American Thoracic Society
MEDICAL SECTION OF THE AMERICAN LUNG ASSOCIATION

Single-breath Carbon Monoxide Diffusing Capacity (Transfer Factor)

Recommendations for a Standard Technique-1995 Update

THIS OFFICIAL STATEMENT OF THE AMERICAN THORACIC SOCIETY WAS ADOPTED BY THE ATS BOARD OF DIRECTORS, JULY 1995.

CONTENTS

BACKGROUND

There has been growing interest in standardizing lung function tests to improve reproducibility and make results more comparable from laboratory to laboratory (1-5). In 1987 the American Thoracic Society (ATS) Committee on Laboratory Proficiency Standards undertook a project to identify and standardize measurement of the carbon monoxide diffusing capacity (D_{LCO}) in an effort to reduce test variability. This resulted in official ATS recommendations for the single-breath D_{LCO} technique, which is the technique most commonly used (6). Since 1987 several new developments in D_{LCO} equipment and computational approaches have occurred. Also, the European Respiratory Society (ERS) and the British Thoracic Society have recently issued separate recommendations regarding D_{LCO} standardization (7, 8). These events prompted the ATS Committee on Laboratory Proficiency Standards to review its original recommendations and produce this 1995 update

D_{LCO} is a measurement of carbon monoxide (CO) transfer from inspired gas to pulmonary capillary blood. This transfer is a complex phenomenon involving the distributional relation of alveolar ventilation to alveolar capillary perfusion, the actual CO transfer properties of the alveolar capillary interface, the capillary volume, the hemoglobin concentration, and the reaction rates between CO and hemoglobin (9-16). Because this process involves more than just diffusion, the measurement of CO uptake is more properly referred to as "CO transfer factor" (7). However, "carbon monoxide diffusing capacity" (D_{LCO}) is the term more commonly used in North America and, therefore, the term used in this document.

The approaches to measuring D_{LCO} include rebreathing, steady state, and a variety of single-breath techniques (7, 10, 16, 17). However, the 10-s single breath-hold technique is still the most commonly used technique in clinical laboratories. Therefore, as in 1987, the committee has restricted this document to issues concerning this method.

Using a single value to summarize individual CO uptake properties of millions of lung units is an inherent limitation in measuring and interpreting D_{LCO}. Even in normal lungs, CO uptake differs between the basilar and apical regions of the lung as a result of gravitational effects on regional blood flow and blood volume (18, 19). Regional differences may become even more pronounced in a number of disease states. Nevertheless, measuring an "overall" CO uptake by the single-breath technique has proved useful in assessing a variety of lung abnormalities that impair alveolar capillary gas transport (Table 1). Moreover, in many diseases, the magnitude of abnormalities in D_{LCO} has been shown to correlate with disease severity and with direct measurements of arterial blood oxygenation, especially during exercise (7, 20-26).

The complexity of the process reflected in the single-breath measurement of CO uptake means that considerable test variability can be expected. The magnitude of interlaboratory variability in D_{LCO} attributable to variations in test technique (breathing maneuvers, timing, methods of gas analysis, and computational techniques) has not been clearly summarized, but Clausen and associates (27) reported an interlaboratory coefficient of variation of 12.7% for D_{LCO} in comparison with 3.4% for FVC. Kan-

This statement was prepared by the Committee on Proficiency Standards for Clinical Pulmonary Laboratories. Members of the Committee were: Robert O. Crapo, M.D., Chair; John L. Hankinson, Ph.D.; Charles Irvin, Ph.D.; Neil R MacIntyre, M.D.; Karen Z. Voter, M.D.; Robert A. Wise, M.D.

D_{LCO} Subcommittee: Neil R. MacIntyre, M.D., Chair; Robert O. Crapo, M.D.; Brian Graham, Ph.D.; Karen Z. Voter, M.D.

Invited Spirometry and D_{LCO} Workshop Participants: Brian Graham, Ph.D.; Carl O'Donnell, Sc.D.; Paolo Padetti, M.D.; Josep Roca, M.D.; Giovanni Viegi, M.D.

Am J Respir Crit Care Med Vol 152. pp 2185-2198, 1995

TABLE 1

PROCESSES ASSOCIATED WITH ALTERATIONS IN D_{LCO}

- Decreases in D_{LCO}
  - Obstructive lung diseases
    - Emphysema
    - Cystic fibrosis
  - Parenchymal lung diseases
    - Interstitial lung disease
      - Caused by fibrogenic dusts, e.g., asbestosis
      - Caused by biologic dusts, e.g., allergic alveolitis
      - Drug reactions, e.g., amiodarone, bleomycin
      - Idiopathic
    - Sarcoidosis
  - Pulmonary involvement in systemic diseases
    - Systemic lupus erythematosus
    - Progressive systemic sclerosis
    - Mixed connective tissue disease
    - Rheumatoid arthritis
    - Dermatomyositis-polymyositis
    - Wegener's granulomatosis
    - Inflammatory bowel disease
  - Cardiovascular diseases
    - Acute myocardial infarction.
    - Mitral stenosis
    - Primary pulmonary hypertension
    - Pulmonary edema
    - Acute and recurrent pulmonary thromboembolism
    - Fat embolization
  - Other
    - Diseases associated with anemia
    - Chronic renal failure
    - Chronic hemodialysis
    - *Marijuana smoking*
    - Acute and chronic ethanol ingestion
    - Freebasing cocaine
    - Cigarette smoking
    - Bronchiolitis obliterans with organizing pneumonia (BOOP)
- Increases in D_{LCO}
  - Diseases associated with polycythemia
  - Pulmonary hemorrhage
  - Diseases associated with increased pulmonary blood flow such as left-to-right intracardiac shunts
  - Exercise

Reproduced with permission from reference 17.

galee and Abboud (28), in a study of one individual tested in 22 different laboratories over 13 yr, found D_{LCO} to vary from 28.7 to 41.7 ml CO/min/mm Hg. In a study in six London laboratories, Saunders (29) showed D_{LCO} values in two individuals ranging from 10.5 to 20.4 ml CO/min/mm Hg, and a more recent study by Wanger and Irvin (30) on five individuals in 13 laboratories showed individual D_{LCO} values ranging from 20.6 to 54.2 ml CO/min/mm Hg. Using computation schemes chosen from published methods, Morris and Crapo (31) demonstrated that D_{LCO} could vary by as much as 41% based on the method of computation alone Gas analyzer inaccuracy introduces yet another source of variability. In a study of 11 laboratories, Cotes (32) found that errors in gas analysis can change D_{LCO} by 53 to 125%. In a follow-up study, Chinn and colleagues (33) found that gas analysis errors had been reduced but not eliminated. Finally, wide variability in D_{LCO} reference equations affects the percent of predicted values and may significantly affect interpretation (17, 34-35). Although the 1987 standards and technical improvements have likely reduced variability, the potential for large *variations in measured* D_{LCO}, *predicted* D_{LCO}, *and percent* predicted D_{LCO} still exists.

This revision will not substantially change the 1987 recommendations for equipment, calibration, and test technique issues, although they are reviewed. Our goals in this revision were to: (*1*) clarify areas of potential confusion, particularly areas where ERS (7) recommendations differ; and (2) address technological developments with potential to reduce D_{LCO} variability. For example, new technology allows CO and tracer gas concentrations to be monitored continuously, eliminating the need to collect gas in a sample bag during the single-breath technique (36-38). Continuous monitoring of flow and gas concentrations also permits new analytic methodologies to be developed. For example, the use of a three-equation computation method analytically accounts for variations in inspiratory and expiratory flow rather than relying on empiric corrections of the measured breath-hold time (36, 37, 39). Another example of a new analytic methodology is the technique of calculating D_{LCO} by eliminating the breath-hold and analyzing CO uptake continuously throughout a slow, controlled expiration (40, 41). Reference values and clinical research demonstrating improved diagnostic sensitivity and/or specificity are not yet available for any of these new analytic approaches. The committee encourages continuing investigation of new analytic methods but is not prepared to recommend that they replace the current single-breath technique in clinical testing at the present time. Similarly, the committee feels that testing under conditions other than with a subject sitting at rest (e.g., testing during exercise or in the supine position [42–44]) needs additional clinical research before widespread use can be recommended.

Standardization is not synonymous with truth. It is certainly not immutable. Standardizing technical issues provides a framework to allow better comparisons between measured values and reference values as well as better comparisons between laboratories. When possible, scientific information was used to guide the committee's decisions. When scientific information was not available, we made arbitrary decisions to complete the recommendations. We expect to update the recommendations as scientific studies become available.

DEFINITIONS AND ABBREVIATIONS

COHb – **Carboxyhemoglobin** concentration in percent (%).

CV – Coefficient of variation (standard deviation/mean value) x 100.

D_{LCO} – Carbon monoxide diffusing capacity; known as the transfer factor (T_{LCO}) in Europe Conventional units are ml CO (STPD)/min/mm Hg; SI units are mmole CO/min/kPa. D_{LCO} in conventional units equals 2.986 x D_{LCO} in SI units.

D_L/V_A – Carbon monoxide diffusing capacity per unit of alveolar volume. In Europe, this is known as T_L/V_A. Conventional units are ml CO (STPD)/min/mm Hg/L (BTPS); SI units are mmoles CO/min/kPa/L (BTPS).

DM-Membrane diffusing capacity (ml CO [STPD]/min/mm Hg).

F_{I_x} – **Fraction** of inspired gas, "x."

F_{A_x} – Fraction of "x" in the alveolar gas. An additional modifier may be added to denote time (e.g., $F_{ACO,0}$ = alveolar fraction of CO at time zero; $F_{ACO,t}$ = alveolar fraction of CO at time t).

FEV, -Forced expiratory volume in 1 s.

FVC -Forced vital capacity.

Hb – Hemoglobin concentration in g/dl.

P_{AO_2} – Alveolar oxygen pressure in mm Hg.

P_B – Barometric pressure in mm Hg.

P_{H_2O} – **Water** vapor pressure in mm Hg.

P_{IO_2} – *Inspired oxygen pressure in* mm Hg.

ATPD – **Ambient** conditions and dry (ambient temperature, ambient PB, P_{H_2O} = 0 mm Hg).

ATPS – Ambient conditions saturated with water vapor (ambient temperature, ambient PB, P_{H_2O} reflecting saturation with water vapor at ambient temperature).

BTPS– Body conditions (normal body temperature [37° C], ambient PB, P_{H_2O} reflecting saturation with water vapor at 37° C [47 mm Hg]).
STPD-Standard conditions (temperature = 0" C, P_B = 760 mm Hg, P_{H_2O} = 0 mm Hg).
t-Breath-hold time in seconds.
TLC – Total lung capacity.
θ–Specific uptake of CO (ml CO [STPD]/min/mm Hg/ml blood).
Tr -Tracer gas, a relatively insoluble and biochemically inert gas used to calculate V_A from its dilution in alveolar gas.
RV – Residual volume.
V_A – Alveolar volume during the test in liters. Conditions (BTPS or STPD) should be specified (e.g., V_A BTPS).
VC – Vital capacity.
Vc – Pulmonary capillary blood volume (ml).
V_D – Dead-space volume in liters. Dead space is of three types: anatomic V_D – dead-space volume of the patient's conducting airways; instrument VD-dead-space volume of the instrument, including mouthpiece, tubing, and connectors; and sample bag V_D – dead-space volume of an alveolar sample bag.
Vr-Inspired volume. The volume of test gas inspired as part of the D_{LCO} test in liters. Conditions (ATPS, ATPD, BTPS, STPD) should be specified (e.g., V_I STPD).
Vs-Volume of the expired sample gas in liters.

