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Rapid and Responsive Monitoring Network for Bioaerosol Emissions: Final Report

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Rapid and Responsive Monitoring Network for Bioaerosol Emissions: Final Report

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Division for Quality of Life

ABSTRACT

This report describes the work undertaken for Defra to design and test parts of a network of instruments for the measurement of airborne particulates and the species *Aspergillus Fumigatus* emitted from municipal composting facilities.

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Approved on behalf of the Managing Director, NPL
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Executive Summary

The treatment of waste, including the composting of biodegradable waste, is now a key part of Defra's strategy to reduce the amount of waste going to landfill. The composting of biowaste also minimises the production of methane, a powerful greenhouse gas, in landfills. New techniques and processes are being developed to respond to an increase in the amount and variety of biowaste being composted. Understanding the emissions from these processes is key to minimising any impact on the environment and human health.

This project has reviewed, selected and tested part of a network of equipment to measure bio-aerosol emissions from composting facilities. The aim has been to improve upon the accuracy and speed of traditional measurement techniques, which are based on culturing biospecies collected onto agar coated plates and laboratory analysis of the cultured colonies. A field test was carried out at a composting site in October 2008 to challenge the network and test the equipment in the field.

The first part of the project was a review, which covers all equipment that could be of use for sampling, general measurement of particulate and identification of fungi and bacteria. It covers equipment that is both on the market and in prototype form. A wide range of equipment for general particulate measurement exists. All commercial equipment for the real time identification of biological species has been developed for defence or health purposes. There is no commercial off-the-shelf technology ready to use for the real time detection of fungi and bacteria from composting facilities.

Three possible network configurations are discussed; the selected network, comprises two tiers of equipment; the first tier detects particulate episodes and the second tier is capable of measuring individual biological species. Equipment to be tested for both tiers, is assessed, ranked and then selected using a list of key criteria, developed in consultation with the Defra Steering Committee.

The Turnkey Instruments Topas was the highest ranked Tier 1 instrument, although equipment ranked second or third would also be suitable. Equipment with the highest ranking for biological species, in particular fungi and bacteria, was the Bertin Technologies Coriolis cyclone sampler with subsequent qPCR determination.

No qPCR assays for fungi or bacteria produced at composting facilities are commercially available. It was therefore decided, after consultation with the Steering Committee, to develop an assay for the priority species *Aspergillus fumigatus.* This species is currently the focus of attention in risk assessments for new composting sites and any routine monitoring required by sites near sensitive receptors

A real time quantitative PCR analysis (qPCR) for *Aspergillus fumigatus* has been successfully developed by NPL and demonstrated in the laboratory, using both pure DNA and DNA extracted from spores grown at the Health Protection Agency (HPA). The assay enables measurement at the femtogram level of DNA which is sufficiently sensitive to enable the detection of the presence of one spore per sample.

The network equipment was tested at the Health Protection Agency in a range of nebulised spray concentrations of *Aspergillus fumigatus* spores. The following instruments and samplers were tested:

- Turnkey Topas (to determine the mass concentration of particulate matter)
- DSTL WIBS2 (fluorescence detector to determine particle number)
- Sartorius MD8 (standard method to sample spores onto a gelatine filter)

- Bertin Coriolis (cyclone sampler to sample particulates into a liquid buffer solution)
- qPCR analysis was carried out at NPL on the Bertin liquid samples.

The testing at HPA showed that the PM_{10} fraction measured by the Topas instrument will provide a non-discriminating indication of the presence of spores, and could therefore be used to trigger a Coriolis sampler to capture this event. In a field implementation of the network, additional information on the wind direction and speed would also be used to help discriminate events which require sampling from those which need not trigger the sampling system. The ability of the qPCR assay to quantify spore counts was also demonstrated. The assay provides a measure of viable and non-viable spores and spore fragments and therefore gives a systematically higher reading than the traditional method, which requires the sample to be cultured. The WIBS2 results showed that within the HPA test chamber there were significantly more non-viable spores than viable spores, and the qPCR is therefore expected to give a higher reading than the cfu measurements.

Field measurements of airborne particles were made at the Veolia composting site at Downend Road, Fareham, over a four week period in October 2008, using three Turnkey Topas, the Bertin Coriolis sampler and further analysis by qPCR at NPL. A trigger unit was designed and built, to demonstrate how a network of general particulate monitors and specialized bioaerosol sampling equipment would be integrated and deployed. This demonstrated how measurements would be recorded automatically if an episode of interest occurs.

The NPL trigger system was set to activate when the wind direction was between 180 and 240 degrees (from the South to South West) i.e. when the wind was blowing across the site to the monitoring position and when the total particulate level reached a preset value. The particulate levels seen peaked around 1200 to 1800 ug/m^3, and the highest levels were observed during periods of turning and shredding on site. It was observed that the actual levels seen on the Topas samplers were very dependant on the location of the samplers with respect to the sources within the site, and therefore the trigger level used is dependant on the sampler location. For this reason, for triggering the system mounted at a distance from the windrows, a much lower trigger level was required. Three levels were used, 100 µg m^-3, 50 µg m^{-3}, and 30 µg m^{-3}. The trigger points and activities being carried at the site, during the period, are summarized below.

Trigger date	**Trigger time**	**Trigger value (µg m^{-3})**	**Topas reading (µg m^{-3})**	**Site Activity**
18/11/2008	13.56	100	124	Shredding & screening
22/11/2008	09.24	100	1183	Shredding & screening
24/11/2008	04.26	30	105	None

Quantification of *Aspergillus fumigatus* was achieved on site, at the boundary fence of the composting site and at distances up to 375m away. PCR inhibition occurred for some samples but this was overcome by a dilution step. Real-time qPCR reports higher values than the conventional microbiological approach. This is not surprising, as qPCR will count non-viable spores while the microbiology approach depends on colony formation. These results are shown below.

Sampling information					Real-time qPCR			Microbiology
Sampling Position	Distance from perimeter (m)	Wind Direction (deg)		Date	Bertin Sample	(1×)	(10×)	cfu/m^3
						spores/m3		
SP1	0	83	Down wind	06/10/08	S28	16591	24842	8320
SP2	70	89	Down wind	06/10/08	S29	17488	13391	5730
SP3	375	86	Up wind	06/10/08	S30	8406	9091	<10
SP4	300	143	Up wind	06/10/08	S31	1437	4838	<10
SP5								460
(SP2)	70	182	Up wind	07/10/08	S37	1476	3938	
(SP4)	300	196	Down wind	07/10/08	S38	—	3020	
(SP4)	300	213	Down wind	09/10/08	S46	9924	20167	
(SP3)	375	262	Up wind	13/10/08	S55	279	491	

Comparison of real-time qPCR and microbiological measurements of spore concentrations. Simultaneous sampling for microbiology and qPCR measurements was made at sites SP1-SP4. Repeat sampling for qPCR was done at sites SP2, 3 and 4 on later days; no qPCR samples were collected at SP5.

A number of recommendations can be made following this study

- Further work should be undertaken to investigate background measurements at a range of different locations. This will allow the levels of spores seen in this study to be placed in a wider context.
- Further refinement of the qPCR methodology to enhance the quantification at very low spore counts and develop a mechanism to identify and account for the observed 'inhibition' seen some field samples.
- Additional field studies at other composting sites to identify whether the levels observed at Downend Road are common to other facilities. This should also include further comparison measurements against the colony counting approach.

NPL would like to thank Veolia for their help and co-operation with this study and Turnkey for the loan of the Topas monitors.

1. A review of instruments for a bio-aerosol sampling network at composting facilities

1.1 Background

The Composting Association has developed and published a protocol for the sampling and analysis of bio-aerosols at composting sites. It uses an Andersen sampler and agar coated plates to sample particulate matter. Collected species are cultured at two temperatures and colonies counted and identified in the laboratory using traditional microscopy. The results from this analysis are a figure for the number of colony forming units of *Aspergillus fumigatus* per cubic metre of air sampled and a figure for the number of colony forming units of mesophilic bacteria per cubic metre of air sampled.

The aim of this review is to carry out a preliminary evaluation of commercially available and near-market equipment, which could be used in a network for real-time measurement of key species, at and around municipal composting sites.

There are a number of issues, which must be examined in the design of a network for sampling and measurement of bio-aerosols, in the presence of other airborne particulate matter. This review covers all equipment that could be of use for sampling, general measurement of particulate and identification of fungi and bacteria. The review covers equipment that is both on the market and in prototype form. It excludes equipment whose primary purpose is generating results for research, but we do refer to equipment which may be commercialised in the future.

The equipment reviewed falls into three broad categories:

- Sampling and measurement of all particulates in terms of mass, number, shape or size:
- Specialised sampling of particulates to enable further analysis and speciation:
- Identification of biological species.

There is a wide range of commercially available equipment for the sampling and measurement of ambient airborne particulate in the size range of interest. This equipment was developed in response to EU regulation covering particulate matter of 10 microns and less. Technology also exists for assessing the air in clean rooms, of importance to the electronics and pharmaceutical industries. The equipment both samples and measures particulate in terms of mass or particle number per unit volume of sampled air.

Specialised sampling of particulates is usually needed to permit the further microbiological analysis for biologically active species, both to preserve their viability and to ensure the compatibility of the sampling medium with the subsequent analysis technique. However, the mass spectrometric and qPCR-based technologies described below, do not require that sample organisms are viable.

The current market in instrumentation for the measurement and detection of bio species is led by equipment developed for military and civil defence applications. These technologies, which were developed for the detection of threat species e.g. *Bacillus anthracis*, are now being made available for other applications, by manufacturers, with the permission of the security authorities. Although these technologies were not developed for the detection of fungi and bacteria from composting, it is likely that their ability to detect and differentiate

naturally occurring species, such as those from waste facilities, from threat species, will have been evaluated.

The techniques and equipment developed for the military are now being adapted to screen for species, e.g. avian flu and MRSA, which have the potential to cause disease in the general human or animal population. The aim is to develop a cheap, quick in-situ screening test to replace the traditional laboratory analyses. Companies are investing research and development funds to access what they believe to be a large market for animal and human health tests, in the developed world.

We have had discussions with all of the major manufacturers or their agents and they have not adapted military instrumentation for the monitoring of species from landfill or composting sites, nor have they seen this, up to now, as a potential market. There is therefore, no off-the-shelf technology ready to use. However, where possible we have indicated the likelihood of adapting techniques and equipment for further assessment and use in this project.

Individual instruments are all reviewed in the following sections and data sheets are included in Appendix 4.

1.2 Methodology

This review has concentrated on commercially available and prototype equipment, which will be available for testing during the timeframe of this project i.e. 2007/08, therefore most contacts have been with instrument manufacturers or their UK agents. NPL already has extensive commercial contacts, not only from our work in type approval testing of environmental measurement equipment, but also from work on security projects. Additional manufacturers of such equipment have been located by carrying out web searches and by contacting members of the sensors Knowledge Transfer Network. Where possible, we have spoken directly to all manufacturers and the timetable of meetings is detailed in Appendix 2. This report focuses on equipment likely to be of use in waste facility monitoring. Other equipment for general particulate monitoring has been investigated but not reported, in order to keep the review focussed on the preferred instrumentation. We have also held a meeting with the Composting Association and notes on the meeting are shown in Appendix 3.

1.3 Instruments to determine particle mass, number, size and shape

This section describes a range of instruments available for the measurement of particle mass, number, size and shape. EU legislation is based on particle mass for a given size range (commonly PM_{10}) but this measurement depends strongly on the conditions of measurement, particularly temperature, due to the presence of semi-volatile constituents in ambient particulate matter. The measurement of particle number over a particular size range is also difficult, due to small variations in the range at low particle diameters between instruments. The instruments described in this section have been included for their potential to measure either mass or number in the correct size range at reasonable cost.

The size of bio-aerosol-containing particulates is highly dependent on a number of factors including the particular organism, its source, the local environmental conditions and the method of aerosolisation. The following table (taken from a report by the University of Birmingham (Brown et al 2005)) summarises the typical size ranges of various types of biological material:

Type of material	Typical diameter	Reference
Pollen (anemophilous plants)	17 – 58 µm	Stanley and Linskens, 1974
Fungal spores	1 – 30 µm	Gregory, 1973
Bacteria	0.25 – 8 µm	Thompson, 1981
Viruses	< 0.3 µm	Taylor, 1988
Plant and animal fragments	various	-

More recent studies have determined the size range of particles upon which biological species are present, often with contrasting results. Schaffer and Lighthart (1997) found that bacteria at inland sites were predominantly associated with particles > 3 µm in diameter, whereas a study of bio-aerosols in schools (Melkin et al 2002) found bacteria and fungal spores to be associated with particles of size ranges 1.1 – 2.1 µm and 1.1 - 4.7 µm respectively. Of more direct relevance to this work, a detailed study of bio-aerosol emissions from composting sites (Environment Agency, 2006) revealed a complex distribution of fungal and bacterial particulate sizes up to 7 µm.

In addition to the instruments described in this section, a range of simple hand-held particle counting devices is also available on the market, for example the TSI DustTrak described in Section 2.2. These devices are in general not suited for landfill applications as they have been designed for occasional use for short periods of time, and would be require major and costly modifications (weatherproof housing, power supplies, communications, etc) to allow them to be used for such purposes. (As described in Section 2.1, the Thermo Fisher Scientific ADR-1200S is based on one of these portable instruments (the pDR-1200) which has been modified for continuous outdoor use.)

The majority of the particle sizing instruments discussed here operate using optical-based technology. The sample is passed through a beam of light and any particles present cause the light to be scattered: the intensity of this scattered light is a function of, amongst other factors, the size and refractive index of the particle. The amount of scattered light from each particle is measured by a photodetector and the particle size calculated from knowledge gained from a prior calibration using spherical polystyrene latex beads of known size and diffractive index.

Measurements of particle shape are more complex and less well developed. The one instrument discussed here which measures particle shape (the Biral Aspect instrument) detects the spatial distribution of scattered light, which is a function of the size, shape and orientation of the particle.

Thermo Fisher Scientific ADR-1200S

The ADR-1200S measures mass concentrations of particulates by means of light-scattering photometry. The system has the capability to collect sampled particles on a membrane filter for analysis, with the possibility of adapting the instrument to sample onto other media (such as agar plates).

The instrument, which is essentially a Thermo pDR-1200 instrument housed in a weatherproof box, is able to detect particles of sizes 0.1 to 10 µm at concentrations between 0.001 to 400 $mg.m^{-3}$, making it well suited to landfill applications. Operation and data logging (via RS232 connections) can be achieved without user intervention and instrument calibration is only required once a year. A 9V power supply is required and the instrument has a 72h battery back-up, so can be operated in the field.

The pDR-1200 instrument (which provides the functionality for the ADR-1200S) is a miniature unit designed for personal exposure monitoring and is one of a range of personal

particle detectors. The next generation pDR instrument (the pDR-1500) is due to be launched in late 2007.

Guide prices: ADR-1200S: £3,900
pDR-1200: £2,300

TSI AeroTrak optical particle counter (Model 8240 or 8260)

The AeroTrak is an optical particle counter that measures particle size and number concentration. Two models are available, the 8240 and 8260: these sample at flow rates of 28.3 $l.min^{-1}$ (equivalent to 1 $ft^3.min^{-1}$) and 50.1 $l.min^{-1}$ respectively. Due to the relatively high concentrations of particles expected around landfill sites, model 8240 is more suited to this application.

The instrument samples particles between of 0.3 to 10 μm and, uniquely for this instrument, allows the user to define six size bins. The counting efficiency for 0.3 μm diameter particles is 50% - this rises to 100% at sizes of 0.45 μm. The maximum total concentration measurable by the 8240 model is approximately 14,000 $particles.l^{-1}$ (400,000 $particles.ft^{-3}$).

The instrument is typically operated using AC Power (110 to 260 V), and a lithium-ion battery (with a lifetime of 3 hours continuous use) is also provided. However, as the instrument was not originally designed for use in the field, the following modifications would be required:

- A weatherproof enclosure. (The sampling efficiency of the instrument has been show to be maintained with up to 6″ of tubing.)
- A heated enclosure (the instrument is designed to operate at 5 - 35°C).
- A communication network. (The instruments have USB connectivity – no analogue instruments are available.)
- A meteorological station (if required).

The instrument can be run continuously in the field (with a daily zero measurement) and only annual calibration is required (at a cost of approximately £450).

Guide prices: AeroTrak model 8240: £4,675
AeroTrak model 8260: £4,795

TSI DustTrak

An alternative solution from TSI is the DustTrak aerosol monitor, a laser photometer which uses 90° light scattering to provide real time measurement of particles at concentrations up to 100 $mg.m^{-3}$. Although the instrument is portable and provides a real-time read out, the facility to partition the particles into a number of size-selective bins is not available and the AeroTrak is therefore the preferred TSI instrument for this landfill monitoring application.

Guide price: DustTrak model 8520: £2,400

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Turnkey Instruments Topas / Osiris

The Topas instrument can measure particles of sizes 0.5 to 20 μm at concentrations up to 60 $mg.m^{-3}$ and records the concentrations of TSP, PM_{10}, $PM_{2.5}$ and PM_1. The devices are currently being used in landfill applications across the UK and also for particulate monitoring by a number of locals Councils - approximately 100 instruments are installed in London alone.

A weatherproof casing heated to 50 °C allows the instrument to be operated in the field. 12V power is required, so mains or battery operation is possible (the battery would need to be

changed approximately every three days). A number of instruments currently in the field are powered by solar or wind energy with battery back-up – in these cases battery needs to be changed every month.

Communications takes place using a GSM modem, but radio modems can be used to send data back to a central PC if there is a direct line of sight. The systems can also be programmed to provide a signal to trigger another operation (e.g. a Tier 2 or 3 instrument) via a voltage out relay. If this option is taken up, one of the usual eight outputs (e.g. temperature or rainfall) will be lost, but if required these parameters could be provided by a central meteorological station, or other Topas instruments on the network.

The instruments can be operated continuously, although an annual calibration is required and the internal GM-A filter needs to be changed every 1-3 months. This filter could also be used as a ready-made sampling device, being removed for analysis whenever a potential bio-aerosol episode has been detected. (If the filter could be replaced with an agar plate, this might be more beneficial.)

The Osiris instrument is almost identical in specification to the Topas, but is not housed in an enclosure suitable for external operation.

Guide price: £5,000 (basic instrument)
£9,000 (including US robotic radio modem and solar / wind power station).

Turnkey Instruments Dustmate

The Dustmate is a hand-held detector designed for short-term sampling, but, like the Osiris, is not suitable for long-term external operation, The Topas is therefore the Turnkey instrument most suited for use in landfill applications.

Guide price: £2,700 (including heated inlet, tripod and software)

Biral Aspect

The Aspect is an aerosol size and shape analyser that provides real-time analysis of particle size and shape for particle concentration of up to 20,000 particles.s^{-1}.

This instrument is based on Biral's ASAS (aerosol size and shape) technology that operates by passing particulates through a laser beam and detecting the spatial distribution of scattered light (the scattering profile) – this is a function of size, shape and orientation of the particle.

Using these means, particles of 0.5 to 20 μm diameter can be detected with a resolution of 0.5 μm. The available range of particle shape detection is an 'asymmetry factor' parameter between 0 (symmetrical particles) and 100 (long fibres).

Guide price: £47,000

(See also the Biral VeroTect instrument in Section 3)

Casella APM950 system (with PM_{10} sampling head)

The APM950 system is an impaction sampler that allows simultaneous real-time monitoring (via infrared light scattering) and gravimetric sampling onto a filter using airstream filtration. By selecting an appropriate sampling head, information can be provided on PM_{10} and $PM_{2.5}$ fractions or the total dust level.

The instrument operates at a flow rate of 16.7 $l.min^{-1}$ and is able to measure particle sizes from 1 – 10 μm at concentrations up to up to 2,000 $\mu g.m^{-3}$. Power can be provided by an AC supply or a rechargeable 6 V lead acid battery and the instrument can operate at temperatures from –10 °C to +40 °C, making and it suited for outdoor applications such as landfill monitoring.

Meteorological information can be provided by the addition of up to four sensors, and communication takes place via RS232 ports. Saved data can also be downloaded to a PC, memory card or remotely via UHF radio or telephone modem.

Guide price: £10,500 (including sampling head)

Grimm EnvironCheck

The EnvironCheck is a light scattering instrument with a detection diode at 90° that continually measures the concentrations of PM_{coarse}, PM_{10}, $PM_{2.5}$ and PM_1. A flow rate of 72 $l.min^{-1}$ is used with a measurable concentration range of 0.1 to 1500 $\mu g.m^{-3}$ and a particle sizes range of 0.25 to 32 μm.

The instrument is housed in a stainless steel casing making it suitable for outdoor use and temperature, pressure and humidity sensors are included. AC power of 110 or 230 V is required. Communication takes place via RS232 ports, but remote data access via a wireless system is also possible.

Guide price: £20,800

Met One 3400 series portable air particle counter

The 3400 series particle counters operate at a flowrate of 28.3 $l.min^{-1}$ or 48.1 $l.min^{-1}$ and samples particles into six size bins between 0.3 to 10 μm or 0.5 to 25 μm. (Two off-the shelf models and a user-defined option are available – the exact specification depends on which of these is chosen.) The instrument operates using 100-240 V AC power or a lithium ion battery (which provides up to 7 hours operating time) and temperature and humidity sensors can be incorporated.

However, as the instruments were designed for use in cleanrooms, waterproof and heated housing would be required for use in the field. Additionally, the maximum measurable particle concentration of the 28.3 $l.min^{-1}$ model is only approximately 14,000 $particles.l^{-1}$, making it unsuitable for studies of emissions from landfill sites.

Guide price: Not known

Met One HHPC-6 handheld particle counter

The HHPC-6 handheld particle counter operates at a flowrate of 2.8 $l.min^{-1}$ and samples particles into six custom-definable size bins from 0.3 to 20 μm. The maximum measurable particle concentration is approximately 70,000 $particles.l^{-1}$, which again makes this unsuited to landfill applications.

Guide price: £2,200

PMT IsoAir

The IsoAir particle sensor comprises a PMT Airnet instrument housed in a weatherproof stainless steel box. As the instrument does not have a display screen, it must be linked to a PC for operation - software is available to facilitate this.

The Airnet instrument is available in a range of different models, selection of which is dependent on the required flow rate and the maximum measurable concentration. The maximum possible measurable particle concentration is approximately 20,000 particles.l^{-1}, which is expected to be much too low for use at landfill sties. A further disadvantage is the limited particle size range of this Airnet 510 model (up to a maximum of 5μm).

The operating costs of this, and the other PMT instruments discussed below is negligible – calibration is only required once a year using latex spheres of known size distribution. Software is available to connect samplers together and it is should also be possible to write simple software to trigger the operation of other instruments when an alarm level is reached.

Guide price: Airnet 510: £2,000

PMT IsoAir Plus

The IsoAir Plus instrument again suffers from a low maximum measurable concentration (15,000 particles.l^{-1}), so is also more suited to cleanroom and pharmaceutical applications. The system comprises of a LASAIR II instrument in resilient stainless steel box. It can be operated continuously, has a pump speed of 28.3 l.min^{-1} and collects particle into six size bins – for the LASAIR II 350L model, these are 0.3, 0.5, 1.0, 5.0, 10.0 and 25.0 μm.

Guide price: LASAIR II 350L: £6,200

PMT HandiLaz 301 / HandiLaz Mini

PMT also produce two hand-held instruments, the HandiLaz 301 and HandiLaz Mini. These instruments operate at a lower flow rate (2.8 l.min^{-1}) than those described above, so would be more likely to avoid exceeding the maximum concentration levels. However, they have a limited size range (bins of 0.3, 0.5 and 5.0 μm) and cannot be operated continuously, so are not suited for this application.

Guide price: HandiLaz 301: £3,000
HandiLaz Mini: £1,600

Cambustion DMS50 & DMS500 instruments

The Cambustion DMS50 & DMS500 instruments are fast, real-time, particulate size spectrometers originally developed for measuring of diesel engine particulates. The instruments have limited particle size range (5 to 560 nm for the DMS50; 5 nm to 2.5 μm for the DMS500). This, and other factors (including their high price) make them unsuitable for landfill monitoring applications.

Guide price: DMS50: £50,000
DMS500: £60,000

Thermo Fisher Scientific 8500 FDMS

The 8500 filter dynamics measurement system (FDMS) is an automated instrument which measures the mass of volatile and particulate matter. Air passes through a size-selective

(PM_{10}, $PM_{2.5}$ or PM_1) inlet and is split isokinetically into main and bypass flows. The main flow passes through a Pallflex 13 mm effective diameter filter mounted on a TEOM (tapered element oscillating microbalance) where a mass transducer provides a continuous direct measurement of the particulate mass collected on the filter. The instrument also compensates for volatilisation effects by adjusting for any change in mass that occurs whilst purged air passes through the filter.

The instrument operates at a flowrate of 1 m^3h^{-1} and a particle mass concentrations of up to 5 x 10^6 $\mu g.m^{-3}$ can be measured with a limit of detection of 0.06 $\mu g.m^{-3}$. When housed in a weather-proof housing, the temperature of air sampled can vary between –30 and + 50°C; the instrument can communicate with a network or other devices via RS232 connections.

Guide price: £20,000.

Summary

A range of instrument for the measurement of particle mass, number, size and shape have been discussed, the first three of which (Thermo Fisher ADR-1220S, TSI AeroTrak and Turnkey Topas) are the most likely candidates for use in a study of emissions from landfill sites. All these instrument are relatively low cost (< £5,000) and each has its advantages and disadvantages (e.g. sensitivity, available particle size ranges, weatherproof housing, ease of communication, power supply, gathering of meteorological data, etc.). If some indication of particle shape is required, the Biral Aspect instrument will be needed, although this is an order of magnitude more expensive.

The selection of a specific instrument will therefore be dependent on the instrument ranking criteria agreed in a later stage of the project.

1.4 Instruments to detect biological material

The instruments discussed in this section have been developed to detect biological material in particulate samples. The first two instruments are commercially available and measure the fluorescence of the sample at an excitation wavelength characteristic of biological material. A number of other devices still in the development stage are then also discussed.

Biral VeroTect

The VeroTect is a real-time biodetector that in addition to measuring particle size and shape (as discussed for Aspect instrument in Section 2) also measures the fluorescence of a bulk sample of the aerosol by use of an innovative light source centred on 280nm - the optimum excitation wavelength for biodetection.

The instrument can analyse to up to 20,000 $particles.s^{-1}$ with sizes of 0.5 to 15 μm and ‘asymmetry factors’ between 0 and 100. It is well suited for outdoor use as it operates at a range of temperatures greater than those experienced in the UK.

Biral’s fluorescence measurement capability is also available as a stand-alone bulk fluorescence chamber.

Guide prices: VeroTect:	£67,700
Bulk fluorescence chamber:	£9,900

ICx Technologies AirSentinel 1000B

The AirSentinel is a continuous real-time continuous monitor which uses UV fluorescence to detect airborne biological species. The response time of the instrument is typically 30 to 120s. A particulate sample is automatically collected when elevated levels of biological material are detected - the threshold of this can be user defined

The instrument can be connected to a network using RS232, RS485, wireless or Ethernet connections, but only has an operational temperature range of 60 to 85 °F meaning that a heated enclosed is required for operation in the field. The required power of 100 V AC can be provided by mains or a lithium-ion battery. The AirSentinel can run continuously without the need for any consumables, the only requirement being that the pump is replaced annually.

Guide price: £8,400

University of Hertfordshire fluorescence detector

A prototype bio-aerosol detector was described by Kaye et al (2004) for unattended deployment in medium to large networks. The Wide Issue Bio-Aerosol sensor (WIBS) used compact xenon flash units and filters to generate alternating flashes of light, at 300 ms intervals, at 260 - 290 nm and 340 - 380 nm. These wavelengths excite the intrinsic fluorescence of the biological fluorophores tryptophan and NADH respectively. Broad bands of emitted fluorescence characteristic of these two fluoprophores were sensed using miniature photomultiplier detectors.

The sensor was successfully tested under laboratory conditions using aerosols of NaCl, ovalbumin and spores of Bacillus subtilis var niger. Kaye et al (2005) subsequently reported a development of this system, the WIBS2 single-particle fluorescence sensor, in which aerosol particles down to approx. 1 μm are sized by a continuous-wave diode laser (660 nm), and then interrogated for intrinsic fluorescence by two xenon sources emitting light at 280 nm and 370 nm. Aerosols containing particles at up to 13,000 particles.l^{-1} can be measured. This new sensor has also been successfully tested under laboratory conditions using a variety of inorganic aerosols and bio-aerosols

Estimated guide price: £2,500 (detector only)

Magee Scientific fluorescence modified aethalometer

A prototype fluorescence detector, based on a standard aethalometer was produced by Magee Scientific for evaluation in military applications. However no details are available on its operation as yet.

EvansCo biological sensing devices

EvansCo Ltd are developing sensor-based devices to detect bio-aerosol emissions and other targets (including bio-warfare agents, environmental contaminants and petroleum products).

The technology is based on physical and chemical reactions that occur at a surface when light is shone at an angle onto one side of that surface, producing a phenomenon known as ‘total internal reflection’. A small proportion of the light energy escapes at the reflection point as a light wave known as an ‘evanescent wave’, and the energy in this evanescent wave may be used to detect molecules that absorb light at the same wavelength. The absorption characteristics can be measured and used to detect and measure the concentration of target

molecules. This is achieved by quantifying changes in evanescent wave characteristics as a result of specific reactions caused by direct absorption of light, producing changes at the interface of the nanosurface.

Tests for different analytes are provided using a disposable probe, which consists of a glass or plastic optical fibre specially prepared with a unique analytical nanosurface. Different analytes require specific individual probes, each chemically modified to produce a receptor surface uniquely designed to recognise that specific target analyte.

Guide price: Not known

Summary

The instruments described above provide an additional level of selectivity to those in Section 2 and will prove to be of use if it is decided that addition functionality (other than just particle size) is required. They have no ability to differentiate between different biological species.

1.5 Particle Samplers

This section describes samplers appropriate for collecting material for subsequent biological analysis. The emphasis is on preserving the viability of species collected. These are divided into larger sensors with limited portability and those that are miniaturised or hand-held.

1.5.1 Limited portability particle samplers

As technologies for the efficient sampling of airborne particulate species are well developed, a wide range of microbial samplers are commercially available for use in workplace environments from cleanrooms to heavy industrial sites. These instruments generally use one of two sampling technologies:

- ***Impactor samplers*.** Impactor samplers are designed to collect particulate matter of a known size range, which is dependent on the dimensions of the impactor. Air is accelerated through a hole, slit, etc. of known dimensions; the particles within the air stream experience a venturi effect and have inertial energy imparted upon them. The larger particles within the stream will be deposited on the impaction surface (usually an agar plate for bio-aerosol applications) and the smaller particles pass through the device. A cascade impactor such as the Andersen sampler (see below) contains a number of impactor plates in series – these devices are used if it is required to sample different particle size ranges simultaneously.

- ***Cyclone samplers.*** In cyclone samplers, air is drawn into the sampler (which contains a known quantity of collection liquid), where the particles within the air stream collide with the internal wall of the device. The location of the impact of the particles is dependent upon the size of the particles – the smaller the particles, the lower down the wall the collision take place. Upon completion of sampling, the liquid is recovered for external analysis.

A comparison of the efficiency of a number of commercially available impactor sampling devices has been reported in the literature (Whyte et al 2007); this section briefly describes these, and other impactor and cyclone samplers.

Thermo Fisher Scientific (Andersen) particle sizing cascade impactors

Andersen particle sizing impactor samplers have been used to sample bio-aerosols in recent studies carried out at composting sites in the UK (SWICEB and ADAS, 2005; Wheeler et al 2001). The six stage impactor is most commonly used – this operates at a flow rate of 28.3 $l.min^{-1}$ and comprises of an aluminium inlet cone, six jet stages, glass Petri dishes, and a base plate held together by three spring clamps and sealed with o-ring gaskets. After sampling, the Petri dishes are then removed, incubated, and analysed by an accepted method.

In addition to the six-stage impactor, two- and single-stage viable (microbial) samplers are also available. (Thermo Fisher Scientific also produces an eight-stage sampler, but this is a non-viable device designed for sampling solid particles and liquid aerosols.) The table below details the particle size cut-off for each sampler:

Sampler	*Particle size range*					
	Stage 1	***Stage 2***	***Stage 3***	***Stage 4***	***Stage 5***	***Stage 6***
Single stage	***> 0.65***	***-***	***-***	***-***	***-***	***-***
Two stage	***> 8.0***	***8.0 - 0.8***	***-***	***-***	***-***	***-***
Six stage	***> 7.1***	***7.1 - 4.7***	***4.7 - 3.3***	***3.3 - 2.1***	***2.1 - 1.1***	***1.1- 0.65***

Guide prices: Single stage: £550 (£825 with a pump)
Two stage: £1,230 (£1,730 with a pump)
Six stage: £2,890 (£3,300 with a pump)

Thermo Fisher Scientific ASAP Model 2800 airborne sample analysis platform

The ASAP system is an impactor sampler designed to sample aerosols in both indoor and outdoor environments and is fully housed in a weatherproof enclosure. Particles between 1 and 10 μm in diameter are sampled onto a polyurethane foam (PUF) cartridge via a slit impaction mechanism.

The system is a compact size (approx 30 cm in all dimensions) and so can be mounted onto a pole or similar structure. An AC (100 – 240 V) or DC (12 - 24 V) power supply is required and the sampler can be made to operated by use a trigger from an external device (e.g. a particle counting instrument). Meteorological sensors can also be used.

Once the samples have been collected and the material extracted from the PUF, analysis can take place can by qPCR or conventional culturing technique - Lovelace Respiratory Research Institute have written a standard operating procedure for this process.

Guide price: £7,200

Vai SMA Atrium

The Atrium is a small (11 cm diameter x 3 cm) impactor sampling unit designed for use for pharmaceutical and biotechnological applications. Air is sampled onto 100 mm agar plates via a route that ensure a capture efficiency of particles greater than 0.5 μm in diameter. The agar plate contained within the device can be filled with 25 ml or 32 ml of nutrient media, providing sampling periods of up to 90 min or 3 h respectively. The samplers operate at a flow rate of 28.3 $l.min^{-1}$.

