7

Electrical methods

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7.1 Introduction: principles

The application of direct electrical methods in analytical microbiology has a long history, extending over 100 years when 'electricity' was being applied to every conceivable scientific problem. Stewart (1899) is generally credited with the first methods, derived from his physiological studies on the circulation of blood in man. However, it is apparent that while he identified some of the problems and proposed valid conclusions, he did not provide a rapid method, experiments extending for many days, and it is difficult with hindsight to decide what he was really measuring. But the principle remains.

The modern interest was rekindled in the 1970s when two instruments were described at the First Symposium on Rapid Methods and Automation in Microbiology and Immunology (Heden and Illeni, 1975). Within a short time there were three instruments competing in the market; now there are four – Bactometer, Malthus, RABIT and BacTrac, each with unique characteristics, operating systems and data presentation but capable of handling multiple samples simultaneously. These electrical methods are concerned with measuring the response of microbial cultures to alternating electrical current (AC) at specific frequencies. There were some developments using direct electrical current (DC) but these were not commercialized. With high currents, the growth medium electrolyses and kills the microbes, and at low currents, mediators such as lipoic acid are needed and the developments have proceeded as biosensors. All types require electrodes to be immersed in the growth medium or food homogenate.

The parameters of importance are impedance and conductance, either alone or in combination. Put simply, impedance is the AC equivalent of resistance to a

DC current. At any frequency (cycles per second, Hz), impedance has two components: capacitance and conductance. Capacitance relates more to the properties and changes at the electrode and conductance to the solution or gel between the electrodes. Resistivity is the reciprocal of conductance. Pure water and solutions of salts have no capacitance and so their impedance is solely conductance. Most microbiological media have capacitance due to their macromolecular content as well as contributions from the inoculum such as food. Changes in impedance are a measure more of the change in biomass in the assay, and conductance changes are due to the effects of metabolism on the charge-carrying capacity of the medium. Large molecules are broken down to small ones which are much better charge carriers; for example amines and ammonia from proteins, lactic acid from carbohydrates, polymers reduced to monomers.

Each growth medium therefore has an impedance value that depends on its composition of defined chemicals and components, and the composition of the inoculum. Salts have a major effect and the operating range of every type of instrument is limited by lack of response to 'high salt' media, and salty foods if not diluted. As some conventional media incorporate high salt levels as selective agents, indirect methods have to be used. Impedance has a high temperature coefficient, about 2 per cent per degree. For sensitive and therefore fast responses to any change, the assay temperature has to be controlled. Manufacturers have different solutions. Bactometer use an uninoculated control alongside the test and measure both. Malthus and RABIT, derived from the same original work and patent, control temperature to within a few thousandths of a degree and make an absolute measurement. BacTrac use four electrodes (terminals) two of which act as controls. All instruments take readings of their chosen parameter(s) every few minutes and store the data or manipulated data on a computer.

All instruments are simple to operate. Microbiologically, they have the unique advantage over other technologies as being hands on/walk away. An extract is prepared from the food source, placed in the test cell with media, and the instrument takes regular readings and announces when the criteria for a positive have been exceeded. They are very economical with trained staff time and effort. They have been on the market for more than 25 years but have not had the uptake experienced by equivalent instruments in automated chemistry. The reasons for this are obscure even allowing for the perceived lack of reliability of early machines when continuous performance of both the 'microbiological' and 'computer' parts of the instruments were pushing the design limits of the technologies. There was an innate resistance of microbiologists to new technologies and industry was not ready for significant capital expenditure in microbiology testing laboratories, being accustomed only to high recurrent costs on media and consumables. There was also the problem of acceptability of the data. Users wanted methods to be approved but were unwilling to participate in the approval system as they faced the costs of running parallel assays, the novel and their conventional assays, and the additional labour was not available.

Despite these problems, electrical tests are probably the most used automated systems in applied microbiology and have been granted approval by most of the leading method accreditation organizations worldwide and are also accepted by laboratory accreditation bodies.

Important reviews include articles by Bolton and Gibson (1994), Silley and Forsythe (1996) and Gibson (2001).

