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

Brian L. Graham[1], Vito Brusasco[2], Felip Burgos[3], Brendan G. Cooper[4], Robert Jensen[5], Adrian Kendrick[6], Neil R. MacIntyre[7], Bruce R. Thompson[8] and Jack Wanger[9]

Affiliations: [1]Division of Respirology, Critical Care and Sleep Medicine, University of Saskatchewan, Saskatoon, SK, Canada. [2]Dept of Internal Medicine, University of Genoa, Genoa, Italy. [3]Respiratory Diagnostic Center, Hospital Clínic, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain. [4]Lung Function and Sleep, Queen Elizabeth Hospital, University of Birmingham, Birmingham, UK. [5]Pulmonary Division, University of Utah, Salt Lake City, UT, USA. [6]Dept of Respiratory Medicine, Bristol Royal Infirmary, Bristol, UK. [7]Pulmonary, Allergy and Critical Care Medicine, Duke University Medical Center, Durham, NC, USA. [8]Allergy, Immunology and Respiratory Medicine, The Alfred Hospital and Monash University, Melbourne, Australia. [9]Consultant, Rochester, MN, USA.

Correspondence: Brian L. Graham, Division of Respirology, Critical Care and Sleep Medicine, University of Saskatchewan, Saskatoon, SK, Canada, S7N 0W8. E-mail: brian.graham@usask.ca

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Summary of updated technical standards for measuring *D*LCO (*T*LCO) including the use of rapid gas analyser systems http://ow.ly/TQVG305g3Zq

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ABSTRACT This document summarises 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. The full standards are also available online as https://doi.org/10.1183/13993003.00016-2016. The major changes in these technical standards relate to *D*LCO measurement with systems using rapidly responding gas analysers for carbon monoxide and the tracer gas, which are now the most common type of *D*LCO instrumentation being manufactured. Technical improvements and the increased capability afforded by these new systems permit enhanced measurement of *D*LCO and the opportunity to include other optional measures of lung function.

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This report was approved by the ATS Board of Directors in August 2016 and endorsed by the ERS Science Council and Executive Committee in September 2016. The full version of these standards is available as https://doi.org/10.1183/13993003.00016-2016.

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Background

A standardised clinical method to determine the diffusing capacity of the lung for carbon monoxide (D_{LCO}), using a tracer gas to determine both the alveolar volume and the alveolar concentration of carbon monoxide at the beginning of breath-holding, was described by Ogilvie *et al.* [1] in 1957. 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 systems and calculations to be "classical". The instrumentation for D_{LCO} measurement has advanced considerably since then, primarily through the use of rapidly responding gas analysers (RGAs) which 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 gas samples in a bag and discard most of the sampled gas data. However, there are several aspects of D_{LCO} measurement that can be improved markedly using all of the data from the continuous measurement technology.

The full technical standards [2], which are published online, are an update of the 2005 European Respiratory Society (ERS)/American Thoracic Society (ATS) standards [3] which, in turn, build upon previous standards [4, 5]. This update reflects the consensus opinions of both of these societies and is designed to: 1) provide an update to the standards required for D_{LCO} systems based on RGA development; 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 D_{LCO} systems can be upgraded and reengineered to meet these new standards for RGA systems. It is expected that as new D_{LCO} systems are designed and built, they will meet and, in many cases, exceed these new standards.

Methods

An application was submitted for a joint ERS/ATS task force to update the 2005 D_{LCO} standards [3] with a particular view to systems using RGAs. The task force co-chairs were approved by the ERS and the ATS while the 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 D_{LCO} 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 used to measure D_{LCO} were sent a survey requesting equipment specifications. Eight of the 13 manufacturers responded. A survey of D_{LCO} 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 a consensus of task force members in regard to the evidence available for various aspects of D_{LCO} measurement (as cited in the document) and reflect the expert opinion of the taskforce members for 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.

Gas analysers and general equipment

Equipment requirements

The equipment specifications and performance standards for D_{LCO} equipment are summarised in table 1.

Flow and volume analysers

Any error in measuring flow and subsequently calculating volume will produce a corresponding equal error in D_{LCO}. However, with the continuing improvement in flow measurement technologies, improved accuracy is being achieved. Flow measurement accuracy over the range −10 to +10 $L \cdot s^{-1}$ must be within ±2%. For calibration with a 3-L syringe (with a specified maximum error of ±0.5%, *i.e.* 2.985–3.015 L), the calibration volume must be within ±2.5% which is equivalent to an error tolerance of ⩽75 mL.

