The main function of the lungs is to establish gas exchange between body tissues and the surrounding air. \( \text{O}_2 \) is taken up and \( \text{CO}_2 \) is eliminated.

This process of gas exchange can be subdivided into three stages:

1. **Ventilation**, which is the mechanism by which the alveolar gas is intermittently freshed with ambient air. As a result the \( \text{O}_2 \) concentration in the alveolar gas remains high, and the \( \text{CO}_2 \) concentration low.
2. **Alveolar-capillary diffusion**, which is the passive passage of gases across the blood-gas barrier.
3. **Perfusion**, which involves the distribution of blood in the lungs and the removal from the lungs by the blood circulation process.

This chapter describes the characteristics of the alveolar to capillary diffusion, the second stage in the classification above (fig. 1).

![Fig. 1. – Schematic illustration of the \( \text{O}_2 \) transfer across the gas-blood barrier. Oxygen uptake (\( V'\text{O}_2 \)) is proportional to the surface area of the membrane and the partial \( \text{O}_2 \) pressure gradient and inversely proportional to the barrier thickness. Finally, \( V'\text{O}_2 \) is proportional to the solubility of \( \text{O}_2 \) in water and inversely proportional to the square root of the molecular mass of \( \text{O}_2 \). \( P_{\text{A},\text{O}_2} \): alveolar oxygen partial pressure; \( P_{\text{c},\text{O}_2} \): capillary oxygen partial pressure.](image-url)
Physiological aspects of gas exchange

In the lung the O$_2$ transport across the gas-blood barrier per unit of time, $V'_{O2}$, is:

$$V'_{O2} \propto \frac{A \cdot K \cdot \alpha}{d} \cdot (P_{A,O2} - P_{C,O2}) \propto T_{L,O2} \cdot (P_{A,O2} - P_{C,O2})$$  \hspace{1cm} (1)

Where:  
$\alpha = \text{proportional to}$  
A = surface area in m$^2$  
K = diffusion coefficient of O$_2$ in m$^2$·s$^{-1}$  
d = distance in m  
$\alpha = \text{Bunsen's solubility coefficient in } \mu\text{mol} \cdot \text{m}^{-3} \cdot \text{kPa}^{-1}$  
$P_{A,O2} = \text{alveolar oxygen partial pressure in kPa}$  
$P_{C,O2} = \text{capillary oxygen partial pressure in kPa}$  
$T_{L,O2} = \text{transfer factor for O}_2 \text{ in } \mu\text{mol} \cdot \text{s}^{-1} \cdot \text{kPa}^{-1}$

The diffusion coefficient, K, depends on the size and the mobility of gas molecules, and therefore on the viscosity of the medium in which diffusion occurs. According to Graham’s law the diffusion coefficient K of any gas at a specific temperature and in a specific medium is proportional to $1/\sqrt{\text{molecular mass}}$. According to FORSTER [1] Graham’s law is valid for respiratory gases dissolved in water. This means that $V'_{O2}$ is proportional to the pressure difference across the alveolar-capillary membrane, with a proportionality constant $T_{L,O2}$. $T_{L,O2}$ is proportional to the surface area A, and inversely proportional to the barrier thickness d. Proper gas transfer therefore requires a large alveolar surface area and a thin gas-blood barrier. Normally, total surface area is 50–150 m$^2$ and the barrier thickness is $\sim$5.10$^{-7}$ m.

In estimating the $T_{L,O2}$, knowledge of the $P_{A,O2}$ and the mean value of $P_{C,O2}$ during the passage of blood through the capillary bed is required. There is a nonlinear increase in the $P_{C,O2}$ of blood during passage along the capillaries, so that the difference in O$_2$ tension across the gas–blood barrier diminishes as a function of time. In healthy volunteers capillary $P_{O2}$ equals $P_{A,O2}$ after about one third of the capillary passage time. If pressure equilibration occurs, diffusion is no longer a limiting factor and $V'_{O2}$ will only depend on the perfusion rate. Because of the non-linear increase in $P_{C,O2}$ the calculation of the $T_{L,O2}$ cannot simply be based on the mean value of mixed venous and end-capillary $P_{O2}$. Therefore, BOHR [2] and KROGH [3] suggested studying the diffusing capacity using carbon monoxide (CO). This gas has an affinity for Hb which is $\sim$230 times larger than that of O$_2$. The calculation of the CO transfer factor $T_{L,CO}$ is based on the assumption that the CO tension in plasma is negligible. In that case the pressure difference across the alveolar-capillary membrane is equal to the CO tension in the alveolar gas ($P_{A,CO}$) and the transfer of CO is independent of the pulmonary perfusion rate. The $T_{L,O2}$ and $T_{L,CO}$ are not numerically identical. According to KROGH [3] $T_{L,O2} = 1.23 T_{L,CO}$. This value of 1.23 is based on the difference in solubility and molecular mass of O$_2$ and CO, respectively. However, because according to ROUGHTON and FORSTER [4] part of the diffusion resistance resides within the erythrocyte and depends on the reaction rate between CO and haemoglobin, this value 1.23 cannot be correct.

ROUGHTON and FORSTER [4] described a model in which the total diffusion resistance 1/$T_{L,CO}$ consists of two resistances in series: the resistance of the alveolar-capillary membrane (1/$D_m$) and the reactive resistance (1/$\theta Q_c[Hb]$) of the blood in the alveolar capillaries.

$$\frac{1}{T_{L,CO}} = \frac{1}{D_m} + \frac{1}{\theta Q_c[Hb]}$$  \hspace{1cm} (2)

Where: $D_m = \text{membrane conductance}$  
$Q_c = \text{effective capillary blood volume, in mL}$
\[ [Hb] = \text{haemoglobin concentration as a fraction of normal} \]
\[ \theta = \text{constant for the rate of CO uptake by the erythrocytes per mL normal blood,} \]
\[ \text{in } \mu\text{mol} \cdot \text{s}^{-1} \cdot \text{kPa}^{-1} \cdot \text{mL}^{-1} \]

The reactive resistance concerns the chemical reaction between haemoglobin and CO and depends on the capillary blood volume and haemoglobin concentration.

