Fundamentals of microbiology in relation to cleansing in the cosmetics industry

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Synopsis—The fundamental MICROBIOLOGICAL aspects of modern methods of CLEANING and STERILIZING equipment with special reference to the HAZARDS of COSMETIC PREPARATIONS.

INTRODUCTION

Almost every operation involving handling of foods and other materials which can be contaminated by organisms of human or other animal origin is in theory a hazard to health. In practice it is fortunately only a few which are known to be a serious hazard, for example contact with a person suffering from a certain disease, rewarming of processed meat dishes, etc. From the hygienic point of view therefore the degree of risk has to be assessed in relation to the cost of preventive measures. Not only the chances of contamination and infection, but the growth-permitting properties of the commodity, the conditions of storage and its intrinsic nature from the microbiological point of view are equally important. The more favourable the conditions for growth of micro-organisms in the product, the more effective should be the preventive measures adopted in the factory.

For the present purpose we may define hygiene as a system of precautions to maintain safety and keeping quality (KQ) in cosmetic products.

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The causative agents in this problem are micro-organisms, so that scientifically it becomes a question of how we should kill them or control their growth. Basically the same principles and methods apply to both pathogens and fault-producing organisms (FPO).

The trend of technology today is clearly towards aseptic packaging of liquid products, and more efficient use of refrigeration to maintain keeping quality. From the bacteriological point of view these two methods imply an increasing importance for heat-resistant spores, psychro-philic (-trophic) organisms and environmental sanitation.

Hitherto industries employing sub-sterilization heat treatments have been particularly concerned with the numbers of thermoduric organisms in their raw materials but with the gradual change to UHT heat treatment methods these types will become of less importance.

The status of hygiene in industry

One of the greatest difficulties with which the new thinkers in industry have had to contend has been the long established general tradition that cleaning of any sort is a menial occupation and of no importance, so that the poorest types of labour were usually employed. This was a fallacy of the first magnitude, a fact which is now realized. No aspect of processing is more important than the cleaning and sterilizing of equipment.

SIGNIFICANCE OF BACTERIA IN COSMETICS

Safety and commercial or keeping quality

The safety (freedom from any pathogens) and KQ of any product are quite distinct properties and must never be confused. A commodity may be safe but of poor KQ and it can be dangerous but commercially satisfactory.

Laboratory control tests must therefore cover both aspects. A product may have such physical and chemical properties that one or both types of test are unnecessary. Dryness and acidity are in practice the dominant conditions in this respect, dryness for all micro-organisms and acidity for all pathogens and most bacteria. Acidity is no deterrent for yeasts and moulds, and in fact often favours them.

In general it may be said that cosmetic preparations, like foods, should either be free from micro-organisms capable of damaging the product, or

should contain a bacteriostatic substance (or condition) capable of preventing their growth.

For pathogens the requirement is different and more exact. Either the product must be free from pathogens or it must contain a substance (or condition) that kills them. This is because a surviving pathogen may not be able to grow in the preparation itself but may be able to proliferate when applied to the skin or mucous membranes, or when inhaled or ingested.

The expression 'free from pathogens' needs definition, and as a standard we suggest failure to recover recognized pathogens, particularly *Staph. aureus*, *Ps. aeruginosa* and *Salmonella* from 100 g using the common standard methods. Coliforms constitute a convenient index group as a measure of quality of raw materials, plant sanitation and hygiene in handling, processing and packaging.

The unpredictability of micro-organisms

The classical textbooks divided bacteria into pathogens and harmless organisms, but unfortunately the true position cannot be so lightly dismissed. During the last 25 years many bacteria previously regarded as harmless commensals or possibly as useful indicator organisms, such as *E. coli*, have been shown to possess pathogenic powers and be capable of causing illness and even death.

Whether this is due to the frequent uncritical and perhaps irresponsible use of antibiotics since 1945 by the medical and veterinary professions must be a matter of opinion, but even some members of these professions are beginning to have doubts about the wisdom of the present day widespread use of antibiotics (1).

Whatever the reason, today organisms such as *Pseudomonas aeruginosa*, *E. coli*, *Aerobacter*, *Flavobacterium*, *Serratia* have been responsible for killing diseases, sometimes in epidemic form. These are all Gram-negative, resistant to disinfectants and antibiotics, and flourish under watery conditions. The irresponsible use of disinfectants, preservatives and antibiotics may even favour the establishment of these types by repressing the Gram-positive organisms, against which they are very effective.

Another aspect of the greatest importance is the considerable degree of variability in any genus, and even in any one species of micro-organism. Textbooks may define the conditions permitting growth and thermal death points, etc., but there will always be exceptions, or in other words, freak organisms. Such atypical strains are often responsible for disease and

defects in products such as cosmetics, pharmaceutical preparations and foods. It follows that assumptions must never be made, risks must never be taken, and a substantial margin of safety always allowed in specifying any heat-treatment, conditions of using a disinfectant and concentration for any preservative until certainty of result has been clearly established by a large number of tests.

The chemist and bacteriological phenomena

The chemist who has not received any biological training often has difficulty in appreciating the wide range of variation in biological behaviour, and the relatively enormous and unavoidable errors in micro-biological testing. For example, the ordinary 'total count', generally the most commonly performed test, has a large error, especially when made on solid materials. In solid and semi-solid materials micro-organisms occur in colonies containing possibly millions of cells. Maceration of the product breaks up the colonies with unpredictable scattering, leading to a large error in the resultant count or very poor reproducibility. Chemical analysis can usually give repeated results agreeing within about 0.1%, but in such tests as the total count it is better to think in terms of logarithmic values, e.g. to allow a difference of $\times$ 10 before asserting that one result is really different from another, or that one sample is better than another. Assuming reliable sampling, two chemical laboratories can usually get reasonable agreement with their analyses. In bacteriological work wide differences may be found, because the method of sampling, handling of the sample, temperature of transport and storage, and technique differences between laboratories may be of tremendous significance. The chemical condition of a substance is usually static whereas the microbiological condition may be quite mobile.

