

## Bacterial colonization as a potential source of nosocomial respiratory infections in two types of spirometer

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*Bacterial colonization as a potential source of nosocomial respiratory infections in two types of spirometer. F. Burgos, A. Torres, J. González, J. Puig de la Bellacasa, R. Rodríguez-Roisin, J. Roca. ©ERS Journals Ltd 1996.*

**ABSTRACT:** The potential risk of spirometers in the transmission of respiratory infections has not been yet established. We performed a prospective cross-sectional study to determine the rate of colonization of a water-sealed spirometer and a pneumotachograph, and the potential risk of cross-transmission of microorganisms to patients using each of these devices.

Fifty four patients (aged  $51 \pm 18$  (mean  $\pm$  SD) yrs) were included in the study. All of them had undergone forced spirometry with bronchodilator response by means of the water-sealed spirometer (n=36) or the pneumotachograph (n=18). None had a clinically apparent respiratory infection at the time of the study. Routine hygiene measures for respiratory equipment were performed before the study protocol. Samples for microbiological cultures of different parts both of the water-sealed spirometer (proximal and distal tubing, bell and water-bell) and pneumotachograph (proximal and distal tubing) were taken daily before and after the usual series of lung function tests during a 5 day period. Pharyngeal swab cultures were obtained before spirometry and 7 days later in each subject.

Thirty six out of a total of 40 (90%) culture samples from the water-sealed spirometer showed microbial growth compared to 4 out of 30 (13%) samples obtained from the pneumotachograph ( $p < 0.0001$ ). Significant colonization of the water-sealed spirometer was apparent after the third day of the study. The microorganisms most frequently isolated were *Penicillium* sp. (62%), *Pseudomonas fluorescens* (32%), and *Burkholderia cepacea* (48%). Distal tubing, water and water-bell were the parts of the water-sealed spirometer that showed higher colonization counts ( $\geq 10^4$  colony-forming units (cfu)·mL<sup>-1</sup>). No transmission sequence of potentially pathogenic microorganisms from equipment to patients or vice versa could be demonstrated.

In summary, the water-sealed spirometer frequently became colonized by microorganisms. The potential hazard of such equipment as reservoirs of microorganisms suggests a need for the implementation of new hygiene measures for their maintenance.

*Eur Respir J*, 1996, 9, 2614–2619.

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Keywords:  
Colonization of respiratory equipment  
forced spirometry  
lung function testing  
prophylaxis of nosocomial infection

Received: January 4 1996  
Accepted after revision August 16 1996

Supported by grants SEPAR/Roussel 1992, FIS (Fondo de Investigación Sanitaria) 93/0605, CIRIT (Comissió Interdepartamental per a la Recerca i Tecnologia), and, ECSC 6975/92 (European Coal and Steel Community).

The contamination of respiratory therapy equipment can lead to the aerosol inoculation of pathogenic microorganisms to the oropharynx, or even directly to the lower airways, in patients undergoing these procedures [1–3]. Spirometers are the most commonly used systems in the pulmonary function laboratory. This type of equipment is transferred from patient to patient several times daily. Thus, these systems should be considered as potential sources of bacterial colonization and subsequent transmission [4]. However, the actual hazard of such equipment in the development of respiratory infections is not well-known. Accordingly, standardized preventive measures have not been validated [5]. HAZALEUS *et al.* [6] reported transmission of *Mycobacterium tuberculosis* from

one patient to 22 others in whom a dry-seal spirometer was used. A simulated model of spirometer colonization suggested that mouthpieces and spirometer tubing may become contaminated with microorganisms and should not be shared among patients [4]. Some authors have suggested that colonization of spirometers could be prevented by using anti-bacterial filters [7].

We performed a sequential study to determine the role of two different commonly used devices: 1) water-sealed spirometers; and 2) pneumotachographs, as reservoirs for bacterial colonization and subsequent transmission. Specifically, we cultured several parts of the equipment throughout a 5 day period. Additionally, the patients who used these instruments were followed-up during a 1 week period.

