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Evaluation of bioaerosol sampling techniques for the detection of *Chlamydophila psittaci* in contaminated air

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Abstract

Chlamydophila (C.) psittaci, a category B bioterrorism agent, causes respiratory disease in birds and psittacosis or parrot fever in man. The disease spreads aerogenically and no vaccines are available for either birds or man. Highly sensitive *C. psittaci* bioaerosol monitoring methods are unavailable. We evaluated: 1) dry filtration for collecting *C. psittaci* from contaminated air using different samplers and membrane filters, 2) impingement into different liquid collection media by use of the AGI-30 impinger and the BioSampler and 3) impaction into newly designed *C. psittaci* media utilizing the MAS-100 aerosol impactor. For personal bioaerosol sampling, we recommend the use of a gelatin filter in combination with the IOM inhalable dust sampler at an airflow rate of 2 L/min. This allowed the detection of 10 organisms of *C. psittaci* by both PCR and culture. For stationary bioaerosol monitoring, sampling 1000 liters of air in 10 minutes with the MAS-100 impactor and ChlamyTrap 1 impaction medium was most efficient and made it possible to detect 1 and 10 *C. psittaci* organisms by PCR and culture, respectively. ChlamyTrap 1 in combination with the MAS-100 impactor might also be applicable for bioaerosol monitoring of viruses.

Keywords

***Chlamydophila psittaci* / bioaerosol / filtration / impingement / impaction**

1. Introduction

Chlamydophila (C.) psittaci causes respiratory disease in birds and can be transmitted to humans. Today, *C. psittaci* has been demonstrated in about 465 bird species comprising 30 different bird orders (Kaleta and Taday, 2003). Human exposure usually occurs when dried fecal droppings or eye and nostril secretions of birds containing the organisms are aerosolized and inhaled by a susceptible host causing psittacosis or parrot fever (Burkhart and Page, 1971; Cole, 1990). Environmental monitoring and control could prevent transmission to humans, especially in risk areas such as: 1) hospitals involved in the direct care of psittacosis patients, 2) laboratories processing clinical samples, 3) research laboratories handling the agent, 4) quarantine stations for imported pet birds and 5) workplaces in the pet bird and poultry industry. Additionally, recent bioterrorist events have emphasized the need to detect and identify biothreat agents such as *C. psittaci* (http://www.bt.cdc.gov). No vaccines are available for either birds or man.

Highly sensitive *C. psittaci* bioaerosol monitoring methods are unavailable. Currently, bioaerosol sampling is based on: 1) dry filtration using membrane filters placed in sampling cassettes, 2) impingement into liquid, or 3) impaction onto agar (Jensen et al., 1992; Muilenberg, 2003).

Dry filtration is generally not recommended for bioaerosol monitoring of viable microorganisms due to desiccation effects (DeCosemo et al., 1992), but it has the advantage that it can be used for stationary bioaerosol monitoring, checking workplaces as well as for personal bioaerosol monitoring as the sampling cassette can be attached to clothing and connected to a small portable pump.

Viable aerosol particles can also be collected by liquid impingement. A major

disadvantage however with conventional impingers, such as the all glass impinger (AGI-30), is the considerable amount of collection fluid that is lost due to violent bubbling in order to achieve sufficient high physical collection efficiency. Moreover, bubbling creates aerosols, allowing the impinged microorganisms to escape again from the collection fluid (Grinshpun et al., 1997; Willeke et al., 1998; Lin et al., 1999). Additionally, the bubbling process might inactivate viruses (Agranovski et al., 2004) and probably other small microorganisms such as *Chlamydiaceae* spp. Some problems associated with the impingers have been addressed with the recently developed swirling aerosol collector, which is commercially available as the Biosampler (Willeke et al., 1998). The Biosampler utilizes a viscous, non-evaporative collection fluid based on mineral oil (ViaTrap) for long-term sampling at an airflow rate of 12.5 L/min. However, ViaTrap cannot be used for detecting live obligate intracellular organisms such as viruses and *Chlamydiaceae* spp. as the collection fluid is toxic to cell cultures and embryonated chicken eggs, which are routinely used to cultivate these organisms. Moreover, small microorganisms like viruses (25-400 nm) and *Chlamydiaceae* spp. (200 nm) cannot be concentrated (ultracentrifugation) in ViaTrap as they do not pellet in this collection medium.

