## Results

## **Quality controls**

In the 20 kindergartens a total of 1,249 children aged between 3 and 6 were involved in the study, and the parents' informed consent was retrieved for 1,020 children (81.7%). Out of these, 56 children were absent during the days in which tests were carried out and 4 refused to undergo the lung examination. Spirometries were performed on a total of 960 children, with a cooperation rate of 95.3% (45 non cooperative children).

The cooperation rate (Table 1) was significantly higher in children older than 3 years of age (Chi-square 11.68, p < 0.01) with not significant differences between genders (Chi-square 0.37, p > 0.10).

Among the 915 cooperative subjects, 149 (16.3%) tests results were excluded as they lacked one or more of the acceptability criteria (Table 2): a total of 766 (83.7%) tests results were included for the analysis.

No age nor gender distribution differences were found either in the included or in the excluded subjects (p < 0.05). Three or more acceptable curves, resulting from the validity test, were present in 93% of cases. The frequency of exclusion, among the 3-year old children, was higher but not statistically significant.

The whole group Flow Expiration Time (FET) mean was 1.1 seconds (IC 95% 1.07–1.13) with non significant gender and age variation.

Among the 766 tests included for further analysis, we observed in 278 cases an early termination with the presence of a sharp drop or a cessation in flow from a point in which the flows where <25% of PEF. These 278 subjects were considered only for their  $\text{FEV}_{0.5}$ ,  $\text{FEV}_{0.75}$  and  $\text{FEV}_1$  and not for FVC and flow analysis (see tab3).

The repeatability of FVC and FEVt for all the parameters was fairly good, with a variability = 10% for almost all the children (higher than 99%) and within 5% in 90.8% of cases: the absolute variability among the different manoeuvres of the same subjects was under 100 ml for 98.6% of subjects, and under 150 ml for all subjects. The MEF25, MEF50 and MEF75 repeatability was lower than the volumes repeatability. Among flows, MEF75 had the smallest variability (< = 10% for 84.4% of subjects) (Table 3).

## Reference values

Table 4 reports the anthropometric characteristics of the 766 subjects. The girls were slightly taller and heavier than the boys. The  $5^{th}$  and  $95^{th}$  BMI percentile (mean  $\pm$  1.64 standard deviations) were respectively 12.6 and 18.8 over all (12.9 and 19.1 for females; 12.4 and 18.4 for males), within the normal range reported in literature for these ages [18,19].

Table 5 reports the lung function parameter means and standard deviations by symptom status. In asymptomatic subjects the lung function parameter values were slightly higher than those in symptomatic subjects. The Box-Cox test[22] for regression analysis of the lung function parameters versus anthropometric variables showed that no linear transformation of dependent or independent variables was necessary. Among asymptomatic subjects the multiple regression analysis (Table 6), using gender, age, height and BMI as covariates, demonstrated that the static and the dynamic lung volumes were significantly higher in females than in males. A significant age positive effect was detected for all lung volumes except for FVC and FEV1. A significant positive effect was also detected for all lung volumes which was independent from height and BMI. Body weight was collinear (Variance Inflation Factor, VIF >18) with BMI and it was therefore excluded on the basis of the Likelihood Ratio (LR)[22] test (p = 0.09 for weight p = 0.04 for BMI).

Table 1: Number and proportion of non cooperating subjects at the spirometry tests, divided according to age and sex

		Females		Males					
Age (yrs)	Total	Non cooperating		Total	Non cooperating		Total	Non cooperating	
		N	%		N	%		N	%
3	20	2	10.0	29	6	20.7	49	8	16.3*
4	204	9	4.4	230	12	5.2	434	21	4.8
5	200	6	3.0	222	8	3.6	422	14	3.3
6	20	1	5.0	35	1	2.8	55	2	3.6
Tot	450	18	4.0	509	27	5.3	960	45	4.7

<sup>\*</sup>  $\chi^2$ = 11.68, p < 0.01