## Material and methods

### Equipment

We investigated two types of spirometer: a water-sealed spirometer (Biomedin®; Padova, Italy) and a modified pneumotachograph heated flow transducer (MGC® 1070; Medical Graphics Co., St. Paul, MN, USA), routinely used in our laboratory for the measurement of forced spirometry and the bronchodilator response. The weekly hygiene maintenance for these devices is as follows: tubing and connections both the water-sealed spirometer and the pneumotachograph were sterilized. The water from the reservoir in the water-sealed spirometer was removed and replaced with sterile distilled water from commercial flasks, and the bell was scrubbed with water and soap. The resistance of the pneumotachograph was cleansed with water and soap. Subsequently, it was dried with compressed air. These procedures were performed in full before the study protocol.

### Study protocol

Overall, a mean of 2,500 spirometric evaluations are performed annually in our institution. Twelve routine spirometric evaluations are performed daily on a 1:2 ratio (pneumotachograph:water-sealed). Accordingly, the study was randomized for the pneumotachograph and for the water-sealed spirometer at a ratio of 1:2. This was performed sequentially over a period of 5 days. The study period was randomly chosen, and lasted from Monday until Friday. Patients performed forced spirometric manoeuvres with bronchodilator response according to the American Thoracic Society (ATS) standards [8]. A sterile mouthpiece for tubing connection was used for each patient. Patients with active respiratory infection were excluded from the study, since we wanted to reproduce the routine standard policy of our laboratory. Patients were also evaluated for the development of clinical symptoms of respiratory infection, such as fever, cough, purulent secretions and abnormal pulmonary auscultation, immediately after spirometry had been performed and again 1 week later. In each patient, pharyngeal swab samples were collected according to a standard method before spirometry and 1 week later.

Samples of different parts of the equipment were taken daily just before and after performing a series of spirometric evaluations. Regarding the pneumotachograph, samples of the proximal and distal parts of the tubing connection to mouthpiece, and the resistance were taken for quantitative bacterial cultures (fig. 1). With respect to the water-sealed spirometer, samples were taken both from the proximal and distal parts of the breathing tubing, the water of the reservoir located between the bell and the cylinder, and finally from the internal surface of the bell (fig. 2). All samples, except water, were collected using a moist, sterile, cotton-tipped applicator (Modified Transport Medium, Eurotubo®; Barcelona, Spain). All samples, except water, were resuspended in 1 mL of saline. An undiluted sample from the water reservoir (1 mL) was collected using a sterile plastic syringe. All samples were immediately transported to the microbiology laboratory. The process

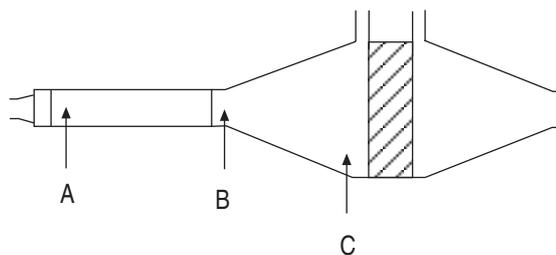


Fig. 1. – Schematic representation of the different parts of the Lilly pneumotachograph. A: proximal; B: distal; C: resistance.

of sampling was always performed by the same person (FB) in a standardized way.

### Microbiological processing

All samples obtained were serially diluted (1:10, 1:100 and 1:1000) using sterile saline. One hundred microlitres of each dilution were plated into the following agar media: 5% sheep blood, chocolate, McConkey, BCYE- $\alpha$ , and Sabouraud-dextrose. All cultures were incubated at 37°C under aerobic conditions and in CO<sub>2</sub>-enriched atmosphere, except Sabouraud-dextrose incubated at 30°C. Cultures were evaluated for growth daily and were discarded, if negative, after 5 days, with the exception of Sabouraud-dextrose which was evaluated weekly and discarded at 4 weeks. Results were expressed as colony-forming units (cfu)·mL<sup>-1</sup>. This represents the concentration of the initial solution. The lower detection limit for bacteria was 10<sup>2</sup> cfu·mL<sup>-1</sup>. The microorganisms isolated were identified according to standardized methods [9].

### Statistical analysis

Results are expressed as mean±sd. Mann-Whitney's test was used to compare means. A p-values of less than 0.05 was considered to be statistically significant [10].

