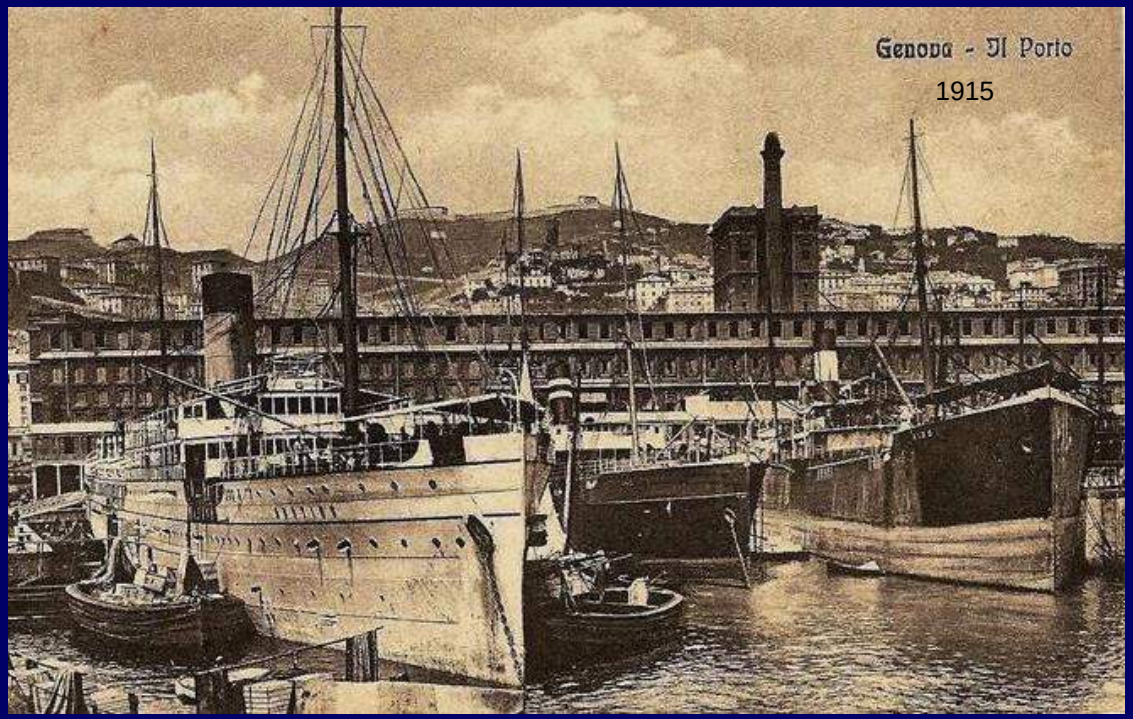
Agenda

Time	Speaker	Topic
13:15 – 13:25	Prof. Dr. med. Vito Brusasco, FERS	Single-breath diffusion capacity for carbon monoxide (DLCO): clinical applications and technical notes
13:25 – 13:55	Prof. Dr. med. Helgo Magnussen	
13:55 – 14:05	Dr. med. Vito Brusasco and Dr. med. Helgo Manussen	Discussion
14:05 – 14:30	Georg Harnoncourt, Christian Buess and Philippe Schlink	Hands-on and QA

Handout content:

Agenda, Test Report (DLCO and Spirometry), PowerPoint Presentation, ERS Standard 2017 (new), ERS Standard 2005 (old), Abstracts of Different Publications

Single-breath diffusion capacity for carbon monoxide (DLco): technical notes



Vito Brusasco

University of Genoa
Italy

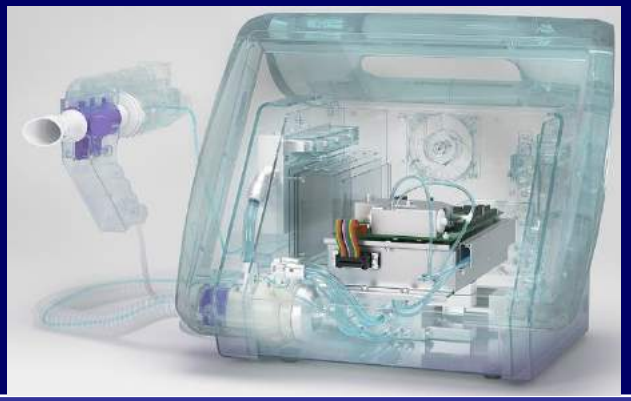
The parents of single-breath DLco (1915)

August and Marie Krogh
(1874-1949) (1874-1943)

Conflict of interest

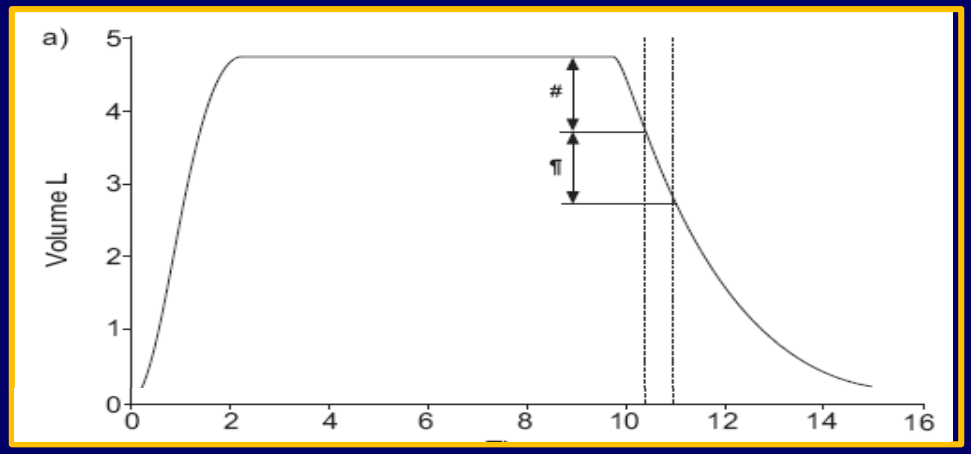
Conflict of interest: None

Recent advancements in single-breath DLCO



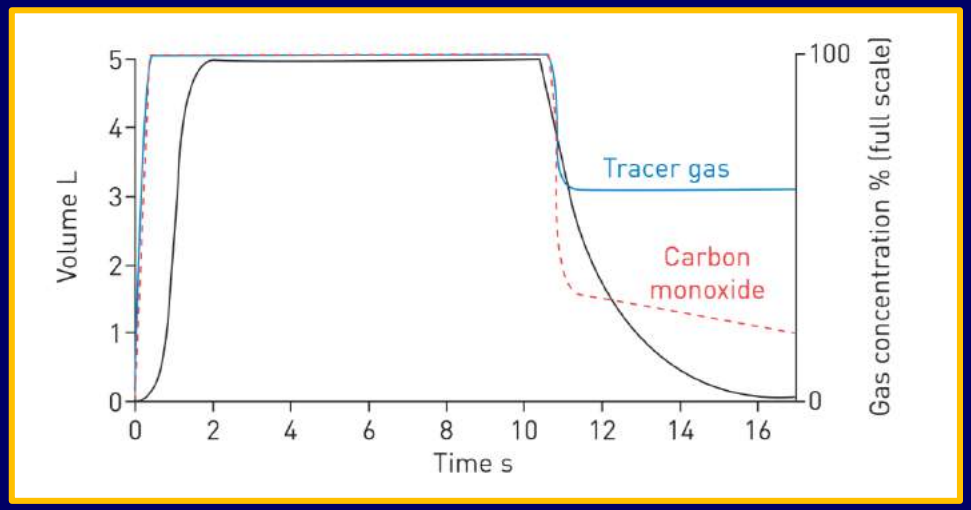
Before	Now
Old technology, slow gas analyzers, bag system	New technology, rapid gas analyzers (RGA, 0-90% response ≤150 ms)
Not robust DLCO measurements	Fast and reliable DLCO measurements
Intense manual quality control necessary	Automatic quality control
	2017 ATS/ERS standards for DLCO with RGA systems
→ No wide use possible	→ Wide use possible

Why rapid gas analyzer?



Single-sample gas-analysis

MacIntyre et al. ERJ 2005

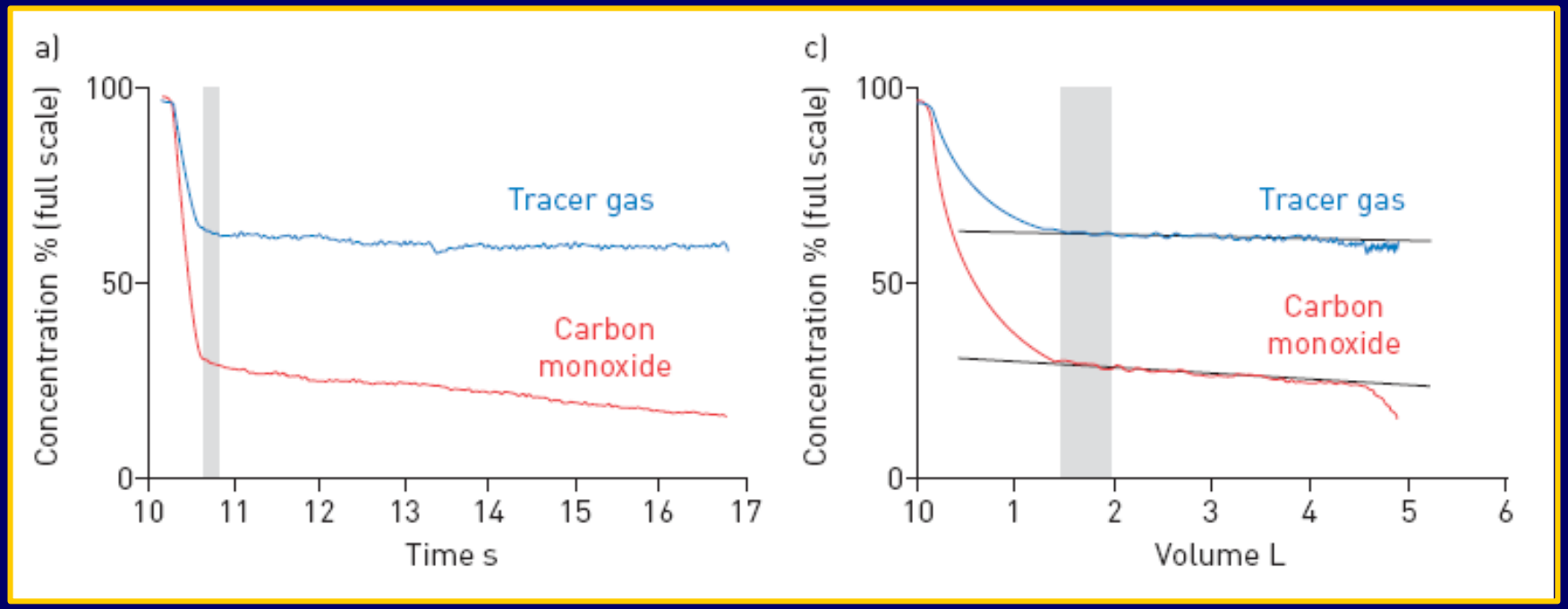


Continuous gas analysis

Graham et al. ERJ 2017

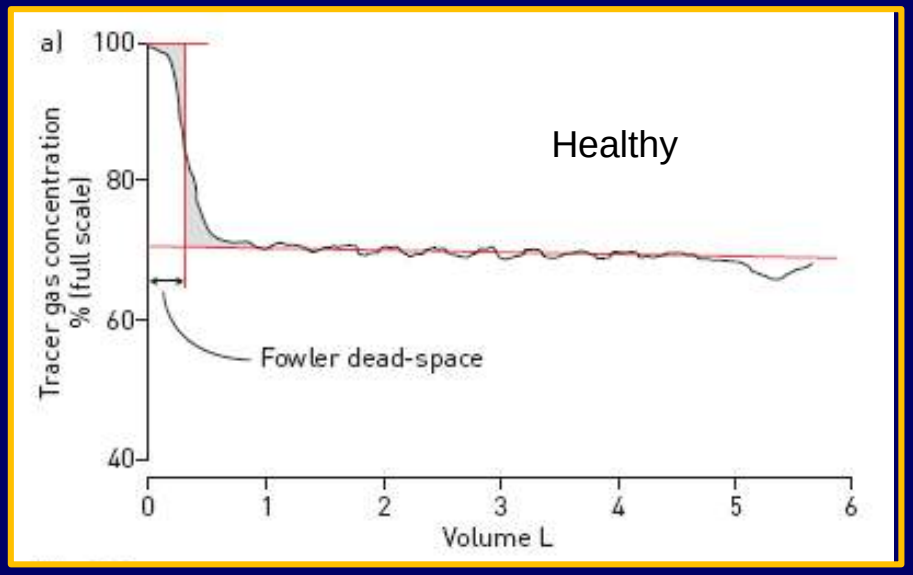
Why rapid gas analyzer?

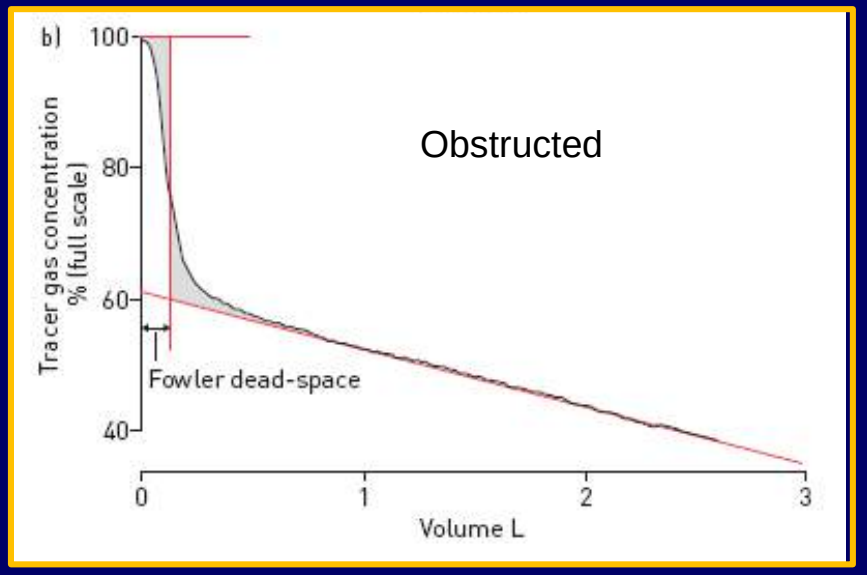
Continuous gas-analysis also allows plotting CO and tracer gas concentrations against expired volume, which makes allocation of gas sampling more reliable



Graham et al. ERJ 2017

Why rapid gas analyzer?





Measurement of all gas expired gives the opportunity to enhance the accuracy of volume determination (Deadspace, alveolar volume)

Graham et al. ERJ 2017

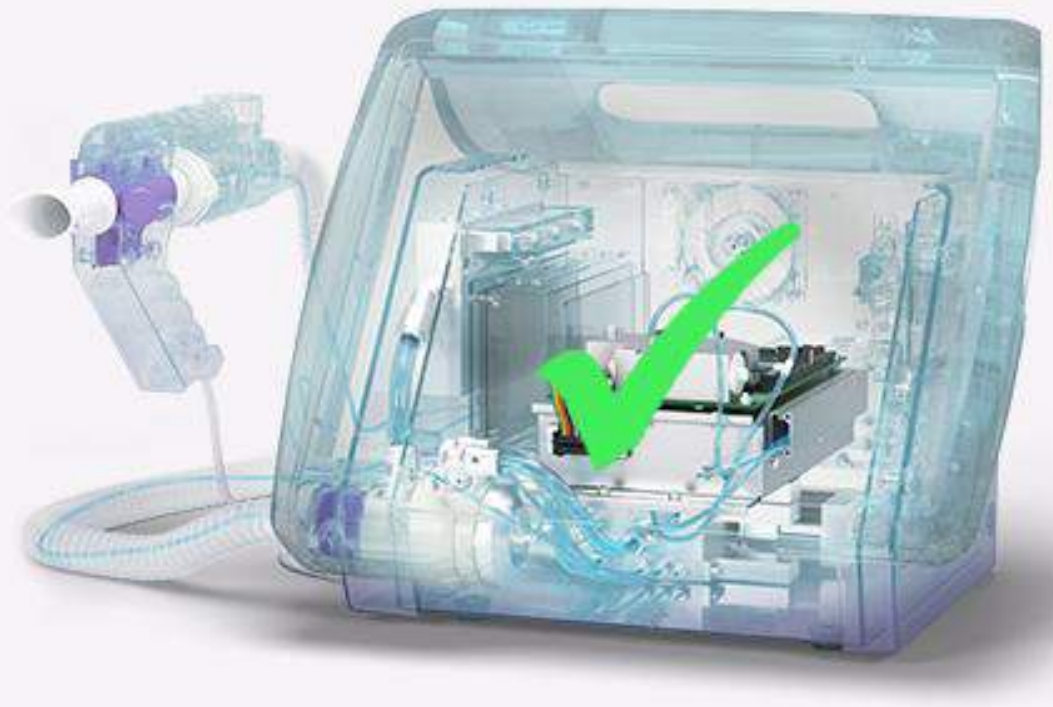
Recommended equipment calibration and quality control for DLco

TABLE 2 Equipment calibration schedule

Calibration technique	Frequency
Flow analyser zeroing	Before each test
Gas analyser zeroing	Before/after each test
Volume calibration check	Daily
Biologic control	Weekly
Calibration syringe *D*Lco check	Weekly
Calibration syringe leak test	Monthly
Linearity check (calibration syringe or simulator)	Monthly

Graham et al. ERJ 2017

Automatic quality control
TrueCheck technology



Quality Control

according to ATS/ERS 2017

- Flow Analyzer Zeroing ✓
- Flushing and Gas Check ✓
- Gas Analyzer Zeroing ✓
- DLCO Calibration Check ✓
- **Gas Analyzer Linearity Check** ✓

powered by TrueCheck™

Automatic quality control TrueCheck technology

- Stability of DLCO without simulator check or calibration before every test (Author name, Respir Care 2017)
- Less handling errors
- Save Time

Reporting of DLco measurements

DLCO

Test Date	21.04.2009 18:20:30	Interpretation	--	Value Selection	Best Value
Post Time		Predicted	Stanojevic (GLI), 2017	BTPS (IN/EX)	1,10/1,02
				User ID	

Parameter	Pred	LLN	Result	Trial 3	Trial 1	Trial 2	%Pred
DLCO [ml/min/mmHg]	32,6	25,3	39,1	40,0	39,1	38,2	120
DLadj [ml/min/mmHg]	32,6	25,3	39,2	40,1	39,2	38,2	120
VA sb [L]	7,07	5,77	6,30	6,37	6,29	6,24	89
DLCO/VA (KCO) [ml/min/mmHg/L]	4,64	3,64	6,21	6,28	6,22	6,12	134
TLC sb [L]	7,22	5,92	6,45	6,52	6,44	6,39	89
VI [L]	-	-	5,22	5,27	5,25	5,13	-
BHT [s]	-	-	-	10,2	10,3	10,1	-

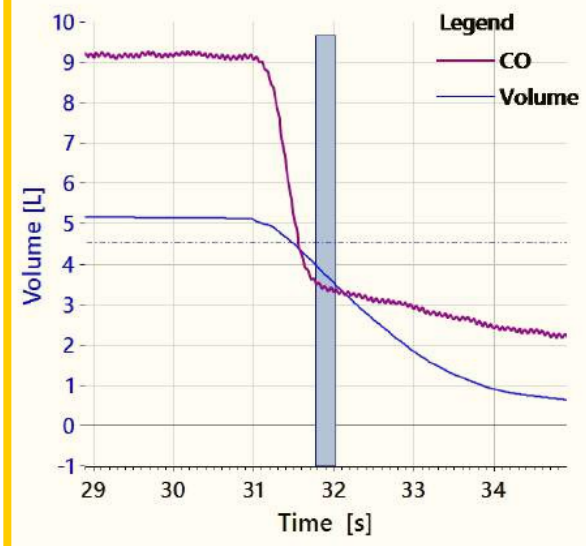
Session Quality A (DLCO Var=0,88ml/min/mmHg (2,2%))

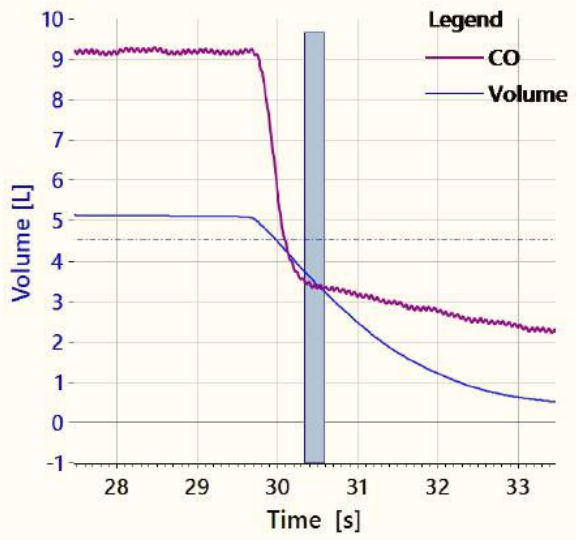
Measured Values

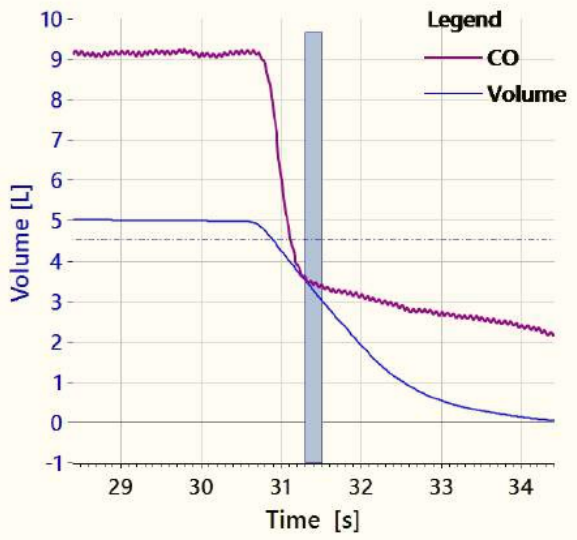
Predicted Values

Quality Grades

Test Graphs

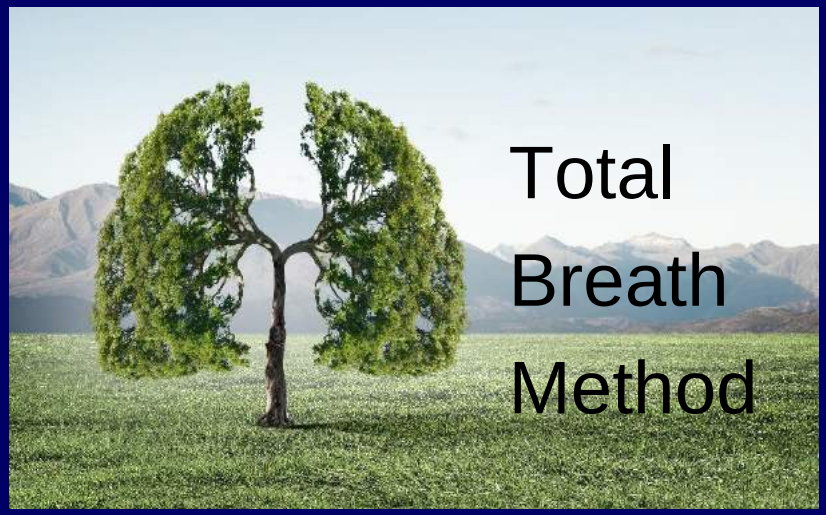






Summary of new recommendations for DLco measurements

Stricter limits for sensors



new reference equations GLI, Stanojevic et al. ERJ 2017

Single breath diffusion capacity for carbon monoxide, DLco: clinical implications

Helgo Magnussen

Pulmonary Research Institute
at LungenClinic Grosshansdorf
Member of German Center for Lung Research

Conflicts of interest

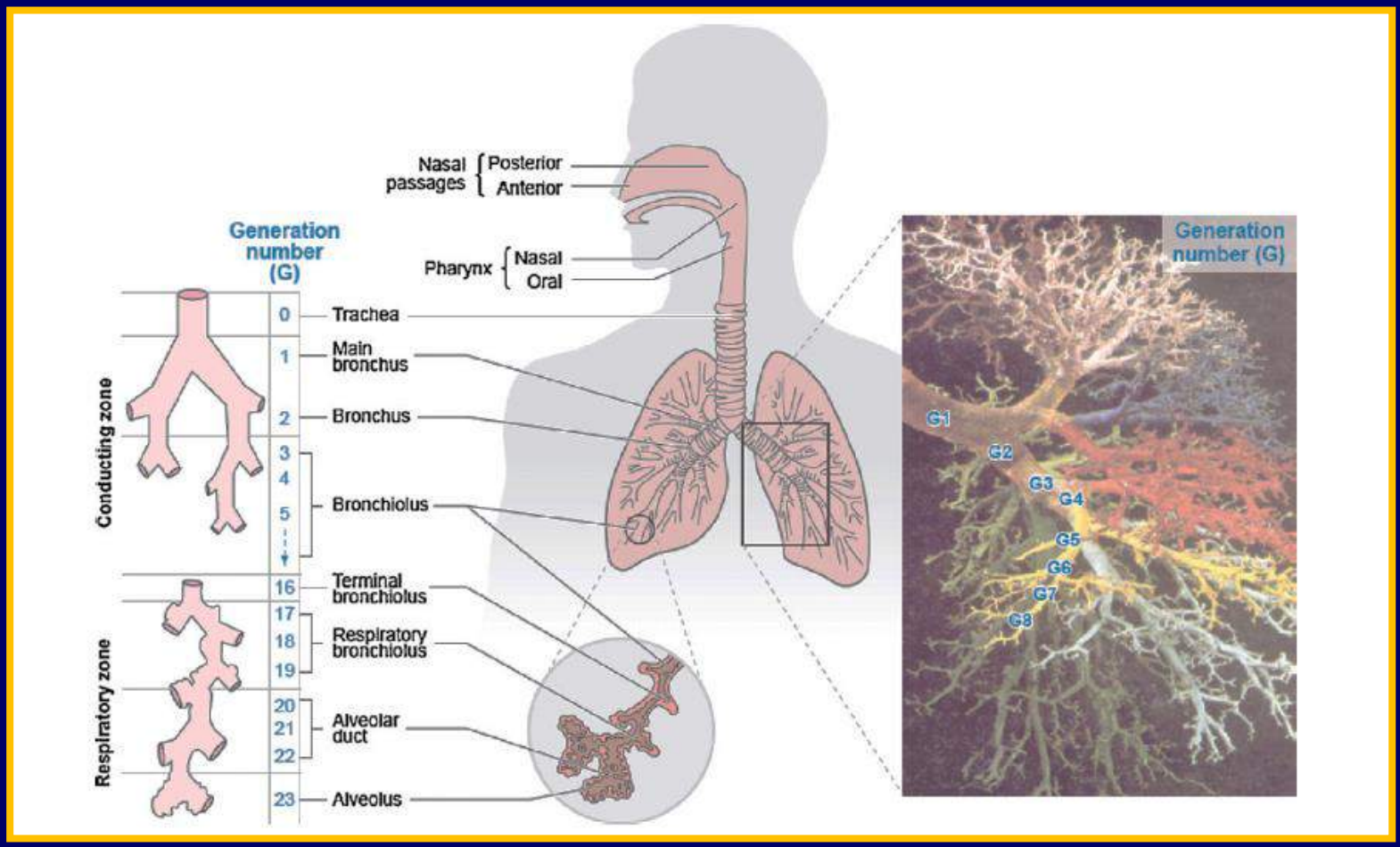
Advisory Board memberships:

Boehringer Ingelheim, AstraZeneca, Merck, Novartis, ndd

Research collaborations:

AstraZeneca, Boehringer Ingelheim, Chiesi, GlaxoSmithKline, Merck, Novartis, Roche, Pfizer

Schematic presentation of the human respiratory system



Assessment of lung function

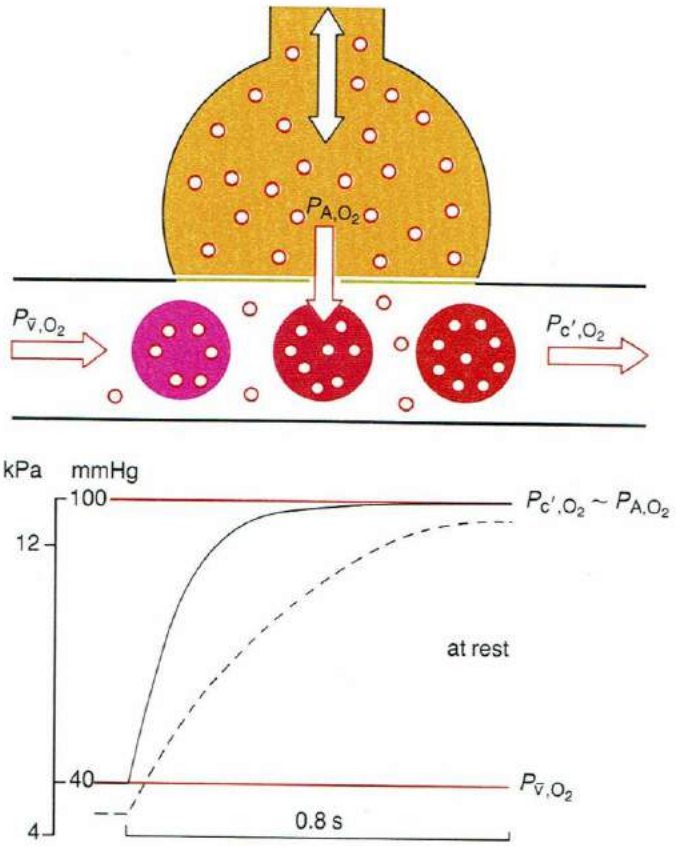
Spirometry is commonly measured

Pulmonary gas transfer is not commonly measured

Assessment of pulmonary gas transfer

Oxygen is our life

Pulmonary transport of oxygen



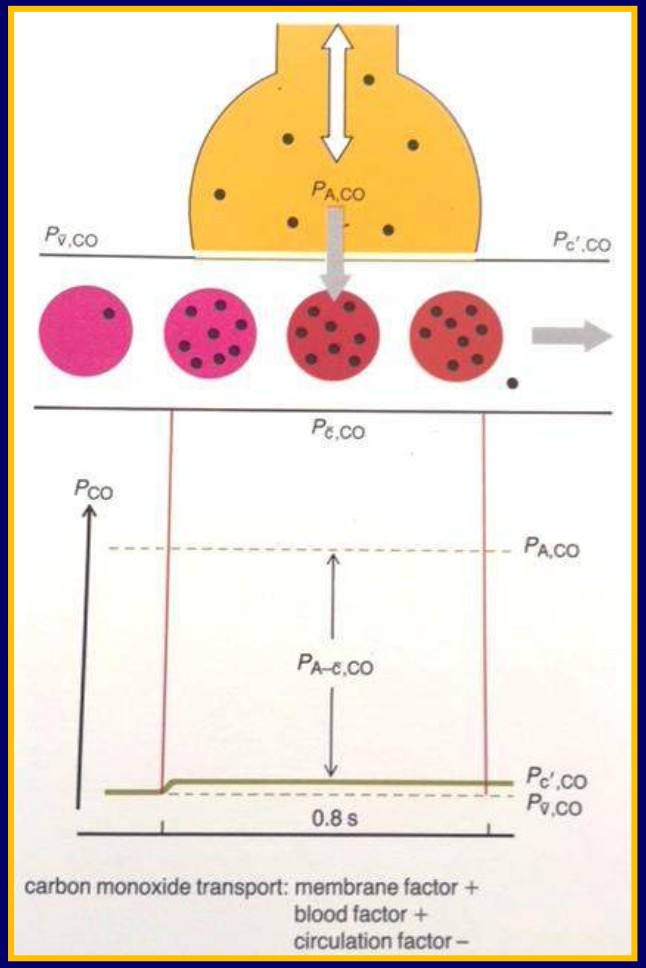
oxygen transport: membrane factor +
blood factor +
circulation factor +
+ = limiting factor