Gas analysers

Rather than collecting a physical sample of exhaled gas, nondispersive infrared carbon monoxide RGAs are used to construct a virtual gas sample from flow and gas concentration data. However, in the signal from such analysers there is both a lag time (the time taken for sampled gas to travel through the sampling tube to the analyser chamber) and an analyser response time (the time taken to reach 90% of the actual measurement from the time the gas sample reaches the analyser) to be considered (figure 1). The gas concentration signal must be precisely shifted in time to align with the flow signal.

TABLE 1 Equipment specifications and performance standards

D_{LCO} System	Specification	
	Required	**Recommended**
Rapid gas analyser systems		
Analyser specification		
0–90% response time (see figure 1)	⩽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 $L \cdot s^{-1}$	
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 (V_A) of 3±0.3 L and D_{LCO} of <0.5 $mL \cdot min^{-1} \cdot mmHg^{-1}$ or <0.166 $mmol \cdot min^{-1} \cdot kPa^{-1}$	
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 V_A and D_{LCO}	Implemented#	
Ensure proper alignment of gas concentration signals and the flow signal	Implemented#	
Measure anatomic dead-space using the Fowler method (see figure 3)	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 2)	Implemented#	
Measure V_A using all of the tracer gas data from the entire manoeuvre in the mass balance equation	Implemented#	
Report the D_{LCO} adjusted for the change in P_{AO_2} due to barometric pressure	Implemented#	
Ability to input simulated digital test data and compute D_{LCO}, V_A, TLC, V_D		Calculate values within 2% of actual values
Report the D_{LCO} adjusted for the change in P_{AO_2} due to P_{ACO_2}, 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 $L \cdot s^{-1}$	
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 (V_A) of 3 ±0.3 L and D_{LCO} of <0.5 $mL \cdot min^{-1} \cdot mmHg^{-1}$ or <0.166 $mmol \cdot min^{-1} \cdot kPa^{-1}$	

D_{LCO}: diffusing capacity of the lung for carbon monoxide; [CO]: carbon monoxide concentration; QA: quality assurance; ATP: ambient temperature, pressure and humidity; V_A: alveolar volume; P_{AO_2}: alveolar oxygen tension; P_{ACO_2}: alveolar carbon dioxide tension; TLC: total lung capacity; V_D: dead-space volume. #: Implemented means that the manufacturer has implemented the designated functionality in the D_{LCO} system.

RGA response time

The response time of the RGA will determine how accurately it is able to track the true carbon monoxide and tracer gas concentrations. Even after the application of an appropriate time shift to correct for the lag

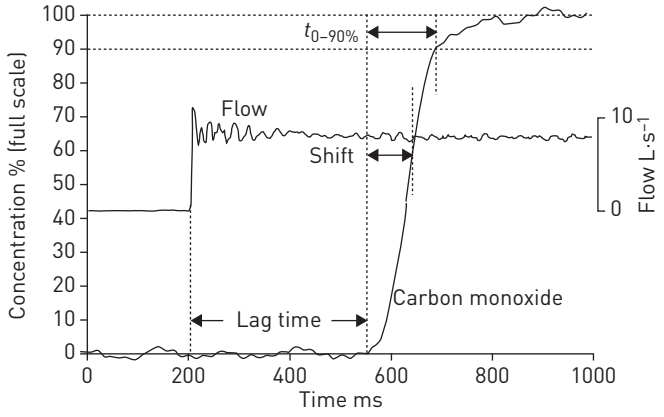

FIGURE 1 Lag and response times for carbon monoxide: the response time of the analyser was estimated by rapidly switching the gas being sampled from zero to full-scale carbon monoxide. The change in the flow signal shows the time at which the switch was made from medical air to test gas. The lag time, the 0–90% response time and the optimal shift are calculated from the resulting response curve.

and response times, there will be a residual error in *D*LCO due to the finite response time. For every 100 ms increase in the 0–90% response time, the error in *D*LCO increases by about 0.7% [6]. Based on the above considerations, the 0–90% response time for RGAs used in *D*LCO systems must be ⩽150 ms.

Linearity and accuracy

The linearity of the gas concentration signals is of primary importance for measuring *D*LCO, since ratios of gas concentrations are considered in the classical calculations [7, 8]. The error in *D*LCO measurement due to nonlinearity in the gas concentration 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 *D*LCO of 13.4 mmol·min^{-1}·kPa^{-1} (40 mL·min^{-1}·mmHg^{-1}) to 1.7% in a subject with a *D*LCO of 3.35 mmol·min^{-1}·kPa^{-1} (10 mL·min^{-1}·mmHg^{-1}) [6]. 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 becomes important when measuring the residual alveolar carbon monoxide concentration and the washout of tracer gas from the previous *D*LCO manoeuvre. The output of a gas analyser must be accurate to within ±1% of full scale.

