) and therefore reported results that were too low. There were also a number of participants returning results that were 10 times lower or higher than the assigned value. These participants were advised to investigate the possibility of calculation error, particularly noting if the 100µl volume has also been taken into consideration.

Figure 3 shows 2 plates of Haemophilus influenza colonies at a dilution of 10^{-2} and 10^{-3}, using 100µl aliquots (originally given in duplicates) on chocolate agar. In the original exercise, participants were asked to return a total of five results, an enumeration of the number of colonies visible on each of the four images, and a final calculation of the total bacterial load in the original sample.

As in the case of the first exercise enumerating yeast and mould colonies, although participants were given the same images to analyse, the counts reported for each of the four images differed quite significantly, for example: the range of results for Figure 3 was from 262 and 295 cfu. In addition to this, participants may not have taken into account all of the dilutions performed and the volume of inoculum used when calculating their final result, as one participant returned results that were consistently ten times too low.

This exercise was also a good demonstration of the use of weighted mean calculations to obtain a more accurate result, as a weighted mean is a more accurate measurement. We would expect participants to use a weighted mean where possible as this minimises the effects of variation between duplicate plates and different dilutions. For example, had the final concentration been calculated solely from Figure 3 then the result based on the median would have been 280,956 cfu/g. This when compared to Figure 4 with a median count of 360,000 cfu/g represents a 22% difference.

Summary

Each of these exercises is a clear demonstration that providing a number of analysts with precisely the same information and removing a wide range of possible sources of error, it does not necessarily follow that these analysts will interpret the information in the same way. Analysts can over or underestimate the number of colonies while counting, can misinterpret morphological signs used for presumptive identification and can make errors during calculation. This can therefore highlight any requirements for further training required in enumeration and calculation of results.