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Standardization of *In-Vitro* Evaluation of Extracorporeal Life Support (ECLS) Devices for Research and Development

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Abstract

Extracorporeal life support (ECLS) technology has witnessed remarkable advancements during the last decades. However, further research and development of devices are required to increase, for example, performance-efficiency, hemocompatibility, and long-term stability. All novel devices, even in early research stages, must undergo rigorous testing and evaluation. Yet, these early evaluations are often conducted under nonstandardized conditions, resulting in data difficult to compare, interpret, or translate into clinical practice. Establishing well-defined, standardized *in-vitro* testing protocols for all ECLS components and devices would represent a major step forward. Such protocols would improve methodological consistency and ensure reproducibility across research groups. This document, developed by an international group of ECLS experts from all disciplines in which such components are designed, developed, and applied, provides clear recommendations and standardized criteria for device testing according to international norms. Adoption of these criteria including the ways of reporting results will foster a unified approach among scientists, engineers, clinicians, and the medical device industry. Ultimately, this common framework will facilitate data interpretation, improve comparability of study results between different groups, making the review of studies more straightforward, as not every aspect of testing requires additional review and discussion, certainly favoring decision-making in the development and application of ECLS technologies.

Keywords: standardization; *in-vitro* testing; extracorporeal membrane oxygenation; ECMO; extracorporeal life support; ECLS; oxygenator; pump; cannula; tubing; blood cell damage.

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The development and extended use of extracorporeal life support (ECLS) has introduced new dimensions in the research of advanced devices. These include blood pumps, hollow fiber membrane lungs (also referred to as oxygenators or blood gas exchangers), 3D membranes, microfluidic membrane lungs, tubing, and cannulae. All these developments demand thorough *in-vitro* verification and *in-vivo* validation testing to prove their proper functioning, efficacy, and safety. *In-vitro* testing aims to evaluate devices in a standardized, reproducible manner across different working points. In contrast, *in-vivo* testing evaluates the device in an environment that should as much as possible reflect its intended use and models the interaction between the living organism and the device. Therefore, the working points in *in-vivo* testing are determined by the physiological needs of the organism and cannot be fully standardized.

Performing *in-vitro* tests in a standardized and reproducible manner enables the comparison of novel devices originating from different research groups, as well as with commercially available devices. It also allows for comparisons across various device sizes and types, provided that results are reported in a standardized format—using units that are not specific to a particular device or size but are broadly comparable. Finally, standardized *in-vitro* testing facilitates both clinical interpretation and the critical appraisal of study quality by peer reviewers.

In the industry, such standards are well-established and globally applied to test devices for market approval. They are developed by experts of the International Organization for Standardization (ISO), endorsed by national standardization bodies, and reviewed every 5 years, updating them as needed to reflect current technological advancements. In the field of ECLS and devices for extracorporeal circulatory support devices, ISO standards specify which tests must be conducted, how they should be conducted, the required duration of testing, the timing and frequency of sample collection, the appropriate test fluids for various assessments, and, in some cases, provide informative guidance on test setups. These standards can also be readily applied in research. However, some requirements such as the proper labeling of the device, primary and secondary packaging, or which information needs to be included in the instructions for use (IfU), may be omitted by researchers during early stages of product development, as they are not essential for preliminary evaluation.

If a device requires deviation from standardized tests (*i.e.*, due to its innovative nature), a clear and well-justified rationale should be provided. Nevertheless, testing should adhere to the standards as closely as possible.

To summarize, internationally accepted test standards should be accepted practice in ECLS research aiming at device prototype development and provide significant advantages (Figure 1)

- the tests and their results are reproducible,
- test setups do not need to be reinvented by each group but can be adapted to the individual device,
- results are universally, directly comparable with other group's reports and commercial devices,
- involving clinicians and translation to industry is easier as tests match those of already marketed devices, and
- the review of articles is more straightforward, as not every aspect of the testing requires additional review and discussion.

In general, a detailed description of the device and the test circuit setup should be provided for all tests. Where possible, this should include both figures and text specifying, for example, the location of temperature, flow, and pressure probes, the materials and the test fluids used, including their respective densities and dynamic

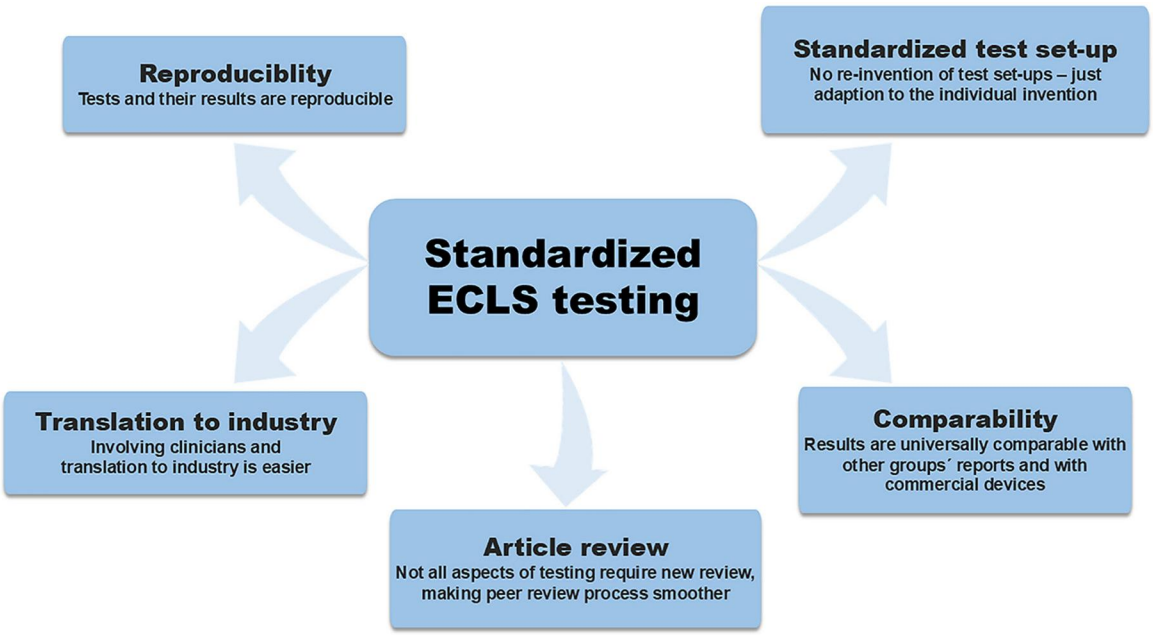

Figure 1. Advantages of standardized testing in ECLS research outlining the aspects of standardized test setup, comparability, reproducibility, article review, and translation to industry. ECLS, extracorporeal life support.

viscosities at the test temperature. The test flow rate and corresponding pressure measurements should be reported, along with the flow profile in case of pulsatile flow.

In the following sections, the different test standards for ECLS devices and recommendations for reporting of results are introduced, including guidance on the selection of appropriate test fluids. This information is intended to support researchers in planning tests of their novel device, to assist reviewers in evaluating the quality of information provided in publications, and to help clinicians better understand published results (for a comprehensive overview, see Table 1). In this study, our research group developed a checklist of items that should be reported to ensure adherence to standardized in-vitro testing of ECLS devices in research (Table 1).

CANNULAE

The testing of cannulae is defined in ISO 18193-Cardiovascular implants and artificial organs-Cannulae for extracorporeal circulation (current version: ISO 18193:2021 and ISO 18193:2021/Amd 1:2025). The standard requires testing of blood pathway integrity, kink resistance, pull strength, connector integrity, radiodetectability, pressure drop, collapse resistance, blood cell damage, integrity (corrosion, abrasion, degradation), among other aspects. For novel devices, the most important tests of the standard are pressure drop, flow characterization, recirculation (for dual-lumen cannula [DLC]), and blood cell damage (see Blood Cell Damage According to ISO Standards).