Drift

The gas analyser 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 ⩽0.5% of full scale over 30 s for tracer gas.

Digitisation

In order for the digitised signal to accurately track the gas concentration signal and 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.

Other equipment considerations

Circuit resistance must be <1.5 $cmH_2O·L^{-1}·s^{-1}$ up to 6 L·s^{-1} flow. If a demand-flow regulator is used on a compressed test gas cylinder, the maximal inspiratory pressure required for 6 L·s^{-1} inspiratory flow through both circuit and valve must be <9 cmH_2O.

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

Equipment calibration and quality control

The considerations for equipment calibration are illustrated in table 2 and are 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.

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 *D*LCO check	Weekly
Calibration syringe leak test	Monthly
Linearity check (calibration syringe or simulator)	Monthly

*D*LCO: diffusing capacity of the lung for carbon monoxide.

2) Each day, prior to testing, there must be a volume calibration check with a 3-L syringe [9]. The syringe should be discharged at least three times to give a range of flows varying between 0.5 and 12 $L \cdot s^{-1}$ (with 3-L injection times of ∼6 s and ∼0.5 s, respectively). The volume at each flow must meet an accuracy requirement of ⩽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 °C or relative humidity changes by more than 15% (absolute). Operators should also perform a calibration check whenever they notice significant discrepancies between inspired volume (V_I) and VC, or between alveolar volume (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 *D*LCO systems using a volume-type spirometer, a spirometer leak test should be performed according to the manufacturer's specifications. Secondly, a *D*LCO 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 is then emptied, filled with 3 L of test gas and then 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 STPD (standard temperature, pressure and dry conditions) to BTPS (body temperature, ambient pressure, saturated with water vapour conditions) correction factor, which is 863/(P_B−47), where P_B is the barometric pressure. 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 *D*LCO must be <0.166 $mmol \cdot min^{-1} \cdot kPa^{-1}$ or <0.5 $mL \cdot min^{-1} \cdot mmHg^{-1}$. Thirdly, a test should be performed on a "standard subject" (biological control) or simulator [10]. Standard subjects are nonsmokers who have been found to have a consistently repeatable *D*LCO (*e.g.* healthy laboratory personnel). If the *D*LCO in a standard subject varies either by >12% or by >1 $mmol \cdot min^{-1} \cdot kPa^{-1}$ (>3 $mL \cdot min^{-1} \cdot mmHg^{-1}$) from the mean of previous values, the test must be repeated. A study of the long-term intersession variability of *D*LCO has found that biological control deviations of >12% or >3 $mL \cdot min^{-1} \cdot mmHg^{-1}$ 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 [11].

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 cmH_2O), 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 [12], or to measure the concentration of a separate high-precision test gas having a certificate of analysis. For systems with independent measurements 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 and *D*LCO checks above 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 D_{LCO} must be <0.166 mmol·min^{-1}·kPa^{-1} or <0.5 mL·min^{-1}·mmHg^{-1}. A review of quality control data for four different D_{LCO} systems between 2006 and 2015 using this procedure found only four outlier points where $|D_{LCO}|$ was >0.13 mmol·min^{-1}·kPa^{-1} (0.4 mL·min^{-1}·mmHg^{-1}). The same data showed that V_A was consistently within 3±0.3 L for 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 D_{LCO} 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 under 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 measurements 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.

Standardisation issues in the single-breath testing technique

The single-breath determination of D_{LCO} 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

Before beginning the test, the manoeuvres must be demonstrated and the subject carefully instructed. The subject must be seated comfortably throughout the test procedure. The test must be performed at a stable, comfortable temperature within manufacturer's equipment specifications.

Inspiratory manoeuvres

Once the mouthpiece and nose clip are in place, tidal breathing must be carried out for sufficient time to ensure that the subject is comfortable with the mouthpiece and that the nose clips and mouthpiece are used appropriately with no leaks. The D_{LCO} 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 so that they can achieve a maximal VC during 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. It is important that the inspired volume of test gas, V_I, be as close to the known VC as possible and 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 V_A is within 200 mL or 5% (whichever is greater) of the highest V_A among acceptable D_{LCO} manoeuvres.

