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Diffusing capacity of the lung for carbon monoxide: updates on recommendations and procedure

Difusión pulmonar de monóxido de carbono: actualizaciones en las recomendaciones y procedimiento

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ABSTRACT. The pulmonary diffusing capacity for carbon monoxide is a test that allows quantitative evaluation of the transfer of oxygen from the alveolar air to its union with hemoglobin through the alveolo-capillary membrane. Since its original description in 1957, the pulmonary diffusing capacity for carbon monoxide test has evolved thanks to the advent of rapid response gas analyzers, as well as standardization efforts and vast improvements in the software and reference values. Currently, the single-breath measurement method is strongly standardized and recommended for clinical purposes. The pulmonary carbon monoxide diffusing capacity with the single breath technique has implications for both the diagnosis and the follow-up and prognosis of patients with chronic diseases not limited to the respiratory system. This document is based on the 2005, 2017 and 2021 European Respiratory Society and American Thoracic Society standards to describe technical recommendations for rapid response gas analyzers-based with the single breath systems. The pulmonary carbon monoxide diffusing capacity is underutilized despite its clinically proven value, which ranks second only to spirometry testing. However, it holds particular relevance for patients with interstitial lung diseases, emphysema, and pulmonary vascular diseases.

Keywords: respiratory function tests, pulmonary diffusing capacity, carbon monoxide, alveolar volume.

RESUMEN. La capacidad de difusión pulmonar de monóxido de carbono es una prueba que permite evaluar cuantitativamente la transferencia de oxígeno del aire alveolar a la hemoglobina sanguínea a través de la membrana alveolocapilar. Desde su descripción original en 1957, la prueba de difusión pulmonar de monóxido de carbono ha evolucionado gracias al advenimiento de los analizadores de gases de respuesta rápida. Actualmente, el método de medición de respiración única está sólidamente estandarizado y es el recomendado con fines clínicos. La prueba de difusión pulmonar de monóxido de carbono de respiración única tiene implicaciones tanto para el diagnóstico como para el seguimiento y el pronóstico de pacientes con enfermedades crónicas no sólo del sistema respiratorio. Este documento se actualiza con información propuesta por la *European Respiratory Society* y de la *American Thoracic Society* en los estándares de los años 2005, 2017 y 2021 e incluye las recomendaciones técnicas para sistemas de respiración única basados en los analizadores de gases de respuesta rápida aceptadas internacionalmente. A pesar de su valor clínicamente comprobado, la difusión pulmonar de monóxido de carbono es subutilizada, aunque se posiciona como la segunda prueba más importante después de la espirometría. Sin embargo, su relevancia es especialmente destacada en pacientes con enfermedades pulmonares intersticiales, enfisema y enfermedades vasculares pulmonares.

Palabras clave: pruebas de función pulmonar, capacidad de difusión pulmonar, monóxido de carbono, volumen alveolar.

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Abbreviations:

ATPD = ambient temperature, atmospheric pressure, dry conditions.
ATPS = ambient temperature, atmospheric pressure, saturated with water vapor conditions.
ATS = American Thoracic Society.
BHT = breath holding time.
BTPS = body temperature, ambient pressure, saturated with water vapour conditions.
CH3 = methane.
COHb = carboxyhemoglobin.
DLCO = pulmonary diffusion of carbon monoxide.
$DLCO_{sb}$ = single-breathing carbon monoxide lung diffusion.
ILD = Interstitial lung disease.
COPD = chronic obstructive pulmonary disease.
ERS = European Respiratory Society.
ERV = expiratory reserve volume.
FRC = functional residual capacity.
FVC = forced vital capacity.
He = helium.
IVC = inspiratory vital capacity.
KCO = pulmonary transfer coefficient for carbon monoxide.
LIN = lower limit of normal.
Ne = neon.
PiO2 = inspired oxygen pressure.
RGA = rapid response gas analyzers.
RV = residual volume.
TI = inspiratory time.
TLC = total lung capacity.
TLCO = pulmonary transfer of carbon monoxide.
VA = alveolar volume.
VIN = inspiratory volume.
V/Q = ventilation to perfusion ratio.

INTRODUCTION

The measurement of pulmonary diffusion of carbon monoxide (DLCO) has undergone significant evolution since its standardization in 1957 by Ogilvie et al.[1] The classical method used small samples of exhaled gas and required several minutes to measure the carbon monoxide concentration. In recent years, rapid response gas analyzers (RGA) have been developed that perform the measurement in less than 150 milliseconds. These advances coupled with rapid microprocessor calculations, and better and more numerous reference values, have led to a revolution in DLCO measurement.[2]

DLCO is a fundamental test for assessing gas exchange at the alveolocapillary membrane, playing a crucial role in the diagnosis, management and prognosis of various diseases not limited to the respiratory system.[3,4] Several techniques have been used to assess carbon monoxide transfer across the alveolocapillary membrane.[5] These include: the multiple-breath (multibreath) method; the intrabreath method, which is performed when maximal inspiration is followed by a slow and uniform maximal exhalation, without a period of apnea in the maneuver;[6] and the «one-breath» method developed by Krogh in 1910.[7] The latter method is widely used and the most standardized.[8,9] In the «one-breath» or «single-breath» ($DLCO_{sb}$) method, a 10-second apnea period is performed during maximal inspiration.[10] In addition to the measurement of $DLCO_{sb}$, simultaneous inhalation of inert gasses, such as helium (He), methane (CH3) or neon (Ne), allow calculation of alveolar volume (VA), total lung capacity (TLC) and residual volume (RV).

This review references the 2005 and 2017 update of the European Respiratory Society (ERS) and American Thoracic Society (ATS) standards,[2,8] as well as the ERS/ATS-2021[11] standard for pulmonary function test interpretation strategies. These standards seek to provide a technical update for DLCO systems based on RGA development and to describe new calculation standards that incorporate continuous gas analysis of the entire exhaled sample, as well as clinical and functional interpretation of test results.

DLCO measurement, performed under standardized conditions and under strict quality control, is a sensitive tool to detect changes in lung function, even less than 10%.[12] Its decrease may indicate chronic lung diseases such as chronic obstructive pulmonary disease (COPD) or interstitial lung disease (ILD), showing a direct correlation with the degree of emphysema, inflammation or fibrosis.[3,13,14] $DLCO_{sb}$ also reflects abnormalities in pulmonary vascular diseases such as pulmonary hypertension,[15] embolism and vasculitis, as well as extrapulmonary diseases such as hemoglobinopathies,[16] obesity,[17] musculoskeletal abnormalities and elevated carboxyhemoglobin (COHb) levels.[18] This broad spectrum of clinical applications highlights the versatility of $DLCO_{sb}$ as a sensitive indicator of multiple conditions affecting lung function and the overall health of the individual.

