



2017 ERS/ATS standards for single-breath carbon monoxide uptake in the lung

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Updated technical standards for measuring DLCO (TLCO) including the use of rapid gas analyser systems <http://ow.ly/QUhv304PMsy>

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ABSTRACT This document provides an update to the European Respiratory Society (ERS)/American Thoracic Society (ATS) technical standards for single-breath carbon monoxide uptake in the lung that was last updated in 2005. Although both DLCO (diffusing capacity) and TLCO (transfer factor) are valid terms to describe the uptake of carbon monoxide in the lung, the term DLCO is used in this document. A joint taskforce appointed by the ERS and ATS reviewed the recent literature on the measurement of DLCO and surveyed the current technical capabilities of instrumentation being manufactured around the world. The recommendations in this document represent the consensus of the taskforce members in regard to the evidence available for various aspects of DLCO measurement. Furthermore, it reflects the expert opinion of the taskforce members on areas in which peer-reviewed evidence was either not available or was incomplete. The major changes in these technical standards relate to DLCO measurement with systems using rapidly responding gas analysers for carbon monoxide and the tracer gas, which are now the most common type of DLCO instrumentation being manufactured. Technical improvements and the increased capability afforded by these new systems permit enhanced measurement of DLCO and the opportunity to include other optional measures of lung function.

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Background

It has been over 100 years since Marie Krogh developed a method to measure the single-breath uptake of carbon monoxide in the lungs [1]. Her experiment was designed to show that passive diffusion could explain oxygen transfer from the alveolar gas to the pulmonary capillary blood, but the methodology became the basis of the test, now in common use, which is called diffusing capacity in North America but is more appropriately called transfer factor in Europe. The abbreviation for transfer factor or diffusing capacity of the lung for carbon monoxide used in this document is *DLCO*, although *TLCO* is an equally valid term.

A standardised clinical method of determining the diffusing capacity of the lung for carbon monoxide was described by OGILVIE *et al.* [2] in 1957 using a tracer gas to determine both the alveolar volume and the alveolar concentration of carbon monoxide at the beginning of breath-holding. This method used the collection of discrete exhaled gas samples from which gas concentrations were measured using gas analysers that took up to several minutes to perform the measurements. In the remainder of this document we will term these “classical” systems and calculations. The instrumentation for *DLCO* measurement has advanced considerably since then, primarily through the use of rapidly responding gas analyser (RGA) systems with gas analysers that have a 0–90% response time of ≤ 150 ms. While RGAs are capable of real-time, continuous gas analysis, most modern systems generally use this advanced instrumentation only to simulate the classical collection of discrete samples of gas in a bag and discard most of the sampled gas data. However, as discussed later, there are several aspects of *DLCO* measurement that can be improved markedly using all of the data provided by this continuous measurement technology.

This document and the accompanying executive summary document [3] are an update of the 2005 American Thoracic Society (ATS) and European Respiratory Society (ERS) standards [4] which, in turn, built upon previous standards [5, 6]. This update reflects the consensus opinions of both of these societies and is designed to: 1) provide an update to the standards required for *DLCO* systems based on RGA systems; and 2) provide new calculation standards that incorporate continuous gas analysis of the entire exhaled sample. It is recognised that classical equipment will remain in use for some time. However, some previously designed *DLCO* systems can be upgraded and re-engineered to meet these new RGA system standards. It is expected that as new *DLCO* systems are designed and built, they will meet and, in many cases, exceed these new standards. This document is meant to function as a stand-alone work but, for certain issues, reference will be made to previous statements. The following recommendations will be restricted to the single-breath technique of measuring the uptake of carbon monoxide in the lung, since this is the most common methodology in use around the world.

Methods

An application was submitted for a joint European Respiratory Society (ERS) and American Thoracic Society (ATS) task force to update the 2005 *DLCO* standards [4] with a particular view to systems using RGAs. The task force co-chairs were approved by the ERS and the ATS. Task force members were scientists and physicians with experience in international guidelines, clinical experience of routine lung function testing and specialist knowledge of gas transfer including research publications. Potential conflicts of interest were disclosed and vetted. The task force consisted of five members of the task force for the 2005 *DLCO* standards and four new members. A search using PubMed for literature published between 2000 and 2015 containing various terms related to diffusing capacity and transfer factor yielded 3637 citations. Task force members reviewed the abstracts and identified 113 as relevant to the project and a further 99 as potentially relevant. All manufacturers of pulmonary function equipment to measure *DLCO* were sent a survey requesting equipment specifications. Eight of 13 manufacturers responded. A survey of *DLCO* equipment specifications published on the manufacturers’ websites was also conducted. Using the 2005 standards as a base document, revisions and additions were made on a consensus basis. The recommendations in this document represent the consensus of task force members in regard to the evidence available for various aspects of *DLCO* measurement (as cited in the document). Furthermore, it reflects the expert opinion of the task force members in areas in which peer-reviewed evidence was either not available or incomplete. The task force also identified areas and directions for future research and development where evidence is lacking.

Determinants of carbon monoxide uptake

The volume of carbon monoxide in the alveolar space is the product of the alveolar volume (V_A) and the alveolar carbon monoxide fraction (F_{ACO} ; *i.e.* the fractional concentration of carbon monoxide in the alveolar space). Thus, at a constant volume, the transfer of carbon monoxide from the lungs into the blood is $V_A \cdot \Delta F_{ACO} / \Delta t$. Furthermore, in the absence of any carbon monoxide back-pressure in the blood, the transfer of carbon monoxide is equal to the product of the alveolar carbon monoxide tension (P_{ACO} ; *i.e.* the partial pressure of carbon monoxide) and the *DLCO*, which is the conductance of carbon monoxide from the inspired test gas in the alveolar space to binding with haemoglobin (Hb) in the blood

(*i.e.* flow = pressure × conductance). The combination of these two formulae gives equation 1, which can be manipulated to give equation 2 for the calculation of D_{LCO} .

$$V_A \cdot \Delta F_{ACO} / \Delta t = P_{ACO} \cdot D_{LCO} \quad (1)$$

$$D_{LCO} = V_A \cdot \Delta F_{ACO} / \Delta t / P_{ACO} \quad (2)$$

The ERS recommends expressing D_{LCO} in SI units ($\text{mmol} \cdot \text{min}^{-1} \cdot \text{kPa}^{-1}$) while the ATS prefers traditional units ($\text{mL} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$) under standard temperature, pressure and dry conditions (STPD). Values in SI units can be multiplied by 2.987 to obtain values in traditional units.

The capacity of the lung to exchange gas across the alveolar–capillary interface is determined by its structural and functional properties [1, 7–25]. The structural properties include the following: lung gas volume; the path length for diffusion in the gas phase; the thickness and area of the alveolar capillary membrane; any effects of airway closure; and the volume of Hb in capillaries supplying ventilated alveoli. The functional properties include the following: absolute levels of ventilation and perfusion; the uniformity of the distribution of ventilation relative to the distribution of perfusion; the composition of the alveolar gas; the diffusion characteristics of the membrane; the concentration and binding properties of Hb in the alveolar capillaries; and the carbon monoxide and oxygen tensions in the alveolar capillaries in that part of the pulmonary vascular bed which exchanges gas with the alveoli.

The process of carbon monoxide transfer from the environment to the pulmonary capillary blood includes six steps, as follows: 1) bulk-flow delivery of carbon monoxide to the airways and alveolar spaces; 2) mixing and diffusion of carbon monoxide in the alveolar ducts, air sacs and alveoli; 3) transfer of carbon monoxide across the gaseous to liquid interface of the alveolar membrane; 4) mixing and diffusion of carbon monoxide in the lung parenchyma and alveolar capillary plasma; 5) diffusion across the red-cell membrane and within the interior of the red blood cell; 6) chemical reaction with constituents of blood Hb [13–19].

The process of carbon monoxide uptake can be simplified into two transfer or conductance properties: 1) membrane conductivity (D_M), which reflects the diffusion properties of the alveolar capillary membrane; and 2) binding of carbon monoxide and Hb. The latter can be represented as the product of the carbon monoxide–Hb chemical reaction rate (θ) and the volume of alveolar capillary blood (V_C). Since these conductances are in series [17], these properties are related as shown in equation 3.

$$1/D_{LCO} = 1/D_M + 1/\theta V_C \quad (3)$$

A number of physiological changes can affect D_M or θV_C to influence D_{LCO} . For example, as the lung inflates D_M increases (largely due to increasing alveolar surface area), while V_C effects are variable (due to differential stretching and flattening of alveolar and extra-alveolar capillaries) [13, 20–27]. The net effect of these changes is that D_{LCO} tends to increase as the lung inflates but the change in D_{LCO} is proportionally less than the change in V_A [22]. Exercise, the supine position and Müller manoeuvres (inspiratory efforts against a closed glottis) can all recruit and dilate alveolar capillaries, thereby increasing V_C and D_{LCO} [28–34]. Alveolar–capillary recruitment also occurs in the remaining lung tissue following surgical resection, since the cardiac output now flows through a smaller capillary network. This causes a less than expected loss of V_C for the amount of lung tissue removed. In contrast, Valsalva manoeuvres (expiratory efforts against a closed glottis) can reduce V_C and thereby reduce D_{LCO} [32].

The measurement of carbon monoxide uptake is also affected by the distribution of ventilation with respect to D_M or θV_C (*i.e.* carbon monoxide uptake can only be measured in lung units into which carbon monoxide was inspired and subsequently expired) [18, 19, 35, 36]. This is particularly important in diseases such as emphysema, where the inhaled carbon monoxide may preferentially go to the better-ventilated regions of the lung and the subsequently measured carbon monoxide uptake will be determined primarily by the uptake properties of these regions. Under these conditions, the tracer gas dilution used to calculate V_A will also reflect primarily regional dilution and underestimate the lung volume as a whole. The resulting calculated D_{LCO} value should thus be considered as primarily reflecting the gas-exchange properties of the better ventilated regions of the lung.

In addition to these physiological and distributional effects on D_{LCO} , a number of pathological states can affect D_M , θV_C , or both and thereby affect D_{LCO} [8, 9, 37–46]. Measurement of D_{LCO} is used when any of these pathological processes are suspected or need to be ruled out. Moreover, measuring changes in D_{LCO} over time in these processes is a useful way of following the course of the disease.

Gas analysers and general equipment

System design

Descriptions of the apparatus and general instructions for performing the single-breath diffusing capacity manoeuvre are available elsewhere [2, 6, 47–50]. Equipment in clinical use varies widely in complexity but the basic principles are the same. All systems have a source of test gas, a method of measuring inspired and expired volume over time and a method of measuring carbon monoxide and tracer gas concentration. Classical discrete-sample gas-analyser *DLCO* systems usually display only volume over time but RGA *DLCO* systems also provide a continuous recording of carbon monoxide and tracer gas concentration during the entire test manoeuvre (figure 1).

Equipment requirements

The performance standards for *DLCO* equipment are summarised in table 1.

Flow and volume analysers

Any error in measuring flow and subsequently calculating volume will produce a correspondingly equal error in *DLCO*. However, with continuing improvement in flow measurement technologies, improved accuracy is being achieved. The flow measurement accuracy over a range of -10 to $+10$ $\text{L}\cdot\text{s}^{-1}$ must be within $\pm 2\%$. For calibration with a 3-L syringe, which has a specified maximum error of $\pm 0.5\%$ (i.e. 2.985 to 3.015 L), the calibration volume must be within $\pm 2.5\%$ which is equivalent to an error tolerance of ≤ 75 mL. The volume measurement accuracy must be maintained over the range of gas compositions and concentrations likely to be encountered during *DLCO* tests.

Gas analysers

For classical discrete sample calculations of *DLCO*, only the ratios of alveolar to inhaled carbon monoxide and tracer gas concentrations are needed. Thus, the analysers must primarily be able to produce an output for measured exhaled carbon monoxide and tracer gas that is a linear extrapolation between the inhaled (test gas) concentrations and zero (no carbon monoxide or tracer gas present in the analysers) [51, 52]. The measurement of carbon monoxide and tracer gas concentrations is also a static measurement when considering a classical discrete sample calculation of *DLCO*. Analyser response time is not an issue and the time of gas sample collection is measured separately. Gas concentration digital signal conditioning is not required to compensate for the response time when calculating *DLCO* using static measurements.

When nondispersive, infrared carbon monoxide RGAs began to be used to construct a virtual gas sample from flow and gas concentration data, rather than collecting a physical sample of exhaled gas, no specifications were mandated other than for the linearity of the gas analysers [5]. However, with RGAs there is both a lag time (due to the travel of the sampled gas through the sampling tube to the analyser chamber) and an analyser response time (the time to reach 90% of the actual measurement signal from the time the gas sample reaches the analyser) to be considered. As such, the gas concentration signal must be precisely shifted in time to align with the flow signal (figure 2).

RGA response time

The response time of the RGA will determine how accurately the analyser is able to track the true carbon monoxide and tracer gas concentrations. The most rapid changes in concentration occur at the start of test

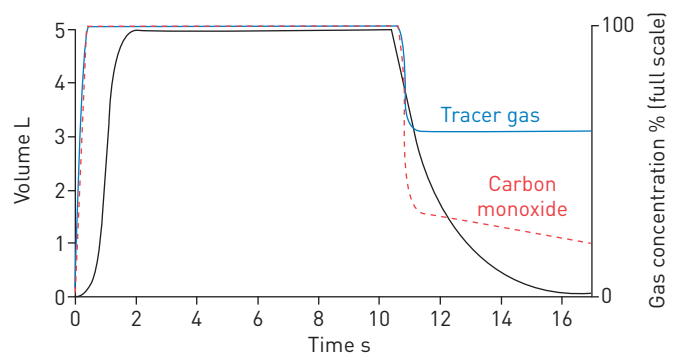


FIGURE 1 Diagram of lung volume and gas concentration during the single-breath manoeuvre to measure the uptake of carbon monoxide. Whereas classical *DLCO* systems only display the volume–time graph, rapid gas analyser (RGA) *DLCO* systems also display the carbon monoxide and tracer gas concentrations throughout the single-breath manoeuvre. Reproduced from [4].