Washout and sample collection

For RGA systems, the point of 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 breakpoint of each phase of the washout (a plot of concentration versus volume) and adding a proportion of the dead-space volume measured by the Fowler technique [13] to the phase II to III breakpoint [14]. Such an approach can be automated. For visual verification of the point of dead-space washout, the tracer gas concentration must be displayed as a function of volume, since using the concentration–time curve can be deceptive because of the higher relative flow at the beginning of exhalation (figure 2). 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 can be calculated for use in measuring D_{LCO}; however, RGA systems are capable of simulating much smaller gas samples although it should be noted that such smaller virtual samples will be more subject to noise in the expired carbon monoxide concentration signal. Indeed, Jones and Meade [15] used gas

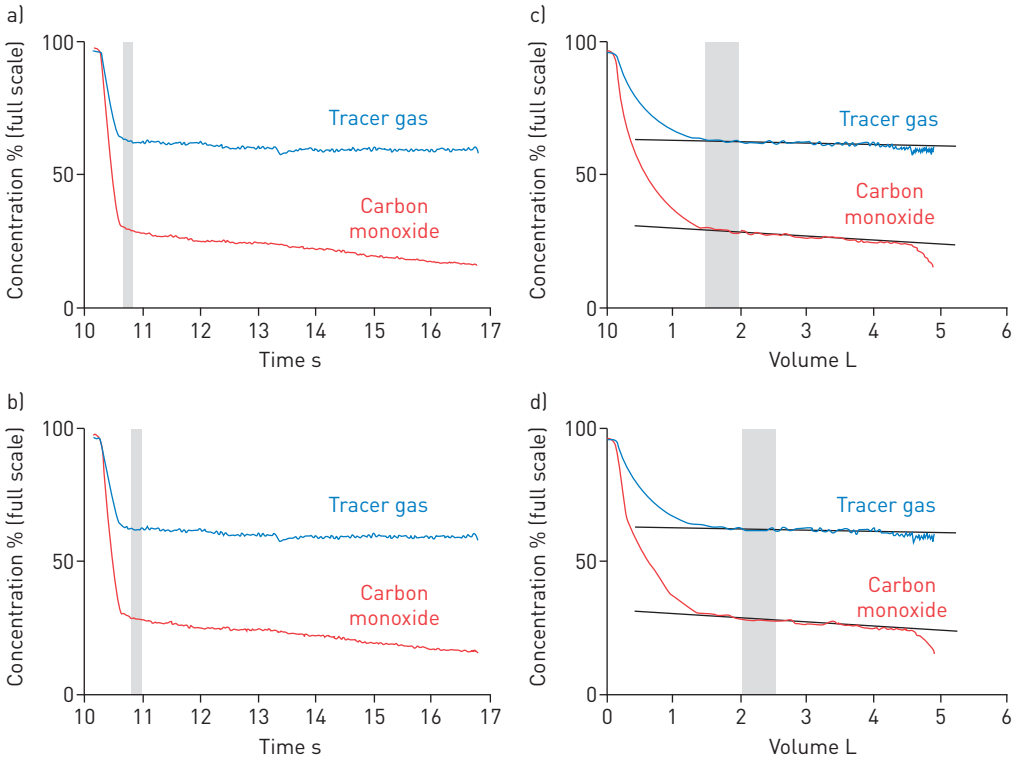

FIGURE 2 Comparison of gas concentration plotted as a function of time (a and b) or volume (c and d) for carbon monoxide and tracer gas. The shaded bar shows the collection of a 500-mL sample of exhaled gas. The upper panels (a and c) show sample collection as selected by computer algorithm (based on 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.

samples of 85 mL in the development of their method and virtual alveolar gas sample volumes of 85–500 mL may be used.

Inspired gas composition

The test gas used to calculate D_{LCO} 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. The inspired carbon monoxide concentration should thus be close to 0.3%. There are two factors determining the rationale for the recommendation of an inspiratory oxygen fraction (F_{IO_2}) of 21% in the test gas for routine D_{LCO} determination. First, the majority of studies developing reference values for D_{LCO}, which are based on the 2005 standards [4], use an F_{IO_2} of 21% (see the section on reference values below). Secondly, the P_{AO_2} (alveolar oxygen tension) following a maximal inhalation will depend on the dead-space volume and the ratio of V_I to V_A for any given 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. P_{AO_2} of 100 mmHg or 13 kPa), it may not do so in all subjects.

