Size distribution and sites of origin of droplets expelled during expiratory activities

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Abstract

A new Expiratory Droplet Investigation System (EDIS) was used to conduct the most comprehensive program of study to date, of the dilution corrected droplet size distributions produced during different respiratory activities.

Distinct physiological processes were responsible for specific size distribution modes. The majority of particles for all activities were produced in one or more modes, with diameters below 0.8 μm. That mode occurred during all respiratory activities, including normal breathing. A second mode at 1.8 μm was produced during all activities, but at lower concentrations.

Speech produced particles in modes near 3.5 μm and 5 μm. The modes became most pronounced during continuous vocalization, suggesting that the aerosolization of secretions lubricating the vocal chords is a major source of droplets in terms of number.

Non-eqilibrium droplet evaporation was not detectable for particles between 0.5 and 20 μm implying that evaporation to the equilibrium droplet size occurred within 0.8 s.

Keywords: expiratory-aerosol, size-distribution, hygroscopic, modality, evaporation

1 Introduction

Sufficient knowledge of droplet size and origin is important for understanding virus transport via the aerosol route. There are many pathways of virus transport, an important one of which being droplet infection, which occurs when aerosol droplets are generated and released during speech, coughing, sneezing, vomiting, or aerosolization of feces during sewage removal and treatment. The dynamics of virus carriage and survival in aerosol droplets, the role of environmental factors and ventilation are poorly understood. As a consequence, understanding of the mechanism of virus spread is less than basic and so is the ability to control and prevent that spread.

Expiratory human activities such as breathing, coughing, sneezing or laughing result in droplet generation by the wind shear forces. Droplet atomization from the respiratory tract arises from the passage of an air-stream at a sufficiently high speed over the surface of a liquid; tongues of liquid are drawn out from the surface, pulled thin and broken into columns of droplets (Hickey 1996). Each of these processes leads to droplets of different sizes and originating from different areas of the upper respiratory tract.

Early investigators (from the 1920s to 1940s) believed that the vast majority of droplets generated through expiratory human activities are in the supermicrometer size (Wells 1934; Jennison 1942; Duguid 1945). This was because the techniques they had available at the time to conduct such studies were insensitive to smaller droplets. The techniques available then were based on counting of large respiratory droplets after collection on a slide or on a culture plate, exposed directly in front of the mouth or by counting droplet images on enlarged, high speed, dark field photographs (Jennison 1942), and for smaller droplets (down to about 1 – 2μm), sampling with a slit sampler after the droplet spray was evenly distributed in the air. Duguid (1945) concluded that in general, 95% particles were smaller then 100 μm, and the majority were in the range from 4 - 8 μm. It can be seen that nearly all of the small droplets originate from the front of the mouth; only relatively few, if any, from the nose or from the throat.

More recent studies, involving optical particle detection techniques capable of measurements down to fractions of a micrometer, suggested that in fact the majority of these particles are in the submicrometer size range (Papineni and Rosenthal 1997). In summary, the study conducted by Papineni and Rosenthal involved five healthy individuals and employed optical particle counters with a particle detection range from 0.3 μm and also electron microscopy as droplet detection methods. Contrary to the earlier studies, this study showed that 80 – 90% of particles from human expiratory activities are smaller than 1μm. The study also showed that the highest droplet concentrations were commonly generated during coughing and the lowest from nasal breathing; however, there was large inter-subject variability in concentrations emitted during various activities. For example, it was shown that coughing in general results (as expected) in many more particles being expelled than mouth breathing; however, for one subject, mouth breathing actually produced a higher concentration of droplets larger than 1μm compared with coughing. One important issue, which was not addressed by the study, was the relationship between the original droplet size and the size measured. Before detection in the instrument,

the droplets spent considerable amount of time in the air, which, as presented below, could have been sufficient for drying of medium size droplets to the droplet residue. Therefore, the measured droplets could in fact have been the dry droplet residue.

Another recent study by Yang et al.(2007) attempted to measure both the dried droplet nuclei size distribution and the initial droplet size distribution produced during coughing. The initial size measurement was performed by having subjects cough into a bag and the resulting captured sample was examined using an aerodynamic particle sizer. Those measurements yielded a mode at 1-2 μm for the dried aerosol and at 8.35 μm.

In summary, from the studies reported so far, there is some understanding of the size of droplets generated directly by humans during various human expiratory activities, and the region in the respiratory tract where they originate. However, since there have been only a handful of studies conducted with the application of modern techniques capable of measuring particle size distribution, it is important that more work is done in this area to develop a better understanding of the mechanism of droplet generation.

The aim of this research was to develop a system for investigating the size distribution of aerosols produced during expiratory activities and to investigate the short term aging of these aerosols in terms of size distribution changes caused by the evaporation of the droplets water component. The system design was to enable measuring the size distribution of expiratory aerosols at a known short time after the aerosol is produced, and able to determine the aerosol concentration and size distribution at the source.

2 Experimental Methods

2.1 Design Criteria

A range of criteria, were considered in the design of an experimental rig to fulfill the aim of the project including; low particle background, the ability to estimate droplet age, avoidance of aerosol re-inspiration, time response, isokinetic sampling, flexibility of instrument accommodation, dilution factor measurement, safety, hygiene and comfort. The design criteria are discussed in more detail below.

Low particle background: The aerosol number concentrations produced during expiratory activities are of the order of 1 cm^{-3} or less in the undiluted state in close proximity to the mouth. These concentrations are much smaller than the average concentrations typically seen in room air for ambient aerosol particles larger than 0.5 μm. Therefore careful attention to the removal of this background aerosol was essential to obtaining a meaningful measurement of expiratory aerosol size distribution.

Ability to select droplet age: Time dependant processes act to modify an expiratory aerosol size distribution, including evaporation and size dependant wall losses due to gravitational settling and diffusion. The evaporation of an aerosol can in principle be detected if the particle size can be measured at two different times before the particles achieve their equilibrium size for the ambient RH at the point of measurement. Therefore the expired aerosol should be analyzed at a well defined time after production so that these effects can be considered. Based on rates of evaporation for pure water, expiratory aerosols are assumed to evaporate very rapidly. This assumption should be tested if possible as the influence of the composition of

expiratory aerosols on evaporation rate is not known. Therefore aerosol size as a function of age should be examined for a range of sizes. Aerosol accumulation in a chamber was to be avoided because such accumulation produces a sample consisting of particles of widely varying age.

Avoidance of aerosol re-inspiration: The method used to obtain the aerosol sample must not involve re-inspiration of the aerosol because size dependant particle losses caused by deposition in the airways and alveoli will modify the original aerosol size distribution.

Real time response: The method should allow the detection of changes in the aerosol concentration in real time to be detected so that the influence of different expiratory activities on aerosol production can be investigated in real time. The length of some activities must be minimized to avoid stressing the volunteer. For example sustained coughing for period in excess of 30 s may be unacceptable to many volunteers. Therefore the aerosol may be available for characterization only briefly. Therefore it is important to be able to alternate between different expiratory activities including breathing, speech and coughing.

