

A comparative study of two different Microbial Air Monitoring Methods: Conventional and Sequential Sampling.

THIS STUDY WAS ACHIEVED IN COLLABORATION WITH

MBV AG

Januar 2006

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Introduction.

The inspectors of the FDA (Food and Drug Administration of the USA) recommend and insist on the necessity to increase the number and the duration of microbiological air sampling per day or as function of specific activities. The difference in quality of Petri dishes (thickness, type of culture medium, rate of drying) influences the efficiency of colony formation as compared to the actual concentration of micro-organisms in the air. Human presence during the air sampling (personal contamination) may also considerably affect the number of colony forming units (CFU). The present study aims to test different influencing parameters on colony formation and to compare two different air sampling methods: conventional and sequential air sampling.

Conventional air sampling consists in collecting air samples at a certain time of the day on one or several Petri dishes. This operation is performed in the presence of a technician. If several samples have to be collected during the day, then this operation is to be repeated at the chosen times of the day on different Petri dishes. The presence of a technician is required for every operation. Fluctuations of the microbiological concentrations in the air in between the times of the sampling can not be measured.

Sequential air sampling consists in taking several samples of microbiologically contaminated air at regular intervals during the whole day or over a 24 hours period. Human presence is only necessary at the beginning and at the end of this period. Samples can be collected on a single Petri dish at time intervals pre-programmed in the microbiological air sampler. Typically 10 or 20 air samples can be collected over a period of 8 to 12 hours (work day) on a single Petri dish.

1 Specific aims.

Since the efficiency of microbiological air sampling in the sequential mode on a single Petri dish could be highly influenced by the drying conditions of 10 to 20 air collections over a prolonged time of sampling (8 to 24 hours), we studied systematically, in a first series of experiments, the rates of drying of Petri dishes in five different situations. The rate of drying was measured as a function of time (8 to 48 hours), as a function of the volume of

the medium (15, 20, 25 and 30 ml of medium per Petri dish), as a function of temperature (variable between 23.4°C and 31.5°C), as a function of the volume of air collected (typically more than 1 m³ per Petri dish) and finally as a function of the number of holes in the grid of the microbiological air sampler (400 holes usually or 300 holes more rarely).

In all cases, the culture medium was the same, i.e. Trypton Soybean Agar (TSA) generously offered by HEIPHA Dr Müller in automatically calibrated volumes of freshly poured CASO Petri dishes. All tests were performed before the date of expiration indicated on the delivered Petri dishes. Packaging conditions are such that mass loss before the date of expiration was negligible.

In a second series of experiments we compared the efficiency of the conventional air sampling method with the efficiency of the sequential air sampling method in a rather highly microbiologically contaminated environment (after the presence of 20 to 25 students in a 100 m³ lecture room) and in a very low contaminated clean room (weighing room for chemical substances used in the pharmaceutical industry).

2 Materials and methods.

The society MBV lend us six microbiological air samplers of which three functioned in the conventional mode and three others in the sequential mode. All Petri dishes were poured with, respectively, 15, 20, 25 or 30 ml of CASO agar, the composition of which is: 15 g of casein peptone, 5 g of soya peptone, 5 g of NaCl and 15 g of agar per litre (pH: 7.30 +/- 0.2). Temperature, relative humidity, atmospheric pressure and time of exposure were routinely registered. The different experiments were carried out at the University of Applied Sciences of Geneva or in a pharmaceutical industry in Geneva. Usually the volumes of air collection were estimated in order to obtain less than 300 CFU/Petri dish. The rate of drying was measured by the variation of mass per time with a Mettler model PM460 balance.

Incubation after microbiological air sampling was carried out for four to five days at room temperature. Colony Forming Units (CFU) were counted directly on the Petri dish and

corrected by Feller statistics (CFU*). The concentration of microbes in the air is expressed in CFU*/m³. All Petri dishes with air collected micro-organisms were recorded with a Sony DSC 75S digital camera.

Statistical analysis was carried out either with the statistical functions of Microsoft Excel 2002 or with Sigmastat 3.1 and Sigmaplot 9.01 software. Averaging, linear regression, single and paired t-Student test and ANOVA (analysis of variation) were the most usual types of statistical analysis. Variations are expressed in all cases with a confidence interval of 95% (CI 95% in Sigmastat 3.10).

3 Rate of drying experiments.

The rate of drying of Petri dishes was measured under four different conditions and on several volumes of culture medium. The four conditions are respectively: Petri dishes that are covered or closed (with plastic cover), Petri dishes that are uncovered or open (without plastic cover), Petri dishes that are covered with a 400 or 300 hole grid and Petri dishes that are covered with a grid and exposed to aspiration of air (1 m³/hr at two frequencies: 1 m³ every 60 minutes or 0,1 m³ every 6 minutes). In all experiments the meteorological conditions were registered (date, time, temperature, relative humidity and atmospheric pressure). Usually, six Petri dishes and six Microbiological Air Samplers (MAS) were used in parallel in order to improve statistical analysis. Six parallel measures in the same conditions are also called one series of measures. This means that one series of measurements can contain up to 72 measures (six Petri dishes over a 12 hours period). Table 1 summarizes all "rate of drying experiments".

Table 1: Rate of drying experiments.

Closed	Open	With grid Without aspiration	With grid With aspiration
15 ml: 2 series	15 ml: 5 series	15 ml: 2 series	15 ml: 2 series
20 ml: 1 series	20 ml: 1 series		
25 ml: 1 series	25 ml: 1 series		
30 ml: 3 series	30 ml: 5 series	30 ml: 2 series	30 ml: 2 series

Most frequently we used the two extreme volumes of culture medium, i.e. 15 ml and 30 ml in order to detect possible significant differences. 20 ml and 25 ml volumes of culture medium were only tested in one series for each.

3.1 Drying rate for different volumes of culture medium in closed Petri dishes.

Six Petri dishes with respectively 15, 20, 25 and 30 ml of culture medium were unpacked, their plastic covers remained in place and their mass was measured at regular intervals of time. The physical parameters were: room temperature between 23.1°C and 25.2°C, relative humidity between 31% and 37%, atmospheric pressure between 1012 and 1018 hPa.

Rate of drying on 15 ml closed Petri dishes

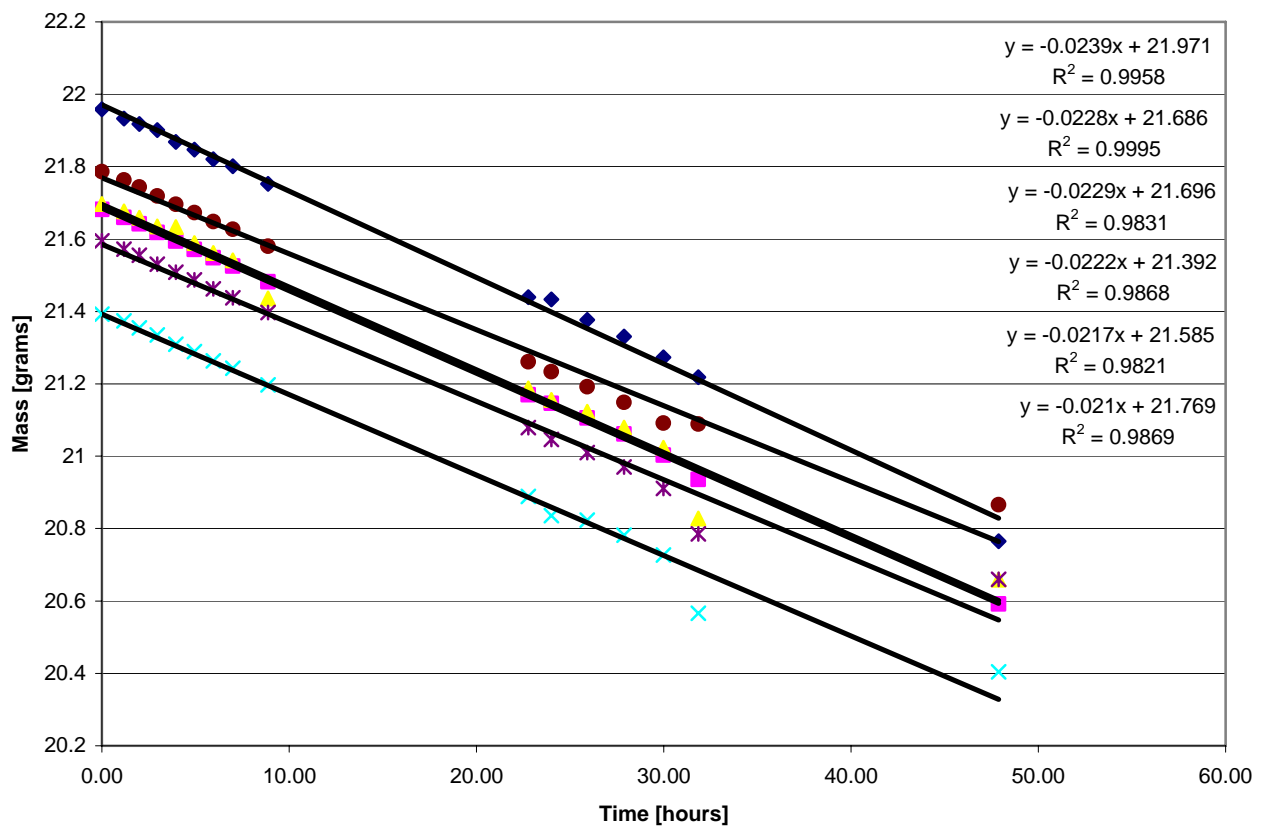


Figure 1.

The mass of six Petri dishes, with their plastic covers remained in place, was measured on a 48 hour interval. The variation of the initial masses is 21.613 +/- 0.202 grams. The average rate of drying is 0.0224 +/- 0.00106 grams/hour.

Table 2: Rates of drying for closed Petri dishes, in the same meteorological conditions and for of all four different volumes of culture medium.

Volume [ml]	Rate of drying [grams/hour]	Initial mass [grams]
15	0.0224 +/- 0.0011	21.613 +/- 0.202
20	0.0233 +/- 0.0007	26.806 +/- 0.392
25	0.0226 +/- 0.0006	31.761 +/- 0.118
30	0.0233 +/- 0.0006	36.317 +/- 0.187

For all four different volumes of initial culture medium we observe that the rate of drying at the physical conditions indicated above is 22.9 +/- 0.7 milligrams/hour. This represents between 1.8% and 3.7% of drying during 24 hours for respectively 30 ml to 15 ml Petri dishes.

In different meteorological conditions these rates of drying may vary. Table 3 shows a summary of all drying rates for uncovered (closed) Petri dishes.

Table3: Rate of drying in different metrological conditions.

Volume [ml]	Meteorological conditions	Rate of drying [grams/hour]	Initial mass [grams]
15	A	0.0224 +/- 0.0011	21.613 +/- 0.202
15	B	0.1120 +/- 0.0621	21.889 +/- 0.326
20	A	0.0233 +/- 0.0007	26.806 +/- 0.392
25	A	0.0226 +/- 0.0006	31.761 +/- 0.118
30	A	0.0233 +/- 0.0006	36.317 +/- 0.187
30	C	0.0311 +/- 0.0057	36.017 +/- 0.160
30	D	0.0347 +/- 0.0024	35.611 +/- 0.433

Drying rates for 30 ml Petri dishes seem to be slightly higher than those for smaller volumes. Condition B should be considered as very atypical because great fluctuations appeared within this series. We omit this experimental result from our conclusive remarks. The meteorological parameters for conditions A, B, C and D are summarized in table 4.

Table 4: Different meteorological conditions.

Meteorological conditions	Temperature [°C]	Relative humidity [%]	Atmospheric pressure [hPa]
A	23.1 to 25.2	31 to 37	1012 to 1018
B	23.3 to 26.2	32 to 33	1017 to 1018
C	26.1 to 29.9	30 to 43	1012 to 1018
D	24.7 to 26.2	32 to 33	1017 to 1018

As far as the influence of the meteorological conditions on the rate of drying, we believe that the interpretation of these results may only lead to unclear speculations. The influence of temperature and relative humidity do not seem to be significant within the ranges present during our experiments.

We conclude that the rate of drying for unpacked and closed Petri dishes varies between 20 mg/hour and 35 mg/hour. This is less than 4% of drying per 24 hours.

3.2 Drying rate for different volumes of culture medium in open Petri dishes.

Six Petri dishes with respectively 15, 20, 25 and 30 ml of culture medium were unpacked, their plastic covers taken off and their mass was measured at regular intervals of time. The physical parameters were the same as for the uncovered Petri dishes: room temperature between 23.1°C and 25.2°C, relative humidity between 31% and 37%, atmospheric pressure between 1012 and 1018 hPa.