Manoeuvre intervals

Using an RGA system, exhaled gas can be monitored as soon as the subject begins breathing through the mouthpiece prior to the inhalation of test gas. This "end-expiratory" tracer gas information will indicate whether or not the washout from a previous manoeuvre is complete, which may occur in less than 4 min in some subjects. The tracer gas concentration at end-exhalation must be $\leqslant$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 compensate the tracer gas concentration data used in the determination of V_A at the beginning of breath-holding.

Calculations

D_{LCO} is calculated using the alveolar volume in litres reduced to STPD conditions (V_{ASTPD}), the breath-hold time in seconds, t_{BH} (calculated by the method of Jones and Meade [15], the barometric pressure in mmHg, P_B, and the fractional concentrations of carbon monoxide and tracer gas in the inspired test gas (F_{ICO} and F_{ITr}) and exhaled alveolar gas (F_{ACO} and F_{ATr}), respectively, as shown in equation 1. The conversion factor of 60 000 arises from the need to convert into the correct units for D_{LCO} (mL·min^{-1}·mmHg^{-1}) using 60 s·min^{-1} and 1000 mL·L^{-1}.

$$D_{LCO} = \frac{V_{ASTPD}}{t_{BH} \cdot (P_B - 47)} \cdot \ln\left(\frac{F_{ICO}}{F_{ACO}} \cdot \frac{F_{ATr}}{F_{ITr}}\right) \cdot 60\,000 \quad (1)$$

If SI units are required, a further conversion factor of 22.4 arises from the conversion of mL(STPD) to mmol, as shown in equation 2.

$$T_{LCO} = \frac{V_{ASTPD}}{t_{BH} \cdot (P_B - 6.28)} \cdot \ln\left(\frac{F_{ICO}}{F_{ACO}} \cdot \frac{F_{ATr}}{F_{ITr}}\right) \cdot 60\,000/22.4 \quad (2)$$

Calculating the alveolar volume

Using RGA systems, the dead-space volume is measured rather than estimated. The total dead-space, V_D, can be obtained from the tracer gas washout curve using the Fowler method (figure 3) [13]. The development of RGA systems allows for the analysis of all of the exhaled gas and, since the tracer gas is being monitored throughout exhalation, there is no need to constrain the measurement of V_A to the discrete

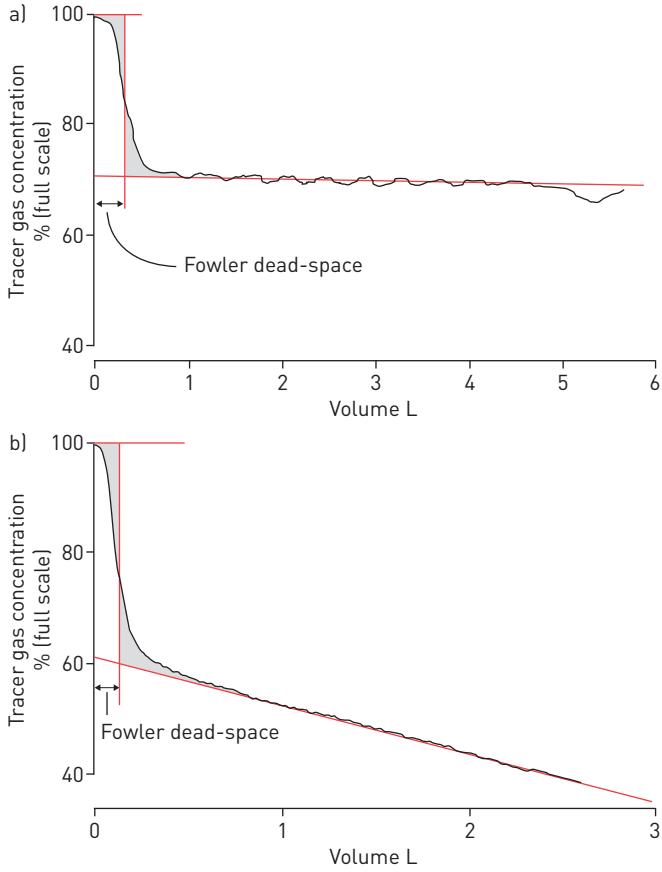

FIGURE 3 Graphical representation of the calculation of the Fowler dead-space volume in a normal, healthy subject (a) and a subject with chronic obstructive pulmonary disease (COPD) (b). The single-breath tracer gas washout is plotted against exhaled lung volume from total lung capacity. The volume at which the shaded area above the tracer gas washout curve equals the shaded area below the curve is the Fowler dead-space [13] which is reported under body temperature, ambient pressure, saturated with water vapour (BTPS) conditions.

sample computationally constructed for determining carbon monoxide uptake. This provides the opportunity to enhance the accuracy of V_A determination and, indeed, using all of the available gas concentration data has been shown to provide a better estimate of V_A [16, 17] than constraining measurement to smaller samples of exhaled gas, as required by the equipment available in 1957 when the clinical single-breath method was developed [1].