Isokinetic sampling: Instrumental and sampling methods for large particle aerosol sizing require isokinetic sampling. Therefore the aerosol sample collection and delivery system should be able to match the sample velocity to the instrument probe. Unfortunately, the velocity of air expelled from the mouth and nose during expiratory activities varies widely not only between activities, but between instances of an activity and even within a given instance of an activity over time. This raises the question of whether isokinetic conditions can be considered an achievable goal at all.

Flexibility of instrument accommodation: The detection limits for available instrumentation necessitate the use of up to four different methods in obtaining a comprehensive characterization of the aerosol. Therefore instruments and methodologies with differing physical requirements were to be accommodated.

Known dilution factor: In order to estimate the total expired particle number as a function of size it was necessary to include the capability to obtain total concentration as a function of size at the source. This could then be used in combination with either a measured or an assumed average expelled air volume to find total emission. In addition to the above physical requirements, a number of requirements facilitate the comfort and cooperation of the participants.

Safety and hygiene: Of primary concern is the issue of volunteer and investigator safety. The experimental design must meet all requirements for ethical clearance. This includes taking into account the need for protection from LASER radiation, and eliminating any possibility of exposure to infectious or toxic materials. Disinfection and cleaning between volunteers necessitates ease of access to the interior fittings.

Comfort: Given that low aerosol concentration may necessitate measurements of extended duration and many repeats, the physical comfort of the participants requires ease of volunteer access to the sampling rig and a comfortable seated position. To minimize psychological discomfort, the volunteer should have control over their physical environment and the freedom to autonomously disengage from the equipment. The equipment should also be as unintimidating as possible and should allow the volunteer to see what is going on around them. Volunteer boredom during

the measurement process can also be a significant problem so the volunteer should be free to engage in passive audiovisual entertainment activity provided it does not interfere with the experiment or compromise safety.

2.2 Expiratory Droplet Investigation System (EDIS)

Overview of the EDIS: The concept adopted for the EDIS is essentially that of a small wind tunnel into which a volunteer can comfortable place their head. Figure shows the basic design of the EDIS. HEPA filtered recirculating air is propelled by a HEPA-filter/fan module past the volunteers head at a low, controlled velocity. The particle free air carries any aerosol emitted by the volunteer to instrument sampling ports positioned inside the duct at a set distance downwind. The wind tunnel is divided into a series of interlocking modules supported on a rack mount. The modules are of different lengths and can be positioned in different ways to vary the configuration of the rig and the distance between the volunteer and instrument probes.

Detailed Description of the EDIS: The modular design provides the needed flexibility to accommodate a variety of instrumentation. Modules designed to accommodate different instrumentation and measurement techniques can be swapped in as required. Continuous variation over a range of several hundred millimeters of the distance between the volunteer and instrument location is achieved through the use of oversized openings sealed by flexible membranes. Furthermore, the spacing modules have lengths which are multiples of the smallest (100 mm). By varying the combination of modules between the volunteer and measurement point it is possible to vary the distance continuously in the range from 10 – 1500 mm. This approach facilitates easy access to all components for cleaning to address the hygiene criterion.

Air flow in the EDIS is generated by a custom made Fan/HEPA module (Rainbow Filters). This module consists of a blower fan, followed by a HEPA filter. Both are contained within a sealed housing, with 400 mm diameter inflow and outflow ports. The ports connect to opposite ends of the EDIS tunnel via sections of 400 mm diameter flexible metalized polyester ducting, thereby creating a closed loop. An adjustable butterfly valve on the inlet (low pressure) side of the HEPA blower module allows a controlled inflow of room air to the otherwise closed system. This filtered inflow of air pressurizes the duct slightly with respect to the environment outside the EDIS. The slight overpressure inside the duct ensures that any leakage through the boots is directed outwards rather inwards, thereby ensuring that no ambient aerosol enters the tunnel. The appropriate valve setting is established with a dummy volunteer in place, by introducing a test smoke at vulnerable points such as the boot seals to verify that the smoke is gently expelled at these points and not drawn into the duct. This continual inflow of ambient air also ensured that CO_2 concentration in the EDIS remained within acceptable limits. The recirculating configuration reduces both the impact of changes in the external relative humidity and temperature on the environment inside the EDIS as well as the filtration demands placed on the HEPA filter. This HEPA filtered air with overpressure feature ensures that the criteria of providing particle free background air and avoiding aerosol re-inspiration were both met.

Flow straightening modules, consisting of 35 mm thick honeycomb discs comprised of horizontally stacked 3.5 mm diameter polycarbonate tubes (Polycore PC3.5-90)

were located at both ends of the tunnel. These suppress residual velocity components generated by the air delivery system which can be perpendicular to the duct axis. The discs are covered by fine stainless steel wire mesh screens which induce slight pressure drops across the flow straighteners and thereby induce uniform airflow across the duct at both ends. Vortices induced by the presence of the volunteer are however unavoidable, and these will result in a degree of longitudinal mixing and consequent retardation and lengthening of the aerosol plume. By minimizing as far as practicable large scale inhomogeneities and non axial flows in the tunnel, the flow straighteners address the criteria for defined droplet age at the sample probe.

The volunteer module follows the upstream flow straightening module. The volunteer is seated below the module, positioned so that their head is centered inside. Head access is through an opening under the module. Not shown in the diagram is a motorized seat lift which allows the volunteer to elevate or lower themselves into and out of the duct using a hand control attached to the chairs arm-rest. This arrangement gives the volunteer the security of knowing they are always in control of their situation thereby addressing the safety and comfort criteria.

The volunteer module is followed by three spacer modules and an instrument module arranged in any order to achieve a desired distance between the volunteer and instrument probe. The instrument module accommodated the Aerodynamic Particle Sizer (APS) measurements discussed below and also provided access for a hygrometer.

All access ports including the volunteer module were equipped with membranes to inhibit air loss. In the case of the volunteer access port, the membrane was lightly weighted at the lower hem to ensure that it remained in contact with the volunteer's upper body, to minimize leakage without causing discomfort to the volunteer.

All tunnel modules are composed of clear cast acrylic (polymethyl methacrylate). Static charge on the tubing is minimized by treatment with an optically clear antistatic cleaning fluid. The choice of a clear material for the duct maximizes visibility both for the comfort of the volunteer and as an aid to the investigators in monitoring volunteer behavior, thereby addressing the criteria for psychological comfort and safety. All design criteria were therefore addressed.

2.3 Instrumentation

The EDIS can accommodate a range of instrumentation; however specific design measures were taken to allow for the APS TSI model 3312A). Although the EDIS can physically accommodate size distribution measurements in the lower sub-micrometer range using the Scanning Mobility Particle Sizer (TSI SMPS) such measurements were found to be impractical because of the excessive times intervals needed to obtain a useful size distribution when concentrations are very low. With a typical starting concentration of less than 0.4 cm^{-3} the SPMS will detect a total of less than four particles per 2 minute activity.