7.2 Instruments

Bactometer (bioMerieux UK Ltd, Grafton Way, Basingstoke RG22 6HY, UK) was one of the first on the market. It was described at the First Symposium on Rapid Methods and Automation in Microbiology and Immunology, Stockholm, 1973 (Cady, 1975) along with the Biobridge (Ur and Brown, 1975), which is no longer produced. It measures overall impedance, capacitance and/or conductance. Samples are held in disposable plastic modules holding eight 2 ml assays and control samples in air incubators. The frequency used for measurement, 2 kHz, favours capacitance more than conductance and so electrode effects predominate. This gives an advantage over other instruments for detecting yeasts and moulds, but in the present context, as pathogenic fungi are rarely measured in foods (their toxins are assayed chemically), there is no advantage.

Malthus (Malthus Instruments Ltd, IDG plc, Topley House, 52 Wash Lane, Bury BL9 6AU, UK) developed from work carried out at the Torry Research Station, Aberdeen and the University of Aberdeen, UK (Richards *et al*., 1978). The author was part of the team. The work derived from experience devising and developing electrical gadgets for measuring fish quality. The Malthus measures purely conductance, minimizing contributions from capacitance by the choice of frequency (10 kHz), and the samples are held in water incubators whose temperature is controlled accurately to within ±0.005ºC. While capacitance was minimized by the use of platinum electrodes in the prototypes (and inert metals in production models), experimental data showed that the conductance changed sooner than capacitance in assays after inoculation with extracts containing bacteria, and conductance was not affected by debris from the inoculum. The fish quality meters use four-terminal electrodes but it was decided that for the size of the changes in conductance of interest there was no advantage and it introduced unnecessary complications to the instrumentation.

The RABIT (Rapid Automated Bacterial Impedance Technique, Don Whitley Scientific Ltd, 14 Otley Road, Shipley BD17 7SE, UK) is also based on the Aberdeen work. It uses solid metal block incubators instead of the water baths and close fitting electrode cells to maintain good contact with the metal block. It measures at 2 kHz and thus has more capacitance included than the Malthus.

The BacTrac (Sy Lab GMBH, Purkensdorf, Austria) uses four-terminal measurements and offers medium and electrode impedance measurements but does not reveal the electrode composition. They also have a small capacity conductance instrument.

All instruments can handle multiple samples simultaneously – up to 500 – with free access to the incubator and thus infinite start times for each assay, or in the case of the Bactometer each module of eight assays. The integral computers display the status of each assay and also when assays have exceeded the parameters chosen for a positive result or when the time limit for a negative result has been exceeded.

A major advantage of these electrical methods is that they do not require optically clear inocula or media. They can make measurements on cloudy solutions containing particles such as hamburger homogenates without any clarification. The only dilution factor is that required to reduce any antimicrobial factors in the foodstuff, for example as with fresh milk or blood. For example, large quantities, representing 10–20 g of food and 90 ml of liquids, can be inoculated into large Malthus bottles. Where debris might coat electrodes as with the Bactometer and RABIT where the electrodes protrude from the base, the electrodes can be coated with a weak agar gel without affecting the speed of response. At the end of the assay, the test cell can be sampled for further confirmation tests. Indeed, one manufacturer of molecular methods praised the electrical methods to the author as a means of screening out negatives before using expensive reagents for further studies.

7.3 Data presentation

The electrical parameter selected is initially presented as a plot of the change in parameter with time. Data gathered in the first 20–30 min of the assay are often discarded as the assay solution is equilibrating to the set temperature of the incubator. The plot resembles a growth curve but with many more points than is possible by traditional microbiological techniques. Numerous studies have shown good correlations between the microbial count of cultures and the conductance and impedance changes with time since inoculation. Thus, the electrical methods are regarded as an alternative to counts methods. A critical parameter is the point on the curve where it deviates from the baseline. Ideally, baselines are linear and flat, but a certain amount of deviation is tolerated, based on experience with the assay. When viewing a complete growth curve it is easy to see, in retrospect, when the curve deviated and this point is termed the 'detection time', the time when growth was first reliably detected. Detection times can be defined empirically, for example when there has been a change in conductance or >10 μS after the first reading following temperature equilibration. Plots of detection times against the logarithm of microbial numbers in the inocula are linear. Thus after calibrating the tests against conventional microbiology, detection times can be used as a measure of numbers or counts. This has been shown to have wide ranging validity and the reproducibility of the electrical assay is better as there is less sample manipulation, for example as there is no need to prepare a dilution series.