GAS ANALYZERS AND GENERAL EQUIPMENT

System Design

Descriptions of the apparatus and general instructions for performing the single-breath diffusing capacity maneuver are available elsewhere (2-5, 7, 17, 45, 46). 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 analyzers (single-sample analyzers or continuous analyzers). Single-sample *gas* analyzer systems usually display volume over time (Figure 1, *upper panel*). Continuous gas analyzer systems may also provide a continuous tracing of CO and tracer gas concentrations (Figure 1, lower *panel*).

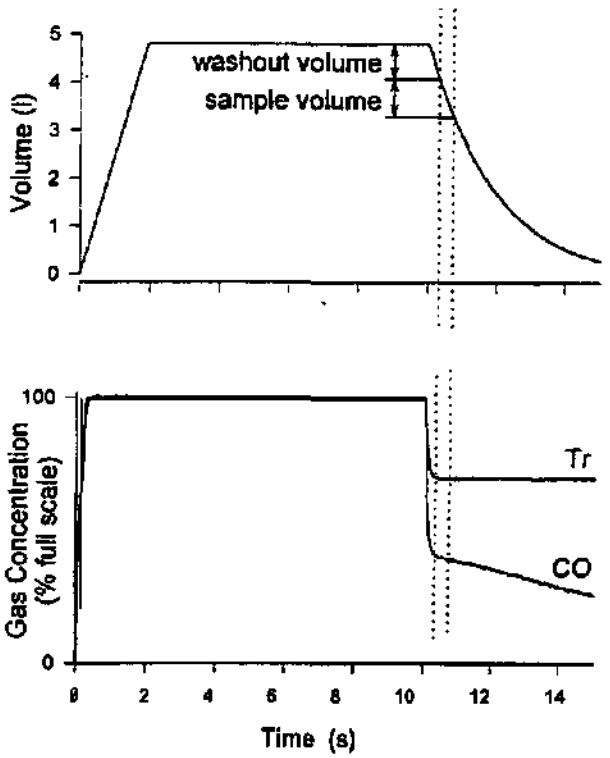

Figure 1. A schematic of lung volume versus time (*upper panel*) and carbon monoxide (CO) and tracer gas (Tr) concentrations versus time (*lower panel*) during a standard D_{LCO} measurement using a continuous analyzer. The dotted lines indicate when the alveolar sample occurs.

Equipment Requirements

Minimum standards for equipment include (Table 2):

1. Volume measurement accuracy should be the same as that established by the ATS for spirometry (47); that is, ± 3% volume accuracy over an 8-L volume range, with test gases present in concentrations likely to be encountered during D_{LCO} tests. Some pneumotachometer devices are sensitive to different gas concentrations. These devices should maintain the required volume accuracy when test gases are used (e.g., when they are inhaled or exhaled by a subject) (48).
2. Gas analyzer accuracy is important in some circumstances, such as measuring CO "back pressure." However, in calculating D_{LCO} only the *ratios* of CO and the tracer gas are used, and the accuracy of the gas analyzers is not as important as the ability to provide a linear output. Linearity should be within 1% of full scale (i.e., once the analyzers have been adjusted to zero with no test gas present and then set to an arbitrary full scale value at the test gas concentrations, the combined effects of zero drift, gain drift, and system nonlinearity on measurements of known dilutions of test gas should be no more than 1% of full scale). The gas analyzers should be stable so linearity is maintained over the test interval. Manufacturers are encouraged to provide a display of the gas concentrations measured by the analyzers so linearity can be confirmed.
3. Circuit resistance should be less than 1.5 cm H_2O/L/s at 6 L/s flow. If a demand flow regulator is used on a compressed test gas cylinder, the maximal inspiratory pressure required for 6 L/s inspiratory flow through both circuit and valve should be less than 10 cm H_2O.
4. The timing device in the D_{LCO} 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.
5. Dead space for both inspired test gas and the alveolar sample should be known, and their role in all data computation algorithms identified and documented. The dead space of the valve and mouthpiece should total less than 0.100 L.
6. If CO, and/or H_2O will interfere with gas analyzer performance, they must be removed from the test gases before passage through the gas analyzers. Water is commonly absorbed by anhydrous $CaSO_4$ or by other products. Selectively permeable tubing can also be used to remove water. Water-vapor permeable tubing has a limited life expectancy. The calculation of D_{LCO} may be significantly affected when such tubing fails to properly equilibrate water vapor. Manufacturers should provide a replacement schedule for water-vapor permeable tubing and a method for checking its function. Users of such tubing should be aware of the problem and have a plan for

TABLE 2

EQUIPMENT SPECIFICATIONS

Volume accuracy	± 3% accuracy over an 8 L volume using test gases
Gas analyzers	Linear from zero to full span within ± 1% of maximal value over duration of test
Circuit resistance	< 1.5 cm H_2O/L/s at flow of 6 L/s
Demand valve sensitivity (if compressed gas source used)	< 10 cm H_2O required for 6 L/s flow through valve and circuit
Timer	± 1.0% over 10 s
Apparatus dead space	< 0.1 L

replacing the tubing at appropriate intervals and for checking the tubing function. A monthly check of tubing function is recommended. One method of checking water vapor permeable tubing is to compare gas concentration measurements made with both dry and humidified test gas. Absorption of CO, can be achieved with either $Ba(OH)_2$ or NaOH. Both generate H_2O when combining with CO_2. Therefore, if aCO_2 absorber is used, it must precede the water absorber in the gas analyzer circuit.

7. The system must be leak-free This is particularly important for D_{LCO} systems that aspirate gas samples at subatmospheric pressure through the gas analyzers (rather than blowing the gas samples at supra-atmospheric pressure). 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 (Table 3)

1. Each day:
   a. Volume calibration with a calibrated 3-L syringe (47-49).
   b. Leak testing (47, 49).
2. Each quarter:
   a. Gas analyzer linearity should be assessed. A straightforward approach is to measure known serial dilutions of test gas, similar to the methods suggested for gas chromatography by Okubo and Lenfant (50) and recommended by the ERS (7).
   b. The timer should be assessed for accuracy (49).
   c. Standard subject(s) should be tested to assure overall stability of the system. Standard subjects are healthy nonsmokers (eg., healthy laboratory personnel). If the D_{LCO} in a standard subject varies more than 10% from known previous values, the test should be repeated. If the repeat test confirms the finding, the D_{LCO} system should be evaluated carefully for the possibility of leaks, nonlinear analyzer function, volume and time inaccuracy, etc When sufficient data on a standard individual are obtained, laboratories may choose to establish their own outlier criteria to serve as indicators of potential problems with their D_{LCO} systems.
3. 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 options for quality control measurements and quality control data management.

TABLE 3

EQUIPMENT QUALITY CONTROL

Volume accuracy and leak testing	Tested daily
Gas analyzer linearity	Tested quarterly
Timer	Tested quarterly
Tests on laboratory personnel	Tested quarterly

Infection Control

The major goal of infection control is to prevent the transmission of infection to patients and staff during pulmonary function testing. Infection may be transmitted by two modes:

1. *Direct contact.* Through direct contact with potential pathogens, there is the potential for transmission of upper respiratory disease, enteric infections, and blood-borne infections. Although infection with hepatitis and HIV are unlikely via saliva, the possibility exists when there is hemoptysis, open sores on the oral mucosa, or bleeding gums. The most likely surfaces for contact are mouthpieces and the immediately proximal surfaces of valves or tubing.
2. *Indirect contact.* Contaminated aerosol droplets have the potential for transmitting tuberculosis, various viral infections, and possibly opportunistic infections and nosocomial pneumonia to susceptible patients. Mouthpieces and proximal valves and tubing are likely candidates for contamination by aerosols.

Pulmonary function equipment has not been directly implicated in the transmission of infections. However, there is indirect evidence that infection may be transmitted during pulmonary function testing. Organisms from the respiratory tract of test subjects have been recovered from mouthpieces and from the proximal surfaces of tubing through which subjects breathe (51, 52). This does not seem to pose an appreciable threat to patients with competent immune systems, although there is some potential for cross-contamination. There is one case report of tuberculosis skin test conversion after exposure to a spirometer used to test a patient with documented tuberculosis (53). There is also circumstantial evidence implicating contaminated pulmonary function equipment in the increasing prevalence of infections due to pseudomonas (especially *Pseudomonas cepacia)* among cystic fibrosis patients at one center (54). Finally, it is well documented that community and hospital water supplies can be contaminated with *legionella, mycobacteria, and pseudomonas organisms (55-57).* Thus, there is a potential for patients and health care workers to deposit microorganisms onto pulmonary function testing circuit surfaces (including mouthpieces, nose clips, tubing, and any internal or external machine surface); these microorganisms could subsequently come into direct or indirect contact with other patients.

Concerns for the protection of immunocompromised hosts, along with increased public and provider awareness of hospital infection control issues over the past decade have led many laboratory directors to use in-line filters routinely as a means of reassuring patients and laboratory personnel that adequate consideration has been given to protection (58, 59). The extent to which in-line filters affect the measurements needed to calculate D_{LCO} is undocumented. One study has shown that a low impedance barrier device did not have a significant impact on spirometric indices such as the FVC and FEV, (60).

Recommendations:

1. Prevention of infection transmission to technicians exposed to contaminated equipment surfaces can be accomplished through proper hand washing or use of barrier devices (latex gloves). To avoid technician exposure and cross-contamination, hands must be washed immediately after direct handling of mouthpieces, tubing, breathing valves, or interior equipment surfaces. Gloves must be worn when handling potentially contaminated equipment if there are any open cuts or sores on technicians' hands. Hand washing must always be performed between patients. Indications and techniques for hand washing during pulmonary function testing have recently been reviewed by Tablan and colleagues (61).

2. To avoid cross-contamination, reusable mouthpieces, breathing tubes, valves, and manifolds should be disinfected or sterilized regularly. Mouthpieces, nose clips, and any other equipment coming into direct contact with mucosal surfaces should be disinfected or sterilized after each use. The optimal frequency for disinfection or sterilization of tubing, valves, or manifolds has not been established. However, any equipment surface with visible condensation from expired air must be disinfected or sterilized before reuse.
3. In settings where tuberculosis or other diseases spread by droplet nuclei are likely to be encountered, proper attention to engineering controls, such as ventilation, air filtration, or ultraviolet decontamination of air, must be used to prevent disease transmission (62).
4. Special precautions must be taken when testing patients with hemoptysis, open sores on the oral mucosa, or bleeding gums. Tubing and breathing valves must be decontaminated before reuse, and internal device surfaces must be decontaminated with accepted disinfectants for blood-transmissible agents.
5. Extra precautions may be undertaken for patients with known transmissible infectious diseases. Possible precautions include: (a) reserving equipment for the sole purpose of testing infected subjects; (b) testing patients at the end of the day to allow time for disassembly and disinfection; and (c) testing patients in their own room or in rooms with adequate ventilation and easily cleaned surfaces.
6. Many devices currently used to measure D_{LCO} incorporate complex valving mechanisms that are located proximal to breathing tubes. These are often difficult to disassemble and disinfect between subjects. In settings where routine disassembly of these mechanisms is not possible, in-line filters may be effective in preventing equipment contamination (59). The economy of using in-line filters as compared with tubing and valve changes will depend on the equipment in use. If an in-line filter is used, the measuring system must meet the minimal recommendations listed above for system accuracy, flow resistance, back pressure, and dead space with the filter installed. Moreover, the interpretation of results should allow for the possibility that the filter might affect equipment performance Appropriate adjustments must be made for additional dead space due to a filter, and equipment must be calibrated with a filter installed.
7. Manufacturers of D_{LCO} equipment are encouraged to design instrumentation that can be easily disassembled for disinfection or find other solutions to hygiene concerns.