PHYSIOLOGICAL BASIS

DLCO is a test that measures the properties of the alveolocapillary membrane for oxygen exchange from alveolar air to erythrocytes in the alveolar capillaries, thus involving not only the physiological mechanism of pulmonary diffusion *(Figure 1)*, but also ventilation, perfusion and the ventilation to perfusion ratio (V/Q). For this reason, especially in Europe, it is more appropriately called pulmonary transfer of CO (TLCO). If you would like to learn more about the physiological basis of pulmonary carbon monoxide diffusion, please refer to the supplementary material.

INDICATIONS AND CONTRAINDICATIONS OF THE TEST

In general, the main indication for $DLCO_{sb}$ testing is the diagnostic evaluation and follow-up of lung parenchymal

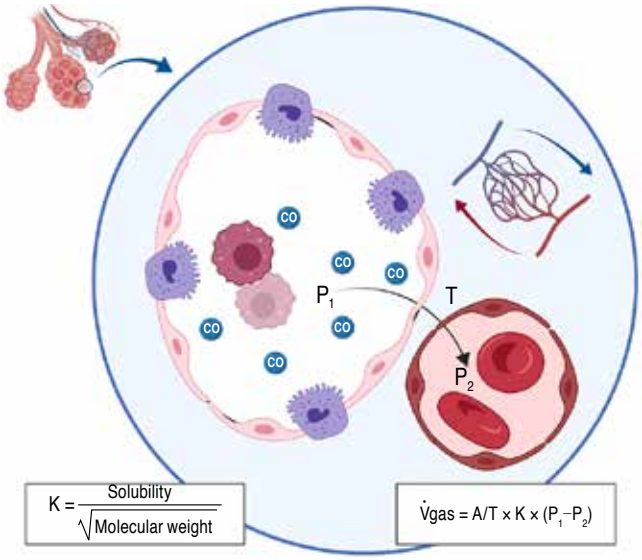

Figure 1: Fick's Law. Fick's law describes the factors that determine the diffusion of a gas through a given surface (A), the thickness of the tissue (T), and the diffusion constant of the gas (K), which corresponds to the solubility and the molecular weight of the test gas, as well as the partial pressure difference across the tissue (P1-P2). The diffusion constant is proportional to the solubility of a gas and is inversely proportional to the square root of the molecular weight of the gas.
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diseases *(Table 1)*. The updated contraindications for the $DLCO_{sb}$ test are listed in *Table 2*.[2,3,19]

DLCO EQUIPMENT AND SUPPLIES

DLCO equipment should meet the international technical recommendations issued by ATS/ERS 2017,[2] with the following recommended minimum requirements for volume measurements and rapid gas analyzer, which can be found in the equipment user manual and supplementary material.

PRE-TEST PREPARATION

Preparation of technical personnel prior to testing

During the COVID-19 pandemic, disease transmission became more prominent in order to mitigate risks. Rigorous implementation of safety and disinfection measures are required *(Table 3)*.[20]

Quality control and equipment calibration[2,21]

1. Daily calibration check: start with zero flow before each maneuver. Verify volume calibration with a 3 L syringe, performing at least three different flows (low, medium and high) between 0.5 and 12 L/s, meeting an accuracy requirement ≤ 2.5%.
2. Weekly procedures or in case of problems: should be performed with a calibrated 3 L syringe, ensuring that the VA calculation is within ± 300 mL of the expected value and DLCO is < 0.166 mmol/min/kPa or < 0.5 mL/min/mmHg. The biological control test should not have deviations > 12% or > 3 mL/min/mmHg, because it could indicate quality control problems.
3. Monthly testing: leak testing of the 3 L calibration syringe is recommended. If it does not return within 10 mL of full fill, it should be sent for repair. Also, a linearity evaluation of the gas analyzer should be performed using known dilutions of the test gas or using a high precision test gas. However, automation of linearity by manufacturers is preferred.
4. General recommendations: In the absence of a high accuracy DLCO and gas simulator, system checks

Table 1: Indications for the $DLCO_{sb}$ test.

1. Obstructive diseases: a. COPD (decreased proportionally to the degree of emphysema) b. Asthma (usually normal or increased) c. Cystic fibrosis (decreased in advanced stage) d. Chronic bronchitis (usually normal or slightly decreased)
2. Evaluation and follow-up of restrictive diseases: a. Interstitial diseases (frequently decreased) b. Extrapulmonary restrictive diseases (usually normal)
3. Pulmonary vascular diseases (frequently decreased): a. Chronic pulmonary thromboembolism b. Pulmonary hypertension c. Pulmonary vasculitis
4. Preoperative evaluation: a. Resection for lung cancer b. Volume reduction surgery c. Lung transplantation
5. Impairment and disability evaluation: a. COPD, interstitial diseases, others b. Prediction of arterial desaturation during exercise in some patients with lung disease c. Assessment of pulmonary effects of chemotherapeutic agents and other drugs known to cause lung damage, as well as radiotherapy
6. Other useful clinical applications of DLCO measurement: a. Evaluation of pulmonary hemorrhage (usually elevated) b. Evaluation of some diffuse pulmonary infectious diseases (e.g., *Pneumocystis pneumonia*) c. Timely diagnosis and follow-up in respiratory surveillance programs in occupational medicine, especially in subjects exposed to inorganic dust

DLCO = pulmonary diffusion of carbon monoxide. COPD = chronic obstructive pulmonary disease.

Table 2: Contraindications to the $DLCO_{sb}$ test.

Absolute
1. Severe hypoxemia (SpO_2 < 75%) In this case its performance can be evaluated according to the altitude where the test is being performed, and always under medical supervision 2. Elevated carboxyhemoglobin levels (COHb > 10 to 15%)
Relative
1. Confusion or poor muscle coordination that prevents the proper maneuver from being performed 2. Acute or decompensated cardiovascular disease (infarction, heart failure, cerebrovascular disease) 3. Pneumothorax in the last three months 4. Risk of bleeding due to hemoptysis or aneurysms 5. Surgery (thorax, abdomen, eye, ear) in the last month 6. Acute respiratory infections in the last two weeks (influenza, common cold) 7. Active pulmonary tuberculosis 8. Advanced or complicated pregnancy 9. Patients with tracheostomy or pleural probes 10. Patients who cannot withhold supplemental oxygen for at least 10 minutes 11. Patients with VC or FVC less than the minimum volumes required by the equipment

COHb = carboxyhemoglobin. $DLCO_{sb}$ = single-breath carbon monoxide lung diffusion. FVC = forced vital capacity. SpO_2 = pulse oximetry oxygen saturation. VC = vital capacity.

