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A simple and rapid test for quality control of liquid media, using the bioscreen microbiological growth analyser

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Abstract

Most commercially produced dehydrated media are rigorously tested by the suppliers, but it is vital that performance is also checked by the consumer following reconstitution and sterilisation. Poor quality control (QC) of laboratory media can adversely affect the results of an entire microbiology laboratory, yet with simple tests the performance of culture media can be easily monitored. The performance of liquid media is generally assessed by comparing test and control broths for approximately equal bacterial growth and turbidity is usually measured by eye. This method is time consuming, involving many serial dilutions, as well as being very subjective. An ideal QC test would be rapid and automated, removing as much human error as possible. This study details trials performed using the Bioscreen to compare growth of organisms in test and control broths. This proved to be a cheap, rapid and reliable means of assessing the quality of liquid media. © 1998 Elsevier Science B.V.

Keywords: Bioscreen; Liquid media; Rapid methods; Quality control

1. Introduction

It is imperative that all media produced in a microbiology department are performing to an acceptable standard, allowing optimum growth of specific organisms. Most commercially produced dehydrated culture media are rigorously tested by suppliers for quality and reproducibility [1]. The performance of the culture media should, however, be tested again in the microbiology laboratory, once each batch has been rehydrated and sterilised. This should detect any deterioration of the dehydrated media during storage, as well as weighing errors, fluctuations in distilled water quality, contaminated glassware, incorrect pH adjustment and problems associated with overheating or underheating in the autoclave [2].

Poor quality control (QC) of prepared media can adversely affect the performance and results of an entire microbiology department [3] yet with relatively simple QC tests performance of culture media can be easily monitored. The Microbiology Department at Unilever Research Laboratory (Colworth House, Sharnbrook, Bedfordshire, UK). has adopted Mossel's ecometric streaking technique for solid media [4–6], but incorporating a spiral plater for speed and accuracy [7,8]. The ecometric technique is based on streaking an inoculum to extinction by a standardised procedure. Results can be compared with previous, quality-tested batches of the same agar. This proved to be a relatively simple, quick,

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cheap and reliable method. The performance of liquid media is generally assessed by comparing test and control broths for approximately equal bacterial growth and the turbidity is usually measured by eye [2,9,10]. Initial trials proved that this popular method was very time consuming, involving serial dilutions to 10^{-12} as well as being very subjective when visually estimating the turbidity of tubes. In a busy media preparation laboratory an ideal test would be rapid and automated, removing as much human error as possible. This study details initial trials performed using the Bioscreen (an automated microbiological growth analyser from Labsystems Oy, Helsinki, Finland) for assessing the quality of liquid media. The Bioscreen has been used for many applications in the field of microbiology, including general research [11,12], food microbiology research [13,14], dairy applications [15], food QC for measuring total counts [16], veterinary microbiology [17] and assessing the effects of chemicals on microorganisms [18]. The Bioscreen has not been used for the QC purposes described in this article.

In this QC study the Bioscreen was used to compare cell numbers of microorganisms after growth in test and control broths. The software converts kinetically measured optical density (OD) data into growth curves and, by constructing appropriate calibration graphs, the data can be interpreted as bacterial counts. The Bioscreen sample plates are sterile, disposable and can take up to 200 samples per test, enabling replication to be easily performed. This method eliminates the need for time consuming serial dilutions, whilst also reducing the amount of test materials required. OD measurement proved to be a cheap, fast and reliable method for assessing the quality of liquid media.

2. Materials and methods

2.1. Preparation of bacterial/yeast suspensions

The following strains were used in all QC tests of liquid media: *Pseudomonas aeruginosa* NCTC 10662, *Lactobacillus plantarum* URLCH 9-217, *Candida albicans* NCPF 3179, *Escherichia coli* NCTC 10418, *Staphylococcus aureus* NCTC 6571, *Listeria monocytogenes* NCTC 11994. With the exception of the *Lactobacillus plantarum* they were all obtained from the Public Health Laboratory Service (Colindale Avenue, London, UK). The *Lactobacillus* used was from the culture collection at Unilever Research Laboratory.

Stock cultures of organisms were maintained on the appropriate agar slopes at 4°C. Cultures were checked for purity and subcultured on a monthly basis.

2.2. Media tested

The following dehydrated media were reconstituted and sterilised according to the manufacturers instructions: Difco heart infusion broth (HIB), Difco all purpose tryptone (APT), Oxoid malt extract broth (MEB), Oxoid MacConkey broth (MacC), Oxoid nutrient broth, Difco brain heart infusion broth (BHIB), Oxoid tryptose phosphate broth (TPB), Oxoid Fraser broth, Oxoid buffered Listeria enrichment broth (BLEB).