STANDARDIZATION ISSUES

The issues addressed by the committee are categorized as technique factors, calculation factors, and interpretation factors. Each issue is summarized and discussed, and a recommendation for a standard approach given.

Technique Factors

Patient conditions for measurement. In general, conditions that may affect pulmonary capillary blood volume and, therefore, measured D_{LCO} should be avoided. They include exercise and heavy meals.

D_{LCO} increases with a change from the seated to the supine position and decreases from the seated to the standing position **(42–46, 63).** Estimates of the magnitude of the change in D_{LCO} with position change are variable (eg., 5 to 30% from sitting to supine) **(42, 43).** D_{LCO} also increases with exercise **(41–44, 64).**

Recommendations. Before beginning the test, the maneuvers should be demonstrated and the subject carefully instructed. The subject should be seated for at least 5 min before testing and remain seated throughout the test procedure. The test should be performed at least 2 hr after a light meal and with the subject having refrained from recent strenuous exercise.

Inspiratory maneuver. Once the mouthpiece is in place, four to five tidal volumes are recorded to determine a regular end-expiratory baseline. The D_{LCO} maneuver then begins with exhalation to residual volume (RV). At RV the subject's mouthpiece is connected to a source of test gas and the subject inhales rapidly to total lung capacity (TLC). The volume of test gas inhaled is V_I. D_{LCO} should be measured near TLC. The 1987 ATS recommendation was that V_I be > 90% of the largest previously measured vital capacity. The ERS recommends that the V_I should be adequate to achieve 90 to 95% TLC (7).

Inspiratory time in healthy subjects averages 1.5 to 2 s (46, 65). In healthy subjects, 95% of inspiratory times are less than 2.5 s (R. O. Crapo, personal communication). In patients with moderate to severe airflow obstruction **($FEV_1/VC < 0.5$), inspiratory** times average about 2 s, with 95% less than 4 s. If the time of inspiration is increased to one half of the breath-hold time (approximately 5 s), the D_{LCO} is reduced by 13% when breath-hold time is measured using the Ogilvie technique (see CALCULATIONS) (46, 65).

Calculating inspiratory time is difficult when the beginning and end of inspiration are not clear. Until more is known, the committee recommends that the back extrapolation technique be used to establish time zero because it is a standard technique in spirometry (1, 47, 66). Since the end of inspiration may be less readily identified, the committee recommends the time when 90% of the V_I has been inspired as a reasonable end point for the purpose of meeting the inspiratory time criteria only.

The rate of inspiration will depend in part upon the resistance of the inlet circuit and the demand valve characteristics (if present). If inlet circuit resistance is too high, the demand valve too insensitive/unresponsive, or if a patient has a high central airway resistance, a rapid inspiratory maneuver may effectively be a Muller maneuver (inspiratory effort against a closed airway), which will falsely increase D_{LCO} (67-69).

Recommendations. The inspiration should be rapid. Ninety percent of V_I should be inspired in less than 2.5 s in healthy subjects and in less than 4.0 s in patients with moderate to severe airway obstruction. The V_I should be at least 90% of the largest previously measured vital capacity, both expressed at BTPS conditions.

Condition of the breath-hold. Valsalva (expiratory efforts against a closed airway) and Muller maneuvers (inspiratory efforts against a closed airway) during the breath-hold will decrease and increase D_{LCO}, respectively (67-69). The 1987 ATS recommendation was to have subjects relax against a closed glottis or valve (6). The rationale was that it was felt this technique would produce the least variation in intrathoracic pressure This maneuver, however, may be more difficult to perform than simply having the subject voluntarily maintain full inspiration using only the minimal effort necessary.

Recommendation. After inspiring the test gas, the subject should either try to relax against a closed glottis or a closed valve during the breath-hold or else maintain a full inspiratory position without straining. Excessive positive or negative intrathoracic pressure (i.e., obvious Valsalva or Muller maneuvers) should be avoided during breath-hold.

Expiratory maneuver. Recommendation. At the end of the breath-hold period, the expiratory maneuver should be smooth, without hesitation or interruption. Sample collection time should not exceed 4 s.

Washout volume. During expiration, a volume of gas must be expired and discarded to clear anatomic and mechanical dead space before the alveolar sample is collected **(17, 46).** The Intermountain Thoracic Society (ITS) (3) and the ERS (7) both recommend washout volumes of 0.75 L. Ogilvie and colleagues (46)

reported a 10% increase in D_{LCO} as the washout volume increased from 1.0 to 2.5 L. Huang and MacIntyre (38) demonstrated with a continuous analyzer that an 0.75 L washout volume was appropriate in more than 90% of patients referred to a pulmonary function laboratory.

Recommendation. In a single-sample system, the washout volume should be 0.75 to 1.0 L. If the patient's vital capacity is < 2.00 L, the washout volume may be reduced to 0.50 L; any such change should be noted in the report. If a continuous gas analyzer system is used, computerized or manual inspection of the expired CO and tracer gas curves may be used to adjust washout volume to assure dead space clearance (Figure 1).

Sample collection volume. Sample volume depends on analyzer requirements as well as assurance that a representative alveolar sample is obtained. The current ERS (7) and ITS (3) recommendations for sample volume are 0.50 to 1.00 L. The Epidemiologic Standardization Project (ESP) recommends a 1-L sample (0.50 L if the vital capacity is < 2.00 L). Continuous gas analyzers can determine alveolar CO concentrations on much smaller volumes (38).

Ogilvie and colleagues (46) recommended that the duration of the alveolar collection be < 2 to 3 s. Kanagami and associates (65) suggested that the total collection time (washout and alveolar sample collection) not exceed 4 s. With the Ogilvie method of calculating breath-hold time (see CALCULATIONS), D_{LCO} increases with increased sample collection time (46, 65). This effect may not be seen with the method by Jones and Meade (70), ESP (2) timing method, or the three-equation technique (36, 37).

Recommendation. With single-sample D_{LCO} systems, a sample volume of 0.50 to 1.00 L should be collected in less than 4 s. If continuous analyzers are used, computerized or visual inspection of the expired CO and tracer gas curves may be used to adjust sample volume to assure an appropriate alveolar sample (38).

Inspired oxygen pressure and alveolar P_{O_2}. *Alveolar* P_{O_2} (P_{AO_2}) and measured D_{LCO} are inversely related. Since P_{AO_2} fluctuates over the ventilatory cycle, the consensus in the European community (7) is that a more stable P_{AO_2} during the D_{LCO} maneuver is achieved with a test gas fraction of inspired oxygen (F_{IO_2}) of 0.17 and variability in measured D_{LCO} should therefore be reduced. In the United States, an F_{IO_2} of 0.21 is generally used, although some systems use test gas mixtures containing CO and He with "balance air." If such a mixture contains about 10% helium, 0.3% CO, and balance air, the F_{IO_2} will be about 0.19. When using other tracer gases, the test gases may constitute less than 1% of the total gas mixture. No current data are available to suggest a preference for the European (F_{IO_2} = 0.17) or American (F_{IO_2} = 0.21) methods.

Measured D_{LCO} will increase as altitude increases (and P_{IO_2} and P_{AO_2} decrease). Kanner and Crapo (71) demonstrated that commonly encountered alveolar oxygen pressures at higher altitudes will cause D_{LCO} to increase an average of 0.35% for each mm Hg decrease in alveolar P_{O_2}. This finding was confirmed by Gray and associates (72) using a hypobaric chamber to simulate altitude changes when they found an average D_{LCO} increase of 0.31% per mm Hg decrease in P_{IO_2}. Both groups argue that increasing test gas P_{IO_2} is an acceptable way to adjust D_{LCO} for altitude.

The use of supplemental oxygen will alter P_{AO_2} and therefore D_{LCO}. The magnitude of the effect and the method of adjusting D_{LCO} in individual patients using supplemental oxygen are unknown and this is an area where further research is clearly needed. Until more is known, it is probably best to measure D_{LCO} with subjects breathing room air as long as it is considered to be clinically safe.

Recommendations. Until more information is available, the test gas in the American community should contain 21% oxygen at sea level. It is recognized that this is controversial and differs from the standard practice in the European community. At altitudes other than sea level, either the test gas F_{IO_2} can be adjusted accordingly (adjust F_{IO_2} to produce a P_{IO_2} of 150 mm Hg) or, if the test gas contains 21% oxygen, the results should be adjusted for altitude or sample bag P_{O_2} (P_{AO_2}) (71, 72) as part of the interpretation (see INTERPRETING THE RESULTS).

Supplemental oxygen should be discontinued at least 5 min before beginning the test. If this cannot be done safely, the interval of time off oxygen should be adjusted appropriately and the resulting D_{LCO} interpreted with caution (see previous comments on the effects of alveolar P_{O_2}).

Interval between tests. Although Ogilvie and colleagues (46) demonstrated essentially complete elimination of test gas in 2 min in three healthy subjects and one emphysematous subject, it is reasonable to expect that a longer interval may be required in some patients with severe maldistribution of ventilation. Johns and associates (73) found no difference between the first and second tests in 129 patients when the interval between tests was 4 min.

Recommendation. At least 4 min should be allowed between tests to allow adequate elimination of test gas from the lungs. The subject should remain seated during this interval. In patients with obstructive airway disease, several deep inspirations during this period may help 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. Subsequent tests should not begin until the end-tidal tracer gas concentration is less than 1% of full scale.

Miscellaneous factors. There may be diurnal variation in D_{LCO}. Cinkotai and Thomson (74) found that D_{LCO} fell progressively throughout the day. Between 9:30 A.M. and 5:30 P.M., the decrease was 1.2%/h, and from 5:30 P.M. to 9:30 P.M., the decrease was 2.2%/h. 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. Frey and colleagues (75), however, explained D_{LCO}'s diurnal variation by increases in CO back pressure and by diurnal variation in hemoglobin concentration.

A 13% change in D_{LCO} during the menstrual cycle has been reported (76). The highest value was observed just before the menses and the lowest was on the third day of menses. This may reflect a hemoglobin effect.

Peavy and colleagues (77) have shown that D_{LCO} is reduced about 15% 90 min after ingestion of 15 to 30 ml of 95% ethanol. The reason for the change is unknown, and the finding has not been confirmed. However, it may reflect analyzer behavior in the presence of ethanol because similar changes have been noted in ketosis.

Cigarette smoking can have profound effects on the measurement of CO uptake. Lung pathology caused by long-term use of cigarettes is known to cause significant reductions in D_{LCO} (21, 78–83). However, there also appears to be a mild, acute, and reversible decrease in D_{LCO} during acute cigarette smoking (80–82). This acute effect is thought to be largely due to the effects on CO back pressure and the "anemia effect" due to increased carboxyhemoglobin concentration (see CALCULATIONS, carboxyhemoglobin and hemoglobin corrections).

Recommendations. Subjects should be asked to refrain from smoking for 24 h before the test. The time of the last cigarette smoked should be recorded. A correction for CO back pressure should be made for recent or heavy cigarette smoking (see CALCULATIONS, carboxyhemoglobin adjustment). Subjects should avoid alcohol for at least 4 h before testing.

Calculations

Basic formula for calculating D_{LCO}:

$$D_{LCO} = V_A\,(STPD) \times (1/t) \times [1/(P_B - 47)] \times \ln(F_{ACO,0}/F_{ACO,t}) \times 60{,}000$$

where $F_{ACO,0} = F_{ICO} \times (F_{ATr}/F_{ITr})$

and $V_A = (V_I - V_D) \times F_{ITr}/F_{ATr}$.