## Results

### Patients' characteristics

Fifty four patients (mean±sd age 51±18 yrs) programmed for forced spirometry and bronchodilator response

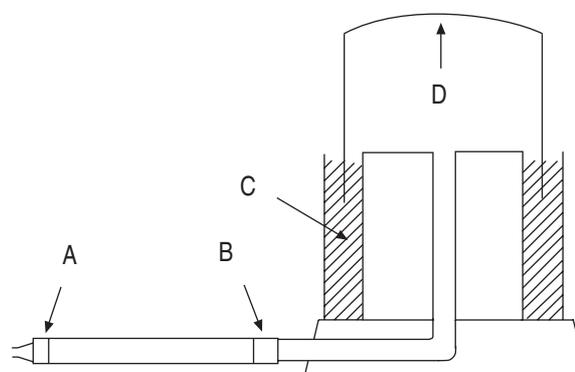


Fig. 2. – Schematic representation of the different parts of the water-sealed spirometer. A: proximal; B: Distal; C: water; D: water-bell.

Table 1. – Microorganisms isolated from the water-sealed spirometer (n=40)

Group	Microorganism	n
Gram-negative cocci	<i>Neisseria</i> sp.	1
Gram-positive cocci	<i>Staphylococcus epidermis</i>	3
	<i>Streptococcus viridans</i>	1
Gram-negative bacilli	<i>Burkholderia cepacea</i>	19
	<i>Pseudomonas fluorescens</i>	13
	<i>Pseudomonas aeruginosa</i>	5
	<i>Xanthomonas maltophilia</i>	5
	<i>Enterobacter cloacae</i>	5
	<i>Pseudomonas vesicularis</i>	1
Fungi	<i>Penicillium</i> sp.	25
	<i>Candida</i> sp.	1

Table 2. – Number of microorganisms isolated in different parts of the water-sealed spirometer in relation to quantitative cultures

	Neg	Microorganisms cfu·mL <sup>-1</sup>				
		10 <sup>3</sup>	10 <sup>3</sup> –10 <sup>4</sup>	10 <sup>4</sup> –10 <sup>5</sup>	10 <sup>5</sup> –10 <sup>6</sup>	10 <sup>6</sup>
Proximal tubing	2	5	-	1	2	-
Distal tubing	-	2	2	2	3	1
Water	1	2	3	-	4	-
Water-bell	1	1	2	3	2	1

The data represent the number of isolations. cfu: colony-forming units; Neg: negative.

in our laboratory during a 1 week period in springtime were included in the study. Thirty one patients were male and 23 female. According to the routine policy of our laboratory, forced spirometry was performed with the water-sealed spirometer in 36 patients (67%). In the remaining 18 (33%) patients, it was performed using a pneumotachograph. The clinical indications for forced spirometry were control of the evolution of respiratory illness in 48 patients ((asthma (21), chronic obstructive pulmonary disease (COPD) (17) and pulmonary fibrosis (10)), and preoperative evaluation in 6 patients. None of the patients had a clinically apparent pulmonary infection.

### Water-sealed spirometer

At least one microorganism was isolated in 36 out of 40 (90%) samples, as shown in table 1. The most frequently isolated microorganisms were *Penicillium* sp. (62%), *Pseudomonas fluorescens* (32%) and *Burkholderia cepacea* (48%). In all but four instances, microorganisms were always concomitantly isolated from at least two samples from different parts of the spirometer. Table 2 shows microbial concentrations cultured in different parts of the water-sealed spirometer. Nineteen samples (48%) showed microorganisms yielding counts  $\geq 10^4$  cfu·mL<sup>-1</sup>, 17 (42%)  $\leq 10^4$  cfu·mL<sup>-1</sup>, and four were negative (two samples from the proximal tubing, one from the water, and one from the bell). Proximal tubing cultures showed the lowest degree of colonization compared to the remaining samples (5 out of 10 samples had counts  $\leq 10^3$  cfu·mL<sup>-1</sup> and two were negative) (table 2).