While the value 1.23 is still discussed, there is consensus that it is constant and therefore nowadays intrapulmonary gas transfer is mostly measured using CO.

**Methods to determine the diffusing capacity**

Several methods have been developed to estimate \( T_{L,CO} \). The most commonly used techniques are the single breath, the intrabreath and multiple breath tests.

**Single breath method**

The single breath method is applied as follows. The patient is breathing via a two-way valve system (fig. 2). After a maximal expiration the subject is asked to inspire as deeply as possible a gas mixture of \( \sim0.3\% \) CO and 5–10\% helium (He) from a bag or gas container. Flows are measured with a flow transducer (Lilly, Fleish, etc.) and the inspired and expired volumes are obtained by integration of the flow signal in time. After a breath-holding time of 10 s at total lung capacity (TLC) the subject exhales, and an alveolar gas sample is collected (fig. 3). Alveolar fractions of CO and He are usually measured in a 750 mL gas sample after discarding the first 750 mL for washout of airways and apparatus dead space.

This technique was first described by Krogh [3]. It is based on the assumption that after inspiring a gas mixture containing CO, the alveolar CO fraction or pressure decreases exponentially with time during breath-holding as CO diffuses into the blood. If the alveolar CO fraction (\( F_{A,CO} \)) is known at the beginning and end of a time interval, it

![Fig. 2. – Schematic representation of equipment for measuring the single breath diffusing capacity. \( F_{I,CO} \): CO fraction in the inspired gas; \( F_{I,He} \): helium fraction in the inspired gas; \( F_{A,CO} \): alveolar CO fraction; \( F_{A,He} \): alveolar He fraction; \( V^* \): flow; \( V \): volume.](image-url)
is possible to calculate the exponential decay constant \(k_{CO}\) of the relationship:

\[
F_{A,CO_t} = F_{A,CO_0} e^{-k_{CO}(t-0)}
\]

(3)

Where: 
- \(0 = \) start time in s
- \(t = \) end time in s
- \(F_{A,CO_t} = F_{A,CO} \) at time \(t\)
- \(F_{A,CO_0} = F_{A,CO} \) at time 0

**Forster et al.** [5] modified the single breath technique by adding the inert gas He to the inspired gas mixture. They measured the He fraction both in the inspired gas, and in the expired gas. Assuming He is insoluble in blood and tissues, they calculated alveolar volume \(V_A\) from the He dilution and the inspired volume \(V_I\). In a mass balance the total volume of He in \(V_A\) is equal to the inspired volume of He:

\[
V_A \cdot F_{A,He} = F_{I,He} \cdot (V_I - V_D)
\]

(4)

Where: 
- \(V_A = \) alveolar volume in litres BTPS (at body temperature and ambient pressure, and saturated with water)
- \(F_{I,He} = \) He fraction in the inspired gas
- \(F_{A,He} = \) alveolar He fraction at time \(t\)
- \(V_I = \) inspired volume in litres BTPS
- \(V_D = \) total dead space in litres BTPS

Because the He analyser is sensitive to CO\(_2\), the last is absorbed prior to both He and CO analysis. The remaining gas concentrations are usually corrected for an absorbed amount of 5% CO\(_2\) [6].

Usually \(V_I\) is equal to the inspiratory vital capacity (IVC), and the maximum alveolar volume \(V_{A,max}\) is calculated according to:

\[
V_{A,max} = \frac{F_{I,He}}{F_{A,He}} \cdot (IVC - V_D)
\]

(5)

**Forster et al.** [5] assumed that He and CO are diluted in a comparable way, which is
still generally accepted. Then, the initial fraction of CO \( F_{A,CO_0} \) can be approximated from the measured inspired CO fraction and the degree to which He is diluted by residual volume (RV), according to:

\[
\frac{F_{A,He}}{F_{I,He}} \approx \frac{F_{A,CO_0}}{F_{I,CO}}
\]

(6)

Where:
- \( F_{I,CO} \) = CO fraction in the inspired gas
- \( F_{I,He} \) = He fraction in the inspired gas
- \( F_{A,He} \) = alveolar He fraction (after t sec)
- \( F_{A,CO_0} \) = alveolar CO fraction at zero time

Another modification was made by Jones and Meade [7], who demonstrated that the effective breath-holding time was not equal to the time the subjects held their breath at TLC. The effective breath-holding time starts when 1/3 of the vital capacity is inspired and lasts until half of the alveolar sample is collected.

In equation (3) \( k_{CO} \) (s\(^{-1}\)) represents:

\[
k_{CO} = \frac{T_{L,CO}(P_B - P_{H_2O,sat})}{K_{STPD} \cdot V_{A,max}}
\]

(7)

Where:
- \( T_{L,CO} \) is in \( \mu \text{mol} \cdot \text{s}^{-1} \cdot \text{kPa}^{-1} \)
- \( P_B \) = barometric pressure and \( P_{H_2O,sat} \) = the saturated water vapour pressure at body temperature (usually 37°C) both in kPa
- \( V_{A,max} \) = the alveolar volume at TLC level in litres BTPS
- \( K_{STPD} \) = the conversion factor for the conversion from litres BTPS to STPD (a volume of gas at standard temperature of 0°C and pressure of 760 mmHg that contains no water vapour) and from L to \( \mu \text{mol} \).