TABLE 1 Equipment specifications and performance standards

DLCO System	Specification	
	Required	Recommended
Rapid gas analyser systems		
Analyser specification		
0–90% response time [see figure 2]	≤150 ms	
Maximum nonlinearity	±1% of full scale	
Accuracy	Within ±1% of full scale	
Interference from 5% carbon dioxide or 5% water vapour	≤10 ppm error in [CO]	
Drift for carbon monoxide	≤10 ppm over 30 s	
Drift for tracer gas	≤0.5% of full scale over 30 s	
Flow accuracy		
	Within ±2% over the range of –10 to +10 L·s ⁻¹	
Volume accuracy (3-L syringe check)		
	Within ±75 mL	
Barometric pressure sensor accuracy		
	Within ±2.5%	
Ability to perform a QA check (3-L syringe; ATP mode; inhaling ~2 L test gas)		
	Calculate total volume (VA) of 3±0.3 L and DLCO of <0.5 mL·min ⁻¹ ·mmHg ⁻¹ or <0.166 mmol·min ⁻¹ ·kPa ⁻¹	
Sample and store data with adequate resolution		
	Digitise at ≥100 Hz per channel with ≥14 bit resolution	Digitise at 1000 Hz
Monitor and report end-expiratory tracer gas and carbon monoxide concentrations (alert operator if washout is incomplete)		
	Implemented [#]	
Compensate for end-expiratory gas concentrations prior to test gas inhalation in the calculation of VA and DLCO		
	Implemented [#]	
Ensure proper alignment of gas concentration signals and the flow signal		
	Implemented [#]	
Measure anatomic dead-space using the Fowler method (see figure 6)		
	Implemented [#]	
Display a graph of gas concentration versus expired volume to confirm the point of dead-space washout and report the amount of manual adjustment if done (see figure 4)		
	Implemented [#]	
Measure VA using all of the tracer gas data from the entire manoeuvre in the mass balance equation		
	Implemented [#]	
Report the DLCO adjusted for the change in PAO₂ due to barometric pressure		
	Implemented [#]	
Ability to input simulated digital test data and compute DLCO, VA, TLC, Vb		
		Calculate values within 2% of actual values
Report the DLCO adjusted for the change in PAO₂ due to PACO₂, if the carbon dioxide concentration signal is available		
		Implemented [#]
Classical discrete sample systems		
Analyser specification		
Maximum nonlinearity	±1% of full scale	
Accuracy	Within ±1% of full scale	
Interference from 5% carbon dioxide or 5% water vapour	≤10 ppm error in [CO]	
Drift for carbon monoxide	≤10 ppm over 30 s	
Drift for tracer gas	≤0.5% of full scale over 30 s	
Flow accuracy		
	Within ±2% over the range of –10 to +10 L·s ⁻¹	
Volume accuracy (3-L syringe check)		
	Within ±75 mL	
Ability to perform a QA check (3-L syringe; ATP mode; inhaling ~2 L test gas)		
	Calculate total volume (VA) of 3±0.3 L and DLCO of <0.5 mL·min ⁻¹ ·mmHg ⁻¹ or <0.166 mmol·min ⁻¹ ·kPa ⁻¹	
DLCO: diffusing capacity of the lung for carbon monoxide; [CO]: carbon monoxide concentration; QA: quality assurance; ATP: ambient temperature, pressure and humidity; VA: alveolar volume; PAO ₂ : alveolar oxygen tension; PACO ₂ : alveolar carbon dioxide tension; TLC: total lung capacity; Vb: dead-space volume. #: Implemented means that the manufacturer has implemented the designated functionality in the DLCO system.		

gas inhalation and at the start of exhalation following the breath-hold. Even after the application of an appropriate time shift (see below) to correct for lag time and analyser response time, there will be a residual error in DLCO due to the finite response time. For every 100 ms increase in the 0–90% response

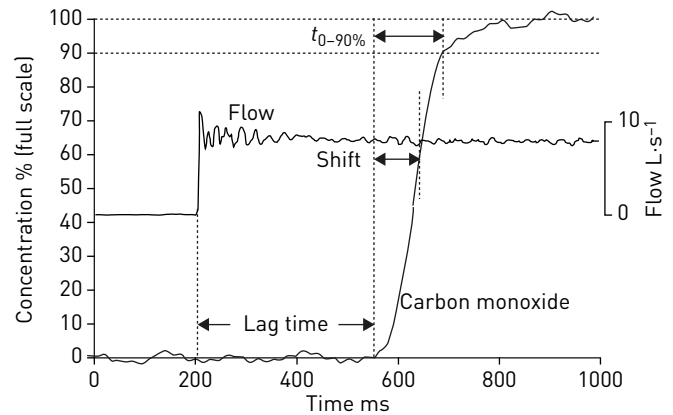


FIGURE 2 Lag and response times for carbon monoxide: the response time of the analyser was estimated by rapidly switching the gas being sampled from zero to full-scale carbon monoxide. The change in the flow signal shows the time at which the switch was made from medical air to test gas. The lag time, the 0–90% response time and the optimal shift are calculated from the resulting response curve.

time, the error in *DLCO* increases by about 0.7% [53]. Based on the above considerations, the 0–90% response time for RGAs used in *DLCO* systems must be ≤ 150 ms.

Response time can be improved by reducing the volume of the analyser chamber and increasing the sample aspiration rate: however, such measures can cause a deterioration of the signal by creating more noise. The use of signal conditioning to simulate a more rapid analyser response may also introduce more noise and errors into the signal. Digital conditioning techniques should only be used to digitally enhance response time if they do not compromise signal quality and accuracy and serve to preserve or improve *DLCO* measurement accuracy.

Linearity and accuracy

The linearity of gas concentration signals is of primary importance in measuring *DLCO* since the ratios of the gas concentrations are considered in the classical calculations [50, 52]. The error in *DLCO* measurement due to nonlinearity in these signals depends on the size of the lungs and the rate of uptake of carbon monoxide. A nonlinearity of 0.5% of full scale can cause errors ranging from 0.5% in a subject with a *DLCO* of $13.4 \text{ mmol}\cdot\text{min}^{-1}\cdot\text{kPa}^{-1}$ ($40 \text{ mL}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$) to 1.7% in a subject with a *DLCO* of $3.35 \text{ mmol}\cdot\text{min}^{-1}\cdot\text{kPa}^{-1}$ ($10 \text{ mL}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$) [53]. The manufacturer specification for analyser linearity is that any nonlinearity must not exceed 0.5% of full scale once zero and full scale values have been set. The accuracy of the gas analyser signal also becomes important when measuring the residual background alveolar carbon monoxide concentration and the washout of tracer gas from the previous *DLCO* manoeuvre. The output of the gas analyser must be accurate to within $\pm 1\%$ of full scale.

Interference and noise

Nondispersive, infrared carbon monoxide analysers typically have some cross sensitivity to carbon dioxide and water vapour. Strategies to reduce and/or compensate for cross sensitivity are required such that the water vapour and carbon dioxide in exhaled gas (up to 5% each; *i.e.* water vapour pressure ($P_{\text{H}_2\text{O}}$) $6.28 \text{ kPa}/47 \text{ mmHg}$) contribute a less than 10 ppm error in the measured carbon monoxide signal. Measuring the exhaled gas from the subject prior to the inhalation of test gas can also provide an offset due to carbon dioxide and water vapour measurements that can be used to adjust the concentration signal.

Drift

Gas analysers should have only minimal drift in zero and gain, such that output is stable over the test interval. Gas analyser drift must be ≤ 10 ppm over 30 s for carbon monoxide and $\leq 0.5\%$ of full scale over 30 s for tracer gas. It is recommended that manufacturers provide an optional test mode to display the measured gas concentrations so that stability can be confirmed. Any drift must be determined by comparing the carbon monoxide and tracer values measured in room air immediately prior to and immediately following the single-breath manoeuvre. The gas concentration signals used in the calculation of *DLCO* must be compensated for drift, assuming a linear change over the measurement interval.

Aspiration flow

Depending upon the design of the breathing circuit, the gas analyser sampling port and the gas analyser aspiration flow, gas may be entrained into the sampling line from room air or from the test gas when the

exhaled flow decreases to near zero at the end of exhalation. Clearly, when the subject's exhaled flow drops below the aspiration flow, the sample will entrain other gas that is not part of the exhaled gas. DLCO instrument manufacturers are required to determine the lowest exhaled flow at which the gas sampling line will not entrain gas other than exhaled gas. This flow must be reported in the system specifications. In the analysis of the exhaled gas concentration data, measurements of gas concentration below the specified flow must not be included in either the determination of washout of tracer gas from a previous manoeuvre (see the section on interval between manoeuvres below) or the calculation of absolute end-expiratory lung volume (V_{ee}) in equations 22 and 25 below.

Digitisation

In order for the digitised signal to accurately track the gas concentration signal and in order to provide adequate opportunity for signal processing for data alignment, the minimum signal sampling rate must be ≥ 100 Hz per channel; however, a rate of 1000 Hz is recommended. The analogue to digital converter resolution must be ≥ 14 bits.

Other equipment considerations

Circuit resistance must be $< 1.5 \text{ cmH}_2\text{O} \cdot \text{L}^{-1} \cdot \text{s}^{-1}$ up to $6 \text{ L} \cdot \text{s}^{-1}$ flow. If a demand-flow regulator is used on a compressed test gas cylinder, the maximal inspiratory pressure required for $6 \text{ L} \cdot \text{s}^{-1}$ inspiratory flow through both the circuit and the valve must be $< 9 \text{ cmH}_2\text{O}$.

Equipment dead-space volume (V_{Dequip}) for both inspired test gas and the alveolar sample must be known and their role in all data computation algorithms must be identified and documented. For adults, the V_{Dequip} including the breathing circuit proximal to the gas analyser sampling point, filter and mouthpiece must be $< 200 \text{ mL}$. Smaller dead-space volumes are recommended for paediatric applications and people with a vital capacity (VC) of less than 2 L.

The system must be leak free; this is particularly important for DLCO systems that aspirate gas samples through the gas analyser at sub-atmospheric pressures. When samples are aspirated, leaks in tubing, fittings and other locations allow room air to be drawn into the gas circuit, thus diluting the sample and reducing the concentrations of carbon monoxide and tracer gases.

Equipment calibration and quality control

The considerations for equipment calibration and quality control are illustrated in table 2. There are a number of regular procedures to apply, summarised as follows:

- 1) Flow and gas analysers must be zeroed prior to each manoeuvre. After each manoeuvre, a new zeroing procedure must be carried out to account for analyser drift during the previous test.
- 2) Each day, prior to testing, there must be a volume calibration check with a 3-L syringe [54]. The syringe should be discharged at least three times to give a range of flow rates varying between 0.5 and $12 \text{ L} \cdot \text{s}^{-1}$ (with 3-L injection times of $\sim 6 \text{ s}$ and $\sim 0.5 \text{ s}$, respectively). The volume at each flow rate must meet an accuracy requirement of $\leq 2.5\%$ error. For devices using disposable flow sensors, a new sensor from the supply used for patient tests must be tested each day. The calibration check may need to be repeated during the day if ambient conditions change. Newer systems monitor ambient conditions and make adjustments as necessary or produce a calibration alert when needed. Older systems may require a calibration check if room temperature changes by more than $3 \text{ }^\circ\text{C}$ or relative humidity changes by more than 15% (absolute). Operators should also perform a calibration check whenever they notice significant

TABLE 2 Equipment calibration schedule

Calibration technique	Frequency
Flow analyser zeroing	Before each test
Gas analyser zeroing	Before/after each test
Volume calibration check	Daily
Biologic control	Weekly
Calibration syringe DLco check	Weekly
Calibration syringe leak test	Monthly
Linearity check (calibration syringe or simulator)	Monthly

DLco: diffusing capacity of the lung for carbon monoxide.

discrepancies between the inspired volume (V_I) and VC, or between V_A and total lung capacity (TLC), which might suggest volume calibration problems.

3) Each week, or whenever problems are suspected, the following procedures must be followed. First, for those $DLCO$ systems using a volume-type spirometer, a spirometer leak test should be performed according to the manufacturer's specifications. Secondly, a $DLCO$ test should be performed with a calibrated 3-L syringe by attaching the syringe to the instrument in the normal patient test mode. The syringe should then be emptied, filled with 3 L of test gas and emptied into the mouthpiece after the 10 s breath-hold. The calculation of V_A must be within 300 mL of 3 L times the ATPD to BTPS (body temperature, ambient pressure, saturated with water vapour conditions) correction factor, which is $310/T_A \cdot P_B/(P_B-47)$, where T_A is the ambient temperature in degrees kelvin and P_B is the barometric pressure in mmHg. It should be noted that a 3-L calibration syringe will have an additional dead-space which, depending on the connection to the mouthpiece, is typically ~ 50 mL and must be considered in the V_A calculation. The absolute value of the calculated $DLCO$ must be $<0.166 \text{ mmol}\cdot\text{min}^{-1}\cdot\text{kPa}^{-1}$ or $<0.5 \text{ mL}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$. Thirdly, a test should be performed on a "standard subject" (biological control) or simulator [55]. Standard subjects are nonsmokers who have been found to have a consistently repeatable $DLCO$ (e.g. healthy laboratory personnel). If the $DLCO$ in a standard subject varies either by $>12\%$ or by $>1 \text{ mmol}\cdot\text{min}^{-1}\cdot\text{kPa}^{-1}$ ($>3 \text{ mL}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$) from the mean of previous values, the test must be repeated. A study of the long-term intersession variability of $DLCO$ has found that biological control deviations either $>12\%$ or $>3 \text{ mL}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$ from the average of the first six tests indicate that the instrument is not within quality control limits and must be carefully evaluated before further patient testing [56]. For a digital system check of the $DLCO$ calculation algorithm, standardised digital data for flow, volume and carbon monoxide and tracer gas concentration will be developed by the task force and made available with a sample rate of 1 kHz as an xml or csv file. It is strongly recommended that manufacturers provide the ability to input data from such a file and generate test results to compare measured *versus* known $DLCO$ and V_A values. For systems failing the above testing, the $DLCO$ system must be evaluated carefully for the possibility of leaks, nonlinear analyser function, and volume and time inaccuracy, *etc.* When sufficient data on a standard individual have been obtained, laboratories should establish their own outlier criteria to serve as indicators of potential problems with their $DLCO$ systems. Manufacturers are encouraged to develop automated quality-control software to assist and enhance the utility of these steps.

