

Sampling plans for situations involving direct hazard from pathogens

Judgments about hazards from pathogens are based largely on bacteriological analysis, but the results of such analysis, and conclusions derived therefrom, depend on the sensitivity of the method used.

Early methods of detecting *Salmonella* in faeces or blood of a diseased person were not really suitable for food analysis. When more sensitive or discriminating media were used, some foods previously thought to be generally free of *Salmonella* frequently proved to be contaminated. Highly sensitive methods have not yet been developed for *Vibrio cholerae*, *Shigella dysenteriae*, several zoonotic bacterial pathogens, or viruses in foods. Hence, opinions on frequency of contamination by such species may need review as more sensitive procedures become available or if more 'durable' strains arise, (e.g., the El-Tor strain of *V. cholerae*).

The sensitivity of examination for *Salmonella* has been improved further by increasing the quantity of material actually examined. Previously, it was customary to examine samples of food weighing 1 g, or 10 g; now it is usual to examine 25 g, or 50 g, occasionally even more. The importance of this change will become evident below.

A. THE IDEA OF ZERO TOLERANCE

In the past, it has been customary to specify that pathogens 'shall be absent' from foods, and the Commission proposed that for them no numerical tolerance should be expressed. At least three considerations challenge this ideal:

(i) No feasible sampling plan can ensure complete absence of a particular organism (Chapter 5, Section L). Even when $c = 0$ (i.e.,

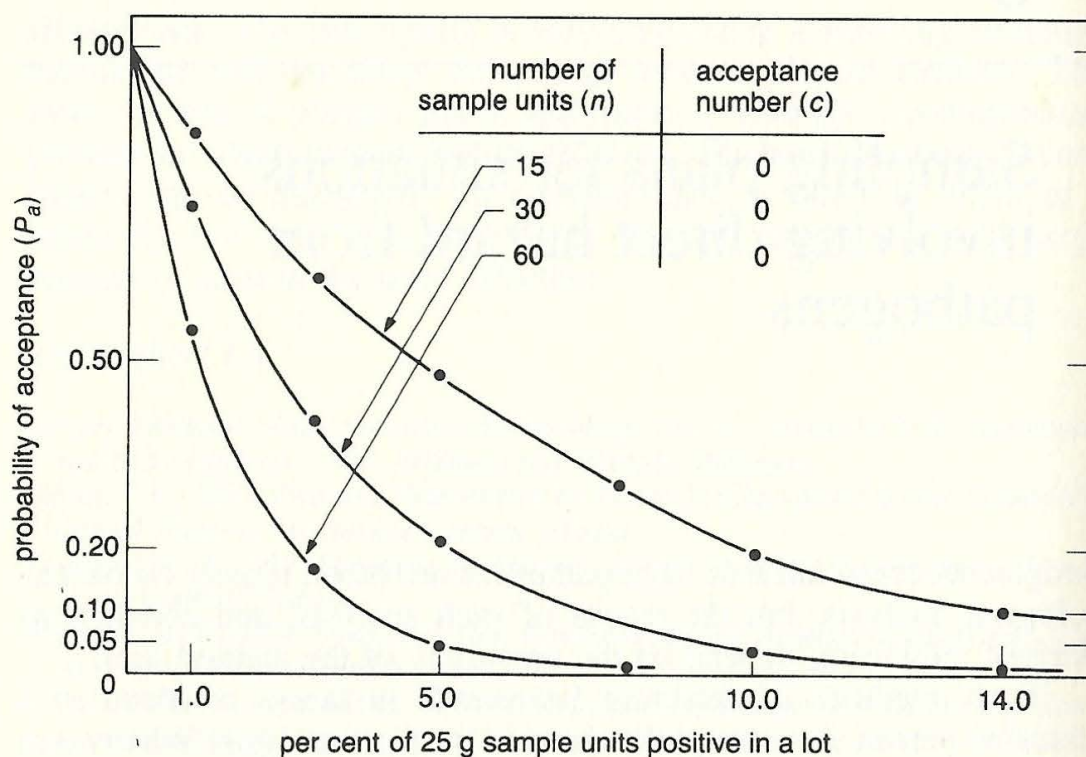


Figure 5 Relations between the sampling plan and the degree of security conferred – OC curves for three sampling plans proposed for *Salmonella*

the lot will be rejected if the pathogen is found in any sample examined), it cannot be guaranteed that the lot is completely free of the organism, no matter how large the number of sample units n . However, what can be found is the probability of acceptance (P_a) for lots of various qualities, as a function of the given n and c . (For example, $n = 60$, $c = 0$ has $P_a = 0.5$, i.e., one chance in two of being accepted, for lots in which 1% of the sample units are defective, i.e., $p = 0.01$; see Fig. 5.)

(ii) Plans in which $c = 0$ are not necessarily the most exacting. For example, if one sets a limit of 5% defectives in a lot, the plan $n = 95$, $c = 1$ will accept 'bad' lots less often, and 'good' lots more often, than will $n = 60$, $c = 0$. In other words, the former plan is the more discriminating, even though it admits the presence of the pathogen. Preference for $c = 0$ is influenced by the wish to emphasize that absence is the desired objective (although it cannot be guaranteed), and by the knowledge that pathogens such as *Salmonella* may be found by direct examination of certain foods and, having been demonstrated, are difficult to overlook.

(iii) It is not yet commercially possible to market some foods

completely without pathogens. For example, most raw meats and poultry contain *Salmonella* at relatively high frequency, and no immediate practical solution is in sight. A sampling plan adjusted to this situation would seem more realistic and satisfactory than one based on an unmeasurable ideal of complete absence (i.e., zero tolerance).

The need for compromise

The foregoing illustrates the difficulty of trying to make a rational compromise between a desire to eliminate pathogens completely to protect consumers and what have been considered practicable methods of production. In this field, past decisions have been arbitrary, and some of them illogical. Thus, no restriction is currently enforced on the enormous quantities of poultry and carcass meats which often are regularly contaminated with *Salmonella*, though these foods are clearly shown by epidemiological evidence to be major vehicles for outbreaks of salmonellosis. In contrast, drastic and costly action has been taken on finding *Salmonella* in sugar confectionery and chocolate, though few outbreaks due to such products have been reported. The risk entailed by the regularly contaminated foods is probably as great as that entailed by an operator who is an infective carrier and whose detection would lead to his removal. Therefore, sampling procedures are needed which remove the more highly contaminated lots without completely strangling production.

To encourage a realistic choice of sampling plans for hazardous pathogens, and because of the current international effort to control *Salmonella* in foods, the problem is considered in further statistical detail with particular reference to *Salmonella*. Similar considerations might conceivably be applied, for example, to the *Arizona* group, *E. coli*, or *Shigella*.

B. SAMPLING PLANS FOR *Salmonella*

Any member of the genus *Salmonella* presents some degree of hazard to human health. Some, like *S. gallinarum* and *pullorum*, are of minor significance for humans; others, notably *S. typhi*, are a severe hazard. But the majority, to which this discussion relates, represent a moderate direct hazard which, through cross-contamination and multiplication, can become widespread readily via foods.

The numbers and incidence of these organisms can and should be low. Foods entailing a minimal disease hazard are examined for *Sal-*

monella only when there is unusual cause for concern. Such examination is needed to decide whether a product is acceptable in any of the following circumstances: (a) the food is of a kind commonly involved in salmonellosis; (b) the food is intended for a sensitive population (see p. 51 and Table 8, p. 55); (c) there is no knowledge of the manufacturing processes, or of the bacteriological record of earlier consignments; (d) there is special reason to suspect that the product of a particular factory may contain *Salmonella*.

Because most food control efforts should be directed to the areas of greatest risk, foods are assigned to categories according to the degree of hazard they present (see Tables 6 and 10, pp. 43 and 74). To each category is attached an appropriately stringent method of deciding the acceptability of the finished product. Case 10 recognizes a relatively mild hazard such as exists when a food may contain *Salmonella* but will be used by ordinary consumers after cooking (e.g., red meats, poultry). Case 12 expresses a greater risk, where the conditions of use may favour spread and/or multiplication, and hence needs more stringent sampling. Case 11 is intermediate. Because absence of *Salmonella* is the preferred criterion of acceptance ($c = 0$), the stringency of examination is normally increased by raising the number of sample units n (see p. 72). Table 14 shows recommended sampling plans for the various categories, with degrees of stringency related to hazard expressed by cases.

