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SERIES "ATS/ERS TASK FORCE: STANDARDISATION OF LUNG FUNCTION TESTING"
Edited by V. Brusasco, R. Crapo and G. Viegi
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Standardisation of the single-breath determination of carbon monoxide uptake in the lung

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CONTENTS

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BACKGROUND

This joint statement is based on the previous statements from the American Thoracic Society (ATS) and the European Respiratory Society (ERS), and much of the material was taken from these statements [1, 2]. It has been updated according to new scientific insights and revised to reflect consensus opinions of both of these societies. This document is meant to function as a stand-alone document, but, for certain issues, references will be made to the previous statements. Although there are other ways to measure carbon monoxide (CO) uptake (*e.g.* steady-state, intra-breath and rebreathing techniques) [3–9], the following recommendations will be restricted to the single-breath technique, since this is the most common methodology in use around the world.

The capacity of the lung to exchange gas across the alveolar-capillary interface is determined by its structural and functional properties [3–22]. 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 blood in capillaries supplying ventilated alveoli. The functional properties include the following: absolute levels of ventilation and perfusion; the uniformity of their distribution with respect to each other; the composition of the alveolar gas; the diffusion characteristics of the membrane; the concentration and binding properties of haemoglobin (Hb) in the alveolar capillaries; and the gas tensions in blood entering the alveolar capillaries in that part of the pulmonary vascular bed which exchanges gas with the alveoli.

Definitions

The rate of CO uptake from the lungs is the product of alveolar partial pressure of CO in excess of any back pressure in the blood (the driving pressure) and a rate constant. This is for CO in the whole lung per unit of driving pressure. For practical reasons, using the single-breath method described below the CO uptake from the lung (K_{CO}) is measured as a concentration fall in alveolar CO per unit time per unit CO driving pressure ($P_{A,CO}$):

$$K_{CO} = \Delta[CO]/\Delta t/P_{A,CO} \quad (1)$$

When K_{CO} is multiplied by the volume of gas in the lung containing CO (alveolar volume (V_A)), the total uptake of CO by the lung per unit of time per unit driving pressure is obtained. This product, $K_{CO} \times V_A$, has been termed transfer factor of the lung for CO by the European community and diffusing capacity of the lung for CO ($D_{L,CO}$) by the North American community. The former term recognises that the measurement of CO uptake reflects a number of processes (not just diffusion), and is a submaximal value and, thus, not truly a ''capacity''. However, the latter term has considerable historical significance and, for the sake of uniformity, the ERS and ATS agreed to use the expression $D_{L,CO}$ in this document.

The ERS recommends expressing $D_{L,CO}$ in the SI units $mmol \cdot min^{-1} \cdot kPa^{-1}$, while the ATS prefers the traditional units mL (standard temperature, pressure and dry (STPD))$\cdot min^{-1} \cdot mmHg^{-1}$. In fact, this is not an important issue, providing the same set of units is used throughout all calculations. Values in SI units should be multiplied by 2.987 to obtain values in traditional units.

Determinants of CO uptake

The process of CO transfer from the environment to the pulmonary capillary blood includes: 1) bulk flow delivery of CO to the airways and alveolar spaces; 2) mixing and diffusion of CO in the alveolar ducts, air sacs and alveoli; 3) transfer of CO across the gaseous to liquid interface of the alveolar membrane; 4) mixing and diffusion of CO in the lung parenchyma and alveolar capillary plasma; 5) diffusion across the red cell membrane and within the interior of the red blood cell; and 6) chemical reaction with constituents of blood Hb [10–16].

The process of CO uptake can be simplified into two transfer or conductance properties: membrane conductivity (D_M), which reflects the diffusion properties of the alveolar capillary membrane; and the binding of CO and Hb. The latter can be represented as the product of the CO–Hb chemical reaction rate (θ) and the volume of Hb in alveolar capillary blood (V_c). Since these are conductances in series [14], these properties are related by:

$$1/D_{L,CO} = (1/D_M) + (1/\theta V_c) \quad (2)$$

A number of physiological changes can affect D_M or θV_c to influence $D_{L,CO}$. As the lung inflates, D_M increases (due to unfolding membranes and increasing surface area), while V_c effects are variable (due to differential stretching and flattening of alveolar and extra-alveolar capillaries) [10, 17–24]. The net effect of these changes is that $D_{L,CO}$ tends to increase as the lung inflates. Exercise, the supine position and Mueller manoeuvres (inspiratory efforts against a closed glottis) can all recruit and dilate alveolar capillaries, thereby increasing V_c and $D_{L,CO}$ [25–31]. 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 $D_{L,CO}$ [29].

The measurement of CO uptake is also affected by the distribution of ventilation with respect to D_M or θV_c (*i.e.* CO uptake can only be measured in lung units into which CO was inspired and subsequently expired) [15, 16, 32, 33]. This is particularly important in diseases such as emphysema, where the inhaled CO may only go to the better-ventilated regions of the lung and the subsequently measured CO uptake will be determined primarily by uptake properties of those 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 $D_{L,CO}$ should thus be considered to be primarily reflecting the gas-exchange properties of the ventilated regions of the lung.

In addition to these physiological and distributional effects on $D_{L,CO}$, a number of pathological states can affect D_M, θV_c, or both, and thereby affect $D_{L,CO}$ (table 1) [5, 6, 34–43]. Measurement of $D_{L,CO}$ is indicated when any of these pathological processes are suspected or need to be ruled out. Moreover, measuring changes in $D_{L,CO}$ over time in these processes is a useful way of following the course of disease.

TABLE 1 Physiological and pathological changes that affect the carbon monoxide diffusing capacity of the lung ($D_{L,CO}$)

Extrapulmonary reduction in lung inflation (reduced V_A) producing changes in D_M or θV_c that reduce $D_{L,CO}$
Reduced effort or respiratory muscle weakness
Thoracic deformity preventing full inflation
Diseases that reduce θV_c and thus reduce $D_{L,CO}$
Anaemia
Pulmonary emboli
Other conditions that reduce θV_c and thus reduce $D_{L,CO}$
Hb binding changes (*e.g.* HbCO, increased F_{I,O_2})
Valsalva manoeuvre (increased intrathoracic pressure)
Diseases that reduce (in varying degrees) D_M and θV_c and thus reduce $D_{L,CO}$
Lung resection (however, compensatory recruitment of θV_c also exists)
Emphysema
Interstitial lung disease (*e.g.* IPF, sarcoidosis)
Pulmonary oedema
Pulmonary vasculitis
Pulmonary hypertension
Diseases that increase θV_c and thus increase $D_{L,CO}$
Polycythaemia
Left-to-right shunt
Pulmonary haemorrhage (not strictly an increase in θV_c, but effectively an increase in lung Hb)
Asthma
Other conditions that increase θV_c and thus increase $D_{L,CO}$
Hb binding changes (*e.g.* reduced F_{I,O_2})
Muller manoeuvre (decreased intrathoracic pressure as in asthma, resistance breathing)
Exercise (in addition, a possible D_M component)
Supine position (in addition, possibly a slight increase in D_M)
Obesity (in addition, a possible D_M component)

V_A: alveolar volume; D_M: membrane conductivity; θ: carbon monoxide (CO)–haemoglobin (Hb) chemical reaction rate; V_c: volume of pulmonary capillary blood; F_{I,O_2}: inspired fraction of oxygen; IPF: idiopathic pulmonary fibrosis; Hb: haemoglobin.

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, 44–48]. Equipment in clinical use varies widely in complexity, but the basic principles are the same. All systems have a source of test gas (bag-in-box, spirometer, compressed gas cylinder), a method for measuring inspired and expired volume over time (spirometers with kymographs, pneumotachometers near the mouthpiece or near a bag-in-box), and gas analysers (single-sample analysers or continuous high-speed analysers). Single-sample gas-analyser systems usually display only volume over time (fig. 1a). Continuous gas-analyser systems also provide a continuous tracing of CO and tracer gas concentrations during the test (fig. 1b).

Equipment requirements

Performance standards for equipment

The performance standards for equipment are as follows (table 2). 1) The volume-measurement accuracy should be the same as that established by the ATS/ERS for spirometry [49]; that is, $\pm$3% volume accuracy ($\pm$3.5% accounting for 0.5% testing syringe error) over an 8-L volume range with test gases present in concentrations likely to be encountered during $D_{L,CO}$ tests. Pneumotachometer devices for sensing flow and volume during the $D_{L,CO}$ manoeuvre may be sensitive to different gas compositions, concentrations or pulsatile flow changes created by demand valves [50]. All devices should maintain the required volume accuracy, regardless of the gas mixture, direction of gas flow (*e.g.* inhaled or exhaled), or pulsatile flow pattern. 2) Gas-analyser accuracy is important in some circumstances, such as measuring CO ''back pressure'' (the expired fraction of CO when no CO has been inhaled). However, in calculating $D_{L,CO}$, only the ratios of alveolar to inhaled CO and tracer gas are needed. Thus, the analysers must primarily be able to produce an output for measured exhaled CO and tracer gas that is a linear extrapolation between the inhaled (test gas concentrations) and zero (no CO or tracer gas present in the analysers) [51, 52]. This is often referred to as a linear response. Since measured $D_{L,CO}$ is very sensitive to errors in relative gas concentration, nonlinearity for the analysers should not exceed 0.5% of full scale (*i.e.* once the analysers have been adjusted to zero, with no test gas present and scaled to full scale using test gas concentrations, system nonlinearity on measurements of known dilutions of test gas should be no more than 0.5% of full scale). For example, if 0.300% CO is used for the test gas, then the maximum error on any dilution should be no more than $\pm$0.0015%. 3) The gas analysers should have only minimal drift in zero and gain, so that output is stable over the test interval. Manufacturers are encouraged to provide a display of the measured gas concentrations so that stability can be confirmed. If significant