2.3. QC test of the liquid media

A loopful of test organism was transferred from each agar slope into 9 ml 0.1% peptone water, which was vortexed thoroughly to distribute the cells. A 100 μl volume of each cell suspension was used to inoculate 10 ml of the corresponding test and control broths (new batch of broth compared to the previously made, quality tested batch). Broths were incubated for 6 h at 30°C, to compare growth of each specific organism in test and control broths. The 6 h broth cultures (40 μl) were used to inoculate 360 μl of the appropriate growth medium in the wells of a Bioscreen plate. The Bioscreen plate was filled in a laminar flow cabinet, Envair Class II microbiological safety cabinet, to maintain sterility. The growth medium used was the corresponding, previously tested batch of broth (known to support optimum growth of the test organism). Each well of the Bioscreen plate was thoroughly mixed and the plate was run in the Bioscreen for 24 h at 30°C. The machine was programmed to read the OD of test wells every 15 min at a wavelength of 600 nm, shaking the plate with medium intensity for 20 s before each reading.

2.4. Analysis of Bioscreen data

Growth curves produced by the Bioscreen are presented as OD plotted against time. A detection time (determined from a point where there is a rapid change in OD) can be related to cell number. The detection time for cells grown in the test broth can be compared to the detection time for cells grown in the control broth. The detection times can be converted to cell numbers using calibration graphs. The pass limit of the QC test was taken to be <1 $\log_{10}$ difference between cell numbers in test and control media. This is a more stringent criteria than that of the tube dilution method.

2.5. Construction of calibration graphs to convert OD data into viable cell counts

Calibration graphs were constructed by inoculating Bioscreen plates with a tenfold dilution series of each test organism in its specific broth. Original cell numbers were determined from a Thoma slide count. The detection times for each dilution were used to plot calibration graphs designed to convert detection time into cell number. The detection time was taken to be the time to an increase of 0.1 OD units at 600 nm.

3. Results

3.1. Bioscreen calibration graphs

Growth curves were produced in duplicate for different cell concentrations of *S. aureus* in nutrient broth, using the Bioscreen. The mean of the two sets of data was calculated using Microsoft Excel version 5.0 and the resulting growth curves were plotted (Fig. 1). It can be seen that the higher the cell number, the faster the change in OD, so the faster the detection time. The detection time is determined from a point where there is a rapid increase in OD