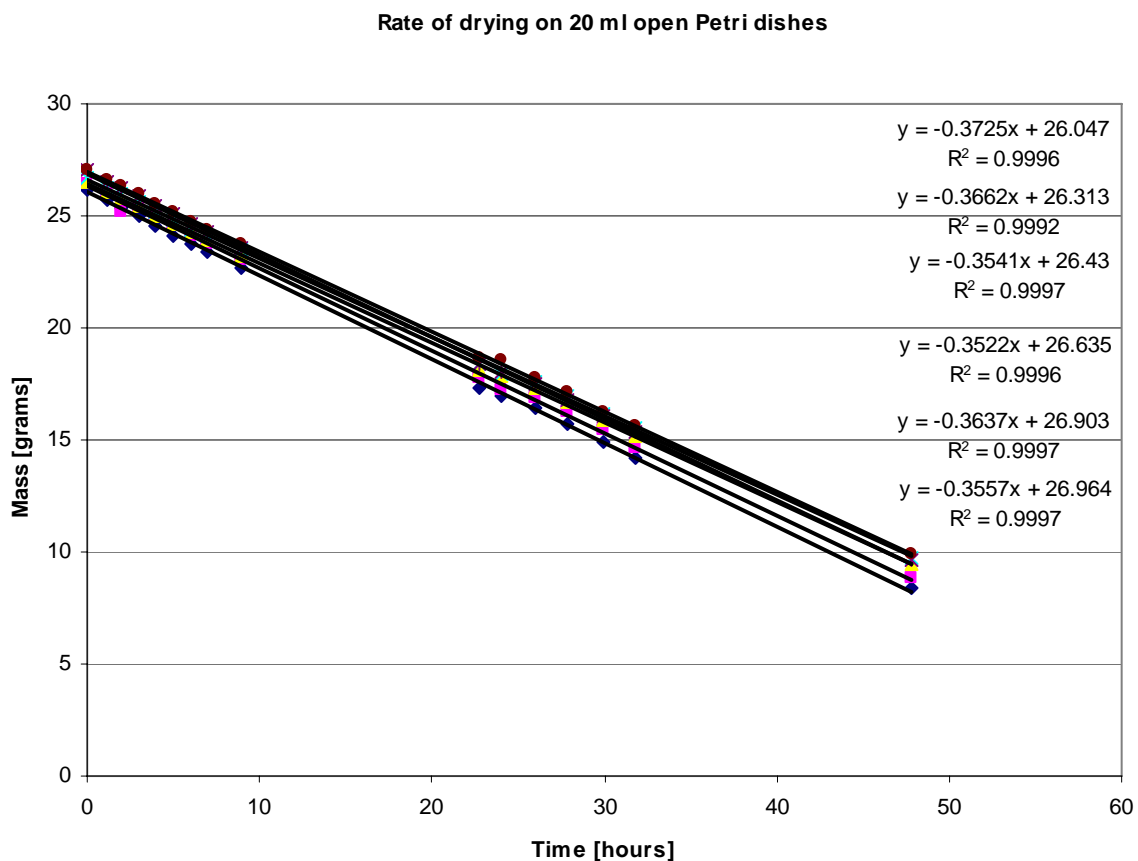


Figure 2.

The mass of six Petri dishes, with their plastic covers taken off, was measured on a 48 hour interval. The variation of the initial masses is 26.549 +/- 0.372 grams. The average rate of drying is 0.361 +/- 0.008 grams/hour.

Table 5: Rates of drying for open Petri dishes, in the same meteorological conditions and for of all four different volumes of culture medium.

Volume [ml]	Rate of drying [grams/hour]	Initial mass [grams]
15	0.380 +/- 0.021	21.619 +/- 0.107
20	0.361 +/- 0.008	26.549 +/- 0.372
25	0.392 +/- 0.013	31.619 +/- 0.368
30	0.453 +/- 0.026	36.300 +/- 0.180

For all four different volumes of initial culture medium we observe that the average rate of drying at the physical conditions indicated above is 0.397 +/- 0.063 grams/hour. This represents between 31.7% and 63.5% of drying during 24 hours for respectively 30 ml to 15 ml Petri dishes.

Furthermore, except for the 15 ml Petri dishes, we observe that the rate of drying depends on the volume of the culture medium as shown in figure 3.

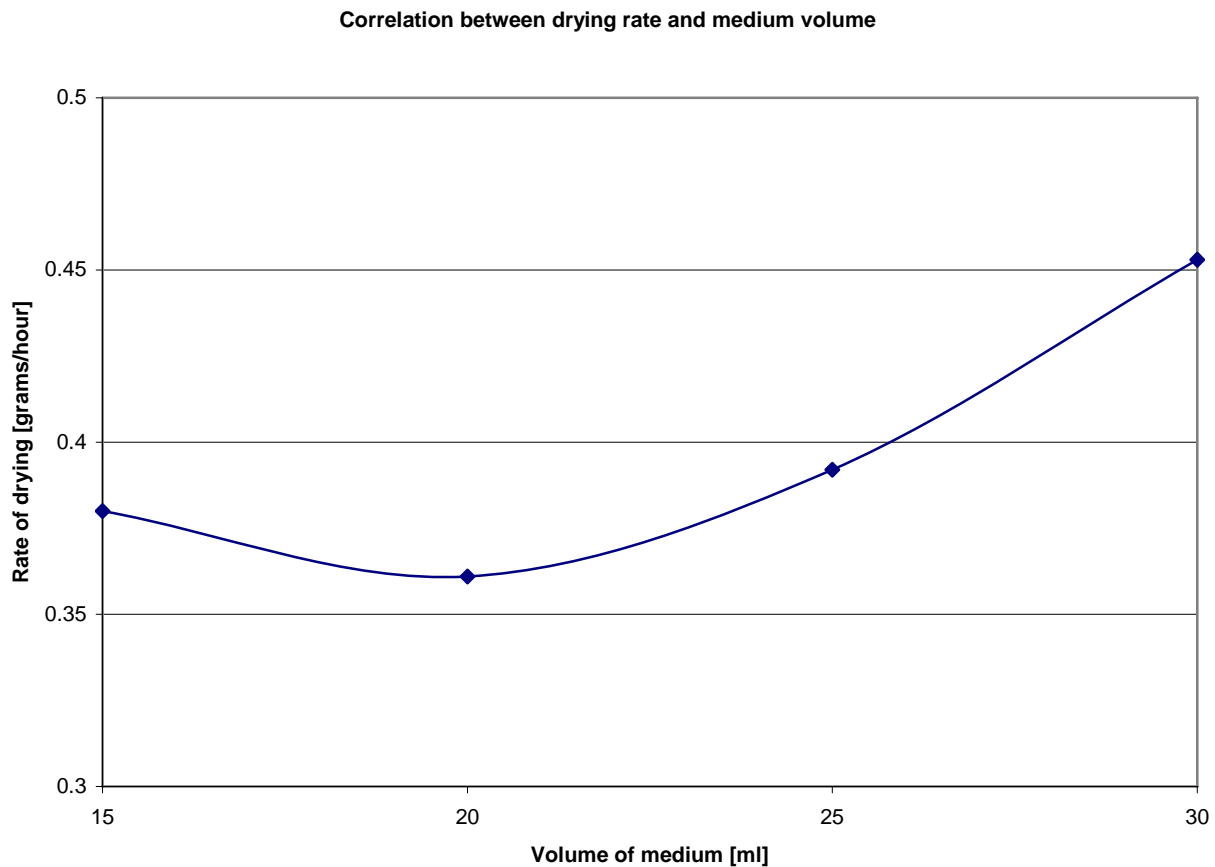


Figure 3

The rate of drying increases with the volume of the culture medium. Between 20 ml and 30 ml of medium this increase corresponds to approximately 9.2 milligrams/hour/ml.

This indicates that the diffusion rate of water within the volume of the culture medium is faster or at least compensates the evaporation rate at the surface of the Petri dish.

We measured the meteorological conditions for nine different days during which experiments were carried out (from A to J). The influence of temperature and relative humidity variations on the rate of drying on open Petri dishes are summarised, for all four volumes of culture medium, in table 6.

Table 6: Rate of drying in different meteorological conditions.

Volume [ml]	Meteorological conditions	Rate of drying [grams/hour]	Initial mass [grams]
15	A	0.380 +/- 0.021	21.619 +/- 0.107
15	B	0.401 +/- 0.012	21.704 +/- 0.295
15	H	0.549 +/- 0.064	20.577 +/- 1.697
15	F	0.446 +/- 0.037	21.422 +/- 0.098
15	J	0.519 +/- 0.055	20.852 +/- 0.376
20	A	0.361 +/- 0.008	26.549 +/- 0.372
25	A	0.392 +/- 0.013	31.619 +/- 0.368
30	A	0.453 +/- 0.026	36.300 +/- 0.180
30	C	0.537 +/- 0.015	35.131 +/- 0.376
30	D	0.695 +/- 0.138	35.684 +/- 0.366
30	E	0.802 +/- 0.020	34.730 +/- 1.065
30	F	0.454 +/- 0.013	35.205 +/- 0.207
30	G	0.556 +/- 0.077	34.870 +/- 0.429

Drying rates for 30 ml Petri dishes are higher than those for smaller volumes. The meteorological parameters for conditions A, B, C, D, E, F, G, H and J are summarized in table 7.

Table 7: Different meteorological conditions.

Meteorological conditions	Temperature [°C]	Relative humidity [%]	Atmospheric pressure [hPa]
A	23.1 to 25.2	31 to 37	1012 to 1018
B	23.3 to 26.2	32 to 33	1017 to 1018
C	26.1 to 29.9	30 to 43	1012 to 1018

D	24.7 to 26.2	32 to 33	1017 to 1018
E	26.3 to 31.5	30 to 39	1008 to 1010
F	25.4 to 29.9	32 to 37	1006 to 1009
G	23.4 to 24.9	27 to 28	1021 to 1022
H	23.1 to 23.8	27	1012 to 1013
J	24.4 to 25.2	36 to 46	1017 to 1018

We observe that condition E (highest temperature range: 26.3 to 31.5°C) corresponds to the highest rate of drying (0.802 +/- 0.020 [grams/hour]). The second highest rate of drying correspond to condition D with a lower temperature range but admittedly also lower relative humidity. Conjunction of both may influence on the rate of drying.

However, we believe that the interpretation of the influence of the meteorological conditions on the rate of drying may only lead to unclear speculations.

We conclude that the rate of drying for uncovered (open) Petri dishes varies between 350 mg/hour and 800 mg/hour. This is more than 55% of drying per 24 hours. The influence of temperature and relative humidity, within our experimental ranges, are not interpretable.

3.3 Drying rate for Petri dishes which are covered by a grid.

Six Petri dishes with 15 ml or 30 ml of culture medium were unpacked and placed in six microbiological air samples each covered with a 400 hole or a 300 hole grid. Then their mass loss was measured at regular intervals of time. Since we have seen above that a correlation between meteorological conditions and the rate of drying would be purely speculative, we neglected these parameters.

Drying rate of 15 ml Petri dishes covered with a 400 hole grid.

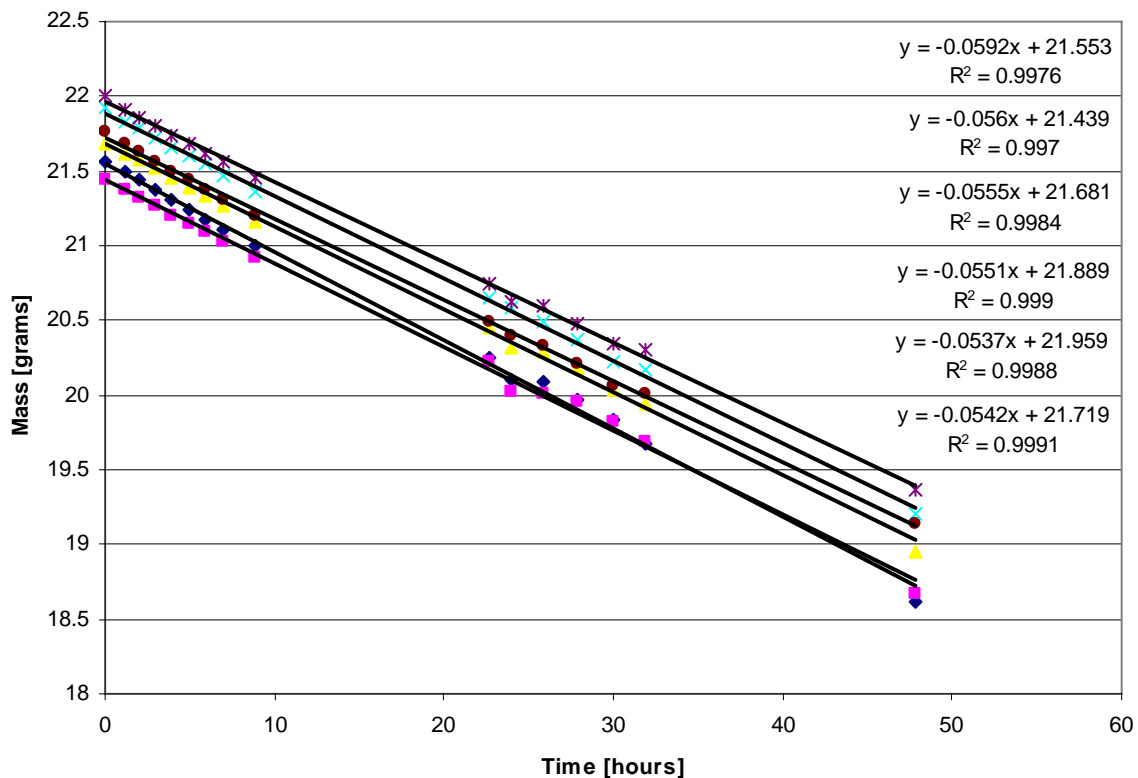


Figure 4.