The sequential pattern of colonization in samples isolated from different parts of the water-sealed spirometer during the 5 day study period is illustrated in figure 3. Peaks of colonization caused by Gram-negative and Gram-positive cocci (*Neisseria* sp. and *Staphylococcus epidermidis*) were observed in distal tubing (on Day 3) and water bell cultures (on Day 5) (fig. 3a). A peak of colonization by fungi (*Penicillium* sp.) was detected on Day 3 of the study in distal tubing cultures (fig. 3b). Figure 3c shows a colonization peak caused by Gram-negative bacilli in proximal tubing cultures at Day 4 (*P. fluorescens* and *B. cepacea*). The level of colonization decreased at Day 5 in the same samples (fig. 3c). Colonization by gram-negative bacilli in the remaining parts of the water-sealed spirometer (proximal, water, water bell) was also observed on the last 2 days of the study. Specifically, at Day 5 the microorganisms isolated were the following: 1) proximal tubing: *P. fluorescens*, *P. cepacea* and *Pseudomonas aeruginosa*; 2) bell: *P. fluorescens*, *B. cepacea*, *P. aeruginosa* and *Enterobacter cloacae*; 3) water-bell: *P. fluorescens*, *B. cepacea*, *P. aeruginosa*; and *E. cloacae*.

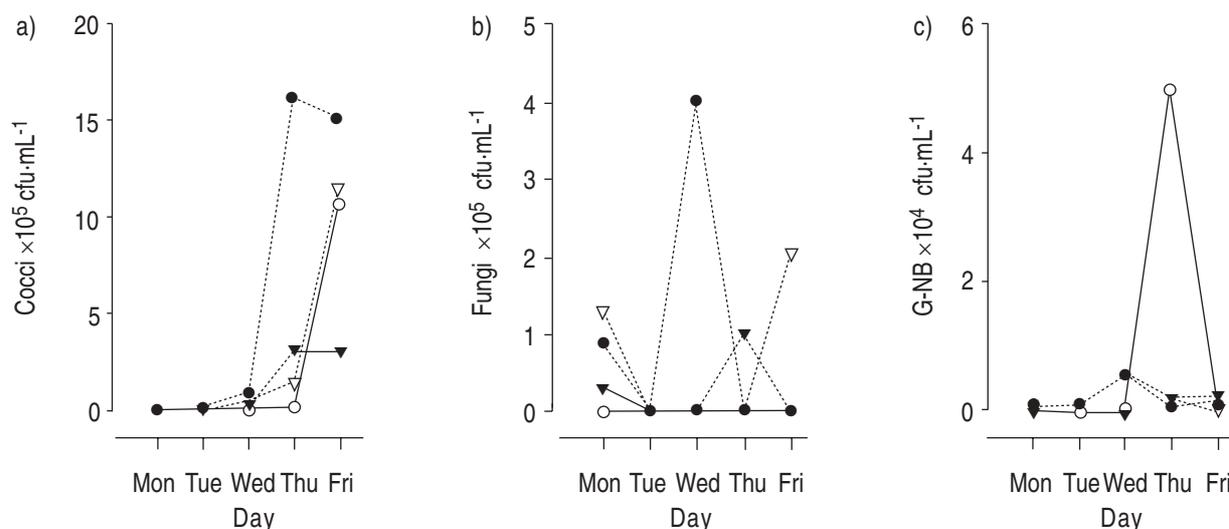


Fig. 3. – Mean concentration of: a) Gram-negative and Gram-positive cocci; b) fungi; and c) Gram-negative bacilli (G-NB) isolated from the different parts of the water-sealed spirometer during the 5 day study period. —○—: proximal tubing; —●—: distal tubing; —▽—: water-bell; —▼—: water.

Table 3. – Presence of microorganisms in pharyngeal swabs of patients

Microorganism	Before spirometry n <sup>+</sup>	1 week later n <sup>+</sup>	Colony count (1 week later)		
			Increase n <sup>†</sup>	Decrease n <sup>†</sup>	No change n <sup>†</sup>
<i>Neisseria</i> sp.	35	36	15	17	4
<i>Staphylococcus epidermidis</i>	35	36	12	11	13
<i>Streptococcus viridans</i>	35	36	16	16	4
<i>Corynebacterium</i> sp.	3	8	6	4	1

+: Number of cases in which each microorganism was isolated; †: number of cases in which the colony count increased, decreased or was unchanged, respectively.

#### Pneumotachograph

Colonization was observed in only 4 out of 30 (13%) cultured samples obtained from the pneumotachograph compared to 36 out of 40 (90%) from the water-sealed spirometer ( $p < 0.0001$ ). Furthermore, the colonization never exceeded  $10^3$  cfu·mL<sup>-1</sup>. The microorganisms isolated were *S. epidermidis* and *Penicillium* sp. at Days 1, 3 and 4 of the study.