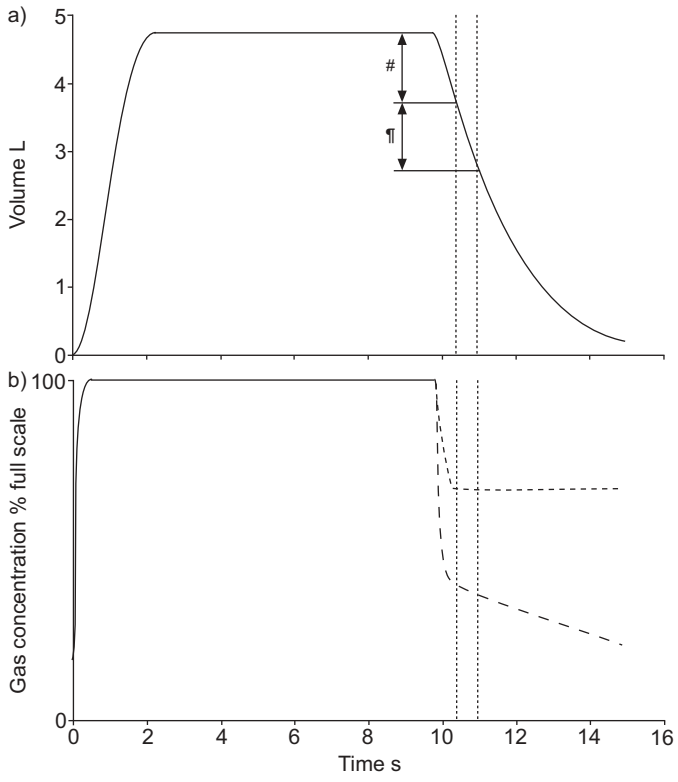

FIGURE 1. Schematic of lung volume (a) and gas concentrations (b) during the single-breath diffusing capacity of the lung for carbon monoxide. The gas-sampling period occurs between the two dotted lines. -----: tracer gas; – – –: carbon monoxide. #: dead space washout; ¶: sample collection. Modified from [1].

drift is present over the time scale of a test (~30 s), then adjustment algorithms should be devised to compensate for the analyser drift from measured data. Gas-analyser stability should be ±0.001% absolute for CO and ±0.5% of the full-scale reading for the tracer gas. 4) If CO_2 and/or H_2O interfere with gas-analyser performance, there are two remedies. First, the CO_2 and/or H_2O can be removed from the test gases before passage through the gas analysers. H_2O is commonly absorbed by anhydrous $CaSO_4$ or by other products. Absorption of CO_2 can be achieved with either $Ba(OH)_2$ or NaOH. Both generate H_2O when combining with CO_2. Therefore, if a CO_2 absorber is used, it must precede the H_2O absorber in the gas-analyser circuit. Selectively permeable tubing can also be used to remove water vapour; however, this tubing may only reduce the water vapour to near ambient levels, and remaining H_2O can still interfere with the gas-analyser performance. Furthermore, water vapour-permeable tubing has a limited life expectancy. One method of checking water vapour-permeable tubing is to compare gas-concentration measurements made with both dry and humidified test gas, and make adjustments described as follows. Manufacturers should provide a replacement schedule for water vapour-permeable tubing and/or a method for checking its function. The second remedy for CO_2 and/or H_2O analyser interference is to characterise the effect of these gases on analyser output, and then adjust the output of the analysers for the presence of the interfering gas species. Two approaches are often employed as follows: assume constant concentrations of the interfering gases and apply a fixed correction factor across all tests; or directly measure the CO_2 and/or H_2O for each test and make proportional adjustments in the analyser output based on the measured concentrations for CO_2 and/or H_2O (see CO_2, H_2O and temperature adjustment for V_A calculations section). 5) Circuit resistance should be <1.5 $cmH_2O \cdot L^{-1} \cdot s^{-1}$ at 6 $L \cdot s^{-1}$ flow. If a demand-flow regulator is used on a compressed test gas cylinder, the maximal inspiratory pressure required for 6 $L \cdot s^{-1}$ inspiratory flow through both circuit and valve should be <10 cmH_2O. 6) The timing device in the $D_{L,CO}$ apparatus should be accurate to within 1% (100 ms over 10 s). The timing technique used for calculation should be identified. If an instrument provides automatic data computation, the accuracy of breath-hold time computation should be documented. 7) Dead space volume (V_D) for both inspired test gas and the alveolar sample should be known, and their role in all data-computation algorithms identified and documented. For adults, the V_D of the valve, filter and mouthpiece should total <0.350 L. Smaller V_D volumes may be needed for paediatric applications. 8) The system must be leak free. This is particularly important for $D_{L,CO}$ systems that aspirate gas samples at subatmospheric pressure through the gas analysers. When samples are aspirated, leaks in tubing, fittings and other locations allow room air to be drawn into the gas circuit, diluting the sample and reducing the concentrations of test gases.

Equipment quality control

The considerations for equipment quality control are as follows (table 3). 1) Prior to each test, gas analysers should be zeroed. After each test, a new zeroing procedure should be carried out to account for analyser drift during the test. 2) Each day, there should be a volume calibration with a 3-L syringe [53]. Technicians should also note significant discrepancies between inspired volume (V_I) and vital capacity (VC), or V_A and total lung capacity (TLC) that might suggest volume-calibration

TABLE 2 Equipment specifications

Volume accuracy	ATS/ERS standards (currently 3.5% accuracy over an 8-L volume using test gases, with a testing syringe accuracy of 0.5%)
Gas analysers	Linear from zero to full span within ±0.5% of full span. Stable over the duration of the test with drift <±0.5% of a measured gas
Circuit resistance	<1.5 $cmH_2O \cdot L^{-1} \cdot s^{-1}$ at a flow of 6 $L \cdot s^{-1}$
Demand-valve sensitivity	<10 cm H_2O required for 6 $L \cdot s^{-1}$ flow through valve and circuit (if compressed gas source used)
Timer	±1.0% over 10 s (100 ms)
Apparatus/valve filter V_D	<0.350 L

ATS: American Thoracic Society; ERS: European Respiratory Society; V_D: dead space volume.

TABLE 3 Equipment quality control

Gas-analyser zeroing	Done before/after each test
Volume accuracy	Tested daily
Standard subject or simulator testing	Tested at least weekly
Gas-analyser linearity	Tested every 3 months
Timer	Tested every 3 months

problems. 3) Each week, or whenever problems are suspected, the following procedures should be carried out. First, leak testing should be done if it is appropriate to the instrument being used. Secondly, a $D_{L,CO}$ test with a calibrated 3.0-L syringe should be used, which is performed by attaching the syringe to the instrument in the test mode. Test gas is withdrawn from the $D_{L,CO}$ machine by the syringe and then reinserted at the end of the breath-hold. The measured $D_{L,CO}$ should be near zero and the measured V_I should be ~3.3L (3.0 L × the body temperature, ambient pressure, saturated with water vapour (BTPS) factor). This procedure checks the inhaled volume accuracy in the $D_{L,CO}$ test mode, which may be in error when spirometry measurements are not. Thirdly, a test could be performed on a ''standard subject'' (biological control) or simulator [54]. Standard subjects are healthy nonsmokers (*e.g.* healthy laboratory personnel). If the $D_{L,CO}$ in a standard subject varies >10% from known previous values, the test should be repeated. If the repeat test confirms the finding, the $D_{L,CO}$ system should be evaluated carefully for the possibility of leaks, nonlinear analyser function, volume and time inaccuracy, *etc*. When sufficient data on a standard individual are obtained, laboratories should establish their own outlier criteria to serve as indicators of potential problems with their $D_{L,CO}$ systems. Manufacturers are encouraged to develop automated quality-control systems to assist and enhance the utility of these steps. 4) Gas-analyser linearity should be assessed every 3 months. A straightforward approach is to measure known serial dilutions of the test gas [55], or measure the concentration of a separate high-precision test gas having a certificate of analysis. At least one intermediate concentration should be used to check linearity. Manufacturers should be encouraged to automate this function. In addition, the timer should be assessed for accuracy every quarter. 5) Records of equipment checks and standard subject tests should be dated, signed and kept in a laboratory log book. Manufacturers are encouraged to provide software and test equipment options for quality-control measurements and quality-control data management.

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 $D_{L,CO}$ equipment and procedures [49, 56].

SINGLE-BREATH TESTING TECHNIQUE STANDARDISATION ISSUES

The single-breath determination of $D_{L,CO}$ involves measuring the uptake of CO from the lung over a breath-holding period. To minimise variability as much as possible, the following recommendations for the standardisation of testing techniques are offered.

Patient conditions for measurement

Factors that affect V_C (*e.g.* exercise, body position, and Hb affinity for CO, such as alveolar oxygen partial pressure (P_{A,O_2}), and carboxyhaemoglobin (COHb)) should be standardised. If clinically acceptable, the subject should not breathe supplemental oxygen for 10 min prior to a standard test. When using exercise or the supine position to assess the ''recruitability'' of $D_{L,CO}$ [15, 25–28], the level of exercise and/or the duration of the supine position should be noted.

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

COHb produces an acute and reversible decrease in $D_{L,CO}$ [57–60], largely due to the effects on CO back pressure and the ''anaemia effect'' from decreased Hb binding sites for CO from the test gas. As cigarette smoking is the most common source of COHb, subjects should be asked to refrain from smoking or other CO exposures on the day of the test. The time of the last cigarette smoked should be recorded and noted for the interpretation. A correction for CO back pressure should be made for recent or heavy cigarette smoking (see Adjustment for carboxyhaemoglobin concentration and CO back pressure section). Manufacturers are encouraged to provide the capability to do this easily.

Inspiratory manoeuvre

Once the mouthpiece and nose clip are in place, tidal breathing should be carried out for a sufficient time to assure that the subject is comfortable with the mouthpiece. Deep inspirations should be avoided during this period as they can increase subsequent CO uptake [61]. The $D_{L,CO}$ 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 should be limited to 6 s, a time consistent with using the forced expiratory volume in six seconds manoeuvre as a surrogate for VC [49]. 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 (*i.e.* less than the known VC) can affect CO uptake, depending upon whether it is a result of an initial suboptimal exhalation to RV (test performed at TLC) or whether it is due to a suboptimal inhalation from RV (test performed below TLC) [19–22]. In the former case, the calculated V_A and $D_{L,CO}$ will accurately reflect lung volume and the CO uptake properties of the lung at TLC. In the latter case, the V_A will be reduced and $D_{L,CO}$ measurement will be affected (see Adjustment for lung volume section).

Due to these effects, it is important that the V_I be as close to the known VC as possible. Data from a large patient population have shown that the V_I during $D_{L,CO}$ measurements averages ~90% of the VC [19], but that as many as 32% of subjects may

fall below this target [62]. A more recent study of >6,000 $D_{L,CO}$ measurements in a university laboratory demonstrated that 72, 86 and 92% of these patients could achieve V_I targets of 90, 85 and 80%, respectively, of the known VC [63]. Since it appears that V_I reductions of as much as 15% of the known VC will reduce the $D_{L,CO}$ <5% [19], a V_I target of 85% of the largest-known VC seems both reasonable and attainable.