Aerosol impactors based on the Andersen principle (Andersen, 1958) capturing microorganisms on agar can be used to sample 1000 liters of air in only 10 minutes. A disadvantage however is the selectiveness of impaction based on the direct collection of airborne microbes on agar, which makes it of limited use for monitoring ambient air for the presence of obligate intracellular pathogens such as *C. psittaci*, as these organisms do not grow on agar. In the present study, we designed two *C. psittaci* impaction media and

evaluated their use for identifying *C. psittaci* in contaminated air.

Thus, in the present study, we examined the efficiencies of dry filtration by the IOM (SKC Ltd., Dorset, U.K.) and PAS6 (University of Wageningen, The Netherlands) filter cassettes using different membrane filters. Secondly, we evaluated the AGI-30 impinger and the BioSampler using eight different liquid collection media specially adapted for collecting *C. psittaci*. Thirdly, the MAS-100 'Andersen' impactor was evaluated using ChlamyTrap 1 and 2, two novel *C. psittaci* collection media. All air samples were examined by both PCR and culture.

2. Material and methods

2.1. Chlamydophila psittaci

C. psittaci genotype D strain 92/1293 (Vanrompay et al., 1993) was used to evaluate the bioaerosol sampling methods. *C. psittaci* was grown in Buffalo Green Monkey (BGM) cells as previously described (Vanrompay et al., 1992). Bacterial titration was performed by the method of Spearman and Kaerber (Mayr et al., 1974) to determine the 50 % tissue culture infective dose ($TCID_{50}$).

2.2. Experimental set-up

All *C. psittaci* bioaerosol monitoring experiments were performed in PC3 negative pressure (150 Pa) isolators (IM 1500, Montair Sevenum, the Netherlands) of 2 m^3. Isolators were operated under controlled biosafety conditions (21°C, 48% relative humidity) and were fumigated (Johnson Diversey, Fontenay Sous Bois Cedex, France) and subsequently ventilated for 48 hours in between two measurements. A freshly

prepared *C. psittaci* suspension of 10^9 bacteria in 10 ml of DMEM (Invitrogen) was completely aerosolized over one hour using a Cirrus™ nebulizer (Laméris, Aartselaar, Belgium) connected to a membrane pump (2 bar; VWR, Leuven, Belgium) generating a particle size of 2 to 5 µm. All sampling devices operated continuously during *C. psittaci* nebulization.

2.3. Dry filtration

Two filtration devices were evaluated: the standard IOM personal inhalable dust sampler (SKC Inc., Eighty Four, PA, USA) and the PAS-6 sampler, manufactured at the University of Wageningen (The Netherlands). Both sampling devices were evaluated with five different filter types of 25 mm diameter consisting of: 1) mixed cellulose ester with support pad (MCES; 0,8 µm pore size, SKC), 2) mixed cellulose ester without support pad (MCE; 0,8 µm pore size, Millipore, Brussels, Belgium), 3) glass fibers (1.0 µm pore size, SKC), 4) gelatin (3 µm pore size, SKC) and 5) polycarbonate (0.1 µm pore size, Millipore). All filters, except for the polycarbonate filter, were evaluated over 60 minutes at two constant airflow rates of 1 and 2 L/min. Due to its small pore size, the polycarbonate filter could only be tested at a constant airflow rate of 1 L/min.