The cannula's material, including its material properties, as well as its geometrical characteristics, must be reported to enable comparison of experimental and/or numerical results across different research groups. In accordance with ISO 18193, this encompasses the inner and outer diameter of the cannula, along with the number of side holes (depending on the design), side hole diameter, the position, and length over which the side holes are distributed. These items should be clearly indicated in a figure and provided in the text. The positioning of measuring devices (flow and pressure probes) should be detailed, including the distance from the cannula's inlet and outlet.

Definitions

- Lumen = internal channel allowing for flow. Drainage lumen may in a DLC consist of one proximal genuine lumen (venopulmonary [VP] DLC), or a combination of a proximal and distal drainage sites (venovenous [VV] DLC).
- Drainage zone = region of one or more cannula holes, end and/or side holes that connects to one drainage lumen. A DLC may have two drainage zones, both communication with the same inner lumen.
- Return zone = usually one end hole with number of side holes (ports) providing return flow (usually in proximity to each other). It could also be a single end hole as for neonatal/pediatric return cannulae, or a port placed on the side of the cannula (VV DLC).
- Cannula size is defined by diameter and length. Diameter given as outer diameter in French (Fr); 3 Fr = 1 mm. The cannula inside diameter shall also be provided. Length is given as effective length, that is, the part of the cannula that can be maximally inserted into the vessel. This part usually determines the pressure gradient for flow. Length is given in millimeters.

Dual- Versus Single-Lumen Cannula

A DLC is designed to provide both drainage and return flow in a relatively close anatomical entity, for example, in VV extracorporeal membrane oxygenation (ECMO) as a cavoatrial or bicaval configuration; or in VP ECMO for venous drainage in the right atrium with return in the pulmonary artery. Tests of any DLC require equal flow in both luminae as pressure differences between them might influence the separating wall (septum) and thus the cross-section geometries of the flow paths.

Approval is according to the intended use by the manufacturer of combined drainage and return *via* a single device, that is, drainage *via* drainage lumen and return *via* the return lumen. Usage for other purposes, for example, using both luminae (usually marked red and blue) for drainage, is questionable since the development of the device is based on combined drainage and return flow functionality, for example, differential pressure over a thin collapsable septum.

A single-lumen cannula is either a drainage or a return cannula for use in VV, VP, or venoarterial (VA) ECMO. These cannulae can be used in single or multisite configurations (*e.g.*, femoral and jugular).

Pressure Drop

The induced pressure drop of a cannula is a principal design point. It should be as low as possible, as centrifugal blood pumps are sensitive to both pre- and afterload, that is, a lower pressure drop implies lower friction losses within the system.

The measurement of pressure drop needs to be performed using a blood analog fluid, with a viscosity of $(3.2 \pm 0.2) \times 10^{-3}$ Pa·s (3.2 ± 0.2 cP) (Table 1). However, for basic numerical simulation studies and their validation testing, water may be used, as it allows easier control of fluid properties.

Pressure sensor (PS) ports should be placed as close as possible to the inlet and outlet of the tested device. The measurements should be repeated, providing information of measurement data variability. The differential pressure, or more specific pressure drop, shall be measured at different flow rates over the entire intended range. A standard method for displaying the results is to plot the pressure (y-axis) against the flow rate (x-axis). Pressure difference and its corresponding measuring sites should be clearly indicated in a figure (Figure 2).

The difference over the cannula should be taken according to intended cannula flow direction. For a

Table 1. Comprehensive Overview of the Most Relevant Standards, Most Important Tests, Test Fluids, Test Conditions, and Recommended Reporting of Results for Cannulae, Tubing, Blood Pumps, Oxygenators, and Hemocompatibility Testing

Device/Standard	Test	Test Fluid	Test Conditions	Result Reporting
Cannula/ISO 18193	Pressure drop (single- and dual-lumen)	Blood analog, with viscosity of 3.2×10^{-3} Pa·s $\pm 0.2 \times 10^{-3}$ Pa·s (3.2 cP ± 0.2 cP)	– Measure pressures at proximal and distal end – For dual-lumen cannula: test each lumen – Test at for ≥3 flow rates over full range of blood flow rates	Report as graph: Δp (pressure difference between the inlet and outlet of the cannula, in mm Hg on y-axis) over blood flow rate (in L/min on x-axis)
	Recirculation (dual-lumen)	Water	– Insert cannula into a model of that part of the cardiovascular system intended to be cannulated – In the model, set flow through the superior vena cava to 40% of the flow rate mimicking body circulation. Typical body circulation flow rates: 0.8 L/min (neonates), 3 L/min (pediatrics), and 6 L/min (adults) – Test over full range of blood flow rates	Report as graph: recirculation (in % of extracorporeal flow rate on y-axis) over blood flow rate (in L/min on x-axis)
Tubing/ISO 15676	Priming volume determination (see Priming Volume Determination under Tubing Packs) Note: Priming volume can also be calculated	Anticoagulated whole blood, water, or saline solution	– Measure in a representative length of tubing (*e.g.*, 1 m) or entire length/tubing set for intended use – Temperature of test liquids: (37 ± 1) °C	Report in ml/m or in ml for entire tubing set
	Pressure drop (see Pressure Drop under Tubing Packs)	Blood analog, with viscosity of 3.2×10^{-3} Pa·s $\pm 0.2 \times 10^{-3}$ Pa·s (3.2 cP ± 0.2 cP)	– Measure pressures at proximal and distal end – Measure in a representative length of tubing (*e.g.*, 1 m) or entire length/tubing set for intended use – Test at for ≥3 flow rates over full range of blood flow rates	Report as graph: Δp (pressure difference between the inlet and outlet of the tubing, in mm Hg on y-axis) over blood flow rate (in L/min on x-axis)
	Spallation testing of roller pump tubing (see Spallation Testing of Roller Pump Tubing)	Water-glycerol solution simulating blood viscosity (2.0×10^{-3} Pa·s (2.0 cP) to 5.0×10^{-3} Pa·s (5.0 cP)	– Pre-filter test solution with 5 μm filter – Fluid temperature: match intended use – Run circuit for 1 h intervals with the longest test lasting 6 h – For CPB, sample at 1, 2, 4, 6 h – For ECLS circuits, sample at least every 24 h thereafter for the length of intended use	Report weight of generated particles (in mg) per test time (in h)
Pump/ISO 18242	Pump performance (see Pump Performance (Pulsatile and Continuous))	Blood analog or anticoagulated whole blood	– Temperature of test liquids: (37 ± 1) °C – Test over full range of pump speeds, afterloads, and blood flow rates	Report as graph: Δp (pressure difference between the inlet and outlet of the pump, in mmHg on y-axis) over blood flow rate (in L/min on x-axis). Depict curves of different pump speeds (in r/min)
			– Additionally, for pulsatile pumps: – Test over the full operating range of the pump for ≥3 typical intended combinations of frequency and flow amplitude. – Measure the time-dependent inlet and outlet pressures and the corresponding flow rates for at least the min.	Report as 2 graphs: mean Δp (mean pressure difference between the inlet and outlet of the pump, in mm Hg on the y-axis) over mean blood flow rate (in L/min on the x-axis). Both blood flow rate (in L/min on