The inspiration should be rapid, since the $D_{L,CO}$ calculations assume ''instantaneous'' lung filling [24, 64–70]. Slower lung filling decreases the amount of time the lung is at full inspiration with a consequent reduction in CO uptake. Although various sample timing techniques address the issue of lung filling and emptying time, it is still reasonable to expect that 85% of V_I should be inspired in <4.0 s. If longer inspiratory times are needed to achieve the 85% V_I goal, this should be noted on the test report.

Condition of the breath-hold and expiratory manoeuvre

Valsalva (expiratory efforts against a closed airway) and Muller manoeuvres (inspiratory efforts against a closed airway) during the breath-hold, by decreasing and increasing thoracic blood volume, respectively, will decrease and increase $D_{L,CO}$, respectively [29, 71, 72] 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 effort necessary. The breath-hold time should be 10 ± 2 s, a target easily achieved in the vast majority of subjects [62].

As with inspiration, the $D_{L,CO}$ calculation assumes instantaneous lung emptying [24, 64–69]. Although various sample timing techniques address the fact that emptying is not instantaneous, it is still reasonable to expect that the expiratory manoeuvre should be smooth, unforced, without hesitation or interruption, and total exhalation time should not exceed 4 s (with sample collection time <3 s). In subjects who require a longer expiratory time to provide an appropriate alveolar gas sample, the expiratory time should be noted in the test report. Common errors that can occur during the inspiration, breath-hold and expiration manoeuvres are given in figure 2.

Washout and sample collection volume

The $D_{L,CO}$ calculations (see Calculations section) require alveolar gas samples. During expiration, a volume of gas must be expired and discarded to clear anatomic and mechanical V_D before the alveolar sample is collected (fig. 1). Contamination of the alveolar gas sample with V_D gas will cause an underestimation of true CO uptake. In general, the washout volume should 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. Newer devices can provide a graphical display of exhaled gas concentrations to assure that V_D gas is not present in the alveolar sample (fig. 1). Using such an analyser, HUANG *et al.* [71] showed that the standard approach noted above adequately cleared V_D in >90% of adults.

The sample gas volume (V_S) is the volume of gas used to analyse alveolar CO and tracer gas concentrations at the end of the breath-hold. In subjects with good gas mixing and uniform ventilation and CO uptake properties, virtually any gas sample after V_D washout will be a good reflection of the lung as a whole. However, in subjects with poor gas mixing or marked sequential emptying of various lung regions, the gas sample collected will only reflect the properties of the regions contributing to that sample. V_S collection time will also affect the measurement of breath-hold time (see below). In order to standardise the collection process, a V_S of 0.50–1.00 L should be collected for analysis. In patients with VC <1 L, a V_S <0.50L may be used if it can be assured that the V_D has been cleared.

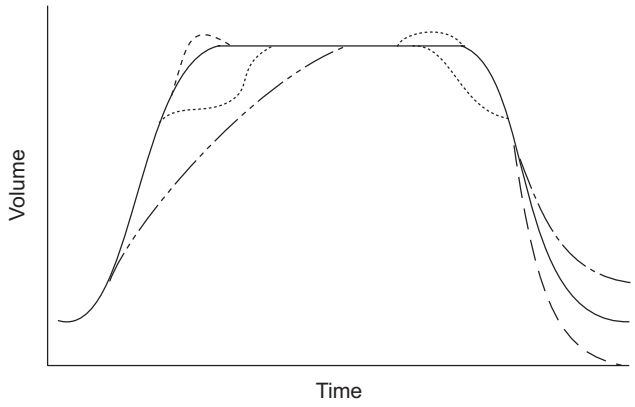

FIGURE 2. Potential problems with the single-breath diffusing capacity of the lung for carbon monoxide breathing manoeuvre that can lead to measurement errors. ·····: stepwise inhalation or exhalation; – - –: exhaled gas leak; – -- –: inhalation too slow; – – –: exhaled volume larger than inhaled volume; ------: transient overshoot from high flows and changing gas temperatures. Adapted from [2].

If continuous analysers with graphical displays are used, computerised or visual inspection of the expired CO and tracer gas curves may be used to adjust washout and the V_S if needed (fig. 1) [71]. These adjustments may be useful in subjects with VC <1 L who are unable to meet the minimum V_D washout and V_S recommended previously (*e.g.* paediatric patients, or adult patients with severe restrictive processes). These adjustments may also be useful in subjects with a large V_D in whom the recommended value range of 0.75–1.0 L is inadequate. For these adjustments to be achieved properly, the displays must represent actual gas concentrations that occurred at the mouth, synchronised for delays in gas transport and adjusted for gas-analyser response. In making such adjustments, the start of the V_S (end of the washout) must clearly be at a point where the tracer gas has started to plateau after the immediate fall from its inspiratory concentration, and the CO curve has ceased its immediate fall and started a smooth gradual decline (fig. 1). Furthermore, reports must indicate that manual adjustments were used to select washout volumes and V_S, so the interpreter can review and verify the adjustments.

Inspired gas composition

The test gases used to calculate $D_{L,CO}$ include a tracer gas to measure V_A, as well as CO. The remainder of the test gas mixture includes O_2 and N_2.

The tracer gas should be relatively insoluble and chemically and biologically inert. Since the tracer gas is used to determine the initial alveolar CO concentration, as well as the V_A from

which CO uptake is occurring, its gaseous diffusivity should be similar to CO. It should not interfere with the measurement of CO concentration. The tracer gas should not ordinarily be present in alveolar gas or else be present at a known, fixed concentration (*e.g.* argon).

Commonly used tracer gases are helium (He) and methane (CH_4). While He meets most of the previous criteria, its gaseous diffusivity is considerably higher than CO. CH_4 is commonly used as a tracer gas for systems that continuously sample expired gas. Its gaseous diffusivity is closer to CO, but it has a slightly higher liquid solubility than He. As new tracer gases are introduced, manufacturers should demonstrate that they produce V_A and $D_{L,CO}$ values equivalent to those measured using He, as this is the tracer gas that is used to derive most of the available reference equations.

The inspired CO should nominally be 0.3%. However, as ratios are more important than absolute values, exact concentrations are not critical. The assumption in calculating CO uptake is that capillary blood does not contain CO. Thus, corrections are needed in patients who have significant COHb (see Adjustment for COHb concentration and CO back pressure section).

Since P_{A,O_2} fluctuates over the ventilatory cycle [72] and can affect CO uptake by affecting θ, a more stable P_{A,O_2} during the $D_{L,CO}$ manoeuvre would seem desirable and, theoretically, can be achieved with a test gas fraction of inspired oxygen (F_{I,O_2}) of 0.17. Most current systems use either a F_{I,O_2} of 0.21 (with fractional concentrations of tracer gases such as CH_4 of <0.01), or gas mixtures containing CO and 10% He with ''balance air'' (an effective F_{I,O_2} of 0.19). Since $D_{L,CO}$ will increase 0.31 to 0.35% for each 0.133 kPa (1 mmHg) drop in P_{A,O_2} [73, 74], the increase in $D_{L,CO}$ that would be expected as the F_{I,O_2} is decreased from 0.21 to 0.17 (P_{A,O_2} decreased ~3.7 kPa (~28 mmHg)) is 8–9%. It is recommended that laboratories use gas mixtures with inspired oxygen partial pressure (P_{I,O_2}) values similar to the reference set used in the interpretation (table 4) [75–82], or make appropriate adjustments of measured or predicted $D_{L,CO}$ for the P_{I,O_2}.

TABLE 4 Inspired gas mixtures used during measurements of normal carbon monoxide (CO) uptake for commonly used reference equations

Author [Ref.]	Gas mixture#
Teculescu [75]	1.5% He, balance air (F_{I,O_2} 0.20)
Van Ganse [76]	14–15% He, balance air (F_{I,O_2} 0.18)
Frans [77]	10% He, 18% O_2
Crapo [78]	10% He, 25% O_2 (comparable to 21% at sea level)
Paoletti [79]	10% He, 20% O_2
Knudson [80]	10% He, 21% O_2
Roca [81]	13% He, 18% O_2
Huang [25]	0.3% CH_4, 0.3% C_2H_2, balance air (F_{I,O_2} 0.20)
Miller [82]	10% He, ?balance air

He: helium; F_{I,O_2}: inspired oxygen fraction; CH_4: methane; C_2H_2: acetylene. #: in addition to 0.3% CO.

By measuring $D_{L,CO}$ at several different levels of P_{A,O_2}, the two components of $D_{L,CO}$ (D_M and V_c) can be distinguished. This is accomplished by using the Roughton–Forster relationship noted previously (equation 2) and varying θ (the reaction rate of O_2 and Hb) by altering the P_{I,O_2}. Subsequently, $1/D_{L,CO}$ is plotted against $1/\theta$ at the different P_{I,O_2} levels. The slope of this relationship is $1/V_c$ and the intercept is $1/D_M$.

Interval between tests

At least 4 min should be allowed between tests to allow an adequate elimination of test gas from the lungs. The subject should remain seated during this interval. In patients with obstructive airway disease, a longer period (*e.g.* 10 min) should be considered. Several deep inspirations during this period may help to clear test gases more effectively. If continuous monitoring of expired gas concentrations is available, the washout of tracer gas from the previous test may be confirmed by observing end-tidal gas concentrations before beginning the next test.

Miscellaneous factors

There may be diurnal variation in $D_{L,CO}$, since one study has found that $D_{L,CO}$ fell 1.2–2.2% per hour throughout the day [83]. The reason for the change was not clear and was not explained by CO back pressure or changes in V_A, V_I or breath-hold time. One explanation is a combination of changes in CO back pressure and diurnal variation in Hb concentration [84]. A 13% change in $D_{L,CO}$ during the menstrual cycle has been reported [85]. The highest value was observed just before the menses, and the lowest was on the third day of menses. It is not clear, however, 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 $D_{L,CO}$ [86]. The mechanisms involved are not clear, although it is known that some fuel-cell CO analysers are sensitive to exhaled ethanol and ketones. In obstructive lung disease subjects, after administration of a bronchodilator, $D_{L,CO}$ may increase up to 6% [87]. Bronchodilators can affect V_A, vasomotor tone, *etc.*, and their use prior to testing could conceivably optimise these factors. Use of a bronchodilator should be noted in the interpretation [88].