The samplers were designed for cleanroom applications and up to ten of the devices can be controlled by a single Control Centre unit, with allows simultaneous sampling at different locations.

Guide prices: Atrium: £500 - £900 each (depending on type)
Control Centre [from]: £2,600 (one port, without pump)
[to]: £23,500 (ten ports, with pump)

Biotrace Air Trace environmental air sampler

The Air Trace sampler samples air through a slit impactor at a rate of 28.3 $l.min^{-1}$ on to a 140 or 150 mm agar plate (which can be rotated at an adjustable speed). The sampling time can be user-adjusted between 2 min and 4 h but is typically 35.3 min (equivalent to sampling $1m^3$ of air).

The instrument was designed to sample particles of sizes from < 1 μm to > 40 μm and is validated to meet these requirements by the Centre for Applied Microbiological Research (part of the Health Protection Agency). Its main applications are in cleanroom and pharmaceutical environments.

Guide price: £6,000

University of Hokkaido honeycomb filter sampler

A novel sampler technology using a honeycomb filter, originally developed at the University of Hokkaido Japan for blood-related applications, has had a successful preliminary field assessment for waste industry applications (see Appendix 5). The efficiency of capture / viability of captured micro-organisms was examined in comparison to conventional methods, for *Aspergillus fumigatus* and *Actinomycetes*. The effectiveness of capturing *Actinomycetes* was the same in both cases (ca. 10^5 $cfu.m^{-3}$), although the honeycomb filter proved less efficient at capturing *A. Fumigatus* – possibly because of the larger pore size. The honeycomb filter captured ca. 10^3 $cfu.m^{-3}$ while the conventional filter captured 10^4 $cfu.m^{-3}$.

Guide price: Not known

Bertin Technologies Coriolis μ

The Coriolis μ instrument is a portable air cyclone sampler designed for the capture of airborne bacteria, fungi and pollen. Air is sampled into a cone where the particles are centrifuged on to the walls being trapped into up to 16 ml of a liquid medium – this can then be analysed by qPCR, immunoassay, or any other routine detection technology.

Particles between 0.5 and 20 μm in diameter are sampled (no further size selection exists) over a definable period of up to 10 min. The sampling rate can be varied from 100 to 300 $l.min^{-1}$ and the instrument's power is provide by a battery or 100 – 240 V mains AC.

Guide price: £6,000

Bertin Technologies Coriolis MS

The Coriolis MS instrument was designed for military applications and uses the same cyclone and sample collection technology as the Coriolis μ instrument discussed above. The MS can however sample a much greater range of particle sizes (0.5 to 300 μm, although again, no further size selection exists) and uses a higher flow rate (360 to 630 $l.min^{-1}$). Up to three sampling modules can be operated simultaneously and samples can be collected for up to 15 min (using the internal battery), 2 h (using a power supply module) or 6 h (using the

mains supply). The increased flexibility of the MS system means that it is likely to be the Bertin instrument most suited for landfill applications.

Guide price: £20,000

Sceptor SpinCon 45010A

The SpinCon 45010A is a wet-concentrator cyclone air sampler that operates at a flow rate of 450 $l.min^{-1}$. The 'wet concentrator' technology is claimed to provide more concentrated samples than the more common wet cyclone methods, thus increasing sensitivity of trace biological species.

Samples are collected for a period of 5 min to 6 h into a sample bottle containing water, PBS or a user-defined solution. Particles as small as 0.2 μm diameter are captured. For a concentration of 10^6 $particles.l^{-1}$, the sampling collection rate is presented as 1.2 x 10^8 $partciles.min^{-1}$ (for particles of diameter 0.5 μm), increasing to over 3.0 x 10^8 $particles.min^{-1}$ for particles 2 μm and above. The instrument in housed in a protective carrying case.

Guide price: £20,500

Sceptor Omni 3000

The Omni 3000 is a portable air sampler, which uses similar cyclone collection technology as the SpinCon instrument. The system operates at a flow rate of 300 $l.s^{-1,}$ and provides particle count data for size ranges > 0.5 μm and > 2.0 μm and provides measurement of meteorological data.

Data is available describing the efficiency of the sampler for Aspergillus fumigatus, where an average collection efficiency of 60% was demonstrated.

Guide price: £6,200

Dycor XMX-CV

Dycor's XMX-CV instrument is a civilian version of an aerosol collection device originally produced for military applications. It has been deigned specifically for outdoor applications and is thus entirely weatherproof.

Particles of between 1 and 10 μm in size are impinged on an (off-the shelf) 50 ml sample vial containing 5 ml of a user-defined liquid. This can be subsequently analysed using any standard biological method. If dry particle collection is required, a dry filter collection option is available.

The instrument operates using 100 V or 230 V AC power and has an operating temperature range of 0 to + 50 °C

Guide price: £10,000

Innovatex BioGuardian air sampler

The BioGuardian is a cyclone sampler that uses centrifuge technology to sample particles of size range 1 to 10 μm into approximately 10 ml of liquid contained with a 50 ml sample vial. Any liquid lost through evaporation is automatically replaced.

The sampler is available in four models:

Model 12.03-1000 (which operates at a flow rate of 1000 l.min^{-1})
Model 5.03-450 (450 l.min^{-1})
A 1000 l.min^{-1} model designed for agricultural use
A low (100 l.min^{-1}) flow rate model

The system has been demonstrated to have a sampling efficient of ≥ 70% for *Bacillius* spores.

Guide prices: Model 12.03-1000: £16,500
Model 5.03-450: £13,500

The SSI air samplers are designed for use in a number of applications and are not specifically designed for the sampling of bio-aerosols species.

Samples can be collected onto a 47mm diameter filter or other medium and the model 2100B allows up to four samples can be taken simultaneously (up to two for the model 2000B). Real-time meteorological conditions are monitored.

Guide prices: Model 2000B: £9,000
Model 2100B: £8,500

Summary

Of the instruments discussed above, the Andersen cascade impactor is the only device that allows simultaneous sampling of multiple particle-size fractions, thus allowing examination of the distribution of the biological target species with particle size. These devices are also the least expensive of those investigated.

The other impactor and cyclone samplers discussed are generally more expensive and can only sample one particle size range, although the use of some of these devices may be preferred if, for example, samples are require on a non-agar medium. The properties of all these instruments should be considered against those of handheld and miniaturised devices discussed in the following section.

1.5.2 Handheld/miniaturised particle samplers

Handheld and miniaturised particle samplers typically operate using the same principles of impaction as the fixed-location samplers discussed above – air is drawn through an head containing a series of holes and onto a contact plate containing agar or other medium. The transportability of these devices make them useful tool for sampling bio-aerosol species in a range of occupational exposure environments; a report has recently been published comparing the performances of portable impactor samplers (Yao and Mainelis, 2007]. A number of smaller cyclone-based samplers are also available.

The technology underpinning handheld samplers is mature and a wide range of commercial instruments is available on the market. Most of these devices have been developed for use in cleanrooms and relatively non-contaminated environments (e.g. hospitals and facilities of the production of pharmaceutical, electronics, food and beverages), so the capacity of these instruments should be assessed carefully before using in a landfill environment.

This section describes in brief a number of these commercially available devices, but first, a miniature cyclone sampling device recently reported in the literature is discussed.

Two-stage cyclone device for personal bio-aerosol sampling

A team working at the National Institute for Occupational Safety and Health in West Virginia has recently reported a personal sampling device that uses a two-stage cyclone to collect bio-aerosols into disposable 1.5 ml micro-centrifuge tubes (Lindsley et al 2006). The system compromises of two such tubes, the first of which has an inlet of 2 mm diameter tilted at an angle of 40° to the horizontal. This is connected to the second tube by a channel with an internal diameter of 3 mm – after bending through an arc of 130°, this reduces to a diameter of 1.3 mm and enters the second tube at an angle of 40°. Finally, a back-up filter is used to prevent particulate matter entering the sample pump. The device is very small and lightweight, being approximately 68 mm wide by 97 mm tall and weighing 86 g (without the pump).

Performance tests showed that at a flow rate of 2.0 $l.min^{-1}$, the first and second stages of the sampler had particle cut-off diameters of 2.6 μm and 1.6 μm respectively – at 3.5 $l.min^{-1}$, these cut-off diameters were 1.8 μm and 1.0 μm respectively. The sampler is therefore well suited for the collection of fungi and bacteria. Sampling efficiency was shown to be ≥ 98% for particles of 3.1 μm diameter and 89 or 96% for particles of 6.2 μm diameter (at flowrates of 2.0 $l.min^{-1}$ and 3.5 $l.min^{-1}$ respectively).

The developers of the instrument suggest that in addition to being used for personal monitoring, the design could be adapted for larger samplers to be used for ambient air applications.

Guide price: Not known

ICx BioCapture 650

The BioCapture is a portable air sampler which uses a rotating impactor to sample particles into a chamber containing 2 to 5 ml of liquid – the technology appears to be very similar to that used in the Bertin Coriolis μ instrument described above.

Particles of sizes 0.5 to 10 μm are collected for a period of 5, 15, 30 or 60 min at a flow rate of 200 $l.min^{-1}$. Power is provided by a lithium-ion battery and the sampler can be operated in temperatures of 2 to 43 °C and in rainfall of up to 45 $mm.h^{-1}$.

Guide price: £7,600

ICx BioBadge 100

The BioBadge is a personal air sample designed to be worn on the body which collects a dry sample of particulates onto a collection disk. A field extraction kit can then be used to provide a wet sample for immediate or later analysis.

Guide price: £1,850

PMT Check Air model 4100

The Check Air model 4100 microbial air sampler is a lightweight handheld device which samples aerosols onto 55 mm contact agar plates. The device can be tripod mounted, operates at a flow rate of 1,000 $l.min^{-1}$ and is powered from an internal battery providing running times of up to 5 h. No particle size selection is available.

Guide price: £1,850

Microbio MB2 air sampler

The Microbio MB2 air sampler is a small (20 x 10 x 4 cm) impactor instrument for the collection of bio-aerosol aerosol species onto a 55 mm agar plate attached to the back of the device.

A volume of air between 25 and 10,000 l is sampled at a rate of 100 $l.min^{-1.}$ Power is provided by four rechargeable AA batteries, which enable a total volume of 30,000 l of air to be sampled on a single charge.

Guide price: £1,250

SAS IAQ

The SAS IAQ sampler is a low-cost hand-held impactor sampler designed to be used at, amongst other locations, compositing sites. Samples can be taken on 90 mm Petri dishes or any type of contact plates. The operating flow rate of the device is 100 $l.min^{-1}$, the volume of air sampled can be user-defined up to 1,000 l and power is provided by an internal battery (which provides over six hours operation). The IAQ is one of a range of SAS portable samplers, and is the most suitable of the range for this application.

Guide price: £1,650

Millipore M Air T sampler

A portable impactor sampler capable of taking a 1 m^3 sample of air in 7 min. The device samples onto pre-filled cassettes approx 7.5cm diameter which contain approximately 20g of agar at a minimum depth of 6 mm – the relatively large quantity of agar reduces the amount of water evaporated during the sampling cycle. The device is battery powered (battery lifetime = 7 h) and a calibration kit can be used to ensure that the flow rate is consistent, thus allowing and reproducible results to be obtained.

Guide price: £2,500

Vai SMA Microportable viable air sampler

The SMA microportable sampler is a rechargeable battery operated sampler designed for use in cleanrooms and other similar environments. The unit incorporates the Atrium sampler discussed in Section 4.1. Flow rates of 28.3 or 141.5 $l.min^{-1}$ can be used and the device is powered by a rechargeable battery.

Guide price: Not known

Biotest HYCON RCS high flow sampler

Also designed for use in the pharmaceutical, cosmetic, food and beverage industries, the RCS high flow cyclone sampler which uses centrifugal impactor technology to sample bio-aerosols onto a user–defined medium. The high flowrate of the instrument (100 $l.min^{-1}$) allows samples of up to 1,000 l of air to be taken in short time periods.

Guide price: Not known

MAS-100 air monitoring system

The MAS-100 is a portable impactor sampler. Also designed for use in cleanroom environments, the device samples at a rate of 100 $l.s^{-1}$ (with an accuracy of ± 2.5% onto either a 100mm Petri dish or a 60mm contact plate. Sampling volumes of up to 2,000 l are achievable. Power is provides by a rechargeable NiMH battery that provides over 7 h of continuous use between charges, and data can be transferred to a PC using a communication cable. The user can carry out calibration of the system by use of a digital anemometer calibration system.

Guide price: £3,800

Buck BioCulture

The Buck BioCulture (model B30120) is a simple impactor sampler which samples onto 90 mm diameter agar-filled Petri dishes at a flow rate of 120 $l.min^{-1}$. Sampling periods of 1, 2, 5 or 10 min are available and the unit is powered by internal NiCd batteries which provide sufficient power for 6h of sampling. A calibration head device is available to calibrate the flow rate of the pump.

Guide price: £700

Microflow 60 air sampler

The Microflow 60 is a handheld impactor sampler device which samples onto 60 mm diameter agar-filled Petri dishes at one of five flow rates: 30, 60, 90, 100 or 120 $l.min^{-1}$. The volume of air to be sampled can be programmed (up to a maximum of 1,000 l) and power is provided by a rechargeable NiMH battery.

Guide price: £1,600

Summary

In comparison to the samplers outlined in the Section 4.1, the handheld and miniaturised devices discussed above are lower cost option. Although they only sample a single particle size range, their highly portable nature and simple operation is likely to be beneficial for an application where only occasional samples are required. However, as discussed in the introduction to this section, care should be taken that the capacity of any sampler used is suitable for the high concentration of particulate matter likely to be emitted from a landfill site.

1.6 Techniques for biological species identification

At present, the identification of microorganisms for the composting industry involves the application of traditional microbiology – growth on selective media and visual identification. Other more precise techniques / technologies are now becoming available to make this process more specific. This section of the report describes some of these new technologies for the identification of microorganisms in bio-aerosols, where they are, or are planned to be and whether they are sufficiently semi-automated and rugged to be used in the field.

Immunosensor systems such as Tradeways Ltd Raptor and Biohawk biodetectors and the Smiths Detection Bio-detector are well-established technologies in the biodetection area. However, antibody technology requires the generation of specific antibodies for a given target microorganism, and this can be a lengthy and expensive process. The relatively small-

scale activity of composting aerosol analysis will probably not allow the investment necessary for assays to be developed for suitable composting target organisms. For this reason, antibody-based detection systems are not described in this report. However, if suitable antibodies become available, one potentially interesting hand-held instrument using antibody technology and resonance acoustic profiling which is currently under development may be of interest to the composting industry in the future and it is described below (section 5.3).

The two technology areas described in detail this report are Mass spectrometry (MS), and PCR (Polymerase Chain Reaction). Both are, theoretically, capable of identifying microorganisms to the species level, and both are actively under development for the identification of microorganisms in bio-aerosols. Neither requires the presence in an aerosol sample of viable microorganisms.

1.6.1 Mass spectrometry Technology

In principle, mass spectrometry can deliver an ultrasensitive and rapid (almost real-time) identification of airborne microorganisms. It can also be reagent-free, and can operate in a continuous unmanned mode. A number of mass spectrometers have been developed, or are under development, to address this task. However, only a single instrument, the Bruker Daltonics CBMS (Chemical, Biological Mass Spectrometer) is available commercially. This collects a sample of airborne particles and analyses them by pyrolysis (Py) mass spectrometry. Although it is the only commercial instrument, a number of other pyrolysis-based mass spectrometers are also under development for bio-aerosol analysis, including the Py-MS and the Py-GC-IMS / Py-GC-IMS-MS.

A single-particle aerosol mass spectrometer is available commercially (ATOFMS) for the analysis of inorganic aerosol particles. However, there are a number of other single-particle instruments currently under development which have been reported as being investigated for bio-aerosol detection. These include the laser-based mass spectrometers ATOFMS, BAMS / SPAMS, MALDI-ATOFMS. For a review of the technical considerations in the design of single-particle laser mass spectrometers see Murphy, D.M (2007).

All of the instrument configurations mentioned above are described below.

Bruker CBMS

The Bruker CBMS is a fully automatic, ruggedised, production instrument, designed for military use. The system consists of [a] a Virtual impactor to collect and concentrate airborne particles into a sample tube, [b] an IR-Pyroliser where sample is heated to decomposition and introduced via a membrane inlet into [c] an ion trap mass spectrometer with fragmentation (MS/MS) capability. The hardware is relatively light (65 kg) and operates from a 24V DC power source.

System control is PC-based via a touch screen and analysis results are displayed using a simple graphical interface (“Bacteria found”, “Spore found” “Nothing detected”). In automatic mode, no user actions are required. However, detailed spectra can be downloaded via a serial port to a separate computer for detailed analysis. The response time from sample acquisition to result is 3 minutes.

The limits of detection for the instrument are 15 Agent Containing Particles per litre of air, for particles over 2 μm diameter. Aerosolised agents detected include Ovalbumin (in place of toxins which would be too dangerous to test), and the pathogenic micro-organisms *Bacillus anthracis*, *Brucella melitensis*, *Brucella suis*, *Yersinia pestis* and *Francisella tularensis* type B.

Two major field trials of the system have been undertaken by the UK and the US governments. However, the reports are governed by a security classification and are not available for public access.

Guide price £245,000

Bruker Biotyper software

Although not designed to be used in conjunction with a 'fieldable' mass spectrometer, and therefore outside of the immediate scope of this report, the Biotyper software may nevertheless be a useful tool for the identification of microorganisms sampled from bio-aerosols. For this reason a description is included here.

Biotyper software is designed to be used on a MALDI-TOF mass spectrometer, for the "*fast and reliable strain-specific identification of bacteria, yeast and fungi"* in the clinical diagnostic, food processing and environmental sectors. In a typical scenario, test samples are cultured overnight, applied to a MS target plate, overlaid with matrix and analysed by MALDI-MS. Identification of unknown microorganisms can take as little as 5 minutes from sample application for a single colony, and 1.5 hours for 100 colonies. The sensitivity of the analysis is approximately 10,000 – 100,000 cells, depending on the MS target plate technology employed and the purity of the sample. Mixtures of two species can be successfully analysed providing the ratio of organisms does not exceed 10:1.

MS analysis gives a characteristic and reproducible pattern of peak masses and intensities, principally generated from high abundance ribosomal proteins. This pattern is analysed by PCA, and compared with a library of reference spectra to allow the identification of unknown organisms. Currently there are in excess of 2,000 entries in the organism reference library

Guide price: £4,000

ATOFMS

Two versions of a single-particle Aerosol Time-of-Flight Mass Spectrometer (ATOFMS) instrument are available commercially from TSI Incorporated (Shoreview, Minnesota, USA) - the Series 3800 ATOFMS models 3800-030 and 3800-100. Originally based on a laboratory-sized instrument, the commercial instrument is based on a subsequent portable version. However, this description is misleading, since the instrument is over 1.5 meters in length and weighs 360 kg.

The instrument can detect and analyse single aerosol particles with diameters in two ranges; 30-300 nm and 300-3000 nm. To overcome the reduced sampling efficiency of small particles, it employs an Aerodynamic Focusing Lens which focuses aerosol particles into a tight beam, after which they are accelerated to a terminal velocity proportional to their aerodynamic diameter. The particle velocity is measured as it crosses two orthogonal continuous-wave laser beams at a know distance between. The velocity is used to predict triggering of an Nd:YAG UV laser which desorbs and ionises components of the particles in the centre of the mass spectrometer ion source (laser desorption ionisation or LDI). Two complete mass spectra (m/z range 1- 800 daltons) are generated for each particle using separate reflectron Tof tubes – a positive ion spectrum and a negative ion spectrum. Ions are detected using a micro-channel plate detectors. The instrument control software package records the particle detection time, aerodynamic diameters and mass spectra. Separate analysis software allows the classification of particles based on the mass spectrometry data. Typically, if the instrument is running all day, between 1 and 8 gigabytes of data are acquired.

The broad capability of the instrument has allowed it to be used for a wide variety of applications, including environmental monitoring, indoor-air-quality monitoring, aerosolised drug-delivery research, characterisation of bio-mass burning, diesel exhaust measurements, smoke stack measurements, industrial hygiene, pharmaceutical applications and tobacco smoke analysis.

TSI market their instruments with a clear bias towards detecting simple inorganic or organic components in aerosols. However, considerable research outside of TSI has examined the ability of enhanced versions of the instrument to analyse bio-aerosol components. These enhanced versions utilise fluorescent sizing / discrimination of biological particles, higher m/z TOF ranges and libraries of bio-aerosol signature spectra.

Guide price: £243,000

BAMS and SPAMS

The BAMS (Bio-Aerosol Mass Spectrometry System) has been developed at the Lawrence Livermore National Laboratory in the USA, for matrix-free ("reagent-free") real-time detection and identification of bio-aerosol components, and is based on developments of the ATOFMS instrument. In general, with this technology, particle LDI mass spectra are recorded within 1 millisecond of a particle leaving the bulk aerosol, and less than 1,000 bacterial particles need to be analysed to give good mass spectra.

Early work with matrix-free laser desorption / ionisation (D/I) allowed the identification and differentiation, under laboratory conditions, of closely related species of *Bacillus*. The system also differentiated between non-microbial background particles such as ragweed pollen and road dust (Ullom et al 2001) Mass spectra from UV laser D/I. were found to be inferior to those from infrared D/I.

Development work with a commercial TSI 3800 instrument showed that the quality of the MS data acquired using *Bacillus* spores was heavily dependent on the variability of the laser power profile (Steele et al 2003: Steele et al 2005). However, it was possible to reproducibly distinguish between spores of *Bacillus atrophaeus* (*B. subtilis var.niger : B. globigii*) and spores of *B. thuringiensis*, albeit using low m/z spore components such as dipicolinate and amino acids, even in the presence of foot powder, gelatine, growth medium, baking soda, sugar and cigarette and wood smoke (Fergenson et al 2004). A comprehensive analysis of mass spectra from B. atrophaeus spores has been undertaken (Czerwieniec et al 2005a: Srivastava et al 2005, Tobias et al 2006).

The first quantitative assessment of a TSI 3800 with modified laser D/I (now referred to as a BAMS instrument), allowed the specific identification of *Mycobacterium tuberculosis* in bio-aerosol particles at > 40 cfu per litre of air in a background-free environment. However, the sensitivity of the instrument depended greatly on the size of the particles in the bio-aerosol – particles <1 μm and >3 μm in diameter gave low transmission efficiencies (Tobias et al 2005).

Modifications to the instrument design, such as the use of an electrostatic ion guide, delayed ion extraction and a linear TOF configuration were shown to greatly improved the instrument sensitivity at high m/z (Czerwieniec et al 2005b).

In a recent publication describing this technology and focussing on the detection of low molecular weight chemical warfare agents (CWAs) (Martin et al 2007), the acronym used for the instrument has changed from BAMS to SPAMS (Single-particle aerosol mass spectrometry). However, a recent oral presentation at the Royal Society of Chemistry (McJimpsey et al 2007) describing the BAMS technology again addressed issues of

microorganism identification, and problems associated with the identification of *Bacillus* spores in bio-aerosols. Two field deployments at the San Francisco International airport were described, the results of which indicated the continuing difficulty in distinguishing between biological and non-biological particles. Interestingly, the BAMS system described now appears to utilise the fluorescence signature of particles to specifically trigger MS analysis.

Guide price: Not available

MALDI-ATOFMS

The basic configuration and operation of the MALDI-ATOFMS (van Wuijckhuijse et al 2000) is similar to that of BAMS/SPAMS. Air is drawn into the instrument and particles are, again, sized by examining the transit time between two continuous lasers. Particles are then examined for fluorescence emission as a result of excitation by 266 nm laser light (Stowers et al 2006), and positive fluorescence triggers particle ionisation by a 308 nm laser. Positive ion mass spectra only are recorded for individual particles. However, in contrast to the BAMS/SPAMS instrument, aerosol particles are coated in-line with a MALDI matrix prior to analysis, considerably improving the quality and m/z range of spectra recorded using this instrument compared to those acquired using “hard” laser desorption/ionisation e.g. BAMS / SPAMS. MALDI-ATOFMS can generate high resolution spectra which cover an m/z range up to 85 kDaltons. Specific proteinaceous markers for the identification of microorganisms often have molecular masses in the tens of thousands. Promising performance by MALDI-ATOFMS for microorganism identification has been demonstrated using *B. subtilis var niger* spores (Stowers et al 2000; Wuijckhuijse et al 2005) and *Erwinea herbicola* (Kleefsman et al 2007). The number of particles necessary to analyse for good mass spectra is similar to that for the BAMS system.

The instrument has been developed by Delft University of Technology and TNO Prins Maurits Laboratory in the Netherlands. It is not yet commercially available.

An alternative and simpler approach being investigated employing MALDI MS for microorganism identification has been reported (Kim et al, 2005). These authors have shown that *E. coli* bio-aerosols can be collected directly onto MALDI target plates positioned in an Andersen six-stage impactor bio-aerosol collector for subsequent MS analysis. A comparison of the use of uncoated plates and plates pre-coated with matrix, showed that collection on pre-coated target plates followed by post-collection solvent addition gave good quality mass spectra over a wide m/z range up to approximately 10,000. However, no indication of sensitivity was reported.

Guide price: Not Available

Py-MS and Py-GC-IMS / Py-GC-IMS-MS

Pyrolysis-gas chromatography-ion mobility spectrometry / mass spectrometry (Snyder et al 2004) has not been shown to have the capability for species identification from bioarosols, but has been shown in field trials to be able to distinguish between Gram + ve and Gram – ve bacteria on the basis of cell wall components detected. Py-MS has been used to identify spores of *Bacillus* species under laboratory conditions (Goodacre et al, 2004).

Other MS-based approaches

Selected ion flow tube-mass spectrometry (SIFT-MS) uses chemical ionisation to detect and quantify volatile organic compounds (VOCs). These are produced during the growth of medically important fungi such as *Aspergillus flavus, Aspergillus fumigatus, Candida albicans, Mucor racemosus, Fusarum solani* and *Cryptococcus neoformans*. The technique is being assessed in the hope that it may form the basis of a species-specific identification for these fungi (Scotter et al 2005).

Summary

The Bruker CBMS, although commercially available, cannot currently identify the most probable target microorganisms for this study, and so should not be considered for the experimental section of this programme. In contrast, the Bruker Biotyper software may well be a useful product to trial for environmental testing. Although not a real-time analysis tool, it may well prove a very valuable replacement to traditional methods for microorganism identification.

Of the single particle mass spectrometers described here, the basic commercial ATOFMS is not suitable for bio-aerosol work. The BAMS /SPAMS and MALDI-ATOFMS experimental instruments are suitable, and while we have been unable to generate a response from the American researchers developing the BAMS/SPAMS instrument with regards to its use in this trial, the Dutch developers of the MALDI-ATOFMS instrument may be interested in participating. We are continuing a dialogue with the Dutch group with this in mind.

1.6.2 Polymerase chain reaction (PCR) Technology

PCR utilises a thermostable DNA polymerase to amplify specific regions of target DNA during successive rounds of DNA thermal denaturation, primer annealing and primer extension. The ability of the PCR process to specifically identify a given microorganism is dependent on the design of the primer oligonucleotides employed. They must anneal to species-specific regions of the host DNA.

Traditionally, the product DNA at the end of a PCR reaction has been fractionated using agarose gel electrophoresis, and semi-quantified by staining with a fluorescent dye. Known quantities of similar sized DNA is fractionated in parallel for comparison.

In more recent years, real-time PCR has developed to allow the rapid and more quantitative analysis of PCR reaction products. In this method, the amplification of target DNA is detected by the fluorescent signal from a fluorescent reporter molecule included in the PCR reaction. The point at which the fluorescent signal is measured in order to calculate the initial template quantity can either be at the end of the reaction (endpoint semi-quantitative PCR) or while the amplification is still progressing (real-time qPCR). The more sensitive and reproducible method of real-time qPCR measures the fluorescence at each cycle as the amplification progresses. This is the method employed by three of the instruments described here – the Cepheid GeneXpert, the Cepheid SmartCycler and the Enigma FL. The Smiths Detection Bio-Seeq PLUS employs an endpoint system.

Cepheid GeneXpert

The GeneXpert system is a fully automatic sample preparation and real-time qPCR analysis system, developed to test for three Biothreat agents – *Bacillus anthracis*, *Yersinia pestis*,

and *Francisella tularensis*. Tests for other agents (e.g. group B Streptococcus and Enterovirus) are currently under development. User-requested tests would need a throughput of 4,000 analyses / year for the development of such tests to be considered commercial by Cepheid. It is the only commercially available fully automatic real-time qPCR detection system currently available (but see Enigma FL below). It has been incorporated in the US Postal Service Biohazard Detection System for the detection of anthrax spores in air samples generated during mail sorting (US Patent Application US 2004/0063197 A1: April 1, 2004), and has to-date performed over 4 million anthrax qPCR tests. A peer-reviewed assessment of the GeneXpert system for the detection of *B. anthracis* has been published (Ulrich et al, 2006).

The system design is modular, with the instrument initially configured to house up to 4 independent detection modules (self-contained cartridges), each capable of 4 channel multiplexing (3 test and 1 control assay). Detection is fluorometric. Additional modules can be added later if required, up o a maximum of 16 for each instrument. Independent modules allow samples to be analysed as required, rather than in batches, and allows different analyses to be performed with each module. Analysis of results requires the use of a computer.

Operation can be carried out by non-laboratory personnel, and simply requires the user to apply a sample and two reagents to a test cartridge, and inserting the cartridge into the instrument. Total hands-on time of ~ 2 minutes. DNA extraction and analysis steps are fully automatic. A test cartridge incorporates a syringe drive, a rotary drive and a sonic horn. The sonic horn is used to lyse sample components using ultrasound, while the syringe drive and rotary drive transfer samples between cartridge compartments in order to wash, purify, concentrate and finally amplify sample DNA.

Each test requires the use of two detection cartridges. The first screens the sample for the presence of one or more of the three threat agents, and a second cartridge is used to identify the specific agent present. Identification employs detection of virulence genes for each organism – a different gene for each species in each of the two cartridges (for *B. anthracis* pX02, pX01; for *F.tularensis* Tul4 and fopA; for *Y. pestis* CAF1 and PLA). The system is highly specific, giving the minimum of false positives, and is rapid – a single cartridge test takes 35 minutes, while a complete analysis to detect a specific agent using two cartridges takes 70 minutes. The sensitivity of the system allows the detection of 200 cfu for Y*. pestis*, < 200 cfu for *B. anthracis* and < 100 cfu for *F. tularensis*.

Although a 4 channel instrument is small (D311mm, W292mm, H385mm) it requires a 100-240V power source for operation – a limitation for a potentially 'fieldable' instrument.

Guide price: 4 module system is approximately £20,000 - £30,000.
A single detection module / test costs £20-£30.

Cepheid SmartCycler

Although not a fully automatic real-time qPCR instrument, the Cepheid SmartCycler is a rugged instrument designed for field use, operating from a 12-24V power source and weighing 10kg. The basic instrument contains 16 independent PCR modules (iCycler modules), which, as above, allows samples to be analysed as required, rather than in batches, and allows different analyses to be performed with each module. Detection of amplicons is fluorometric, and up to 4 assays may be multiplexed in each sample tube (3 test and 1 control). Reaction conditions and results are displayed on a suitable PC. Up to 96 modules can be independently controlled and monitored from a single PC. Results can be available in 40 minutes or less from sample addition.

In contrast to the GeneXpert system, target DNA isolation is performed separately from the instrument using a suitable procedure. Target DNA is then transferred to an Eppendorf tube, and PCR reagents, primers, probes or intercalating dyes added. The complete reaction mix is then transferred to SmartCycler reaction tubes for analysis. This instrument is therefore not designed for use by non-laboratory staff. However, PCR reagents are available from Cepheid as lyophilised reagent mastermix beads (SmartMix, OmniMix Hot Start), stable at 2-8^{o}C, which can be dispensed from a hand-held dispenser into reaction tubes, and hydrated by the addition of sample, primers and probes. The complete reaction mix can then be transferred into the cycler as above.

Despite the limitations on its use by non-laboratory staff, the instrument is still a valuable field tool for micro-organism identification by trained personnel (Schaad et al 2000), or for the field validation of qPCR protocols designed for transfer to fully automatic instruments.

Guide price: A complete 16 channel system housed in a rugged transportation box (Smart Cycler TD) is £25,000-£35,000 and weighs 33.5 kg. The price for a single PCR reaction is in the region of £2 to £5, depending on DNA isolation procedure and detection chemistry selected.

Smiths Detection Bio-Seeq PLUS

The Smiths Bio-Seeq PLUS is another manual PCR instrument designed for use in the field, using samples collected from surfaces with a swab. It is a simple small, hand-held, battery powered devise, which can run up to 6 independent assays simultaneously (20 assays on a single charge). Assay reagents are contained in single-use cartridges, and PCR results are available in 50 minutes. The unit is designed to be used by an inexperienced operator. As in the case of the Cepheid SmartCycler system, sample target DNA isolation is performed separately from the instrument using a suitable procedure. Commercial assays are currently available for only the usual small range of Biological warfare agents; *Bacillus anthracis*, *Yersinia pestis*, *Francisella tularensis* and an Orthopox virus. However, it should be possible to design assays more suitable for the programme of work anticipated for this project.

Guide price: £20,000

Enigma FL

The Enigma FL (Field Laboratory) was developed by Enigma Diagnostics Ltd. as a 'fieldable' instrument to confirm, using an orthogonal technique (real-time PCR), the identification of microorganisms initially carried out using immunological methods. It is ruggedised for military use, and operates using a 12-24V DC power source. However, the instrument market includes point-of-care clinical testing, pen-side veterinary testing and industrial process testing (detection of contaminating nucleic acids). The analysis procedure is completely automatic after the addition of sample. Necessary training for field operatives is minimal - estimated at being 10 minutes after extensive pen-side trials organised by the Veterinary Laboratories Agency, Weybridge. A GPS system is included in the instrument to automatically confirm the location of tests. The instrument was not available commercially at the time of this review.

An advantage over the GeneXpert system is that it is possible for users to utilise their own primers, during a pause in the instrument programme, in what otherwise is a fully automatic analysis. This is helpful in the early stages of assay development. A drawback in comparison to the GeneXpert system is that the instrument only has a single sample capability, although it is possible to multiplex up to 6 assays (5 test and 1 control) in the same sample. In order to overcome this limitation, Enigma Diagnostics are currently developing a multi-sample instrument targeted at the hospital laboratory market.

