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FOOD MICROBIOLOGY

How it used to be before the 1950s and what it may become in the 1980s

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Food Microbiology had purely empirical origins like most branches of Science. Pasteur laid the foundations about 1860 and since that time Science, Medicine, Veterinary Medicine and Agriculture have all contributed something without really identifying with Food Microbiology. In practice it therefore remained limited to a Cinderella topic which might well be characterized as *'plate count and coliform boy scouting'*.

An improvement occurred about 1950. Buttiaux (France) greatly contributed to analytical Food Microbiology, Dack (Chicago) to the pathogenesis of food-transmitted diseases and, with Sir Graham Wilson, to their prevention, while Ingram founded and developed the modern concept of Microbial Ecology of Foods. Although the legitimate child of no particular Science, Food Microbiology at least emerged from the Orphanage well provided. It has since developed into an adult Science of its own. Its present achievements and probable future developments are briefly reviewed in this contribution.

THE FABLE AND FACTS OF QUALITY ASSURANCE

Sampling examination can never protect the consumer against microbiological hazards in food logistics.[1] Similarly monitoring cannot prevent potential loss of food quality. These goals can only be attained by laying down and adhering to Good Manufacturing and Distribution Practices (GMP). This principle, too long ignored has been condensed in a most explicit way by C. L. Goldman a few years ago.[2] He wittily remarked that monitoring of final products is like a pregnancy test, it indicates the possibility of a problem, whereas GMP is similar to taking a contraceptive preparation which prevents the problem happening. GMPs have four essential elements[3]: the use of the best possible raw materials; adequate processing; safe distribution and storage of marketed products; sound culinary preparation and handling of the latter. Epidemiological data[4] demonstrate that most instances of loss of microbiological integrity occur at the distribution stage–particularly in catering and private homes. This prompted Dack as early as the 1950s[5] to stress the importance of *longitudinal extension* of Microbiological Quality Assurance beyond the factory gate.

Once GMPs are faithfully practised, monitoring commences to serve a useful purpose, i.e. in *assessing* rather than trying to *attain* quality of final products. The reason for this is that by the introduction of GMPs quality fluctuations will be dramatically reduced (Figure 1). Hence the probability that incidental quality loss due to human failure will be detected by sampling examination is essentially increased.

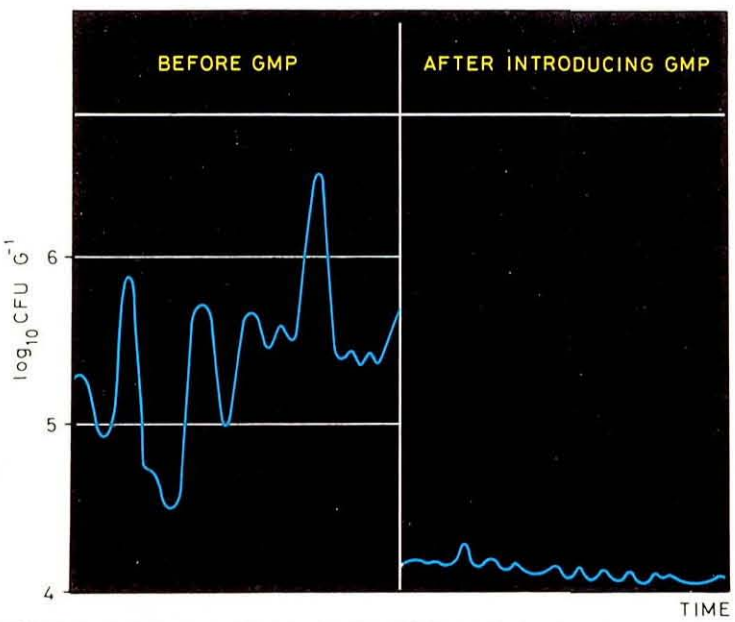

FIGURE 1. The influence of the introduction of GMPs on the level and fluctuation of microbial colonization of foods.

MICROBIOLOGICAL MONITORING: *CAVEANT CONSULES...*

Microbiological examination of food samples has at least three aspects that require thorough training and experience by its practitioners. These include the choice of criteria and assessment of 'standards', in addition to the central subject of this paper: adequate methodology.