Adapted from Tammeling G.J and Quanjer H 1982

Measurement of diffusion capacity for oxygen

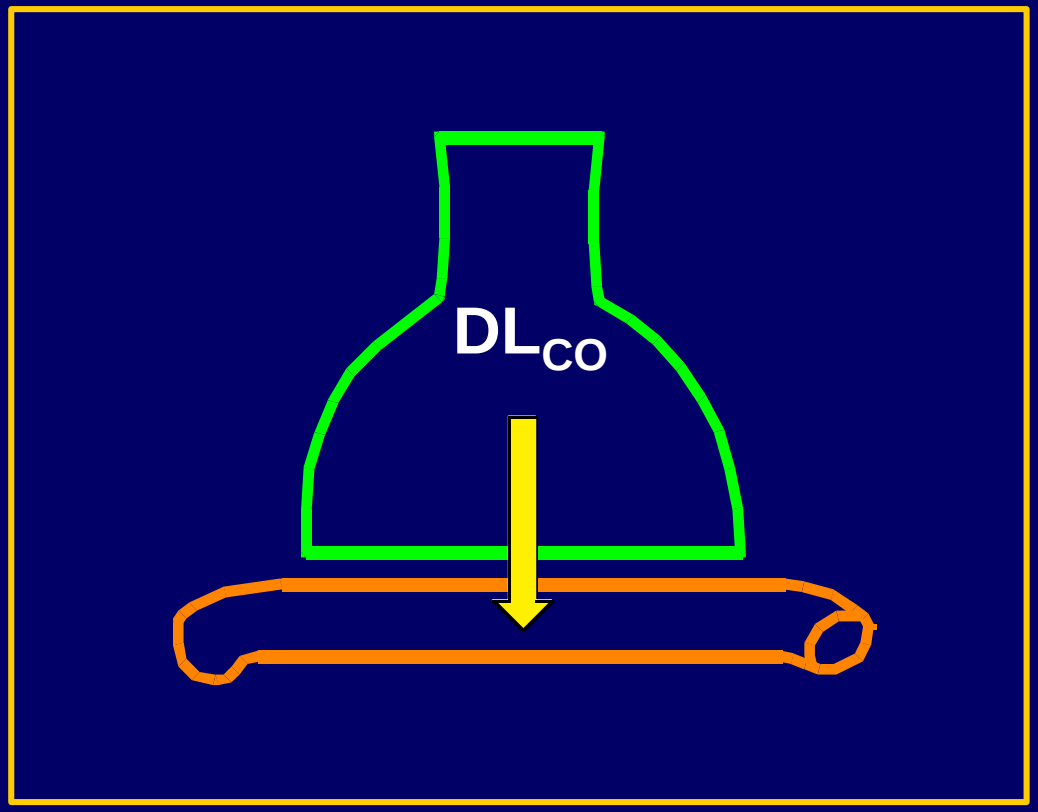
Measurement of end-capillary and mean capillary oxygen pressure can not be measured directly

Pulmonary transport of CO

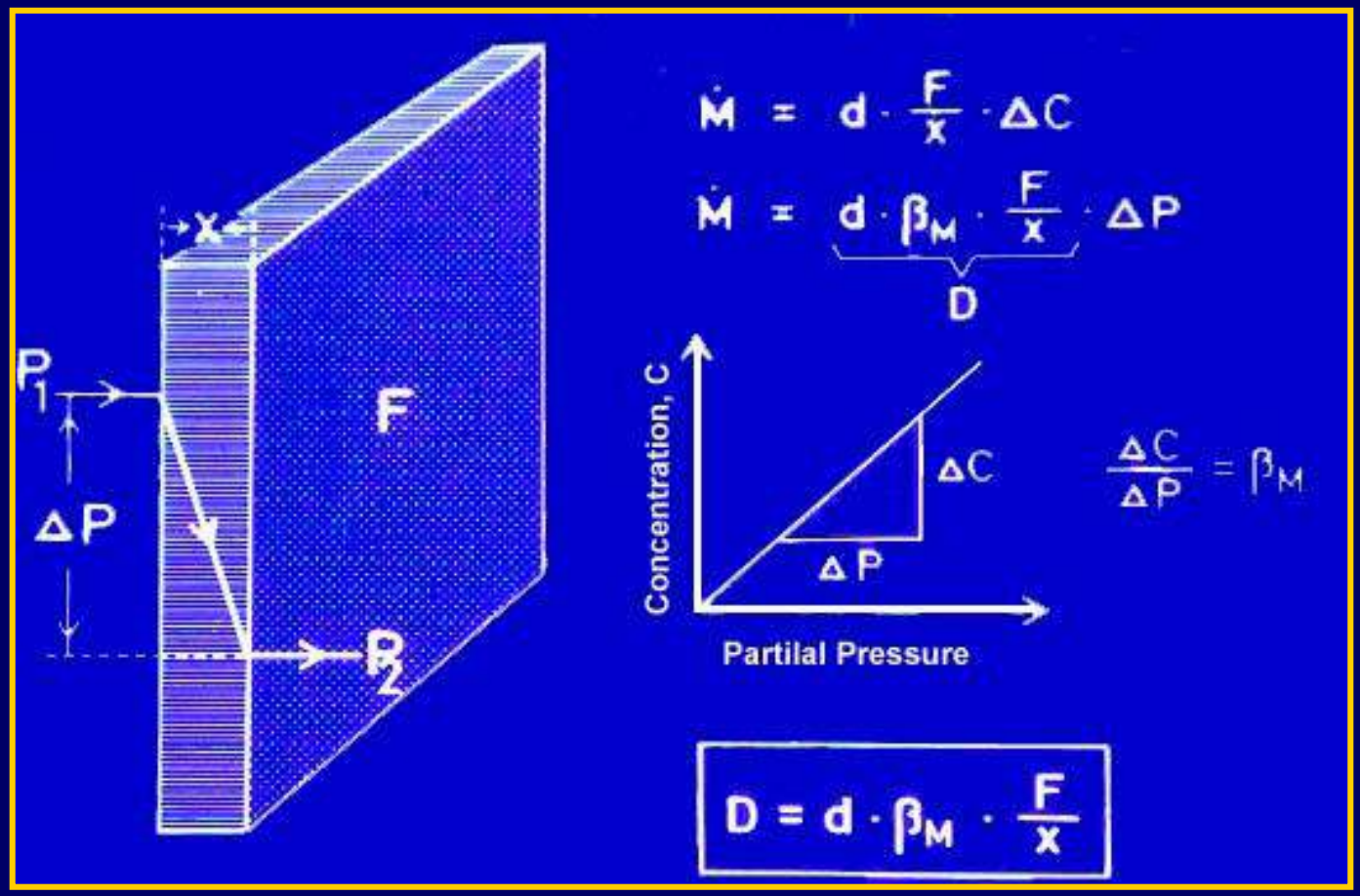


adapted from Tammeling G.J and Quanjer H 1982

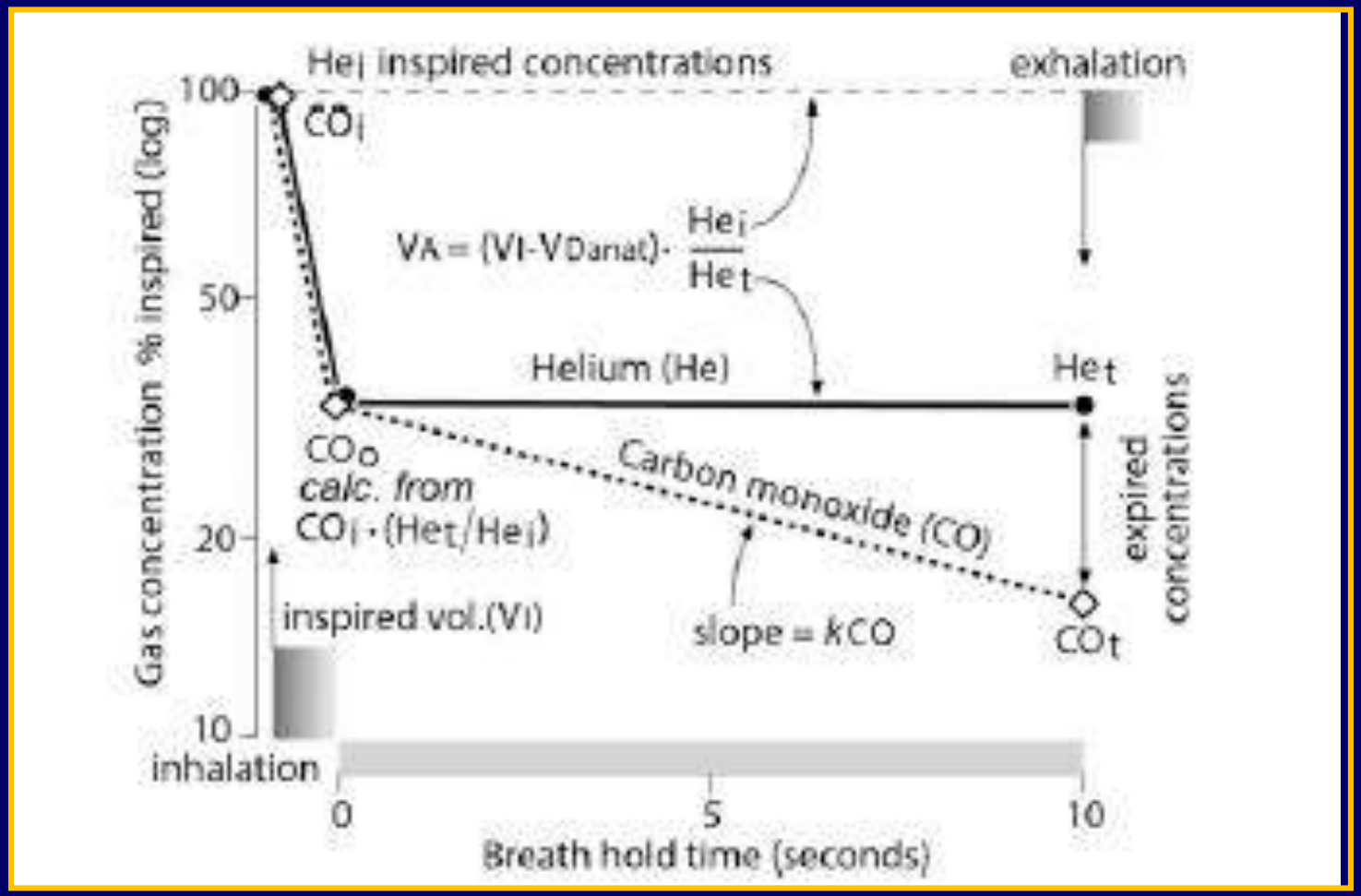
Lung model for calculation of DLco



Diffusion through a barrier: Ficks law

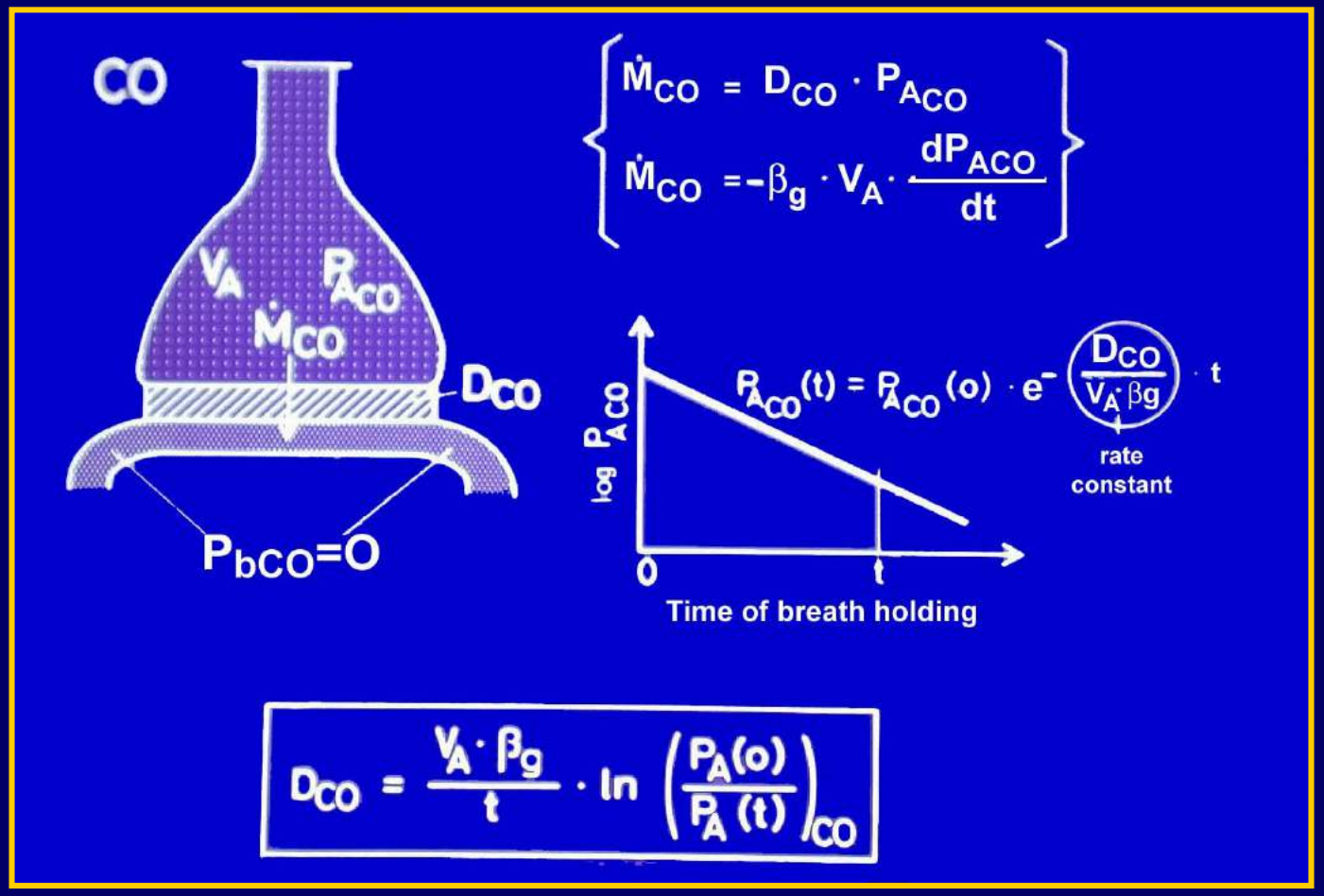


Parameters derived from a single breath maneuver during DLco measurement

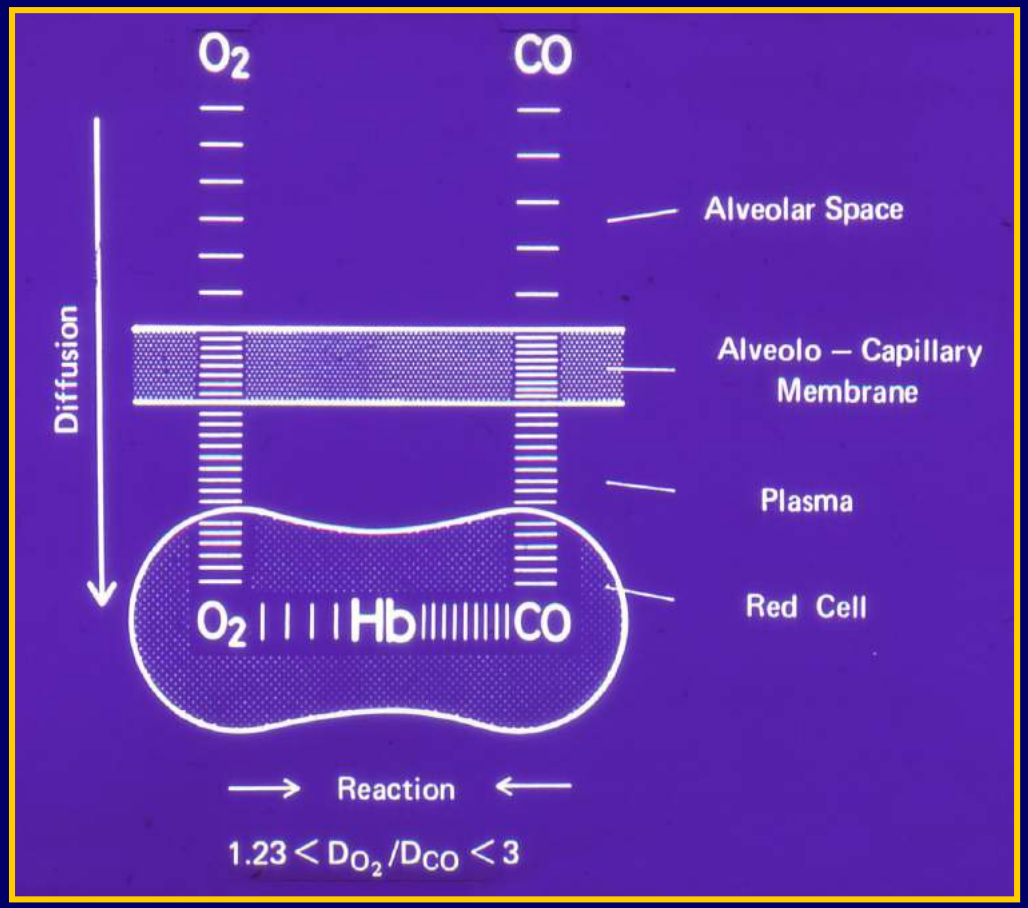


Hughes JM and N Pride AJRCCM 2012

Calculation of single breath diffusion capacity for CO



Partitioning of the diffusion capacity of the lung



Roughton and Foster 1957

Clinical Applications for DLCO

- COPD
- Pulmonary Hypertension
- Cardiovascular diseases
- Interstitial lung disease
- Pulmonary Toxicity

Clinical Applications for DLCO

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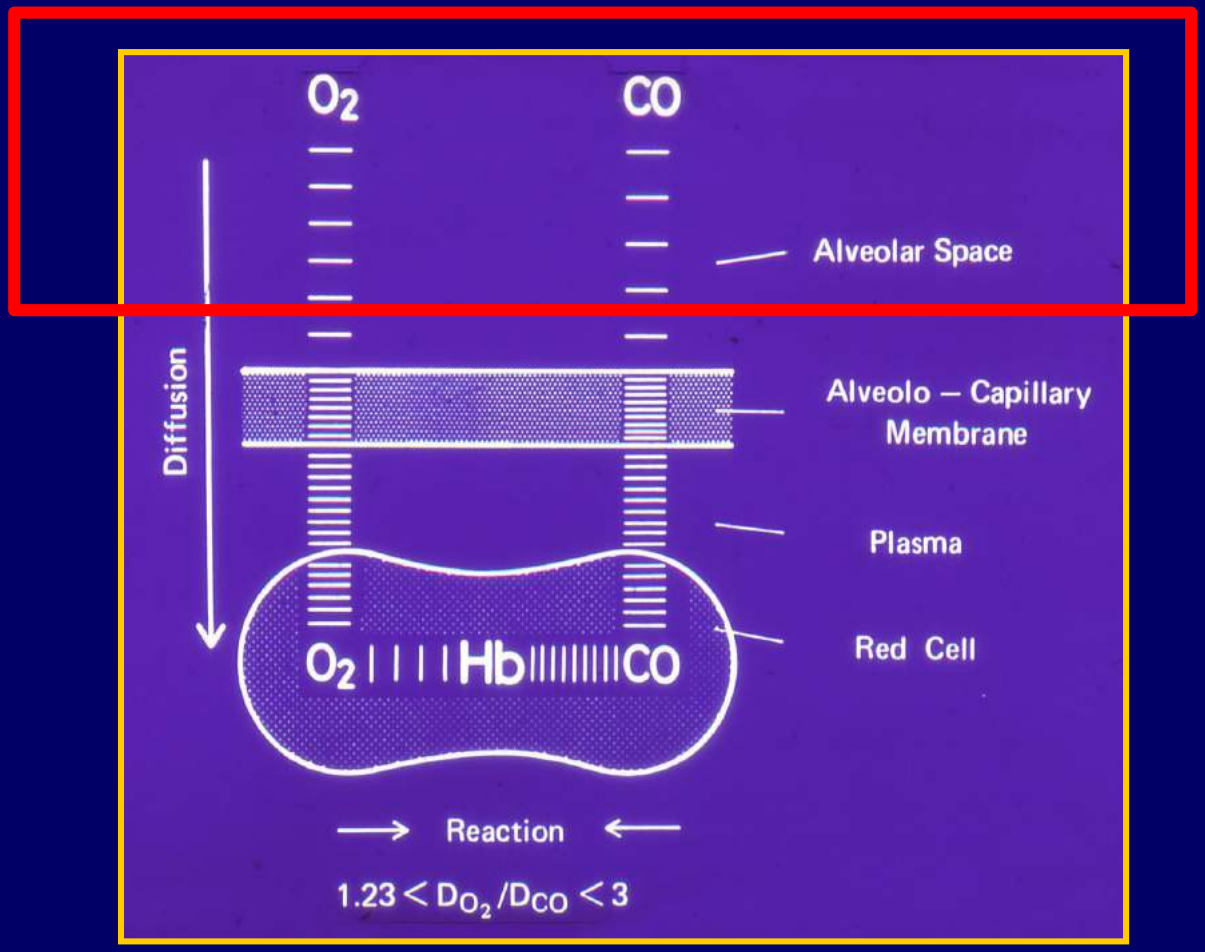
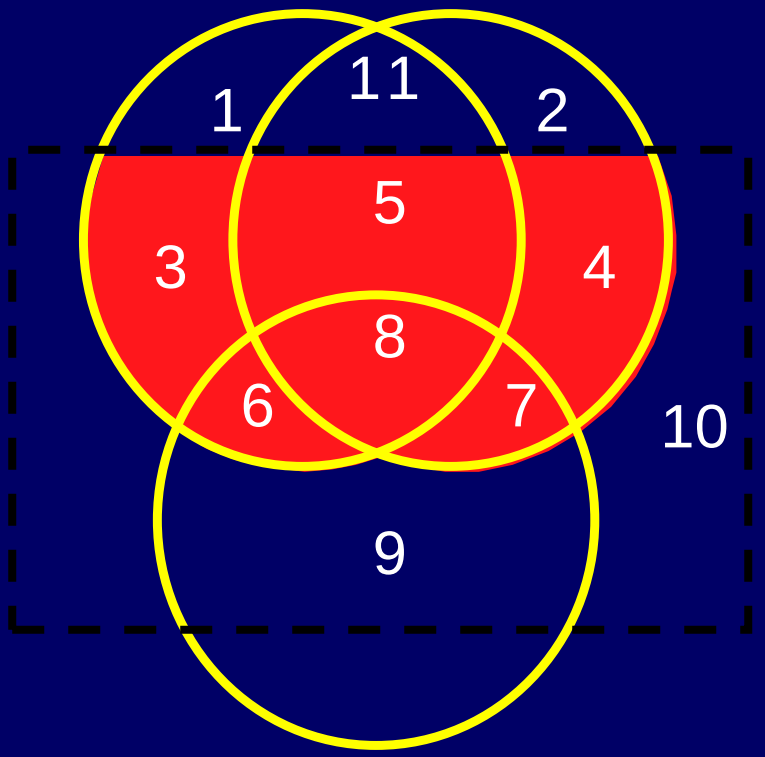
Partitioning of the diffusion capacity of the lung



Roughton and Foster 1957

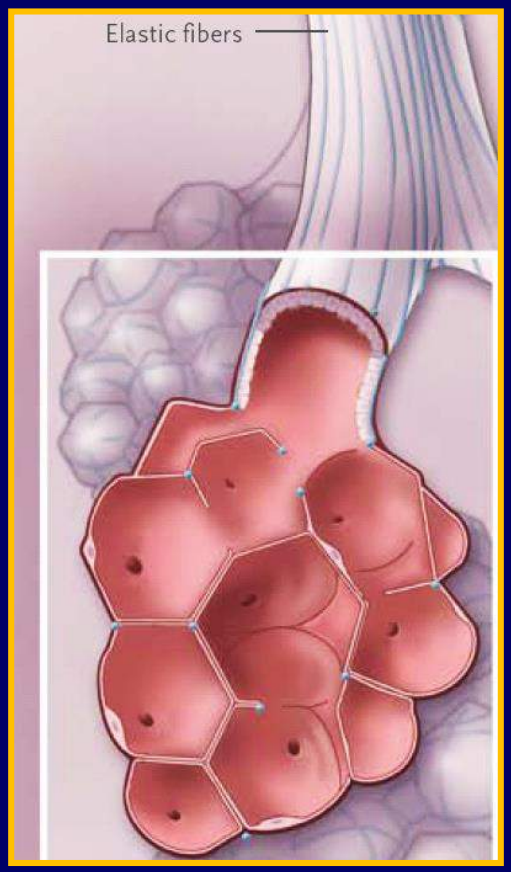
Phenotyping of COPD

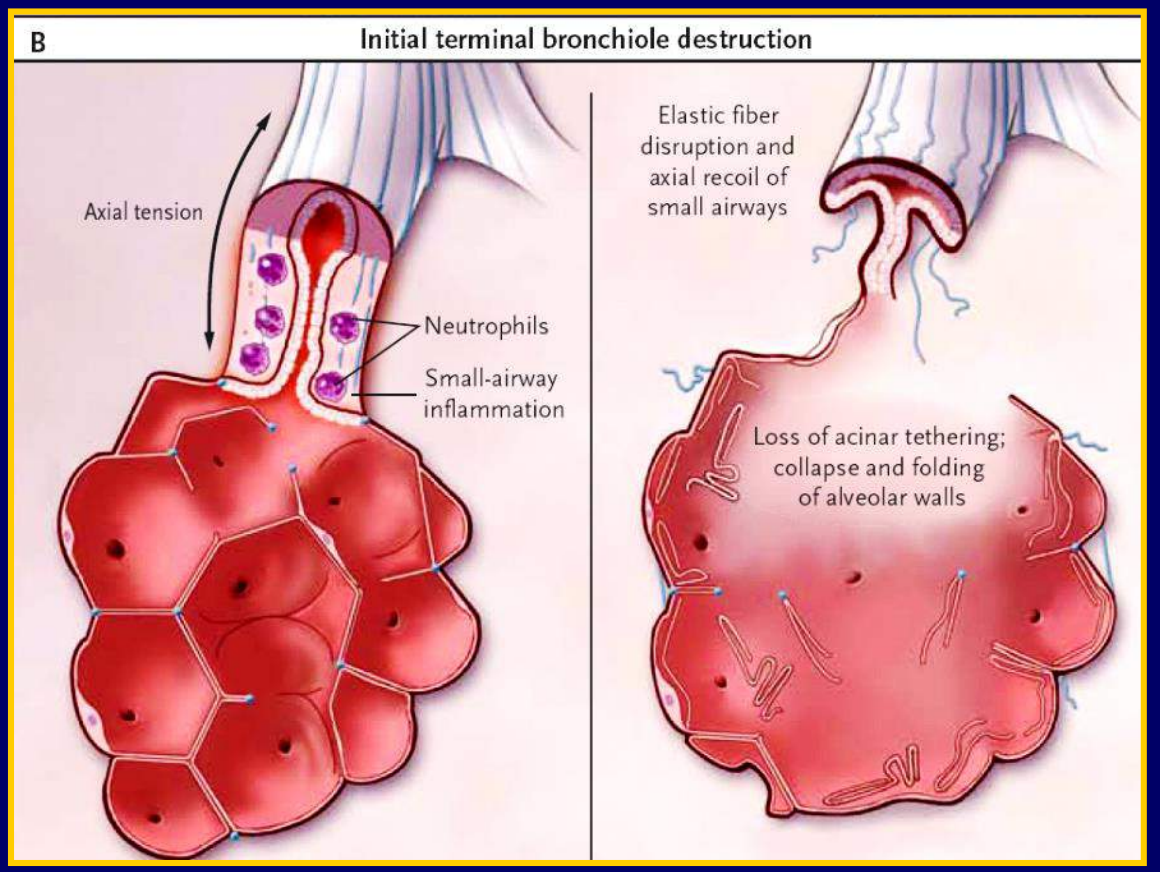


1 chronic bronchitis without obstruction
2 emphysema without obstruction
3 chronic bronchitis with obstruktion
4 emphysema with obstruktion
5 chronic bronchitis and emphysema with obstruction
6 asthma with signs of chronic bronchitis
7 emphysem with signs of asthma
8 chronic bronchitis and emphysema with signs of asthma
9 asthma
10 other obstructive airway diseases
11 chronic bronchitis and emphysema without obstruktion

modified AJRCCM 152: S77-120 (1995)

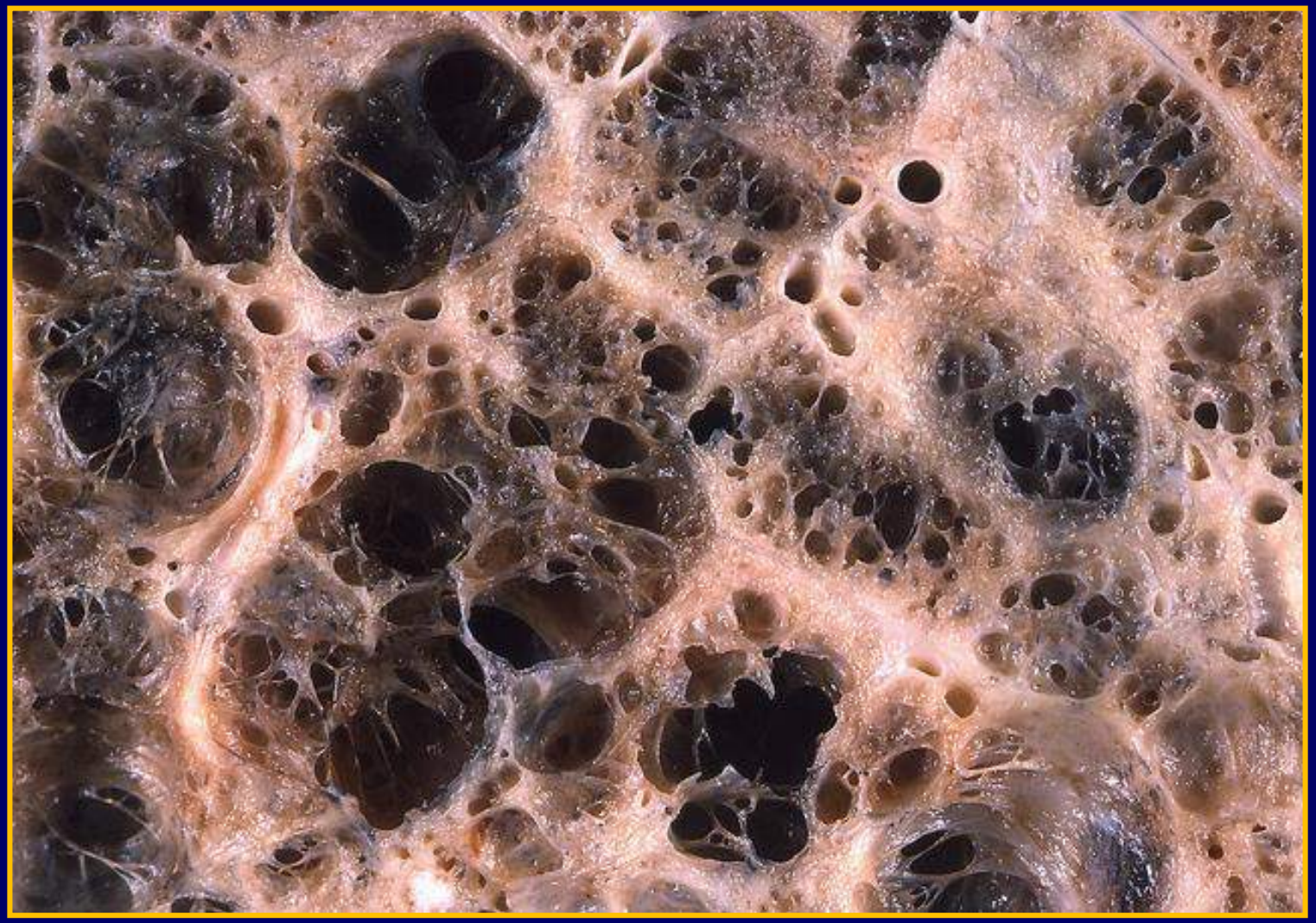
Emphysema – a disease of small airways or lung parenchyma