Continued

Table 1. Continued

Device/Standard	Test	Test Fluid	Test Conditions	Result Reporting
			– and max. operating conditions over 10 pumping cycles	y-axis) and Δp (in mm Hg on y-axis) over time (in s on x-axis), depict curves of at least minimum and maximum operating conditions
	Priming volume determination (see Priming Volume Determination under Blood Pumps)	Anticoagulated whole blood, water or saline solution	– Temperature of test liquids: (37 ± 1) °C	Report in ml
	Pump durability (see Pump Durability)	Blood analog or anticoagulated whole blood	– Test at worst-case scenario with regard to pump speed, pressure head, temperature, *etc*.	
Oxygenator/ISO 7199	Gas exchange testing (pulsatile and continuous) (see Gas Exchange Testing (Pulsatile and Continuous))	Anticoagulated whole blood	– Oxyhemoglobin percentage: (65 ± 5) % – Hemoglobin: (120 ± 10) g/L – Base excess: (0 ± 5) mmol/L – pCO_2 in blood: (6.0 ± 0.7) kPa (≈[45 ± 5] mm Hg) – Temperature of test liquids: (37 ± 1) °C – Test over full range of blood and gas flow rates	Report as 2 graphs: ΔcO_2 or ΔcCO_2 (normalized to 1 L blood in ml_{Gas}/L_{blood} on y-axis) over blood flow rate (in L/min on x-axis). Include the gas-to-blood flow ratio
	Priming volume determination (see Priming Volume Determination under Membrane Lungs)	Anticoagulated whole blood, water, or saline solution	– Temperature of test liquids: (37 ± 1) °C	Report in ml
	Pressure drop (see Pressure Drop under Membrane Lungs)	Anticoagulated whole blood (or blood analog with the correct dynamic viscosity)	– Temperature of test liquids: (37 ± 1) °C – Test over full range of blood flow rates	Report as graph: Δp (in mm Hg on y-axis) over blood flow rate (in L/min on x-axis)
	Heat exchanger perfomance factor (see chapter 5.4)	Anticoagulated whole blood or water	– Blood inlet temperature: (30 ± 1) °C – Water inlet temperature: (40 ± 1) °C – Test over full range of blood flow rates	Report as graph: heat exchanger performance factor (in % on y-axis) over blood flow rate (in L/min on x-axis)
Blood cell damage	Oxygenator (ISO 7199)	Anticoagulated whole blood	– Max. 2 L fluid/ circuit, ± 3 % difference between circuits – Blood flow: worst-case scenario testing, ± 5 % between circuits – pCO_2: (5.3. ± 0.7) kPa – Base excess: (0 ± 5) mmol/L – Glucose: (10 ± 5) mmol/L – Hemoglobin: (120 ± 10) g/L – Blood temperature: (37 ± 2) °C – 6 h test duration – Min. n = 5 repetitions	pfHb in mg/dl NIH in g/100 L White blood cells reduction in % Platelet count reduction in %
	Pump (ISO 18242)	Anticoagulated whole blood	– Max. 1 L fluid/ circuit, ± 3 % difference between circuits – Blood flow: worst-case scenario testing, ± 5 % between circuits – Glucose: (10 ± 5) mmol/L – Hemoglobin: (120 ± 10) g/L – Blood temperature: representative of a range of the intended temperatures during clinical use of the device	pfHbin mg/dl NIH in g/100 L White blood cells reduction in % Platelet count reduction in %

Continued

Table 1. Continued

Device/Standard	Test	Test Fluid	Test Conditions	Result Reporting
			– 6 h test duration – Min. n = 5 repetitions	
	Pump (ASTM 1841)	Anticoagulated whole blood, animals (ovine, bovine or porcine) or human donors	– Max. 500 mL fluid/ circuit – Maximum blood flow rate, ±5% between circuits – Initial pfHb ≤ 50 mg/dL – Hematocrit: (35 ± 2)% – 6 h test duration – Min. n = 5 repetitions	pfHb in mg/dL NIH in g/100 L MIH
	Cannulae (ISO 18193)	Anticoagulated whole blood	– Max. 2 L fluid/ circuit, ± 3% difference between circuits – Maximum blood flow rate, ±5% between circuits – Base excess: (0 ± 5) mmol/L – Glucose: (10 ± 5) mmol/L – Hemoglobin: (120 ± 10) g/L – Blood temperature: (37 ± 2) °C – 6 h test duration – Min. n = 5 repetitions	pfHb in mg/dl NIH in g/100 L White blood cells reduction in % Platelet count reduction in %

CPB, cardiopulmonary Bypass; ECLS, Extracorporeal Life Support; MIH, modified index of hemolysis; NIH, normalized index of hemolysis; pCO_2, partial pressure of CO_2; pfHb, plasma-free hemoglobin.

single-lumen cannula, this implies the following PS positions (Figure 2):

- Return cannula: $PS1_R$ at cannula inlet (connection to tubing) and $PS2_R$ at cannula tip
- Drainage cannula: $PS1_D$ at cannula tip and $PS2_D$ at suction side (connection closest to pump).

Using this convention, $p_1 - p_2$ will provide pressure drop; otherwise, the results should be referred to as differential pressure.

The same convention should be applied to the drainage and return lumen of the DLC.

The SI unit (International System of Units) for pressures is Pascal (Pa); however, current practice is to report hydrostatic pressures in millimeters of mercury (mm Hg), as this is the standard unit used in clinical practice. The following conversion factors apply:

$$1\ \text{Pa} = 0.0075\ \text{mm Hg}; 1\ \text{kPa} = 7.5\ \text{mm Hg}; 1\ \text{mm Hg} = 0.133\ \text{kPa}.$$

Flow Characterization *In Vitro* and *In Silico*

The ISO standard specifies that a blood analog should be used for testing pressure drop. However, for providing data for validation, water can be used to obtain controlled fluid properties (density, viscosity). If blood analog fluids are used, their properties should be controlled throughout the measurement to assess potential drift in viscosity.

Information regarding the temporal and spatial resolution inherent to the measurement technique applied (*e.g.*, particle image velocimetry [PIV], laser-induced fluorescence [LIF], magnetic resonance imaging [MRI], ultrasound, *etc.*) needs to be reported to allow for evaluation of the flow field and the stresses generated by the flow, as well as to assess data with respect to sensitivity. This allows for a robust evaluation of the fluid stresses developing in the flow and enables comparison between experimental and numerical results. For both experimental (*in vitro*) and numerical (*in silico*) studies (*e.g.*, computational fluid dynamics [CFD]), the following should be provided:

- Geometry characteristics (both *in vitro* and *in silico*)
  - Patient-derived geometry: geometry rendering approach and reconstruction
  - Material characteristics (rigid or compliant)
- Experimental setting (*in vitro*)
  - Hardware (pressure probes, flow meters, high-speed cameras, lasers, *etc.*): manufacturer and product model. Provide calibrated range when adequate
  - High-speed camera or MRI: spatial resolution in all direction (pixel/mm)
  - Laser-based techniques (PIV, LIF):
    - laser type, energy, and frequency (temporal resolution/sampling rate)
    - laser sheet thickness and interrogation window
    - tracer particles (type, size/diameter)
  - Sampling times for data averaging: mean and standard deviation
- Numerical setting (*in-silico*)
  - Mode of numerical simulation: Reynolds-averaged Navier-Stokes (RANS; steady or unsteady solver) or large Eddy simulations (LES)
  - Fluid model (Newtonian/non-Newtonian viscosity)
  - Numerical discretization used in time and space
  - Grid sensitivity assessment: this should be carried out for the specific parameter (velocity and/or gradient) under investigation, and not limited to global values such