The number of microbiological *criteria* used in examining foods should always be limited to the strict minimum. This allows optimal use of available laboratory capacity. In particular this guards against the loss of credibility of Food Microbiology when it tries to advocate and practice wholly useless examination of commodities. The essential criteria are dictated by (i) epidemiological figures, i.e. foods most frequently involved in outbreaks[6] where health risks are concerned; (ii) ecological data on the spoilage organisms specific for each particular food – its so-called 'spoilage association'.[7]

Ever since the middle 1950s voluminous literature has been published on Microbiological 'Standards' for Foods. Many of these papers are conflicting and quite a few show an emotional rather than a rational approach to the subject. Yet, a fully scientific treatment of 'Standards' is possible. All this requires is that the scientist chooses to wear the doctor's white coat rather than the policeman's badge. For over 50 years it has been customary to use so-called *Reference Values* for clinical laboratory data in human and veterinary medicine. Exactly the same approach is feasible in the case of drafting microbiological Reference Values for foods. They are assessed by experimental surveys on valid samples, obtained from manufacturing industries operating GMPs that have been *verified, prior* to drawing samples[8] (Figure 2).

Generally results of laboratory examinations carry little weight unless carefully defined and validated *methods* are used. This applies particularly to the microbiological examination of foods for the following reasons:

(i) most of the available reliable methods have only been designed about 1950 or much later, and few have been validated by collaborative testing;

(ii) with an occasional exception such as skim milk and ice-cream, micro-organisms are distributed very unevenly in foods and may be attached to food tissues; therefore they require carefully designed and validated release techniques before microbiological counts can be meaningful;

(iii) alarmingly little attention is paid to the true performance of purchased selective culture media or ingredients recommended for the determination of a given group of micro-organisms.

In the following section an attempt will be made to suggest some improvements for the future, in the practice of Food Microbiology.

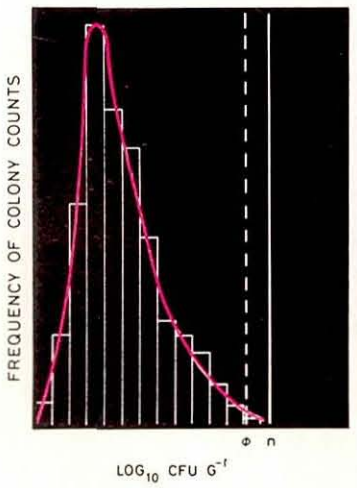

FIGURE 2. The assessment of Reference Values for the microbiological condition of foods, using surveys on end-product samples taken from manufacturing industries where GMPs have been verified *before* the samples were taken.

THE CHALLENGE FOR THE 1980s

Release of Organisms from Foods

When carrying out an examination to determine heterogeneity in the distribution of micro-organisms in various foods, two phenomena can be distinguished. First there is the unequal distribution at a *macroscopic* level. Examples are (a) the scattering of bacteria over the surface of carcass meats, particularly their concentration on 'predilection sites'[9] and (b) the virtual limitation of micro-organisms to the aqueous phase of emulsified foods.[10] The second instance of heterogeneity is clumping on a *microscopic* scale, which can be observed, e.g. on meat and poultry surfaces when they are examined by scanning electron microscopy.[11]

Inaccuracies due to these phenomena can easily be overcome by using proper sampling selection and release methods. In the examination of emulsified foods, the aqueous phase should be recovered by a treatment that spares the micro-organisms.[10]. When sampling carcasses the localization of the most contaminated areas should be determined.[9] Furthermore, under no condition should surface impression methods be used for recovering contaminating bacteria from these sites on the carcass; instead dissection techniques should be used.[12, 13] Such measures should become routine in the 1980s.

Occurrence and Control of Sublethal Lesions in Micro-Organisms from Foods

Soon after World War II many authors reported considerable losses of recovery when organisms released from processed foods were plated directly into or onto selective culture media. This occurred despite the fact that such media had been found to fully recover populations, freshly emulsified from slopes of non-selective media. Further studies revealed that this resulted from lesions incurred by micro-organisms in foods exposed to modes of processing such as heating, drying, freezing or preservation by acidification to pH <4.5. The extent of such sublethal lesions and hence the losses in recovery in selective media depends on many factors. These include: (i) types of stress; (ii) intensity of stress; (iii) characters of the cytoplasm of the cells exposed to stress; (iv) properties of the selective culture medium used for the ultimate enumeration of stressed populations.[14, 15] Consequently the degree of underestimation of microbial loads in processed foods, resulting from direct plating in selective media, can vary from less than one to more than six log cycles.[14]