Our recommendations in Table 14 are similar in principle to those of the report of the *Salmonella* Committee of the NAS-NRC (1969), in that both increase the stringency of the sampling plan with increase in the degree of hazard caused by the conditions of food use. An obvious difference is that the present proposals involve less stringent plans. For this there are two reasons:

(i) The sampling plans proposed by NAS-NRC were not intended for routine examinations, but for use when the question arises whether a product contains *Salmonella*. Routine examinations will from time to time raise this question, and the NAS-NRC plans were intended for the special investigation it entails. For such investigations, the Commission agrees (Chapter 5, Section 0) that plans are desirable in which the degree of stringency is as great as the NAS-NRC recommended (see Table 14).

(ii) If a few highly pathogenic types such as *S. typhi* are regarded separately, the remaining *Salmonella* types which occur commonly in foods (to which Table 14 refers) usually involve mild to moderate disease risks. The NAS-NRC report does not make a similar distinction

TABLE 14

Stringency of sampling for *Salmonella* for foods with different categories of disease hazard (all 2-class plans with $c = 0$)

ICMSF				NAS/NRC ^a	
Hazard ^c in use	Case ^c	<i>n</i> values ^b		Category	<i>n</i> values ^b
		Normal ^c routine	Special ^d		
Reduced	10	5	15	B	13
Unchanged	11	10	30	C	29
Increased	12	20	60	D	60

^a Based on the Report of the *Salmonella* Committee US NAS/NRC (1969).

^b n = number of sample units.

^c Compare Tables 6 and 10 (pp. 43 and 74).

^d E.g., where the food will be eaten by susceptible consumers (Table 8, p. 55), or for tightened inspection (Table 11, p. 77).

and considers all types of *Salmonella* together, implying a high degree of risk. The Commission agrees that where circumstances (e.g., unusual susceptibility in the probable consumers) create a higher risk, sampling plans of higher stringency are desirable (compare p. 51; and Table 8, p. 55).

Figure 5 gives the operating characteristic curves which express the relation between the degree of security conferred and the sampling plans proposed. For instance, consider the curve for the plan $n = 30$, $c = 0$. If the proportion of sample units defective (p) is 0.05 (i.e., 5% are defective), the probability of acceptance (P_a) is about 0.25, i.e., 1 in 4; hence 3 out of 4 such lots would be rejected. Suppose the units are 25 g samples – a realistic supposition with a food like dried or frozen egg. They are defective if examination shows them to contain any *Salmonella*. Hence, if there are 5% of defective units (i.e., 1 defective in 20) there is, on average, *Salmonella* contamination in $20 \times 25 \text{ g} = 0.5 \text{ kg}$ of food. That is to say, the plan $n = 30$, $c = 0$, with analytical units of 25 g, will reject 3 out of 4 lots in which the incidence of contamination is about two analytical units positive for *Salmonella* per kg.

To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory

investigation undertaken for ICMSF (Silliker and Gabis, 1973) has indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis and Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) shows comparable reliability in the use of composite analytical units; for example, examination of three composites of 20×25 g analytical units has given the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing analytical units. Alternatively, such compositing offers the prospect of large increases in the number of analytical units, n , with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. No reduction in the cost of collecting the necessary number of sample units can be expected, since analytical units to be composited would still have to be chosen randomly.

C. PROBLEMS IN THE IMPLEMENTATION OF STRINGENT SAMPLING PLANS

(a) *Conceivable alternative plans*

As indicated above, for a pathogen like *Salmonella* there are strong reasons for adopting sampling plans in which $c = 0$:

(i) It is objectionable to some people to admit the presence of a recognized pathogen.

(ii) There is the very great technical advantage of combining many analytical units for bacteriological examination in systems with $c = 0$, because a single positive decides the outcome.

(iii) The plan with $c = 1$ (or more) would always require the examination of (many) more analytical units, for equal probability of acceptance, than a plan with $c = 0$.

(iv) Even if the indicated probabilities are the same, their implications are not. For example, when a lot is accepted with the plan $n = 60$, $c = 0$, it is possible (though not certain!) that the lot may perhaps not be contaminated at all. But using the plan $n = 95$, $c = 1$ and accepting one positive, it is known that the lot certainly is contaminated (though the probability that a lot with 5% of analytical units contaminated would be accepted is in both instances $P_a = 0.05$).

(b) *Fallacious procedures*

It might be suggested, for example, that, if the plans $n = 60, c = 0$ and $n = 95, c = 1$ provide equivalent probability (for lots having 5% of units defective), an operator finding one positive in 60 analytical units might then proceed to examine another 35 (total $n = 95$) in the hope of clearing the lot if all the latter proved negative. But such a procedure is in reality a two-stage plan $n_1 = 60, c_1 = 1$, plus $n_2 = 35, c_2 = 0$, which has a greater probability of accepting an unsatisfactory lot than $n = 95, c = 1$. In fact, this probability P_a is 0.07, compared with 0.05 for the one-stage plan. Although in this example the difference is not great, there are situations where such two-stage procedures cause more serious error. Where two-stage sampling plans are actually being used, their OC curves should be computed and the resulting probabilities of acceptance evaluated.

A similar fallacy can arise where a plan requires a large number of analytical units, which would be unusually costly. Suppose the plan is $n = 95, c = 1$, but for economy a group of only 20 units is tested initially. If one unit should fail to comply in this initial group, an analyst might examine the remainder (in this case 75) with the idea that if he does not find any defectives in the second group, he may ignore the first and concentrate on the finding of no defective out of 75 units. Nevertheless, the plan actually corresponding to the tests made is $n_1 = 20, c_1 = 1$ and $n_2 = 75, c_2 = 0$. No justification exists for 'preferring' the results from the second series. Similarly, if the plan were $n = 60, c = 0$, and the analyst felt uncertain about accepting his initial finding of a defective unit in the first 20 analytical units examined, and if the product were accepted on the basis of the second set of results, the actual plan applied would be the double sampling plan $n_1 = 20, c_1 = 1; n_2 = 40, c_2 = 0$, which actually accepts more defective lots than would $n = 60, c = 0$.

D. RELATION TO CURRENT COMMERCIAL PRACTICE

Stringent sampling plans like those just suggested represent an ideal not yet attainable for products like poultry or carcass meats, which are found to be frequently contaminated by *Salmonella* when specially sensitive methods of detection are used. At present it is often recommended to use procedures like rinsing or swabbing of the whole carcass, where in effect the analytical unit is most of the carcass surface and internal cavity, and a positive may correspond to levels