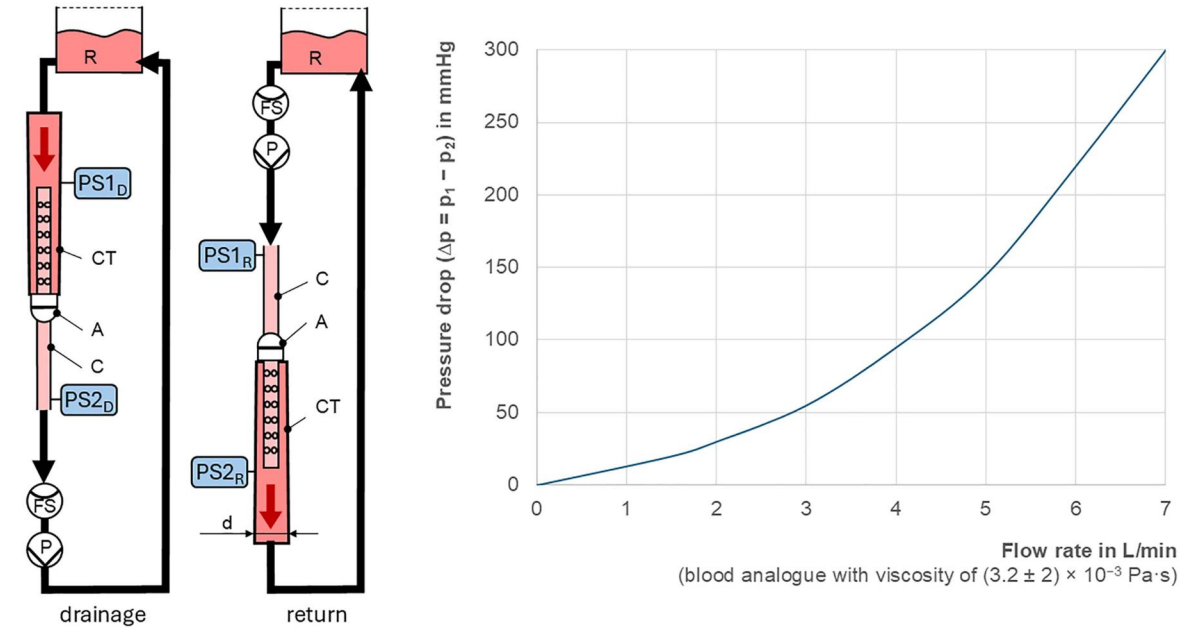

Figure 2. Testing ECLS cannula. Left: Example of test circuit for pressure drop measurement in drainage and return cannula. Right: Graph with exemplary test results. A, access port with seal; C, cannula under test; CT, cylindrical test chamber with $d = 2 \times$ diameter of cannula under test; FS, flow sensor; P, pump; $PS1_D$ and $PS2_D$, pressure sensors 1 and 2 for drainage cannula; $PS1_R$ and $PS2_R$, pressure sensors 1 and 2 for return cannula; *R*, reservoir for blood analog.

as pressure drop. The grid should be assessed in regions of interest where the most challenging flow is expected to develop.

For both *in-vitro* and *in-silico* assessment, boundary conditions should be clearly specified. For both experimental and numerical data, validation or verification data against data from literature should be provided, if available. Confirm that the approach chosen is adequate for the respective research question, *that is*, sufficient spatial and temporal resolution if assessing stress dynamics.

All data should be presented using SI units for time, space, velocity, forces, energy, *etc*.

Recirculation (Dual-Lumen Cannula)

The procedure for assessing recirculation is described in ISO 18193. The test fluid should be water (Table 1). While this results in a flow situation that differs dynamically from that of a more viscous fluid (blood analog), the larger flow structures are expected to be similar. Measurements should be performed at different flow rates covering the entire intended operating range. Results should be reported as a percentage (%) of the extracorporeal circuit flow rate (y-axis) plotted against the blood flow rate (L/min, x-axis).

TUBING PACKS

The testing of tubing packs is defined in ISO 15676-Cardiovascular implants and artificial organs-Requirements for single-use tubing packs for cardiopulmonary bypass and ECMO (current version: ISO 15676:2016). This standard requires testing of blood pathway integrity, connections, tubing life, spallation, priming volume determination, and other relevant parameters. The following paragraphs outline the most important aspects (Table 1).

Priming Volume Determination

All tubing used in ECLS is specified by its inner lumen diameter in inches (3/16"; 1/4," 3/8," or 1/2"). The priming volume per length unit can be calculated from diameter and length or measured by sealing off one end and measuring the filling volume of a known length of tubing. For precise measurement, the latter method carries less error given nonconstant inner tubing diameter for same size. This issue has become more pronounced for various manufacturers over the last decade.

Pressure Drop

The pressure drop is calculated as the difference between the inflow and the outflow pressures of the total tubing length, otherwise refer to as differential pressure (see Pressure Drop). For a laminar flow in a straight tube, pressure drop can be calculated *via* the Hagen-Poiseuille's law. However, this relation requires steady (laminar) flow of a Newtonian fluid (constant viscosity). The equation considers the inner diameter (cross-sectional area), the length of the tube, and viscosity of the fluid. The clinical experience says that using Hagen-Poiseuille's law for determination of resistance and flow is likely to deviate less from reality and manufacturer's information compared with cannula according to earlier studies from this working group.[1–3] The ISO standard does not require pressure drop measurements.

Spallation Testing of Roller Pump Tubing

Spallation occurs when material is lost from a device surface (shedding) from mechanical stress or impact, typically exemplified by the roller pump constantly grinding the tubing. For tubing used in the roller pump's raceway, this is particularly important, and specialized polymer compounds have been developed (*e.g*., Tygon, Super Tygon). Despite these improvements, material fatigue still occurs, and tubing life span is limited typically to 100 hours in clinical practice. The proportion of the tubing exposed to the raceway is thus regularly moved (walked) for a tubing section not earlier exposed. After "walking the tubing" another 100 hours are possible. If this procedure is not performed, the risk of tubing rupture is imminent.

Methods for detecting particles used in scientific literature include high accuracy laser diode particle counting, scanning electron microscope, Coulter counter, and microfilters.

ISO 15676:2016 determines the fluid to be a water-glycerol solution to simulate blood viscosity to test spallation. The fluid is prefiltered with a 5 μm filter. The circuit volume is kept low and needs two Y-connectors and in one of these limbs, a "fine filter." At each prespecified time point, the whole circuit volume is passed through the filter. The filter is then dried and weighed, and the cumulative mass of released particles then reported as milligrams per time point. Tests for extracorporeal circulation devices are performed for at least 6 hours, and for ECLS at least 24 hours. A failed test is defined by a leak in tubing wall.

The prefiltering of the fluid allows for particles <5 μm to confound the early assessments. The "fine filter" particle cutoff is not defined by the applied standard but typically referred to as filtering particles (1–10) μm. Thus, the method used in the ISO standard is coarse and risks to miss $\geq$50% of the particles given an average size of (0.83 ± 0.03) μm.

BLOOD PUMPS

The testing of blood pumps is defined in ISO 18242-Cardiovascular implants and artificial organs-Centrifugal blood pumps (current version: ISO 18242:2016 and ISO 18242:2016/Amd 1:2023). This standard requires testing of blood pathway integrity, connector integrity, hydraulic performance, pump durability, blood cell damage, *etc*., and covers test conditions for pulsatile pumps. For novel

devices, the most important aspects are hydraulic performance (main function of the pump), priming volume, pump durability (to ensure reliable functioning over the entire intended duration of use), and blood cell damage (see Blood Cell Damage According to ISO Standards).

The pump is an integral component of the ECLS circuit, facilitating blood circulation outside the body. Accurate reporting of pump-related parameters is critical to ensure reproducibility, comparability, and proper interpretation of results across experimental studies. This section outlines the minimum reporting standards for pump systems in ECLS research and refers to ISO 18242 for centrifugal pumps.

Pump Performance (Pulsatile and Continuous)

ISO 18242 states that the test fluid for hydraulic performance shall be either a blood analog or anticoagulated blood. For more information on the influence of viscosity on centrifugal pump performance, see Ippen.[4] The testing covers a combination of different pump speeds (revolutions per minute [r/min]), afterloads, and flow rates (Table 1). For pumps intended for pulsatile flow, both frequency and amplitude should also be varied over the entire intended operating range.

The preferred and standardized way to present the results is a graph with pump performance curves, covering the entire rated operating range of (mean) pump speeds (in r/min), with the (mean) pressure head (pressure difference between the inlet and outlet of the pump, typically in mmHg) on the y-axis and the (mean) flow rate on the x-axis (in L/min) (Figure 3).

Millimeters of mercury (mm Hg), although not an SI unit, is the preferred unit for reporting the pressure head generated by the pump, as it aligns with clinical application and can therefore be correctly interpreted by clinicians.

Priming Volume Determination

For priming volume determination, water is accepted as test fluid. The determination can be performed volumetrically or gravimetrically. One way is to inject water (*e.g.*, using a syringe) into a dry device while carefully deairing it, and then determining the volume of the injected fluid. The resulting priming volume should be reported in milliliters (Table 1).