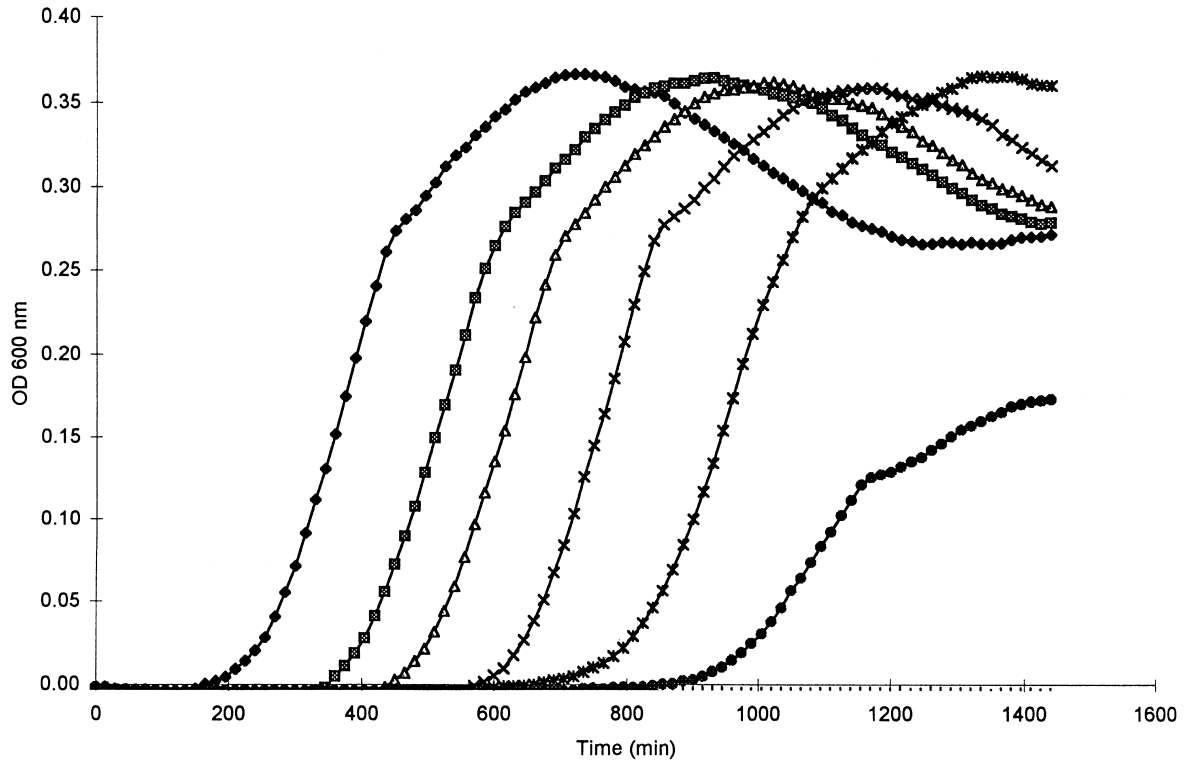

Fig. 1. Determination of detection times for various cell numbers of *S. aureus*.

and can be related to the number of cells present. It was taken to be the time to an increase of 0.1 OD units at 600 nm. Having determined the detection time for each cell number, these were used to construct a calibration graph (Fig. 2). These results show that there is very good correlation between OD data and cell number. Using *S. aureus* in nutrient broth, $r^2=0.983$. Calibration graphs were constructed for each type of broth medium which is regularly used by the Colworth Microbiology Department, using appropriate test strains. All showed good correlation between cell number and OD. Providing these same strains are used in future QC studies, these curves can always be utilised to convert the Bioscreen data into viable cell counts.

3.2. Testing the performance of liquid media using the Bioscreen

Bioscreen growth curves were produced in duplicate for *S. aureus* after growth in test and control broths. The mean of the two sets of data was calculated and the resulting graphs were plotted (Fig. 3). The test broth was the most recently made batch, which was compared to the control (a previous, quality tested batch). These results show that the shape of the test and control curves were almost identical. When the time to a change of 0.1 OD was read from the calibration graph, it was found that there was only 0.48 $\log_{10}$ difference between growth in the test and the control broths (Table 1). This means that the newly made medium was performing within the acceptable standards chosen by the Colworth Microbiology Department (<1 $\log_{10}$ difference). A pass/fail limit for the test needed to be assigned, to establish which broths should be retested and possibly rejected. The value of <1 $\log_{10}$ difference between growth in test and control broths was chosen because this allows for some experimental error, whilst still giving a good indication of whether the test broth is capable of promoting early and rapid growth similar to that of the control broth. Different

