

Online supplementary material

Method supplementary 1

Standard operating procedure for TFV loop measurements

The equipment consisted of the lung function machine Exhalyzer® D, infant face mask (size 2), dead space reducer set 1 (DSR1) for babies up to 15 kilograms, bacteria filter, Spirette, calibration syringe 100 milliliters for babies up to 15 kilograms, nafion tube for gas sampling, flow head, CO2 adapter with capnostat sensor, SF6 gas tube, and disinfectant. The study personnel were qualified to perform lung function measurements after individual training. For safety considerations, the Exhalyzer® D and the study personnel's hands were disinfected and non-single use equipment was sterilized after each measure. The required equipment was assembled, the Exhalyzer® D connected to the air and oxygen through the central gas supply, calibrated for atmospheric pressure and room temperature (maximum deviation: temperature ± 0.5 °C, atmospheric pressure ± 5 hPa), flow (maximum deviation: inspiratory and expiratory flow 0.99%), channel (maximum deviation: ± 20 milliliter/second from the desired flow of 200 milliliter/second) and tracer gas prior to lung function measurements, according to the manufacturer's instructions. Prior to measurements, name, gender and date of birth was recorded. Length and weight of the baby was measured after the lung function testing, to ensure calm babies, and later merged with lung function variables. Lung function was measured while the infants were held in a semi-declined or declined position in parent's arms or lap lying on a firm pillow or lying flat in stroller to ensure similar positioning of the child regardless of placement. Essential requirements were that the infants had to be calm, preferably not fed right before the measure, lying comfortable, and were able to breathe freely and unrestrictedly. The mask was held with a stable grip with the whole hand around the mask placed over the mouth and nose of the infant, using fingers to control minimal leakage from the mask. When the infant breathed calmly and evenly into the mask, the tidal breath flow-volume loop (TFV) loop measurement was started. A series of at least 10 consecutive breaths was aspired. Arousal state, time to last feeding prior to measure, recently used inhalations or other medications and deviations from the standard operating procedure were documented.

Method supplementary 2

Standard operating procedure for quality assessment of TFV loop measurements

Spiroware® software version 3.2.1 was used for lung function analysis. For automatic selection of loops by the software, the automatic breath acceptance criteria were set to exclude curves $\pm 10\%$ from median V_T and the valid minute ventilation range was set to minimum 0.5 and maximum 2.5 l/min as default by the manufacturer.

The following TFV loop parameters were included for manual analysis: time to peak tidal expiratory flow divided by expiratory time (t_{PTEF}/t_E), expiratory time (t_E), time to peak tidal expiratory flow (t_{PTEF}), expiratory volume (V_E), tidal volume (V_T), respiratory rate (RR) and volume at peak expiratory flow (V_{PTEF}).

When analyzing a test run, all breaths were visualized and selected parameters as mentioned above displayed on the screen for inspection for each individual breath. Firstly, all loops that were technically unacceptable (uneven shape with no clear peak tidal expiratory flow (PTEF) or double PTEF, kinks, notches, aborted or uneven flow towards PTEF, incomplete inspiration or expiration phase, deviant shapes or volumes) were removed.

Secondly, each breath was evaluated, with particular focus on the expiratory phase. The breaths ought to have a reproducible shape, only one PTEF and justly be at the same place on the time-flow loop as the rest of the selected curves, fairly even shapes with reproducible ratios and similar volumes, little deviation of volumes in inspiratory and expiratory phase from mean V_T , minor deviation of flow in inspiration and expiration from the mean of remaining curves, while still allowing normal variation, as the breathing pattern of healthy children may vary. Ideally consecutive breaths of equal shape should be saved when there were few (<10) loops, but also non-consecutive breaths could be saved when reproducible. Loops with abnormally high RR were excluded, with the aim of preferably reproducible RR. With all uncertainty, the three investigators erred on the side of normality.

Explicit criteria for exclusion of loops were loops with kink, notches, aborted or uneven flow towards PTEF and no clear PTEF or double PTEF.

Finally, the measurements were concluded, saved and reported. Upon report, the rater reported the quality of the test: “Successful” for tests that are of good, reproducible quality and preferably include at least 10 curves. “Partly successful” for tests that include few curves or when reproducibility in variables is uncertain. “Not successful” for tests of bad quality or uncertainty around whether the test represents the way the child breathes, or when no loops are saved.

Table S1 Tests deemed unsuitable for analysis by at least one of the three raters and thus excluded from the ICC analyses.

Infant	Rater	Quality of test	Initial number of loops	Loops after selection	t_{PTEF}/t_E	V_T/kg (ml)
3	1	Not successful	36	0	-	-
	2	Not successful		1	0.84	3.1
	3	Not successful		0	-	-
7	1	Not successful	24	0	-	-
	2	Not successful		0	-	-
	3	Not successful		0	-	-
8	1	Partly successful	32	8	0.40	2.4
	2	Partly successful		8	0.37	2.4
	3	Not successful		0	-	-
25	1	Not successful	33	0	-	-
	2	Not successful		2	0.72	3.6
	3	Not successful		0	-	-
39	1	Partly successful	14	3	0.44	3.3
	2	Partly successful		4	0.47	3.8
	3	Not successful		0	-	-

Abbreviations:

t_{PTEF}/t_E : ratio of time to peak tidal expiratory flow to expiratory time

V_T/kg : Tidal volume per kilogram

Table S2 ICC for tests by all raters

Included loops (range)	Infants (n)	t_{PTEF}/t_E	RR	V_T/kg	V_E
2-67	52	0.97 (0.92-0.98)	0.99 (0.99-1.0)	0.98 (0.97-0.99)	0.98 (0.97-0.99)
10-67 [†]	37	0.98 (0.95-0.99)	0.99 (0.99-1.0)	0.99 (0.98-1.0)	0.99 (0.97-0.99)

[†]Tests including 10 loops or more

Abbreviations:

t_{PTEF}/t_E : ratio of time to peak tidal expiratory flow to expiratory time

RR: respiratory rate

V_T/kg : tidal volume per kilogram

V_E : expiratory volume