

JFM 00037

Introduction and prospective

D.A.A. Mossel

*Department of the Science of Food of Animal Origin, Faculty of Veterinary Medicine, University of Utrecht,
3508 TD Utrecht, The Netherlands*

(Received 31 December 1984; accepted 14 January 1985)

The activities of the Working Party on Culture Media (WPCM) of the International Union of Microbiological Societies (IUMS) since 1978 are reviewed. The assignments at hand include (1) drawing up a list of media used on a large scale and therefore qualifying for a pharmacopoeia-type monograph, making serious attempts to eliminate bias resulting from parochial preference; (2) requirements for the functioning of these media, i.e. productivity and selectivity, with regard to the type of specimen being examined.

Key words: Selective culture media; Monitoring; Selectivity; Productivity

Introduction

Culture media are used in large quantities throughout the world. It is therefore not surprising that occasionally disappointing diagnostic results are obtained due to malfunctioning of purchased media ('extramural aetiology'). In addition, errors are sometimes made 'in house' which adversely affect the functioning of essentially satisfactory preparations. It is a matter of common interest to microbiologists and medium manufacturers alike that such occurrences are reduced to a minimum (Mossel, 1982).

It has been a point of debate for a long time, however, whether practising bacteriologists should be concerned about the first aspect. In other words, when culture media are invariably purchased from large, ethical, internationally operating manufacturers, it is felt that they should be responsible for ensuring that media are always fully adequate for use, e.g., in the way the car industry provides customers with safe vehicles. Another group of bacteriologists is of the opinion that the ultimate responsibility for a valid diagnosis, including the use of well-functioning media, rests with the diagnostician, who should therefore also perform some medium monitoring activities.

This author supports the latter view. Even the car industry suffers from the problem that minor deficiencies in the final product sometimes escape the most accurate quality control systems, resulting in the well-known recalls of vehicles to dealers. Is it not generally accepted that even Homer occasionally nods? Another reason for this point of view is that numerous reports in the international literature

(Reuter, 1968; Prier et al., 1975; Annino, 1978; Mossel, 1982) and observations of the author's Institute (Mossel, 1971; Mossel et al., 1974; Mossel et al., 1977; Mossel et al., 1978; Mossel et al., 1979a, b; Mossel et al., 1980a, b; Mossel et al., 1983; Visser et al., 1985) illustrate that marked differences in functioning may occur between (i) different brands of a medium which are of exactly the same composition according to their labels; and (ii) successive batch numbers of the same brand of the same medium, none of which was near the expiry date when tested.

However this may be ultimately resolved for the time being it is recommended that both parties should at least realize their responsibility and see to a reasonable number of measures for assuring the quality of culture media. As always this applies not only to monitoring, but also, as the first priority, to preventive measures, such as careful practices of preparation, pH adjustment, decontamination, distribution, inoculation and incubation of media (Mossel, 1971).

One element of the use of culture media remains clearly the full responsibility of the bacteriologist, viz. the choice of a medium for a particular diagnostic purpose. Unfortunately, here a personal bias is often observed: a given medium is preferred and other formulae are rejected without the preference always being supported by adequate experimental data. Now it is also true that some bacteriologists can make certain media function perfectly, whereas others seem to be unable to score equally satisfactory results with that same medium (Kendall, 1982). Altogether, however, comparability of data obtained in different parts of the world – and sometimes even in laboratories within the same area or country – would greatly benefit from the use of the same set of media for the detection or enumeration of the most important taxa.

During the World Congress of Bacteriology held in Munich in 1978, the International Union of Microbiological Societies (IUMS) agreed to (i) consider adoption of the latter approach as the preferable one; (ii) favour its acceptance by carefully codifying the composition, use and monitoring particularly of selective culture media for that purpose. A Working Party on Culture Media (WPCM-IUMS) was constituted to attain this goal. The organisation of the London Symposium was its fourth activity.

A short review of the past WPCM-IUMS activities

At the first symposium held in Mallorca in 1979 (Corry, 1982) an inventory of selective culture media in current use was presented, together with an attempt to separate facts from fables about every particular one. Testing methods suitable for assessing the functioning of solid and liquid media were also reviewed. In the latter area at least four criteria were suggested for the evaluation of any medium. These included: (i) physical and chemical properties, such as colour, transparency, gel strength, pH and E_h , where applicable (Mossel, 1971); (ii) productivity, defined as the recovery of strains the medium is supposed to grow, expressed as the \log_{10} -fraction of the numbers of colonies obtained on a suitable non-selective control medium; (iii) selectivity: similar \log_{10} -fraction for organisms that should, in principle, be more

or less completely inhibited on, or in, the medium; (iv) electivity, defined as clearness of typical traits demonstrated by organisms for which a differential medium was designed, in order to discriminate macroscopically between sought and 'background' organisms. A summary of methods that could be used to determine the microbiological characteristics of a selective medium is presented in Table I.