Equation 3 can be rewritten as:

\[
\ln \left( \frac{F_{A,CO_0}}{F_{A,CO_t}} \right) = k_{CO} \cdot (t - 0) = \frac{T_{L,CO}(P_B - P_{H_2O,sat})}{K_{STPD} \cdot V_{A,max}} \cdot t
\]

(8)

Rearrangement gives:

\[
T_{L,CO} = V_{A,max} \cdot \frac{1}{t} \cdot \frac{K_{STPD}}{(P_B - P_{H_2O,sat})} \cdot \ln \left( \frac{F_{A,CO_0}}{F_{A,CO_t}} \right)
\]

(9)

The exponential decay constant \( k_{CO} \) is the primary variable; it is proportional to \( T_{L,CO}/V_A \). \( T_{L,CO} \) is therefore obtained by multiplying \( T_{L,CO}/V_A \) with \( V_A \). Both \( T_{L,CO} \) and \( T_{L,CO}/V_A \) are used to describe the diffusion properties of the gas–blood barrier.

Ogilvie et al. [8] described the single breath method in detail with respect to dead space wash-out volume, breath-holding time, effects of changes in intrathoracic pressure, body position and lung volume and studied the reproducibility of the test. The modern single breath test is based on Ogilvie’s paper and on European Respiratory Society (ERS) [9] and American Thoracic Society (ATS) [10] guidelines. To minimise variability the ERS and ATS give recommendations to deal with factors that affect pulmonary capillary blood volume, CO back tension, submaximal inspired volume, prolonged inspiration or expiration times and not optimal breath-holding conditions. Several of these sources of error are discussed in the section entitled "Factors influencing the diffusion measurement".

**Three equations method.** The conventional single breath method assumes fast inspiration and expiration. In the case of reduced inspiratory and/or expiratory flows, the accuracy and reproducibility of the single breath test are improved by implementing
the three equations method (DL,COSB-3EQ) [11]. When using rapidly responding CO and inert gas analysers, different algorithms can be used for inhalation, breath-holding (the Krogh equation [3]) and exhalation, respectively. Such a refinement makes the single breath test a more useful marker of disease, in particular in obstructive patients who inhale and exhale slowly. Graham et al. [12] reported an improved precision and accuracy of TL,CO estimates using the DL,COSB-3EQ method. The ATS Epidemiology Standardization Project [6] recommended this technique when single breath manoeuvres are performed with reduced flows and/or at reduced breath-holding time. The DL,COSB-3EQ method appears to be comparable with the traditional single breath test when inhalation and exhalation are forced and breath-holding time is ~10 s.

Advantages and limitations. The single breath method is considered the "gold standard" to determine transfer factor. The fact that breath-holding occurs at TLC level is an advantage as well as a disadvantage of the single breath method. The advantage is that TLC is a reproducible reference point. A disadvantage of breath-holding at TLC is that diffusing capacity is measured at a non-physiological lung volume. Another disadvantage is that not every patient is capable to perform the single breath procedure. Either the patient cannot hold his breath at TLC for 10 s, or cannot deliver the required 1.5 L exhaled volume (0.75 L for washout of dead space and 0.75 L alveolar gas sample).

Traditionally the single breath method utilises a single alveolar gas sample, which is assumed to be representative of the entire lung. Implicitly the lung is assumed to be one single, well-mixed compartment with one TL,CO and TL,CO/VA value, respectively. However, CO uptake occurs in a large number of acini, each with their own relative contribution. In obstructive patients inhaled CO will be preferentially distributed to the better-ventilated lung areas and the single breath transfer factor will accordingly be weighted towards these well-ventilated areas.

Predicted values single breath transfer factor. The ERS [9] reported predicted values for Caucasians, which are dependent on age, stature and sex. The predicted values for TL,CO were derived from studies carried out with comparable equipment and techniques, which seemed to be compatible with the recommendations. The equations are a summary of the mean from literature. The corresponding TL,CO/VA predicted values should be calculated from TL,CO and TLC predicted values. The ethnic component of the transfer factor is small and for clinical purposes unimportant [9]. When using a specific set of predicted values, investigators should be sure the measurement conditions are comparable with laboratory conditions (e.g. percentage of O₂ in the inspiratory gas mixture). Stam et al. [13] described predicted values for children from 6–18 yrs of age. TL,CO increases and TL,CO/VA decreases exponentially with height. Because TLC is also exponentially related with height, both TL,CO and TL,CO/VA are linearly related with TLC.

Intrabreath method

The intrabreath method attempts to obtain information on the distribution of TL,CO/VA. One uses a rapid responding CO and CH₄ analyser [14–16] or a mass spectrometer for fast He analysis. After a maximal inspiration TL,CO is measured continuously after a brief breath-holding time (1–2 s) during one single, slow and maximal exhalation performed at a relatively constant flow. A flow restrictor and/or an on-screen flow indicator can be used to maintain the desired flow (0.3–0.6 L·s⁻¹ or 0.5–1 L·s⁻¹). Using the traditional TL,CO equation (9), TL,CO is repeatedly calculated during the entire exhalation manoeuvre from 10% increments of exhaled volume using the CO fractions at the beginning and end of each volume increment.
Advantages and limitations. An advantage is that this method will result in a $T_L,\text{CO}/V_A$, which is varying during the exhalation and which might explain regional differences in diffusion characteristics. Furthermore, a vital capacity $<1.5$ L is no longer a limitation, and the breath-holding time needs to be brief only. A disadvantage is that not every patient will be able to produce a low and constant expiratory flow. The use of a flow restrictor to obtain a constant expiratory flow has the disadvantage of increasing intrathoracic pressure if the expiration attempt is too forced, causing a decreased effective capillary blood volume and therefore a decreased diffusing capacity.