4) Each month a leak test of the 3-L calibration syringe should be performed. If the calibration syringe does not have a volume scale on the shaft, mark 50 mL below full by measuring the excursion of the shaft from 0 to 3 L and marking it at a distance that is 0.017 of the full excursion. Fill the syringe and place a stopper at the syringe input. Push the syringe in to the 50 mL mark (which generates a pressure of about 17 cmH₂O), hold for 10 s and release. If the syringe does not return to within 10 mL of the full position, it should be sent for repair. The procedure is then repeated starting with the syringe at 50 mL below full, applying the stopper and pulling the syringe to the full position.

5) Each month, gas-analyser linearity should be assessed. A straightforward approach is to measure known serial dilutions of the test gas [57], or to measure the concentration of a separate high-precision test gas having a certificate of analysis. Manufacturers must be encouraged to automate this function. For systems with independent measurement of carbon monoxide and tracer gas, the analyser linearity may also be assessed by comparing the ratio of carbon monoxide and tracer gas concentration to arbitrary dilutions of test gas with room air. A third type of calibration syringe test, which differs from the volume check in point two and the $DLCO$ check in point three by using the 3-L syringe in ambient temperature, pressure and humidity (ATP) mode, may also reveal problems with analyser linearity. With approximately 1 L of air in the syringe, the test begins by filling the remaining volume with test gas. Following a 10 s "breath-hold" the syringe is then emptied. The calculation of V_A must be within 300 mL of 3 L with the syringe dead-space being used for the anatomic dead-space in the V_A calculation. The absolute value of $DLCO$ must be $<0.166 \text{ mmol}\cdot\text{min}^{-1}\cdot\text{kPa}^{-1}$ or $<0.5 \text{ mL}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$. A review of quality control data for four different $DLCO$ systems between 2006 and 2015 using this procedure found only four outlier points where $|DLCO|$ was $>0.13 \text{ mmol}\cdot\text{min}^{-1}\cdot\text{kPa}^{-1}$ ($>0.4 \text{ mL}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$). The same data showed that V_A was consistently within 3 ± 0.3 L for the four systems (unpublished data from B.R. Thompson). Gas mixing in the syringe can be improved by using low flow rates and extending the breath-hold time. The effects of incomplete mixing in the syringe can be minimised by using a larger sample volume. In the absence of a $DLCO$ simulator and high-precision test gases, system checks must be performed using a 3-L calibrating syringe in ATP mode. Manufacturers must provide this test option, which will be the same as the usual testing procedure for a patient, with the exception that V_A will be reported in ATP rather than BTPS.

6) A record of equipment checks and standard subject tests should be dated and kept in a laboratory log book or digital file folder. Manufacturers are encouraged to provide software and test equipment options for quality control measurement and quality control data management. In addition, manufacturers may provide equipment-specific, quality-control measures in addition to the foregoing points. If water vapour

permeable tubing is used to either remove water vapour or equilibrate water vapour with room air, such tubing must be replaced according to manufacturer recommendations to ensure that it is functioning properly. Chemical gas analyser cells will have a replacement schedule. Manufacturers may also have preventative maintenance schedules for various other system components (e.g. balloon valves) which will require testing and replacement as necessary.

Quality control for RGA systems

Modern DLCO systems are completely integrated and do not use stand-alone gas analysers that can be tested separately. Specifications for manufacturers are required to facilitate a uniform testing and calibration strategy across all systems. Quality-control requirements include analogue testing with devices such as a simulator [58], the option to operate in full ATP mode and a digital calibration option to verify the computational algorithms. The digital calibration option should use simulated flow, carbon monoxide concentration and tracer gas concentration data from standardised manoeuvres with a known DLCO.

Infection control

The major goal of infection control is to prevent the transmission of infection to patients and staff during pulmonary function testing. The recommendations in the ATS/ERS documents for spirometry and general considerations for pulmonary function testing also apply to DLCO equipment and procedures [59–61].

Standardisation issues in the single-breath testing technique

The single-breath determination of DLCO involves measuring the uptake of carbon monoxide from the lung over a breath-holding period. To minimise variability as much as possible, the following specifications for the standardisation of testing techniques are provided.

Patient condition

Factors that affect VC (e.g. exercise, body position, Hb affinity for carbon monoxide, alveolar oxygen tension (PAO_2), and level of carboxyhaemoglobin (COHb)) must be standardised. If clinically acceptable, the subject should not breathe supplemental oxygen for ≥ 10 min prior to a DLCO manoeuvre. In addition, when using exercise or the supine position to assess the ability of the lung to increase gas transfer [18, 28–31], the level of exercise and/or the duration of the supine position must be noted. Before beginning the test, the manoeuvres must be demonstrated and the subject carefully instructed. Furthermore, the subject must be seated comfortably throughout the test procedure, which must be performed at a stable, comfortable temperature within the manufacturer's equipment specifications.

COHb produces an acute, reversible decrease in DLCO [62–66], largely due to its effects on carbon monoxide back-pressure and the “anaemia effect” from decreased Hb binding sites for test gas carbon monoxide. As cigarette smoking is the most common source of COHb, subjects must be asked to refrain from smoking or other sources of carbon monoxide exposure on the day of the test. The time of the last cigarette smoked must be recorded and noted for the interpretation. A correction for carbon monoxide back-pressure must be made for recent or heavy cigarette smoking (see the section on adjustment for COHb concentration and carbon monoxide back-pressure below). Air pollution may also result in higher COHb levels and exposure to high levels of air pollution should be noted.

Inspiratory manoeuvres

Once the mouthpiece and nose clip are in place, tidal breathing must be carried out for a sufficient time to assure that the subject is comfortable with the mouthpiece and that the nose clips and mouthpiece are used appropriately with no leaks. The DLCO manoeuvre begins with unforced exhalation to residual volume (RV). In obstructive lung disease, where exhalation to RV may require a prolonged period, a reasonable recommendation is that this portion of the manoeuvre must be limited to < 12 s. Exhalation times of up to 12 s will allow most patients with airflow obstruction to exhale sufficiently such that they can achieve a maximal VC for the subsequent inhalation of test gas. Submaximal inhalation occurs most frequently in patients with airflow obstruction who are not given adequate time to exhale prior to the inhalation of test gas.

At RV, the subject's mouthpiece is connected to a source of test gas, and the subject inhales rapidly to TLC.

A submaximal inspired volume of test gas (i.e. less than the known VC) can affect carbon monoxide uptake depending upon whether it is a result of an initial suboptimal exhalation to RV (manoeuvre performed at TLC) or whether it is due to a suboptimal inhalation from RV (manoeuvre performed below TLC) [22–25]. In the former case, the calculated VA and DLCO will accurately reflect lung volume and the carbon monoxide uptake properties of the lung at TLC. In the latter case, the VA will be reduced and DLCO measurement will be affected.

Due to these effects, it is important that the inspired volume of test gas, V_i , be as close to the known VC as possible. Data from a large patient population have shown that the V_i during DLCO measurement averages ~90% of the VC [22]. Since the introduction of the 2005 guidelines and subsequent implementation of quality-control checks by equipment manufacturers, there has been an improvement in test quality such that 90% of the largest known VC as the lower limit of acceptability for V_i has been shown to be attainable [67]. Furthermore, as noted above, V_i will be improved by allowing up to 12 s for exhalation prior to inhalation of test gas. V_i must be at least 90% of the largest VC in the same pulmonary function testing session. However, a manoeuvre may be deemed to be acceptable if V_i is within 85% of the largest VC and the V_A is within 200 mL or 5% (whichever is greater) of the highest V_A among acceptable DLCO manoeuvres.

The inspiration must be rapid, since the DLCO calculations assume instantaneous lung filling [27, 68–74]. Slower lung filling decreases the amount of time the lung is at full inspiration with a consequent reduction in carbon monoxide uptake. Although various sample timing techniques address the issue of lung filling and emptying time, inspiration of test gas should be sufficiently rapid such that that 85% of V_i must be inspired in <4.0 s. If longer inspiratory times are needed to inspire 85% of V_i , this must be noted on the test report.

Breath-hold and expiratory manoeuvres

During the breath-hold, both the Valsalva and Müller manoeuvres (expiratory or inspiratory efforts against a closed glottis, respectively) can affect DLCO calculation by decreasing or increasing thoracic blood volume, respectively, resulting in a corresponding decrease or increase in DLCO, respectively, for each manoeuvre [32, 75, 76]. The intrapulmonary pressure during the breath-hold should thus be near atmospheric and this is best accomplished by having the subject voluntarily maintain full inspiration using only the minimal necessary effort. The breath-hold time must be 10 ± 2 s, a target easily achieved in the vast majority of subjects [77].

As with inspiration, the DLCO calculation assumes instantaneous lung emptying [27, 68–72]. Although various sample timing techniques address the fact that emptying is not instantaneous, it is still reasonable to expect that the expiratory manoeuvre must be smooth, unforced and without hesitation or interruption.

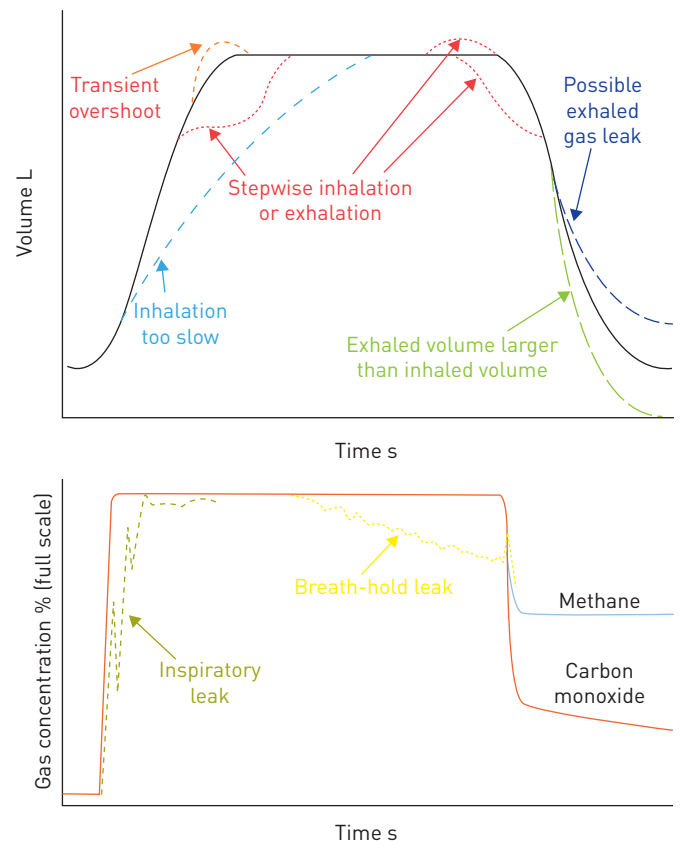


FIGURE 3 Potential problems with the breathing manoeuvre for single-breath diffusing capacity of the lung for carbon monoxide that can lead to measurement errors. Reproduced from [4].

For classical systems, the exhalation time for washout and discrete sample collection should not exceed 4 s. In subjects who require a longer expiratory time to provide an appropriate alveolar gas sample, the expiratory time must be noted in the test report. For RGA systems, exhalation should continue to RV, with a maximum exhalation time of 12 s, which provides improved measurement of V_A as noted in the data analysis for RGA systems section below. The results of common errors that can occur during the inspiration, breath-hold and expiration manoeuvres are illustrated in figure 3.

Washout and sample collection manoeuvres

$DLCO$ calculations (see the calculations section below) are performed by analysis of discrete alveolar gas samples containing carbon monoxide and tracer gas. During expiration, a volume of gas must be expired to clear the total anatomical and equipment dead-space volume (V_D) and then discarded before the alveolar sample is collected (figure 1). Collecting an alveolar gas sample before the point of dead-space washout will underestimate $DLCO$, while delaying sample collection beyond the point of dead-space washout will overestimate $DLCO$ [68, 72].

Washout and sample collection in classical systems

The washout volume must be 0.75–1.0 L (BTPS). If the patient's VC is <2.00 L, the washout volume may be reduced to 0.50 L. The discrete-sample gas volume (V_s) is the volume of gas collected following the breath-hold and used to analyse alveolar carbon monoxide and tracer gas concentrations. V_s collection time will affect the measurement of breath-hold time (see below). For discrete sample systems that require larger sample volumes, a V_s of 0.5–1 L should be collected for analysis. In patients with a VC <1 L, a V_s <0.5 L may be used if it can be confirmed that the dead-space has been cleared.