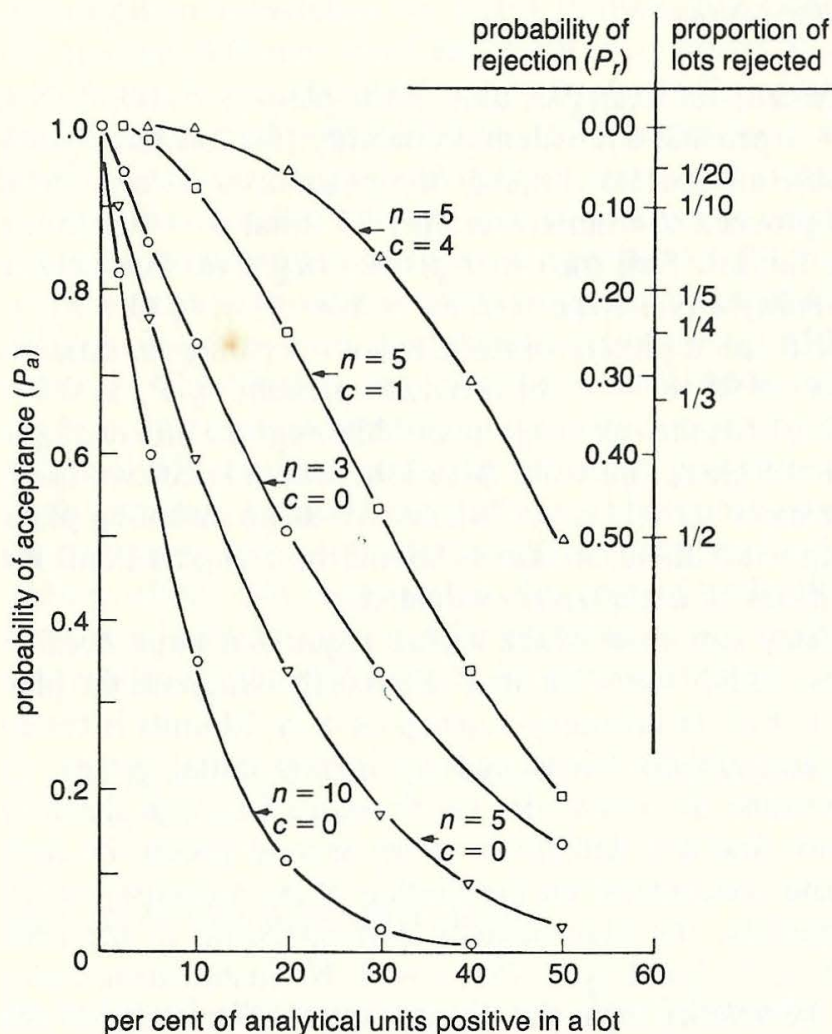


Figure 6 Operating characteristic curves for sampling plans with small numbers of analytical units (data collected from Tables 2 and 3, pp. 34–35)

of contamination of the order of one *Salmonella* cell per kg for poultry, or even one per 100 kg for carcass meats. Using such criteria, one finds slaughter lines producing carcasses found to be contaminated with a frequency of 10% or more. For lots with 10% defectives (i.e., $p = 0.10$), the relations shown in Section A of Table 15 are valid. The sampling plan $n = 60, c = 0$ would reject virtually all such material; $n = 5, c = 0$ would reject one-third of it (Fig. 6). Even the plan $n = 5, c = 1$ would reject 1 in 12 lots, a commercially severe rate of rejection. If, as a result of rejections on the basis of $n = 5, c = 1$, the quality of lots improved to the point where only 2% instead of 10% of carcasses were found to be contaminated, the relations would be as in Section B of Table 15. If 2% of carcasses are contaminated,

TABLE 15

Comparison of some operating characteristics of several sampling plans relevant to examination for pathogens (data collected from Tables 2 and 3, pp. 34 and 35)

Sampling plan	A. For lots with $p = 0.10$, i.e., 10% of samples contaminated			B. For lots with $p = 0.02$, i.e., 2% of samples contaminated		
	P_a^a	P_r^b	Approximate proportion of lots rejected	P_a^a	P_r^b	Approximate proportion of lots rejected
$n = 60, c = 0$	<0.005	0.995	199/200	0.30	0.70	2/3
$n = 10, c = 0$	0.35	0.65	2/3	0.82	0.16	1/6
$n = 5, c = 0$	0.59	0.41	1/3	0.90	0.10	1/10
$n = 3, c = 0$	0.73	0.27	1/4	0.94	0.06	1/16
$n = 5, c = 1$	0.92	0.08	1/12	1.00	<0.005	<1/200

a P_a means probability of acceptance of lot.

b P_r means probability of rejection of lot.

the indicated average concentration of *Salmonella* cells is of the order of one per 50 kg for poultry, and one per 5000 kg or more for beef. If a 2% frequency of contamination were still thought to be too great, a similar pressure for further improvement (rejecting about 1 in 10) could be reintroduced by changing the sampling plan from $n = 5$, $c = 1$ to $n = 5$, $c = 0$.

The above illustrates how, by periodical adjustment of the stringency of the sampling plan, a steady pressure to improve could be applied without placing catastrophic restrictions upon the supply of the commodity at any time.

The sampling plan $n = 5$, $c = 1$ has $P_a =$ about 0.65 for lots with $p = 0.24$ (see Fig. 6); that is this plan would reject 1 out of 3 lots in which one-quarter of the units were contaminated. If these units were poultry carcasses weighing about 1 kg, one-quarter contaminated corresponds to a level of contamination of the order of one *Salmonella* cell per 4 kg of food; while with beef carcasses, the figure would be of the order of one per 400 kg. This kind of situation is very different from the examination of analytical units of order 25 g (e.g., of egg). The plan $n = 60$, $c = 0$ rejects about one-third of lots in which 0.7% of analytical units are defective, which (these being 25 g analytical units) corresponds to a minimum level of contamination of about 1 cell per 3 kg. That is to say, the plan $n = 60$, $c = 0$, applied even to 25 g analytical units (e.g., of egg), actually provides less protection than the plan $n = 5$, $c = 1$ applied to whole-carcass samples of poultry of effective weight about 1 kg; even the latter plan would reject a substantial proportion of current production of poultry carcasses.

In such circumstances, the immediate application of the more stringent sampling plans to poultry, and still more to larger carcasses, seems at present unrealistic. The application of even so loose a plan as $n = 5$, $c = 1$ would immediately require a substantial improvement on current levels of contamination; and that improvement could then be continued by proceeding to plans of successively greater stringency. It is important to understand the difference between the sampling plans for some raw meats and those for other foods as regards *Salmonella*, and to note the effect of the size of the analytical unit on stringency of examination, as mentioned at the beginning of this chapter.

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Collecting and handling sample and analytical units

The objective of sampling is to obtain a representative sample of a food and to submit sample units to the laboratory in a condition bacteriologically unchanged from that existing at the time of sampling. All persons concerned with sampling should take appropriate measures to prevent, as far as possible, any contamination of either the food consignment or the sample units and any microbial growth or death within the sample units during transport to the laboratory and during subsequent storage and handling.

The sampling directions given in this chapter are considered good practice and should be followed whenever possible. The directions are those that apply generally, in collecting, labelling, transporting, and storing sample units, and in preparing them for laboratory analysis. It is, of course, difficult to lay down fixed rules to be followed in every situation, and in particular circumstances they may require some modification. Specific sampling directions relating to a given food or class of food are given in Chapters 11–26.

A. SAMPLE UNIT VS ANALYTICAL UNIT

A sample may consist of one or more sample units. A sample unit is the individual portion or container of food randomly taken as part of the overall sample; an analytical unit is that part of the sample unit actually used in an individual analysis in the laboratory. The two may be the same. In many situations, sample units will consist of unopened containers (e.g., hermetically sealed cans, consumer-size packages of frozen foods) holding many times the amount of food needed for the analytical unit.

Each sample unit provides only one result for each test carried out.

The food homogenate prepared from one analytical unit (ICMSF, 1978, pp. 108–111) will often provide enough material for a number of different microbial tests, but occasionally it will be necessary to take two or more analytical units from the same sample unit. For example, when tests for coliforms, staphylococci, and *Salmonella* are being run on the same food, one analytical unit is required for the preparation of the homogenate (to be used for tests for both the coliforms and staphylococci) and one other for *Salmonella* enrichment.

B. GENERAL DIRECTIONS

(a) *Materials*

1 *Sample containers.* Use clean, dry, sterile, leakproof containers such as wide-mouth glass or plastic jars or bottles, stainless metal cans, or disposable plastic bags, whose capacity is adequate for the sample unit desired. Usually they have a capacity of at least 8 oz (227 g). Containers to be reused should be of a quality suitable for repeated sterilizations (see (b) below). Use jars, bottles, and cans with screw-type caps. Insoluble non-absorbent cap liners are necessary; impervious liners made of pressed pulp faced with vinylite are satisfactory. Moulded rubber, plastic, or cork stoppers may be used, but should be covered with an inert material such as aluminum foil or a plastic sheet before they are pressed into the sample container. Disposable plastic bags must be sealed securely after filling so that they cannot leak or become contaminated during normal handling. It is a good practice to place one bag inside another (double thickness) to resist puncture.

2 *Instruments for opening food packages.* Sterile scissors, knives, can-openers, saws, as required, can be used to open food packages.

3 *Sampling devices.* Sterile scoops, spoons, triers, probes, drill bits, tongs, forks, cork-borers, pipettes, and swabs can be used for collecting sample units. Sterile scissors or knives may be needed to cut portions from large items of food.