For assays in selective media after a pre-enrichment stage, the recoding of a detection time means that the target microorganism is present. It is not possible to relate the count at detection time to the numbers of microbes in the original food sample, but there is a normal relationship to the numbers inoculated from the pre-enrichment. When large volume flasks are used, low numbers of bacteria can be detected – perhaps 1 microbe per inoculum which can be >10 g. As mentioned earlier, positive cultures are often subjected to confirmation tests. Using cultures from electrical tests, the physiological state of the culture can be estimated from the progress of the growth curve, and the cell concentration estimated. This can be useful for some tests when it is useful to know that the sample taken is not dead. Stationary cultures containing many dead cells or cells coated in slime may give poor results in some secondary tests.

7.4 Pathogen assays: introduction

Electrical assays are growth assays and growth depends on the culture obtaining nutrients and energy from the medium and assay environment. Thus all assays are based on traditional methods and conventional information about the growth requirements and characteristics of the culture. The difference lies in the interpretation of the knowledge base. In conventional microbiology, the end point is usually visual detection of a colony on the surface of an agar plate or in the medium, or turbidity of a broth. In the former, clearly separated, well-defined colonies are the aim, with little interference from debris from the food homogenate. In electrical assays, the only consideration is good metabolism of electrically neutral substrates into charge carriers. Dehydrated media of a single type can yield quite different conductance responses from a named organism that has the same colony appearance. Not all peptones and tryptones are the same and media manufacturers do select particular batches for particular purposes or for defined media. This is known in the microbiology community when aliquots from the same batch of media are often distributed to participants in collaborative trials. One UK manufacturer tests media components for conductance response even when the end use is conventional microbiology as it was found that uniformity from production batch to batch was enhanced. With the introduction of ISO 17025 for laboratory media, manufacturers can take the responsibility for quality control of batches of media and so divert lots with good electrical responses to customers with conductance/impedance instruments.

The initial capacitance and/or conductance reading of the media in the test cell must be within the measuring range of the instrument. This is a limiting factor as some assays for *Salmonella, Listeria, Staphylococcus*, etc. utilize media with high salt contents. It is known from physico-chemical studies that the relationship between chemical concentration and conductivity is better at low concentrations (approaching infinite dilution) than at high where doubling the concentration of an ion has little effect. The solution to this problem has been the development of indirect conductance methods (Owens *et al.*, 1989).

Table 7.1 Conductance and impedance methods for pathogens associated with foods

Microorganism	Reference
Salmonella spp.	Easter and Gibson (1985) Gibson (1987b) Arnott *et al*. (1988) Ogden (1988) Pugh *et al*. (1988) Bullock and Frodsham (1989) Pless *et al*. (1995) Quinn *et al*. (1995)
Escherichia coli and coliforms	Gibson *et al*. (1984) Dupont *et al*. (1994) Colquhoun *et al*. (1995) Dupont *et al*. (1996) Timms *et al*. (1996) Gibson (1997) Edminston and Russell (1999, 2000)
Enterobacteriaceae	Petitt (1983) Wood and Williams (2000)
Clostridium spp.	Gibson (1987a) Dromigny *et al*. (1997)
Listeria spp.	Philips and Griffiths (1989) Bolton and Gibson (1994)
Faecal streptococci	Neaves *et al*. (1988)

During metabolism, all cells produce CO_2 (and water). The first CO_2 produced dissolves in the medium. As the concentration increases, there is soon a significant level in the headspace above the culture medium and in equilibrium with it. In indirect conductance, the electrodes are placed in an alkaline solution within the assay so that the CO_2 can diffuse into the solution, and react to form carbonates, which produce a change in conductance. In theory, every conventional medium could be used as the primary medium and growth detected in the secondary medium. In practice users prefer the direct assay.