In these calculations volumes are in liters, breath-hold time (t) in seconds, barometric pressure (PB) in mm Hg; 47 represents water vapor pressure at 37° C. F_{ICO}, $F_{ACO,t}$, F_{ITr}, and F_{ATr} are the fractional concentrations of carbon monoxide and the tracer gas in the inspired and alveolar gas samples, respectively. V_A is alveolar volume, V_I is inspired volume, and V_D is dead-space volume (anatomic and instrument). The factor 60,000 converts L/s to ml/min. This equation (15) is technically valid only for the breath-holding portion of the single-breath maneuver but is usually applied to the entire maneuver.

Breath-hold time-measurement and duration. Inspiration and expiration times are finite and must be considered in the calculation of breath-hold time.

Three widely used methods of measuring breath-hold time for D_{LCO} are illustrated in Figure 2. The "classic" or Ogilvie method *(46)* measures-breath-hold time from the beginning of inspiration to the beginning of sample collection. Jones and Meade (70) proposed a second method, theoretically more accurate and reproducible than the classic method. Breath-hold time was measured to include 0.7 of the inspiratory time and 0.5 of the sample collection time This approach computes breath-hold time based on the expected CO concentration profile in the alveolar space from a theoretical instantaneous inspiration compared with a typical pattern of inspiration. The ESP (2) recommended measuring breath-hold time from the point at which one half of V_I had been inspired to the beginning of sample collection. There is little difference in calculated D_{LCO} using either the Ogilvie or the Jones and Meade breath-hold time technique in normal subjects *(84-86)*, but the latter method provides the least overestimation of D_{LCO} when airflow obstruction is present (87). The ESP technique produces a significantly shorter breath-hold time, and thus a larger D_{LCO} in normal subjects and patients with airflow limitation (86). The 1987 ATS recommendations (6) accepted either the Jones and Meade or Ogilvie techniques but discouraged use of the ESP technique.

A theoretically more accurate way to account for volume changes over time during inspiration and expiration is to use three separate equations for D_{LCO} during inspiration, breath-hold, and expiration (the "three-equation" technique [36, 37]). This algorithm is commercially available but its clinical advantages are not yet clear.

Recommendation. The Jones and Meade method of determining the breath-hold time is the preferred method. The start and end of inspiration is determined by extrapolation of the best-fit linear regression of volume versus time during inspiration. The technician should take care to assure that the breathing maneuver does not have stepwise changes in inspiration, breath-holding, or expiration. Breath-hold time should be between 9 to 11 s. Alternative breath-hold timing algorithms may be appropriate to maintain consistency (eg., longitudinal studies), but these measurements should be recognized as outside ATS recommendations.

Alveolar volume, method of measurement. Because D_{LCO} reflects CO uptake from alveolar gas, a measurement of alveolar volume (V_A) is required. V_A can be calculated by adding V_I to a separately measured residual volume and using appropriate adjustments for dead space. More frequently, V_A is calculated by measuring the tracer gas dilution during the breath-hold (see below). If the tracer gas dilution method is used to calculate V_A, tracer gas properties and the various dead spaces must be considered.

The *tracer gas* should be relatively insoluble and be chemically and biologically inert. Because the tracer gas is being used

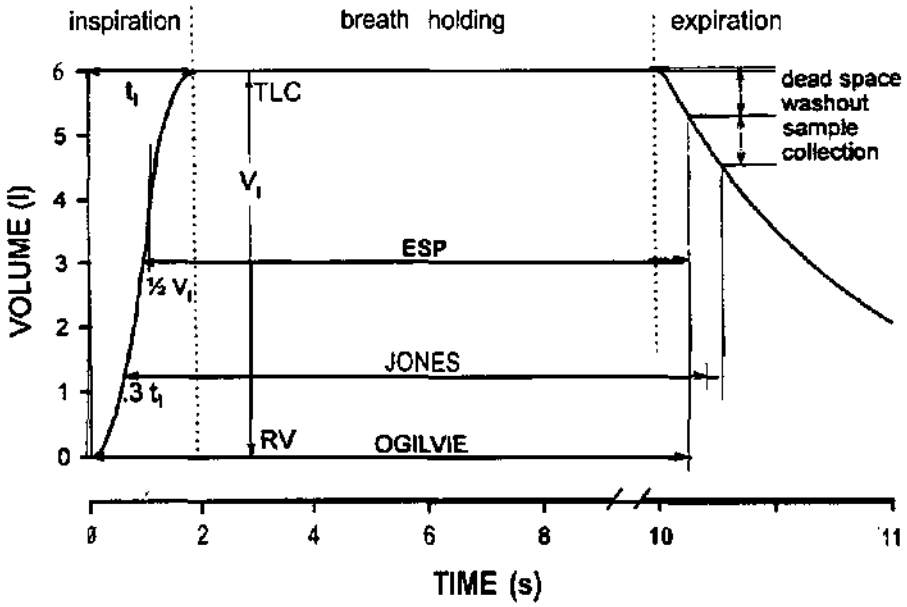

Figure 2. Schematic illustration of different methods of measuring breath-hold time for the single-breath D_{LCO}. V_I = inspired volume; t_I = time of inspiration. The Ogilvie or "classic" method (46) measures breath-hold time from the beginning of inspiration to the beginning of alveolar sample collection. The Jones and Meade method (70) includes 0.70 of inspiratory time and half of sample time. The Epidemiologic Standardization Project (2) measures breath-hold time from the time of 50% of V_I to the beginning of alveolar sample collection.

to determine the V_A from which CO uptake is occurring, its density, viscosity, and gaseous diffusivity should be similar to CO. It should approximately follow the ideal gas law and should not interfere with the measurement of CO concentration. The tracer gas should not ordinarily be present in alveolar gas (e.g., He) or be present at a known, fixed concentration (e.g., Ar). The tracer gas used most frequently is helium (He). While He meets most of the criteria, being relatively insoluble and inert, its gaseous diffusivity and viscosity are higher than air. Studies comparing single-breath He dilution lung volumes to other methods of measuring lung volumes have shown good agreement in normal subjects but underestimation of V_A in patients with obstructive lung disease (attributed to incomplete gas mixing) (2, 39). Other noble gases, including neon (Ne) and argon (Ar), have been used as tracer gases. There are also reports of using small concentrations (0.3%) of methane (CH_4) as a tracer gas (38, 42). As new tracer gases are introduced, manufacturers should demonstrate that they produce V_A and D_{LCO} values equivalent to those measured using traditional tracer gases.

Deadspace occurs in three areas: instrument dead space (volume of the mouthpiece, filters [if used], and connections within the valving system), anatomic dead space (volume in the conducting airways that does not participate in gas exchange), and in single sample systems with collection bags, sample bag dead space (residual volume in the sample bag and connectors).

Calculations of V_A from single-breath tracer gas dilution must account for these dead-space volumes by subtracting both instrument and anatomic dead space from inspired volume The calculation of V_A is thus: $V_A = F_{ITR}/F_{ATR} \times (V_I -$ instrument $V_D -$ anatomic VD). Instrument V_D should be specified by the manufacturer but may vary as the user alters the system (e.g., addition of a filter). Anatomic V_D is usually estimated from body size (88) but can vary with lung volume and breath-hold time (89, 90). The 1987 ATS recommendation (6) was to use a fixed value of 150 ml for anatomic V_D. This value, however, does not work well for small adults or children. Cotes (45) suggests an adjustment of 2.2 ml/kg body weight for anatomic VD. If a continuous gas analyzer is used, anatomic V_D may be measured using the technique described by Fowler (90).

Sample bag dead space (sample bag VD) dilutes the sample gas and alters the measured concentrations of expired gases. The size and direction of the error depend on sample volume (Vs), the dead-space volume of the sample bag and its connectors (sample bag VD), and sample bag v_D gas content. Sample bag V_D could contain test gas, room air, or expired gas from a subject (after a D_{LCO} test). When sample bag V_D contains room air, the effect of sample bag v_D is to reduce the measured concentrations of expired gases. The $F_{A_{Tr}}$ term in the calculation of v_A should be adjusted for this dead space When sample bag V_D contains room air, the adjustment is as follows (the adjustment will differ when sample bag V_D contains gases other than room air):

$$\text{Adjusted } F_{A_{Tr}} = \text{measured } F_{A_{Tr}} \times (Vs/[Vs - \text{sample bag } V_D])$$

No adjustment is required in the $F_{ACO,0}/F_{ACO,t}$ term because the adjustment factor occurs in both the numerator and denominator and cancels. Estimates of the potential change in D_{LCO} in existing systems when no adjustment is made for sample bag dead space range from 0.3 to 8%, depending on sample bag size and sample bag V_D (31).

Alveolar volumes calculated by the single-breath tracer gas dilution techniques or by adding V_I to a separately measured RV (after accounting for anatomic VD) are usually comparable in normal subjects. Indeed, several investigators (91, 92) have found that differences between single-breath and closed-circuit tracer gas dilution methods were small and of little consequence in normal subjects as well as patients with mild air flow obstruction ($FEV_1/VC > 0.5$). As airflow obstruction worsens, however, impaired gas mixing and distribution during the breath-hold can lead to significantly lower values for simultaneously measured single-breath v_A than for V_A obtainedby other methods (93, 94). This discrepancy can lead to substantial differences in calculated D_{LCO} in patients with airflow obstruction, depending on which measurement of V_A is used (95). If the simultaneous single-breath V_A is used, the calculated D_{LCO} could be considered as the D_{LCO} in the regions of the lung into which test gas was distributed. If the sum of V_I and a separately measured RV are used as V_A, the resulting D_{LCO} should be considered the D_{LCO} that would exist if that entire V_A had CO transfer properties similar to the average of the lung regions into which test gas was distributed (i.e., the lung regions measured by the simultaneous single-breath method of measurement of V_A).

Recommendations. Manufacturers should report instrument and sample bag dead space. Efforts should be made to reduce instrument dead space (including filters) to less than 100 ml. The instrument dead space (including sample bag) must be flushed with room air (or if membrane component of diffusion [D_M] and pulmonary capillary blood volume [Vc] are to be calculated, appropriate levels of oxygen) before the single-breath maneuver so that it will not contain expiratory gas from a previous subject. Sample bag dead space should be less than 2% of the sample volume or 10 ml, whichever is larger. An appropriate adjustment for sample bag dead space should be made to the measurement of $F_{A_{Tr}}$. The adjustment will vary depending on the gas in sample bag VD. Anatomic dead space should be calculated as 2.2 ml per kg of average body weight expressed at ATPD conditions.

The V_A used to calculate single-breath D_{LCO} should always be determined using single-breath tracer gas dilution. Other methods of estimating V_A in the calculation of D_{LCO} are also acceptable but must be reported in addition to the single-breath method and the method of V_A measurement identified.

Inspired gas conditions. Though inspired gas is commonly assumed to be at ATPS conditions (2), 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, test gas inspired from a bag-in-box system or through a pneumotachometer from a bag or a compressed gas cylinder with a demand valve is a dry gas (less than 10 ppm water) and thus at ATPD conditions (3). When D_{LCO} is calculated with V_I at ATPS rather than at ATPD (assuming the actual conditions are ATPD), D_{LCO} is underestimated by 3% at sea level and by 4% at 1400 m altitude (31).

Recommendation. The inspired gas conditions should be correctly determined and the proper conversion factors used (Table 4). Manufacturers should specify and document inspired gas conditions for each instrument.