The mass of six Petri dishes, covered with a 400 hole grid, was measured on a 48 hour interval. The variation of the initial masses is 21.707 +/- 0.206 grams. The average rate of drying is 0.0556 +/- 0.0020 grams/hour.

The rates of drying for 15 ml or 30 ml Petri dishes as well as for Petri dishes covered by MAS grids with 400 holes or 300 holes are summarised in table 8.

Table 8: Drying rates for Petri dishes covered by a MAS grid.

Volume [ml]	Grid [number of holes]	Rate of drying [grams/hour]	Initial mass [grams]
15	400	0.0556 +/- 0.00204	21.707 +/- 0.206
30	400	0.0686 +/- 0.00249	35.688 +/- 0.188
15	300	0.0614 +/- 0.00451	21.0756 +/- 0.332
30	300	0.0654 +/- 0.00681	35.543 +/- 0.572

We observe that the rate of drying is slightly higher for the 30 ml Petri dishes.

Petri dishes covered by both types of grids show a rate of drying varying between 55 mg/hour and 70 mg/hr. These rates are about two times faster than the rates of drying for closed Petri dishes and more than ten times slower than the rates of drying for open Petri dishes.

All three rates of drying for 30 ml Petri dishes are summarised in Figure 5.

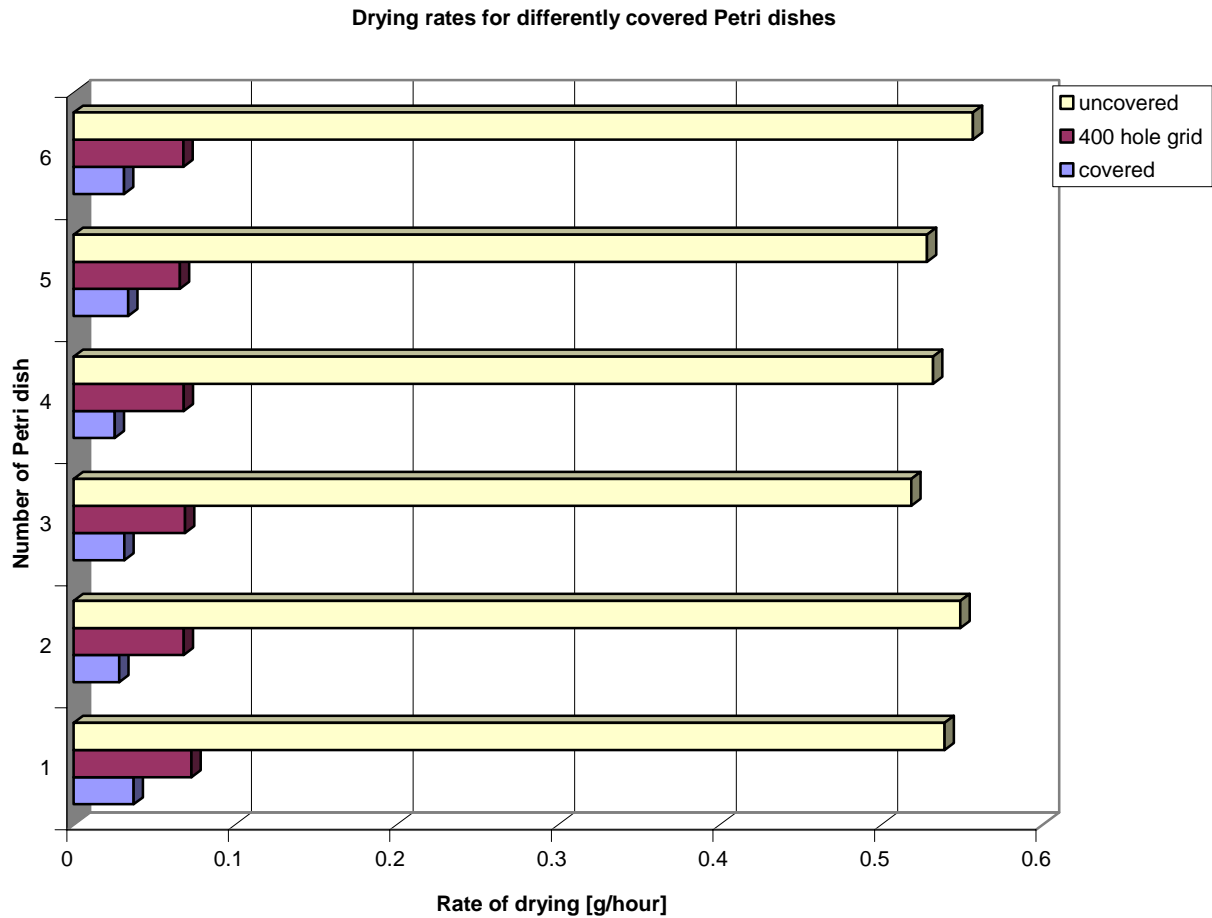


Figure 5.

The mass of six 30 ml Petri dishes, uncovered, covered with a 400 hole grid and totally uncovered, was measured on a 48 hour interval. The average rates of drying are respectively 31.1 mg/hour, 68.6 mg/hour and 537 mg/hour, i.e. respectively 2.5%, 5.5% and 43% of mass loss in 24 hours.

We conclude that the rate of drying for Petri dishes covered by a MAS grid varies between 55 mg/hour and 70 mg/hour, which is about two times faster than those for covered (closed) Petri dishes. This represents less than 10% of drying per 24 hours.

3.4 Drying rate for Petri dishes which are covered by a grid and which are exposed to the aspiration of air.

15 ml or 30 ml Petri dishes, covered by a MAS grid with either 400 holes or 300 holes, were exposed to an aspiration of 1 m³/hour of air. Although the rate of aspiration is fixed at 100 litres/minute, the frequency of aspiration can be varied. We used two frequencies: 1 m³ every 60 minutes and 0.1 m³ every 6 minutes. The rate of exposure, i.e. 1 m³/hour is the same. The influence of the meteorological parameters was neglected in all circumstances.

Figure 6 shows typical rates of drying for 15 ml Petri dishes covered with a 400 hole grid and exposed during 7 hours.

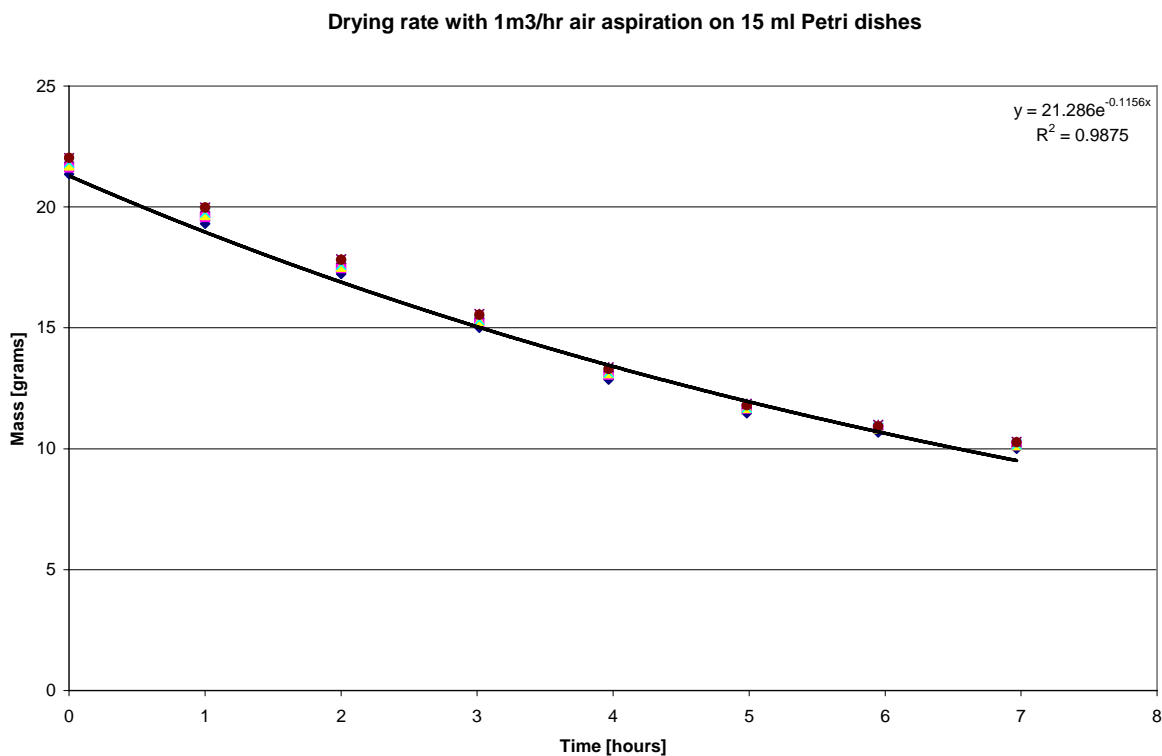


Figure 6.

Six 15 ml Petri dishes were exposed during seven hours to an aspiration of air of 1m³/hour 10 minutes of aspiration (rate: 100L/minute) were followed by 50 minute "waiting intervals".

We observe that mass loss is not linear. In order to avoid interpretations of exponential type of curves, we subdivided the drying period in two intervals of time. A first interval concerns up to 4 m³ of aspiration and a second interval from 5 m³ to 7 m³ of aspiration.

Table 9: Rates of drying for 15 ml Petri dishes which were exposed to 7 m³ aspiration of air.

15 ml Petri dishes	Rate of drying [mg/hr]	Drying [%]	Initial mass [grams]
1 to 4 m ³ of aspiration	2173 +/- 24	58	21.845 +/- 0.280
5 to 7 m ³ of aspiration	979 +/- 28	20	idem
1 to 7 m ³ of aspiration	1741 +/- 28	81	idem

The aspiration of air in the first four hours interval is 2173 +/- 24 mg/hour. This corresponds to almost 60% of drying of the 15 ml Petri dishes. In the last three hour interval, the rate of drying drastically drops to 979 +/- 28 mg/hour, which represents another 20% of drying of the 15 ml Petri dishes. The average rate of drying is 1741 +/- 28 mg/hour, which represents 80% of drying of the 15 ml Petri dishes.

The rates of drying for 15 ml or 30 ml Petri dishes as well as for Petri dishes covered by MAS grids with 400 holes or 300 holes and exposed to aspiration of are summarised in table 10. The time of interval indicates the frequency of air aspirations. A 60 minutes interval is composed of 10 minutes of aspiration at 100L/minute, i.e. 1 m³ followed by a 50 minutes pause before the next aspiration. A 6 minutes interval is subdivided in 1 minute of aspiration, i.e. 100 litres followed by a 5 minutes pause; the total volume aspired in one hour is also 1 m³.

Table 10: Drying rates for Petri dishes covered by a MAS grid and exposed to aspiration of air.

Volume [ml]	Grid [number of holes]	Time of interval [minutes]	Interval [m3]	Rate of drying [mg/hour]
15	400	60	0 to 4	2173 +/- 24
15	400	60	5 to 7	979 +/- 28
15	400	60	0 to 7	1741 +/- 28
30	400	60	0 to 10	2268 +/- 78
15	400	6	0 to 5	1823 +/- 44
30	400	6	0 to 5	2165 +/- 438
15	300	60	0 to 5	1782 +/- 72
30	300	60	1 to 5	2771 +/- 75

We observe that the aspiration of air on 15 ml Petri dishes induces an average rate of drying of about 1.8 grams/hour. This corresponds to 60% of drying after 5 m³ (within 5 hours) for 15 ml Petri dishes. The aspiration of air on 30 ml Petri dishes leads to an average rate of drying of about 2.4 grams/hour, which corresponds to 40% of drying after 5 m³ (within 5 hours) for 30 ml Petri dishes.

We conclude that the rate of drying for Petri dishes covered by a MAS grid and exposed to the aspiration of air at a rate of 1 m³/hour varies between 1750 mg/hour and 2750 mg/hour which is about 4 times faster than the rate of drying for open Petri dishes. This represents about 50% of drying per 5 m³ (within 5 hours).

A summarised conclusion, were we omit the meteorological conditions as well as the influence of the volume of culture medium, is shown in table 11, figure 7 and figure 8.