Table 3: Preparation of technical personnel before performing the $DLCO_{sb}$ test.

1. Barrier devices and cleaning procedures: – Use of barrier devices, such as filters, should be made to prevent cross transmission of diseases – Despite the use of in-line filters, the ATS and ALAT standards stress the continuous need for regular cleaning and decontamination of equipment
2. Personal hygiene: – Health personnel should follow the recommended standards of hand washing, either by hand washing (40-60 seconds) or by rubbing with alcohol gel (20-30 seconds) – The 5 moments of hand washing/hand hygiene, according to World Health Organization (WHO) guidelines, should be followed
3. Personal Protective Equipment (PPE): – PPE includes gown for activities that may generate splashes or sprayable liquids from blood, organic fluids, secretions or excretions – Use of disposable gloves in procedures with potential contact with infectious material, changing them between tasks and procedures, and washing gloves with alcohol gel before removing them – Use of surgical mask or N95 during patient care, and eye protection to prevent splashes, in case of contagious diseases
4. Disinfection and sterilization: – Before caring for each patient, disinfection and/or sterilization of equipment, instruments and surfaces must be carried out

These measures, based on ALAT (Latin American Thorax Association) recommendations, are essential to ensure a safe testing environment and minimize the risk of disease transmission.[20]
ATS = American Thoracic Society. $DLCO_{sb}$ = single-breath pulmonary diffusion of carbon monoxide.

should be performed using a 3 L calibration syringe in ambient temperature, atmospheric pressure, saturated with water vapour (ATPS) conditions, with VA reporting in ambient temperature, atmospheric pressure, dry (ATPD) conditions instead of body temperature, ambient pressure, saturated with water vapour (BTPS) conditions. A digital calibration option should be available to verify the computational algorithms of the system. This option should use simulated flow data, CO concentration and tracer gas concentration from standardized maneuvers with a known DLCO.

Instructions for the patient

To minimize variability, the following pre-test specifications, pre-test patient instructions *(Table 4)* and patient preparation for the test *(Table 5)* should be considered.

PROCEDURE[2,8,22,23]

1. In the diffusing equipment system the patient data will be placed, for the interpretation of $DLCO_{sb}$ values an adjustment for equipment dead space and barometric

pressure (altitude) is required, which should be done by the equipment software before calculating the predicted values.
2. Perform spirometry to obtain a forced vital capacity (FVC) maneuver according to the latest international standards.
3. The individual is positioned correctly, holding the mouthpiece and positioning the nose clip appropriately. A new mouthpiece with a filter should always be used on each patient and check that there are no leaks through the mouthpiece or nose.
4. As illustrated in *Figure 2*, start with two to three breaths at tidal volume, maintaining a stable functional residual capacity (FRC).[24]
5. From FRC, the patient is asked to exhale in a relaxed manner until RV (expiratory reserve volume maneuver, ERV), where a plateau (< 25 mL) of at least one second should be achieved, and then the valve is activated.
   a. In obstructive patients, where exhalation to RV may require more time, it is recommended that

Table 4: Instructions for the patient before the $DLCO_{sb}$ test.

1. Avoid smoking or vaping on the day of the test, write down the time of last consumption
2. Avoid using chest-restrictive garments (vests, corsets or very tight clothing)
3. It is not necessary to suspend basic medication
4. Fasting is not required for the test, light eating is recommended
5. Avoid intense exercise, at least four hours before (if the patient has exercised, it is necessary to specify it)
6. Do not use supplemental oxygen for ≥ 10 minutes, if the patient's clinical status allows it
7. Avoid consuming alcohol on the day of the test
8. If a nitrogen flushing test must also be performed, it is recommended that the carbon monoxide diffusion test be performed first; If carried out later, wait twice as long as the duration of the nitrogen flushing test[2]
9. In case of suspicion of high COHb levels (smokers, firefighters, etc.), it is recommended to measure them[18,24]
10. It is recommended to apply a brief medical history questionnaire, which includes:
    a. History of current or past smoking and vaping, total number of years of smoking, and average daily number of cigarettes per day
    b. History of occupational exposure to fumes or dusts, total number of years of exposure and average hours per day
    c. History of respiratory symptoms: dyspnea, wheezing, cough, and expectoration
    d. Contraindications of the test: acute cardiovascular disease, acute or active respiratory infections (influenza, common cold, tuberculosis, etc.), advanced or complicated pregnancy

COHb = carboxyhemoglobin. $DLCO_{sb}$ = single-breath pulmonary diffusion of carbon monoxide.

Table 5: Patient preparation to perform the $DLCO_{sb}$ test.

1. Reception and confirmation of identity:
   – The technician or doctor in charge receives the patient and confirms his or her full name and date of birth, ensuring that they match the medical request and the file or registration number, if applicable
   – Contraindications are reviewed, and if any are present, the test is not performed unless authorized in writing by the treating physician or approved by the laboratory medical director
2. Anthropometric measurements of weight and height will be carried out in accordance with what is established in other pulmonary function tests
3. Demographics:
   – The patient's age in years on the day of the test and other demographic data such as ethnicity are recorded
   – The altitude of the region where the test is performed should be recorded for adjustments, if necessary
   – These data are entered into the equipment data program
4. Additional information:
   – Tobacco or vaping consumption, previous intense physical exercise and the use of inhaled bronchodilators are recorded
   – The patient rests sitting for at least 10 minutes before the test
5. Instructions for the maneuver:
   – The maneuver is explained in clear and easy language for the patient, ensuring that they understand each step
   – The use of the nose clip is explained and the patient is instructed on the proper use of the mouthpiece
   – Instructions include holding the mouthpiece with your teeth, without biting, sealing it with your lips around it, and avoiding inserting your tongue into the hole
   – Place the patient in the correct position: sitting, with both feet resting on the floor, trunk upright and head slightly elevated (maintain this position throughout the maneuver)

$DLCO_{sb}$ = single-breath pulmonary diffusion of carbon monoxide.

