1 Introduction

This annex is intended to provide information concerning bioburden in condom manufacturing, to recommend bioburden limits for foiled product and to give guidance for manufacturers in controlling and monitoring bioburden.

This annex has also been prepared to provide an accurate explanation regarding bioburden and microbial control for male latex condoms for national regulatory authorities, programme managers, procurement bodies and other interested parties.

2 Bioburden, limits and rationale

2.1 Bioburden

Control of microbial contamination on medical devices is essential to ensure the consistency and safety of the product. The total population of viable aerobic microorganisms on the product and inner foil is termed the bioburden. Micro-organisms normally associated with contamination during manufacturing are bacteria, yeasts and fungi. Although some viruses can survive for varying times, they cannot multiply outside the body and are therefore not normally included in bioburden counts.

A number of sources, such as raw materials, process water, manufacturing equipment, packaging materials, personnel and the environment, can contribute to the total bioburden count of a final foiled product. In order to control and minimize bioburden, regular monitoring of these sources should be maintained.

Because bioburden control is a requirement of Good Manufacturing Practice (GMP) (1–3) of medical device manufacture, this monitoring may also be used as part of the quality control system to ensure adequate environmental control, efficacy of cleaning procedures, efficacy of monitoring and adherence to GMP.

It is recommended that bioburden levels be determined periodically—for example, at quarterly or, ideally, weekly intervals.

Standard operating procedures (SOPs) for bioburden monitoring should include limits for each stage of the manufacturing process and should include equipment and the environment.

2.2 Condom bioburden limits

Searches of the scientific literature have failed to identify any reports of genito-urinary infections linked to use of condoms that may have been contaminated during manufacture. Nevertheless, limits for bioburden levels on final foiled product are recommended in order to ensure product quality and user safety.

Final foiled product should have a bioburden maintained at less than 100 colony forming units (cfu) per item. Occasional excursion above this recommended limit may occur but, if so, should not be allowed to exceed 500 cfu per item. If the higher end of the limit is exceeded, immediate action should be taken to reduce the bioburden, for example, by undertaking cleaning and sanitizing procedures.

There should be an absence of all pathogenic organisms and in particular of Staphylococcus aureus, Pseudomonas aeruginosa and Enterobacteriaceae, including Escherichia coli. There should also be no growth of fungi and yeasts.

Routine testing of condoms may be conducted using the Total Viable Count (TVC) methods (4–6) and the Specified Organism Test (7, 8).

2.3 Rationale

Staphylococcus aureus organisms, which are carried by 25% to 32% of the general population (9, 10), may be pathogenic and can cause severe infections of skin, wounds, respiratory tract, and the urinary tract as well as sepsicaemia (11–13). Some specific Staphylococci produce an enterotoxin (14, 15) that, if ingested, causes gastric upset and food poisoning.

Staphylococci may also produce a toxin (TSST) (16) that causes toxic shock syndrome (17). This has never been implicated in condom use, however. It was classically associated with prolonged insertion of highly absorbent tampons, but it is now known that other medical conditions are related to the syndrome (18).
Poor GMP may facilitate cross-contamination with *Staphylococci* from manufacturing operators to the product.

*Pseudomonas* species are opportunistic pathogens that are often resistant to many of the commonly used antibiotics. They may cause a range of infections, particularly in people whose immune systems are compromised. They have been known to cause lung, ear, eye, and urinary tract infection and sepsicaemia (19–21). This organism is frequently found in water supplies. Therefore, strict contamination control of all process water is required.

*Enterobacteriaceae* including *Escherichia coli*, may cause infections of many sites including the genital and urinary tracts, brain, blood and gastrointestinal tract (22, 23). Specific *Escherichia coli* strains produce an enterotoxin that causes food poisoning and may lead to kidney and liver failure (24). Poor personal hygiene, especially inadequate hand-washing after using the toilet, will cause cross-infection with *Escherichia coli* by transfer between people touching surfaces, materials and products.

Some fungal species have been shown to cause slow deterioration of latex products and should therefore be absent from the final packed product (25–27). Yeast species may cause infections of skin, mouth and genital areas. Therefore, these also should be absent from final foiled product (28, 29).

### 3 Test methods

Bioburden testing should be conducted at regular intervals—for example, quarterly, but preferably weekly. It is recommended that results are regularly monitored so that bioburden trends may be analysed in order to validate microbiological control measures and to monitor environment and product for significant changes. Regular monitoring of results will also enable any necessary remedial actions to be completed as soon as possible.

Bioburden testing should be conducted in accordance with written procedures. The methods used must be adequate to extract bioburden from the test sample, including surfaces, and must maintain the viability of the organisms extracted. The culture media used must support growth of the extracted bioburden. To allow comparisons over time, sampling systems should be consistent.

