GUIDELINES ON TEST METHODS FOR ENVIRONMENTAL MONITORING FOR ASEPTIC DISPENSING FACILITIES

PRODUCED BY

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General Introduction [1], [2], [3]

This document was originally compiled in response to a need from the Scottish Aseptic Services Specialist Interest Group (ASSIG) and Quality Assurance Specialist Interest Group (QASIG) membership for more specific guidance on the various environmental monitoring techniques used to demonstrate conformance to accepted clean room standards and the requirements of current Good Manufacturing Practice (cGMP). This revised version takes into account the many changes that have occurred to standards since the original was produced. Since publication of the first edition parts of BS EN ISO 14644 "Cleanrooms and associated controlled environments" have come into existence and those parts that have been issued for use have been considered in the preparation of this guidance document. It should be reiterated that this document is intended as an interpretation of relevant standards. If there is any doubt over a specific area then reference should always be made to the original standard.

Monitoring of both physical and microbiological contamination remains essential in aseptic operations to provide ongoing information on the maintenance of a stable and suitable environment for the aseptic preparation of products for administration to patients. This guidance document contains general information on physical and microbiological test methods and should be used in conjunction with the original source documents where more detailed information may be obtained. Sample monographs are provided here containing guidance on all aspects of the specific test methods. The layout of this document is in accordance with the "Guidelines on environmental monitoring for aseptic dispensing facilities" [1] with section 1 covering physical test methods and section 2 covering microbiological test methods. It is recommended that monitoring performed should be at least at the minimum specified frequencies in order to provide the necessary information.

Senior personnel within the aseptic preparation department must have an understanding of clean room and clean air device technology together with a thorough knowledge of all the features in their department, e.g. ventilation systems, position and grade of HEPA filters, types of clean air devices etc. It is preferable for certain tests, e.g. airborne particle counting, active air sampling or settle plates, that sampling is performed in the operational condition rather than the at rest state. It should be stressed that such sampling should not interfere with critical work zone protection. Results from monitoring should be considered (even in retrospect) when reviewing documentation for finished product release. It is recommended when monitoring in the operational state, where an active sampling method is used e.g. particle counting or active air sampling, that the testing is performed during routine operator or process simulation tests, in order not to compromise the quality of any products prepared that may be administered to patients. Testing in the at rest state can be carried out to monitor baseline contamination levels. Surfaces and personnel should also be monitored at the frequencies stated. A report of the test data indicating the significance of the results and any corrective action must be brought to the attention of all relevant staff (at team meetings) and full records kept on file for future reference. Particular importance should be placed on obtaining meaningful results, monitoring trends, setting "in-house" standards, where appropriate, and action levels.

Information obtained from all monitoring, by in-house or external contractors, should be actively and knowledgeably assessed by either the Responsible Pharmacist or the Quality Assurance/Control Pharmacist, and any necessary action taken and not merely filed for record purposes.

Test methodologies [4]

Testing physical parameters as a means of measuring the performance of equipment/facilities is accepted practice. The physical tests performed are reproducible and accurate and can be used to validate the facilities/equipment's performance against accepted criteria e.g. airborne particulate contamination, pressure differentials, clean room air change rates. Testing microbiological parameters as a means of evaluating equipment/facilities performance is less accurate as the means of determination are highly variable. Microbiological levels in the environment are not uniformly distributed in a given area and fluctuate with time.

It is therefore vital that test methodologies exist as part of the environmental monitoring programme. Each test method selected for routine monitoring should be validated. If changes are made to test methods, variations may introduce unknown variability and comparisons between data are spurious. It is essential that test methodologies are defined and complied with so that trends in contamination levels may be compared. It is important to emphasise that failure to comply with the methodologies may render the samples collected invalid. Each test result should be reviewed in the context of previous and subsequent samplings with the major emphasis on detecting patterns within the data obtained.

SECTION 1: PHYSICAL TESTS

Introduction

Testing may be carried out by internal staff (Aseptic/QA/QC) or by external contractors. If external contractors carry out testing then they should be registered or accredited to a recognised qualification e.g. Cleanroom Testing and Certification Board (CTCB) of the Scottish Society for Contamination Control. In either case it is essential that testing is carried out using recognised test methods, by trained staff and is in accordance with an agreed test specification, which outlines which tests are to be carried out. Test sites have the responsibility for ensuring that clean air devices are in a clean and safe condition, e.g. free from cytotoxic contamination, before internal staff or external contractors test them.

The tests outlined in section 1 are physical tests that may be carried out for monitoring conventionally ventilated rooms housing one or more clean air devices. The clean air supply to these rooms is generally through one or more grilles in the ceiling (often with HEPA filters in place) and air is extracted by loss to adjacent rooms or through extract grilles (usually at low level). The clean room operates on the principle that the air supplied is of sufficient quantity to dilute or remove the contamination generated in the room.

Equipment used for any tests should be calibrated and traceable to national standards. Where the test is performed by an external service contractor a valid certificate of calibration for the test equipment used should be supplied with the test report. Steps should be taken to ensure that equipment used by in-house staff or external contractors for performing monitoring tests has been suitably cleaned and maintained prior to any testing being carried out.

Entry and exit of all personnel and equipment to the clean room(s) or device(s) under test must be in accordance with the site standard operating procedure(s). Clean air devices and other areas where contamination may have occurred must be cleaned according to site standard operating procedure(s) on completion of testing.

1. NON-VIABLE PARTICLE COUNTS [1], [3], [5], [6], [7], [8]

1.1. Introduction

Control of airborne contamination requires certain measures to be adopted, which are usually dictated by the type of product required at the end of the preparation process. Pharmacy clean room facilities are designed, constructed and operated to minimise the risk of microbiological and physical contamination by non-viable airborne particulates to the products prepared. Measurement and determination of the number and size of airborne particulate contamination is essential to ensure that a suitable environment is maintained for the preparation of aseptically prepared products. In the event of an air handling system or clean air device failure or shutdown (controlled) it is important to know when the room or device environment is back within specification. Steps should be taken to determine when the facility or device has returned to its normal operating grade i.e. how quickly the facility or equipment cleans-up. Guidelines for determination of clean-up time for clean rooms and clean air devices following shut down of air handling units/devices are contained in a separate document. The guideline covers many of the aspects described below for routine monitoring.

1.2. Equipment

It is standard practice to utilise modern technology and use an optical particle counter where the air sample is drawn into the instrument and passed through a light scattering device. The signal that this generates is electronically processed to display particle counts at different size ranges. The sampling rate can vary but the most likely sample volumes are $0.1ft^3$ or $1ft^3$. Particle counters can count a range of particle sizes ranging from 0.3µm up to 25µm. Pharmaceutical standards quote limits for the total number of particles equal to or greater than 0.5µm and 5µm, and these are the particle sizes that are usually measured. Counts of other particle sizes may be useful for investigation in the event of problems occurring.

1.3. Sample locations and volumes

1.3.1. Clean rooms

The operational condition is the most important condition to be measured as it reflects the actual contamination when the area is working; it should be noted that monitoring in the operational condition will give the highest particle counts.

It is necessary to take sufficient samples within the clean room to have confidence that the room is performing within the limits set by the standards. A statistical technique may be employed to give a mathematical basis to this confidence. Using this technique the number of sampling locations reflects the size of the room and its cleanliness e.g. the larger and cleaner the room the more sampling locations that must be taken. However the most common method for selection of the number of sampling locations can be determined using the formula [7]:

$$N_L = \sqrt{A}$$

where N_L is the minimum number of sampling locations (rounded up to the next whole number).
A is the floor area of the clean room in m^2.

The sample locations selected should be evenly distributed within the area under test and at a position related to the working activity (typically at bench height 1m from the floor). A site plan should be prepared indicating sampling locations.

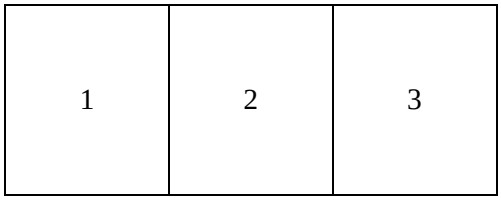
The minimum volume to be sampled is 27L (approx $1ft^3$). The sampling rate can vary but most particle counters operate at a fixed sampling rate of either $0.1ft^3$ or $1ft^3$ per min. The sampling rate and the volume of air sampled should be recorded.

Where the sampling rate is $1ft^3$ per min, 1-minute samples should be taken and this should be repeated at least five times per sample location. It is common to take more samples at each location to allow equipment to settle down following the initial start-up.

Where the sampling rate is $0.1ft^3$ per min, at least $1ft^3$ of air should be sampled at each location.