Pump Durability

When developing a new pump, the intended duration of use should be defined and considered in the choice and design of the bearing principle (mechanical, hydrodynamical, magnetically levitated, or hybrid versions). Therefore, the prototype needs to be tested for durability to prove that the device will retain its function over the entire rated operating lifetime. For this, the worst-case scenario for the pump needs to be determined. This often corresponds with the highest flow rate at the highest pressure head, but it can also be other variations of flow rates and pressure heads, especially in low flow or pulsatile pumping mode. Bearing wear, unintended contacts of a hydrodynamic, magnetically levitated or hybrid bearing, or similar events can be detected by visual (magnified) inspection but require thorough consideration regarding their quantification.

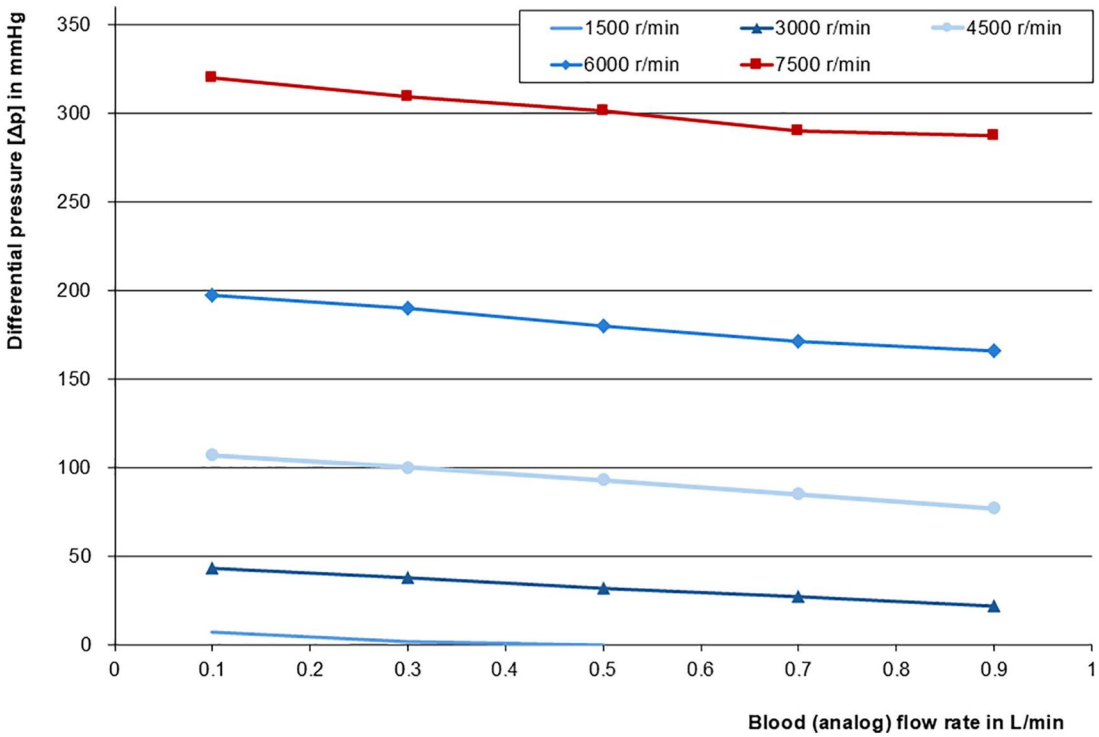

Figure 3. Plot of exemplary pump performance curves of a pump for neonatal applications (adapted from Arens[5]).

MEMBRANE LUNGS

The testing of membrane lungs is defined in ISO 7199-Cardiovascular implants and artificial organs-Blood gas exchangers (oxygenators) (current version: ISO 7199:2024). This standard requires testing of blood pathway integrity, gas exchange function, connector integrity, priming volume determination, blood cell damage, *etc*. For novel devices, the most important parts are

1. gas exchange testing, the core function of an oxygenator,
2. priming volume determination, to minimize blood dilution in the patient (especially for neonatal and pediatric devices), as this influences the oxygen transport capacity of the blood,
3. pressure drop over the oxygenator, as it affects the working point of an associated blood pump (higher pressure drop means higher afterload for the pump and requires higher pump rotational speed), and
4. blood cell damage (as described in Blood Cell Damage According to ISO Standards).

Gas Exchange Testing (Pulsatile and Continuous)

Oxygen is mainly transported by red blood cells (RBCs) as it chemically binds to the hemoglobin (Hb) contained in the RBCs. Only a small fraction is physically dissolved in the blood plasma.

The oxygen concentration in blood (in ml/L) can be calculated using equation 1 (note: clinically, the here defined "oxygen concentration" is also referred to as "oxygen content"):

$$cO_2 = 1.34\frac{ml_{O2}}{g_{Hb}}\cdot cHb\cdot SO_2 + 0.03\frac{ml_{O2}}{L_{Blood}\cdot mm\ Hg}\cdot pO_2 \quad (1)$$

In this formula, 1.34 ml_{O2}/g_{Hb} being the so-called Hüfner number (the amount of oxygen in ml that can be bound by 1 g Hb), and 0.03 $ml_{O2}/(L_{blood}$ mm Hg) as the solubility coefficient of oxygen in blood plasma. Using the oxygen dissociation curve (Figure 4) and Eq. 1, saturation and oxygen content can be calculated from partial pressures, and *vice versa*. However, the shape of the oxygen dissociation curve depends on the temperature, partial pressure of CO_2 (pCO_2), and pH. It shifts to the left (becomes steeper) at lower temperature, lower pCO_2, and higher pH, and shifts to the right (becomes flatter) at higher temperature, higher pCO_2, and lower pH. Therefore, it is important to keep these blood parameters within the ranges defined in the standard during testing (Table 1).

Gas exchange testing must always be performed with whole blood, as the mass of oxygen chemically bound to Hb by far exceeds the mass of oxygen physically dissolved in plasma or water (Figure 4). Typically, fresh whole blood from a slaughterhouse is used (*e.g.*, porcine or bovine). If using stored blood, for example, (nearly) expired blood from a blood bank, it needs to be carefully evaluated, if the age of the RBCs affects the results due to the different oxygen binding and releasing behavior of aged RBCs.[6]