FACTORS CONTROLLING BACTERIAL GROWTH

The most important conditions controlling micro-organisms are (1) availability of food for growth and as a source of energy, (2) warmth or a certain range of temperature, (3) moisture, water activity or relative humidity, and (4) absence of lethal factors.

Food

Micro-organisms can flourish with such minute amounts of food that

scrupulous cleansing is necessary to free any item of equipment from these food traces. Protein of animal origin may be described as the favoured food for human pathogens, and skin secretions, mucus, etc. afford excellent nutrients for bacteria, assuming conditions are favourable.

Temperature

Broadly speaking, pathogens flourish best at about blood heat, but most can grow over the range 16–45°C. Where conditions are unavoidably favourable for bacterial growth, e.g. a nutrient liquid at pH 7, the best, simplest and least objectionable way to prevent or delay growth is to hold the product at a low temperature, e.g. about 0°C. Cold does not kill bacteria *per se*, but if organisms cannot grow they tend to die out.

It is impossible to generalize about temperature conditions in respect of organisms producing defects; they may vary from psychrophils (psychrotrophs) growing well at –5 to +5°C to the obligate thermophils which grow only above 37°C and flourish happily at 55-63°C. Cosmetic preparations are often, or attempt to be, antiseptic in character, i.e. they contain one or more ingredients possessing some disinfectant power. Unfortunately these may have little efficacy against *Pseudomonas*, etc., so that low temperature storage of cosmetics cannot be relied upon to prevent growth of these types. All *pseudomonads* and many Gram-negative bacteria, yeasts and moulds can grow down to low temperatures, some moulds down to –23°C. Even extreme cold, e.g. —100°C, cannot be relied upon to kill micro-organisms, although they usually die off slowly.

Moisture

A certain 'moisture' or water activity (a_w) is one of the major conditions for bacterial proliferation. On surfaces it is customary to speak in terms of relative humidity, but the essential feature is the water activity (osmotic pressure) of the medium in which the micro-organisms are contained. This may be a protein-fat film of minute thickness, perhaps only a few microns.

The simplest way of restraining growth of all micro-organisms is to keep the product or equipment dry. Thus in powders, even foods, micro-organisms will die out if the moisture content is low, e.g. below 5%, and they will also die out in tanks, fillers, pipelines, homogenizers, containers, etc., if these are kept dry. The one requisite is that they must be absolutely clean

i.e. entirely devoid of organic matter. If they are not, then organisms will survive but not grow, and so sterilization becomes ineffective.

There is one important point to bear in mind in this connection. Bacteria are classified in two broad groups on the basis of the gram stain. gram-positive organisms, such as *Streptococcus, Staphylococcus, Corynebacterium*, are more susceptible to disinfectants and antibiotics than the gram-negative (*Pseudomonas, Salmonella, Shigella*, coliforms) but are more resistant to drying, especially if protected by traces of organic matter. Thus the *streptococci* of scarlet fever, basically very delicate bacteria, can survive for years in particles of skin shed by patients, be hidden in such places as bed equipment, and cause an infection years later.

The hygiene 'probability equation'

Developing the theme of the factors controlling bacterial growth we may express the situation in highly condensed form as:

P_t = probable bacterial count at time t

= f $(N_o, T, a_w$ (or RH),$F_{qn}, F_n, L^{-1},t)$

where N_o = initial number

T = temperature

a_w = water activity in product

RH = relative humidity in equipment

F_{qn} = amount of nutrients available (e.g. soil in equipment)

F_n = nature or type of product

L = lethality factor (e.g. uv, sunlight, acidity, high osmotic pressure)

t = time of observation after production

This means simply that the probable count in a product or in a piece of equipment after time t will be a function of the factors discussed above.

If all factors except two, e.g. T and t, are standardized it may be possible to estimate P_t with a fair degree of accuracy, using data previously obtained.

Acidity

In general, micro-organisms flourish best in the pH range 5–8.5. Pathogens are usually more sensitive to extreme values and many grow well only between pH 5.5 and 8. Any product, including even foods, which is below pH 4.5 can be regarded as safe because no ordinary pathogens can

grow, and any present will die out. Yeasts and moulds are a significant exception, and the few pathogenic species (e.g. *Candida albicans*) are much more resistant to acids than bacteria. Thus *C. albicans* grows best over the range 5.1–6.4 and can grow over a wide pH range (2).

This acidity factor is of special interest to cosmetic chemists because the secretions of the skin are acid, being usually about pH 5.4. Moreover the free fatty acids may exercise a germicidal effect *per se*. This natural protective effect probably plays an important role in protecting the skin against invading organisms. These can do little harm against the intact skin but may invade if the skin is damaged. In extreme cases the pH of the skin may be as low as 4, and this secretory mechanism has been called the 'acid mantle' of the skin. It follows that frequent washing with a strongly alkaline soap is not to be recommended, and prolonged soaking in hot baths is also inadvisable for the same reason. Scientifically the skin should be cleansed with a preparation at about pH 5.4 rather than with an alkaline material. Basically it is always sounder to assist and stimulate the body's natural protective mechanism rather than try and kill bacteria by 'disinfectants' and other means. There will always be the occasion when a virulent organism will get through. To be really effective an antiseptic must have strong biocidal properties, and unfortunately there is usually a good correlation between biocidal power and traumatic effect on animal tissue.