Table 11: Rate of drying for all four conditions of exposure to air:

Condition of drying	Rate of drying [mg/hour]	Percentage of drying [%/interval of time]
Closed	20 – 35	< 4% / 24 hours
Open	350 – 800	> 55% / 24 hours
With grid	55 – 70	< 10% / 24 hours
With grid + aspiration	1750 – 2750	+/- 50% / 5 hours (5 m ³)

If we take an average value for the rates of drying and a common interval of time (5 hours) for the percentage of drying, then we obtain Figure 7 and Figure 8.

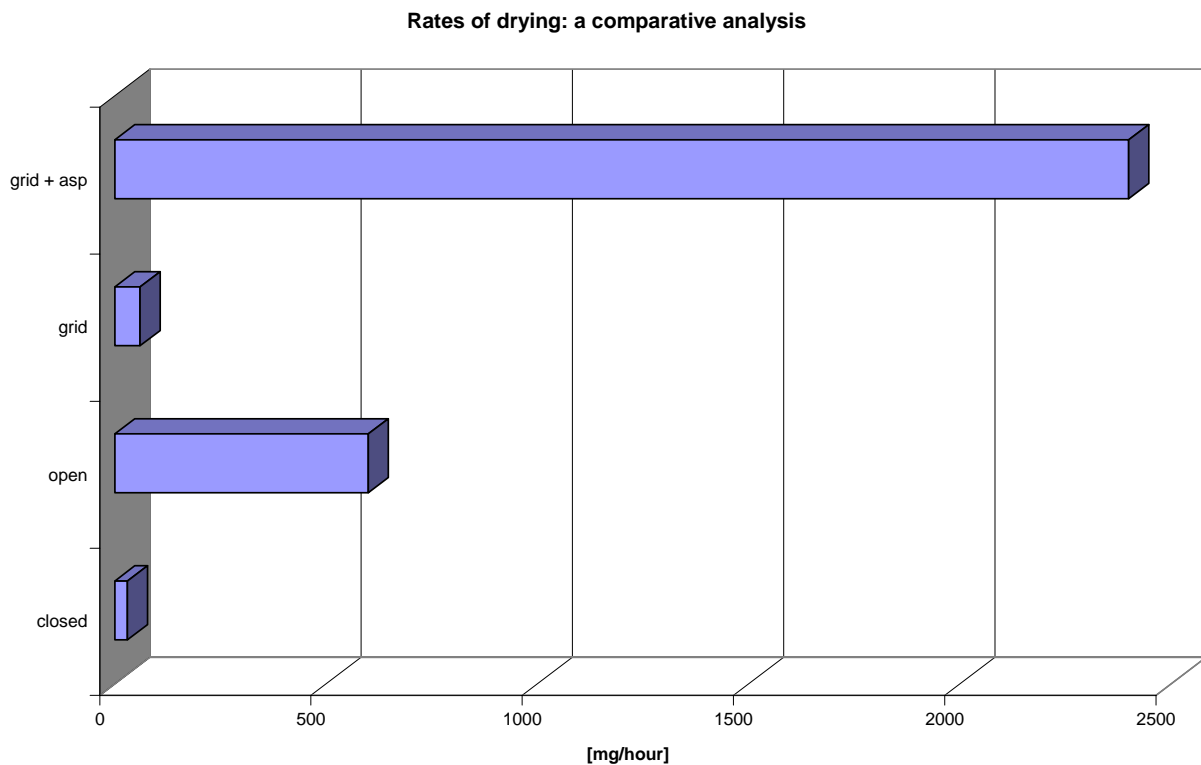


Figure 7.

For all four drying conditions (closed, open, with grid and with grid and aspiration) the rates of drying were averaged and compared. Rates are expressed in milligrams per hour.

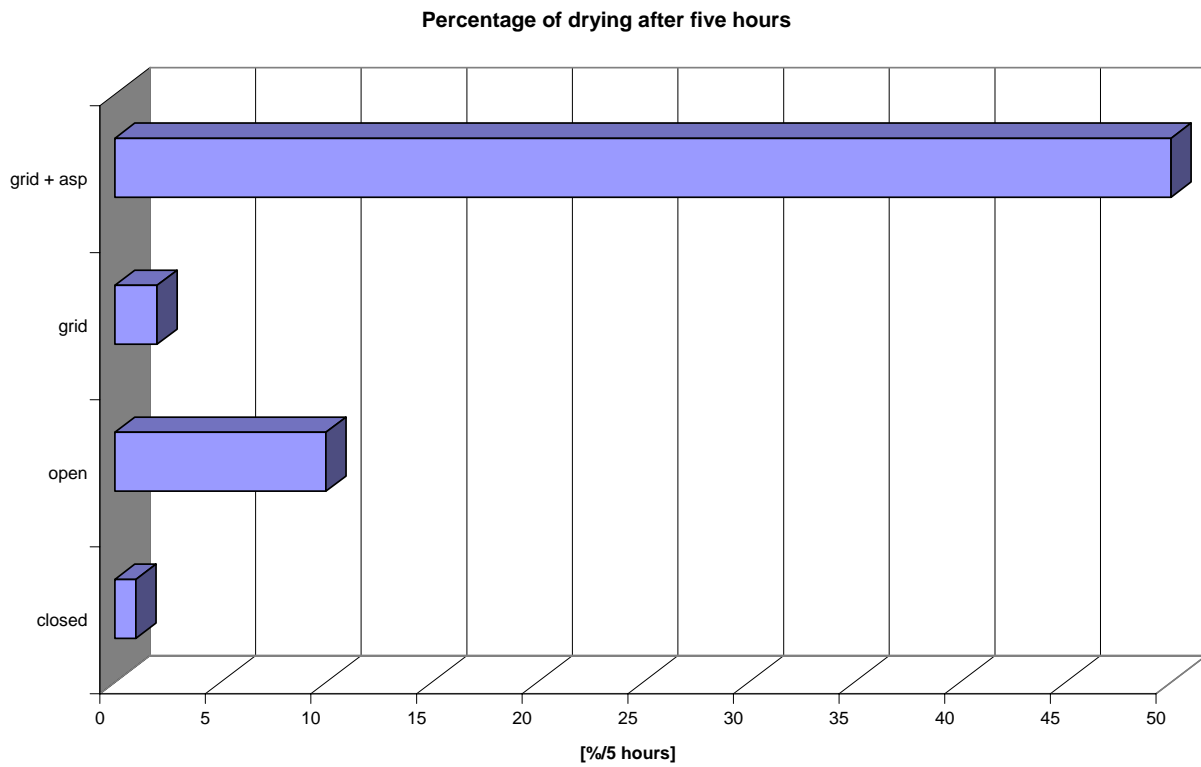


Figure 8.

For all four drying conditions (closed, open, with grid and with grid and aspiration) the percentages of drying were averaged and compared. Percentages were calculated over a 5 hour interval, which corresponds to the aspiration of 5 m³ of air.

Our final conclusion for the rates of drying in the four different conditions:

- **Closed = unpacked but covered with the plastic cover.**
- **Open = with the plastic cover removed.**
- **Grid = Petri dish installed in an air sampler and covered with a grid (400 or 300 holes)**
- **Grid + aspiration = Petri dish installed in a an air sampler, covered with a grid and exposed to the aspiration of air at a rate of 100L/minute for 1 or 10 minutes at regular intervals (respectively 6 to 60 minutes).**

indicates that the rate of drying with aspiration of air is 4 times faster than with open Petri dishes, 40 times faster than with Petri dishes covered with a grid and 80 times

faster than closed Petri dishes. The rate of drying increases slightly with the volume of culture medium (figure 3, page 12).

4 Conventional and sequential air sampling.

We call conventional air sampling a procedure during which a predetermined volume of air is collected on a Petri dish. If the microbiological contamination in the air is to be followed over a working day period (8 to 12 hours), than several samples have to be collected, each on a separate Petri dish, in the presence of a technician at regular intervals of time. Typically, three measures a day (morning, noon and afternoon) require the collection of air, in the presence of an operator, on three different Petri dishes.

Sequential air sampling is a procedure in which a total predetermined volume of air can be collected over a working day period (8 to 12 hours) on a single Petri dish at regular intervals of time at a frequency of up to 20 samples which is automatically pre-programmed in the microbiological air sampler. Typically, 10 to 20 air samples can be collected in the absence of a technician, over a 10 hour period at intervals of respectively 1 hour or 30 minutes.

With the conventional method, the rate of drying during a single collection of air on the appropriate Petri dish is usually negligible for air volumes below 1 to 2 m³.

With the sequential method, the different rates of drying between the sampling (“waiting intervals”) and during the air sampling (“active air collection intervals”) are essential. Fortunately, the preceding studies on the rates of drying showed that: ***the rate of drying with aspiration of air is 4 times faster than with open Petri dishes, 40 times faster than with Petri dishes covered with a grid and 80 times faster than closed Petri dishes.***

In other words, drying between air sampling is 40 times slower than during the sampling activity. Hence, the influence of “waiting intervals” can be ignored over a several days period of air collection!

This second series of experiments, on conventional and sequential air sampling, can be subdivided into three different sections.

First, we compared microbiological air sampling at a total volume of 160 litres, but at different frequencies with microbes collected on open Petri dishes without aspiration.

Second, we compared the conventional and sequential modes in a rather highly contaminated lecture room (without air conditioning) at the University of Applied Sciences of Geneva.

Third, we compared the conventional and sequential modes in an over pressurised clean room (where culture media ingredients for pharmaceutical purposes are measured with precision balances).

General methodology was exposed in the material and method section, but the more specific experimental conditions are described in each subsequent section.

4.1 Microbiological air sampling at different frequencies and on open Petri dishes.

Four experiments were designed. At all instances the total volume of collected air was fixed to 160 litres over a day period of eight hours. The closed, open and air collecting Petri dishes were placed as shown in figure 9.

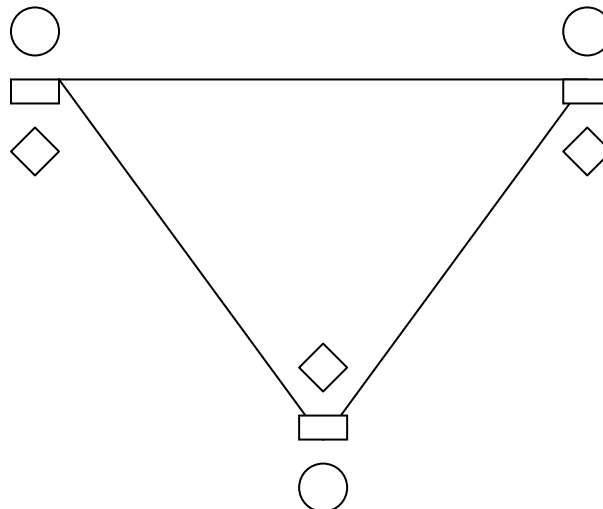


Figure 9

The three microbiological air samplers (circles) are placed at the summits of a triangle at 2 metre distances. The closed (rectangles) and open (diamond shaped) Petri dishes are placed at 15 cm and 30 cm from the air samplers.

In the first experiment, the experimental design shown in figure 9 was placed in a lecture room (A104) during 8 hours in the absence of students. With the microbiological air samplers we collected 160 litres of air at two frequencies. Frequency f2 represents 8 cycles of 20 litres at 1 hour intervals. Frequency f3 represents 4 cycles of 40 litres at 2 hour intervals. Unfortunately, frequency f1 (16 cycles of 10 litres at 30 minute intervals) could not be realised because of battery failure.

Typical colony forming units are shown in figure 10.