#### Pharyngeal swabs

A total of 108 pharyngeal swab samples were obtained. Seventy two were from patients who used the water-sealed spirometer and the remaining 36 were from patients using the pneumotachograph. Table 3 shows the different microorganisms isolated in the initial and final samples of pharyngeal swabs. There were no qualitative changes in pharyngeal colonization when comparing final (1 week after the spirometry) to initial samples (before spirometry). Exceptionally, nonpotentially pathogenic microorganisms, such as *Neisseria* sp., *S. epidermidis* and *S. viridans*, were isolated from the distal tubing, water-bell and water-bell, respectively, and concomitantly from pharyngeal swabs. None of the patients showed changes or new respiratory signs and/or symptoms suggestive of respiratory infection during 1 week after spirometry.

### Discussion

The main finding from the present study is that bacterial colonization was virtually absent in the Lilly pneumotachograph but occurred frequently and in different parts of the water-sealed spirometer. Although we could not prove changes in the pharyngeal flora after spirometry, the colonization of this equipment should be considered as a potential risk factor for transmission of respiratory infections.

In the aetiopathogenic sequence of nosocomial respiratory infections, inhaling contaminated aerosols from respiratory therapy equipment is one of the accepted mechanisms [11–13]. The later is not infrequent in critically-ill intubated patients. Contaminated aerosols have been demonstrated to originate from contaminated nebulizers [2], water condensate in ventilator tubing [1], and even peak flow meters [14] and resuscitator bags [15].

Information regarding the potential risk of transmission due to colonization of equipment for measuring respiratory function in pulmonary laboratories is scanty or anecdotal [4, 6, 16]. For instance, HAZALEUS and BERDISCHEWSKY [6] reported a small outbreak caused by *M. tuberculosis*

contaminating apparatus for pulmonary function testing. RUTALA *et al.* [4] found colonization of mouthpieces of dry spirometers from patients with active pulmonary infections, although the remaining parts of the equipment remained uncolonized, suggesting that it is unnecessary to routinely disinfect interior surfaces of spirometers after use by infected patients. HOUSTON *et al.* [16] investigated the bacterial pathogens isolated from the unsterilized tube and spirometer bellows of a Vitalograph® used by 1,000 patients over a 4 week period. The most common organisms isolated were: *Acinetobacter* sp., *Flavobacterium* sp., *S. epidermidis*, *Streptococcus* sp., and fungi.

We found no colonization in the pneumotachograph. This could be explained by the high temperatures [17] obtained around the proximal tubing, inhibiting bacterial and fungal growth. However, the water-sealed spirometer became colonized in various parts. This colonization was important in quantitative terms, indicating a high level of contamination. However, there are some considerations in the present study that deserve particular attention regarding the latter finding. Firstly, patients with active pulmonary infections were not studied, since we wanted to reproduce the standard policy of our laboratory. It is possible that the risk of transmission from spirometers to patients may be higher when studying patients with active respiratory infections. However, RUTALA *et al.* [4], studying bacterial colonization of dry-rolling seal spirometers in active pulmonary infections, did not find contamination inside the spirometers.

Secondly, the study was performed sequentially during a 5 day period, and colonization of the equipment occurred after the third day in spite of the use of routine hygiene measures. Thirdly, some isolated microorganisms (*e.g.* *P. aeruginosa* or *E. cloacae*) are frequent pulmonary pathogens. Nevertheless and although we cannot discard transient pharyngeal colonization, we could not isolate these microorganisms from the oropharynx of the patients. However, a possible limitation of our study regarding transmission from spirometers to patients is that we did not examine sputum cultures, due to the difficulty of obtaining these respiratory samples.

Finally, although some contaminants of the spirometer (*e.g.* *Neisseria* sp., *S. epidermidis*, *Streptococcus viridans*, *Candida albicans* and *E. cloacae*) could have been transmitted from patients, most of the microorganisms isolated were environmental, such as *Penicillium* sp. and *P. fluorescens*. The high frequency of *Penicillium* sp. is an uncommon fact. Most surprisingly this fungus was isolated at an early stage in a disinfected instrument (fig. 3b), again suggesting easy environmental contamination.