Contemporary medical thought is veering in this direction. For example, it is now the practice not to remove the natural protective material (*vernix caseosa*) on the new born infant and wash it by orthodox means (alkaline soap) immediately after birth. The warm water and soap cleansing is left over for a few days, thus allowing the natural mechanisms of the skin to protect the infant against any fortuitous contamination in early extra-uterine existence.

The other major example of a natural acid protective mechanism is the acidity of the stomach. In healthy adults the pH of the active gastric secretion is about 2, at which no ordinary pathogens can survive. Sub-acid secretions (pH 2–5) allow acid-resistant types such as *Str. salivarius* to pass through into the bowel, and in cases of gross acid deficiency (pH > 5) most pathogens can survive easily. This is the main reason why young infants and very old people are much more susceptible to food poisoning or infective enteritis than healthy adults. In general young infants and old people are much more susceptible to infections of any type. The young have not acquired much immunity and in old people there is a lowered efficiency of all biochemical mechanisms in the body.

The quantitative approach–the 'communal phenomenon'

All studies and control tests in bacteriology should be quantitative; in other words an attempt should always be made to estimate the number involved, although the error may be large. The reason is that the onset of an infection, or the development of a microbiological defect in any product, is dependent on the initial number of organisms concerned. Apart from a few exceptions, it is doubtful whether a single bacterial cell ever did anyone any harm. It appears to be necessary for at least a few organisms to establish themselves and adapt their environment for growth (the 'communal effect'). This is clearly illustrated by figures for the *minimum infective dose* for well recognized diseases (*Table I*). It follows that any reduction in numbers of micro-organisms is worthwhile, even if a complete kill is impossible for practical reasons.

Table I.
Minimum infective doses (approx.) for pathogens in human beings

Disease	Number of cells
Typhoid fever	3
Tuberculosis	100
Cutaneous moniliasis	100 000
Salmonelloses (other than typhoid fevers)	100 000 to 1 000 000

Pseudomonas aeruginosa

This organism, also called *Ps. pyocyanea* and the 'green pus organism', has assumed considerable importance in medicine, surgery, pharmaceutics and cosmetic preparations in recent years. Not only is it capable of being a virulent pathogen but it is so resistant to commonly used disinfectants and antiseptic preparations that it can often be isolated from them in startlingly large numbers.

It is now being found in almost all watery environments in hospitals, nurseries, kitchens and other places where food is prepared and human beings work.

Many interesting and disturbing examples are continually coming to light. Quite recently severe cases of mastitis in a valuable dairy herd were found to be caused by a *Pseudomonas aeruginosa* contamination of the water used for washing the cows' udder. This was ultimately traced to a dead rat

in a water tank. The water had been assumed to be pure because it came from the mains.

Type of product and potential contamination

Each type of product is liable to its own specific kind of contamination and microbiological growth according to
   (i) chemical nature (protein, fat, carbohydrate and mixtures of these);
   (ii) pH value;
   (iii) oxygen tension;
   (iv) surface tension;
   (v) other biocidal factors operating;
   (vi) presence of biocidal substances.
In practice one organism (e.g. *Pseudomonas*, yeast, mould) may so establish itself that it 'gets away' and the material becomes virtually a pure culture. If this organism is dangerous or affects the life of the product, disaster is inevitable.

PRACTICAL ASPECTS: PROCEDURES IN THE FACTORY

Control of raw materials

All materials used in cosmetics should be checked for quality visually, chemically and where appropriate microbiologically. Specifications to buyers should include microbiological standards where necessary. It may be possible to blend, improve or 'top up' the chemical or functional quality of a crude ingredient, but it is often not possible or practicable to improve the microbiological quality without damaging the product. A defect of this nature can persist right through the processing and packaging to retail sale and use.

Formulation and preservation

In general it is always better to prevent microbiological growth by formulation rather than by relying on preservatives. These, like anti-oxidants, are rarely completely satisfactory for a prolonged period, especially with warm ambient temperatures, whereas control by formulation (i.e. by physical and chemical means) lasts indefinitely. No organism can grow without nutrients but almost anything organic can act as a nutrient for some organisms. Physical factors are highly specific for particular types of organism, e.g. acidity for most bacteria but not for yeasts or moulds,

absence of oxygen for obligate aerobes, aerobic conditions for anaerobes. A useful measure of control can be exercised by designing formulation, processing and cleansing methods with particular reference to the type of infection to which the product is most vulnerable.

Temperature control

Temperature is in practice the most important factor controlling the growth of bacteria, and so the safety and keeping quality of the product, assuming satisfactory hygiene. Two of the biggest mistakes made in factory practice are to assume that dial thermometers and recorders are always accurate, and that calculations made in respect of heat transfer under ordinary conditions also apply in hot weather. Both these fallacious assumptions have led to major catastrophes in more than one industry.

All working (dial) thermometers should be checked against a known accurate thermometer *in the laboratory*. Mercury in glass thermometers should never be used in the factory.

All cooling systems should be calculated allowing for an atmospheric temperature of 27°C and a mains water temperature of 20°C, or alternatively provision made for additional cooling capacity in hot weather.

Design of equipment

In the food industries it took a whole generation to convince engineers that micro-organisms existed and could spoil a product. Early types of equipment were often a paradise for bacteria with their multiplicity of dead ends, crevices, unhygienic joints, indiscriminate use of absorbent materials, etc. and the impossibility of cleaning and sterilizing them.

In early educational work we laid down as a basic principle that all equipment for materials of biological perishability had to be dismantled daily and each item individually cleaned before sterilization. There was even equipment on the market for this purpose which could not be dismantled.

Fortunately engineers have now received the message and equipment in this field today is practically all well designed and hygienically constructed.

Sterilization of equipment by heat

Heat is usually applied as hot water, steam at atmospheric pressure

(e.g. in a steam chest) or under pressure (e.g. in an autoclave) usually at 15 lb/sq in.

