

Performance Assessment of Prefilters and Filters Using a Test Bench



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Test Report

Caroline Duchaine¹, Ph.D.
Valérie Létourneau¹, Ph. D.
Jacquelin Labrecque²
François Pouliot², Eng., MBA

¹ Centre de recherche de l'Institut universitaire de cardiologie et de pneumologie de Québec (CRIUCPQ)

² Centre de développement du porc du Québec inc. (CDPQ)



CENTRE DE RECHERCHE
INSTITUT UNIVERSITAIRE
DE CARDIOLOGIE
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DE QUÉBEC

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Project Team

Project Director

Francis Pouliot, Eng., MBA.
Centre de développement du porc du Québec inc.

Project Investigator

Caroline Duchaine, Ph.D.
Centre de recherche de l'Institut universitaire de cardiologie
et de pneumologie de Québec

Project Coordinator

Marie-Aude Ricard, Eng.
Centre de développement du porc du Québec inc.

Writing Team

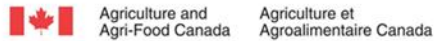
Valérie Létourneau, Ph.D.,
Centre de recherche de l'Institut universitaire de cardiologie
et de pneumologie de Québec

Jacquelin Labrecque
Centre de développement du porc du Québec inc.

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Table of Contents

Introduction	3
Objectives	4
Material and Methods.....	4
Design of a test bench to measure the efficiency of the filters	4
Generation and Sampling of Test Bench Viral Aerosols	7
Results and Discussion	9
Objective A. Design and validation of a test bench that can be used to assess the efficiency of different filtration units.....	9
Objective B. Assessment of the re-emission of phages Phi6 through the different filtration units during purges (background noise)	11
Objective C. Assessment of efficiency in capturing particles of different diameters and phages Phi6 through different filtration units.....	11
Conclusion	16
References	18

List of Table

Table 1	Validating the uniformity of air speed in the test bench.....	9
Table 2	Air flow rate at the iris for different fan frequencies.....	10
Table 3	Difference of air flow measurement between the anemometer and the iris.....	10

List of Figure

Figure 1	Test bench diagram from the ANSI/ASHRAE 52.2 standard.....	5
Figure 2	Test bench used to study the various filtration units.....	6
Figure 3	Experimental device for nebulizing viral aerosols.....	7
Figure 4	Experimental design for sampling viral aerosols	8
Figure 5	Dispersion of measurement points in the test bench cross-section	9
Figure 6	Efficiency in capturing phages Phi6 through different filtration units	12
Figure 7	Efficiency in capturing total particles through different filtration units.....	13
Figure 8	Efficiency in capturing 0.3 μm particles through different filtration units	14
Figure 9	Efficiency in capturing 0.5 μm particles through different filtration units	14
Figure 10	Efficiency in capturing 1 μm particles through different filtration units	15
Figure 11	Efficiency in capturing 3 μm particles through different filtration units	15
Figure 12	Efficiency in capturing 5 μm particles through different filtration units	16

Note

It should be noted that the results of the present study refer to the experimental design, more specifically, to the use of a six-jet nebulizer generating viral particles and salts with a median mass aerodynamic diameter of about 1 μm (experimental data, unpublished). The equipment available did not allow generating a broader spectrum of particles. However, as part of the assessment of filter efficiency in a bioexclusion context, the hypothesis is that this population of particles proves fairly representative of particles coming from surrounding buildings, which are favourable to the transport of viruses between sites. The hypothesis, nonetheless, remains to be confirmed in future studies. It should also be considered that viral aerosols produced artificially in the test bench do not represent the group encountered within swine buildings, i.e., particles with a median mass aerodynamic diameter of 11-14 μm (O'Shaughnessy et al., 2002). Therefore, for a biocontainment concept, for which the spectrum of particles to be filtered is much broader, we cannot assess the efficiency of filters with certainty with the experimental design used in this study. Consequently, the results obtained should be interpreted carefully as they essentially allow validating the design of the test bench. The size of particles on which the PRRSV is carried both in the herd building's perimeter and outside is still unknown to date and it is impossible, for the time being, to certify that viruses are not carried on larger particles than the spectrum of particles achieved with the present experimental design. Consequently, under no circumstances can the results described herein be used to explain or settle problems associated with viral aerosols encountered in the field before subsequent studies on the determination of the size of particles carrying swine viruses in the air are carried out. The present study allowed establishing the bases and the foundation necessary for laboratory reproduction of viral aerosols and the development of new filtration methods adapted to swine buildings.

Introduction

The herd of Canadian swines is associated with a good health quality allowing more than 125 importing countries to deal with Canada. In Quebec, the *Fédération des producteurs de porcs du Québec* (FPPQ) has assessed the farmgate value of hog farms to be more than 1.2 billion dollars (FPPQ, 2012). Canadian herds, however, are weakened by the porcine reproductive and respiratory syndrome virus (PRRSV) as well as by the Influenza virus. The Faculty of Veterinary medicine of the University of Montréal, besides, has estimated that up to 90% of Quebec herds are infected by the PRRSV in high animal density areas (FMV, 2007). The PRRS is the costliest swine disease in Quebec with 150 million dollars annually (FMV, 2007).

The viruses associated with the PRRS and Influenza are enveloped and single-stranded RNA. There are several possible paths of contamination, including live animals, transportation vehicles, humans, equipment and bioaerosols. Bioaerosols within buildings originate from food, litter, skin debris, building materials, and mainly manure (Nehmé *et al.*, 2008). Bioaerosols in hog barns have a median aerodynamic diameter of 11-14 μm (O'Shaughnessy *et al.*, 2002) and total dust is present at concentrations of 2 to 6 mg/m^3 (Duchaine *et al.*, 2000). The emission and dissemination of the PRRS and Influenza viruses through bioaerosols are insufficiently characterized even if air is a possible path of contamination for swine herds. For example, following an outbreak of PRRS in the United States, Lager and Mengeling (2000) have concluded, through sequencing of the ORF5 region, the presence of identical PRRSV strains in unrelated farms located in the same area (the most distant being at 33 km) (Lager and Mengeling, 2000). Finally, mathematical models strongly support the path of transmission of Influenza as being aerosols (Davis *et al.*, 2009; Tellier, 2009). Davis *et al.* (2009) have concluded, from a recent outbreak of equine Influenza A (H₃) in Australia (Queensland), that Influenza viruses are spread over distances of 1 to 2 km through aerosols (Davis *et al.*, 2009). Indeed, the Influenza epidemics affected 438 horse farms, among which 81% were not contiguous to other infected farms. Generally, the infected farms were several kilometers distant (up to 13 km) from one another. The dispersion pattern of the Influenza viruses coincided with that of prevailing winds at the time (Davis *et al.*, 2009).