The APS measures aerodynamic diameter of particles in the diameter range 0.5-20μm and detects particles as small as 0.3 μm.

Additional instrumentation included a relative humidity probe (Hygropalm Hycroclip) incorporated into the APS probe tube to determine the water vapor concentration in the sample stream, hot wire anemometer probe (TSI Velocicheck™ 8340) which determined the air velocity in the duct during aerosol measurements and a digital oscilloscope used to determine delay between aerosol emission and sizing.

The results reported here are for droplets in the size range below 20 μm. They were obtained using the APS with the EDIS.

2.4 Measurement of transit times from source to measurement

Transit times for the aerosol were determined by measuring the delay between the commencement of a pulse-like expiratory activity and the detection/measurement of particles produced by the activity. To achieve this, a volunteer was asked to perform a momentary cough or burst of a voiced expiratory activity which can be described as the briefest possible vocalization of the "word" "aah". The sound was used to trigger a timed recording of the analog output of the APS particle detection signal. For this purpose the volunteer was fitted with a lapel microphone from which the signal was amplified and applied as the trigger input to a digital storage oscilloscope (CRO). The CRO then produced a trace of the particle detection signal from the APS analog output which was recorded at high temporal resolution. The trace was examined to identify pulses representing particles detected by the APS and the time delay between the commencement of the trace and the pulses was accurately measured. Accuracy was maximized by using the briefest possible vocalizations in the case of speech and in the case of coughing single isolated coughs, in each case waiting for all particles to be removed before repeating the process. Nevertheless a number of particles were detected over an extended period for each sound. This is due to turbulence and boundary layer entrapment delaying some particles.

These measurements yield a minimum particle age of 0.79±0.1s for aerosols produced 10 mm from the entrance to the sample probe. This measurement is based on the first particles to be detected after the vocalization.

2.5 Dilution correction of concentrations

Size distributions for the expiratory activities were corrected in terms of concentration for dilution by using a factor derived using water vapor as trace gas. The water vapor concentration was determined continuously in the sample stream at the APS probe inlet during each activity. The regular bypass (bp) activity provided the background water vapor concentration. The water vapor concentration at the source was derived from reported values of relative humidity and temperatures in the expiratory tract during expiration as summarized in Table .

The dilution factor (D) for the aerosol sample at the probe was calculated using Equation 1 and Equation 2.

Equation 1: Calculation of the sample dilution factor from water vapor concentration

$$D = \frac{AH_0}{AH_s - AH_{BG}}$$

where

AH_0 = water vapor concentration in the respiratory tract

AH_s = water vapor concentration in the sample

AH_{BG} = background air water vapor concentration in the EDIS

Equation 2: Calculation of absolute humidity or water vapor concentration from relative humidity and temperature

$$AH(T, RH) = \frac{RH}{100}\left(\frac{p_{sat} MW_{H_2O}}{RT}\right)$$

RH = relative humidity (%)

$p_{sat}(T)$ = saturation vapour pressure of water at T

MW_{H_2O} = molecular weight of water

R = gas constant

T = temperature (K)

2.6 Size correction to approximate initial size

The equilibrium size distribution of a hygroscopic aerosol (eg one containing water soluble salts) depends on the RH provided the aerosol has deliquesced. Deliquescence will certainly have occurred if the aerosol was generated from an aqueous solution and the RH has not fallen below the aerosols efflorescence RH. The tendency of a hygroscopic aerosol droplet to be larger than its dry constituents at equilibrium is often referred to as hygroscopic growth (Katoshevski et al. 1999; Johnson et al. 2005). Hygroscopic aerosols show decreasing hygroscopic growth as the RH deceases.

A simplified model of hygroscopic behavior has been adopted by some researchers for expiratory aerosols. For example Nicas et al.(Nicas et al. 2005) assumed that the equilibrium size is always (for 30%≤RH≤70%) one half of the initial diameter, regardless of the specific RH. Nevertheless the authors of the current study consider the incomplete existing data on the hygroscopic behavior of respiratory fluid aerosols to be far too uncertain to apply a meaningful size distribution correction. The size distributions presented in this manuscript are therefore shown with droplet sizes as they were measured, ie at lower RH than occurs in the expiratory tract. Therefore the size distributions do not include the hygroscopic growth that would exist in that saturated environment.

2.7 Modal diameter versus age of aerosol

Existing instrumentation does not permit two successive size measurements for a specific droplet so measurements intended to detect the non-equilibrium evaporation process, were therefore conducted on separate aerosol samples. The size distribution of the aerosol for a given activity is variable, so repeated measurements are needed to reduce the error of the mean aerosol size distribution in order to conduct a meaningful comparison of the size distribution at two different droplet ages. Furthermore the

uncertainty in the age of the droplet is increased by the turbulence near the volunteer's face. This manifests as an increase in the time taken for an aerosol bolus to pass the APS intake probe. Such a range of ages will produce a broadening of the size distribution if evaporation is still occurring.

Assuming that it is possible to overcome these difficulties by conducting a sufficient number of repeated measurements the experimenter is still faced with the fact that differences in the position of modes in the average size distribution for a given activity can occur because of RH differences at the two distances.

Provided the ambient air temperature is not very low, RH decreases as distance from the volunteer increases, due to dilution of the aerosol water vapor content by somewhat warm ambient air. If the aerosol is slightly hygroscopic, as would be expected due to the natural presence of salts such as NaCl in bodily fluids, the equilibrium droplet diameter will vary with the RH at the point of measurement when the RH is above the deliquescence RH of the solute. Therefore, a direct conclusion cannot be reached from measurements of the effect of time delay on modal diameter without first establishing the magnitude of the hygroscopic effect in such measurements.

We did not attempt to measure the hygroscopic behavior of the aerosol directly. Nevertheless, conclusions concerning the equilibrium of droplet size with respect to RH during size distribution measurements conducted at two distances (and hence different RH) can be arrived at for a multimodal aerosol by comparing the changes in position of two modes if we assume that the hygroscopic property of the aerosol is similar at all sizes. This approach is discussed in the results section.

2.8 Measurement Protocol

The study was fully scrutinised and cleared by QUT's Human Research Ethics Committee. Fifteen volunteers were recruited on the basis of age (≤35 years), via a broadcast email invitation offering a small financial reward. They were instructed to self exclude if they were smokers, experiencing illness, asthma sufferers, had recently experienced expiratory problems or were likely to experience discomfort in confined spaces.

The following expiratory activities were defined. As discussed further in the results, these activities emphasize and contrast different aerosol production processes occurring during normal expiratory processes:

- b-n-m: breathing in through the nose and out through the mouth
- b-n-n: breathing in through the nose and out through the nose
- aah-w-p: un-modulated whisper (whispered "aah") with regular pauses for recovery to prevent drying of the mouth or labored breathing.
- aah-v-p: un-modulated vocalization (voiced "aah") with regular pauses for recovery
- c-w-p: whispered counting with regular pauses for recovery
- c-v-p: voiced counting with regular pauses for recovery
- cough

2.9 Statistical Analysis

Comparisons were made using parametric or non-parametric statistical methods as appropriate. The 0.05 level of significance was used throughout and all reported p-values are two-sided unless otherwise stated.