Hot air requires 160°C for 3 h or 170°C for 2 h to ensure sterility of equipment or powders in thin layers.

Although heat, especially autoclaving, is still the preferred method in medical work, it is often inconvenient or impractical in industry for one or more of the following reasons:

  (i)   it is expensive;
  (ii) it causes deterioration of materials (e.g. plastics and delicate fabrics);
  (iii) it causes distortion in equipment (e.g. pipelines and gaskets);
  (iv) considerable time is taken to heat and to cool;
  (v)   residues may be baked on unless the equipment is thoroughly cleaned;
  (vi) inefficient heating may result in the incubation of micro-organisms in inaccessible parts of the equipment.

In general, it may be asserted that heating a liquid for a few seconds at 75°C will destroy non-thermoduric vegetative cells, at 90°C all vegetative cells, and at 130-140°C all cells including spores. When considering surfaces, even of such easily cleansed materials as glass and polished stainless steel, a more drastic treatment is necessary, mainly because of the possible presence of very thin, invisible films of soiling matter which are often present although the utensil or equipment appears to be clean. Rubber and similar materials are extremely difficult to clean and sterilize. Thus Anderson, Sage and Spaulding (3) showed that it is necessary to hold contaminated rubber nipples in boiling water for 5 min to destroy *C. albicans*, although a few seconds at 100°C is sufficient to destroy it in milk (4).

Sterilization by chemicals

Broadly speaking there are four ways of chemically sterilizing equipment:

  (i)   cleaning by a detergent (e.g. alkali) and then sterilization by a sterilant (e.g. hypochlorite) or a quaternary ammonium compound (QAC),
  (ii) cleaning by a stronger concentration of a detergent-sterilant followed by sterilization by a weaker concentration (e.g. iodophors)
  (iii) cleaning and sterilizing by a detergent-sterilant (e.g. QAC+alkali) followed by a 'sterile rinse' (e.g. QAC or hypochlorite).

(iv) using a single substance which has powerful cleaning and sterilizing properties (e.g. sodium hydroxide or nitric acid) followed by a sterile rinse.

Inter-relationship between detergents and sterilants

In practice it is impossible to dissociate the action of a detergent from that of a sterilant. All detergents have some killing power in addition to their ability to remove most of the micro-organisms from a surface by cleaning which effects mechanical removal. Some sterilants exert a detergent action by chemically attacking a constituent of the soil, e.g. hypochlorites accelerate the degradation of proteins, and dilute acids can dissolve the calcium salts in heated milk deposits and hard water scale and so disintegrate a strongly adhering film.

The action of a detergent is complex. The main aspects are chemical hydrolysis of fat and protein, wetting of the equipment surface, solution of certain constituents, and, when an oxidizing agent such as chlorine, iodine, nitric acid, percarbonate or hydrogen peroxide is present, destruction of substances by oxidation. The combined effect of these activities is to disintegrate and loosen the soil so that it can be washed away.

Bactericidal action of detergents

Many detergents have marked germicidal properties although they are used primarily as detergents, and this may be the only claim made by manufacturers of proprietary products. Hot water at 60-80°C will kill most or all vegetative cells but few spores. A detergent will always enhance the killing effect of heat. Probably the best example is sodium hydroxide. A treatment of 63°C for 30 min in water will kill all bacteria except thermoduric ones and spores, but a 1–3% NaOH solution under these conditions will kill all thermoduric cells and a considerable proportion of spores.

Detergents are almost invariably used hot and so act by enhancing the bactericidal effect of heat. This effect is especially valuable against spores in those industrial applications, e.g. bottle-washing followed by cold filling, where excessive temperatures have to be avoided.

Detergent-sterilants are particularly useful where high temperatures cannot be used, as in manual dishwashing or because of delicacy of the material.

According to Monori and Varga (5) even apparently innocuous detergents such as the anionics, non-ionics and trisodium phosphate can exert a powerful killing effect against most pathogens but not against tubercle bacilli or spores.

The effectiveness of alkaline detergents can be improved by incorporation of anionic wetting agents if foaming problems are not likely to arise. The alkyl-aryl sulphonates may improve not only wetting, emulsifying and deflocculating but also the bactericidal action (6).

It is generally accepted that there is no reliable method of testing efficiency of detergent action suitable for application under all conditions or relevant to all problems, although standard test methods for *detergents* as such can be devised.

Comparison of sterilants

All sterilants have their characteristic advantages and disadvantages, and it is quite unsound to attempt to compare them unless consideration is given to the conditions of use.

The following factors should be taken into consideration when deciding on the best method for sterilizing equipment:

(a) Material of construction. Stainless steel and glass are best from the hygiene point of view (7).

(b) Adequacy of supply of steam and/or hot water (85-90 °C).

(c) Time available for cleaning and sterilizing. A quick turnover (e.g. for a tanker) may make the use of steam impossible.

(d) Type of equipment, e.g. large tank, long pipeline, equipment susceptible to heat distortion.

The advantages and disadvantages of heat and chemical sterilizing methods are summarized in *Table II* (8).

Recommendations for choice of sterilant are given in *Table III* (8).

Chlorine compounds are particularly indicated where a quick drastic action is required.

The QAC are not recommended where serious gram-negative contamination is possible, or where rinsing is difficult, e.g. where surfaces are rough or absorbent. One danger with QAC preparations is that they may be used as detergents although they are sold only as a sterilant.

Table II.