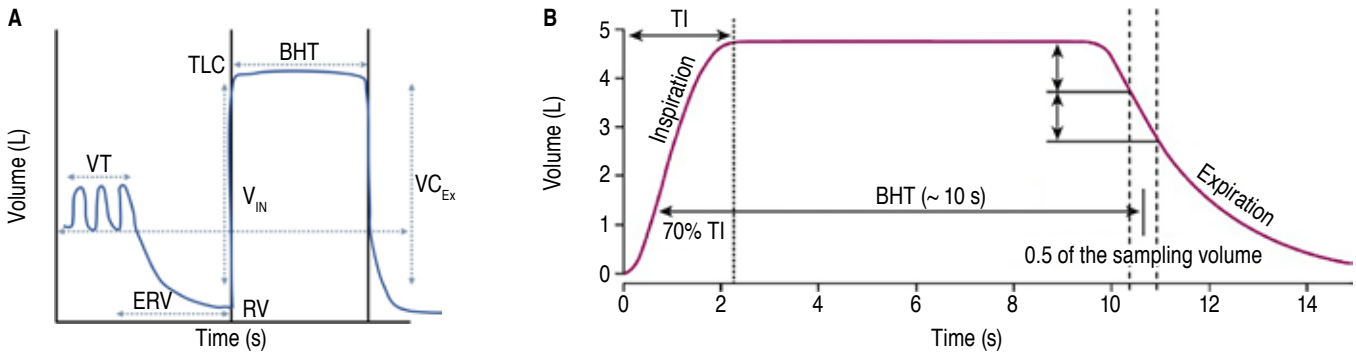

Figure 2: $DLCO_{sb}$ maneuver, single breath. **A)** The maneuver begins with stable breathing at tidal volume (VT) followed by a relaxed expiratory reserve volume (ERV) maneuver. Upon reaching residual volume (RV), the subject must complete the inspiratory vital capacity that determines the inspiratory volume (VIN) of the maneuver in less than four seconds, until reaching total lung capacity (TLC). After this, an apnea of 10 ± 2 seconds is performed followed by unforced expiration (VCEx) for at least four seconds. **B)** breath holding time (BHT) is calculated by the Jones-Mead method, which includes 70% of the inspiratory time (TI) up to half of the alveolar sampling time.
$DLCO_{sb}$ = single-breath pulmonary diffusion of carbon monoxide.
Modified from: DeCato TW.[23]

this part of the maneuver be limited to < 12 s, allowing this group of patients to exhale enough to achieve maximum vital capacity on the subsequent inhalation.

6. In VR, the subject is asked to inhale rapidly to TLC (where the mouthpiece is connected to the gas source).
7. The maximum inspiratory vital capacity (IVC) maneuver should be performed in less than four seconds, reaching a volume ≥ 90% of the FVC measured by spirometry previously (with a minimum tolerance of 85% for a B quality and 80% for a C quality).
8. The patient is asked to maintain a period of apnea for 10 ± 2 s, avoiding leaks and Valsalva or Müller maneuvers (expiratory or inspiratory effort against a closed glottis, respectively).
9. The subject is instructed to perform an unforced exhalation, without interruptions or hesitations.
10. In rapid systems (RGA), exhalation should be continued to RV, which improves VA measurement.
11. In case of a failed maneuver, instructions and demonstration should be repeated if necessary.
12. The time between maneuvers should be at least four minutes, to allow adequate tracer gas removal, in cases of severe airflow obstruction up to 10 minutes of waiting time is recommended.
13. A minimum of two maneuvers that meet acceptability and repeatability criteria must be completed, with a maximum of five attempts, in order to avoid increasing COHb in the blood (five $DLCO_{sb}$ maneuvers increases 3-3.5% of COHb in the blood).[24]

A submaximal inspiratory volume of the sample gas less than the known vital capacity may affect carbon monoxide inhalation, depending on whether it was from a suboptimal exhalation to RV (performed at TLC) or was from a suboptimal inhalation from RV (maneuver achieved below TLC). In the first case, VA and calculated $DLCO_{sb}$ reliably reflect lung volume and lung properties at TLC. In the second case, VA is reduced and DLCO measurement is affected.

Inspiration must be rapid, as the $DLCO_{sb}$ calculation assumes instantaneous lung filling, this explained because when the lungs fill more slowly, they decrease the amount of time the lung is in full inspiration, with a consequent reduction in carbon monoxide entry.

Valsalva or Müller maneuvers can affect the calculation of $DLCO_{sb}$ by decreasing or increasing intrathoracic blood volume, resulting in an increase or decrease in $DLCO_{sb}$, respectively for each maneuver. *Figure 3* shows some artifacts that may be observed during the maneuver.

RESULTS REVIEW

Acceptability criteria[2]

1. Obtain an inspiratory volume (VIN) ≥ 90% of the largest FVC in the same test session; if this is not achieved, we can obtain an A quality with a VIN ≥ 85% of the largest FVC in the same test session along with a VA within 200 mL or 5% (whichever is greater) of the largest VA from other acceptable maneuvers.
2. Inspiratory time (IT) less than 4 seconds (obtain 85% of the test gas inhaled in < 4 seconds).
3. Stable breath holding time (BHT) for 10 ± 2 seconds with no evidence of leaks or Valsalva/Müller maneuvers during this time.

4. On classical analyzers the exhalation time must be greater than 4 seconds (i.e., sample collection is completed within 4 seconds of the onset of exhalation). In rapid response analyzers the exhalation should continue up to the residual volume, with a maximum exhalation time of 12 seconds, which provides a better measurement of VA.

Repeatability evaluation

The variability of $DLCO_{sb}$ depends more on technical than biological factors. The $DLCO_{sb}$ test should have at least two repeatable maneuvers in two units of $DLCO_{sb}$ in mL/min/mmHg (equivalent to 0.67 unit in mmol/min/kPa). It is considered that more than 95.5% of patients can achieve this repeatability criterion.[2,9]

Quality control of $DLCO_{sb}$ maneuvers

A grade A maneuver is one that meets all acceptability criteria, therefore, the average $DLCO_{sb}$ of two repeatable grade A maneuvers should be reported. If after repeating the test, the operator cannot obtain two repeatable grade A maneuvers, then the values are reported with the warning to the interpreter that the test session was not optimal *(Table 6)*.[2]