The operation of the instrument is based on a disposable single-shot rotating test cartridge (carousel), containing liquid reagents, dried reagents, tools (such as a foil-piercer and a pipettor) and a capillary tube for the PCR reaction. The capillary tube is constructed of a robust electrically conducting polymer (ECP) which heats up in response to an applied voltage, and which allows rapid thermal cycling due to its very low thermal mass. Cycling using hot air or a Peltier block is therefore avoided. Following sample application (up to 1 ml volume), sample lysis under denaturing conditions is automatically carried out, including a sonication step for samples containing spores. Target DNA is extracted and isolated on magnetised silica beads which are then robotically transferred through the remaining stages of sample preparation before the DNA is pipetted into the thermocycling reaction tube. The amplicons are detected fluorgenically, and results are displayed to the operator using a simple traffic-light system (although detailed amplification curves are available for down-load to a remote PC for analysis if required). An internal control target is analysed simultaneously.

Reagents are stabilised for storage at room temperature by freeze-drying in the presence of suitable excipients.

As well as being compact (L240mm, W314mm, H510mm, 20 kg), robust, sensitive and accurate, the system is very fast. Target DNA isolation from a crude sample can take as little as 12 minutes, while a complete 50 cycle analysis can be complete in 40 minutes. Each PCR cycle can take as little as 15 seconds. If using cyber green to detect amplicons, analysis result can be obtained in as little as 20 minutes. Sensitivity will depend on the target DNA extraction procedure required, but typically will be tens to hundreds of target copies.

Guide price: High throughput users, buying freeze-dried reagents from Enigma, will probably have free access to the instrument. However, each test will cost £10-£20 – competitive to the per test cost using the GeneXpert system. Lower throughput users, or those preparing their own reagents, will probably pay £20,000 to £30,000 for an instrument.

Other real-time qPCR instruments

A number of other real-time qPCR systems are available commercially, for example the Roche LightCycler (single sample multiplex assays) and the Roche LightCycler 480 (96 sample or 384 sample mutiplexed assays). However, none of these other systems are fully automatic, and all require separate target DNA isolation prior to assay. In addition, all are designed to be laboratory based - they are not ruggedised and all require mains AC power. Furthermore, where multiple samples analysis is possible, assays cannot be run independently of one another. For these reasons, instruments such as these are considered unsuitable for this application, and will not be described here.

1.6.3 Emerging technologies

Smiths Detection is currently developing a portable (7 kg), battery powered, continuous, rapid (< 5 minutes), real-time optical biosensor system to broadly classify biological agents in air (Smart Bio Sensor). The system classifies agents as bacteria, bacterial spores, toxins and viruses, and has a high tolerance of environmental interferents. They anticipate that the Smart Bio sensor will be used in conjunction with the Bio-Seeq PLUS PCR system to classify and then identify bio-aerosol components.

Antibody technology is combined with resonance acoustic profiling in an instrument currently under development by a joint venture between Akubio and NIAID in the USA. Full details of the instrument are not available, except that it is powered by 6 ‘AA’ batteries and contains 8 detection channels. The prototype measures 12 x 12 x 7 cm and weighs 700g. A project proposal has been submitted to the US Army to ruggedise and field test the instrument. In this format it may be a useful tool for the composting industry. However, the large-scale

laboratory version of the instrument, while available commercially (RAPid4), is not suitable for use in this programme.

A number of other bio-physical approaches for the detection / identification of microorganisms in bio-aerosols are under investigation and may well be significant in the future. These include real-time flow cytometry (Chen and Li, 2007), surface-enhanced Raman spectroscopy (SERS) (Sengupta et al 2005, 2007), Coherent anti-Stokes Raman scattering (CARS) (Petrov et al 2007), FT-IR spectroscopy (Fischer et al 2006), Laser-induced breakdown spectroscopy (LIBS) (Dixon and Hahn, 2005), and the application of micro-chip-sized mass produced portable mass spectrometers (Hauschild et al 2007).

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2. Network configurations for the monitoring of bioaerosols from composting facilities

2.1 Introduction

This section examines the options for assembling the sampling equipment a practical monitoring network. Specific operational requirements such as power and communications will be covered in Section 7.

2.2 Overview of network aims

The aim of this section is to discuss possible configurations for a network of monitors to enable the quantification and identification of bioaerosols emitted from composting facilities.

A number of configurations of networks are considered, based on different monitoring equipment. Each provides a different operational methodology and has varying costs in terms of equipment and operating overhead (including staffing requirements). In addition different network configurations provide data with varying temporal resolutions and response times.

The precise configuration of a monitoring network is dependent on the purpose for which the measurements are required. There are at least three reasons for the operation of such a network:

- Research purposes e.g. to obtain more information on the concentration of specific bioaerosols emitted and the links with site activities
- Control purposes e.g. to replace/augment existing monitoring required by permits to operate
- To investigate the effects on adjacent receptors

The ITT describes a proposed network design consisting of monitoring using a number of tiers of complexity.

These tiers are described below.

Tier 1 This tier is envisaged as a low cost alert level, providing limited measurements of particulate matter. No attempt to identify or quantify biological material is intended at this tier. The previous report has shown that there are a number of fairly low cost instruments measuring particle number, mass and size, which could perform this task.

Tier 2 This tier of instrumentation is intended to be able to provide some identification of the presence of biological material. Techniques such as fluorescence together with aerosol shape determination, as developed for DSTL by Prof Kaye (University of Hertfordshire) may be sufficiently low cost candidates for Tier 2 use, although the commercially available Biral Verotect instrument is likely to be too expensive.

Tier 3 These instruments are able to provide identification and speciation of biological organisms. In the ITT it was assumed that Tier 3 instruments could provide real time or pseudo real time results in the field without requiring separate laboratory analysis. However, the review of available techniques has shown there is only a single fieldable automated

instrument (Enigma FL) currently available in the UK, which, in principle, could be relatively quickly configured to give near real-time analysis of the range of organisms relevant to this programme. This is based on PCR (polymerase chain reaction technology). In practice, transfer of the bioaerosol from the sampler to the instrument would be done manually in the first instance.

There is not currently any commercial mass spectrometry (MS) based equipment which will offer similar performance, but further discussions with TNO in the Netherlands may allow us the examine the usefulness of their experimental MALDI-ATOFMS (matrix-assisted laser desorption/ionisation aerosol time of flight mass spectrometer).

Ancillary monitors These are monitoring equipment, which provide additional data important for the operation of the network. The main example of ancillary monitors are meteorological systems, providing measurements of wind direction and speed.

2.3 Requirements

Following discussions with Defra and the Environment Agency the following requirements for the monitoring network have been defined. The impact these requirements have on the network design process is discussed.

2.3.1 Purpose

The network is not intended at this stage for long term regulatory monitoring. It is intended to be used as a short-term investigation and regulatory tool to assess emissions from specific sites or to be used in scientific studies.

Network design impact:

The network is therefore not required to be entirely automated nor to run unattended for long periods of time. Operation by untrained or semi-trained staff, or by the sites themselves is not seen as a priority. In addition cost drivers are lower as only a limited number of installations will be required. It is likely the network will be moved to different sites, and so flexibility in configuration of the network will be important. Investigative studies will potentially require monitoring for a wide range of bio-material, and possibly the identification of which biological organisms are present beyond a pre-determined list.

2.3.2 Cost

The implications of the discussion above not withstanding, it is understood that cost is still a major driver.

Network design impact:

The option to use large numbers of high specification Tier 3 instruments is too costly to implement. Network designs must aim to reduce the number of high cost instruments needed to a minimum. Similarly, the range of species may need to be limited to keep cost to a minimum (see below).

2.3.2 Target biological species

Composting Association guidelines recommend the enumeration of the specific organism *Aspergillus fumigatus,* and also the enumeration of total bacteria in the group known as mesophilic bacteria. This group contains a large number of species, and current enumeration methods do not differentiate between the individual species of mesophilic bacteria sampled. The methodologies described in this report would allow the detection / enumeration of specific individual species, and so would extend the depth of information available regarding the species of organisms associated with compost degradation.

However, it should be noted that the data obtained from these new technologies may not be directly comparable with data collected by traditional methods, since traditional methods rely on enumerating viable organisms, while MS and PCR technologies will measure both viable and non-viable organisms.

Network design impact:
Both mass spectrometry and PCR can detect a number of species. In the case of PCR, assays for individual species need to be developed separately, so cost increases with complexity. If PCR is employed as the Tier 3 technology then Configuration 2 (see below) would be appropriate, as collected sample could be transported to an on-site facility and give near real-time analysis results (1-2 hrs post collection). If mass spectrometry were selected as the Tier 3 technology, it would be necessary to adopt a Configuration 3 network (see below), as current MS instruments analyse aerosol samples directly from an emission plume.

2.4 Network designs

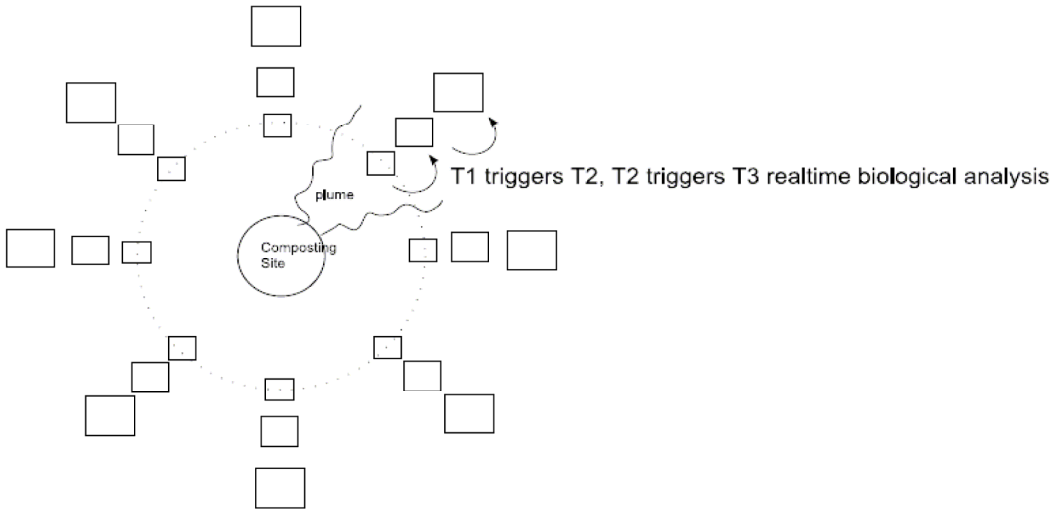
The following sections describe three network configurations, and discuss their capabilities and operation.

Configuration 1
This network design is as described in the ITT.

In operation, the Tier 1 monitors run continuously. When these identify enhanced particulate matter, and if the ancillary meteorological data shows the monitors which have detected the particulates are downwind of the site, then these trigger the operation of the real time Tier 2 instruments. These measure the particulate plume for specific characteristics indicative of biological material, and if present, trigger Tier 3 instruments in the downwind region to sample and analyse the particulate, in order to identify the biological species.

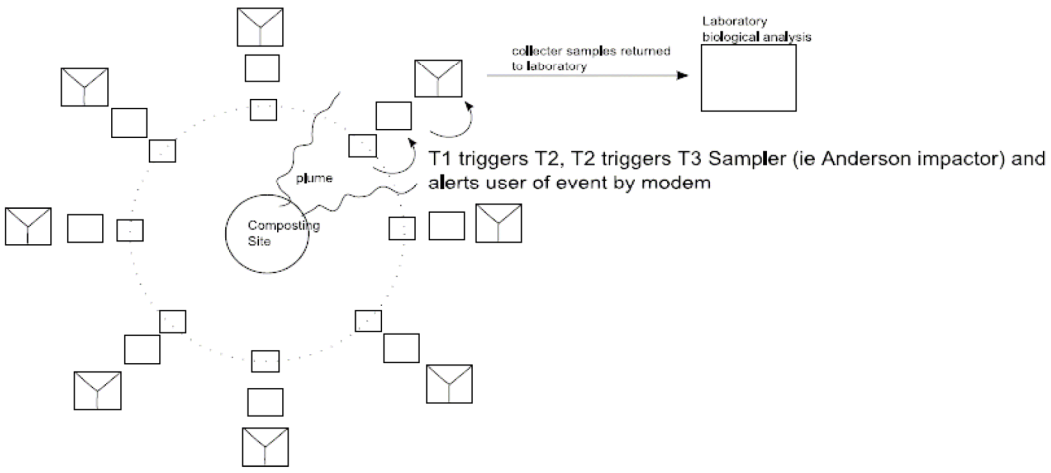
The rationale for the tiered approach is that the higher level instrumentation, Tier 2 and most importantly Tier 3 are expensive to operate. In the case of Tier 3 this may include the use of expensive reagents in the identification process. However, the capital cost of a large number of Tier 3 instruments may make this configuration unrealistic. It has also been noted in our earlier report, that the operational costs of Tier 2 instruments are unlikely to be greater than that for Tier 1, and so Tier 2 instruments could carry out the functions of both Tier 1 and 2.

Another option would be to remove Tier 2, and instead to rely on the wind data and Tier 1 data to trigger Tier 3. This may be an option if the Tier 1 instruments are able to provide some pre-screening based on particle size. However it has been noted that biological material may be present over a range of sizes, and be adhered to particulate matter, so this approach may not be feasible in practice.



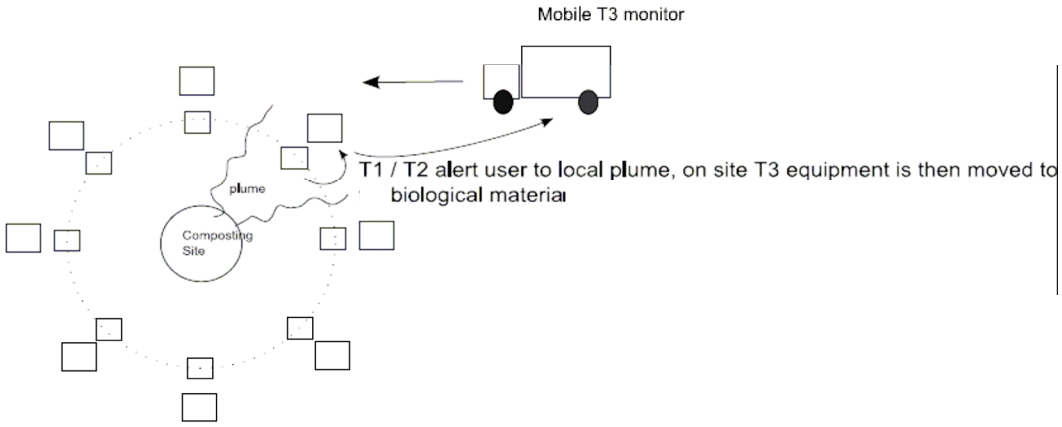
Configuration 2

In this configuration, the expensive Tier 3 instruments are replaced by lower cost samplers. These are again activated by Tier 1 and/or Tier 2 instruments and the meteorological data. (The same discussion on the roles of Tier 1 and Tier 2 is relevant to this configuration too). Once the sampling has completed, the network would alert an operator, who would collect the sampled material. The sampled material would then be sent to a laboratory for analysis. This laboratory could be on site using portable or semi-portable instruments, or could be an off-site analysis laboratory. This configuration requires some manual intervention and does not provide real time data. An enhancement would be the use of multiple sample systems which could store a number of samples collected over different alert periods, reducing the number of visits required by the operator, and removing down time caused by waiting for the collected samples to be removed.



Configuration 3

This configuration is similar to Configuration 1. However in order to keep the costs down, only one Tier 3 analyser is deployed. Once an alert is provided by the Tier 1/Tier 2 stage, the Tier 3 analyser, mounted in a mobile laboratory, is moved to an appropriate monitoring location. This cuts down the capital cost of the network considerably. However, it is possible that by moving the Tier 3 monitor after the Tier 1/Tier 2 alert, the emissions event may be missed and not captured in the Tier 3 monitor. This network also requires the most overhead in terms of operation, with the mobile laboratory needing to be manned for the entire monitoring period.



2.5 Conclusions

A network based on Configuration 2 using Tier 1 and Tier 3 only offers the most feasible and lowest cost option.

3. Bioaerosol sampling network: Define selection criteria and select proposed units

3.1 Introduction

This Section ranks equipment to be tested for the sampling and analysis of bioaerosols at municipal composting facilities. The aim is to assess all reviewed equipment according to a list of key criteria. Criteria cover measurement, ease of operation, fieldability, communications, initial cost, ongoing cost, and commercial status. The criteria are described for each tier of instrumentation/sampler as described in 'A review of instruments for a bio-aerosol sampling network at composting facilities'. A weighting has been given to each criterion, after discussion with the Defra Steering Committee, which is multiplied by the score for each criterion. The scores are summed for each instrument to give an approximate overall ranking of all the equipment for each tier. In many cases, scoring can only be 'high' 'medium' or 'low' and this is converted to a numerical score. Details are given in Appendix 6. This method of ranking equipment seeks to provide a balance between measurement capability, operability and cost.

The conclusions from the scoring are then combined with the description of possible network configurations from Section 2 to recommend the preferred combination(s) of equipment/network configurations to be taken forward for further assessment.

Results from a Defra funded study by the IOM: 'Dose-response relationships for bioaerosol emissions from waste treatment processes: Interpretation of published dose-response relationships', have been used to make a decision on the priority species to be measured, according to toxicology evidence, after discussion with, and approval of, the Defra Steering Committee.

3.2 Tier 1 instruments

Ranking

Tier 1 instruments are those which measure one or more of particle mass, number, size and shape. These are characteristics with may give some indication of the biological activity of the particles. These instruments are discussed in detail in Section 2 of the earlier report: '*a review of instruments for a bioaerosol sampling network at composting facilities'*. As the (mainly optical-based) technology is well developed, all of the instruments are available as commercial products.

Table 3.1, below, assesses each of the Tier 1 instruments against a range of criteria. These criteria, which will be used to select the equipment, have been agreed with the Defra steering committee. A numerical score is attached to each level of each criterion, as described in Appendix 6, and a final ranking has been calculated.

The instruments are referred to by the numbers shown below in the accompanying Tables.

2.1 Thermo Fisher Scientific ADR-1200S
2.2(a) TSI AeroTrak optical particle counter (Model 8240 or 8260)
2.2(b) TSI DustTrak
2.3(a) Turnkey Instruments Topas/Osiris
2.3(b) Turnkey Instruments Dustmate
2.4 Biral Aspect
2.5 Casella APM950 system (with PM_{10} sampling head)
2.6 Grimm EnvironCheck

2.7(a) Met One 3400 series portable air particle counter
2.7(b) Met One HHPC-6 handheld particle counter (standard model)
2.8(a) PMT IsoAir (model 510)
2.8(b) PMT IsoAir Plus (model 350L)
2.8(c) PMT HandiLaz 301 /Handilaz Mini
2.9 Cambustion DMS50 & DMS500 instruments
2.10 Thermo Fisher Scientific 8500 FDMS

Conclusion

The Turnkey Instruments Topas has the highest score as shown in Table 3.1, followed by the Thermo Fischer Scientific ADR-1200s and the TSI AeroTrak Model 8240. Any of the three instruments would be suitable.

Table 3.1- Scoring of Tier 1 instruments

Criterion	Instrument / Evaluation							
	2.1	**2.2(a)**	**2.2(b)**	**2.3(a)**	**2.3(b)**	**2.4**	**2.5**	**2.6**
Number or mass characterisation?	Y	Y	Y	Y	Y	Y	Y	Y
Additional size separation?	N	Y	N	Y	Y	N	N	Y
Shape characterisation?	N	N	N	N	N	Y	N	N
Particle sizes analysed / μm	< 10	0.3 - 10	[(a)]	0.5 -20	0.5 -20	0.5 –20	0.1 – 10	< 10
Continuous operation?	Y	Y	N	Y	N	Y	Y	Y
Minimum measurable concentration or detection rate	1 $\mu g.m^{-3}$	< 0.2 particles.min^{-1}	1 $\mu g.m^{-3}$	0.01 $\mu g.m^{-3}$	0.01 $\mu g.m^{-3}$	?	1 $\mu g.m^{-3}$	1 $\mu g.m^{-3}$
Maximum measurable concentration or detection rate	400 $mg.m^{-3}$	0.4 x 10^6 particles.min^{-1}	100 $mg.m^{-3}$	60 $mg.m^{-3}$	60 $mg.m^{-3}$	1.2 x 10^6 particles.min^{-1}	2 $mg.m^{-3}$	1.5 $mg.m^{-3}$
Flow rate / $l.min^{-1}$	?	28.3	1.7	?	?	?	16.7	72
Ability to sample particles?	N	N	N	Y	Y	N	Y	N
Fieldability [1]	H	M	M	H	L	M	H	H
Meteorological measurements?	N	N	N	Y	N	N	N	Y
Wireless communication?	N	N	N	Y	N	N	N	Y
Number of data points stored	13,000	> 100,000	31,000	?	?	?	?	?
Battery back-up	Y	Y	Y	Y	Y	?	Y	?
Cost (purchase)	£3,900	£4,675	£2,400	£5,000	£2,700	£47,225	£10,500	£20,800
Cost (ongoing)	Annual calib.	Annual calib.	?	Annual calib.	Annual calib.	?	?	?
Commercial status [2]	H	H	H	H	H	H	H	H
Availability for this study [3]	H	H	H	H	H	H	M	M
Total Numerical Score	**53**	**46**	**32**	**61**	**38**	**42**	**44**	**43**
Ranking	**2nd**	**3rd**		**1st**				

Rankings

Unless specified below, **H**: high, **M**: medium, **L**: low, **Y** = yes, **N** = no, **?** = not known

Table continued next page……

[1] **H**: fully fieldable **M**: minor modifications required **L**: major modifications required

[2] **H**: fully commercial **M**: prototype available **L**: under development

[3] **H**: loan agreed with manufacturer **M**: not discussed with manufacturer (instrument can be purchased) **L**: not available

(a) Dependent on size-selective head (PM_{10}, $PM_{2.5}$ or PM_1)

Table 3.1- Scoring of Tier 1 instruments (continued)

Criterion	Instrument / Evaluation						
	2.7(a)	**2.7(b)**	**2.8(a)**	**2.8(b)**	**2.8(c)**	**2.9**	**2.10**
Number or mass characterisation?	Y	Y	Y	Y	Y	Y	Y
Additional size separation?	Y	Y	Y	Y	N	Y	N
Shape characterisation?	N	N	N	N	N	N	N
Particle sizes analysed / μm	< 25	< 5	< 5	< 25	< 5	0.005 – 2.5	[(a)]
Continuous operation?	Y	N	Y	Y	N	Y	Y
Minimum measurable concentration or detection rate	< 0.2 particles.min^{-1}	< 0.2 particles.min^{-1}	< 0.2 particles.min^{-1}	< 0.2 particles.min^{-1}	< 0.2 particles.min^{-1}	?	0.06 μg.m^{-3}
Maximum measurable concentration or detection rate	0.4 x 10^{6} particles.min^{-1}	0.2 x 10^{6} particles.min^{-1}	0.6 x 10^{6} particles.min^{-1}	0.425 x 10^{6} particles.min^{-1}	0.2 x 10^{6} particles.min^{-1}	10^{12} dN/dlogD$_p$/cc	5,000 mg.m^{-3}
Flow rate / l.min^{-1}	28.3	2.83	1.0	1.0	2.83	2.5	1 m^3.h^{-1}
Ability to sample particles?	N	N	N	N	N	N	Y
Fieldability [1]	M	L	M	M	L	M	M
Meteorological measurements?	N	N	N	Y	N	N	N
Wireless communication?	N	N	N	Y	N	N	N
Number of data points stored	5,000	500	?	3,000	10,000	?	1,680
Battery back-up	Y	Y	N	N	Y	?	N
Cost (purchase)	?	£2,200	£2,000	£6,200	£1,600	£60,000	£20,000
Cost (ongoing)	?	?	Annual calib	Annual calib	?	?	?
Commercial status [2]	H	H	H	H	H	H	H
Availability for this study [3]	M	M	M	M	M	M	M
Total Numerical Score	**42**	**32**	**45**	**44**	**28**	**37**	**38**
Ranking							

Rankings
Unless specified below, **H**: high, **M**: medium, **L**: low, **Y** = yes, **N** = no, **?** = not known

[1] **H**: fully fieldable **M**: minor modifications required **L**: major modifications required
[2] **H**: fully commercial **M**: prototype available **L**: under development
[3] **H**: loan agreed with manufacturer **M**: not discussed with manufacturer (instrument can be purchased) **L**: not available

[(a)] Dependent on size-selective head (PM_{10}, $PM_{2.5}$ or PM_1)

3.3 Tier 2 instruments

Ranking

Tier 2 instruments have been developed to detect biological material in particulate samples. The first two instruments listed are commercially available and measure the fluorescence of the sample at an excitation wavelength characteristic of the biological material. The other instruments are still at various stages of development, but should also be considered for use in the study.

Table 3.2 (below) assesses each of the Tier 2 instruments against a range of criteria. As for Tier 1, these criteria, which will be used to select the equipment, have been agreed with the Defra steering committee. A numerical score is attached to each level of each criterion, as described in Appendix 6, and a final ranking has been calculated.

The instruments are referred to by the following numbers:
3.1 Biral VeroTect
3.2 ICx Technologies AirSentinel 1000B
3.3 University of Hertfordshire WIBS2 fluorescence detector
3.4 Magee Scientific fluorescence modified aethalometer
3.5 EvansCo biological sensing devices

Conclusion

The Biral Verotect had the highest score as shown in Table 3.2, followed by the ICx Technologies AirSentinel 1000B and the University of Hertfordshire/WIBS 2 fluorescence detector.

Table 3.2- Scoring of Tier 2 instruments

Criterion	Instrument / Evaluation				
	3.1	**3.2**	**3.3**	**3.4**	**3.5**
Florescence characterisation?	Y	Y	Y	Y	Y
Shape characterisation?	Y	N	Y	?	?
Size characterisation?	Y	N	Y	?	?
Particle sizes analysed / μm	0.5 - 15	?	< 1	?	?
Response time / s	?	30 - 120	?	?	?
Continuous operation?	Y	Y	Y	?	?
Max. measurable concentration	20,000 particles.s^{-1}	?	125 particles.s^{-1}	?	?
Flow rate / l.min^{-1}	33	?	1	?	?
Ability to sample particles?	N	Y	N	?	?
Fieldability [1]	H	L	M	?	?
Wireless communication?	N	Y	N	?	?
Cost (purchase)	£67,670	£8,400	£2,500 [(b)]	?	?
Cost (ongoing)	?	1 pump / year	?	?	?
Commercial status [2]	H	H	M	M	L
Availability for this study [3]	H	M	H	M	L
Total Numerical Score	**51**	**46**	**42**	**?**	**?**
Ranking	**1st**	**2nd**	**3rd**		

Rankings
Unless specified below, H: high M: medium L: low; Y = yes N = no; ? = not known

[1] H: fully fieldable M: minor modifications required L: major modifications required
[2] H: fully commercial M: prototype available L: under development
[3] H: loan agreed with manufacturer M: not discussed with manufacturer (instrument can be purchased) L: not available

[(b)] Detector only

3.4 Tier 3 particle samplers

Ranking

These can be categorised as either 'limited portability' samplers (designed for used in fixed locations) or 'handheld / miniaturised samplers'. Only a few of the instruments in the latter category have a size-selective inlet.

Table 3.3, below, assesses these instruments against a range of criteria. As for the measurement equipment, these criteria, which will be used to select the equipment, have been agreed with the Defra steering committee. A numerical score is attached to each level of each criterion, as described in Appendix 6, and a final ranking has been calculated.

The instruments are referred to by the following numbers:

Limited portability particle samplers	Handheld / miniaturised particle samplers
4.1.1 Thermo Fisher (Andersen) six-stage cascade impactor	**4.2.1** Two-stage cyclone device for personal bioaerosol sampling
4.1.2 Thermo Fisher ASAP Model 2800	**4.2.2** ICx BioCapture 650
4.1.3 Vai SMA Atrium	**4.2.3** ICx BioBadge 100
4.1.4 Biotrace Air Trace environmental air sampler	**4.2.4** PMT Check Air model 4100
4.1.5 University of Hokkaido honeycomb filter sampler	**4.2.5** Microbio MB2 air sampler
4.1.6 Bertin Technologies Coriolis μ	**4.2.6** SAS IAQ
4.1.7 Bertin Technologies Coriolis MS	**4.2.7** Millipore M Air T sampler
4.1.8 Sceptor SpinCon 45010A	**4.2.8** Vai SMA Microportable viable air sampler
4.1.9 Sceptor Omni 3000	**4.2.9** Biotest HYCON RCS high flow sampler
4.1.10 Dycor XMX-CV	**4.2.10** MAS-100 air monitoring system
4.1.11 Innovatex BioGuardian air sampler (model 12.03-1000)	**4.2.11** Buck BioCulture
4.1.12 SSI air samplers	**4.2.12** Microflow 60 air sampler

Conclusion

The particle samplers have been ranked and scored. Individual samplers use different sampling media: agar, filters, liquid or foam. Selection of the best sampler should therefore be driven not only by the ranking but also by the sampling medium required for subsequent analysis. For sampling into liquids, using a limited portability sampler, the Bertin Technologies Coriolis or MS come joint first with the Dycer XMV-CV, as shown in Table 3.3.

Table 3.3- Scoring of Tier 3 particle samplers

Criterion	Limited portability particle samplers: Evaluation											
	4.1.1	**4.1.2**	**4.1.3**	**4.1.4**	**4.1.5**	**4.1.6**	**4.1.7**	**4.1.8**	**4.1.9**	**4.1.10**	**4.1.11**	**4.1.12**
Particle sizes analysed / μm	> 0.65	1 – 10	> 0.5	> 0.5	?	0.5 – 20	0.5-300	> 0.2	> 0.5	1 - 10	1 -10	?
Multi-size selective?	Y	N	N	N	?	N	N	M	N	N	N	N
Flow rate / $l.min^{-1}$	28.3	200	28.3	28.3	?	100-300	360-630	450	300	530	1000	?
Sampling medium [4]	Agar	Foam	Agar	Agar	Filter	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Agar / filter
Fieldability [1]	H	H	L	L	?	M	H	M	M	H	L	L
Meteorological measurements?	Y	N	N	N	?	N	N	N	N	N	N	N
Cost (purchase)	£3,300	£7,200	£3,400[c]	£6,000	?	£6,000	£20,000	£20,500	£6,000	£10,000	£16,000	£9,000
Cost (ongoing)	?	?	?	?	?	?	?	?	?	?	?	?
Commercial status [2]	H	H	H	H	M	H	H	H	H	H	H	H
Availability for this study [3]	M	M	M	M	M	H	H	M	M	M	M	M
Total Numerical Score	**29**	**21**	**15**	**12**	**5**	**16**	**21**	**21**	**16**	**21**	**12**	**12**
Ranking	**1st (Agar)**						**=1st Liquid**	**=1st Liquid**		**=1st Liquid**		

Rankings
Unless specified below, H: high M: medium L: low; Y = yes N = no; ? = not known

[1] H: fully fieldable M: minor modifications required L: major modifications required
[2] H: fully commercial M: prototype available L: under development
[3] H: loan agreed with manufacturer M: not discussed with manufacturer (instrument can be purchased) L: not available

[(c)] For one port with control centre. The cost increases up to £31,000 for ten ports and control centre.

Table continued next page……

Table 3.3- Scoring of Tier 3 particle samplers (continued)

Criterion	Handheld / miniaturised particle samplers: Evaluation											
	4.2.1	**4.2.2**	**4.2.3**	**4.2.4**	**4.2.5**	**4.2.6**	**4.2.7**	**4.2.8**	**4.2.9**	**4.2.10**	**4.2.11**	**4.2.12**
Particle sizes analysed / μm	> 1	0.5 – 10	All	All	All	All	All	All	All	All	All	All
Multi-size selective?	Y	N	N	N	N	N	N	N	N	N	N	N
Flow rate / l.min^{-1}	Variable	200	40	100	100	100	143	28.3/141	100	100	30-120	30-120
Sampling medium	Liquid	Liquid	Filter	Agar	Agar	Agar	Agar	Agar	Agar	Agar	Agar	Agar
Fieldability [1]	L	M	L	L	L	L	L	L	L	L	L	L
Meteorological measurements?	N	N	N	N	N	N	N	N	N	N	N	N
Cost (purchase)	?	£7,600	£1,850	£1,850	£1,250	£1,650	£2,500	?	£2,300	£3,800	£700	£1,600
Cost (ongoing)	?	?	?	?	?	?	?	?	?	?	?	?
Commercial status [2]	M	H	H	H	H	H	H	H	H	H	H	H
Availability for this study [3]	M	M	M	M	M	H	M	M	M	M	M	M
Total Numerical Score	**12**	**16**	**19**	**19**	**19**	**19**	**15**	**15**	**15**	**15**	**19**	**19**
Ranking		**=1st Liquid**	**=1st Filter**	**=1st Agar**	**=1st Agar**	**=1st Agar**					**=1st Agar**	**=1st Agar**

Rankings
Unless specified below, H: high M: medium L: low; Y = yes N = no; ? = not known

[1] H: fully fieldable M: minor modifications required L: major modifications required
[2] H: fully commercial M: prototype available L: under development
[3] H: loan agreed with manufacturer M: not discussed with manufacturer (instrument can be purchased) L: not available

[(c)] For one port with control centre. The cost increases up to £31,000 for ten ports and control centre.

3.5 Tier 3 instruments

Tier 3 instruments are those which are able to identify the species of microorganisms present in a bioaerosol. Those instruments selected fall into two technology areas; mass spectrometry and PCR.

Table 3.4, below, assesses these instruments against a range of criteria. As for other Tiers, these criteria, which will be used to select the equipment, have been agreed with the Defra steering committee. A numerical score is attached to each level of each criterion, as described in Appendix 6, and a final ranking has been calculated.

The instruments are referred to by name.