CALCULATIONS

The transfer factor or diffusing capacity for a gas in the lungs (D_L) equals its rate of exchange across the lung divided by its transfer gradient:

$$D_L = \text{rate of gas uptake} / \text{transfer pressure gradient} \quad (3)$$

The rate of gas uptake is expressed in mL STPD·min^{-1}, and the transfer gradient (the difference between alveolar and pulmonary capillary pressures) in mmHg. Thus, $D_{L,CO}$ has traditional units of mL STPD·min^{-1}·mmHg^{-1} (SI units of mmol·min^{-1}·kPa^{-1}). For CO, the pulmonary capillary CO tension is near zero and thus:

$$\begin{aligned} D_{L,CO} &= \text{total CO uptake over time} / P_{A,CO} \\ &= \Delta[CO] \times V_A / \Delta t / P_{A,CO} \end{aligned} \quad (4)$$

The single-breath $D_{L,CO}$ technique assumes that both CO and the tracer gas (Tr) are diluted comparably on inspiration. Thus,

the initial alveolar partial pressure of CO ($P_{A,CO,0}$) can be calculated by knowing the inspired tracer gas fraction ($F_{I,Tr}$) and fraction alveolar tracer gas ($F_{A,Tr}$):

$$F_{A,CO,0} = F_{I,CO} \times F_{A,Tr}/F_{I,Tr} \quad (5)$$

$$P_{A,CO,0} = P_B \times F_{A,CO,0} \quad (6)$$

where $F_{A,CO,0}$ is the initial alveolar inspired CO fraction, $F_{I,CO}$ is the inspired CO fraction, P_B is the barometric pressure and $F_{A,CO,0}$ is the initial alveolar CO fraction.

Tracer gas dilution is also used to determine the effective V_A as described below. Solving for $D_{L,CO}$ thus yields the equation:

$$D_{L,CO} = (V_A/(t/60 \times (P_B - P_{H_2O})) \times \ln((F_{A,Tr} \times F_{I,CO})/(F_{I,Tr} \times F_{A,CO})) \quad (7)$$

where V_A is in mL STPD, t is breath-hold time in seconds, and P_{H_2O} is water vapour pressure.

Calculating breath-hold time

The ''breath-hold time'' or time of transfer during which CO changes from its initial to final concentration is in the denominator of the $D_{L,CO}$ equation (equation 7). As noted previously, the single-breath measurement of CO 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 by JONES and MEADE [68] (fig. 3) 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 [64].

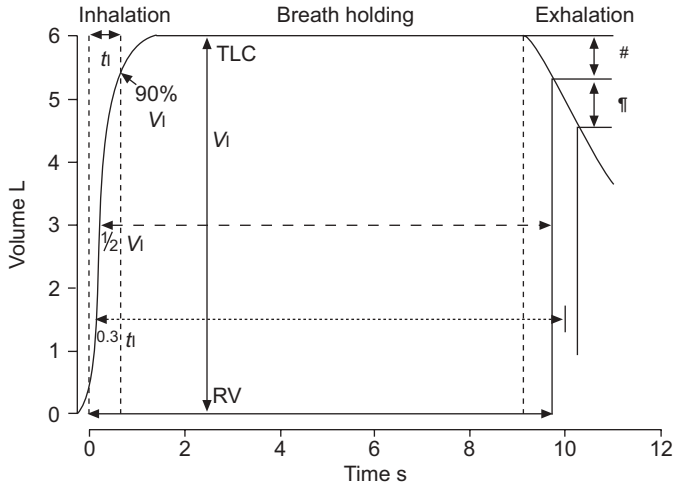

FIGURE 3. Schematic illustration of different methods of measuring breath-hold time for the single-breath diffusing capacity of the lung for carbon monoxide. The method by OGILVIE (———) [48] measures breath-hold time from the beginning of inspiration to the beginning of alveolar sample collection. The method by JONES and MEADE (·····) [68] includes 0.70 of inspiratory time and half of sample time. The Epidemiologic Standardization Project (– – – –) measures breath-hold time from the time of 50% of inspired volume (*VI*) to the beginning of alveolar sample collection. *tI*: time of inspiration (-----; defined from the back-extrapolated time 0 to the time that 90% of the *VI* has been inhaled); TLC: total lung capacity; RV: residual volume. #: dead space washout; ¶: sample collection. Adapted from [1].

With the approach taken by JONES and MEADE [68], breath-hold time equals the time starting from 0.3 of the inspiratory time to the middle of the sample collection time. As in spirometry, the back-extrapolation technique should be used to establish time zero [48, 49]. The time when 90% of the *VI* has been inspired is a reasonable end point for defining inspiratory time (fig. 3).

A theoretically more accurate way to account for volume changes over time during inspiration and expiration is to use three separate equations for $D_{L,CO}$ during inspiration, breath hold and expiration (the ''three-equation'' technique) [24, 64]. This algorithm is commercially available and may be particularly useful in subjects unable to rapidly fill or empty their lungs. However, clinical experience with this approach is limited.

Other breath-hold timing algorithms may be appropriate in maintaining consistency (*e.g.* longitudinal studies), but these measurements should be recognised as less suitable recommendations.

Calculating the alveolar volume

V_A represents an estimate of lung gas volume into which CO is distributed and then transferred across the alveolar capillary membrane [3, 4]. Thus, it is critical in the measurement of $D_{L,CO}$. As noted previously, V_A is measured simultaneously with CO uptake by calculating the dilution of an inert Tr. For normal subjects, this calculated single-breath determination of V_A ($V_{A,sb}$) plus estimated V_D closely matches TLC determined by plethysmography [19, 70]. However, poor gas mixing in patients with maldistribution of inspired volume (*e.g.* obstructed airways patients) can markedly reduce Tr dilution and, thus, lead to values for $V_{A,sb}$ that are markedly less than a V_A determined from the actual total thoracic gas volume (V_{TG}). The observed CO uptake is also affected by poor gas mixing under these conditions, and will primarily reflect the CO transfer properties of the regions into which the test gas is distributed. It has been suggested that a separately determined V_A from a more accurate technique (*e.g.* multiple-breath technique ($V_{A,mb}$) or plethysmography ($V_{A,plethys}$)) could be substituted for $V_{A,sb}$ under these conditions to ''correct'' for the effects of maldistribution. However, the $D_{L,CO}$ calculation (equations 4 and 7) is based on the volume of gas into which the Tr (and CO) distributes, and not the total V_{TG}. Moreover, substituting a larger, separately determined $V_{A,mb}$ or $V_{A,plethys}$ assumes that D_M and V_c properties in the unmeasured lung regions are similar to those in the measured lung regions, an assumption that is difficult to justify. Due to these considerations, a separately measured $V_{A,mb}$ or $V_{A,plethys}$ should not be substituted for $V_{A,sb}$. Instead, when the $V_{A,sb}$ is markedly less than a separately determined $V_{A,mb}$ or $V_{A,plethys}$, this should be reported and the ratio of $V_{A,sb}$ to $V_{A,mb}$ or $V_{A,plethys}$ reported. For the subsequent interpretation of $D_{L,CO}$, it should then be noted that the maldistribution of inspired gas probably contributed to any observed reduction in measured $D_{L,CO}$.

The volume of distribution for the tracer gas can be determined from values for V_I, $F_{I,Tr}$ and $F_{A,Tr}$, and knowing the conditions of the inspired and expired gases. Since the amount of tracer gas in the lung (alveolar plus dead space) equals the amount of inspired tracer gas, and the dead space tracer gas fraction is the same as the inspired fraction (all expressed at BTPS):

$$V_I \times F_{I,Tr} = V_A \times F_{A,Tr} + V_D \times F_{I,Tr} \quad (8)$$

$$V_A = V_I - V_D \times (F_{I,Tr}/F_{A,Tr}) \quad (9)$$

Although V_A is usually expressed under BTPS conditions, it must be converted to STPD conditions to calculate $D_{L,CO}$ in equation 7.

It is essential that V_D is considered in the calculation of V_A. V_D occurs in two areas: instrument V_D (*i.e.* volume of the mouthpiece, filters and connections within the valving system); and anatomic V_D (*i.e.* the volume in the conducting airways that does not participate in gas exchange). Instrument V_D should be specified by the manufacturer, but may vary as the user alters the system (*e.g.* addition of a filter).

There are various methods to estimate anatomic V_D. Examples include a fixed value of 150 mL [1] (although this does not work well for small adults or children), and another of 2.2 mL × kg body weight [47] (although this does not work well for very obese subjects). In studies deriving the commonly used reference equations (table 4), the most commonly used technique was to assume 2.2 mL × kg body weight. However, some investigators ignored anatomic V_D [79, 80, 82], and one used age+2.2mL × kg body weight [78]. If the body mass index is <30, the current authors recommend using an estimate for anatomic V_D of 2.2 mL × kg body weight. In more obese subjects or if the weight is unknown, V_D (mL) can be estimated using the following equation:

$$V_D = 24 \times \text{height} \times \text{height}/4545 \quad (10)$$

where height is measured in cm, or:

$$V_D = 24 \times \text{height} \times \text{height}/703 \quad (11)$$

where height is measured in inches.

In single-sample systems, the sample-bag residual volume (sometimes called a sample-bag dead space) dilutes the sample gas and alters the measured concentrations of 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 V_{SRV} gas content. V_{SRV} could contain test gas, room air or expired gas from a subject (after a $D_{L,CO}$ test). When V_{SRV} contains room air, its effect is to reduce the measured concentrations of expired gases. The following equation adjusts for this:

$$\text{Adjusted } F_{A,Tr} = \text{measured } F_{A,Tr} \times (V_S/(V_S - V_{SRV})) \quad (12)$$

Estimates of the potential change in $D_{L,CO}$ in existing systems when no adjustment is made for sample-bag dead space range from 0.3–8%, depending on sample-bag size and V_{SRV} [89].

Manufacturers should report instrument and sample-bag dead space. Both of these must be flushed with room air (or, if D_M and V_c are to be calculated, appropriate levels of oxygen) before the single-breath manoeuvre so that it will not contain expiratory gas from a previous subject. V_{SRV} should be <2% of the V_S or 10 mL, whichever is larger.

Inspired gas conditions

Though inspired gas is often assumed to be measured at ambient temperature and pressure, saturated with water vapour conditions, this is only true in systems in which the test gas is transferred to a water-sealed spirometer before it is inspired. In most cases, the test gas inspired from a bag-in-box system, through a pneumotachometer from a bag, or a compressed gas cylinder with a demand valve is a dry gas (<10 ppm H_2O) and, thus, at ambient temperature and pressure, dry conditions. The inspired volume needs to be converted to BTPS conditions to use in equations 7, 8 and 9. It is recommended the V_I (BTPS) be reported, and manufacturers should specify and document inspired gas conditions for each instrument.