Some of the samples tested may contain materials that might inhibit growth of micro-organisms. It is recommended that culture media used contain additives that will neutralize these antimicrobial effects (30–32).

When tests are conducted, care must be taken to avoid contamination of samples, culture media and test equipment. Careful control and good aseptic technique will ensure that there is no inadvertent external contamination.

All methods utilized must be validated in order to ensure that test requirements are met. Dilution factors and recovery factors will be calculated from these validation studies and must be incorporated into the test calculations (33).

It may be possible to utilize rapid methods both for routine monitoring of the environment, equipment and materials and for testing of condoms. There will be interference, however, from chemicals and powder used in the manufacturing process. Therefore, an extensive validation programme will be required, particularly if used for condom Total Viable Counts.

When micro-organisms are isolated, some additional testing must be completed to ensure that the isolates are none of the prohibited organisms.

#### 3.1 Routine monitoring

Routine monitoring procedures for the manufacturing environment and equipment may involve the following methods.

##### 3.1.1 Surface testing

Testing of surfaces may be carried out using swabs, contact plates, contact slides or the rapid bioluminescence test.

Contact plates and slides are designed so that the surface of the solid media may be directly applied to the test surface and then incubated. Such tests are quick.
and easy to use, and results are directly related to the contact area. The disadvantage is that possibly not all organisms will adhere to the media, and the plates or slides can be used only on flat surfaces (34, 35).

Determining bioburden by use of swabs is particularly useful for monitoring irregularly shaped equipment and difficult-to-access surfaces. Swabs are normally moistened in a liquid medium and then rubbed across a predetermined area. The swab may be directly applied to agar medium, or the swab may be immersed in liquid media, agitated to remove organisms and the TVC completed as for liquid testing. Direct application of the swab to the agar may not remove all organisms from the swab, whereas using an intermediate liquid stage will improve recovery of the organisms (34).

Bioluminescence testing (36, 37) is especially helpful for examining surface bioburden because results are rapidly obtained and will confirm whether cleaning procedures have been carried out correctly. This will enable a quick response to any problem areas identified, thereby preventing product contamination. The test method utilizes the reaction that occurs between bacterial adenosine triphosphate (ATP) and firefly luciferin/luciferase, resulting in emission of light.

### 3.1.2 Powders and liquids

Microbiological testing of powders and liquids can be achieved utilizing pour plates, spiral plating/spread plates, membrane filtration or the dilution droplet technique of Miles and Misra. In the case of water testing, the Most Probable Number (MPN) method can also be considered (30, 38–41).

A measured amount of powder can be dissolved in either a suitable solvent or in liquid culture media. Testing then proceeds as for liquid samples. Solvents and powder samples may have an inhibitory effect, however. Therefore, suitable dilutions or neutralizing agents should be used.

For the pour plate method, samples of liquid are added to cooled molten agar and mixed, and plates are poured. When set, plates are incubated at appropriate temperatures and times, and colonies are counted.

Alternatively, a liquid sample may be directly applied to the agar surface, spread and then incubated. A smaller sample may be required in order to ensure that discrete colonies are cultured and so enable accurate counting. Samples may be delivered and spread using spiral plating equipment. The number of colonies can be related to the volume of suspension delivered and the total count calculated.

For the Miles and Misra method, a series of dilutions are made from the samples. Then measured drops are placed on the agar surface. A minimum of five separated drops from each dilution is required. Plates are allowed to dry and are incubated, and counts are made.

When large sample volumes are available, particularly as in the case of water testing, the MPN method may be used. A range of dilutions is made in liquid growth medium. The range must be selected so that the lowest dilutions do not show microbial growth. Tables have been produced, such as those by DeMan, using statistical assessments to determine the MPNs of organisms present in the initial sample.

In tests of chlorinated water, any residual antimicrobial effect of the chlorine may be neutralized with sodium thiosulphate.

The membrane filtration technique utilizes a membrane with a sub-micron pore size, large enough to enable large volumes to pass under pressure, but small enough to retain bacteria. The membrane is then placed onto an agar plate and incubated, and colonies are counted. This technique is particularly useful when there are low numbers of microbes or when there may be interfering substances in the liquid sample being tested.

### 3.1.3 Air sampling

Microbiological testing of air samples may be achieved by using settle plates or by active air sampling (42–44). Agar plates are left exposed for a defined period of time in the area under test. They are then incubated and colonies are counted. Whyte has established that, for a bioburden of 100 cfu per m³, a 90 mm diameter plate exposed for an hour will show 10 or 11 cfu (42).

Active sampling systems are also available (45, 46). Air is drawn into a device for a measured period of time. The micro-organisms are deposited onto agar, which is then incubated. Types of active air samplers available are slit
samplers, centrifugal samplers and impaction samplers. The cost of equipment and consumables may be high.