Washout and sample collection in RGA systems

The time point for dead-space washout can be determined from the expired tracer gas concentration data using an objective algorithm. The beginning of the alveolar plateau can be located by determining the

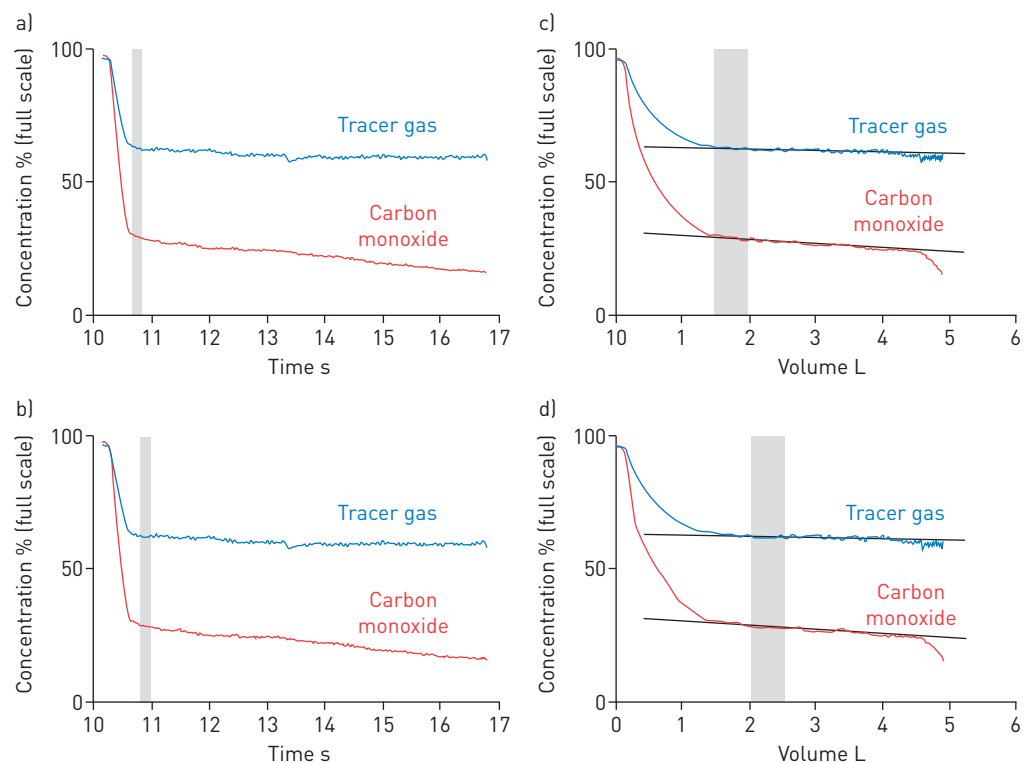


FIGURE 4 Comparison of gas concentration plotted as a function of time (a and b) or volume (c and d) for carbon monoxide and tracer gas. The shaded bar shows the collection of a 500-mL sample of exhaled gas. The upper panels (a and c) show sample collection as selected by computer algorithm (based on gas concentration and lung volume). The lower panels (b and d) show sample collection after manual adjustment by an operator using the concentration versus time plot. Operators tend to be more conservative and may over-shift the sample. When gas concentration is plotted against time, the shift does not appear to be significant; however, when gas concentration is plotted against volume, the degree of shift becomes more apparent.

breakpoint of each phase of the washout on a plot of concentration *versus* volume and adding a proportion of the dead-space volume measured by the FOWLER technique [78] to the phase II to III breakpoint [79]. Such an approach can be automated; however, for visual verification of the point of dead-space washout, the tracer gas concentration must be displayed as a function of volume, since verifying the point of dead-space washout using the concentration *versus* time curve can be deceptive due to the relatively high flow at the beginning of exhalation. This is illustrated in figure 4. If the sample collection point is changed by the operator, it must be recorded in the database and on the report.

With RGA systems, the concentrations of carbon monoxide and tracer gas in a virtual alveolar gas sample are calculated for use in measuring DLCO. The gas concentrations in a virtual sample, that would have been observed in a sample of a given volume had it been collected at a given point during exhalation, are calculated from the flow and gas concentration data. A virtual 200-mL sample analysed by the method of JONES and MEADE [72] has been found to be robust [68]. However, these systems are capable of simulating much smaller gas samples and JONES and MEADE [72] used 85-mL gas samples in the development of their method. Smaller virtual samples will be more affected by noise in the expired carbon monoxide concentration signal. Virtual alveolar gas sample volumes of 85 mL to 500 mL may be used.

Inspired gas composition

The test gas used to calculate DLCO should contain very close to 0.3% carbon monoxide, 21% oxygen, a tracer gas and a balance of nitrogen. The tracer gas must be relatively insoluble and relatively chemically and biologically inert. Since the tracer gas is used to determine the initial alveolar carbon monoxide concentration, as well as the V_A from which carbon monoxide uptake is occurring, its gaseous diffusivity should be similar to carbon monoxide and it should not interfere with the measurement of carbon monoxide concentration. The tracer gas should also not ordinarily be present in alveolar gas or else be present at a known, fixed concentration (*e.g.* argon).

Commonly used tracer gases include helium and methane. Helium meets most of the previous criteria; however, its gaseous diffusivity is considerably higher than that of carbon monoxide. Methane is commonly used for RGA systems; its gaseous diffusivity is closer to carbon monoxide but it has a slightly higher liquid solubility than helium. A recent study has found no clinical difference in DLCO using either helium or methane in normal subjects or patients with COPD [80].

As noted above, the inspired carbon monoxide concentration should be close to 0.3%; however, as ratios are more important than absolute values, exact concentrations are not critical. The assumption in calculating carbon monoxide uptake is that capillary blood does not contain carbon monoxide. Thus, corrections are needed in patients who have significant COHb (see the section on adjustment for COHb concentration and carbon monoxide back-pressure below). There are two considerations influencing the rationale for recommending an inspiratory oxygen fraction (F_{IO_2}) of 21% in the test gas for routine DLCO testing. First, the majority of studies developing reference values for DLCO, which are based on the 2005 standards [4], use an F_{IO_2} of 21% (see the section on reference values below). Secondly, the PAO_2 following a maximal inhalation will depend on the dead-space volume and the ratio of V_I to V_A for any given value of F_{IO_2} in the test gas. Hence, if reducing F_{IO_2} in the test gas is intended to simulate tidal breathing conditions (*i.e.* a PAO_2 of 100 mmHg or 13 kPa), it may not do so in all subjects.

Although not performed routinely, the measurement of DLCO at several different levels of PAO_2 allows the two components of DLCO (DM and θVC) to be distinguished. This is accomplished by using the Roughton-Forster relationship noted previously (equation 3) and varying θ by altering PAO_2 . Subsequently, $1/DLCO$ is plotted against $1/\theta$ at the different PAO_2 levels. The slope of this relationship is $1/VC$ and the intercept is $1/DM$. While there are differences in the proposed value of θ , it is beyond the scope of this report to make recommendations for the value of θ to be used.

Manoeuvre intervals

Manoeuvre intervals in classical systems

The 2005 ERS/ATS recommendations state that at least 4 min must be allowed between manoeuvres to allow for adequate elimination of test gas from the lungs. The subject should remain seated during this interval. In patients with airflow obstruction, a longer period (*e.g.* 10 min) should be considered. Several deep inspirations during this period may help to clear test gases more effectively.

Manoeuvre intervals in RGA systems

Exhaled gas can be monitored as soon as the subject begins breathing through the mouthpiece prior to the inhalation of test gas. If a previous manoeuvre was conducted, the information collected on end-expiratory tracer gas levels will indicate whether or not washout is complete, which may occur in less than 4 min in some subjects. For complete washout, the tracer gas level at end-exhalation must be $\leq 2\%$ of the tracer gas

concentration in the test gas. Occasionally, if a subject has not reached this level of washout after 5 min, the operator may have the option of continuing with the next manoeuvre. However, in either event, the end-expiratory tracer gas concentration must be reported and used to adjust the tracer gas concentration data used in the determination of V_A at the beginning of breath-holding.

The carbon monoxide concentration measured in exhaled gas prior to inhaling test gas can be used for three important purposes [53]: 1) to adjust $DLCO$ calculations for the back-pressure of carbon monoxide, both the ambient level and the increase that occurs with multiple $DLCO$ manoeuvres; 2) to estimate the COHb concentration and adjust the $DLCO$ calculation accordingly; and 3) to compensate for any residual effects of water vapour and carbon dioxide on the carbon monoxide analysers.

Miscellaneous factors

There may be a diurnal variation in $DLCO$, since one study has found that $DLCO$ falls 1.2–2.2% per hour throughout the day [81]. The reason for this change is not clear and is not explained by carbon monoxide back-pressure or changes in V_A , V_I , or breath-hold time. One explanation is a combination of changes in carbon monoxide back-pressure and diurnal variation in Hb concentration [82]. A 13% change in $DLCO$ during the menstrual cycle has been reported [83]. The highest value is observed just before the menses and the lowest is observed on the third day of menses; however, it is not clear if this is simply a Hb effect or whether it reflects other physiological processes (e.g. hormonal changes on pulmonary vascular tone). Ingestion of ethanol has been reported to decrease $DLCO$ [84, 85]. The mechanisms involved are not clear, although it is known that some fuel-cell carbon monoxide analysers are sensitive to exhaled ethanol and ketones.

Pulmonary function test sequence

$DLCO$ manoeuvres are frequently conducted immediately following the administration of 400 µg of salbutamol in the interval between pre- and post-bronchodilator spirometry testing [60]. While an older study in obstructive lung disease subjects found that $DLCO$ may increase by up to 6% after administration of a bronchodilator [86], a newer study has found that the administration of 400 µg of salbutamol has no significant effect on $DLCO$ in normal control subjects or in patients with either reversible or non-reversible airflow obstruction [87]. A further study has found no significant salbutamol effect on $DLCO$ in COPD patients at doses of less than 1000 µg [88]. Therefore, there is no recommendation against use of a bronchodilator prior to $DLCO$ tests.

Spirometry is a form of exercise [59], which could conceivably impact on $DLCO$ values; however, no studies were found which support a recommendation for a rest interval following spirometry. If the order of testing includes measuring absolute lung volumes using nitrogen washout, during which 100% oxygen is inspired [89] prior to $DLCO$ manoeuvres, ample time is required for alveolar oxygen levels to return to normal. For nitrogen to wash back in to normal levels, allow a rest interval equal to twice the time required for the nitrogen washout test to be completed [90]. It is recommended that $DLCO$ measurements be made before any multi-breath nitrogen washout tests.

Calculations

Calculating diffusing capacity

Converting equation 2 to calculus notation and using $P_{ACO} = F_{ACO} \cdot (P_B - P_{H_2O})$, where F_{ACO} is the alveolar carbon monoxide fraction in the dry gas, P_B is the barometric pressure and P_{H_2O} is the water vapour pressure, gives equation 4 as shown below.

$$\frac{d(V_A \cdot F_{ACO})}{dt} = DLCO \cdot F_{ACO} \cdot (P_B - P_{H_2O}) \quad (4)$$

Assuming a constant volume and that the pulmonary capillary carbon monoxide tension is near zero, solving for $DLCO$ gives equation 5, where $F_{ACO,0}$ and $F_{ACO,t}$ are the fractional concentrations of carbon monoxide in the alveolar volume at time 0 and time t, respectively. The rate of gas uptake is expressed in mL(STPD)·min⁻¹ and the transfer gradient (the difference between the alveolar and pulmonary capillary pressures) in mmHg. Therefore, $DLCO$ has traditional units of mL(STPD)·min⁻¹·mmHg⁻¹ and SI units of mmol(STPD)·min⁻¹·kPa⁻¹.

$$DLCO = \frac{V_A}{t \cdot (P_B - P_{H_2O})} \cdot \ln\left(\frac{F_{ACO,0}}{F_{ACO,t}}\right) \quad (5)$$

The single-breath $DLCO$ technique assumes that both carbon monoxide and the tracer gas are diluted equally on inspiration. Thus, the initial alveolar concentration of carbon monoxide at the theoretical start

of breath-holding ($F_{ACO,0}$) can be calculated by knowing the inspired tracer gas fraction (F_{ITr}) and the alveolar tracer gas fraction (F_{ATr}). In this case, if F_{ICO} is the carbon monoxide fraction in the inspired test gas, we can generate equation 6.

$$F_{ACO,0} = F_{ICO} \cdot \frac{F_{ATr}}{F_{ITr}} \quad (6)$$

Tracer gas dilution is also used to determine the effective V_A . If we solve for D_{LCO} we can generate equation 7, where V_A is reported in L (BTPS) and t_{BH} , the breath-hold time, is reported in seconds.

$$D_{LCO} = \frac{V_A}{t_{BH} \cdot (P_B - P_{H_2O})} \cdot \ln\left(\frac{F_{ICO}}{F_{ACO}} \cdot \frac{F_{ATr}}{F_{ITr}}\right) \quad (7)$$

If we convert V_A to STPD conditions we obtain equation 8 for traditional units of D_{LCO} (V_A mL(STPD)·min⁻¹·mmHg⁻¹). The factor of 60 000 arises from the change to the traditional units (60 s to 1 min and 1 L to 1000 mL).

$$D_{LCO} = \frac{V_{ASTPD}}{t_{BH} \cdot (P_B - 47)} \cdot \ln\left(\frac{F_{ICO}}{F_{ACO}} \cdot \frac{F_{ATr}}{F_{ITr}}\right) \cdot 60\,000 \quad (8)$$

If we then convert to SI units we obtain equation 9 (units of T_{LCO} : mmol·min⁻¹·kPa⁻¹), where the factor of 22.4 arises from the conversion of mL(STPD) to mmol.

$$T_{LCO} = \frac{V_{ASTPD}}{t_{BH} \cdot (P_B - 6.28)} \cdot \ln\left(\frac{F_{ICO}}{F_{ACO}} \cdot \frac{F_{ATr}}{F_{ITr}}\right) \cdot 60\,000/22.4 \quad (9)$$

Calculating breath-hold time

The breath-hold time, or time of transfer during which carbon monoxide changes from its initial to its final concentration (t_{BH}), is part of the denominator in the D_{LCO} equation (equation 7). As noted previously, the single-breath measurement of carbon monoxide uptake assumes an instantaneous lung filling and emptying process. However, both inspiration and expiration require up to several seconds, and these periods of changing gas volume in the lung must be accounted for in the calculations. For purposes of standardisation, the method of JONES and MEADE (figure 5) [72] is recommended, since it has the theoretical appeal of empirically accounting for the effects of inspiratory and expiratory time. This method has also been shown to adequately address inspiratory flows as low as 1 L·s⁻¹, breath-hold times as short as 5 s and expiratory flows as low as 0.5 L·s⁻¹ in normal subjects [68] when using an RGA system that

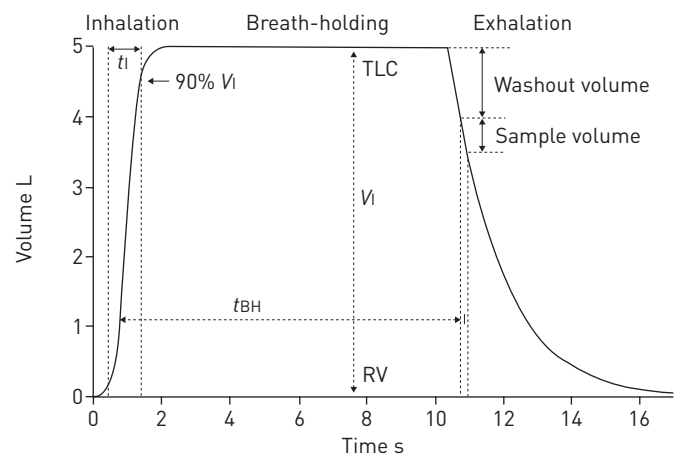


FIGURE 5 Schematic illustration of measuring breath-hold time for the single-breath diffusing capacity of the lung for carbon monoxide. The JONES and MEADE [72] breath-hold time includes 0.7 of inspiratory time and half of sample time. V_i : inspired volume; t_i : time of inspiration (defined from the back-extrapolated time 0 to the time that 90% of the V_i has been inhaled); t_{BH} : breath-hold time; TLC: total lung capacity; RV: residual volume. Reproduced from [4].

measures the dead-space and calculates V_A using the tracer gas concentration data from the entire manoeuvre. With the approach taken by JONES and MEADE [72], breath-hold time equals the time starting from 0.3 of the inspiratory time (t_i) to the middle of the sample collection time. As in spirometry, the back-extrapolation technique must be used to establish time zero [2, 59]. The time when 90% of the V_I has been inspired is a reasonable end-point for defining inspiratory time (figure 5).