At the molecular level various effects of stress have been described. These include the degradation of RNA, DNA and various proteins. However, the common underlying mechanism of all sublethal stress seems to be

damage to the cell envelope, resulting in leakage of ions, peptides and TCA intermediates from the somatic pool. Treatments leading to repair of sublethal damage are often referred to as *resuscitation*.[16]

Since the character and intensity of stresses occurring in practice vary widely, and the same applies to the responses of the different taxa and the degree of inhibitory properties shown by different media to equally injured populations, resuscitation treatments leading to acceptable repair and hence to almost complete recovery on selective media will also differ greatly (Figure 3). It is, however, quite striking that two hours suspension in buffered tryptone soya peptone broth at about 30°C seems to recover most types of injury encountered in practice.[14,15] The exception is in the detection of *Salmonella* sp. in foods,[14] where cells are subsequently to be recovered in highly selective enrichment media.

It will be one of the many tasks of food microbiologists in the 1980s to establish the molecular biological mechanism of this phenomenon and to find procedures for more rapid resuscitation. This is dictated not only because many perishable foods require rapid monitoring, but the longer periods of resuscitation currently found indispensable lead to massive proliferation of commensals which can result in the antagonistic suppression of the likely pathogen.[17]

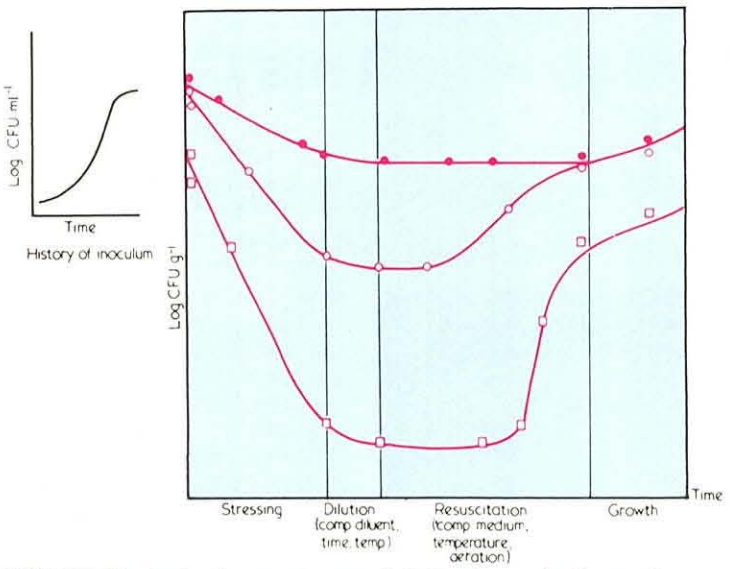

FIGURE 3. The destruction repair curve. ●-Optimal non-selective medium, ○-optimal selective medium; □-suboptimal selective medium.

Occurrence and Prevention of Population Changes During Transportation of Food Samples to the Laboratory

The microbial population of every food is in a constant state of flux.[18] Changes in the microbial load of a food sample must therefore be expected to occur during its transportation from the sampling site to the laboratory. The degree of change depends on (i) the intrinsic properties of a food, such as a_W, pH, Eh and nutrient composition; and (ii) time-temperature profile of transportation. Our experience has shown that the changes may often be dramatic.

In order to avoid such errors food microbiologists should study the possibility of 'taking the laboratory to the sample'. This is particularly essential for developing countries, where the distances involved are often very long. A promising system may be the so-called Agar Impression Plating and Contact (AIPC) slides.[19] It consists of 'dip slides' as currently used for the bacteriological examination of urine by the practising physician, using media adapted to the needs of Food Microbiology.

An additional advantage of AIPC slides is that they also lend themselves very well for resuscitation purposes. Foods or food dilutions are first of all plated out on AIPC slides with tryptone soya peptone agar. After a suitable period of incubation these are replicated on to slides covered with the appropriate selective enumeration media.