3 Results

3.1 Modality and concentration versus activity

We divided the process of voiced speech into components with the potential to produce aerosol during speech. We recorded the aerosol size distribution associated with each of these elements by comparing the aerosol produced during different component combinations. The components are:

1. The exhalation of breath. Breath contains aerosol produced simply by breathing. Production of droplets in the expiratory airflow could occur anywhere in the expiratory tract either during inspiration or expiration.
2. Exhalation through partially adducted vocal folds (whispered or un-voiced "aah"). The narrowing of the opening between the mucus bathed folds results in aerosol production due to the increased air velocity at this restriction during exhalation. The aerosol size distribution in this case will include any aerosol from normal breathing as well as any from the vocal apparatus.
3. Aerosol is produced during exhalation through the vibrating mucus coated vocal folds during vocalization. The aerosol size distribution in this case may be a combination of normal breath aerosol, vocal fold adduction aerosol and vocal fold vibration aerosol.
4. Exhalation during movements of the mouth lips and tongue also produces aerosol from the saliva which is always present in the mouth. Also present will be breath aerosol, vocal fold adduction aerosol, and vocal fold vibration aerosol, depending on whether the subject whispers or voices words during the activity.

Representative particle number size distributions for each activity was found by averaging over all measurements for all subjects after correcting for dilution to show concentration in the upper expiratory tract during expiration T_0 = 37 ˚C and RH_0 = 100 % (Marini and Slutsky 1998). The resulting particle concentrations and size distributions for each activity, are presented in the Figure 2 and 3.

The approach to the analysis was designed to explore the viability of the hypothesis that all expiratory size distributions are a composite of aerosol size distributions produced by the physical components of an expiratory activity, i.e. breathing, vocal chord vibrations and mouth movements. We propose that the intensity of the contributions from each size distribution is related to the intensity of the corresponding physical component in the activity.

A finite mixture model is a weighted combination of two or more statistical distributions. They can be used to simply and flexibly describe heterogeneous data or classify observations into defined subgroups. For example, skew data can be represented as a mixture of normal or t distributions with different means and variances, or as a mixture of normal and gamma distributions. Based on frequentist or Bayesian approaches, observations can be probabilistically assigned to the different distributions(McLachlan and Peel. 2000; MARIN et al. 2005).

Attempts to fit mixture models used the three point smoothed data in order to reduce the influence of measurement noise, while retaining the gross features of the size distribution. As a first step to fitting a mixture model, the most complex of the activities in terms of modal structure c-v-p was examined visually to determine the number of modes required to achieve a good overall fit (R^2>0.98) of a multimodal Gaussian model. Figure 4A shows the PSD for c-v-p aerosol. Four distinct modes (A, B, C and D) were identified, permitting a fit with R^2=0.998. The strengths of the modes are indicated by the area under the size distribution mode representing number concentration.

The process was then repeated with the simplest activity of breathing, initializing the fitting process with the mode parameters developed in the c-v-p case. Figure 4B shows the PSD for bnm. At this point it can be seen that modes B, C and D are more prominent during the vocalized activity.

Figure 4C shows the fit for aah-v-p aerosol. Modes B, C and D are very prominent which is consistent with the fact that vocalization is much more continuous and therefore more active during aah-v-p than during c-v-p.

Good fits were obtained with the four mode model for all activities. Figure 5 summarizes the locations and areas under the four modes for each activity and also shows the average total concentration recorded by the APS ($0.3 \leq D \leq 20\mu m$). The locations of the modes vary by less than 23% in terms of diameter across all activities. Mode A includes both aerodynamically sized particles and particles which although detected by the APS, were too small to be measured for aerodynamic diameter.

Mode B which is more active during the vocalized activities is also very active during coughing. During speech, the vocal folds, which are bathed in lubricating mucus, vibrate vigorously. Physiologically, a cough is produced by abruptly parting the vocal chords to allow a sudden expulsion of air. It is possible that during both of these processes aerosolization of the mucus is responsible for the 1.8μm mode in Figure 5. Modes C and D are also higher during vocalized activities but less so during coughing, suggesting that they may be activated primarily by vocalization.

Mode A is the most prominent in all activities particularly in relative terms during unvocalized activities as is particularly obvious in Figure 5B Mode A must therefore arise from the simple process of breathing, however some of the variations require explanation. For example, why did aah-w-p produce much higher mode A concentration than b-n-m. The answer lies in the location of the mode near the lower boundary of the APS range.

In addition to individually measuring the size of particles, the APS also reported large numbers of additional particles which were detected, but which were too small for a successful diameter measurement to be obtained. It is therefore likely that much of mode A actually lies just below the APS range. The APS response declines as the lower range limit is approached so that a tail similar to that of a Gaussian mode is expected on the left side of a range-truncated mode. For such a mode, the area in the APS range will be highly sensitive to any movement of that modes central diameter. As discussed previously, aerosols produced from bodily fluids are expected to be somewhat hygroscopic, due to their soluble salt content. Examination of the relationship between the area under mode A and RH revealed a significant correlation.

This implies that during high RH conditions mode A moved more fully into the APS size range producing higher concentration reading from the APS.

In summary, strong differences in the mode A concentration as derived from the APS data, may reflect small changes in modal position due to differences in the conditions of inhalation, exhalation or sample RH rather than the amount of aerosol produced by the process itself.

Based on the above, we propose the following four mode model of expiratory aerosol production in the size range below 20 μm:

Mode A is a breath mode which is produced either during inhalation or exhalation for all expiratory activities.

Mode B, C and D, are produced by energetic movements of the vocal folds. They are most prominent during speech and coughing. The modes would also be expected during sneezing, which is physiologically similar to coughing. The activity and perhaps also the central diameter of the mode are expected to show some dependence on the frequency of vibration and therefore on the vocal pitch.

Clear evidence of a droplet mode associated with mouth movements was not found although it is possible that the associated modes occur at larger droplet sizes, outside the APS range, or where the number concentrations are too small for the modes to be significantly resolved in the sample sizes considered here.

3.2 Modal diameter versus droplet age

As discussed previously, droplet age in the EDIS increases with distance from the subject to the probe. Figure 6 shows the dilution corrected droplet size distribution for "aah-v-p" aerosol collected at two distances from the probe. The aerosol RH at the probe is shown also. The aerosol modal diameter clearly decreases with increasing distance. This effect may result either from differences in the equilibrium diameter of the droplets due to RH differences at the two locations or from evaporation toward equilibrium (non-equilibrium evaporation).