CO_2 and H_2O adjustment. When V_A is measured as part of the single-breath maneuver and when gas analyzer properties require that CO, and H_2O be absorbed before gas analysis, the expired tracer gas and CO concentrations are artifactually increased (2). F_{ICO} and $F_{I_{Tr}}$ are unaffected when no CO, or H_2O is present in the test gas. No adjustment for the increase in $F_{ACO,t}$ 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 ($F_{ACO,0}/F_{ACO,t}$) and therefore cancels. An adjustment for the increase in expired tracer gas concentration ($F_{A_{Tr}}$) is necessary when it is used to calculate V_A.

Some newer systems use selectively permeable tubing to either remove water vapor completely or to equilibrate all gas samples with ambient humidity. Proper correction factors must be applied that require knowing how water vapor affects gas concentration measurements.

TABLE 4

ADJUSTMENTS TO TRACER GAS CONCENTRATIONS FOR H_2O, CO_2, AND TEMPERATURE

1. H_2O removed from sampled gas; CO_2 does not interfere with analyzers:

$$V_{A_{BTPS}} = (V_{I_{ATPD}} - V_{D_{INST}} - V_{D_{ANAT}}) \cdot \frac{F_{I_{T_r}}}{F_{S_{T_r}}} \cdot \frac{P_B}{P_B - 47} \cdot \frac{310}{273 + T}$$

$$V_{A_{STPD}} = (V_{I_{ATPD}} - V_{D_{INST}} - V_{D_{ANAT}}) \cdot \frac{F_{I_{T_r}}}{F_{S_{T_r}}} \cdot \frac{P_B}{760} \cdot \frac{273}{273 + T}$$

2. H_2O and CO_2 removed from sampled gas:

$$V_{A_{BTPS}} = (V_{I_{ATPD}} - V_{D_{INST}} - V_{D_{ANAT}}) \cdot \frac{F_{I_{T_r}}(1 + F_{ACO_2})}{F_{S_{T_r}}} \cdot \frac{P_B}{P_B - 47} \cdot \frac{310}{273 + T}$$

$$V_{A_{STPD}} = (V_{I_{ATPD}} - V_{D_{INST}} - V_{D_{ANAT}}) \cdot \frac{F_{I_{T_r}}(1 + F_{ACO_2})}{F_{S_{T_r}}} \cdot \frac{P_B}{760} \cdot \frac{273}{273 + T}$$

If no measurement of F_{ACO_2} is available then it may be assumed to be 0.05.

3. H_2O in sampled gas equilibrated to room air; CO_2 does not interfere with analyzers.
If $F_{I_{T_r}}$ is read by the analyzers, the equations are the same as #1.
If tank values (i.e., dry gas concentrations) are used for $F_{I_{T_r}}$, then:

$$V_{A_{BTPS}} = (V_{I_{ATPD}} - V_{D_{INST}} - V_{D_{ANAT}}) \cdot \frac{F_{I_{T_r}}}{F_{S_{T_r}}} \cdot \frac{P_B - P_{H_2O}}{P_B - 47} \cdot \frac{310}{273 + T}$$

$$V_{A_{STPD}} = (V_{I_{ATPD}} - V_{D_{INST}} - V_{D_{ANAT}}) \cdot \frac{F_{I_{T_r}}}{F_{S_{T_r}}} \cdot \frac{P_B - P_{H_2O}}{760} \cdot \frac{273}{273 + T}$$

4. Neither H_2O nor CO_2 removed from sampled gas, no interference with analyzers, heated sample tubing to prevent condensation:

$$V_{A_{BTPS}} = (V_{I_{ATPD}} - V_{D_{INST}} - V_{D_{ANAT}}) \cdot \frac{F_{I_{T_r}}}{F_{S_{T_r}}} \cdot \frac{310}{273 + T}$$

$$V_{A_{STPD}} = (V_{I_{ATPD}} - V_{D_{INST}} - V_{D_{ANAT}}) \cdot \frac{F_{I_{T_r}}}{F_{S_{T_r}}} \cdot \frac{P_B - 47}{760} \cdot \frac{273}{273 + T}$$

In these calculations, room temperature (T) is measured in Celsius and gas pressures are measured in mm Hg. P_B is barometric pressure; P_{H_2O} is ambient water vapor pressure in these equations. $V_{D_{INST}}$ = instrument dead space, $V_{D_{ANAT}}$ = anatomic dead space. F_{I_T} is the fraction of tracer gas in the inspired test gas. F_{ACO_2} is the fraction of CO_2 in the alveolar sample. $F_{S_{T_r}}$ refers to the measurement of the tracer gas (Tr) in the alveolar sample and may differ from $F_{I_{T_r}}$, depending on the effects of CO_2 and H_2O as noted. In all four cases, V_I is the measured volume of inhaled dry gas and is thus considered under ATPD conditions. The conversion to BTPS and 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. Standard gas condition conversion formulas must therefore be adjusted as described above.

Recommendations. Depending on how CO, and H_2O affect the gas analyzers and the system used to compensate for these gases, different calculations of V_A may be required as outlined in Table 4. Manufacturers must determine and document the appropriate corrections for their systems.

Gas conditions of V_A in D_L/V_A. In the expression D_L/V_A, V_A is variably reported at BTPS (31) or STPD (%, 97) conditions. Most recommendations now suggest that it be expressed at BTPS conditions (3, 4). Recent normal values also uniformly report the D_L/V_A in ml CO (STPD)/min/mm Hg/L (BTPS) (98-101). When V_A is expressed at STPD, D_L/V_A varies with altitude. D_L/V_A is independent of altitude when V_A is expressed at BTPS.

Recommendation. V_A in the denominator of D_L/V_A should be reported at BTPS conditions. The traditional units of D_L/V_A are, therefore, ml CO (STPD)/min/mm Hg/L (BTPS). This recommendation is based on the clinical experience of the conference members and on the independence of D_L/V_A from altitude when V_A conditions are BTPS.

Interpreting the Results

Acceptability, reproducibility, and number of tests. Acceptable tests are defined in Table 5. Reproducibility describes the variation in multiple tests. The common statement that duplicate measurements of D_{LCO} measured within a single testing session (intrasession) should be within 5 to 6% of each other is based on a coefficient of variation (CV) of repeated measurements in normal subjects of 5 to 6%. Intrasession CVs of 3 to 4% can be achieved (30, 102). In contrast, intercession D_{LCO} variability of up to 9% has been documented in normal individuals in repeated measurements over a period of 1 yr (103). Because most intrasession variability is technical rather than physiologic, the mean of acceptable tests is reasonable to report.

Intrasession CV for repeated measures of D_{LCO} increases with increasing airway obstruction (104-106). Therefore, expected reproducibility should be worse in patients with airway obstruction when percent difference between duplicate measurements is used as the only criterion. The exact method by which mea-

TABLE 5

ACCEPTABLE TEST CRITERIA FOR D_{LCO}

1. Use of proper quality controlled equipment.
2. Inspired volume of > 90% VC in less than 4 s.
3. A stable breath-hold for 9-11 s. There should be no evidence of leaks or Valsalva or Muller maneuvers.
4. Expiration in less than 4 s with appropriate clearance of dead space and proper sampling/analysis of alveolar gas.

sured CV can be translated into a standard is not clear. It is clear that using ±1 CV is not statistically justifiable. A more acceptable standard would be ± 2 CV or ± 10%. In patients with a reduced D_{LCO}, even 10% may be too restrictive. Until more information is available, there is no reason to change the 1987 standards (6).

Current standards recommend at least two D_{LCO} tests be performed (6), but research is needed to determine the actual number of tests required to provide a reasonable estimate of average D_{LCO} value for a given person.

Recommendation. Theie should be at least two acceptable tests that meet the reproducibility requirement of being within ± 10% or 3 ml CO (STPD)/min/mm Hg of the average D_{LCO}. The average of at least two acceptable tests that meet this reproducibility requirement should be reported.

Adjustmentfor hemoglobin concentration That D_{LCO} changes as a function of hemoglobin concentration (Hb) is well known. All current methods of adjusting for Hb involve unproved assumptions, and no method has been uniformly accepted. Different methods of adjustment produce widely divergent adjustment factors when changes in Hb are large.

The most commonly used method of adjusting D_{LCO} for Hb is that of Cotes and associates (45, 107). They offered a theoretical approach in which D_{LCO} is adjusted to a standard Hb of 14.6 g/dl using the relationship described by Roughton and Forster (11), where $1/D_L = 1/D_M + 1/\theta Vc$ and θ is assumed to be proportional to the standard hemoglobin and $D_M/\theta Vc$ is assumed to be 0.7. The previous D_{LCO} standards document (6) recommended adjustment of D_{LCO} to a standard hemoglobin of 14.6 g/dl for everyone. On average, this recommendation resulted in a relatively small adjustment factor for adult men whose average Hb is not far from 14.6 g/dl. In contrast, for adult women and children under age 15 whose average Hb is about 13.4 g/dl, the average correction factor was much larger, potentially introducing more error into the adjustment. In this document separate adjustments for these two groups are recommended to minimize the size of the average correction factor applied to measured D_{LCO}. The choice of 13.4 g/dl as a standard Hb for women is slightly different than the normalization to a Hb of 12.8 recommended in the ATS guidelines for evaluation of disability (108).

The equation for adjustment to an Hb of 14.6 g/dl (appropriate for adolescent and adult men) is:

$$\text{Hb-adjusted } D_{LCO} = \text{observed } D_{LCO}\ (10.22 + Hb)/1.7\ Hb$$

The equation for adjustment to an Hb of 13.4 g/dl (appropriate for children under 15 yr of age and women) is:

$$\text{Hb-adjusted } D_{LCO} = \text{observed } D_{LCO}\ (9.38 + Hb)/1.7\ Hb$$

Investigators have offered other equations for the correction for Hb, many of which are similar to the correction factor described by Cotes and colleagues (45,107). Others have suggested incorporation of Hb or hematocrit into reference equations (99, 109).

The ITS (3) and the ERS (7) recommend the equation described by Cotes and coworkers (45, 107). Adjusting either the measured or predicted D_{LCO} will allow interpretative statements to be made about whether the differences between measured and predicted can be explained by differences in hemoglobin concentration.

Recommendation. The equation described by Cotes and colleagues (45, 107) should serve as the basis for hemoglobin adjustments. An adjustment of D_{LCO} for Hb is desirable as part of the interpretation. It is especially important to measure Hb or hematocrit in situations where Hb would be expected to vary from the norm (e.g., hemorrhage, malignancy, or exposure to cytotoxic medications). If an adjustment factor is used, measured D_{LCO} should be adjusted to an Hb of 14.6 g/dl for males over 15 yr of age and to 13.4 g/dl for women and children of either gender under age 15. Both the unadjusted and adjusted values should be reported. If the predicted rather than the measured D_{LCO} is adjusted, both the adjusted and unadjusted predicted values should be reported. If reference equations that incorporate Hb or hematocrit are used, the measured Hb or hematocrit should be reported.