1.3.2. Clean air devices

Measurements must be made in clean air devices where the contamination level is critical. It should be stressed that the sampling procedure must not interfere with work zone air quality or airflows when monitoring in the operational state. It is suggested that a grid approach is adopted as follows for the work zone:



The sampling rate and volume of air sampled should be as specified for clean rooms. Where the sampling rate is $1ft^3$ per min, 1-minute samples should be taken and this should be repeated at least five times per sample location in the clean air device. Where the sampling rate is $0.1ft^3$ per min, at least $1ft^3$ of air should be sampled at each location.

1.4. Frequency of sampling

In order to comply with cGMP the minimum frequency for monitoring of non-viable particulates is three monthly.

Note: It is preferable that sampling takes place with the facility in the operational condition i.e. personnel present and normal operations being carried out or in a condition specified. The operational condition for unidirectional airflow cabinets/isolators and transfer devices can be considered to be when an operator is working in any part of the clean air device. Sampling in the 'at rest' condition may be continued at an agreed frequency to monitor baseline contamination levels.

1.5. Method of sampling

1.5.1 Clean rooms

1. Set up the monitoring equipment to give total particle counts according to the operating procedure.

2. Record the name and number of operators working in the area being tested and their activity at the time of testing, where possible. Any changes in activities must be documented.

3. Carry out sampling at the locations marked on the site plan with the probe positioned facing upwards at normal working height i.e. approximately 1m. If this is not possible, place on the floor and record this. When testing in the "at rest" condition it is preferable if work progresses from "dirty" to clean areas. In all monitoring, steps should be taken to ensure that the sample probe does not pick up particle counts from the motor/exhaust of the test instrument or from adjacent operations.

4. Following the procedure for operating the particle counter, start the count.

5. Observe the first count to ensure the particle counter is operating correctly. Where appropriate check the first printout produced to ensure information is printed clearly. Note: In carrying this out a greater proportion of particles will be observed due to the presence of the operator.

6. Where appropriate write sample location next to the counts on the printout. If equipment is operating satisfactory, continue the count, and vacate the room unless involved in the work session, for sufficient time to allow the appropriate number of samples to be taken.

7. If equipment is not operating satisfactory determine what the fault is by checking the standard operating procedures.

1.5.2. Clean air devices

1. Clean air devices (such as a unidirectional airflow cabinets or safety cabinets), which may have been switched off, should be switched on and allowed to run for the time specified in the local operating procedure or for the validated clean up time for the device before any particle counts are made.

2. Follow the appropriate transfer procedure ensuring the probe, probe stand, tubing and particle counter, where appropriate, are swabbed with sterile alcohol wipes before placing into the clean air device.

3. Set up the monitoring equipment to give total particle counts according to the operating procedure.

4. Record the name of operator(s) working in the clean air device being tested and their activity at the time of testing, where possible. Any changes in activities must be documented.

Note: In order to avoid sampling procedures causing interference to the work zone air quality or air flows during the preparation of products for administration to patients it is recommended that the operational sampling be performed during operator or process simulation testing.

5. Position the probe isokinetically in relation to the air flow (facing the air flow), ensuring the probe is halfway into the device being monitored. Other probe positions, e.g. facing work, may be used for monitoring specific activities, where this is deemed necessary.

6. Following the procedure for operating the particle counter, start the count.

7. Observe the first count to ensure the particle counter is operating correctly. Where appropriate check the first printout produced to ensure information is printed clearly. Note: In carrying this out a greater proportion of particles may be observed due to the presence of the operator.

8. Where appropriate write sample location next to the counts on the printout. If equipment is operating satisfactory then continue the count to allow the appropriate number of samples to be taken.

9. If equipment is not operating satisfactory determine what the fault is by checking the standard operating procedures.

Note for Isolators

Where the isolator has an access port for the sampling tube this should be used and a suitable seal achieved so that the device may be monitored as described above. Where the isolator has no access port it is possible to monitor the device by cutting off one of the glove fingers, passing the sampling tube through this and then sealing it appropriately. This is not ideal as it means that the device cannot be monitored in the operational state (especially if it is a two glove isolator) and the device will require a replacement glove at the end of the test period with a thorough clean down of the device to remove any possible contamination that may have entered by deliberately breaking the barrier. Alternatively small battery operated particle counters can be used to monitor the internal conditions of isolators in the operational state where no access port is available. The volume of air sampled with such equipment should be recorded.

1.6. Results and interpretation of results

1.6.1. Clean rooms

Examine the results for each sample location. Determine the first and last counts in each location and discard as the equipment operator can often influence them. Select five consecutive counts at the end of the sampling period and calculate the mean of the 0.5µm and 5µm counts per m^3 or other particle sizes under consideration. Record these calculations on the worksheet/record sheet and repeat for all sample locations in the clean room.

1.6.2. Clean air devices

Examine the results for each sample location. Determine the first and last counts in each location and discard. Select five consecutive counts at the end of the sampling period and calculate the mean of the 0.5µm and 5µm counts per m^3 or other particle sizes under consideration. Record these calculations on the worksheet/record sheet and repeat for all sample locations.

1.6.3. Interpretation of results

Current clean rooms standards state that the room or device will be accepted as having passed the test if the particle concentration at each of the locations falls below the class limit. Therefore, if the mean number of particles per cubic metre for each particle size under consideration at each sampling position is equal to or less than the appropriate number given in Table 1.1., the controlled space (clean room or clean air device), under the conditions specified, is deemed to have a satisfactory level of environmental cleanliness.

Table 1.1.

EC GMP Grade	Location Examples	Maximum permitted number of particles/m³ equal to or above			
		At rest (c)		Operational	
		0.5µm	5µm	0.5µm	5µm
A	Unidirectional airflow cabinet (UAFC)	3 500	1(d)	3 500	1(d)
	Isolator				
	Transfer device				
B	Background to UAFC	3 500	1(d)	350 000	2 000
	Background to isolator (a)				
C	Clean support room	350 000	2 000	3 500 000	20 000
D	Background to isolator (b)	3 500 000	20 000	NS	200 000

(a) Limits for background environment for negative pressure isolators with type A, B, C1 transfer devices or background environment for positive pressure isolators with type A transfer devices.

(b) Limits for isolators to be sited in a grade D background or the background environment for negative pressure isolators with type C2, D, E, F transfer devices or the background environment for positive pressure isolators with type B, C1, D, E, F transfer devices.

(c) The conditions given for the "at rest" state should be achieved after a short "clean up" period of 15 – 20 minutes (guidance value) in an unmanned state after completion of operations.

(d) These areas are expected to be completely free from particles of size greater than or equal to 5µm. As it is impossible to demonstrate the absence of particles with any statistical significance the limits are set to 1 particle/per m³. During clean room qualification it should be shown that the areas can be maintained within the defined limits.

1.7. Action

It can be appreciated that the airborne contamination level of a given clean room is dependent on the particle generating activities in the room. If the room is at rest, very low particle concentrations can be achieved closely reflecting the quality of the air supplied to the room and hence the efficiency of the filter system. If the room is operational there will be a greater particle concentration, which is wholly dependant on the number of staff and the activities they are performing. It is expected that a drop in classification will occur as a result of those activities. This information should be available when interpreting the data generated during the monitoring as it may allow problems to be pinpointed. It is difficult to replicate conditions when monitoring environments in the operational state and this should be considered when interpreting data and deciding whether or not an investigation and corrective action are necessary. Where limits are exceeded for clean air devices an investigation into the problem should always be carried out. Any corrective action taken as a result of investigations should be recorded. Retesting may be carried out immediately in clean rooms, where there is a known reason for the failure e.g. due to a certain activity or task being carried out, and if the results are acceptable the facility will be considered to have passed.

2. PRESSURE DIFFERENTIALS [5]

2.1. Introduction

The test defined here is an independent check on the accuracy of any continuous monitoring system. It is likely that the test as defined here will not be carried out in-house and will usually be carried out by an external service contractor.

In a controlled environment installation it is important to ascertain that the correct degree of overpressure can be maintained relative to the adjacent areas of lower classification in order that contamination is not drawn into the controlled environment from its surroundings. It is necessary to ensure that air moves from clean areas to less clean areas and not vice versa. Measurement of room pressure is an indirect means of determining this as it may be assumed that air will flow from an area of high pressure to one with lower pressure. The highest quality clean rooms should therefore have a higher pressure than adjacent clean rooms. The pressure differentials between clean rooms are usually monitored on a continuous basis by either gauges (electronic/magnahelic) or incline manometers.

2.2. Equipment

Instrument of choice is likely to be an electronic manometer (portable and easy to use), incline manometer or magnahelic gauge.

2.3. Sample locations

It is only possible to measure the differential between adjacent areas connected either by a door or grille. Some systems measure pressure differentials with respect to one point. When this type of system is being checked the differential pressure must be checked against this reference point.