Calculating the alveolar volume

Alveolar volume in classical systems

V_A represents an estimate of lung gas volume into which carbon monoxide is distributed and then transferred across the alveolar capillary membrane [1, 6] and is thus critical in the measurement of $DLCO$. Classical $DLCO$ equipment collects an actual sample of exhaled gas for analysis and determination of the carbon monoxide and tracer gas concentrations. Since there is only one measurement, the alveolar volume is calculated from the same sample that is used for analysis of carbon monoxide uptake [2]. As noted elsewhere, JONES and MEADE [72] have shown that the sample has to be small (85 mL) to reduce errors in $DLCO$ determination. The calculation of V_A requires an assumption that the alveolar gas is completely mixed at the maximal lung volume and that a small sample of exhaled gas will presumably be representative of the entire lung. In normal subjects this assumption is reasonable and has little effect on the measurement of V_A . However, in patients whose lung disease results in a heterogeneous distribution of ventilation, the size and timing of the sample have a major effect on the measurement of V_A . For classical systems, V_A is determined from values for V_I , F_{ITr} and F_{ATr} (measured in the discrete-sample gas volume, V_S). Since the amount of tracer gas in the lung (alveolar plus dead-space) equals the amount of inspired tracer gas and given that the dead-space tracer gas fraction is the same as the inspired fraction, we can generate equations 10 and 11.

$$V_I \cdot F_{ITr} = V_A \cdot F_{ATr} + V_D \cdot F_{ITr} \quad (10)$$

$$V_A = (V_I - V_D) \cdot F_{ITr} / F_{ATr} \quad (11)$$

V_A is reported under BTPS conditions and then converted to STPD conditions to calculate $DLCO$, as in equations 8 and 9. The inspired volume (V_I) is the measured volume of inhaled dry gas and is thus considered to be under ambient temperature (T), ambient pressure (P_B), dry (ATPD) conditions. The conversion to body temperature, ambient pressure, saturated with water vapour (BTPS) and standard temperature, pressure, dry (STPD) conditions may require conversion factors to compensate for the diluting or concentrating effects of adding or removing water vapour or carbon dioxide at the gas sampling site. Several examples of V_A calculations using such conversion factors are given below.

Where water vapour is removed from the sampled gas and carbon dioxide does not interfere with the analysers we can use equations 12 and 13 as follows, where V_{ABTPS} is the alveolar volume under BTPS conditions and V_{IATPD} is the inspired volume under ATPD conditions.

$$V_{ABTPS} = (V_{IATPD} - V_{Dequip} - V_{Danat}) \cdot \frac{F_{ITr}}{F_{ATr}} \cdot \frac{P_B}{(P_B - 47)} \cdot \frac{310}{(273 + T)} \quad (12)$$

$$V_{ASTPD} = (V_{IATPD} - V_{Dequip} - V_{Danat}) \cdot \frac{F_{ITr}}{F_{ATr}} \cdot \frac{P_B}{760} \cdot \frac{273}{(273 + T)} \quad (13)$$

Where water vapour and carbon dioxide are removed from the sampled gas we can use equations 14 and 15 as follows, where F_{ACO_2} is the fraction of carbon dioxide in the alveolar sample. If no measurement of F_{ACO_2} is available then a value of 0.05 may be assumed.

$$V_{ABTPS} = (V_{IATPD} - V_{Dequip} - V_{Danat}) \cdot \frac{F_{ITr}}{F_{ATr} \cdot (1 - F_{ACO_2})} \cdot \frac{P_B}{(P_B - 47)} \cdot \frac{310}{(273 + T)} \quad (14)$$

$$V_{ASTPD} = (V_{IATPD} - V_{Dequip} - V_{Danat}) \cdot \frac{F_{ITr}}{F_{ATr} \cdot (1 - F_{ACO_2})} \cdot \frac{P_B}{760} \cdot \frac{273}{(273 + T)} \quad (15)$$

Where water vapour in the sampled gas is equilibrated to room air, carbon dioxide does not interfere with the analysers and tank values (*i.e.* the dry gas concentration) are used for F_{ITr} , we can use equations 16

and 17 as shown below. If $F_{I\text{Tr}}$ is read by the analysers, the corrections are the same as in equations 12 and 13 above.

$$V_{\text{ABTPS}} = (V_{\text{IATPD}} - V_{\text{Dequip}} - V_{\text{Danat}}) \cdot \frac{F_{\text{ITr}}}{F_{\text{ATr}}} \cdot \frac{(P_{\text{B}} - P_{\text{H}_2\text{O}})}{(P_{\text{B}} - 47)} \cdot \frac{310}{(273 + T)} \quad (16)$$

$$V_{\text{ASTPD}} = (V_{\text{IATPD}} - V_{\text{Dequip}} - V_{\text{Danat}}) \cdot \frac{F_{\text{ITr}}}{F_{\text{ATr}}} \cdot \frac{(P_{\text{B}} - P_{\text{H}_2\text{O}})}{760} \cdot \frac{273}{(273 + T)} \quad (17)$$

If neither water vapour nor carbon dioxide are removed from the sampled gas, no interference is observed for the analysers and the sample tubing is heated to prevent condensation, we can use equations 18 and 19 as shown below:

$$V_{\text{ABTPS}} = (V_{\text{IATPD}} - V_{\text{Dequip}} - V_{\text{Danat}}) \cdot \frac{F_{\text{ITr}}}{F_{\text{ATr}}} \cdot \frac{310}{(273 + T)} \quad (18)$$

$$V_{\text{ASTPD}} = (V_{\text{IATPD}} - V_{\text{Dequip}} - V_{\text{Danat}}) \cdot \frac{F_{\text{ITr}}}{F_{\text{ATr}}} \cdot \frac{(P_{\text{B}} - 47)}{760} \cdot \frac{273}{(273 + T)} \quad (19)$$

In all four cases, temperature is measured in degrees Celsius and gas pressures are measured in mmHg. It is essential that V_{D} is considered in the calculation of V_{A} . V_{D} occurs in two areas: equipment dead-space, V_{Dequip} (*i.e.* the volume of the mouthpiece, filters and connections within the breathing circuit) and anatomic dead-space, V_{Danat} (*i.e.* the volume in the conducting airways that does not participate in gas exchange). V_{Dequip} must be specified by the equipment manufacturer but may vary as the user alters the system (*e.g.* by adding a filter or using a different filter). A further small correction to V_{D} can be made where V_{Dequip} is assumed to be under ATPD conditions, since it is filled with room temperature, dry test gas at the end of inspiration, whereas V_{Danat} should be assumed to be under BTPS conditions. There are various methods to estimate V_{Danat} . One example uses a fixed value of 150 mL [4, 5], although this does not work well for small adults or children. Another uses a value of $2.2 \text{ mL}\cdot\text{kg}^{-1}$ of body weight [50], although this does not work well for very obese subjects. In the studies which derive the commonly used reference equations, the latter is the most commonly used technique. However, some investigators have ignored V_{Danat} [91–93] and one uses a value derived from $\text{age} + 2.2 \text{ mL}\cdot\text{kg}^{-1}$ of body weight [94]. If body mass index (BMI) is $<30 \text{ kg}\cdot\text{m}^{-2}$, the recommendation is to use an estimate for V_{Danat} of $2.2 \text{ mL}\cdot\text{kg}^{-1}$ body weight. In more obese subjects, or if the weight of the subject is unknown, V_{Danat} (in mL) can be estimated using equation 20 where height (h) is measured in cm.

$$V_{\text{Danat}} = h^2/189.4 \quad (20)$$

With classical discrete-sample systems, which collect the alveolar sample in a collection bag or chamber, the sample-bag residual volume (sometimes called the sample-bag dead-space) dilutes the sample gas and alters the measured concentrations of the expired gases. The size and direction of the error depends on V_{S} , the residual volume of the sample bag and its connectors (V_{SRV}) and the gas content of this residual volume. V_{SRV} could contain test gas, room air, or expired gas from a subject after a *DLCO* manoeuvre. When V_{SRV} contains room air, its effect is to reduce the measured concentrations of the expired gases and equation 21 can be used to adjust for this. Estimates of the potential change in *DLCO*, in existing systems when no adjustment is made for sample-bag dead-space, range from 0.3–8% depending on the sample-bag size and V_{SRV} [95].

$$F_{\text{ATr}}[\text{adjusted}] = F_{\text{ATr}}[\text{measured}] \cdot (V_{\text{S}}/(V_{\text{S}} - V_{\text{SRV}})) \quad (21)$$

For classical systems, manufacturers must report instrument and sample-bag dead-space. Both of these must be flushed with room air or, if DM and VC are to be calculated, appropriate levels of oxygen before the single-breath manoeuvre such that they will not contain expiratory gas from a previous subject. V_{SRV} must be $<2\%$ of V_{S} or 10 mL, whichever is larger. Importantly, when RGAs are used to measure the exhaled sample, there is no residual bag volume to consider (V_{A} is calculated using a mass balance of all inhaled and exhaled gas; equations 22–26 in the next section).

For normal subjects, the classical single-breath determination of alveolar volume (V_{Asb}) described above closely matches TLC determined by plethysmography [22, 74]. However, poor gas mixing in patients with

maldistribution of inspired volume (e.g. patients with obstructed airways) can markedly alter tracer gas dilution leading to values for V_{Asb} that are markedly less than the value of V_A determined from the actual total thoracic gas volume. Observed carbon monoxide uptake is also affected by poor gas mixing under these conditions and will primarily reflect the carbon monoxide transfer properties of the regions into which the test gas is distributed. It has been suggested that a separately determined V_A value from a more accurate method (e.g. multiple-breath technique (V_{Amb}) or plethysmography ($V_{Aplethys}$)) could be substituted for V_{Asb} under these conditions to correct for the effects of maldistribution. However, the $DLCO$ calculation (equation 7) is based on the volume of gas into which the tracer gas (and carbon monoxide) distributes, and not the total thoracic gas volume. Moreover, substituting a larger, separately determined V_{Amb} or $V_{Aplethys}$ value assumes that DM and VC properties in the unmeasured lung regions are similar to those in the measured lung regions, an assumption that is difficult to justify. Additionally, if V_{Asb} is replaced with a different value, the applicability of the $DLCO$ reference equations is compromised.

Due to these considerations, a separately measured V_{Amb} or $V_{Aplethys}$ should not be substituted for V_{Asb} . Instead, when the value of V_{Asb} is markedly less than that determined separately for V_{Amb} or $V_{Aplethys}$, this must be reported and the ratio of V_{Asb} to V_{Amb} or $V_{Aplethys}$ may optionally be included. For the subsequent interpretation of $DLCO$, it should then be noted that the maldistribution of inspired gas probably contributes to any observed reduction in measured values.

Alveolar volume in RGA systems

As mentioned in the previous section, when an RGA system is used the dead-space volume is measured rather than estimated. The total dead-space, V_D , can be measured from the tracer gas washout curve using the FOWLER [78] method (figure 6). A linear regression line estimating the slope of phase III of the tracer gas washout concentration as a function of volume should be calculated using the last half of the expiratory tracing by volume. The Fowler dead-space is the volume where the area between the phase III slope and the

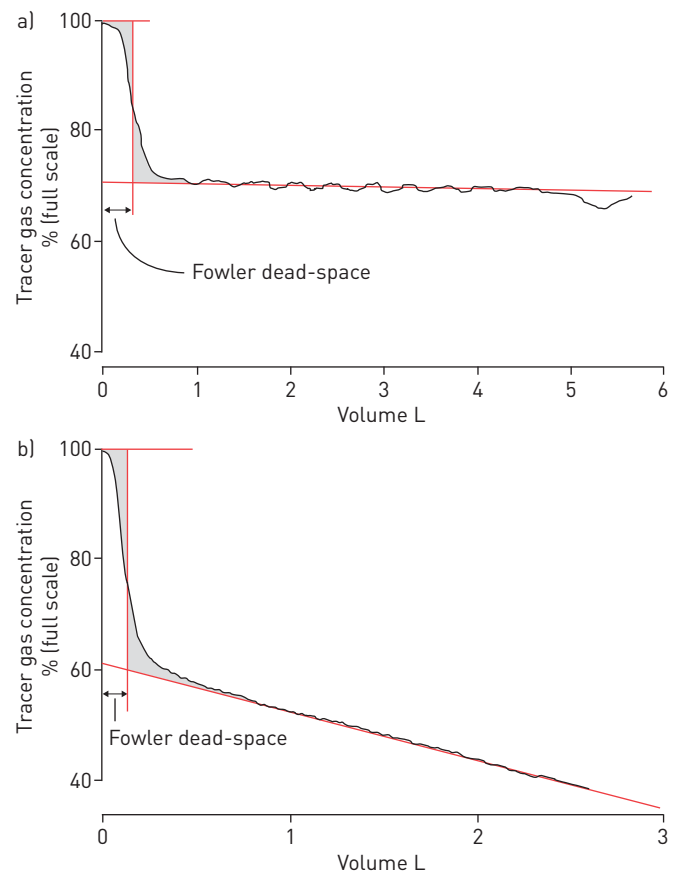


FIGURE 6 Graphical representation of the calculation of the Fowler dead-space volume in a normal, healthy subject (a) and a subject with chronic obstructive pulmonary disease (COPD) (b). The single-breath tracer gas washout is plotted against exhaled lung volume from total lung capacity. The volume at which the shaded area above the tracer gas washout curve equals the shaded area below the curve is the FOWLER dead-space [78] which is reported under body temperature, ambient pressure, saturated with water vapour (BTPS) conditions.

tracer gas washout curve equals the area between the peak tracer gas concentration and the tracer gas washout curve. The anatomic dead-space, V_{Danat} , is equal to the Fowler dead-space minus the equipment dead-space, V_{Dequip} , which includes the filter and/or mouthpiece and which must be supplied by the manufacturer.