CO_2, H_2O and temperature adjustment for V_A calculations

Exhaled gas contains CO_2 and H_2O, 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, and this will raise both CO and tracer gas concentrations. Under these circumstances, adjustments are required for the increase in $F_{A,Tr}$ to calculate V_A (table 5). However, no adjustment for the increase in alveolar inspired CO fraction at time t ($F_{A,CO,t}$) and $F_{A,Tr}$ is necessary in calculating the rate of CO uptake, since the concentration factor appears in both the numerator and the denominator of the expression ($F_{A,CO,0}/F_{A,CO,t}$) and therefore cancels.

Exhaled gas is initially at body temperature. Some systems allow this to cool (gas volume contracts), whereas others will provide heat to maintain the temperature. Adjustments to BTPS conditions may be required depending upon the system design (table 5).

All of these adjustments should be documented by the manufacturer for their particular system.

EVALUATING THE MEASUREMENT OF $D_{L,CO}$

Acceptability, repeatability and number of tests

Acceptable tests are defined in table 6. Repeatability describes the variability on repeated testing with no change in test conditions [90, 91]. In a large university-based laboratory study, a coefficient of variation of repeated measurements in normal subjects was 3.1%, and this increased only slightly (from 4.0 to 4.4%) in patients with abnormal spirometry patterns [63]. In contrast, an inter-session $D_{L,CO}$ variability of up to 9% (reproducibility) has been documented in normal individuals in repeated measurements over a period of 1 yr [92].

Since most intra-session variability is technical rather than physiological, the mean of acceptable tests is reasonable to report. In this report, there should be at least two acceptable tests that meet the repeatability requirement of either being within 3 mL CO (STPD)·min^{-1}·mmHg^{-1} (or 1 mmol·min^{-1}·kPa^{-1}) of each other or within 10% of the highest value. In a large university-based laboratory study, >95% of the patients could meet this criteria [63].

TABLE 5 Corrections for barometric pressure (P_B), ambient water vapour pressure (P_{H_2O}), partial pressure of CO_2 and temperature

H_2O removed from sampled gas; CO_2 does not interfere with analysers

$V_{A,BTPS} = (V_{I,ATPD} - V_{D,INST} - V_{D,ANAT}) \times (F_{I,Tr}/F_{S,Tr}) \times (P_B/(P_B - 47)) \times (310/(273+T))$

$V_{A,STPD} = (V_{I,ATPD} - V_{D,INST} - V_{D,ANAT}) \times (F_{I,Tr}/F_{S,Tr}) \times (P_B/760) \times (273/(273+T))$

H_2O and CO_2 removed from sampled gas

$V_{A,BTPS} = (V_{I,ATPD} - V_{D,INST} - V_{D,ANAT}) \times (F_{I,Tr}(1+F_{A,CO_2})/F_{S,Tr}) \times (P_B/(P_B - 47)) \times (310/(273+T))$

$V_{A,STPD} = (V_{I,ATPD} - V_{D,INST} - V_{D,ANAT}) \times (F_{I,Tr}(1+F_{A,CO_2})/F_{S,Tr}) \times (P_B/760) \times (273/(273+T))$

If no measurement of F_{A,CO_2} is available, then it may be assumed to be 0.05

H_2O in sampled gas equilibrated to room air; CO_2 does not interfere with analysers. If $F_{I,Tr}$ is read by the analysers, the equations are the same as for when H_2O is removed from sampled gas. If tank values (*i.e.* dry gas concentrations) are used for $F_{I,Tr}$, then the following equations are used

$V_{A,BTPS} = (V_{I,ATPD} - V_{D,INST} - V_{D,ANAT}) \times (F_{I,Tr}/F_{S,Tr}) \times ((P_B - P_{H_2O})/(P_B - 47)) \times (310/(273+T))$

$V_{A,STPD} = (V_{I,ATPD} - V_{D,INST} - V_{D,ANAT}) \times (F_{I,Tr}/F_{S,Tr}) \times ((P_B - P_{H_2O})/760) \times (273/(273+T))$

Neither H_2O nor CO_2 removed from sampled gas, no interference with analysers, heated sample tubing to prevent condensation

$V_{A,BTPS} = (V_{I,ATPD} - V_{D,INST} - V_{D,ANAT}) \times (F_{I,Tr}/F_{S,Tr}) \times (310/(273+T))$

$V_{A,STPD} = (V_{I,ATPD} - V_{D,INST} - V_{D,ANAT}) \times (F_{I,Tr}/F_{S,Tr}) \times ((P_B - 47)/760) \times (273/(273+T))$

In these calculations, room temperature (T) is measured in Celsius and gas pressures are measured in mmHg. In all four cases, the inspired volume (V_I) is the measured volume of inhaled dry gas and, thus, is considered under ambient temperature, ambient pressure, and dry (ATPD) conditions. The conversion to body temperature, ambient pressure, saturated with water vapour (BTPS) and standard temperature, pressure and dry (STPD) may require factors to compensate for the diluting or concentrating effects of adding or deleting H_2O or CO_2 at the gas sampling site. Therefore, standard gas condition conversion formulae must be adjusted as described previously. V_A: alveolar volume; $V_{D,INST}$: instrument dead space; $V_{D,ANAT}$: anatomic dead space; $F_{I,Tr}$: fraction of tracer (Tr) gas in the inspired test gas; $F_{S,Tr}$: fraction of the Tr gas in the alveolar sample, which may differ from the fraction of alveolar Tr gas, depending on the effects of CO_2 and H_2O as noted; F_{A,CO_2}: fraction of CO_2 in the alveolar sample.

TABLE 6 Acceptable test criteria for diffusing capacity of the lung for carbon monoxide

Use of proper quality-controlled equipment
V_I of >85% of largest VC in <4 s#
A stable calculated breath hold for 10±2 s. There should be no evidence of leaks, or Valsalva or Mueller manoeuvres
Expiration in <4 s (and sample collection time <3 s)#, with appropriate clearance of V_D and proper sampling/analysis of alveolar gas

V_I: inspired volume; VC: vital capacity; V_D: dead space. #: tests outside these timing limits might still have clinical utility, but these deviations from standard acceptability criteria should be noted and possible impact/correction factors considered.

The average of at least two acceptable tests that meet this repeatability requirement should be reported (*i.e.* outliers excluded). While it is recommended that at least two $D_{L,CO}$ tests should be performed, research is needed to determine the actual number of tests required to provide a reasonable estimate of average $D_{L,CO}$ value for a given person. As noted below, five tests will increase COHb by ~3.5% [84], which will decrease the measured $D_{L,CO}$ by ~3–3.5%. Thus, more than five tests are not recommended at the present time.

Adjustments to the measurement of $D_{L,CO}$ prior to interpretation

$D_{L,CO}$ depends upon a number of physiological factors. Besides varying with age, sex, height and possibly race, $D_{L,CO}$ also changes with Hb, lung volume, COHb, P_{I,O_2} (*e.g.* altitude), exercise and body position. Although these effects may cause changes in $D_{L,CO}$ in opposite directions [93], all should be considered in interpreting the observed CO uptake. Moreover, specific adjustments for three of these factors (Hb, COHb and P_{I,O_2}) should always be made to ensure appropriate interpretation (see below). Consideration could also be given to adjust for a submaximal inspiration resulting in a less than expected V_A.

Adjustment for haemoglobin

Since CO–Hb binding is such an important factor in CO transfer, $D_{L,CO}$ changes can be substantial as a function of Hb concentration [93–97]. The empirical change in $D_{L,CO}$ with Hb change closely matches what is expected from a theoretical approach using the relationship in equation 2, with θ assumed to be proportional to the Hb, $D_M/\theta V_c$ is assumed to be 0.7 [96], and the ''standard'' Hb value is assumed to be 14.6 $g \cdot dL^{-1}$ (9 $mmol \cdot l^{-1}$ SI) in adult males and adolescents and 13.4 $g \cdot dL^{-1}$ (8.26 $mmol \cdot l^{-1}$ SI) in adult females and children <15 yrs. Using these relationships and expressing Hb in $g \cdot dL^{-1}$, the equation for adjusting predicted $D_{L,CO}$ in adolescents and adult males is:

$$D_{L,CO,predicted}\ \text{for Hb} = D_{L,CO,predicted} \times (1.7\,\text{Hb}/(10.22 + \text{Hb})) \quad (13)$$

The equation for adjusting predicted $D_{L,CO}$ in children <15 yrs of age and females is:

$$D_{L,CO,predicted}\ \text{for Hb} = D_{L,CO,predicted} \times (1.7\,\text{Hb}/(9.38 + \text{Hb})) \quad (14)$$

Results from a more recent study in patients with a wide range of Hb abnormalities [97] showed a slightly greater and more

linear relationship, but corrected values were generally consistent with equations 13 and 14.

Adjustments for P_{A,O_2}

As noted previously, P_{A,O_2} affects the measurement of $D_{L,CO}$. P_{A,O_2} changes will occur as a consequence of supplemental O_2 breathing (higher P_{A,O_2}) or performing $D_{L,CO}$ assessments at altitude (lower P_{A,O_2}). As mentioned before, $D_{L,CO}$ will change by ~0.35% per mmHg change in P_{A,O_2} [73, 74] or by ~0.31% per mmHg decrease in P_{I,O_2}. Adjustments to the predicted $D_{L,CO}$ in a subject on supplemental O_2 may be made using a measured P_{A,O_2} and assuming a normal P_{A,O_2} on room air at a sea level of 100 mm Hg, as follows:

$$D_{L,CO,predicted}\ \text{for elevated}\ P_{A,O2} = D_{L,CO,predicted}/(1.0+0.0035(P_{A,O2}-100)) \quad (15)$$

If the adjustment is being made for altitude, assuming a P_{I,O_2} of 150 mmHg at sea level:

$$D_{L,CO,predicted}\ \text{for altitude} = D_{L,CO,predicted}/(1.0+0.0031(P_{I,O2}-150)) \quad (16)$$

Adjustment for COHb concentration and CO back pressure

COHb can affect the measured uptake in the following two ways [98–100]. First, by occupying Hb binding sites, CO produces an ''anaemia effect''. Secondly, CO partial pressure in the blood will reduce the driving pressure for CO transport from alveolar gas to capillary blood.