The biosecurity measures applied in Quebec herds, even though it is internationally recognized, is not able to prevent the transmission of bioaerosols containing PRRS and Influenza viruses. The filtration of bioaerosols at the exhaust fans of farm buildings is a possible solution to prevent the dispersion of aerosol viruses from a swine herd to another. Experimental models have demonstrated that HEPA (High Efficiency Particulate Air) filters, as well as MERV 16 (Minimum Efficiency Reporting Value) classified filters block the transmission of the PRRSV in animals through bioaerosols (Batista *et al.*, 2008; Dee *et al.*, 2005, 2006, 2010; Otake *et al.*, 2010; Spronk *et al.*, 2010). However, the applicability of HEPA filters in the industry is very low since they are expensive, clog rapidly under the effect of the dust contained in the surrounding air, and cause much air restriction. Consequently, the use of MERV 16 filters or combinations of prefilters and filters with a lower MERV value (< 16) should be privileged. They must, however, allow eliminating, inactivating or diluting the viral aerosols emitted by farm buildings.

A test bench has been developed by the *Centre de développement du porc du Québec inc.* (CDPQ) and the *Centre de recherche de l'Institut universitaire de cardiologie et de pneumologie de Québec* (CRIUCPQ) to assess the flow rate through various combinations of prefilters and filters when submitted to a head loss as typically encountered through a swine barn filter, as well as their efficiency in capturing bacterial viruses (phages Phi6, level of containment 1) mimicking the PRRS and Influenza viruses. In total, 9 filtration units were studied, including five combinations of new prefilters, one combination of second-hand prefilter and filter, one combination of new prefilter and second-hand filter, and two new prefilters only. The second-hand prefilters and filters were received from two different Canadian hog barns.

Objectives

- A. Design and validation of a test bench that can be used to assess the efficiency of different filtration units
- B. Assessment of the re-emission¹ of phages Phi6 through the different filtration units during purges (background noise)
- C. Assessment of efficiency in capturing particles of different diameters and phages Phi6 through different filtration units

Material and Methods

Design of a test bench to measure the efficiency of the filters

The Research Triangle Institute (Foarde and Hanley, 1999) has conducted a study in which a test bench complying with the ASHRAE 52.2 standard for inert particles (ANSI/ASHRAE, 2012) was used, following minor modifications, to assess the efficiency in filtering airborne biological particles (Figure 1). Indeed, according to Foarde and Hanley (1999), the filtration characteristic curves of biological particles coincide with those of inert particles, demonstrating the validity of the ASHRAE 52.2 standard for biological particles. Consequently, the test bench developed by the CDPQ and the CRIUCPQ (Figure 2) is made up of an air duct in which the air is blown by a fan and other components ensuring compliance with the ASHRAE 52.2 standard. A biological particle nebulization system, however, replaces the inert dust injection system of the ASHRAE 52.2 standard, and various particle samplers and counters have been added in order to determine the concentration of biological particles in the air.

¹ The concept of re-emission started in this document refers to the release of particles previously blocked and stored by the filters.

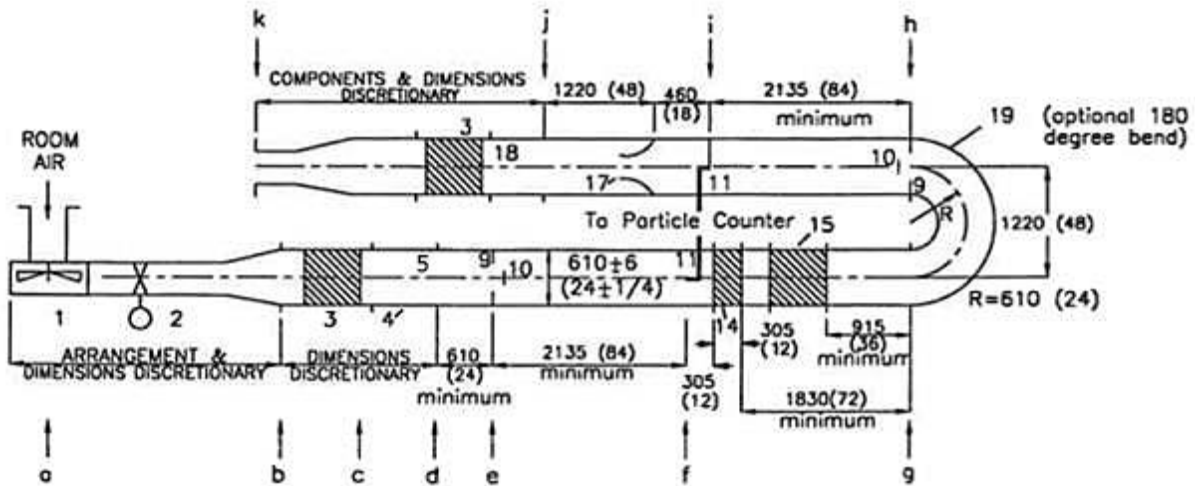


Figure 1 Test bench diagram from the ANSI/ASHRAE 52.2 standard

The different components of the test bench are identified above and listed below:

List of components

1. Fan
2. Air flow control valve
3. HEPA filter bank for eliminating background noise
4. Transition of the HEPA filter bank to the test bench duct
5. Bioaerosol injection tube
6. Aerosol generator (*Not shown in this diagram*)
7. Dust feed pipe (*Not shown in this diagram*)
8. Dust feeder (*Not shown in this diagram*)
9. Mixer inlet for equal virus distribution in the duct
10. Diffuser perforated plate for equal virus distribution in the duct
11. Optical particle counter sampling tubes
12. Static tap (*Not shown in this diagram*)
13. Manometer (*Not shown in this diagram*)
14. Filtration unit to be assessed
15. Final filter (*Not applicable*)
16. Vertical manometer (*Not shown in this diagram*)
17. Iris for measuring the air flow rate in the test bench
18. Transition of the test bench duct to the HEPA filter bank for minimizing release to the environment
19. 180° elbow for limiting the space required for test bench installation



Figure 2 Test bench used to study the various filtration units

The different filtration units were evaluated by reproducing the air pressures found in farm building. The current method for designing air filtration systems in swine buildings consists in minimizing the filter area, in order to reduce costs, while keeping a reasonable maximum pressure in the summer when all ventilation stages are in operation. Thus, in order to be able to compare the filtration units, it was decided to set the filter pressure differential at 0.2 in of water. The filter air speed was then measured when the filter pressure (0.2 in of water) was reached and varied between filtration units as a function of the air restriction caused by the filtering membrane.