The development of RGA systems now allows the analysis of all gas exhaled and provides the opportunity to enhance the accuracy of V_A determinations. Given that the tracer gas can now be monitored throughout exhalation, there is no need to constrain the measurement of V_A to the discrete sample computationally constructed to determine carbon monoxide uptake. Indeed, using all of the available gas concentration data has been shown to provide a better estimate of V_A [71, 96] than constraining the measurement to a smaller sample of exhaled gas (as was required by the equipment available in 1957 when the clinical single-breath method was developed [2]).

This technique uses a mass balance approach to determine V_A in which the volume of tracer gas inhaled and the volume subsequently exhaled are measured and the latter subtracted from the former to determine the volume of tracer gas remaining in the lung at end-exhalation [71, 96]. The volume of tracer gas left in the lung is then divided by the end-expiratory tracer gas concentration to give the absolute end-expiratory lung volume V_{ee} . The TLC is then calculated by adding the expired volume (V_E) to V_{ee} and subtracting V_{Dequip} . If V_E is the volume expired from the maximum volume during breath-holding (to the end of the manoeuvre) then the single-breath total lung capacity (TLC_{sb}) can be defined as $\text{TLC}_{\text{sb}} = V_E + V_{\text{ee}} - V_{\text{Dequip}}$ and $V_A = \text{TLC}_{\text{sb}} - V_{\text{Danat}}$. Residual tracer gas in the lung from a previous manoeuvre can be measured prior to the start of the current manoeuvre and included in the mass balance equation.

In more detail, V_A is calculated using a mass balance equation which states that the tracer gas left in the lung at end exhalation is equal to all of the tracer gas inhaled minus the tracer gas exhaled. The sum of the inhaled and exhaled tracer gas volumes is the integral, with respect to time, of flow \times tracer gas concentration where flow is positive for inhalation and negative for exhalation. In this case, V_{ee} (including V_{Dequip} and V_{Danat}) is thus described by equation 22 where t_0 is the time at the start of test gas inhalation, t_f is the time at the end of exhalation, $\text{Tr}(t)$ is the tracer gas concentration at any time t (adjusted to BTPS conditions), Tr_{ee} is the mean tracer gas concentration at end-exhalation and $\text{flow}(t)$ is the flow at any time t (under BTPS conditions).

$$V_{\text{ee}} = \frac{1}{\text{Tr}_{\text{ee}}} \int_{t_0}^{t_f} \text{Tr}(t) \cdot \text{flow}(t) dt \quad (22)$$

Depending upon the signal to noise ratio, the average value of Tr over the last 250 mL can be used for Tr_{ee} . Furthermore, when $\text{flow}(t)$ is positive during inhalation of dry test gas, it is adjusted by $310/T \cdot P_B / (P_B - 47)$ where T is the ambient temperature. All measurements of tracer gas are assumed to be made with the water vapour pressure in the sample line equilibrated to the water vapour pressure in room air.

The absolute lung volume at any time t , $V(t)$, during the manoeuvre can then be described by equations 23 and 24. The integral of $\text{flow}(t)dt$ from time t_0 to time t_f is the net change in total volume over the entire manoeuvre and will be zero if the inhaled volume, V_I , equals the exhaled volume, V_E . The integral of $\text{flow}(t)dt$ from time t_0 to time t is the net volume change at any time t . Hence, at the end of the single breath manoeuvre, $V(t_f)$ is simply equal to $V_{\text{ee}} - V_{\text{Dequip}}$.

$$V(t) = V_{\text{ee}} + \int_{t_0}^t \text{flow}(t) dt - \int_{t_0}^{t_f} \text{flow}(t) dt - V_{\text{Dequip}} \quad (23)$$

$$V(t) = V_{\text{ee}} - \int_t^{t_f} \text{flow}(t) dt - V_{\text{Dequip}} \quad (24)$$

If the tracer gas has not been completely washed out from a previous $DLCO$ manoeuvre, then the residual alveolar tracer gas concentration (Tr_R) measured just prior to the inhalation of test gas must be considered in the mass-balance equation and V_{ee} is duly described by equation 25.

$$V_{\text{ee}} = \frac{1}{(\text{Tr}_{\text{ee}} - \text{Tr}_R)} \int_{t_0}^{t_f} (\text{Tr}(t) - \text{Tr}_R) \cdot \text{flow}(t) dt \quad (25)$$

The value of V_A to be reported in BTPS conditions is described by equation 26. This value is converted to STPD conditions for use in equation 8 or 9.

$$V_A = V_E + V_{ee} - V_{\text{Danat}} - V_{\text{Dequip}} \quad (26)$$

This method has been compared to plethysmography in normal subjects and in patients with lung disease with various breath-hold times [71, 96]. For normal subjects, there is little difference in $DLCO$ when using either method to measure V_A ; however, V_A measured by the RGA method is significantly higher than V_A measured by the classical method in subjects with COPD or uncontrolled asthma. The resulting $DLCO$ measurements in COPD cases are some 8 to 15% higher. Since reference values for $DLCO$ are developed using normal subjects, existing reference values continue to be applicable using V_A measured by the RGA method. For subjects with COPD the effect of using the RGA V_A value will, in isolation, be to calculate an increased $DLCO$ value. However, the V_A measured from a discrete sample will vary with the sample volume and sample timing [72] such that using the RGA V_A value should improve reproducibility of $DLCO$ in these subjects.

Another significant advantage of calculating absolute lung volume at end-exhalation instead of at maximal inhalation is that the impact of errors due to the assumption of complete gas mixing in the lung is reduced. For example, in a patient with a TLC of 7 L and a RV of 2 L, a 10% error in TLC (700 mL) translates into a 10% error in $DLCO$. However, a 10% error in RV would be 200 mL and when VC is added to RV the volume error at TLC is only 2.9% which translates into only a 2.9% error in $DLCO$.

During the transition from classical systems to RGA systems, some laboratories may wish to report $DLCO$ values using the 2005 ATS/ERS method in combination with those obtained using RGA V_A for comparative purposes. RGA V_A values may alter $DLCO$ in some older normal subjects, who have more heterogeneous distribution of ventilation due to the normal ageing process, and therefore might yield slightly higher $DLCO$ values compared to current classically derived reference values. As with any set of reference values, which must be validated in each laboratory, $DLCO$ values using V_A must be validated using a group of normal, healthy subjects. The Global Lung Function Initiative is in the process of developing all-age predicted values using datasets submitted from 12 countries (www.lungfunction.org).

Inspired gas conditions

In most cases, the test gas inspired from a bag or a compressed gas cylinder with a demand valve is a dry gas and, as such, is considered to be under ATPD conditions. The inspired volume needs to be converted into BTPS conditions for use in equations 10 and 11. It is recommended that the V_I (BTPS) be reported and that manufacturers should specify and document inspired gas conditions for each instrument. Since gas cooling can occur due to decompression through the delivery valve, manufacturers are required to measure the test gas temperature at the pneumotachometer in a typical system in their testing laboratory and provide appropriate compensation for gas cooling if necessary.

Carbon dioxide, water vapour and temperature adjustment for alveolar volume calculations

Exhaled gas contains carbon dioxide and water vapour which were not present in the test gas mixture. As noted previously, some systems remove one or both of these if they interfere with analyser function, raising both carbon monoxide and tracer gas concentrations. Under these circumstances, adjustments are required for the increase in F_{ATr} used to calculate V_A . However, no adjustment for the increase in alveolar inspired carbon monoxide and tracer gas fractions at time t ($F_{ACO,t}$ and $F_{ATr,t}$) is necessary in calculating the rate of carbon monoxide uptake, since the concentration factor appears in both the numerator and the denominator of the expression ($F_{ATr,t}/F_{ACO,t}$) and the effect therefore cancels itself out. Exhaled gas is initially at body temperature and some systems allow this to cool, such that the gas volume contracts, whereas others will provide heat to maintain the temperature. As such, adjustments to BTPS conditions may be required depending upon the system design. All of these adjustments must be documented by the manufacturer for their particular system. The conversion factors used to modify calculations in $DLCO$ manoeuvres are shown in equations 8, 9 and 12–19.

RGA signal alignment

To properly analyse continuous gas samples, the gas concentration signals from the analysers must be properly aligned with the flow signal from the pneumotachometer (figure 2). The first step is to shift the concentration signal ahead in time to compensate for the lag time (the time required for the gas to travel from the aspiration port to the analyser chamber). The lag time is a function of the length and diameter of the tubing and the analyser aspiration rate. The length of the tubing should be minimised to prevent mixing of the aspirated sample within the sampling tube, which can blunt the response time through a process of Taylor dispersion. The amount of mixing will also depend on the configuration of the sampling

circuit, including any valves and junctions, which can create turbulence. It should also be noted that lag time can vary with gas viscosity and, when helium is used as the tracer gas, this may require dynamic compensation during exhalation.

An additional shift of each gas concentration signal relative to the flow signal is also required to compensate for the response time of the analyser. This can be accomplished using an optimal shift equal to the natural logarithm of twice the time constant of the analyser response [97]. Alternatively, alignment may be achieved by other signal processing strategies such as cross-correlation techniques (convolution of signals).

For a more accurate *DLCO* calculation, a third shift equal to the dead-space transit time may be used to translate the gas concentrations measured at the mouth to the gas concentrations in the alveolar space. During inhalation, the gas sampled at the aspiration port will not reach the gas-exchanging alveolar space until at least one dead-space transit time later and, similarly, the gas sampled at the aspiration port during exhalation is gas that was in the alveolar space one dead-space transit time previously. If a system uses this further correction the effective breath-hold time in the alveolar space will be reduced (typically by 0.05–0.15 s) and *DLCO* will be increased (typically by 0.5–1.5%).

Interpolation between data points may be required to achieve optimal shifting of the gas concentration data, particularly if lower digitisation rates are used. To reduce errors introduced by interpolation, a sample rate of 1000 Hz per channel is recommended.

Transfer coefficient of the lung for carbon monoxide

The logarithmic change in carbon monoxide concentration during the breath-hold phase of the single-breath manoeuvre, divided by t_{BH} and the P_B of the dry gas is termed K_{CO} . This is equivalent to the left hand side of equation 5 without the V_A term and, conceptually, *DLCO* is thus equivalent to $V_A \cdot K_{CO}$. The specific calculations for K_{CO} are shown below as equations 27 and 28; however, if values are required in SI units, it is necessary to convert 1000 mL(STPD) to mmol as shown in equations 29 and 30.

$$K_{CO} = \ln\left(\frac{F_{ICO}}{F_{ACO}} \cdot \frac{F_{ATr}}{F_{ITr}}\right) \cdot \frac{1}{t_{BH}/60 \cdot (P_B - P_{H_2O})} \cdot \frac{1000 \text{ mL}}{1 \text{ L}} \cdot \frac{273}{310} \cdot \frac{P_B - P_{H_2O}}{760} \quad (27)$$

$$K_{CO} = \ln\left(\frac{F_{ICO}}{F_{ACO}} \cdot \frac{F_{ATr}}{F_{ITr}}\right) \cdot \frac{1}{t_{BH}} \cdot 69.52 \quad (28)$$

$$K_{CO} = \ln\left(\frac{F_{ICO}}{F_{ACO}} \cdot \frac{F_{ATr}}{F_{ITr}}\right) \cdot \frac{1}{t_{BH}/60 \cdot (P_B - P_{H_2O})} \cdot \frac{1000 \text{ mL}}{1 \text{ L}} \cdot \frac{273}{310} \cdot \frac{P_B - P_{H_2O}}{101.3} \cdot \frac{1 \text{ mmol}}{22.4 \text{ mL}} \quad (29)$$

$$K_{CO} = \ln\left(\frac{F_{ICO}}{F_{ACO}} \cdot \frac{F_{ATr}}{F_{ITr}}\right) \cdot \frac{1}{t_{BH}} \cdot 23.29 \quad (30)$$

It should be noted that the calculation of K_{CO} is completely independent of the gas flow, lung volume and barometric pressure measured during the manoeuvre. Although the units of the logarithmic change in carbon monoxide concentration per unit time and per unit pressure are $\text{min}^{-1} \cdot \text{mmHg}^{-1}$ (or $\text{min}^{-1} \cdot \text{kPa}^{-1}$), K_{CO} is expressed in units of $\text{mL}(\text{STPD}) \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1} \cdot \text{L}(\text{BTPS})^{-1}$ or $\text{mmol} \cdot \text{min}^{-1} \cdot \text{kPa}^{-1} \cdot \text{L}(\text{BTPS})^{-1}$ only because the basic measurement of the logarithmic change in gas concentration over time is multiplied by 1000 mL(STPD) and then divided by 1 L(BTPS) which changes the magnitude of the value of K_{CO} by 1000 times the BTPS to STPD factor [98].

As V_A is not a component of K_{CO} , some users prefer to use K_{CO} as it eliminates the uncertainty in measurement of V_A from the assessment of carbon monoxide uptake. This uncertainty arises from the assumption that F_{ATr} , as measured from the exhaled gas sample, is representative of the entire lung. However, this same assumption is used to estimate the alveolar carbon monoxide concentration at the start of breath-holding and K_{CO} measurement is thus subject to the same uncertainty [99].