Selection of Microbial Groups to be Used in Monitoring Foods

Many, quite often emotional arguments have taken place on the usefulness of examination of foods for pathogens, when contrasted to organisms with supposed or alleged 'marker' function. Once more the answer emerged only after ambiguity and an unscientific approach had been eliminated. Food Microbiology is particularly indebted to the late Professor Ingram for his contribution in this area of Microbial Ecology.[8]

It is quite clear that in all epidemiological investigations a direct search for the pathogen involved can never be omitted. For day-to-day monitoring it was Ingram's suggestion to distinguish between two groups of marker organisms.

First, there is the group whose presence in a food indicates the possible occurrence of an ecologically related pathogenic type. Such organisms could be called *index* organisms. They were introduced in 1892 by Schardinger; *E. coli* was used as the index for *Salmonella* in the monitoring of drinking water.[20] Much later certain groups of bacteria have been introduced with the purpose of revealing deficiencies about the *microbiological condition* of a food *in more general terms*. Ingram suggested designating these as *indicator* organisms. The classical example here is Sir Graham Wilson's interpretation of the occurrence of coli-aerogenes bacteria in pasteurized milk.[21] No less than three unwanted attributes of a consignment of pasteurized milk could be revealed by the presence of numbers of coli-aerogenes bacteria exceeding an experimentally established Reference Value. These include (i) inadequate heat treatment, (ii) post-pasteurization recontamination, and (iii) storage of the final product at too high a temperature. This approach eliminated all the problems that have been so frequently and so furiously discussed in the past, as the following may illustrate.

Many authors have rejected the use of *Enterobacteriaceae* as indicator organisms because these are encountered in appropriate samples quite frequently, whereas *Salmonella* is not detected in the portions usually examined. This is, of course, no real objection! The purpose of the examination of foods for *Enterobacteriaceae* is to check the process for safety, i.e. absence of deficiencies *sensu* Wilson – a situation that is entirely different from the actual isolation of a pathogen.

Others have criticized the use of *Enterobacteriaceae* and other indicator organisms because, despite the absence of these, pathogens have been detected in samples. In Ingram's approach this criticism only applies to the explicit use of *Enterobacteriaceae* as *index* organisms – quite a different proposal, of course. First of all, despite all the problems yet to be solved in their reliable detection (*vide infra*), pathogens should always be looked for directly in foods in which they occur more frequently than seems acceptable from the point of view of consumer protection. *In addition,* there is invariably room for the use of suitably chosen index organisms, because their detection is so much more rapid, reliable and simple.

To increase the usefulness of this complementary testing the so-called ε-factor has been introduced. This is defined as the proportion between numbers of colony forming units of index organisms and the pathogen concerned in the same food.[22] Numerical values for ε-factors vary widely, from just over 1 to 10^6. It would be most helpful if such ε-factors could be determined more systematically and for more pathogens in addition to *Salmonella*. Knowledge of these factors would allow calculation of the risk of accepting consignments of foods contaminated with a given pathogen, despite the absence of the index organism.[23] This approach should also take full account of the need to protect the food manufacturer from being confronted with requirements including unattainably low levels of contamination, even when GMPs are carefully followed.[24] This is one of the most pressing but at the same time challenging areas of research in the Microbial Ecology of Foods.

Whereas enumeration of index and indicator organisms is a relatively well developed area of Analytical Food Microbiology,[8] this cannot be said for the pathogenic species most frequently involved in foodborne disease. Recent studies demonstrate that even the detection and enumeration of the most intensively studied pathogen, *Salmonella*, is fraught with problems that require more profound studies.[25] Also methods for the enumeration of *Staph. aureus* in foods are still being improved. The reliability of the egg yolk reaction for the identification of the species is rather limited.[26] Consequently a modified Baird-Parker medium, relying on the direct demonstration of coagulase activity with pig plasma[26,27] or fibrinogen[28] as the substrate may well be the future development.

The detection of Shigellae other than *Sh. sonnei* in foods is at an undeniably primitive stage, comparable with that of the demonstration of the presence of *Salmonella* in the 1950s.[29] For the more recently recognized groups like enteropathogenic *E. coli*,[30] *V. parahaemolyticus*,[31,32] the other 'non-agglutinating vibrios',[33] *Yers. enterocolitica*[34] and *Campylobacter*[35] the situation is even less reliable. This is another task eagerly awaiting the innovative efforts of the well trained Food Microbiologist!