Figure 6 also shows a multimodal lognormal fit to the data. Denoting the modes from smallest to largest as A, B and C the diameter reduction factors (0.04, 1.6, 1.5 respectively) for modes B and C are similar, as would be expected if the two modes remained in hygroscopic growth equilibrium at each measurement and had similar hygroscopic growth properties. In the case of mode A, the location of the mode cannot be accurately determined because the mode is only partially inside the APS range. Note also that apart from mode A, which is even further outside the APS size range at low RH, the particle concentration (area under the mode) associated with corresponding modes is similar at the two RH's, indicating that no migration of droplets is occurring between modes. Although the D Mode discussed in the previous section is clearly visible at 10 mm, mode D was not fitted. This is because no droplets associated with mode D were detected at 300 mm where the dilution factor was 81. The main reason for this lack of droplets is that the aerosol concentrations in that mode are very low, and the much greater dilution factor (81) occurring at a distance of 300 mm when compared with that immediately in front of the probe (5.3) resulted in a complete absence of particle counts being recorded where mode D should appear.

At the minimum achievable distance of 10 mm, the approximate time taken for the aerosol to reach the APS sizing region is 0.8 s, consisting mostly of the time taken to travel along the probe tube and through the APS nozzle assembly. A 5μm droplet of pure water evaporates in 0.8s at 97% RH and a 3μm droplet will do so in less than 0.33 s. Thus unless the solutes greatly retard the evaporation process, 0.8 s may be sufficient for the solute laden droplets to achieve their equilibrium size distribution even at the highest RH encountered at the probe mouth. Evidence that the aerosol remained in equilibrium, can be discerned from the modal structure of the aerosol. Where the RH increases too fast for the aerosol to remain in hygroscopic growth equilibrium; the modes will merge as the small diameter modes overtake the slower growing larger modes. Subsequent stabilization or reduction in RH will allow the modes to again separate. Such an increase and subsequent decrease occurs as the exhaled air firstly reaches the cooler upper reaches of the expiratory tract and is then released from the mouth or nose to be diluted in ambient air. The extent of these modal spacing changes will depend on the residence time in the supersaturated condition.

In summary, a multimodal aerosol preserves its equilibrium modality during slow RH changes that maintain equilibrium, but the modes will separate during non-equilibrium evaporation and merge during the reverse situation of non-eqilibrium growth due to condensation.

Thus if a multimodal aerosol is found to preserve its equilibrium hygroscopic growth modality at all times throughout a measurement, then the aerosol can be considered to have been in equilibrium at each measurement and non-equilibrium evaporation can not be said to have been observed or measured.

3.3 Comparison with existing work

The results of the current study can be compared with the results of others by examining Table 2.

The results from this study agree on a number of points with the findings of Papineni and Rosenthal (1997). The majority of droplets from human expiratory activities are very small, being in low micrometer and high sub-micrometer ranges. Where Papineni and Rosenthal found that 80-90% of droplets were smaller than 1μm, the current study agrees, showing that these smallest particles are located within an aerosol mode, centered in the range 0.1-1 μm but that the precise location depends on the ambient RH. The study also confirmed that averaged over subjects, the lowest concentrations are obtained from breathing and that coughing results in more particles being expelled than mouth breathing.

The current study results are more consistent with those of Papineni and Rosenthal (1997), Edwards et al.(2004) and Fairchild and Stampfer (1987) than with the results obtained by Yang et al.(2007). The concentrations are however much higher than those of Papineni and Rosenthal and lower than those of Edwards et al. comparing most favorably with those of Fairchild and Stampfer.

Fairchild and Stampfer sampled aerosol from a respirator mask using a laser aerosol spectrometer drawing a sample flow rate of 60 $cm^3.min^{-1}$. The extent to which this sample may have been diluted by the purified air is dependent on the flow rate of air into the respirator mask. No discussion of this point or of dilution correction appears

in the paper. If clean air entered the mask at the same rate at which air was withdrawn (by the subject inhaling plus the instrument flowrate) then the instrument would measure the concentration of the breath although there would be intervals (during inhalation) when the air inside the mask was replaced by filtered air giving rise to some dilution.

It is important to note that the sampling methodology used to determine concentration in the study by Papineni and Rosenthal is questionable, a fact acknowledged in that paper. The authors stated that concentration was determined by continuously measuring total particle count (not concentration) over a series exhaled breaths and dividing this number by the breath volume. This approach would only be accurate if the particle counter drew the all of the exhaled breath. The authors stated that emissions from the mouth were directed into a funnel attached to the particle counter but that the counter drew air at a fixed flow rate of 7 Lpm. The exhalation rate of a typical breath can easily exceed this by an order of magnitude, so it is likely that only a small fraction of the exhaled breath was analyzed, leading to an understatement of the true concentration by an order of magnitude.

The results of Yang et al.(2007) are highly questionable. The aerosol in the respiratory tract is at equilibrium having the same concentration and temperature as the surrounding respiratory fluid from which it was generated. The process where the authors of that study transferred this warm and water vapor saturated aerosol to a dry bag at room temperature would have involved a severe disturbance of the water partitioning and therefore to the original equilibrium size of the aerosol. With the ambient temperature surrounding the bag being lower than that in the respiratory tract, water in the supersaturated sample would have moved from the gas phase to the liquid. This would have occurred on any available surface including the aerosol and the walls of the bag. The size distribution measured would therefore have been much larger than that of the aerosol in the respiratory tract. It is even possible that homogenous nucleation occurred, dramatically increasing the number of droplets. The concentrations reported were many orders of magnitude larger than those reported in other recent studies (Papineni and Rosenthal 1997; Edwards et al. 2004). This may have been due to contamination of the sample with room air. A type of filter mask was used in some experiments but not all experiments, but the low concentrations that have typically been observed by researchers, imply the absolute necessity of a virtually perfect HEPA filtered breathing environment to prevent ambient aerosol contamination from dominating the measurement.

In summary the results of the current study represent a very significant advance in the current knowledge of expired aerosol size distributions and their sites of origin for a range of important expiatory activities. The methodology employed is a significant improvement over previous methods, offering considerably greater accuracy and permitting an account of the aerosol dilution so that concentrations can be determined at the source.

Acknowledgments
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References

Duguid, J. P. (1945). "The numbers and the sites of origin of the droplets expelled during expiratory activities." Edinburgh Medical Journal LII(II): 385 - 401.

Edwards, D. A., Man, J. C., Brand, P., Katstra, J. P., Sommerer, K., Stone, H. A., Nardell, E. and Scheuch, G. (2004). "Inhaling to mitigate exhaled bioaerosols." Proc Natl Acad Sci U S A. 101(50): 17383-8.

Fairchild, C. I. and Stamper, J. F. (1987). "Particle concentration in exhaled breath." Am. Ind. Hyg. Assoc. J. 48: 948.

Hickey, A. J., Ed. (1996). Inhalation aerosols : physical and biological basis for therapy. Lung biology in health and disease, Marcel Dekker Inc.

Jennison, M. W. (1942). "Atomizing of mouth and nose secretions into the air as revealed by high speed photography." Aerobiology 17: 106-128.

Johnson, G. R., Ristovski, Z. D., D'Anna, B. and Morawska, L. (2005). "The hygroscopic behavior of partially volatilized coastal marine aerosols using the VH-TDMA technique." Journal of Geophysical Research 110.