Mathematically, K_{CO} can be calculated as $DLCO/V_{ABTPS}$. However, K_{CO} should not be reported using the term $DLCO/V_A$, as it may be inferred from this term that $DLCO$ can be corrected or normalised for V_A . In fact, the relationship between lung volume and carbon monoxide uptake is complex and studies evaluating the effects of reduced V_I (and thus V_A) show the relationship to be a linear and certainly less than 1:1

(i.e. the fall in $DLCO$ is far less than the fall in lung volume) [20, 21, 98, 100, 101]. This likely reflects the fact that alveolar folding–unfolding and capillary volume changes resulting from lung volume changes do not translate into concomitant and equal changes in $DLCO$. Thus, while the KCO calculation might add insight into carbon monoxide uptake properties of the lung [98], it cannot be used as a simple technique to normalise $DLCO$ for volume.

Optional calculations

Separate equations for inhalation, breath-hold and exhalation

When KROGH [1] developed the measurement of the “diffusion constant” in 1915, she had to design a manoeuvre that would be compatible with an analytical solution of the equation for gas transfer. She achieved this by simulating a pure breath-hold manoeuvre and the measurement of $DLCO$ today continues to be constrained by having the patient perform a rapid inhalation, 10 s of breath-holding and a rapid exhalation. Deviations from a pure breath-hold manoeuvre cause errors in $DLCO$ because the Krogh equation is only valid for this case [68, 72].

The gas transfer equation can also be written for inhalation and exhalation, both of which become equivalent to the breath-holding equation at zero flow. Using data from the continuous monitoring of flow and the concentrations of carbon monoxide and tracer gas throughout the manoeuvre, an algorithm can be used to calculate $DLCO$ by numerical methods [53]. This method accounts analytically for the times of inhalation and exhalation, gives values of $DLCO$ that are not dependent on how rapidly the manoeuvre is performed and returns a fixed value of $DLCO$ over the entire manoeuvre that accounts for the observed uptake of carbon monoxide. All of the exhaled gas data can be used to provide a more representative measurement of $DLCO$ for the whole lung rather than calculating $DLCO$ from a small alveolar gas sample.

Using the “three-equation method” [53] in young, healthy subjects, the standardised manoeuvre gives the same values for $DLCO$ as the ATS standardised manoeuvre. However, when these same subjects perform the manoeuvre with slower flows and/or shorter breath-hold times, similar to those seen in patients with airflow obstruction, the three-equation method gives unchanged $DLCO$ values while the ATS standardised method yields significantly higher $DLCO$ values [68]. KROGH [1] designed her experiment for normal subjects and not for patients with lung disease. The standardised manoeuvre penalises lung disease patients, who cannot perform it adequately; however, with RGA instrumentation it is no longer necessary to use an arduous, demanding manoeuvre to measure $DLCO$.

Indices of heterogeneity of ventilation and gas transfer

As noted above, the heterogeneity of ventilation affects $DLCO$ measurement [102, 103]. Gas concentration data from RGA systems can be used to calculate indices of ventilation nonuniformity, such as the slope of phase III of the alveolar plateau [90, 104]. However, such indices need to be normalised to account for the differences in lung volume and the differences in RV/TLC that occur from person to person [79]. Other measures of mixing efficiency may be calculated from the tracer gas data [53].

Disease processes can also affect the distribution of gas transfer in the lung. Using the three-equation method, the observed decay in carbon monoxide during exhalation can be compared to the carbon monoxide decay that would be predicted for a homogeneous lung in which diffusion occurs uniformly. An index of $DLCO$ heterogeneity has been developed that is capable of distinguishing smokers with normal lung function and normal $DLCO$ from a control group of nonsmokers [105].

Evaluating the measurement of $DLCO$

Acceptability, repeatability and quality control

Acceptable manoeuvres are defined in table 3. The volume–time graph should show a smooth, sharp rise in volume, followed by a stable breath-hold and a smooth, sharp exhalation (figure 3). The gas concentration graph should show a very sharp rise when test gas is introduced and remain stable until exhalation followed by an initial rapid decline with a smooth transition to phase III. Variations from this pattern will indicate leaks. The V_I of test gas must be at least 90% of the largest VC measured in the same pulmonary function testing session. At least 85% of test gas V_I must be inhaled within <4 s. There must be no evidence of a Müller or Valsalva manoeuvre during the breath-hold period. Alveolar sample collection must be completed within 4 s. The calculated breath-hold time must be 10 ± 2 s. For RGA systems, virtual sample collection should be initiated after the completion of dead-space washout. A manoeuvre with a $V_I/VC < 90\%$ but $\geq 85\%$ may be deemed acceptable if the V_A is within 200 mL or 5% (whichever is greater) of the largest V_A from other acceptable manoeuvres.

Repeatability describes intra-session variability on repeated testing when there is no change in test conditions [106, 107]. In a large university-based laboratory study, the coefficient of variation for repeated measurement in normal subjects was 3.1% and this increased only slightly (from 4.0 to 4.4%) in patients

TABLE 3 Acceptability, repeatability and quality control in DL_{CO} testing**Criteria for acceptability**

A $V_I \geq 90\%$ of the largest VC in the same test session; alternatively a $V_I \geq 85\%$ of the largest VC in the same test session and V_A within 200 mL or 5% (whichever is greater) of the largest V_A from other acceptable manoeuvres
 85% of test gas V_I inhaled in <4 s
 A stable calculated breath-hold for 10 ± 2 s with no evidence of leaks or Valsalva/Müller manoeuvres during this time
 Sample collection completed within 4 s of the start of exhalation. For RGA systems, virtual sample collection should be initiated after dead-space washout is complete

Criteria for repeatability

At least two acceptable DL_{CO} measurements within $2 \text{ mL} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$ [$0.67 \text{ mmol} \cdot \text{min}^{-1} \cdot \text{kPa}^{-1}$] of each other

Score	Quality control grading [#]		
	V_I/VC	t_{BH}	Sample collection
A	$\geq 90\%$ [¶]	8–12 s	≤ 4 s
B	$\geq 85\%$	8–12 s	≤ 4 s
C	$\geq 80\%$	8–12 s	≤ 5 s
D	$\leq 80\%$	<8 or >12 s	≤ 5 s
F	$\leq 80\%$	<8 or >12 s	>5 s

V_I : inspired volume; VC: vital capacity; V_A : alveolar volume; t_{BH} : breath-hold time; DL_{CO} : diffusing capacity of the lung for carbon monoxide. [#]: only grade A manoeuvres meet all acceptability criteria. The average DL_{CO} values from two or more grade A manoeuvres that meet the repeatability criterion should be reported. If only one grade A manoeuvre is attained, the DL_{CO} value from that manoeuvre should be reported. If no grade A manoeuvre is obtained, manoeuvres of grades B to D might still have clinical utility. The average of such manoeuvres should be reported but these deviations from the acceptability criteria must be noted to caution the interpreter of the test results. Manoeuvres of grade F are not useable. [¶]: or $V_I/VC \geq 85\%$ and V_A within 200 mL or 5% (whichever is greater) of the largest V_A from another acceptable manoeuvre.

with abnormal spirometry patterns [108]. Studies conducted prior to the publication of the 2005 standards found DL_{CO} variability of up to 9% (reproducibility) in normal individuals in repeated measurement over a period of 1 year [109] and coefficients of variation ranged from 6.2% to 12% in selected UK regions [110].

Repeatability requirement: there must be at least two acceptable manoeuvres that are within $2 \text{ mL} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$ ($0.67 \text{ mmol} \cdot \text{min}^{-1} \cdot \text{kPa}^{-1}$) of each other. A study of 4797 test sessions found that 95.5% of cases met this criterion [67]. Since most intra-session variability is technical rather than physiological, the mean of acceptable manoeuvres is reported. The average of at least two acceptable manoeuvres that meet the repeatability requirement must be reported (*i.e.* outliers excluded). While it is recommended that at least two acceptable DL_{CO} manoeuvres must be performed, research is needed to determine the actual number of manoeuvres required to provide a reasonable estimate of average DL_{CO} for a given person. As noted elsewhere, five manoeuvres will result in an increase of $\sim 3.5\%$ COHb from baseline [66, 82], which will decrease the measured DL_{CO} by $\sim 3\text{--}3.5\%$. Thus, conducting more than five manoeuvres is not a recommended strategy.

There are no quality control grading systems that have been validated using the new standards contained in this document. Until such validation is published, an interim grading system is provided in table 3 and further research is recommended to develop and validate a DL_{CO} grading system.

A grade A manoeuvre meets all acceptability criteria. The average DL_{CO} from two or more grade A manoeuvres that are repeatable (*i.e.* are within $2 \text{ mL} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$ or $0.67 \text{ mmol} \cdot \text{min}^{-1} \cdot \text{kPa}^{-1}$ of each other) should be reported. If, after repeat testing, the operator is unable to obtain two repeatable grade A manoeuvres, then the following values are reported with a caution to the interpreter that the testing session was suboptimal: 1) If two or more grade A manoeuvres that are not repeatable are obtained, then the average DL_{CO} value from the acceptable manoeuvres is reported. 2) If only one grade A manoeuvre is obtained, then the DL_{CO} value from that manoeuvre is reported. 3) If no acceptable manoeuvres are obtained, then the average DL_{CO} value of the manoeuvres with grades B, C or D is reported. 4) If only grade F manoeuvres are obtained, then no DL_{CO} value is reported.

Adjustments to the predicted value of DLCO prior to interpretation

The value of $DLCO$ depends upon a number of physiological factors. Besides varying with age, sex, height and possibly ethnicity, $DLCO$ also changes with Hb, lung volume, COHb, P_{IO_2} (inspired oxygen tension, e.g. altitude), exercise and body position. Although these effects may cause changes in $DLCO$ in opposite directions [111], all should be considered in interpreting the observed carbon monoxide uptake. It is recommended that adjustments for these factors be made in the predicted rather than the measured $DLCO$ value. The predicted $DLCO$ value is derived from measurements in normal subjects who are disease free, have normal Hb levels and minimal COHb, are sitting at rest, and breathing room air. If any of these conditions are not met, then the predicted value should be adjusted accordingly.

Adjustment for haemoglobin

Since carbon monoxide–Hb binding is such an important factor in carbon monoxide transfer, $DLCO$ changes can be substantial as a function of Hb concentration [111–115]. The empirical effect upon $DLCO$ with change in Hb closely matches what is expected from a theoretical approach using the relationship in equation 3, with θ assumed to be proportional to Hb, $DM/\theta VC$ assumed to be 0.7 [113] and the “standard” Hb value assumed to be $14.6 \text{ g}\cdot\text{dL}^{-1}$ ($9 \text{ mmol}\cdot\text{L}^{-1}$) in adult and adolescent males and $13.4 \text{ g}\cdot\text{dL}^{-1}$ ($8.26 \text{ mmol}\cdot\text{L}^{-1}$) in adult females and children <15 years old. Using these relationships and expressing Hb in $\text{g}\cdot\text{dL}^{-1}$, the predicted $DLCO$ in adolescents and adult males can be adjusted using equation 31, while that in children <15 years of age and females is adjusted using equation 32. Results from a more recent study in patients with a wide range of Hb abnormalities [115] show a slightly greater and more linear relationship; however, corrected values are generally consistent with equations 31 and 32.

$$D_{LCO}[\text{predicted for Hb}] = D_{LCO}[\text{predicted}] \cdot (1.7\text{Hb}/(10.22 + \text{Hb})) \quad (31)$$

$$D_{LCO}[\text{predicted for Hb}] = D_{LCO}[\text{predicted}] \cdot (1.7\text{Hb}/(9.38 + \text{Hb})) \quad (32)$$

The measurement of Hb in the American population [116] found deviation from these standard values, especial in males, children and seniors; differences were also found between Caucasian and African-Americans. Furthermore, the survey found that Hb levels in the general population are changing over time. If a more appropriate reference Hb level (Hb_{ref}) is available then the predicted $DLCO$ is adjusted using equation 33.

$$D_{LCO}[\text{predicted for Hb}] = D_{LCO}[\text{predicted}] \cdot (1.7\text{Hb}/(0.7\text{Hb}_{\text{ref}} + \text{Hb})) \quad (33)$$

Adjustments for alveolar oxygen tension

As noted previously, PAO_2 affects the measurement of $DLCO$ and changes to PAO_2 (such as supplemental oxygen breathing that gives higher PAO_2 values) will have a correlating effect on $DLCO$ values. The value of $DLCO$ will change by $\sim 0.35\%$ for each 1 mmHg change in PAO_2 or $\sim 2.6\%$ for each 1 kPa change in PAO_2 [117, 118]. Adjustments to the predicted $DLCO$ in a subject on supplemental oxygen may be made using a measured PAO_2 and assuming a normal PAO_2 breathing room air at sea level of 100 mmHg (13.3 kPa). This is shown in equation 34 below or equation 35 if SI units are preferred.

$$D_{LCO}[\text{predicted for elevated } P_{AO_2}] \approx D_{LCO}[\text{predicted}]/(1.0 + 0.0035(P_{AO_2} - 100)) \quad (34)$$

$$T_{LCO}[\text{predicted adjusted for } P_{AO_2}] \approx T_{LCO}[\text{predicted}]/(1.0 + 0.026(P_{AO_2} - 13.3)) \quad (35)$$

Some pulmonary function systems include measurement of carbon dioxide. In these systems, the end-expiratory carbon dioxide concentration can be used to estimate the alveolar oxygen partial pressure using the simplified alveolar gas equation. In patients who have higher carbon dioxide levels (higher P_{ACO_2}) and consequently lower PAO_2 values, the predicted $DLCO$ can be corrected to compensate for the increase in $DLCO$ that arises. For example, at a barometric pressure of 760 mmHg (101.3 kPa), if the P_{ACO_2} in a patient retaining carbon dioxide was 50 mmHg (6.67 kPa) then the PAO_2 would be 86 mmHg (11.5 kPa) and the predicted $DLCO$ would be 4.8% higher than if the P_{ACO_2} were 40 mmHg (5.33 kPa). However, there are many assumptions inherent in this approach and more research is needed to determine the validity of such an adjustment.