Mitzner W N Engl J Med; 2011:365:1637-1639

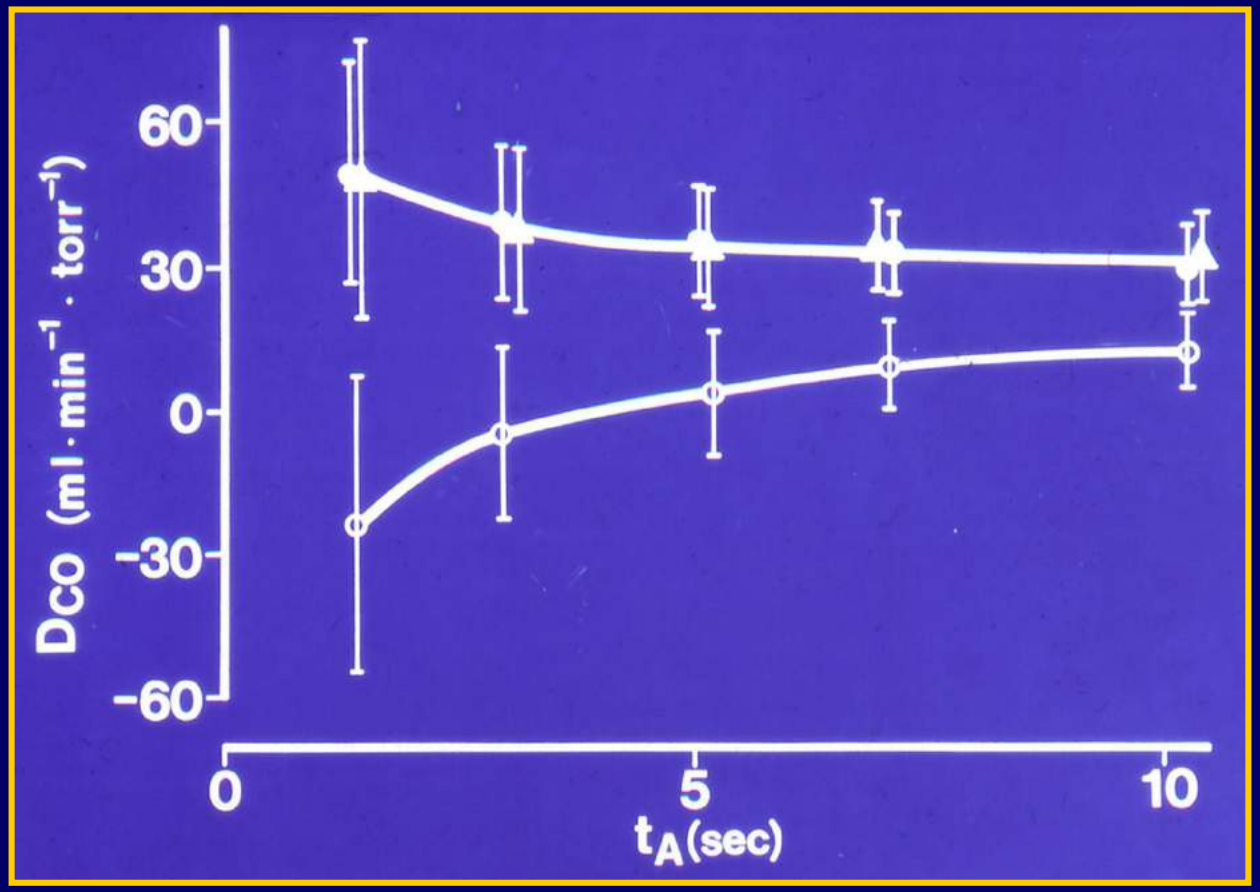
Morphology Emphysem



Mechanism for impaired Dlco in pulmonary emphysema

Gas	molecular weight
Helium	4
CO	28

Effect of breath-hold time on DLco in patients with airway obstruction



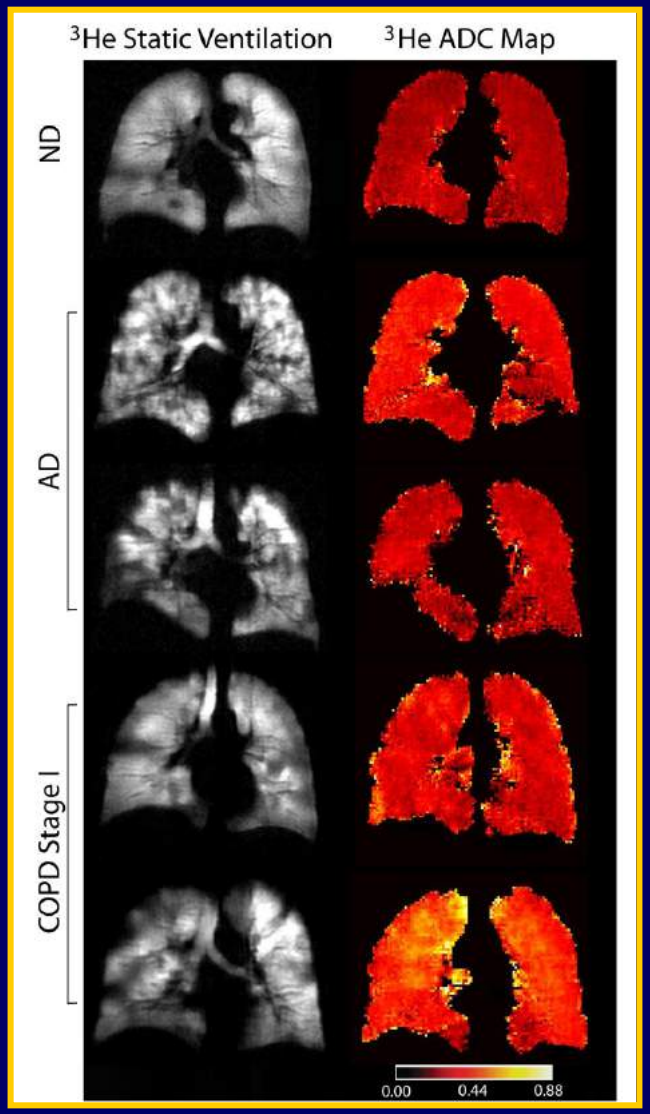
Magnussen et al Respiration 1979

Effect of breath-hold time on DLCO(SB) in patients with airway obstruction

DLCO is limited by the reduced transport of CO from the inspired gas through the alveolar gas prior to alveolar-capillary gas exchange

Graham BL et al J Appl Physiol 1985

Abnormal DLco in ex-smokers without airflow limitation



Kirby M et al Thorax 2013

Abnormal DLco in ex-smokers without airflow limitation

In ex-smokers with normal spirometry and CT but abnormal DLCO, there were significantly worse symptoms, 6MWD and (3)He ADC compared with ex-smokers with normal DLCO.

Kirby M et al Thorax 2013

DLCO Influences Morbidity Beyond Spirometry and CT Evidence of Emphysema in COPDGene

Meredith McCormack MD MHS[1], Aparna Balasubramanian MD[1], Neil MacIntyre MD[2], Rob Henderson[1], Robert Jensen PhD[3], Greg Kinney MPH PhD [4], William Stringer MD[5], Craig P. Hersch MD[6], Russell Bowler MD[7], Richard Casaburi MD[8], MeiLan Han MD MS[9], Janos Porszasz MD PhD[8], Graham Barr MD DrPH[9], Barry Make MD[4], Robert Wise MD[1]

JOHNS HOPKINS MEDICINE

COPDGene® COPD Genetic Epidemiology

INTRODUCTION

- Spirometry is the cornerstone of COPD diagnosis and recent initiatives incorporate symptoms and radiographic features to classify and manage the disease.
- DLCO, a major component of pulmonary function testing, is inconsistently utilized as a tool in COPD assessment.
- The COPD Gene study provides an opportunity to determine the independent relationship between DLCO and key COPD outcomes.

OBJECTIVE

- To explore whether lower DLCO is associated with greater COPD morbidity, independent of FEV_1 and emphysema, and whether the combination of a severe reduction in DLCO and FEV_1 is associated with worse outcomes than either condition in isolation.

METHODS

- 5-year visit was analyzed in 1892 COPD Gene participants

RESULTS

Participant Characteristics

Characteristic	(N=1325)
Age	68 (63, 75)
Male, N (%)	719 (54%)
Black, N (%)	226 (17%)
BMI kg/m²	28 (24, 31)
Obese (BMI≥30), N (%)	434 (33%)
ATS pack years	45 (34, 63)
Smoking Status, N (%)	
Former smoker	788 (59%)
Current to former smoker	156 (12%)
Current smoker	381 (29%)
FEV_1 percent predicted	62 (45, 78)
DLCO percent predicted	63 (48, 80)
CAT	13 (7, 20)
SGRQ, Median	28 (13, 45)
SF-36 Physical	42 (33, 50)
SF-36 Mental	55 (47, 63)
6MWD (meters)	1279 (1000, 1502)
% Emphysema	4 (1,15)
Emphysema >5%, N (%)	644 (49%)
Resting oxygen saturation %	96 (94, 97)

The Association between DLCO and FEV_1 and COPD Morbidity

	DLCO % predicted		FEV_1 % predicted	
Outcomes	Regression Coefficient (95% CI)	p-value	Regression Coefficient (95% CI)	p-value
CAT score	-0.70 (-0.89, -0.52)	<0.001	-1.23 (-1.46, -1.01)	<0.001
SGRQ score	-1.92 (-2.44, -1.4)	<0.001	-3.21 (-3.81, -2.6)	<0.001
Activity	-2.78 (-3.58, -1.98)	<0.001	-4.48 (-5.32, -3.64)	<0.001
Impact	-1.49 (-2.03, -0.95)	<0.001	-2.41 (-2.86, -1.94)	<0.001
Symptom	-1.77 (-2.33, -1.22)	<0.001	-3.51 (-3.87, -3.15)	<0.001
SF-36 Physical Function	1.05 (0.73, 1.38)	<0.001	1.47 (1.12, 1.81)	<0.001
SF-36 Mental	-0.01 (-0.41, 0.4)	0.974	0.05 (-0.31, 0.43)	0.764
6MWD (meters)	46.35 (36.79, 55.9)	<0.001	51.54 (44.21, 58.87)	<0.001
Resting oxygen saturation %	0.25 (0.06, 0.44)	0.010	0.21 (0.16, 0.27)	<0.001
Hospitalization Rate (Rate Ratio)	0.80 (0.71, 0.90)	<0.001	0.77 (0.73, 0.82)	<0.001

SF-36 Physical Function

Six Minute Walk Distance

- We speculate that the association between DLCO and COPD morbidity, independent of spirometry and CT emphysema, may reflect the presence of subclinical pulmonary vascular injury and its impact on clinical outcomes, an area that is underappreciated in the assessment of patients with COPD.
- DLCO is a widely available, inexpensive, minimal risk test that may be an underutilized tool in COPD assessment and future studies investigating the integration of DLCO into multi-dimensional assessment approaches are warranted.

Funding sources: COPDGene

OBJECTIVE

- To explore whether lower DLCO is associated with greater COPD morbidity, independent of FEV_1 and emphysema, and whether the combination of a severe reduction in DLCO and FEV_1 is associated with worse outcomes than either condition in isolation.

DLCO Influences Morbidity Beyond Spirometry and CT Evidence of Emphysema in COPDGene

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JOHNS HOPKINS MEDICINE

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- Spirometry is the cornerstone of COPD diagnosis and recent initiatives incorporate symptoms and radiographic features to classify and manage the disease.
- FEV_1 is associated with worse outcomes than either condition in isolation.

METHODS

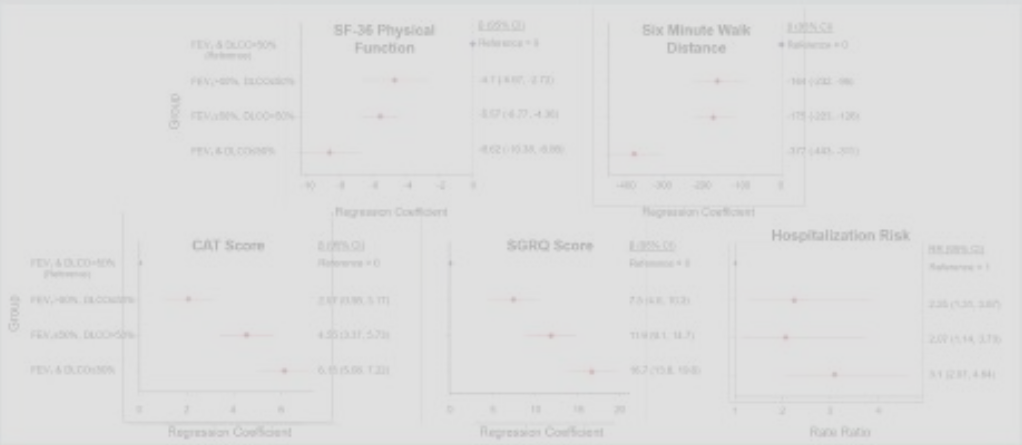
- 5-year visit was analyzed in 1892 COPD Gene participants GOLD Stages 1-4
- DLCO % predicted was calculated using Miller non-smoking reference equations, adjusting for hemoglobin and altitude. FEV1 % predicted was calculated using NHANES reference equations
- A categorical variable was created to represent four possibilities: 1) FEV_1 and DLCO both > 50% predicted, 2) $FEV_1 \leq 50\%$ and DLCO > 50%, 3) DLCO $\leq$ 50% and FEV_1 > 50% and 4) both $\leq$ 50%
- Outcomes included CAT, SGRQ, SF-36, 6 minute walk distance, resting oxygen saturation, COPD hospitalization rate
- Multivariable models were created adjusting for age, sex, obesity, race, education, pack-years of smoking, smoking status, diabetes, FEV_1 and emphysema

RESULTS

Participant Characteristics

Characteristic	(N=1325)
Age	68 (63, 75)
Male, N (%)	719 (54%)

The Association between DLCO and FEV_1 and COPD Morbidity



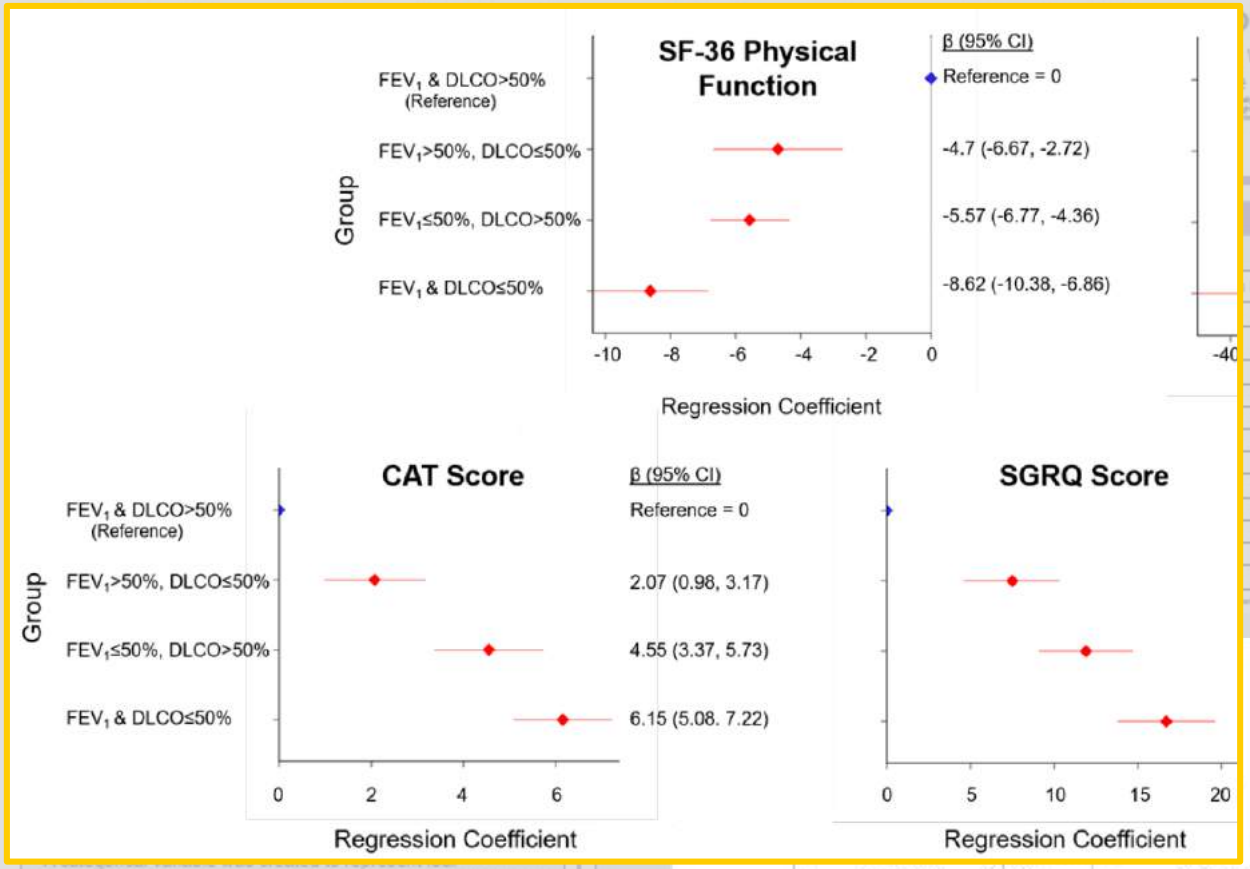
METHODS

- 5-year visit was analyzed in 1892 COPD Gene participants GOLD Stages 1-4

CONCLUSIONS

- Impairment in gas transfer, represented by a reduction in DLCO, was associated with increased COPD morbidity across domains of symptoms, quality of life, functional status, and risk of hospitalization, even after accounting for spirometry and CT evidence of emphysema.
- Severe impairment in both FEV_1 and DLCO was associated with worse symptoms, quality of life, and functional exercise capacity compared to severe impairment in either alone.
- We speculate that the association between DLCO and COPD morbidity, independent of spirometry and CT emphysema, may reflect the presence of subclinical pulmonary vascular injury and its impact on clinical outcomes, an area that is underappreciated in the assessment of patients with COPD.
- DLCO is a widely available, inexpensive, minimal risk test that may be an underutilized tool in COPD assessment and future studies investigating the integration of DLCO into multi-dimensional assessment approaches are warranted.

Funding sources: COPDGene



f Emphysema in COPDGene

William Stringer MD[5], Craig P. Hersch MD[6], Russell Bowler MD[7], MD[4], Robert Wise MD[1]

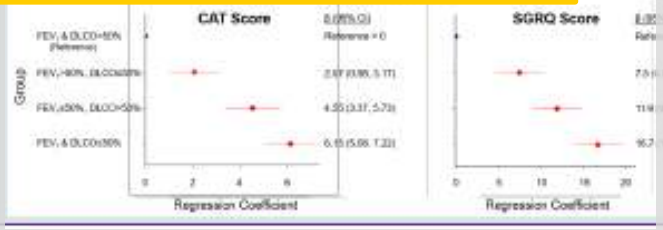
COPDGene®
COPD Genetic Epidemiology

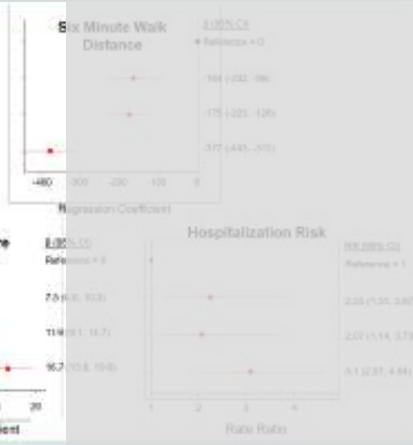
between DLCO and FEV_1 and COPD Morbidity

	DLCO % predicted		FEV_1 % predicted	
	Regression Coefficient (95% CI)	p-value	Regression Coefficient (95% CI)	p-value
	-0.70 (-0.89, -0.52)	<0.001	-1.23 (-1.46, -1.01)	<0.001
	-1.92 (-2.44, -1.4)	<0.001	-3.21 (-3.81, -2.6)	<0.001
	-2.78 (-3.68, -1.88)	<0.001	-4.48 (-5.32, -3.64)	<0.001
	-1.49 (-2.03, -0.95)	<0.001	-2.41 (-2.88, -1.94)	<0.001
	-1.77 (-2.33, -1.22)	<0.001	-3.51 (-3.87, -3.15)	<0.001
	1.05 (0.73, 1.38)	<0.001	1.47 (1.12, 1.81)	<0.001
	-0.01 (-0.41, 0.4)	0.974	0.05 (-0.31, 0.43)	0.764
	46.35 (36.79, 55.9)	<0.001	51.54 (44.21, 58.87)	<0.001
	0.25 (0.06, 0.44)	0.010	0.21 (0.16, 0.27)	<0.001
	0.80 (0.71, 0.90)	<0.001	0.77 (0.73, 0.82)	<0.001

possibilities: 1) FEV_1 and DLCO both > 50% predicted, 2) FEV_1 ≤50% and DLCO > 50%, 3) DLCO ≤ 50% and FEV_1 > 50% and 4) both ≤ 50%

- Outcomes included CAT, SGRQ, SF-36, 6 minute walk distance, resting oxygen saturation, COPD hospitalization rate
- Multivariable models were created adjusting for age, sex, obesity, race, education, pack-years of smoking, smoking status, diabetes, FEV_1 and emphysema





CONCLUSIONS

- Impairment in gas transfer, represented by a reduction in DLCO, was associated with increased COPD morbidity across domains of symptoms, quality of life, functional status, and risk of hospitalization, even after accounting for spirometry and CT evidence of emphysema.
- Severe impairment in both FEV_1 and DLCO was associated with worse symptoms, quality of life, and functional exercise capacity compared to severe impairment in either alone.
- We speculate that the association between DLCO and COPD morbidity, independent of spirometry and CT emphysema, may reflect the presence of subclinical pulmonary vascular injury and its impact on clinical outcomes, an area that is underappreciated in the assessment of patients with COPD.
- DLCO is a widely available, inexpensive, minimal risk test that may be an underutilized tool in COPD assessment and future studies investigating the integration of DLCO into multi-dimensional assessment approaches are warranted.

Funding sources: COPDGene

DLco influences morbidity beyond spirometry and CT evidence of emphysema in COPD

Conclusion

Impairment of DLco was associated with increased COPD morbidity across symptoms, quality of life, and risk of hospitalization even after accounting for spirometry and CT evidence of emphysema

JOHNS HOPKINS MEDICINE

COPDGene® COPD Genetic Epidemiology

DLco influences morbidity beyond spirometry and CT evidence of emphysema in COPD

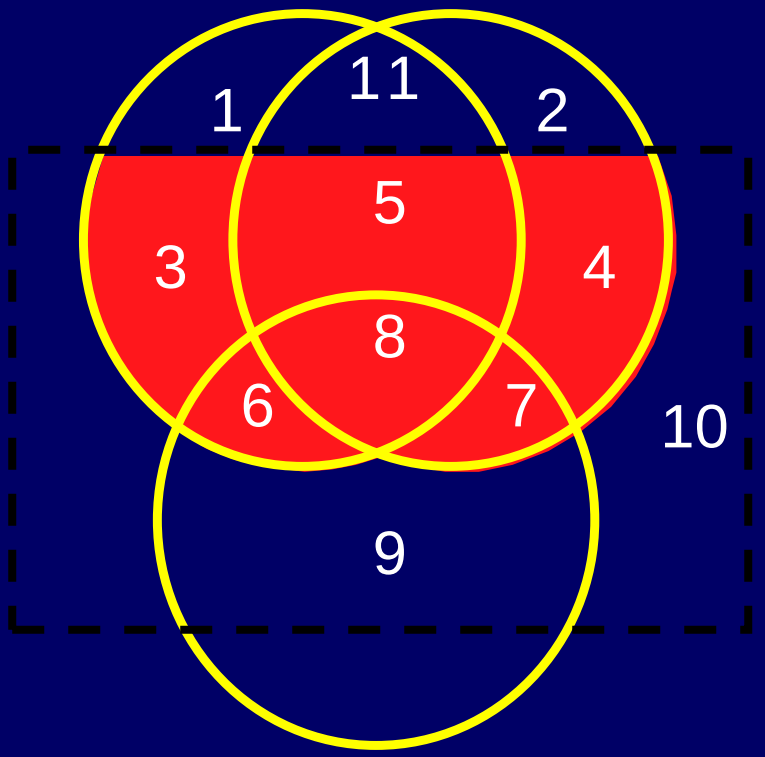
Conclusion

association between DLco and COPD morbidity independent of spirometry and CT emphysema may reflect the presence of subclinical pulmonary vascular injury

JOHNS HOPKINS MEDICINE

COPDGene® COPD Genetic Epidemiology

Phenotyping of COPD



1 chronic bronchitis without obstruction
2 emphysema without obstruction
3 chronic bronchitis with obstruktion
4 emphysema with obstruktion
5 chronic bronchitis and emphysema with obstruction
6 asthma with signs of chronic bronchitis
7 emphysem with signs of asthma
8 chronic bronchitis and emphysema with signs of asthma
9 asthma
10 other obstructive airway diseases
11 chronic bronchitis and emphysema without obstruktion

modified AJRCCM 152: S77-120 (1995)

Are COPD and asthma different diseases ?

	Asthma $n = 11$	COPD $n = 11$	P-value
age (yr)	54.5 (7.4)	56.6 (8.3)	n.s.
height (cm)	173.2 (10.6)	174.9 (6.3)	n.s.
weight (kg)	78.3 (14.1)	76.6 (13.9)	n.s.
VC (l)	3.65 (0.99)	3.23 (1.25)	n.s.
FEV_1 (l)	1.82 (0.81)	1.85 (0.81)	n.s.
FEV_1 (%pred)	56.0 (18.1)	54.8 (18.7)	n.s.
TIC (l)	6.33 (1.08)	7.40 (1.08)	0.03
ITGV (l)	4.05 (0.60)	5.35 (0.91)	< 0.001
ITGV (%pred)	120.1 (16.0)	154.7 (21.9)	< 0.001
DL_{CO} (%pred)	99.0 (18.6)	67.4 (11.8)	< 0.001

Magnussen H, Richter K and C Taube Clinical and Experimental Allergy 1998

Clinical Applications for DLCO

- COPD
- Pulmonary Hypertension
- Cardiovascular diseases
- Interstitial lung disease
- Pulmonary Toxicity

Pulmonary Hypertension WHO Classification

Group 3: pulmonary hypertension due to lung disease and/or chronic hypoxia

Group 3 includes pulmonary hypertension resulting from lung diseases or shortage of oxygen in the body (hypoxia). The common diseases associated with group 3 pulmonary hypertension are:

- Chronic obstructive pulmonary disease (COPD)
- Interstitial lung disease
- Sleep-disordered breathing, a group of diseases that affect breathing during sleep like obstructive sleep apnea (OSA)
- Chronic high-altitude exposure
- Lung developmental abnormalities
- Alveolar hypoventilation disorders

More on idiopathic pulmonary arterial hypertension with a low diffusing capacity

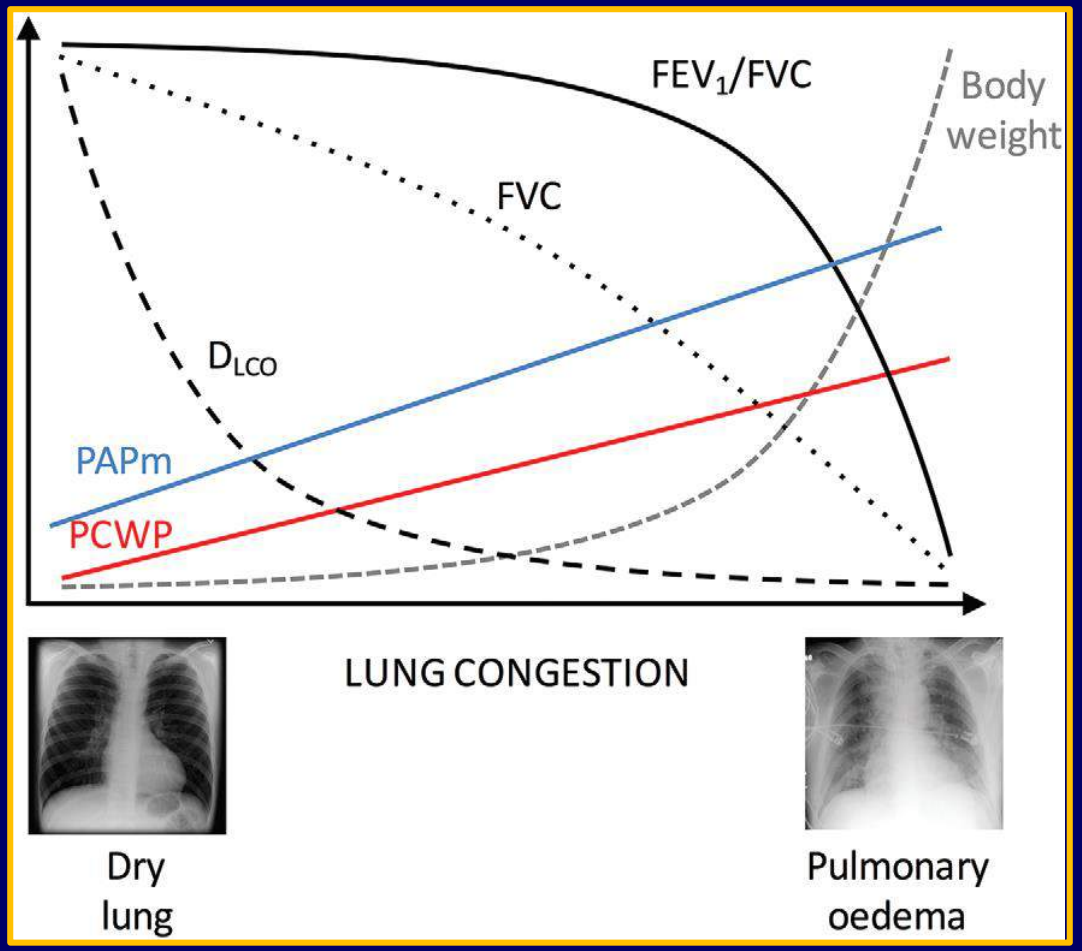
Characteristic	Disease		p-value
	IPAH (n=22)	CPFE (n=13)	
Age years	74±6	72±6	0.389
Gender male	16 (73)	13 (100)	0.039
Smoking habits			
Never smoked	2 (9)	1 (8)	–
Former or current smoker	20 (91)	12 (92)	–
Smoking duration pack-years	50 (35–60)	50 (40–80)	0.103
Cardiovascular comorbidities			
Coronary heart disease	17 (77)	8 (62)	0.319
Hypertension	10 (77)	21 (96)	0.096
Pulmonary function			
FVC % predicted	95±12	85±14	0.029
FEV_1 % predicted	90±11	77±15	0.007
FEV_1/FVC %	76±8	68±10	0.025
RV % predicted	98±9	101±15	0.457
TLC % predicted	94±10	84±9	0.008
RV/TLC %	42±4	44±7	0.379
FRC % predicted	98±12	95±16	0.564
D_{LCO} % predicted	30±8	22±7	0.007
D_{LCO}/V_A % predicted	33±10	27±9	0.050