The most widely used tests for pathogens and indicator organisms in foods are those for *Salmonella* spp., *Enterobacteriaceae*, *Escherichia coli* and coliforms, vibrios, *Listeria* spp., and to some extent *Campylobacter* spp. The scope of the assays for pathogens is shown in Table 7.1. The performance of many of the media discussed here are given by Bolton and Gibson (1994).

7.5 Assays for *Salmonella*

There have been many assays devised for *Salmonella* spp. in foods: indeed they have been the driving force for the adoption of the conductance methods because the economic case for investment in instruments was easier to justify than for

other organisms. Also, the reduction in assay time was significant and made salmonella testing worthwhile as recalls based on positive results were feasible and could be complete before the food had reached the retailers and been consumed. The assays are based on more traditional media than some of the current popular growth media because they can contain either active compounds that do not give much of a conduction/impedance change or ion concentrations fall outside the range of measurement of the instruments. The sensitivity and assay times for conductance assays are on a par with those using molecular methods especially when the time for initial cultures are taken into consideration (Gibson, 1998).

7.5.1 Easter-Gibson medium

Easter and Gibson (1985) devised their medium based on their work on fish spoilage. They had noted that trimethylamine-N-oxide, an osmoregulator in fish, acted as an electron acceptor during the growth of bacteria, being reduced to trimethylamine, which has the odour of stale fish, and that this reduction was accompanied by a large change in conductance. The substrate is a neutral compound with no net charge and the product is as basic as ammonia. Trimethylamine-N-oxide was added to many commercially available media. These were inoculated with *Salmonella* spp., recent isolates from foods in a local public health laboratory, and their electrical responses in a prototype Malthus recorded. By far the best response was obtained with Liefson's selenite-cysteine medium and this was further developed. The recipe is shown in Table 7.2. Conductance changes of >700 μS were obtained; in development work for spoilage bacteria, changes of ~100 μS were regarded as 'good'. Many other strains of bacteria from the same source were tested; the only false positives were from some strains of *Citrobacter freundi*. As it can cause some gastrointestinal infection, it was not regarded as a serious false positive.

Liefson used 0.2, 0.4 and 0.8 per cent sodium selenite in his medium depending on the source of the sample. While Easter and Gibson found that 0.4

Table 7.2 Recipe for Easter and Gibson's *Salmonella* detection medium

Ingredient	Quantity per litre
Peptone	5
$Na_2HPO_4.2H_2O$	10
Dulcitol	5
Trimethylamine oxide dihydrate*	5.6
Sodium biselenite	4

The pH is adjusted to pH 7.2. The medium is steamed for 10 min. Immediately before use, 10 ml of L-cystine stock (0.1 g in 15 ml N-NaOH made up to 100 ml with water) is added to each litre.
* Originally, the hydrochloride salt was used. It was extremely acidic and caused problems if technicians forgot to adjust the pH of the medium. The dihydrate is now widely available and is neutral.

per cent selenite was best in their studies, they said that users should test the other concentrations for particular applications. One major factor is the concentration of phosphate as Liefson had shown that the toxicity of selenite was greatly increased in the presence of phosphate. Besides the presence of phosphate in some foods, even the fortuitous addition of phosphate in buffers used in sample preparation can have an effect making the medium toxic even for *Salmonella* spp.

The source of the peptone can have an effect on the response of the medium. Easter and Gibson originally used a batch of Evan's peptone and obtained excellent assay results. Media manufacturers had some difficulty reproducing the responses and had to test many batches of peptones to reproduce the results. For traditional growth assays where the endpoint is a visible colony on the surface of a plate or turbidity in a broth, the precise peptone used is usually of little importance. But in electrical assays, careful selection is important and users may well find that it is better to buy commercially prepared media rather than to make it in their own laboratory. The author has since heard that media manufacturers put considerable effort into the preparation and selection of peptones for specific media and that the lack of reproducibility of results with published media may be due to the particular properties of the constituents of the medium used in the originators' laboratory. Under the provisions of ISO 17025, manufacturers can ensure the quality of complete batches of media reducing the need for quality control checks by the user.

