TangenX® TFF Cassette Air Integrity Testing

Introduction

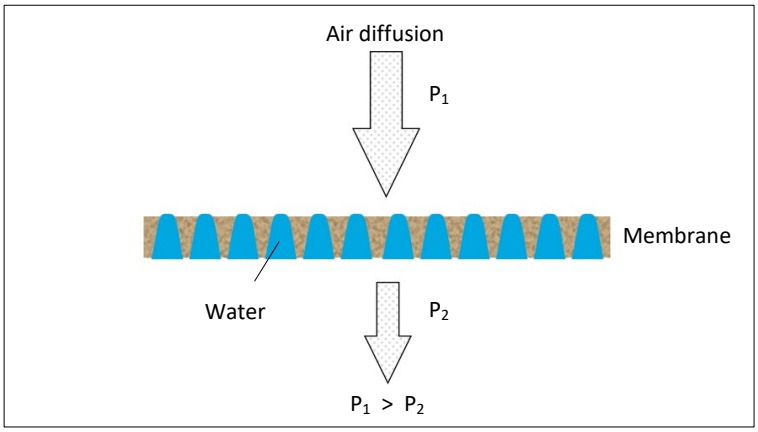
The purpose of cassette integrity testing is to provide a non-destructive method for verifying the integrity of a TangenX® Tangential Flow Filtration (TFF) Cassette. Each cassette manufactured by Repligen undergoes strict release testing, including an air integrity test. Repligen recommends performing an air integrity test on each cassette prior to use. This application note provides basic information about integrity testing, the cassette air integrity test procedure and troubleshooting information and applies to TangenX® SIUS single-use TFF cassettes and to TangenX® PRO reusable TFF cassettes.

Figure 1. TangenX® SIUS Single-use and TangenX® PRO Reusable TFF Cassettes

Cassette integrity background

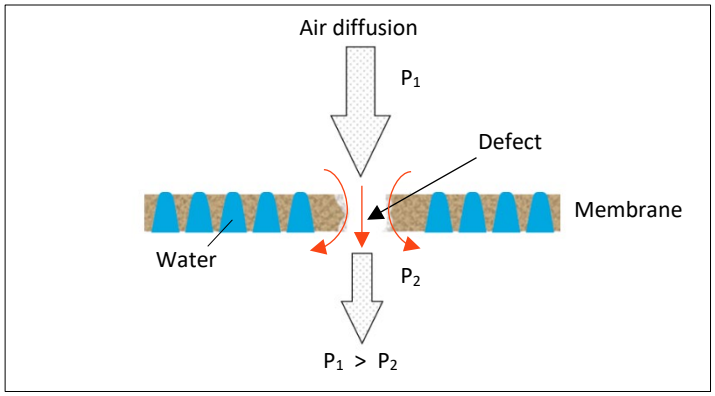
Integrity testing is a non-destructive method used to assure that the device will retain target molecules with little effect on yield or recovery. An integrity test confirms that there are no major defects in the membrane or cassette and that they are correctly installed in the holder.

Figure 2. Air Integrity testing for TFF cassette



Air Integrity is measured at a pressure below the membrane's bubble point. Only minimal amount of air diffusing through the liquid will pass from the feed to permeate.

Figure 3. Air Integrity membrane defect testing for TFF cassette



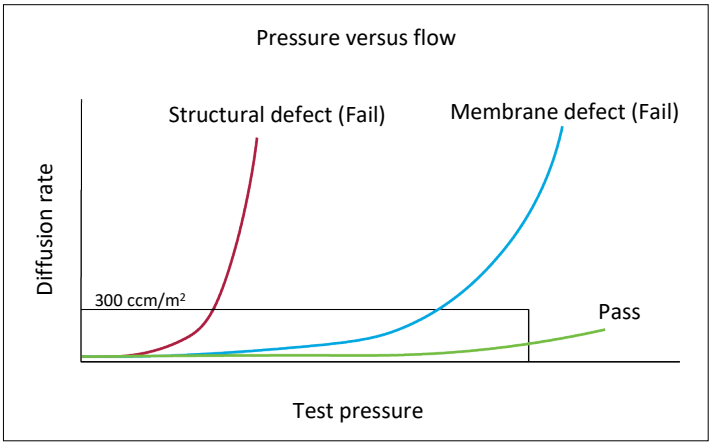
When a defect is present in the TFF cassette, air will easily pass through the opening. The increased air flow is easily detected and indicates a defect is present.