2.4. Impingement

Two sampling devices were evaluated: 1) the standard AGI-30 liquid impinger (VWR) and the BioSampler (SKC), being a second generation impinger. Both impingers operated for 60 minutes at the recommended airflow rate of 12.5 L/min (Willeke et al., 1998). Each impinger was tested with eight different collection fluids: 1) 100% distilled

water, 2) 50 % distilled water + 50 % mineral oil (Acros Organics, Geel, Belgium), 3) 40 % distilled water + 60 % glycerol (Acros Organics), 4) 16 % distilled water + 84 % glycerol, 5) 100 % glycerol, 6) 100 % Chlamydia transport medium [sucrose 74.6 g/L (Acros Organics); KH_2PO_4 5,1 g/L and K_2HPO_4 1,2 g/L (Sigma, Bornem, Belgium); L-glutamic acid mono potassium salt 0,9 g/L and gentamycin 50 µg/ml (Invitrogen, Merelbeke, Belgium); vancomycin 100 µg/ml and streptomycin 100 µg/ml (Soenen, Merelbeke, Belgium); nystatin 25000 U/ml (Sigma) pH 7], 7) Chlamydia transport medium + 2 % carboxymethylcellulose sodium salt (CMC) (Sigma) and finally 8) 50 % Chlamydia transport medium + 50 % mineral oil. Collection media were selected based on literature and on our experience with chlamydophila preservation and stirrer cell cultures. Glycerol, distilled water and mineral oil have been used before (Willeke et al., 1998; Näsman et al., 2008). Carboxymethylcellulose is generally used in stirrer vessels. It augments the viscosity of cell culture medium preventing mechanical cell damage by the magnetic stirrer (Moreira et al., 1995). Like mineral oil, CMC could prevent bubbling of collection media during sampling. Transport medium is excellent for chlamydial preservation. During sample processing, mineral oil was again removed to avoid cell culture toxicity. All impingers were fumigated, rinsed with desinfectol (Chem-Lab NV, Zedelgem, Belgium) and distilled water and subsequently autoclaved in between two aerosol measurements.

2.5. Impaction

The efficiency of the MAS-100 impactor (Merck, Darmstadt, Germany) was evaluated using ChlamyTrap 1 (semi-solid) and 2 (solid), two in house-made collection media.

ChlamyTrap 1 was prepared by adding sucrose (74.6 g), KH_2PO4 (5.1 g), K_2HPO_4 (1.2 g), L-glutamic acid mono potassium salt (0.9 g), fetal calf serum (20 % v/v, Invitrogen), vancomycin (200 µg/ml), streptomycin (200 µg/ml), nystatin (50.000 U/ml) and gentamicin (100 µg/ml) to 500 ml sterile MQ water. This sucrose phosphate buffer was subsequently filter (0.22 µm) sterilized. Thereafter a methocel solution was prepared by adding 5 g methocel®MC (Sigma) to 225 ml boiling MQ water. The methocel solution was shaken overnight (4°C) where after 5 ml sterile $NaHCO_3$ (5.6%, Sigma), 20 ml sterile MQ water and 250 ml sucrose phosphate buffer were added. ChlamyTrap 1 was subsequently poured in Petri dishes. ChlamyTrap 2 is a 1 % (w/v) solution of agar (Applichem, Darmstadt, Germany) in sucrose phosphate buffer poured in Petri dishes. The MAS-100 impactor was evaluated at a constant flow rate of 100 L/min (Meier and Zingre, 2000) during 0.5; 1.0; 1.5; 2.5; 5.0; 7.5 or 10 minutes, sampling respectively 50, 100, 150, 250, 500, 750 or 1000 liters of *C. psittaci* contaminated air.

2.6. Processing of samples prior to analyses

Collection fluids and filters were gently removed from stationary and personal sampling devices respectively and transferred to sterile recipients. MAS-100 Petri dishes were transported as such. All samples were transported on ice. Collection fluids were divided in two equal parts. For the gelatin filter, 10 ml of sterile distilled water was added followed by incubation at 37 °C until the filter completely dissolved. Subsequently, the filter solution was divided in two equal parts. Both parts were provided with either 5 ml Chlamydia transport medium for chlamydial isolation or 5 ml DNA stabilization buffer (Roche, Brussels, Belgium) for nested PCR. All other filters were cut in half and parts 1