Sterilizing agents—advantages and disadvantages

	Steam	Hot water (90°C)	Chlorine-releasing compounds	Quaternary ammonium compounds	Iodophors
Cost	Varies	Varies	Low	High	Intermediate
Convenience	Depends on lay-out	Recirculatory system necessary	Very convenient	Very convenient	Very convenient
Penetration	Good if adequate supply	Good	Clean plant essential	Clean plant essential	Has detergent action
Heating effect	Tanks, etc. may require hours to cool. Undesirable stresses may be set up	Less than steam	None	According to temperature	None
Suitability	Very suitable for enclosed systems and small articles in chests	Very suitable for pipelines	All purposes	All purposes	All purposes
Persistence	Not persistent	Not persistent	Not persistent	Persistent	Not persistent
Corrosion	None	None	Very corrosive unless maintained at pH 9 or above	None	Not corrosive if thoroughly rinsed away
Odour	None	None	Marked	None	None below 50°C
Rinsing	Unnecessary	Unnecessary	Good rinsing essential	Good rinsing essential	Good rinsing essential

All chemical sterilants can be corrosive if improperly used, e.g. at too high a concentration, at too high a temperature, for too long, and/or not adequately rinsed away.

Table III.

Recommendations on types of sterilant

Sterilant	Circumstances where indicated	Circumstances where inadvisable
Hypochlorites, chlorocyanuric acids and sodium phosphate hypochlorite.	Where drastic action required. Where low cost is important. Where all types of micro-organisms are likely to be encountered. Where alkaline detergent required	Where odour inadvisable. Where corrosion likely

TABLE III.—*Continued*

Sterilant	Circumstances where indicated	Circumstances where inadvisable
Quaternary ammonium compounds	Where odour, taste and toxicity to be avoided. Where safety in use important. Where gram-positive organisms chief danger. Where alkaline detergent desired.	Where low cost important. Where gram-negative organisms a likely danger. Where rinsing difficult (e.g. rough surfaces)
Iodophors	Where calcium scale a problem	With galvanized iron
Ortho-phenyl-phenol	For equipment not coming into contact with food	
Nitric acid	Where calcium scale a problem	Where equipment not all stainless steel. Where strong acid dangerous. Where fat-protein films a special problem
Hexachlorophene	Where contact with hands, etc. possible. In toilet preparations, etc.	Not for food equipment
Chlorhexidine	As 'antiseptic'.	Where cost important
Amphoterics	Where neutral conditions required	,,

Detergent-sterilants and their use

A detergent-sterilant is required to carry out the following operations:

(i) Remove all soil.

(ii) Remove or kill all pathogens and potential pathogens.

(iii) Remove or kill all fault-producing organisms (FPO).

(iv) Reduce bacteria to 1 per sq cm surface area or per ml cubic capacity.

The main fields of application for detergent-sterilants are given in *Table IV*.

Table IV.

Applications of detergent-sterilants for sanitizing equipment

1. In general for all cleansing purposes where heat cannot be used, e.g. walls, floors, wooden and plastic table tops, refrigerators, cold stores, etc.
2. The food industries, particularly for equipment for perishable foods such as milk and the more vulnerable foods such as meat, poultry, fish and eggs.
3. Medical and surgical activities: hospitals, clinics, surgeries, etc.
4. Sanitary aspects of communal activities: schools, colleges, catering, swimming and shower baths, public lavatories, public transport and all equipment communally used.
5. Institution maintenance cleaning.
6. Domestic: dishwashing, babies' and children's items particularly in nurseries, etc.
7. Cleaning or washing operations where heat cannot be employed: manual operations, delicate fabrics, plastics, etc.
8. Agriculture: particularly dairy farms, animal pens and all equipment wherever animals are involved.
9. Sanitation generally: wherever potentially dangerous material is handled as in slaughter houses, disposal of offal and refuse, etc.
10. Sewage disposal and all operations involving obnoxious material.

In general, detergent-sterilants, like chemical sterilants, are particularly useful where for any reason heat cannot be used, or is expensive or inconvenient.

The value of changing methods and sterilants

Particular methods, and particular chemical formulations are often specially good for certain purposes and against particular types of organism. The consistent use of one method and/or one formulation may lead to a weakness in the overall system because of a slow film build-up (too slight to be noticed) or acclimatization by a particular organism, e.g. coli, spore-former or yeast.

The types of organism surviving on equipment depend on the nature of the soil and the sanitizing method used. It is therefore strongly recommended that at intervals, say once weekly, a different method should be used. For in-place cleaning systems it may not be convenient or economic to change the system drastically, e.g. use steam or hot water instead of hypochlorite, but it is usually possible to change the type of detergent and/ or sterilant.

In-place or circulation cleaning

The scale of operations in factories today and the shortage and cost of manpower have revolutionized our attitude to cleaning and sterilizing. The classical idealist methods would be impossible today, and all relevant industries have gone, or are rapidly going over to in-place cleaning. Provided *all* the equipment is suitably designed for this purpose, the method can be entirely successful. The usual system is a closed circuit with spray devices for tanks. A minimum velocity to give turbulent flow in pipelines, and properly formulated detergents and sterilants are essential. Permanent tanks for these solutions, regular laboratory control and automation allow such systems to operate with very little man-power although capital cost may be high.

The problem of emulsions

In addition to the purely physical and chemical problems associated with the formulation of emulsions there will often be microbiological problems. Three phases may be involved—the continuous phase (usually aqueous), the discontinuous phase (usually fatty) and possibly an adsorbed layer phase which may have considerable significance for organisms. This

third phase may act as a powerfully adsorbing film for bacterial nutrients and a focus for bacterial clumps or colonies.

Homogenization may lead to biological troubles because clusters of micro-organisms are broken up and the cells dispersed throughout the medium. Homogenizers are also difficult to clean and sterilize.

Emulsions of water in oil are much more stable microbiologically than the reverse, as the organisms are confined to their own water globule. If these globules are very small (a few microns) growth appears to be halted rapidly and the organisms die out.