Adjustment for carboxyhemoglobin concentration and CO backpressure. The routine D_{LCO} computation assumes that carbon monoxide back pressure (i.e., CO partial pressure in the blood) is zero. However, cigarette smoke as well as other environmental sources can produce measurable levels of CO back pressure and carboxyhemoglobin (COHb). Exposure to ordinary environmental CO and endogenous production of CO as a byproduct of hemoglobin catabolism commonly result in measured COHb levels of 1 to 2% (110). Small increases in COHb occur when CO is inspired in the D_{LCO} test. Frey and associates, for example, found that COHb increased about 0.7% with each single-breath D_{LCO} test (75). Ogilvie and associates (46) estimated that a COHb level of 10% would result in an 8% underestimation of D_{LCO} but recommended no adjustment because it was difficult to measure COHb at that time. Instead, they suggested subjects minimize the COHb level by not smoking on the day of the test. Cadigan and coworkers (111), Frans and associates (112), and Mohsenifar and Tashkin (113) have shown that the effect of COHb in reducing D_{LCO} is greater than would be predicted by the back pressure effect alone. Frans and associates (112) suggest that as COHb increases, the effective hemoglobin mass decreases, thereby decreasing D_{LCO} in what they call an "anemia" effect. They reported that D_{LCO} decreased about 1.2% for each percent increase in COHb; about 60% of the decrease is due to the effect of back pressure and 40% is due to the "anemia" effect. Mohsenifar and Tashkin (113) showed that D_{LCO} is decreased about 1% for each 1% increase in COHb. Estimates from the data of Cadigan and coworkers (111) suggest the effect of COHb is also about a 1% decrease in D_{LCO} for each 1% increase in COHb.

Carboxyhemoglobin concentrations can now be easily measured using CO oximeters, and CO back pressure can be measured in expired gas. Alternatively, simple methods of estimating CO content in blood have been published (114-116). Adjusting for CO back pressure is now easier and reasonable. Remember, however, that adjustment for only back pressure will not fully adjust D_{LCO} for the total effect of COHb. Leech and colleagues (86) emphasized the importance of adjusting D_{LCO} for the effect of COHb, especially in an epidemiologic setting where small changes in D_{LCO} between groups of people are important.

The 1-2% baseline COHb levels attributable to endogenous production of CO and ordinary environmental exposures are already incorporated into reference values based on healthy nonsmoking subjects.

Recommendations. An adjustment for COHb is not required but is recommended for interpretative purposes when COHb is elevated. Measured D_{LCO} can be adjusted for COHb and CO back pressure in one of two ways:

TABLE 6

D_{LCO} REFERENCE EQUATIONS FOR ADULTS

Reference NO.	N	Equation	r2	SEE	Smoking Status
Men					
96	84	6.8 − 0.238A + 15.5BSA	.	5.04	
45	227	0.325H − 0.200A − 17.6	.	5.10	
102	-	3.75V_A − 0.153A + 19.93	.	.	.
98†	123	0.410H − 0.210A − 26.31	0.60	4.02	NS
83	74	0.1646H − 0.229A + 12.9113	0.46	4.84	NS
101	80	0.441 H − 0.1936A − 31.3822	0.32	5.79	NS
99	71	0.3551 H − 0.2741 A − 11.3527	0.67	4.57	NS
4	‡	0.3319H − 0.1971A − 18.006	0.79	4.21	
119	194	0.3674H − 0.1961 A − 21.8982	0.45	4.40	NS
Women					
96	51	0.5 − 0.117A + 15.5BSA	.	5.04	
120	41	0.212H − 0.156A − 2.66	.	3.69	.
102		5.38V_A − 0.083A + 7.72	.	.	.
98§	122	0.256H − 0.144A − 8.36	0.56	3.57	NS
83	159	0.1602H − 0.1111 A + 2.2382	0.54	3.95	NS + ES
101	291	0.1569H − 0.0677A + 5.0767	0.09	4.31	NS
99	99	0.1872H − 0.1460A + 3.8821	0.38	4.50	NS
4	‡	0.2441 H − 0.1463A − 8.20	0.44	3.49	.
119	167	0.1369H − 0.1233A + 0.0917W + 1.8879	0.37	2.91	NS

Definition of abbreviations: V_A = alveolar volume in L STPD; H = height in cm; A = age in years; W = weight in kg; BSA = body surface area; ECCS = European Community for Coal and Steel; NS = nonsmokers; ES = ex-smokers; r2 = coefficient of determination; SEE = standard error of the estimate. Estimates of regression variability are listed under SEE regardless of how the author labeled the variability.
Modified with permission from reference 17.

. Information not available in reference.
† Adjusted to a standard hemoglobin concentration of 14.6 g/dL.
‡ Summary equations from several studies.
§ No adjustment for hemoglobin (Hb) concentration; average Hb for the study population was 13.3 g/dl.

1. Measure **COHb** directly or estimate the **COHb**. The following equation empirically corrects for both CO back pressure and the "anemia" effect of **COHb** on D_{LCO} (113):

COHb-adjusted D_{LCO} = measured D_{LCO} (1 + [% COHb/100])

2. CO back pressure can be measured in expired gas before a D_{LCO} maneuver or estimated using one of several available techniques (113-116). D_{LCO} can then be recalculated after subtracting the estimated CO back pressure from both $F_{ACO,0}$ and $F_{ACO,f}$. Units must be consistent before making the subtraction (ie, back pressure must be estimated in the same units [% or mm Hg] as the measured CO levels in the calculation of D_{LCO}). This method will not adjust D_{LCO} for the "anemia" effect of **COHb** and is less preferable than method 1.

Observed and predicted D_{LCO} values should always be reported. If an adjustment is made for CO back pressure, the method of adjustment should be specified and the adjusted measured or predicted value reported consistent with the adjustment for hemoglobin.

Adjustments for altitude-induced changes. Given a constant F_{IO_2}, increasing altitude will result in a decrease in P_{IO_2} and increase D_{LCO} about 0.35% per mm Hg decrease in alveolar sample P_{O_2} (P_{AO_2}) (71) or about 0.31% per mm Hg decrease in P_{IO_2} *(72).* Adjustments to a standard P_{AO_2} of 120 mm Hg may be made using a measured P_{AO_2} as follows:

Altitude-adjusted D_{LCO} =
measured D_{LCO} x (1.0 + 0.0035[P_{AO_2} − 120])

Alternatively, an average adjustment for interpretative purposes only can be made for a given altitude as follows, assuming an average P_{IO_2} of 150 mm Hg at sea level (72):

Altitude-adjusted D_{LCO} =
measured D_{LCO} x (1.0 + 0.0031[P_{IO_2} − 150])
(estimated P_{IO_2} = 0.21 [PB − 47])

Adjustments based on average altitudes may be more variable than adjustments based on individual measurements of P_{AO_2}. Altitude effects on increasing Hb concentration will also affect the measurement of D_{LCO} (see above). It is not known whether increasing altitude has other effects on D_{LCO}.

Recommendations. The uncorrected D_{LCO} should always be reported. Adjustment for altitude is permitted but not required. The choice of adjusting the measured or the predicted value and the method of reporting should be consistent with the adjustments of other factors in the measurement and reporting of D_{LCO}.

Reference equations. Selecting reference equations for D_{LCO} remains a problem. Large differences have been observed among different reference equations and among different laboratories (Table 6). Currently, most reference equations use height, sex, and age to predict D_{LCO}. Alternative equations are available that use alveolar volume (V_A) in the prediction equation (102). The use of V_A in a prediction equation provides a form of normalization for lung volume exposed to the test gas.

Until more information is available, it is important for every laboratory to ensure that normal D_{LCO} and D_L/V_A values measured in their laboratory match the predicted values they use. Physicians should be alert to the possibility that reference values in the laboratory they use may be inappropriate for their patient clientele Predicted values consistently inappropriate for the clinical situation should lead to a re-examination of both the test technique and the reference equations. Formal comparison of measured and predicted values will identify the most appropriate reference equations (117). The committee recommends that a number of healthy individuals (asymptomatic, nonobese, nonsmokers with a normal physical examination of the chest and abdomen and normal hemoglobin concentrations) be tested. The measured and predicted values should be compared by calculating residuals (measured minus the predicted) for each subject. The reference equation producing the sum of residuals closest to zero will probably provide the best fit, although there is no current research to suggest what index of best fit is most appropriate. The number of subjects needed to provide an accurate estimate of fit is also unknown. Preliminary work (117) suggests that five is not enough and that 15 to 20 may be a reasonable compromise between accuracy and effort. We suggest that at least 15 healthy subjects of each sex be tested and compared with reference equations to establish which equation will be most appropriate for a given laboratory. The subjects should be of varying heights and ages to define an adequate range Further research is needed to guide the proper selection of reference equations.

Few good ethnic comparisons are available for D_{LCO} and, therefore, little is known about its ethnic variation. One abstract reports a lower value in blacks (118). As this information becomes available, it will be important to include it in the selection of reference equations.

Recommendations. Each laboratory should select reference equations appropriate for the methods used and the population tested. Reference equations demonstrated to be appropriate for a given laboratory are especially important in the interpretation of lung function tests used to evaluate impairment or disability (25).

Interpretation when test specifications are not met. The specifications for test performance are fairly rigidly set and not all patients will be able to meet them. When standards are too rigid, valid data may be excluded if tests are rejected outright (for example, if the inspired volume is 85% of the largest vital capacity and the patient cannot meet the 90% requirement with three or four tries).

Recommendation. Such data should be reported with the caveat that the data are suboptimal. The interpretation should identify the discrepancy as well as the direction and magnitude of the potential error involved. Such errors may or may not be important in clinical decision making.