2.4. Frequency of sampling

Whilst it is recommended that the pressure differential between clean rooms and adjacent areas is monitored continuously by gauges/manometers and recorded on at least a daily basis, it is important that the accuracy of the measuring system be determined independently from gauge/manometer readings on at least an annual basis using the method below.

2.5. Method of sampling

1. The test must be carried out with the installation unmanned and operating at its designed flow rate (for both supply and extract) and in a balanced condition. To check the pressure differentials the air supply system must be running at design or as per any modification, and therefore supplying and extracting the correct volumes of air.

2. All doors in the facility/suite must be closed.

3. A tube from the measuring device is passed under the door, or through a by-pass grille or damper connecting the two areas being tested, and into the room.

Note: The tube and manometer device must be well clear of the door such that it is free from obstruction and so that no pressure is registered from air movement along the floor near the door.

4. Following the procedure for operating the equipment record the reading.

2.6. Results and interpretation of results

If the pressure difference is equal to or greater than the appropriate minimum pressure drop, the controlled environment installation shall be deemed to have maintained a satisfactory degree of pressure difference. The pressure difference between clean rooms and adjacent areas shall be equal to or greater than the values specified below:

> 10 Pa between classified area and adjacent area of lower classification
> 15 Pa between classified and unclassified area

The results obtained may be used to check the accuracy of any in-house measuring system of gauges or manometers.

2.7. Action

Where the pressure differential does not meet the requirements an investigation into the problem should be carried out. Low pressure differentials may be addressed by replacing blocked (or partially blocked) HEPA filters, increasing fan speeds, increasing airflows to specific areas by altering damper positions or by rebalancing air supplies. Major changes to the air supply system e.g. alterations of room layouts, addition of extra trunking/HEPA filters, must only be undertaken by authorised personnel. Any corrective action taken as a result of the investigation should be recorded.

3. AIRFLOW VELOCITY [1], [3], [5], [6], [9]

3.1. Introduction

All critical operations in aseptic units must be carried out in clean air devices, which are designed, constructed and operated in such a manner as to provide localised clean conditions where the particulate and microbiological contamination is reduced to a pre-defined level. The operational requirements of different devices requires that specific air velocities are delivered through HEPA filters in order to ensure the localised environment is fit for its intended purpose. Airflow velocities are determined for clean air devices supplying unidirectional airflow to the work zone. Clean air devices with turbulent or non unidirectional air supply to the work zone e.g. turbulent flow isolators, have the air supply volume or air change rate determined rather than the airflow velocity.

3.2. Equipment

The equipment required for measuring the airflow velocity is a thermal anemometer, vane anemometer or equivalent.

3.3. Sample locations

The work zone for which airflow velocities are measured is characterised by airflow at right angles to the supply HEPA filter, often referred to as the entrance plane of the airflow. Sample locations for measuring the airflow should be such that the work zone entrance plane is divided up into a grid of equal areas. The following are suggested grids for each type of clean air device:

(i) UNIDIRECTIONAL AIRFLOW CABINET/UNIDIRECTIONAL AIRFLOW ISOLATOR

1	2	3
4	5	6
7	8	9

Horizontal or Vertical Velocity

Reading should be taken 10cm from the surface of filter in the middle of the grid

(ii) SAFETY CABINET

1	2	3	4
5	6	7	8

Vertical Velocity

Reading should be taken in the horizontal plane of the work zone 10cm above the top edge of the working aperture

9	10	11	12

Inward Velocity

The vane should be aligned with inward airflow
Take readings in the plane of the front aperture

(iii) ISOLATOR PASS THROUGH HATCH (POSITIVE OR NEGATIVE PRESSURE)

1		2
	3	
4		5

Reading should be taken 2 – 3cm from the surface of the pre-filter in the middle of the grid. Ensure the anemometer head is pointing in the direction of airflow.

Where a grid or screen to test air velocities which is less than those suggested is used, then steps should be taken to show uniformity of airflows across the entire filter face.

3.4. Frequency of sampling

The test should be carried out at least three monthly.

3.5. Method of sampling

1. Swab the anemometer head and connecting cable with sterile alcohol wipes. In addition swab instrument outside casing if it is removed from its carrying case. Take care with vanes.

2. Place anemometer head in cabinet.

3. Support the anemometer such that the airflow is unobstructed.

4. Position the sample head perpendicular to the airflow at a distance of not more than 10cm from the HEPA filter or as directed.

5. Follow the procedure for operating the anemometer.

6. Record results for the indicated test positions.

Note: Where necessary, correct the results according to the instrument correction factor.

3.6. Results and Interpretation of results

The mean of the air velocities obtained at each sample position should be determined and be within the limits specified in Table 3.1.

Table 3.1. :

Clean Air Device	Airflow	Limits m/s
Unidirectional airflow cabinet	Horizontal airflow Vertical airflow	0.45± 0.1* 0.30± 0.05
Safety cabinet	Vertical airflow Inward airflow	0.25-0.50* Not less than 0.4
Unidirectional airflow isolator	Vertical airflow	0.30-0.60*

* No value may deviate from the mean by more than ± 20%

The air velocities measured for pass through hatches should be used to determine air change rates in accordance with the design specification for the device under test. Likewise the air velocities measured in the critical work zone of the isolator can used to determine the air change rate for the main work area in accordance with the device design specification.

All determined mean values should be within limits. Air velocities exceeding the stated value may cause excessive air movement and affect work zone protection. Air velocities below the limit may be insufficient to maintain critical work zone protection.

3.7. Action

Where the values obtained are outwith limits an investigation into the problem should be carried out. Excessive variation across the measured values may be caused by poor device design or uneven blockage of the filter. Alteration of fan speed or replacement of the HEPA filter may solve the problem. Major changes to the air supply system must only be undertaken by authorised personnel. Any corrective action taken as a result of the investigation should be recorded.

4. AIR CHANGE RATE (ACR) [2], [3]

4.1. Introduction

Conventional clean rooms operate on the principle that the air supplied to the room is of sufficient quantity to dilute or remove the contamination generated within the room. Measurement of the air supply volume and determination of the air change rate (ACR) is a measure of the frequency of air turnover in the clean room. This gives some idea as to how quickly contamination may be removed from the clean room provided there is acceptable mixing of air in the room. The ACR can be determined by measuring the mean air velocity at the supply HEPAs or grilles and calculating the air change rate based on the mean air supply volume or by using a flow measuring hood (balometer), which collects all of the air from the supply and gives an air supply volume directly. Whichever method is used to determine the ACR it should be consistent, as the two techniques will give different readings. Always compare like with like. Using a flow measuring hood will give a more accurate air change rate reading, whilst an anemometer will allow a gross measurement that can be used for trend analysis.

4.2. Equipment

The equipment required for measuring the air velocity is a thermal anemometer, vane anemometer or equivalent. A flow measuring hood with appropriate measuring device can be used.

4.3. Sample locations

Where an anemometer is used to determine the mean air velocity sufficient measurements should be made across the HEPA filter or grille face. It is suggested that at least four positions are tested across the filter or grille face to obtain the mean supply air velocity. Where a flow measuring hood is used the flow hood opening should completely cover the filter or diffuser grille and three readings taken to obtain an average of the air supply volume from the filter or grille. It should be noted that when using a flow measuring hood, cross-reference to air velocity readings in the duct is required to calculate the correction factor for the equipment.

4.4. Frequency of sampling

The test should be carried out at least three monthly using the same test method each time in order to allow comparison of test results.

4.5. Method of sampling

4.5.1. Using an anemometer

1. Support the anemometer such that the airflow is unobstructed.

2. Position the sample head of the anemometer such that it is against the supply air grille and approximately 10cm from the HEPA filter, where installed. If the filter is set back behind a grille it is acceptable to take the readings on or at the surface provided the vanes or eyelashes of the grille are not moved. The effect of the non-uniform velocity across the supply area can be minimised by taking more readings per unit area.

3. Repeat for each supply filter or grille.

4. Calculate the mean air velocity (m/s) at each HEPA filter/grille and then calculate the air supply volume (m^3/s) for each HEPA/grille by multiplying the mean air velocity by the HEPA filter/grille area (m^2).

4.5.2. Using a flow measuring hood

1. Place the flow measuring hood opening completely over the filter or grille, seating the face of the hood against a flat surface to prevent air bypass and inaccurate readings.

2. Measure and record the mean air supply volume (m^3/s) by taking three readings at each supply HEPA or grille.

4.6. Results and interpretation of results

The ACR (per hour) can be calculated using the following formula:

$$\text{ACR} = \frac{\text{air supply volume } (m^3/s) \times 3600}{\text{Room volume } (m^3)}$$

Where there is more than one supply HEPA in a room the air supply volume for each filter should be determined and the volumes summed (to give a total air supply volume) before multiplying by 3600 and dividing by the room volume. The ACR in some facilities may be determined using secondary spillover air from door grilles in addition to primary air supplied from HEPA filters. The air supply from grilles should be treated in the same manner as an additional HEPA filter and the air volume summed to give a total air supply before calculating the ACR.