Unsuspected reservoirs of contamination

When all reasonable precautions have been taken but there still occur sporadic and serious outbreaks of infection the cause is probably an unsuspected reservoir of contamination. The following explanations have been found in factory practice:
  (i) poor communication between management and staff;
  (ii) poor supervision, especially early in the morning;
  (iii) bad hygienic design of equipment or lay-out;
  (iv) changes in cleaning/sterilizing procedure to reduce costs;
  (v) rapid staff turnover;
  (vi) making assumptions without laboratory tests to check them.

Filtration and clarification

When a liquid product has to be cleared of suspended particles, filtration is usually adopted because clarification is expensive and may be impossible. It should be realized however that, if the product is of poor microbiological stability, filtration may do nothing to improve this, and may even worsen it because all the material is forced through a layer of suspended matter which may be building up a substantial bacterial population. Filter cloths or pads should be renewed frequently for this reason, and double alternating filters preceded by fine mesh strainers are to be recommended where the nature of the product makes this desirable.

The size of micro-organisms

One difficulty in teaching hygiene to operatives, and even to factory floor management, is to convince people of the size of bacteria. To talk about

microns is meaningless to them. A simple analogy is to point out that if a bacterium were magnified to the size of a man, then the man would have to be magnified to the size of Great Britain to maintain the proportion. No system of filtration practicable for large volumes of liquids can be relied on to remove all bacteria, although yeasts and moulds, being about 10 times the size (diameter) can usually be removed to a considerable extent.

Factory water supplies

The bacteriological purity of water is generally judged on the basis of the Ministry of Health Memo No. 71 which assesses potability by the presumptive and faecal coli tests, supported by total colony counts at 22 and 37°C. Many years' experience has proved the validity of this method, but potability is not the same as quality for a particular industrial purpose. Defects in cosmetic and other preparations may be caused by *Pseudomonas* and similar Gram-negative bacteria, yeasts, moulds and other types of no public health significance. The hazards for water, which is drunk, and for cosmetics, which are applied to *and remain on the skin*, are quite different. The latter include *Staphylococcus aureus, Pseudomonas aeruginosa*, and various skin pathogens which are usually completely ignored in public health water bacteriology. A further fallacy is the assumption that water as used *in* the factory is as pure as water supplied *to* the factory. Storage in tanks and passage through pipelines, pumps, filters, softeners, etc. may easily result in gross contamination. Unless constant testing shows that the water is of adequate purity, mild chlorination (2-5 ppm) is recommended. For a survey of problems and control methods see Davis (9).

Hands as a source of infection

Apart from obviously bad air conditions, contamination or infection of a product is always caused by contact. Of all the ways in which this can occur, there is little doubt that in practice the hands are the commonest means whereby a product becomes contaminated by a pathogenic organism. Skin is impossible to sterilize and the bacterial load may vary from a few thousands to a few millions. *Staphylococci* and enterobacteria are nearly always found (10).

Biological control of pathogens and fault-producing organisms

Considerable success has been achieved in agriculture and in some

branches of medicine by using biological control to eliminate pests. The usual method is to liberate one type of living organism that is predatory on the pest. Little has been done in this direction in industry, although it is well known that by the use of 'starters', e.g. in cheesemaking, undesirable organisms can usually be suppressed. It has been observed in some fields of work that there may be an inverse relationship between the numbers of a harmful and of an innocuous organism. Thus we have observed this type of inverse relationship between *Staph. aureus* and *Staph. albus* in foods, and such a relationship may also be found on the skin. This type of approach to problems of infection is largely an unexplored field.

Hygienic packaging

The glass bottle is still the favoured container in the cosmetics, milk and other industries, and likely to remain so for some time. Glass is the most hygienic (i.e. most easily cleansed) of all common materials (7) although it is heavy, fragile and susceptible to neglect and sabotage. It has the advantages of transparency, cheapness (if re-used) and does not have to be imported.

Blow-moulded plastics bottles are gaining favour in the soft drinks and other industries. They are very light, cheap, strong, non-fragile, reasonably rigid and initially sterile by virtue of their method of manufacture which involves a temperature of *ca.* 180°C. Their use is certain to increase.

The plastics sachet is the lightest and cheapest of all single service containers, but is experiencing some consumer resistance, as of course do all new ventures. It is likely to be the container of the future for many liquid and solid products.

Some progress has been made in the use of sterilizable plastics sachets (11).

General precautions in hygiene, etc.

The most efficient cleansing of equipment is easily invalidated if unhygienic methods are practised subsequently.

The following points should be observed:

(i) Check the quality of all raw materials.
(ii) Unpack raw materials in a separate building, especially if embedded in sawdust, cotton waste, straw, etc.
(iii) Apply a biocidal treatment where necessary if this is practicable. Controlled heat-treatment is usually the simplest and most reliable.

(iv) Control the quality of water *as used in the factory*.
(v) Check the bacterial purity of the air near fillers, etc.
(vi) Check the hygienic condition of all containers.
(vii) Remove all residues, broken or split containers, etc., as soon as possible.
(viii) Do not use cloths for mopping up spillages unless these are maintained in a clean condition. Such cloths can be a menace. Paper towels are much better (12).
(ix) Thoroughly clean equipment *immediately after* use.
(x) Sterilize or cleanse all equipment, as may be necessary, *immediately before* use.

Common fallacies in hygiene

Some common fallacies experienced in commercial life are the assumptions that:
(i) splashing disinfectant over floors, etc. solves the hygiene problem,
(ii) forcing steam through a circuit with the production of great clouds of 'steam' and considerable noise necessarily sterilizes the equipment,
(iii) if equipment looks clean, then it must be clean. This is not true microbiologically,
(iv) 'Window dressing devices' such as making people wear white coats and caps, UV lamps, use of disinfectant aerosols, may serve some useful purpose and undoubtedly exert a psychological influence, but in terms of real worth are not quantitatively very important. Thorough cleaning of all equipment and prevention of contact contamination constitute 99% of factory hygiene.