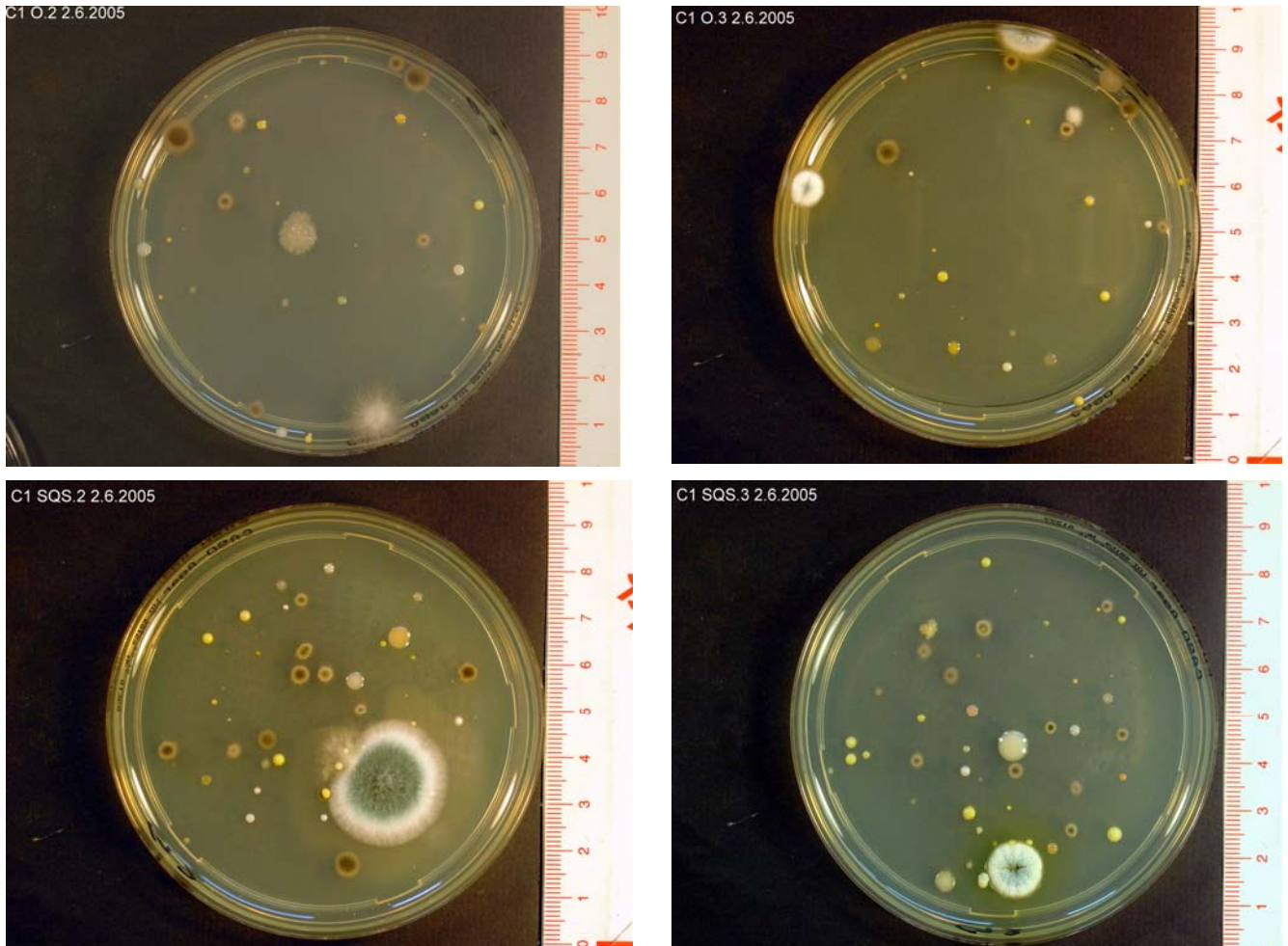


Figure 10.

Petri dishes which were left open (upper two photos) or exposed to 160 litres of air collection (lower two photos) at two different frequencies: f2 and f3 (SQS 2 and SQS 3)

Figure 11 illustrates a comparison of colony forming units collected on open Petri dishes and those collected with an aspiration of 160 litres of air at two different frequencies: f2 and f3.

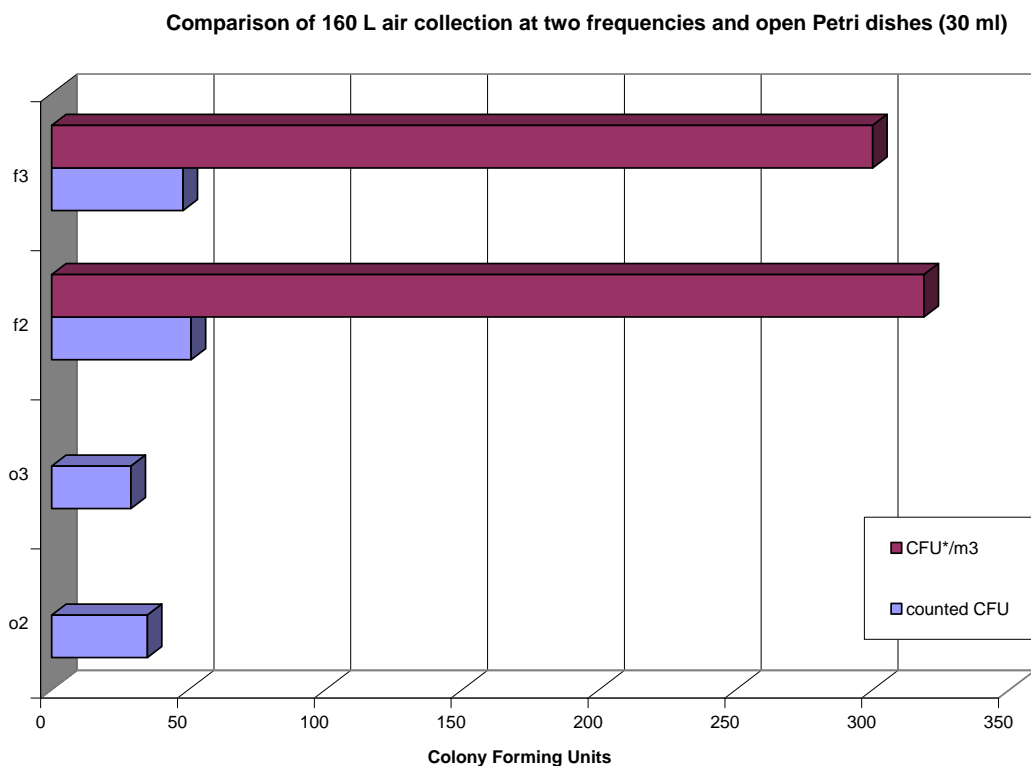


Figure 11

Colony forming units (CFU) were counted on the Petri dish and normalised to a concentration of CFU*/m³. The CFU on the open Petri dishes can not be normalised since the volume of collected air is unknown.

In the second experiment, the experimental design was the same as in the first experiment. With the microbiological air samplers we collected 160 litres of air at three frequencies. Frequency f1 represents 16 cycles of 10 litres at 30 minute intervals, frequency f2 represents 8 cycles of 20 litres at 1 hour intervals and frequency f3 represents 4 cycles of 40 litres at 2 hour intervals.

Figure 12 summarises the results.

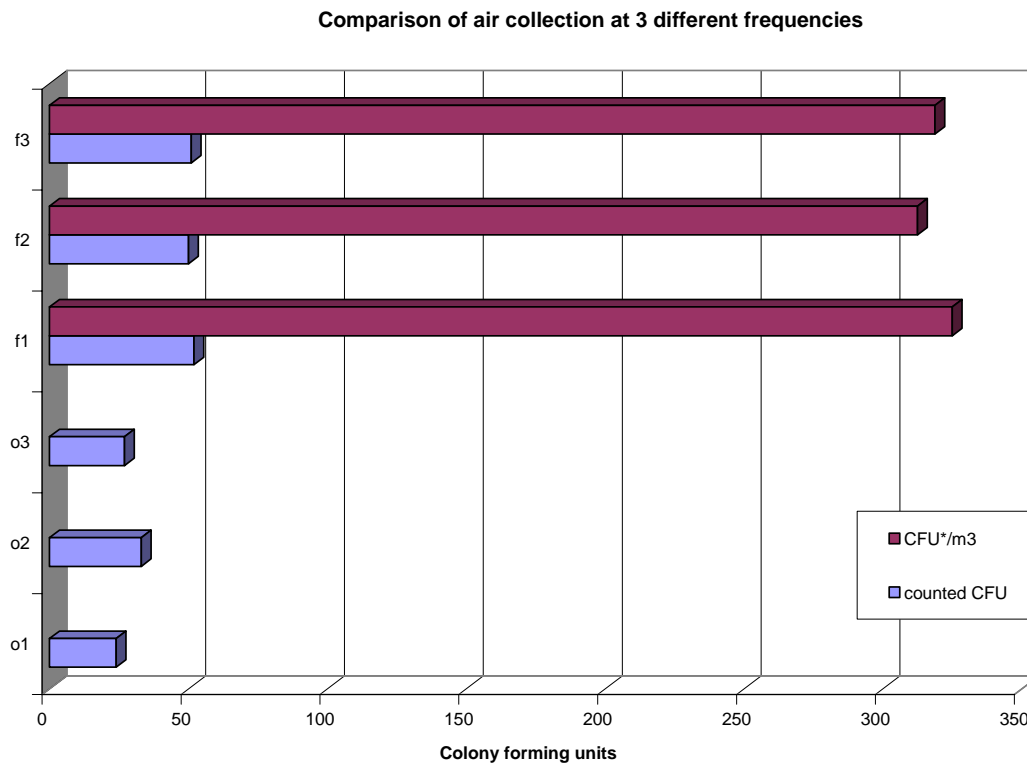


Figure 12

The number of colony forming units found after 8 hour exposure to open Petri dishes are shown in o1, o2 and o3. F1, f2 and f3 show the number of colony forming units after 8 hours of sequential air collection at three different frequencies, respectively: 16 cycles of 10 litres at 30 minute intervals, 8 cycles of 20 litres at 1 hour intervals and 4 cycles of 40 litres at 2 hour intervals.

We observe that 160 litres of sequential air collection at different frequencies during 8 hours corresponds to a number of colony forming units (50.4 ± 1.9) about 2 times higher than those collected during the same time on open Petri dishes (296 ± 5.5). Closed Petri dishes were kept sterile. The three different frequencies show no significant variation on the number of colony forming units.

In the third and fourth experiment, the experimental design was the same as in the first experiment. With the microbiological air samplers we collected 160 litres of air at a one week interval and at only one frequency (f1: 16 cycles of 10 litres at 30 minute intervals).

The experimental results are summarised in figure 13.

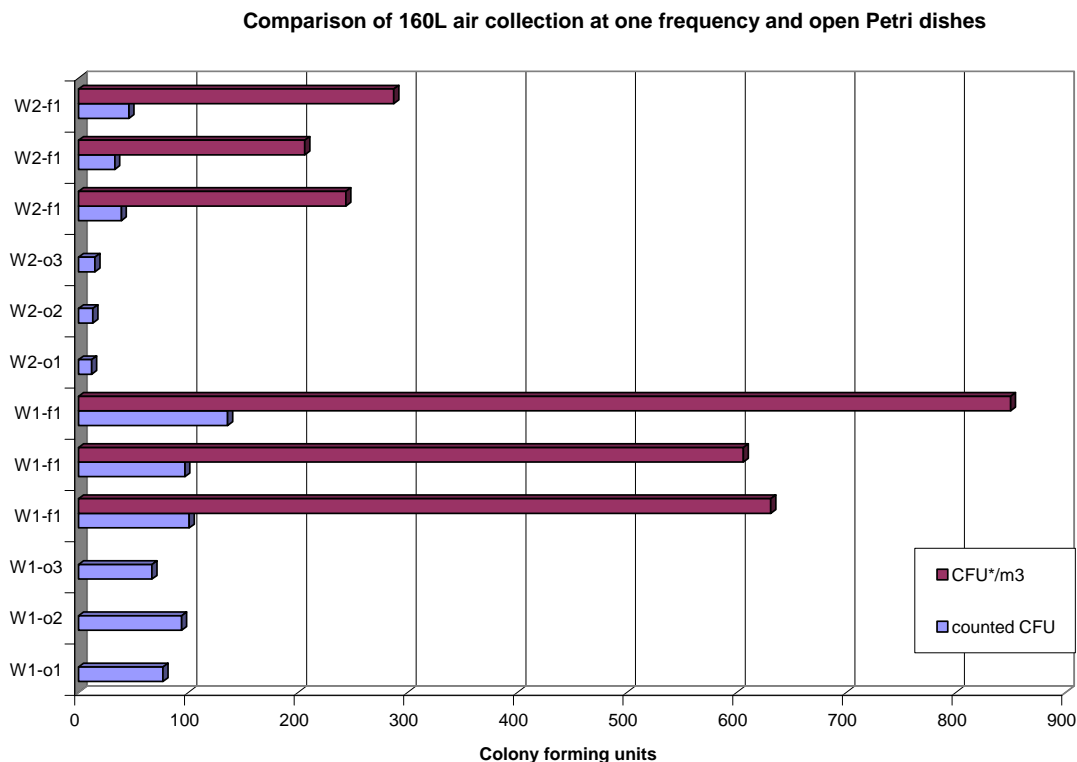


Figure 13.

160 L of air was collected during 8 hours at the frequency (f1) of 16 cycles of 10L at 30 minute intervals. W1 and W2 indicate respectively weeks 1 and 2. O1, o2 and o3 refer to three open Petri dishes. Air collection of known volume (160L) is also normalized to CFU*/m³.

We observe that 160 litres of air collection corresponds to a number of colony forming units which vary by a factor 2 from one week to the other. Again, counts are higher for 160L of air collection than for open Petri dishes. In table 12 we summarise the average counts for 160 litres of collected air at different frequencies and the average counts obtained without aspiration on open Petri dishes.

Table 12: Average CFU counts for 160 L aspiration of air and open Petri dishes.