References

1. American Thoracic Society. 1979. Snowbird workshop on standardization of spirometry. *Am. Rev. Respir. Dis.* 119:831–838.
2. Ferris, B. G. 1978. Epidemiology Standardization Project. *Am. Rev. Respir. Dis.* 118(Suppl.):62–72.
3. Morris, A. H., R. E. Kanner, R. 0. Crapo, and R. M. Gardner. 1984. Clinical Pulmonary Function Testing: A Manual of Uniform Laboratory Procedures, 2nd ed. Intermountain Thoracic Society, Salt Lake City.
4. Quanjer, PhH. 1983. Standardized lung function testing. Bull. *Eur. Physiopathol. Respir.* 19(Suppl. 5):39–44.
5. Clausen, J. L., and L. P. Zarins, editors. 1982. Pulmonary Function Testing Guidelines and Controversies: Equipment, Methods and Normal Values. Academic Press, New York.
6. American Thoracic Society. 1987. Single breath carbon monoxide diffusing capacity (transfer factor): recommendations for a standard technique. *Am. Rev. Respir. Dis.* 136:1299–1307.
7. European Respiratory Society. 1993. Standardization of the measurement of transfer factor (diffusing capacity). *Eur. Respir. J.* 6(Suppl. 16):41–52.
8. British Thoracic Society and the Association of Respiratory Technicians and Physiologists. 1994. Guidelines for the measurement of respiratory function. *Respir. Med.* 88:165–194.
9. MacIntyre, N. R., N. E. Leatherman, J. L. Deitz, R. Wagner, and M. Friedman. 1986. Distribution and uptake of helium, carbon monoxide and acetylene in the lungs during high frequency oscillatory ventilation. *Respir. Physiol.* 63:201–212.
10. Comroe, J. H., Jr. 1975. Pulmonary diffusing capacity for carbon monoxide (D_{LCO}). *Am. Rev. Respir. Dis.* 111:225–240.
11. Roughton, F. J. W., and R. E. Forster. 1957. 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.* 11:290–302.
12. Weibel, E. R. 1971. Morphometric estimation of pulmonary diffusion capacity, I: model and method. *Respir. Physiol.* 11:54–75.
13. Forster, R. E., W. S. Fowler. D. V. Bates, and B. Van Lingen. 1954. The absorption of carbon monoxide by the lungs during breathholding. *J. Clin. Invest.* 33:1135–1 145.
14. Forster, R. E. 1957. Exchange of gases between alveolar air and pulmonary capillary blood: pulmonary diffusing capacity. *Physiol. Rev.* 37:391–452.
15. Krogh, M. 1914. The diffusion of gases through the lungs of man. *J. Physiol.* 49:271–300.
16. MacIntyre, N. R. 1989. Diffusing capacity of the lung for carbon monoxide. *Respir. Care* 34:489–499.
17. Crapo, R. O., and R. E. Forster. 1989. Carbon monoxide diffusing capacity. *Clin. Chest Med.* 10:187–198.
18. Michaelson, E. D., M. A. Sackner, and R. L. Johnson. 1973. Vertical distribution of pulmonary diffusing capacity and capillary blood flow in man. *J. Clin. Invest.* 52:359–365.
19. Macintyre. N. R., and J. A. Nadel. 1982. Regional diffusing capacity in normal lungs during a slow exhalation. *J. Appl. Physiol. 52:* 1487–1492.
20. Epler, G. R., F. A. Saber, and E. A. Gaensler. 1980. Determination of severe impairment (disability) in interstitial lung disease. *Am. Rev. Respir. Dis.* 121:647–659.
21. Viegi, G., P. Paoletti, R. Prediletto, F. Di Pede, L. Carrozzi, G. Carmignani, U. Mammini. M. D. Lebowitz, and C. Giuntini. 1990. Carbon monoxide diffusing capacity, other indices of lung function and respiratory symptoms in a general population sample. Am. *Rev.* Respir. *Dis.* 141:1033–1039.
22. Nordenfelt, I., and G. Svensson. 1987. The transfer factor (diffusing capacity) as a predictor of hypoxaemia during exercise in restrictive and chronic obstructive pulmonary disease. *Clin. Physiol.* 7:423–430.
23. Gelb, A. F., W. M. Gold, R. R. Wright, H. R. Bruch, and J. A. Nadel. 1973. Physiologic diagnosis of subclinical emphysema. *Am. Rev. Respir. Dis.* 107:50–63.
24. Rosenberg, E., and R. C. Young, Jr. 1979. Potential value of diffusing capacity per liter of lung volume (DL/VA) for early detection of alveolar capillary defects. *Lung* 157:23–29.
25. Renzetti, A. D., E. R. Bleecker, G. R. Epler, R. N. Jones, R. E. Kanner, and L. H. Repsher. 1986. Evaluation of impairment/disability secondary to respiratory disorders: statement of the American Thoracic Society. *Am. Rev. Respir. Dis.* 133:1205–1209.
26. Owens, G. R., R. M. Rogers, B. E. Pennock, and D. Levin. 1984. The diffusing capacity as a predictor of arterial oxygen desaturation during exercise in patients with chronic obstructive pulmonary disease. N. *Engl. J.* Med. 310:1218–1221.
27. Clausen, J., R. Crapo, and R. Gardner. 1984. Interlaboratory comparisons of pulmonary function testing (abstract). *Am. Rev. Respir. Dis.* 129(Suppl.):A37.
28. Kangalee, K. M., and R. T. Abboud. 1992. Interlaboratory and intralaboratory variability in pulmonary function testing. *Chest* 101: 88-92.
29. Saunders, K. B. 1977. Current practice in six London lung function laboratories. *Proc. Royal Soc. Med.* 70:162–163.
30. Wanger, J., and C. Irvin. 1991. Comparability of pulmonary function results from 13 laboratories in a metropolitan area. *Respir. Care* 36:1375–1382.
31. Morris, A. H., and R. 0. Crapo. 1985. Standardization of computation of single-breath transfer factor. Bull. *Eur. Physiopathol. Respir.* 21:183–189.
32. Cotes, J. E. 1963. Effect of variability in gas analysis on the reproducibility of the pulmonary diffusing capacity by the single breath method. *Thorax* 18:151–154.
33. Chinn, D. J., Y. Naruse, and J. E. Cotes. 1986. Accuracy of gas analysis in lung function laboratories. *Thorax* 41:133–137.
34. Make, B., A. Miller, G. Epler, and J. B. L. Gee. 1982. Single breath diffusing capacity in the industrial setting. *Chest* 82:351–356.
35. Crapo, R. O., and R. M. Gardner. 1986. D_{LCO} reference equations: a perspective. *Am. Rev. Respir. Dis.* 134:856.
36. Graham, B. L., J. A. Dosman, and D. J. Cotton. 1980. A theoretical analysis of the single breath diffusing capacity for carbon monoxide. *IEEE Trans. Biomed. Eng.* 27:221–227.
37. Graham, B. L., J. T. Mink, and D. J. Cotton. 1981. Improved accuracy and precision of single-breath CO diffusing capacity measurements. *J. Appl. Physiol.* 5 1: 1306-13 13.
38. Huang, Y-C., and N. R. MacIntyre. 1992. Real-time gas analysis improves the measurement of single-breath diffusing capacity. *Am. Rev. Respir. Dir.* 146:946–950.
39. Graham, B. L., J. T. Mink, and D. J. Cotton. 1985. Effect of breathhold time on $DLCO_{SB}$ in patients with airway obstruction. *J. Appl. Physiol.* 58:1319–1325.
40. Wilson, A. F., J. Hearne, M. Brennen, and R. Alfonso. 1994. Measurement of transfer factor during constant exhalation. *Thorax* 49:1121–1126.
41. Newth, C. J. L., D. J. Cotton, and J. A. Nadel. 1977. Pulmonary diffusing capacity measured at multiple intervals during a single exhalation in man. *J. Appl. Physiol.* 43:617–623.
42. Huang, Y-C., M. J. Helms, and N. R. MacIntyre. 1994. Normal values for single exhalation diffusing capacity and pulmonary capillary blood flow in sitting, supine positions and during mild exercise. *Chest* 105:501–508.
43. Stokes, D. L., N. R. MacIntyre, and J. A. Nadel. 1981. Non-linear increases in diffusing capacity during exercise by seated and supine subjects. *J. Appl. Physiol.* 51:858–863.
44. Johnson, R. L., W. S. Spicer, J. M. Bishop, and R. E. Forster. 1960. Pulmonary capillary blood volume, flow and diffusing capacity during exercise. *J. Appl. Physiol.* 15:893–902.
45. Cotes, J. E. 1993. Lung Function, 5th ed. Blackwell Scientific Publications, London.
46. Ogilvie, C. M., R. E. Forster, W. S. Blakemore, and J. W. Morton. 1957. A standardized breathholding technique for the clinical measurement of the diffusing capacity of the lung for carbon monoxide. *J. Clin. Invest.* 36:1–17.
47. American Thoracic Society. 1995.1994 Update on Standards of Spirometry. *Am. J. Respir. Crit. Care Med.* 152:1107–1136.
48. Yeh, M. P., T. D. Adams, R. M. Gardner, and F. G. Yanowitz. 1984. Effect of O_2, N_2, and CO, composition on the non-linearity of Fleisch pneumotachograph characteristics. *J. Appl. Physiol.* 56:1423–1425.
49. Gardner, R. M., J. L. Clausen, R. 0. Crapo, G. R. Epler, J. L. Hankinson, R. L. Johnson, and A. L. Plummer. 1986. Quality assurance in pulmonary function laboratories. ATS position paper. *Am. Rev. Respir. Dis.* 134:625–627.