and 2 were provided with 5 ml Chlamydia transport medium or 5 ml DNA stabilization buffer, respectively. Semi-solid MAS-100 media were also divided in two equal parts. Solid MAS-100 media were divided in two and each part was sampled with a Dacron-tipped aluminium-shafted swab (Fiers, Kuurne, Belgium). The first swab was stored in 2 ml Chlamydia transport medium while the second was stored in 2 ml DNA stabilization buffer. All samples were stored at -80 °C until tested. Filter parts and swabs were shaken (300 rpm) for one hour at 4 °C for the ones in Chlamydia transport medium and at room temperature for the ones in DNA stabilization buffer. All samples were ultracentrifuged (45,000 x g, 45 minutes, 4 °C). Pellets for bacterial isolation were suspended in 500 µl Chlamydia transport medium while the ones for nested PCR were suspended in 180 µl DNA extraction buffer (QIAamp DNA Mini Kit, Qiagen, Hilden, Germany). All samples were stored at –80°C until tested.

2.7. C. psittaci nested PCR

DNA extraction with the QIAamp DNA Mini Kit was performed according to the manufacturer's guidelines. The presence of the *C. psittaci* outer membrane protein A (*ompA*) gene was examined using a nested PCR as previously described (Van Loock et al., 2005) generating a PCR product of 472 bp. Results were visualized after agarose (1.2 %) gel electrophoresis. The density of the bands was evaluated using Quantity One software (Bio-Rad Laboratories). An amount of 12 ng *ompA* was amplified by PCR and was used as a reference to score the samples after background subtraction. Relatively to the reference sample value of 1, samples with density values of 0.01-0.25; 0.26-0.50; 0.51-0.75 and > 0.75 are indicated by '+', '++', '+++' and '++++', respectively. Score '–'

208 is indicative of a negative result and score '++++' indicates a high-yield PCR
209 amplification.
210
211 *2.8. C. psittaci isolation*
212 The presence of viable *C. psittaci* was examined by isolation in BGM cells and direct
213 immunofluorescence staining at day 3 post inoculation (IMAGEN™, Dakocytomation,
214 Denmark) (Vanrompay et al., 1994). The number of *C. psittaci* positive cells was counted
215 in five randomly selected microscopic fields (400x, Nikon Eclipse TE2000-E, Japan). A
216 score from 0 to 4 was given. Score 0 means that there were no *C. psittaci* positive cells.
217 Score 1 was given when a mean of 1-5 non-replicating elementary bodies was present.
218 Scores 2, 3 and 4 were given when a mean of 1-5, 6-10 and >10 inclusion positive cells
219 could be observed.
220
221 *2.9. Sensitivity*
222 Bioaerosol monitoring techniques, performing superior in both culture and nested
223 PCR, were selected to determine their *C. psittaci* detection limit. Therefore, 1, 10 and 100
224 $TCID_{50}$ of *C. psittaci* cell culture harvest were nebulized separately in negative pressure
225 isolators. Following nebulization, *C. psittaci* contaminated air was collected and
226 examined by culture as well as nested PCR. All samples were analyzed in duplicate.
227
228 **3. Results**
229 *3.1. Dry filtration*

Culture and nested PCR results for the various filter types tested by both the IOM personal inhalable dust sampler and the PAS6 sampler at an airflow rate of 1 and 2 L/min are shown in Table 1. Overall, the IOM sampler performed better than the PAS-6 device as only one negative PCR result was obtained compared to four negatives when using the PAS-6 sampler. There was a benefit of using an airflow rate of 2 L/min instead of 1 L/min, especially for nested PCR as scores were augmented in five of eight measurements. Isolation scores were augmented in only three of eight measurements. However, culture scores never diminished when doubling the airflow rate. Overall, the gelatin filter with the IOM sampling cassette performed best in both culture (score 3) and nested PCR (++++). The MCES filter also performed well in nested PCR (++++) but rather bad in culture (score 1). Thus, the gelatin filter in combination with the IOM sampler evaluated at an airflow rate of 2 L/min was the optimal dry filtration technique for detecting chlamydial DNA as well as viable *C. psittaci*.