In this study, it is impossible to ascertain whether there was transient pharyngeal and/or airway colonization from equipment to patients, since pharyngeal swabs were not collected sequentially, but if colonization occurred it was not of clinical relevance. With the exception of anecdotal reports [6], there is no evidence of transmission from contaminated spirometers to non-critically ill patients and the results of the present study support this statement. However, water-sealed spirometers may be potential risk factors for transmission in immunosuppressed patients with altered humoral and cellular defences. Taking the examples of this study, the recovery of bacteria on the surface of spirometer tubing or from water does not necessarily imply that these bacteria will be aerosolized during subsequent inspiratory manoeuvres. Future, experimental studies should answer this question.

Prophylactic measures against colonization of spirometers should consider enhancement of the routine hygiene measures and strict control of patients who undergo the test. Unfortunately, these measures have never been clearly standardized and the optimal frequency for disinfection or sterilization of tubing, valves and manifolds has not been established. Some recommendations have been suggested in a recent ATS statement, that include, at least, decontamination of breathing tubes between patients when using a closed-circuit technique [8].

Another prophylactic measure could be the inclusion of in-line antibacterial filters. Some authors have suggested that the use of antibacterial filters may prevent bacterial contamination of spirometers and also have antiviral barrier properties [18]. For instance, WAßER *et al.* [7] demonstrated that by using filters the bacterial burden of the spirometers decreased dramatically from 45,200 to 50 cfu·mL. Another study demonstrated a high efficiency of these filters in removing exhaled bacteria after spirometry [18]. However, controversy exists regarding the potential alteration of pulmonary function test measurements when using these filters [19]. Significant differences between pulmonary function test measurements with and without the use of filters were found by FUSO *et al.* [20]. However, the authors did not consider this to be clinically significant, since changes due to the filter were within the intraindividual variability of repeated measurements.

The present study was not designed to evaluate viral contamination or other types of microorganism difficult to culture on standard media, such as *M. tuberculosis*. In fact, patients with suspicion of pulmonary tuberculosis were excluded from this study, since individual chest radiographs were always examined according to the routine of our laboratory. It seems logical to anticipate that spirometers (particularly during inspiratory manoeuvres) may serve as viral or mycobacterial transmitters, since aerosolized droplets containing contaminated particles are the primary means of transmission of viral or mycobacterial pulmonary infections [21]. A strict control of patients, avoiding the study of those subjects with clinical suspicion of active respiratory infection, could be an effective preventive approach for viral and mycobacterial respiratory infections.

Respiratory technicians are cautious regarding the performance of spirometric evaluation on patients infected with human immunodeficiency virus (HIV). Although

the virus has been found in saliva, there is no proven case of transmission by this type of body fluid. Nevertheless, theoretically, this possibility cannot be ruled out. Reserving particular equipment for use by infected people is difficult. Moreover, carriers of HIV are underdiagnosed or may not wish to be recognized. Since screening for HIV presents legal and ethical problems, DENISON *et al.* [22] advocate the universal application of safeguards against cross-infection, including the use of disposable components.

*Legionella pneumophila* colonizes portable water, and respiratory equipment filled or rinsed with tap water may serve as a secondary reservoir for these infections [23]. Contaminated aerosols generated by respiratory equipment could then transmit airborne *L. pneumophila* to the respiratory tract of patients. Hypothetically, this could be the case with water-sealed spirometers. *L. pneumophila* did not grow in any of the samples taken in our study, although our bacteriological technique (serial dilution) is not the most adequate for culturing *L. pneumophila* and we could not rule out that this microorganism may be more relevant. We consider it to be advisable to perform a periodic surveillance for *Legionella* spp. in the equipment, particularly water-sealed spirometers, and also recommend the systematic use of distilled water.

In summary, colonization of the pneumotachograph is uncommon. On the other hand, water-sealed spirometers can easily be contaminated with environmental flora and potentially pathogenic microorganisms. Although we could not demonstrate transmission of respiratory infection from this equipment, water-sealed spirometers have a potential risk in the development of these infections. The implementation of new and daily hygiene measures for the maintenance of such equipment is desirable.

**Acknowledgements:** The authors are grateful to C. Martinez, M. El-Ebiary and the technical staff of our Service for their co-operation in this study.

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