4 *Labels or markers.* Containers can usually be labelled by marking them with a felt-point indelible marking pen. However, light-coloured waterproof cardboard tags with reinforced eyelet holes and wire or cord ties or gum-backed paper labels or adhesive-backed tape can be used to identify samples. Labels should be big enough to carry all relevant data (see (e) below).

5 *Sterilizing and disinfecting equipment.* In the laboratory, sampling containers or implements should be sterilized in either an autoclave or

an oven capable of reaching a temperature of 170°C. In the field, a pressure-cooker, a steam-chamber, or a hydrocarbon (propane or butane) torch or burner is necessary for sterilization. Disinfecting agents, such as alcohol (95% ethyl or isopropyl), which is subsequently flamed, or hypochlorite solution, which is not a sterilant (made up to 100 ppm and discarded when it falls below 50 ppm), may be used in the field when the above procedures are not practical.

6 *Storage and transport of samples.* A plastic-foam or other insulated container of sufficient capacity is needed for holding and transporting chilled or frozen sample units. Appropriate racks may be needed to secure sample units or prevent leakage or contamination of them during transit. Either ice or plastic or metal containers filled with refrigerant or water and frozen are needed to keep sample units cool. Dry ice is needed to keep frozen sample units frozen.

7 *Cold storage of samples.* In the field, sample units should be stored in either a refrigerator in the establishment from which the samples are being collected or an insulated container with ice or cold containers as described above. Sample units may be chilled first under running water or in ice- or water-baths. In the laboratory, a refrigerator capable of maintaining the sample units at 0–5°C is essential. A freezer or freezer compartment of a refrigerator, depending on the volume of frozen samples received, capable of maintaining samples at –10°C is also required.

8 *Temperature-measuring device.* A device such as a thermometer or thermocouple probe and potentiometer is needed to measure temperatures of food and storage units. Many varieties of digital or recording potentiometers with one or more thermocouple probes are available; these offer more flexibility than thermometers. Metal bayonet (at least 5 inches long), dial-faced (graduated at intervals of not less than 2°C and covering a range of –10°C to 110°C) thermometers have proved useful for measuring food temperatures in the field.

(b) *Sterilizing sampling containers and utensils*

1 Sterilize all sample containers and utensils that are to come in contact with a food. As a rule, sterilize beforehand in the laboratory by one of the following methods:

a Autoclaving at 121°C for 15 minutes. Use this method for all materials containing water and materials likely to be damaged by dry heat (e.g., rubber).

- b* Exposing glassware, metal containers, and implements and similar materials to hot air (170°C) in an oven for at least 1 hour.
- 2 Try to bring enough pre-sterilized sampling utensils from the laboratory to collect the desired number of sample units. If additional samples are to be collected after the pre-sterilized utensils have been used, clean (wash, rinse, and dry) used ones at the site and sterilize or disinfect them by using one of the following methods:
- a* Autoclave them in a small (20–30 litre) pressure-cooker at 121°C for 15 minutes.
 - b* Expose them in a chamber to steam at 100°C for 1 hour.
 - c* Heat them in a small, portable hot-air oven at 170°C for 1 hour (assuming power is available).
 - d* Flame the part of the utensil that will contact food thoroughly with a propane or butane torch or Bunsen burner (taking care not to overheat sampling equipment).
 - e* Immerse them in 95% ethyl or isopropyl alcohol for 3 or more seconds and flame to burn off the alcohol. Repeat this procedure twice more, making sure on each occasion that the flame is out before reimmersing the utensils in the alcohol.
 - f* Immerse them for at least 30 seconds in hypochlorite solution containing not less than 50 ppm of available chlorine or other acceptable halogen sanitizers which are of a concentration equivalent to at least 50 ppm hypochlorite (not a sterilant).

Do not use method *d* or *e* if there is danger of fire or explosion. Methods *b* through *f* may not kill all spores.

(c) Collecting sample units

- 1 Only an authorized person properly trained in the appropriate techniques should collect sample units. This person may be assisted by other persons under his or her supervision.
- 2 If possible, collect sample units during unloading of a cargo of food (from a ship, truck, airplane) randomly from all parts of the load. Otherwise, collect samples randomly from the 'frame' (see Chapter 3, Section D).
- 3 If all individual packages are accessible, choose those to be sampled by use of random numbers (see Chapter 3, Section C).
- 4 When sampling large cartons containing smaller packages, choose the desired number of cartons at random, and from each of these, again

at random, take the desired number of smaller packages. At each stage, use random numbers (see Chapter 3, Section C).

5 Submit sample units to the laboratory in original unopened containers whenever practicable. This will reveal the condition of the product as prepared or altered by subsequent storage and, in the case of samples taken at the retail level, as offered to the public.

6 Take at least twice the amount required for analysis in the laboratory (i.e., in most situations, about 200 grams) to provide a reserve portion in case of accident or later enquiry. Store the reserve under conditions in which its microbial condition will not change appreciably.

7 If the product is in containers too large to be easily transported to the laboratory, transfer representative sample units to separate sterile containers under aseptic conditions. Remove surface contamination on appropriate areas of the food package and the sample container by wiping with 70% alcohol, after removing gross dirt by washing or sponging with 70% alcohol or, in the case of paper containers, by removing the outer ply. Open sealed food packages carefully with a sterile cutting instrument such as scissors or a knife, as appropriate. Use a separately sterilized instrument for each package to avoid cross-contamination. Exercise special care where the risk of cross-contamination is high (e.g., with light powders or pressurized packages). Do not open hermetically sealed cans under field conditions. Submit such cans to the laboratory as sample units.

8 If the food is in bulk, sample consistently from various places within the container(s) rather than, say, just from the top or bottom (unless experience assures complete mixing, or unless sampling from some particular place(s) has become accepted as a result of long experience), or choose the sampling site randomly.

9 When sampling from an outlet or sampling port of a bulk container, allow some material to pass through to 'flush' the port before collecting the sample.

10 Avoid contamination when taking a sample unit from bulk or from a food package and when transferring the material to the sample container.

11 Use an instrument appropriate to the physical state of the food for collecting sample units. For example, triers may be useful for sampling blocks of hard cheese, a drill and bit for frozen products, scoops or hollow probes for dried foods, pipettes or dippers for liquids, and spoons for mixed foods (see recommendations for particular foods in Chapters 11–26).

12 Strive always to obtain a representative sample. Where possible,

agitate liquid, free-flowing, or suspended materials until the product is homogeneous. If feasible, unwieldy brittle material, such as certain frozen liquids, may be placed in a large sterile container (e.g., a strong plastic bag) and broken into smaller pieces with a blunt instrument. If this procedure is not feasible, lay the material on a sterile surface and aseptically break it into smaller fragments using a suitable instrument such as a sterile hammer and sterile chisel. Take sample units from the crushed material.

13 Measure the air temperature of the storage room or shipping vehicle as well as the temperature of the food from which the sample is drawn. Do not insert a thermometer into the food until after the sample has been taken. Disinfect the thermometer or thermocouple between measurements to avoid cross-contamination. In instances where small packages are sent unopened to the laboratory, record the temperature of the food in an adjacent package in the same case or carton. The temperature of boneless moist food or dried foods collected in plastic bags can be taken by folding the filled bag around the stem of the thermometer or sensor of a thermocouple and waiting until the temperature reading stabilizes.

(d) Number of sample units

Draw the number of sample units specified in the sampling plan used. See Chapters 11–26 for recommendations for individual foods and cases; also Chapter 3, Sections B and C, for what constitutes a representative sample and recommendations on random sampling, and Section B of this chapter for recommendations on the amount of food to take for each sample unit.

(e) Labelling sample containers

1 Label all sample unit containers immediately before or after the sample unit is taken. Fix the label to prevent accidental removal during subsequent handling.

2 Number the sample unit container or write the number on the label. Make a related record including all the information necessary to identify the sample (see (j) below).

3 When the sample unit is taken from a larger container such as a bag or carton, identify this container with the corresponding number of the sample unit, in the event a subsequent examination is required.