Olsson K et al ERJ 2017

Clinical Applications for DLCO

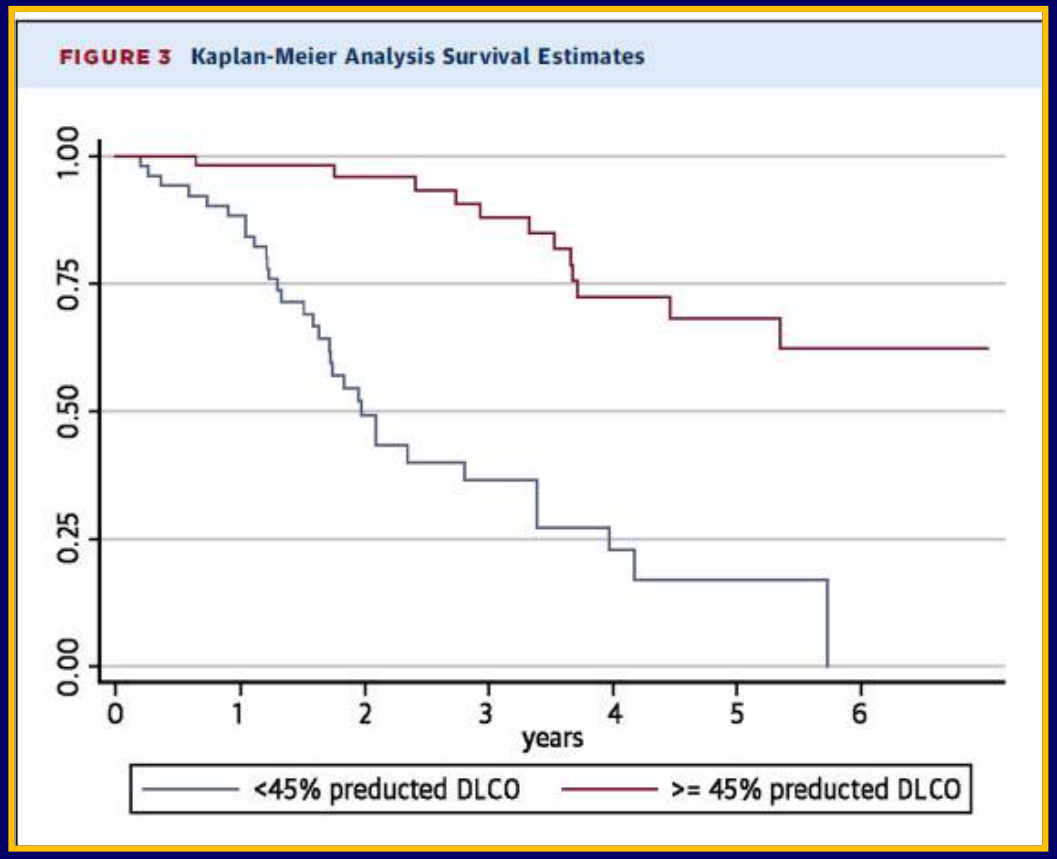
- COPD
- Pulmonary Hypertension
- Cardiovascular diseases
- Interstitial lung disease
- Pulmonary Toxicity

Single breath diffusion capacity for carbon monoxide, DLco in heart failure



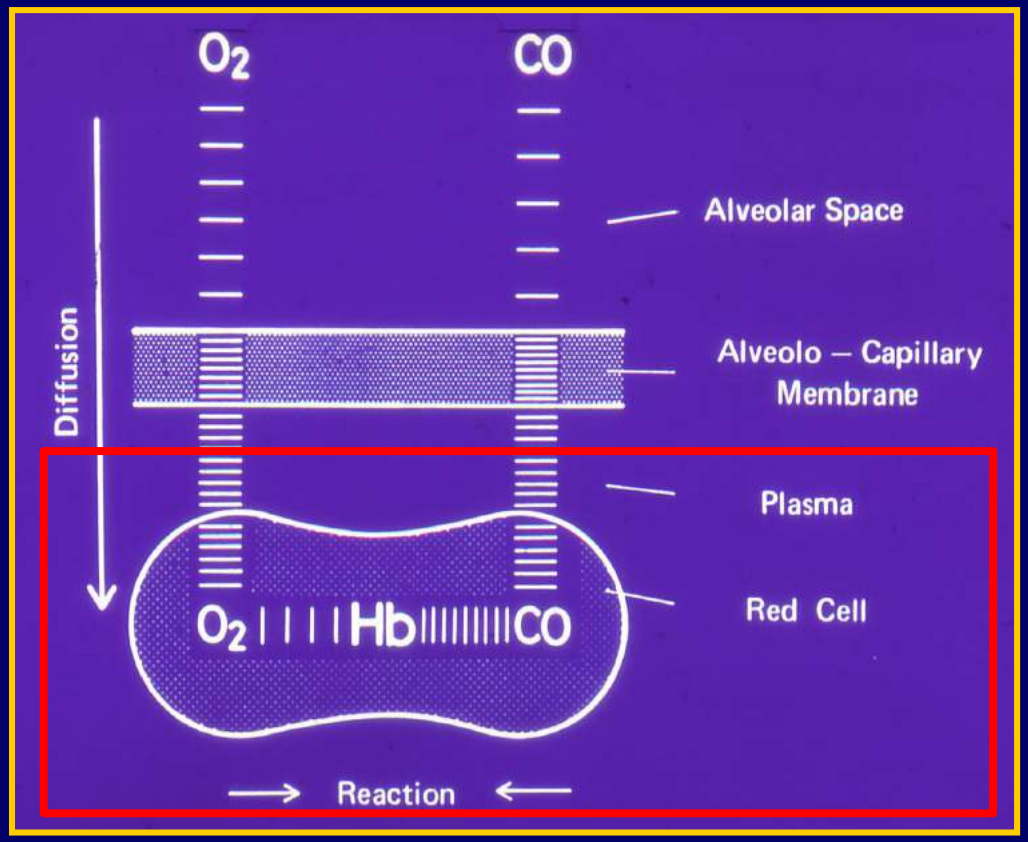
Magnussen, H. et all., 2017

DLco and mortality in pulmonary hypertension due to heart failure with preserved ejection fraction



Hoeper M et al JACC: HEART FAILURE 2016

Partitioning of the diffusion capacity of the lung



Roughton and Foster 1957

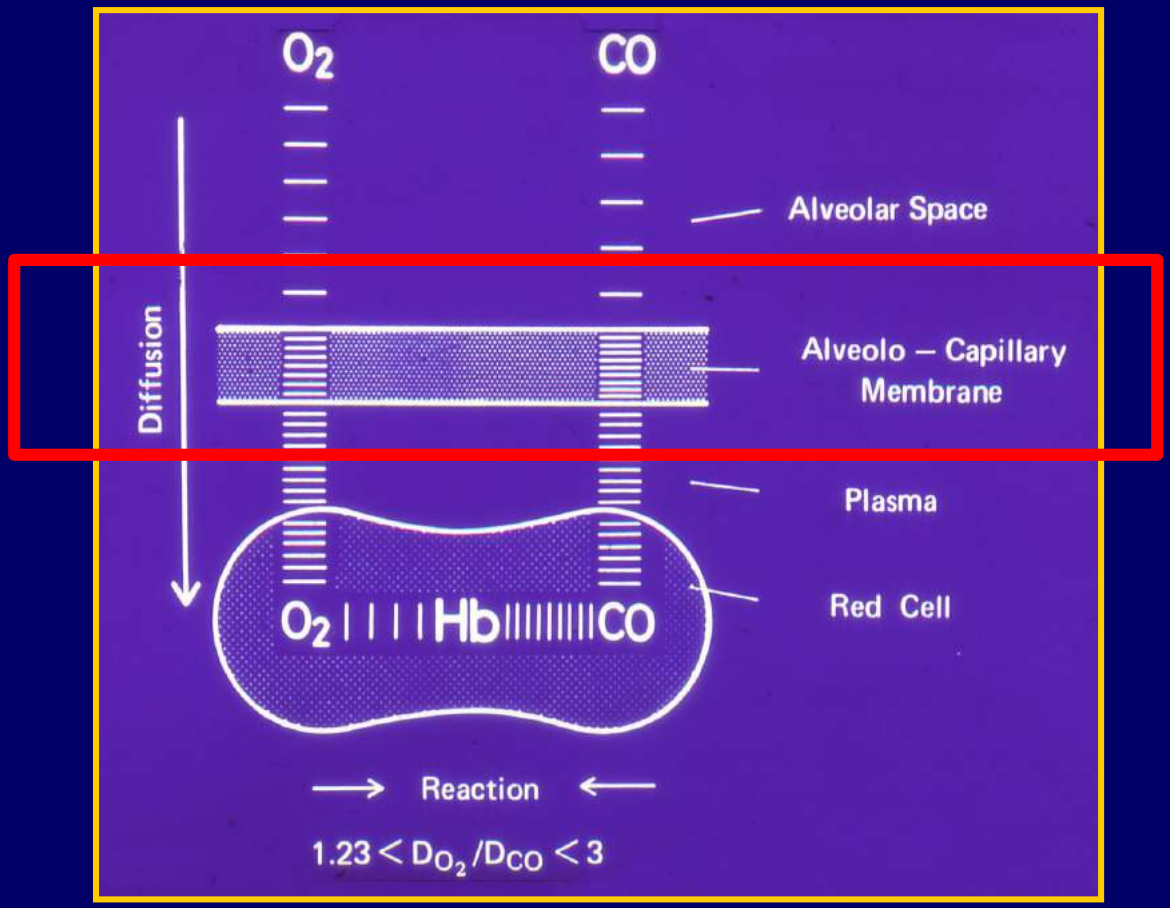
Limitation of CO Transport in red cells

- Small pulmonary vessel disease in pulmonary hypertension

Clinical Applications for DLCO

- COPD
- Pulmonary Hypertension
- Cardiovascular diseases
- Interstitial lung disease
- Pulmonary Toxicity

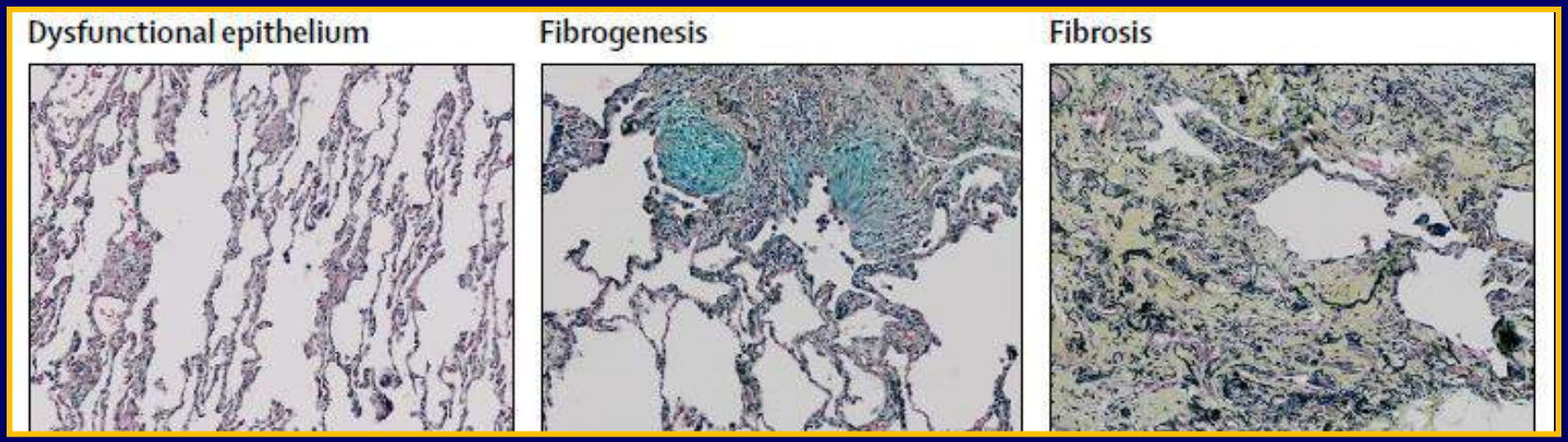
Partitioning of the diffusion capacity of the lung



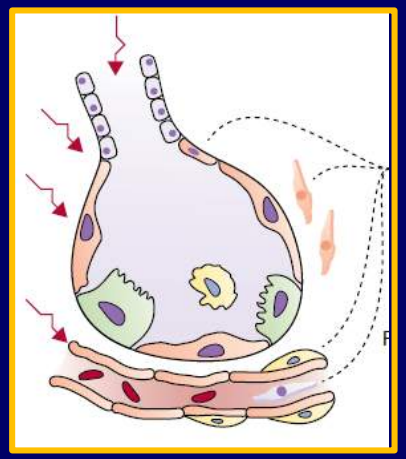
Roughton and Foster 1957

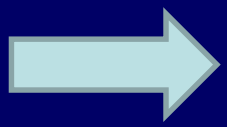
Pathogenesis of idiopathic pulmonary fibrosis

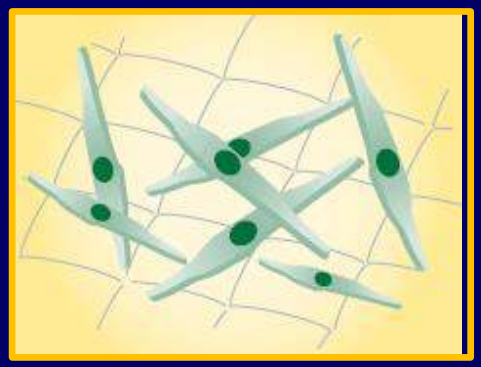
(modified from Richeldie L et al Lancet 2017)



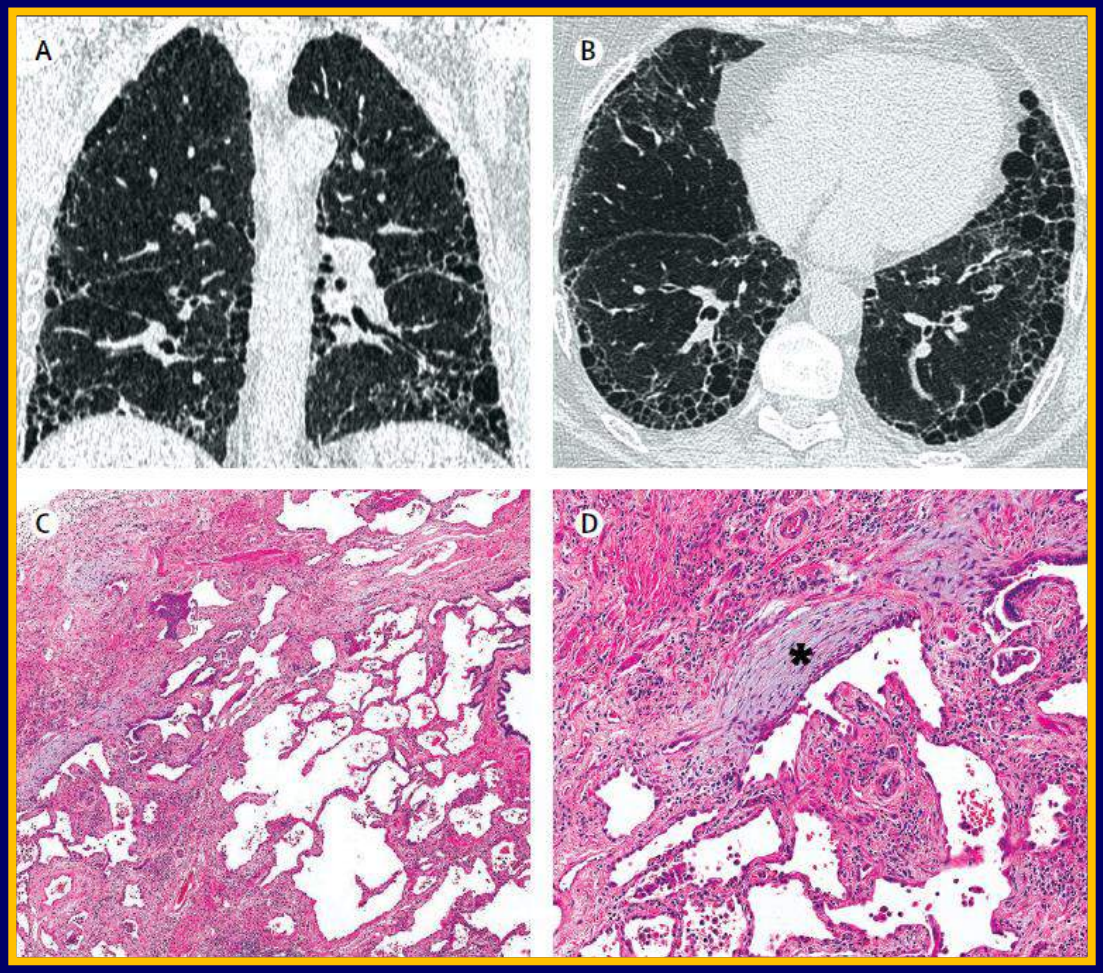
Activation and proliferation







Radiological and histological patterns of usual interstitial pneumonia



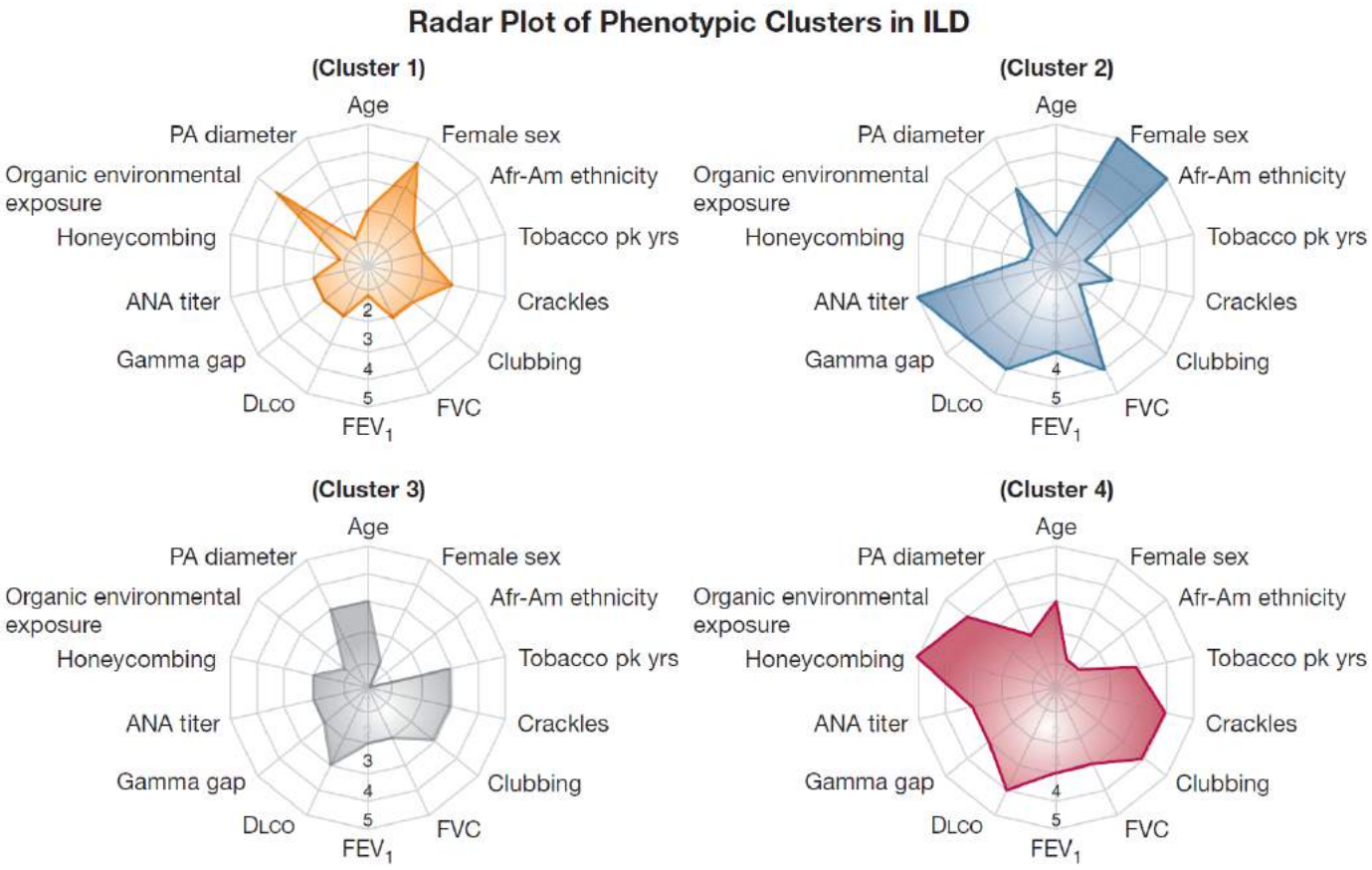
Richeldie L et al Lancet 2017

Phenotypic Clusters Predict Outcomes in a Longitudinal Interstitial Lung Disease Cohort

Disease entities	Number
Connective tissue disease associated interstitial lung disease CTD-ILD	173
Chronic hypersensitivity pneumonitis CHP	119
Idiopathic pulmonary fibrosis IPF	286
Interstitial pneumonia with autoimmune features IPAF	156
unclassifiable	36

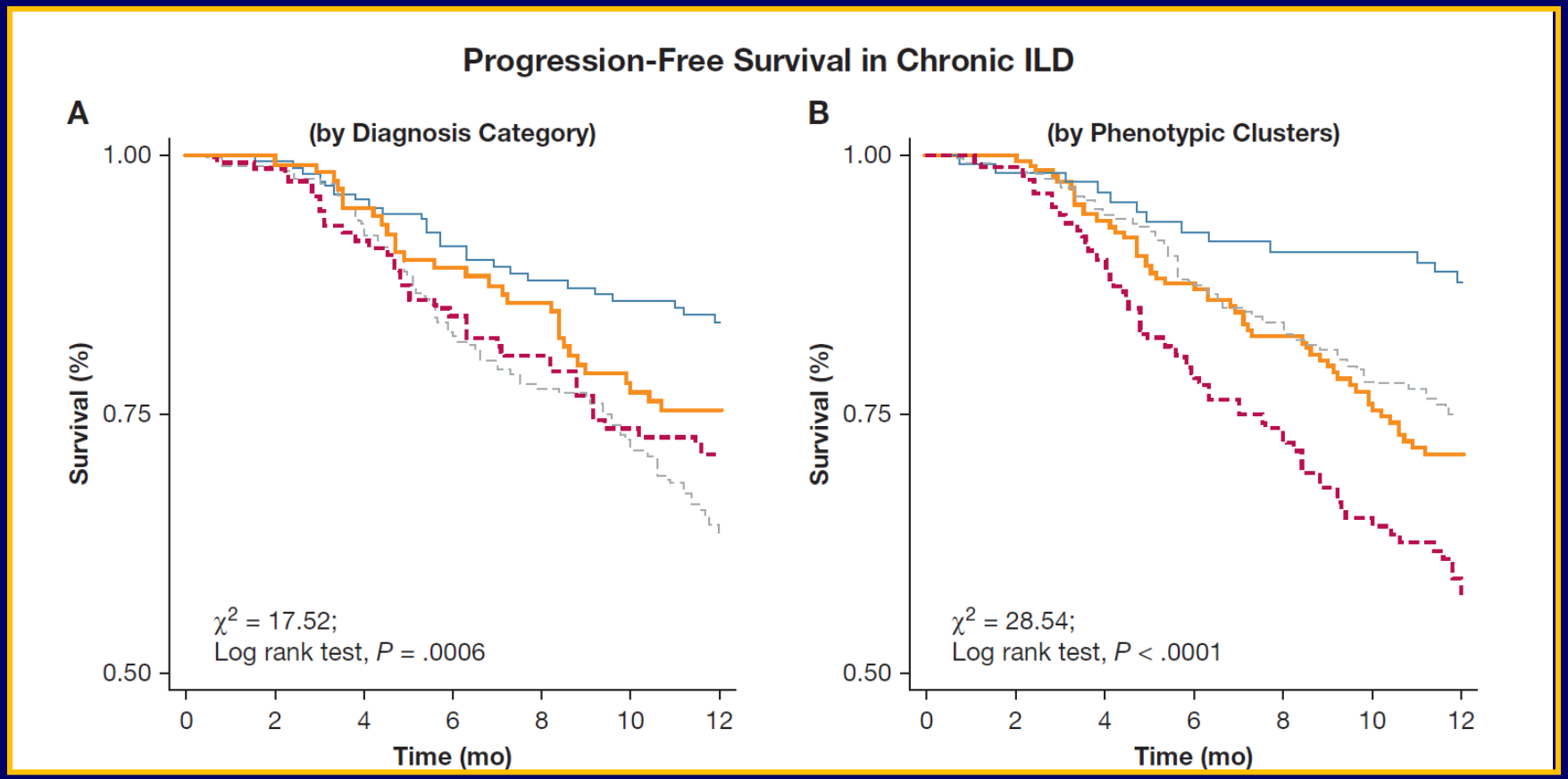
Adegunsoye A et al CHEST2018

Phenotypic Clusters Predict Outcomes in a Longitudinal Interstitial Lung Disease Cohort



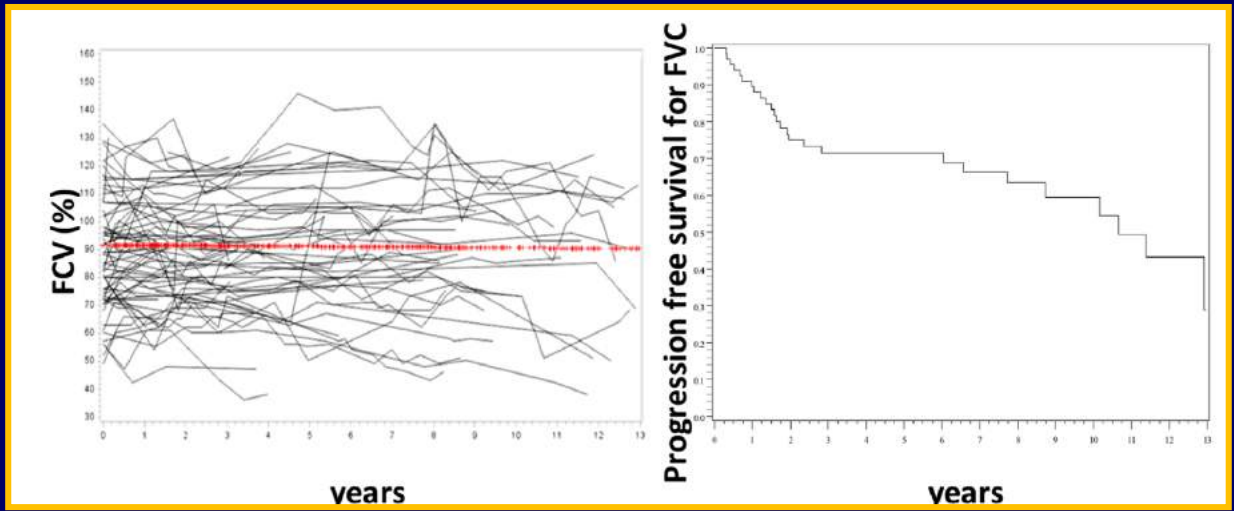
Adegunsoye A. et al CHEST2018

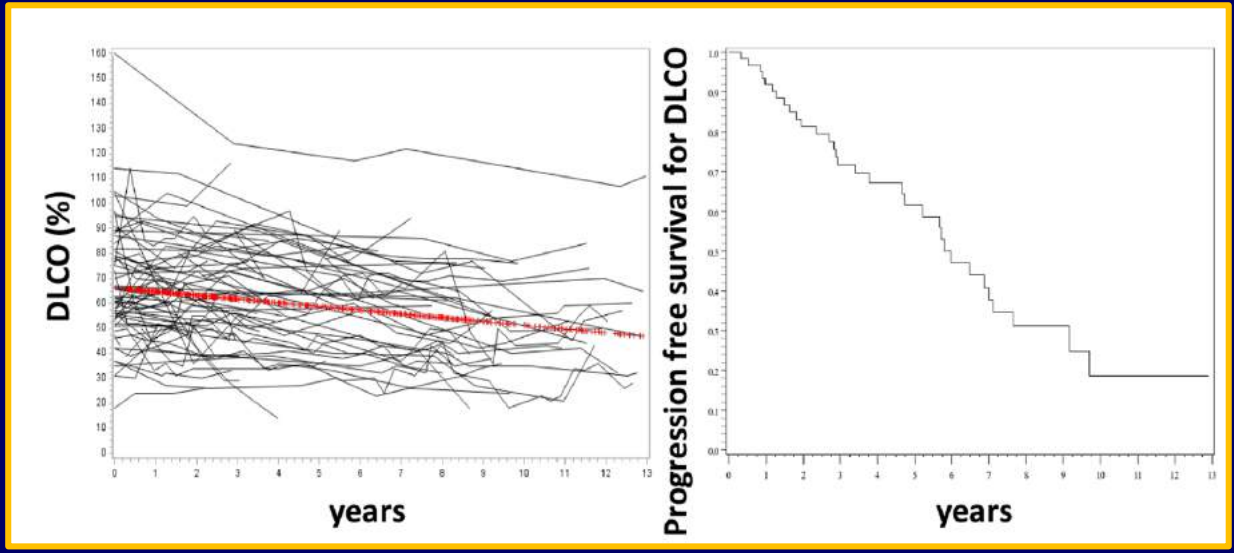
Phenotypic Clusters Predict Outcomes in a Longitudinal Interstitial Lung Disease Cohort



Adegunsoye A. et al CHEST2018

Predictors of lung function test severity and outcome in systemic sclerosis-associated interstitial lung disease





Le Gouellec N et al
PLoS ONE 2017

Predictors of lung function test severity and outcome in systemic sclerosis-associated interstitial lung disease

Conclusion

"In this SSc-ILD population, FVC was therefore stable while DLCO significantly declined over time."

Le Gouellec N et al, PLoS ONE 2017

Clinical Applications for DLCO

- COPD
- Pulmonary Hypertension
- Cardiovascular diseases
- Interstitial lung disease
- Pulmonary Toxicity

Relevant areas for pulmonary toxicity

- Bone marrow transplant
- Rheumatology
- Cardiac treatment

Single breath diffusion capacity for carbon monoxide, DLco in drug induced toxicity

Any cancer therapy should be followed by serial lung function measurements

What about pneumotoxic effects ?

Cancer prevention 1

Cardiotoxic effects of anthracycline-based therapy: what is the evidence and what are the potential harms?

Bennett E Levis, Phillip F Binkley, Charles L Shapiro

Lancet Oncol 2017; 18: e445–56

DLco Measurements Summary

- simple and rapidly to perform
- reliable
- reproducible

DLco add important informations for the diagnosis and prediction of outcomes of various pulmonary and extrapulmonary diseases

Discussion

Hands-on & QA

Georg Harnoncourt

CEO

Christian Buess Ph.D

CTO (Chief Technology Officer)

Philippe Schlink

Director Global Product Management

Definitions

- DLCO – The diffusing capacity for carbon monoxide (DLCO) is also known as the transfer factor for carbon monoxide or TLCO. It is a measure of the conductance of gas transfer from inspired gas to the red blood cells.
- VA – The alveolar volume (VA) can be considered the number of contributing alveolar units and is measured during the single breath DLCO by use of a tracer gas (eg, helium).
- KCO – The carbon monoxide transfer coefficient (KCO is approximately kCO/barometric pressure in mL/minute/ mmHg/L) is often written as DLCO/VA. It is an index of the efficiency of alveolar transfer of carbon monoxide.