Multiple breath methods

In children and very ill adults, the single breath manoeuvre is difficult to perform. Multiple breath methods have been developed to avoid the necessity of breathholding manoeuvres and a minimum vital capacity (VC) of 1.5 L.

Steady state method. In the steady state technique (Filley et al. [17] and Bates et al. [18]) subjects breathe during a certain time from a gas container filled with a gas mixture with a low CO concentration. Mixed expired CO is monitored until a steady state is reached. The diffusing capacity under steady state conditions is estimated from:

$$T_L,\text{CO} = \frac{V'_{\text{CO}}}{P_{\text{A,CO}}}$$

Where $V'_{\text{CO}}$ is the CO uptake, which is calculated from inspired and expired amount of CO, and $P_{\text{A,CO}}$ is the alveolar CO tension. The reproducibility is low and its importance is limited because the results of this method depend on the minute volume. Furthermore, the CO load of the measurement is high.

Rebreathing method. Another multiple breath method is the rebreathing technique introduced by Krühoffer [19]. The subjects hyperventilate for $\sim 30$ s from a bag containing a gas mixture with a low CO and inert gas concentration. Breathing must be performed at a large tidal volume and at a rate of $\sim 30$ breaths per minute. The gas in the lungs is assumed to be well mixed with the gas in the rebreathing device. An inert gas is added to measure lung volume and the total volume of the rebreathing system. $T_L,\text{CO}$ is calculated from initial and final CO fractions, comparable to the single breath method. As in the steady state method results of this method are also dependent on breathing pattern. An advantage over the steady state method is the smaller CO load, because the inspiratory CO fraction decreases during the measurement.

Usually the rebreathing technique does not entail CO$_2$ absorption or O$_2$ supplementation. As a consequence, the measurement time must be brief. Furthermore, the measurements are usually performed during voluntary hyperventilation to approximate one compartment for the alveolar volume, the dead space and the volume in the rebreathing device. Patients who are too ill to perform a single breath test will also have problems with such a hyperventilation procedure. Therefore, Stam et al. [20] developed a rebreathing method at normal resting ventilation in which CO$_2$ is absorbed and O$_2$ supplied. The system consists of a bellows in which the O$_2$ concentration is kept between 20–22% (figs 4 and 5). The ventilation of the subject is measured with a displacement transducer connected to the bellows. He, CO and O$_2$ concentrations are analysed continuously. The patient is connected to the rebreathing system at functional residual capacity (FRC) level. During the measurement the CO fraction decreases both by dilution with alveolar gas and by diffusion across the alveolar-capillary membrane. FRC is estimated by monitoring the dilution of He (fig. 6). By comparing the exponential
decay of He and CO in time, the dilution independent time constant of the CO disappearance, $k_{CO}$, can be calculated from the linear decay of the natural logarithm of the CO fraction in time (fig. 7). This time constant is representative of the average $TL_{CO}/VA$ during resting ventilation. Prediction equations in adults are based on $VA$, alveolar ventilation ($V'A$) and age, in children on $VA$, $V'A$ and height.

**Advantages and limitations.** An advantage of the rebreathing technique developed by Stam et al. [20] is that the transfer factor can be obtained in patients with very small lung...
volumes while breathing normally. Because $k_{CO}$ depends on $V'/A$, minute ventilation needs to remain as constant as possible during the test.

**Determining the components of the transfer factor**

As Roughton and Forster [4] described in their model (equation 2), the total diffusion resistance, $1/T_{L,CO}$, is subdivided in its components $1/D_m$ and $1/\theta Q_c$ [Hb]. Because the rate $\theta$ for the reaction between CO and Hb depends on the $O_2$ tension, $T_{L,CO}$ also varies with the $O_2$ tension. The estimation of $D_m$ and $Q_c$ is based on single breath $T_{L,CO}$ measurements at two different $O_2$ levels and thus two different reaction rates. The
values of $1/T_{L,CO}$ are linearly related with $1/\vartheta$ with constants for $1/D_m$ and $1/Q_c$. $1/D_m$ and $1/Q_c$ are determined from the intercept with the ordinate and the slope of the linear relationship, respectively (fig. 8). According to ROUGHTON and FORSTER [4] $\vartheta$ can be calculated from the ideal alveolar oxygen tension.

**Simultaneous CO and NO measurement.** BORLAND and HIGGENBOTTAM [21] and GUENARD et al. [22] described another technique to separate the transfer factor into its components. They measured $T_{L,CO}$ and transfer factor for NO ($T_{L,NO}$) simultaneously, using the single breath method. $1/T_{L,CO}$ is influenced by both the membrane resistance $1/D_m$ and the reactive resistance $1/Q_c$. Since NO reacts much faster with haemoglobin, $T_{L,NO}$ is much less influenced by the reaction rate with haemoglobin and is therefore a good estimate of $D_m$. As a result, it is possible to calculate $D_m$ and $Q_c$ from simultaneous CO and NO measurements.

**Advantages and limitations.** An advantage of determining the components of the transfer factor is that it is possible to differentiate whether diffusion disturbances are caused by interstitial or capillary pathology. $T_{L,CO}$ can be lowered because of an alteration in $D_m$, in $Q_c$ or a combination of both. The accuracy of the estimation of $D_m$ with the graphical method with various $O_2$ tensions is limited, because $1/D_m$ is about zero. A normal variation in $T_{L,CO}$ at both $O_2$ levels might lead to an admittedly small variation in $1/D_m$, but a large variation in $D_m$. It is even possible to obtain a negative $D_m$. A limitation of the simultaneous CO and NO method is the fast disappearance of NO. Breath-holding time has to be reduced to $\leq 5$ s for the estimation of $T_{L,NO}$, while for the estimation of $T_{L,CO}$ a breath-holding time $<5$ s is too short.