3.2. Impingement

Culture and nested PCR results for the various collection fluids used for the AGI-30 and BioSampler are shown in Table 2. Bioaerosol sampling by means of the AGI-30 was inefficient in capturing *C. psittaci*. All cultures were negative and nested PCR was only (weakly) positive when using Chlamydia transport medium. As expected, the BioSampler obtained better results. However, collection media supplemented with mineral oil or glycerol were (nearly) culture negative. Collection fluids based on Chlamydia transport medium performed best in culture while distilled water supplemented with mineral oil performed best in nested PCR.

253

254 *3.3. Impaction*

255 Results of ChlamyTrap 1 and 2, used for the MAS-100 impactor, are presented in
256 Table 3. *C. psittaci* bioaerosol monitoring by means of the MAS-100 impactor was
257 superior to impingement. Increasing the amount of aspirated air resulted in augmented
258 scores in both culture and nested PCR. Especially, ChlamyTrap 1 performed extremely
259 well for collecting viable *C. psittaci* as well as chlamydial DNA. Testing 1000 liters of air
260 in 10 minutes by use of ChlamyTrap 1 gave the best results in culture and nested PCR.

261

262 *3.4. Sensitivity*

263 The sensitivities of the best *C. psittaci* bioaerosol sampling techniques, namely the
264 IOM inhalable dust sampler in combination with a gelatin membrane filter, and of the
265 MAS-100 impactor in combination with ChlamyTrap 1 were evaluated (Table 4). Both
266 were tested at their technically allowed maximum airflow rate of 2 L/min and 100 L/min,
267 respectively. Airflow rates higher than 2 L/min resulted in disruption of the gelatin
268 membrane filter. The airflow rate of 100 L/min was the maximum airflow rate capable of
269 keeping semi-solid medium inside the Petri dish during sampling. The IOM sampler was
270 utilized for no longer than one hour to prevent desiccation, sampling a total of 120 liters
271 of contaminated air. For comparison, the MAS-100 sampling time was adjusted to 1.2
272 min in order to collect the same amount of air. Finally, the impactor was also allowed to
273 collect 1000 liters of contaminated air during 10 minutes, as in the experimental set up.

274 Overall, the sensitivity of both bioaerosol monitoring techniques was comparable
275 when collecting 120 liters of air, as filtration and impaction by means of a gelatin filter

276 and ChlamyTrap 1, respectively resulted in a detection limit of 10 organisms. However,
277 impaction into ChlamyTrap 1 was more sensitive for collecting live organisms, as culture
278 scores for detecting 10 bacteria were higher. Interestingly, after the impaction of 1000
279 liters of air, as few as one bacterium could be detected by nested PCR, while 10
280 organisms needed to be present to be detected by culture resulting in a high isolation
281 score of 3 (Fig. 1).
282
283 **4. Discussion**
284 As the concern about bioaerosol exposure has noticeably increased over the last few
285 decades, it is very important that reliable and highly sensitive monitoring techniques are
286 available to investigate the risk of exposure to infectious substances. *Chlamydophila* (*C.)*
287 *psittaci* bioaerosol monitoring could provide important information on infection pressure
288 in farm houses as well as in slaughterhouses, which could result in improved farm
289 management, optimization of disinfection methods, and personal preventive
290 measurements. As there are no methods described for *C. psittaci* bioaerosol monitoring,
291 our primary goal was to evaluate dry filtration, impingement and impaction techniques
292 currently being used for bioaerosol monitoring of non-obligate intracellular bacteria or
293 viruses.
294 We evaluated the efficiency of MCES, MCE, glass fiber, gelatin and polycarbonate
295 membrane filters placed in IOM and PAS6 filter cassettes, both of which are currently
296 used for bioaerosol monitoring. *C. psittaci* bioaerosol monitoring by means of the IOM
297 filter cassette was most efficient. This might be ascribed to the fact that the PAS6 filter
298 cassette was actually designed to characterize exposure to inhalable, non-biological dust

299 particles and fumes, for instance in the rubber industry (Vermeulen et al., 2000; de Vocht
300 et al., 2006) and to measure dust exposure levels in bakeries (Houba et al., 1997; Bulat et
301 al., 2004). The IOM sampler, on the other hand, has already been optimized for detecting
302 microbes in contaminated air (Kenny et al., 1998).