At the second Colloquium held by the WPCM-IUMS in Dallas, Texas, in 1981 these activities were expanded (Proceedings, 1982). A few more recently developed

TABLE I

Criteria recommended for use in the evaluation of selective media

1. Productivity for pure cultures

Definition: N_s^s/N_0^s

where: N_s^s = number of colonies of sought type obtained on selective medium.

N_0^s = number of colonies obtained on control medium which, ideally, is the selective medium without selective agents.

Assessment: Spiking with representative test strains, including robust and fastidious types.

Requirement: $\log_{10} N_0^s - \log_{10} N_s^s < 0.5$

2. Selectivity for pure cultures

Definition: N_s^i/N_0^i

where: N_s^i = number of colonies of interfering types on selective medium.

N_0^i = number of colonies obtained on control medium.

Assessment: Spiking with refractory and sensitive interfering strains.

Requirement: $\log_{10} N_0^i - \log_{10} N_s^i > 5$.

3. Electivity:

Definition: easy recognition of colonies of different organisms, based on either intrinsic properties of organisms, or specific responses to the medium under study.

Assessment: Ranking ease of differentiation, avoiding parochial bias.

Interpretation: Rank correlation.

4. Performance with natural food samples:

Definition: Extent of recovery of sought organisms, and degree of inhibition of interfering organisms, when the test medium is compared to a similar, though different one.

Assessment: By direct inspection, microscopy or cultural methods.

Interpretation: Grouping of recoveries of sought and non-sought organisms in series of ranges $10^x - 10^{x+y}$ and analysis by rank correlation methods.

5. Taxobias:

Definition: Extent to which a medium selects or suppresses a particular species or biotype within a selectively enumerated taxogroup.

Assessment: Using natural food samples, investigation of taxobias by microscopy or biochemical examination of a representative selection of colonies, substantiated by reinoculation of colonies.

Interpretation: Rank correlation.

media were presented along with improved modifications of classical ones. For their evaluation in most instances the criteria suggested by the present author during the meeting in Mallorca (Table I) were used. At the conclusion of this second Colloquium the participants felt that only one aspect had to be dealt with experimentally before the Working Party could embark upon its main task of drafting Pharmacopoeia-like protocols (Bartl, 1982), for the monitoring of more generally accepted culture media. This concerned a collaborative assay of one of the newer, simplified testing methods, recommended for the monitoring of solid media, i.e. the ecometric technique. This relies on streaking standardized inocula of challenge strains onto media under test by a procedure allowing ever decreasing numbers of colony forming units per unit of surface area to be achieved, as in the mode of dilution attained in spiral plating (Mossel et al., 1983).

An evaluation of this method was carried out in January 1982 at Utrecht University, The Netherlands (Corry et al., 1985). It was linked to assessing the suitability of machines for the preparation of poured plates. From this study it appeared that ecometry, provided it was carefully standardized would indeed lead to good reproducibility when used by bacteriologists of various scientific backgrounds. Moreover, plating machines were found to work well, though they should preferably be operated in a clean environment, and with a filtered air supply.

Desiderata of the third Symposium

It follows from the previous section that the London colloquium should make a brave attempt to draft a few dozen pharmacopoeia-like protocols on the most frequently used selective media in food, water and environmental microbiology. It is only natural that a few media in current use in clinical microbiology will also be included, because those which have proved reliable for the detection of, e.g., pathogenic bacteria in faeces, in many instances are also suitable for the examination of foods for the same pathogens (Mossel et al., 1983).