Membrane filtration may also be used. Air samples are passed through a sub-micron membrane filter pad for a designated time. The membrane is subsequently placed on an agar surface and then cultured to determine bacterial numbers present in the air sample.

In the case of all air sampling techniques, loss of viability may occur due to desiccation of the organisms. Hence, prolonged sampling times should be avoided.

3.1.4 Identification of micro-organisms
On completion of the primary testing, additional tests may be required to identify any organisms isolated, in order to confirm that none of the prohibited organisms are present. A gram stain, coagulase test and oxidase test will indicate whether species identification is required. Biochemical profiles may be used to identify organisms to species level.

3.1.5 Rapid test methods
There are rapid test methods available that may be considered for testing materials and product and for environmental testing. Rapid test technology obtains bioburden measurement by utilizing turbidity, bioluminescence, conductance or impedance (35, 36, 47, 48).

The advantage of using rapid methods for routine monitoring is that any increase in bioburden will be detected early, thus allowing action to be taken quickly to prevent continuing contamination of product.

The disadvantage of using rapid methods is that there may be interference from some samples that could nullify the use of these techniques. There must be extensive validation programmes, and initial outlay for equipment will be high. Dependant on the particular rapid test method used, ongoing supplies of consumables may also be expensive.

4 Guidance for controlling microbial contamination
Cleaning and sanitizing procedures and bioburden limits should be established for all manufacturing procedures and for environmental monitoring. A period of preliminary testing will determine baseline counts to enable routine test limits to be established.

When defined, the recommended limits must be at a level that will ensure product safety. After bioburden limits have been established, routine testing programmes can be installed for all stages of the manufacturing process.

4.1 Equipment
All manufacturing equipment, including tote bins, should be cleaned and sanitized at regular intervals to a written schedule. Cleaning should be microbiologically validated using surface test methods to ensure the efficacy of the cleaning procedures and to ensure that there is no cross-contamination onto product.

4.2 Environment
The manufacturing environment should be controlled to minimize microbial contamination and to ensure that pests such as rodents, birds and insects do not gain access to any manufacturing areas. This is especially important in the manufacturing stages after final drying. It is recommended that air sampling be regularly conducted, particularly in areas where the condoms are most vulnerable to microbial contamination, until product has been foiled.

4.3 Personnel
Microbial contamination may also arise from personnel. When standing still, a person will normally shed 100,000 particles per minute. Moving may increase this to more than one million particles per minute. These particles will contain microbes normally present on skin. Coughing, sneezing and touching product or equipment will also greatly add to the bioburden. Suitable protective clothing and gloved hands will give a measure of protection against this contamination. GMP training will help to enforce correct handling procedures to ensure that contact and cross-contamination between personnel and product are minimized.

4.4 Raw materials
All raw materials, including water and packaging materials, should be tested at regular intervals. Some materials may have an inherent antimicrobial effect. If this
has been confirmed, then monitoring may continue at a much reduced rate on these particular materials.

4.4.1 Water
Water is a major material used during manufacturing, and so it must be controlled microbiologically and chemically.

Some incoming water supplies may contain extremely high bioburden levels, particularly in adverse local weather conditions such as very heavy rainfall or drought, and must be treated before storage. Treatment methods may include filtration, reverse osmosis (RO) (49, 50), ultraviolet irradiation (UV) or chemical treatment. It should be noted that chemical treatment may interfere with production processes and, depending on the chemicals used, may also cause adverse reactions in personnel.

After initial treatment the stored water should be kept under controlled conditions to minimize any further contamination or growth of micro-organisms.

Additional treatment of water may be necessary to produce deionised (DI) or softened water. Many microorganisms find favourable conditions for growth on the DI resin beds and on RO membranes. It is essential, therefore, that the servicing protocols be followed rigorously to prevent colonization of the equipment with microbes.

If UV irradiation is used, monitoring of the system to confirm correct UV emission is essential in order to ensure that the UV lamps have not become partially obscured and therefore ineffective.

4.5 Dipping, stripping and drying
Dipping lines utilize large volumes of process water, which is sometimes recirculated at certain points of the process. It is recommended that there is no recirculation, but, if recirculation is necessary, it should be kept to a minimum and only recirculated for short periods of time or for a single re-use. Microbiological testing will confirm whether bioburden is being properly controlled under these circumstances.

All equipment should be regularly monitored, using surface testing methods that will confirm the efficacy of cleaning.

Handling of condoms should always be carried out with gloved hands. Whenever necessary, gloves should be either sanitized with antimicrobial wipes or else replaced. Any antimicrobial materials used for sanitizing must not interfere with process or product or adversely affect personnel.

It is recognized that heat and drying will inactivate many micro-organisms. Nevertheless, there are species that can survive such treatment, and, therefore, dryers should be cleaned and sanitized at intervals and included in the monitoring programme.