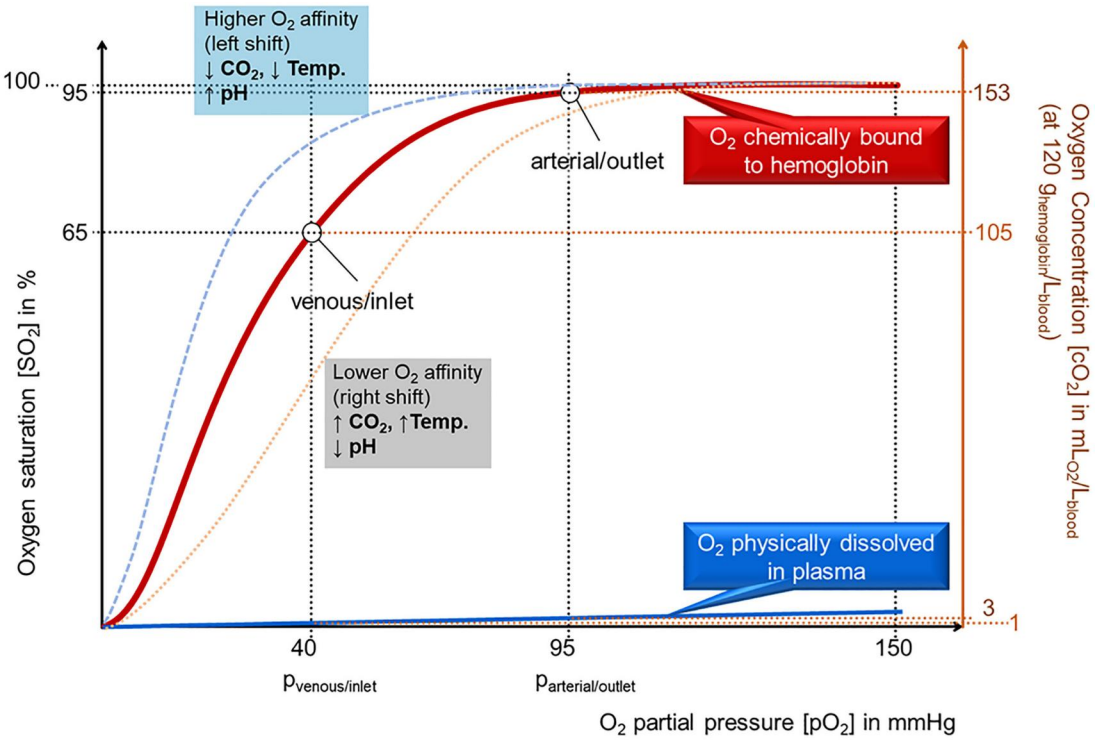

Figure 4. Oxygen dissociation curve (not applicable to neonatal blood since fetal Hb has a significantly higher oxygen binding affinity) relating oxygen saturation and oxygen concentration in blood to partial pressure of oxygen. Changes in pCO_2, temperature, and pH influence the oxygen binding affinity and result in a left (↓ CO_2, ↓ temperature, ↑ pH, dashed blue line) or right (↑ CO_2, ↑ temperature, ↓ pH, dotted orange line) shift of the curve. Oxygen concentrations are dependent on Hb concentration: here the values were calculated for a Hb concentration of 120 g/L, a pO_2 of 95 mm Hg and a saturation of 95%. Hb, hemoglobin.

Physiologically, an oxygenator should be capable of oxygenating blood to a saturation of at least 95% at a Hb concentration of 120 g/L from a starting saturation of 65%. More specifically, ISO 7199 requires a SO_2 of (65 ± 5) % as an inlet condition. Taking these conditions into account means that any oxygenator should be capable of exchanging a minimum of 50 ml_{O2}/L_{blood}:

$$\Delta cO_2 = 1.34\ \frac{ml_{O2}}{g_{Hb}} \cdot 120\ \frac{g_{Hb}}{L_{blood}} \cdot (95\% - 65\%) + 0.03 \frac{ml_{O2}}{L_{blood} \cdot mm\ Hg} (95\ mm\ Hg - 40\ mm\ Hg)$$
$$\Rightarrow \Delta cO_2 = 50\ ml_{O2}/L_{blood}$$

For determining the basic requirement of CO_2 elimination, the respiratory quotient (RQ) has to be taken into account. This defines the volume of CO_2 which needs to be eliminated by ventilation over the volume of oxygen absorbed and is dependent on the metabolic production rate, which is influenced, among other factors, by the patient's diet. For a pure carbohydrate diet, the RQ is 1; for fat, it is 0.7; and for protein 0.8. For an adult with a mixed diet of carbohydrates and proteins, metabolizing 50 ml/L_{blood} oxygen results in the need to eliminate a minimum of 40 ml/L_{blood} of CO_2 *via* ventilation. In neonatal and pediatric patients on a predominantly carbohydrate diet, the RQ can be estimated to be 1, requiring a minimum of 50 ml_{CO2}/L_{blood} to be eliminated.

Both requirements for oxygenation and CO_2 elimination are solely based on physiological considerations and normalized to 1 L of blood; thus, they are independent of the type or size of the oxygenator or its intended blood flow rate. Therefore, the unit milliliter of exchanged gas per liter of blood (ml_{Gas}/L_{blood}) is the preferred way of reporting gas exchange capability as a function of the

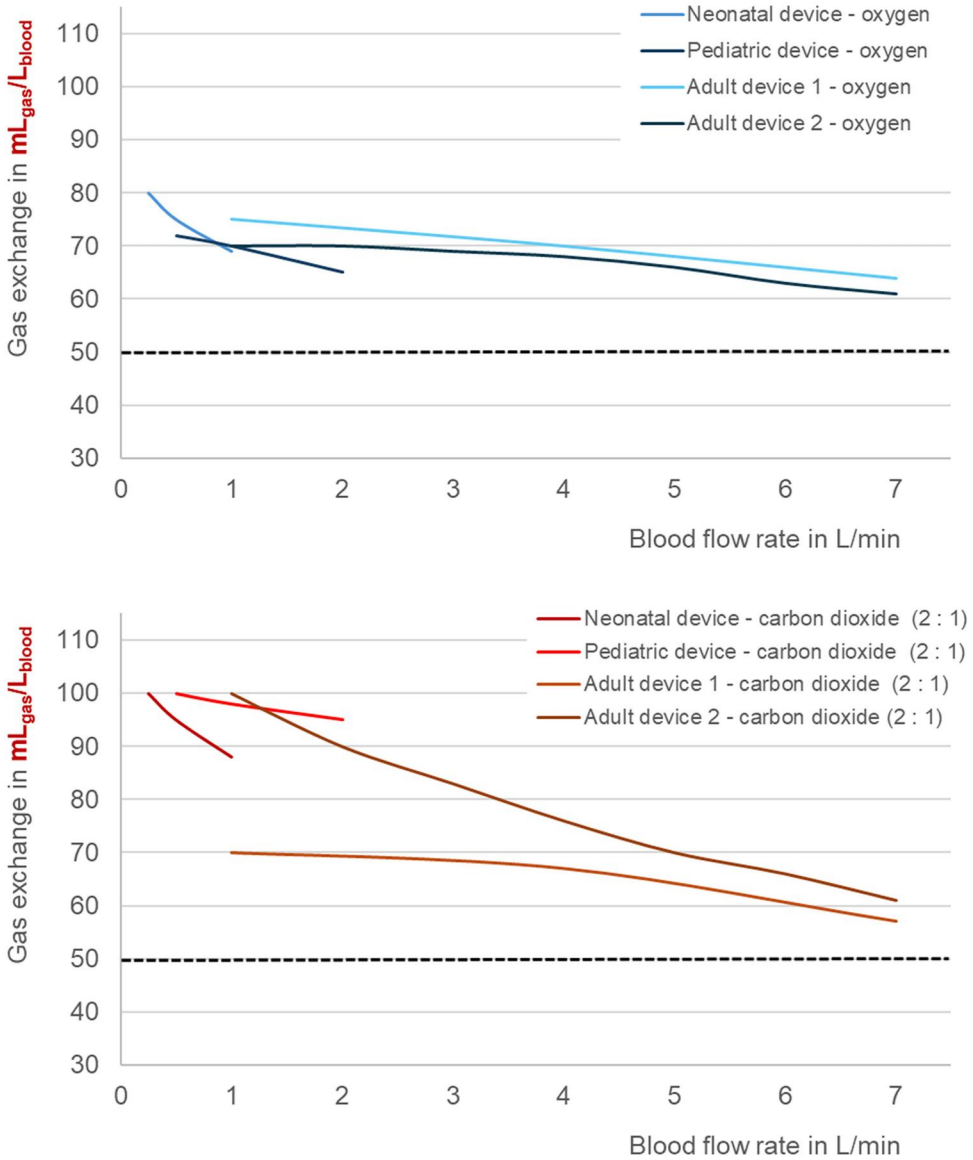

Figure 5. Exemplary depiction of gas exchange (in ml_{gas}/L_{blood}) over a range of blood flow rates for oxygen (top graph) and carbon dioxide (bottom graph; at a gas-to-blood flow ration of 2: 1). The lower limit of 50 ml_{gas}/L_{blood} for oxygen exchange (and for carbon dioxide exchange at a respiratory quotient of 1) is marked as dashed horizontal line.

blood flow rates at which the device was tested, as depicted in Figure 5. It allows:

- to capture at one glance if physiological requirements are met at all blood flow rates and
- to easily compare oxygenators of different types and sizes with each other.

The CO_2 elimination rate of an oxygenator is mainly dependent on the sweep gas flow rate.[7] Therefore, CO_2 elimination should be tested at different sweep gas-to-blood flow ratios (*e.g.*, 1:1, 2:1, 3:1). When reporting the results, the respective sweep gas-to-blood ratio must always be specified (Figure 5).