Exposure to ordinary environmental CO and endogenous production of CO as a byproduct of Hb catabolism commonly results in measured COHb levels of 1–2% [98]. The 1–2% baseline COHb levels that are attributable to endogenous production of CO and ordinary environmental exposures are already incorporated into reference values based on healthy nonsmoking subjects. Cigarette smoke and other environmental sources, however, can produce measurable levels of CO back pressure and COHb that may need to be considered in the measurement of CO uptake [99]. Small increases in COHb also occur when CO is inspired in the $D_{L,CO}$ test. FREY *et al*. [84], for example, found that COHb increased by ~0.7% with each single-breath $D_{L,CO}$ test.

CO back pressure can be measured in expired gas before a $D_{L,CO}$ manoeuvre or estimated using one of several available techniques [100–103]. For example, CO back pressure can be calculated from COHb from the following equation:

$$\text{alveolar}\ [CO] = (COHb/O_2Hb) \times (\text{alveolar}\ [O_2])/210 \quad (17)$$

$D_{L,CO}$ can then be recalculated after subtracting the estimated CO back pressure from both the initial and final alveolar CO. Units must be consistent before making the subtraction. However, this method will not adjust $D_{L,CO}$ for the ''anaemia'' effect of COHb.

Several studies have evaluated both the empirical and theoretical effects of COHb on $D_{L,CO}$ and incorporated both the back pressure and the ''anaemia'' effects of COHb. In general, a 1% increase in COHb reduces the measured $D_{L,CO}$ by ~0.8–1% from both effects [13, 14]. Using this approach, the following equation empirically reduces predicted $D_{L,CO}$ by 1% for each per cent COHb >2%:

$$D_{L,CO,predicted}\ \text{for COHb} = D_{L,CO,predicted} \times (102\% - \text{COHb}\%) \quad (18)$$

An adjustment for COHb is not required, but is recommended for interpretative purposes when COHb is elevated/suspected. No adjustment is required if COHb <2%, since reference equations already incorporate this.

Adjustment for lung volume

As noted previously, $D_{L,CO}$ decreases as the lung deflates as a function of both membrane and capillary configuration changes [17–24, 104–111]. The relationship is complex, however, and is probably nonlinear [108, 110]. In normal subjects with experimental reductions in V_I (and, thus, V_A), adjustment equations for this effect have been derived [18, 19, 109, 111] and a recent representative example consists of the following:

$$D_{L,CO}\ (\text{at}\ V_{Am}) = D_{L,CO}\ (\text{at}\ V_{Ap}) \times (0.58+0.42(V_{Am}/V_{Ap})) \quad (19)$$

$$K_{CO}\ (\text{at}\ V_{Am}) = K_{CO}\ (\text{at}\ V_{Ap}) \times (0.42+0.58/(V_{Am}/V_{Ap})) \quad (20)$$

where V_{Am} represents measured V_A and V_{Ap} represents predicted V_A at normal TLC.

It should be noted that this $D_{L,CO}$ adjustment for a reduced V_I (and V_A) from a submaximal effort is substantially less than a 1:1 $D_{L,CO}/V_A$ adjustment (*i.e.* the fall in $D_{L,CO}$ as lung volumes are reduced is much less than the fall in V_A). As a consequence, the $D_{L,CO}/V_A$ ratio will rise with a reduced V_I from a submaximal effort. Thus, if this ratio is used to adjust (''correct'') $D_{L,CO}$ for the effects of a reduced V_A from a submaximal V_I, it will markedly ''overcorrect''.

It is important to emphasise that the V_A effects on $D_{L,CO}$ discussed above were derived from studies in normal subjects with submaximal V_I. These V_A effects (and consequent $D_{L,CO}$ adjustments for V_A) have not been validated in lung diseases where lung pathology has reduced CO uptake properties, as well as V_I and V_A. In some of these diseases (*e.g.* status post-pneumonectomy), the reduction in $D_{L,CO}$ may be less than the reduction in V_A (high $D_{L,CO}/V_A$); in others (*e.g.* pulmonary vascular disease), the reduction in $D_{L,CO}$ may be greater than the reduction in V_A (low $D_{L,CO}/V_A$) [17]. In many disease states, however, the ratio of pathological reductions in $D_{L,CO}$ and V_A may be quite variable and of unclear physiological or clinical significance. Thus, although the $D_{L,CO}/V_A$ relationship can be used to describe the relative reductions in CO uptake properties and alveolar gas volumes in lung disease [17, 19, 107, 112], drawing more specific clinical or pathological conclusions based upon V_A (or any other volume) adjustments should be made with caution. This is especially true if the adjustment leads to the implication that CO uptake properties of the lung are normal. Further study is clearly needed on the interactions of CO uptake and alveolar gas volume in lung disease before more specific volume-adjustment recommendations can be made.

Reporting values

Several values are measured with the single-breath $D_{L,CO}$ and many factors affect $D_{L,CO}$. It is important that the report

includes the results needed for optimal interpretation. The average of at least two acceptable tests should be reported (*i.e.* outliers excluded).

The report should always include the unadjusted measured $D_{L,CO}$, the predicted and per cent predicted $D_{L,CO}$, and the predicted and per cent predicted $D_{L,CO}/V_A$ (K_{CO}). Any adjustments (*e.g.* for Hb, COHb, P_{I,O_2}, or lung volume) should also be reported along with the data used to make the adjustment. The average V_A should be reported along with the predicted V_A (the predicted TLC minus predicted V_D) and per cent predicted V_A. The average V_I should also be noted. If a separately measured VC is available, it should be reported to serve as a reference for the adequacy of the V_I. In addition, comments relevant to the quality of the measurements should be included.

ABBREVIATIONS

Table 7 contains a list of abbreviations and their meanings, which will be used in this series of Task Force reports.

TABLE 7 List of abbreviations and meanings

ATPD	Ambient temperature, ambient pressure, and dry
ATPS	Ambient temperature and pressure saturated with water vapour
BTPS	Body temperature (*i.e.* 37°C), ambient pressure, saturated with water vapour
C	Centigrade
CFC	Chlorofluorocarbons
cm	Centimetres
COHb	Carboxyhaemoglobin
$D_{L,CO}$	Diffusing capacity for the lungs measured using carbon monoxide, also known as transfer factor
$D_{L,CO}/V_A$	Diffusing capacity for carbon monoxide per unit of alveolar volume, also known as K_{CO}
D_M	Membrane-diffusing capacity
DT	Dwell time of flow >90% of PEF
EFL	Expiratory flow limitation
ERV	Expiratory reserve volume
EV	Back extrapolated volume
EVC	Expiratory vital capacity
$F_{A,X}$	Fraction of gas X in the alveolar gas
$F_{A,X,t}$	Alveolar fraction of gas X at time t
$FEF_{25–75\%}$	Mean forced expiratory flow between 25% and 75% of FVC
$FEF_{X\%}$	Instantaneous forced expiratory flow when X% of the FVC has been expired
FEV_1	Forced expiratory volume in one second
FEV_t	Forced expiratory volume in t seconds
$F_{E,X}$	Fraction of expired gas X
$FIF_{X\%}$	Instantaneous forced inspiratory flow at the point where X% of the FVC has been inspired
$F_{I,X}$	Fraction of inspired gas X
FIVC	Forced inspiratory vital capacity
FRC	Functional residual capacity
FVC	Forced vital capacity
H_2O	Water
Hb	Haemoglobin
Hg	Mercury
Hz	Hertz; cycles per second
IC	Inspiratory capacity
IRV	Inspiratory reserve volume
IVC	Inspiratory vital capacity
K_{CO}	Transfer coefficient of the lung (*i.e.* $D_{L,CO}/V_A$)
kg	Kilograms
kPa	Kilopascals
L	Litres
$L \cdot min^{-1}$	Litres per minute
$L \cdot s^{-1}$	Litres per second
lb	Pounds weight
$MEF_{X\%}$	Maximal instantaneous forced expiratory flow where X% of the FVC remains to be expired
MFVL	Maximum flow–volume loop
mg	Milligrams
MIF	Maximal inspiratory flow
mL	Millilitres
mm	Millimetres
MMEF	Maximum mid-expiratory flow
ms	Milliseconds
MVV	Maximum voluntary ventilation
P_{A,O_2}	Alveolar oxygen partial pressure
P_B	Barometric pressure
PEF	Peak expiratory flow
P_{H_2O}	Water vapour partial pressure
P_{I,O_2}	Inspired oxygen partial pressure
θ (theta)	Specific uptake of CO by the blood
RT	Rise time from 10% to 90% of PEF
RV	Residual volume
s	Seconds
STPD	Standard temperature (273 K, 0°C), pressure (101.3 kPa, 760 mmHg) and dry
TB	Tuberculosis
TGV (or V_{TG})	Thoracic gas volume
t_I	Time taken for inspiration
TLC	Total lung capacity
Tr	Tracer gas
t_{tot}	Total time of respiratory cycle
TV (or V_T)	Tidal volume
V_A	Alveolar volume
$V_{A,eff}$	Effective alveolar volume
VC	Vital capacity
V_c	Pulmonary capillary blood volume
V_D	Dead space volume
V_I	Inspired volume
V_S	Volume of the expired sample gas
μg	Micrograms