(f) *Sealing of sample containers*

In certain situations, such as where the food is the subject of a dispute or is suspect in an outbreak of food-borne illness, it may be necessary to place an 'official' seal on the container from which the sample unit was taken, preferably in the presence of persons authorized to represent all parties concerned. The seal may be glued paper or other material which makes it impossible to tamper with the contents or the label without irreparably damaging it. Identify the seal with the date, sample number, and identifying mark of the person collecting the sample. When, for official purposes, multiple samples are required, treat each sample unit identically.

(g) *Transporting and storing samples*

1 Examine sample units as soon as practicable after they are taken. Otherwise transport and/or store sample units under conditions which minimize the possibility of change.

2 Transport sample units to the laboratory for analysis as rapidly as practicable. If the product is canned (shelf-stable) or is in a dry condition, cooling the sample units is unnecessary but avoid temperatures above 40°C. Canned products considered likely to 'blow' should be refrigerated.

3 If the product is perishable and unfrozen, cool sample units rapidly to 0 to 5°C (e.g., in an ice or a water bath) and maintain this temperature during transport (see (a) above). Do not freeze the sample. In transporting sample units ensure that the containers will be held vertically and that liquid from thawed ice in direct contact with the containers does not contaminate the sample units.

4 Until analysis maintain sample units of frozen products in the frozen state.

(h) *Collecting samples during investigations of outbreaks of food-borne disease*

Sampling during the investigation of disease outbreaks (investigational sampling) may be quite different from routine sampling. Using professional judgment, samples of food or equipment surfaces should be collected at the point of operation considered most likely to yield food-borne pathogens; that is, where contamination, survival, or growth might occur (Bryan, 1980; Bryan et al., 1976).

Check storage facilities for food left from the suspect meal or lot. If none are available, collect sample units of food prepared in a manner similar to that used in the preparation of the suspect food as well as of the corresponding raw food (particularly that of animal origin) and ingredients used.

Collect samples of each component of a food (e.g., meringue and filling for custard pies). These have different ingredients and they could have been prepared by different persons or prepared or stored in different ways prior to final assembly.

If the mode of spread or source of aetiological agent is sought, and if definitive typing of isolates is contemplated, swabs of food contact surfaces and other environmental samples can be taken. When collecting swab samples, rub sterile swabs or sponges randomly over large areas of foods of animal origin and over contact surfaces of utensils and equipment used to process, store, or prepare suspect foods. Sample units should be labelled, sealed, transported, and stored as stated in Sections (e) to (g) above.

(i) *Legal requirements*

Before examining foods likely to be the subject of litigation, it is essential that the legal requirements for sampling be determined and followed.

(j) *Sampling report*

1 Complete a detailed sampling report, signed by the person responsible for collecting the samples, and countersigned by representatives of the other parties concerned, if they are present. This report should contain the following information relevant to each lot in the consignment:

- a* name and address of the person collecting the samples
- b* names and addresses of appropriate representatives of the parties concerned
- c* date, place, and time of sampling
- d* reason(s) for sampling
- e* nature of the food
- f* names of the manufacturer, importer, seller, buyer, as appropriate
- g* number and size of the units constituting the lot
- h* number and marking of the lot

- i* type and identification of the transport vehicle
- j* origin of shipment
- k* place of destination
- l* dates of shipment and arrival of the lot
- m* number of the bill of lading or contract
- n* method of sampling (random throughout lot, random throughout accessible units, or otherwise)
- o* size, number, and reference numbers of sample units
- p* temperature of the product at the moment of sampling
- q* means of transporting samples to the laboratory and by whom
- r* name and address of laboratory analysing samples
- s* tests to be made (e.g., APC, *Salmonella*, *Clostridium perfringens*)

2 The report should also include information on factors, conditions, or circumstances which may have influenced sampling or be pertinent to the investigation; for example, the condition of the individual packages, breakage, sanitation, humidity.

(k) *Preparing sample units for analysis*

Withdraw a representative analytical unit from the sample unit.

- 1 Begin the analysis as soon as possible after the sample material arrives at the laboratory.
- 2 If the sample is frozen, use one of the following methods:
(a) If the frozen material can be easily comminuted, do so and proceed without thawing. (b) Partially thaw sample units in their original containers (or in the containers in which they were received at the laboratory) in a refrigerator at 2 to 5°C.
- 3 Agitate thoroughly any food that is liquid, suspended, or free-flowing, before opening the sample container.
- 4 Avoid contamination when opening the sample container. Destroy surface contamination on and near the lid area of the container (or where the opening is to be made, in the case of foods in closed packages) by (a) flaming or (b) wiping with 70% alcohol and air-drying or burning away the excess alcohol, as appropriate.
- 5 Take special care in opening sample containers or packages of powdered materials to avoid contaminating the working area and other samples. If practicable open such containers in a room separate from the testing area or in a cabinet where the powder can be contained. Avoid sudden movements and keep doors and windows closed.
- 6 When a product is composed of several components or layers (e.g.,

turkey and dressing, cream pie with meringue topping), it is desirable to determine the microbial population or contamination of each component. Such testing can reveal possible sources of contamination or identify an area where improper processing, handling, and/or storage has occurred. Therefore, sample the parts separately while preventing cross-contamination as much as possible.

7 Observe any abnormal appearance or aroma of the sample units and note observations on the record form.

8 Use aseptic technique to withdraw and transfer samples to a sterile blender or stomacher bag.

9 For preparation and dilution of the food homogenate, follow directions given on pages 108–111 of ICMSF, 1978.

10 Store unused portions of the sample units in a refrigerator or a freezer as in (g) above, for replicate analyses if required.

11 Clean and disinfect all surfaces used.

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Sampling plans for raw meats

World trade in raw meats, both chilled and frozen, continues to be significant in terms of volume despite their potential hazard to health. Total world red meat production in 1979 was more than 78 million tonnes, with about 9% being traded internationally.

A. REASONS FOR SAMPLING

Raw meats are important sources of *Salmonella* and *Clostridium perfringens*, which are often incriminated in outbreaks of food-borne disease (ICMSF, 1980, p. 354). They are also sources of *Staphylococcus aureus*, *Campylobacter fetus* subsp. *jejuni*, and *Yersinia enterocolitica*. Salmonellae are found more often in pork and in veal from young calves than in other meats (ICMSF, 1980, p. 347). In the first edition of this book criteria for salmonellae were included as guidelines to assist control of this organism in raw meat. It was hoped that such guidelines would lead to a reduction in the contamination rate and a drop in the incidence of food-borne disease. However, outbreaks of salmonellosis due to meat have continued with little evidence of diminution. In some instances salmonellosis has been caused by the consumption of raw or inadequately cooked meat products but a more common hazard arises through cross-contamination from raw to cooked meat or other foods, and subsequent time-temperature abuse.

Clostridium perfringens is ubiquitous, and although it occurs on carcass meat, usually in low numbers, it cannot be controlled by any known means. The majority of outbreaks of *C. perfringens* gastroenteritis attributable to meat result from inadequate storage of the cooked product. Prevention involves attention to the time/temperature con-

ditions of cooking and, more important, to hot holding, cooling, and reheating before consumption.

Staphylococcus aureus may occur on raw meat although usually only in low numbers. Contamination by animal strains of *Staph. aureus*, which are thought to have a low enterotoxin-forming potential, is probably of less consequence than contamination from human sources. *Staphylococcus aureus* competes poorly with the normal microbial flora of raw meat and constitutes a health hazard only when this competing flora is restricted and there is temperature abuse of the product.

Campylobacter fetus subsp. *jejuni* is often present in the intestinal flora of healthy animals used for food production (Blaser, 1982). However, the numbers present on red meat are generally low and the organism has only a limited potential for growth or survival on refrigerated or cooked meat. Food-borne outbreaks of *Campylobacter* enteritis appear to arise almost exclusively from eating raw or undercooked food (Blaser, 1982).