Adjustment for carboxyhaemoglobin concentration and carbon monoxide back-pressure

As noted previously, COHb can affect the measured uptake of carbon monoxide in the following two ways [119–121]. First, by occupying Hb binding sites, carbon monoxide produces an “anaemia effect”. Secondly, carbon monoxide partial pressure in the blood will reduce the driving pressure for carbon monoxide transport from alveolar gas to capillary blood. Exposure to ordinary environmental carbon monoxide and endogenous production of carbon monoxide as a byproduct of Hb catabolism commonly results in measured COHb levels of 1–2% [119]. However, cigarette smoke and other environmental sources can produce measurable levels of carbon monoxide back-pressure and COHb that may need to be considered in the measurement of carbon monoxide uptake [119].

The inhalation of carbon monoxide in the single-breath manoeuvre causes COHb to increase by 0.6–0.7% for each manoeuvre [66, 82]. The adjustment of the predicted $DLCO$ value for carbon monoxide was found to be -0.938% per 1.0% increase in COHb [122]. For RGA systems, carbon monoxide back-pressure can be measured in expired gas prior to the inspiration of test gas in the $DLCO$ manoeuvre [62] and can be compensated for analytically. For classical systems, carbon monoxide back-pressure can be estimated using one of several available techniques [121, 123–125]. For example, carbon monoxide back-pressure can be calculated from COHb using equation 36, where COHb and O_2Hb are the fractions of carbon monoxide and oxygen-bound haemoglobin, respectively.

$$F_{ACO} = (COHb/O_2Hb) \cdot (F_{AO_2})/210 \quad (36)$$

$DLCO$ can then be recalculated after subtracting the estimated carbon monoxide back-pressure from both the initial and final alveolar carbon monoxide partial pressures (units must be consistent before making the subtraction). Unfortunately, this method will not adjust $DLCO$ for the “anaemia effect” of COHb; however, several studies have evaluated both the empirical and theoretical effects of COHb on $DLCO$ and incorporated both the back-pressure and the “anaemia effect” of COHb into the adjustment. In general, a 1% increase in COHb reduces the measured $DLCO$ by ~ 0.8 –1% from both effects [16, 17]. Using this approach, equation 37 empirically reduces predicted $DLCO$ by 1% for each percentage point of COHb $> 2\%$.

$$D_{LCO}[\text{predicted for COHb}] = D_{LCO}[\text{predicted}] \cdot (102\% - COHb\%) \quad (37)$$

A more recent study using an RGA system to measure alveolar carbon monoxide concentration, combined with venous measurement of COHb, found that the effect of carbon monoxide back-pressure and the “anaemia effect” are almost equal and the combined effect is a 2% decrease in $DLCO$ for each 1% increase in COHb [62]. These findings were verified in a discrete sample system [66]. In these studies, where the carbon monoxide back-pressure was measured and used in the calculation of $DLCO$, equation 38 was used to further correct for the “anaemia effect” where F_{ACOb} is the alveolar carbon monoxide fraction in ppm measured at the end of exhalation to residual volume, just prior to the inhalation of test gas.

$$D_{LCO}[\text{corrected}] = D_{LCO} \cdot (1 + F_{ACOb}/560) \quad (38)$$

As endogenous COHb (1–2%) was present in the healthy nonsmoking subjects from whom prediction equations were generated, an adjustment for COHb is only recommended for interpretative purposes when COHb levels are known to be elevated or levels above 2% are suspected. Methaemoglobin (MetHb) will not bind carbon monoxide meaning there is, effectively, a reduced amount of haemoglobin available and leading to a similar “anaemia effect”. Since there is effectively less Hb to bind with carbon monoxide during the $DLCO$ manoeuvre, the measured $DLCO$ is reduced. An adjustment for MetHb has been proposed [126] and is shown in equation 39.

$$Hb[\text{adjusted}] = Hb \cdot (100 - MetHb/100) \quad (39)$$

Adjustment of DLco for barometric pressure

For factors such as Hb that are related to the individual subject, the recommended adjustment is made to the predicted $DLCO$ value. However, barometric pressure (P_B) is an environmental factor that is

independent of the individual and therefore the adjustment should be made to the measured $DLCO$ value to simulate standard pressure conditions. The variation in $DLCO$ due to the typical range in high and low pressure cells at a given altitude is approximately $\pm 1.5\%$. P_B decreases with altitude (such that P_{IO_2} decreases) and $DLCO$ increases by about 0.53% for each 100 m of increase in altitude. Moreover, the applicability of using a reference value data set from a different location is improved if both the measured $DLCO$ and the predicted value of $DLCO$ are adjusted to standard pressure (760 mmHg or 101.3 kPa). The adjustment for P_B [4, 117] assumes a P_{IO_2} of 150 mmHg (20 kPa) at standard pressure and can be calculated using equations 40 (P_B in mmHg) and 41 (P_B in kPa).

$$D_{LCO}[P_B \text{ adjusted}] \approx D_{LCO}(0.505 + 0.00065 P_B) \quad (40)$$

$$D_{LCO}[P_B \text{ adjusted}] \approx D_{LCO}(0.505 + 0.00488 P_B) \quad (41)$$

For $DLCO$ reference values that do not provide P_B data, the altitude of the centre in which the reference values were obtained can be used to estimate P_B [127] using equations 42 and 43 where a is the altitude above sea level in metres. It should be noted that the relationship between $DLCO$ and P_B has not been confirmed using an RGA system. Further research is needed to validate the use of equations 40 and 41.

$$P_B[\text{mmHg}] = 760 (1 - 2.25577 \cdot 10^{-5} \cdot a)^{5.25588} \quad (42)$$

$$P_B[\text{kPa}] = 101.325 (1 - 2.25577 \cdot 10^{-5} \cdot a)^{5.25588} \quad (43)$$

Reporting values

This document is intended to establish technical standards which, in terms of reporting, will require $DLCO$ systems to be able to report the variables listed in table 4. It is not intended to specify which variables end users should include in the report forms used in their laboratories, nor is it intended to address the interpretation of $DLCO$. Although work is ongoing towards establishing a standardised pulmonary function laboratory report form, there is no current standard. A $DLCO$ system must be able to report the unadjusted measured $DLCO$, the $DLCO$ adjusted for P_B , the lower limit of normal and z-score, predicted, and percentage of predicted $DLCO$, KCO , the lower limit of normal and z-score, predicted, and percentage of predicted KCO . Any adjustments (e.g. for Hb, COHb, P_{IO_2} or lung volume) must also be reported along with the data used to make them. The average V_A must be reported along with the predicted V_A (the predicted TLC minus the predicted V_D) and percentage of predicted V_A . If available, a separately measured TLC and V_A/TLC ratio may be reported, although this is optional. The average V_I must also be noted. If a separately measured VC is available, it must be reported to serve as a reference for the adequacy of V_I . In addition, comments relevant to the quality of the measurements recorded must be included. A complete list of specifications for which variables and measurements that $DLCO$ systems are able to report is given in table 4. While the use of z-scores is favoured in the interpretation of pulmonary function results, given the continuing use of “percentage of predicted” values in many laboratories, the ability to report both z-scores and percentage of predicted values is recommended.

Specifications for reports and output of results

Although standardised reporting forms are being considered, manufacturers have diverse options for reporting of results. This is due, in a large measure, to the insistence of various pulmonary function laboratories on having a customised report that matches their historic format. A common option for accommodating electronic medical records, is the provision of a pdf document; however, a universal format for data output in the form of a csv or xml formatted data file has been proposed. This format should include the results and demographic/environmental data for each test in a format that will permit the user to import data to and their own reporting format export to their particular electronic record. The data file must include all data necessary to calculate the variables listed in table 4. For RGA systems, the data arrays for flow, carbon monoxide and tracer gas must be included and must be adjusted for auto-zero and calibration factors, with the optimal shift applied to the concentration data. Flow data must be converted to BTPS conditions and the data must include the equipment dead-space, the washout volume, the alveolar sample volume, the barometric pressure and the carbon monoxide and tracer gas concentrations at end-exhalation prior to the inhalation of test gas. Manufacturers must provide the format details to permit users to import the data. The complete specifications for the data file are given in the supplementary materials.

TABLE 4 DLCO reporting requirements

Variable [#]	Requirement
DLCO (unadjusted)	Required
DLCO (adjusted for P_B)	Required
DLCO (LLN and/or z-score)	Required
DLCO (predicted)	Required
DLCO (adjusted,predicted)	Optional (required if any adjustments made-specify adjustments)
DLCO (% of predicted)	Required
V_A (BTPS)	Required
V_A (LLN and/or z-score)	Required
V_A (% of predicted)	Optional
K_{CO}	Required
K_{CO} (LLN and/or z-score)	Required
K_{CO} (predicted)	Required
K_{CO} (% of predicted)	Required
P_B	Required
t_{BH}	Required
V_I (BTPS)	Required
Fowler (anatomic) dead-space	Required for RGA systems
TLC_{sb}	Required for RGA systems
Reference values source	Required
Test quality grade	Recommended (include % variability in DLCO acceptable manoeuvres)
Operator comments	Required (number of manoeuvres, number of acceptable manoeuvres)
Graphs	Required (full manoeuvre and exhaled gas concentration <i>versus</i> volume with sample collection indicated for RGA systems)
Hb	Optional (required if used to adjust DLCO)
COHb	Optional (required if used to adjust DLCO)
Alternative calculations (e.g. three-equation DLCO, normalised slope of phase III)	Optional

BTPS: body temperature, ambient pressure, saturated with water vapour; LLN: lower limit of normal; DLCO: diffusing capacity of the lung for carbon monoxide; V_A: alveolar volume; K_{CO}: transfer coefficient of the lung for carbon monoxide; P_B: barometric pressure; t_{BH}: breath-hold time; V_I (BTPS): inspired volume under BTPS conditions; V_A (BTPS): alveolar volume under BTPS conditions; TLC_{sb}: single-breath total lung capacity; Hb: haemoglobin; COHb: carboxyhaemoglobin; RGA: rapidly responding gas analyser. [#]: for DLCO, V_A, K_{CO}, t_{BH}, V_I, V_{Danat} and TLC_{sb} the average values from the acceptable and repeatable manoeuvres are reported.

Reference values

The Global Lung Health Initiative (GLI) is currently working on the development of global reference values for DLCO which will very likely be in a similar structure to the GLI spirometry reference values [128]. Implementation of these reference values requires more complexity than simply inserting coefficients for polynomials and a DLCO system must be able to implement this method of calculating reference values. A list of reference values for DLCO developed using methods that adhere to the 2005 DLCO standards is provided in table 5 [4].

Summary

It is not the intention of the new standards to render older equipment or instrumentation with alveolar sample chambers or bags, that is still in current use, obsolete. The 2005 ATS/ERS standards address this type of instrumentation. It is recognised that some equipment which meets the 2005 standards will continue to be used but the expectation is that new equipment will meet or exceed the new standards. Some of the systems currently available will be able to meet the new standards with software upgrades.

As already noted, the changes in DLCO standards will not impact the applicability of reference values. In general, pulmonary function measurements are more accurate and precise in normal, healthy subjects than in patients with lung disease so that changes which improve the measurement of DLCO will have less impact on normal, healthy subjects, which favours the continued applicability of reference values derived using older systems. There are already systematic differences among reference value sets for DLCO which are related to the equipment and methodology which impact their applicability. Some reference values

TABLE 5 Reference values for *DLCO* from studies that complied with the 2005 American Thoracic Society/European Respiratory Society *DLCO* standards

Author [#]	Year	Country	Age	Subjects
Thompson [129]	2008	Australia	45–71 years	498 male/474 female
Koopman [†] [130]	2011	Netherlands	7–18 years	278 male/265 female
Garcia-Rio [†] [131]	2012	Spain	65–85 years	169 male/262 female
Kim [132]	2012	USA and Australia	5–19 years	225 male/254 female
Thomas [133]	2014	Denmark	5–17 years	male/female (297 total)
Michailopoulos [134]	2015	Greece	18–91 years	234 male/233 female
Verbanck [135]	2016	Belgium	20–80 years	128 male/124 female

DLCO: diffusing capacity of the lung for carbon monoxide. [#]: only studies with at least 100 males and 100 females are included. All of these reference values were derived using caucasian subjects. [†]: test gas contained 19% oxygen (all other studies used test gas with 21% oxygen).

currently in use were developed prior to the publication of the 2005 ATS/ERS standards [4]. Hence, there is already a pressing need for reliable, comprehensive reference values for *DLCO*.

Advances in technology have outpaced guidelines and standards. These revisions to the *DLCO* standards are required to make optimal use of existing, clinically available technology. Guidelines and standards should not constrain progress in the improvement of pulmonary function measurements but should serve to continually improve the quality of *DLCO* measurements.

Recommendations for research directions

In the course of developing these technical standards, the following areas were identified as candidates for research studies to fill knowledge gaps and provide more specific guidelines.

- 1) Conduct studies of *DLCO* in normal, healthy subjects in ethnicities other than Caucasian over a wide age range to either validate the use of Caucasian reference values or develop ethnicity-specific reference values.
- 2) Develop a standardised common report form for pulmonary function testing that can be the default for all laboratories and electronic medical record systems.
- 3) Determine the effect of barometric pressure on *DLCO* in normal subjects and COPD patients over a range of pressures reflecting altitudes from sea level to 2500 m to either confirm or replace equations 40 and 41.
- 4) Determine the effects of obesity on $V_{D_{an}}$, TLC_{sb} and *DLCO*.
- 5) Determine whether Hb measured by skin prick or venipuncture is more appropriate for *DLCO* adjustment and conduct studies to confirm or revise the relationship between Hb and *DLCO* expressed in equations 31 and 32.
- 6) Determine normal Hb levels in populations of different ethnicity and geographical location.
- 7) Test the proposed *DLCO* grading scale in large clinical databases for both classical and RGA *DLCO* systems.
- 8) Determine the impact of carbon dioxide retention on *DLCO* measurements.
- 9) Determine the sensitivity and action levels for the assessment of gas analyser linearity using the dilution of test gas in a calibration syringe.
- 10) Determine the repeatability of calculating V_A and TLC_{sb} by the method in equation 26 which uses all of the tracer gas concentration data throughout the manoeuvre.

In addition to these research directions, there is also a need to update the guidelines for the interpretation of pulmonary function tests in general and of *DLCO* in particular.

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