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REFERENCES

1 American Thoracic Society. Single-breath carbon monoxide diffusing capacity (transfer factor). Recommendations for a standard technique: 1995 update. *Am J Respir Crit Care Med* 1995; 152: 2185–2198.
2 Cotes JE, Chinn DJ, Quanjer PH, Roca J, Yernault JC. Standardization of the measurement of transfer factor (diffusing capacity). Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. *Eur Respir J* 1993; 6: Suppl. 16, 41–52.
3 Krogh M. The diffusion of gases through the lungs of man. *J Physiol (Lond)* 1914; 49: 271–300.
4 Forster RE. Exchange of gases between alveolar air and pulmonary capillary blood: pulmonary diffusing capacity. *Physiol Rev* 1957; 37: 391–452.
5 MacIntyre NR. Diffusing capacity of the lung for carbon monoxide. *Respir Care* 1989; 34: 489–499.
6 Crapo RO, Forster RE. Carbon monoxide diffusing capacity. *Clin Chest Med* 1989; 10: 187–198.
7 Wilson AF, Hearne J, Brennen M, Alfonso R. Measurement of transfer factor during constant exhalation. *Thorax* 1994; 49: 1121–1126.
8 Leathart GL. Steady-state diffusing capacity determined by a simplified method. *Thorax* 1962; 17: 302–307.
9 Meyer M, Scheid P, Riepl G, Wagner H-J, Piiper J. Pulmonary diffusing capacities for CO_2 and CO measured by a rebreathing technique. *J Appl Physiol* 1981; 51: 1643–1650.
10 Weibel ER. Morphometric estimation of pulmonary diffusion capacity. I. Model and method. *Respir Physiol* 1971; 11: 54–75.
11 Forster RE, Fowler WS, Bates DV, Van Lingen B. The absorption of carbon monoxide by the lungs during breath-holding. *J Clin Invest* 1954; 33: 1135–1145.
12 MacIntyre NR, Leatherman NE, Deitz JL, Wagner R, Friedman M. Distribution and uptake of helium, carbon monoxide and acetylene in the lungs during high frequency oscillatory ventilation. *Respir Physiol* 1986; 63: 201–212.
13 Comroe JH Jr. Pulmonary diffusing capacity for carbon monoxide (DLCO). *Am Rev Respir Dis* 1975; 111: 225–240.
14 Roughton FJW, Forster RE. Relative importance of diffusion and chemical reaction rates in determining rate of exchange of gases in the human lung, with special reference to true diffusing capacity of pulmonary membrane and volume of blood in the lung capillaries. *J Appl Physiol* 1957; 11: 290–302.
15 Michaelson ED, Sackner MA, Johnson RL. Vertical distribution of pulmonary diffusing capacity and capillary blood flow in man. *J Clin Invest* 1973; 52: 359–365.
16 MacIntyre NR, Nadel JA. Regional diffusing capacity in normal lungs during a slow exhalation. *J Appl Physiol* 1982; 52: 1487–1492.
17 Hughes JMB, Pride NB. In defense of the carbon monoxide transfer coefficient KCO (TL/VA). *Eur Respir J* 2001; 17: 168–174.
18 Stam H, Versprille A, Bogaard JM. The components of the carbon monoxide diffusing capacity in man dependent on alveolar volume. *Bull Eur Physiopath Respir* 1983; 19: 17–22.
19 Johnson DC. Importance of adjusting carbon monoxide diffusing capacity (DLCO) and carbon monoxide transfer coefficient (KCO) for alveolar volume. *Respir Med* 2000; 94: 28–37.
20 Filley GF, MacIntosh DJ, Wright GW. Carbon monoxide uptake and pulmonary diffusing capacity in normal subjects at rest and during exercise. *J Clin Invest* 1954; 33: 530–539.
21 Leech JA, Martz L, Liben A, Becklake MR. Diffusing capacity for carbon monoxide: the effects of different durations of breath-hold time and alveolar volume and of carbon monoxide back pressure on calculated results. *Am Rev Respir Dis* 1985; 132: 1127–1129.
22 McGrath MW, Thomson ML. The effect of age, body size and lung volume change on alveolar-capillary permeability and diffusing capacity in man. *J Physiol (Lond)* 1959; 146: 572–582.
23 Newth CJL, Cotton DJ, Nadel JA. Pulmonary diffusing capacity measured at multiple intervals during a single exhalation in man. *J Appl Physiol* 1977; 43: 617–623.
24 Graham BL, Dosman JA, Cotton DJ. A theoretical analysis of the single breath diffusing capacity for carbon monoxide. *IEEE Trans Biomed Eng* 1980; 27: 221–227.
25 Huang YC, Helms MI, MacIntyre NR. Normal values for single exhalation diffusing capacity and pulmonary capillary blood flow in sitting, supine positions and during mild exercise. *Chest* 1994; 105: 501–508.
26 Stam H, Kreuzer FJA, Versprille A. Effect of lung volume and positional changes on pulmonary diffusing capacity and its components. *J Appl Physiol* 1991; 71: 1477–1488.
27 Stokes DL, MacIntyre NR, Nadel JA. Non-linear increases in diffusing capacity during exercise by seated and supine subjects. *J Appl Physiol* 1981; 51: 858–863.
28 Johnson RL, Spicer WS, Bishop JM, Forster RE. Pulmonary capillary blood volume, flow and diffusing capacity during exercise. *J Appl Physiol* 1960; 15: 893–902.
29 Smith TC, Rankin J. Pulmonary diffusing capacity and the capillary bed during Valsalva and Muller maneuvers. *J Appl Physiol* 1969; 27: 826–833.
30 Cotes JE, Snidal DP, Shepard RH. Effect of negative intra-alveolar pressure on pulmonary diffusing capacity. *J Appl Physiol* 1960; 15: 372–376.
31 Cotton DJ, Mink JT, Graham BL. Effect of high negative inspiratory pressure on single breath CO diffusing capacity. *Respir Physiol* 1983; 54: 19–29.

32 Cotton DJ, Prabhu MB, Mink JT, Graham BL. Effects of ventilation inhomogeneity on DLCO SB-3EQ in normal subjects. *J Appl Physiol* 1992; 73: 2623–2630.

33 Cotton DJ, Prabhu MB, Mink JT, Graham BL. Effect of ventilation inhomogeneity on ''intrabreath'' measurements of diffusing capacity in normal subjects. *J Appl Physiol* 1993; 75: 927–932.

34 Epler GR, Saber FA, Gaensler EA. Determination of severe impairment (disability) in interstitial lung disease. *Am Rev Respir Dis* 1980; 121: 647–659.

35 Viegi G, Paoletti P, Prediletto R, *et al.* Carbon monoxide diffusing capacity, other indices of lung function and respiratory symptoms in a general population sample. *Am Rev Respir Dis* 1990; 141: 1033–1039.

36 Nordenfelt I, Svensson G. The transfer factor (diffusing capacity) as a predictor of hypoxemia during exercise in restrictive and chronic obstructive pulmonary disease. *Clin Physiol* 1987; 7: 423–430.

37 Gelb AF, Gold WM, Wright RR, Bruch HR, Nadel JA. Physiologic diagnosis of subclinical emphysema. *Am Rev Respir Dis* 1973; 107: 50–63.

38 Rosenberg E, Young RC Jr. Potential value of diffusing capacity per liter of lung volume (DL/VA) for early detection of alveolar capillary defects. *Lung* 1979; 157: 23–29.

39 Renzetti AD, Bleecker ER, Epler GR, *et al.* Evaluation of impairment/disability secondary to respiratory disorders. Statement of the American Thoracic Society. *Am Rev Respir Dis* 1986; 133: 1205–1209.

40 Owens GR, Rogers RM, Pennock BE, Levin D. The diffusing capacity as a predictor of arterial oxygen desaturation during exercise in patients with chronic obstructive pulmonary disease. *N Engl J Med* 1984; 310: 1218–1221.

41 Morrison NJ, Abboud RT, Ramadan F, *et al.* Comparison of single breath carbon monoxide diffusing capacity and pressure-volume curves in detecting emphysema. *Am Rev Respir Dis* 1989; 139: 1179–1187.

42 Gould GA, Redpath AT, Ryan M, *et al.* Lung CT density correlates with measurements of airflow limitation and the diffusing capacity. *Eur Respir J* 1991; 4: 141–146.

43 Bates DV. Uptake of CO in health and emphysema. *Clin Sci* 1952; 11: 21–32.

44 Clausen JL, Zarins LP. Pulmonary function testing guidelines and controversies: equipment, methods and normal values. New York, Academic Press, 1982.

45 Quanjer PH. Standardized lung function testing. *Bull Eur Physiopathol Respir (Clin Respir Physiol)* 1983; 19: Suppl. 5, 39–44.

46 Morris AR, Kanner RE, Crapo RO, Gardner RM. Clinical pulmonary function testing: a manual of uniform laboratory procedures. 2nd Edn. Salt Lake City, Intermountain Thoracic Society, 1984.

47 Cotes JE. Lung function. 5th Edn. London, Blackwell Scientific Publications, 1993.

48 Ogilvie CM, Forster RE, Blakemore WS, Morton JW. A standardized breath-holding technique for the clinical measurement of the diffusing capacity of the lung for carbon monoxide. *J Clin Invest* 1957; 36: 1–17.

49 Miller MR, Hankinson J, Brusasco V, *et al.* Standardisation of spirometry. *Eur Respir J* 2005; 26: 319–338.

50 Yeh MP, Adams TD, Gardner RM, Yanowitz FG. Effect of O_2, N_2, and CO_2 composition on the non-linearity of Fleisch pneumotachograph characteristics. *J Appl Physiol: Respir Environ Exercise Physiol* 1984; 56: 1423–1425.

51 Cotes JE. Effect of variability in gas analysis on the reproducibility of the pulmonary diffusing capacity by the single breath method. *Thorax* 1963; 18: 151–154.

52 Chinn DJ, Naruse Y, Cotes JE. Accuracy of gas analysis in lung function laboratories. *Thorax* 1986; 41: 133–137.

53 Gardner RM, Clausen JL, Crapo RO, *et al.* Quality assurance in pulmonary function laboratories. ATS position paper. *Am Rev Respir Dis* 1986; 134: 625–627.

54 Glissmeyer EW, Jensen RL, Crapo RO, Greenway LW. Initial testing with a carbon monoxide diffusing capacity simulator. *J Invest Med* 1999; 47: 37A.

55 Okubo T, Lenfant C. Calibration of gas chromatograph without standardized gas mixtures. *Respir Physiol* 1968; 4: 255–259.

56 Miller RM, Crapo R, Hankinson J, *et al.* General considerations for pulmonary function testing. *Eur Respir J* 2005; 26: 153–161.

57 Graham BL, Mink JT, Cotton DJ. Effects of increasing carboxyhemoglobin on the single breath carbon monoxide diffusing capacity. *Am J Respir Crit Care Med* 2002; 165: 1504–1510.

58 Sansores R, Pare PD, Abboud RT. Acute effect of cigarette smoking on the carbon monoxide diffusing capacity of the lung. *Am Rev Respir Dis* 1992; 146: 951–958.

59 Knudson RJ, Kaltenborn WT, Burrows B. Effects of cigarette smoking and smoking cessation on the carbon monoxide diffusing capacity of the lung in asymptomatic patients. *Am Rev Respir Dis* 1989; 140: 645–651.

60 Sansores R, Pare PD, Abboud RT. Effect of smoking cessation on pulmonary carbon monoxide diffusing capacity and capillary blood volume. *Am Rev Respir Dis* 1992; 146: 959–964.