**Factors influencing the diffusion measurement**

The ROUGHTON and FORSTER [4] model clearly illustrates that the diffusing capacity depends on: haemoglobin concentration, oxygen tension and effective capillary blood volume. Therefore, an estimate of the diffusing capacity without knowledge of the Hb concentration is of limited value, and a Hb correction is always required. The diffusion indices are corrected for abnormal Hb concentrations according to the procedure
described by the European Community for Coal and Steel (ECCS) [9].

\[
T_{L,CO}(corr) = T_{L,CO}(obs) \cdot \frac{a + \theta \cdot [\text{Hb}]}{(a + \theta) \cdot [\text{Hb}]}
\]  

(11)

Where: 

\( T_{L,CO} (corr) \) = \( T_{L,CO} \) corrected to reference Hb concentration 
\( T_{L,CO} (obs) \) = the observed \( T_{L,CO} \) at the actual Hb concentration 
\( a \) = the ratio of membrane conductance and capillary blood volume (in traditional units mL, min and mmHg \( \sim 0.7 \) and in SI units mmol, min and kPa 230) 
\( \theta \) = the reaction rate for the CO Hb reaction at an oxygen pressure of 110 mmHg 
\( [\text{Hb}] \) = haemoglobin as a fraction of normal.

The \( \text{O}_2 \) dependence of the reactive resistance implies that patients on supplementary \( \text{O}_2 \) have to be disconnected from the oxygen supply for at \( \sim 10 \) mins prior to a diffusion measurement [9]. Arising from the dependence of the transfer factor on the effective capillary blood volume, the body position needs to be upright during the measurement, because reference values were determined in that posture. The lung is more equally perfused in the supine position, resulting in a larger effective capillary blood volume and a larger diffusing capacity [23]. This might be due to a shift of blood from the systemic circulation into the pulmonary circulation when changing from upright to recumbent posture. According to Lewis et al. [24], capillaries are simply endothelial tubes that open fully if transmural pressure exceeds a critical opening pressure. As a consequence capillaries in the basal parts of the lungs in the sitting position will be fully open, whereas in the apex the majority of the capillaries are closed. In the supine position gravitational effects have less effect, resulting in a more uniform perfusion and therefore in a larger effective capillary blood volume and blood flow [Bryan et al. [25] and Stam et al. [23]). These data suggest that the lung is an overdimensioned gas exchanger with a large reserve of capillary blood volume. An increase in effective \( Q_c \) results in an increased \( T_{L,CO} \) and \( T_{L,CO}/V_A \) in the supine position. A lack of response to a change in body position in various pulmonary or cardiac diseases seems to be an indication that the capillaries in the upper lung zones are fully recruited in both positions [26, 27]. When cardiac output is increased due to physical activity, effective capillary blood volume is also increased due to distension and recruitment of capillaries. Therefore, a patient needs to take a rest for \( \sim 5 \) min before starting the diffusion test.

Furthermore, alveolar pressure should be near atmospheric during the breath-holding time. A Valsalva manoeuvre decreases and a Muller manoeuvre increases capillary blood volume and therefore \( T_{L,CO} \) and \( T_{L,CO}/V_A \) [28].

Furthermore, the results of a diffusion measurement are influenced by: alveolar volume at which the measurement is performed; the CO back tension in the capillary blood; and washout time of test gases in between the various measurements.

As mentioned, the \( T_{L,CO} \) is proportional to the surface area \( A \) of the blood–gas barrier. A decrease in lung volume will cause a decrease in surface area \( A \) and consequently, in \( T_{L,CO} \). However \( T_{L,CO}/V_A \) is higher at reduced alveolar volumes, compared with reference values estimated at a normal TLC [9], because \( V_A \) (proportional with the radius to the third power) is decreasing faster than \( T_{L,CO} \) (proportional with surface area and thus with the radius to the second power). Therefore, it is important that the inspired volume during the single breath procedure is as close as possible to the known \( V_C \).

Because transfer factor is measured using CO and it is assumed that the capillary CO pressure equals zero, the number of successive single breath tests is limited to a maximum of five measurements a day. If, for some reason, this number is exceeded, corrections should be made for CO back tension. Failing to correct for back tension, in smokers the
transfer factor will be underestimated. Similarly, after a recent cigarette CO back tension correction is required or the test should be postponed.

In between measurements, a minimal interval of 4 min is required to allow elimination of test gases from the lung. During this interval the patient should remain at rest and seated.

Comparison of single breath and rebreathing method during rest ventilation

The absolute values of TL,CO and TL,CO/VA obtained with the various methods are not the same. The main reason is that with the single breath method diffusion parameters are estimated at TLC, whereas with the steady state or rebreathing methods they are estimated at a smaller lung volume (FRC+1/2 tidal volume).

As described by Stam et al. [13, 29, 30] there is a linear relationship between single breath TL,CO/VA and VA. Extrapolation to the lung volume range at which the various rebreathing measurements were performed results in the shaded area in figure 9a. Because CO disappears in the alveoli only, the VA, VD and rebreathing system constitute separate compartments. Theoretically, these compartments can be regarded as one compartment at infinite ventilation only. Figure 9b illustrates that above an alveolar ventilation of 30 L·min⁻¹ the absolute values of rebreathing TL,CO/VA are comparable with those of the single breath TL,CO/VA at the same volume level.

Therefore, predicted values of rebreathing diffusing capacity during rest ventilation are not only age dependent, but should also depend on alveolar volume and alveolar ventilation.