This approach uses a mass balance technique in which the volume of tracer gas inhaled and subsequently exhaled are measured, such that the volume of tracer gas remaining in the lung at end-exhalation is known [16, 17]. The volume of tracer gas left in the lung is divided by the end-expiratory tracer gas concentration to measure the absolute end-expiratory lung volume. Total lung capacity is then calculated by adding the expired volume to the end-expiratory volume and subtracting equipment dead-space. The residual tracer gas in the lung from a previous manoeuvre, if any, can be measured prior to the start of the manoeuvre and included in the mass balance equation.

RGA signal alignment

To properly analyse continuous gas samples, the gas concentration signal from the analyser must be properly aligned with the flow signal from the pneumotachometer. The first step is to shift the concentration signal ahead in time to compensate for the lag time (figure 1). The lag time refers to the time required for the gas to travel from the aspiration port to the analyser chamber and 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 or junctions that create turbulence. Lag time can also vary with the viscosity of the gas and as such, when helium is used as the tracer, dynamic compensation of the lag time may be required during exhalation. An additional shift of each gas concentration signal relative to the flow signal must also be performed to compensate for the response time of the analyser. This adjustment can be performed using an optimal shift equal to the natural logarithm of twice the time constant of the analyser response [18]. Alternatively, alignment may be achieved by other signal processing techniques, such as cross-correlation (convolution of signals).

Evaluating the measurement of D_{LCO}

Acceptability, repeatability and quality control

The volume–time graph for a manoeuvre should show a smooth, sharp rise in volume, followed by a stable breath-hold and a smooth, sharp exhalation. 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. Acceptable manoeuvres are defined in table 3. The V_I of test gas must be at least 90% of the highest VC measured in the same pulmonary function testing session. At least 85% of test gas V_I must be inhaled 4 s and there must be no evidence of a Müller or Valsalva manoeuvre during the breath-hold period. The calculated breath-hold time must be 10±2 s and the alveolar sample collection must be completed within 4 s. For RGA systems, the virtual sample collection must be initiated after the completion of dead-space washout. A manoeuvre with a V_I/VC <90% but ⩾85% may be deemed acceptable if the V_A is within 200 mL or 5%, whichever is greater, of another acceptable manoeuvre.

Repeatability describes the intra-session variability on repeat testing when there is no change in test conditions [19, 20]. In a large university-based laboratory study, the coefficient of variation for repeat measurement in normal subjects was reported as 3.1% and this increased only slightly (from 4.0–4.4%) in patients with abnormal spirometry patterns [21]. Studies conducted prior to the publication of the 2005 standards found D_{LCO} variability of up to 9% (reproducibility) in normal individuals in repeat measurement over a period of 1 year [22] and coefficients of variation ranged from 6.2–12% for selected UK regions [23].

Repeatability requirement: there must be at least two acceptable manoeuvres that are within 2 mL·min^{-1}·mmHg^{-1} (0.67 mmol·min^{-1}·kPa^{-1}) of each other. A study of 4797 test sessions found that 95.5% of cases met this criterion [24]. 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 D_{LCO} manoeuvres must be performed, research is needed to determine the actual number of manoeuvres required to provide a reasonable estimate of average D_{LCO} for a given person. As noted elsewhere, five manoeuvres will result in an increase of ~3.5% carboxyhaemoglobin (COHb) from baseline [25, 26], which will decrease the measured D_{LCO} by ~3–3.5%. Thus, conducting more than five manoeuvres is not a recommended strategy.