61 Prabhu MB, Mink JT, Graham BL, Cotton DJ. Effect of a deep breath on gas mixing and diffusion in the lung. *Respir Physiol* 1990; 79: 195–204.

62 Welle I, Eide GE, Bakke P, Gulsvik A. Applicability of the single-breath carbon monoxide diffusing capacity in a Norwegian community study. *Am J Respir Crit Care Med* 1998; 158: 1745–1750.

63 Punjabi NM, Shade D, Patel AM, Wise RA. Measurement variability in single breath diffusing capacity of the lung. *Chest* 2003; 123: 1082–1089.

64 Graham BL, Mink JT, Cotton DJ. Improved accuracy and precision of single-breath CO diffusing capacity measurements. *J Appl Physiol* 1981; 51: 1306–1313.

65 Graham BL, Mink JT, Cotton DJ. Overestimation of the single-breath carbon monoxide diffusing capacity in patients with air-flow obstruction. *Am Rev Respir Dis* 1984; 129: 403–408.

66 Cotton DJ, Soparkar GR, Grahan BL. Diffusing capacity in the clinical assessment of chronic airflow limitation. *Med Clin North Am* 1996; 80: 549–564.

67 Graham BL, Mink JT, Cotton DJ. Effect of breath-hold time on DLCO (SB) in patients with airway obstruction. *J Appl Physiol* 1985; 58: 1319–1325.

68 Jones RS, Meade F. A theoretical and experimental analysis of anomalies in the estimation of pulmonary diffusing capacity by the single breath method. *Q J Exp Physiol Cogn Med Sci* 1961; 46: 131–143.

69 Chinn DJ, Harkawat R, Cotes JE. Standardization of single-breath transfer factor (TLCO); derivation of breath-holding time. *Eur Respir J* 1992; 5: 492–498.

70 Ferris BG. Epidemiology Standardization Project (American Thoracic Society). *Am Rev Respir Dis* 1978; 118: 1–120.

71 Huang Y-C, MacIntyre NR. Real-time gas analysis improves the measurement of single-breath diffusing capacity. *Am Rev Respir Dis* 1992; 146: 946–950.

72 Comroe J. Physiology of respiration. Chicago, Year Book Medical Publisher, 1974.

73 Kanner RE, Crapo RO. The relationship between alveolar oxygen tension and the single-breath carbon monoxide diffusing capacity. *Am Rev Respir Dis* 1986; 133: 676–678.

74 Gray C, Zamel N, Crapo RO. Effect of a simulated 3,048 meter altitude on the single-breath transfer factor. *Bull Eur Physiopath Respir* 1986; 22: 429–431.

75 Teculescu DB, Stanescu DC. Lung diffusing capacity. Normal values in male smokers and nonsmokers using the breath-holding technique. *Scand J Respir Dis* 1970; 51: 137–149.

76 Van Ganse WF, Ferris BG Jr, Cotes JE. Cigarette smoking and pulmonary diffusing capacity. (Transfer factor). *Am Rev Respir Dis* 1972; 105: 30–41.

77 Frans A, Stanescu DC, Veriter C, Clerbaux T, Brasseur L. Smoking and pulmonary diffusing capacity. *Scand J Respir Dis* 1975; 56: 165–183.

78 Crapo RO, Morris AH. Standardized single breath normal values for carbon monoxide diffusing capacity. *Am Rev Respir Dis* 1981; 123: 185–189.

79 Paoletti P, Viegi G, Pistelli G, *et al.* Reference equations for the single breath diffusing capacity: a cross-sectional analysis and effect of body size and age. *Am Rev Respir Dis* 1985; 132: 806–813.

80 Knudson RJ, Kaltenbom WT, Knudson DE, Burrows B. The single-breath carbon monoxide diffusing capacity: reference equations derived from a healthy nonsmoking population and effects of hematocrit. *Am Rev Respir Dis* 1987; 135: 805–811.

81 Roca J, Rodriguez-Roisin R, Cobo E, *et al.* Single breath carbon monoxide diffusing capacity prediction equations from a Mediterranean population. *Am Rev Respir Dis* 1990; 141: 1026–1032.

82 Miller A, Thornton JC, Warshaw R, Anderson H, Teirstein AS, Selikoff U. Single breath diffusing capacity in a representative sample of the population of Michigan, a large industrial state. Predicted values, lower limits of normal, and frequencies of abnormality by smoking history. *Am Rev Respir Dis* 1983; 127: 270–277.

83 Cinkotai FF, Thomson ML. Diurnal variation in pulmonary diffusing capacity for carbon monoxide. *J Appl Physiol* 1966; 21: 539–542.

84 Frey TM, Crapo RO, Jensen RL, Elliott CG. Diurnal variation of the diffusing capacity of the lung: is it real? *Am Rev Respir Dis* 1987; 136: 1381–1384.

85 Sansores RH, Abboud RT, Kennell C, Haynes N. The effect of menstruation on the pulmonary carbon monoxide diffusing capacity. *Am J Respir Crit Care Med* 1995; 151: 381–384.

86 Peavy HH, Summer WR, Gurtner C. The effects of acute ethanol ingestion on pulmonary diffusing capacity. *Chest* 1980; 77: 488–492.

87 Iversen ET, Sorensen T, Heckscher T, Jensen JI. Effect of terbutaline on exercise capacity and pulmonary function in patients with chronic obstructive pulmonary disease. *Lung* 1999; 177: 263–271.

88 Chinn DJ, Askew J, Rowley L, Cotes JE. Measurement technique influences the response of transfer factor (TLCO) to salbutamol in patients with airflow obstruction. *Eur Respir J* 1988; 1: 15–21.

89 Morris AH, Crapo RO. Standardization of computation of single-breath transfer factor. *Bull Eur Physiopath Respir* 1985; 21: 183–189.

90 Wanger J, Irvin C. Comparability of pulmonary function results from 13 laboratories in a metropolitan area. *Respir Care* 1991; 36: 1375–1382.

91 Gaensler EA, Smith AA. Attachment for automated single breath diffusing capacity measurement. *Chest* 1973; 63: 136–145.

92 Hathaway EH, Tashkin DP, Simmons MS. Intra-individual variability in serial measurements of DLCO and alveolar volume over one year in eight healthy subjects using three independent measuring systems. *Am Rev Respir Dis* 1989; 140: 1818–1822.

93 Viegi G, Baldi S, Begliomini E, Ferdeghini EM, Pistelli F. Single breath diffusing capacity for carbon monoxide: effects of adjustment for inspired volume dead space, carbon dioxide, hemoglobin and carboxyhemoglobin. *Respiration* 1998; 65: 56–62.

94 Mohsenifar Z, Brown HV, Schnitzer B, Prause JA, Koerner SK. The effect of abnormal levels of hematocrit on the single breath diffusing capacity. *Lung* 1982; 160: 325–330.

95 Clark EH, Woods RL, Hughes JMB. Effect of blood transfusion on the carbon monoxide transfer factor of the lung in man. *Clin Sci* 1978; 54: 627–631.

96 Cotes JE, Dabbs JM, Elwood PC, Hall AM, McDonald A, Saunders MJ. Iron-deficiency anaemia: its effect on transfer factor for the lung (diffusing capacity) and ventilation and cardiac frequency during sub-maximal exercise. *Clin Sci* 1972; 42: 325–335.

97 Marrades RM, Diaz O, Roca J, *et al.* Adjustment of DLCO for hemoglobin concentration. *Am J Respir Crit Care Med* 1997; 155: 236–241.

98 Coburn RF, Forster RE, Kane PB. Considerations of the physiological variables that determine the blood carboxyhemoglobin concentration in man. *J Clin Invest* 1965; 44: 1899–1910.

99 Viegi G, Paoletti P, Carrozzi L, *et al.* CO diffusing capacity in a general population sample: relationship with cigarette smoking and air-flow obstruction. *Respiration* 1993; 60: 155–161.

100 Mohsenifar Z, Tashkin DP. Effect of carboxyhemoglobin on the single breath diffusing capacity: derivation of an empirical correction factor. *Respiration* 1979; 37: 185–191.

101 Gaensler EA, Cadigan JB, Ellicott MF, Jones RH, Marks A. A new method for rapid precise determination of carbon monoxide in blood. *J Lab Clin Med* 1957; 49: 945–957.
102 Henderson M, Apthorp CH. Rapid method for estimation of carbon monoxide in blood. *Br Med J* 1960; 2: 1853–1854.
103 Jones RH, Ellicott MF, Cadigan JB, Gaensler EA. The relationship between alveolar and blood carbon monoxide concentrations during breath-holding. *J Lab Clin Med* 1958; 51: 553–564.
104 Cassidy SS, Ramanathan M, Rose GL, Johnson RL Jr. Hysteresis in the relation between diffusing capacity of the lung and lung volume. *J Appl Physiol* 1980; 49: 566–570.
105 Cotes JE, Meade F, Sanders MJ. Effect of volume inspired and manner of sampling the alveolar gas upon components of the transfer factor (diffusing capacity of the lung) by the single breath method. *J Physiol (Lond)* 1965; 181: 73–75.
106 Cadigan JB, Marks A, Ellicott MF, Jones RH, Gaensler EA. An analysis of factors affecting the measurement of pulmonary diffusing capacity by the single breath method. *J Clin Invest* 1961; 40: 1495–1514.
107 Chinn DJ, Cotes JE, Flowers R, Marks AM, Reed JW. Transfer factor (diffusing capacity) standardized for alveolar volume: validation, reference values and applications of a new linear model to replace KCO (TL/VA). *Eur Respir J* 1996; 9: 1269–1277.
108 Cotton DJ, Taher F, Mink JT, Graham BL. Effect of volume history on changes in DLCO SB-3EQ with lung volume in normal subjects. *J Appl Physiol* 1992; 73: 434–439.
109 Frans A, Nemery B, Veriter C, Lacquet L, Francis C. Effect of alveolar volume on the interpretation of single breath DLCO. *Respir Med* 1997; 91: 263–273.
110 Huang Y-C, O'Brien SR, MacIntyre NR. Intrabreath diffusing capacity of the lung in healthy individuals at rest and during exercise. *Chest* 2002; 122: 177–185.
111 Stam H, Hrachovina V, Stijnen T, Versprille A. Diffusing capacity dependent on lung volume and age in normal subjects. *J Appl Physiol* 1994; 76: 2356–2363.
112 Stam H, Splinter TAW, Versprille A. Evaluation of diffusing capacity in patients with a restrictive lung disease. *Chest* 2000; 117: 752–757.