Generation and Sampling of Test Bench Viral Aerosols

The various filtration units were studied in triplicate for their capacity in blocking the emission of particles of different diameters and viral particles artificially generated by a six-jet nebulizer (Collison Nebulizers, BGI Incorporated, MA, U.S.A.). The purge of the test bench (in the absence of aerosolization of phages Phi6) was similarly studied to evaluate if there was any re-emission of phages Phi6 from the filtration units following nebulizations.

Inside a test bench, a 1X phage buffer solution (50 mM Tris-HCl pH7.5, 100 mM NaCl, 8 mM MgSO₄) containing 10⁸ phages Phi6 per millilitre was aerosolized for 20 minutes with a jet nebulizer (20 psig, 12 L/min, 6-jet nebulizer, BGI Incorporated, Waltham, MA, U.S.). In parallel to the aerosolization of phages Phi6 (Figure 3), the air of the test bench was sampled upstream and downstream of the air filtration units studied by impingers containing each 20 mL of 1X phage buffer (Figure 4, 12.5 L/min, 20 minutes, All-Glass Impinger 30 [AGI-30], Ace Glass Incorporated, NJ, U.S.) In addition, air particles with a diameter of 0.3, 0.5, 1, 3, 5 and 10 µm present upstream and downstream of filtration units were counted by an optical particle counter (Figure 4, 28.03 L/min, 5 minutes, Particle Counter Model 3315, Met One, Loveland, CO, U.S.). Concentrations were expressed in number of particles per cubic metre of air. The sampling was done in an isokinetic manner, i.e., the air speed at the inlet of the suction duct (in the duct of the test bench) of the AGI-30 and the particle counter and the air speed in the test bench were the same at more or less 10%. The isokinetic sampling avoids disrupting the air flow in order to ensure representative sampling of particles passing through the test bench duct. Sharp tips of different diameters were then designed according to the anticipated air speed spectrum and the ingoing air flow rate on the sampling equipment. The tips were changed manually depending on the filter air flow rate.



Figure 3 Experimental device for nebulizing viral aerosols

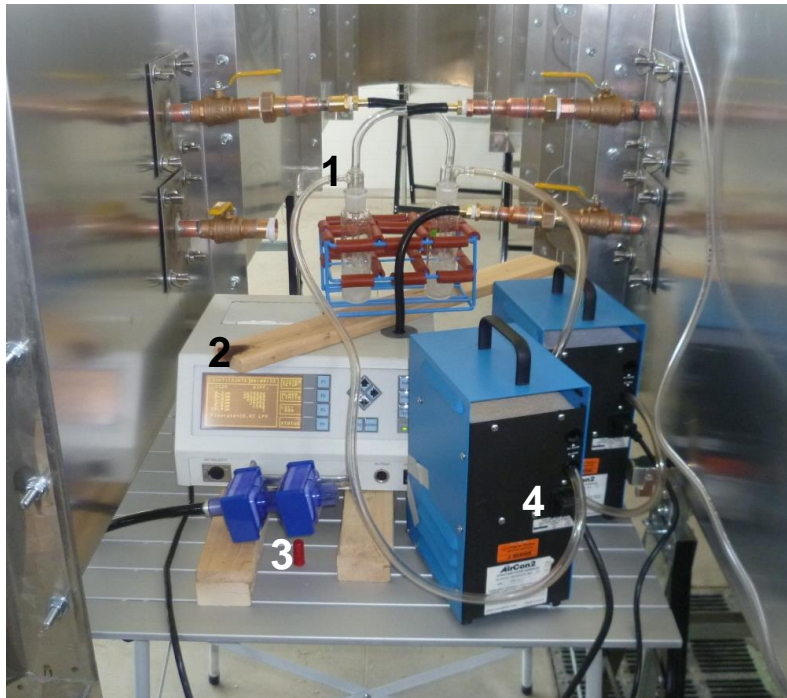


Figure 4 Experimental design for sampling viral aerosols

List of equipment

- 1: AGI-30 impingers
- 2: Optical particle counter
- 3: Device for purging the particle counter
- 4: Two very-high-flow pumps for operating the AGI-30 impingers

Nine filtration units were evaluated: five combinations of new prefilters and filters (A to E), one combination of second-hand prefilter and filter (F), one combination of new prefilter and second-hand filter (G) and two new prefilters only (H and I).

Following the sampling, the solutions from the AGI-30 impingers were kept at -80°C up to extraction of the viral ribonucleic acid (RNA). The $140\ \mu\text{L}$ viral RNA (representing about 2 L of air) was extracted using the viral RNA Extraction Kit (QIAGEN, Mississauga, ON, Canada) as per manufacturer's instructions. The viral RNA was then transcribed into complementary deoxyribonucleic acid (cDNA) using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA, U.S.A.) The cDNA quantification of phages Phi6 was performed through polymerase chain reaction (PCR) according to a previously published protocol (target: ORF8, Gendron *et al.*, 2010). The DNA amplification was done using the iQ Supermix reagents (Bio-Rad) and the DNA Engine Opticon 2 system (Bio-Rad). Data were acquired through the Opticon Monitor software (Bio-Rad) then analyzed with the \log_{10} equation (number of targeted copies) = f (threshold cycle) and a plasmidic standard curve containing a copy of target open reading frame 8 (ORF8) gene. The PCR amplification efficiency was determined using the equation $E = 10^{-(\text{slope})-1}$. Concentrations in phages Phi6 were expressed in number of phages Phi6 per cubic metre of air.

Results and Discussion

Objective A. Design and validation of a test bench that can be used to assess the efficiency of different filtration units

Test bench calibration and validation

The design of the test bench was validated on the uniformity of air speed inside the duct, the accuracy of the flow rate measurement using the iris, and airtightness.