To achieve the level of cleanliness in an aseptic room and a clean support room the ACR should be greater than 20 air changes per hour.

4.7. Action

Where a facility has been designed to achieve a specific ACR this should be determined. The supply volume will drop as HEPA filters block and as a result the ACR will fall. Where the ACR does not meet design requirements an investigation into the problem should be carried out. Any corrective action taken as a result of the investigation should be recorded. The corrective action may require filters to be changed and the air supply system to be re-balanced to achieve the desired ACR. Major changes to the air supply system must only be undertaken by authorised personnel.

5. OPERATOR PROTECTION FACTOR (OPF) [9]

5.1. Introduction

Class II safety cabinets are designed to control airborne contamination of the product and reduce the exposure of the operator to any airborne contamination generated within the cabinet from work procedures. The work surface of the safety cabinet is flushed with a unidirectional filtered downward airflow and is constructed to minimise, by means of an inward airflow through the working aperture, the escape of airborne contamination generated within the cabinet. Ideally there should be no escape of hazardous aerosol from a safety cabinet and the protection factor provided to the operator should be infinite. In reality safety cabinets do not give complete protection as, in use, operators disturb airflows and hence the protection provided by the cabinet. The test to determine the protection factor involves generating an aerosol of particles e.g. potassium iodide generated by a spinning disc, in the cabinet and the effect of an operators arms on the airflows and hence the effect on the protection factor. The following is a brief overview of the KI discus test used to determine the operator protection factor (OPF) described in BS EN 12469: 2000, Annex C. It is described here in order to allow an interpretation of test results. It is likely that this test will not be carried out in-house and will usually be carried out by an external service contractor.

5.2. Equipment

The equipment required includes an aerosol generator assembly for producing the potassium iodide aerosol (concentration specified) and the detection system (air samplers) where filter papers are used to detect KI aerosol that has escaped from the cabinet. Also included are metal cylinders, which simulate the presence of operator's arms during the test and the likely disturbance that they may cause to the airflows. Equipment used should be calibrated and traceable to national standards. Where the test is performed by an external service contractor a valid certificate of calibration for the test equipment used should be supplied with the test report.

5.3. Sample locations

For safety cabinets up to 1m wide, 5 replicate protection factor tests are taken in the centre of the working aperture according to the test procedure. Cabinets larger than this will require additional sample positions.

5.4. Frequency of sampling

The test need only be carried out annually as the test is usually performed by an external contractor with the necessary equipment and expertise.

5.5. Method of sampling

The method of sampling is defined in the standard. With the cabinet running a volume of KI solution is generated into an aerosol and the air sampler detection system is operated. Aerosol escape will be picked up by the filter membranes and show up as brown spots. The number of brown spots counted can be used in a formula to determine the operator protection factor. For example a protection factor of 1×10^5 would correspond to 62 spots on the filter membrane. The OPF is taken to be the worst figure achieved by the cabinet in the 5 tests.

5.6. Results and interpretation of results

The cabinet is considered to provide sufficient operator protection where all values of the OPF are greater than 1×10^5, when tested in accordance with the standard. Values less than this mean that the escape of contamination from the cabinet may be greater than is acceptable.

5.7. Action

Where a safety cabinet fails to meet requirements for the OPF an investigation into the problem should be carried out. The OPF can be influenced by poor siting of the cabinet, air flows/currents causing turbulence near the aperture, cabinet performance (fan speed, filter blockage etc) and work activities in/around the cabinet. Steps should be taken to eliminate each of these potential causes from the situation. Any corrective action taken as a result of the investigation should be recorded. The test may then be repeated.

6. FILTER INTEGRITY TESTING [5], [10]

6.1. Introduction

Filter integrity testing (sometimes referred to as DOP – dispersed oil particulate – testing) is carried out to ensure that filters comply with the standard required for efficiency. The following is a brief overview of the test used to determine the filter integrity as described in BS 5295. It is described here in order to allow an interpretation of test results. It is likely that this test will not be carried out in-house and will usually be carried out by an external service contractor.

6.2. Equipment

The equipment required to carry out the test will comprise of the oil generator, suitable tubing, compressor motor, CO_2 cylinder and photometer. Equipment used should be calibrated and traceable to national standards. Where the test is performed by an external service contractor a valid certificate of calibration for the test equipment used should be supplied with the test report.

6.3. Sample location/positions

Every filter in the cleanroom facility must be tested. This includes all ceiling terminal HEPA filters and clean air device filters.

6.4. Frequency of testing

The test should be carried out at least annually. More frequent testing may be carried out if necessary.

6.5. Method of testing

All smoke detectors present must be isolated prior to testing as the oil (smoke) challenge will set them off.
The method of sampling is defined in the standard. The oil generator is used to heat a reservoir of mineralised oil (Ondina oil) to above 300°C. A stream of carbon dioxide is passed over the reservoir and fine droplets of oil are collected producing a suspension in the carrier carbon dioxide gas that resembles a “smoke”. The smoke challenge is introduced as close as possible upstream to the filter under test at a concentration of between 50 and 100microgram/L.

The downstream side of the HEPA filter is scanned using a photometer. The photometer operates on the light scattering principle and any “smoke” passing through the HEPA filter under test will enter the detection chamber of the photometer where the “smoke” concentration is measured. The concentration detected will be presented as a reading on the photometer. In order to calculate the efficiency of the filter subtract the reading from 100%. For isolators where it is impossible to scan the filter, e.g. dual in line exhaust filters, a volumetric method can be used as detailed in DIS ISO 14644 –3.

Any residue of “smoke” (oil) used in the testing will evaporate from the filter media but it is essential to thoroughly clean work surfaces before re-commencing aseptic activities.

6.6. Results and interpretation of results

The efficiency of the filter must be > 99.999% for those filters providing Grade A/B conditions
The efficiency of the filter must be > 99.99% for those filters providing Grade C/D conditions

6.7. Action

If the calculated filter efficiency is less than the limits above then action should be taken to replace the leaking filter. Where the leaking filter is in a clean air device this may necessitate taking the device out of use. Sealing leaks detected in filters is not advocated, but in the case of isolator hatch filters and cleanroom terminal filters there may be scope for sealing the leak until a new filter can be purchased and installed, provided an intact seal can be produced. The time period between finding a leak and installing the new filter must be kept to a minimum (as guidance this should not exceed four to six weeks).

SECTION 2: MICROBIOLOGICAL TESTS

Introduction

A major consideration in the operation of clean room technology for aseptic dispensing is the monitoring of viable contamination within clean environments. It is important to stress that statistically microbiological monitoring is not as reliable as physical monitoring and that examination of trends or patterns of contamination must be carried out. The tests defined in section 2 provide guidance on how to conduct microbiological monitoring procedures, which are commonly practised in environmental monitoring programmes.

Some of the information is common to all of the test methodologies and is dealt with in the following section.

General Information [11], [12], [13]

Sample equipment

Solid growth media (e.g. settle and contact plates), prepared according to a recognised formulation, may be obtained from or through the hospital Microbiology Department. It may be preferable however, if materials are purchased from a commercial manufacturer since they will be manufactured to a suitable consistency and are available in a variety of formats (e.g. irradiated). Commercially available materials are batch prepared to an appropriate specification and quality controlled to test the growth capabilities of the media. Agar strips specific to certain test devices should be purchased from the manufacturer. Whether purchasing materials from an outside supplier or obtaining through the hospital Microbiology Department certificates of analysis/fertility (Appendix I) should be requested for each batch of product supplied. Growth media should also be demonstrated as 'growth supporting' for the time and conditions under which exposure occurs.

The quality of test materials should be checked thoroughly prior to using them. Spurious high counts may arise from the use of inadequately controlled microbiological test materials. Consideration should be given to purchasing irradiated materials for use in critical zones. Irradiated materials need not be pre-incubated and may be safely transferred to the critical zones before opening. If materials are obtained that are not irradiated from a commercial source then consideration should be given to pre-incubating to check media sterility prior to use.

It is preferable to use a growth medium with low selectivity i.e. capable of supporting a broad spectrum of microorganisms including aerobes, anaerobes, fungi, yeast and moulds. The media types listed below have been found to be suitable:

Nutrient agar
Tryptone Soya Agar (TSA)
Blood agar
Columbia agar with horse/sheep blood

When necessary to detect or search for a particular type of microorganism a selective culture medium should be used - consult with medical microbiology for advice and recommendations.

The recommended size of solid media is 90 mm in diameter (approximate internal area 64 cm^2) for settle plates and 55 mm (surface area 25 cm^2) for contact plates.