Table 3.4- Scoring of Tier 3 instruments

Criterion	**Instrument / Evaluation**								**Key / possible responses**
	Bruker CBMS	**TSI**	**BAMS**	**MALDI-**	**Cepheid GeneXpert**	**Cepheid SmartCycler**	**Smiths Bio-Seeq PLUS**	**Enigma FL**	
		ATOFMS	**SPAMS**	**ATOFMS**					
Real-time measurement parameters	Pyrolysis ion m/z MS/MS +ve ion mode	Laser ablation m/z MS +ve and -ve ion mode	Laser ablation m/z MS +ve and -ve ion mode	MALDI m/z MS +ve ion mode	Fluorescence	Fluorescence	Fluorescence	Fluorescence	-
Biological discrimination possible?	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	**Yes** or **No**
Biological speciation possible? Multiple simultaneous species?	Yes - limited / ?	No	Limited	Limited	Yes / 3+control per sample	Yes / 3 + control per sample	Yes / ?	Yes / 5 + control per sample	**Yes, No, Limited**
Sampling time	2 min	N/A	N/A	N/A	N/A	N/A	N/A	N/A	**N/A =** not applicable
Response time	3 min	1 second	1 second	1 second	70 min	40 min +	50 min +	20 min	**"+"** = needs sample prep before assay
Real-time operation mode	D	C	C	C	D	D	D	D	**C:** continuous **D:** discontinuous
Cost (purchase)	£245k	£243k	N/A	N/A	£20-30k	£25-35k	£20k	£20-30k	-
Cost (ongoing / per test)	?	?	?	?	£20-30	£2-5	?	£10-20	-
Complexity / user intervention	L	L	L	L	L	S	S	L	**S =** significant intervention
									L = little intervention
									O = ongoing support required
Selectivity / specificity	H	H	H	H	H	H	H	H	**H =** high **L =** low
Known interferents	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	-
Detection limits	15 ACP/litre air	10E-18 mol PAHs	1,000 bacteria	2,100 bacteria	100-200 cfu	100-200 cfu	100-200 cfu	100-200 cfu	-

Table continued next page……

Criterion	**Instrument / Evaluation**								**Key / possible responses**
	Bruker CBMS	**TSI**	**BAMS**	**MALDI-**	**Cepheid GeneXpert**	**Cepheid SmartCycler**	**Smiths Bio-Seeq PLUS**	**Enigma FL**	
		ATOFMS	**SPAMS**	**ATOFMS**					
Max. measurable concentration	NK	NK	NK	NK	D	D	D	D	**D** = dilute sample **NK** = not known
Level of quantification	H	H	H	H	H	H	H	H	**H** = high **P** = poor
Particle size range	> 2 um	30-300 nm or 100-3000 nm	optimum 1-3 um	0.1 - 10 um	N/A	N/A	N/A	N/A	**N/A** = not applicable
Multiple sample capability	N/A	N/A	N/A	N/A	up to 16 multiplexed	up to 16 multiplexed	up to 6 multiplexed	1 multiplexed	
Flow rate	?	0.1 litres/min	1.0 litre/min	NK	N/A	N/A	N/A	N/A	**N/A** = not applicable
Configuration	P	P	P	P	N/A	N/A	N/A	N/A	**Po:** point **Ar:** area (sample)r
Sampling mode	A	A	A	A	N/A	N/A	N/A	N/A	**A:** active **P:** passive
Sampling directions	O	D	D	D	N/A	N/A	N/A	N/A	**D:** directional **O:** omni-directional
Field operability	SC	E	?	?	N/A	SC + PC +battery	SC	SC + battery	**H:** housing required **SC:** self contained **E:** external services required
Unattended operation period	OR	OR	OR	OR	OR, assay period	OR, assay period	OR, assay period	OR, assay period	**OR** = operator required
Operational conditions	Fieldable	10-35 deg C, 0-75% RH	NK	NK	NK	NK	NK	NK	**NK** =not known
Services required	None	Mains electricity	Mains electricity	Mains electricity	Mains electricity	None	None	None	
Stability of sampled material	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	**N/A** = not applicable
Viable / non-viable measurement	viable + non-viable	N/A	viable + non-viable	viable + non-viable	viable + non-viable	viable + non-viable	viable + non-viable	viable + non-viable	
Connectivity	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	**USB** / **RS232** / **W**: wireless
Availability for this study	No	No	No	No	No	Yes	Yes	Yes	
Commercial status	Commercial	Commercial	Experimental	Experimental	Commercial	Commercial	Commercial	Pre-production	
Total	**68**	**29**	**47**	**47**	**74**	**92**	**82**	**94**	
Ranking						**2nd**	**3rd**	**1st**	

3.6 Choice of Priority Species for Measurement

A Defra funded literature study by the IOM, ('Dose-response relationships for bioaerosol emissions from waste treatment processes: Interpretation of published dose-response relationships'), has concluded is that there is insufficient health information to be able to identify key marker species. Whilst the microbial mix is clearly important, there is little quantitative information linking species mix to effects. Most health studies report dust and endotoxin, some report beta (1->3) glucan, total fungi/microbes/bacteria/acetinomycetes or viable fungi.

Actinomycetes species appear to be important, but only two studies provide any kind of quantitative information. *Aspergillus fumigatus* is clearly important but comes from multiple sources and may not be the key species for particular waste activities. Adverse effects occur at background levels of exposure.

From health studies, the only bioaerosol component for which there exists exposure-response information is endotoxin.

After discussion with the Defra steering committee it has been decided to concentrate monitoring activity on *Aspergillus fumigatus*, as it is one of the main focuses of current bioaerosol monitoring at composting facilities.

3.7 Conclusions

The Turnkey Instruments Topas was the highest ranked Tier 1 instrument, although equipment ranked second or third would also be suitable. The Tier 3 equipment with the highest ranking for biological species, in particular fungi and bacteria, was the Bertin Technologies Coriolis (m or MS) with subsequent PCR determination.

The measurement of the priority species, *Aspergillus fumigatus*, by PCR is not currently offered commercially. The development of the assay is described in Section 4.

The use of mass spectrometry and/or detection based on the fluorescence of the sample at an excitation wavelength characteristic of the biological material should be investigated in parallel.

4. PCR assay development: Aspergillus fumigatus

4.1 Introduction

This Section details the initial laboratory work carried out at NPL to develop a real time quantitative PCR analysis (RT-qPCR) for *Aspergillus fumigatus* using both pure DNA and DNA extracted from spores grown at the Health Protection Agency (HPA). Section 5 describes the optimisation of the DNA extraction procedure.

4.2 Assay Development

We have used different sources of DNA materials with different purity and interfering substances in the sample matrix. In its purest form, we purchased some pure *Aspergillus fumigatus* genomic DNA from the Fungal Genetics Stock Center (FGSC). As an intermediate purity, we are extracting DNA from *Aspergillus fumigatus* spores grown at the Health Protection Agency (HPA) The "crudest" DNA source will come from air sampling that should contain particles impurities, PCR inhibitors and interfering chemical substances.

The three different purities of DNA source enable us to establish better experimental conditions and methodology for spore DNA extraction and RT-qPCR and identify the sources of error in the assay and correct for them. This will enable the final assay for use "in the field" to predict the number of *Aspergillus* spores per cubic metre of air with known confidence.

4.2.1 Background information about the RT-qPCR assay

The assay is based on the detection of a specific and unique DNA sequence on *Aspergillus fumigatus* mitochondrial genome. The Polymerase Chain Reaction (PCR) amplifies the short sequence of DNA through a series of thermal cycles. One cycle is composed of a denaturation step and a hybridization/elongation step. During the hybridization step a specific probe containing a fluorescent dye and a quencher is hybridized and digested by the polymerase. The degradation of this probe leads to an increase in the distance between the fluorescent dye and the quencher that generates a fluorescent signal. Fluorescence intensity is monitored and recorded in "real time" during each cycle by the instrument. The moment when the fluorescent signal reaches a threshold value is commonly used to quantify the number of DNA entities present initially in the reaction.

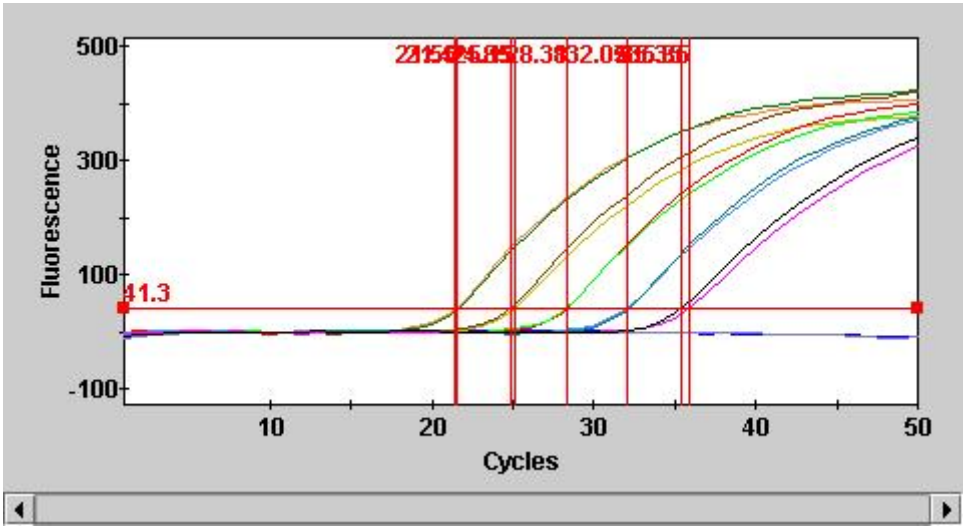
4.2.2 Evaluation of the assay performance using pure *Aspergillus fumigatus* genomic DNA

Performance and specificity of the PCR is mainly due to the design of primers and probes. As a first concern, we investigated if primers are specific to the DNA target i.e. they match perfectly with a unique complementary DNA sequence under reaction conditions.

Serial dilutions of a commercial supply of *Aspergillus fumigatus* genomic DNA were performed to evaluate the limit of detection of the assay and its specificity. The amount of DNA per reaction was selected in the range of 20ng to 2pg per ml of stock solution as this is the expected concentration range of DNA that is predicted to be extractable from air samples.

The plot below shows the fluorescent intensity recorded during cycles for serial dilutions of DNA; the horizontal red line represents the fluorescent threshold value. When a sample reaches this value the intercept on the x-axis gives us a Cycle threshold value that could be correlated to a DNA quantity or spores number. The RT-qPCR amplification is exponential, so a sample with high starting quantity of DNA would give smaller Cycle threshold (Ct) value as it reaches this concentration after fewer amplification cycles, while at the opposite, a

sample with low starting amount of DNA will reach the fluorescent threshold value later, so its Cycle threshold value will be higher.



Results are positive for DNA samples from 20ng to 2pg per reactions, blanks (water instead of DNA) are negative that is represented by a flat line below the fluorescent threshold value. The standard curve plot shows an R^2 value of 0.9998. At this time, the assay shows a limit of detection from 20ng to 2pg of *Aspergillus* genomic DNA with %CV values below 1%.

The specificity of the reaction was determined by direct visualisation of the PCR amplification product on an agarose gel stained with ethidium bromide.

A band between 100bp and 200bp could be observed on the agarose gel, no additional bands could be detected with EtBr staining. The PCR appears to be sequence specific; also experimental conditions do not generate unspecific PCR products. When DNA is replaced by water in the reaction, no bands could be detected that mean primers and probes do not form self-assembly structures during the reaction that could generate inappropriate fluorescent signals and so lead to the generation of false positive results.

4.2.3 Evaluation of the assay performance using *Aspergillus fumigatus* spores

During this project, *Aspergillus fumigatus* was grown at the collaborating HPA laboratory. After 21 days of culture, spores were collected from plates and re-suspended in ultrapure

water containing 0.05% Tween 80, and then spores in solution were sent to NPL to provide a control of understanding the performance of the assay when the DNA is collected directly from the spores.

Fungal cell walls consist of a thick layer of chitin, (1-3)- β-D glucan, (1-6)- β glucan, lipids and peptides. Due to this, cell walls are difficult to disrupt using common techniques, and this step required optimisation to increase recovery and yield of DNA.

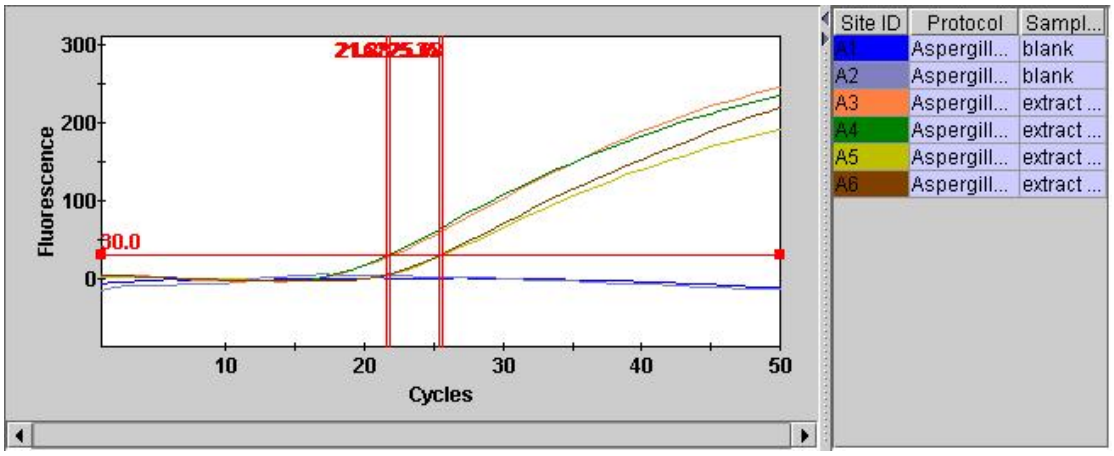
The extraction of mitochondrial DNA from spores is composed of 2 steps:

- Enzymatic digestion using zymolase (or lyticase)
- Follow by a mechanical disruption of the cell wall using glass beads and a bead beater

In the first instance, we used a protocol described in the literature that consists of:

- Spores are incubated with zymolase during 2 hours at 37°C at 180rpm
- After incubation, the equivalent volume of 100-150 L of glass beads is added in the tube and placed in the mini-bead beater (3min at 4800 oscillations per min).

After extraction, the sample is directly added to the RT-qPCR mix and thermocycled.



Results are positive for both spore's DNA extract with an average Ct values of 21.75 and 25.51 (%CV is comparable to pure genomic DNA with values below 1%). Blank samples (water instead of DNA) are negative which reflects the absence of contamination during the process.

4.2.4 Quantification

Aspergillus fumigatus DNA from FGSC (Fungal Genetics Stock Center) contains genomic and mitochondrial DNA. *A. fumigatus* genome is composed of 8 chromosomes (roughly 28.9Mb) and mitochondrial DNA (32kb). The sequence targeted by the probe is located in the mitochondrial DNA part that represents 0.11% of the genome size. The estimated DNA concentration provided by FGSC includes the full genome and the mitochondrial DNA. It is difficult reliably to estimate the ratio of mitochondrial DNA to genomic DNA at this stage. So there will need to be further refinement of the PCR assay to determine how reliably it will measure number of spores. The present assay will determine between the presence and absence of spores and give a measure of quantity of spores but with scope for improved accuracy.

The limit of detection of the RT-qPCR assay is very low, close to the femtogram of DNA per reaction, so handling and assay preparation need to be in line with best practice guide in this area to avoid any contamination.

5. PCR assay optimisation: Aspergillus fumigatus

5.1 Introduction

This Section describes the development and performance evaluation of the Polymerase Chain Reaction (PCR) method for the detection of fungal spores in bioaerosols. The method has been specifically developed to measure *Aspergillus fumigatus*. However, the method developed is equally applicable to other biological species of relevance to composting monitoring. Many sorts of DNA target could be used to detect the presence of pathogenic agents in airborne samples. The technique described in the following section will target mitochondrial DNA.

5.2 Technical Approach

Literature reports the use of different genomic material to detect *Aspergillus fumigatus* in airborne sample, blood, bronchoalveolar lavage fluids or tissue biopsies. Some of them present advantages such as the use of multi-copy genes (e.g. rRNA genes) that could decrease the detection limit. On the other side, these multi-copy genes are highly conserved within evolution between species, which makes sequence discrimination harder. Mitochondrial DNA assays were described as a potential target to detect pathogen agents; also each cell contains multiple mitochondria, which is an additional benefit to increase the technique sensitivity.

Three batches of spores of *A. fumigatus* (ATCC –42203) were grown at HPA for this project using two incubation times (7 and 21 days). Then spore suspensions were shipped to NPL for analysis.

Batch ID	Spores count in cfu/mL	Incubation time
AF1502/08	3.25×10^7 cfu/mL	7 days
AF2802/08	3.2×10^7 cfu/mL	21 days
AF2003/08	1.7×10^7 cfu/mL	21 days

Table 5.1: Batches of Aspergillus fumigatus grown at HPA

Then, the spore suspensions were counted at NPL using a haemocytometer. The extraction technique of *Aspergillus fumigatus* mitochondrial DNA involves two sequential steps. Firstly, enzymatic digestion of the cell wall is carried out using zymolase (or lyticase) for two hours at 37°C. Then mechanical disruption of the cell wall is achieved using a mini-bead beater for 3 minutes at 4800 oscillations/min.

The PCR assays have been developed using SmartBeads that contain the polymerase, dNTPs and Magnesium. The PCR assay for spores extract is performed as described below:

SmartBead 1 bead
Primers AF7 (10µM) 1µL
Primers AF8 (10µM) 1µL
Probe AF9 (10µM) 1µL
Molecular grade H_2O 37µL
Spores extract 10µL

The PCR reaction mix was then thermocycled during 50 cycles as follows:

95°C 120 s
95°C 20 s
65°C 60 s
50 cycles

The Smartcycler instrument is used to monitor fluorescence during the annealing/elongation step of thermocycling (excitation at 450-495 nm and emission at 510-527 nm).

The Smartcycler software assigns Cycle Threshold values (C_t) that correspond to an increase of the fluorescent intensity, above the fluorescent threshold value. The fluorescent threshold value was set to 30, which represents more than 10 times the fluorescent background signal.

The CFU (colony forming unit) number is obtained by counting colonies that grow on a specific agar medium after an incubation period. This CFU count appears to be underestimated compared to haemocytometer count. Of the spores present in solution, only a proportion are viable, and the other spores could be dead or damaged during sampling which may not reflect the potential pathogenicity . No obvious correlation has been identified between spore count and CFU values; the difference between both counting methods is nearly one order of magnitude, but this difference varies from batch to batch.

For this work we will be counting spores using the haemocytometer and correlating to a standard curve of reference DNA, assuming one spore of *Aspergillus fumigatus*, containing genomic and mitochondrial DNA, represents 24.4 femtogram of reference DNA.

5.3 Performance characteristics of the method

To perform RT-qPCR assays in alignment with Health and Safety regulations and Good Laboratory Practice, we have contained critical methodology steps in different areas. *Aspergillus fumigatus* was grown at HPA (Health Protection Agency); spore suspensions were sent in a safe container to NPL for analysis. Due to the detection sensitivity of the reaction, we had to perform the different steps of the analysis method in three dedicated locations:

1. Handling of spores and extraction were performed under a Class II cabinet.
2. Master mix and PCR preparation
3. Thermocycling

By splitting into these steps, we minimize contamination and prevent any hazards for operators. This section describes the experimental evaluation of the method carried out at NPL, using Genomic reference DNA and spores. The following characteristics have been assessed and quantified.

Repeatability of the RT-qPCR using Genomic reference DNA

This is defined as the standard deviation of successive measurements of the same sample. It has been determined from repeated measurements of the same sample. From the Genomic reference DNA stock solution purchased from the Fungal Genetics Stock Center (FGSC), we performed a series a 10 time serial dilutions to get from 20ng to 20fg per reaction.

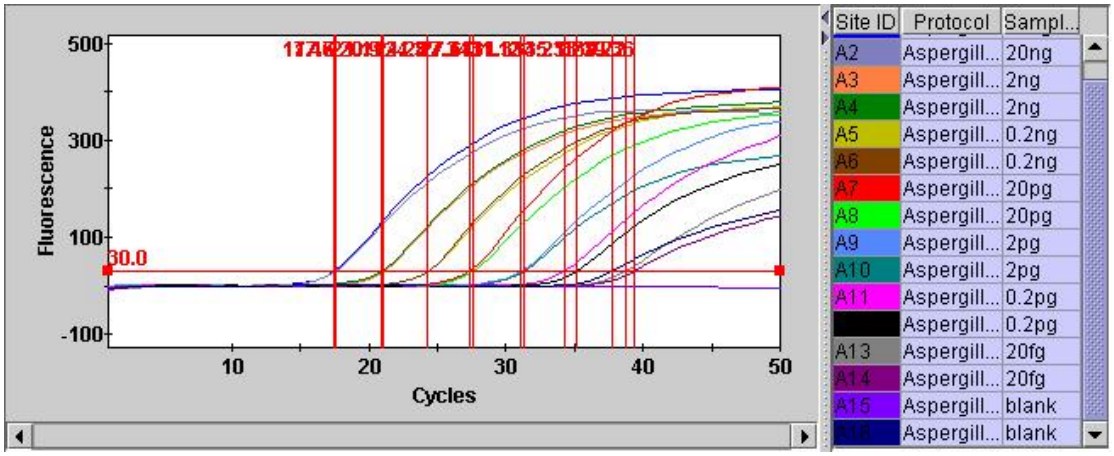


Figure 5.1: Repeats of serial dilutions of genomic DNA from 20ng to 20fg per reaction

The graph above shows repeats of serial dilutions of genomic DNA from 20ng to 20fg per reaction.

Two operators have performed five repeats of the following standard curve. Results show a good reproducibility with a coefficient of variation (%CV) below 0.6% for concentrations range from 20ng to 20pg. Also, the %CV reaches 3% for concentrations between 0.2ng and 20fg. The main source of variation may be attributed to DNA low concentration stability at 4°C; further details are provided in the following section.

Quantity of Genomic DNA per reactions in fg	Average of 5 repeats	Standard deviation	%CV
20,000,000	17.59	0.08	0.45
2,000,000	21.06	0.10	0.48
200,000	24.69	0.09	0.36
20,000	27.91	0.15	0.53
2,000	32.62	1.02	3.12
200	34.97	0.55	1.59
20	38.44	0.96	2.50

Table 5.2: Repeatability Tests

Repeatability of the assay is good for genomic concentration between 20ng and 20pg. A shift in Ct values was observed with the time for lowest concentration of the standard curve (for further details see the sample and reagents stability section).

Repeatability of Extraction + QPCR

This is the repeatability of the whole analysis stage including the extraction of the DNA from the spores. It is defined as the standard deviation of repeated measurements of the same sample.

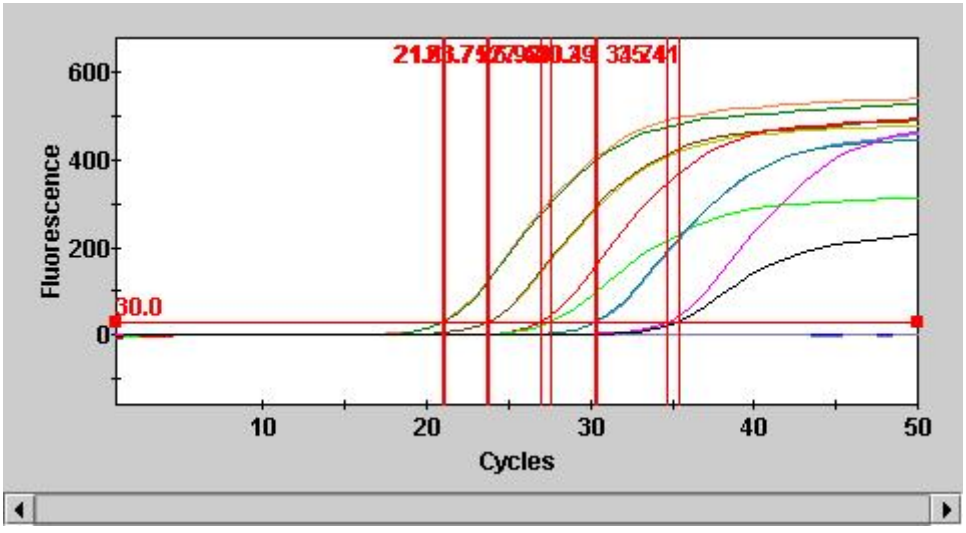


Figure 5.2: Repeatability of Extraction + QPCR

Spores count	Ct values (Average of 4 repeats)	Standard deviation	%CV
$1.7x10^6$	21.05	0.07	0.34
$1.7x10^5$	23.73	0.03	0.12
$1.7x10^4$	27.305	0.46	1.68
$1.7x10^3$	30.36	0.10	0.33
$1.7x10^2$	35.075	0.47	1.35

Table 5.3: Repeatability of RT-qPCR performed on spores extract

The table above shows repeatability of RT-qPCR performed on spores extract. Ct values show a %CV below 1.5% for ten times dilutions of spore stock solution.

Preliminary Reproducibility

Preliminary reproducibility of the PCR has been assessed for the Genomic reference DNA dilution. The same methodology was performed by two operators at different time using the same stock solution of DNA and same equipments for PCR preparation (e.g. pipettes, consumables.)

Variability of Ct values due to the operator is below 1.6% for a quantity of DNA between 20ng and 200fg. Variability of Ct values reaches 2.8% for the lowest DNA concentration.

Quantity of Genomic DNA per reactions in fg	Operator 1	Operator 2	%CV
20,000,000	17.59	17.60	0.01
2,000,000	21.10	20.92	0.59
200,000	24.87	24.34	1.54
20,000	28.04	27.63	1.05
2,000	32.48	32.90	0.91
200	34.89	35.14	0.49
20	38.94	37.46	2.74

Table 5.4: Variability between operators

Linearity of the RT-qPCR assay

Linearity of the assay is defined as the maximum residual from a linear fit of Ct values to DNA quantity. Linearity has been assessed with 7 dilutions of Genomic reference DNA for each experiment blanks that contain no DNA have been performed. Linearity of the RT-qPCR assay is good with overall R^2 value equals to 0.994.

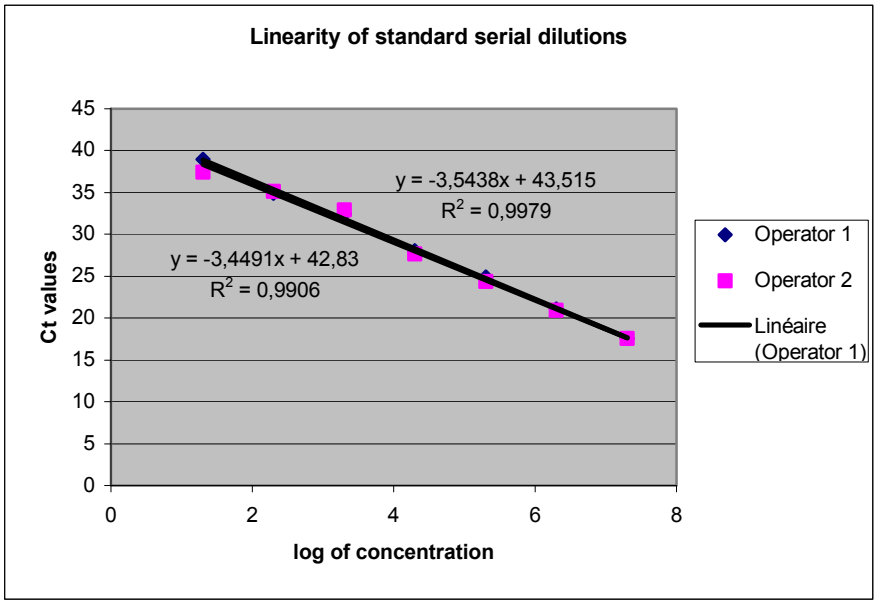


Figure 5.3: Linearity of standard serial dilutions

The graph shows a very good linearity of the standard DNA dilutions performed by two operators.

Linearity of Extraction + RT-qPCR

Linearity has been assessed using 5 serial dilutions of spores solutions for each experiment. Linearity of the RT-qPCR assay is good with overall R^2 value up to 0.99.

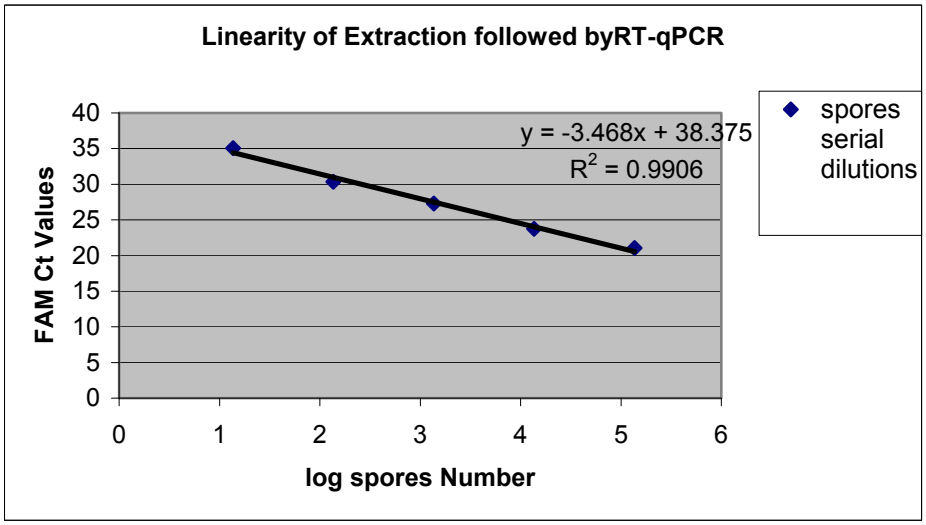


Figure 5.4: Linearity of Extraction followed by RT-qPCT

Limit of detection (sensitivity) of PCR stage

The limit of detection (LOD) is defined as the lowest concentration detected compared to background signal. Background signal is derived from unbound probe, free dye or similar. The Smartcycler software performs automatically background subtraction that occurs in two steps. The first step corrects for any drift (positive or negative slope) in the background. The second calculates the average signal and subtracts that signal from each data point so that the resultant growth curve represents amplified product only.
From our experimental data the LOD for DNA is equal to 20fg of genomic material per RT-qPCR reaction.

Extraction Efficiency

The extraction efficiency is a key parameter to obtain good repeatability of the assay. This parameter has been calculated assuming one spore genome copy is equal to 24.4fg; so by correlating the spores numbers to DNA quantities and Ct values from the standard DNA serial dilution plot, we estimate the extraction efficiency to be around 50%.

Limit of detection (sensitivity) of Extraction + PCR stage

From our experimental data the LOD for spore extraction is equal to 1.8 spores per RT-qPCR reaction.

Sensitivity to external parameters

Laboratory work did not reveal external parameters that could an impact on the RT-qPCR sensitivity.

Specificity

DNA probes and primers sequences were designed to be specific to *Aspergillus fumigatus*; no cross-reaction with sequences from other fungal species has been reported in the scientific literature. Fungal spores are rich in nucleases that potentially can degrade the target DNA sequence when the spores are broken open. This could artificially lower the concentration of DNA measured and thus the apparent number of spores per sample. Experiments on the extraction of DNA from *Aspergillus fumigatus* spores have shown that this is not a significant cause of error in this assay.

Cross contamination tests

All experimental steps have been designed to minimize and avoid cross-contamination across samples. Critical steps are performed in different locations to prevent contamination. Also, use of disposable consumables reduces the risk of carry-over contaminants.

For each experiment, we performed blanks that contain no DNA; the assay is validated when no detectable fluorescent signal is observed after 50 cycles of amplification.

Sample and reagents stability

During repeatability assessment of the method, all materials were stored at 4^{o}C for one week. During this period, we noticed some drift of C_t values that may be due to stability problems pertaining to:

1. Low concentration DNA dilutions. C_t values for Genomic reference DNA dilutions between 2 pg and 20 fg increased during time (corresponding to decrease in DNA). This stability issue could explain the high %CV for those concentrations.

2. Dilutions of probes at working concentration appear to be not stable after brief storage at 4^{o}C. We observed a decrease of fluorescence signal with time that is correlated to an increase in the background signal.

6. *Aspergillus fumigatus* tests carried out at the Health Protection Agency (HPA)

6.1 Introduction

This Section describes experiments carried out at the Health Protection Agency (HPA) to quantify the efficiencies of selected particulate sampling and analysis methods, compared with standard methods, for the measurement of airborne *A. fumigatus* spores.

6.2 Tests carried out with the Turnkey Topas instrument

A total of 51 samples were taken at a range of theoretical nebuliser spray concentrations between 6.5 x 10^4 and 2.6 x 10^7 cfu.ml^{-1}. For all samples, the number of colony forming units (cfu) per unit volume of air was determined by the Coriolis and MD8 samplers.

As described in the HPA report, the nebuliser contained 10-15 ml of solution, which was released into the chamber at 0.1-0.2 ml.min^{-1} using a flow rate of air of 4.5 l.min^{-1}. The Coriolis sampled into a liquid medium of 15-17 ml volume at a flow rate of 630 ml.min^{-1}, and the MD8 sampled onto a membrane, which was subsequently dissolved into 20 ml of sterile distilled water.

The Turnkey Topas instrument provided measurements of the mass concentrations of a range of particle size fractions. An example of the data obtained by the Turnkey Topas instrument is shown in Figure 6.1, where the measured concentrations of PM_1, $PM_{2.5}$, PM_{10} and total particulate matter (TPM) for samples 20-27 are plotted against time.

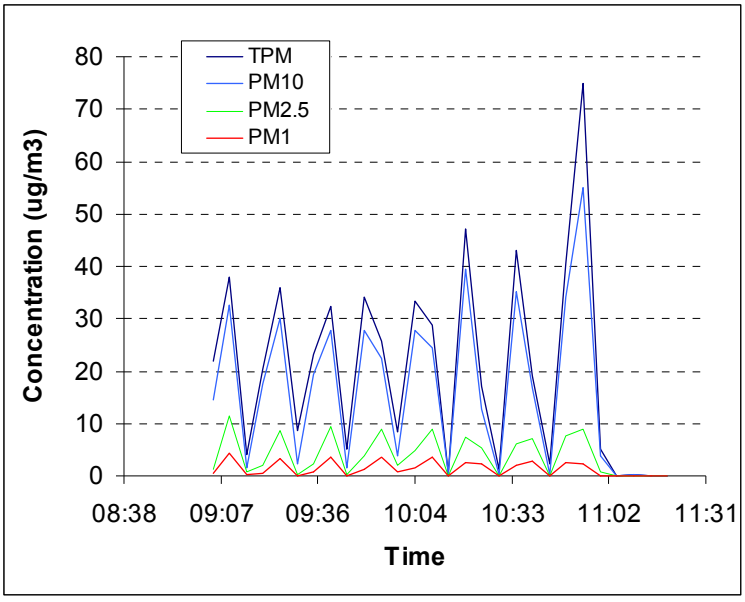


Figure 6.1: Plot of particle concentration (determined by the Topas) against time for samples 20-27.