Easter and Gibson's medium has been modified. Gibson (1987b) substituted mannitol for dulcitol with excellent results. Dulcitol-negative strains of *Salmonella* spp. are very rare. Mannitol is a better inducer of the enzymes of dulcitol metabolism than dulcitol itself. He also used dimethylsulphoxide as electron acceptor. This was a very efficient and a cheap ingredient but the odour of the endproduct, dimethyl sulphide, proved to be too obnoxious for general use.

Some customers reported to media suppliers some false positives from some products, especially some meats. Antibiotic mixes have been devised to reduce their incidence, rather than modifying the medium by varying the concentration of selenite or the selenite/phosphate ratio. An example is given in Table 7.3. Such mixtures are better obtained from suppliers than prepared in the user's laboratory as testing their selectivity and stability can be onerous.

Positive results from pre-enriched cultures are often seen after 6 h incubation. Generally, incubation times of 16 h are used with a maximum of 30 h. A positive result after 48 h was reported to the author some years ago. Extended incubation

Table 7.3 Selective agents used in *Salmonella* media

Novobiocin	0.01 mg/ml
Cefsulodin	Not disclosed
Additional sodium selenite	1–2 g/l

times are generally not productive and better use can be made of the instrument by increasing the number of samples tested.

The medium is not toxic to *Salmonella* spp., after growth is complete. Subcultures can be taken for further testing. In the early days, technicians were reluctant to report a positive result without carrying out confirmation tests in case they activated an expensive product recall in error. With the adoption of the precautionary principle, this may change especially as there are rapid confirmatory tests on the market.

7.5.2 Ogden's lysine decarboxylase test

Ogden (1988) developed his medium to be complementary to the Easter-Gibson medium especially with respect to dulcitol-negative strains and citrobacters. At the time a few strains of the former had been reported although the published incidence was <1:10000. His medium is shown in Table 7.4. It also uses selenite as selective agent. The conductance response is proportional to the lysine concentration. While the recipe appears simple, this medium has proved difficult to make commercially (and in other laboratories) for unknown reasons. The author is not aware of any instances where this medium has yielded a positive result when the result with the Easter-Gibson medium has been negative. However, as it is part of the protocols for approved methods (see later) it has to be used.

7.5.3 Bullock and Frodsham's LINCR medium

Bullock and Frodsham (1989) devised a medium for detecting *Salmonella* spp. in confectionery (Table 7.4). It was based on an existing medium. While somewhat empirical, it is likely that some of the ingredients are useful in

Table 7.4 Composition (g/l) of lysine-based media for *Salmonella*

	Ogden (1988)	Bullock and Frodsham (1989)	Arnott *et al.* (1988)
Lactalbumin hydrolysate	5		
Glucose	10		1
L-lysine HCl	10	10	5
Na biselenite	4		0.8
Yeast extract		3	3
Tryptone		5	
L-cystine		0.1	
Mannitol		5	
Salicin		1	
Ferric ammonium citrate		0.5	
Nathiosulphate		0.1	
Neutral red		0.025	
Mandelic acid (0.9 mg/l)		1	
Novobiocin		0.15 μg/ml final	

neutralizing inhibitors in the foods and being substrates for the microorganisms not readily available in the specialized test material. It contains an antibiotic to reduce the incidence of false positives, thought to be citrobacters from dairy products.

7.5.4 Arnott's lysine medium

Also working with confectionery, Arnott *et al*. (1988) published a lysine medium. They used it alongside Easter and Gibson's medium and the combination gave more true positive isolates from their test materials than conventional methods and in a third of the assay time.

7.6 Assays for *Enterobacteriaceae, Escherichia coli* and coliforms

This family, species and group of microorganisms probably represent the largest field of testing by electrical methods. They include the specific species and variants as well as the generic 'indicator' organisms. Indicators have been used for two main reasons, firstly because the test for the groups they indicate are long and cumbersome, and secondly because they give quick results. Rapid methods obviate the latter. As coliform tests were originally used in place of, *inter alia*, *Salmonella* tests, conductance/impedance assays now permit as fast reporting of results as conventional assays.