303 Focusing on the results of the IOM sampler, the gelatin membrane filter performed
304 best in culture as well as PCR. This filter demonstrated its suitability for detecting DNA
305 of 10 *C. psittaci* and as few as 10 live chlamydial organisms. Kenny et al. (1998).have
306 previously demonstrated the usefulness of gelatin membrane filters for detecting
307 microorganisms, such as *Escherichia coli*, *Saccharomyces cerevisiae* and *Penicillium*
308 *expansum* in air.. Also the recovery of *Bacillus* bacteria and virus-containing aerosols by
309 gelatin filters was shown to be satisfactory (Jaschhof, 1992; Li et al., 1999; Tseng and Li,
310 2005; Burton et al., 2007). Moreover, an airflow rate of 2 L/min for the IOM sampler was
311 recommended to aspirate the inhalable and hence infectious fraction (Kenny et al., 1998).

312 In addition to dry filtration, gravitational sedimentation, impingement and impaction
313 are also used for bioaerosol monitoring. As the size of the infectious *C. psittaci* is similar
314 to that of viruses, it is not advisable to use gravitational sedimentation for capturing on
315 collection plates, as gravitational forces alone are not sufficient for capturing these
316 organisms for subsequent detection and identification. In theory, impingement and
317 impaction are more promising techniques for *C. psittaci* bioaerosol monitoring.

318 The conventional AGI-30 impinger and the BioSampler have been found to perform
319 extremely well when sampling for total bacteria and fungi (Thorne et al., 1992; Terzieva
320 et al., 1996; Lin et al., 1999). Furthermore, impingement into liquid is generally thought
321 to be better than dry filtration, especially for counting live bacteria or fungi (Li et al.,

322 1999). However, our results indicate that the AGI-30 is not appropriate for capturing *C.*
323 *psittaci*. Turbulence and bubbling of the collection medium most likely enhanced
324 evaporation significantly, resulting in the re-aerosolisation and, therefore, inefficient
325 collection of *C. psittaci*. The BioSampler gave better results, especially when using *C.*
326 *psittaci* transport medium with or without the viscosity augmenting CMC. Nevertheless,
327 BioSampler results were a bit disappointing, especially for PCR. Overall, poor results of
328 the AGI-30 impinger and the swirling aersosol collector or BioSampler could also be due
329 to the fact that *C. psittaci* is too small to be captured by liquid impingement. Accordingly,
330 recent viral bioaerosol sampling experiments with the AGI-30 and the BioSampler indeed
331 showed that they are inadequate in collecting sub-micrometer and ultra-fine virus
332 particles. Both samplers had low viral collection efficiencies and the virus viability could
333 be lost during collection (Hogan et al., 2005).

334 We also evaluated the MAS-100 single stage impactor (Meier and Zingre, 2000).
335 Former research indicated that the MAS-100 was suitable for routine evaluation of
336 bacterial and fungal air contamination (Nesa et al., 2001; Engelhart et al., 2006; Yao and
337 Mainelis, 2007). We prepared two novel collection media, especially adapted for
338 collecting live *C. psittaci* as well as chlamydial DNA and evaluated them in the MAS-
339 100 impactor. The semi-solid ChlamyTrap 1 medium was superior in entrapping *C.*
340 *psittaci* and, interestingly, results were better than for impingement and dry filtration. The
341 current technique was extremely sensitive since as few as 1 and 10 organisms could be
342 detected by PCR and culture, respectively. The fact that the solid ChlamyTrap 2 medium
343 led to less optimal results might be ascribed to injury effects during collection. Stress and
344 injury depend on the degree to which the microorganisms are embedded in the collection