Priming Volume Determination

For priming volume determination, water is an accepted test fluid. The process may be performed volumetric or gravimetric. One option is to measure the weight of the empty, dry device, fill it completely with water (from the inlet tubing connection to the outlet tubing connection), carefully deaerate it, and then measure the weight of the fluid-filled device. Finally, the difference between dry and filled weight of the device needs to be calculated and translated into a volume. The determined priming volume shall be reported in milliliters.

Ratio of priming volume in active and passive parts of the oxygenator

Emerging research on microfluidic oxygenators shows efficient gas exchange in microfluidic cells, with some studies claiming a higher area-specific gas exchange efficiency compared with currently used hollow fiber devices.[8] Unfortunately, comparisons between microfluidic and hollow fiber devices often lack standardized test results that are truly comparable and neglect that the entire blood contacting area of an oxygenator is much larger than the membrane area alone. However, higher area efficiency may be achieved for small units of microfluidic devices, but these often require to be scaled up by multiplying the single unit to provide sufficient gas exchange area for, for example, adult patients.[9] Thus, the implementation of blood distribution channels at the inlet and outlet of the full-sized device is needed. These distribution channels do not contribute to the gas exchange, but contain a certain amount of priming volume and therefore contribute to hemodilution caused by the device.[10] Also, other designs of oxygenators require structures to distribute the blood from the connective tubing to the gas exchange membranes which do not contribute to the gas exchange.

Therefore, for all developments of novel oxygenators, the ratio of the priming volume of the passive blood distribution geometries to the overall priming volume (active and passive parts) should be determined and reported.

Pressure drop

The induced pressure drop of an oxygenator is a principal design point of an oxygenator. It needs to be very low, if the oxygenator is intended to be used in a pumpless mode (arterio-venous cannulation in which the patient's heart pumps the blood through the oxygenator), for example, in an artificial placenta application.[11] A low resistance of the oxygenator is also advantageous for circuits that use a blood pump, as centrifugal blood pumps are afterload-sensitive, that is, a lower pressure drop of the oxygenator allows for lower pump rotational speeds and thus lower shear stress induced by the pump.

Measurement of pressure drop needs to be performed with full blood or a blood analog. Due to the lower viscosity of water compared to blood, pressure drop cannot be accurately measured using water. Pressure sensors should be positioned as close as possible to the inlet and outlet of the device. The differential pressure, or pressure drop, should be measured at different flow rates over the entire intended range. A standard method for presenting the results is to plot the pressure (y-axis) against the flow rate (x-axis, Figure 6).

HEMOCOMPATIBILITY

Blood Cell Damage According to ISO Standards

In the context of ECLS, blood cell damage mainly describes the destruction of RBCs (hemolysis) due to elevated shear stresses in pump, oxygenator, or cannula. As a consequence of hemolysis, oxygen transport is hampered since ~95% of transported oxygen is normally bound to Hb within the RBCs. Further, plasma-free Hb (pfHb) interacts with the hemostatic system, causing platelet (PLT) activation and mediating binding of von Willebrand Factor to PLTs.[13,14] To avoid hemolysis and the accompanying adverse events, hemolysis testing is mandatory for cannula, pumps, and oxygenators used in ECLS circuits. Measurement of PLT and leucocyte counts is included in the tests as well to account for damage to all blood cells. For each device, the specific standard covers blood cell damage. However, specification of test conditions, blood sampling, and analyses is streamlined between the following standards:

- Oxygenators (ISO 7199:2024),
- Centrifugal blood pumps (ISO 18242:2016 and ISO 18242:2016/Amd 1:2023), and
- Cannula (ISO 18193:2021 and ISO 18193:2021/Amd 1:2025)

The principal test setup, experiment execution, and analyses of blood samples are similar for all three devices. Most importantly, all blood cell damage tests must be performed with anticoagulated whole blood with a Hb concentration of (120 ± 10) g/L in a comparative manner. This means that there are always two identical test loops run at the same conditions filled with blood from the same donor, one equipped with the test device and the other equipped with a predicate or comparator device (a device similar to the subject device that is a legally marketed device, recognized-to-be-safe and is used for the same intended clinical use). Additionally, a static

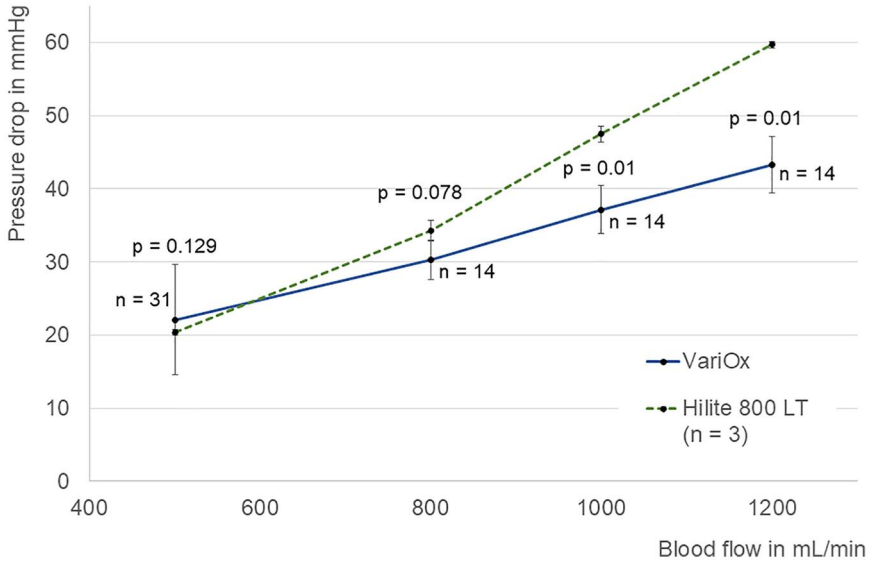

Figure 6. Plot of mean pressure drops [Δp] against blood flow rate including the number of tests [n] of device under test and comparator device (adapted from Arens *et al*.[12]).

Table 2. Initial Parameters for Blood Cell Damage Testing (ISO 7199:2024, ISO 18193:2021, ISO 18242.2016/Amd 1:2023)

Parameter	Target Value	Maximum Deviation
Base excess	0 mmol/L	±5 mmol/L
Blood glucose	10 mmol/L	±5 mmol/L
Hemoglobin	120 g/L	±10 g/L
Circuit volumes (oxygenator and cannula)	Max. 2 L	±3% between parallel circuits
Circuit volumes (pumps)	Max. 1 L	±3% between parallel circuits

reference accounting for the auto-hemolysis of the blood is included for blood pump and oxygenator testing.

Since the maximum circuit priming volume is 1 L for blood pumps and 2 L for oxygenators and cannula, the use of a single human blood donation for a referential test including the static reference is not feasible. As a surrogate, the use of animal blood, particularly slaughterhouse blood, is common practice. Appropriate animal blood models for hemolysis testing are pig and cattle (ASTM F1830:2019). Although the overall degree of hemolysis using animal (slaughterhouse) blood might differ from human hemolysis, comparative testing reveals the same differences between test and predicate device.[15]

The general test setups for ECLS component testing include a blood reservoir, the test device, a blood pump in the case of oxygenator and cannula testing and tubing to connect the components and allow for flow and PSs as well as sampling and deairing ports. The total circuit volume should be kept as small as possible to increase the signal-to-noise ratio. Although only explicitly stated in ISO 7199:2024, test temperature shall be (37 ± 1) °C; therefore, a water bath, temperature-controlled chamber or a heat exchanger shall be included as well.

Before filling the test loops with blood, it is recommended to prime the circuit with a blood-compatible fluid (*e.g.*, saline solution) to deair the complete circuit. After priming the test loops with blood, any remaining air should be removed, and the initial blood values as specified in the standards should be maintained (Table 2).