Handout

- Agenda of Workshop
- Power Point
- DLCO Standard 2017 (and older Version) Publications (COPD → Poster COPDGene, Heart Failure – main pub,
- Prof. Magnussen will send:
- Hyperthension Fibrosis, Rheumatology, Toxicology)
- Printout Report sample (Spiro + DLCO combined)

ndd Medical Technologies

Zurich, Switzerland

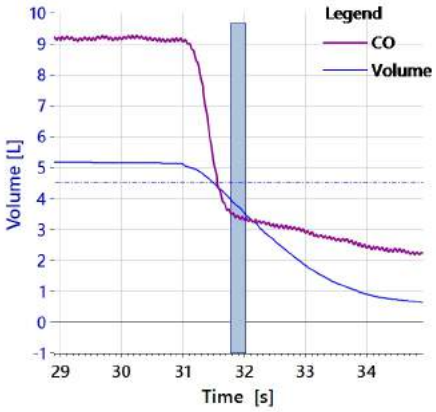
Smith, Peter ID: PSM-11213 Age: 40 (08.11.1968)

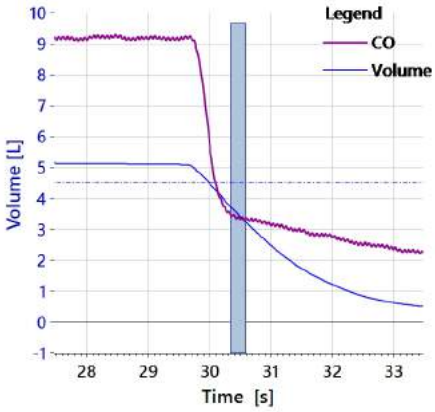
Gender	Male	Height	182 cm	COPD	--
Ethnicity	Caucasian	Weight	80 kg BMI 24.2		
Referred by	Test	Ordered by	Test Test		
Smoker	FORMER				
Comment	SpO2 test HR test				

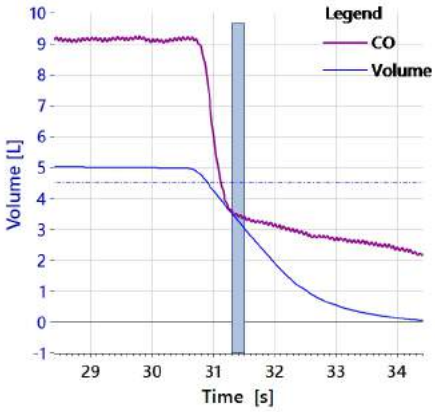
DLCO

Test Date	21.04.2009 18:20:30	Interpretation	--	Value Selection	Best Value
Post Time		Predicted	Stanojevic (GLI), 2017	BTPS (IN/EX)	1,10/1,02
				User ID	

Parameter	Pred	LLN	Result	Trial 3	Trial 1	Trial 2	%Pred
DLCO [ml/min/mmHg]	32,6	25,3	39,1	40,0	39,1	38,2	120
DLadj [ml/min/mmHg]	32,6	25,3	39,2	40,1	39,2	38,2	120
VA sb [L]	7,07	5,77	6,30	6,37	6,29	6,24	89
DLCO/VA (KCO) [ml/min/mmHg/L]	4,64	3,64	6,21	6,28	6,22	6,12	134
TLC sb [L]	7,22	5,92	6,45	6,52	6,44	6,39	89
VI [L]	-	-	5,22	5,27	5,25	5,13	-
BHT [s]	-	-	-	10,2	10,3	10,1	-
Session Quality	A (DLCO Var=0,88ml/min/mmHg (2,2%))						







8005 28.06.2018

Test data updated by: 8005 (14.11.2017)
- Predicted 'Nhanes III'->'Quanjer 2012'
- DLCO Predicted recalculated 'Nhanes'

Test data updated by: 8005 (14.11.2017)
- Predicted recalculated 'Quanjer 2012'
- DLCO Predicted recalculated 'Nhanes'

Test data updated by: 8005 (18.05.2018)
- Smoker 'No'->'Former'
- Asthma 'Yes'->'None'
- COPD 'No'->'None'
- Predicted recalculated 'Quanjer 2012'
- DLCO Predicted recalculated 'Nhanes'

Test data updated by: 8005 (28.06.2018)
- Predicted recalculated 'Quanjer 2012'
- DLCO Predicted 'Nhanes'->'GLI 2017'

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

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

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

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

@ERSpublications
Updated technical standards for measuring *D*LCO (*T*LCO) including the use of rapid gas analyser systems http://ow.ly/QUhv304PMsy

Cite this article as: Graham BL, Brusasco V, Burgos F, *et al*. 2017 ERS/ATS standards for single-breath carbon monoxide uptake in the lung. *Eur Respir J* 2017; 49: 1600016 [https://doi.org/10.1183/13993003.00016-2016].

ABSTRACT This document provides an update to the European Respiratory Society (ERS)/American Thoracic Society (ATS) technical standards for single-breath carbon monoxide uptake in the lung that was last updated in 2005. Although both *D*LCO (diffusing capacity) and *T*LCO (transfer factor) are valid terms to describe the uptake of carbon monoxide in the lung, the term *D*LCO is used in this document. A joint taskforce appointed by the ERS and ATS reviewed the recent literature on the measurement of *D*LCO and surveyed the current technical capabilities of instrumentation being manufactured around the world. The recommendations in this document represent the consensus of the taskforce members in regard to the evidence available for various aspects of *D*LCO measurement. Furthermore, it reflects the expert opinion of the taskforce members on areas in which peer-reviewed evidence was either not available or was incomplete. The major changes in these technical standards relate to *D*LCO measurement with systems using rapidly responding gas analysers for carbon monoxide and the tracer gas, which are now the most common type of *D*LCO instrumentation being manufactured. Technical improvements and the increased capability afforded by these new systems permit enhanced measurement of *D*LCO and the opportunity to include other optional measures of lung function.

This article has supplementary material available from erj.ersjournals.com

Received: Jan 04 2016 | Accepted after revision: July 24 2016

This report was approved by the ATS Board of Directors in August 2016, and endorsed by the ERS Science Council and Executive Committee in September 2016. An executive summary of these standards is available as https://doi.org/10.1183/13993003.E0016-2016.

Support statement: This report was supported by the American Thoracic Society (grant: FY2015) and the European Respiratory Society (grant: TF-2014-19). Funding information for this article has been deposited with the Open Funder Registry.

Conflict of interest: None declared.

Background

It has been over 100 years since Marie Krogh developed a method to measure the single-breath uptake of carbon monoxide in the lungs [1]. Her experiment was designed to show that passive diffusion could explain oxygen transfer from the alveolar gas to the pulmonary capillary blood, but the methodology became the basis of the test, now in common use, which is called diffusing capacity in North America but is more appropriately called transfer factor in Europe. The abbreviation for transfer factor or diffusing capacity of the lung for carbon monoxide used in this document is D_{LCO}, although T_{LCO} is an equally valid term.

A standardised clinical method of determining the diffusing capacity of the lung for carbon monoxide was described by Ogilvie *et al.* [2] in 1957 using a tracer gas to determine both the alveolar volume and the alveolar concentration of carbon monoxide at the beginning of breath-holding. This method used the collection of discrete exhaled gas samples from which gas concentrations were measured using gas analysers that took up to several minutes to perform the measurements. In the remainder of this document we will term these "classical" systems and calculations. The instrumentation for D_{LCO} measurement has advanced considerably since then, primarily through the use of rapidly responding gas analyser (RGA) systems with gas analysers that have a 0–90% response time of ⩽150 ms. While RGAs are capable of real-time, continuous gas analysis, most modern systems generally use this advanced instrumentation only to simulate the classical collection of discrete samples of gas in a bag and discard most of the sampled gas data. However, as discussed later, there are several aspects of D_{LCO} measurement that can be improved markedly using all of the data provided by this continuous measurement technology.

This document and the accompanying executive summary document [3] are an update of the 2005 American Thoracic Society (ATS) and European Respiratory Society (ERS) standards [4] which, in turn, built upon previous standards [5, 6]. This update reflects the consensus opinions of both of these societies and is designed to: 1) provide an update to the standards required for D_{LCO} systems based on RGA systems; and 2) provide new calculation standards that incorporate continuous gas analysis of the entire exhaled sample. It is recognised that classical equipment will remain in use for some time. However, some previously designed D_{LCO} systems can be upgraded and re-engineered to meet these new RGA system standards. It is expected that as new D_{LCO} systems are designed and built, they will meet and, in many cases, exceed these new standards. This document is meant to function as a stand-alone work but, for certain issues, reference will be made to previous statements. The following recommendations will be restricted to the single-breath technique of measuring the uptake of carbon monoxide in the lung, since this is the most common methodology in use around the world.

Methods

An application was submitted for a joint European Respiratory Society (ERS) and American Thoracic Society (ATS) task force to update the 2005 D_{LCO} standards [4] with a particular view to systems using RGAs. The task force co-chairs were approved by the ERS and the ATS. Task force members were scientists and physicians with experience in international guidelines, clinical experience of routine lung function testing and specialist knowledge of gas transfer including research publications. Potential conflicts of interest were disclosed and vetted. The task force consisted of five members of the task force for the 2005 D_{LCO} standards and four new members. A search using PubMed for literature published between 2000 and 2015 containing various terms related to diffusing capacity and transfer factor yielded 3637 citations. Task force members reviewed the abstracts and identified 113 as relevant to the project and a further 99 as potentially relevant. All manufacturers of pulmonary function equipment to measure D_{LCO} were sent a survey requesting equipment specifications. Eight of 13 manufacturers responded. A survey of D_{LCO} equipment specifications published on the manufacturers' websites was also conducted. Using the 2005 standards as a base document, revisions and additions were made on a consensus basis. The recommendations in this document represent the consensus of task force members in regard to the evidence available for various aspects of D_{LCO} measurement (as cited in the document). Furthermore, it reflects the expert opinion of the task force members in areas in which peer-reviewed evidence was either not available or incomplete. The task force also identified areas and directions for future research and development where evidence is lacking.

Determinants of carbon monoxide uptake

The volume of carbon monoxide in the alveolar space is the product of the alveolar volume (V_A) and the alveolar carbon monoxide fraction (F_{ACO}; *i.e.* the fractional concentration of carbon monoxide in the alveolar space). Thus, at a constant volume, the transfer of carbon monoxide from the lungs into the blood is $V_A \cdot \Delta F_{ACO}/\Delta t$. Furthermore, in the absence of any carbon monoxide back-pressure in the blood, the transfer of carbon monoxide is equal to the product of the alveolar carbon monoxide tension (P_{ACO}; *i.e.* the partial pressure of carbon monoxide) and the D_{LCO}, which is the conductance of carbon monoxide from the inspired test gas in the alveolar space to binding with haemoglobin (Hb) in the blood

(*i.e.* flow = pressure × conductance). The combination of these two formulae gives equation 1, which can be manipulated to give equation 2 for the calculation of D_{LCO}.

$$V_A \cdot \Delta F_{ACO}/\Delta t = P_{ACO} \cdot D_{LCO} \quad (1)$$

$$D_{LCO} = V_A \cdot \Delta F_{ACO}/\Delta t/P_{ACO} \quad (2)$$

The ERS recommends expressing D_{LCO} in SI units ($mmol \cdot min^{-1} \cdot kPa^{-1}$) while the ATS prefers traditional units ($mL \cdot min^{-1} \cdot mmHg^{-1}$) under standard temperature, pressure and dry conditions (STPD). Values in SI units can be multiplied by 2.987 to obtain values in traditional units.

The capacity of the lung to exchange gas across the alveolar–capillary interface is determined by its structural and functional properties [1, 7–25]. The structural properties include the following: lung gas volume; the path length for diffusion in the gas phase; the thickness and area of the alveolar capillary membrane; any effects of airway closure; and the volume of Hb in capillaries supplying ventilated alveoli. The functional properties include the following: absolute levels of ventilation and perfusion; the uniformity of the distribution of ventilation relative to the distribution of perfusion; the composition of the alveolar gas; the diffusion characteristics of the membrane; the concentration and binding properties of Hb in the alveolar capillaries; and the carbon monoxide and oxygen tensions in the alveolar capillaries in that part of the pulmonary vascular bed which exchanges gas with the alveoli.

The process of carbon monoxide transfer from the environment to the pulmonary capillary blood includes six steps, as follows: 1) bulk-flow delivery of carbon monoxide to the airways and alveolar spaces; 2) mixing and diffusion of carbon monoxide in the alveolar ducts, air sacs and alveoli; 3) transfer of carbon monoxide across the gaseous to liquid interface of the alveolar membrane; 4) mixing and diffusion of carbon monoxide in the lung parenchyma and alveolar capillary plasma; 5) diffusion across the red-cell membrane and within the interior of the red blood cell; 6) chemical reaction with constituents of blood Hb [13–19].

The process of carbon monoxide uptake can be simplified into two transfer or conductance properties: 1) membrane conductivity (D_M), which reflects the diffusion properties of the alveolar capillary membrane; and 2) binding of carbon monoxide and Hb. The latter can be represented as the product of the carbon monoxide–Hb chemical reaction rate (θ) and the volume of alveolar capillary blood (V_C). Since these conductances are in series [17], these properties are related as shown in equation 3.

$$1/D_{LCO} = 1/D_M + 1/\theta V_C \quad (3)$$

A number of physiological changes can affect D_M or θV_C to influence D_{LCO}. For example, as the lung inflates D_M increases (largely due to increasing alveolar surface area), while V_C effects are variable (due to differential stretching and flattening of alveolar and extra-alveolar capillaries) [13, 20–27]. The net effect of these changes is that D_{LCO} tends to increase as the lung inflates but the change in D_{LCO} is proportionally less than the change in V_A [22]. Exercise, the supine position and Müller manoeuvres (inspiratory efforts against a closed glottis) can all recruit and dilate alveolar capillaries, thereby increasing V_C and D_{LCO} [28–34]. Alveolar–capillary recruitment also occurs in the remaining lung tissue following surgical resection, since the cardiac output now flows through a smaller capillary network. This causes a less than expected loss of V_C for the amount of lung tissue removed. In contrast, Valsalva manoeuvres (expiratory efforts against a closed glottis) can reduce V_C and thereby reduce D_{LCO} [32].

The measurement of carbon monoxide uptake is also affected by the distribution of ventilation with respect to D_M or θV_C (*i.e.* carbon monoxide uptake can only be measured in lung units into which carbon monoxide was inspired and subsequently expired) [18, 19, 35, 36]. This is particularly important in diseases such as emphysema, where the inhaled carbon monoxide may preferentially go to the better-ventilated regions of the lung and the subsequently measured carbon monoxide uptake will be determined primarily by the uptake properties of these regions. Under these conditions, the tracer gas dilution used to calculate V_A will also reflect primarily regional dilution and underestimate the lung volume as a whole. The resulting calculated D_{LCO} value should thus be considered as primarily reflecting the gas-exchange properties of the better ventilated regions of the lung.

In addition to these physiological and distributional effects on D_{LCO}, a number of pathological states can affect D_M, θV_C, or both and thereby affect D_{LCO} [8, 9, 37–46]. Measurement of D_{LCO} is used when any of these pathological processes are suspected or need to be ruled out. Moreover, measuring changes in D_{LCO} over time in these processes is a useful way of following the course of the disease.

Gas analysers and general equipment

System design

Descriptions of the apparatus and general instructions for performing the single-breath diffusing capacity manoeuvre are available elsewhere [2, 6, 47–50]. Equipment in clinical use varies widely in complexity but the basic principles are the same. All systems have a source of test gas, a method of measuring inspired and expired volume over time and a method of measuring carbon monoxide and tracer gas concentration. Classical discrete-sample gas-analyser *D*LCO systems usually display only volume over time but RGA *D*LCO systems also provide a continuous recording of carbon monoxide and tracer gas concentration during the entire test manoeuvre (figure 1).

Equipment requirements

The performance standards for *D*LCO equipment are summarised in table 1.

Flow and volume analysers

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

Gas analysers

For classical discrete sample calculations of *D*LCO, only the ratios of alveolar to inhaled carbon monoxide and tracer gas concentrations are needed. Thus, the analysers must primarily be able to produce an output for measured exhaled carbon monoxide and tracer gas that is a linear extrapolation between the inhaled (test gas) concentrations and zero (no carbon monoxide or tracer gas present in the analysers) [51, 52]. The measurement of carbon monoxide and tracer gas concentrations is also a static measurement when considering a classical discrete sample calculation of *D*LCO. Analyser response time is not an issue and the time of gas sample collection is measured separately. Gas concentration digital signal conditioning is not required to compensate for the response time when calculating *D*LCO using static measurements.

When nondispersive, infrared carbon monoxide RGAs began to be used to construct a virtual gas sample from flow and gas concentration data, rather than collecting a physical sample of exhaled gas, no specifications were mandated other than for the linearity of the gas analysers [5]. However, with RGAs there is both a lag time (due to the travel of the sampled gas through the sampling tube to the analyser chamber) and an analyser response time (the time to reach 90% of the actual measurement from the time the gas sample reaches the analyser) to be considered. As such, the gas concentration signal must be precisely shifted in time to align with the flow signal (figure 2).

RGA response time

The response time of the RGA will determine how accurately the analyser is able to track the true carbon monoxide and tracer gas concentrations. The most rapid changes in concentration occur at the start of test

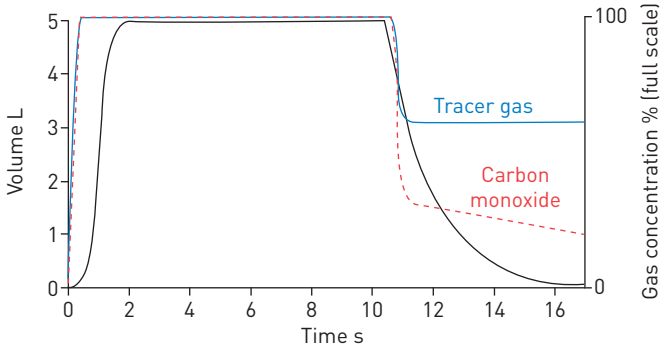


FIGURE 1 Diagram of lung volume and gas concentration during the single-breath manoeuvre to measure the uptake of carbon monoxide. Whereas classical *D*LCO systems only display the volume–time graph, rapid gas analyser (RGA) *D*LCO systems also display the carbon monoxide and tracer gas concentrations throughout the single-breath manoeuvre. Reproduced from [4].

TABLE 1 Equipment specifications and performance standards

D_{LCO} System	Specification	
	Required	Recommended
Rapid gas analyser systems		
Analyser specification		
0–90% response time (see figure 2)	⩽150 ms	
Maximum nonlinearity	±1% of full scale	
Accuracy	Within ±1% of full scale	
Interference from 5% carbon dioxide or 5% water vapour	⩽10 ppm error in [CO]	
Drift for carbon monoxide	⩽10 ppm over 30 s	
Drift for tracer gas	⩽0.5% of full scale over 30 s	
Flow accuracy	Within ±2% over the range of −10 to +10 $L \cdot s^{-1}$	
Volume accuracy (3-L syringe check)	Within ±75 mL	
Barometric pressure sensor accuracy	Within ±2.5%	
Ability to perform a QA check (3-L syringe; ATP mode; inhaling ~2 L test gas)	Calculate total volume (V_A) of 3±0.3 L and D_{LCO} of <0.5 $mL \cdot min^{-1} \cdot mmHg^{-1}$ or <0.166 $mmol \cdot min^{-1} \cdot kPa^{-1}$	
Sample and store data with adequate resolution	Digitise at ⩾100 Hz per channel with ⩾14 bit resolution	Digitise at 1000 Hz
Monitor and report end-expiratory tracer gas and carbon monoxide concentrations (alert operator if washout is incomplete)	Implemented#	
Compensate for end-expiratory gas concentrations prior to test gas inhalation in the calculation of V_A and D_{LCO}	Implemented#	
Ensure proper alignment of gas concentration signals and the flow signal	Implemented#	
Measure anatomic dead-space using the Fowler method (see figure 6)	Implemented#	
Display a graph of gas concentration *versus* expired volume to confirm the point of dead-space washout and report the amount of manual adjustment if done (see figure 4)	Implemented#	
Measure V_A using all of the tracer gas data from the entire manoeuvre in the mass balance equation	Implemented#	
Report the D_{LCO} adjusted for the change in P_{AO_2} due to barometric pressure	Implemented#	
Ability to input simulated digital test data and compute D_{LCO}, V_A, TLC, V_D		Calculate values within 2% of actual values
Report the D_{LCO} adjusted for the change in P_{AO_2} due to P_{ACO_2}, if the carbon dioxide concentration signal is available		Implemented#
Classical discrete sample systems		
Analyser specification		
Maximum nonlinearity	±1% of full scale	
Accuracy	Within ±1% of full scale	
Interference from 5% carbon dioxide or 5% water vapour	⩽10 ppm error in [CO]	
Drift for carbon monoxide	⩽10 ppm over 30 s	
Drift for tracer gas	⩽0.5% of full scale over 30 s	
Flow accuracy	Within ±2% over the range of −10 to +10 $L \cdot s^{-1}$	
Volume accuracy (3-L syringe check)	Within ±75 mL	
Ability to perform a QA check (3-L syringe; ATP mode; inhaling ~2 L test gas)	Calculate total volume (V_A) of 3±0.3 L and D_{LCO} of <0.5 $mL \cdot min^{-1} \cdot mmHg^{-1}$ or <0.166 $mmol \cdot min^{-1} \cdot kPa^{-1}$	

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

gas inhalation and at the start of exhalation following the breath-hold. Even after the application of an appropriate time shift (see below) to correct for lag time and analyser response time, there will be a residual error in D_{LCO} due to the finite response time. For every 100 ms increase in the 0–90% response

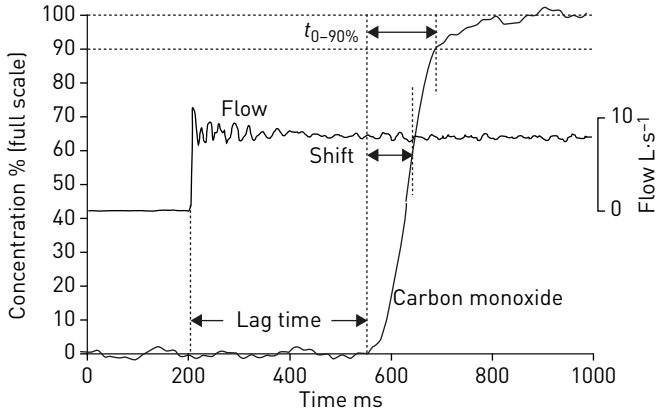


FIGURE 2 Lag and response times for carbon monoxide: the response time of the analyser was estimated by rapidly switching the gas being sampled from zero to full-scale carbon monoxide. The change in the flow signal shows the time at which the switch was made from medical air to test gas. The lag time, the 0–90% response time and the optimal shift are calculated from the resulting response curve.

time, the error in *D*LCO increases by about 0.7% [53]. Based on the above considerations, the 0–90% response time for RGAs used in *D*LCO systems must be ⩽150 ms.

Response time can be improved by reducing the volume of the analyser chamber and increasing the sample aspiration rate: however, such measures can cause a deterioration of the signal by creating more noise. The use of signal conditioning to simulate a more rapid analyser response may also introduce more noise and errors into the signal. Digital conditioning techniques should only be used to digitally enhance response time if they do not compromise signal quality and accuracy and serve to preserve or improve *D*LCO measurement accuracy.

Linearity and accuracy

The linearity of gas concentration signals is of primary importance in measuring *D*LCO since the ratios of the gas concentrations are considered in the classical calculations [50, 52]. The error in *D*LCO measurement due to nonlinearity in these signals depends on the size of the lungs and the rate of uptake of carbon monoxide. A nonlinearity of 0.5% of full scale can cause errors ranging from 0.5% in a subject with a *D*LCO of 13.4 mmol·min^{-1}·kPa^{-1} (40 mL·min^{-1}·mmHg^{-1}) to 1.7% in a subject with a *D*LCO of 3.35 mmol·min^{-1}·kPa^{-1} (10 mL·min^{-1}·mmHg^{-1}) [53]. The manufacturer specification for analyser linearity is that any nonlinearity must not exceed 0.5% of full scale once zero and full scale values have been set. The accuracy of the gas analyser signal also becomes important when measuring the residual background alveolar carbon monoxide concentration and the washout of tracer gas from the previous *D*LCO manoeuvre. The output of the gas analyser must be accurate to within ±1% of full scale.

Interference and noise

Nondispersive, infrared carbon monoxide analysers typically have some cross sensitivity to carbon dioxide and water vapour. Strategies to reduce and/or compensate for cross sensitivity are required such that the water vapour and carbon dioxide in exhaled gas (up to 5% each; *i.e.* water vapour pressure (P_{H_2O}) 6.28 kPa/47 mmHg) contribute a less than 10 ppm error in the measured carbon monoxide signal. Measuring the exhaled gas from the subject prior to the inhalation of test gas can also provide an offset due to carbon dioxide and water vapour measurements that can be used to adjust the concentration signal.

Drift

Gas analysers should have only minimal drift in zero and gain, such that output is stable over the test interval. Gas analyser drift must be ⩽10 ppm over 30 s for carbon monoxide and ⩽0.5% of full scale over 30 s for tracer gas. It is recommended that manufacturers provide an optional test mode to display the measured gas concentrations so that stability can be confirmed. Any drift must be determined by comparing the carbon monoxide and tracer values measured in room air immediately prior to and immediately following the single-breath manoeuvre. The gas concentration signals used in the calculation of *D*LCO must be compensated for drift, assuming a linear change over the measurement interval.

Aspiration flow

Depending upon the design of the breathing circuit, the gas analyser sampling port and the gas analyser aspiration flow, gas may be entrained into the sampling line from room air or from the test gas when the

exhaled flow decreases to near zero at the end of exhalation. Clearly, when the subject's exhaled flow drops below the aspiration flow, the sample will entrain other gas that is not part of the exhaled gas. D_{LCO} instrument manufacturers are required to determine the lowest exhaled flow at which the gas sampling line will not entrain gas other than exhaled gas. This flow must be reported in the system specifications. In the analysis of the exhaled gas concentration data, measurements of gas concentration below the specified flow must not be included in either the determination of washout of tracer gas from a previous manoeuvre (see the section on interval between manoeuvres below) or the calculation of absolute end-expiratory lung volume (V_{ee}) in equations 22 and 25 below.

Digitisation

In order for the digitised signal to accurately track the gas concentration signal and in order to provide adequate opportunity for signal processing for data alignment, the minimum signal sampling rate must be ⩾100 Hz per channel: however, a rate of 1000 Hz is recommended. The analogue to digital converter resolution must be ⩾14 bits.

Other equipment considerations

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

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

The system must be leak free; this is particularly important for D_{LCO} systems that aspirate gas samples through the gas analyser at sub-atmospheric pressures. When samples are aspirated, leaks in tubing, fittings and other locations allow room air to be drawn into the gas circuit, thus diluting the sample and reducing the concentrations of carbon monoxide and tracer gases.

Equipment calibration and quality control

The considerations for equipment calibration and quality control are illustrated in table 2. There are a number of regular procedures to apply, summarised as follows:

1) Flow and gas analysers must be zeroed prior to each manoeuvre. After each manoeuvre, a new zeroing procedure must be carried out to account for analyser drift during the previous test.

2) Each day, prior to testing, there must be a volume calibration check with a 3-L syringe [54]. The syringe should be discharged at least three times to give a range of flow rates varying between 0.5 and 12 $L \cdot s^{-1}$ (with 3-L injection times of ~6 s and ~0.5 s, respectively). The volume at each flow rate must meet an accuracy requirement of ⩽2.5% error. For devices using disposable flow sensors, a new sensor from the supply used for patient tests must be tested each day. The calibration check may need to be repeated during the day if ambient conditions change. Newer systems monitor ambient conditions and make adjustments as necessary or produce a calibration alert when needed. Older systems may require a calibration check if room temperature changes by more than 3 °C or relative humidity changes by more than 15% (absolute). Operators should also perform a calibration check whenever they notice significant

TABLE 2 Equipment calibration schedule

Calibration technique	Frequency
Flow analyser zeroing	Before each test
Gas analyser zeroing	Before/after each test
Volume calibration check	Daily
Biologic control	Weekly
Calibration syringe D_{LCO} check	Weekly
Calibration syringe leak test	Monthly
Linearity check (calibration syringe or simulator)	Monthly

D_{LCO}: diffusing capacity of the lung for carbon monoxide.

discrepancies between the inspired volume (V_I) and VC, or between V_A and total lung capacity (TLC), which might suggest volume calibration problems.

3) Each week, or whenever problems are suspected, the following procedures must be followed. First, for those D_{LCO} systems using a volume-type spirometer, a spirometer leak test should be performed according to the manufacturer's specifications. Secondly, a D_{LCO} test should be performed with a calibrated 3-L syringe by attaching the syringe to the instrument in the normal patient test mode. The syringe should then be emptied, filled with 3 L of test gas and emptied into the mouthpiece after the 10 s breath-hold. The calculation of V_A must be within 300 mL of 3 L times the STPD to BTPS (body temperature, ambient pressure, saturated with water vapour conditions) correction factor, which is $863/(P_B-47)$, where P_B is the barometric pressure. It should be noted that a 3-L calibration syringe will have an additional dead-space which, depending on the connection to the mouthpiece, is typically ~50 mL and must be considered in the V_A calculation. The absolute value of the calculated D_{LCO} must be <0.166 mmol·min^{-1}·kPa^{-1} or <0.5 mL·min^{-1}·mmHg^{-1}. Thirdly, a test should be performed on a "standard subject" (biological control) or simulator [55]. Standard subjects are nonsmokers who have been found to have a consistently repeatable D_{LCO} (*e.g.* healthy laboratory personnel). If the D_{LCO} in a standard subject varies either by >12% or by >1 mmol·min^{-1}·kPa^{-1} (>3 mL·min^{-1}·mmHg^{-1}) from the mean of previous values, the test must be repeated. A study of the long-term intersession variability of D_{LCO} has found that biological control deviations either >12% or >3 mL·min^{-1}·mmHg^{-1} from the average of the first six tests indicate that the instrument is not within quality control limits and must be carefully evaluated before further patient testing [56]. For a digital system check of the D_{LCO} calculation algorithm, standardised digital data for flow, volume and carbon monoxide and tracer gas concentration will be developed by the task force and made available with a sample rate of 1 kHz as an xml or csv file. It is strongly recommended that manufacturers provide the ability to input data from such a file and generate test results to compare measured *versus* known D_{LCO} and V_A values. For systems failing the above testing, the D_{LCO} system must be evaluated carefully for the possibility of leaks, nonlinear analyser function, and volume and time inaccuracy, *etc.* When sufficient data on a standard individual have been obtained, laboratories should establish their own outlier criteria to serve as indicators of potential problems with their D_{LCO} systems. Manufacturers are encouraged to develop automated quality-control software to assist and enhance the utility of these steps.

4) Each month a leak test of the 3-L calibration syringe should be performed. If the calibration syringe does not have a volume scale on the shaft, mark 50 mL below full by measuring the excursion of the shaft from 0 to 3 L and marking it at a distance that is 0.017 of the full excursion. Fill the syringe and place a stopper at the syringe input. Push the syringe in to the 50 mL mark (which generates a pressure of about 17 cmH_2O), hold for 10 s and release. If the syringe does not return to within 10 mL of the full position, it should be sent for repair. The procedure is then repeated starting with the syringe at 50 mL below full, applying the stopper and pulling the syringe to the full position.

5) Each month, gas-analyser linearity should be assessed. A straightforward approach is to measure known serial dilutions of the test gas [57], or to measure the concentration of a separate high-precision test gas having a certificate of analysis. Manufacturers must be encouraged to automate this function. For systems with independent measurement of carbon monoxide and tracer gas, the analyser linearity may also be assessed by comparing the ratio of carbon monoxide and tracer gas concentration to arbitrary dilutions of test gas with room air. A third type of calibration syringe test, which differs from the volume check in point two and the D_{LCO} check in point three by using the 3-L syringe in ambient temperature, pressure and humidity (ATP) mode, may also reveal problems with analyser linearity. With approximately 1 L of air in the syringe, the test begins by filling the remaining volume with test gas. Following a 10 s "breath-hold" the syringe is then emptied. The calculation of V_A must be within 300 mL of 3 L with the syringe dead-space being used for the anatomic dead-space in the V_A calculation. The absolute value of D_{LCO} must be <0.166 mmol·min^{-1}·kPa^{-1} or <0.5 mL·min^{-1}·mmHg^{-1}. A review of quality control data for four different D_{LCO} systems between 2006 and 2015 using this procedure found only four outlier points where $|D_{LCO}|$ was >0.13 mmol·min^{-1}·kPa^{-1} (>0.4 mL·min^{-1}·mmHg^{-1}). The same data showed that V_A was consistently within 3±0.3 L for the four systems (unpublished data from B.R. Thompson). Gas mixing in the syringe can be improved by using low flow rates and extending the breath-hold time. The effects of incomplete mixing in the syringe can be minimised by using a larger sample volume. In the absence of a D_{LCO} simulator and high-precision test gases, system checks must be performed using a 3-L calibrating syringe in ATP mode. Manufacturers must provide this test option, which will be the same as the usual testing procedure for a patient, with the exception that V_A will be reported in ATP rather than BTPS.