Quality Assurance of Culture Media

No result of a microbiological examination of foods can ever be better than the culture media with which it is obtained. It has been the vexing experience of many clinical as well as food bacteriologists that batches of commercially available selective culture media neither seem to recover the organisms they are designed for, even after these have been fully resuscitated, nor adequately suppress the 'background' flora of different foods they are supposed to inhibit. Our own confrontation with this sad state of affairs occurred in the early 1960s. Batches of brilliant green bile enrichment broth, where lactose has been replaced by glucose to recover all *Enterobacteriaceae*, to our great surprise gave lower confirmed cfu counts than the original lactose based ones. A painstaking investigation revealed that batches of the 'best' bile salts and triphenyl methane dyes, ie. inhibitory to non-*Enterobacteriaceae* but non-toxic to *Enterobacteriaceae* were invariably used for the conventional lactose containing media. However, in the new glucose based media, which were clearly not yet established, more than one manufacturer incorporated completely unchecked batches of the critical inhibitory agents, making the media toxic for very low numbers (2–7) of cfu of many *Enterobacteriaceae*. Surveys carried out in 1977 and 1978 demonstrated that quite a few media for *Enterobacteriaceae* were still showing such deficiencies.[8,23]

This situation compels food quality assurance microbiologists to calibrate the performance of every newly purchased batch of selective medium. This applies even when previous evidence has been obtained that a given manufacturer generally supplies media of satisfactory performance. The following procedures have been found useful for liquid enrichment, solid isolation and enumeration media. In all instances at least three and preferably five test strains of both the organisms to be recovered and the types to be suppressed should be used. They should be of varying robustness in both instances.

Liquid Media: taxa that should freely develop are inoculated at levels from 2–7 cfu per tube up to about 10^3. Background strains that should not grow are inoculated in densities 10^3–10^7 per tube.[36] Controls are made, in both instances, in rich non-selective media, such as tryptone soya broth with yeast extract added. The results are expressed in 'titres', i.e. the negative logarithm of the highest decimal dilution showing growth.[36] An acceptable medium should show high titres for the organisms to be detected and very low ones only for taxa to be suppressed.

Solid Media: the reference method is the Miles-Misra technique in the more accurate modification of Slack & Weldon[37] (Figure 4). Organisms of the group for which the medium was designed should be recovered almost quantitatively. The 'background' flora of foods should be suppressed by a factor 10^3–10^4.

For mass monitoring, when large amounts of different culture media are to be examined, the ecographic technique (Figure 5) can be used. Overnight cultures of test strains of both groups are streaked in parallel lines onto solid media or liquid media, solidified by the addition of agar at approximately 15 g litre^{-1}. Taxa supposed to develop quantitatively on a given medium should demonstrate growth at least to level 4 (Figure 5) whereas 'background' flora should remain in sector 1 to 2.[38]

Collaborative testing of the methods just described is an essential element in attempts to standardize the use of selective culture media at international level.

The Impact of the Introduction of Less Labour-Intensive Methods

As indicated before, the microbiological examination of foods is a relatively young branch of Science – certainly when compared with analytical Food Chemistry. Therefore, it is not surprising that mechanization and automation were introduced

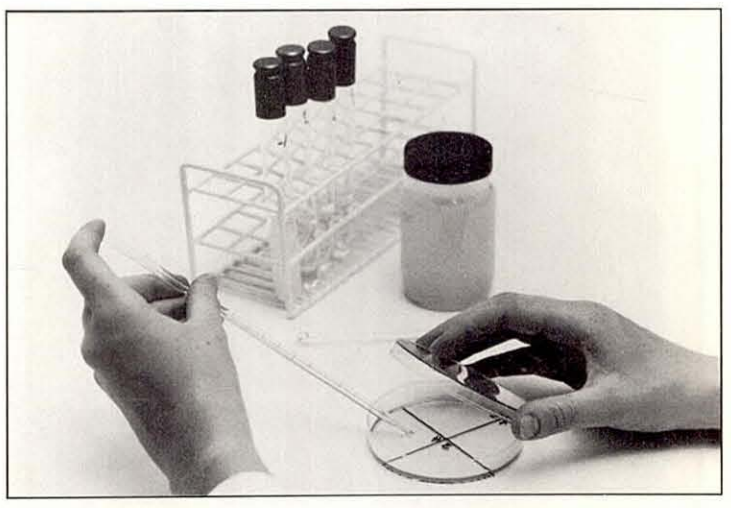

FIGURE 4. The monitoring of selective culture media using the Miles-Misra technique as modified by Slack & Weldon.[37] Pipettes are used to deliver 0.01 ml aliquots instead of droplets.