Katoshevski, D., Nenes, A. and Seinfeld, J. H. (1999). "A study of processes that govern the maintenance of aerosols in the marine." Journal of Aerosol Science. 30(4): 503-532.

MARIN, J.-M., MENGERSEN, K. and ROBERT, C. P. (2005). Bayesian Mixtures. Handbook of Statistics. D. Dey; and C. R. Rao, Elsevier. 25: 50.

Marini, J. J. and Slutsky, A. S. (1998). Physiological Basis of Ventilatory Support, Informa Health Care.

McFadden, E. R., Pichurko, B. M., Bowman, H. F., Ingenito, E., Burns, S., Dowling, N. and Solway, J. (1985). "Thermal Mapping of the Airways in Humans." J. Appl. Physiol. 58: 564-70.

McLachlan, G. J. and Peel., D. (2000). Finite Mixture Models., Wiley.

McRae, R. D. R., Jones, A. S., Young, P. and Hamilton, J. (1995). "Resistance, humidity and temperature of the tracheal airway." Clinical Otolaryngology 20(4): 355-356.

Nicas, M., Nazaroff, W. W. and Hubbard, A. (2005). "Toward Understanding the Risk of Secondary Airborne Infection: Emission of Respirable Pathogens." Journal of Occupational and Environmental Hygiene 2(3): 143 - 154.

Papineni, R. S. and Rosenthal, F. S. (1997). "The size distribution of droplets in the exhaled breath of healthy human subjects." Journal of Aerosol Medicine 10(105-116).

Wells, W. F. (1934). "On air-born infection. Study II. Droplet and droplet nuclei." American Journal of Hygine 20: 611-618.

Yang, S., Lee, G. W. M., Chen, C.-M., Wu, C.-C. and Yu, K.-P. (2007). "The Size and Concentration of Droplets Generated by Coughing in Human Subjects." Journal of Aerosol Medicine 20(4): 484-494.