In order to validate the uniformity of air speed inside the duct, nine equidistant points were selected on its cross-section (Figure 5).

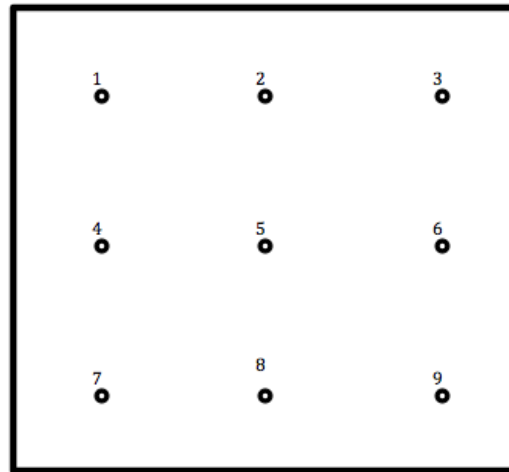


Figure 5 Dispersion of measurement points in the test bench cross-section

To provide access to these nine points, three holes were drilled on the duct side. Using a hot-wire anemometer with a telescopic probe (KIMO VT 200, Montpon, France) and a level for ensuring appropriate height of measurements, the air speed was measured by making a 10-second average at the nine points for different fan frequencies. The results are reported in Table 1.

Table 1 Validating the uniformity of air speed in the test bench

Fan frequency (Hz)	Air speed (ft/min)									Average speed (ft/min)	Average flow rate (ft ³ /min)	Maximum variation from the mean (MAX 15%)
	Position in the duct*											
	High			Mean			Low					
	1	2	3	4	5	6	7	8	9			
60	397.9	415.2	383.7	430.2	469.6	456.3	377.9	430.5	392.4	417.1	1668.3	12.6%
55	335.2	357.4	366.5	368.4	371.3	428.5	319.0	356.7	393.2	366.2	1465.0	17.0%
50	275.3	328.4	335.2	351.9	359.6	362.0	332.7	321.9	304.5	330.2	1320.7	16.6%
45	254.6	272.1	300.6	282.3	314.9	320.5	265.7	282.3	300.9	288.2	1152.8	11.7%
40	232.7	251.7	238.0	263.7	264.6	231.3	217.9	248.4	223.8	241.3	965.4	9.7%
35	207.6	202.6	183.8	223.8	222.0	220.2	191.9	195.1	175.6	202.5	810.0	13.3%
30	168.3	171.4	147.1	167.7	183.1	167.4	168.0	168.3	150.0	165.7	662.8	11.2%
25	117.0	127.5	123.2	127.3	131.1	139.8	117.0	130.2	122.8	126.2	504.8	10.8%
20	84.6	77.5	83.8	81.5	82.9	95.7	80.0	87.1	79.4	83.6	334.4	14.5%

* see Figure 5

According to the ANSI/ASHRAE 52.2 standard, the maximum permitted variation from the mean is 15%. The maximum variation from the mean slightly exceeded the maximum allocated for frequencies of 50 and 55Hz (Table 1). The slight exceedance can, however, be explained by a lack of precision in the handling. For example, the mere fact of slightly turning the probe containing the anemometer's heating wire caused changes in the measurements. Considering only one value exceeded the maximum allowed in each case, it was not necessary to make major changes to the design of the test bench. In addition, the test bench was never used at such high flow rates in the course of the study.

To validate the accuracy of the iris, the flow rate of the latter (Table 2) was compared with the average flow rate obtained from the measurements taken using the hot-wire anemometer for the different fan frequencies (Table 3).

Table 2 Air flow rate at the iris for different fan frequencies

Fan frequency (Hz)	Iris		
	K	Iris ΔP (in of water)	Flow rate (ft ³ /min)
60	2154	0.4772	1488
55	2154	0.3862	1339
50	2154	0.3042	1188
45	2154	0.2371	1049
40	2154	0.1798	913
35	2154	0.1217	751
30	1525	0.1654	620
25	1294	0.1281	463
20	806	0.1398	301

Like for the validation of the uniformity of the airflow, the validation of the iris accuracy includes data slightly above the 10% maximum error prescribed in the ANSI/ASHRAE 52.2 standard (Table 3). Again, the same factors may explain the differences. Therefore, the design of the test bench was kept and the iris was used to measure the air flow rate during the study.

Table 3 Difference of air flow measurement between the anemometer and the iris

Fan frequency (Hz)	Error (%) (MAX 10%)
60	12.1%
55	9.4%
50	11.2%
45	9.9%
40	5.7%
35	7.8%
30	6.9%
25	9.0%
20	11.0%

Objective B. Assessment of the re-emission of phages Phi6 through the different filtration units during purges (background noise)

No phages Phi6 was detected in the air when purging new or second-hand filtration units (filtration units A to G). However, for filtration units H and I, two prefilters seem to have reemitted phages Phi6. Nonetheless, concentrations in phages Phi6 are below or near the detection limit, therefore other replicates must be made to confirm the data obtained. Very few total particles (a few hundred at the most) were detected during purges as compared with $(4.34 \pm 0.45) \times 10^7$ of total particles per cubic metre of air present during the aerosolization of salts and phages Phi6.

Although there does not seem to have been a high level of re-emission, it should be considered that the purges involve no nebulization, not even for salt particles without biological particles. The air used during the purges was previously filtered through a HEPA filter and no intervention was made upstream of the filtration unit section. A hypothesis to be considered is, therefore, that there might be more re-emission of inert and biological particles when newly emitted particles and particles captured by the filter collide. Such collisions might potentially foster the separation between particles previously captured by the filters and the filter membrane, thus resulting in a re-emission.