Reporting of results and interpretation

It is important that the report includes the following data for optimal interpretation (table 1). The diffusion measurement should be performed at least twice. At Erasmus University in Rotterdam at least three measurements are performed (columns 1, 2 and 3) with the average in column 4. Column 7 is the predicted value and the last column is the standard deviation in the predicted value. In column 5 the percentage of predicted and in column 6 the standard deviation score (SDS) or Z-score is reported. The first four rows are concerning the volumes at which the single breath test is performed. The next four rows give TL,CO and TL,CO/VA respectively, in which the values with the subscript c correspond with the diffusion indices corrected to a normal Hb concentration. As mentioned earlier, a correct interpretation of the diffusion data is only possible if the Hb concentration is known. In row 9 TL,CO/VA is not compared with a predicted value at predicted TLC, but with a TL,CO/VA predicted value at the actual TLC [29].

In row 4 the measured VC during the single breath manoeuvre (VCsb) is compared with the known VC from spirometry (VCspir). If the patient exhaled and inhaled maximally the ratio between VCsb and VCspir is ~1. Ventilation distribution unequality is evaluated based on the ratio between TLC determined with the single breath test (TLCsb) and TLC determined with the multiple breath He washin method (TLCmb). A TLCsb/TLCmb ratio >0.85 has been regarded as an indication for normal ventilation distribution (Roberts et al. [31]). Conclusions about unequal ventilation are only valid when the VCsb/VCspir ratio is ~1. If the ratio VCsb/VCspir <1 and the TLCsb/TLCmb <0.85, then unequal ventilation cannot be excluded, but TLCsb may be measured partly too low due to submaximal inspiration. If VCsb/VCspir<1 and TLCsb/TLCmb=1, then
Fig. 9. – The single breath $T_{L,CO}/V_A$ as function of $V_A$ (a) and the rebreathing $T_{L,CO}/V_A$ as function of alveolar ventilation ($V_A^*$) (b) in a healthy volunteer. $V_A^*$ is the alveolar volume range between the mean $\pm$ 2 SD obtained from the rebreathing manoeuvres. The shaded area in a) represents the range in single breath $T_{L,CO}/V_A$ corresponding to the volume range of $V_A^*$. This area corresponds with the shaded area in b). The dashed line in b) is the linear regression line for the $T_{L,CO}/V_A$ versus $V_A$ relationship up to a $V_A$ of 20 L·min$^{-1}$.

Table 1. – Layout of report of the diffusion measurement

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Average</th>
<th>% pred</th>
<th>Z-score</th>
<th>Pred</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLCsb L</td>
<td>6.15</td>
<td>6.22</td>
<td>6.22</td>
<td>6.19</td>
<td>89</td>
<td>-1.13</td>
<td>6.98</td>
<td>0.70</td>
</tr>
<tr>
<td>TLCsb/TLCmb</td>
<td></td>
<td></td>
<td></td>
<td>0.69</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VCsrb L</td>
<td>4.85</td>
<td>4.91</td>
<td>4.90</td>
<td>4.89</td>
<td>106</td>
<td>0.46</td>
<td>4.63</td>
<td>0.56</td>
</tr>
<tr>
<td>VCsrb/VCspir</td>
<td></td>
<td></td>
<td></td>
<td>0.89</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_{L,CO}$ µmol·s$^{-1}$·kPa$^{-1}$</td>
<td>43.02</td>
<td>46.96</td>
<td>48.84</td>
<td>46.32</td>
<td>29</td>
<td>-4.62</td>
<td>161.9</td>
<td>25.00</td>
</tr>
<tr>
<td>$T_{L,CO}$ µmol·s$^{-1}$·kPa$^{-1}$</td>
<td>43.02</td>
<td>46.96</td>
<td>48.84</td>
<td>43.90</td>
<td>27</td>
<td>-4.72</td>
<td>161.9</td>
<td>25.00</td>
</tr>
<tr>
<td>$T_{L,CO}/V_A$ µmol·s$^{-1}$·kPa$^{-1}$·L$^{-1}$</td>
<td>7.18</td>
<td>7.75</td>
<td>8.06</td>
<td>7.68</td>
<td>34</td>
<td>-4.47</td>
<td>22.67</td>
<td>3.35</td>
</tr>
<tr>
<td>$T_{L,CO}/V_A$ µmol·s$^{-1}$·kPa$^{-1}$·L$^{-1}$</td>
<td>7.18</td>
<td>7.75</td>
<td>8.06</td>
<td>7.28</td>
<td>32</td>
<td>-4.59</td>
<td>22.67</td>
<td>3.35</td>
</tr>
<tr>
<td>$T_{L,CO}/V_{AC}$ RCL µmol·s$^{-1}$·kPa$^{-1}$·L$^{-1}$</td>
<td>32</td>
<td></td>
<td></td>
<td>-4.66</td>
<td>23.93</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb mmol·L$^{-1}$</td>
<td>10.30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TLC: total lung capacity; sb: single breath test; mb: multiple breath test; VC: vital capacity; spir: spirometry; $T_{L,CO}$: transfer factor for carbon monoxide; c: corrected to standard Hb concentration; $V_A$: alveolar volume; $T_{L,CO}/V_{AC}$ RCL: predicted value at actual TLC.
VCsb is too small at the expiration side (residual volume is not reached). In that case the single breath test is performed at TLC level and the $T_{L,CO}$ and $T_{L,CO}/V_A$ results are not influenced by this decreased VCsb.

Most pulmonologists use percentage of predicted when comparing the results with predicted values. The diffusion indices are normally distributed and a normal range between $z_{-1.64 \, SD}$ and $z_{1.64 \, SD}$ from predicted is assumed (90% of the healthy volunteers). A SDS- or Z-score, *i.e.* the deviation in SD from predicted ((measured-predicted)/SD), is used to detect the severity of the pathology. The Z-score is related to the chance that the index is normal. In the Erasmus University in Rotterdam it is agreed that a deviation from predicted is judged as in table 2.