The media may be modified and contain neutralising agents to inactivate residual surface disinfectant present on the surface to be tested. Other media may be used provided the growth supporting capability of the media has been tested.

Sampling conditions

Sampling should be undertaken at the frequencies and locations specified. It is preferable that sampling be carried out in the operational state with any process equipment running and personnel performing normal operations or in a specified condition. It should be stressed that such sampling should not interfere with critical work zone protection or compromise the quality of any products prepared that may be administered to patients. The operational condition for unidirectional airflow cabinets/ isolators and transfer devices can be considered to be where an operator is working in any part of the clean air device. Sampling in the at rest condition may be continued at an agreed frequency to monitor baseline contamination levels. The operational conditions and the activities being performed at the time of testing should be recorded.

Sample locations

A sampling plan should be prepared to provide on-going information on the maintenance of a stable and suitable environment. Areas where a microbiologically controlled environment is specified should be monitored. These include critical zones of clean air devices, transfer devices, background environments for clean air devices and changing rooms.

Sample locations are classified according to the risk to the product during the aseptic preparation process, Table A:

Table A:

Zone Grade	Location/Area
Critical: (Grade A zones)	Local zone for high risk operations e.g. filling zone, work surfaces of unidirectional airflow cabinets/isolators/safety cabinets (immediate work zone where product is exposed to atmosphere or where materials come into direct contact with equipment or work surfaces)
Intermediate: (Grade B zones)	Any area in the clean room that may come into contact with product e.g. bench in clean room, transfer hatch (of room), transfer device of isolator. In the case of aseptic preparation the background environment for grade A zones.
Non-critical: (Grade C & D zones)	Any area external to the clean room e.g. where product/materials may be stored in a clean support room. Less critical stages in the aseptic preparation process e.g. labelling, documentation, checking. In the case of aseptic preparation using isolators grade D is the minimum acceptable background environment.

Sample positions within background environments should include areas where there is personnel activity or specific operations are carried out e.g. adjacent to bench areas where trays that have been passed into the clean room are held before being transferred into a clean air device.

Sample locations, therefore, must be considered as part of the test and monitoring strategy.

Incubation conditions

Following testing the samples should be incubated as soon as possible (within 24 hours of sampling, same day is preferred) and should be held at room temperature with the medium uppermost until incubated or manipulated. If the medium is dropped or touched by an operator then this should be reported, the sample should be marked accordingly and treated as usual. Under no circumstances should samples that have been taken be refrigerated.

Incubation of samples, inverted, at 30 - 35°C for at least 2 days is suitable for the growth of bacteria. Incubation of samples, inverted, at 20 - 25°C for at least 5 days is suitable for the growth of mould and fungi.

Other incubation conditions may be used if it can be shown that the conditions promote the growth of (all) microorganisms that may have been recovered during the sampling procedure. Incubation conditions should be monitored to ensure that the appropriate incubation temperature is maintained throughout the incubation phase.

Results and reading of samples

After appropriate incubation microbiological contamination should grow into discrete macroscopic colonies that can be enumerated and the number of discrete colony forming units (cfu) can be counted on each sample. Record the number (per unit surface area) on the appropriate report. Separate colony counts may be tabulated for mould and bacteria. Colony types may be identified if this is considered appropriate. If cfu are not discrete (coincidence) entities or are Too Numerous To Count (TNTC - usually greater than 300 cfu per sample), record the result as TNTC. If one type of cfu tends to grow in a spreading manner, count this as "one spreading colony" and record it as such. All samples (contaminated or not) should be disposed of according to local procedures.

Note: For plates used in the single sieve to agar sampler, it is necessary to correct the cfu reading for the statistical possibility of multiple particles passing through the same hole. A correction table is provided in the operating manual for the sampler.

The following details should be recorded:

1. Sample location.
2. Date sample taken (length of time plate exposed, if appropriate, for settle plates).
3. Number of colony forming units (cfu) per sample.
4. Batch number and expiry of media.
5. Operator responsible for exposure of samples.
6. Operation being undertaken in cleanroom.
7. Operator reading the result and date read.
8. Person reviewing/approving/accepting results (Responsible Pharmacist/Quality Controller)

It is important that there is knowledge of the 'normal' background flora of a cleanroom facility and therefore a suitably qualified person should identify organisms. Any unusual organisms or deviation from 'normal' flora may require action.

Action levels

Due to the natural imprecision of the test method, the expected low levels of contamination and the natural variability of the levels, the data obtained requires most careful analysis if it is to be of value in assessing the level of control of critical processes.

The use of statistical models to analyse monitoring data requires considerable care and cannot be recommended unreservedly. Alternatively, trends in the data should be identified. Recommended action levels are given in the individual test methodologies.

Action to be taken in the event of a result being on or over the action level

Exceeding action levels on isolated occasions may not require more action than examination of control systems. However, the frequency of exceeding the limit should be examined and should be low. If the frequency is high or shows an upward trend then action should be taken which may include an increase in frequency of testing, observation of operator technique or investigation of cleaning procedures.

Identification of the causative microorganism(s) may aid tracing the source of the contamination.

Successive results greater than the action levels demand that appropriate action be taken to reduce contamination to within limits. Where a problem has been observed the contaminating microorganisms should be identified. Isolated instances require no more action than examination of control systems.

If repeated contamination appears an investigation into the problem should take place and corrective action should be carried out which will rectify the problem. The corrective action should investigate the root cause of the problem and identify steps, with time scale, that will be taken to reduce the contamination levels to "normal". A record of all action taken should be made in an "Abnormal Event Log" (Appendix II).

The following areas should be examined where action levels are repeatedly breached:

Identify
1. Possible cause
2. Contaminating microorganisms

Investigate
1. Whether isolated sample or whole area involved
2. Personnel - operator status (grade), level of training, health, technique, wash up
3. Cleaning procedures
4. Changing procedure
5. HEPA filter integrity of room/clean air device
6. Processes carried out
7. Previous test results for trends or other identified problems.

Liase with
1. Aseptic personnel
2. Microbiology personnel
3. QA/QC personnel

7. USE OF SETTLE PLATES [1], [2], [3], [11]

7.1. Introduction

Settle plate sampling is a direct method of assessing the likely number of microorganisms depositing onto the product or surface in a given time. It is based on the fact that, in the absence of any kind of influence, airborne microorganisms, typically attached to larger particles, will deposit onto open culture plates. Microorganisms are usually found in the air of occupied rooms rafted onto skin cells with very few present on their own. The average size of microbial particle will deposit, by gravity, onto surfaces at a rate of approximately 1 cm/s.

In settle plate sampling Petri dishes containing agar medium are opened and exposed for a given period of time, thus allowing microbe-bearing particles to deposit onto them. Petri dishes which are 90 mm in diameter (approximate internal area 64 cm^2) are most commonly used. The number of microbe bearing particles deposited onto the agar surface of the plate over the period of exposure is ascertained by incubation of the plate and counting the number of microbial colonies, more commonly known as colony forming units (cfu). The microbial deposition rate may be reported as the number depositing in a given area per unit time.

7.2. Limitations of the test method

Settle plates allow continuous sampling throughout a given work period, although they cannot indicate variation of contamination levels throughout the sampling period. They are insensitive unless a long exposure period is adopted in order to detect the low levels of airborne microorganisms. If this is not carried out the results are biased to give favourable data. If this is not practicable then plates should be monitored for successive work sessions and the incidence of contamination analysed.

It is important to know that the plates used are capable of sustaining the growth of microorganisms after the period of exposure since drying of the medium surface may be a problem.

7.3. Sample equipment

See General Information

7.4. Sample locations

Areas where a microbiologically controlled environment is specified should be monitored. An assessment of the controlled environment should identify the processes taking place and all potential microbiological hazards, which may be expected to occur or to be introduced.

The critical work zone of clean air devices should be monitored as these are the areas where previously sterilised materials are brought together and manipulated (processed) into finished dosage forms. Sample locations of plates in the critical work zone should be selected with reference to the actual work area and the position of filters. Monitoring critical areas should be carried out under "worst case" conditions for contamination with process equipment running and personnel performing normal operations. It is recommended that 2 settle plates are exposed for the whole working session.

Sample locations for settle plates in clean rooms should include areas where there is little air movement (i.e. "dead spaces") or where airflows converge or are excessively turbulent. Areas where these conditions are most likely to occur are:

- adjacent to doors
- in pass through hatches
- at low level return air grilles
- between HEPA's in clean rooms
- in corners of rooms

Areas within the clean room where there is personnel activity or specific operations are carried out should also be subject to monitoring e.g. adjacent to bench areas where trays that have been passed into the clean room are held before being transferred into a clean air device.