Many of the testing methods for these organisms lead to definitions of the hierarchy rather than, as logic would have it, the other way round. Thus, coliforms and faecal coliforms are defined, even in EU Directives, in terms of their response on and in certain media under fixed times and temperatures of incubation. This led to some spurious correlations in some early publications on comparison of results from electrical and conventional assays done at different temperatures with different growth enhancers and inhibitors present. As different microorganisms would be selected and predominate in the assays, the correlations reported would be fortuitous and could not be reproduced in other laboratories. The EU now allow for methods regarded as equivalent to the old conventional assays to be used but have not yet set the criteria for comparisons.

Inhibitors are used in media to improve the selectivity of the assay. While they have a great effect on non-target bacteria, it is often forgotten that they can also have a minor effect on the bacteria of interest and slow their growth. The assays that have proved worthwhile for the enterics are those that select the fastest growing bacteria by inclusion of the substrates most favoured by them and those with specific inhibitors. For true rapid methods, the former is the more scientific approach. Generally, to negate this approach, very high numbers of competing bacteria need to be present. In a processed product where only low numbers of bacteria are expected, this is an additional virtue rather than a drawback of the method.

Many researchers have adapted conventional media recipes to suit the products they are interested in. All manufacturers produce or recommend a 'coliform' medium. While conventional media rely on carbohydrate metabolism to produce colour changes, the electrical responses are generally due to the metabolism of specific peptones specially chosen to give a good response in the presence of some selective agents. There are no reports comparing these media either with one or all instruments. Details of the media can easily be obtained via the Internet (search for 'manufacturer's name + coliforms' and their website).

For many foods, the enteric count is done by MPN methods, as this is the only statistically accepted manner of testing where the population is large and the expected numbers of microorganisms is low. Grades are set with reference to MPN tables. Electrical methods are calibrated to give numbers per unit volume, except when pre-enrichments have been done. Because large inoculum volumes can be used in some electrical assays, for example the Malthus 100 ml test cell, there is no need for MPN techniques. Good correlations between such assays and MPN grades have been found (Gibson, 1997).

A complicating factor in comparing methods is the need to allow enterics to recover from 'injury, caused by freezing, heating, etc.' Most of the enteric assays are done under reduced oxygen conditions. These favour recovery of such bacteria, especially when compared with highly aerobic assays such as plate assays in the presence of inhibitors, and the electrical assays can give higher recoveries than conventional approaches such that the actual correlations between methods may appear to be lower.

Some examples of the kind of methods and results are described in more detail below. Some very good protocols have been presented at scientific meetings but not fully published and so cannot be given here. However, with the long experience of many users, it is clear that the electrical methods perform very well with a wide range of foods, both raw and processed, and the limiting factors are sample size and microbe distribution, and the limitations of the comparative conventional method.

7.6.1 IFREMER system for *Escherichia coli* in shellfish

The French authority for the bacteriological quality of bivalve shellfish, IFREMER, has developed a procedure to test for compliance with EU Directive EEC 492/1993 for *E. coli*. The Directive mentions the MPN technique but allows for the use of any method shown to be equivalent. Dupont *et al*. (1994) first developed a protocol using the Malthus Coliform Broth and showed that the medium had the required specificity and sensitivity. There were some potential problems when cells were inoculated with high numbers of *Klebsiella* spp. but these would not be a concern as shellfish producers would not want products with that contamination. The team then compared the results found on actual samples by conductance assay and with their own in-house three-tube MPN method (brilliant green lactose bile broth) (Dupont *et al*., 1996). For all shellfish,

the correlation was $r = -0.968$. The sensitivities of the methods were similar and the conductance method showed better repeatability than the MPN. The specificity for the Malthus method was excellent; 96 per cent of the positive responses were due to *E.coli* and only 0.7 per cent of them failed to respond within 20 h, quite acceptable within the limitations of conventional microbiology and the sampling schemes used. Results were obtained within 5–9 h, compared with 3 days for the MPN method, and from the food safety point of view, batches containing significant numbers of *E.coli* could be intercepted before reaching markets. IFREMER has sited a considerable number of conductance instruments around the coast of France for regulating the shellfish industry and there is anecdotal evidence that the bacteriological safety of such shellfish to the consumer has improved.

7.6.2 Inter-laboratory study

As a result of the IFREMER study, the EU funded a project on shellfish testing by various rapid methods involving nine laboratories from eight countries (Ogden *et al*., 1998). Statistical analysis was not straightforward as the conductance method detected more confirmed positive samples than the MPN, but in general the results confirmed that conductance methods were satisfactory for enforcement of the EU Directive.