This shows that an obvious 'spike' can be observed relating to each individual sample and the measured concentration for the first seven samples shows good repeatability. (The seemingly large concentration measured for the final sample is not a real effect and is caused by stopping the experiments – the 'real' concentration for the final sample of each day can be taken to be that recorded approximately 5 min after starting sampling.)

The particles detected are predominately in the PM_{10} size fraction and the relative proportion of PM_1 and $PM_{2.5}$ particles is similar for sets of samples taken at the same spray concentration, but varies to some extent between sets of samples. The TPM concentrations used here are therefore very similar to the PM_{10} concentrations – spores are expected to exist in the PM_{10} size fraction. The total PM concentration measured by the Topas is compared against the viable spore concentration measured by the Coriolis and MD8 samplers, and the theoretical nebuliser spray concentration in Figure 6.2:

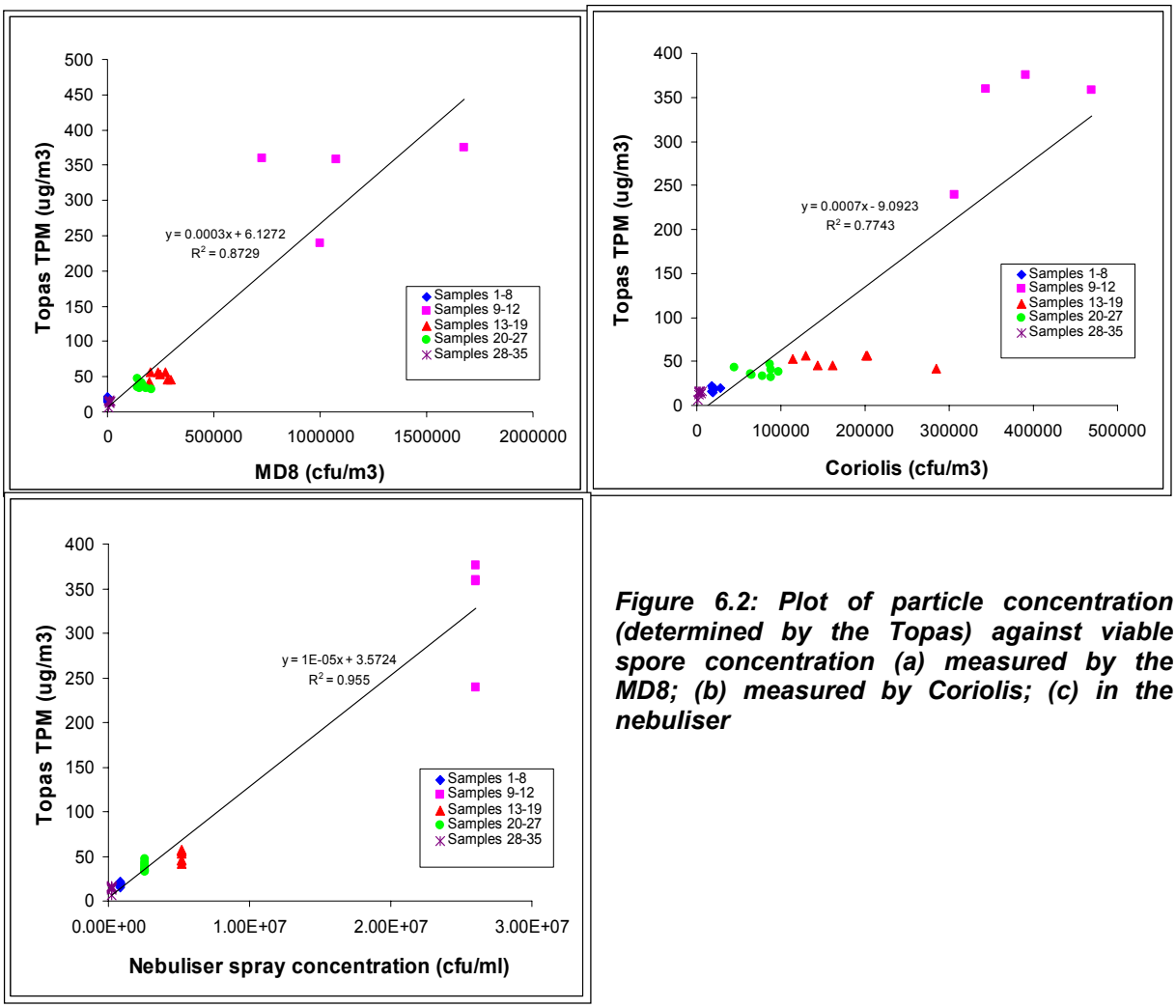


Figure 6.2: Plot of particle concentration (determined by the Topas) against viable spore concentration (a) measured by the MD8; (b) measured by Coriolis; (c) in the nebuliser

The relationships between the particulate concentration measured by the Topas and MD8, and that between the Topas and nebuliser concentration exhibit some linearity. The relationship between the Topas and Coriolis data shows much less of a linear trend.

HPA have calculated the 'sampling efficiency' of the Coriolis for each sample by comparing the measured spore concentration (in $cfu.m^{-3}$) with that from the MD8 sampler (the standard method). These data are shown in Figure 3. The average 'sampling efficiency' calculated from each set of repeat samples is very variable (between 36 % and 92 %) with a standard deviation which is also very large – between 21 % and 48 % relative.

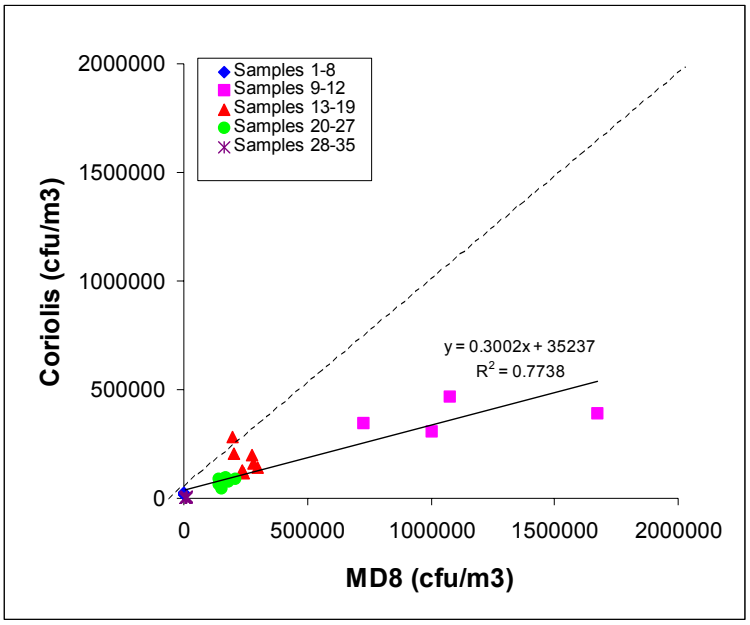


Figure 6.3: Comparison of spore concentrations measured by the MD8 and Coriolis samplers. The dashed diagonal line indicates 100% 'sampling efficiency' of the Coriolis with respect to the MD8 sampler (the standard method).

6.3 Correlation between qPCR assays, particle concentration (Topas) and cfu values (Coriolis and MD8 Samplers)

Samples collected at HPA with Coriolis samplers were sent to NPL the same day to perform qPCR assays. To allow comparison with the other techniques, the results have been plotted against spores per cubic metre; those values were obtained by extrapolating previous qPCR results in spores per assays to spores per cubic metre, using the known sample volume for the Coriolis sampler. Colony–forming units were obtained by growing aliquots of samples recovered from Coriolis and MD8 samplers on Saboraud dextrose agar.

Figure 6.4a

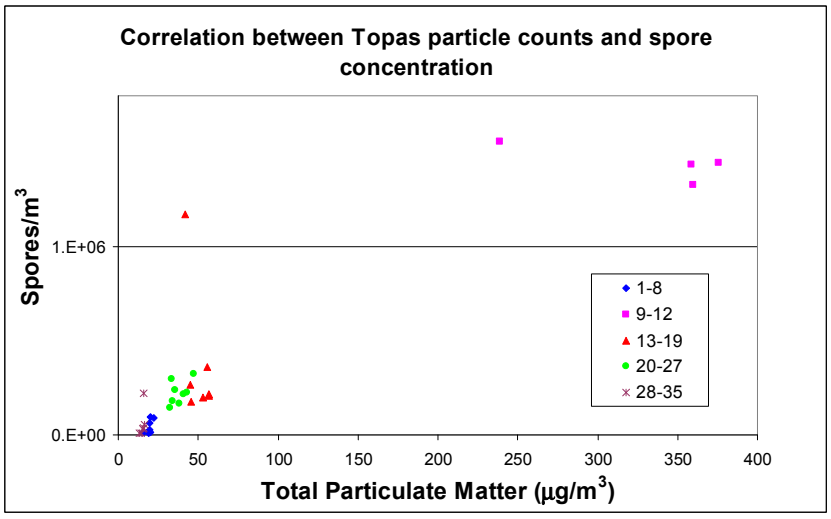


Figure 6.4b

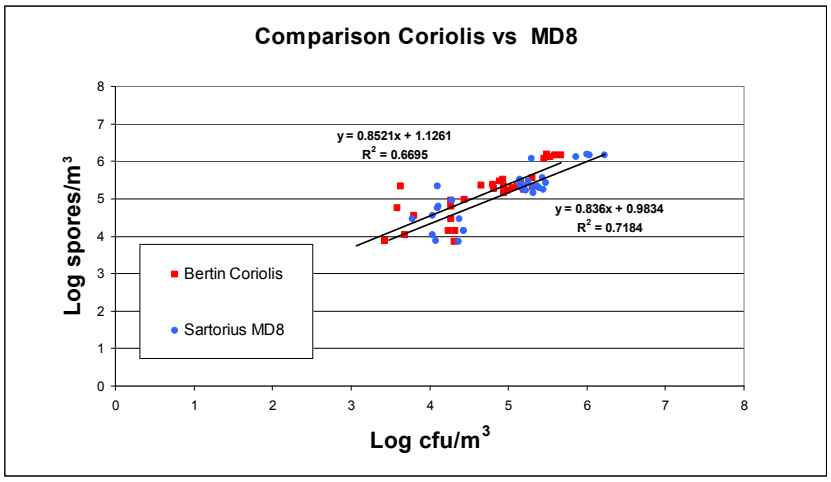


Figure 6.4: Correlation between Topas Particulate Matter and spore concentration per cubic metre (Figure 6.4a). Comparison of spore concentration and cfu values per cubic metre obtained with Coriolis and MD8 (Figure 6.4b).

Spores concentrations obtained by RT-PCR show a correlation with total particulate matter measured by Topas instrument. Two outliers can be observed for high nebuliser spray concentrations (Figure 6.4a).

Figure 6.4b shows a linear relationship between spore concentrations and the cfu values per cubic meter obtained from both samplers. Both sampling methods display large variability for lower nebuliser spray concentrations (2.6×10^5 to 8.67×10^5 cfu.ml^{-1}). The trendlines from the Coriolis and MD8 data are parallel (indicating that the sensitivity of the PCR technique is similar for both sets of samples), although the MD8 trendline lies to the right of the Coriolis trendline - this shift represents the different viability of spores after sampling. The settings of the Coriolis sampler (high air flow rate and the use of cyclone technology) have been seen to damage spores due to an increase of mechanical forces - microscopic observations of spore suspensions contain a large quantity of cellular debris. The MD8 sampler is less destructive and results in higher spore viability.

The qPCR results for Coriolis samples reflect the amount of mitochondrial DNA present in the collection tube that should be a mixture of viable and 'dead' spores.

6.4 Tests carried out with the WIBS2 instrument

A total of 24 samples were taken – four each at six theoretical nebuliser spray concentrations between 8.13×10^4 and 3.25×10^7 cfu.ml^{-1}. The number of colony forming units per unit volume of air sampled was determined by the Coriolis and MD8 samplers, which were operated using the same parameters as described above. The WIBS2 instrument used a sampling rate of 275 ml.min^{-1} and a total flow rate of 3100 ml.min^{-1}.

The raw data from the WIBS2 sampler has been processed to give the total number of particles measured in one minute time intervals. An example of the data is shown in Figure 6.5, which shows the number of particles measured for samples 117-120 (using a nebuliser spray concentration of 8.13×10^4 cfu.ml^{-1}) and for samples 121-124 (using a nebuliser spray concentration of 3.25×10^7 cfu.ml^{-1}).

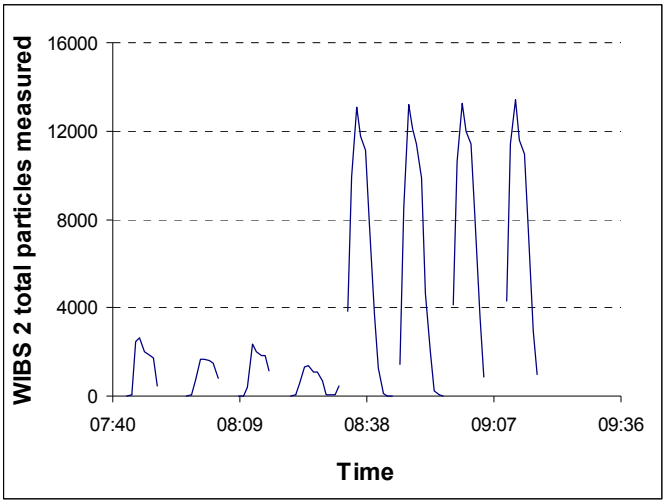


Figure 6.5: Plot of total particles measured (determined by the WIBS2) against time for samples 117-120 (nebuliser spray concentration = 8.13 x 10^4 cfu.ml^{-1}) and samples 121-124 (spray concentration = 3.25 x 10^7 cfu.ml^{-1}).

Plots comparing the total number of particles counted by the WIBS2 for each sample against the results the Bertin sampler, MD8 sampler, and the theoretical nebuliser spray concentration are shown in Figure 6.6:

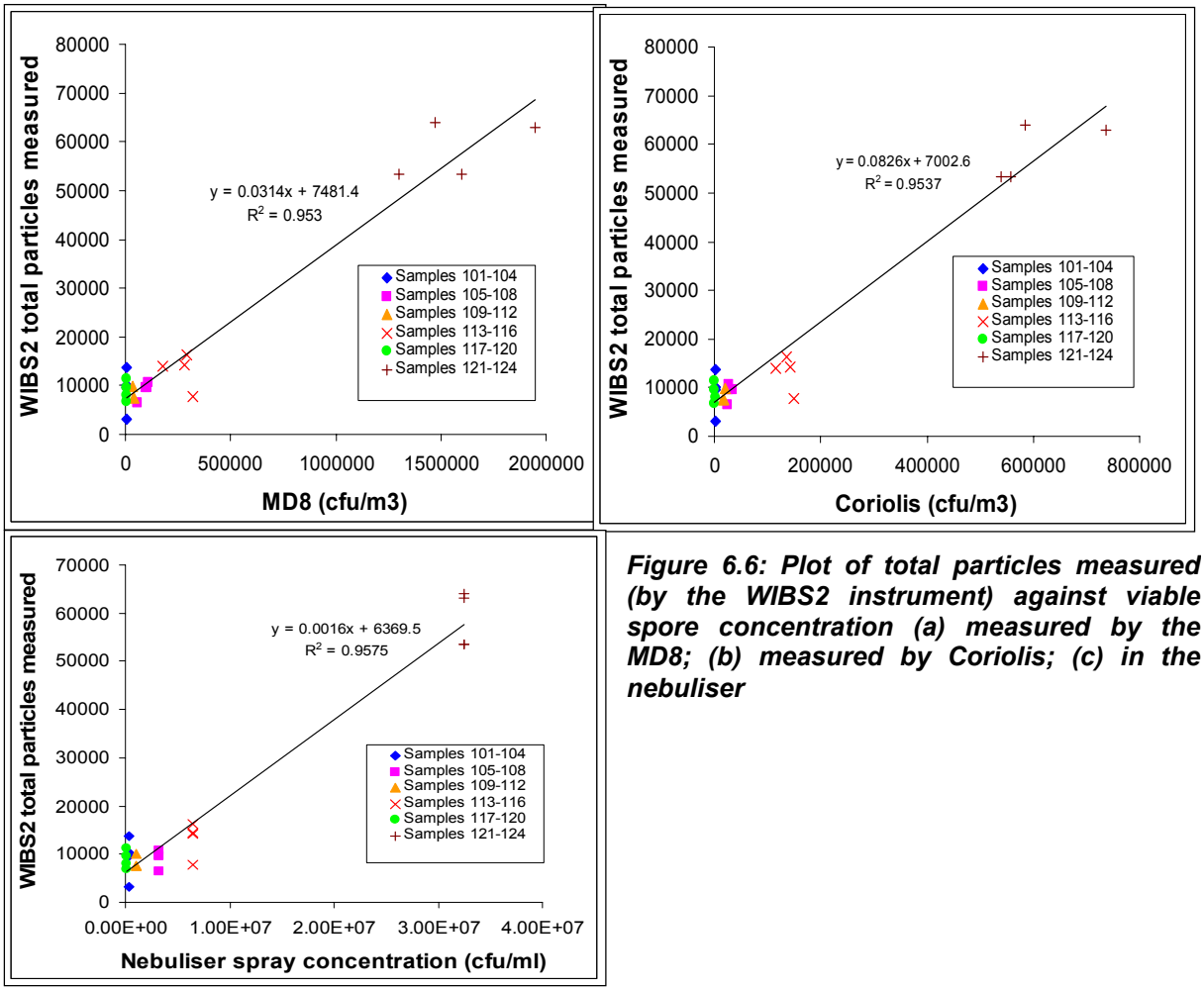


Figure 6.6: Plot of total particles measured (by the WIBS2 instrument) against viable spore concentration (a) measured by the MD8; (b) measured by Coriolis; (c) in the nebuliser

A linear relationship does exist between the number of particles measured by the WIBS2 and the concentration of colony forming units determined by the samples (or the theoretical nebuliser spray concentration). The plots are consistently more linear than the equivalent for the Topas instrument.

The data in Figure 6.6 can also be re-plotted with the particulate concentrations determined by the WIBS2 on the y-axis. To calculate these values, the total number of particles measured by the WIBS2 during a period of 7 min was divided by the approximate total volume of air sampled (1.925 l). These plots are shown in Figure 6.6, where it can be seen that the number of particles per cubic metre of air is typically between one and four orders of magnitude higher than the number of colony forming units per cubic metre of air. This suggests that the vast majority of particles sampled during this study were not viable spores.

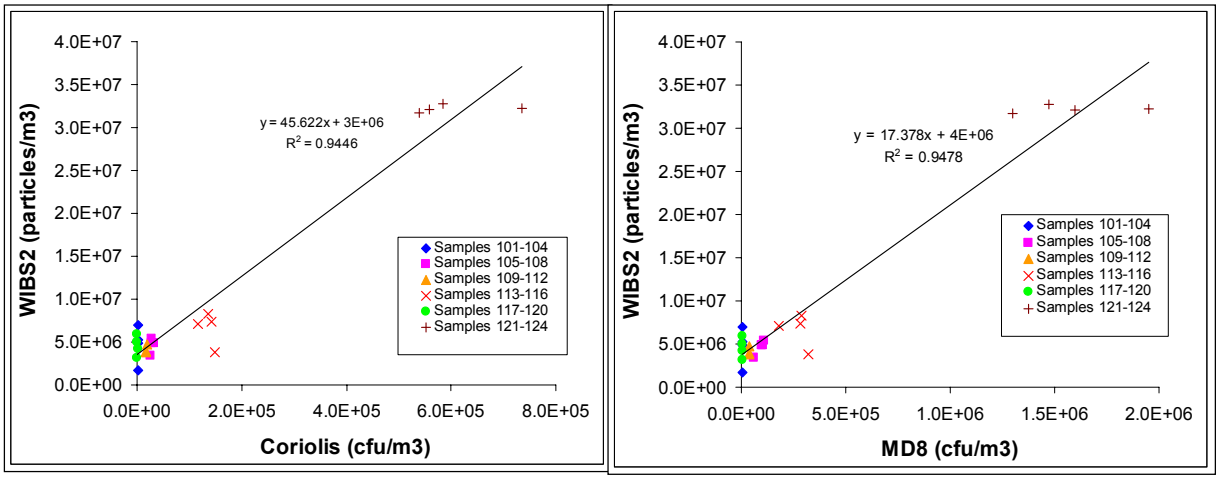


Figure 6.7: Plot of particles per cubic metre of air measured (by the WIBS2 instrument) against viable spore concentration (a) measured by the MD8; (b) measured by Coriolis

The between-sample variability in the measured Coriolis : MD8 'sampling efficiency' (25 % to 52 %) is less than for the experiments carried out with the Topas, and the relative standard deviations also show an improvement, although these are still quite large – between 7 % and 25 %. A comparison of the spore concentrations measured by the Coriolis and MD8 sampler is shown in Figure 6.8.

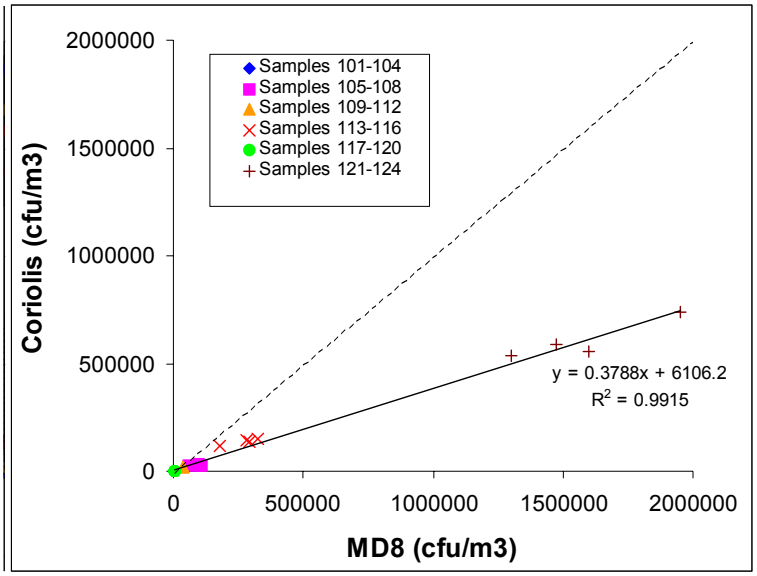


Figure 6.8: Comparison of spore concentrations measured by the MD8 and Coriolis samplers. The dashed diagonal line indicates 100% 'sampling efficiency' of the Coriolis with respect to the MD8 sampler (the standard method).

6.5 Correlation between qPCR assays and total particle with the WIBS2 and cfu values (Coriolis and MD8 Samplers)

Samples collected at HPA were sent to NPL the same day to perform qPCR assays.

Figure 6.9a:

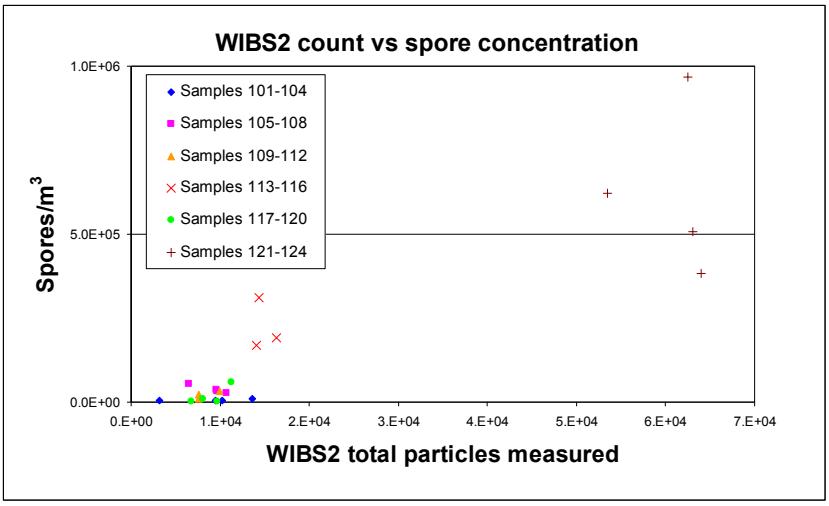


Figure 6.9b:

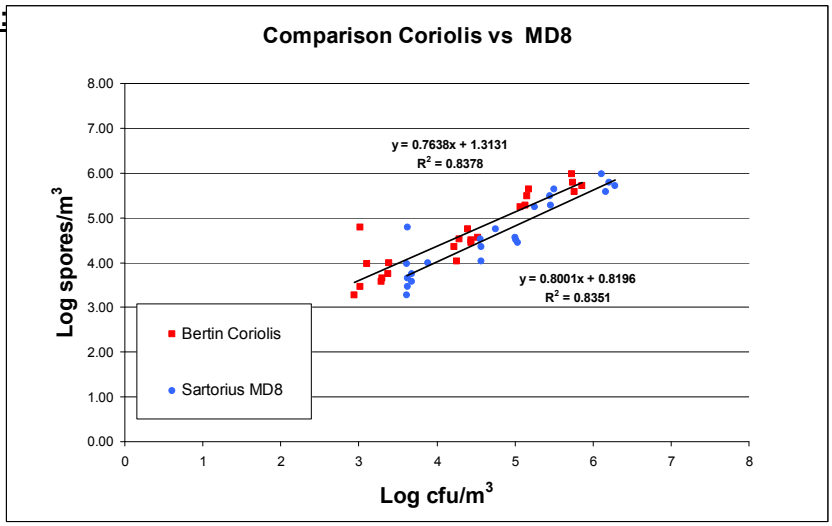


Figure 6.9: Spores concentrations in cubic metre, obtained by qPCR are plotted against Total particles measured by the WIBS2 instrument (Fig. 9a). Correlations between spores concentration per m^3 and $cfu.m^{-3}$ values obtained from MD8 and Coriolis samplers are plotted in Fig. 6.9b

For spray concentrations up to 1.06 x 10^6 $cfu.ml^{-1}$, the number of particles detected by the WIBS2 instrument was independent of nebuliser spray concentration (approximately 9 x 10^3 particles for all samples). The relative standard deviation for those concentrations varies from 21.9 % to 47.5 %. As a result, Figure 6.9a shows no obvious relationship between the total number of particles counted and spores concentrations. However, a linear correlation is observed for the Coriolis and MD8 samplers over the range of nebuliser spray concentrations studied (Figure 6.9).

The lower precision in the number of total particles counted by the WIBS2 instrument for the low concentration samples is likely to be a result of the small volume of air sampled. The sampling rate of the WIBS2 (275 ml.min^{-1}) was more than three orders of magnitude less than the Coriolis (630 l.min^{-1}) and more reproducible results would have therefore been achieved with an extended sampling time.

Also, as previously mentioned, the Coriolis sampler shows lower cfu values for the same theoretical spore concentration, this is the result of cell damage that occurs during sampling (Figure 6.9b)

6.6 Summary

- A range of nebulised spray concentrations of *A.fumigatus* spores were generated and sampled in a chamber at HPA with the following instruments and samplers:
    - Turnkey Topas (to determine the mass concentration of particulate matter)
    - WIBS2 (fluorescence detector to determine particle number)
    - Sartorius MD8 (standard method to sample spores onto a gelatine filter)
    - Bertin Coriolis (cyclone sampler to sample particulates into a liquid buffer solution)
    - qPCR (polymerase chain reaction analysis carried out at NPL on the Bertin liquid samples)
- Table 6.10 compares colony-forming unit (cfu) concentrations obtained with both samplers (MD8 and Coriolis) to the mass concentration of total particulate matter (TPM) measured by the Topas instrument and qPCR results expressed in spores per cubic metre.
- Under experimental conditions, the TPM measured by the Topas shows a linear relationship with values obtained with the reference method.
- The reference growing method shows a wide standard relative standard deviation from 14% to 47.5% over the range of nebuliser spray concentration. Relative standard deviation decreases, respectively below 25% using the Topas particle counter.
- Spores concentrations per cubic metre obtained by RT-PCR are higher than cfu values for the same sample. This is as expected and reflects the spore damage occurring during sampling with the Coriolis. PCR will identify and measure damaged and non-viable spores.

Sample ID	Average TPM Topas		MD8		Coriolis		Coriolis qPCR	
	□g/m3	RSD	cfu.m^{-3}	RSD	cfu.m^{-3}	RSD	Spores.m^{-3}	RSD*
1-8	**18.9**	12.2%	**2.20 x 10^4**	21.9%	**2.03 x 10^4**	15.8%	**4.43 x 10^4**	78.0%
9-12	**333.2**	19.0%	**1.12 x 10^6**	35.8%	**3.77 x 10^5**	18.6%	**1.44 x 10^6**	6.5%
13-19	**50.7**	12.3%	**2.49 x 10^5**	15.9%	**1.77 x 10^5**	33.1%	**3.71 x 10^5**	89.2%
20-27	**38.1**	13.7%	**1.62 x 10^5**	14.0%	**7.66 x 10^4**	22.5%	**2.24 x 10^5**	27.8%
28-35	**13.7**	24.7%	**9.50 x 10^3**	37.3%	**3.50 x 10^3**	47.5%	**5.99x 10^4**	134.7%

Table 6.10: Summary of results obtained for samples 1 to 35 with three instruments: Topas, MD8, Coriolis and qPCR. The Relative Standard Deviation (RSD) represents the ratio of the standard deviation and the mean. (RSD* values obtained by qPCR are high due to the conversion of the PCR results to spore counts, that involve conversion of logarithmic values, the RSD values for the qPCR assays are below 6% over the range of concentrations. Further analysis need to be performed to optimise results extrapolations and to assess the sampling effects)

The WIBS2 instrument was evaluated over a range of theoretical nebuliser spray concentrations between 8.13 x 10^4 and 3.25 x 10^7 cfu.ml^{-1}. For spray concentrations up to 1.06 x 10^6 cfu.ml^{-1}, the number of particles detected by the WIBS2 instrument was independent of nebuliser spray concentration (approximately 9 x 10^3 particles for all samples). This may be a 'residual' or 'background' value.

Sample ID	WIBS2		MD8		Coriolis		Coriolis qPCR	
	Total particles	RSD	cfu.m^{-3}	RSD	cfu.m^{-3}	RSD	Spores.m^{-3}	RSD*
101-104	**9.14 x 10^3**	47.5%	**5.38 x 10^3**	29.8%	**2.18 x 10^3**	10.9%	**5.88 x 10^3**	46.0%
105-108	**9.12 x 10^3**	20.2%	**9.19 x 10^4**	25.2%	**2.84 x 10^4**	12.4%	**3.78 x 10^4**	31.3%
109-112	**8.39 x 10^3**	16.1%	**3.36 x 10^4**	20.0%	**1.64 x 10^4**	21.2%	**2.18 x 10^4**	49.9%
113-116	**1.31 x 10^4**	28.4%	**2.68 x 10^5**	23.4%	**1.36 x 10^5**	10.6%	**2.76 x 10^5**	43.3%
117-120	**8.95 x 10^3**	21.9%	**4.19 x 10^3**	1.1%	**1.06 x 10^3**	15.1%	**1.87 x 10^4**	150%
121-124	**6.08 x 10^4**	8.1%	**1.58 x 10^6**	17.4%	**6.04 x 10^5**	14.8%	**6.20 x 10^5**	40.5%

Table 6.11: Summary of results obtained for samples 101 to 124 with four instruments: WIBS2, MD8, Coriolis and qPCR. (RSD* values obtained by qPCR are high due to the conversion of the PCR results to spore counts, that involve conversion of logarithmic values, the RSD values for the qPCR assays are below 6% over the range of concentrations. Further analysis needs to be performed to optimise results extrapolations and to assess the sampling effects)

The test programme has shown that the PM_{10} fraction measured by the Topas instrument will provide a non-discriminating indication of the presence of spores, and could therefore be used to trigger a Coriolis sampler to capture this event. The Topas measurement would not (and is not intended to) discriminate spores from any other PM_{10} event. In a field implementation of the network, additional information on the wind direction and speed would also be used to help discriminate events which require sampling from those which need not trigger the sampling system.

The test programme has demonstrated the ability of the qPCR assay to quantify spore counts. The assay provides a measure of viable and non-viable spores and spore fragments and therefore gives a systematically higher reading than the approaches that require the sample to be cultured. The WIBS2 results showed that within the test chamber there were significantly more non-viable spores than viable spores, and the qPCR is therefore expected to give a higher reading than the cfu measurements.

7. A prototype network integration system for bio-aerosols monitoring

7.1 Introduction

The preferred network design consists of two stages of monitoring systems:

- Real-time continuous monitors measuring particulate concentration, which are used to trigger the periodic samplers when there is a particulate event. (Tier 1)

- Short-term periodic sampling systems, collecting particulate and aerosol matter for subsequent laboratory analysis for *Aspergillus fumigatus* spore content using qPCR (Tier 3).

In addition, meteorological information, wind speed and direction, is used to provide additional screening of the trigger events, to ensure only samplers which are downwind of the composting facility are triggered.

A complete sampling network will consist of a number, (nominally 12), of the Tier 1 and Tier 3 instruments arranged around the site to allow automated sampling under different wind conditions. In order to test the network integration, a sub-set of a complete network has been configured and tested. This subset consists of a paired Tier 1 aerosol monitor (Turnkey Topas) and Tier 3 aerosol sampling system (Bertin Coriolis), which were connected to a Skye Instruments MiniMet for wind speed and direction via the NPL developed trigger system described below.

The Bertin Coriolis sampler is a single shot system, requiring the sample to be manually removed and reset in preparation for the next sample. It was therefore decided that the trigger system should inform the operator of a trigger event by sending a text message to a mobile phone. This allows an operator to be offsite during the network operation.