TABLE 3 Acceptability, repeatability and quality control in D_{LCO} testing

Criteria for acceptability			
A V_I ⩾90% of the largest VC in the same test session; alternatively, a V_I ⩾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 D_{LCO} measurements within 2 mL·min^{-1}·mmHg^{-1} (0.67 mmol·min^{-1}·kPa^{-1}) of each other			
Quality control grading[#]			
Score	**V_I/VC**	**t_{BH}**	**Sample collection**
A	⩾90%[¶]	8–12 s	⩽4 s
B	⩾85%	8–12 s	⩽4 s
C	⩾80%	8–12 s	⩽5 s
D	⩽80%	<8 or >12 s	⩽5 s
F	⩽80%	<8 or >12 s	>5 s

V_I: inspired volume; VC: vital capacity; V_A: alveolar volume; t_{BH}: breath-hold time; D_{LCO}: diffusing capacity of the lung for carbon monoxide. [#]: only grade A manoeuvres meet all acceptability criteria. The average D_{LCO} 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 D_{LCO} 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⩾85% and V_A within 200 mL or 5% (whichever is greater) of the largest V_A from another acceptable manoeuvre.

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 D_{LCO} grading system.

A grade A manoeuvre meets all acceptability criteria. The average D_{LCO} from two or more grade A manoeuvres that are repeatable (*i.e.* are within 2 mL·min^{-1}·mmHg^{-1} or 0.67 mmol·min^{-1}·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 D_{LCO} value from the acceptable manoeuvres is reported. 2) If only one grade A manoeuvre is obtained, then the D_{LCO} value from that manoeuvre is reported. 3) If no acceptable manoeuvres are obtained, then the average D_{LCO} value of the manoeuvres with grades B, C or D is reported. 4) If only grade F manoeuvres are obtained then no D_{LCO} value is reported.

Adjustments to the predicted value of D_{LCO} prior to interpretation

The value of D_{LCO} depends upon a number of physiological factors. Besides varying with age, sex, height and possibly ethnicity, D_{LCO} also changes with lung volume, Hb (haemoglobin) and COHb level, P_{IO_2} (inspired oxygen tension, *e.g.* altitude), exercise and body position.

Adjustment for haemoglobin

Using the Cotes equations [27] and assuming the "standard" Hb value to be 14.6 g·dL^{-1} in adult and adolescent males and 13.4 g·dL^{-1} in adult females and children <15 years of age, the adjustment to the predicted D_{LCO} value of the former is described by equation 3 while the adjustment to the latter is

described by equation 4.

$$D_{LCO}[\text{predicted for Hb}] = D_{LCO}[\text{predicted}] \cdot (1.7\text{Hb}/(10.22 + \text{Hb})) \quad (3)$$

$$D_{LCO}[\text{predicted for Hb}] = D_{LCO}[\text{predicted}] \cdot (1.7\text{Hb}/(9.38 + \text{Hb})) \quad (4)$$

Measurement of Hb in the American population [28] indicated difference from these "standard" values, especial in males, children and seniors; differences were also reported between Caucasian and African-Americans. Furthermore, the survey found that Hb levels in the general population were changing over time. If a more appropriate reference Hb level (Hb_{ref}) is available, then the predicted D_{LCO} value is adjusted as in equation 5.

$$D_{LCO}[\text{predicted for Hb}] = D_{LCO}[\text{predicted}] \cdot (1.7\text{Hb}/(0.7\text{Hb}_{ref} + \text{Hb})) \quad (5)$$

Adjustment for carboxyhaemoglobin concentration and carbon monoxide back-pressure

The level of COHb can affect the measured uptake of carbon monoxide in two ways [29–31]. 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.

For RGA systems, carbon monoxide back-pressure can be measured in expired gas prior to the inspiration of test gas in the D_{LCO} manoeuvre [32] and analytically compensated for by subtracting the estimated carbon monoxide back-pressure from both the initial and final alveolar carbon monoxide partial pressures. However, this method will not adjust D_{LCO} for the "anaemia effect" effect of COHb.

A recent study using an RGA system to measure alveolar carbon monoxide concentration combined with venous measurements 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 D_{LCO} for each 1% increase in COHb [32]. These findings were verified in a discrete-sample system [26]. In these studies, where the carbon monoxide back-pressure was measured and used in the calculation of D_{LCO}, equation 6 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 gas volume, just prior to the inhalation of test gas.

$$D_{LCO}[\text{corrected}] = D_{LCO} \cdot (1 + F_{ACOb}/560) \quad (6)$$

Adjustment of D_{LCO} for barometric pressure

While the preceding adjustments are made to the predicted value of D_{LCO}, it is recommended that the measured value of D_{LCO} be adjusted for barometric pressure. As P_B decreases, P_{IO_2} decreases and D_{LCO} increases. For factors such as Hb that are related to the individual subject, the recommended adjustment is made to the predicted D_{LCO} value. However, P_B is an environmental factor that is independent of the individual and therefore the measurement of D_{LCO} should be adjusted to simulate standard pressure conditions. The variation in D_{LCO} due to the typical range of high and low pressure cells at a given altitude is approximately ±1.5%. As mentioned above, as P_B decreases with altitude, D_{LCO} increases. The rate of change is 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 D_{LCO} and the predicted value of D_{LCO} are adjusted to a standard pressure (101.3 kPa or 760 mmHg). The adjustment for P_B [3, 33] assumes a P_{IO_2} of 20 kPa (150 mmHg) at standard pressure and the adjusted value of D_{LCO} is thus described by equation 7 (P_B in mmHg) or equation 8 (P_B in kPa).