Objective C. Assessment of efficiency in capturing particles of different diameters and phages Phi6 through different filtration units

The experimental design put in place enabled the generation of aerosols containing on average $(7.7 \pm 6.7) \times 10^6$ phages Phi6 per cubic meter of air in the test bench. For comparison purposes, circoviruses have previously been detected at concentrations of 10^7 per cubic metre of air in several swine buildings in Quebec (Verreault *et al.*, 2010). The aerosols of phages Phi6 were reduced more efficiently by filtration units A ($95.8 \pm 1.6\%$), D ($96.8 \pm 1.5\%$) and E ($98.1 \pm 1.1\%$) (Figure 6). Increased variability in the replicates was observed for filtration units B and H, stressing the importance of making more than three replicates per filtration unit in the future. The second-hand versions of filtration unit C ($92.9 \pm 4.8\%$), i.e., filtration units F ($88.4 \pm 7.4\%$) and G ($85.5 \pm 2.6\%$) seemed slightly less efficient in capturing viral aerosols than new units. The results remain to be confirmed through the study of more than three replicates. Filtration unit H is little efficient in blocking the emission of phages Phi6 ($-7.8 \pm 33.1\%$). The negative filtration efficiency percentages associated with filtration unit H (Figure 6) and other filtration units (Figures 7 to 10) can be explained by 1) a better mix of the Phi6 aerosol cloud in the air downstream of the filtration unit, and therefore a better efficiency of AGI-30 impingers in sampling particles; 2) the re-emission of viral particles accumulated in the filtration membranes and; 3) the fragmentation of larger particles (such as 3 and 5 μm , Figures 11 and 12) when passing through the prefilters, filters or the test bench duct.

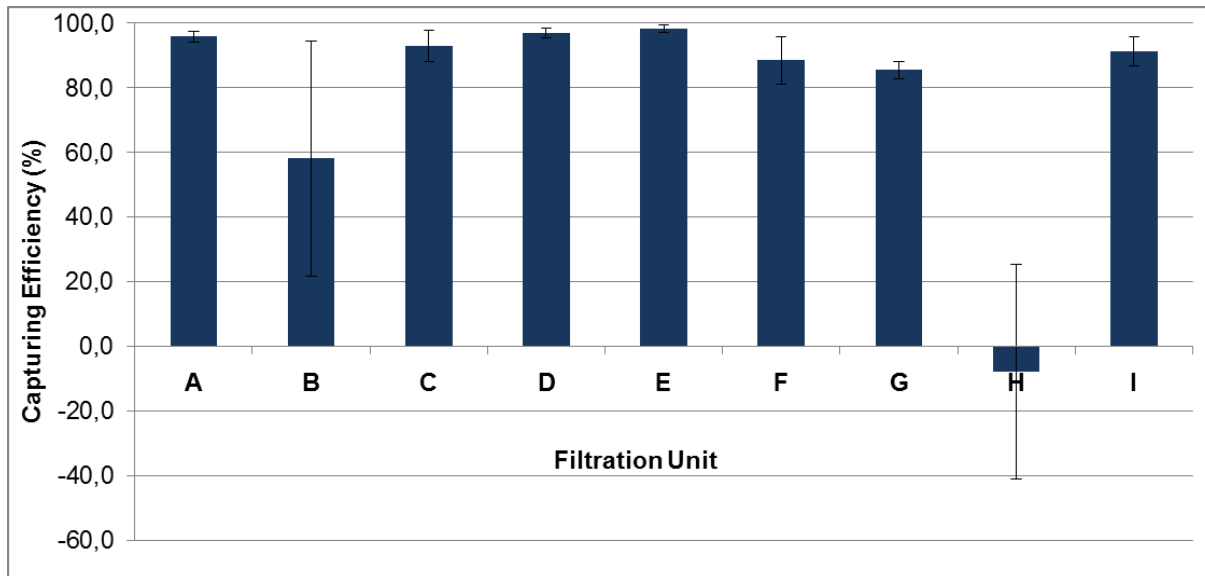


Figure 6 Efficiency in capturing phages Phi6 through different filtration units

The experimental design put in place enabled the generation of aerosols containing on average $(4.34 \pm 0.45) \times 10^7$ of total particles per cubic meter of air in the test bench. Concerning the efficiency in capturing total particles (Figure 7), on average, the highest efficiency percentages are associated with filtration units C ($17.4 \pm 2.8\%$), D ($58.7 \pm 5.5\%$) and E ($76.6 \pm 2.7\%$). Filtration units D and E were thus among the most efficient in capturing phages Phi6 and total particles. Filtration unit A, although it was efficient in reducing concentrations of phages Phi6, did not prove very efficient in capturing total particles. Most particles in the test bench being $0.3 \mu\text{m}$ in diameter, the lowest efficiency of filtration unit A in capturing total particles can be explained by a low efficiency in filtering $0.3 \mu\text{m}$ particles, i.e., -85.3% . The fragmentation of large particles into fine particles within filtration unit A or the re-emission of $0.3 \mu\text{m}$ particles accumulated in the filtration membrane may explain such results. The same conclusion can be drawn similarly for other filtration units and explains the key differences between the efficiency for total particles and for phages Phi6. It is therefore not advisable to compare the efficiency in capturing total particles between new and second-hand versions for the same filtration unit.

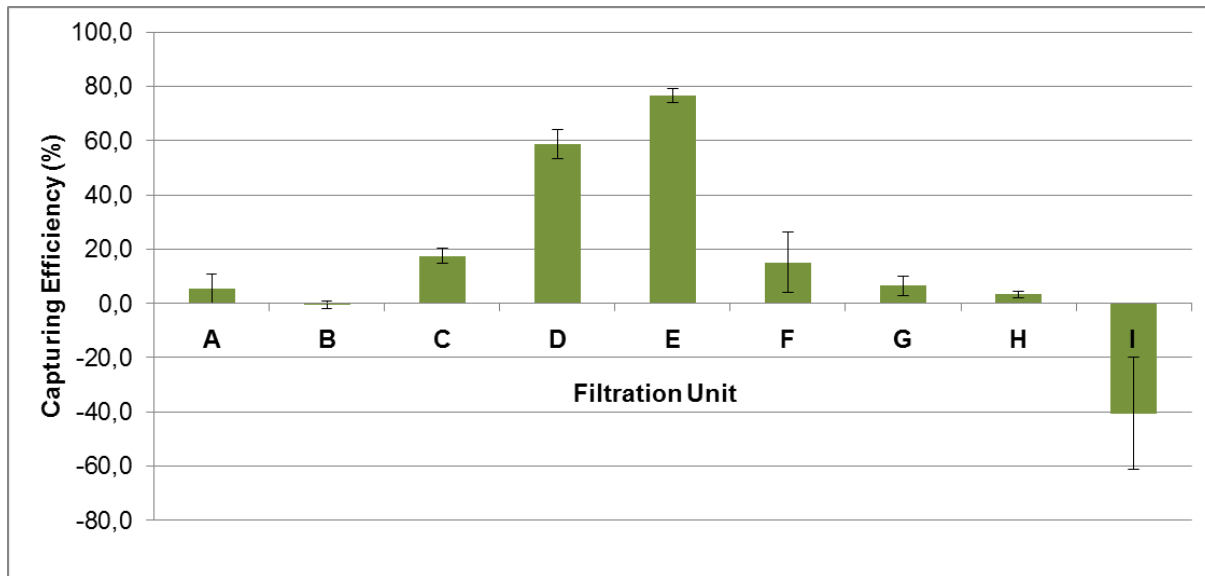


Figure 7 Efficiency in capturing total particles through different filtration units