The use of microbiological criteria as a control measure for reducing the incidence of disease arising from the consumption of meat carrying *Salmonella*, *C. perfringens*, and *Staph. aureus* was considered by a FAO/WHO Working Group (FAO/WHO, 1979) in light of the Codex 'General Principles for the Establishment and Application of Microbiological Criteria for Foods' (Codex, 1981). According to these principles a microbiological criterion should be established and applied only where there is a definite need and where it is both practical and likely to be effective. Such criteria should form part of, or be related to, a Codex Code of Practice for the particular product. The presence of *Salmonella* and *C. perfringens* on raw meats is generally more a reflection of their incidence in the live animal than of a breakdown of hygiene. In present circumstances the occurrence of salmonellae and the other pathogens in raw meat cannot be entirely prevented by the application of codes of hygienic practice (ICMSF, 1980, p. 347). Also, the extreme variability of distribution of pathogens such as salmonellae in meats prevents the establishment of practical sampling plans which would ensure with any degree of confidence the absence of salmonellae in meats. Consequently the Working Group concluded that the application of limits for pathogenic microorganisms at port of entry was not appropriate in terms of the Codex 'General Principles.'

In view of the importance of *Salmonella* as a cause of food-borne disease and meat as a vehicle for its transmission there is much to be gained from regular in-plant monitoring to establish the extent of the problem in particular areas and to detect increases in prevalence. Prod-

ucts that have a history of being implicated as causes of salmonellosis should be sampled at appropriate points during production and distribution to determine the prevalence of contamination and to trace its source on the farm as well as to look at the effects of travel, holding, and processing of stock. The aim is to collect information that will help to motivate the producer to eliminate salmonellae in the live animal and thus control the problem at its source.

The ultimate solution to the *Salmonella* problem lies in producing *Salmonella*-free animals. The provision of *Salmonella*-free foodstuffs for animals would be a major advance towards this aim (see Chapter 14, Feeds of Animal Origin and Pet Foods). Until this is achieved treatments that destroy salmonellae in the product (e.g., irradiation) may provide some protection for the consumer. In the absence of effective control measures, environmental and line sampling are more useful than testing the end-products. Although improvement of slaughter hygiene is incapable of eliminating contamination with salmonellae, neglect of hygiene can make the situation much worse. A comprehensive monitoring program in the processing plant can provide a check on the prevalence of infection in livestock and the effectiveness of measures of hygiene in controlling spread of contamination.

The FAO/WHO Working Group (FAO/WHO, 1979) concluded also that the number of indicator organisms in meat neither reflects adherence to a code of hygienic practice nor indicates presence or absence of pathogens. Hence criteria based on indicator organisms were not justified for raw meat. These conclusions, and those concerned with pathogens, have been adopted by the Codex Committee on Food Hygiene (Codex, 1981).

B. SAMPLING PLANS

In the virtual absence of studies relating health risks to surface counts of aerobic organisms on carcass meats it is not appropriate to reject consignments of product for public health reasons solely on the basis of high aerobic plate counts. However, aerobic plate counts (APC) can be used to monitor Good Commercial Practice (GCP), and criteria based on such examinations are a valuable aid to in-plant quality control. Microbiological quality control of meat-processing involves development and use of processing methods designed to keep microbial numbers low by reducing contamination and preventing growth. Microbiological monitoring of product and plant at critical control points

TABLE 19

Sampling plans and recommended microbiological limits for raw meat^a

Product ^b	Test	Method reference ^c	Case	Plan class	n	c	Limit per cm ² or gram	
							m	M
Carcass meat, before chilling	APC	118	1	3	5	3	10 ⁵	10 ⁶
Carcass meat, chilled	APC	118	1	3	5	3	10 ⁶	10 ⁷
Edible offal, chilled	APC	118	1	3	5	3	10 ⁶	10 ⁷
Carcass meat, frozen	APC	118	1	3	5	3	5 × 10 ⁵	10 ⁷
Boneless meat, frozen (beef, veal, pork, mutton)	APC	118	1	3	5	3	5 × 10 ⁵	10 ⁷
Comminuted meat, frozen	APC	118	1	3	5	3	10 ⁶	10 ⁷
Edible offal, frozen	APC	118	1	3	5	3	5 × 10 ⁵	10 ⁷

^a **Not for use at port of entry** but for in-plant quality control. Refer to Section B for appropriate applications.

^b Unfrozen carcasses and primal cuts, swab counts per cm²; other meats and meat products, per gram.

^c Refers to page number in ICMSF, 1978.

can be used to assess the effectiveness of sanitation factors in limiting microbial growth. These counts are compared with APC criteria established for particular products prepared under specific conditions. Sampling plans and microbiological criteria for certain raw meat commodities are listed in Table 19.

The proposals for raw meats are based on limited data collected mainly from production and under a restricted range of circumstances. The criteria have been established as guidelines and some tolerance must be allowed in their application; for example, meat marginally acceptable, or even rejected, by these guidelines could be used in heated products, but would have very limited shelf-life as fresh meat, even under good refrigeration. Meat prepared according to the *Recommended International Code of Hygienic Practice for Fresh Meat* (CAC/RCP 11-1976, FAO, Rome) may on occasion exceed these limits, which should not be used as legal standards. No attempt has been made to distinguish between meat from different species although it has been noted that sheep carcasses normally have a slightly higher

level of initial contamination than those of beef and numbers of bacteria tend to be higher again on pig carcasses (ICMSF, 1980, p. 342).

Although there are considerable quantities of horse meat in international trade, criteria have not been included because of lack of data.

The FAO/WHO Working Group (FAO/WHO, 1979) considered that the only point at which APC could be used for chilled meat, to evaluate the hygienic conditions under which it was produced, was at the slaughterhouse, and because of its perishable nature it would not be possible to set APC limits for use in guidelines or specifications for chilled meats at port of entry. This consideration applies to meat that is vacuum-packaged in an oxygen-impermeable plastic film as well as to chilled meat packaged in other ways. Vacuum-packaging and modified-atmosphere packaging bring about changes in the bacterial flora, and the storage life depends more on the nature of the flora that develops than on the numbers of bacteria present after processing.

Case 1 with a 3-class plan for the APC is appropriate for utility tests for general contamination of a product that is normally cooked before consumption. The values for m reflect current commercial attainment based on the examination of meat from different sources, mainly at point of production, and are supported by experience that meat with plate counts lower than the value set for m usually has not been subject to excessive contamination or undue faulty handling. A normal shelf-life would be anticipated. Overall data from a large number of samples have been analysed but not all products have been tested to a similar degree.

The M values for APC are based on experience that meat with APC values in excess of 10^7 is either grossly contaminated or has been exposed to conditions permitting microbial growth to a level not far short of the point at which incipient spoilage can be detected. Immediately after slaughter an M value of 10^6 is justified on the basis that counts below this level can be readily achieved under GCP. At this level some tolerance is allowed for further processing which could involve slight increase in microbial numbers.

Microbiological criteria applied to frozen meat should be similar to those applied to chilled meat. The criteria proposed for frozen carcass meat and frozen cuts assume that the meat is sampled in the frozen state and thawed quickly before examination of samples. Spoilage occurs at about the same level; hence the M values are the same. Reflecting the small decrease in numbers due to freezing and storage m values are 5×10^5 instead of 10^6 . Commercially thawed meat

usually has a higher count than the frozen product, and the criteria for chilled carcass meat could apply.

The microbiological quality of comminuted meat reflects the situation in the meat from which it is prepared. Counts in general are 10 – to 100 – fold greater in commercial minced meat than on the equivalent carcass (ICMSF, 1980, p. 370). If scrap meat and trimmings from the outer surface of carcasses are used, the counts in the mince will be higher than if whole cuts are minced. Mechanically deboned meat derived from good-quality raw material can be microbiologically equivalent to or better than minced, manually boned meat provided strict temperature control is exercised, but the process needs careful control in respect to the material being boned and sanitation of the equipment. There have been many proposals for microbiological criteria for minced meat but there is no evidence that a criterion has any relevance to health hazard. Accordingly criteria similar to those for carcass meat are proposed.

There is little information available on the microbiology of edible offals but the considerations are similar to those for carcass meat and the criteria proposed are the same.

C. SAMPLING PROCEDURES

(a) *Carcasses and primal cuts*

For chilled or frozen carcass meat and large bone-in or boneless cuts, as $n = 5$ for all criteria suggested, take subsamples from 5 individual carcasses or cartons of cuts (sample units). If the lot is distributed in several shiploads or freightcars take the sample units from more than one transportation unit.