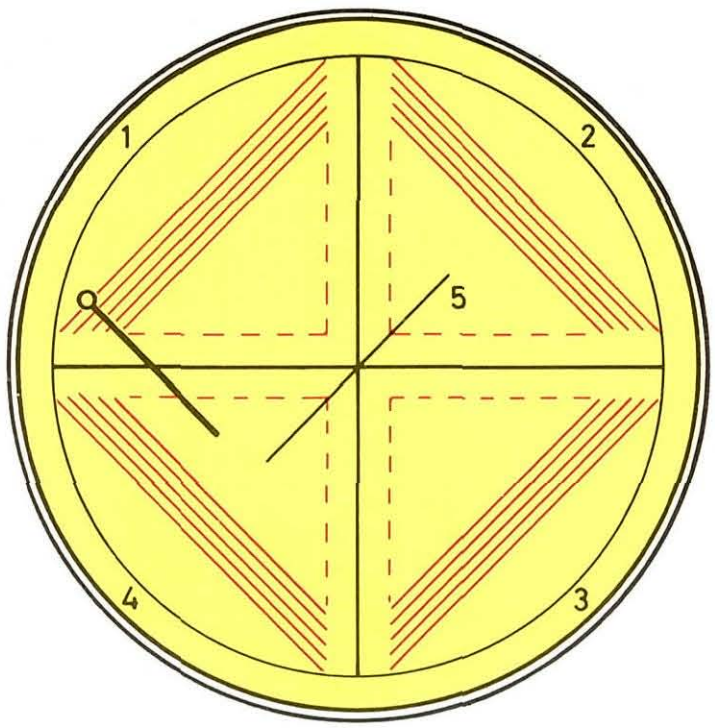

FIGURE 5. The 'ecometric' monitoring of culture media. Test plates are streak-inoculated with series of parallel lines (sectors 1–5) using test strains cultured in brain/heart infusion broth.

in the former area of Food Science so much later than elsewhere. Even so, with the increasing international trade in processed food products, the need for labour saving, rapid and more economical procedures in the microbiological monitoring of food is pressing. To date only relatively few techniques have been developed and validated. The progress made so far and further research needs will be briefly reviewed.

Enumeration Methods: first, a great many mechanized variants of *classical* procedures are now available. These include replacing poured plates by the inoculated droplet technique,[39] spread drop plates by mechanized inoculation equipment and the introduction of optical methods for colony counting.[40] Attempts in validation of these techniques have demonstrated that, not surprisingly, all that is required is improved and more reliable *engineering.*

Amongst the *non-conventional* techniques the following merit particular attention. The use of radioactive substrates, particularly glucose[41] and lysine[42] is certainly promising. However, it is clear that the use of reactive media that have been made selective is indispensable. The same applies to the use of the measurement of changes of impedance as a result of microbial proliferation.[43] Quite an amount of fundamental research remains to be done before this principle seems applicable to foods.

The use of the ATPase/luciferin bioluminescent method for assessing cfu levels of specific organisms in food is hampered by exactly the same lack of basic knowledge.[40] Finger-printing of broad classes of micro-organisms of significance in foods by automated determination of characteristic metabolites, such as pyruvic and lactic acid[44] and ethanol,[40] is well developed as far as chemical specificity is concerned; the ecological relevance of these methods has yet to be assessed in more detail.[44] Another biochemical method which shows some promise is the *Limulus* lysate endotoxin assay.[45] A striking lack of progress is noted in the area of immunological methods. Fluorescent antibody testing of salmonellae is quite well developed[46] and the use of ELISA for the detection of staphylococcal enterotoxins seems to be promising.[47] Yet, much more could and should be done in this quite versatile, classical area of microbial identification and enumeration.