List of Tables & Figures

Figures

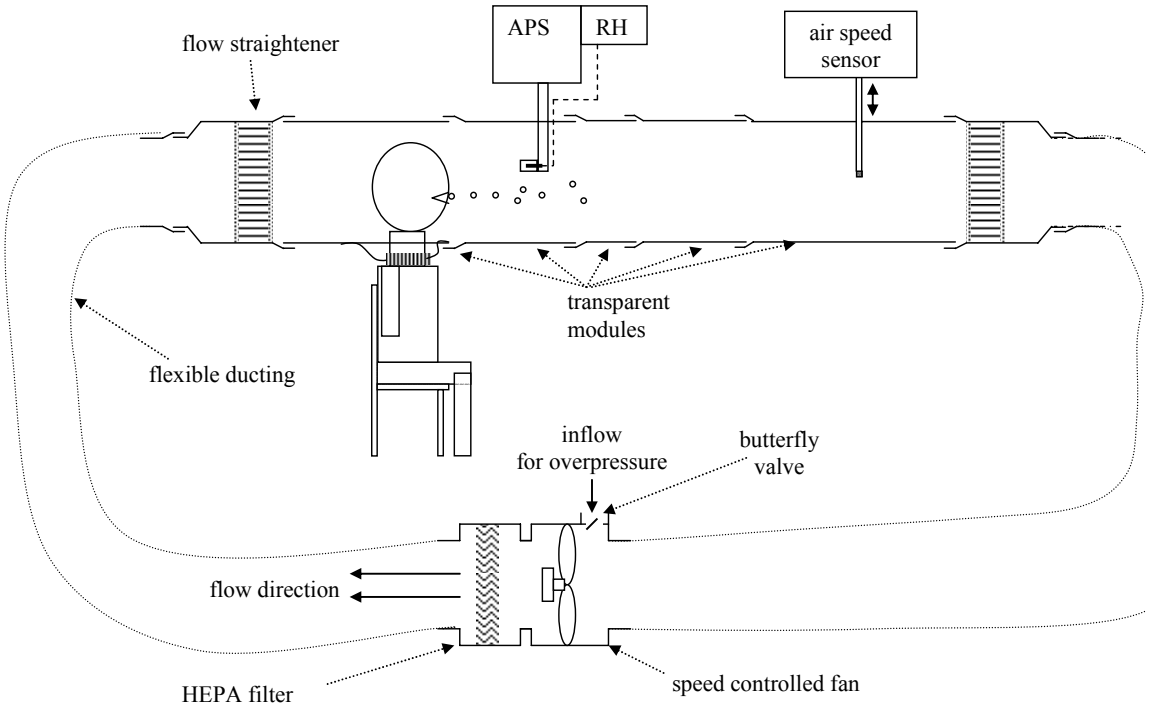


Figure 1

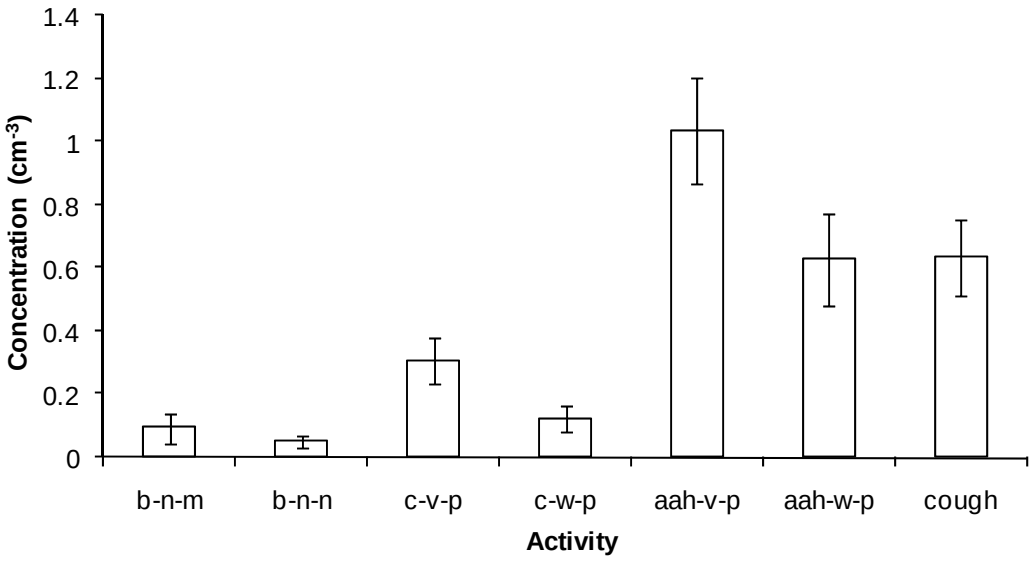


Figure 2

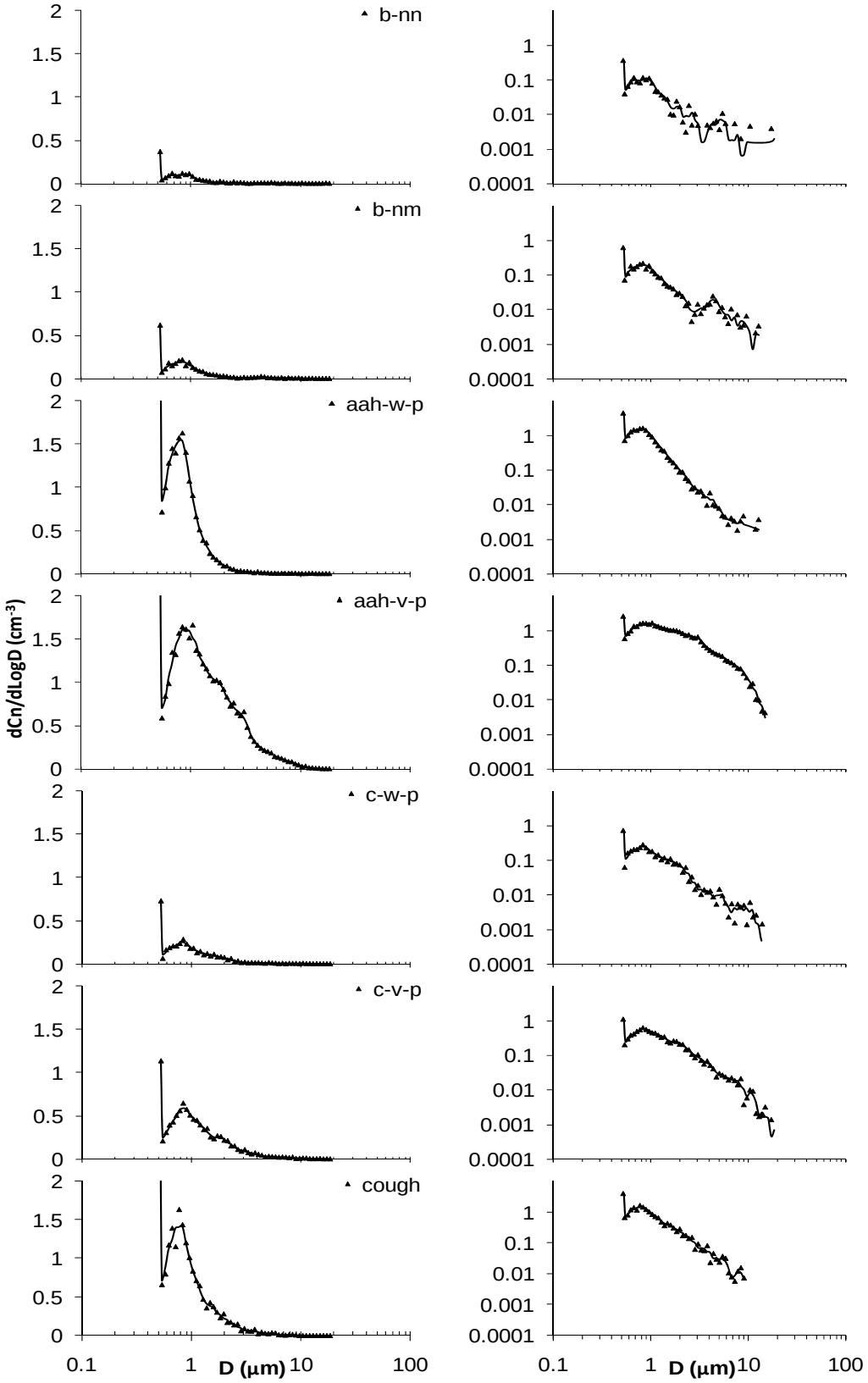


Figure 3

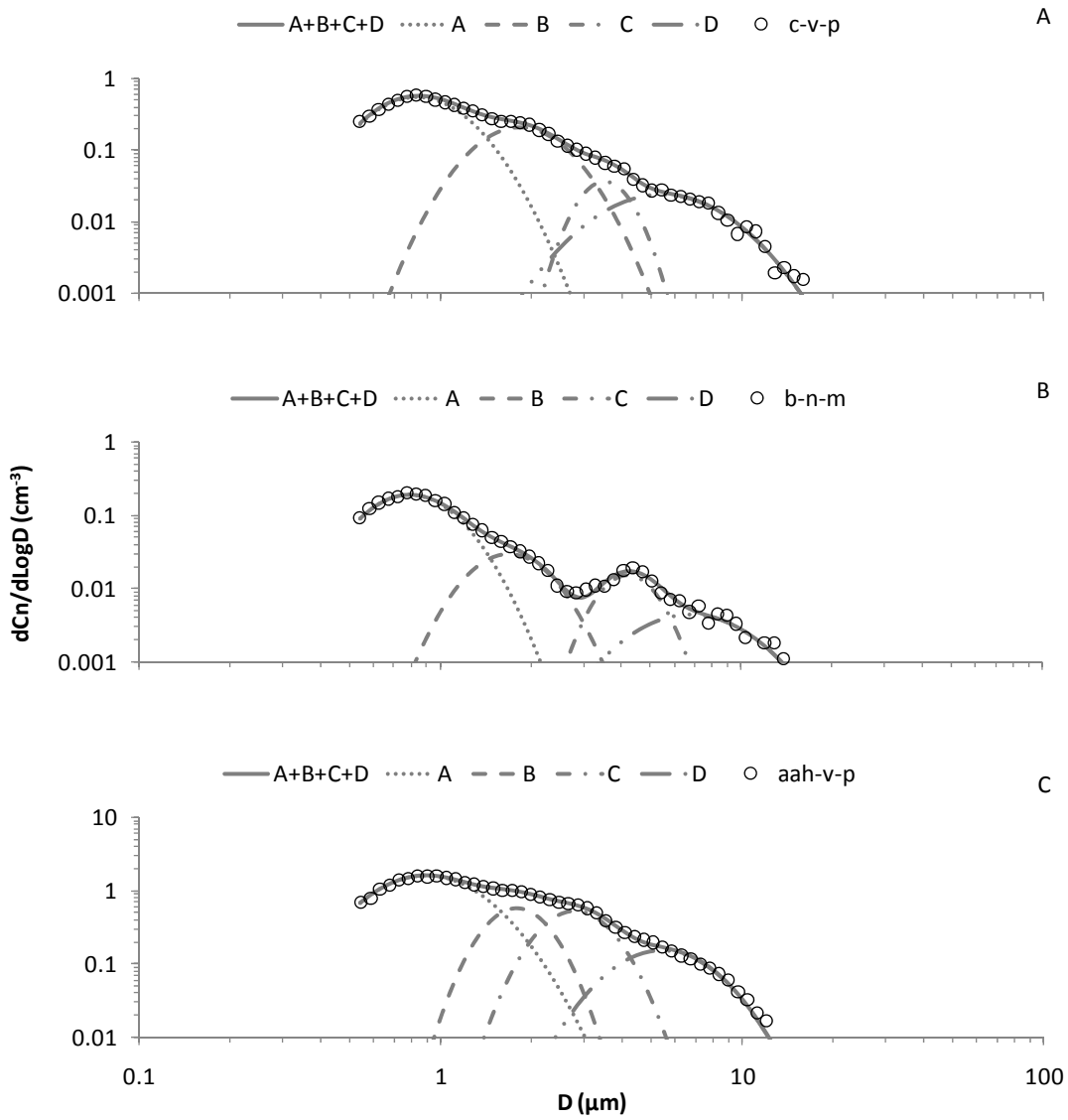
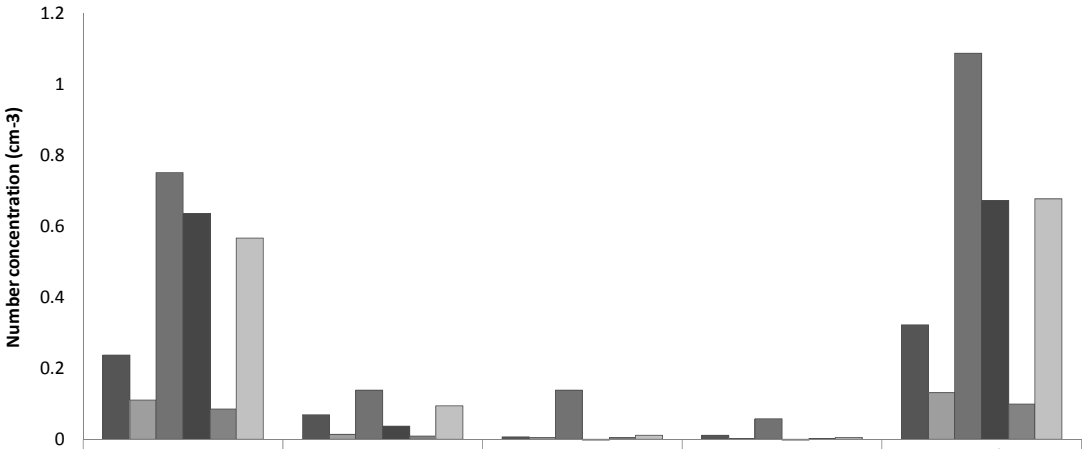