Because contamination of meat is often very uneven, a number of subsamples should be taken from different parts of the carcass or cut, including areas known to be subject to contamination or particularly favourable for microbial growth. The subsamples from a carcass or carton may be pooled and thoroughly mixed to form a composite analytical unit, or each may be treated as a separate analytical unit.

Bacterial contamination of carcasses or cuts is generally on the surface, and this should be recognized in the collection of subsamples. Because defacing of carcasses or cuts is often economically undesirable, the surface swab technique is recommended for the APC on unfrozen meat. Samples should be taken at sites likely to be contaminated

(see, e.g., Roberts et al., 1980). Lamb and pork carcasses should be swabbed at at least two points (leg and brisket), and beef and horse at three (leg, flank, and neck). Sampling of cuts should include the original exterior surface and a cut surface.

For a comparison of swabbing and excision methods see ICMSF, 1980, p. 359.

(b) Boneless bulk cuts, comminuted meat, and edible offals

Except for the offals it is usually not possible to identify the original surface. From each of 5 cartons or packages remove a sample unit of about 200 g of tissue, which should comprise subsamples from a number of different parts of the pack. This composite sample unit should be mixed thoroughly and the appropriate analytical unit withdrawn.

(c) Retail packages

These include chilled or frozen consumer-portion packed cuts and offals as well as comminuted meat. The sampling procedure entails the examination of 5 packages from the lot.

D. SAMPLE COLLECTION

Remove any wrappings from the carcass or package carefully without handling the meat. Use sterile swabs and templates for taking surface swab samples. Mark the sampling area with sterile metal guides (e.g., 5, 50, or 100 cm²). Use two sterile swabs to sample each area. Moisten the first swab with sterile peptone water and rub firmly across the exposed area several times in all directions. Use the second swab dry and rub over the same area. Introduce both swabs into a bottle containing 3 or 4 glass beads and an appropriate known volume of diluent (e.g., 0.1% peptone, 0.9% NaCl). Shake vigorously.

To avoid cross-contamination when removing portions of meat, use sterile instruments (knives for unfrozen meat; saws, cleavers, and special drills for frozen meat), and transfer the piece aseptically to sample containers.

Samples of frozen meat should remain frozen during transport to the laboratory. Samples from chilled carcasses should not be frozen.

E. TEST PROCEDURES

(a) *Aerobic plate counts*

Comminute, blend, or stomach the various portions of meat composing the sample unit and withdraw the analytical unit. Combine the swabs to form an analytical unit, or treat each separately. Prepare dilutions and perform the APC test according to the directions given in ICMSF, 1978, p. 118. Spread plates are preferable to pour plates when examining raw meats.

The temperature used for incubation of plates should be related to the material being examined. To estimate numbers of mesophilic contaminants on freshly dressed carcasses it is advisable to incubate plates at 35 or 37°C. For meat that has been held under chilled conditions, plates should be incubated at 25°C to include psychrotrophs and thus give a better indication of the microbial growth that has occurred. Counts at 20°C give similar results after a longer incubation time.

(b) *Salmonella*

The test for salmonellae requires an analytical unit of 25 g and is based on positive or negative results using the method described in ICMSF, 1978, p. 163. For frozen products use a non-selective enrichment before selective enrichment and plating on selective agar media. Sample units should be taken from appropriate parts of carcasses, cuts of meat, and offals.

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Sampling plans for processed meats

Processed meats comprise a number of meat products, manufactured by many different technologies, including heat-processing, curing, drying, fermenting, acidifying, and packaging (ICMSF, 1980).

Processing changes the eating quality of meat. It also changes the composition of the microflora, and some processes kill or prevent growth of pathogens. Basically processing is intended to prolong the shelf-life of the products. The increase in shelf-life may range from a few days to several years. Processed meats with only a slight increase of shelf-life would not be candidates for international trade, while those given some degree of heat-treatment or curing combined with an adequate storage temperature move in international trade.

According to the FAO (1981), international trade during 1980 of processed meat and meat preparations amounted to about 1 million tonnes, of which sausages comprised about 115,000 tonnes. These FAO figures relate mainly to three groups of products: (a) shelf-stable cured canned meats; (b) perishable cured canned meats; and (c) cooked or uncooked, fermented, dried, or semi-dried sausages.

A. REASONS FOR SAMPLING

In the following section processed meats will be treated according to the outline in ICMSF, 1980, pp. 378–409. Discussion will deal with hazards (public health or spoilage), international trade of the products, and the possibility and feasibility of instituting means of control other than by microbiological criteria.

TABLE 20

Sampling plans and recommended microbiological limits for processed meats^a

Product	Test	Method reference ^b	Case	Plan class	n	c	Limit per gram	
							m	M
Dried blood, plasma, and gelatin	<i>Staph. aureus</i>	220	8	3	5	1	10 ²	10 ⁴
	<i>C. perfringens</i>	264	8	3	5	1	10 ²	10 ⁴
	<i>Salmonella</i>	163 ^c	11	2	10	0	0	
'Roast' beef ^d	<i>Salmonella</i>	163 ^c	12	2	20	0	0	
Pâté	<i>Salmonella</i>	163 ^c	12	2	20	0	0	

^a Refer to text for appropriate applications.^b Refers to page numbers in ICMSF, 1978.^c See also ISO 6579 (1981).^d Includes beef cooked in water baths; see Section B(f).

B. SAMPLING PLANS

(a) *Dried meats and dried animal products*

Dried meats like jerky, charqui, and biltong are mainly produced and consumed locally. Most dried meats found in international trade are intended for further processing. Further processing would normally consist of dry-blending with other food ingredients (e.g., dried soups) or rehydration and canning. In both cases the dried meats are subjected to conditions that either would not change or would reduce the hazards.

In international trade no evidence of hazard has been demonstrated, and no microbiological criteria are proposed.

Dried animal products such as dried whole blood, blood plasma, and gelatin are used as ingredients for formulated foods. When these ingredients are used in further processed products that are not subjected to adequate heat-treatment it is proposed to include sampling plans for *Staphylococcus aureus*, *Clostridium perfringens*, and *Salmonella* (Table 20).

For *Staph. aureus* and *C. perfringens* 3-class sampling plans, case 8 with $n = 5$, $c = 1$, $m = 10^2$, $M = 10^4$, are proposed. *Salmonella* should be considered in case 11 with a 2-class sampling plan with $n = 10$, $c = 0$.

(b) *Raw cured meats, high a_w*

High a_w meat products are defined as those with an a_w above 0.92, such as occurs in bacon produced by a Wiltshire or similar process (ICMSF, 1980, pp. 383–388).

In Wiltshire curing sides or parts of sides of pork, with or without bones, are injected with brine before either tank- or dry-curing. Smoke may be applied, but the product is not heated. There are many other types of manufacture of bacon, but this is the most common for bacon in international trade.

The microbiology of bacon is characterized by a rapid change from the initial gram-negative flora to gram-positive salt-tolerant micrococci and lactic acid bacteria. Only where curing is inadequate or the pH of the meat (usually the collar) is high will putrefactive spoilage dominate. Otherwise, spoilage is characterized by sliming.

Staphylococci could be considered to be of concern but are not able to compete with the normal saprophytic flora. Applying microbiological criteria at manufacture can make little contribution to the control of spoilage.

Salmonella is not a major concern because the product is cooked immediately before consumption.

(c) *Raw, cured, or salted meats and natural casings, low a_w*

Low a_w meat products are defined (ICMSF, 1980) as those having a water activity of 0.92 or below. This group includes whole cuts of meat (e.g., Bündener Fleisch, Parma ham, Westphalian ham, Smithfield ham, Prosciutti), non-fermented dried sausages, and natural casings, all of which are in international commerce.

Although some of the raw cured or salted meats have been implicated in outbreaks of food-borne illness, particularly botulism, the incidents have usually involved home-prepared products. Commercial products have had a very good record of safety. Growth of non-pathogenic bacteria occurs during the process of salting, curing, and drying of many of these products and can contribute to their flavour characteristics. Hence, microbiological criteria need not be applied to these commercial products. Cured, dried non-fermented sausages are in international trade but the only spoilage problem appears to be mould formation on the casing. No public hazards have been identified, and no microbiological criteria are proposed.