345 medium (Stewart et al., 1995). The viability of the organisms probably decreased as a
346 result of the impaction onto the solid surface. Moreover, desiccation of the bacteria on the
347 ChlamyTrap 2 surface during collection could contribute to the loss of viability. On the
348 contrary, ChlamyTrap 1 allowed *C. psittaci* to be incorporated into the semi-solid
349 medium. Therefore, desiccation was probably minimal and viability remained.
350 In conclusion, for personal bioaerosol sampling, we recommend the use of the gelatin
351 filter in combination with the IOM sampler at a flow rate of 2 L/min for the collection
352 and subsequent detection of *C. psittaci* by both culture and/or PCR. However, for
353 stationary bioaerosol monitoring, sampling 1000 liters of air in 10 minutes with the
354 MAS-100 impactor is preferred. The MAS-100 is compact, portable and extremely easy
355 to calibrate. The novel ChlamyTrap 1 medium allowed sample processing for both
356 culture and PCR. ChlamyTrap 1 in combination with the MAS-100 impactor might also
357 be applicable for collecting other obligate intracellular microorganisms like viruses from
358 contaminated air. However, further research is still needed to confirm this.
359

360 **Conflict of interest statement**

361 None of the authors (Caroline Van Droogenbroeck, Marleen Van Risseghem, Lutgart
362 Braeckman and Daisy Vanrompay) has a financial or personal relationship with other
363 people or organizations that could inappropriately influence or bias the paper entitled
364 "Evaluation of bioaerosol sampling techniques for the detection of *Chlamydophila*
365 *psittaci* in contaminated air".
366

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Figure caption

Fig. 1. Illustration of culture scores (400x). Pictures A, B, C and D represent *C. psittaci* isolation scores of 1, 2, 3 and 4 respectively. Score 0 means that there were no *C. psittaci* positive cells. Score 1 was given when a mean of 1-5 non-replicating elementary bodies was present. Scores 2, 3 and 4 were given when a mean of 1-5, 6-10 and >10 inclusion positive cells could be observed.

Table 1
Culture and nested PCR scores for the IOM and PAS6 air samplers

Membrane filter (pore size)	Airflow (l/min)	IOM sampler		PAS6 sampler	
		Culture[a]	Nested PCR[b]	Culture	Nested PCR
MCES (0,8 µm)	1	1	++	0	+
	2	1	++++	0	++++
MCE (0,8 µm)	1	1	+	0	-
	2	2	++	1	-
Glass Fiber (1,0 µm)	1	1	++	0	-
	2	1	++	0	++
Gelatin (3,0 µm)	1	2	+++	1	+
	2	3	++++	1	+
Polycarbonate (0,1 µm)	1	1	-	1	-

[a] Isolation score 0: no *C. psittaci* positive cells. Score 1: mean of 1-5 non-replicating elementary bodies. Scores 2, 3 and 4: mean of 1-5, 6-10 and >10 inclusion positive cells.

[b] A nested PCR score of '+' to '++++' corresponds to the density of the band on the agarose gel. A score of '-' is indicative for a negative result.

Table 2

Culture and nested PCR scores for different collection fluids used for the AGI-30 and BioSampler

Collection fluids	AGI-30		BioSampler	
	Culture[a]	Nested PCR[b]	Culture	Nested PCR
Distilled water	0	-	2	+
Distilled water + mineral oil	0	-	1	++
40% distilled water / 60% glycerol	0	-	1	-
16% distilled water/ 84% glycerol	0	-	0	-
Glycerol	0	-	0	-
Transport medium	0	+	3	+
Transport medium + 2% CMC	0	-	3	-
Transport medium + mineral oil	0	-	0	-

[a,b] Scores: as for Table 1.

Table 3

Culture and nested PCR scores for ChlamyTrap 1 and 2 tested in the MAS-100 impactor

Aspirated air volume (liter)	ChlamyTrap 1		ChlamyTrap 2	
	Culture[a]	Nested PCR[b]	Culture	Nested PCR
50	1	-	0	-
150	1	-	1	-
250	2	-	1	+
500	3	+	1	+
750	3	++++	1	+
1000	4	++++	2	++