$$D_{LCO}[P_B \text{ adjusted}] \approx D_{LCO}(0.505 + 0.00065P_B) \quad (7)$$

$$D_{LCO}[P_B \text{ adjusted}] \approx D_{LCO}(0.505 + 0.00488P_B) \quad (8)$$

Reporting values

This document is intended to establish technical standards which, in terms of reporting, will require D_{LCO} systems to be able to report the variables shown in table 4. It is not intended to specify which variables

TABLE 4 D_{LCO} reporting requirements

Variable[#]	Requirement
D_{LCO} (unadjusted)	Required
D_{LCO} (adjusted for P_B)	Required
D_{LCO} (LLN and/or z-score)	Required
D_{LCO} (predicted)	Required
D_{LCO} (adjusted,predicted)	Optional (required if any adjustments made-specify adjustments)
D_{LCO} (% 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 D_{LCO} acceptable manoeuvres)
Operator comments	Required (number of manoeuvres, number of acceptable manoeuvres)
Graphs	Required (full manoeuvre and exhaled gas concentration *versus* volume with sample collection indicated for RGA systems)
Hb	Optional (required if used to adjust D_{LCO})
COHb	Optional (required if used to adjust D_{LCO})
Alternative calculations (*e.g.* three-equation D_{LCO}, normalised slope of phase III)	Optional

BTPS: body temperature, ambient pressure, saturated with water vapour; LLN: lower limit of normal; D_{LCO}: 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 D_{LCO}, V_A, K_{CO}, t_{BH}, V_I, V_{Danat} and TLC_{sb} the average values from the acceptable and repeatable manoeuvres are reported.

end users should include in the report forms used in their laboratories, nor is it intended to address the interpretation of D_{LCO}. Although work is ongoing towards establishing a standardised pulmonary function laboratory report form, there is no current standard. A D_{LCO} system must be able to report the unadjusted measured D_{LCO}, the D_{LCO} adjusted for P_B, the lower limit of normal and z-score, predicted, and percentage of predicted D_{LCO} values. They must also be able to report the carbon monoxide transfer coefficient, K_{CO}, which is the logarithmic decay of carbon monoxide concentration over t_{BH} per unit of pressure, as well as the lower limit of normal and z-score, predicted, and percentage of predicted K_{CO} values. Any adjustments (*e.g.* for Hb, COHb, P_{IO_2}, or lung volume) must also be reported along with the data used to make the adjustment. 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 optionally be reported. The average V_I must also be noted. If a separately measured VC is available, it can be reported to serve as a reference for the adequacy of the V_I value. In addition, comments relevant to the quality of the measurements must be included. A complete list of specifications for which variables and measurements D_{LCO} systems should be 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.

The Global Lung Health Initiative (GLI) is currently working on the development of global reference values for D_{LCO} which will very likely be of a similar structure to the GLI spirometry reference values [34].

Implementation of these reference values requires more complexity than simply inserting coefficients for polynomials and a *D*LCO system must be able to implement this method of calculating reference values.

Summary

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

As already noted, the changes in *D*LCO standards will not impact the applicability of reference values. In general, pulmonary function measurement is more accurate and precise in normal, healthy subjects than in patients with lung disease. As such, changes which improve the measurement of *D*LCO will have less impact on normal, healthy subjects and will favour the continued applicability of reference values derived using older systems. There are already systematic differences amongst reference value sets for *D*LCO, which are related to the equipment and methodology used and which impact upon their applicability. Some reference values currently in use were developed prior to the publication of the 2005 ERS/ATS standards [3]. Hence, there is already a pressing need for reliable, comprehensive reference values for *D*LCO. A list of recommended research directions is given in the full standards document [2].

Advances in technology have outpaced guidelines and standards. These revisions to the *D*LCO standards are required to make optimal use of existing, clinically available technology. After all, guidelines and standards should not constrain progress in pulmonary function measurement but should serve to promote continual improvement.

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