50. Okubo, T., and C. Lenfant. 1968. Calibration of gas chromatograph without standardized gas mixtures. *Respir. Physiol.* 4:255–259.
51. Rutala, D. R., W. A. Rutala, D. J. Weber, and C. A. Thomann. 1991. Infection risks associated with spirometry. *Infect. Control Hosp. Epidemiol.* 12:89–92.
52. Leeming, J. P., A. H. Kendrick, D. Pryce-Roberts, D. Smith, and E. C. Smith. 1993. Use of filters for the control of cross-infection during pulmonary function testing. *J. Hosp. Infect.* 23:245–246.
53. Hazaleus, R. E., J. Cole, and M. Berdischewsky. 1981. Tuberculin skin test conversion from exposure to contaminated pulmonary function testing apparatus. *Respir. Care* 26:53–55.
54. *Isles*, A., I. Maclusky, M. Corey, R. Gold, C. Prober, P. Flemming, and H. Levison. 1984. *Pseudomonas cepacia* in cystic fibrosis: an emerging problem. *J. Pediafr.* 104:206–210.
55. du Moulin, G. C., K. D. Stottmeier, P. A. Pelletier, A. Y. Tsang, and J. Hedley-White. 1988. Concentration of *Mycobacterium avium* by hospital hot water systems. *J.A.M.A.* 260:1599–1601.
56. von Reyn, C. F., R. D. Waddell, T. Eaton, R. D. Arbeit, J. N. Maslow, T. W. Barber, R. J. Brindle, C. F. Gilks, J. Lumio, J. Lahdevirta, A. Ranki, D. Dawson, and J. 0. Falkinham, III. 1993. Isolation of *Mycobacterium avium* complex from water in the United States, Finland, Zaire, and Kenya. *J. Clin. Microbiol.* 31:3227–3230.
57. Eichorn, J. H., M. L. Bancroff, H. Laasberg, G. C. du Moulin, and A. J. Saubermann. 1977. Contamination of medical gas and water pipelines in a new hospital building. *Anesthesiology* 46:286–289.
58. Chaisson, R. E., and S. McAvinue. 1991. Control of tuberculosis during aerosol therapy administration. *Respir. Care* 36:1017–1025.
59. Kirk, Y. L., K. Kenday, H. A. Ashworth, and P. R. Hunter. 1992. Laboratory evaluation of a filter for the control of cross-infection during pulmonary function testing. *J. Hosp. Infect.* 20:193–198.
60. Denison, D. M., D. S. Cramer, and P. J. V. Hanson. 1989. Lung function testing and AIDS. *Respir. Med.* 83:133–138.
61. Tablan, 0. C., W. W. Williams, and W. J. Martone. 1985. Infection control in pulmonary function laboratories. *Infect. Control* 6:442–444.
62. U.S. Department of Health and Human Services. 1990. Guidelines for prevention of tuberculosis in health care settings with special focus on related issues. *MMWR* 39:20–22.
63. Daly, W. J., S. T. Giammona, and J. C. Ross. 1965. The pressure volume relationship of the normal pulmonary capillary bed. *J. Clin. Invest.* 44:1261–1269.
64. Krogh, A., and M. Krogh. 1909. On the rate of diffusion of carbonic oxide into the lungs of man. *Skandinavisches Archiv fur Physiologie* 23:236–247.
65. Kanagami, H., T. Katsura, K. Shiroishi, K. Baba, and T. Ebina. 1961. Studies on the pulmonary diffusing capacity by the carbon monoxide breath holding technique, I: normal subjects. *Acta Med. Scand.* 169:583–594.
66. Chinn, D. J., R. Harkawat, and J. E. Cotes. 1992. Standardization of single-breath transfer factor (T_{LCO}): derivation of breathholding time. *Eur. Respir. J.* 5:492–498.
67. Cotes, J. E., D. P. Snidal, and R. H. Shepard. 1960. Effect of negative intra-alveolar pressure on pulmonary diffusing capacity. *J. Appl. Physiol.* 15:372–376.
68. Smith, T. C., and J. Rankin. 1969. Pulmonary diffusing capacity and the capillary bed during Valsalva and Muller maneuvers. *J. Appl. Physiol.* 27:826–833.
69. Cotton, D. J., J. T. Mink, and B. L. Graham. 1983. Effect of high negative inspiratory pressure on single breath CO diffusing capacity. *Respir. Physiol. 54: 19-29.*
70. Jones, R. S., and F. Meade. 1961. A theoretical and experimental analysis of anomalies in the estimation of pulmonary diffusing capacity by the single breath method. Q. *J. Exp. Physiol.* 46:131–143.
71. Kanner,R. E., and R. 0. Crapo. 1986. The relationship between alveolar oxygen tension and the single-breath carbon monoxide diffusing capacity. *Am. Rev. Respir. Dis.* 133:676–678.
72. Gray, C., N. Zamel, and R. 0. Crapo. 1986. Effect of a simulated 3,048 meter altitude on the single-breath transfer factor. *Bull. Eur. Physiopathol. Respir.* 22:429–431.
73. Johns, D. P., P. D. Rochford, C. E. Barter, and J. A. Streeton. 1983. Duplicate measurements of carbon monoxide transfer factor and alveolar volume. *Breath 18: 14-15.*
74. Cinkotai, F. F., and M. L. Thomson. 1966. Diurnal variation in pulmonary diffusing capacity for carbon monoxide. *J. Appl. Physiol.* 21:539–542.
75. Frey, T. M., R. 0. Crapo, R. L. Jensen, and C. G. Elliott. 1987. Diurnal variation of the diffusing capacity of the lung: is it real? *Am. Rev. Respir. Dis.* 136:1381–1384.
76. Sansores, R. H., R. T. Abboud, C. Kennell, and N. Haynes. 1995. The effect of menstruation on the pulmonary carbon monoxide diffusing capacity. *Am. J. Respir. Crit. Care Med.* (In press)
77. Peavy, H. H., W. R. Summer, and C. Gurtner. 1980. The effects of acute ethanol ingestion on pulmonary diffusing capacity. *Chest* 77:488–492.
78. Viegi, G., P. Paoletti, M. Vellutini, L. Carroui, F. Di Pede, S. Baldacci, P. Modena, M. Pedreschi, C. DiPede, and C. Giuntini. 1991. Effects of daily cigarette consumption on respiratory symptoms and lung function in a general population sample of north Italian *men. Respiration* 58:282–286.
79. Viegi, G., P. Paoletti, L. Carrozzi, S. Baldacci, P. Modena, M. Pedreschi, F. DiPede, U. Mammini, and C. Giuntini. 1993. CO diffusing capacity in a general population sample: relationship with cigarette smoking and airflow obstruction. *Respiration 60:* 155–161.
80. Sansores, R., P. D. Pare, and R. T. Abboud. 1992. Acute effect of cigarette smoking on the carbon monoxide diffusing capacity of the lung. *Am. Rev. Respir. Dis.* 146:951–958.
81. Knudson, R. J., W. T. Kaltenborn, and B. Burrows. 1989. Effects of cigarette smoking and smoking cessation on the carbon monoxide diffusing capacity of the lung in asymptomatic patients. *Am. Rev. Respir. Dis.* 140:645–651.
82. Sansores, R., P. D. Pare, and R. T. Abboud. 1992. Effect of smoking cessation on pulmonary carbon monoxide diffusing capacity and capillary blood volume. *Am. Rev. Respir. Dis.* 146:959–964.
83. Miller, A., J. C. Thornton, R. Warshaw, H. Anderson, A. S. Teirstein, and I. J. Selikoff. 1983. Single breath diffusing capacity in a representative sample of the population of Michigan, a large industrial state. *Am. Rev. Respir. Dis.* 127:270–277.
84. Teculescu, D., and D. Stanescu. 1970. Classic versus effective timing of breathholding lung diffusing capacity determinations. *Rev. Roum. Med. Int.* 7:361–367.
85. Teculescu, D. B., and D. C. Stanescu. 1970. Lung diffusing capacity: normal values in male smokers and nonsmokers using the breath-holding technique. *Scand. J. Respir. Dis.* 51:137–149.
86. Leech, J. A., L. Martz, A. Liben, and M. R. Becklake. 1985. Diffusing capacity for carbon monoxide: the effects of different durations of breathhold time and alveolar volume and of carbon monoxide back pressure on calculated results. *Am. Rev. Respir. Dis. 132:* 1127-1129.
87. Beck, K. C., K. P. Offord, and P. D. Scanlon. 1994. Comparison of 4 methods for calculating diffusing capacity by the single breath methods. *Chest* 105:594–600.
88. Radford, E. P. 1955. Ventilation standards for use in artificial respiration. *J. Appl. Physiol.* 7:451–460.
89. Anthonisen, N. R., and J. A. Fleetham. 1987. Ventilation: total, alveolar, and dead space. In Fihman, A. P., editor. Handbook of Physiology, Section 3, Vol. 4. Waverly Press. Baltimore. 113-129.
90. Fowler, W. S. 1948. Lung function studies; II: the respiratory dead space. *Am. J. Physiol.* 154:405–416.
91. Mitchell, M. M., and A. D. Renzetti. 1968. Application of the single-breath method of total lung capacity measurement to the calculation of the carbon monoxide diffusing capacity. *Am. Rev. Respir. Dis.* 97:581–584.
92. Paoletti, P., G. Viegi, L. Carrozzi, F. DiPede, P. Modena, L. Ballerin, S. Baldacci, M. Pedreschi, G. Pistelli, and C. Giuntini. 1992. Residual volume in a general population: effects of body size, age, cigarette smoking, and respiratory symptoms. *Chest 102: 1209-1215.*
93. Teculescu, D. B., and D. C. Stanescu. 1969. Total lung capacity in obstructive lung disease: comparative determinatives by single and multiple breath helium dilution. *Bull. Eur. Physiopathol. Respir.* 5:453–464.
94. Mikus, J. P., A. G. Robins, G. W. Burke, et *al.* 1978. Comparison of five methods for determination of FRC and TLC in 100 persons. *Am. Rev. Respir. Dis.* 118(part 2):Appendix 6.
95. Chinn, D. J., J. Askew, L. Rowley, and J. E. Cotes. 1988. Measurement technique influences the response of transfer factor (T_{LCO}) to salbutamol in patients with airflow obstruction. *Eur. Respir. J.* 1:15–21.
96. Burrows, B., J. E. Kasik, A. H. Niden, and W. R. Barclay. 1961. Clinical usefulness of the single-breath pulmonary diffusing capacity test. *Am. Rev. Respir. Dis.* 84:789–806.
97. Ayers, L. N., M. L. Ginsberg, J. Fein, and K. Wasserman. 1975. Diffusing capacity, specific diffusing capacity and interpretation of diffusion defects. *West. J. Med.* 123:255–264.
98. Crapo, R. O., and A. H. Morris. 1981. Standardized single breath nor-

mal values for carbon monoxide diffusing capacity. *Am. Rev. Respir. Dis.* 123:185–189.

99. Knudson, R. J., W. T. Kaltenborn, D. E. Knudson, and B. Burrows. 1987. The single-breath carbon monoxide diffusing capacity: reference equations derived from a healthy nonsmoking population and effects of hematocrit. *Am. Rev. Respir. Dis.* 135:805–811.
100. Salorinne, Y. 1976. Single-breath pulmonary diffusing capacity. *Scand. J. Respir. Dis.* 96(Suppl.):1–84.
101. Paoletti, P., G. Viegi, G. Pistelli, F. DiPede, P. Fazzi, R. Polato, M. Saetta, R. Zambon, G. Carli, C. Giuntini, M. D. Lebowitz, and R. Knudson. 1985. Reference equations for the single breath diffusing capacity: a cross sectional analysis and effect of body size and age. *Am. Rev. Respir. Dis.* 132:806–813.
102. Gaensler, E. A., and A. A. Smith. 1973. Attachment for automated single breath diffusing capacity measurement. *Chest* 63:136–145.
103. Hathaway, E. H., D. P. Tashkin, and M. S. Simmons. 1989. Intraindividual variability in serial measurements of D_{LCO} and alveolar volume over one year in eight healthy subjects using three independent measuring systems. *Am. Rev. Respir. Dis.* 140:1818–1822.
104. Graham, B. L., J. T. Mink, and D. J. Cotton. 1984. Overestimation of the single-breath carbon monoxide diffusing capacity in patients with airflow obstruction. *Am. Rev. Respir. Dis.* 129:403–408.
105. Cotton, D. J., M. B. Prabhu, J. T. Mink, and B. L. Graham. 1992. Effects of ventilationinhomogeneity on D_{LCOSB} in normal subjects. *J. Appl. Physiol.* 73:2623–2630.
106. Crapo, R. O. 1980. Effect of breath-hold time measurement on the reproducibility of D_{LCO} (abstract). *Am. Rev. Respir. Dis.* 121(Suppl.): 330.
107. Cores, J. E., J. M. Dabbs, P. C. Elwood, A. M. Hall, A. McDonald, and M. J. Saunders. 1972. Iron-deficiency anaemia: its effect on transfer factor for the lung (diffusing capacity) and ventilation and cardiac frequency during submaximal exercise. *Clin. Sci.* 42:325–335.
108. American Thoracic Society. 1986. Evaluation of impairment/disability secondary to respiratory disorders. *Am. Rev. Respir. Dis. 133: 1205-1209.*
109. Mohsenifar, Z., H. V. Brown, B. Schnitzer, J. A. Prause, and S. K. Koerner. 1982. The effect of abnormal levels of hematocrit on the single breath diffusing capacity. Lung 160:325–330.
110. Coburn, R. F., R. E. Forster, and P. B. Kane. 1965. Considerations of the physiological variables that determine the blood carboxyhemoglobin concentration in man. *J. Clin. Invest.* 44:1899–1910.
111. Cadigan, J. B., A. Marks, M. F. Ellicott, R. H. Jones, and E. A. Gaensler. 1961. An analysis of factors affecting the measurement of pulmonary diffusing capacity by the single breath method. *J. Clin. Invest.* 40:1495–1514.
112. Frans, A., D. C. Stanescu, C. Veriter, T. Clerbaux, and L. Brasseur. 1975. Smoking and pulmonary diffusing capacity. *Scand. J. Respir. Dis.* 56:165–183.
113. Mohsenifar, Z., and D. P. Tashkin. 1979. Effect of carboxyhemoglobin on the single breath diffusing capacity: derivation of an empirical correction factor. *Respiration* 37:185–191.
114. Gaensler, E. A., J. B. Cadigan, M. F. Ellicott, R. H. Jones, and A. Marks. 1957. A new method for rapid precise determination of carbon monoxide in blood. *J. Lab. Clin. Med.* 49:945–957.
115. Henderson, M., and C. H. Apthorp. 1960. Rapid method for estimation of carbon monoxide in blood. *B.M.J.* 2:1853–1854.
116. Jones, R. H., M. F. Ellicott, J. B. Cadigan, and E. A. Gaensler. 1958. The relationship between alveolar and blood carbon monoxide concentrations during breathholding. *J. Lab. Clin. Med.* 51:553–564.
117. American Thoracic Society. 1991. Lung function testing: selection of reference values and interpretative strategies. *Am. Rev. Respir. Dis.* 144:1202–1218.
118. Weisman, I. M., and R. J. Zeballos. 1987. Lower single breath diffusing capacity in black subjects compared to caucasians (abstract). *Chest 92: 1423.*
119. Roca, J., R. Rodriguez-Roisin, E. Cobo, F. Burgos, J. Perez, and J. L. Clausen. 1990. Single breath carbon monoxide diffusing capacity prediction equations from a Mediterranean population. *Am. Rev. Respir. Dis.* 141:1026–1032.
120. Billiet, L., W. Baiseier, and J. P. Naedts. 1963. Effect de la taille, du sexe et de l'age sur la capacite de diffusion pulmonaire e l'adulte normal. *J. Physiol.* 55:199–200.