Two additional Turnkey Topas instruments were deployed to provide additional information on upwind particulate sources. Further samples were taken using the Bertin Coriolis at locations within the site, at the boundary fence and at 200m from the site, to provide a fuller data set of emissions of particulate and *A. fumigatus* at the site. These data will be presented in a subsequent report, and will be used to assess the correlation between particulate events and the aerosol/particulate level used to trigger the sampling.

7.2 Field tests of Network

Measurements of airborne particles were made at the Veolia composting site at Downend Road, Fareham, over a four week period in October 2008. The network integration system was tested between 15th and 24th October. The Turnkey Topas, the Bertin Coriolis and the Skye Instruments MiniMet instruments were linked by an in-house NPL designed trigger control unit. The requirement of this system is such that measurements may be recorded automatically if an episode of interest occurs during times when no operating staff are present.

Three instruments are used in this configuration strategically placed with respect to the working composting area. A Turnkey Topas particulate sensor is used to detect an excess on a specific set level of concentration of aerosol particles falling within a particular size range or total particulate. This information is combined with selected parameters of wind speed and direction from the Skye Instruments MiniMet to generate a trigger signal that activates a measurement cycle of a Bertin Coriolis aerosol sampler. Each sample is then returned to NPL for laboratory analysis for the species of interest.

Figure 7.1 shows an overview of the system layout.

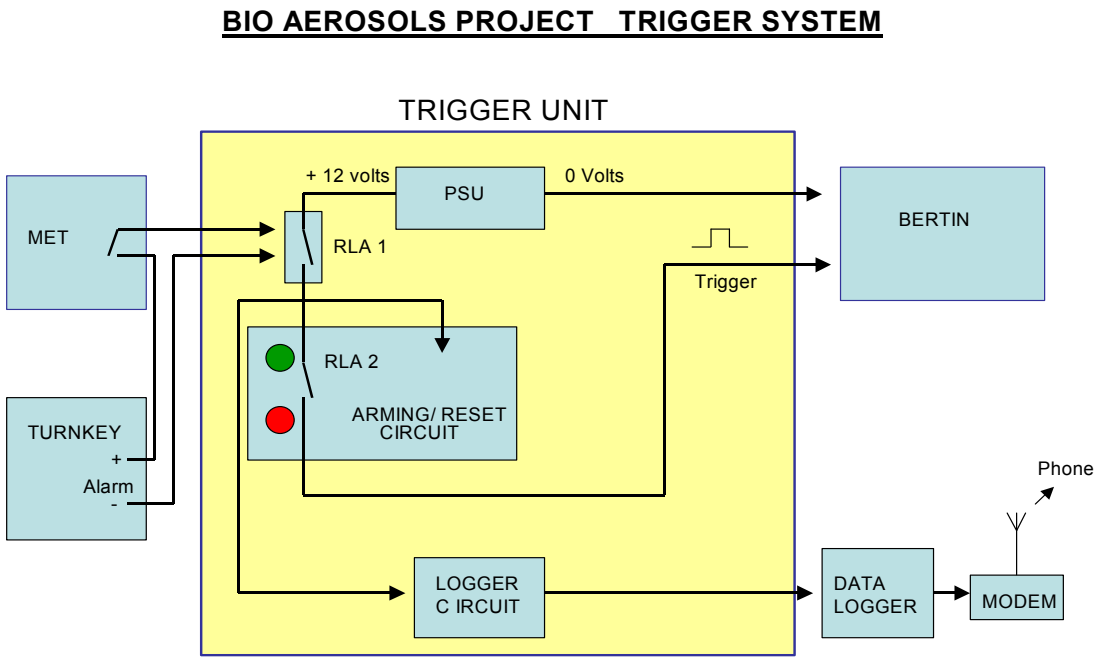


Figure 7.1: Overview of trigger system layout

The Trigger Unit is set "ARMED" ready for use by closing the internal relay (RLA2). This state is indicated by displaying a green lamp. When the conditions of both the required wind speed and direction are met, and the Turnkey has detected an appropriate concentration of aerosol, a signal is then sent to activate the control system input relay (RLY1). This completes the circuit and generates a 12-volt trigger to start the Bertin measurement sample. At this point in time a monostable circuit produces a delayed pulse that resets RLA2 and removes the Bertin trigger after a time period of two seconds. This state is displayed by replacing the green lamp with an illuminated red lamp. No more Bertin triggers can take place until an operator replaces the sample flask in the Bertin and re ARMS the trigger unit using a manual green push button. This act will remove the red display and once again illuminates the green lamp showing that the system is ready for the next measurement.

Each time that conditions are valid for a Bertin measurement, a "DATA VALID" signal is generated within the trigger unit that is compatible with the analogue input range of a Datataker logger. This signal is recorded by the logger and is in turn fed to a modem that sends a telephone text message to the operating staff. This draws attention to the fact that a site visit is required and the Bertin sample requires removal.

The control box, built by NPL, incorporating this circuitry is shown below in Figure 7.2. The detailed circuit diagram is shown in Appendix 7. The monitoring locations and equipment are shown in Figures 7.3, 7.4 and 7.5. The trio of instruments comprising the Turnkey Topas, the Bertin Coriolis and the Skye Instruments MiniMet were placed in the North East corner of the site. The Minimet was placed on top of a container to measure wind direction typical of the whole site.

Figure 7.2: NPL trigger system

Figure 7.3: Monitoring Position

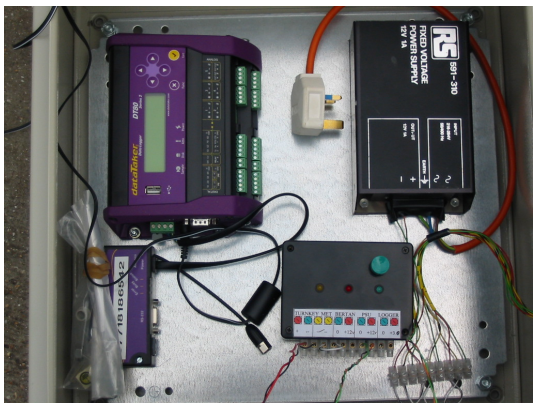


Figure 7.4: Close- ups of instrumentation

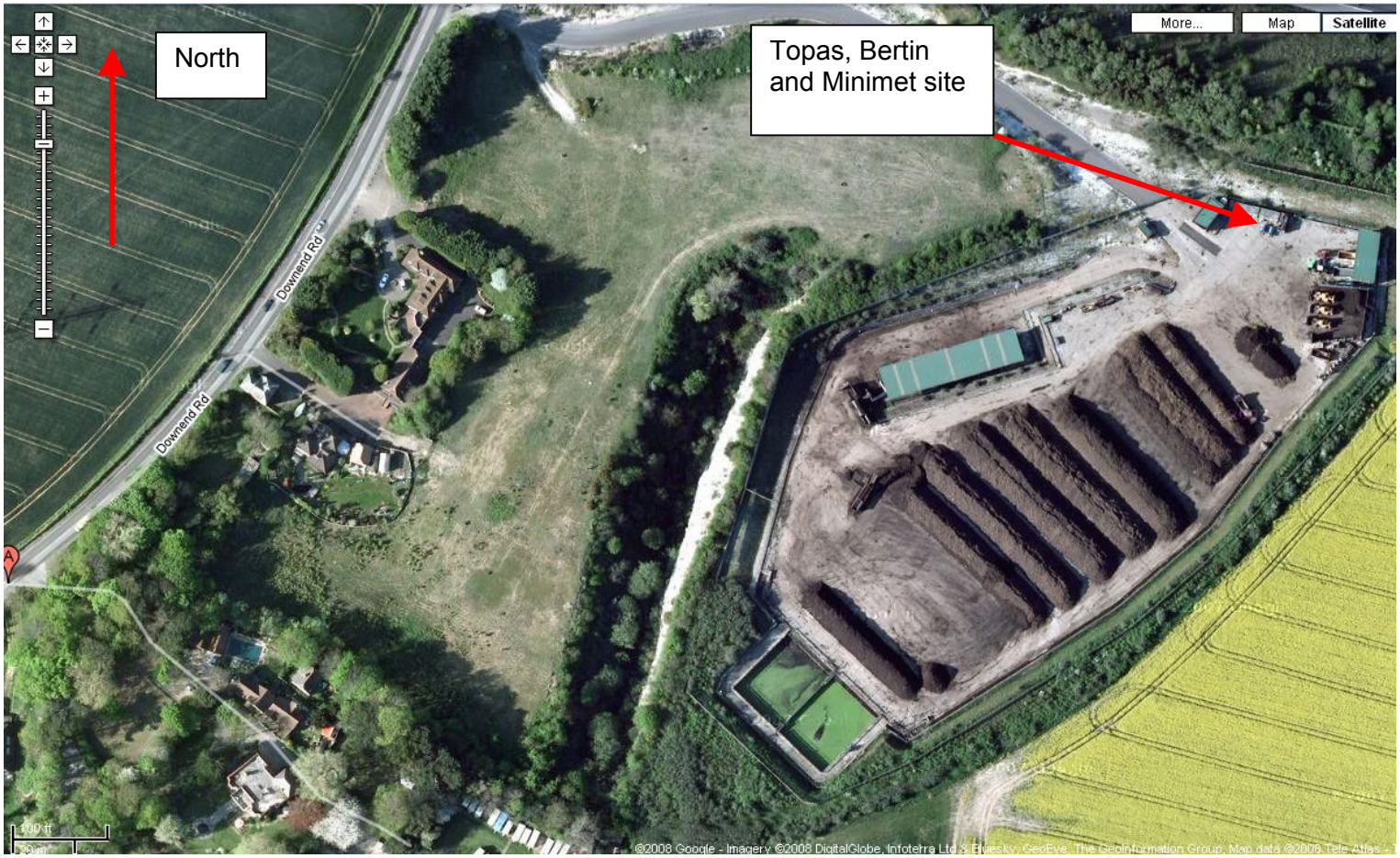


Figure 7.5: Location of monitoring point on Downend Road site

The NPL trigger system was set to activate when the wind direction was between 180 and 240 degrees (from the South to South West) i.e. when the wind was blowing across the site to the monitoring position and when the total particulate level reached a preset value. The effect of changing the preset particulate trigger level was investigated. Three levels were used, 100 µg m-3, 50 µg m^{-3}, and 30 µg m^{-3}. The trigger points and activities being carried at the site, during the period, are summarized below. The Bertin data associated with these events will be presented in a further report.

Trigger date	**Trigger time**	**Trigger value (µg m^{-3})**	**Topas reading (µg m^{-3})**	**Site Activity**
18/11/2008	13.56	100	124	Shredding & screening
22/11/2008	09.24	100	1183	Shredding & screening
24/11/2008	04.26	30	105	

7.3 Acknowledgements

NPL would like to thank Veolia for their help and co-operation with this study and Turnkey for the loan of the Topas monitors.

8. Field Trial at Downend Road Composting Site

8.1 Introduction

Field measurements of airborne particles were made at the Veolia composting site at Downend Road, Fareham, during a visit in July and over a four week period in October 2008, using three Turnkey Topas, the Bertin Coriolis sampler and further analysis of collected samples by qPCR at NPL. Sixty samples were collected and analysed for the presence and quantity of *Aspergillus fumigatus* spores.

The aims of the field trial were as follows:

- Test the new qPCR method on real airborne samples collected on site and downwind of a composting facility
- Challenge a small part of a prototype network for bioaerosol monitoring (described in Section 7).
- Gain a representative data set to compare the new PCR-based method with traditional monitoring
- Correlate site activities with general particulate and *Aspergillus fumigatus* generation on site and in the immediate location of the composting facility
- Assess the levels of *Aspergillus fumigatus* at 250m from the boundary fence of the composting facility.

8.2 The Downend Road Site

The Downend Road site, operated by Veolia, processes municipal green waste in open windrows. It is situated in open farmland, apart from a waste transfer station at a distance of 150 m and a motorway at 100m. There is some habitation within 250m of the site. The site has an odour suppression system around the perimeter fence. The site has PAS100 certification and compost is turned about once a month. The turning process lasts about a week, depending on wind direction. An enzyme can be added to accelerate the composting process and reduce the requirement for turning.

NPL installed power to two fixed sites where Turnkey Topas instruments were operating throughout the trial. A weather station was also operated continuously to provide wind direction and speed. The third Turnkey Topas and the Bertin sampler were moved to different sampling locations on and off site. The locations of the fixed sites are shown on the picture of the site below.

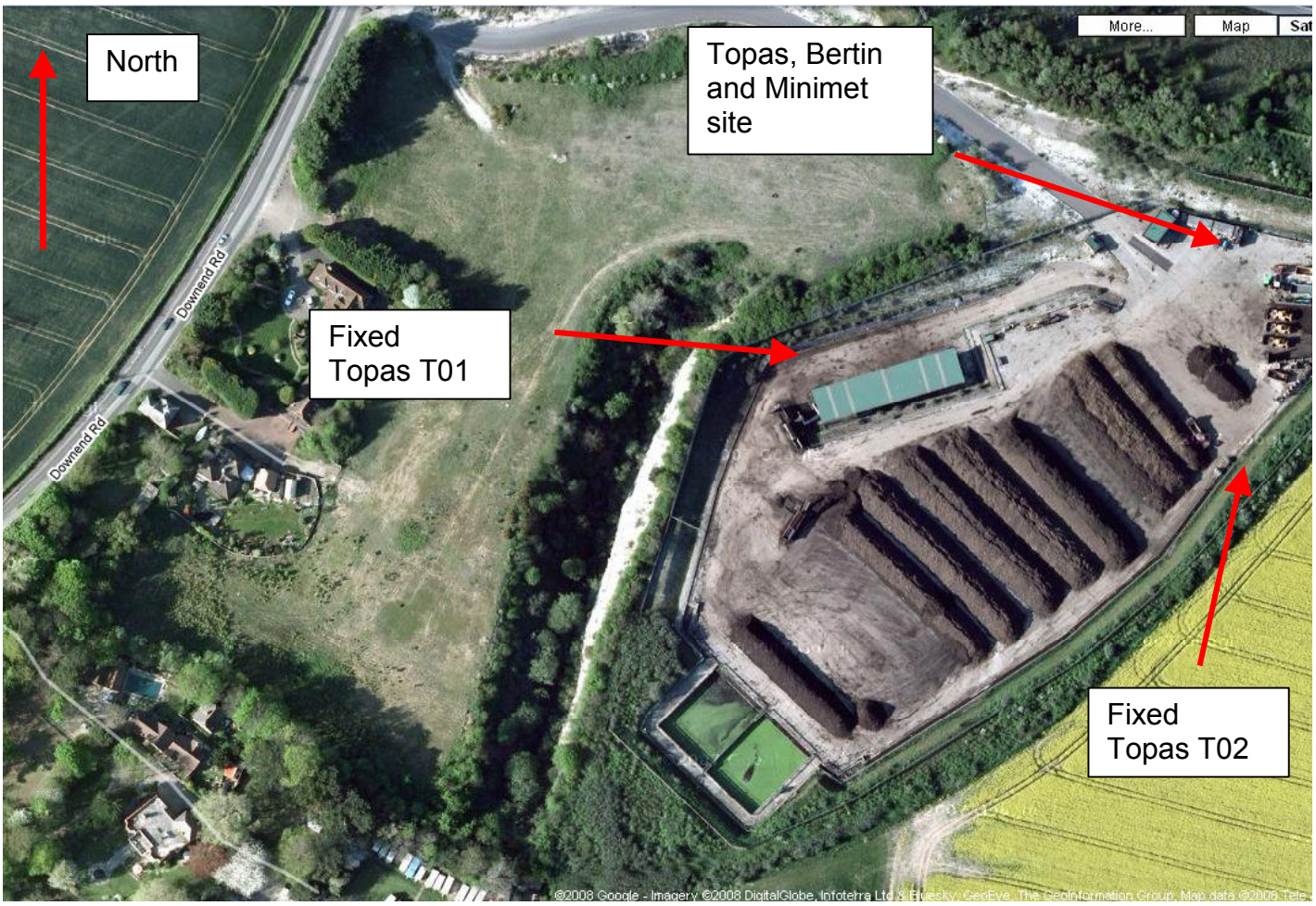


Figure 8.1: Fixed sampling locations at the Downend Road Site

The following activities were occurring at the site during the second field trial:

Day	Date	Green **In** Tonne	Progrow **Out** Tonne	Operations
Friday	26-Sep-08	128.76	84.26	Shredding and screening
Saturday	27-Sep-08	60.48	1.48	Shredding until 14:30
Sunday	28-Sep-08	76.36	0?	
Monday	29-Sep-08	122.08	8.56	Turning 8:00-15:45, Shredding
Tuesday	30-Sep-08	128.3	36.2	Turning 8:00-15:45, Shredding
Wednesday	1-Oct-08	88.68	27.58	Turning 8:00-15:45, Shredding
Thursday	2-Oct-08	74.76	34.3	Turning 8:00-15:45, Shredding
Friday	3-Oct-08	88.72	5.36	Turning 8:00-15:45, Shredding
Saturday	4-Oct-08	47.78	1.72	Shredding
Sunday	5-Oct-08	33.3	0	No Activity
Monday	6-Oct-08	78.96	28.26	Turning 8:00-15:45, Shredding
Tuesday	7-Oct-08	78.78	36.54	Turning 7:45-15:50, Shredding
Wednesday	8-Oct-08	67.68	56.12	Turning 8:00-12:00, Shredding
Thursday	9-Oct-08	81.66	28.02	Turning 8:00-15:45, Shredding
Friday	10-Oct-08	99.92	142.68	Turning 8:00-15:00, Shredding
Saturday	11-Oct-08	59.96	1.74	Shredding
Sunday	12-Oct-08	47.6	1.14	No Activity
Monday	13-Oct-08	98.98	3.92	Shredding and screening
Tuesday	14-Oct-08	123.66	29.62	No Operation
Wednesday	15-Oct-08	112.58	57.26	No Operation
Thursday	16-Oct-08	98.6	5.4	Shredding from 10:45
Friday	17-Oct-08	126.56	32.36	
Saturday	18-Oct-08	15.46	2.34	No Operation
Sunday	19-Oct-08	55.72	1.72	No Operation
Monday	20-Oct-08	116.48	28.5	Shredding and screening
Tuesday	21-Oct-08	117.9	9.14	Shredding and screening
Wednesday	22-Oct-08	75.36	33.44	Shredding and screening
Thursday	23-Oct-08	116.82	126.72	Shredding and screening
Friday	24-Oct-08	108.14	86.02	Shredding and screening

8.3. Real-Time qPCR Analysis of Field Samples

Sampling

20 ml samples were collected using a Bertin sampler, running at a flow rate of 630 l/min. The samples were stored frozen until needed, and then aliquots of the samples were processed according to the protocol previously described.

Interpretation of Real Time qPCR results

Real-time qPCR works by monitoring the fluorescence from an amplicon[1]-specific probe at every cycle in the amplification process. Because qPCR amplification is exponential, with the number of amplicon molecules doubling at each cycle of the reaction, it is possible to infer the original number of molecules present from the cycle at which the fluorescence crosses a threshold value. This number, the threshold cycle or C_t value, is the most useful output from the amplification process. The threshold value is chosen so that it falls in the exponential part of the amplification process.

Figure 8.2 shows typical fluorescence traces from a batch of amplification reactions, taken from the analysis of the samples of the second field trial. Here the fluorescence is that from the dye FAM, and is plotted in arbitrary fluorescence units. The threshold value used was 30 “intensity units”. Of the 16 reactions, 7 showed amplification in this instance, giving meaningful C_t values. The remaining reactions gave no amplification, and the C_t values were recorded arbitrarily as 0. S20 was a field blank and was negative for both dilutions, as were the two qPCR blanks.

[1] An amplicon is the DNA molecule that is produced by an amplification process, such as PCR.

S1 was the only sample that resulted in amplification at the 1× dilution; note the increase in C_t from the 1× to the 10× curve from 31.53 to 34.88 (a shift of 3.35 cycles, very close to the expected shift for a tenfold dilution of $\log_2(10)$ cycles or 3.32). See the results of the second field trial below for an explanation of the rationale behind the dilutions.

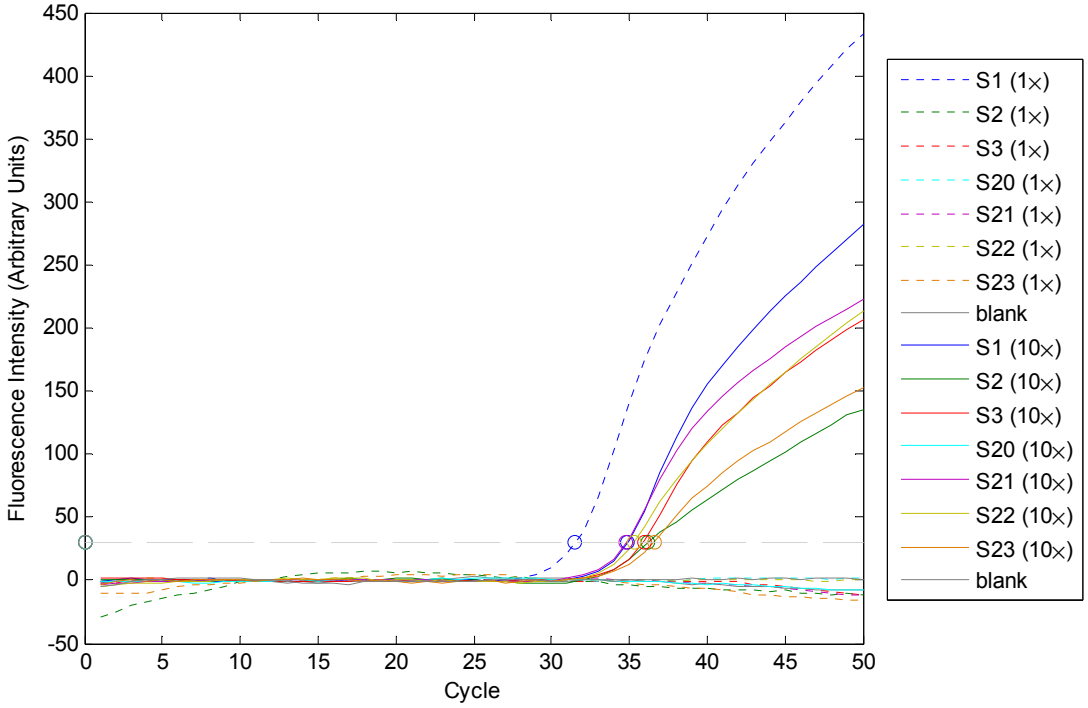


Figure 8.2: Example fluorescence traces from real-time qPCR reactions. The circles indicate the C_t values for each trace. Dashed traces are for 1× samples, solid lines for 10×. The horizontal dashed line indicates the threshold value of 30. Note that trajectories fall into two classes; those showing amplification and those that remain at background levels.

Calibration Curve

To enable quantification of spores it is necessary to convert the output of the real-time qPCR process (a C_t value) into the number of spores in 1 m^3 of air. For this we require two pieces of information:

1. The volume of air corresponding to 1 ml of the sample buffer. This is given by the flow rate and duration of the sampling process, and the volume of the buffer.
2. A calibration curve for the RT-PCR reaction relating the observed C_t values to the number of spores per ml in the sample.

To this end, we generated a calibration curve using laboratory-grown spores of *Aspergillus fumigatus*. The number of spores in a suspension was counted using a haemocytometer and a dilution series of this suspension was made. Each stage of this dilution series was then analysed by using the preparation method and RT-PCR protocol developed in our previous work. The range of dilutions covered was from 6.0×10^{2} ml^{-1} to 6.0×10^{6} ml^{-1}. The results obtained are presented in Table 8.1 and plotted in Figure 8.3 and Figure 8.4.

Because the qPCR amplification process is initially exponential, it is not surprising that it shows a logarithmic response to the spore concentration. In Figure 8.2, the C_t values are plotted against the base 2 logarithm of the spore concentration in ml^{-1}. The data are well fit by a straight line, with an R^2 of 0.99. This linear calibration curve spans four orders of magnitude.

Intuitively, since the amount of the amplicon present doubles with each cycle, the number of cycles taken to reach the threshold value would be expected to decrease by approximately 1 for a two-fold increase in spore count. Indeed, in Figure 8.3, it can be seen that the slope of the calibration curve is very close to –1. The *y* intercept value is 44.075 cycles. Alternatively, base 10 logarithms may be used (Figure 8.4), in which case the slope is –3.468 whilst the other parameters remain the same; this is more intuitive.

Note that the use of a 0 value for the C_t in the absence of amplification is potentially confusing as this corresponds to a spore count of 2^{44} or 1.8×10^{13} ml^{-1}!

This calibration curve relates to spores as counted using a haemocytometer slide. It should be noted that spores are distinct from “colony forming units” (cfu) as not all spores may be viable. This is particularly the case if there is some damage to the spores as a result of the sampling process.

Sample ID	Spore Count (ml^{-1})	Log_2 spore count	FAM Ct value
1	Blank	Blank	0
			0
2	6.0×10^{6}	4.493	21.00
			21.10
3	6.0×10^{5}	4.263	23.75
			23.71
4	6.0×10^{4}	3.988	26.98
			27.63
5	6.0×10^{3}	3.650	30.43
			30.29
6	6.0×10^{2}	3.206	34.74
			35.41

Table 8.1: Real Time qPCR Data used to construct calibration curve.

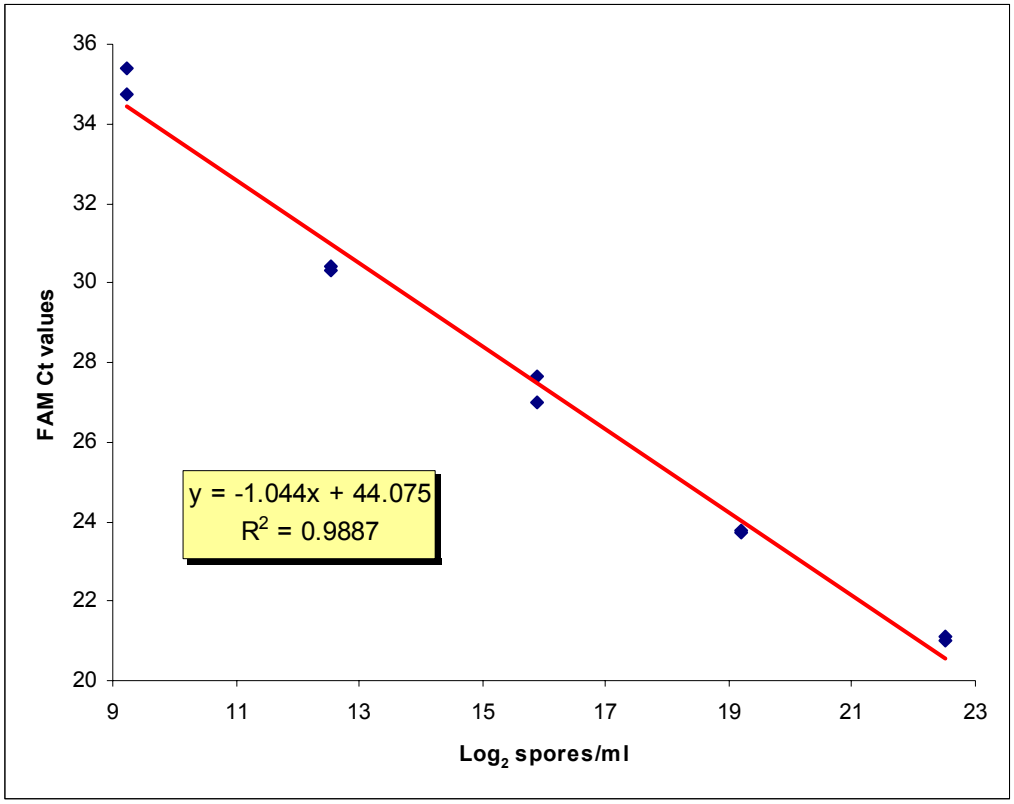


Figure 8.3: Calibration Curve for Real Time qPCR using dilution series of spores (base 2 log).

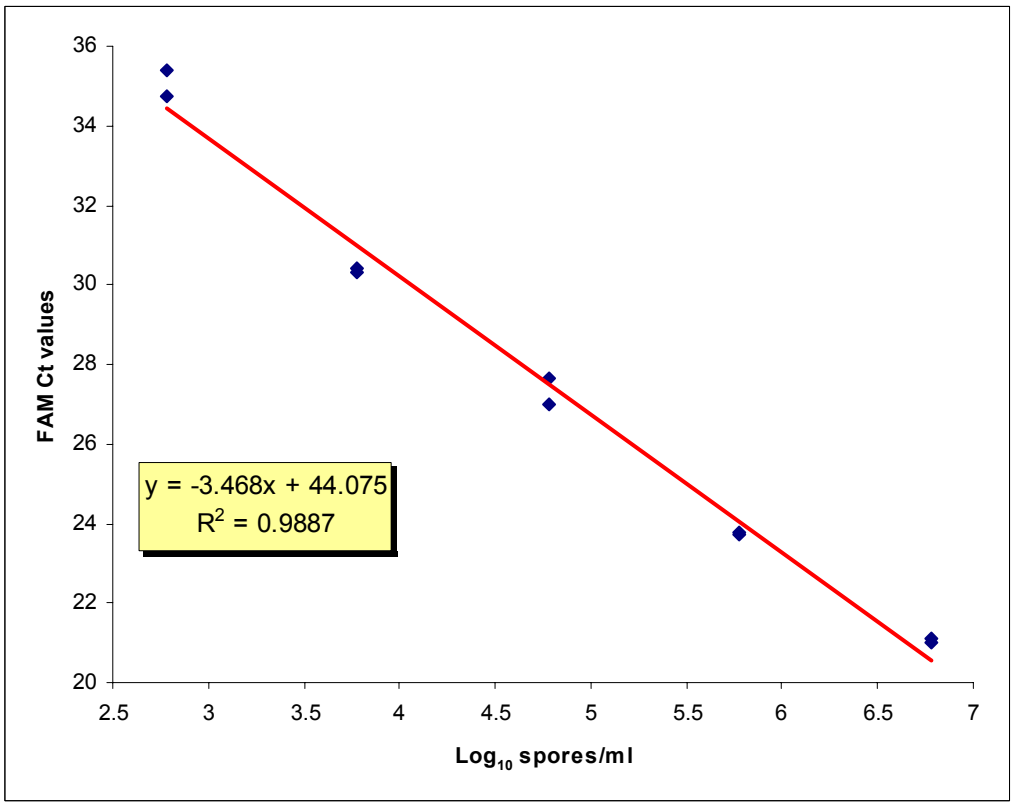


Figure 8.4: Calibration Curve for Real Time qPCR using dilution series of spores (base 10 log).

8.4 Preliminary field test – July 2008

A preliminary visit was carried out at Downend Road whilst turning was being carried out, to assess the levels of *Aspergillus fumigatus*, the optimum sampling time and to assist in planning of the full field trial in October. All samples were taken a few metres from the office

at the entrance to the site, as this was the only location with an accessible power supply. NPL laid power lines to the sampling locations for the second month long field trial.

Results

The results of the preliminary field test, conducted at the Fareham site on the 23rd July 2008, are presented in the table below. Duplicate qPCR reactions were performed for each sample. The volume of air samples is based on a flow rate of 630 l/min (0.63 m^3/min). The air samples were captured in 20 ml of buffer solution. Spore counts are calculated using the calibration curve; all the samples fell within the range of the curve.

Sampling duration (min)	Sampling volume (m^3)	Sampling time	FAM C_t		Spores/ml		Spores/m^3	
			1	2	1	2	1	2
5	3.15	11:21	31.7	31.9	3700	3240	23494	20572
5	3.15	11:41	30.4	30.2	9008	9755	57193	61936
10	6.3	11:55	29.4	29.3	17614	18330	55917	58190
10	6.3	12:14	31.3	32.2	4699	2708	14919	8598
20	12.6	12:33	28.6	28.8	28981	26234	46002	41641
20	12.6	13:03	24.8	24.4	368555	461893	585007	733163
10	6.3	13:32	27.9	27.8	46128	50959	146438	161773
10	6.3	14:22	25.5	26.0	223995	162866	711096	517035

Table 8.2: Real-time qPCR results from 23/07/08 field test. The C_t values from the real-time qPCR were used to calculate the spore number concentrations in the solutions and thereby the number concentrations in the air samples.

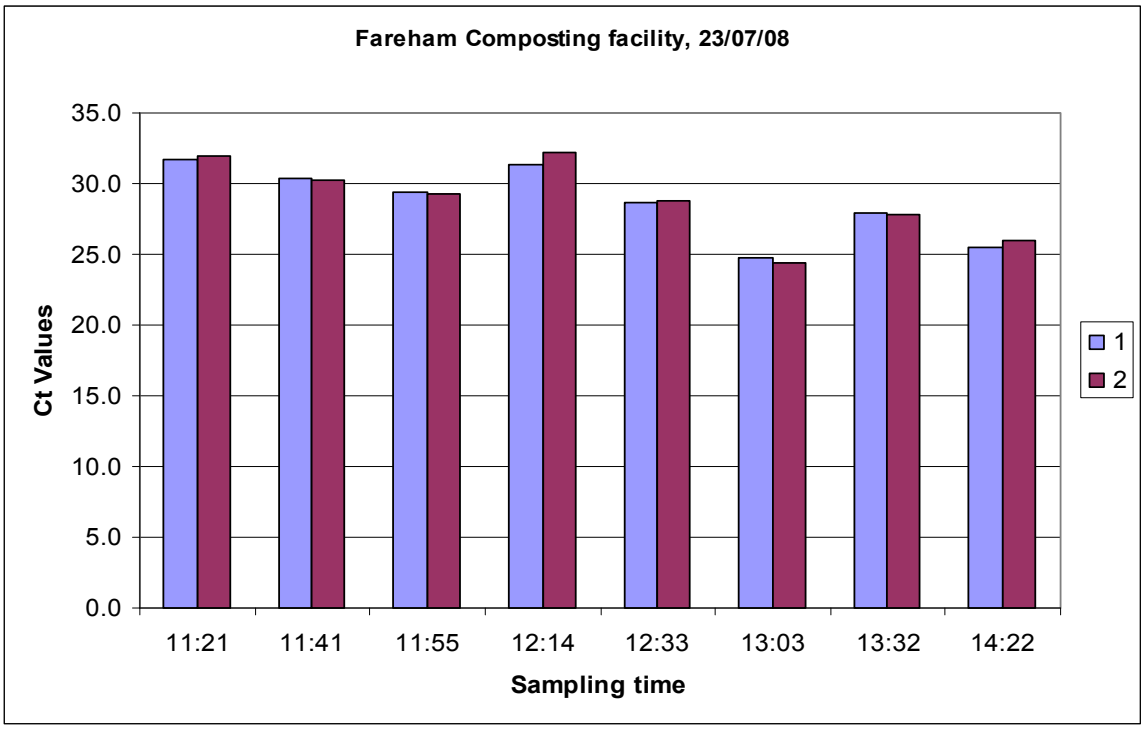


Figure 8.5: Real-time qPCR results. The results are broadly consistent between the replicate qPCR reactions.