7.5. Frequency of sampling

(a) At least sessionally - at all test sites within unidirectional airflow cabinet (UAFC)/isolator and transfer devices as practicable.
(b) Weekly - at all test sites in background environment and change facilities.

Sampling in the 'at rest' condition may be continued at an agreed frequency to monitor baseline contamination levels.

7.6. Method of sampling

The following specimen procedure for use and exposure of settle plates may be adopted.

1. Examine the plates for contamination prior to use.

2. Assemble the plates required and ensure that the correct information is written on the base of the plate (the part containing the media) with ink or other marker. Do not mark the lid of the plate, as there is always a possibility of lids coming off and being replaced on the incorrect sample plate. The following details may be marked on each plate or recorded separately:

- operator who collected sample
- date and time of day sample taken
- area/location of sample
- position/sample number

3. Transfer the plates into the area/room/cabinet where they are to be exposed as outlined in the appropriate transfer procedure.

4. Enter the area to be tested by the appropriate procedure, if required.

5. Place the plates in the appropriate positions with the lids still on.

6. Raise lids to expose the surface of the medium, rest the lid on the very edge of the plate so that the entire agar surface is completely exposed. Take care not to put fingers on plates. Avoid passing anything over the top of plates being exposed, where possible.

7. Leave plates exposed for the full work session. The exposure time should be recorded before sending the plates for incubation.

8. After exposure:

- Replace lids of plates.
- Swab areas where plates have been exposed with a suitable disinfectant (e.g. sterile IMS 70% solution) to remove any trace of media or condensation from the lids, which may contaminate the clean room.
- Remove from area/room/cabinet
- Collect all plates exposed, and return to QA or microbiology for incubation. Ensure plates are secured in a suitable container.

9. Complete and enclose the necessary documentation.

The SOP should also include:

- Outline process of how settle plates are obtained for your hospital or a brief statement on where they are obtained or collected from.
- Settle plates for later use should be stored in the recommended storage conditions with the medium uppermost. If refrigerated, the plates should be removed from the refrigerator half an hour before they are due to be exposed. Care should be taken to ensure that plates are used before the expiry date on the label. Plates should not be used after this date.
- Outline details of how settle plates are returned to the Microbiology department, where used. Details of transport and any other arrangements should be described. Also outline method of destruction of exposed plates with/without growth.

7.7. Incubation conditions

See General Information.

7.8. Results and reading of samples

See General Information.

7.9. Action levels

Recommended action levels are given in Table 7.1.

Table 7.1.: Diameter 90mm, 4hr exposure. Note 1.

EC GMP Grade	Location Examples	At rest (cfu)	Operational (cfu)
A	Unidirectional airflow cabinet (UAFC)	1 per 2 plates	1 per 2 plates
	Isolator	1 per 2 plates	1 per 2 plates
	Transfer device	<1	5
B	Background to UAFC	<1	5
	Background to Isolator (a)	<1	5
C	Clean support room	5	50
D	Background to Isolator (b)	50	100

(a) Limits for background environment for negative pressure isolators with type A, B, C1 transfer devices or background environment for positive pressure isolators with type A transfer devices.

(b) Limits for isolators to be sited in a grade D background or the background environment for negative pressure isolators with type C2, D, E, F transfer devices or the background environment for positive pressure isolators with type B, C1, D, E, F transfer devices.

Note 1: Values shown are average values. Individual settle plates may be exposed for less than 4 hours. If the settle plates are exposed for less than 4 hours, the action levels should be adjusted accordingly.

7.10. Action

See General Information

8. DETERMINATION OF AIRBORNE MICROBIOLOGICAL CONTAMINATION BY ACTIVE AIR SAMPLING [1], [2], [3], [11]

8.1. Introduction

Control of viable particles or microorganisms, which are typical of certain types of manufacturing environments or products, is essential. Whilst there are finite limits and standard methods for the evaluation of non-viable particles within the pharmaceutical industry, as yet there is no definitive standardisation of methods for the microbiological evaluation of airborne particles.

Active air samplers all work on the principle of sucking or blowing a stream of air at a sufficiently high velocity to cause any microorganisms in the sample to be impacted against a chosen medium.

8.2. Limitations of the test methods

None of the recognised methods have universal acceptance or approval and each has its own merits and demerits. Viable microbiological counts can be highly variable for a number of reasons, for example:

- non-random distribution of microorganisms in the environment;
- imprecision of the sampling technique employed;
- the rate of sampling - at low velocities air samples and the microorganisms they contain may be deflected around the sampling medium and never be detected whereas at high velocities desiccation of the microorganisms may occur.

8.3. Sample equipment

A variety of equipment and methods is available for active air sampling, however the two main types of equipment in use in hospital pharmacies are the centrifugal and the single sieve to agar samplers. In all cases after the specified sampling time, the agar strip, plate or filter in the sampler is removed, incubated under appropriate conditions then examined for microbiological growth. The number of colony forming units (cfu) is recorded.

8.3.1. Centrifugal sampler

Operating principle. The air sample is drawn into the sampling head by means of an impeller. The impeller then directs the air onto an agar strip fitted around the circumference of the sampling head.

Microbiological medium - see General Information.

8.3.2. Single sieve to agar sampler

Operating principle. Air is aspirated at a fixed speed for variable time through a cover, which has been machined with a series of small holes of special design. The resulting airflow is directed onto the surface of a settle or contact plate containing agar medium.

Microbiological medium **–** see General Information.

8.3.3 Gel membrane filtration

Operating principle. Air is drawn through a sterile gelatine filter mounted on a stand. The filter is then removed and placed on agar in a petri dish using a no touch technique.

Microbiological medium – see General information

8.3.4 Slit samplers

Operating principle. Air is drawn through a slit, which rotates across the surface of an agar plate around a central axis. The speed of rotation can be set e.g. so that the whole surface of the plate is covered within one hour. It is possible to match levels of contamination with time periods and activities being carried out.

Microbiological medium – see General Information

8.4. Sample locations

Areas where a microbiologically controlled environment is necessary should be specified in a sampling plan and should be monitored. These include critical zones of clean air devices, transfer devices, background environments for clean air devices and changing rooms and areas where there is personnel activity or specific operations are carried out e.g. adjacent to bench areas where trays that have been passed into the clean room are held before being transferred into a clean air device.

The number and volume of samples taken in each area should be considered. The number of samples will depend on the size and the grade of the area being monitored. In total, a minimum volume of 1000 litres (1 m^3) should be sampled. This may be obtained from one sample or in larger areas from a number of samples.

8.5. Frequency of sampling

Active microbiological monitoring should be undertaken monthly in the operational state in the locations outlined above.

8.6. Method of sampling

For full details of operation of equipment, consult the operating manual. SOP's should define how sampling is performed in clean rooms and clean air devices, taking into account any limitations of test equipment. Set out below are important points to be considered which should be included in SOP's for carrying out sampling:

- media strips and plates should be examined prior to use for signs of contamination;
- equipment which is battery powered should be checked to ensure the battery status before use;
- equipment must be subject to routine calibration;
- all equipment must be sterilised or disinfected according to normal SOP's prior to transfer into clean areas;
- all media strips and plates must be handled carefully to avoid inadvertent contamination;
- all media strips and plates must be appropriately labelled.

8.7. Incubation conditions

See General Information.

8.8. Results and reading of samples

See General Information.

8.9. Action Levels

Recommended action levels are given in Table 8.1.

Table 8.1.:

EC GMP Grade	Location Examples	At rest (cfu/m^3)	Operational (cfu/m^3)
A	Unidirectional airflow cabinet (UAFC)	<1	<1
	Isolator	<1	<1
	Transfer device	<1	10
B	Background to UAFC	<1	10
	Background to Isolator (a)	<1	10
C	Clean support room	10	100
D	Background to Isolator (b)	100	200

(a) Limits for background environment for negative pressure isolators with type A, B, C1 transfer devices or background environment for positive pressure isolators with type A transfer devices.

(b) Limits for isolators to be sited in a grade D background or the background environment for negative pressure isolators with type C2, D, E, F transfer devices or the background environment for positive pressure isolators with type B, C1, D, E, F transfer devices.

8.10. Action

See General Information

9. USE OF FINGER DAB PLATES [1], [2], [3], [11]

9.1. Introduction

While this method does not actually measure environmental contamination, the data it generates is as important as those generated by other environmental monitoring techniques.

Finger dab plates can be used to show a breakdown in operator aseptic technique where the operator touches a contaminated surface e.g. face when adjusting mask and contamination is transferred to the operators hand and then to products or materials that are handled in the critical work zone. They may also show a breakdown in the transfer process disinfection. Poor technique may lead to items being insufficiently disinfected and transferred into the clean room or clean air device whilst still contaminated. The contamination may then be transferred to the operators gloved hands and then from there to product and materials. In addition finger dab plates can be used to evaluate operator training.