Particles with a 0.3, 0.5, 1, 3, 5 and 10 μm diameter were respectively found at concentrations of $(2.0 \pm 0.5) \times 10^7$, $(1.50 \pm 0.1) \times 10^7$, $(7.8 \pm 1.7) \times 10^6$, $(2.5 \pm 1.1) \times 10^5$, $(1.4 \pm 0.6) \times 10^4$ and $(2 \pm 2) \times 10^1$ of particles per cubic metre of air in the test bench. Concerning the efficiency in capturing particles 0.3, 0.5, 1, 3, and 5 μm in diameter (Figures 8 to 12), on average, the highest efficiency percentages are associated with filtration units D and E, i.e., the best to reduce the emission of phages Phi6. Filtration unit I is one of the most efficient for larger particles i.e. 3 and 5 μm (Figures 11 and 12), whereas filtration unit D stands out for capturing the finest particles, i.e. 0.3 and 0.5 μm in diameter (Figures 8 and 9). Filtration units F and G (second-hand versions of filtration unit C) have an efficiency percentage for filtering 0.3 μm particles lower than that of filtration unit C. Filtration efficiencies for particles 0.5 to 5 μm in diameter are, however, higher than for filtration unit C. This could be explained by more particle fragmentation events in the presence of second-hand filtration units or emissions of particles contained in the filtering membranes due to collisions of the latter with viral aerosols. An other hypothesis was that the more the filtration unit has been used, the more it is clogged and retains larger particles. The hypothesis can be partly verified by comparing the air flow rates for second-hand and new filtration units. Indeed, the new filtration units had a higher flow rate than the second-hand units for the same static pressure, meaning that they created less air restriction. Therefore, it is possible to assume that they also created less particle restriction. It should be noted that very few particles were present downstream of second-hand filtration units in the absence of nebulization (during the purges of the test bench and hence in the absence of possible collisions between viral particles emitted and particles already present in the filtering membranes), i.e., in the presence of air purified by the test bench HEPA filter.

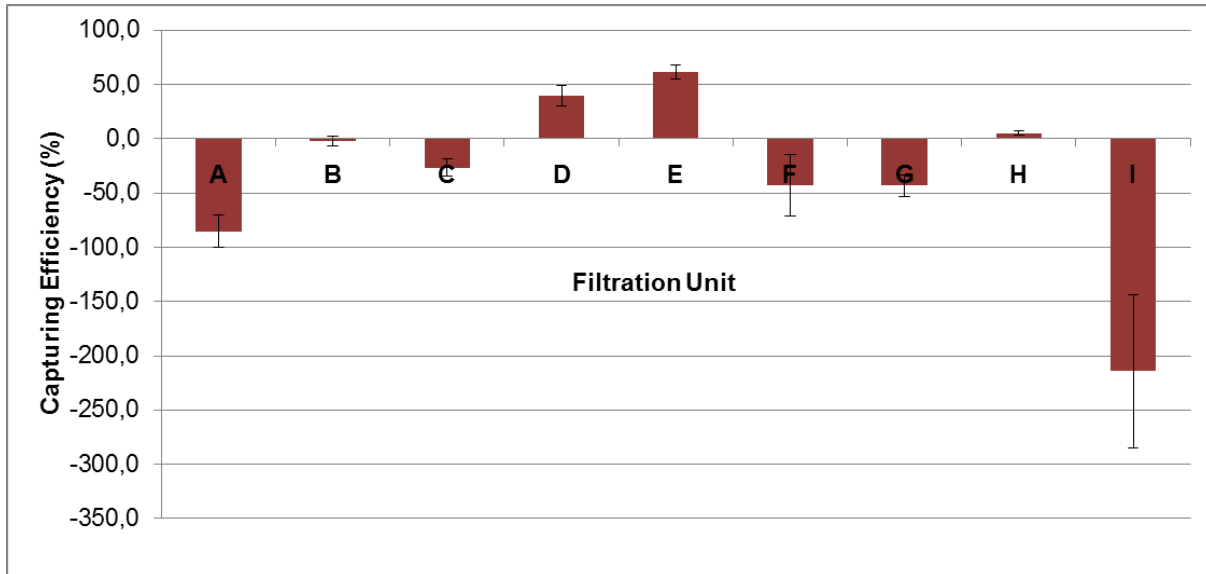


Figure 8 Efficiency in capturing 0.3 µm particles through different filtration units