Natural casings from international commerce are generally packed

in salt and have not been identified as a vehicle of food-borne illness. Survival and growth of some salt-tolerant bacteria occurs in salted casings, but *Salmonella* indigenous to the casings does not survive for more than two or three weeks after salting. Microbiological criteria are unnecessary after salting.

(d) *Fermented sausages*

Cured fermented sausages include both semi-dry and dry types. Both types undergo a fermentation process which should rapidly lower the product pH to <5.5 . At this point semi-dry products may or may not be heated but if the pork used contains *Trichina* heating to 58.3°C may be mandatory. Other semi-dry sausages may only receive light heating (e.g., Thuringer). Dry sausage is placed in "dry rooms" with controlled humidity for an extended period after fermentation. During this ageing process, the product loses moisture and the a_w falls to c. 0.88, which is sufficiently low to make the heat-treatment to control *Trichina* unnecessary.

In both semi-dry and dry fermented sausages the main hazard is the growth of *Staph. aureus* during the initial stages of the fermentation before the pH of the mix falls sufficiently to inhibit its growth.

It has been suggested that microbiological criteria for *Staph. aureus* (not more than 100,000 per gram) be applied at the conclusion of the fermentation process or at the beginning of the drying process. More benefit could be obtained by a controlled acidulation to below pH 5.4 (using starter cultures or chemical acidulants such as glucono-delta-lactone) than by microbiological testing. *Salmonella* derived from the raw ingredients may survive processing but is unlikely to multiply. Total counts criteria are inappropriate because of growth of fermenting organisms.

The American Meat Institute has recently formulated Good Manufacturing Practices in connection with the production of fermented sausages (AMI, 1982). A maximum pH of 5.3 is prescribed in both dry and semi-dry fermented sausages.

(e) *Cured meats packed under vacuum*

Some of these products support the growth of *Clostridium botulinum* (Pivnick and Barnett, 1965) and *Salmonella* (Davidson and Webb, 1972) at abuse temperatures. The developing flora in vacuum-packaged cured meats is composed almost entirely of lactic acid bacteria. Large

numbers of these organisms may develop without objectionable organoleptic changes. Despite the fact that these products are often abused through inadequate refrigeration, outbreaks of staphylococcal food-poisoning are seldom traced to them, probably because staphylococci fail to grow in competition with other microorganisms. Salmonellae are capable of survival during protracted storage of these products at 5°C and can grow when the meats are incubated at ambient temperature (Goepfert and Chung, 1970), but these products have not proved to be a significant *Salmonella* hazard.

It is assumed that sliced bacon will be heated prior to consumption and, in addition, that the growth of lactic acid bacteria will afford protection against growth of *Staph. aureus*. There is no evidence of food-borne disease from sliced bacon and thus criteria are not proposed.

Ham is sometimes involved in food-borne illness due to *Staph. aureus*, usually as a consequence of contamination during slicing or handling, followed by temperature abuse. Vacuum-packed sliced hams are rarely implicated. During storage of vacuum-packed meats, including hams, under refrigeration a large population of lactic acid bacteria develops in whose presence staphylococci fail to grow. There is no evidence that criteria at manufacture or at port of entry would reduce the hazard.

Frankfurters and bologna have also been implicated occasionally in food-borne illness, but control of the production process affords greater assurance of safety than would the application of criteria.

(f) *Cooked uncured meats*

Cooked uncured meats (e.g., roast-beef joints and cuts) are increasingly important in international trade. Their involvement in food-borne salmonellosis could be avoided by proper heat-processing, prevention of post-process contamination, rapid cooling, and subsequent appropriate time-temperature control in the home or food-service establishment. A criterion for *Salmonella* is proposed: case 12, $n = 20$, $c = 0$ (Table 20). There is no evidence that aerobic plate count (APC) limits would serve a useful purpose.

Considerable international trade exists in pasteurized comminuted meat products such as pâtés. At port of entry they frequently have high APCs and are occasionally associated with food-borne illness. Control is by adequate heat-processing, prevention of recontamination, and appropriate chill storage. Microbiological data are inadequate, but these products may contain *Salmonella*. If there is reason for concern,

the appropriate sampling plan for *Salmonella* would be case 12, $n = 20$, $c = 0$ (Table 20).

(g) *Perishable cured canned meats*

The most important meat products of this kind in international trade are pasteurized canned cured hams and pork shoulders. A high proportion of these products are heated to centre temperatures of only 65 to 75°C. They should be transported under refrigeration, and should be labelled 'perishable, keep under refrigeration' or with words to similar effect. While the importance of testing such products is recognized, the high price of canned hams and similar foods is a serious obstacle to intensive examination. For these products, data on thermal processing, water supply, seam inspection, and chemical composition should be available, together with records of temperature during shipping. If all such data are satisfactory, no testing is necessary.

The Codex Alimentarius Committee for Processed Meat and Poultry Products is currently considering sampling plans for this category of products. Until the results become available the following is offered for guidance.

1. Sampling procedure at port of entry

If adequate production records are not available, sampling should be done according to the following scheme (see also Table 21).

- a* Measure the air temperature in the space between containers, preferably with an electronic measuring device.
- b* Take 10 containers at random from the lot. Identification of individual containers at this point is unnecessary. Examine the 10 containers for 'swells' and defects (see footnote, Table 21).
- c* If the temperature does not exceed 10°C or any specified lower temperature and if no swells or defective containers are found accept the lot.

If one or more defective or swollen containers are found, hold the lot and determine the cause by more extensive sampling (see Chapter 5, Section N, Investigational Sampling).

If the temperature exceeds 10°C or any specified lower temperature, or if there is reason to suspect that these temperatures have been exceeded during transport, proceed to step *d*.

TABLE 21

Sampling plans for perishable canned cured meats (to be used when processing data are not available or are unsatisfactory)

Test	Case	Plan class	<i>n</i>	<i>c</i>	Limit per gram		Acceptance
					<i>m</i>	<i>M</i>	
Step 1 Visual inspection for swells and defective containers*	—	2	10	0	—	—	Reject if 1 or more defective cans are found. Proceed to step 2 if no defective cans are found.
Step 2 Measurement of air temperature between cans	—	2	10	0	—	—	Accept if temperature is below 10°C. If higher proceed to step 3.
Step 3 Determination of aerobic plate count in centre and periphery	—	3	5	2	10 ³	10 ⁴	See Section G of this chapter for interpretation of the presence of micro-organisms.

* A defective container is a container which is of faulty manufacture, is improperly closed, or has been damaged in such a manner that may permit contamination of the contents following the heat-process (post-processing contamination).

d Take 5 containers from the warmer places in the lot and hold the lot. Proceed to step *e*.

e Identify the 5 containers selected under *d* and send them to a laboratory for microbiological examination. Transport should take place under refrigeration, at 10°C or less.

2. Laboratory analysis

a In the laboratory draw sample units from the 5 containers aseptically so as to obtain one unit from the centre and one from the periphery of each container.

b Examine for APC (ICMSF, 1978, p. 118). A direct Gram stain and an anaerobic count may sometimes be helpful. Rarely, the only bacteria present in large numbers are anaerobes.

- c Reject if an analytical unit from one or more of the 5 containers has a viable count exceeding 10^4 per gram. Also reject if one or two analytical units from 3 or more containers show a viable count higher than 10^3 per gram. Otherwise, accept.
- d When rejection is indicated an investigation into the cause of the problem might be appropriate and the supplier informed of the results. It should be emphasized that the values for m and M in this context do not necessarily reflect any spoilage or health hazard, but merely indicate degrees of undesirable commercial practice. The limits have been established from data derived from very large numbers of analyses over several years and are known to be attainable under GCP. It has been found that at export counts are normally less than 10^2 per gram. If the lot does not meet this criterion, the decision on the action to be taken should be one of the options given in Chapter 2.

(h) *Shelf-stable cured or uncured canned meats*

Shelf-stable meats have undergone a treatment sufficient to guarantee the subsequent safety and stability of the product at normal ambient temperatures. Canned cured and canned uncured meat products are considered with other low-acid canned foods in Chapter 26.

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