4.6 Slurry treatment
The slurry is a rich medium normally stored at a temperature that is optimal for microbial growth. Therefore, great care must be taken firstly in the cleaning and sanitizing of the mixing vessels, the reservoir tanks and the processors, and secondly in the choice of materials used to make the slurry. The water quality used in this process is particularly important. Ideally, slurry should not be recycled. Bioburden should be regularly monitored using TVC tests for liquids.

If necessary, consideration may be given to antimicrobial treatment of the slurry. Depending on the biocide used, it is possible that this may interfere with processing, cause skin reactions in operators and users and possibly be ineffective at the pH of the slurry. If a biocide is used, a full risk assessment must be completed.

4.7 Electronic testing, lubrication and foiling
After drying, condoms will be electronically tested by either wet or dry methods and will then be rolled, ready for lubrication and foiling. Bioburden of the liquids, the water in the testing baths in the case of wet testing and the lubricant and the lubricating and foiling equipment should be frequently monitored.

These processes should be maintained in a controlled environment. Personnel in these manufacturing areas should wear protective clothing and gloves and maintain a high standard of GMP.

All batches of foil should be microbiologically tested before release for use.
Once the condoms are sealed in their individual packs, they are protected from contamination, and any further operations do not necessarily have to be carried out in controlled environments. If any further operations are carried out where un-foiled condoms are stored, however, appropriate controls remain necessary.

References

3 ISO 13485:2003, 6.4, 7.5, 2. 1 Medical devices—quality management systems.
4 See Appendix 1, following, Method 1.
5 See Appendix 1, following, Method 2.
6 German Pharmacopoeia (DAB), 1996: V2.1.8.1 and V2.1.8.2.
18 Herzer CM. Toxic shock classically associated with prolonged tampon use; now other conditions have been related to this syndrome. Journal of the American Board of Family Practitioners, 2001, 14(2):131–136.
ANNEX II, Appendix 1

Method 1. Enumeration of total population of micro-organisms on foiled condoms

1. Using sterile forceps and scissors, aseptically remove a condom from the foil, cut the bead in two places, unroll the condom with sterile forceps and place in 10 ml of peptone water with added 0.3% lecithin and 3% polysorbate (Tween 80). Lecithin and polysorbate are added to neutralize any residual inhibitory effects from the condoms. Letheen broth may be used as an alternative extraction medium.

2. In order to remove the bioburden from the condom, mix the contents with a shaker, stomacher or vortex mixer for the time previously determined from validation studies. Care must be taken not to mix too vigorously, as this may kill some microbes.

3. Using a sterile pipette, transfer 1 ml of the extraction fluid into 20 ml of molten Sabourauds Dextrose Agar (SDA) kept at 40 °C. Mix gently to disperse the sample throughout the media and pour into a sterile Petri dish. Allow to set.

4. Repeat with another SDA and with 2x 20 ml of Tryptone Soya Agar (TSA).

5. Incubate the SDA plates at 20 °C for five days and incubate TSA plates at 30 °C for three days.

6. Inspect the TSA plates at three days and count colonies. Re-incubate and count at five days.

7. Count the number of colonies on each TSA plate and find the average of the two counts. Examine the SDA plates and count fungal and yeast colonies. Calculate the average count as above. Make corrections for dilution and recovery factors (previously determined by the validation tests).

The total viable aerobic count is determined by adding together the corrected averages of the fungal and bacterial counts.

8. Some identification of bacteria is required in order to confirm the absence of the specified organisms. Gram stain and colonial morphology will identify gram positive cocci and gram negative...
bacilli. A positive coagulase test will indicate probable *Staphylococcus aureus*, whilst the oxidase test will indicate possible *Pseudomonas* species. Results from these short tests will determine if further identification is required. Biochemical profiling will identify organisms to species level.

**Method 2. Enumeration of total viable aerobic microbial population and tests for specified microorganisms on foiled condoms**
1. Using aseptic technique, remove 13 condoms from packaging with sterile forceps into a large sterile dish, and cut up the condoms using sterile scissors.

2. Weigh 10 grams of material and place it in 100 ml of extraction media in either a 150 ml bottle or a stomacher bag. The extraction media should be capable of neutralizing any residual antimicrobial effect from the condoms. Suggested media is peptone water with the addition of 3% polysorbate (Tween 80) and 0.3% lecithin.

3. Stomach or mix the sample for the time required to remove the bioburden, as previously determined by validation testing.

4. Using a sterile pipette, transfer 10 ml of the condom extraction fluid into 100 ml of Soya casein digest broth and gently mix.

5. Proceed using the methods described in *United States Pharmacopeia*, 31 Microbiological tests <61> and <62> or in the *German Pharmacopoeia* (DAB) V2.1.8.1 and V.2.1.8.2 in order to determine Total Viable Count and absence of *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. 