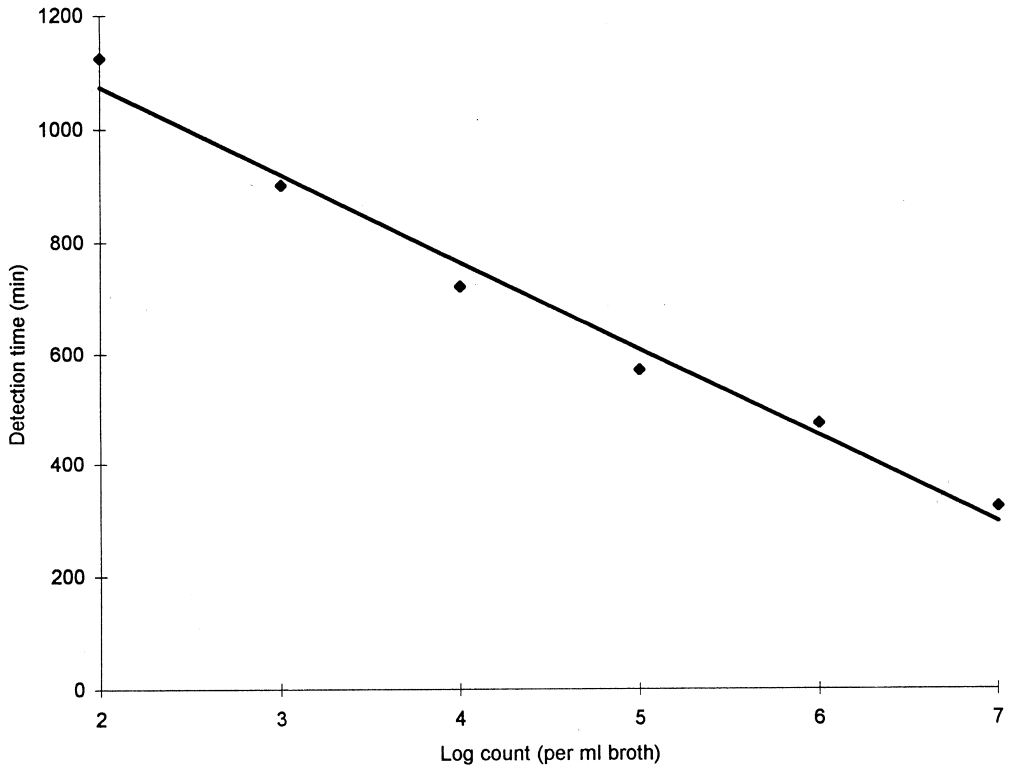

Fig. 2. Calibration curve: detection times plotted against initial cell numbers.

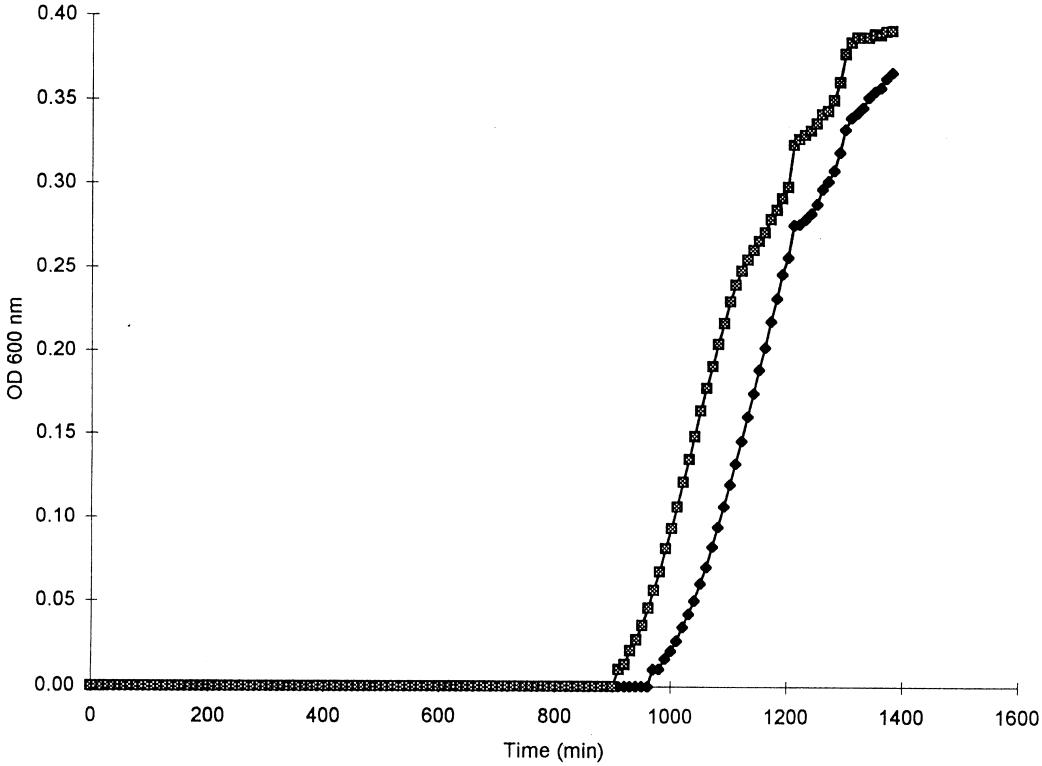

Fig. 3. QC test for nutrient broth.

laboratories can set their own acceptance/rejection criteria dependant on their media types and applications. In the Miles–Misra tube dilution technique, approximately equal growth (measured by eye) produced at similar inoculum levels is used to indicate satisfactory performance compared to the standard [1]. We decided that this was too subjective which is why the Bioscreen data was converted to cell counts in test and control broths, giving a more quantitative test.