7.6.3 Specific assays for *E. coli*

Ogden (1993) developed a specific assay for all *E. coli* including the entero-haemorrhagic O157 strains that do not give conventional responses and detection on standard plated media. He utilized their ability to ferment D-glucuronic acid, a compound and test that is part of the taxonomic definition of the species. He included trimethylamine-N-oxide as electron acceptor as its reduction gives a large and rapid conductance change (Table 7.5). Only one strain tested failed to give the normal response and some *Citrobacter* spp. gave false positive responses in respect of the total conductance change but not the rate of change. As mentioned earlier, manufacturers select batches of peptones that give good conductance responses. In the author's experience, peptones with molecular weights of 10,000–20,000 with a high proportion of neutral amino acid residues have been best. In this medium, Ogden uses lactalbumin hydrolysate, perhaps an expensive component but one that gives a good response for rapid growth. Ogden *et al.* (2000) have also increased the sensitivity of their assay by concentrating *E. coli* O157 from large volumes using immunomagnetic beads.

Edminston and Russell (2000) have assessed the specificity of the Bactometer *E. coli* method for counting the bacteria on fresh chicken carcasses in the presence of genetically similar species. When incubated at 44ºC, *C. freudii* did not grow and only high numbers (>106) of *S. enteritidis* and of *Shigella sonnei* would interfere with the enumeration. As such numbers are highly unlikely to be

Table 7.5 Ogden's GT conductance medium for *E. coli* (g/l)

Lactalbumin hydrolysate	5
Trimethylamine-N-oxide.$2H_2O$	5
NaCl	5
K_2HPO_4	1
$MgSO_4.7H_2O$	1
NH_4Cl	0.5
Na D-glucuronic acid	5

found in poultry samples and should result in rejection of the lot, *E. coli* detection and enumeration on poultry by conductance methods was regarded as a satisfactory method.

There have been reports of additions to conductance media to give optical changes useful for confirmation of the species present. Thus methyl umbelliferyl glucuronide has been added as an assay for β-glucuronidase. This approach can be developed further.

7.7 Assays for other pathogens

7.7.1 *Listeria*

There have been many attempts to develop media for testing foods for *Listeria* spp., in particular *L. monocytogenes*. A major difficulty has been the transition from laboratory studies with pure cultures to assays on real foods, preferably naturally contaminated but more usually spiked, as the methods used in the conventional microbiological tests for comparison are problematic. The indirect conductance method has been used with some success by Bolton (1990) as some of the more recently proposed conventional media use high concentrations of salts as selective agents such that the media are outside the normal measurement range of the instruments. Also some media have included a mixture of antibiotics; these work well with pure laboratory cultures but the presence of foods or food extracts can affect the results.

One successful approach was that of Bolton for Malthus. From the taxonomic descriptions of listeria, he incorporated hippuric acid as the key differentiation substrate of the medium with excellent results, both in the microbiological and conductance sense. Bolton and Gibson (1994) discuss the results further. In brief the results from electrical assays are as good as those from conventional tests in that no single method seems able to distinguish all positives, probably in part due to the low numbers of listeria in food samples and their irregular distribution. However, the 'hands off' approach of the electrical methods allow for far more samples to be analysed and the resulting cultures are ideal for confirmatory tests, both conventional and molecular biological.

7.7.2 Campylobacter

All manufacturers offer media for campylobacters. One problem is the lack of an approved method for comparison. Another is the use of special gas atmospheres. Bolton found that when he filled the Malthus tubes to the brim with his Preston medium, the medium contained suitable levels of the required gases. His experience, and that of others, is that the conductance methods are suitable for screening foods for campylobacters and give more positives, later confirmed by alternative means, than conventional testing. The protocol is given by Bolton and Gibson (1994).

7.7.3 *Clostridia*

Gibson (1987a) showed that the growth of *Clostridium botulinum* in pork slurries could be monitored using conductance measurements. Dromigny *et al*. (1997) devised a medium for detecting clostridia in foods. One problem encountered by many researchers is that the signal breaks up due to the copious production of gas bubbles by some clostridia. This means that there is not a typical growth curve. As the production of gas in the selective media is a characteristic of these microbes, the time taken to break up of signal is a measure of the numbers present and occurs sooner in the assay than the visible appearance of gas.