9.2. Limitations of the test method

All test methods possess shortcomings. Below are details of some of the limitations in using settle or contact plates for finger dabs:

- only sample a small part of the operators hands (usually only operators finger tips);
- efficiency of the sampling method is low (plates will lift only a percentage of the actual surface contamination present);
- problem with coincidence with certain microorganisms if agar is overly wet or test surface is wet;
- residual medium must be removed from operators gloves after testing.

9.3. Sample equipment

See General Information

Finger dabs can be performed using either standard 90 mm settle plates or 55 mm contact plates (with a raised media surface).

9.4. Sample locations

Samples must be taken in the grade A zone, as defined below.

Grade A zones	Critical work zone e.g. work area of unidirectional airflow cabinets/isolators/safety cabinets.

9.5. Frequency of sampling

Each operator should be tested sessionally (left and right hand on separate plates). More frequent monitoring may be carried out if the operator is new or a specific problem is identified through investigations.

9.6. Method of sampling

The sample technique will be similar irrespective of whether settle or contact plates are used. The following specimen procedure for finger dab plates may be adopted.

1. Examine the plates for contamination prior to use.

2. Transfer suitably labelled plates to the operator to be tested. It may be convenient for these plates to be supplied at the same time as materials for the last product to be made in that session or at the end of a lengthy operation. This will also ensure that all disinfectant has evaporated from the external surface of the plates prior to commencing the test.

3. Sampling should take place at the end of a work session prior to the operator carrying out any cleaning or tidying operations. Before performing the test the operator should ensure that gloves are dry and free of any disinfectant.

4. The operator should lift the lid of the plate, with the opposite hand to that being tested, and keeping hold of the lid in the other hand, touch the agar surface with the tips of all fingers then the thumb (in the gap on the plate behind where fingers were tested) on the hand being tested. A firm and even pressure should be applied for approximately 5 to 10 seconds, taking care not to damage the agar surface. Replace the lid of the plate.

5. Repeat the process for the other hand.

6. Ensure that the glove surfaces tested are cleaned with a suitable disinfectant to remove any possible traces of residual agar before performing any other operations.

9.7. Incubation conditions

See General Information

9.8. Results and reading of samples

See General Information

9.9. Action levels

Recommended action levels are given below:

Glove print 5 fingers, cfu/glove <1 (in Grade A zones)

The average should be less than 1. On an infrequent basis the limits may be exceeded.

9.10. Action

See General Information

10. USE OF CONTACT PLATES [1], [2], [3], [11]

10.1. Introduction

Surfaces may become contaminated in a number of ways e.g. microorganisms settling out from the environment or from the direct touch by an operator. One of the objectives of surface sampling is to determine the efficiency of routine cleaning procedures in removing contamination. Therefore, sampling should be done before and after cleaning to determine the effectiveness of the cleaning procedure.

Standard contact plates (RODAC: replicate organism detection and counting) should contain sufficient agar growth media to create a raised media surface, and may have a grid scored on the base. The convex agar meniscus allows direct application to test surfaces (e.g. walls, floors, equipment) for hygiene control. The medium used may contain neutralising agents, which inactivate any residual disinfectants on the surface to be tested and therefore enable comparative results before and after cleaning. Contact plates are best used for detecting microorganisms that may be present on flat surfaces in relatively low numbers.

10.2. Limitations of the test method

All test methods possess shortcomings, therefore the sampling regimen used and analysis of the subsequent data is crucial. Below are details of some of the limitations in using contact plates:

- not suitable for inaccessible or irregular surfaces;
- must be used prior to drying up of the agar;
- problem with coincidence with certain microorganisms if agar is overly wet or test surface is wet;
- residual medium must be removed from surface area tested;
- contact plates have a low sample recovery and plates will lift only a percentage of the actual surface contamination present;
- the test is only sampling a very small area (sample population).

10.3. Sample equipment

See General Information

10.4. Sample locations

Sample locations should be selected on the basis of risk to the product during the aseptic preparation process, in accordance with Table A, Section 2. For example, the sampling location in a grade A zone for high risk aseptic operations will be the filling zone work surface of the clean air device.

The number of plates used will be related to the size and grade of the area under test (e.g. one plate in a mini-isolator and up to three plates in a 2 metre unidirectional airflow cabinet).

10.5. Frequency of sampling

For surfaces the recommended minimum frequency of testing is weekly at marked sites in clean rooms and controlled areas. The frequency of sampling can vary and will depend on the scale of activity carried out in the aseptic facility. It should be tied in with other monitoring techniques so that a snapshot of all aspects of the aseptic environment can be obtained. Monitoring of specific zones may be carried out more frequently. cGMP states when "the unit is in use the critical zone of the controlled workspace should be monitored on a sessional basis". It is preferable if all surfaces within critical areas are tested to evaluate the level of their microbial cleanliness on a frequent basis according to the scale of aseptic operations.

10.6. Method of sampling

The following specimen procedure for use of contact plates may be adopted.

1. Examine the plates for contamination prior to use.

2. Assemble the plates required and ensure that the correct information is written on the base of each plate (the part containing the media) using indelible ink. Do not mark the lid of the plate as there is a possibility of lids coming off and being replaced on the incorrect sample plate. The following details may be marked on each plate or recorded separately:

- operator who collected sample
- date and time of day sample taken
- area/location of sample
- position/sample number

3. Transfer the contact plates into the area to be tested, as described in the appropriate transfer procedure.

4. Enter the area to be tested by the appropriate procedure, if required.

5. Before testing any surface ensure that it is dry.

6. When sampling surfaces with contact plates, remove the lid and place the agar surface in maximum contact with the sampling site for 10 seconds by applying a constant force spread evenly over the whole contact plate without twisting or sliding and avoiding the creation of bubbles.

7. After contact with the sample surface, remove and replace the lid, avoiding any unnecessary hand movement near or over the agar surface.

8. Clean the surface tested with a suitable disinfectant to remove any possible traces of residual agar.

9. Repeat as required for each sampling location.

10. After all the samples are taken gather all sample plates together, remove from the area/room/cabinet and incubate as directed.

11. Complete and enclose the necessary documentation.

10.7. Incubation conditions

See General Information

10.8. Results and reading of samples

See General Information

10.9. Action levels

Recommended action levels are given in Table 10.1.

Table 10.1.:

EC GMP Grade	Location Examples	At rest (cfu/25cm²)	Operational (cfu/25cm²)
A	Unidirectional airflow cabinet (UAFC)	<1	<1
	Isolator	<1	<1
	Transfer device	<1	5
B	Background to UAFC	<1	5
	Background to Isolator (a)	<1	5
C	Clean support room	5	25
D	Background to Isolator (b)	25	50

(a) Limits for background environment for negative pressure isolators with type A, B, C1 transfer devices or background environment for positive pressure isolators with type A transfer devices.

(b) Limits for isolators to be sited in a grade D background or the background environment for negative pressure isolators with type C2, D, E, F transfer devices or the background environment for positive pressure isolators with type B, C1, D, E, F transfer devices.

10.10. Action

See General Information

11. USE OF SWABS [1], [2], [3], [11]

11.1. Introduction

Surfaces may become contaminated in a number of ways e.g. microorganisms settling out from the environment or from the direct touch by an operator. One of the objectives of surface sampling is to determine the efficiency of routine cleaning procedures in removing contamination. Therefore, sampling should be performed before and after cleaning to determine the effectiveness of the cleaning procedure.

Swabs can be used to quantitatively analyse the level of contamination by using a template of known dimensions e.g. a flat square aluminium plate (or stainless steel) with a 5 cm x 5 cm hole cut out may be used. The inner edges of the hole should be bevelled (no right angles) to allow complete sampling of the surface. The area inside the template is swabbed thus giving a reproducible sample size.

For uneven surfaces or awkward areas e.g. door handles, curved surfaces, where contact plates or the swab template cannot be used, swabs can still be used to give a qualitative analysis of the cleaning procedure i.e. what type of microorganism is present.

11.2. Limitations of the test method

It must be noted that there are limitations with the use of swabs to validate cleaning. No matter whether it is used as a quantitative or qualitative method, it is dependant on the ability of the swabbing technique to remove microorganisms present on the sample surface. This should be given due consideration when comparing results.

11.3. Sample equipment

11.3.1. Swabs

There are a variety of swab types available from different manufacturers. The type of swab used will affect the sampling method and plating out technique employed. Ordinary dry sterile swabs which require to be moistened with sterile 0.9% Sodium Chloride (NaCl) Injection BP before use are one type and the transport swab is another type. The latter is a swab with a plastic shaft and a synthetic tip - with Amies medium, which is capable of sustaining microbial growth until the swab can be plated out.