A+B+C+D
A
B
C
D
c-v-p
A
1
0.1
0.01
0.001
A+B+C+D
A
B
C
D
b-n-m
B
dCn/dLogD (cm-3)
1
0.1
0.01
0.001
A+B+C+D
A
B
C
D
aah-v-p
C
10
1
0.1
0.01
0.1
1
10
100
D (µm)

Figure 4



	A, CMD≤0.80±0.08 µm	B, CMD=1.8±0.3 µm	C, CMD=3.5±0.7 µm	D, CMD=5.5±1 µm	Total
cvp	0.236	0.068	0.007	0.011	0.322
cwp	0.110	0.014	0.004	0.002	0.130
aahvp	0.751	0.139	0.139	0.059	1.088
aahwp	0.636	0.037	0.000	0.000	0.672
bnm	0.084	0.009	0.003	0.002	0.098
cough	0.567	0.093	0.012	0.006	0.678

Figure 5

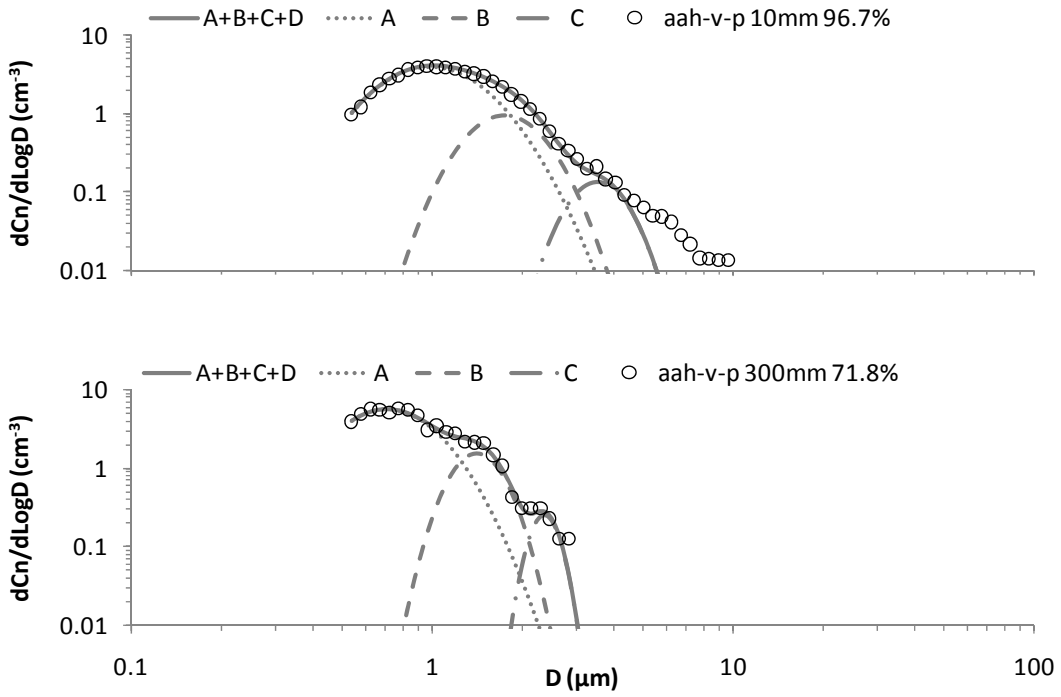


Figure 6

Tables

Table 1

Location	**RH (%)**	**T (˚C)**	**AH ($kg.m^{-3}$)**	**Source**
Trachea during expiration (Normal upper expiratory tract)	99.2 (98.8-99.7)	36.2 (35.8-36.4)	$4.2x10^{-2}$	(McRae et al. 1995)
Upper airway	100	37	$4.4x10^{-2}$	(Marini and Slutsky 1998)
Upper Airway	*100*	37	$4.4x10^{-2}$	(McFadden et al. 1985)

Table 2

Concentration (cm^{-3})

Activity	This Study[§]	Papineni and Rosenthal(Papineni and Rosenthal 1997)[*]	Edwards et al. (Edwards et al. 2004)	Yang et al. (Yang et al. 2007)[‡]	Fairchild and Stampfer(Fairchild and Stamper 1987)[*]
Coughing	0.64[#]	0.024-0.22		881-2355	0.6
Breathing	0.092 b-n-m 0.05 b-n-n	0.0027-0.033 mouth 0.001-0.0125 nose	0.014-0.32		0.1 cm^{-3}
Speech	0.307 c-v-p	0.0063-0.0355			0.6
Modal Diameters (μm)					
Breathing, coughing and speech	≤0.8, 1.8, 3.5, 5.5	≤0.8, 1-1.5[**]		1-2[†]	

[§] Average concentration, corrected for dilution. [#] Average cough interval: 1.2 s. [*] result was not corrected for sample dilution. [‡] Possible contamination of sample by ambient aerosol. [**] Based on visual inspection of Fig 5 in Papineni and Rosenthal (1997). [†] Droplet nuclei.