7.7.4 Vibrios

Gibson *et al*. (1984) showed that vibrios were detected in low salt TCBS ahead of any enteric bacteria. By virtue of their extremely rapid growth relative to any competing bacteria, they were easily detected. They ran the assays along with a coliform assay. When the detection times were shorter in the coliform assay than the TCBS tube, coliforms were present and not vibrios. It should be remembered that many of the differential media are not wholly selective for a particular species of group, and detection within a specified time can give excellent screening results.

7.7.5 *Yersinia*

While *Yersinia* are not normally assayed in foods except for epidemiological purposes, Walker's (1989) medium is noteworthy in that hydrolysis of urea is a key feature and this reaction gives a relatively large conductance change, the urea having no charge and the ammonia a very high charge. The trimethylamine-N-oxide/trimethylamine couple gives a greater change.

7.7.6 Gram positive bacteria

Electrical assays have not been so well developed and used for Gram positives. For example, none of the traditional assays for *Staphylococcus aureus* have

really converted well to conductance assays but some are slightly better in impedance assays. Work needs to be done on determining the best concentration(s) of selective agent(s) and inhibitors of competing flora. However, with near sterile products where any contamination is likely to be *Stap. aureus* suitable assays have been devised. The problem lies with the lack of a universal medium for general food use and this was also the case for many years with traditional approaches.

Provided that their metabolism can be tweaked to give copious amounts of CO_2, the indirect method should be suitable for detecting most microbes. In practice, it seems that the bacteria with the most active decarboxylases are those easily detected by direct assay.

7.8 Accreditation of electrical methods

The Malthus system for *Salmonella* spp. in all foods was the first pathogen assay using electrical methods to obtain an international approval, in this case from the AOAC International, the independent US approvals body (Gibson *et al*., 1992), and Final Action Status was later granted on the basis that there was satisfaction and no complaints from users. This was a landmark, in that it was the first automated hands-off system to be approved. The approval is ring-fenced and applies only to the Malthus system. It involves using the two media, TMAO-dulcitol-based and Ogden's lysine media after pre-enrichment. Positive results require, for AOAC purposes, to be confirmed by their conventional procedures. In retrospect, a single medium assay is all that is needed. Because there were a few reports at the time of dulcitol-negative salmonellas, the company played safe by using the lysine medium in tandem. Since then, there have been so few isolates of such bacteria, that it would be better to use the capacity of the instruments to double the number of samples as the heterogeneity of the distribution of salmonellas in food is a greater risk for missing them than the occurrence of a dulcitol-negative strain.

The *Salmonella* system has since been approved for all conductance assays by the International Standards Organization (ISO) and many of its component bodies. The International Dairy Federation (IDF) ran a large trial with instruments from different manufacturers and gave the assay approval. Some details of the trials have been published (Prentice *et al*., 1990). The shellfish assays for enteric bacteria are used by the official bodies for monitoring bivalves in France. Many laboratories using electrical methods have been granted accredited status by their national authorities. In addition, such laboratories are normally members of proficiency testing programmes and their results on distributed samples have been within the norm expected.

7.9 Conclusions and future trends

Of all the rapid automated methods in food microbiology the electrical methods have come of age. They are accepted by their users and their customers and the brand names are quoted in scientific conversation as understood by all. There has been a dearth of recent publications describing methods but this is mainly due to the maturity of the existing methods as practised in thousands of laboratories worldwide servicing the public demand for safe food. The plot of the change of conductance with time can yield a wealth of information. Jason and Jason (1998) have devised algorithms to identify species and subspecies from the data from *E.coli* growth curves. This approach – detection and identification in a single assay – deserves further study. Linkage with other evolving technologies both at the preliminary stages of the assays such as separating and concentrating the target microorganisms with selective magnetic beads, and confirmation of positive screening assays with molecular biological methods are to be encouraged. No other hands-off screening method yields such a mass of cells for further work.

7.10 References

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