Air

Most quality controllers in factories have their views as to how the purity of air should be maintained, both for the staff and the product. An air conditioning system which controls temperature, humidity and removes micro-organisms by filtration and/or electrostatic means is by far the best, but is quite expensive.

For the product, as distinct from the staff, an aerosol or fog treatment is the simplest and cheapest, but the choice of biocide is a tricky problem. Chlorine (as hypochlorite) is cheap and effective, but very corrosive.

Formaldehyde is also cheap and effective but very unpleasant for the staff, and somewhat corrosive. It causes eye, nose and throat reactions at 2-3 ppm. Up to 5 ppm can be tolerated for a few minutes, but at 10 ppm it becomes almost unbearable (13).

Fogs of QAC solutions appear to be both effective, cheap and without objection. Little or nothing is known of the effect of repeated inhalation of minute concentrations of QAC (e.g. 1 ppm) but in general they can be regarded as non-toxic. There has been some scare in medical circles about the use of benzene compounds in hygienic measures on account of possible carcinogenic effects, but there appears to be no real evidence for this. For example, the toxicity of benzoic acid is low. It there is any apprehension over the benzyl QAC a twin chain (C_8 or C_{10}) compound may be equally or even more effective.

Walls, roofs and air in factories

These problems are inter-related because whatever organisms may occur on one will be found on the others. Clearly the factory air cannot be free from contamination which occurs on walls and roofs, or is present in air outside the factory.

Moulds, yeasts and algae grow readily on any surface if the RH is over 70% and the merest trace of nutrients is present. The first precaution is therefore to maintain adequate ventilation and at all cost to prevent condensation at any time. Such condensate can be teeming with gram-negative bacteria including coliforms and *Pseudomonas*. The simplest and best treatment for soiled walls and other surfaces is to wet them and 1 h later to apply a penetrative, non-foaming, alkaline detergent by a spray or other suitable means. This will clean the surfaces and exercise a substantial killing effect, but in order to obtain an effective kill a suitable bactericide-fungicide must be incorporated in the detergent. If the wall, etc., has been allowed to get into bad condition, repeated treatments will be necessary.

Needless to say, all walls, etc. in a cosmetics factory should have a smooth, impervious and washable surface.

Methodology in hygiene

Practical recommendations for cleaning, sterilizing and hygiene in public health and various industries are given in (14–19).

One of the most serious problems in the teaching of the principles of

hygiene and their application is that there is no glamour, no excitement and no romance in such a subject.

Heart transplants, human organ banks, the fertilization of the human ovum outside the uterus and similar topics hit the headlines, but at the most these advances save an occasional life or prolong a few lives for a few months. On the other hand hygiene has saved millions of lives and prevented many millions of cases of disease. The dramatic rise in the expectation of life during the past 100 years is mainly due to the application of hygienic principles. No branch of medicine or science has contributed more to human health and happiness.

LABORATORY CONTROL

Laboratory control of cleaning and sterilizing

The choice of a particular method is less important than regular testing. The ultimate criterion must always be the keeping quality and safety of the product, and it is usually easy to link the hygiene aspects in the factory with the quality of the products. The 'first product through' test, swabbings, rinsings or any other sound method is adequate for both staff training and protecting the quality of the product (16).

An important feature in hygiene control is that tests should be surprise tests; the staff should never know when a particular piece of equipment is going to be checked. A rigid and known time-table for plant control defeats its own object.

Laboratory tests are useless unless they are acted upon. I have known of a case where the head of the laboratory put his reports on the manager's desk daily, but it was not until the poor quality of the product was revealed later that the manager studied the reports.

The testing of disinfectants, antiseptics and preservatives

The high degree of competitiveness in this field results in many far-reaching claims being made by manufacturers and salesmen. Unfortunately it is not possible to assess the validity of these claims without a careful examination by an expert with adequate bacteriological facilities, a process which is time-consuming and expensive. There is no single, reliable test which can be applied to any of these preparations. Results vary considerably according to the technique used, and the cynic might with some reason assert that one can get almost any result one likes by selection of the

conditions of testing. The only ultimate criterion which is of any value to the user is whether the substance fulfils the claims made for it under the condition of use. Any laboratory test used should therefore simulate as closely as possible these conditions.

Choice of end-point

It should be realized that there is nothing definite about disinfectant studies; everything is arbitrary. Such terms as 'complete kill' or '99.99 per per cent kill' are quite arbitrary and can only be defined in terms of the particular technique used.

There are many factors capable of affecting the result of any disinfectant test, irrespective of its nature (*Table V*). In a laboratory test, these can at least be standardized but under conditions of use such factors may

Table V.

Factors influencing the result of any disinfectant test

1. Type of organism (e.g. *Staph. aureus*)
2. Particular strain used
3. Physiological condition—long term (e.g. how long in artificial culture)
4. Physiological condition—short term (e.g. 24 h at 37°C)
5. Composition of medium used for growth (e.g. nutrient broth)
6. Physical state of medium used for growth (e.g. agar medium)
7. Temperature, humidity, etc. of growth
8. Concentration of disinfectant
9. Stability of disinfectant to moisture, oxygen, etc.
10. Temperature of test
11. Time of contact
12. pH value
13. Osmotic pressure
14. Synergistic effects with other substances, e.g. acids, surfactants, salts.
15. Concomitant substances (e.g. in hard water); compatibility with other substances present
16. Physical nature of suspending fluid, e.g. emulsion
17. Surface tension of suspending liquid (e.g. Teepol)
18. Nature of solvent (e.g. part isopropyl alcohol)
19. Method of mixing disinfectant and cells
20. Type, physical nature and concentration of soiling matter
21. Choice and concentration of inactivator (e.g. Lubrol W)
22. Medium for growing survivors
23. Incubation temperature for plates
    Surface film tests
24. Soiling material used (e.g. whole milk)
25. Method of fixing to surface (e.g. drying at 37°C)
26. Chemical nature of surface used (e.g. stainless steel)
27. Condition of surface (e.g. highly polished)

vary considerably, e.g. temperature, time of contact, nature and amount of soilage, hardness of water.