In the effort to arrive at such protocols for media the following fundamental *microbiological* consideration should be taken into account. Analysis of the functioning of a medium should never be divorced from the substrate that is inoculated onto or into it. This is less important in clinical microbiology, where (i) only three or four rather similar types of specimens are examined: blood, cerebrospinal fluid, exudates and pus and stool; (ii) a specimen is often either sterile or contains a pathogen. On the other hand, foods differ tremendously, both in chemical composition and in colonization. These affect the functioning of a given medium in the following ways:

- constituents of the food under examination may markedly change the composition of the medium and thereby its functioning, particularly in surface plating and liquid media; e.g., Folpmers' (1935) excellent glutamate minerals medium for the detection of *Escherichia coli* in water will not function well when inoculated with food macerates;
- the 'background' microbial community structure of the food sample may greatly affect a medium's functioning: it is, for instance, very hard to suppress large

numbers of Lancefield group D streptococci in media designed for the enumeration of clostridia;

- there is often a substantial effect of the composition, processing and/or storage of a food sample on the vitality of its microbial association (Mossel and van Netten, 1984) and consequently on the functioning of a given medium. In most instances these effects are so serious that populations released from such foods can only be inoculated into or onto selective media after a revitalization treatment known as resuscitation (Allen et al., 1952) has been carried out.

Moreover, a few *educational* aspects of diagnostic microbiology should definitely not be overlooked. The assignment of the WPCM-IUMS must, clearly, be entrusted to an international group of microbiologists who are often graduates of quite different 'schools'. This applies not only to national elements in one branch, e.g. medicine, but also to completely different disciplines. It is well known that whereas in one country veterinarians and agricultural microbiologists are trained at the same college, in other areas of the world these two disciplines have very little training in common. The same holds true for academic education in medicine and pharmacy, which in some countries is entrusted to the same faculty, but in many universities is completely distinct.

Furthermore, there is a *psychological* element that should be taken into account. Often a strong preference in certain institutes and even entire countries is observed for a given medium, which seems to result from bias because the same medium is virtually never used in any other country. In the interest of diagnostic microbiology, collaborative international trials of the more popular media should be undertaken where required. Where this has been done in the past, e.g., in the detection of salmonellae (Edel and Kampelmacher, 1973; van Schothorst et al., 1978) only a few methods and media have passed the stringent test. There is little doubt that the same would be observed if such trials were undertaken in other areas of diagnostic microbiology provided of course (i) the evaluation criteria of the WPCM-IUMS summarized in Table I were to be carefully observed; (ii) the ecological essentials expounded earlier in this section are not ignored.

There is, hence, a fascinating challenge ahead for a really internationally oriented fraternity of professional microbiologists. As far as 'cost-benefit' quotients of such efforts are concerned it seems likely that these are infinitesimally low. Instead of having to choose from a momental Manual of over 1000 pages (Difco, 1984), the food and water microbiologist could in future rely on a recommendation of perhaps 25 pages. And the endless and extremely expensive discussions about which medium and method to use by manufacturers and purchasers, particularly during disputes, which are even more costly, would be completely avoided.

Finally for the microbiologist, agreement on monitoring methods would allow ample time and staff to study other areas much more interesting than medium development and validation. In food microbiology this would mean elaborating effective modes of processing foods and water for microbiological safety so that, at last in the long term, intervention leading to a markedly reduced morbidity of food- and water-borne disease could be introduced at the required scale (Kayser and Mossel, 1984).