6) A record of equipment checks and standard subject tests should be dated and kept in a laboratory log book or digital file folder. Manufacturers are encouraged to provide software and test equipment options for quality control measurement and quality control data management. In addition, manufacturers may provide equipment-specific, quality-control measures in addition to the foregoing points. If water vapour

permeable tubing is used to either remove water vapour or equilibrate water vapour with room air, such tubing must be replaced according to manufacturer recommendations to ensure that it is functioning properly. Chemical gas analyser cells will have a replacement schedule. Manufacturers may also have preventative maintenance schedules for various other system components (*e.g.* balloon valves) which will require testing and replacement as necessary.

Quality control for RGA systems

Modern D_{LCO} systems are completely integrated and do not use stand-alone gas analysers that can be tested separately. Specifications for manufacturers are required to facilitate a uniform testing and calibration strategy across all systems. Quality-control requirements include analogue testing with devices such as a simulator [58], the option to operate in full ATP mode and a digital calibration option to verify the computational algorithms. The digital calibration option should use simulated flow, carbon monoxide concentration and tracer gas concentration data from standardised manoeuvres with a known D_{LCO}.

Infection control

The major goal of infection control is to prevent the transmission of infection to patients and staff during pulmonary function testing. The recommendations in the ATS/ERS documents for spirometry and general considerations for pulmonary function testing also apply to D_{LCO} equipment and procedures [59–61].

Standardisation issues in the single-breath testing technique

The single-breath determination of D_{LCO} involves measuring the uptake of carbon monoxide from the lung over a breath-holding period. To minimise variability as much as possible, the following specifications for the standardisation of testing techniques are provided.

Patient condition

Factors that affect V_C (*e.g.* exercise, body position, Hb affinity for carbon monoxide, alveolar oxygen tension (P_{AO_2}), and level of carboxyhaemoglobin (COHb)) must be standardised. If clinically acceptable, the subject should not breathe supplemental oxygen for $\geqslant$10 min prior to a D_{LCO} manoeuvre. In addition, when using exercise or the supine position to assess the ability of the lung to increase gas transfer [18, 28–31], the level of exercise and/or the duration of the supine position must be noted. Before beginning the test, the manoeuvres must be demonstrated and the subject carefully instructed. Furthermore, the subject must be seated comfortably throughout the test procedure, which must be performed at a stable, comfortable temperature within the manufacturer's equipment specifications.

COHb produces an acute, reversible decrease in D_{LCO} [62–66], largely due to its effects on carbon monoxide back-pressure and the "anaemia effect" from decreased Hb binding sites for test gas carbon monoxide. As cigarette smoking is the most common source of COHb, subjects must be asked to refrain from smoking or other sources of carbon monoxide exposure on the day of the test. The time of the last cigarette smoked must be recorded and noted for the interpretation. A correction for carbon monoxide back-pressure must be made for recent or heavy cigarette smoking (see the section on adjustment for COHb concentration and carbon monoxide back-pressure below). Air pollution may also result in higher COHb levels and exposure to high levels of air pollution should be noted.

Inspiratory manoeuvres

Once the mouthpiece and nose clip are in place, tidal breathing must be carried out for a sufficient time to assure that the subject is comfortable with the mouthpiece and that the nose clips and mouthpiece are used appropriately with no leaks. The D_{LCO} manoeuvre begins with unforced exhalation to residual volume (RV). In obstructive lung disease, where exhalation to RV may require a prolonged period, a reasonable recommendation is that this portion of the manoeuvre must be limited to <12 s. Exhalation times of up to 12 s will allow most patients with airflow obstruction to exhale sufficiently such that they can achieve a maximal VC for the subsequent inhalation of test gas. Submaximal inhalation occurs most frequently in patients with airflow obstruction who are not given adequate time to exhale prior to the inhalation of test gas.

At RV, the subject's mouthpiece is connected to a source of test gas, and the subject inhales rapidly to TLC.

A submaximal inspired volume of test gas (*i.e.* less than the known VC) can affect carbon monoxide uptake depending upon whether it is a result of an initial suboptimal exhalation to RV (manoeuvre performed at TLC) or whether it is due to a suboptimal inhalation from RV (manoeuvre performed below TLC) [22–25]. In the former case, the calculated V_A and D_{LCO} will accurately reflect lung volume and the carbon monoxide uptake properties of the lung at TLC. In the latter case, the V_A will be reduced and D_{LCO} measurement will be affected.

Due to these effects, it is important that the inspired volume of test gas, V_I, be as close to the known VC as possible. Data from a large patient population have shown that the V_I during D_{LCO} measurement averages ~90% of the VC [22]. Since the introduction of the 2005 guidelines and subsequent implementation of quality-control checks by equipment manufacturers, there has been an improvement in test quality such that 90% of the largest known VC as the lower limit of acceptability for V_I has been shown to be attainable [67]. Furthermore, as noted above, V_I will be improved by allowing up to 12 s for exhalation prior to inhalation of test gas. V_I must be at least 90% of the largest VC in the same pulmonary function testing session. However, a manoeuvre may be deemed to be acceptable if V_I is within 85% of the largest VC and the V_A is within 200 mL or 5% (whichever is greater) of the highest V_A among acceptable D_{LCO} manoeuvres.

The inspiration must be rapid, since the D_{LCO} calculations assume instantaneous lung filling [27, 68–74]. Slower lung filling decreases the amount of time the lung is at full inspiration with a consequent reduction in carbon monoxide uptake. Although various sample timing techniques address the issue of lung filling and emptying time, inspiration of test gas should be sufficiently rapid such that that 85% of V_I must be inspired in <4.0 s. If longer inspiratory times are needed to inspire 85% of V_I, this must be noted on the test report.

Breath-hold and expiratory manoeuvres

During the breath-hold, both the Valsalva and Müller manoeuvres (expiratory or inspiratory efforts against a closed glottis, respectively) can affect D_{LCO} calculation by decreasing or increasing thoracic blood volume, respectively, resulting in a corresponding decrease or increase in D_{LCO}, respectively, for each manoeuvre [32, 75, 76]. The intrapulmonary pressure during the breath-hold should thus be near atmospheric and this is best accomplished by having the subject voluntarily maintain full inspiration using only the minimal necessary effort. The breath-hold time must be 10±2 s, a target easily achieved in the vast majority of subjects [77].

As with inspiration, the D_{LCO} calculation assumes instantaneous lung emptying [27, 68–72]. Although various sample timing techniques address the fact that emptying is not instantaneous, it is still reasonable to expect that the expiratory manoeuvre must be smooth, unforced and without hesitation or interruption.

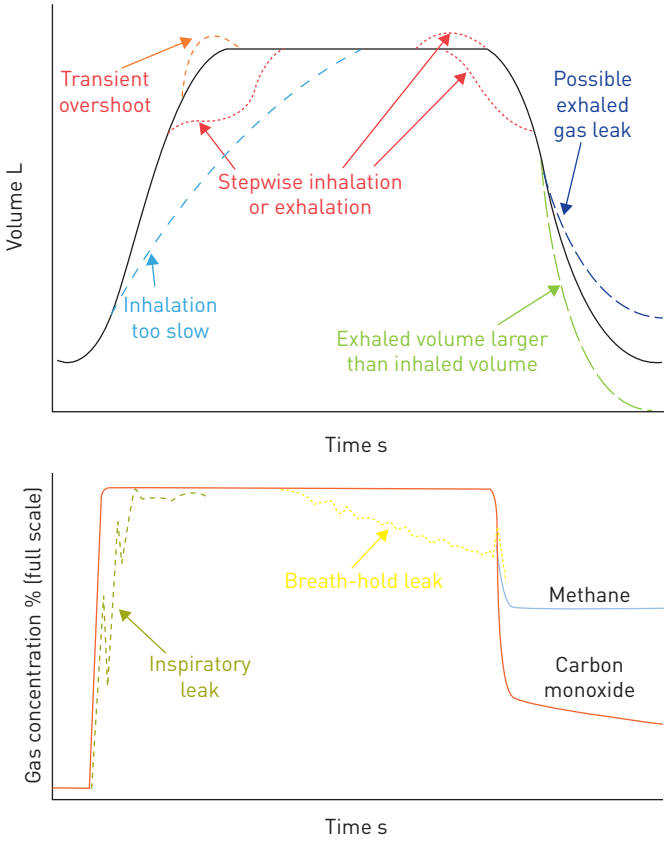


FIGURE 3 Potential problems with the breathing manoeuvre for single-breath diffusing capacity of the lung for carbon monoxide that can lead to measurement errors. Reproduced from [4].

For classical systems, the exhalation time for washout and discrete sample collection should not exceed 4 s. In subjects who require a longer expiratory time to provide an appropriate alveolar gas sample, the expiratory time must be noted in the test report. For RGA systems, exhalation should continue to RV, with a maximum exhalation time of 12 s, which provides improved measurement of V_A as noted in the data analysis for RGA systems section below. The results of common errors that can occur during the inspiration, breath-hold and expiration manoeuvres are illustrated in figure 3.

Washout and sample collection manoeuvres

D_{LCO} calculations (see the calculations section below) are performed by analysis of discrete alveolar gas samples containing carbon monoxide and tracer gas. During expiration, a volume of gas must be expired to clear the total anatomical and equipment dead-space volume (V_D) and then discarded before the alveolar sample is collected (figure 1). Collecting an alveolar gas sample before the point of dead-space washout will underestimate D_{LCO}, while delaying sample collection beyond the point of dead-space washout will overestimate D_{LCO} [68, 72].

Washout and sample collection in classical systems

The washout volume must be 0.75–1.0 L (BTPS). If the patient's VC is <2.00 L, the washout volume may be reduced to 0.50 L. The discrete-sample gas volume (V_S) is the volume of gas collected following the breath-hold and used to analyse alveolar carbon monoxide and tracer gas concentrations. V_S collection time will affect the measurement of breath-hold time (see below). For discrete sample systems that require larger sample volumes, a V_S of 0.5–1 L should be collected for analysis. In patients with a VC <1 L, a V_S <0.5 L may be used if it can be confirmed that the dead-space has been cleared.

Washout and sample collection in RGA systems

The time point for dead-space washout can be determined from the expired tracer gas concentration data using an objective algorithm. The beginning of the alveolar plateau can be located by determining the

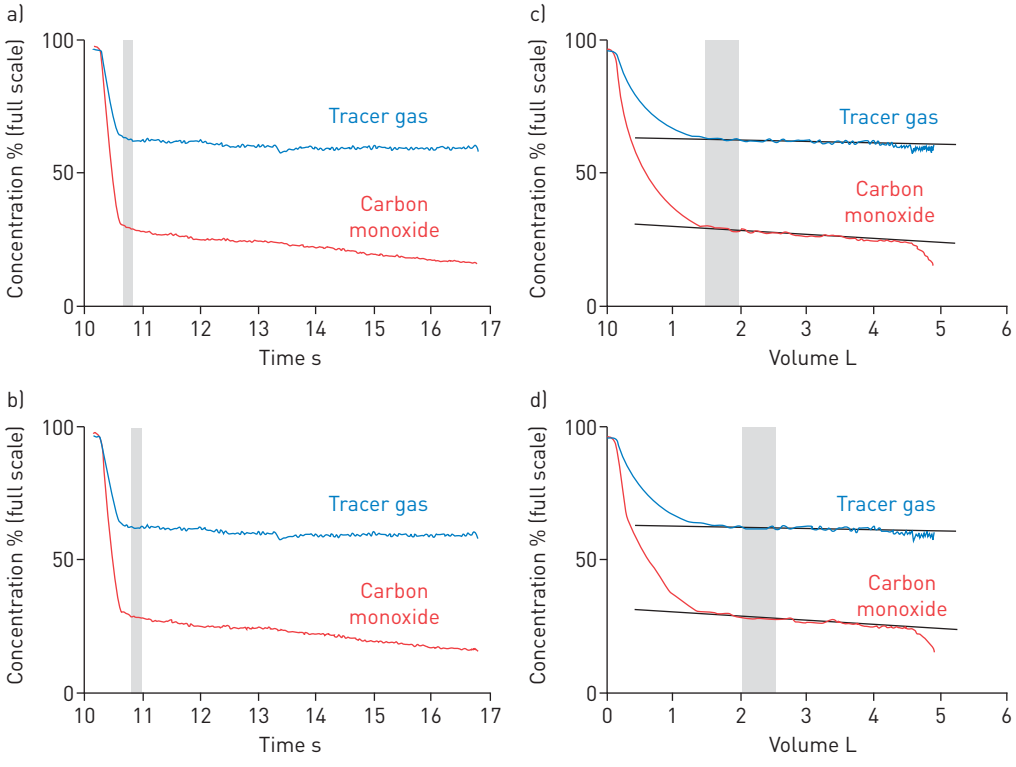


FIGURE 4 Comparison of gas concentration plotted as a function of time (a and b) or volume (c and d) for carbon monoxide and tracer gas. The shaded bar shows the collection of a 500-mL sample of exhaled gas. The upper panels (a and c) show sample collection as selected by computer algorithm (based on gas concentration and lung volume). The lower panels (b and d) show sample collection after manual adjustment by an operator using the concentration versus time plot. Operators tend to be more conservative and may over-shift the sample. When gas concentration is plotted against time, the shift does not appear to be significant; however, when gas concentration is plotted against volume, the degree of shift becomes more apparent.

breakpoint of each phase of the washout on a plot of concentration *versus* volume and adding a proportion of the dead-space volume measured by the FOWLER technique [78] to the phase II to III breakpoint [79]. Such an approach can be automated; however, for visual verification of the point of dead-space washout, the tracer gas concentration must be displayed as a function of volume, since verifying the point of dead-space washout using the concentration *versus* time curve can be deceptive due to the relatively high flow at the beginning of exhalation. This is illustrated in figure 4. If the sample collection point is changed by the operator, it must be recorded in the database and on the report.

With RGA systems, the concentrations of carbon monoxide and tracer gas in a virtual alveolar gas sample are calculated for use in measuring D_{LCO}. The gas concentrations in a virtual sample, that would have been observed in a sample of a given volume had it been collected at a given point during exhalation, are calculated from the flow and gas concentration data. A virtual 200-mL sample analysed by the method of JONES and MEADE [72] has been found to be robust [68]. However, these systems are capable of simulating much smaller gas samples and JONES and MEADE [72] used 85-mL gas samples in the development of their method. Smaller virtual samples will be more affected by noise in the expired carbon monoxide concentration signal. Virtual alveolar gas sample volumes of 85 mL to 500 mL may be used.

Inspired gas composition

The test gas used to calculate D_{LCO} should contain very close to 0.3% carbon monoxide, 21% oxygen, a tracer gas and a balance of nitrogen. The tracer gas must be relatively insoluble and relatively chemically and biologically inert. Since the tracer gas is used to determine the initial alveolar carbon monoxide concentration, as well as the V_A from which carbon monoxide uptake is occurring, its gaseous diffusivity should be similar to carbon monoxide and it should not interfere with the measurement of carbon monoxide concentration. The tracer gas should also not ordinarily be present in alveolar gas or else be present at a known, fixed concentration (*e.g.* argon).

Commonly used tracer gases include helium and methane. Helium meets most of the previous criteria; however, its gaseous diffusivity is considerably higher than that of carbon monoxide. Methane is commonly used for RGA systems; its gaseous diffusivity is closer to carbon monoxide but it has a slightly higher liquid solubility than helium. A recent study has found no clinical difference in D_{LCO} using either helium or methane in normal subjects or patients with COPD [80].

As noted above, the inspired carbon monoxide concentration should be close to 0.3%; however, as ratios are more important than absolute values, exact concentrations are not critical. The assumption in calculating carbon monoxide uptake is that capillary blood does not contain carbon monoxide. Thus, corrections are needed in patients who have significant COHb (see the section on adjustment for COHb concentration and carbon monoxide back-pressure below). There are two considerations influencing the rationale for recommending an inspiratory oxygen fraction (F_{IO_2}) of 21% in the test gas for routine D_{LCO} testing. First, the majority of studies developing reference values for D_{LCO}, which are based on the 2005 standards [4], use an F_{IO_2} of 21% (see the section on reference values below). Secondly, the P_{AO_2} following a maximal inhalation will depend on the dead-space volume and the ratio of V_I to V_A for any given value of F_{IO_2} in the test gas. Hence, if reducing F_{IO_2} in the test gas is intended to simulate tidal breathing conditions (*i.e.* a P_{AO_2} of 100 mmHg or 13 kPa), it may not do so in all subjects.

Although not performed routinely, the measurement of D_{LCO} at several different levels of P_{AO_2} allows the two components of D_{LCO} (D_M and θV_C) to be distinguished. This is accomplished by using the Roughton–Forster relationship noted previously (equation 3) and varying θ by altering P_{AO_2}. Subsequently, $1/D_{LCO}$ is plotted against $1/\theta$ at the different P_{AO_2} levels. The slope of this relationship is $1/V_C$ and the intercept is $1/D_M$. While there are differences in the proposed value of θ, it is beyond the scope of this report to make recommendations for the value of θ to be used.

Manoeuvre intervals

Manoeuvre intervals in classical systems

The 2005 ERS/ATS recommendations state that at least 4 min must be allowed between manoeuvres to allow for adequate elimination of test gas from the lungs. The subject should remain seated during this interval. In patients with airflow obstruction, a longer period (*e.g.* 10 min) should be considered. Several deep inspirations during this period may help to clear test gases more effectively.

Manoeuvre intervals in RGA systems

Exhaled gas can be monitored as soon as the subject begins breathing through the mouthpiece prior to the inhalation of test gas. If a previous manoeuvre was conducted, the information collected on end-expiratory tracer gas levels will indicate whether or not washout is complete, which may occur in less than 4 min in some subjects. For complete washout, the tracer gas level at end-exhalation must be $\leqslant$2% of the tracer gas

concentration in the test gas. Occasionally, if a subject has not reached this level of washout after 5 min, the operator may have the option of continuing with the next manoeuvre. However, in either event, the end-expiratory tracer gas concentration must be reported and used to adjust the tracer gas concentration data used in the determination of V_A at the beginning of breath-holding.

The carbon monoxide concentration measured in exhaled gas prior to inhaling test gas can be used for three important purposes [53]: 1) to adjust D_{LCO} calculations for the back-pressure of carbon monoxide, both the ambient level and the increase that occurs with multiple D_{LCO} manoeuvres; 2) to estimate the COHb concentration and adjust the D_{LCO} calculation accordingly; and 3) to compensate for any residual effects of water vapour and carbon dioxide on the carbon monoxide analysers.

Miscellaneous factors

There may be a diurnal variation in D_{LCO}, since one study has found that D_{LCO} falls 1.2–2.2% per hour throughout the day [81]. The reason for this change is not clear and is not explained by carbon monoxide back-pressure or changes in V_A, V_I, or breath-hold time. One explanation is a combination of changes in carbon monoxide back-pressure and diurnal variation in Hb concentration [82]. A 13% change in D_{LCO} during the menstrual cycle has been reported [83]. The highest value is observed just before the menses and the lowest is observed on the third day of menses; however, it is not clear if this is simply a Hb effect or whether it reflects other physiological processes (*e.g.* hormonal changes on pulmonary vascular tone). Ingestion of ethanol has been reported to decrease D_{LCO} [84, 85]. The mechanisms involved are not clear, although it is known that some fuel-cell carbon monoxide analysers are sensitive to exhaled ethanol and ketones.

Pulmonary function test sequence

D_{LCO} manoeuvres are frequently conducted immediately following the administration of 400 µg of salbutamol in the interval between pre- and post-bronchodilator spirometry testing [60]. While an older study in obstructive lung disease subjects found that D_{LCO} may increase by up to 6% after administration of a bronchodilator [86], a newer study has found that the administration of 400 µg of salbutamol has no significant effect on D_{LCO} in normal control subjects or in patients with either reversible on non-reversible airflow obstruction [87]. A further study has found no significant salbutamol effect on D_{LCO} in COPD patients at doses of less than 1000 µg [88]. Therefore, there is no recommendation against use of a bronchodilator prior to D_{LCO} tests.

Spirometry is a form of exercise [59], which could conceivably impact on D_{LCO} values; however, no studies were found which support a recommendation for a rest interval following spirometry. If the order of testing includes measuring absolute lung volumes using nitrogen washout, during which 100% oxygen is inspired [89] prior to D_{LCO} manoeuvres, ample time is required for alveolar oxygen levels to return to normal. For nitrogen to wash back in to normal levels, allow a rest interval equal to twice the time required for the nitrogen washout test to be completed [90]. It is recommended that D_{LCO} measurements be made before any multi-breath nitrogen washout tests.

Calculations

Calculating diffusing capacity

Converting equation 2 to calculus notation and using $P_{ACO}=F_{ACO}\cdot(P_B-P_{H_2O})$, where F_{ACO} is the alveolar carbon monoxide fraction in the dry gas, P_B is the barometric pressure and P_{H_2O} is the water vapour pressure, gives equation 4 as shown below.

$$\frac{d(V_A \cdot F_{ACO})}{dt} = D_{LCO} \cdot F_{ACO} \cdot (P_B - P_{H_2O}) \quad (4)$$

Assuming a constant volume and that the pulmonary capillary carbon monoxide tension is near zero, solving for D_{LCO} gives equation 5, where $F_{ACO,0}$ and $F_{ACO,t}$ are the fractional concentrations of carbon monoxide in the alveolar volume at time 0 and time t, respectively. The rate of gas uptake is expressed in mL(STPD)·min^{-1} and the transfer gradient (the difference between the alveolar and pulmonary capillary pressures) in mmHg. Therefore, D_{LCO} has traditional units of mL(STPD)·min^{-1}·mmHg^{-1} and SI units of mmol(STPD)·min^{-1}·kPa^{-1}.

$$D_{LCO} = \frac{V_A}{t \cdot (P_B - P_{H_2O})} \cdot \ln\left(\frac{F_{ACO,0}}{F_{ACO,t}}\right) \quad (5)$$

The single-breath D_{LCO} technique assumes that both carbon monoxide and the tracer gas are diluted equally on inspiration. Thus, the initial alveolar concentration of carbon monoxide at the theoretical start

of breath-holding ($F_{ACO,0}$) can be calculated by knowing the inspired tracer gas fraction (F_{ITr}) and the alveolar tracer gas fraction (F_{ATr}). In this case, if F_{ICO} is the carbon monoxide fraction in the inspired test gas, we can generate equation 6.

$$F_{ACO,0} = F_{ICO} \cdot \frac{F_{ATr}}{F_{ITr}} \quad (6)$$

Tracer gas dilution is also used to determine the effective V_A. If we solve for D_{LCO} we can generate equation 7, where V_A is reported in L (BTPS) and t_{BH}, the breath-hold time, is reported in seconds.

$$D_{LCO} = \frac{V_A}{t_{BH} \cdot (P_B - P_{H_2O})} \cdot \ln\left(\frac{F_{ICO}}{F_{ACO}} \cdot \frac{F_{ATr}}{F_{ITr}}\right) \quad (7)$$

If we convert V_A to STPD conditions we obtain equation 8 for traditional units of D_{LCO} (V_A mL(STPD)·min^{-1}·mmHg^{-1}). The factor of 60 000 arises from the change to the traditional units (60 s to 1 min and 1 L to 1000 mL).

$$D_{LCO} = \frac{V_{ASTPD}}{t_{BH} \cdot (P_B - 47)} \cdot \ln\left(\frac{F_{ICO}}{F_{ACO}} \cdot \frac{F_{ATr}}{F_{ITr}}\right) \cdot 60\,000 \quad (8)$$

If we then convert to SI units we obtain equation 9 (units of T_{LCO}: mmol·min^{-1}·kPa^{-1}), where the factor of 22.4 arises from the conversion of mL(STPD) to mmol.

$$T_{LCO} = \frac{V_{ASTPD}}{t_{BH} \cdot (P_B - 6.28)} \cdot \ln\left(\frac{F_{ICO}}{F_{ACO}} \cdot \frac{F_{ATr}}{F_{ITr}}\right) \cdot 60\,000/22.4 \quad (9)$$

Calculating breath-hold time

The breath-hold time, or time of transfer during which carbon monoxide changes from its initial to its final concentration (t_{BH}), is part of the denominator in the D_{LCO} equation (equation 7). As noted previously, the single-breath measurement of carbon monoxide uptake assumes an instantaneous lung filling and emptying process. However, both inspiration and expiration require up to several seconds, and these periods of changing gas volume in the lung must be accounted for in the calculations. For purposes of standardisation, the method of Jones and Meade (figure 5) [72] is recommended, since it has the theoretical appeal of empirically accounting for the effects of inspiratory and expiratory time. This method has also been shown to adequately address inspiratory flows as low as 1 L·s^{-1}, breath-hold times as short as 5 s and expiratory flows as low as 0.5 L·s^{-1} in normal subjects [68] when using an RGA system that

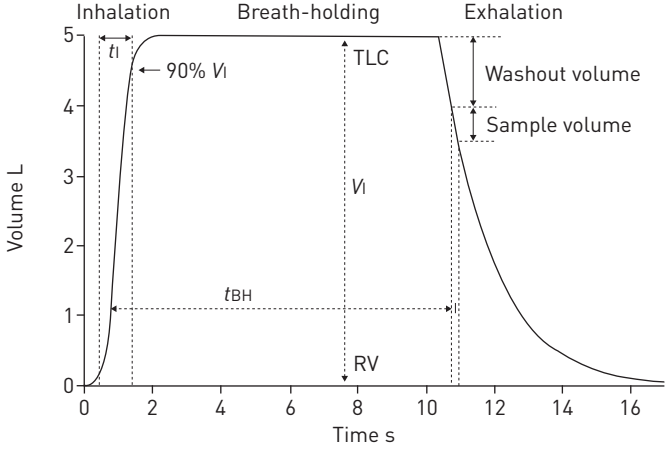


FIGURE 5 Schematic illustration of measuring breath-hold time for the single-breath diffusing capacity of the lung for carbon monoxide. The Jones and Meade [72] breath-hold time includes 0.7 of inspiratory time and half of sample time. V_I: inspired volume; t_I: time of inspiration (defined from the back-extrapolated time 0 to the time that 90% of the V_I has been inhaled); t_{BH}: breath-hold time; TLC: total lung capacity; RV: residual volume. Reproduced from [4].

measures the dead-space and calculates V_A using the tracer gas concentration data from the entire manoeuvre. With the approach taken by JONES and MEADE [72], breath-hold time equals the time starting from 0.3 of the inspiratory time (t_I) to the middle of the sample collection time. As in spirometry, the back-extrapolation technique must be used to establish time zero [2, 59]. The time when 90% of the V_I has been inspired is a reasonable end-point for defining inspiratory time (figure 5).

Calculating the alveolar volume

Alveolar volume in classical systems

V_A represents an estimate of lung gas volume into which carbon monoxide is distributed and then transferred across the alveolar capillary membrane [1, 6] and is thus critical in the measurement of D_{LCO}. Classical D_{LCO} equipment collects an actual sample of exhaled gas for analysis and determination of the carbon monoxide and tracer gas concentrations. Since there is only one measurement, the alveolar volume is calculated from the same sample that is used for analysis of carbon monoxide uptake [2]. As noted elsewhere, JONES and MEADE [72] have shown that the sample has to be small (85 mL) to reduce errors in D_{LCO} determination. The calculation of V_A requires an assumption that the alveolar gas is completely mixed at the maximal lung volume and that a small sample of exhaled gas will presumably be representative of the entire lung. In normal subjects this assumption is reasonable and has little effect on the measurement of V_A. However, in patients whose lung disease results in a heterogeneous distribution of ventilation, the size and timing of the sample have a major effect on the measurement of V_A. For classical systems, V_A is determined from values for V_I, F_{ITr} and F_{ATr} (measured in the discrete-sample gas volume, V_S). Since the amount of tracer gas in the lung (alveolar plus dead-space) equals the amount of inspired tracer gas and given that the dead-space tracer gas fraction is the same as the inspired fraction, we can generate equations 10 and 11.

$$V_I \cdot F_{ITr} = V_A \cdot F_{ATr} + V_D \cdot F_{ITr} \tag{10}$$

$$V_A = (V_I - V_D) \cdot F_{ITr}/F_{ATr} \tag{11}$$

V_A is reported under BTPS conditions and then converted to STPD conditions to calculate D_{LCO}, as in equations 8 and 9. The inspired volume (V_I) is the measured volume of inhaled dry gas and is thus considered to be under ambient temperature (T), ambient pressure (P_B), dry (ATPD) conditions. The conversion to body temperature, ambient pressure, saturated with water vapour (BTPS) and standard temperature, pressure, dry (STPD) conditions may require conversion factors to compensate for the diluting or concentrating effects of adding or removing water vapour or carbon dioxide at the gas sampling site. Several examples of V_A calculations using such conversion factors are given below.

Where water vapour is removed from the sampled gas and carbon dioxide does not interfere with the analysers we can use equations 12 and 13 as follows, where V_{ABTPS} is the alveolar volume under BTPS conditions and V_{IATPD} is the inspired volume under ATPD conditions.

$$V_{ABTPS} = (V_{IATPD} - V_{Dequip} - V_{Danat}) \cdot \frac{F_{ITr}}{F_{ATr}} \cdot \frac{P_B}{(P_B - 47)} \cdot \frac{310}{(273 + T)} \tag{12}$$

$$V_{ASTPD} = (V_{IATPD} - V_{Dequip} - V_{Danat}) \cdot \frac{F_{ITr}}{F_{ATr}} \cdot \frac{P_B}{760} \cdot \frac{273}{(273 + T)} \tag{13}$$

Where water vapour and carbon dioxide are removed from the sampled gas we can use equations 14 and 15 as follows, where F_{ACO_2} is the fraction of carbon dioxide in the alveolar sample. If no measurement of F_{ACO_2} is available then a value of 0.05 may be assumed.

$$V_{ABTPS} = (V_{IATPD} - V_{Dequip} - V_{Danat}) \cdot \frac{F_{ITr}}{F_{ATr} \cdot (1 - F_{ACO_2})} \cdot \frac{P_B}{(P_B - 47)} \cdot \frac{310}{(273 + T)} \tag{14}$$

$$V_{ASTPD} = (V_{IATPD} - V_{Dequip} - V_{Danat}) \cdot \frac{F_{ITr}}{F_{ATr} \cdot (1 - F_{ACO_2})} \cdot \frac{P_B}{760} \cdot \frac{273}{(273 + T)} \tag{15}$$

Where water vapour in the sampled gas is equilibrated to room air, carbon dioxide does not interfere with the analysers and tank values (*i.e.* the dry gas concentration) are used for F_{ITr}, we can use equations 16

and 17 as shown below. If F_{ITr} is read by the analysers, the corrections are the same as in equations 12 and 13 above.

$$V_{ABTPS} = (V_{IATPD} - V_{Dequip} - V_{Danat}) \cdot \frac{F_{ITr}}{F_{ATr}} \cdot \frac{(P_B - P_{H_2O})}{(P_B - 47)} \cdot \frac{310}{(273 + T)} \quad (16)$$

$$V_{ASTPD} = (V_{IATPD} - V_{Dequip} - V_{Danat}) \cdot \frac{F_{ITr}}{F_{ATr}} \cdot \frac{(P_B - P_{H_2O})}{760} \cdot \frac{273}{(273 + T)} \quad (17)$$

If neither water vapour nor carbon dioxide are removed from the sampled gas, no interference is observed for the analysers and the sample tubing is heated to prevent condensation, we can use equations 18 and 19 as shown below:

$$V_{ABTPS} = (V_{IATPD} - V_{Dequip} - V_{Danat}) \cdot \frac{F_{ITr}}{F_{ATr}} \cdot \frac{310}{(273 + T)} \quad (18)$$

$$V_{ASTPD} = (V_{IATPD} - V_{Dequip} - V_{Danat}) \cdot \frac{F_{ITr}}{F_{ATr}} \cdot \frac{(P_B - 47)}{760} \cdot \frac{273}{(273 + T)} \quad (19)$$

In all four cases, temperature is measured in degrees Celsius and gas pressures are measured in mmHg. It is essential that V_D is considered in the calculation of V_A. V_D occurs in two areas: equipment dead-space, V_{Dequip} (*i.e.* the volume of the mouthpiece, filters and connections within the breathing circuit) and anatomic dead-space, V_{Danat} (*i.e.* the volume in the conducting airways that does not participate in gas exchange). V_{Dequip} must be specified by the equipment manufacturer but may vary as the user alters the system (*e.g.* by adding a filter or using a different filter). A further small correction to V_D can be made where V_{Dequip} is assumed to be under ATPD conditions, since it is filled with room temperature, dry test gas at the end of inspiration, whereas V_{Danat} should be assumed to be under BTPS conditions. There are various methods to estimate V_{Danat}. One example uses a fixed value of 150 mL [4, 5], although this does not work well for small adults or children. Another uses a value of 2.2 mL·kg^{-1} of body weight [50], although this does not work well for very obese subjects. In the studies which derive the commonly used reference equations, the latter is the most commonly used technique. However, some investigators have ignored V_{Danat} [91–93] and one uses a value derived from age+2.2 mL·kg^{-1} of body weight [94]. If body mass index (BMI) is <30 kg·m^{-2}, the recommendation is to use an estimate for V_{Danat} of 2.2 mL·kg^{-1} body weight. In more obese subjects, or if the weight of the subject is unknown, V_{Danat} (in mL) can be estimated using equation 20 where height (h) is measured in cm.

$$V_{Danat} = h^2/189.4 \quad (20)$$

With classical discrete-sample systems, which collect the alveolar sample in a collection bag or chamber, the sample-bag residual volume (sometimes called the sample-bag dead-space) dilutes the sample gas and alters the measured concentrations of the expired gases. The size and direction of the error depends on V_S, the residual volume of the sample bag and its connectors (V_{SRV}) and the gas content of this residual volume. V_{SRV} could contain test gas, room air, or expired gas from a subject after a D_{LCO} manoeuvre. When V_{SRV} contains room air, its effect is to reduce the measured concentrations of the expired gases and equation 21 can be used to adjust for this. Estimates of the potential change in D_{LCO}, in existing systems when no adjustment is made for sample-bag dead-space, range from 0.3–8% depending on the sample-bag size and V_{SRV} [95].

$$F_{ATr}[\text{adjusted}] = F_{ATr}[\text{measured}] \cdot (V_S/(V_S - V_{SRV})) \quad (21)$$

For classical systems, manufacturers must report instrument and sample-bag dead-space. Both of these must be flushed with room air or, if D_M and V_C are to be calculated, appropriate levels of oxygen before the single-breath manoeuvre such that they will not contain expiratory gas from a previous subject. V_{SRV} must be <2% of V_S or 10 mL, whichever is larger. Importantly, when RGAs are used to measure the exhaled sample, there is no residual bag volume to consider (V_A is calculated using a mass balance of all inhaled and exhaled gas; equations 22–26 in the next section).