The test was carried out on a selection of broth media which were kept as stock items in the

Table 1
QC tests on stock items of liquid media

Broth	Organism	Calibrations r^2	Log_{10} count/ml control broth	Log_{10} count/ml test broth	Log_{10} difference in counts
HIB	*Pseudomonas*	0.997	4.93	5.01	0.08
APT	*Lactobacillus*	0.997	2.36	2.88	0.52
MEB	*Candida*	0.990	4.23	4.50	0.27
MacC	*E. coli*	0.992	3.35	3.34	0.01
Nutrient	*S. aureus*	0.983	1.96	2.44	0.48
BHIB	*Listeria*	0.997	4.10	4.30	0.20
TPB	*Listeria*	0.996	3.37	4.15	0.78
Fraser	*Listeria*	0.998	4.77	4.71	0.06
BLEB	*Listeria*	0.993	3.17	3.33	0.16

List of abbreviations in Section 2.1 Section 2.2.

Colworth Microbiology Department (Table 1). It can be seen that all media were performing to an acceptable standard, with growth in test and control broths being very similar in all cases. This table also shows the extremely good correlation coefficients for each calibration graph. This means that OD data can be related quite accurately to cell numbers, so the Bioscreen is a suitable technique for all of these particular strains grown in these specific broths.

4. Discussion

The calibration curves produced for each broth using the QC test organisms showed very good correlation between OD data produced by the Bioscreen and cell number. These graphs can be used in all future QC tests of liquid media to convert Bioscreen data, for these test organisms, into cell counts. Even if a new batch of broth varies very slightly in colour (broth colour between batches is not always consistent and may change slightly during autoclaving or storage at 4°C) this will not affect the results. The original calibrations can still be used, because it is the change from initial OD which is used to calculate detection times. Any slight colour variation (not always visible by eye) can be simply accounted for by always running an uninoculated broth from the same batch alongside each test, and taking all OD readings against this broth blank.

In this trial, all stock items of liquid media tested in the Colworth Microbiology Department were found to be performing to an acceptable standard. The number of organisms after 6 h growth was chosen because one of the fundamental performance parameters of a culture medium is its ability to promote early and rapid growth of microorganisms [2]. Using 6 h also means that the manual part of the test is completed within a working day. A more complex test can be performed, using the Bioscreen to monitor growth in test and control broths throughout the entire growth curve. This is done by inoculating old and new batches of broth in the wells of the plate. This allows comparison of lag phase, growth rate and final cell number achieved. These kinetic parameters are not generally recommended as units of measure in a simple QC test, because criteria for acceptance/rejection of liquid media based on such parameters are not yet available [10]. This type of test can, however, give good indication (by visual examination of graphs) whether the control and test broths are performing to a similar standard. Accumulation of such data over time should eventually make it possible to estimate what the acceptance/rejection criteria for these kinetic parameters should be. For the purpose of a simple and fast QC test, a single point determination (i.e. cell number after 6 h growth) is adequate to give a good indication of the performance of liquid media.

In this trial, the performance of all stock media was found to be acceptable. If, however, one of the broths had failed the test, it would be important to retest the media, holding the rest of the ready-to-use batch in the refrigerator, until it is confirmed whether the batch is acceptable for use. Repeating the test should establish whether failure of the initial QC test was due to human/experimental error, for example, inadequate mixing of test inoculum etc. If the broth fails a second time, the batch of prepared media is unacceptable and should be removed from storage and discarded. Another batch needs be prepared from the original container of dehydrated media. Testing this will help to determine whether the problem was with the raw ingredients from the media supplier, or whether there was a mistake in the reconstitution and sterilisation of the batch. Records should be kept for autoclave temperatures during sterilisation, water quality, pH etc. and these should also be carefully analysed if a batch of media fails the QC test.

In these trials, only one QC test organism was used for each broth. The test can be further refined depending on the exact application of each medium. For example, selective broths such as BLEB and Fraser should not only support the growth of *Listeria*, but their supplements should also suppress the growth of other competitors. One such competitor could be run in the QC test of these broths as well as *Listeria*. If a differential medium, such as MacConkey broth is tested, the Bioscreen method can still be used. The Neutral Red indicator changes this broth from purple to yellow as acid is produced by the coliforms. This was found to cause a decrease in OD but did not affect calibration graphs because the colour change was proportional to cell number, still resulting in good correlation between detection time and coliform counts ($r^2 = 0.992$).

Using the method detailed in this paper, a Microbiology department can assess the performance of all their liquid media in a single Bioscreen run, on a regular basis. The method is rapid, automated and less subjective than certain other QC tests based on OD measurements where turbidity is estimated by eye. Collection and analysis of QC data over time should also give a better understanding of how the age or storage temperature of each broth might affect its performance. This should allow better determination of the shelf-life of various items of liquid media and ensure each one allows optimum growth of appropriate organisms.

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