Identification Techniques: given the drudgery involved in the preparation of endless series of media customarily used to assess the dissimilative and assimilative pattern of isolates with the purpose of their identification, it was not at all surprising that prefabricated aids would sooner or later appear on the market. From the vast series of publications in this area and our own experience it has emerged that two systems seem to be the most promising *ones for reasons of versatility and* information cost quotient.

The first is the use of micro plastic tubes with conventional media or substrates for enzymatic reactions, e.g. the API system. The second is the diffusion principle incorporated in the 'Minitek' system.[48] It should not be concealed that other techniques also show potential merits that require validation.

The API, Minitek and similar systems allow a tremendous saving of work and materials. However, they are fraught with one extremely dangerous 'trap'. We have recently observed that unless isolates are previously examined for the taxonomic key criteria morphology, catalase and oxidase reaction and mode of attack on glucose, grave errors may be made. However, this is in essence a matter of proper training of staff. This illustrates once more that post-graduate training in advanced Food Microbiology is another *desideratum* for the 1980s. The remaining minor problems in the area of labour-saving techniques require the attention of biochemists and, *once more engineers. They will* certainly be resolved within the coming decennium.

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Culture Media Ingredients

Bile and Bile Salts

Bile is a product of the liver. The composition of the yellow to green fluid varies according to its animal source and state of preservation. It contains bile pigments, bile salts, fatty acids and their salts, cholesterol, mucin, lecithin, inorganic salts, ethereal sulphates, glycuronic acids, urea and porphyrins. Fresh bile contains approximately 10% w/v solids. An analysis of ox bile expressed as percentage of total solids is as follows:

Glycocholic acid	37.3
Taurocholic acid	28.9
Taurodihydroxycholanic acids	7.1
Glycodihydroxycholanic acids	6.1
Unconjugated bile acids	Trace

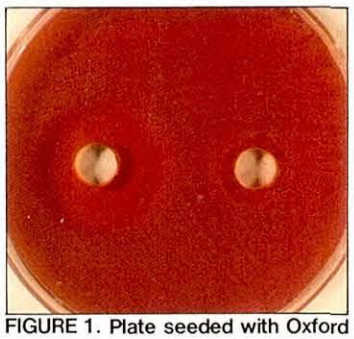
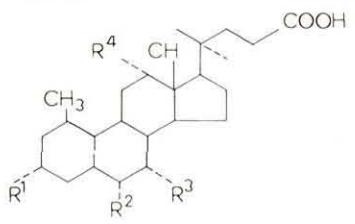


FIGURE 1. Plate seeded with Oxford strain of *Staph. aureus* showing large zone of inhibition in presence of more than 0.02% deoxycholic acid.

A *bile salt* is usually considered to be the sodium salt of a conjugated bile acid. A *conjugated bile acid* is a peptide formed from a *bile acid* and *glycine* (H_2NCH_2COOH) or *taurine* ($H_2NCH_2CH_2SO_3H$). As far as is known, all bile acids present in normal (fresh) animal bile are conjugated. The structure of bile acids may be represented as follows:



Acid	R¹	R²	R³	R⁴
Lithocholic	OH	H	H	H
Hyodeoxycholic	OH	OH	H	H
Chenodeoxycholic	OH	H	OH	H
Deoxycholic	OH	H	H	OH
Hyocholic	OH	OH	OH	H
Cholic	OH	H	OH	OH

The principal acids in ox and sheep bile are cholic and deoxycholic, whereas in pig bile they are hyodeoxycholic and hyocholic. Human bile contains large amounts of chenodeoxycholic acid. Acidification of an aqueous solution of bile salt will precipitate the conjugated bile acid. Alkaline hydrolysis of a bile salt or conjugated bile acid followed by acidification precipitates the bile acid.

Desiccated Bile

Freshly collected or preserved animal bile is collected at the abattoir, filtered, concentrated at low temperatures and dried. When reconstituted it should form clear solutions with a brown-green colour at 10% w/v concentration. This was the first bile additive used in culture media. Bile is a nutritive environment for a number of organisms and putrefaction quickly occurs in the abattoir unless it is chilled or preserved with chemical solutions. The bacterial spoilage leads to deconjugation of the bile salts and releases varying amounts of free bile acids. As these acids are more toxic to bacteria than the conjugates, there is considerable scope for variation in the toxicity of bile in culture media. The presence of bile pigments, which may cause confusion in media with indicator dyes, plus the variation in quality caused the search for more refined products.