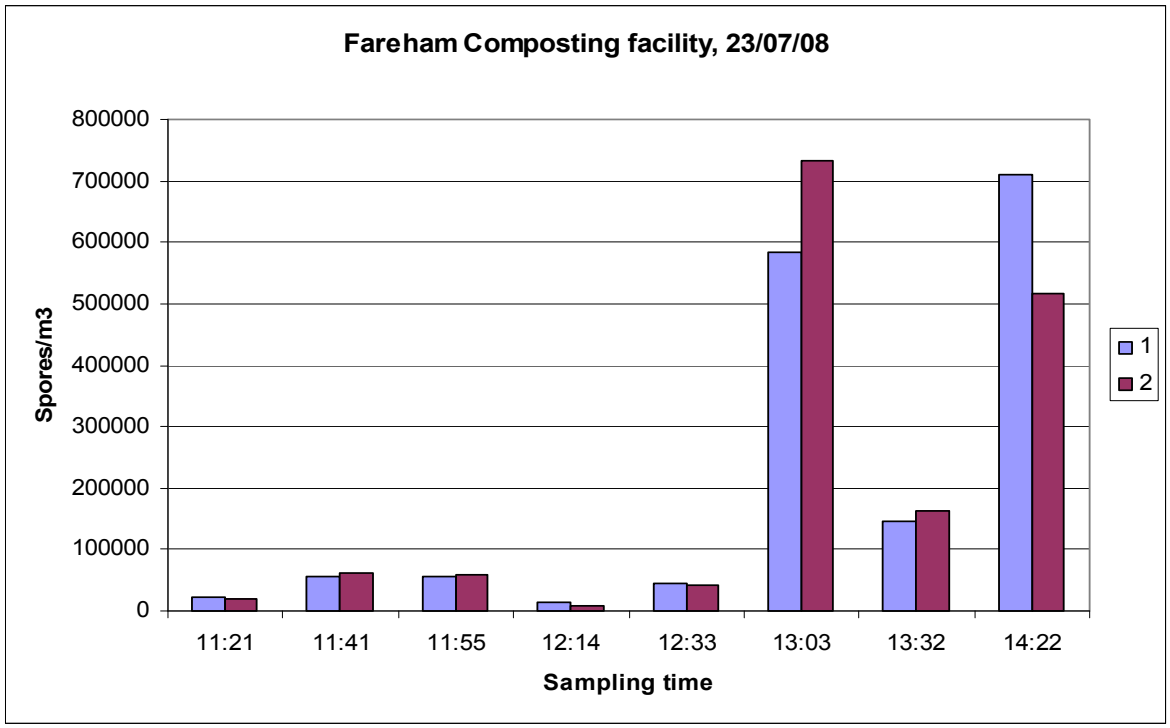


Figure 8.6: Spore number concentrations in air samples, with linear scaling. Note that the samples collected at 13:03 and 14:22 have a markedly higher level of spores detected than the samples collected at other times.

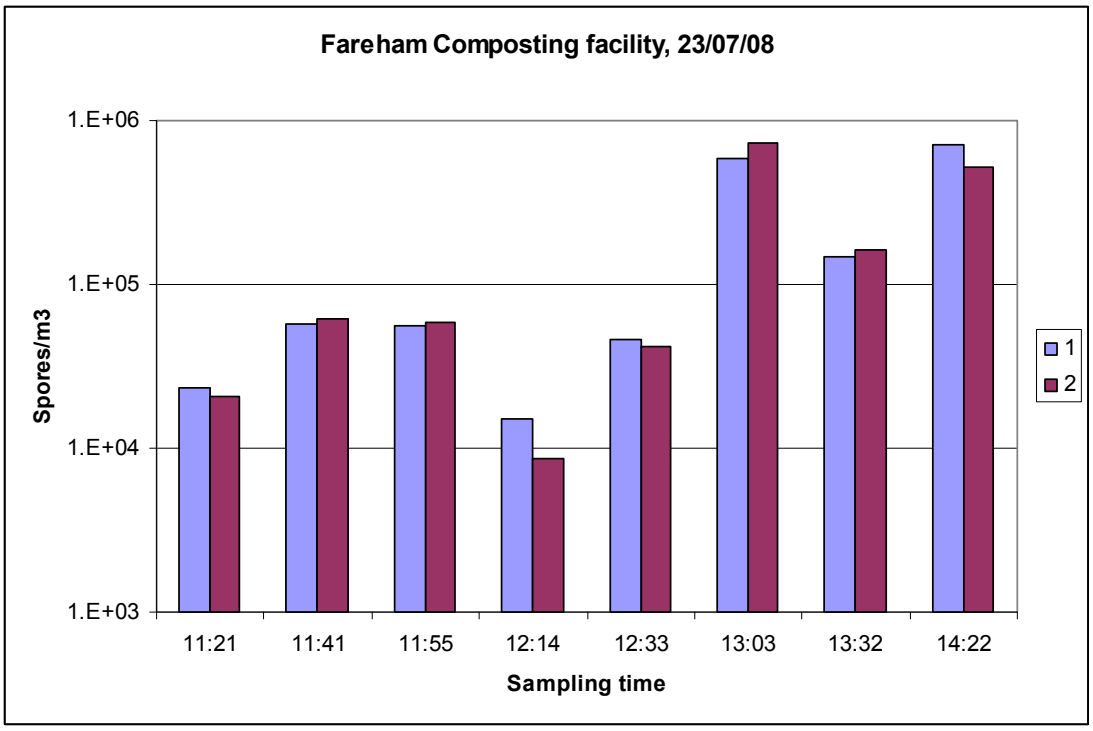


Figure 8.7: Spore number concentrations in air samples, with logarithmic scaling

Conclusion

The real-time qPCR reaction performed well with the samples from the field trial. All the reactions gave positive results. The replicate reactions gave consistent results. Furthermore, for all the sampling periods used, (5-20 minutes) the C_t values fell within the range of the calibration curve. No PCR inhibition effects were observed. PCR inhibition is a concern with environmental samples, which may be contaminated with a variety of materials that may prevent successful PCR.

8.5 Second field test – September-October 2008

The second field test was carried out at the Downend Road composting site from 26th September through 24th October 2008. As before, samples were collected at a flow rate of 630 l/min into a volume of 20 ml of buffer. Samples were collected at a number of locations, including some off site, and a record was kept of site activity and weather conditions. Some samples were collected in parallel with a conventional microbiological methodology to permit comparison. The picture below shows the location of the off site sampling points.

Figure 8.8: Location of off site sampling points during second field test.

8.5.1 Real-time qPCR results

Amplification of samples at 1× concentration

Initially, samples were analysed in the normal way without dilution. Of the 61 samples analysed, 5 were field blanks and no spores were detected by qPCR. However, of the remaining 56, only 30 resulted in amplification (see Figure 8.9). Therefore 26 samples failed to yield any product. This could have been because spores were absent, but it was suspected that this result might be due to PCR inhibition resulting from some component present in the field samples. To investigate this further, the qPCR assay was repeated using 10× dilutions of the samples.

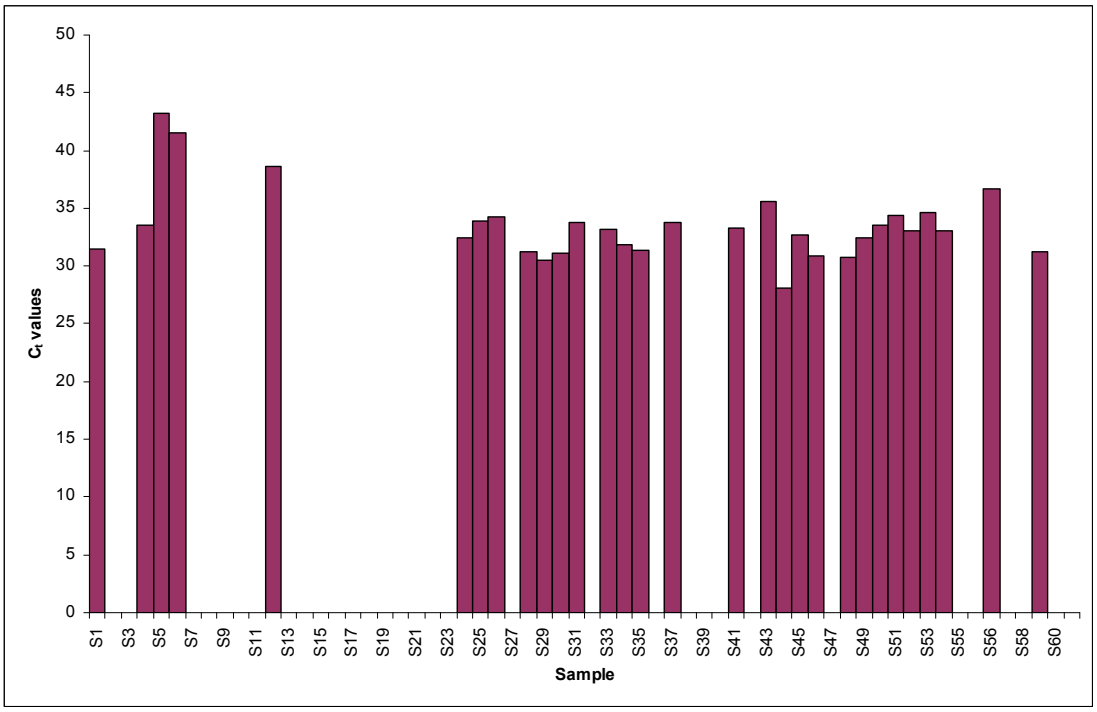


Figure 8.9: Real-time qPCR Results (C_t values) from undiluted samples. Missing (zero) values indicate no amplification. Samples S20, S39, S47, S55A and S61 were field blanks and gave a negative result. Sample S57 was not available for analysis.

Amplification of 10× diluted samples

When the diluted samples were analysed by real-time qPCR, amplification was successful for all samples (other than the blanks); the C_t values are presented in Figure 8.10. This strongly suggests that PCR inhibition was responsible for the failure of many of the undiluted samples. It will also be apparent that a number of the samples exceeded the maximum C_t value of ~35 cycles seen in the calibration curve. This means that we must be cautious in using our calibration curve to quantify the level of spores present in these cases, although the amplification behaviour is expected to be linear down to relatively low copy numbers. This is partly because the necessity of performing a 10× dilution will have shifted the Ct values upwards by $\log_2(10)$ cycles or 3.32.

Since results were obtained for all non-blank samples when the 10× dilution was applied, these results were used for all further analysis, bearing in mind the caveat noted above. Calculated spore counts are shown with linear scaling in Figure 8.11, and logarithmic scaling in Figure 8.10. The linear scaling tends to emphasise the higher values, and it is immediately obvious that samples S9 and S44 show markedly higher levels than the others. With the logarithmic scaling the lower values are also apparent. Most of the samples fell between 1000 m^{-3} and 100,000 m^{-3}. However, samples S26 and S55 gave markedly lower levels of 572 m^{-3} and 5 m^{-3} respectively.

Two aspects of these samples were investigated further; the nature of the PCR inhibition effect and the interpretation of the extremely low value obtained for sample S55.

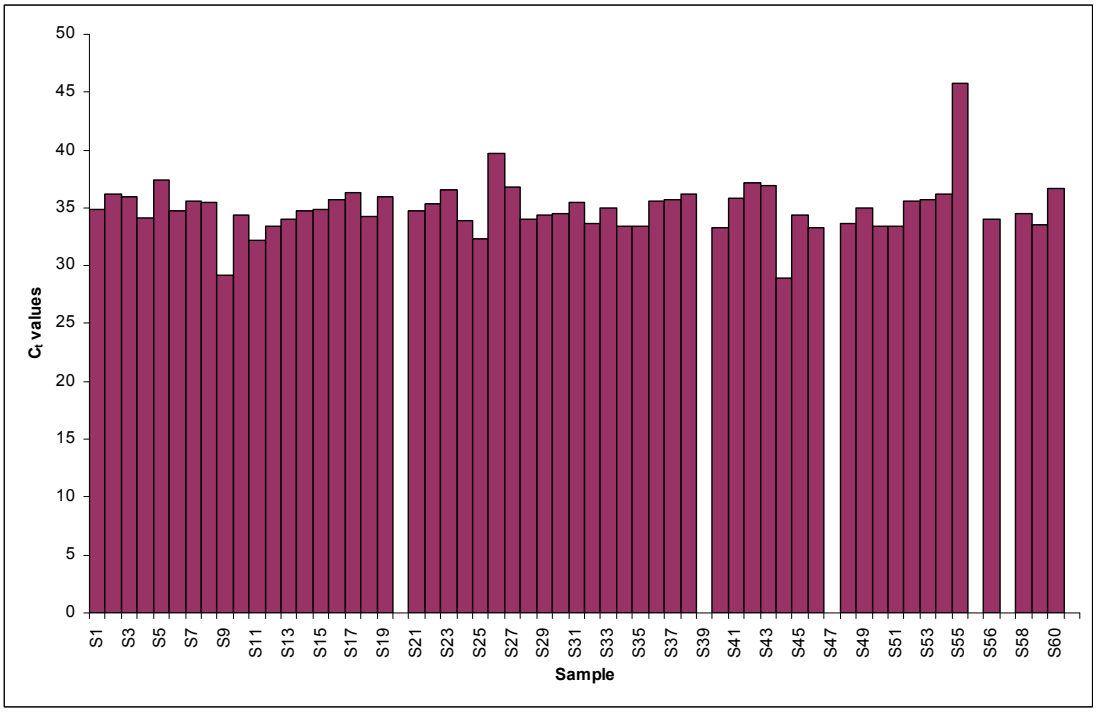


Figure 8.10: Real-time qPCR results (C_t values) from 10× diluted samples. Samples S20, S39, S47, S55A and S61 were field blanks and gave a negative result. Sample S57 was not available for analysis.

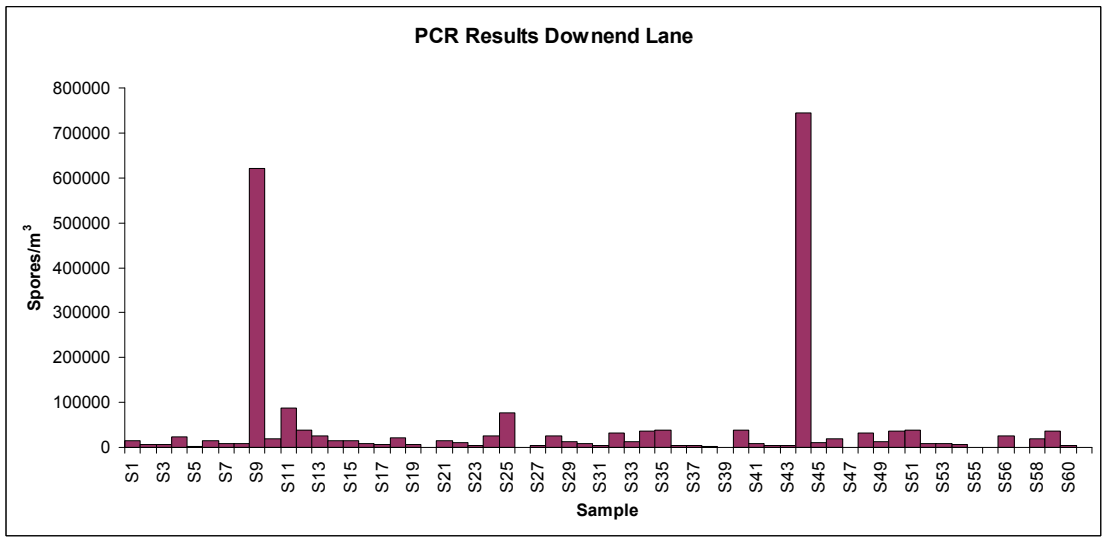


Figure 8.11: Spore number concentrations in air samples. Note that the majority of samples showed spore counts less than 100,000 m^{-3}; however, samples S9 and S44 showed much higher levels (622,000 m^{-3} and 744,000 m^{-3} respectively).

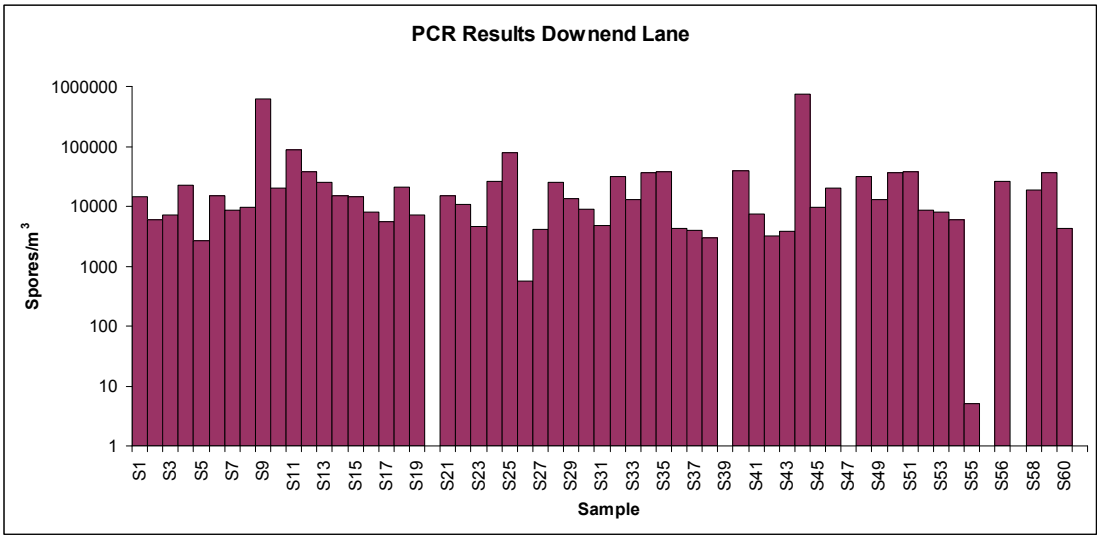


Figure 8.12: Spore number concentrations in air samples (log scaling). Note that the variability in spore levels is more apparent with the log scaling. Most of the samples gave spore levels between 1000 m^{-3} and 100,000 m^{-3}. However, samples S26 and S55 gave lower levels of 572 m^{-3} and 5 m^{-3} respectively.

PCR Inhibition effects

To gain a better understanding of the PCR inhibition effect observed with the undiluted samples, and specifically to investigate whether the effect was quantitative as well as qualitative, some repeat amplifications were performed. Two samples were chosen; one which failed to amplify when undiluted (S2) and one that was successfully amplified (S54). The threshold cycle values are given in Table 8.3. It is immediately apparent that the sample S54 that was successfully amplified previously was also amplified successfully again at the 1× and 10× dilutions; however in this case the amplification was not successful at 100× and 1000×. In this case it may be that there was insufficient material present at the higher dilutions. Surprisingly, the S2 sample was amplified successfully at all dilutions on the second attempt. This result is hard to explain without further investigations, but it may be that the interfering agent is not uniformly dispersed throughout the samples; for example, it might be present in a particulate form.

Experiment		**Original**		**Repeat**			
Dilution factor		**1**	**10**	**1**	**10**	**100**	**1000**
Sample No.	**S2**	—	36.19	31.8	34.58	37.84	34.56
	S54	33.06	36.17	34.68	35.84	—	—

Table 8.3: Real-time qPCR repeats for samples to investigate apparent qPCR inhibition. Original results with dilution factors

Figure 8.13 shows the C_t values graphically. It is apparent that despite a degree of scatter, the C_t values increase for the lower concentrations as expected. The exception to this is the 1000× dilution of S2. However, more variability is expected at the lower concentrations, as smaller actual numbers of DNA molecules are present in the PCR reactions. It is also obvious that the amount of scatter seen here is larger than seen for the spore dilutions used in constructing the calibration curve, or indeed for the measurements made in the first field trial. This may be due to the presence of higher levels of interferents in the samples from the second field trial; in any case the variation in C_t seems to be ± 1 cycle (corresponding to a four-fold range of spore concentrations).

When the C_t values are converted to spore counts in m^{-3}, taking into account the dilution factors applied to the samples, the results appear to be relatively consistent down to 100× dilution (Figure 8.14). This reinforces the view that the PCR inhibition is “all or nothing” in nature, although more data are required to confirm this.

It is not clear why the samples from the second field trial experienced problems with PCR inhibition whereas those from the first trial did not, particularly as the nature of the inhibiting material is not clear. Possibilities include the siting of the sampler, the activity or the nature of the material at the site, and the different season. For example, one might expect a higher background level of (non-target) mould spores in the autumn.

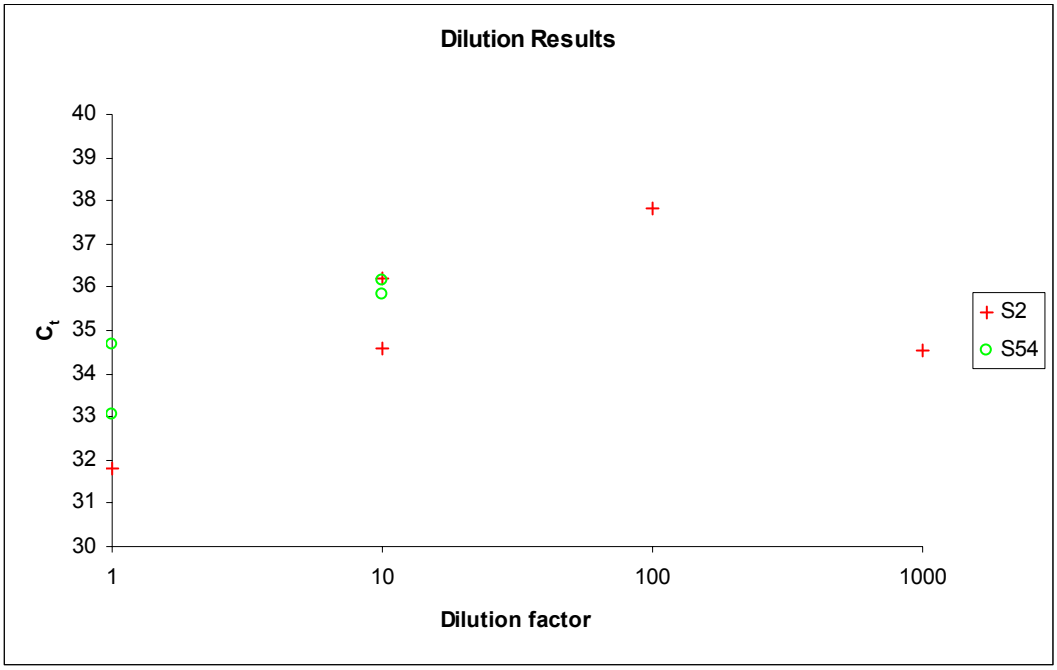


Figure 8.13: Real-time qPCR results for dilutions of samples exhibiting apparent PCR inhibition. Reactions which failed to give an amplification product are not shown.

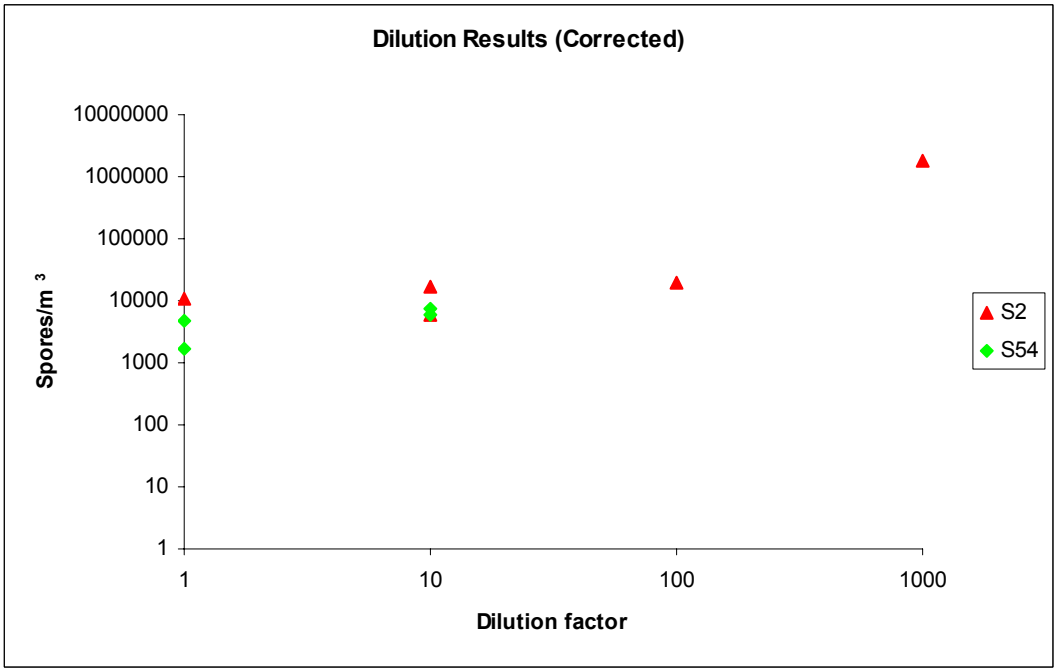


Figure 8.14: Calculated spore counts (corrected for dilution factor). Successful amplifications gave consistent results down to a dilution factor of 100.

Low-copy number samples

Sample S55 gave the lowest spore count observed (5 m^{-3}) with a threshold cycle of 45.81, well beyond the range of the calibration curve. It is also expected that at such low levels the reproducibility of the measurement will suffer due to the sampling effects and the small

numbers of spores being sampled. It should also be noted that this reaction was one of those that did not result in amplification for the undiluted sample, and that the reported value is based on the 10× dilution. Furthermore this corresponds to 3 spores/ml in the sample, and implies that the qPCR detection (which uses much less than 1 ml) is reaching sensitivity to less than one spore. This is not as impossible as it may sound, as each spore contains many copies of the mitochondrial DNA which could be dispersed either by the preparation of the spores or damage when the spores are collected. However, because of the statistical variability that is likely at such low copy numbers, we repeated the analysis of S55 to ensure that the results were not a statistical fluke, or the result of PCR inhibition.

The results of these repeat measurements are given in Table 8.4. It is striking that the undiluted samples were successfully amplified in the repeat runs and gave C_t values consistent to within less than half a cycle. The results with the 10× dilution were much more variable; one of the repeat runs failed to amplify and the other two differed by nearly 8 cycles. The failure to amplify in this case is unlikely to be due to PCR inhibition (as this did not occur in any of the 10× dilutions) and this variability is more likely to reflect the extremely low copy number and consequent statistical fluctuations in the number of template molecules present in the PCR reaction.

The corresponding values for the number concentration of spores in the original air sample are plotted in Figure 8.16. The values for the undiluted sample may be influenced by PCR inhibition, whereas those for the 10× dilutions are subject to statistical variations due to low copy number. However, the consistency of the undiluted sample results suggests that they may be more reliable, and the spore concentration is somewhere in the region of 100 m^{-3} to 200 m^{-3}.

		C_t		Spores/ml		Spores/m^3	
Dilution Factor		1	10	1	10	1	10
Repeat	1	—	45.81	—	3	—	5
	2	36.09	37.87	201	615	318	977
	3	36.52	—	151	—	239	—
Average						279	491

Table 8.4: Repeat real-time qPCR analysis of low copy-number sample S55. Repeat 1 is the result described above.

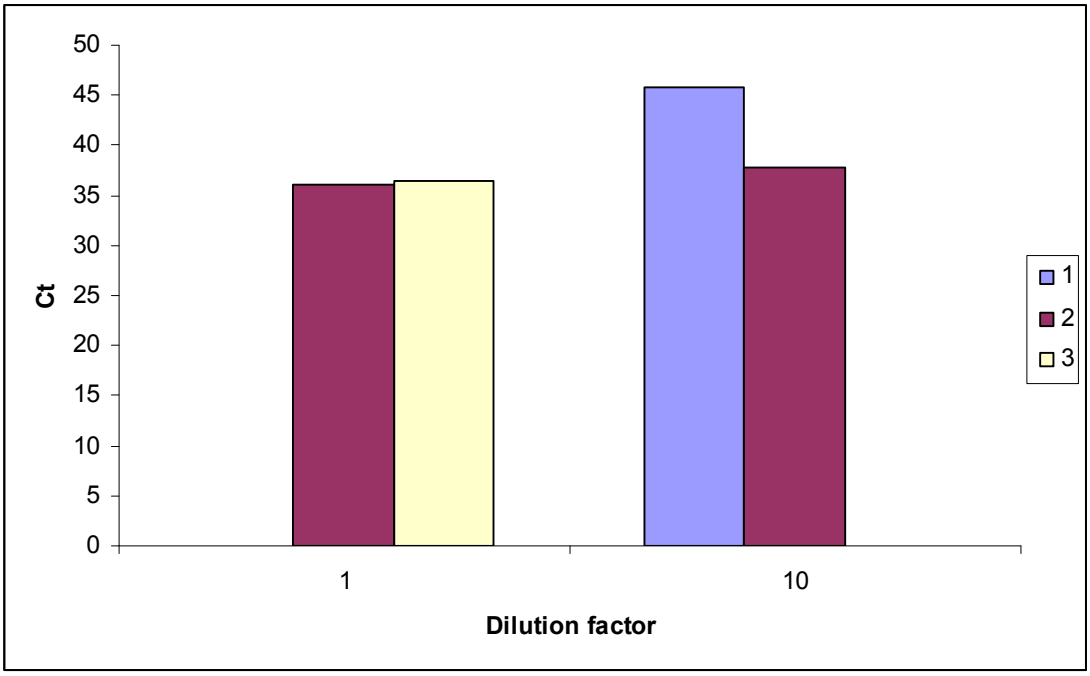


Figure 8.15: Real-time qPCR results from repeats of S55 analysis. Legend indicates repeat numbers; missing bars are where amplification did not occur.

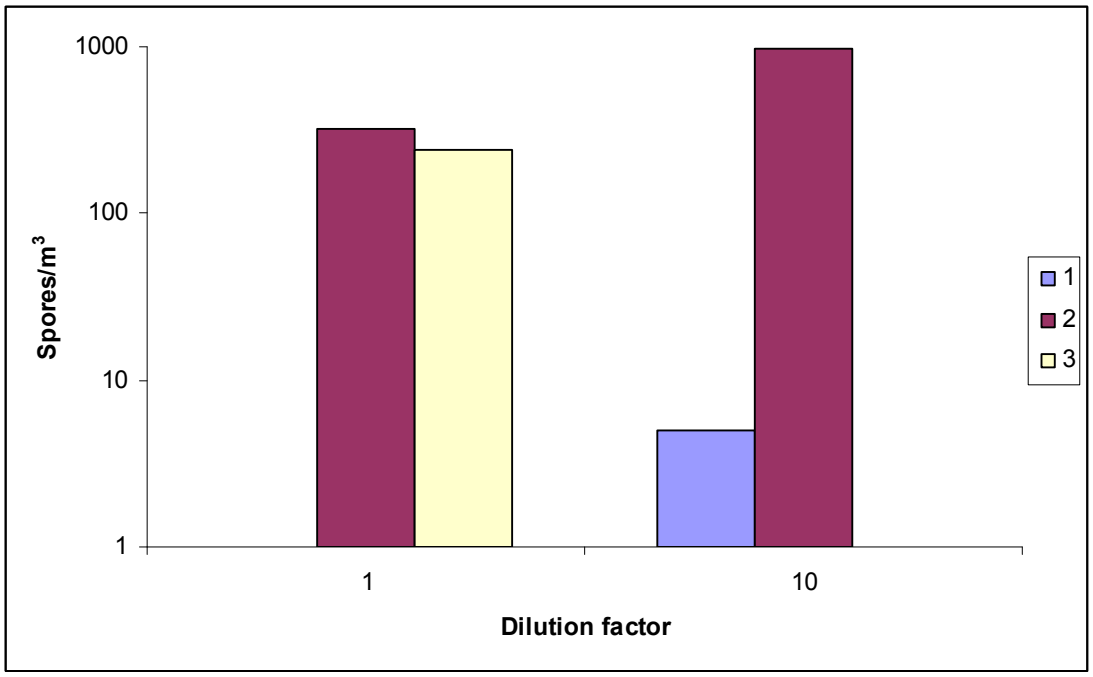


Figure 8.16: Calculated spore number concentrations in air for repeated analyses of S55

8.5.2 Comparison with Microbiology Results

To compare the real-time qPCR results with the current standard method, which relies upon the impaction of air samples onto agar plates followed by colony counting, the second field study included some simultaneous sampling by both methods. Simultaneous samples were collected at four locations; repeat samples were collected at three of these locations. Due to access difficulties it was not possible to collect samples using the Coriolis sampler at location S5 and to no parallel tests were carried out at this location. The results are summarised in Table 8.5 and plotted in Figure 8.17.

The microbiological method relies upon the germination and growth of the spores and hence only counts viable spores; values are reported in colony forming units or cfu. Since the real-time qPCR method counts all spores, it is expected that the numbers reported will be somewhat higher. This is indeed what was observed.

The nominally down-wind sites, SP1 and SP2, gave relatively high values by both methods, with in excess of 10,000 spores/m^3 by the real-time qPCR approach, and ~8000 and ~6000 cfu/m^3 by the microbiology approach. In contrast, at the nominally up-wind sites, SP3 and SP4, which were also at much greater distances from the site, the microbiology results were very low, reporting less than 10 cfu/m^3. The levels recorded by the real-time qPCR were rather lower than the down-wind sites, but in this case were notably higher than the microbiology results. The results obtained by the repeat qPCR measurements on later days were similar, except for the SP3 measurement on the 13th, which gave a result of 5 spores/m^3 (however, repeat analyses of this sample suggest a more accurate value would be 100-200 spores/m^3; see the previous section on low-copy number samples).