Reports of microbiological examinations

In my opinion many reports and claims for disinfectant power, etc. by manufacturers, are unscientific and even sloppy. Such statements as 'kills typhoid in 2 min', 'coli absent', etc., are meaningless, and in fact may even be misleading. The conditions of testing should always be stated, and reports should state results in a form such as 'coli not detected in 1 ml', etc.

Some recommended microbiological control methods for cosmetic products are given in *Table VI.*

Table VI.
Recommended bacteriological control methods for cosmetic products

Organism	Medium	Temperature °C
Total (public health)	Blood agar	37
Total (general)	Glucose tryptone agar	27
Staphylococcus aureus	Mannitol salt (21)	37
	Egg yolk (22)	37
	Phenol phthalein phosphate (23)	37
Pseudomonas aeruginosa	Acetamide agar (24)	37 (42)
	Cetrimide agar (39)	37
Presumptive coliforms	MacConkey broth	30
	Eosin methylene blue agar (25) ⎫ *Escherichia* and	30
	Violet red bile agar (26) ⎬ *Aerobacter* can be differentiated on these media	30
Faecal coli (follow-up tests)	⎰ MacConkey broth (both must be used)	44
	⎱ Peptone water	44
Candida albicans, yeasts and moulds	Malt agar pH 5.4	27
Yeasts and moulds	Malt or buffered beerwort agar pH 3.5 (27)	27
	Buffered citric acid agar pH 3.5 (28)	27
Streptococcus faecalis	Crystal violet azide blood agar (29)	37
Salmonella	Selenite broth (for enrichment) (30)	37 (43)
	Desoxycholate (31)	
	Brilliant green (32) or	
	Bismuth sulphite agar (33)	37
	Kligler iron agar (32), (35)) or	37
	Kohn-Gillies broths (36), (37)	
	Final serological confirmation	
Clostridium spores	Meat broth	37
	Reinforced clostridial medium (38)	37

Oxoid or Difco media (see Manuals issued by these firms)

Microbiological standards

There is now considerable interest in microbiological standards for foods and some other products. This is a subject into which which no-one should enter without experience of the product as well as a knowledge of microbiology. It is very easy to lay down standards which are impracticable, unnecessary or even just ridiculous. The basic requirements are

    (i) careful standardization of all aspects of sampling, transporting and storage of the sample,

   (ii) standardization of laboratory testing,

  (iii) consideration of age of product,

  (iv) allowance for error of test,

   (v) selection of appropriate tests,

  (vi) proper interpretation of results.

Standards should only be advisory, and are mainly a matter between buyer and seller. They can be particularly useful for purchasers of basic materials. For general quality control purposes standards set for production in the factory must be related to the microbiological behaviour of the product between production and final use.

Some suggested standards are given in *Table VII*.

In U.S.A. the F.D.A. have become very concerned with the problem of microbiological contamination of cosmetics, especially in relation to *Ps. aeruginosa*. It can be anticipated that they will shortly issue standards for this purpose, which will obviously be of the greatest importance for the British export trade.

Table VII.
Tentative microbiological standards for cosmetic preparations g^{-1} or ml^{-1}

	Satisfactory	Doubtful	Unsatisfactory
Total colony count*	<1 000	1 000-10 000	> 10 000
Presumptive coliforms*	< 10	10- 100	> 100
Faecal coli	< 1	1- 10	> 10
*Staph. aureus**	< 1	1- 10	> 10
*Ps. aeruginosa**	< 1	1- 10	> 10
Salmonella	Not detectable in 100 g		
Fault producing organisms	Impossible to generalise		

* =first priority in laboratory control work.

Tests and standards for the efficiency of cleansing

Cleansing consists of two treatments—physical cleaning and sterilization or disinfection. There is a steady trend towards combining these.

Cleaning or detergency

There is usually no need to assess this alone, or to set standards.

If required, an assessment of residual organic matter can be obtained by allowing contact with a reactive chemical, e.g. chlorine, for a given time and then determining the loss in available chlorine.

A simple 'spot test' is to take advantage of the fact that iodine, as in iodophors, stains organic matter a yellowish colour. Iodophors do *not* stain clean glass, stainless steel, etc., although allegations have been made to this effect. Any staining indicates a greasy or protein film, hard water scale, etc.

Residual bacteria or 'sterility'

If equipment has been properly cleaned and 'sterilized' the number of bacteria left will not exceed 1 per cm^2 area by a swab test or 1 per ml capacity by a rinse test (14). These tests are therefore quite adequate to assess the efficiency of cleansing in a general sense. It can be assumed under ordinary working conditions that if these results are satisfactory (i.e. less than 1 colony per cm^2 or ml) then all pathogens will have been killed or removed. It is also unlikely that FPO will have survived in sufficient numbers to cause trouble, but as a safeguard 1 000 cm^2 may be swabbed and the 20 ml Ringer solution added to a suitable enrichment medium (*Table VI*) for the cultivation of specific pathogens or FPO.

Alternatively 5 ml could be added to
  (i) mannitol salt broth (for *Staphylococcus*),
  (ii) selenite broth (for *Salmonella*),
  (iii) acetamide broth (for *Pseudomonas aeruginosa*),
  (iv) buffered citric acid broth pH 3.5 (for yeasts and moulds),

    (the appropriate confirmatory tests must be made) but such a refinement is only necessary when a relevant problem arises.

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