Test conditions refer to the worst-case conditions as specified by the manufacturer for oxygenators and blood pumps and to the maximum blood flow rate for cannula. Especially for blood pumps, the worst-case condition does not necessarily refer to the maximum pump speed but may also be reached in a low-flow off-design operation point.[16,17] The allowed maximum deviation from the values for all devices is ± 5%.

The total test duration is 6 hours for all devices and includes blood sampling at least at the specified time points described in the respective standards. Blood analyses include the following parameters:

- PfHb
- White blood cells (WBCs)
- PLTs
- Blood gas values
  - All: pH and base excess
  - Oxygenators only: pO_2 and pCO_2
- Hb/hematocrit (Hct)
- Activated clotting time (ACT)

Additionally, the following test parameters shall be measured:

- Temperature
- Flow rates
- Pump speed (blood pumps only)
- Pressure (inflow and outflow)
- Circuit volume change

The tests must be repeated at least five times under identical setting, only using blood from five different donors to account for blood-inherent variations in blood cell damage response. The results for blood cell damage

should be reported as the mean ± standard deviation from all test repetitions. Hemolysis results should be reported as pfHb in mg/dl over time and normalized index of hemolysis (NIH) in g/(100 L) using Eq. 2.

WBC and PLT: $\Delta(t_0 - t_6)$ in %

$$NIH = \frac{V}{\dot{V}} \cdot \frac{\Delta PfHb}{\Delta t} \cdot \left(1 - \frac{Hct}{100}\right) \quad (2)$$

With V = volume of blood in test circuit in L, $\dot{V}$ = blood flow rate in L/min, ΔpfHb = change in pfHb concentration in mg/dl, Δt = time difference in minutes, Hct = in %

All target values shall be defined by the manufacturers.

Blood Cell Damage According to the American Society for Testing and Material Standard Practice

With regard to blood pump hemolysis testing, the American Society for Testing and Materials (ASTM) has published a standard practice for the assessment of hemolysis in continuous flow blood pumps (F1841 –19) and for the collection and preparation of blood for blood pump hemolysis testing (F1830 – 19). The ASTM standard practice provide additional information and requirements compared with ISO.

For instance, ASTM F1830 – 19 specifies different anticoagulation agents and its concentrations for the use in hemolysis testing as well as blood collection and transportation details. Further, a maximum initial pfHb of 50 mg/dl before testing is defined.

While the test procedure of comparative testing over 6 hours with a minimum repetition of five times is similar in ASTM F1841:2019 to ISO 18242:2016, a mandatory value of Hct = 35% ± 2% is introduced instead of a cHb = (120 ± 10) g/L. Further, the total test circuit volume per loop is limited to 500 ml, which is half of the ISO restriction. The ASTM standard provides detailed procedures for prepriming and loop filling as well as sample drawing including discarding of stagnant blood from the sample port beforehand. Sampling schedule and recorded values differ slightly from the ISO.

For evaluation and analysis of the hemolysis tests, ASTM gives more details on statistical analysis and data presentation, that is, including a linear regression for pfHb over time which should be at least $R^2 = 0.95$. In addition to pfHb and NIH (see Eq. 2), the modified index of hemolysis (MIH) shall be calculated. The unitless MIH takes the total circuit Hb into account and can be calculated as follows (Eq. 3):

$$MIH = \frac{NIH}{Hb_{total}} = \frac{V}{\dot{V}} \cdot \frac{\Delta PfHb}{\Delta t \cdot Hb_{total}} \cdot \left(1 - \frac{Hct}{100}\right) \quad (3)$$

Thrombogenicity

In contrast to hemolysis testing, thrombogenicity testing of ECLS devices is still subject to research and currently far away from standardization, although being one of the major unsolved limitations of medical devices in general and ECLS in particular.[18–21] The United States Food and Drug Agency (FDA) identified the development of new *in-vitro* methods for thrombogenicity evaluation and the standardization of suitable methods for, for example, oxygenator thrombogenicity testing as an "important area of research."[22] However, only material thrombogenicity testing is addressed in the ISO 10993-4:2017, providing several possible test setups and analysis parameters, but lacking clear, comparable, and reproducible instructions for thrombogenicity testing.

Regarding ECLS components, no *in-vitro* thrombogenicity tests are demanded in the respective standards, which, as a consequence, leads to thrombogenicity evaluation of the devices in *in-vivo* animal trials for the first time. This procedure is questionable for several reasons. First, animal trials should be performed with the least possible number of tests, therefore using the best possible design of the device. These requirements cannot be fulfilled with a device never been tested for thrombogenicity before. Second, animal trials are usually performed at a late development stage. Stepping back after negative *in-vivo* thrombogenicity results entails high costs and time for the device development. Therefore, *in-vitro* thrombogenicity tests are favorable and urgently required. But there are several challenges and limitations that hamper the development of suitable *in-vitro* test methods.

Since there is a large variety of ECLS circuits differing in, for example, oxygenator size, integration of pump, allowed priming volume (pediatric *versus* adult), tubing sets, every possible test setup is highly specific for the device to be tested, making results hardly transferable and comparable *per se*. Additionally, one inherent limitation of dynamic *in-vitro* thrombogenicity tests is the poor test surface/circuit surface as well as test surface/blood volume ratio. Large test setups with high priming volumes might impact the measured thrombogenicity more than the device under test does. Additionally, slight differences in thrombogenic response between two test devices might perish within the test circuit noise.

The required blood volumes present another challenge of *in-vitro* thrombogenicity assessment, most often exceeding the volume of a human blood donation (max. 450 ml). Whereas hemolysis can be well evaluated using animal blood, the hemostatic system of pig, cattle, or sheep is not 100% comparable to that of humans. The transferability of thrombogenicity results obtained with animal blood to the human application is another topic of current research activities and was summarized in a recent review.[23]

Despite the challenges, suitable *in-vitro* thrombogenicity tests for ECLS devices are urgently needed and present the next step toward the improvement of device hemocompatibility.

LIMITATIONS OF *IN-VITRO* TESTING

One of the main limitations of *in-vitro* testing is the inability to capture the complex reactions of the organism to ECLS initiation and the contact of blood with the large artificial surfaces of the circuit and its components. This contact initiates and propagates a complex immediate inflammatory response and activates coagulation.

Furthermore, the complexity of critical illness and the patient's reaction to it, which may further imbalance hemostasis, is not addressed in *in-vitro* testing. Moreover, systemic anticoagulation may have different systemic effects in the human or animal organism compared with the *in-vitro* model, complicating the delicate balance between anticoagulatory and procoagulant factors observed *in vivo*. It is likely that due to the absence of inflammatory and acute-phase reaction factors in the test blood, the thrombogenic potential of the blood and the device cannot be comprehensively evaluated. Additionally, *in-vitro* test durations are limited to approximately 6 hours due to the lacking metabolism and decreasing viability of the test blood. Since the average time of clinical use of an ECLS circuit is considerably longer, long-term effects cannot be depicted in the *in-vitro* mode.

Finally, systematic reviews and meta-analyses of high-quality research represent the highest level of evidence necessary to assess and make final recommendations on the methods or technologies used. These reviews are only possible if the rationale, methodology, results, and outcomes are well-organized and uniformly reported. To capture the advances in research and reporting of results, the establishment and standardization of minimal reporting criteria in experimental ECLS research was necessary.

CONCLUSIONS/OUTLOOK

The purpose of this study is to define minimal, standardized testing and reporting criteria related to *in-vitro* evaluation of ECLS devices in research and development, covering test fluids to be used. We anticipate that these minimum, standardized testing and reporting criteria will benefit researchers and clinicians in their laboratory work and in their roles as authors, editors, and peer reviewers by ensuring the quality and reproducibility of tests as well as comparability of results. Additionally, they are intended to help different users such as guideline developers, policy makers, and other stakeholders, as well as healthcare providers and their patients, in comparing and interpreting *in-vitro* methods and results. Ultimately, we hope that implementation of these recommendations will lead to more transparent, complete, organized, and accurate reporting of *in-vitro* evaluations, thus facilitating better evidence-based decision-making.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to report.

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Guidelines