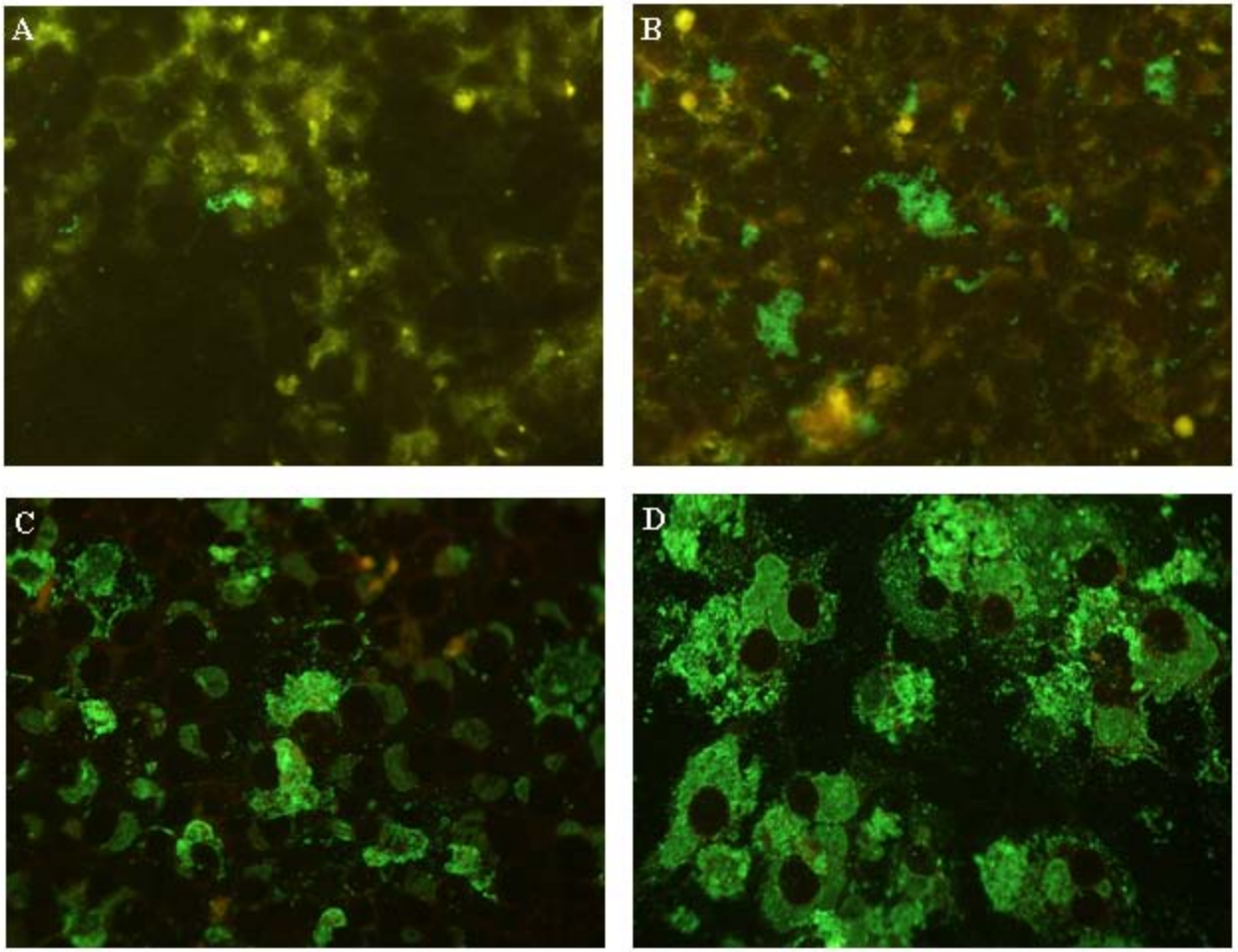
[a,b] Scores: as for Table 1.

Table 4

Sensitivities of dry filtration and impaction for collecting *C. psittaci* contaminated air

Amount of aerosolized bacteria	Bioaerosol monitoring by					
	IOM (120 liter)		MAS-100 (120 liter)		MAS-100 (1000 liter)	
	Gelatin membrane filter		ChlamyTrap 1		ChlamyTrap 1	
	Culture[a]	Nested PCR[b]	Culture	Nested PCR	Culture	Nested PCR
10^0	0	-	0	-	0	+
10^1	1	+	2	+	3	+
10^2	2	+	2	+	4	++

[a,b] Scores: as for Table 1.


A
B
C
D