Experiment Number	CFU/160L	CFU/open	Ratio 160L/open
1 and 2	50.4 +/- 1.9	29.6 +/- 5.5	1.4 to 2.1
3 (week 1)	111.3 +/- 53.3	79.3 +/- 33.9	1.0 to 2.4
4 (week 2)	39.3 +/- 16.2	13.3 +/- 3.8	1.4 to 5.8

We conclude that the air collection of 160 litres during 8 hours is independent of the frequency of collection. The air collection of 160 litres during 8 hours gives about 2 times more colony forming units than those obtained by the exposure of open Petri dishes during the same time but without aspiration of air.

4.2 Conventional and sequential air sampling at high concentrations of colony forming units.

Two experiments were designed. In both experiments we used 20 ml Petri dishes and the overall circumstances were identical at one day interval (June 30th and July 1st, 2005). At all instances the total volume of collected air was fixed to 160 litres over a day period of eight hours. The closed, open and air collecting Petri dishes were placed as shown in figure 14.

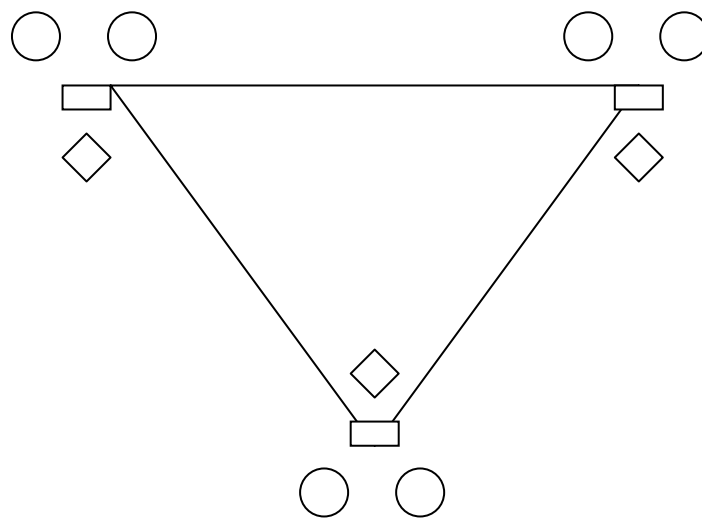


Figure 14.

The six microbiological air samplers (circles), of which three functioned in the conventional mode and three in the sequential mode, were placed at the summits of a triangle at 2 metre distances. The closed (rectangles) and open (diamond shaped) Petri dishes were placed at 15 cm and 30 cm from the air samplers. Conventional air sampling was always performed before or after sequential air sampling operations.

In both experiments, the experimental design shown in figure 14 was placed in a lecture room (A104) during 8 hours in the absence of students. With the conventional microbiological air samplers we collected nine times 20 litres of air at 1 hour intervals. The first sample was taken before the beginning of the sequential mode and the last sample was taken after the end of the sequential mode. Sequential mode was functioning for 8 cycles of 20 litres at 1 hour intervals, thereby collecting 160 litres of air on a single Petri dish. Three open (o1, o2 and o3) and closed Petri dishes were disposed close to the air

samplers and three other open Petri dishes (o4, o5 and o6) were placed at more peripheral distances (about 50 cm from the central triangle).

In figure 15 we show the colony forming units collected (without aspiration) on open Petri dishes.

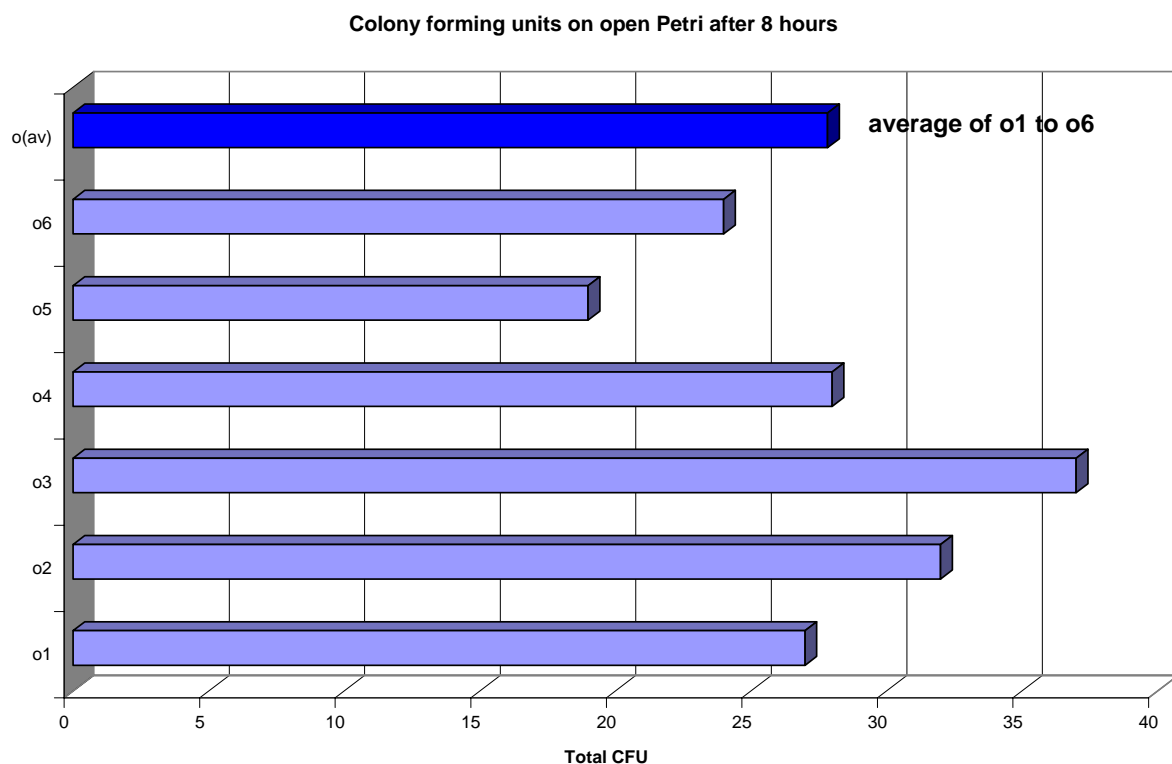


Figure 15.

Six open Petri dishes were exposed during 8 hours to ambient micro-organisms. O4, o5 and o6 are the more peripheral Petri dishes. An average of all six measures is shown in o (av).

The peripheral Petri dishes seem to have collected slightly less colony forming units than the more centrally exposed Petri dishes (respectively: 24 +/- 11 versus 32 +/- 12). This difference is statistically not significant. The closed Petri dishes were kept sterile (no colony forming units).

In figure 16, we show the colony forming units collected in the sequential mode on three different microbiological air samplers and which are placed on the summits of a triangle at 2 meter distance.

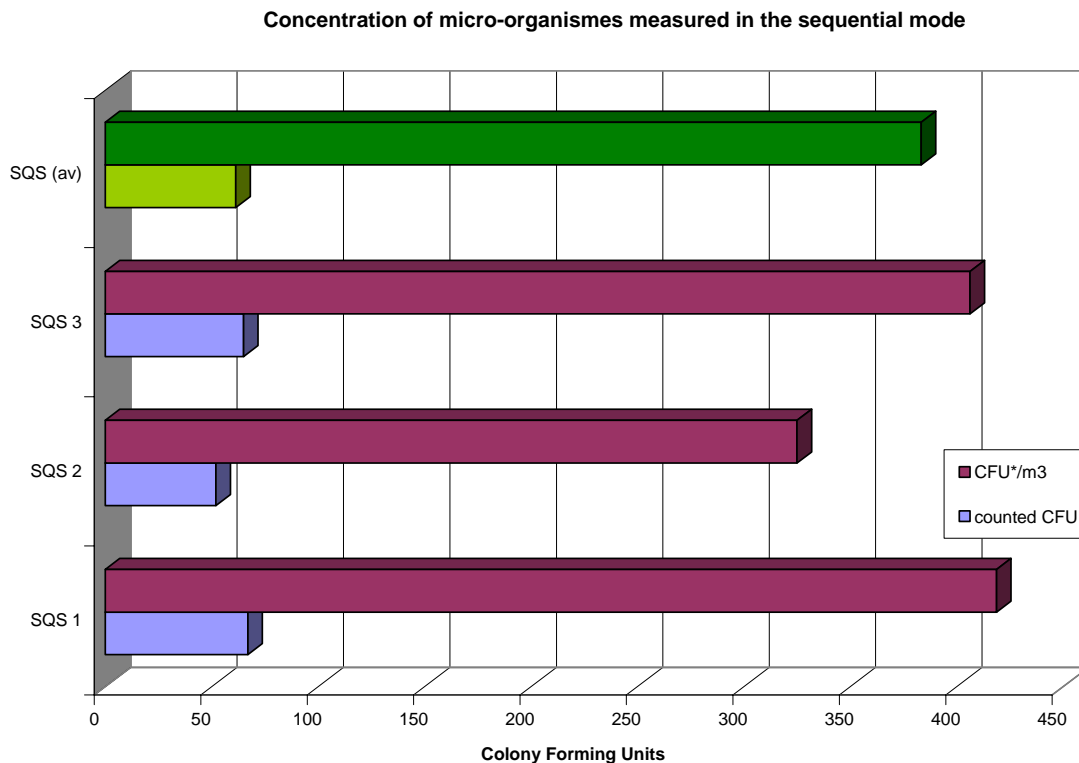


Figure 16

Three microbiological air samplers collected 160 litres of air during 8 cycles of 20 litres at 1 hour intervals. Colony forming units were counted after 5 days of incubation at room temperature. The green bars represent an average of all three sequential measures (SQS 1, SQS 2 and SQS 3).

Again, the collection of 160 litres of air is about **2 times** higher than the air collection on open Petri dishes. The average concentration of micro-organisms per cubic metre was: $383 \pm 126 \text{ CFU}^*/\text{m}^3$.

In figure 17, we show the results of a comparative study between the conventional and sequential modes of air sampling performed on three parallel measurements in each mode. The results of the sequential air sampling were shown in figure 16. The average

value of three parallel measurements is represented in figure 17 by the “green bar” noted SQS. Three conventional air samplings were performed every hour (either before or after the three automatic sequential air sampling operations) on three separate Petri dishes each time. The first conventional samples were taken 5 minutes before the first three sequential sampling operations and the last conventional samples were taken about 5 to 10 minutes after the last three sequential sampling operations at 16h45.

A comparative study between conventional and sequential air sampling.

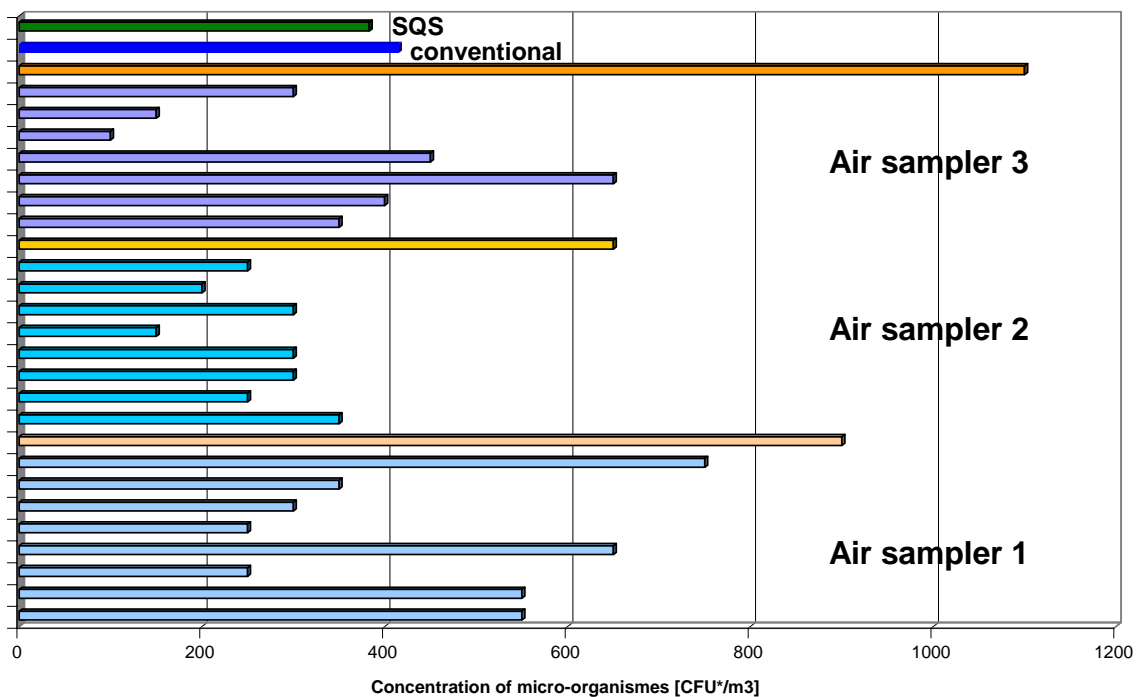


Figure 17

Three conventional air samplers (air sampler 1, 2 and 3) collected every hour 20 litres of air on 9 different Petri dishes and three sequential air samplers collected automatically 8 cycles of 20 litres during 8 hours on a single Petri dish. The two sampling operations were shifted by a 5 to 10 minute interval in such a way that the sequential air sampling was always in the absence of an operator. The orange bars show the last three conventional air samples of the day taken at 16h45.