In case of a $TLC_{sb}/TLC_{spir} > 0.85$ (equal ventilation distribution) and a decreased $TLC_{sb}$, $T_{L,CO}/V_A$, is compared with predicted values at predicted TLC (row 8), as well as with predicted values at the actual disease limited TLC (row 9).

### Clinical indications

**Chronic obstructive pulmonary disease**

Chronic obstructive pulmonary disease (COPD) is one of the major causes of death worldwide. Loss of alveolar surface area and dysfunction of the alveolar membrane as in emphysema lead to a decreased transfer factor. Measurement of the transfer factor can be of importance in the (early) detection of COPD.

**Interstitial lung disease**

Thickening of the alveolar membrane and a diminished total lung capacity due to interstitial processes may lead to a severe decline in transfer factor. The acinus is disrupted and the diffusion pathway is lengthened. Typical diseases are extrinsic allergic alveolitis, pulmonary vasculitis syndromes, systemic lupus erythematosus, and of course, interstitial fibrosis.

**Pulmonary bleeding disorders**

Measurement of the transfer factor can be helpful in detecting intrapulmonary bleeding in patients with disorders such as primary pulmonary haemosiderosis, Wegener’s disease or Goodpasture’s syndrome. Because of the high affinity between CO and Hb the $T_{L,CO}$ and $T_{L,CO}/V_A$ can be increased appreciably in alveolar haemorrhage, because CO will react with Hb without the need to pass the gas–blood barrier. A typical feature is the gradual decrease in diffusing capacity after several measurements, because the blood in the alveoli becomes saturated with CO.

<table>
<thead>
<tr>
<th>Table 2. – Deviation from predicted values each measured value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>Mildly decreased</td>
</tr>
<tr>
<td>Moderately decreased</td>
</tr>
<tr>
<td>Moderately severe</td>
</tr>
<tr>
<td>Severe</td>
</tr>
</tbody>
</table>

140
Pre-operative screening

It is important to screen the transfer factor pre-operatively prior to any major surgery to predict whether problems can be expected during anaesthesia or in the post-operative phase. It is also recommended to measure the transfer factor prior to any lung surgery (e.g. resection due to lung cancer), because resection will result in loss of surface area.

Interpretation of diffusing capacity in:

Restrictive disease

$T_{L,CO}$ and $T_{L,CO}/VA$ are usually compared with predicted values, which are determined in healthy volunteers, who by definition have a normal TLC. Thus the current predicted values relate to measurements made at normal TLC [9]. In patients with a restrictive ventilatory defect (i.e. a reduced TLC) or with a larger than normal TLC, a comparison with predicted values at predicted TLC can lead to erroneous conclusions. A decrease in lung volume will cause a decrease in surface area $A$ and consequently in $T_{L,CO}$. However $T_{L,CO}/VA$ is higher at reduced alveolar volumes, compared with predicted values estimated at a normal TLC, because $VA$ (proportional to the radius to the third power) is decreasing faster than $T_{L,CO}$ (proportional to the surface area and thus to the radius to the second power).

Stam et al. [29] suggested that in restrictive pulmonary disease $T_{L,CO}$ and $T_{L,CO}/VA$ should be compared with predicted values at a lung volume equal to the patients actual TLC. Therefore, they derived reference values for $T_{L,CO}/VA$ as a function of alveolar volume. Their results were corroborated by Chinn et al. [32] and Frans et al. [33], who found a comparable relationship between $T_{L,CO}$ and $VA$. Johnson [34] proposed a procedure to correct predicted values of $T_{L,CO}$ and $T_{L,CO}/VA$ at predicted normal TLC to a symptom limited TLC. However, a disadvantage of such a method is that both predicted values of $T_{L,CO}$ and $T_{L,CO}/VA$ at predicted TLC, and the volume correction procedure, have their own variability. The standard deviations of the calculated $T_{L,CO}$ and $T_{L,CO}/VA$ predicted values at lower $VA$ levels are considerably larger than at normal TLC, and therefore conclusions concerning the severity of pathology are more difficult.

However, Hughes et al. [35] criticised a voluntary volume reduction model in normal subjects. They stated that this model describes a restriction of extra pulmonary origin or due to respiratory muscle weakness only. Because this model assumes uniform changes and in interstitial pulmonary disease structural and functional changes are nonuniform, they stated that a restriction due to interstitial lung disease is not comparable with voluntary volume reduction in normals. However, Stam et al. [30] studied a group of males without previous pulmonary disease before and after treatment with bleomycin for a germ cell tumour. All of the studied subjects developed a diffusion disturbance and half of them also developed a restriction. It was observed that the slope in $T_{L,CO}$ and $T_{L,CO}/VA$ with change in $VA$ was similar before and after the treatment with bleomycin. This supports the contention that the extent of diffusion disturbance was assessed more correctly, and appeared greater, if $T_{L,CO}$ and $T_{L,CO}/VA$ were compared with reference values at actual TLC, rather than to values at predicted or pretreatment TLC. Furthermore, Hughes et al. [35] stated that voluntary volume reduction is not comparable with pneumonectomy. They described another model for loss of alveolar units, which is based on the work of Hsia et al. [36], who described an increase in $T_{L,CO}/VA$ during exercise due to a larger pulmonary blood flow, causing a more equal perfusion by capillary recruitment. Hughes et al. [35] assumed that total pulmonary
blood flow remains at pre-resection level, so that after resection the flow to the remaining lung will increase about two-fold. They describe that this situation is comparable with the dependance of $T_{L,CO}/VA$ on cardiac output as described by Hsia et al. [36]. Corris et al. [37] established an empirical relationship for the increase in $T_{L,CO}/VA$ (post-pre pneumonectomy) based on the percentage of flow to the resected lung pre-operatively. These predictions are comparable with the Hughes model.