$DLCO_{SB}$ REPORT

Special considerations and limitations for $DLCO_{sb}$

1. For interpretation of $DLCO_{sb}$ results, equipment dead space adjustment should be performed.[11] The dead space adjustment should include the respiratory circuit proximal to the sampling point, the filter, and the gas analyzer mouthpiece, which should be < 200 mL. Smaller dead space volumes are recommended for pediatric-aged patients and adults with a vital capacity of less than 2 L.[25]
2. $DLCO_{sb}$ increases at higher altitudes because of lower oxygen competition due to lower PiO2.[26]
3. Hemoglobin, COHb and backpressure concentrations or increased carbon monoxide outflow resistances may affect the $DLCO_{sb}$ test, and should be considered when interpreting.[11,16]
4. Diurnal variation in the DLCO result has been reported (1.2%/hour drop from 9:30 a.m. to 5:50 p.m.).[27]
5. There is a change of up to 13% during menstrual cycles. The highest value of $DLCO_{sb}$ is just before menstruation and the lowest value on the third day of menstruation.[28]
6. There may be a reduction of up to 15% of the $DLCO_{sb}$ value within 90 minutes of ingesting alcohol.[29,30]
7. Smoking affects test results; a prevalence of a $DLCO_{sb}$ below the lower limit of normal (LLN) in patients without airway obstruction has been observed in 26.7% when they are active smokers, and 14.4% in those who have quit smoking.[31]
8. An increase in $DLCO_{sb}$ during pregnancy (first trimester) has been described but not consistently found in other studies.[32,33]
9. The Valsalva maneuver can decrease $DLCO_{sb}$ because it decreases the amount of blood in the pulmonary capillaries.[12]
10. In subjects with obstructive disease, bronchodilator use increases $DLCO_{sb}$ by up to 6%, so the use of these drugs should be recorded by the technician.[34] However, recent studies have found no significant effects on DLCO with doses lower than 1,000 μg of salbutamol, so the use of bronchodilators before DLCO testing is not inadvisable.[2]

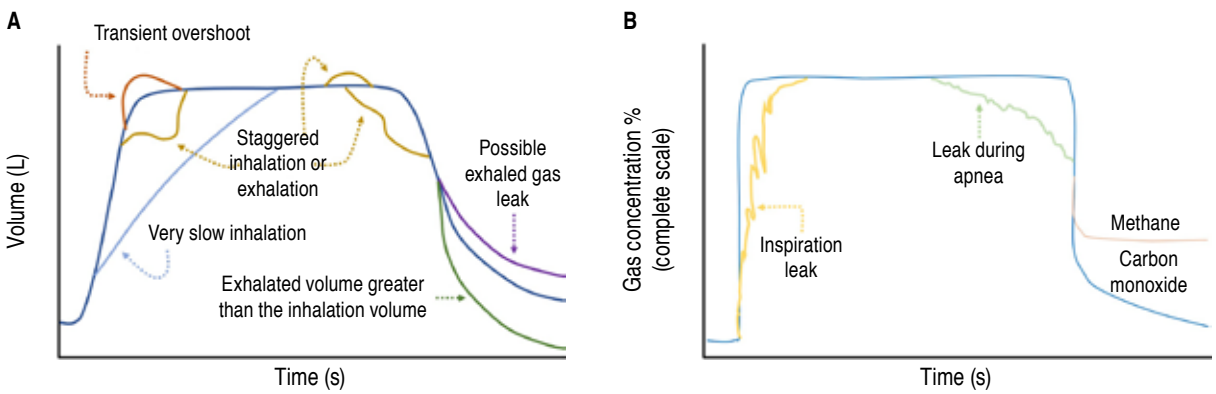

Figure 3: DLCO artifacts. **A** and **B)** Problems that can occur during the maneuver for $DLCO_{sb}$ that can lead to measurement errors are shown. DLCO = pulmonary diffusion of carbon monoxide. $DLCO_{sb}$ = single-breath pulmonary diffusion of carbon monoxide.
Modified from: Graham BL, et al.[2]

Table 6: Classification of quality control.

Quality	V_{IN}/FVC (%)	T_A	Sample collection time* (seconds)
A	≥ 90‡	8-12 s	≤ 4
B	≥ 85	8-12 s	≤ 4
C	≥ 80	8-12 s	≤ 5
D	≤ 80	< 8 o > 12 s	≤ 5
F	≤ 80	< 8 o > 12 s	> 5
Quality A: meets all acceptability criteria. Report the average $DLCO_{sb}$ of two repeatable quality A maneuvers.			
Unsuccessful repetition (if two repeatable quality A maneuvers are not obtained):			
1. Two or more maneuvers A not repeatable, but acceptable: Report the average $DLCO_{sb}$ of those acceptable maneuvers 2. Only one maneuver A is obtained: Report the $DLCO_{sb}$ value of that maneuver 3. Maneuvers A are not obtained: report the average $DLCO_{sb}$ of the maneuvers with grades B, C or D 4. Only F maneuvers are obtained: Do not report any $DLCO_{sb}$ value			

Note: In each of these situations, these deviations from the acceptability criteria should be noted to alert the interpreter of the test results.
$DLCO_{sb}$ = single-breath pulmonary diffusion of carbon monoxide. FVC = forced vital capacity. T_A = apnea time. VA = alveolar volume. V_{IN} = inspired volume.
* Only in classic analyzers, in fast response analyzers it is necessary to reach the residual volume with a maximum of 12 seconds.
‡ V_{IN}/FVC ≥ 85% and alveolar volume within 200 mL or 5% (whichever is greater) of the largest VA of another acceptable maneuver.

ADJUSTMENTS IN THE $DLCO_{SB}$ VALUE

There are physiological factors that can affect the $DLCO_{sb}$ measurement, inducing changes in opposite directions, therefore, current standards recommend four adjustments: Hb, COHb, the inspired pressure of oxygen (PiO_2) or altitude adjustment and VA adjustment. It is suggested to adjust for these factors in the predicted value of $DLCO_{sb}$ rather than the measured value. This predicted value is calculated from measurements in healthy individuals without disease, with normal Hb and COHb levels, performed at rest and breathing at room air. If any of these conditions are not met, corresponding adjustments to the predicted value are advised and can be found in the supplementary material.[2,11]

BASIC INTERPRETATION PROCESS

1. The primary measurements are the pulmonary transfer coefficient for carbon monoxide (KCO) (carbon monoxide concentration change measured over time per unit volume and pressure) and VA, its product (DLCO = KCO × VA) is the key index interpreted for gas transfer.
2. Define the alveolocapillary membrane diffusion pattern according to the $DLCO_{sb}$ concentrations proposed by the 2022 ATS/ERS interpretation strategies technical standard,[11] algorithm in *Figure 4*.
3. For severity grading it is recommended to use the $DLCO_{sb}$ Z-score, i.e., the DLCO measurement expressed in standard deviations outside that predicted by reference values for individuals of the same height, age and sex generating the following categories:

   a. Normal DLCO: ± 1.645 SD.
   b. Mild decrease: from –1.645 to –2.5 SD.
   c. Moderate decrease: from –2.51 to –4.0 SD.
   d. Severe decrease: < –4.1 SD.