We observe that the last three conventional air samples (orange bars) are reproducibly and significantly higher than all other conventional air samples taken during the day. This significant difference was also confirmed in the second identical experiment performed the

next day under the same circumstances. We explain this higher air concentration of micro-organisms by the fact that more than 100 students leave their lecture rooms at 16h30. This leads to a high concentration of human presence in a rather small corridor at a peak period of about 10 to 15 minutes. During the access to our lecture room with the six air samplers, an important flux of colony forming units could have increased the overall concentration of micro-organisms just before the last conventional air sampling operation.

Figure 18 shows the same data as in figure 17, except that the last conventional air sampling measures have been omitted from the average of all conventional measures taken during the day.

A comparative study between conventional and sequential air sampling.

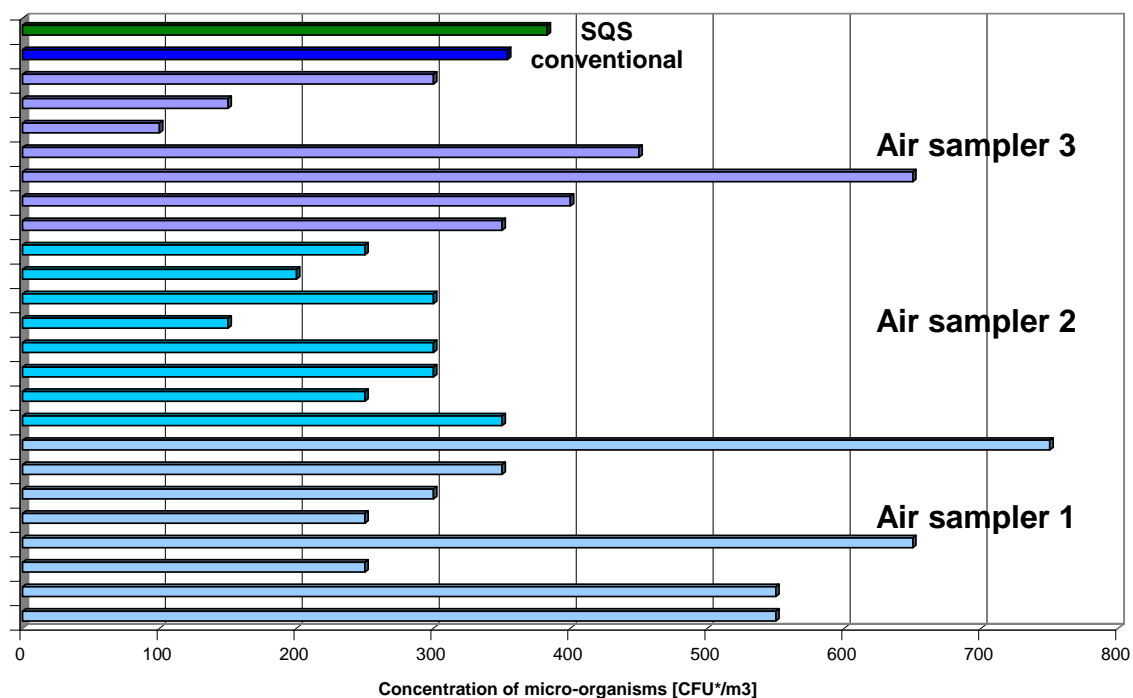


Figure 18

Three conventional air samplers (air sampler 1, 2 and 3) collected every hour 20 litres of air on 8 different Petri dishes and three sequential air samplers collected automatically 8 cycles of 20 litres during 8 hours on a single Petri dish. The two sampling operations were shifted by a 5 to 10 minute interval in such a way that

the sequential air sampling was always in the absence of an operator. The last conventional air samples, taken at 16h45, were omitted.

We observe that the average of three sequential air samplings gives a slightly lower concentration of micro-organisms than the overall average of the three conventional air sample operations taken 9 times during the day. If however the atypical measures of the last conventional samples, taken at 16h45, are omitted, we observe a slightly higher concentration as measured by the sequential mode (SQS).

In table 13 we summarise the experimental results of the two comparative studies performed on two consecutive days in identical environmental conditions.

Table13: A comparison between conventional and sequential air sampling.

Experiment	Method of sampling	Number of samples	Concentration [CFU*/m ³]
1	Sequential	3	383 +/- 126
	Conventional	9	415 +/- 99
	Conventional	8	354 +/- 74
2	Sequential	3	321 +/- 158
	Conventional	9	424 +/- 115
	Conventional	8	348 +/- 72

We observe that sequential and conventional air sampling lead to very similar results. Care should be taken at particular environmental influences (in our case the high concentration of human presence at a specific time of the day). As we can see, this can lead to important although statistically non significant differences as well in the average concentrations as in the "95% error intervals". "95% error intervals" are always higher for the sequential air sampling mode, but one atypical measure in the conventional mode increased this "95% error interval" also considerably (from about +/- 73 to about +/- 108).

We conclude that sequential air sampling of 160 litres in 8 cycles of 20 litres during 8 hours on a single Petri dish gives similar results to those obtained by 8 conventional air samples of 20 litres collected on 8 different Petri dishes over the same period of time. The “95% error intervals” are slightly higher for sequential air sampling, but an atypical environmental influence can considerably modify the results obtained with the conventional air sampling mode. Sequential air sampling decreases such environmental influences, because sampling is performed in the absence of an operator and external environmental changes.

4.3 Conventional and sequential air sampling at low concentrations of colony forming units.

Five experiments took place in an over pressurised clean room (where culture media ingredients for pharmaceutical purposes are measured with precision balances). Both conventional and sequential air sampling were performed on six microbiological air samplers of which three were operating in each mode.

The first experiment was a preliminary experiment (with only conventional air sampling) in order to measure the microbiological air contamination in four different zones:

Zone A1: class A / ISO 5 or 100 (less than 100 particles/foot³, i.e. 3530 particles/m³)

Zone A2: class A / ISO 5 or 1000 (less than 1000 particles/foot³, i.e. 35300 particles/m³)

Zone B: class C / ISO 7 or 10000 (less than 10000 particles/foot³, i.e. 353000 particles/m³)

Zone C: class C / ISO 7 or 10000 (less than 10000 particles/foot³, i.e. 353000 particles/m³)

(A particle should be smaller than 0.5 microns)

Zones A1 and A2 have an over pressure of 45 [Pa] compared to atmospheric pressure and a laminar flow from bottom to top (floor to ceiling). Zones B and C have air conditioned from the top (ceiling) and over pressures of 30 [Pa] and 15 [Pa], respectively.

In the zones A1 and A2, we collected 500 litres and 1 m³ of air on two separate Petri dishes. In the zones B and C, we collected 200, 400, 600, 800 and 1000 litres of air on five separate Petri dishes.

The results of this experiment are summarised in table 14.

Table 14: Concentration of micro-organisms in four different zones of an over pressurised clean room (August 24th, 2005 between 10h00 and 11h30).

Localisation	Collected volume [litres]	Counted colonies [CFU]	Concentration [CFU*/m ³]	Average concentration [CFU*/m ³]
Zone A1: 100	500	1	2	1
	1000	0	0	
Zone A2: 1000	500	4	8	6
	1000	4	4	
Zone B: 10000	200	7	35	15
	400	3	8	
	600	7	12	
	800	15	19	
	1000	3	3	
Zone C: 10000	200	3	15	27
	400	7	18	
	600	17	28	
	800	34	44	
	1000	29	30	

We observe that zone C is about **2 times** more contaminated than zone B, which is about **2 times** more contaminated than zone A2. Zone A1 is very clean with about 1 CFU/m³.

These average concentrations should be considered only as indicative, because no serious statistical analyses can be performed on these small numbers.

Taking into account these preliminary results, we decided to perform our comparative study between conventional and sequential air sampling in zone C at three different localisations. Site 1 was in a corner, site 2 was in the middle, close to the longest wall and

site 3 was close to a wash-hand basin (see also photos). All air monitoring was close to the floor.



Photos of site 1, site 2 and site 3 (from left to right).

In figure 19 we show the microbiological air concentrations measured at the three different sites described above. On each site, three conventional air samples of 1 m³ were taken in the morning, at noon and in the afternoon. The sequential air sampling was pre-programmed for 10 automatic cycles of 100 litres. Both, conventional and sequential air samplers were at 30 cm distance. All air sampling was programmed or performed in such a way to avoid simultaneous air monitoring.

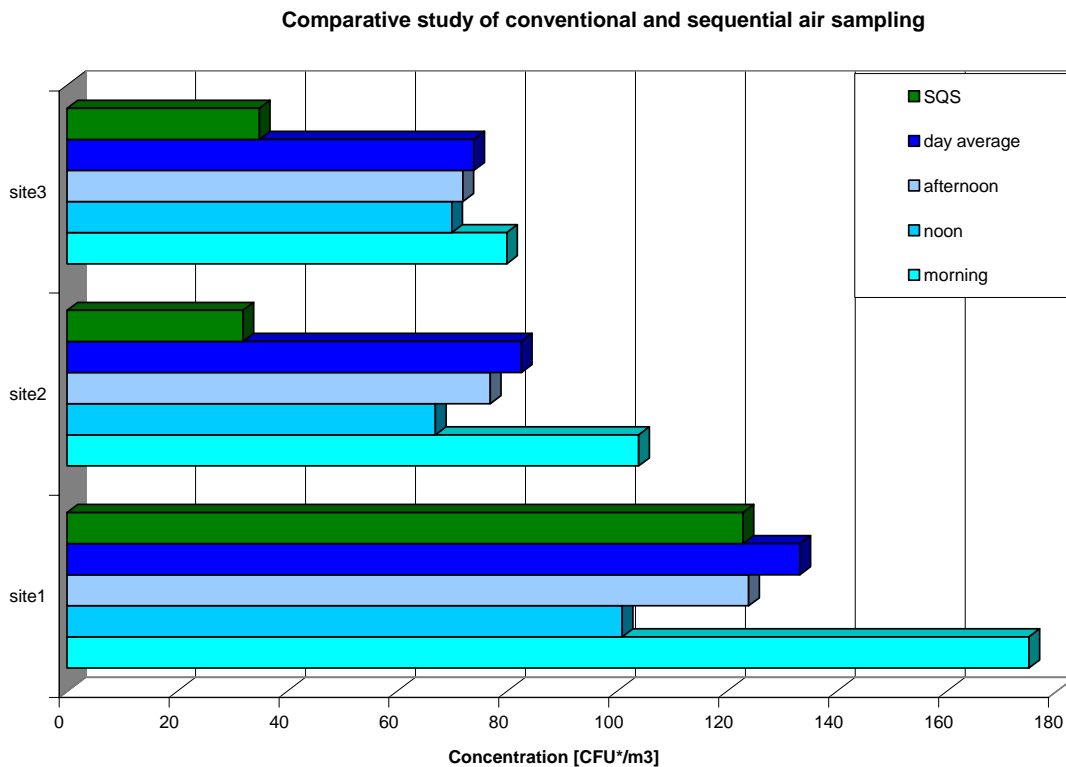


Figure 19.

At three different sites in zone C, three conventional air samples of 1 m³ were taken between 8h23 and 17h29 at 4 hour intervals (morning, noon and afternoon). At slightly shifted times three sequential air samples were taken at a frequency of 10 cycles of 100 litres during 9 hours. Day averages were calculated for each site (dark bleu bars).

We observe that conventional and sequential air sampling show **the same** concentrations for site 1. At site 2 and site 3, the average concentrations of conventional air sampling are about **2 times** higher than the cumulative concentrations measured by sequential air sampling. This variation can be essentially due to considerable air fluxes caused by the over pressure system along the larger wall and close to the hand-wash basin. Indeed, site 1 was situated in a corner with less air flux. During conventional air sampling with the presence of an operator, air flux was minimised because no doors were opened. We did not measure the frequency of door opening, at the three different entrances of zone C, during sequential air sampling, but we know that considerable handling and transport of

goods across zone C have taken place during the day. This could have minimized the sampling efficiency during sequential sampling.

The last three experiments (A, B and C) were situated in zones A1 and A2 which are separated by a lamellar plastic curtain.

The localisation of the air samplers is illustrated in figure 20.