For normal subjects, the classical single-breath determination of alveolar volume (V_{Asb}) described above closely matches TLC determined by plethysmography [22, 74]. However, poor gas mixing in patients with

maldistribution of inspired volume (*e.g.* patients with obstructed airways) can markedly alter tracer gas dilution leading to values for V_{Asb} that are markedly less than the value of V_A determined from the actual total thoracic gas volume. Observed carbon monoxide uptake is also affected by poor gas mixing under these conditions and will primarily reflect the carbon monoxide transfer properties of the regions into which the test gas is distributed. It has been suggested that a separately determined V_A value from a more accurate method (*e.g.* multiple-breath technique (V_{Amb}) or plethysmography ($V_{Aplethys}$)) could be substituted for V_{Asb} under these conditions to correct for the effects of maldistribution. However, the D_{LCO} calculation (equation 7) is based on the volume of gas into which the tracer gas (and carbon monoxide) distributes, and not the total thoracic gas volume. Moreover, substituting a larger, separately determined V_{Amb} or $V_{Aplethys}$ value assumes that D_M and V_C properties in the unmeasured lung regions are similar to those in the measured lung regions, an assumption that is difficult to justify. Additionally, if V_{Asb} is replaced with a different value, the applicability of the D_{LCO} reference equations is compromised.

Due to these considerations, a separately measured V_{Amb} or $V_{Aplethys}$ should not be substituted for V_{Asb}. Instead, when the value of V_{Asb} is markedly less than that determined separately for V_{Amb} or $V_{Aplethys}$, this must be reported and the ratio of V_{Asb} to V_{Amb} or $V_{Aplethys}$ may optionally be included. For the subsequent interpretation of D_{LCO}, it should then be noted that the maldistribution of inspired gas probably contributes to any observed reduction in measured values.

Alveolar volume in RGA systems

As mentioned in the previous section, when an RGA system is used the dead-space volume is measured rather than estimated. The total dead-space, V_D, can be measured from the tracer gas washout curve using the Fowler [78] method (figure 6). A linear regression line estimating the slope of phase III of the tracer gas washout concentration as a function of volume should be calculated using the last half of the expiratory tracing by volume. The Fowler dead-space is the point where the area between the phase III slope and the

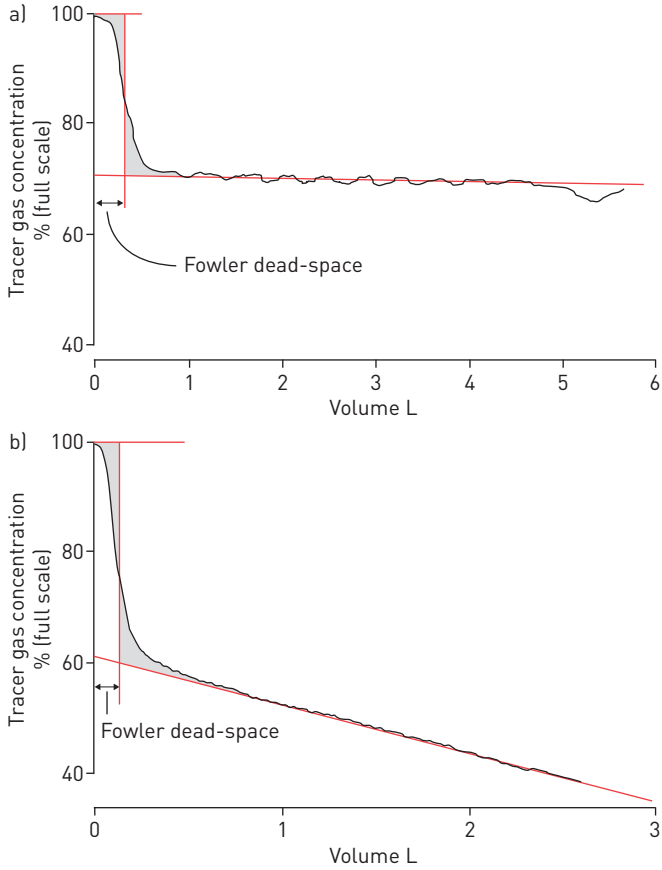


FIGURE 6 Graphical representation of the calculation of the Fowler dead-space volume in a normal, healthy subject (a) and a subject with chronic obstructive pulmonary disease (COPD) (b). The single-breath tracer gas washout is plotted against exhaled lung volume from total lung capacity. The volume at which the shaded area above the tracer gas washout curve equals the shaded area below the curve is the Fowler dead-space [78] which is reported under body temperature, ambient pressure, saturated with water vapour (BTPS) conditions.

tracer gas washout curve equals the area between the peak tracer gas concentration and the tracer gas washout curve. The anatomic dead-space, V_{Danat}, is equal to the Fowler dead-space minus the equipment dead-space, V_{Dequip}, which includes the filter and/or mouthpiece and which must be supplied by the manufacturer.

The development of RGA systems now allows the analysis of all gas exhaled and provides the opportunity to enhance the accuracy of V_A determinations. Given that the tracer gas can now be monitored throughout exhalation, there is no need to constrain the measurement of V_A to the discrete sample computationally constructed to determine carbon monoxide uptake. Indeed, using all of the available gas concentration data has been shown to provide a better estimate of V_A [71, 96] than constraining the measurement to a smaller sample of exhaled gas (as was required by the equipment available in 1957 when the clinical single-breath method was developed [2]).

This technique uses a mass balance approach to determine V_A in which the volume of tracer gas inhaled and the volume subsequently exhaled are measured and the latter subtracted from the former to determine the volume of tracer gas remaining in the lung at end-exhalation [71, 96]. The volume of tracer gas left in the lung is then divided by the end-expiratory tracer gas concentration to give the absolute end-expiratory lung volume V_{ee}. The TLC is then calculated by adding the expired volume (V_E) to V_{ee} and subtracting V_{Dequip}. If V_E is the volume expired from the maximum volume during breath-holding (to the end of the manoeuvre) then the single-breath total lung capacity (TLC_{sb}) can be defined as $TLC_{sb}=V_E+V_{ee}-V_{Dequip}$ and $V_A=TLC_{sb}-V_{Danat}$. Residual tracer gas in the lung from a previous manoeuvre can be measured prior to the start of the current manoeuvre and included in the mass balance equation.

In more detail, V_A is calculated using a mass balance equation which states that the tracer gas left in the lung at end exhalation is equal to all of the tracer gas inhaled minus the tracer gas exhaled. The sum of the inhaled and exhaled tracer gas volumes is the integral, with respect to time, of flow×tracer gas concentration where flow is positive for inhalation and negative for exhalation. In this case, V_{ee} (including V_{Dequip} and V_{Danat}) is thus described by equation 22 where t_0 is the time at the start of test gas inhalation, t_f is the time at the end of exhalation, $Tr(t)$ is the tracer gas concentration at any time t (adjusted to BTPS conditions), Tr_{ee} is the mean tracer gas concentration at end-exhalation and $flow(t)$ is the flow at any time t (under BTPS conditions).

$$V_{ee} = \frac{1}{Tr_{ee}} \int_{t_0}^{t_f} Tr(t) \cdot flow(t)\, dt \tag{22}$$

Depending upon the signal to noise ratio, the average value of Tr over the last 250 mL can be used for Tr_{ee}. Furthermore, when $flow(t)$ is positive during inhalation of dry test gas, it is adjusted by $310/T \cdot P_B/(P_B-47)$ where T is the ambient temperature. All measurements of tracer gas are assumed to be made with the water vapour pressure in the sample line equilibrated to the water vapour pressure in room air.

The absolute lung volume at any time t, $V(t)$, during the manoeuvre can then be described by equations 23 and 24. The integral of $flow(t)dt$ from time t_0 to time t_f is the net change in total volume over the entire manoeuvre and will be zero if the inhaled volume, V_I, equals the exhaled volume, V_E. The integral of $flow(t)dt$ from time t_0 to time t is the net volume change at any time t. Hence, at the end of the single breath manoeuvre, $V(t_f)$ is simply equal to V_{ee}-V_{Dequip}.

$$V(t) = V_{ee} + \int_{t_0}^{t} flow(t)\, dt - \int_{t_0}^{t_f} flow(t)\, dt - V_{Dequip} \tag{23}$$

$$V(t) = V_{ee} - \int_{t}^{t_f} flow(t)\, dt - V_{Dequip} \tag{24}$$

If the tracer gas has not been completely washed out from a previous D_{LCO} manoeuvre, then the residual alveolar tracer gas concentration (Tr_R) measured just prior to the inhalation of test gas must be considered in the mass-balance equation and V_{ee} is duly described by equation 25.

$$V_{ee} = \frac{1}{(Tr_{ee} - Tr_R)} \int_{t_0}^{t_f} (Tr(t) - Tr_R) \cdot flow(t)\, dt \tag{25}$$

The value of V_A to be reported in BTPS conditions is described by equation 26. This value is converted to STPD conditions for use in equation 8 or 9.

$$V_A = V_E + V_{ee} - V_{Danat} - V_{Dequip} \quad (26)$$

This method has been compared to plethysmography in normal subjects and in patients with lung disease with various breath-hold times [71, 96]. For normal subjects, there is little difference in D_{LCO} when using either method to measure V_A; however, V_A measured by the RGA method is significantly higher than V_A measured by the classical method in subjects with COPD or uncontrolled asthma. The resulting D_{LCO} measurements in COPD cases are some 8 to 15% higher. Since reference values for D_{LCO} are developed using normal subjects, existing reference values continue to be applicable using V_A measured by the RGA method. For subjects with COPD the effect of using the RGA V_A value will, in isolation, be to calculate an increased D_{LCO} value. However, the V_A measured from a discrete sample will vary with the sample volume and sample timing [72] such that using the RGA V_A value should improve reproducibility of D_{LCO} in these subjects.

Another significant advantage of calculating absolute lung volume at end-exhalation instead of at maximal inhalation is that the impact of errors due to the assumption of complete gas mixing in the lung is reduced. For example, in a patient with a TLC of 7 L and a RV of 2 L, a 10% error in TLC (700 mL) translates into a 10% error in D_{LCO}. However, a 10% error in RV would be 200 mL and when VC is added to RV the volume error at TLC is only 2.9% which translates into only a 2.9% error in D_{LCO}.

During the transition from classical systems to RGA systems, some laboratories may wish to report D_{LCO} values using the 2005 ATS/ERS method in combination with those obtained using RGA V_A for comparative purposes. RGA V_A values may alter D_{LCO} in some older normal subjects, who have more heterogeneous distribution of ventilation due to the normal ageing process, and therefore might yield slightly higher D_{LCO} values compared to current classically derived reference values. As with any set of reference values, which must be validated in each laboratory, D_{LCO} values using V_A must be validated using a group of normal, healthy subjects. The Global Lung Function Initiative is in the process of developing all-age predicted values using datasets submitted from 12 countries (www.lungfunction.org).

Inspired gas conditions

In most cases, the test gas inspired from a bag or a compressed gas cylinder with a demand valve is a dry gas and, as such, is considered to be under ATPD conditions. The inspired volume needs to be converted into BTPS conditions for use in equations 10 and 11. It is recommended that the V_I (BTPS) be reported and that manufacturers should specify and document inspired gas conditions for each instrument. Since gas cooling can occur due to decompression through the delivery valve, manufacturers are required to measure the test gas temperature at the pneumotachometer in a typical system in their testing laboratory and provide appropriate compensation for gas cooling if necessary.

Carbon dioxide, water vapour and temperature adjustment for alveolar volume calculations

Exhaled gas contains carbon dioxide and water vapour which were not present in the test gas mixture. As noted previously, some systems remove one or both of these if they interfere with analyser function, raising both carbon monoxide and tracer gas concentrations. Under these circumstances, adjustments are required for the increase in F_{ATr} used to calculate V_A. However, no adjustment for the increase in alveolar inspired carbon monoxide and tracer gas fractions at time t ($F_{ACO,t}$ and $F_{ATr,t}$) is necessary in calculating the rate of carbon monoxide uptake, since the concentration factor appears in both the numerator and the denominator of the expression ($F_{ATr,t}/F_{ACO,t}$) and the effect therefore cancels itself out. Exhaled gas is initially at body temperature and some systems allow this to cool, such that the gas volume contracts, whereas others will provide heat to maintain the temperature. As such, adjustments to BTPS conditions may be required depending upon the system design. All of these adjustments must be documented by the manufacturer for their particular system. The conversion factors used to modify calculations in D_{LCO} manoeuvres are shown in equations 8, 9 and 12–19.

RGA signal alignment

To properly analyse continuous gas samples, the gas concentration signals from the analysers must be properly aligned with the flow signal from the pneumotachometer (figure 2). The first step is to shift the concentration signal ahead in time to compensate for the lag time (the time required for the gas to travel from the aspiration port to the analyser chamber). The lag time is a function of the length and diameter of the tubing and the analyser aspiration rate. The length of the tubing should be minimised to prevent mixing of the aspirated sample within the sampling tube, which can blunt the response time through a process of Taylor dispersion. The amount of mixing will also depend on the configuration of the sampling

circuit, including any valves and junctions, which can create turbulence. It should also be noted that lag time can vary with gas viscosity and, when helium is used as the tracer gas, this may require dynamic compensation during exhalation.

An additional shift of each gas concentration signal relative to the flow signal is also required to compensate for the response time of the analyser. This can be accomplished using an optimal shift equal to the natural logarithm of twice the time constant of the analyser response [97]. Alternatively, alignment may be achieved by other signal processing strategies such as cross-correlation techniques (convolution of signals).

For a more accurate D_{LCO} calculation, a third shift equal to the dead-space transit time may be used to translate the gas concentrations measured at the mouth to the gas concentrations in the alveolar space. During inhalation, the gas sampled at the aspiration port will not reach the gas-exchanging alveolar space until at least one dead-space transit time later and, similarly, the gas sampled at the aspiration port during exhalation is gas that was in the alveolar space one dead-space transit time previously. If a system uses this further correction the effective breath-hold time in the alveolar space will be reduced (typically by 0.05–0.15 s) and D_{LCO} will be increased (typically by 0.5–1.5%).

Interpolation between data points may be required to achieve optimal shifting of the gas concentration data, particularly if lower digitisation rates are used. To reduce errors introduced by interpolation, a sample rate of 1000 Hz per channel is recommended.

Transfer coefficient of the lung for carbon monoxide

The logarithmic change in carbon monoxide concentration during the breath-hold phase of the single-breath manoeuver, divided by t_{BH} and the P_B of the dry gas is termed K_{CO}. This is equivalent to the left hand side of equation 5 without the V_A term and, conceptually, D_{LCO} is thus equivalent to $V_A \cdot K_{CO}$. The specific calculations for K_{CO} are shown below as equations 27 and 28; however, if values are required in SI units, it is necessary to convert 1000 mL(STPD) to mmol as shown in equations 29 and 30.

$$K_{CO} = \ln\left(\frac{F_{ICO}}{F_{ACO}} \cdot \frac{F_{ATr}}{F_{ITr}}\right) \cdot \frac{1}{t_{BH}/60 \cdot (P_B - P_{H_2O})} \cdot \frac{1000\,\text{mL}}{1\,\text{L}} \cdot \frac{273}{310} \cdot \frac{P_B - P_{H_2O}}{760} \tag{27}$$

$$K_{CO} = \ln\left(\frac{F_{ICO}}{F_{ACO}} \cdot \frac{F_{ATr}}{F_{ITr}}\right) \cdot \frac{1}{t_{BH}} \cdot 69.52 \tag{28}$$

$$K_{CO} = \ln\left(\frac{F_{ICO}}{F_{ACO}} \cdot \frac{F_{ATr}}{F_{ITr}}\right) \cdot \frac{1}{t_{BH}/60 \cdot (P_B - P_{H_2O})} \cdot \frac{1000\,\text{mL}}{1\,\text{L}} \cdot \frac{273}{310} \cdot \frac{P_B - P_{H_2O}}{101.3} \cdot \frac{1\,\text{mmol}}{22.4\,\text{mL}} \tag{29}$$

$$K_{CO} = \ln\left(\frac{F_{ICO}}{F_{ACO}} \cdot \frac{F_{ATr}}{F_{ITr}}\right) \cdot \frac{1}{t_{BH}} \cdot 23.29 \tag{30}$$

It should be noted that the calculation of K_{CO} is completely independent of the gas flow, lung volume and barometric pressure measured during the manoeuvre. Although the units of the logarithmic change in carbon monoxide concentration per unit time and per unit pressure are $min^{-1}{\cdot}mmHg^{-1}$ (or $min^{-1}{\cdot}kPa^{-1}$), K_{CO} is expressed in units of $mL(STPD){\cdot}min^{-1}{\cdot}mmHg^{-1}{\cdot}L(BTPS)^{-1}$ or $mmol{\cdot}min^{-1}{\cdot}kPa^{-1}{\cdot}L(BTPS)^{-1}$ only because the basic measurement of the logarithmic change in gas concentration over time is multiplied by 1000 mL(STPD) and then divided by 1 L(BTPS) which changes the magnitude of the value of K_{CO} by 1000 times the BTPS to STPD factor [98].

As V_A is not a component of K_{CO}, some users prefer to use K_{CO} as it eliminates the uncertainty in measurement of V_A from the assessment of carbon monoxide uptake. This uncertainty arises from the assumption that F_{ATr}, as measured from the exhaled gas sample, is representative of the entire lung. However, this same assumption is used to estimate the alveolar carbon monoxide concentration at the start of breath-holding and K_{CO} measurement is thus subject to the same uncertainty [99].

Mathematically, K_{CO} can be calculated as D_{LCO}/V_{ABTPS}. However, K_{CO} should not be reported using the term D_{LCO}/V_A, as it may be inferred from this term that D_{LCO} can be corrected or normalised for V_A. In fact, the relationship between lung volume and carbon monoxide uptake is complex and studies evaluating the effects of reduced V_I (and thus V_A) show the relationship to be alinear and certainly less than 1:1

(*i.e.* the fall in D_{LCO} is far less than the fall in lung volume) [20, 21, 98, 100, 101]. This likely reflects the fact that alveolar folding–unfolding and capillary volume changes resulting from lung volume changes do not translate into concomitant and equal changes in D_{LCO}. Thus, while the K_{CO} calculation might add insight into carbon monoxide uptake properties of the lung [98], it cannot be used as a simple technique to normalise D_{LCO} for volume.

Optional calculations

Separate equations for inhalation, breath-hold and exhalation

When Krogh [1] developed the measurement of the "diffusion constant" in 1915, she had to design a manoeuvre that would be compatible with an analytical solution of the equation for gas transfer. She achieved this by simulating a pure breath-hold manoeuvre and the measurement of D_{LCO} today continues to be constrained by having the patient perform a rapid inhalation, 10 s of breath-holding and a rapid exhalation. Deviations from a pure breath-hold manoeuvre cause errors in D_{LCO} because the Krogh equation is only valid for this case [68, 72].

The gas transfer equation can also be written for inhalation and exhalation, both of which become equivalent to the breath-holding equation at zero flow. Using data from the continuous monitoring of flow and the concentrations of carbon monoxide and tracer gas throughout the manoeuvre, an algorithm can be used to calculate D_{LCO} by numerical methods [53]. This method accounts analytically for the times of inhalation and exhalation, gives values of D_{LCO} that are not dependent on how rapidly the manoeuvre is performed and returns a fixed value of D_{LCO} over the entire manoeuvre that accounts for the observed uptake of carbon monoxide. All of the exhaled gas data can be used to provide a more representative measurement of D_{LCO} for the whole lung rather than calculating D_{LCO} from a small alveolar gas sample.

Using the "three-equation method" [53] in young, healthy subjects, the standardised manoeuvre gives the same values for D_{LCO} as the ATS standardised manoeuvre. However, when these same subjects perform the manoeuvre with slower flows and/or shorter breath-hold times, similar to those seen in patients with airflow obstruction, the three-equation method gives unchanged D_{LCO} values while the ATS standardised method yields significantly higher D_{LCO} values [68]. Krogh [1] designed her experiment for normal subjects and not for patients with lung disease. The standardised manoeuvre penalises lung disease patients, who cannot perform it adequately; however, with RGA instrumentation it is no longer necessary to use an arduous, demanding manoeuvre to measure D_{LCO}.

Indices of heterogeneity of ventilation and gas transfer

As noted above, the heterogeneity of ventilation affects D_{LCO} measurement [102, 103]. Gas concentration data from RGA systems can be used to calculate indices of ventilation nonuniformity, such as the slope of phase III of the alveolar plateau [90, 104]. However, such indices need to be normalised to account for the differences in lung volume and the differences in RV/TLC that occur from person to person [79]. Other measures of mixing efficiency may be calculated from the tracer gas data [53].

Disease processes can also affect the distribution of gas transfer in the lung. Using the three-equation method, the observed decay in carbon monoxide during exhalation can be compared to the carbon monoxide decay that would be predicted for a homogeneous lung in which diffusion occurs uniformly. An index of D_{LCO} heterogeneity has been developed that is capable of distinguishing smokers with normal lung function and normal D_{LCO} from a control group of nonsmokers [105].

Evaluating the measurement of D_{LCO}

Acceptability, repeatability and quality control

Acceptable manoeuvres are defined in table 3. The volume–time graph should show a smooth, sharp rise in volume, followed by a stable breath-hold and a smooth, sharp exhalation (figure 3). The gas concentration graph should show a very sharp rise when test gas is introduced and remain stable until exhalation followed by an initial rapid decline with a smooth transition to phase III. Variations from this pattern will indicate leaks. The V_I of test gas must be at least 90% of the largest VC measured in the same pulmonary function testing session. At least 85% of test gas V_I must be inhaled within <4 s. There must be no evidence of a Müller or Valsalva manoeuvre during the breath-hold period. Alveolar sample collection must be completed within 4 s. The calculated breath-hold time must be 10±2 s. For RGA systems, virtual sample collection should be initiated after the completion of dead-space washout. A manoeuvre with a V_I/VC <90% but ⩾85% may be deemed acceptable if the V_A is within 200 mL or 5% (whichever is greater) of the largest V_A from other acceptable manoeuvres.

Repeatability describes intra-session variability on repeated testing when there is no change in test conditions [106, 107]. In a large university-based laboratory study, the coefficient of variation for repeated measurement in normal subjects was 3.1% and this increased only slightly (from 4.0 to 4.4%) in patients

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

Criteria for acceptability

A V_I ⩾90% of the largest VC in the same test session; alternatively a V_I ⩾85% of the largest VC in the same test session and V_A within 200 mL or 5% (whichever is greater) of the largest V_A from other acceptable manoeuvres

85% of test gas V_I inhaled in <4 s

A stable calculated breath-hold for 10±2 s with no evidence of leaks or Valsalva/Müller manoeuvres during this time

Sample collection completed within 4 s of the start of exhalation. For RGA systems, virtual sample collection should be initiated after dead-space washout is complete

Criteria for repeatability

At least two acceptable D_{LCO} measurements within 2 mL·min^{-1}·mmHg^{-1} (0.67 mmol·min^{-1}·kPa^{-1}) of each other

	Quality control grading[#]		
Score	**V_I/VC**	**t_{BH}**	**Sample collection**
A	⩾90%[¶]	8–12 s	⩽4 s
B	⩾85%	8–12 s	⩽4 s
C	⩾80%	8–12 s	⩽5 s
D	⩽80%	<8 or >12 s	⩽5 s
F	⩽80%	<8 or >12 s	>5 s

V_I: inspired volume; VC: vital capacity; V_A: alveolar volume; t_{BH}: breath-hold time; D_{LCO}: diffusing capacity of the lung for carbon monoxide. [#]: only grade A manoeuvres meet all acceptability criteria. The average D_{LCO} values from two or more grade A manoeuvres that meet the repeatability criterion should be reported. If only one grade A manoeuvre is attained, the D_{LCO} value from that manoeuvre should be reported. If no grade A manoeuvre is obtained, manoeuvres of grades B to D might still have clinical utility. The average of such manoeuvres should be reported but these deviations from the acceptability criteria must be noted to caution the interpreter of the test results. Manoeuvres of grade F are not useable. [¶]: or V_I/VC⩾85% and V_A within 200 mL or 5% (whichever is greater) of the largest V_A from another acceptable manoeuvre.

with abnormal spirometry patterns [108]. Studies conducted prior to the publication of the 2005 standards found D_{LCO} variability of up to 9% (reproducibility) in normal individuals in repeated measurement over a period of 1 year [109] and coefficients of variation ranged from 6.2% to 12% in selected UK regions [110].

Repeatability requirement: there must be at least two acceptable manoeuvres that are within 2 mL·min^{-1}·mmHg^{-1} (0.67 mmol·min^{-1}·kPa^{-1}) of each other. A study of 4797 test sessions found that 95.5% of cases met this criterion [67]. Since most intra-session variability is technical rather than physiological, the mean of acceptable manoeuvres is reported. The average of at least two acceptable manoeuvres that meet the repeatability requirement must be reported (*i.e.* outliers excluded). While it is recommended that at least two acceptable D_{LCO} manoeuvres must be performed, research is needed to determine the actual number of manoeuvres required to provide a reasonable estimate of average D_{LCO} for a given person. As noted elsewhere, five manoeuvres will result in an increase of ~3.5% COHb from baseline [66, 82], which will decrease the measured D_{LCO} by ~3–3.5%. Thus, conducting more than five manoeuvres is not a recommended strategy.

There are no quality control grading systems that have been validated using the new standards contained in this document. Until such validation is published, an interim grading system is provided in table 3 and further research is recommended to develop and validate a D_{LCO} grading system.

A grade A manoeuvre meets all acceptability criteria. The average D_{LCO} from two or more grade A manoeuvres that are repeatable (*i.e.* are within 2 mL·min^{-1}·mmHg^{-1} or 0.67 mmol·min^{-1}·kPa^{-1} of each other) should be reported. If, after repeat testing, the operator is unable to obtain two repeatable grade A manoeuvres, then the following values are reported with a caution to the interpreter that the testing session was suboptimal: 1) If two or more grade A manoeuvres that are not repeatable are obtained, then the average D_{LCO} value from the acceptable manoeuvres is reported. 2) If only one grade A manoeuvre is obtained, then the D_{LCO} value from that manoeuvre is reported. 3) If no acceptable manoeuvres are obtained, then the average D_{LCO} value of the manoeuvres with grades B, C or D is reported. 4) If only grade F manoeuvres are obtained, then no D_{LCO} value is reported.

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

The value of D_{LCO} depends upon a number of physiological factors. Besides varying with age, sex, height and possibly ethnicity, D_{LCO} also changes with Hb, lung volume, COHb, P_{IO_2} (inspired oxygen tension, *e.g.* altitude), exercise and body position. Although these effects may cause changes in D_{LCO} in opposite directions [111], all should be considered in interpreting the observed carbon monoxide uptake. It is recommended that adjustments for these factors be made in the predicted rather than the measured D_{LCO} value. The predicted D_{LCO} value is derived from measurements in normal subjects who are disease free, have normal Hb levels and minimal COHb, are sitting at rest, and breathing room air. If any of these conditions are not met, then the predicted value should be adjusted accordingly.

Adjustment for haemoglobin

Since carbon monoxide–Hb binding is such an important factor in carbon monoxide transfer, D_{LCO} changes can be substantial as a function of Hb concentration [111–115]. The empirical effect upon D_{LCO} with change in Hb closely matches what is expected from a theoretical approach using the relationship in equation 3, with θ assumed to be proportional to Hb, $D_M/\theta V_C$ assumed to be 0.7 [113] and the "standard" Hb value assumed to be 14.6 g·dL^{-1} (9 mmol·L^{-1}) in adult and adolescent males and 13.4 g·dL^{-1} (8.26 mmol·L^{-1}) in adult females and children <15 years old. Using these relationships and expressing Hb in g·dL^{-1}, the predicted D_{LCO} in adolescents and adult males can be adjusted using equation 31, while that in children <15 years of age and females is adjusted using equation 32. Results from a more recent study in patients with a wide range of Hb abnormalities [115] show a slightly greater and more linear relationship; however, corrected values are generally consistent with equations 31 and 32.

$$D_{LCO}[\text{predicted for Hb}] = D_{LCO}[\text{predicted}] \cdot (1.7\text{Hb}/(10.22 + \text{Hb})) \quad (31)$$

$$D_{LCO}[\text{predicted for Hb}] = D_{LCO}[\text{predicted}] \cdot (1.7\text{Hb}/(9.38 + \text{Hb})) \quad (32)$$

The measurement of Hb in the American population [116] found deviation from these standard values, especial in males, children and seniors; differences were also found between Caucasian and African-Americans. Furthermore, the survey found that Hb levels in the general population are changing over time. If a more appropriate reference Hb level (Hb_{ref}) is available then the predicted D_{LCO} is adjusted using equation 33.

$$D_{LCO}[\text{predicted for Hb}] = D_{LCO}[\text{predicted}] \cdot (1.7\text{Hb}/(0.7\text{Hb}_{\text{ref}} + \text{Hb})) \quad (33)$$

Adjustments for alveolar oxygen tension

As noted previously, P_{AO_2} affects the measurement of D_{LCO} and changes to P_{AO_2} (such as supplemental oxygen breathing that gives higher P_{AO_2} values) will have a correlating effect on D_{LCO} values. The value of D_{LCO} will change by ~0.35% for each 1 mmHg change in P_{AO_2} or ~2.6% for each 1 kPa change in P_{AO_2} [117, 118]. Adjustments to the predicted D_{LCO} in a subject on supplemental oxygen may be made using a measured P_{AO_2}, where $P_{AO_2}=F_{IO_2}\cdot(P_B-47)$ and assuming a normal P_{AO_2} in room air at a sea level of 100 mmHg (13.3 kPa). This is shown in equation 34 below or equation 35 if SI units are preferred.

$$D_{LCO}[\text{predicted for elevated } P_{AO_2}] \approx D_{LCO}[\text{predicted}]/(1.0 + 0.0035(P_{AO_2} - 100)) \quad (34)$$

$$T_{LCO}[\text{predicted adjusted for } P_{AO_2}] \approx T_{LCO}[\text{predicted}]/(1.0 + 0.026(P_{AO_2} - 13.3)) \quad (35)$$

Some pulmonary function systems include measurement of carbon dioxide. In these systems, the end-expiratory carbon dioxide concentration can be used to estimate the alveolar oxygen partial pressure using the simplified alveolar gas equation. In patients who have higher carbon dioxide levels (higher P_{ACO_2}) and consequently lower P_{AO_2} values, the predicted D_{LCO} can be corrected to compensate for the increase in D_{LCO} that arises. For example, at a barometric pressure of 760 mmHg (101.3 kPa), if the P_{ACO_2} in a patient retaining carbon dioxide was 50 mmHg (6.67 kPa) then the P_{AO_2} would be 86 mmHg (11.5 kPa) and the predicted D_{LCO} would be 4.8% higher than if the P_{ACO_2} were 40 mmHg (5.33 kPa). However, there are many assumptions inherent in this approach and more research is needed to determine the validity of such an adjustment.