Although larger numbers were expected from the qPCR than from the microbiology, a greater difference between the two methods is shown for the down-wind than the up-wind sites. The implication is that the viability of the spores collected at SP3 and SP4 was much less than at SP1 and SP2. Further work is needed to confirm this result.

Sampling information				Real-time qPCR			Microbiology
Sampling Position	Distance from perimeter (m)	Wind Direction (deg)	Date	Bertin Sample	(1×)	(10×)	cfu/m^3
					spores/m3		
SP1	0	83	06/10/2008	S28	16591	24842	8320
SP2	70	89	06/10/2008	S29	17488	13391	5730
SP3	375	86	06/10/2008	S30	8406	9091	<10
SP4	300	143	06/10/2008	S31	1437	4838	<10
SP5							460
(SP2)	70	182	07/10/2008	S37	1476	3938	
(SP4)	300	196	07/10/2008	S38	—	3020	
(SP4)	300	213	09/10/2008	S46	9924	20167	
(SP3)	375	262	13/10/2008	S55	279	491	

Table 8.5: Comparison of real-time qPCR and microbiological measurements of spore concentrations. Simultaneous sampling for microbiology and qPCR measurements was made at sites SP1-SP4. Repeat sampling for qPCR was done at sites SP2, 3 and 4 on later days; no qPCR samples were collected at SP5. The values for sample S55 are the revised values based on the repeat measurements conducted in the preceding section.

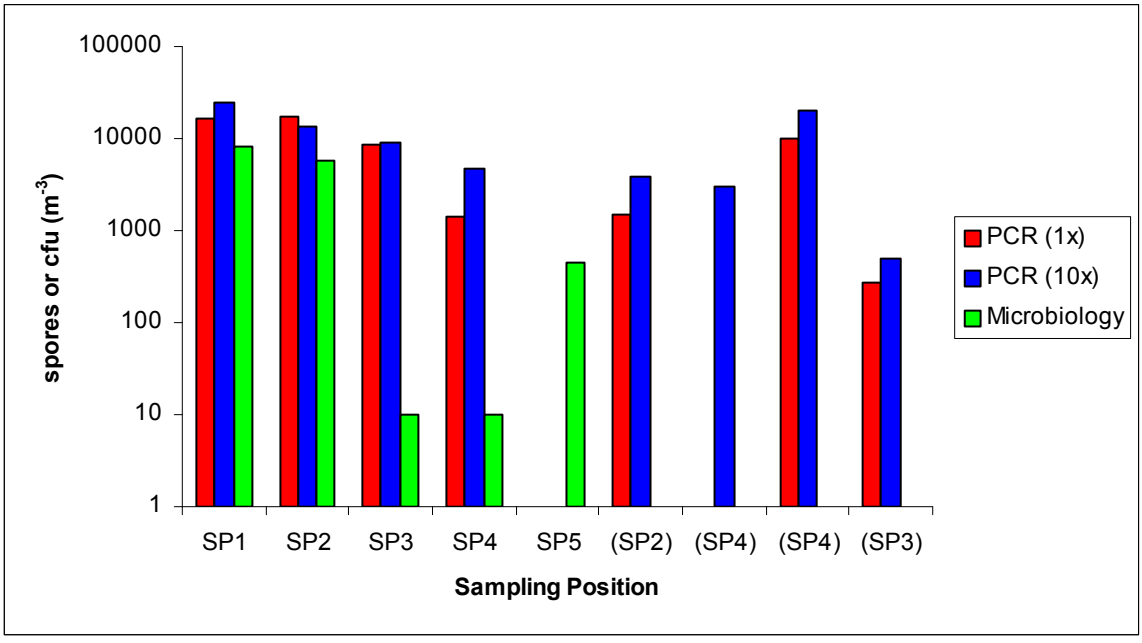
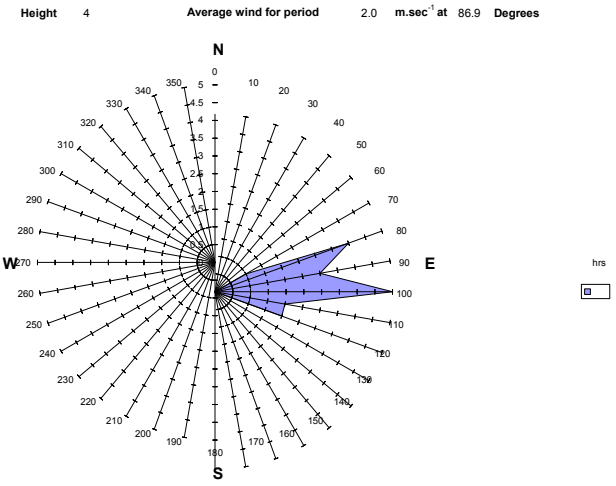
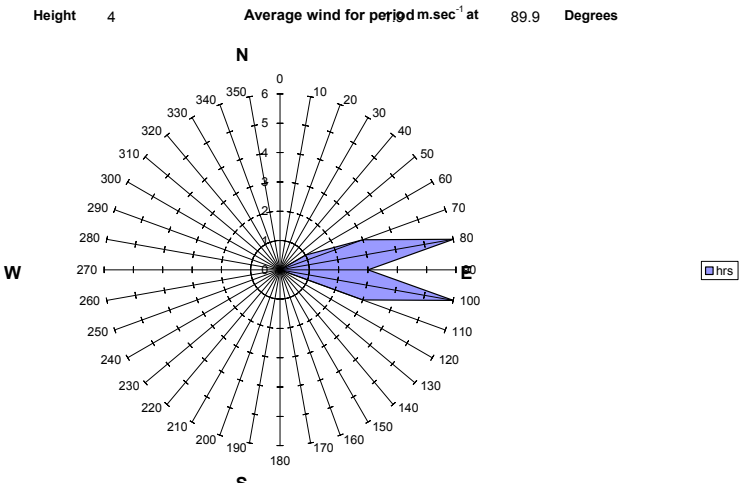


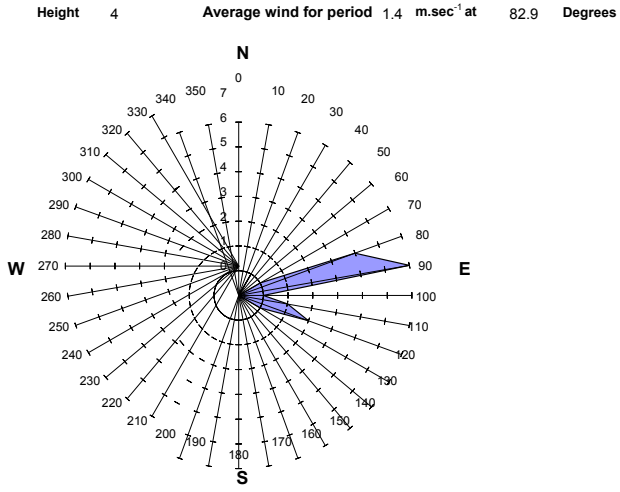
Figure 8.17: Graphical comparison of real-time qPCR and microbiological measurements. Microbiology values reported as <10 are plotted as 10. Real time qPCR results are in spores/m^3; microbiology results are in cfu/m^3. The values for sample S55 are the revised values based on the repeat measurements conducted in the preceding section.



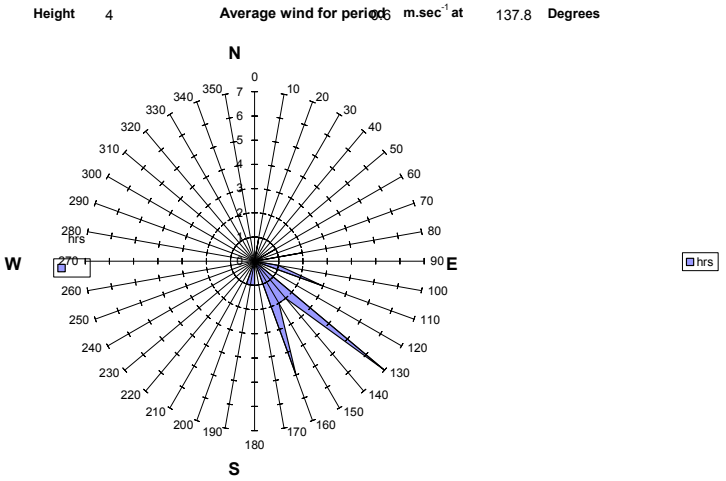
SP1, S28 Wind Rose



SP2, S29 Wind Rose



SP3, S30 Wind Rose



SP4, S31 Wind Rose

Figure 8.18 shows the wind direction during the off site sampling at SP1, SP2, SP3, SP4

8.5.3 Particulate Measurements

The Turnkey Topas provided valuable data on general particulate levels at the boundary fence throughout the field trial. One of the Topas instruments was also coupled to the Met. station and the Bertin Coriolis sampler to test the concept of integrating the instruments into an automatic network. This is described in Section 7.

The levels of particulate measured throughout the trial are shown below, together with a weekly wind rose.

The primary aim of the particulate measurements was to show how the Tier one measurement technique, in this case the Topas instruments, could be used to trigger the Coriolis aerosol samplers. The aim was therefore to show that combining the wind direction and speed information with real time aerosol measurements, would allow discrimination of the emissions from the site. The measurements also provided information on the particulate levels in the vicinity of the composting facility. From the preliminary measurements the PM_{10} fraction was selected as the measurement best suited to use for triggering the bioaerosol sampling.

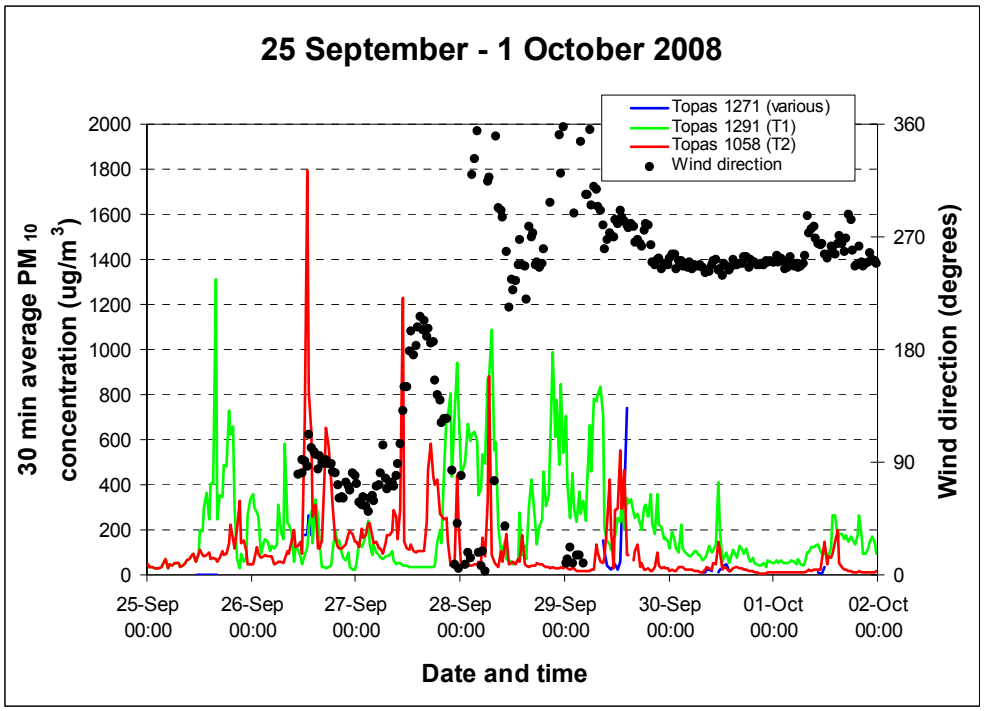
Two fixed sampling locations were used for Topaz particulate monitors, T01 and T02. T01 was close to the western boundary fence, T02 was to the east. Both locations were within 50 m of the windrows. For much of the sampling period a third Topas was operated together with a Bertin Coriolis sampler, at location T03, at the north of the site (See Figure 8.1). During a period of windrow turning, from the 29th September to the 3rd October, the Bertin-Coriolis and Topaz sampler pair were used off site, at locations SP1, SP2, SP3 and SP4 (See Figure 8.8).

The following Figures (8.19 to 8.22), present a summary of the continuous particulate (PM_{10}) data collected during the whole campaign. The wind direction is also shown.

The data from the T01 sampler, to the west of the site, is generally higher throughout the period. This may be because the wind was more often from the east, and also may have been due to the location of the samplers with respect to the particulate sources on site. Both samplers saw peaks of particulate during shredding and turning periods. In most cases in which there were no recorded site activities (shredding and turning), such as on the 5th October and 15th to 19th October, the particulate measurements were low. However, in some cases, observable particulate levels do not correspond to recorded site activities, such as that observed on Sunday the 12th. As the site was closed, this aerosol probably arose from re-suspended particulate blown from the site, or from off site sources. During the period from the 9th to the 13th, the wind was blowing more from the west, and the higher levels seen on the Topas at T02 reflect this. The Topas samplers generally give higher readings when they are downwind of the site, indicating that they are primarily monitoring emission from the site.

8.5.4 Summary

The particulate levels seen peaked around 1200 to 1800 ug/m^3, and the highest levels were observed during periods of turning and shredding on site. It was observed that the actual levels seen on the Topas samplers was very dependant on the location of the samplers with respect to the sources within the site, and therefore the trigger level used is dependant on the sampler location. For this reason although at T01 and T02 levels of over 1000 ug/m3 were routinely seen, for triggering the system mounted further from the windrows at T03, a much lower trigger level was required.



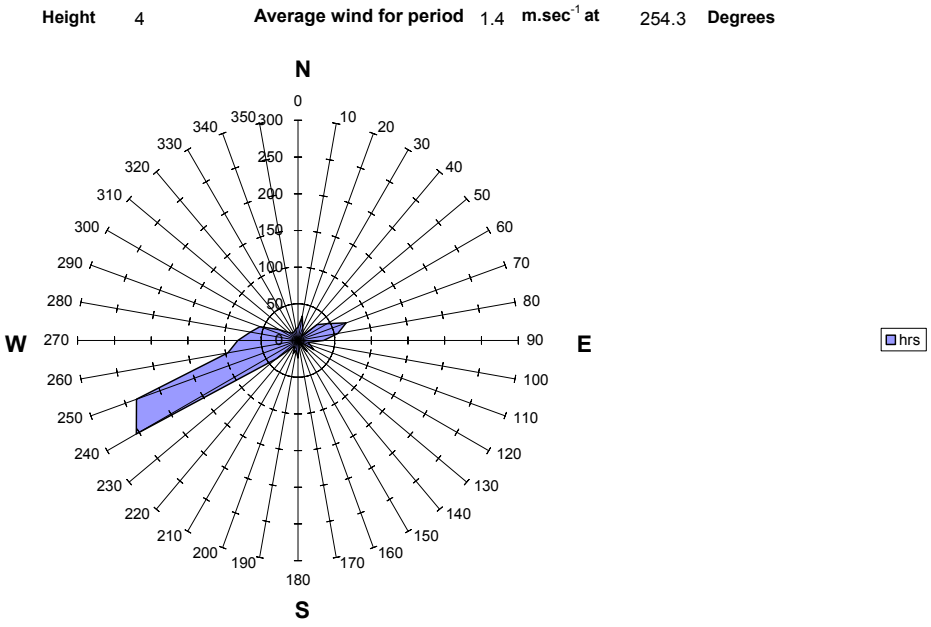
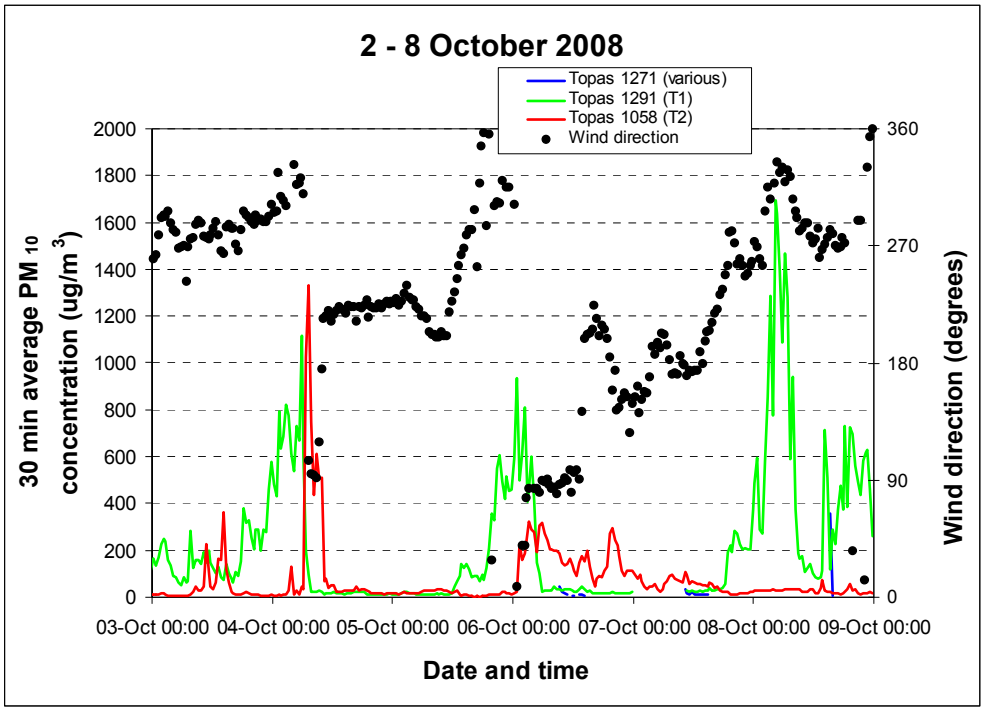


Figure 8.19 Particulate data and Wind Rose w/c 25th September 2008



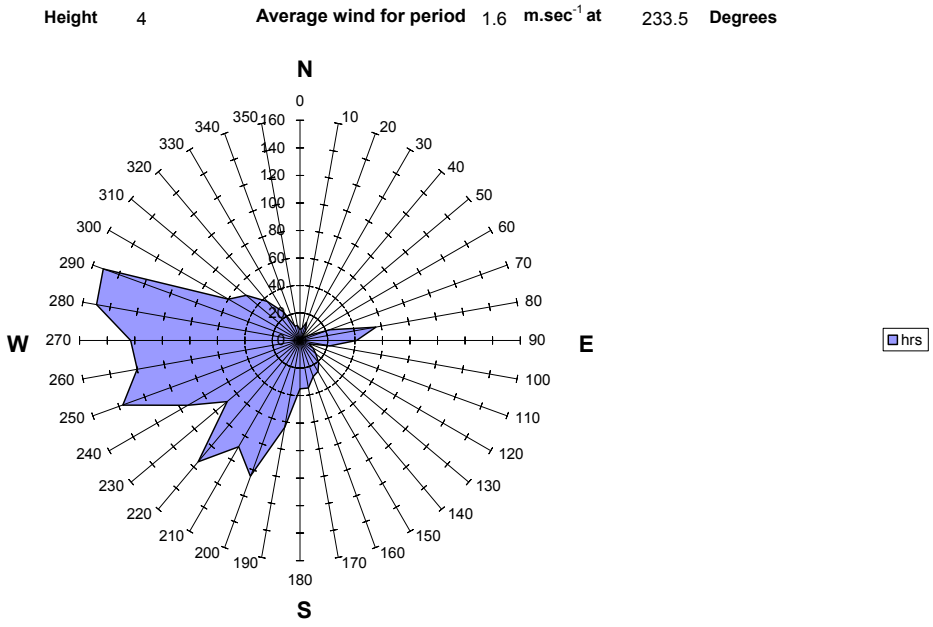
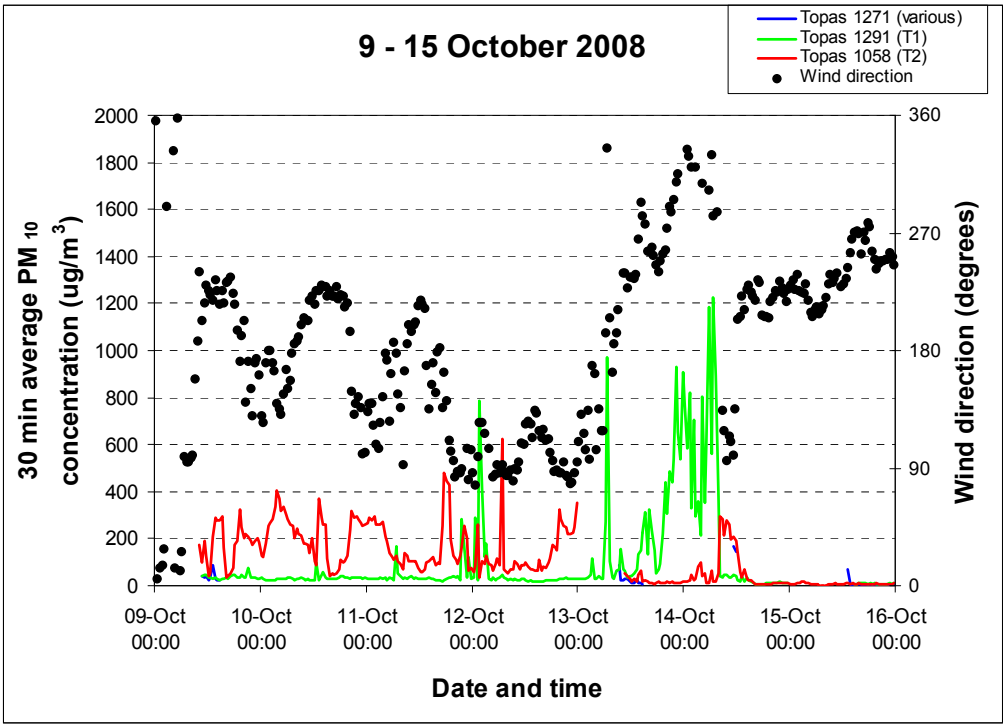


Figure 8.20 Particulate data and Wind Rose w/c 2nd October 2008



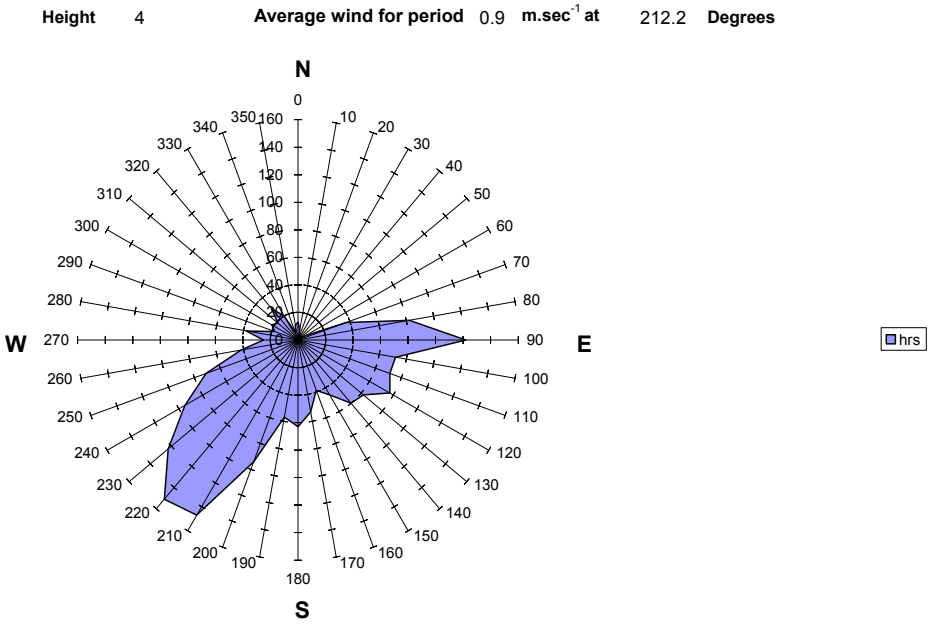
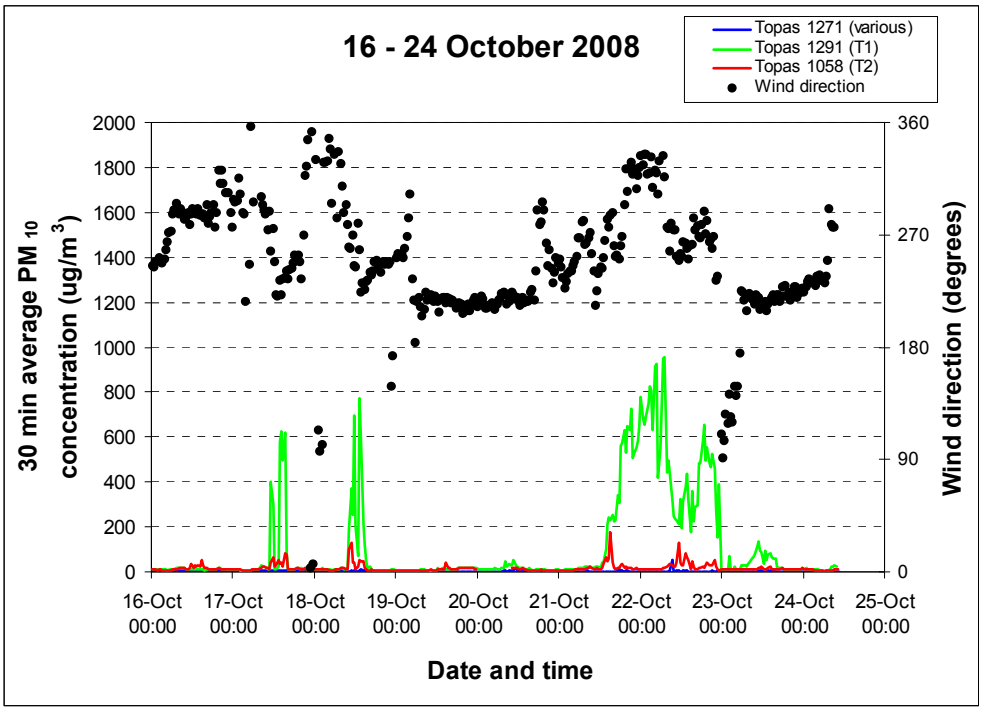


Figure 8.21 Particulate data and Wind Rose w/c 9th October 2008



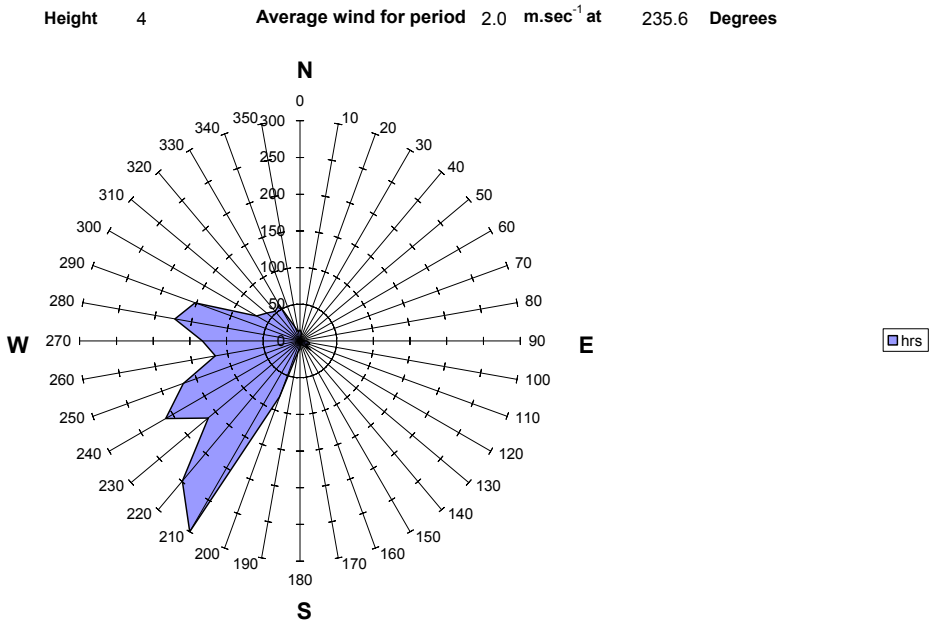


Figure 8.22 Particulate data and Wind Rose w/c 16th October 2008

8.6 Conclusions from the field trials

The particulate levels at the boundary fence peaked around 1200 to 1800 ug/m^3, and the highest levels were observed during periods of turning and shredding on site.

Reliable quantification of *Aspergillus fumigatus* in air samples, using the NPL developed real time qPCR assay has been demonstrated.

PCR inhibition occurred for some samples. It is not clear why there is more of a problem in the samples collected in the second trial, nor whether the inhibition is a feature of the air samples from the compost site or of air samples more generally. It is likely that the inhibition affect can be overcome using dilution, as shown by the repeat experiments, but this requires further study.

Real-time qPCR reports higher values than the conventional microbiological approach. This is not surprising, as qPCR will count non-viable spores while the microbiology approach depends on colony formation.

The assay is extremely sensitive, although quantification becomes unreliable at low spore concentrations.

More information on background levels of *Aspergillus fumigatus* would be useful.

9. Conclusions and Recommendations

This project has involved the development and trial of a novel monitoring methodology measuring bioaerosols from composting facilities. An initial decision was made to focus on the detection of specific bioaerosols, namely *Aspergillus fumigatus*.

The ITT for the project specified a network design utilising three Tiers of instruments:

- Tier 1 being low cost screening analysers monitoring particulate levels in real time,
- Tier 2 being screening analysers (able to discriminate biological material from other particulates
- Tier 3 being automated biological analysis and quantification

The initial phases of the project reviewed available commercial off-the-shelf (COTS) equipment able to fulfil the requirements of the different Tiers, and reviewed of possible network designs. These reviews concluded that a 3 Tier configuration of the type originally considered would not be feasible, owing to the inhibitive cost of the Tier 3 instrumentation. An alternative configuration was developed, in which the Tier 3 instruments are replaced by automated sampling systems, which collect material during potential events, and this is then subsequently analysed. This configuration was subsequently developed and trialled in the field.

An objective assessment of the commercially available equipment was then made against the requirements for the network configuration, using a set of criteria. Specific models of real-time aerosol samplers and bioaerosol sampling systems were selected to be used in the field assessment of a prototype of the network. The Turnkey Instruments Topas was the highest ranked Tier 1 instrument, although equipment ranked second or third would also be suitable. The Tier 3 equipment with the highest ranking for biological species, in particular fungi and bacteria, was the Bertin Technologies Coriolis (m or MS) with subsequent qPCR determination. A prototype real-time optical instrument for the identification of biological material (A Tier 2 instrument) was also identified, the WIBS 2 instrument developed at DSTL.

The analysis of the collected material was then investigated, and quantitative PCR analysis qPCR, was identified as the most promising technique, enabling cost effective development of targeted assays for specific bioaerosols of interest. A method was then successfully developed to enable qPCR analysis of *Aspergillus fumigatus*. This included the development of suitable approaches to enable the extraction of the DNA material from the spores. A series of laboratory experiments were carried out to characterise the method, these included measurements of genomic material and whole spores cultured by the HPA. This showed that the sensitivity and linearity of the method were very promising.

A series of laboratory tests were carried out using cultured spores in a test chamber at the HPA. This involved the selected TOPAS particulate real time monitor, the Bertin Coriolis aerosol sampler and the WIBS 2 instrument, together with a standard sampler of the type routinely used for sampling onto agar plates. The test programme demonstrated that the PM_{10} fraction measured by the Topas instrument could provide a non-discriminating indication of the presence of spores, and could therefore be used to trigger a Bertin Coriolis sampler to capture this event. The Topas measurement would not (and is not intended to) discriminate spores from any other PM_{10} event.

The test programme demonstrated the ability of the qPCR assay to quantify spore counts. The assay provides a measure of viable and non-viable spores and spore fragments and therefore gives a systematically higher reading than the approaches that require the sample to be cultured. The WIBS2 results confirmed that within the test chamber there were significantly more non-viable spores than viable spores, and the qPCR is therefore expected to give a higher reading than the cfu measurements.

A prototype network, consisting of one segment of a proposed 12 segment network was tested at the Downend composting facility. This included a paired Bertin Coriolis sampler and a Topas instrument, together with wind speed and direction sensors. Additional Topas instruments were also deployed to check particulate levels at other sectors of the network. An automated system was developed to trigger the Bertin sampler when the Topas instrument detected high particulate levels. This trigger also took into account the wind direction and speed, to only trigger when the sampler was downwind of the composting facility. The network was also configured to send an automated text message to the network operator, to alert them to the trigger events. This enables the operator to attend the network and retrieve the collected aerosols, and reset the Bertin sampler. Sampling was undertaken during periods where the compost was being turned and during normal operation. A limited number of parallel measurements were made against the 'standard' agar plate and subsequent culture and colony counting methodology.

The collected samples were analysed using the qPCR assay developed within this project. The study showed that the qPCR approach had very good sensitivity and was able to quantify spore counts for *Aspergillus fumigatus* in the field. A number of observations were made. The first is that high levels of spores were measured, consistently higher levels than the conventional approach, even at the 250m 'regulatory' distance. The qPCR approach measures all spores including those which are not viable, and would therefore not be measured by the colony counting approach. However, it should be borne in mind that non-viable material is still considered a potential risk to health. The qPCR assay was shown to be extremely sensitive, however, a possible inhibition due to an as yet unknown interferent was discovered.

It is therefore the conclusion of this work that the methodology developed within this project has the potential to offer a cost effective approach to allow the near real time quantification of biological aerosols from composting facilities.

A number of recommendations can be made following this study:

- Further work should be undertaken to investigate background measurements at a range of different locations. This will allow the levels of spores seen in this study to be placed in a wider context. In particular, a second field trial of the new qPCR technique at a different composting site is recommended to provide a further comparison with the traditional technique and more data on background and 250 m levels of *Aspergillus fumigatus.* This should also include further comparison measurements against the colony counting approach.

- Further refinement of the qPCR methodology should be carried out, to enhance the quantification at very low spore counts and develop a mechanism to identify and account for the observed 'inhibition' seen some field samples. This includes spiking studies (adding spores or DNA to “control” environmental samples). This work could also include the development of an internal or external amplification control to detect, and/or compensate for, the presence of qPCR inhibition in a reaction

- The involvement of qPCR manufacturers should be sought again, once a requirement and hence a market for this analysis is established.