4. It is also useful to compare VA with TLC measured by body plethysmography to analyze whether maldistribution of the test gas that may contribute to lower $DLCO_{sb}$ (i.e., carbon monoxide uptake can only be analyzed for regions where the test gasses are distributed). The normal value for the VA/TLC ratio in adults is approximately 0.85-0.90.[35] Values significantly below this suggest that deficiencies in the gas mixture are likely to contribute to a low measured $DLCO_{sb}$. In the absence of lung volume data by plethysmography, the presence of a steep downward slope in the inert gas tracing during exhalation suggests the possibility of gas maldistribution; however, there are no ideal ways to adjust for these conditions.[11]
5. Quality grading according to *Table 6*.
6. The choice of reference equation may affect the final interpretation. Each laboratory must select the most appropriate equation for the methods and population selected. This is essential since large differences between reference equations have been described.[11,26,36]
   In Mexico we have two reference equations, which were mostly performed in Mexico City (2,240 m above sea

level), in a pediatric population (4 to 20 years of age) by Gochicoa et al.[37] and in an adult population (22 to 83 years of age) by Vázquez et al. the latter includes adjustment for altitude.[38]

7. For interpretation, the relevant adjusted values for altitude (PiO2), Hb value and COHb should be considered.

CONCLUSIONS

DLCO is a pulmonary function test that assesses gas exchange and plays a crucial role in the diagnosis, monitoring and prognosis of multiple diseases. It is crucial to note that DLCO cannot be assessed in isolation; its constituent components, such as VA and KCO, need to be considered. Ignoring these variables may result in the loss of relevant clinical information. Furthermore, the importance of performing an integrated analysis of DLCO in conjunction with other pulmonary functional tests and available clinical data is emphasized.

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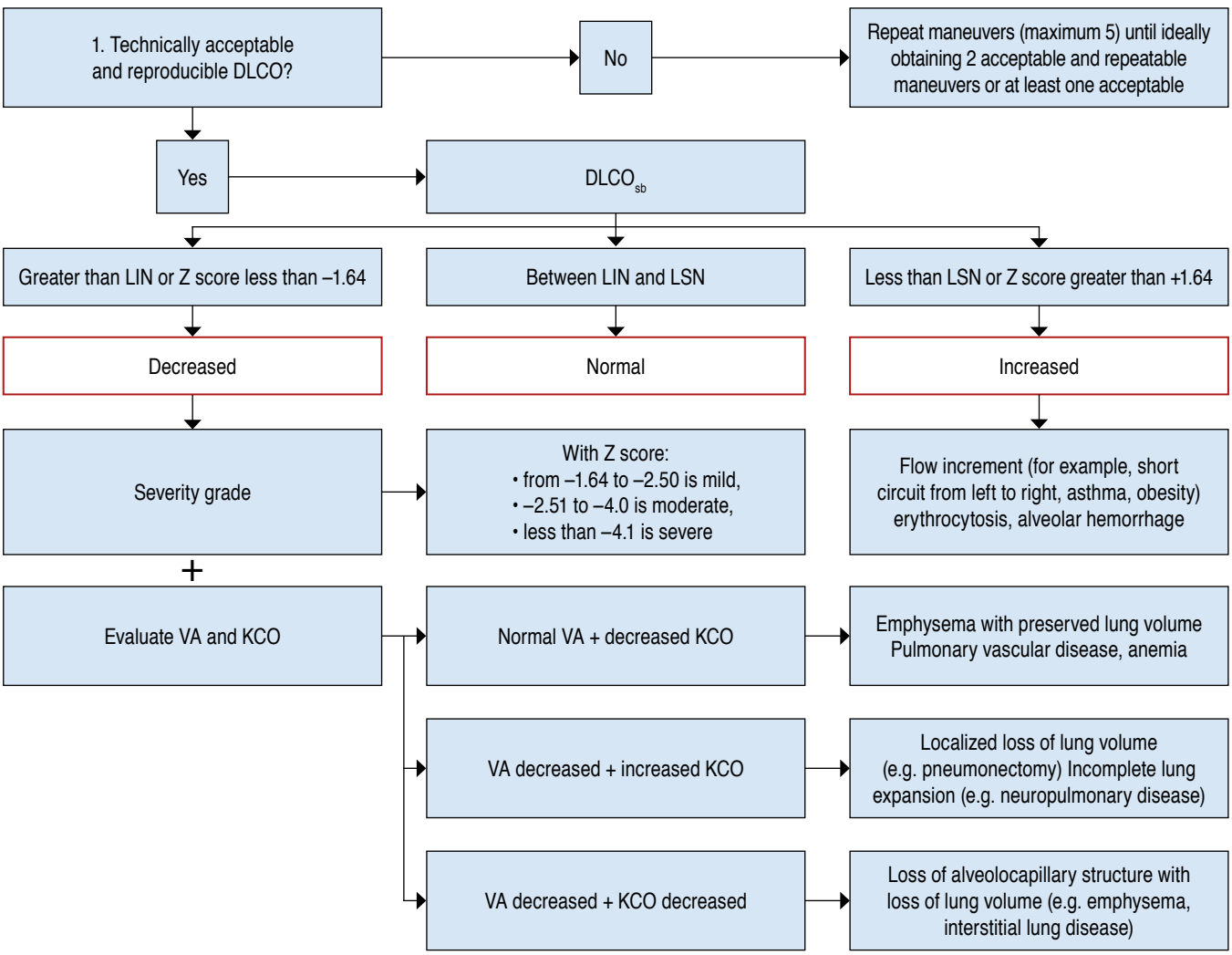

Figure 4: Algorithm for interpreting abnormal $DLCO_{sb}$ patterns. The interpretation of $DLCO_{sb}$ involves, first, determining whether it is low or high in relation to the 5th and 95th percentiles of the reference values. An elevated $DLCO_{sb}$ generally indicates increased pulmonary blood volume, erythrocytosis, or free hemoglobin in the airways. To understand a low $DLCO_{sb}$, the components are examined: alveolar volume (VA) and CO transfer coefficient (KCO). Normal VA suggests pulmonary vascular involvement, emphysema with preserved volume, or anemia. A low VA with low or normal KCO indicates loss of alveolocapillary structure, as in emphysema or interstitial lung disease (ILD). With low VA and high KCO, a state of low lung volume is suggested.
Note: the interpretation of this algorithm refers to presumptive clinical diagnoses, they should not be considered definitive diagnoses.
DLCO = pulmonary diffusion of carbon monoxide. $DLCO_{sb}$ = single-breath pulmonary diffusion of carbon monoxide.