Adjustment for carboxyhaemoglobin concentration and carbon monoxide back-pressure

As noted previously, COHb can affect the measured uptake of carbon monoxide in the following two ways [119–121]. First, by occupying Hb binding sites, carbon monoxide produces an "anaemia effect". Secondly, carbon monoxide partial pressure in the blood will reduce the driving pressure for carbon monoxide transport from alveolar gas to capillary blood. Exposure to ordinary environmental carbon monoxide and endogenous production of carbon monoxide as a byproduct of Hb catabolism commonly results in measured COHb levels of 1–2% [119]. However, cigarette smoke and other environmental sources can produce measurable levels of carbon monoxide back-pressure and COHb that may need to be considered in the measurement of carbon monoxide uptake [119].

The inhalation of carbon monoxide in the single-breath manoeuvre causes COHb to increase by 0.6–0.7% for each manoeuvre [66, 82]. The adjustment of the predicted D_{LCO} value for carbon monoxide was found to be −0.938% per 1.0% increase in COHb [122]. For RGA systems, carbon monoxide back-pressure can be measured in expired gas prior to the inspiration of test gas in the D_{LCO} manoeuvre [62] and can be compensated for analytically. For classical systems, carbon monoxide back-pressure can be estimated using one of several available techniques [121, 123–125]. For example, carbon monoxide back-pressure can be calculated from COHb using equation 36, where COHb and O_2Hb are the fractions of carbon monoxide and oxygen-bound haemoglobin, respectively.

$$F_{ACO} = (\mathrm{COHb}/\mathrm{O_2Hb}) \cdot (F_{AO_2})/210 \quad (36)$$

D_{LCO} can then be recalculated after subtracting the estimated carbon monoxide back-pressure from both the initial and final alveolar carbon monoxide partial pressures (units must be consistent before making the subtraction). Unfortunately, this method will not adjust D_{LCO} for the "anaemia effect" of COHb; however, several studies have evaluated both the empirical and theoretical effects of COHb on D_{LCO} and incorporated both the back-pressure and the "anaemia effect" of COHb into the adjustment. In general, a 1% increase in COHb reduces the measured D_{LCO} by ~0.8–1% from both effects [16, 17]. Using this approach, equation 37 empirically reduces predicted D_{LCO} by 1% for each percentage point of COHb >2%.

$$D_{LCO}[\text{predicted for COHb}] = D_{LCO}[\text{predicted}] \cdot (102\% - \mathrm{COHb}\%) \quad (37)$$

A more recent study using an RGA system to measure alveolar carbon monoxide concentration, combined with venous measurement of COHb, found that the effect of carbon monoxide back-pressure and the "anaemia effect" are almost equal and the combined effect is a 2% decrease in D_{LCO} for each 1% increase in COHb [62]. These findings were verified in a discrete sample system [66]. In these studies, where the carbon monoxide back-pressure was measured and used in the calculation of D_{LCO}, equation 38 was used to further correct for the "anaemia effect" where F_{ACOb} is the alveolar carbon monoxide fraction in ppm measured at the end of exhalation to residual volume, just prior to the inhalation of test gas.

$$D_{LCO}[\text{corrected}] = D_{LCO} \cdot (1 + F_{ACOb}/560) \quad (38)$$

As endogenous COHb (1–2%) was present in the healthy nonsmoking subjects from whom prediction equations were generated, an adjustment for COHb is only recommended for interpretative purposes when COHb levels are known to be elevated or levels above 2% are suspected. Methaemoglobin (MetHb) will not bind carbon monoxide meaning there is, effectively, a reduced amount of haemoglobin available and leading to a similar "anaemia effect". Since there is effectively less Hb to bind with carbon monoxide during the D_{LCO} manoeuvre, the measured D_{LCO} is reduced. An adjustment for MetHb has been proposed [126] and is shown in equation 39.

$$\mathrm{Hb}[\text{adjusted}] = \mathrm{Hb} \cdot (100 - \mathrm{MetHb}/100) \quad (39)$$

Adjustment of D_{LCO} for barometric pressure

For factors such as Hb that are related to the individual subject, the recommended adjustment is made to the predicted D_{LCO} value. However, barometric pressure (P_B) is an environmental factor that is

independent of the individual and therefore the adjustment should be made to the measured D_{LCO} value to simulate standard pressure conditions. The variation in D_{LCO} due to the typical range in high and low pressure cells at a given altitude is approximately ±1.5%. P_B decreases with altitude (such that P_{IO_2} decreases) and D_{LCO} increases by about 0.53% for each 100 m of increase in altitude. Moreover, the applicability of using a reference value data set from a different location is improved if both the measured D_{LCO} and the predicted value of D_{LCO} are adjusted to standard pressure (760 mmHg or 101.3 kPa). The adjustment for P_B [4, 117] assumes a P_{IO_2} of 150 mmHg (20 kPa) at standard pressure and can be calculated using equations 40 (P_B in mmHg) and 41 (P_B in kPa).

$$D_{LCO}[P_B \text{ adjusted}] \approx D_{LCO}(0.505 + 0.00065\,P_B) \quad (40)$$

$$D_{LCO}[P_B \text{ adjusted}] \approx D_{LCO}(0.505 + 0.00488\,P_B) \quad (41)$$

For D_{LCO} reference values that do not provide P_B data, the altitude of the centre in which the reference values were obtained can be used to estimate P_B [127] using equations 42 and 43 where a is the altitude above sea level in metres. It should be noted that the relationship between D_{LCO} and P_B has not been confirmed using an RGA system. Further research is needed to validate the use of equations 40 and 41.

$$P_B[\text{mmHg}] = 760\ (1 - 2.25577 \cdot 10^{-5} \cdot a)^{5.25588} \quad (42)$$

$$P_B[\text{kPa}] = 101.325\ (1 - 2.25577 \cdot 10^{-5} \cdot a)^{5.25588} \quad (43)$$

Reporting values

This document is intended to establish technical standards which, in terms of reporting, will require D_{LCO} systems to be able to report the variables listed in table 4. It is not intended to specify which variables end users should include in the report forms used in their laboratories, nor is it intended to address the interpretation of D_{LCO}. Although work is ongoing towards establishing a standardised pulmonary function laboratory report form, there is no current standard. A D_{LCO} system must be able to report the unadjusted measured D_{LCO}, the D_{LCO} adjusted for P_B, the lower limit of normal and z-score, predicted, and percentage of predicted D_{LCO}, K_{CO}, the lower limit of normal and z-score, predicted, and percentage of predicted K_{CO}. Any adjustments (*e.g.* for Hb, COHb, P_{IO_2} or lung volume) must also be reported along with the data used to make them. The average V_A must be reported along with the predicted V_A (the predicted TLC minus the predicted V_D) and percentage of predicted V_A. If available, a separately measured TLC and V_A/TLC ratio may be reported, although this is optional. The average V_I must also be noted. If a separately measured VC is available, it must be reported to serve as a reference for the adequacy of V_I. In addition, comments relevant to the quality of the measurements recorded must be included. A complete list of specifications for which variables and measurements that *D*LCO systems are able to report is given in table 4. While the use of z-scores is favoured in the interpretation of pulmonary function results, given the continuing use of "percentage of predicted" values in many laboratories, the ability to report both z-scores and percentage of predicted values is recommended.

Specifications for reports and output of results

Although standardised reporting forms are being considered, manufacturers have diverse options for reporting of results. This is due, in a large measure, to the insistence of various pulmonary function laboratories on having a customised report that matches their historic format. A common option for accommodating electronic medical records, is the provision of a pdf document; however, a universal format for data output in the form of a csv or xml formatted data file has been proposed. This format should include the results and demographic/environmental data for each test in a format that will permit the user to import data to and their own reporting formand export to their particular electronic record. The data file must include all data necessary to calculate the variables listed in table 4. For RGA systems, the data arrays for flow, carbon monoxide and tracer gas must be included and must be adjusted for auto-zero and calibration factors, with the optimal shift applied to the concentration data. Flow data must be converted to BTPS conditions and the data must include the equipment dead-space, the washout volume, the alveolar sample volume, the barometric pressure and the carbon monoxide and tracer gas concentrations at end-exhalation prior to the inhalation of test gas. Manufacturers must provide the format details to permit users to import the data. The complete specifications for the data file are given in the supplementary materials.

TABLE 4 D_{LCO} reporting requirements

Variable[#]	Requirement
D_{LCO} (unadjusted)	Required
D_{LCO} (adjusted for P_B)	Required
D_{LCO} (LLN and/or z-score)	Required
D_{LCO} (predicted)	Required
D_{LCO} (adjusted,predicted)	Optional (required if any adjustments made-specify adjustments)
D_{LCO} (% of predicted)	Required
V_A (BTPS)	Required
V_A (LLN and/or z-score)	Required
V_A (% of predicted)	Optional
K_{CO}	Required
K_{CO} (LLN and/or z-score)	Required
K_{CO} (predicted)	Required
K_{CO} (% of predicted)	Required
P_B	Required
t_{BH}	Required
V_I (BTPS)	Required
Fowler (anatomic) dead-space	Required for RGA systems
TLC_{sb}	Required for RGA systems
Reference values source	Required
Test quality grade	Recommended (include % variability in D_{LCO} acceptable manoeuvres)
Operator comments	Required (number of manoeuvres, number of acceptable manoeuvres)
Graphs	Required (full manoeuvre and exhaled gas concentration *versus* volume with sample collection indicated for RGA systems)
Hb	Optional (required if used to adjust D_{LCO})
COHb	Optional (required if used to adjust D_{LCO})
Alternative calculations (*e.g.* three-equation D_{LCO}, normalised slope of phase III)	Optional

BTPS: body temperature, ambient pressure, saturated with water vapour; LLN: lower limit of normal; D_{LCO}: diffusing capacity of the lung for carbon monoxide; V_A: alveolar volume; K_{CO}: transfer coefficient of the lung for carbon monoxide; P_B: barometric pressure; t_{BH}: breath-hold time; V_I (BTPS): inspired volume under BTPS conditions; V_A (BTPS): alveolar volume under BTPS conditions; TLC_{sb}: single-breath total lung capacity; Hb: haemoglobin; COHb: carboxyhaemoglobin; RGA: rapidly responding gas analyser. [#]: for D_{LCO}, V_A, K_{CO}, t_{BH}, V_I, V_{Danat} and TLC_{sb} the average values from the acceptable and repeatable manoeuvres are reported.

Reference values

The Global Lung Health Initiative (GLI) is currently working on the development of global reference values for D_{LCO} which will very likely be in a similar structure to the GLI spirometry reference values [128]. Implementation of these reference values requires more complexity than simply inserting coefficients for polynomials and a D_{LCO} system must be able to implement this method of calculating reference values. A list of reference values for D_{LCO} developed using methods that adhere to the 2005 D_{LCO} standards is provided in table 5 [4].

Summary

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

As already noted, the changes in D_{LCO} standards will not impact the applicability of reference values. In general, pulmonary function measurements are more accurate and precise in normal, healthy subjects than in patients with lung disease so that changes which improve the measurement of D_{LCO} will have less impact on normal, healthy subjects, which favours the continued applicability of reference values derived using older systems. There are already systematic differences among reference value sets for D_{LCO} which are related to the equipment and methodology which impact their applicability. Some reference values

TABLE 5 Reference values for D_{LCO} from studies that complied with the 2005 American Thoracic Society/European Respiratory Society D_{LCO} standards

Author[#]	Year	Country	Age	Subjects
Thompson [129]	2008	Australia	45–71 years	498 male/474 female
Koopman[¶] [130]	2011	Netherlands	7–18 years	278 male/265 female
Garcia-Rio[¶] [131]	2012	Spain	65–85 years	169 male/262 female
Kim [132]	2012	USA and Australia	5–19 years	225 male/254 female
Thomas [133]	2014	Denmark	5–17 years	male/female (297 total)
Michailopoulos [134]	2015	Greece	18–91 years	234 male/233 female
Verbanck [135]	2016	Belgium	20–80 years	128 male/124 female

D_{LCO}: diffusing capacity of the lung for carbon monoxide. [#]: only studies with at least 100 males and 100 females are included. All of these reference values were derived using caucasian subjects. [¶]: test gas contained 19% oxygen (all other studies used test gas with 21% oxygen).

currently in use were developed prior to the publication of the 2005 ATS/ERS standards [4]. Hence, there is already a pressing need for reliable, comprehensive reference values for D_{LCO}.

Advances in technology have outpaced guidelines and standards. These revisions to the D_{LCO} standards are required to make optimal use of existing, clinically available technology. Guidelines and standards should not constrain progress in the improvement of pulmonary function measurements but should serve to continually improve the quality of D_{LCO} measurements.

Recommendations for research directions

In the course of developing these technical standards, the following areas were identified as candidates for research studies to fill knowledge gaps and provide more specific guidelines.

1) Conduct studies of D_{LCO} in normal, healthy subjects in ethnicities other than Caucasian over a wide age range to either validate the use of Caucasian reference values or develop ethnicity-specific reference values.
2) Develop a standardised common report form for pulmonary function testing that can be the default for all laboratories and electronic medical record systems.
3) Determine the effect of barometric pressure on D_{LCO} in normal subjects and COPD patients over a range of pressures reflecting altitudes from sea level to 2500 m to either confirm or replace equations 40 and 41.
4) Determine the effects of obesity on V_{Danat}, TLC_{sb} and D_{LCO}.
5) Determine whether Hb measured by skin prick or venipuncture is more appropriate for D_{LCO} adjustment and conduct studies to confirm or revise the relationship between Hb and D_{LCO} expressed in equations 31 and 32.
6) Determine normal Hb levels in populations of different ethnicity and geographical location.
7) Test the proposed D_{LCO} grading scale in large clinical databases for both classical and RGA D_{LCO} systems.
8) Determine the impact of carbon dioxide retention on D_{LCO} measurements.
9) Determine the sensitivity and action levels for the assessment of gas analyser linearity using the dilution of test gas in a calibration syringe.
10) Determine the repeatability of calculating V_A and TLC_{sb} by the method in equation 26 which uses all of the tracer gas concentration data throughout the manoeuvre.

In addition to these research directions, there is also a need to update the guidelines for the interpretation of pulmonary function tests in general and of D_{LCO} in particular.

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SERIES "ATS/ERS TASK FORCE: STANDARDISATION OF LUNG FUNCTION TESTING"
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Standardisation of the single-breath determination of carbon monoxide uptake in the lung

N. MacIntyre, R.O. Crapo, G. Viegi, D.C. Johnson, C.P.M. van der Grinten, V. Brusasco, F. Burgos, R. Casaburi, A. Coates, P. Enright, P. Gustafsson, J. Hankinson, R. Jensen, R. McKay, M.R. Miller, D. Navajas, O.F. Pedersen, R. Pellegrino and J. Wanger

CONTENTS

KEYWORDS: Alveolar-capillary permeability, carbon monoxide, carbon monoxide diffusing capacity of the lungs, carbon monoxide transfer factor of the lungs, gas exchange, inspiratory manoeuvres

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BACKGROUND

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

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

Definitions

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

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

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

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

Determinants of CO uptake

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

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

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

A number of physiological changes can affect D_M or θV_c to influence $D_{L,CO}$. As the lung inflates, D_M increases (due to unfolding membranes and increasing surface area), while V_c effects are variable (due to differential stretching and flattening of alveolar and extra-alveolar capillaries) [10, 17–24]. The net effect of these changes is that $D_{L,CO}$ tends to increase as the lung inflates. Exercise, the supine position and Mueller manoeuvres (inspiratory efforts against a closed glottis) can all recruit and dilate alveolar capillaries, thereby increasing V_c and $D_{L,CO}$ [25–31]. Alveolar-capillary recruitment also occurs in the remaining lung tissue following surgical resection, since the cardiac output now flows through a smaller capillary network. This causes a less than expected loss of V_c for the amount of lung tissue removed. In contrast, Valsalva manoeuvres (expiratory efforts against a closed glottis) can reduce V_c and thereby reduce $D_{L,CO}$ [29].

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

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

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

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

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

GAS ANALYSERS AND GENERAL EQUIPMENT

System design

Descriptions of the apparatus and general instructions for performing the single-breath diffusing capacity manoeuvre are available elsewhere [2, 44–48]. Equipment in clinical use varies widely in complexity, but the basic principles are the same. All systems have a source of test gas (bag-in-box, spirometer, compressed gas cylinder), a method for measuring inspired and expired volume over time (spirometers with kymographs, pneumotachometers near the mouthpiece or near a bag-in-box), and gas analysers (single-sample analysers or continuous high-speed analysers). Single-sample gas-analyser systems usually display only volume over time (fig. 1a). Continuous gas-analyser systems also provide a continuous tracing of CO and tracer gas concentrations during the test (fig. 1b).

Equipment requirements

Performance standards for equipment

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

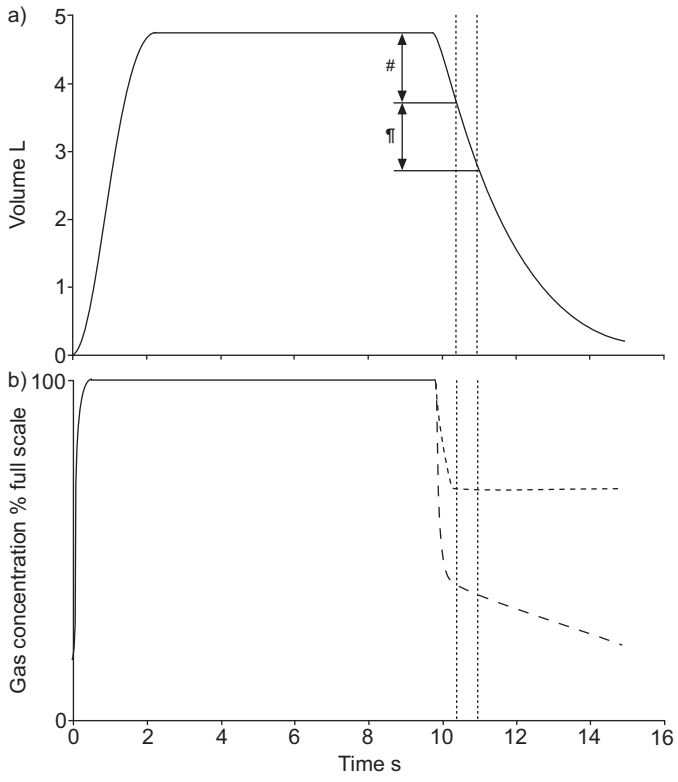

FIGURE 1. Schematic of lung volume (a) and gas concentrations (b) during the single-breath diffusing capacity of the lung for carbon monoxide. The gas-sampling period occurs between the two dotted lines. -----: tracer gas; – – –: carbon monoxide. #: dead space washout; ¶: sample collection. Modified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

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

Equipment quality control

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

TABLE 2 Equipment specifications

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

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

TABLE 3 Equipment quality control

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

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

Infection control

The major goal of infection control is to prevent the transmission of infection to patients and staff during pulmonary function testing. The recommendations in the ATS/ERS documents for spirometry and general considerations for pulmonary function testing also apply to $D_{L,CO}$ equipment and procedures [49, 56].

SINGLE-BREATH TESTING TECHNIQUE STANDARDISATION ISSUES

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

Patient conditions for measurement

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

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

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

Inspiratory manoeuvre

Once the mouthpiece and nose clip are in place, tidal breathing should be carried out for a sufficient time to assure that the subject is comfortable with the mouthpiece. Deep inspirations should be avoided during this period as they can increase subsequent CO uptake [61]. The $D_{L,CO}$ manoeuvre begins with unforced exhalation to residual volume (RV). In obstructive lung disease, where exhalation to RV may require a prolonged period, a reasonable recommendation is that this portion of the manoeuvre should be limited to 6 s, a time consistent with using the forced expiratory volume in six seconds manoeuvre as a surrogate for VC [49]. At RV, the subject's mouthpiece is connected to a source of test gas, and the subject inhales rapidly to TLC.

A submaximal inspired volume (*i.e.* less than the known VC) can affect CO uptake, depending upon whether it is a result of an initial suboptimal exhalation to RV (test performed at TLC) or whether it is due to a suboptimal inhalation from RV (test performed below TLC) [19–22]. In the former case, the calculated V_A and $D_{L,CO}$ will accurately reflect lung volume and the CO uptake properties of the lung at TLC. In the latter case, the V_A will be reduced and $D_{L,CO}$ measurement will be affected (see Adjustment for lung volume section).

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

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

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

Condition of the breath-hold and expiratory manoeuvre

Valsalva (expiratory efforts against a closed airway) and Muller manoeuvres (inspiratory efforts against a closed airway) during the breath-hold, by decreasing and increasing thoracic blood volume, respectively, will decrease and increase $D_{L,CO}$, respectively [29, 71, 72] The intrapulmonary pressure during the breath hold should thus be near atmospheric, and this is best accomplished by having the subject voluntarily maintain full inspiration using only the minimal effort necessary. The breath-hold time should be 10 ± 2 s, a target easily achieved in the vast majority of subjects [62].

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

Washout and sample collection volume

The $D_{L,CO}$ calculations (see Calculations section) require alveolar gas samples. During expiration, a volume of gas must be expired and discarded to clear anatomic and mechanical V_D before the alveolar sample is collected (fig. 1). Contamination of the alveolar gas sample with V_D gas will cause an underestimation of true CO uptake. In general, the washout volume should be 0.75–1.0 L (BTPS). If the patient's VC is <2.00 L, the washout volume may be reduced to 0.50 L. Newer devices can provide a graphical display of exhaled gas concentrations to assure that V_D gas is not present in the alveolar sample (fig. 1). Using such an analyser, HUANG *et al.* [71] showed that the standard approach noted above adequately cleared V_D in >90% of adults.

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

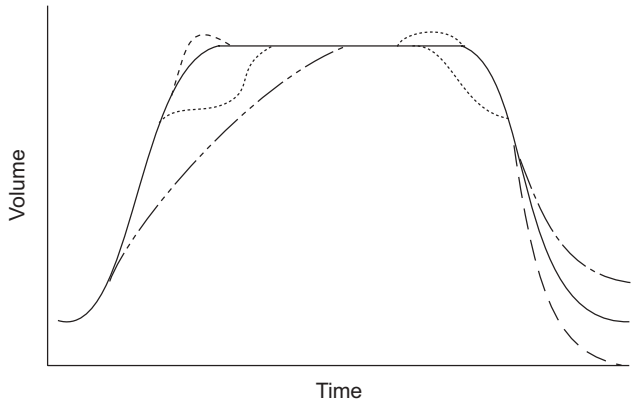

FIGURE 2. Potential problems with the single-breath diffusing capacity of the lung for carbon monoxide breathing manoeuvre that can lead to measurement errors. ·····: stepwise inhalation or exhalation; – - –: exhaled gas leak; – -- –: inhalation too slow; – – –: exhaled volume larger than inhaled volume;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

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

Inspired gas composition

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

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

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

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

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

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

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

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

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

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

Interval between tests

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

Miscellaneous factors

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

CALCULATIONS

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

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

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

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

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

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

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

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

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

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

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

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

Calculating breath-hold time

The ''breath-hold time'' or time of transfer during which CO changes from its initial to final concentration is in the denominator of the $D_{L,CO}$ equation (equation 7). As noted previously, the single-breath measurement of CO uptake assumes an ''instantaneous'' lung filling and emptying process. However, both inspiration and expiration require up to several seconds, and these periods of changing gas volume in the lung must be accounted for in the calculations. For purposes of standardisation, the method by JONES and MEADE [68] (fig. 3) is recommended, since it has the theoretical appeal of empirically accounting for the effects of inspiratory and expiratory time. This method has also been shown to adequately address inspiratory flows as low as 1 L·s^{-1}, breath-hold times as short as 5 s, and expiratory flows as low as 0.5 L·s^{-1} in normal subjects [64].

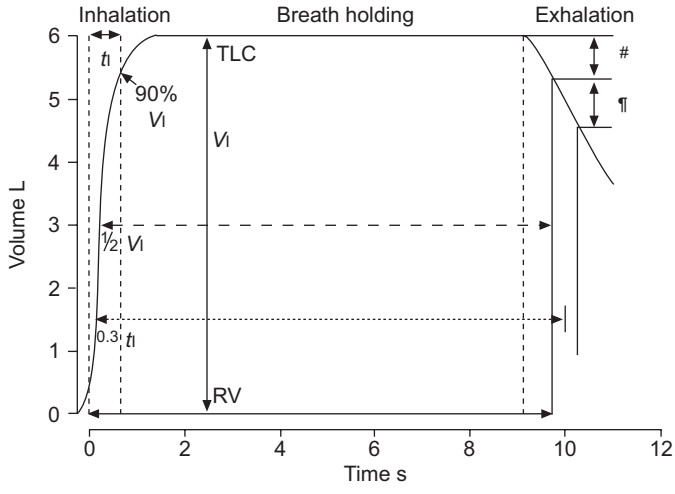

FIGURE 3. Schematic illustration of different methods of measuring breath-hold time for the single-breath diffusing capacity of the lung for carbon monoxide. The method by OGILVIE (———) [48] measures breath-hold time from the beginning of inspiration to the beginning of alveolar sample collection. The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

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

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

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

Calculating the alveolar volume

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

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

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

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

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

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

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

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

where height is measured in cm, or:

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

where height is measured in inches.

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

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

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

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

Inspired gas conditions

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

CO_2, H_2O and temperature adjustment for V_A calculations

Exhaled gas contains CO_2 and H_2O, which were not present in the test gas mixture. As noted previously, some systems remove one or both of these if they interfere with analyser function, and this will raise both CO and tracer gas concentrations. Under these circumstances, adjustments are required for the increase in $F_{A,Tr}$ to calculate V_A (table 5). However, no adjustment for the increase in alveolar inspired CO fraction at time t ($F_{A,CO,t}$) and $F_{A,Tr}$ is necessary in calculating the rate of CO uptake, since the concentration factor appears in both the numerator and the denominator of the expression ($F_{A,CO,0}/F_{A,CO,t}$) and therefore cancels.

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

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

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

Acceptability, repeatability and number of tests

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

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

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

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

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

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

H_2O and CO_2 removed from sampled gas

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Adjustment for haemoglobin

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

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

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

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

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

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

Adjustments for P_{A,O_2}

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

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

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

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

Adjustment for COHb concentration and CO back pressure

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

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

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

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

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

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

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

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

Adjustment for lung volume

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

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

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

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

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

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

Reporting values

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

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

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

ABBREVIATIONS

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

TABLE 7 List of abbreviations and meanings

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

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Idiopathic pulmonary fibrosis

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ABSTRACT

Idiopathic pulmonary fibrosis is a prototype of chronic, progressive, and fibrotic lung disease. Healthy tissue is replaced by altered extracellular matrix and alveolar architecture is destroyed, which leads to decreased lung compliance, disrupted gas exchange, and ultimately respiratory failure and death. In less than a decade, understanding of the pathogenesis and management of this disease has been transformed, and two disease-modifying therapies have been approved, worldwide. In this Seminar, we summarise the presentation, pathophysiology, diagnosis, and treatment options available for patients with idiopathic pulmonary fibrosis. This disease has improved understanding of the mechanisms of lung fibrosis, and offers hope that similar approaches will transform the management of patients with other progressive fibrotic lung diseases.

On the role of abnormal DLCO in ex-smokers without airflow limitation: symptoms, exercise capacity and hyperpolarised helium-3 MRI

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ABSTRACT

Background
The functional effects of abnormal diffusing capacity for carbon monoxide (DLCO) in ex-smokers without chronic obstructive pulmonary disease (COPD) are not well understood. Objective We aimed to evaluate and compare well established clinical, physiological and emerging imaging measurements in ex-smokers with normal spirometry and abnormal DLCO with a group of ex-smokers with normal spirometry and DLCO and ex-smokers with Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage I COPD.

Methods
We enrolled 38 ex-smokers and 15 subjects with stage I COPD who underwent spirometry, plethysmography, St George's Respiratory Questionnaire (SGRQ), 6 min Walk Test (6MWT), x-ray CT and hyperpolarised helium-3 (3He) MRI. The 6MWT distance (6MWD), SGRQ scores, 3He MRI apparent diffusion coefficients (ADC) and CT attenuation values below −950 HU (RA950) were evaluated.

Results
Of 38 ex-smokers without COPD, 19 subjects had abnormal DLCO with significantly worse ADC (p=0.01), 6MWD (p=0.008) and SGRQ (p=0.01) but not RA950 (p=0.53) compared with 19 ex-smokers with normal DLCO. Stage I COPD subjects showed significantly worse ADC (p=0.02), RA950 (p=0.0008) and 6MWD (p=0.005), but not SGRQ (p=0.59) compared with subjects with abnormal DLCO. There was a significant correlation for 3He ADC with SGRQ (r=0.34, p=0.02) and 6MWD (r=−0.51, p=0.0002).

Conclusions
In ex-smokers with normal spirometry and CT but abnormal DLCO, there were significantly worse symptoms, 6MWD and 3He ADC compared with exsmokers with normal DLCO, providing evidence of the impact of mild or early stage emphysema and a better understanding of abnormal DLCO and hyperpolarised 3He MRI in ex-smokers without COPD.

Diffusion Capacity and Mortality in Patients With Pulmonary Hypertension Due to Heart Failure With Preserved Ejection Fraction

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ABSTRACT

OBJECTIVES
This study sought to investigate the prognostic importance of a low diffusion capacity of the lung for carbon monoxide (DLCO) in patients with a catheter-based diagnosis of pulmonary hypertension due to heart failure with preserved ejection fraction (PH-HFpEF).

BACKGROUND
In patients with pulmonary arterial hypertension, a low DLCO is associated with poor outcome. It is unclear whether the same is true in patients with PH-HFpEF.

METHODS
This study retrospectively analyzed clinical characteristics, smoking history, lung function measurements, chest computed tomography, hemodynamics, and survival in 108 patients with PH-HFpEF. The presence of post-capillary PH was determined by right heart catheterization. Patients with moderate or severe lung function abnormalities were excluded.

RESULTS
On the basis of previous studies and receiver-operating characteristic curve analysis, the study cohort was divided into patients with a DLCO <45% of the predicted value (DLCO<45%, low DLCO; n ¼ 52) and patients with a DLCO $45% of the predicted value (DLCO$45%; n ¼ 56). DLCO<45% was associated with male sex (odds ratio [OR]: 2.71; 95% confidence interval [CI]: 1.05 to 6.99; p ¼ 0.039) and smoking history (OR: 5.01; 95% CI: 1.91 to 13.10; p < 0.001). There were no correlations between DLCO and other lung function parameters and hemodynamics. Compared with patients with DLCO$45%, patients with DLCO<45% had a significantly worse outcome (survival rate at 3 years 36.5% vs. 87.8%, p < 0.001 by log-rank analysis). Cox proportional hazard analysis identified DLCO<45% as an independent predictor of death (hazard ratio: 6.6; 95% CI: 2.6 to 16.9; p < 0.001).

CONCLUSIONS
In patients with PH-HFpEF, a low DLCO is strongly associated with mortality. (J Am Coll Cardiol HF 2016;-:-–-)