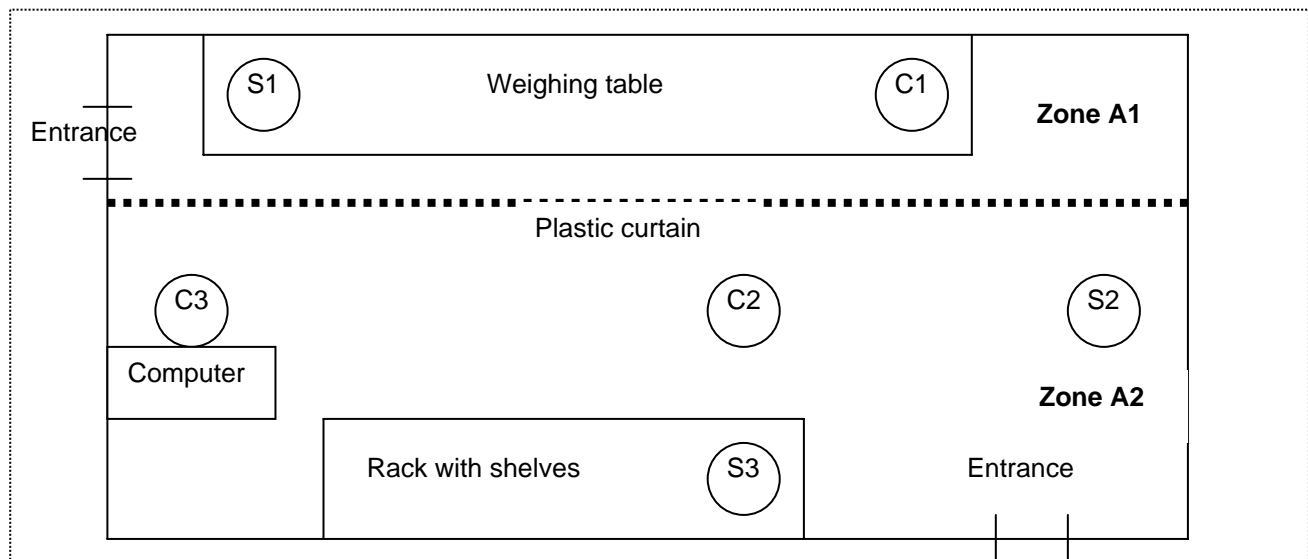


Figure 20.

Three conventional air samplers (C1, C2 and C3) and three sequential air samplers (S1, S2 and S3) were placed as indicated. Conventional air samplers collected 1 m³ three (morning, noon and afternoon) or two times (morning and afternoon) a day. Sequential air samplers collected 10 cycles of 100 litres (S1 and S3) or 20 cycles of 50 litres (S2) every hour or half-hour, respectively, during 9 hours. Two people were present in zone A1 and one person worked in zone A2 between the computer and the rack with shelves.

The clean room with zones A1 and A2 were ventilated in 45 [Pa] over pressure by a laminar flow at a speed of 0.45 [m/s] and circulating from the floor to the ceiling. Clothing is completely changed and includes hair protection, mask and gloves.

The 25 ml Petri dishes with CASO agar used for air collection were incubated at 25°C for 5 days.

Experiment A took place on October 4th, 2005. The results are shown in figure 21.

Conventional and sequential air sampling in a clean room (Exp A)

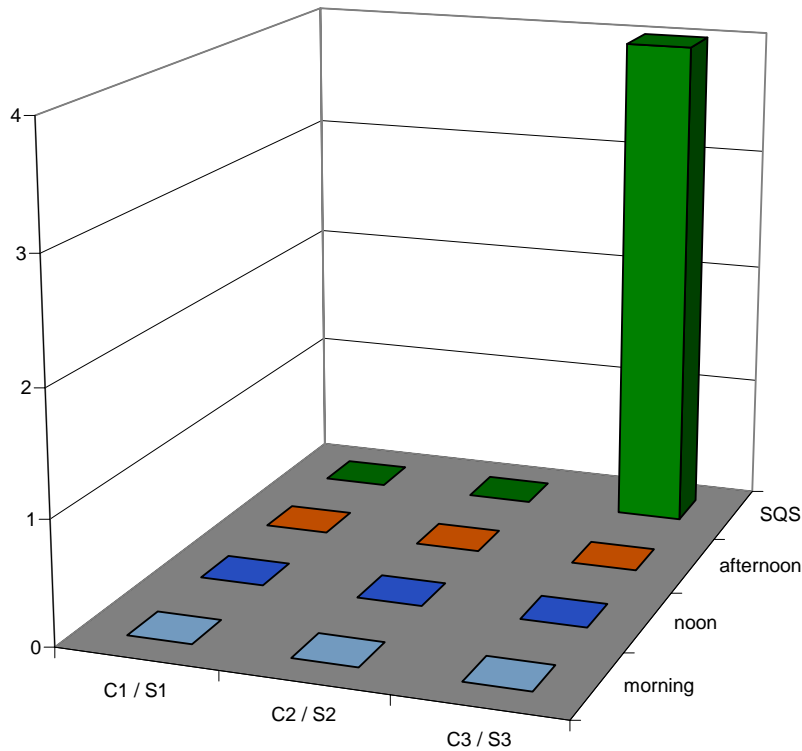


Figure 21.

Three conventional air samplers (C1, C2 and C3) collected 1 m³ three times a day (morning, noon and afternoon) and three sequential air samplers (S1, S2 and S3) collected 10 cycles of 100 litres (S1 and S3) or 20 cycles of 50 litres (S2) every hour or half-hour, respectively, during 9 hours. Petri dishes were incubated for 5 days at 25°C.

All air samples showed zero colony forming units (CFU) except for the sequential air sampling at S3 (4 CFU) which was situated between two shelves on a metal rack.

Experiment B took place on October 6th, 2005. The results are shown in figure 22.

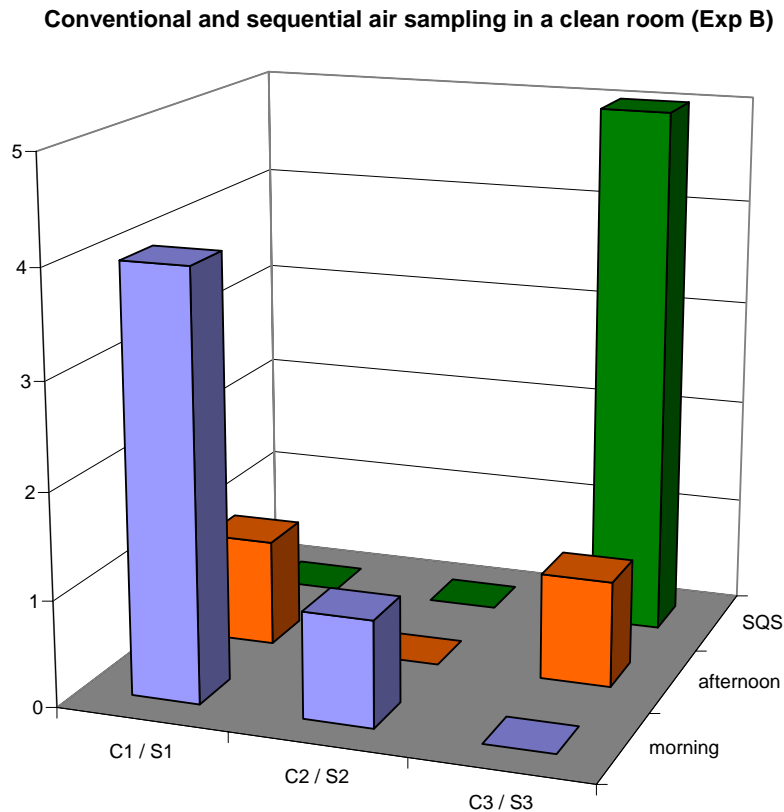


Figure 22.

Three conventional air samplers (C1, C2 and C3) collected 1 m³ two times a day (morning and afternoon) and three sequential air samplers (S1, S2 and S3) collected 10 cycles of 100 litres (S1 and S3) or 20 cycles of 50 litres (S2) every hour or half-hour, respectively, during 9 hours. Petri dishes were incubated for 5 days at 25°C.

On the conventional air sampler (C1) placed on the weighing table we found 4 CFU in the morning and 1 CFU in the afternoon. For the conventional air samplers C2 in the middle of zone A2 and C3 on the metal rack we measured, respectively, 1 CFU in the morning and 1 CFU in the afternoon. Only the sequential air sampler S3 placed between the shelves on the metal rack collected 5 CFU. Both S1 and S2 showed no CFU after the collection of 1 m³

Experiment C took place on October 25th, 2005. The results are shown in figure 23.

Conventional and sequential air sampling in a clean room (Exp C).

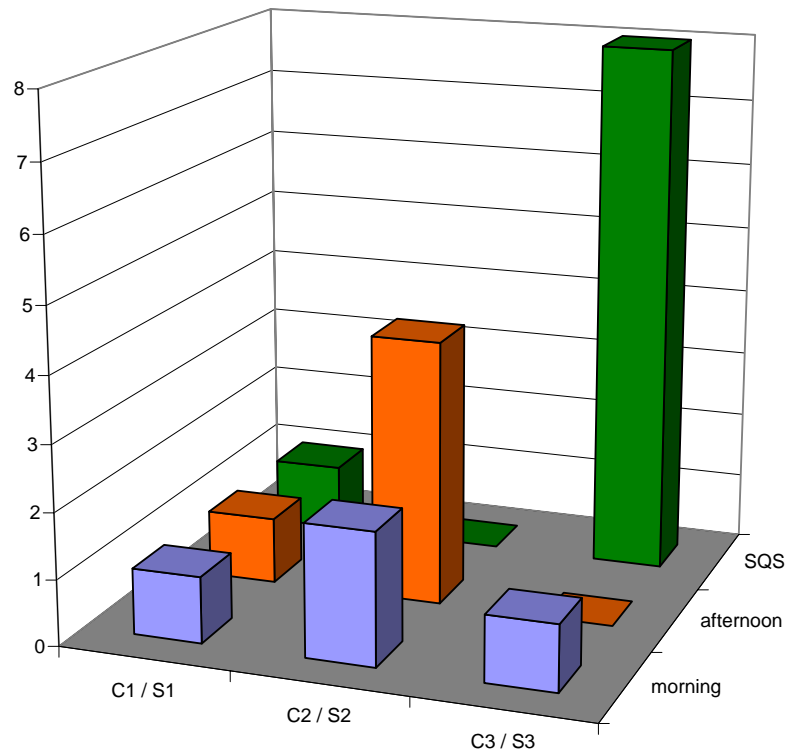


Figure 23.

Three conventional air samplers (C1, C2 and C3) collected 1 m³ two times a day (morning and afternoon) and three sequential air samplers (S1, S2 and S3) collected 10 cycles of 100 litres (S1 and S3) or 20 cycles of 50 litres (S2) every hour or half-hour, respectively, during 9 hours. Petri dishes were incubated for 5 days at 25°C.

All air samplers showed a microbiological air concentration between 1 and 8 CFU/m³ except for S2, placed in zone A2 at 1,20 metre from the floor and C3 placed next to the computer in the afternoon.

We observe that very few colony forming units were found in all three experiments (A, B and C) performed in zones A1 and A2 of a clean room with laminar air flow from the floor to the ceiling. Sequential air sampling detected highest contamination on the rack with

shelves. This could be interpreted that laminar air flow is less efficient between the shelves on this rack. With such low counts, any statistical interpretation is of course useless.

We conclude that sequential air sampling of 1 m³ in 10 cycles of 100 litres or 20 cycles of 50 litres during 9 hours on a single Petri dish gives similar results to those obtained by 3 conventional air samples of 1 m³ collected either three or two times on either 9 or 6 separate Petri dishes over the same period of time. Statistical analysis is in all cases insignificant.

5 General conclusion and perspectives.

The rates of drying under different circumstances show that in the sequential sampling mode, the drying of Petri dishes between air sampling operations is about 40 times slower than during air sampling at a rate of 1 m³/hour.

About 50% of mass loss due to drying occurs after 5 m³ of air aspiration on 20 to 30 ml Petri dishes. 15 ml Petri dishes should be avoided because the rate of drying implies a percentage of drying above 60% after 5 m³ of air aspiration.

If one admits that 50% of drying is approximately the upper limit for efficient air sampling, than sequential air sampling of small volumes (up to a total of 1 m³) could be carried out over 5 day periods (working week).

Sequential air sampling is a good alternative to conventional air sampling, because the average values obtained in the sequential mode are in good agreement with the average values obtained in the conventional mode.

The advantages of sequential air sampling are:

- the use of less Petri dishes
- the possibility to monitor air over a longer observation period and in the absence of human presence (technician)
- the possibility to avoid atypical environmental influences caused by the coming and going of an operator whose presence is unavoidable in the conventional operating mode.

Quantitative measurements on the correlation between the influence of drying of a Petri dish and the efficiency of air sampling would clearly define the upper limits of sequential air sampling as well in time as in sampling volume.

It is essential to confirm these limits for the sequential operating mode if this new technique is to be used in a larger domain of applications. A better understanding between drying percentage and the efficiency microbiological air sampling would open new perspectives in this field.