Bile Salts

A further refinement in the use of bile is to precipitate the bile salts and separate them from the bile pigments and other substances present in crude bile.

The product bile salts, may be a mixture of conjugates and free bile acids, depending on the quality of the bile used in the process. Using thin-layer absorption chromatography (TLC) the following results were obtained:

Bile Acids	Oxoid Bile Salts	Difco Bile Salts
Cholic acid	+++	–
Unknown acid	–	+
Hyodeoxycholic acid	–	–
Chenodeoxycholic acid	–	+
Deoxycholic acid	+	–
Lithocholic acid	–	+
Taurocholic acid	+	–
Taurodeoxycholic acid	+	+
Glycocholic acid	+	–
Unknown	–	+
Glycochenodeoxycholic acid	+	+++

– no spot, + small spot, +++ large spot

(Northolt, M.D. (1972). *Anton. van Leeuwenhoek*, **38**, 632)

The presence of more than 0.02% w/w deoxycholic acid in bile or bile salts indicates a degree of toxicity that may be unacceptable in some culture media (Figure 1).

In Oxoid laboratories, strains of staphylococci are used as biological indicators of deoxycholic acid. Raw bile can be tested in 10% w/v solutions by diffusion techniques from wells cut into agar plates seeded with sensitive strains of staphylococci. Zones of inhibition up to 30 mm in diameter can be seen with highly "toxic" biles. When tested with TLC such biles usually show large "spots" of deoxycholate.

Although bile-containing media are formulated to suppress Gram-positive organisms and to select the Gram-negatives, bile salts (free from bile acids) will allow staphylococci and streptococci to grow. Particular advantage is taken of this characteristic in the U.K., where MacConkey Agar (e.g. Oxoid CM7) is used as a general purpose medium for clinical bacteriology. The growth of Group D streptococci and *Staphylococcus aureus* would be looked for on this medium when it is used for culture of urine, neonatal faeces and purulent material obtained from patients. To prevent the growth of such organisms and Gram-positive sporing rods, aniline dyes are often added to bile-containing media (e.g. Violet Red Bile Agar, Brilliant Green Bile Broth and Brilliant Green MacConkey Agar). Bile salts should be purchased as a fine, white powder and, when reconstituted as a 2% w/v solution in distilled water, should be a clear, bright, slightly yellow solution at pH 7.0. When incorporated into culture media, it should not affect the colour of the indicator dyes or their subsequent change in colour. It should not form a surface scum or deposit in the medium after storage.

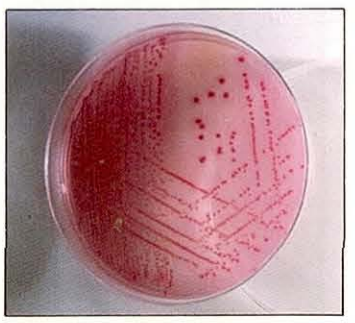


FIGURE 2. Example of bile salt halo around colonies of *E. coli.* on VRBA medium.

Bile Salts No. 3

It has been stressed that free bile acids may be considered too toxic for some organisms; nevertheless, there are circumstances in which free bile acids are deliberately chosen for inclusion in culture media. Such media are designed to be very selective and they may receive large quantities of commensal organisms in the inoculum. Bile Salts No. 3, bile salts mixture, etc., contain cholic and deoxycholic acid.

As opposed to normal concentrations of bile salts in culture media at 5 g/l, hydrolysed bile salts are used at 1.5 g/l.

A characteristic of bile acids in culture media is the precipitation of the acids as an opaque halo around the colony if the pH drops below a critical value. The growth of *E. coli* on Violet Red Bile Agar is indicated by the presence of red colonies (indicating lactose fermentation) and a purple halo (which indicates a greater production of organic acids than red colonies without the halo) (Figure 2).

One of the most important factors in the role of deoxycholate-citrate in culture media is the effect of magnesium. The activity of deoxycholate is enhanced in the absence of magnesium, and the role of citrate is to act as a chelate. The addition of magnesium to deoxycholate-citrate medium reduces the inhibitory effect and improves the growth of shigellae.

It can be seen from this brief description that bile is a complex highly variable selective agent. Only close chemical control of the raw material and subsequent processing can introduce standardization into the product.

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