11.3.2. Plates

See General Information

11.4. Sample locations

Sample locations should be selected on the basis of risk to the product during the aseptic preparation process, in accordance with Table A, Section 2. For example, the sampling location in a grade A zone for high risk aseptic operations will be the filling zone work surface of the clean air device.

The number of swabs taken will be related to the size and grade of the area under test (e.g. one swab in a mini-isolator and up to three swabs in a 2 metre unidirectional airflow cabinet).

11.5. Frequency of sampling

For surfaces the recommended minimum frequency of testing is weekly at marked sites in clean rooms and controlled areas. The frequency of sampling can vary and will depend on the scale of activity carried out in the aseptic facility. It should be tied in with other monitoring techniques so that a snapshot of all aspects of the aseptic environment can be obtained. Monitoring of specific zones may be carried out more frequently. cGMP states when “the unit is in use the critical zone of the controlled workspace should be monitored on a sessional basis”. It is preferable if all surfaces within critical areas are tested to evaluate the level of their microbial cleanliness on a frequent basis according to the scale of aseptic operations.

11.6. Method of sampling

The following method is suggested for using swabs.

Equipment for carrying out test

Sterile transport swabs
10 ml syringe and needle
10 ml ampoule of 0.9% Sodium Chloride (NaCl) Injection BP
Aluminium plate (with 5 cm x 5 cm hole)
Disinfectant spray/sterile swabs (for cleaning plate/surfaces tested)
Settle plates for plating out swabs

Procedure for taking a swab sample using transport swabs

1. Assemble the swabs required and ensure that the correct information is written on the label of each tube. The following details should be indicated:

- operator who collected swab sample
- date and time of collection
- area/site of collection
- position/sample number

2. Before testing any surface ensure that it is dry.

3. Where used, disinfect the aluminium plate and allow disinfectant to evaporate before placing on the surface to be tested, if required.

4. Assemble the needle and 10 ml syringe. Open the ampoule of 0.9% NaCl Injection BP and draw up the contents.

5. Push a small amount of liquid (about 0.5 ml) onto the surface to be tested. If the surface to be tested is uneven or not horizontal then it is permissible to apply a small amount of liquid from the syringe directly onto the swab. Do not allow the needle tip to come into contact with the swab.

6. Open the swab package and take out the swab. Keep transport tube in hand.

7. Wipe the swab over the sample area in close parallel streaks, using firm even pressure and rotating the swab between fingers to maximise sample pick-up. The swab should be held at a 30° angle to the contact surface. With the same swab, repeat this process at right angles to the first streaks to ensure that the entire sample area is swabbed.

8. Replace swab into transport tube ensuring that swab comes into contact with transport medium by pushing down hard.

9. Remove the aluminium plate from the surface. Clean the surface tested and the aluminium plate, where used, with a suitable disinfectant e.g. IMS 70% to remove any possible contamination.

10. Repeat as required for each sampling position.

11. A negative control swab should be carried out at the end of sampling by applying a small amount of liquid from the syringe directly onto the swab and immediately placed into the transport tube. A positive control swab should be carried out in a similar way by sampling a known “dirty" surface.

12. Gather all swab samples together and manipulate further as directed below under “plating out method for swabs”.

Note: The operational conditions at the time of testing should be recorded. In quantitative testing if a non-standard size of area is sampled an approximation of the area tested should be recorded so that the colony count per approximate area obtained can be related to an action level.

Plating out method for swabs

Examine the plates for contamination prior to use.

A standard streaking out method should be used when plating out the swab. The method should ensure that the swab is rotated as it is run over the surface of the media to ensure that any microorganisms recovered from the surface sample are deposited onto the surface of the plate.

Note: Swabs should be plated out as soon as possible after sampling. If there is a delay the swab should be stored at room temperature.

11.7. Incubation conditions

See General Information.

11.8. Results and reading of samples

See General Information.

11.9. Action levels

Recommended action levels are given in Table 11.1.

Table 11.1.:

EC GMP Grade	Location Examples	At rest (cfu/25cm²)	Operational (cfu/25cm²)
A	Unidirectional airflow cabinet (UAFC)	<1	<1
	Isolator	<1	<1
	Transfer device	<1	5
B	Background to UAFC	<1	5
	Background to Isolator (a)	<1	5
C	Clean support room	5	25
D	Background to Isolator (b)	25	50

a) Limits for background environment for negative pressure isolators with type A, B, C1 transfer devices or background environment for positive pressure isolators with type A transfer devices.

(b) Limits for isolators to be sited in a grade D background or the background environment for negative pressure isolators with type C2, D, E, F transfer devices or the background environment for positive pressure isolators with type B, C1, D, E, F transfer devices.

11.10. Action

See General Information

Definitions [2], [3], [5], [12]

Action levels - microbial quality levels or ranges which, when exceeded signal an apparent drift from normal operating conditions and which require action.

Antimicrobial - a compound or formulation used to retard the growth of or to kill microorganisms.

At rest - where production equipment and materiel are installed in a clean room and functioning but no personnel are present.

Clean room - a room with control of particulate contamination constructed and used in such a way as to minimise the introduction, generation and retention of particle inside the room and in which the temperature, humidity and pressure shall be controlled as necessary.

Clean air device - a small enclosure e.g. unidirectional airflow cabinet, safety cabinet or isolator, that has its own filtered air supply and which may or may not be located in another controlled space, e.g. a clean room.

Controlled workspace - a clean zone of a clean room or clean air device.

cfu (colony forming unit) - either one or an aggregate of many microbial cells which, when cultivated on solid media, will develop into a single visual colony.

Critical work zone - the area in a clean air device where aseptic manipulations are performed.

Disinfection - any process, chemical or physical, designed to kill microorganisms.

Flora - the types of microorganisms found in an environment.

Isolator - a containment device that utilises barrier technology for the enclosure of a controlled workspace.

Operational - where the installation is functioning in its working mode and with a specified level of personnel present.

Particle size - for measurement using light scattering instruments, the diameter of a sphere having the same optical response as that of the particle being measured.

Pathogen - a microorganism capable of causing disease.

Residual activity - residue, which may or may not be active, that remains on surfaces after treatment with an antimicrobial substance.

Sterile - the condition of lacking all forms of microbiological life.

Transfer device - a device, which can be fixed or removable, which allows material to be transferred into and out of an isolator.

Unidirectional airflow – where the plane of the air supply from a HEPA filter is moving in one direction, although air velocity within the plane of movement may vary. This term was previously referred to as laminar airflow e.g. to describe the airflow in horizontal or vertical clean air devices.

Viable particle - a particle that is capable of reproduction; an organism.

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~ The introduction of BS EN ISO 14644 - 1, 1999, has superseded parts 1 and 4 of BS 5295. In addition to this parts 0, 2 and 3 of BS 5295 have been amended as an interim measure, pending the publication of further parts of the BS EN ISO 14644 series, whereupon Parts 0, 2 and 3 of BS 5295 will also be withdrawn. BS 5295 Part 1 was superseded by PD 6609:2000 – Environmental cleanliness in enclosed spaces – Guide to test methods. The test methods defined in this document are essentially the same as those in BS 5295 Part 1.

Appendix I - Example of a Certificate of Analysis/Fertility

Microbiological Laboratories Inc.

Certificate of Analysis

D.O.M. 29/01/2004
D.O.E. 29/04/2004

Product Description: **Tryptone Soya Agar 90mm**

Product Code: 8084 Batch Number: 5828

Date of Analysis: 29/01/2004 QC Number: 04/300

1. PHYSICAL PARAMETERS

(✓ = as spec) 1 a. Appearance : ✓

1 b. Volume : ✓ 1 c. pH : 7.3

2. STERILITY (5 days)

(✓ = no growth) 25ºC ☑ 37ºC ☑ 45ºC ☑

3. MICROBIAL CHALLENGE	Counts	
Organism	Test	Control
Bacillus subtilis ATCC 6633	63	60
Candida albicans ATCC 10231 NCPF 3179	23	28
Escherichia coli ATCC 8739 NCIB 8545	12	10
Pseudomonas aeruginosa NCIB 8626	27	26
Staphylococcus aureus ATCC 6538P NCTC 7447	23	26
Streptococcus faecalis ATCC 19433	25	21

4. LABELS AND PACKAGING

(✓ = present)

PLATE LABEL	BOX/PACK LABEL
Product Code ☑	Product Code ☑
Product Name ☑	Product Name ☑
Batch Number ☑	Batch Number ☑
	Expiry Date ☑

THIS PRODUCT MEETS THE SPECIFICATION AS DETAILED ABOVE

SIGNED: John Smoltz DATE: 31/01/2004

(Quality Manager)

Appendix II

Abnormal Event Log Sheet No.:

Date of Test	Test	Result of Test	Action Taken	Initials and Date