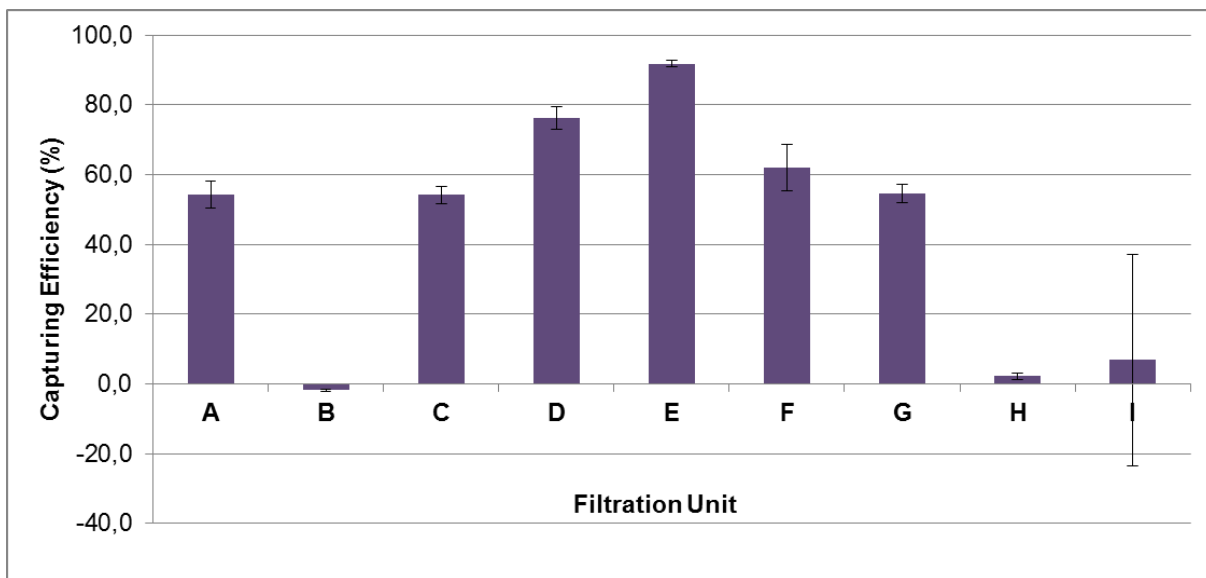


Figure 9 Efficiency in capturing 0.5 µm particles through different filtration units

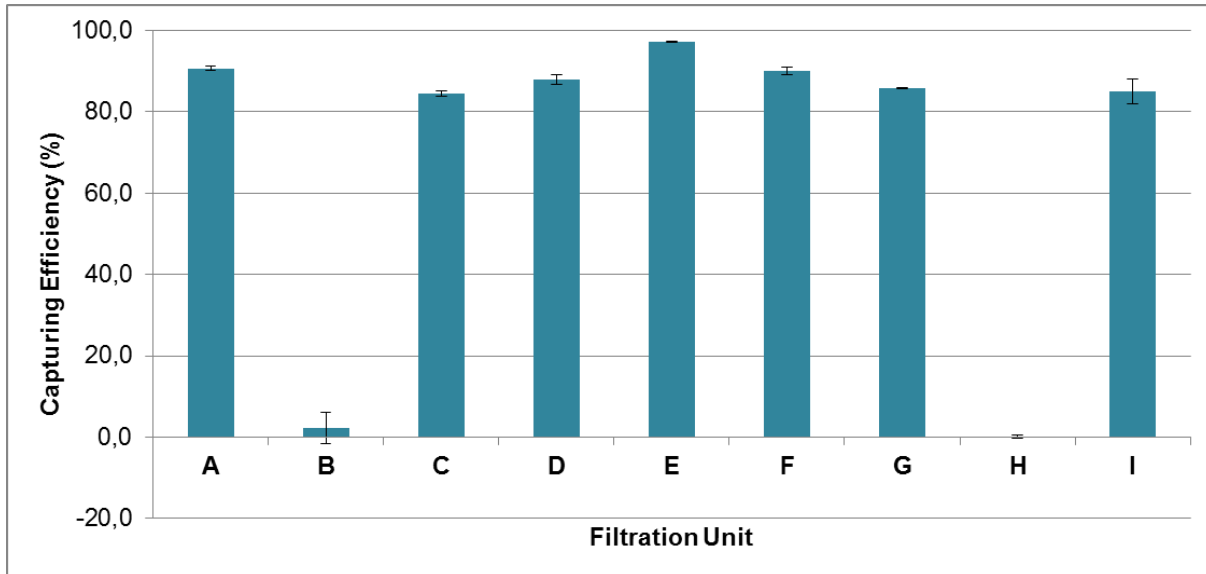


Figure 10 Efficiency in capturing 1 µm particles through different filtration units

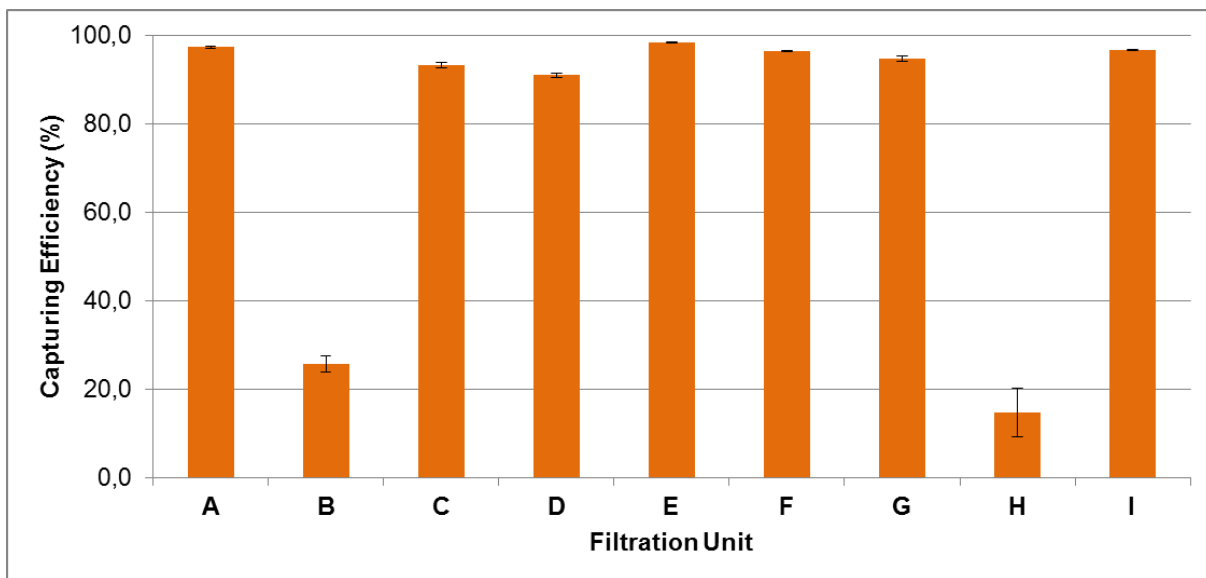


Figure 11 Efficiency in capturing 3 µm particles through different filtration units