References

- Allen, L.A., S.M. Pasley and M.S.F. Pierce, 1952. Conditions affecting the growth of bacterium coli on bile salts media. Enumeration of this organism in polluted waters. *J. Gen. Microbiol.* 7, 257-267.
- Annino, J.S., 1978. What does laboratory 'quality control' really control? *N. Engl. J. Med.* 299, 1130-1131.
- Bartl, V., 1982. What can be expected from the pharmacopoeia approach to quality assurance of culture media. *Arch. Lebensmittelhyg.* 33, 174-175.
- Corry, J.E.L., Editor, 1982. Quality assurance and quality control of microbiological culture media. G.I.T.-Verlag, Darmstadt.
- Corry, J.E.L., H. Leclerc, D.A.A. Mossel, N. Skovgaard, G. Terplan and P. van Netten, 1985. A collaborative study of the quality of media prepared and poured by an automated system. *J. Appl. Bacteriol.*, in press.
- Edel, W. and E.H. Kampelmacher, 1973. Comparative studies on the isolation of 'sublethally injured' *Salmonellae* in nine European laboratories. *Bull. W.H.O.* 48, 167-174.
- Folpners, T., 1935. Vergelijkende resultaten, verkregen door vervanging van pepton (Witte) door glutaminezuur etc. als stikstofbron bij de Eijkmanproef met oppervlaktewater in verschillende reinigingsstadia. *Antonie van Leeuwenhoek* 2, 343-357.
- Kayser, A. and D.A.A. Mossel, 1984. Intervention sensu Wilson: The only valid approach to microbiological safety of food. *Int. J. Food Microbiol.* 1, 1-4.
- Kendall, M., 1982. Bismuth sulphite agar for the isolation of salmonellae. In: *Quality assurance and quality control of Microbiological culture media*, edited by J.E.L. Corry, G.I.T.-Verlag, Darmstadt, pp. 133-139.
- Mossel, D.A.A., 1971. Microbiological culture media as ecosystems. 5. Ecometric evaluation of media. In: *Effects of sterilization on components in nutrient media*. Wageningen. Agricultural University, Miscellaneous Papers Series Nr. 9, pp. 29-31.
- Mossel, D.A.A., G.A. Harrewijn and C.F.M. Nesselrooy-van Zadelhoff, 1974. Standardization of the selective inhibitory effect of surface active compounds used in media for the detection of Enterobacteriaceae in foods and water. *Health Lab. Sci.* 11, 260-267.
- Mossel, D.A.A., I. Eelderink and J.P. Sutherland, 1977. Development and use of single, 'polytropic' diagnostic tubes for the approximate taxonomic grouping of bacteria isolated from foods, water and medicinal preparations. *Zentralbl. Bakteriol. Parasitenkd. Abt. I, Orig.* A 238, 66-79.
- Mossel, D.A.A., P.G.H. Bijker and I. Eelderink, 1978. Streptococci of Lancefield groups A, B and D and those of buccal origin in foods: their public health significance, monitoring and control. In: *Streptococci*, edited by F.A. Skinner and L.B. Quesnel, Academic Press, London, pp. 315-334.
- Mossel, D.A.A., I. Eelderink, M.T.A.G.F. Koopmans and F. van Rossem, 1979a. Influence of carbon source, bile salts and incubation temperature on recovery of Enterobacteriaceae from foods, using MacConkey-type agars. *J. Food Protect.* 42, 470-475.
- Mossel, D.A.A., F. van Rossem and A. Rantama, 1979b. Ecometric monitoring of agar immersion plating and contact (AIPC)-slides used in assuring the microbiological quality of perishable foods. *Lab. Pract.* 28, 1185-1187.
- Mossel, D.A.A., K.E. Dijkmann and M. Koopmans, 1980a. Experience with methods for the enumeration and identification of yeasts occurring in foods. In: *Biology and activities of yeasts*, edited by F.A. Skinner, S.M. Passmore and R.R. Davenport, Academic Press, London, 279-288.
- Mossel, D.A.A., F. van Rossem, M. Koopmans, M. Hendriks, M. Verouden and I. Eelderink, 1980b. Quality control of solid culture media. A comparison of the classic and the so-called ecometric technique. *J. Appl. Bacteriol.* 49, 439-454.
- Mossel, D.A.A., 1982. Ecological essentials of the use of selective culture media in Public Health Microbiology. In: *Quality assurance and quality control of microbiological culture media*, edited by Corry, J.E.L., G.I.T.-Verlag, Darmstadt, pp. 11-19.
- Mossel, D.A.A., T.M.G. Bonants van Laarhoven, A.M.Th. Ligtenberg-Merkus and M.E.B. Werdler, 1983. Quality assurance of selective culture media for bacteria, moulds and yeasts. An attempt at standardisation at the international level. *J. Appl. Bacteriol.* 54, 313-327.

- Mossel, D.A.A. and P. van Netten, 1984. Harmful effects of selective media on stressed micro-organisms: Nature and remedies. In: The revival of injured micro-organisms, edited by M.H.E. Andrew and A.D. Russell, Academic Press, London, pp. 329-369.
- Prier, J.E., J.T. Bartola and H. Friedman, Editors, 1975. Quality control in microbiology. University Park Press, Baltimore.
- Proceedings of the Second International Symposium on Quality Assurance of Microbiological Culture Media, 1982. Arch. Lebensmittelhyg. 33, No. 6, 137-175.
- Reuter, G., 1968. Erfahrungen mit Nährböden für die selektive mikrobiologische Analyse von Fleischerzeugnissen. Arch. Lebensmittelhyg. 19, 53-57; 84-89.
- Schothorst, M. van, R.J. Gilbert, R.W.S. Harvey, O. Pietsch and E.H. Kampelmacher, 1978. Comparative studies on the isolation of Salmonella from minced meat. Zentralbl. Bakteriол. Parasitenkd. Abt. I, Orig., B, 167, 138-145.
- Visser, I.J.R., F. Jaisly and D.A.A. Mossel, 1985. The effect of properties of dried preparations of sulphide iron motility (SIM) agar on the result of motility readings. J. Appl. Bacteriol., in press.