It is important to use an appropriate model for reference values depending on the origin of the restriction. In interstitial pulmonary disease an appropriate model is not obvious, but comparison with predicted values at predicted TLC will lead to an overestimation of $T_{L,CO}/VA$. Therefore, it is important to perform more extensive research in this particular field.

**Obstructive disease**

In healthy volunteers the assumption that a small sample of air early during the exhalation is representative of the entire lung seems to be acceptable, but in patients with uneven ventilation and uneven distribution of $T_{L,CO}/VA$ the analysis of only one small gas sample might lead to erroneous conclusions. This is because primarily the CO uptake in the well ventilated parts of the lung will be estimated, while a different $T_{L,CO}/VA$ can be expected in the poorly ventilated lung areas. An indication of this ventilation unequality is a ratio $TLC_{sb}/TLC_{mb}$, which is $<0.85$ [31]. Not only unequal ventilation, but uneven distribution of gas transfer $T_{L,CO}/VA$ might occur. $T_{L,CO}/VA$ will change during the exhalation and here methods such as the intrabreath method come into play [14–16].

**Improvement and validation of diffusion equipment**

Worldwide, there are many manufacturers of equipment for measuring the transfer factor of the lung. Diffusion data measured with the apparatus of the various manufacturers differ significantly. Recently, Gissmeyer et al. [38] and Jensen and Crapo [39] developed a single breath $T_{L,CO}$ simulator. By producing gas mixtures of CO and an inert gas with air, this equipment creates a constant and adjustable TLC, $T_{L,CO}$ and $T_{L,CO}/VA$. A diffusion simulator will not only be valuable in comparing equipment, but it will also be valuable in the regular calibrating of equipment instead of the customary biological calibration.

**Conclusion**

The transfer factor of the lung has become a major lung function index and is an important diagnostic index in COPD, interstitial pathology, etc. Several methods to determine the transfer factor were developed in the last century. Each method has its own advantages and limitations. The single breath method became the most generally accepted method worldwide. Standardisation is important to diminish the variability of the single breath method and, therefore, the ERS and ATS recommended guidelines. However, in the case of unequal ventilation or unequal distribution of diffusion characteristics the traditional single breath test is insufficient. One of the possible prospects, when using fast responding gas analysers, is to obtain more information about unequal distribution of transfer factor and ventilation. Techniques such as the intrabreath or three equations method will probably be more important in the near future.

For patients who are not able to perform the single breath test or have a too small VC,
multiple breath diffusion tests have been developed. The rebreathing diffusion test is recommended in these situations. Especially when measuring young children, a rebreathing technique during rest ventilation is recommended.

The ECCS report [9] warns: "The association between $T_{L,CO}/V_A$ and lung volume can lead to difficulty in interpretation, particularly during childhood and adolescence, in non-Caucasians and in patients in whom the total lung capacity is reduced". In patients with restricted lung pathology the traditional comparison with predicted values for the single breath diffusing capacity at predicted TLC is not correct. Dependent on the origin of the restriction different ways are described to take the diminished alveolar volume into account. This emphasises the importance of using an appropriate set of predicted values. Further research on this particular issue is needed. The most advanced equipment may be used to measure the transfer factor, but the results will be poor if the measured data are not interpreted correctly!

### Summary

The main function of the lungs is to establish exchange of O$_2$ and CO$_2$ between the environment and the capillary blood. The gas transport across the alveolar-capillary membrane can be measured by the transfer of carbon monoxide (CO). CO has a high affinity for haemoglobin and is assumed to be absent in pulmonary capillary blood. After inspiration, CO diffuses by the partial CO pressure gradient over the gas–blood barrier from the alveoli into the capillary blood and disappears from the alveolar gas. The decrease in CO fraction in the alveolar gas in a fixed time interval quantifies the diffusing capacity of the lung. As not only diffusion but also chemical reactions affect the CO transfer, the term "transfer" ($T$) rather than diffusion ($D$) is used.

Traditionally, gas transfer across the alveolo-capillary membrane is described in the USA by the diffusing capacity for CO ($D_{L,CO}$) and in Europe it is called the transfer factor ($T_{L,CO}$). However, $D_{L,CO}$ and $T_{L,CO}$ describe the same variable and are interchangeable. Methods to determine the transfer factor $T_{L,CO}$ are the single breath, the intrabreath and multiple breath methods. Each has its advantages and limitations. The most important limitation of the single breath technique is the required lung volume. Vital capacity (VC) has to be $>1.5$ L in order to obtain reliable results. Traditional single breath measurements are inaccurate in the case of severe airway obstruction due to inadequate time for equilibration of gases in the lung. Using equipment that is based on fast responding gas analysers, conclusions of unequal distribution of the diffusion characteristics may be drawn. A minimal VC of 1.5 L is not required when using fast gas analysers. At reduced lung volume $T_{L,CO}/V_A$ increases and this may lead to erroneous interpretation of data in patients with a restrictive lung disease. For the interpretation, it is important to take the possible influence of a reduced $V_A$ or the influence of severe airway obstruction into consideration. In patients who are not able to perform the single breath test and in small children the transfer factor is determined with multiple breath methods. From the multiple breath methods the rebreathing method is traditionally performed during hyperventilation. Patients who are too ill to perform a single breath test, will also have problems with a hyperventilation procedure. Therefore, a rebreathing method during normal, spontaneous ventilation was developed. When measuring the rebreathing transfer factor during rest ventilation, it is important to realise that results are dependent on alveolar ventilation and alveolar volume.

To minimise the variability in the diffusion measurement it is important to standardise.
these tests with respect to e.g. haemoglobin correction, body position, effect of O\textsubscript{2} etc. An important step forward is the use of European Respiratory Society/American Thoracic Society guidelines.

**Keywords:** Intrabreath, multiple breath, obstructive disease, restrictive disease, single breath, transfer factor.

**References**