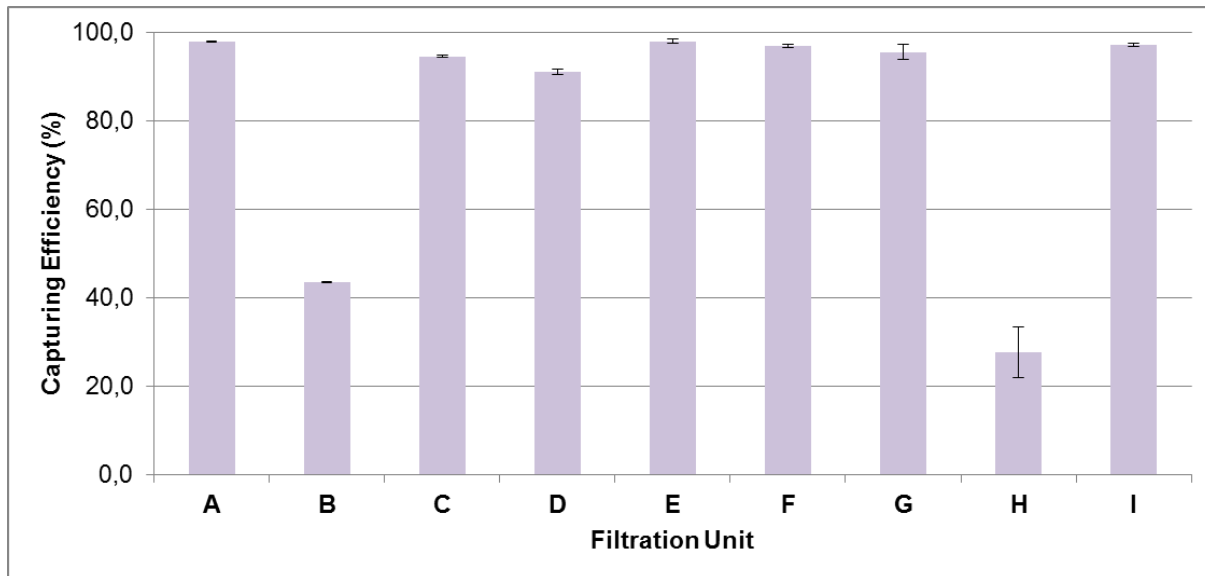


Figure 12 Efficiency in capturing 5 μm particles through different filtration units

Filtration unit E seems to be the best choice based on all particles evaluated. Experiments will have to be repeated to confirm such results. The efficiency in capturing 10 μm particles was not considered during the study, given that their number was near the detection limit of the particle counter (generally, less than 10 particles of 10 μm in diameter as compared with 10^6 particles of 0.3 or 0.5 μm in diameter for 5 minutes of sampling).

By comparing the results obtained with the MERV values provided by the manufacturers of the different prefilters and filters assessed, very significant differences can be observed. Besides, given different particles can divide by passing through the filtration units or be reemitted from the filtering membrane, it seems impossible to us to compare the measurements achieved with the MERV values provided by the manufacturers.

Conclusion

To conclude, the results obtained seem to be much more accurate to assess the efficiency of filtration units with regard to phages than particles, since the fragmentation and re-emission of the latter from filtering membranes make the measurements difficult to interpret. These two suppositions explaining the results obtained in this study will need to be confirmed by a subsequent study. In terms of efficiency in blocking particles of different diameters, there is a marked difference with the characteristics provided by the manufacturers. It would be possible to solve this difficulty by conducting efficiency tests according to the ANSI/ASHRAE 52.2 standard, i.e., by evaluating the efficiency of filtration units in blocking each size of particles individually and weighing the samples taken upstream and downstream instead of counting particles. Weighing samples collected upstream and downstream would also probably enable a more reliable correlation between the efficiency of the filtration unit in blocking Phi6 and total particles. Indeed, according to the results, some filtration units are very efficient in blocking phages, but weaker to block total particles, which is contradicting in itself.

The present study enabled the development of a test bench for assessing the efficiency of different filtration units regarding viruses and particles encountered in swine barns. Phages Phi6 were used to mimic the PRRS virus in the different trials.

It should be noted that as mentioned at the beginning of the report, the results obtained are related to the experimental design, more specifically, to the use of a six-jet nebulizer generating viral particles and salts with a median mass aerodynamic diameter of about 1 μm (experimental data, unpublished). The equipment available did not allow generating a broader spectrum of particles carrying biological particles (phages Phi6). However, as part of the assessment of the efficiency of filtration units in a bioexclusion context, the hypothesis is that this population of particles proves fairly representative of all particles coming from surrounding buildings, which are favourable to the transport of viruses between sites. The hypothesis, nonetheless, remains to be confirmed in future studies. It should also be considered that viral aerosols produced artificially in the test bench do not represent the group encountered within swine buildings, namely particles with a median mass aerodynamic diameter of 11-14 μm (O'Shaughnessy *et al.*, 2002). Therefore, for a biocontainment concept, for which the spectrum of particles to be filtered is much broader, we cannot assess the efficiency of filters with certainty using the experimental design used in this study. Consequently, the results obtained should be interpreted carefully with the purpose of validating the design of the test bench. The size of particles on which the PRRSV is carried both in the herd building's perimeter and outside is still unknown to date and it is impossible, for the time being, to certify that viruses are not carried on larger particles than the spectrum of particles achieved with the present experimental design. Consequently, under no circumstances can the results described herein be used to explain or settle problems associated with viral aerosols encountered in the field before subsequent projects on the determination of the size of particles carrying swine viruses in the air are carried out.

The present study allowed establishing the bases and the foundation necessary for laboratory reproduction of viral aerosols and the development of new filtration methods adapted to swine buildings. Improvements will be required to the nebulization method (e.g., improve the concentration and aerodynamic diameter of particles and foster the survival of viruses during nebulization and sampling) as well as other experimental aspects. For example, more replicates will be required in the future in order to achieve statistically significant results for all filtration units. Trials on different filters from an individual manufacturer and of an individual model could also be performed to evaluate variations between production lots. The uniform distribution of the particle cloud carrying the phages will have to be validated to ensure representative sampling upstream and downstream. Once the size of inert particles carrying the viruses found in the swine industry is known, confirmation will be required that phages Phi6 closely represent animal viruses. An MSc project has been submitted to the Canadian Swine Health Board (CSHB) and to Swine Innovation in order to optimize both the test bench and the experimental methodologies.

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**Centre de développement du porc du Québec inc.
Place de la Cité, tour Belle Cour
2590, boulevard Laurier, bureau 450
Québec (Québec) G1V 4M6**

**☎ 418 650-2440 • 📠 418 650-1626
cdpq@cdpq.ca • www.cdpq.ca**