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SUPPLEMENTARY MATERIALS

Pulmonary diffusion of carbon monoxide: updates in recommendations and procedure

PHYSIOLOGICAL BASES

The extraordinary affinity of carbon monoxide (CO) for hemoglobin allows this gas to be useful for evaluating gas exchange in the alveolocapillary membrane. This measurement reflects both the diffusion of CO and the rate of absorption by hemoglobin (Hb). There are several processes by which the transfer or uptake of CO from the outside to the Hb is interfered with, these processes are determined by Fick's diffusion law, which describes the flow of a gas through a semipermeable barrier, formula 1:

$$\text{Gas flow } (\dot{V}) = (A/T) \times (P1 - P2) \times K$$
(Formula 1)

The amount of gas transferred per unit of time ($\dot{V}$) is directly proportional to the diffusion area or surface (A), the gas diffusion constant (K) and the partial pressure gradient of the gasses across the membrane (P1 – P2); and inversely proportional to the thickness of the membrane (T). Applying this equation to pulmonary gas transfer, P1 and P2 are the gas concentrations in the alveolus and pulmonary capillary, respectively. Since it is not possible to specify the alveolar area (A), the membrane thickness (T) and the diffusion constant (K) of the alveolocapillary membrane for the entire lung, these variables are replaced by a single constant (DL), which represents the diffusion capacity for the lung as a whole[1] *(Figure 1S)*.

Measurement of CO transfer capacity is preferred over oxygen (O_2) for several reasons. Although both gases diffuse easily through the alveolocapillary membrane and combine with Hb, CO has a higher affinity than O_2; that is, about 210 times more related by Hb. The measurement of CO, being easily detectable, provides a more precise evaluation of pulmonary diffusion properties. When CO is measured, the P2 in the equation is assumed to be zero due to the high affinity of CO for Hb,[2,3] in contrast, the partial pressure of oxygen (PO_2) in the capillary increases as the erythrocyte It travels along the pulmonary capillary, so that, under normal conditions of rest and cardiac output, the oxygen pressure in the alveolus and capillary comes to a near equilibrium when the erythrocyte is only one-third the length of the capillary. At this point, no more O_2 can be transferred. However, if more blood flows through the capillary, more O_2 can be taken up, making O_2 uptake both «diffusion-limited» and «perfusion-limited».[4]

Although there are situations in which O_2 transfer may be diffusion limited, ventilation-perfusion imbalance and shunt are much more important causes of resting hypoxemia than changes in membrane thickness. alveolocapillary.[5,6] On the other hand, diffusion can more easily reach its maximum and limit oxygen transfer during exercise and at altitude.

The initial partial pressure of CO in the alveolus (PACO) can be analyzed assuming that CO is diluted to the same extent as the inhaled inert gas (such as helium), which is used to calculate the instantaneous dilution of inhaled CO by volume residual. DLCO is expressed as the volume of CO (in milliliters) transferred per minute per millimeter of mercury of alveolar partial pressure of CO (mL/min/mmHg).

As illustrated in *Figure 1S*, the CO diffusion pathway requires passing through the alveolocapillary membrane. Roughton and Forster[2] simplified this process into two steps: 1) diffusion of CO, described as the membrane component (Dm), and 2) binding of CO to Hb, described as the chemical reaction rate of COHb (θ) multiplied by pulmonary capillary blood volume (Vc), formula 2:

$$DLCO = Dm + \theta Vc$$
(Formula 2)

This basic equation for DLCO is a conductance, flow divided by pressure change ($\dot{V}/\Delta P$). The uptake of CO can be simplified to two properties of gas conductance. First, the CO conductance across the alveolocapillary membrane (Dm), which reflects the diffusion capacity of the membrane; and second, the binding capacity of CO to Hb (θVC). These two conductances are in series and are summarized in formula 3:

$$1/DLCO = (1/Dm) + (1/\theta Vc)$$
(Formula 3)

Starting from formula 3, the conductances through which the molecules of a gas in the alveolocapillary membrane have to pass are represented as the reciprocals of the resistances, so that they can be added in series.

The Dm depends on: 1) surface area and thickness of the alveolocapillary membrane, 2) the thickness and surface area of the erythrocyte membrane contained in the alveolar capillaries and 3) the thickness of the plasma barrier, including all its components. The product of θVC is also called reactive conductance. Theta (θ) is the product of the ratio of the chemical reaction between CO and Hb, expressed as a ratio of 1 mL of blood (with a standard hemoglobin concentration); and Vc is the volume of Hb in the alveolar capillary blood.

Understanding this formula is important for interpretation purposes. Alveolar recruitment due to lung hyperinflation affects Dm, while capillary recruitment, as occurs in changes in body position, for example, supine position or during the Müller maneuver (deep inspiration with closed glottis), increases θVc.

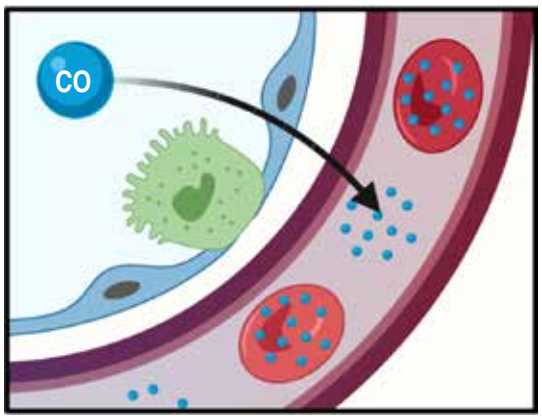

Figure 1S: Representation of carbon monoxide diffusion from the alveolus to hemoglobin; it must pass through the alveolocapillary membrane (consisting of the alveolar epithelium, basement membrane, a potential interstitial space and capillary endothelium), a thin plasma layer and the erythrocyte membrane, until it binds with hemoglobin.
«Created with BioRender.com»

DLCO EQUIPMENT AND CONSUMABLES

DLCO equipment should meet the international technical recommendations issued by the American Thoracic Society and the European Respiratory Society (ATS/ERS 2017),[7] with the following recommended minimum requirements for volume measurements and rapid gas analyzer, which can be found in the equipment user manual:

1. The equipment must meet the flow and volume measurement requirements established by ATS/ERS 2019 for spirometry.[8] Flow measurement accuracy should be in the range of –10 to +10 L/s, which should be within ± 2%.
2. Calibration with 3 L syringe, with specified maximum error of ± 0.5% (i.e., 2.985 to 3.015 L), the calibration volume should be within ± 2.5%, which is equivalent to an error tolerance ≤ 75 mL. This volume measurement accuracy must be maintained over the entire range of gas composition and concentration.
3. The response time from 0 to 90% should be ≤ 150 ms.
4. The CO and tracer gas analyzer should have a linear response from zero concentration to full concentration of the test gas. The error in the linear response of the analyzer should not exceed more than 0.5% on the full scale.
5. The gas analyzer output should be accurate to within ± 1% of full scale.
6. The gas analyzer should be stable throughout the test, maintaining a minimum zero offset (measured in ppm and percent) and minimum gain offset. The gas analyzer offset should be ≤ 10 ppm in 30 seconds for carbon monoxide and ≤ 0.5% of full scale in 30 seconds for tracer gas.
7. The presence of carbon dioxide (CO_2) and water vapor should not interfere with the gas analyzer. If so, the equipment should remove these gasses before the sample passes through the analyzer or the equipment makes adjustments to the gas measurement according to the concentration of CO_2 and H_2O vapor present.
8. Circuit resistance should be < 1.5 cmH_2O/L/s at a flow rate of 6 L/s, if the test gas tank uses a flow demand regulator, the maximum inspiratory pressure across the circuit and valve should be < 10 cmH_2O.
9. The device timer should be accurate to 1% (100 ms over 10 seconds).
10. Monitor and report tracer gas and CO concentrations at end-expiration (alert operator if flushing is incomplete).
11. Ensure correct alignment of gas concentration signals and flow signal.
12. The equipment should measure the anatomical dead space using the Fowler method; failure to do so may result in an estimate of the anatomical dead space, but with the risk of inaccurate results.[9]
13. Display a graph of gas concentration versus exhaled volume to confirm the dead space washout point and report the amount of manual adjustment if performed.
14. Report DLCO adjusted for the change in PAO2 due to barometric pressure.
15. Ability to enter simulated digital test data and calculate DLCO, VA, TLC, VD.
16. Compensate for end-expiratory gas concentrations prior to test gas inhalation in the calculation of VA and DLCO.
17. The equipment dead space volume (DV) for both the inspired test gas and the alveolar sample should be known, its role in all data computation algorithms should be identified and documented. For adults, the VD should be < 200 mL, including the breathing circuit proximal to the gas analyzer sampling point, filter, and mouthpiece. Smaller dead space volumes are recommended for pediatric population and persons with a vital capacity (VC) < 2 L.
18. The system should be free of leaks.
19. For the digitized signal to accurately follow the gas concentration signal and provide adequate opportunity for signal processing for data alignment, the minimum signal sampling rate should be ≥ 100 Hz per channel with > 14 bits of resolution; however, a rate of 1,000 Hz is recommended.
20. The accuracy of the barometric pressure sensor should be within ± 2.5%.
21. Must have the capability to perform a quality check (with a 3 L syringe, under ATPS conditions and inhalation of ~2 L of test gas), the equipment must calculate total volume (VA) of 3 ± 0.3 L and DLCO of < 0.5 mL/min/mmHg or < 0.166 mmol/min/kPa.

Other equipment and consumables

1. Gas mixture tank for medical use; example: 0.27-0.33% CO, 9-11% helium, 18-25% oxygen and the rest nitrogen.
2. Computer and printer, according to device requirements.
3. Scales for weight and height measurement and tape measure for arm extension measurement, when required.
4. Environmental thermometers with an accuracy of 1 °C.
5. Disposable in-line filter nozzle with > 99% efficiency for filtration of viruses, bacteria and mycobacteria; dead space < 100 mL and resistance less than 1.5 cmH_2O at a flow rate of 6 L/s.
6. Infection control attachments:
7. Access to hand washing and disinfectant gel.
8. Surgical mask for general protection, and when N95 mask is required it must have a leakage of less than 10% and a filtration efficiency of > 95% at a flow of 50 L/min.

Adjustment for hemoglobin

Because Hb is the binding site for CO, $DLCO_{sb}$ can change significantly depending on the Hb concentration in the blood. Better results are obtained with Hb measured on the same day, particularly in suspected polyglobulia, anemia or long-term measurements. Using these relationships and expressing Hb in g/dL, the predicted DLCO in adolescents and adult men can be adjusted using the following equation:

$$DLCO\ [predicted\ for\ Hb] = DLCO\ [predicted] \times (1.7 \times Hb/(10.22 + Hb))$$

While that of children under 15 years of age and women is adjusted using the following equation:[7]

$$DLCO\ [predicted\ for\ Hb] = DLCO\ [predicted] \times (1.7Hb/(9.38 + Hb))$$

Adjustment for carboxyhemoglobin

CO binds to Hb, and DLCO depends on the amount of Hb, therefore, DLCO is reduced if COHb increases. Adjustment for COHb is not routinely required, but is recommended, if COHb levels are suspected to be high, usually in smokers. Smokers have COHb of 5-10%, while nonsmokers < 3%. If COHb is < 2% no adjustment is required. Adjustment of DLCO for COHb is performed following the following equation.[7,10,11]

$$DLCO\ [predicted\ for\ COHb] = DLCO\ [predicted] \times (102\text{-}COHb\%)$$

It should be remembered that CO inhalation in the single-breath maneuver causes COHb to increase by 0.6 to 0.7% for each maneuver.[7,12]

Alveolar Oxygen Pressure Adjustment (P_AO_2)

Oxygen and CO compete for the same binding sites with Hb, so P_AO_2 affects DLCO. If P_AO_2 is high, DLCO decreases and vice versa. The first adjustment to this level is a concentration of 21% oxygen in the test gas. The DLCO value will change by approximately 0.35% for every 1 mmHg change in P_AO_2 or approximately 2.6% for every 1 kPa change in P_AO_2.[8]

Altitude adjustment

Altitude also affects P_AO_2. The higher the altitude, the higher the DLCO because P_AO2 decreases. Adjustment for altitude could be made in two ways:

1. DLCO [adjusted PB] = DLCO (0.505 + 0.00065 PB)
2. Altitude-adjusted DLCO = measured DLCO × [1 + 0.0031 (PiO_2-150)].

Where estimated IOP_2 = 0.21 (barometric pressure –47), or predicted values can be adjusted.

$$The\ P_AO_2 = 0.21\ (PB\text{-}47)$$

Example: BP in Mexico City averages 585 mmHg, therefore:

$$IOP_2 = 113\ mmHg$$

The adjustment in Mexico City would correspond to:

$$DLCO\ CDMX = DLCO\ (0.885)$$

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