Figure 4. Air diffusion through a membrane equation

J Air flow (cm^3/min)
D Diffusivity of air in water (cm^2/sec)
H Solubility of air in water (cm^2/L x atm)
P Differential pressure
A Area of membrane (cm^2)
C Membrane constant
L Equal to pore length

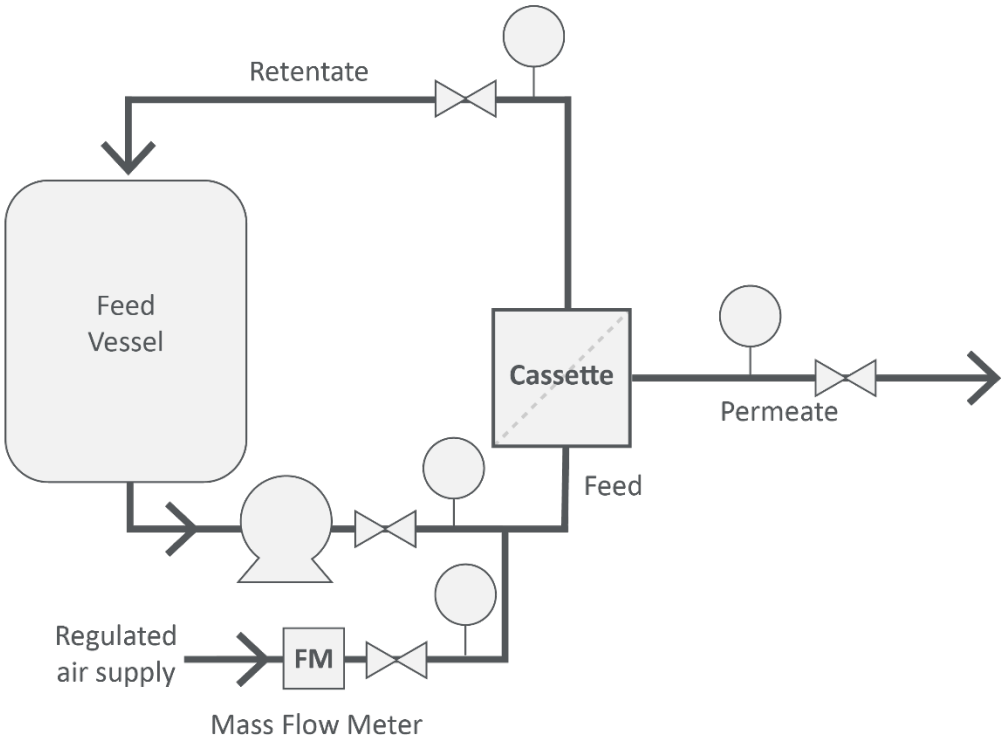
$$J = \frac{D \times H \times P \times A \times C}{L}$$

Figure 5. Air diffusion rate: Pressure versus flow defect testing for TFF cassette



Air Integrity testing for ultrafiltration cassettes can identify both membrane defects as well as structural cassette defects at 300 ccm/m^2 and 1 bar (14.5 psi).

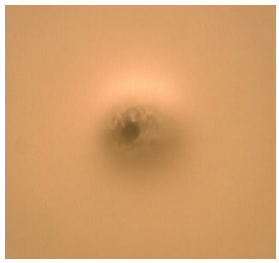
Figure 6. Air Integrity testing set-up for ultrafiltration cassettes



Cassette integrity specificity

A TangenX® SIUS® PD Cassette was tested for integrity using an air diffusion test, where the upstream side of the cassette was pressurized with air. The wetted membrane did not allow a significant amount of air to pass through the membrane due to the surface tension of the liquid in the pores. The result of the initial integrity test is found in Table 1. The specificity of the method was demonstrated by creating a defect (pinhole) in a cassette and measuring airflow before and after the defect was created.

Figure 7. 100X magnification of a membrane



An artificially created defect (pinhole) in the membrane surface demonstrates the specificity of the air integrity test. An image of the defect obtained at 100 X magnification is shown in Figure7. The result of the integrity test following creation of the defect to the cassette is found in Table 1. The pinhole in the membrane allowed air to pass through the membrane and the flow was measured. The difference in the airflow between the initial sample and the modified sample was nearly 100 times greater. The difference was specific to the air diffusion rate and not the liquid cross flow rate. The difference between the two liquid flow rates was not affected and no difference in liquid flow was detected.

Table 1. Integrity test with defect added

	Cassette status: Initial	Cassette status: Modified
Air diffusion rate (ccm)	24	2196
Liquid flow rate (mL/min)	621	620
Results within specification	Yes	No
Difference observed - Air diffusion	N/A	Yes
Difference observed - Flow rate	N/A	Yes

Cassette integrity precision

Method repeatability was shown by testing a minimum of five measurements of one cassette on a single instrument. Repeatability data was obtained by analyzing one cassette five times on the same day using the QC method procedure TX1001-POQ-132. The results of this precision study demonstrate the method is repeatable. For each set of data generated (Table 2), air diffusion rates were within specification and the deviation between replicates was less than 1%.

Table 2. Method repeatability results

		Air diffusion		
Test #	Operator	Air diffusion rate (ccm)	% difference	Within specification
1	A	5	0.45	Yes
2	A	5		
3	A	5		
4	A	6		
5	A	5		

Intermediate precision was shown by having multiple analysts, on multiple days, in one area, perform the testing. The two operators on each of the two days used the same cassette and raw materials. Intermediate precision results were used to identify which of the above factors contributed significant variability to the final result. Each set of data generated for air diffusion (Table 3) were within specification and the deviation between replicates was less than 1%.

Table 3. Intermediate precision results

		Air diffusion		
Test #	Operator	Air diffusion rate (ccm)	% difference	Within specification
1	A	6	0.50	Yes
1	B	5		
2	A	5		
2	B	5		

Integrity test procedure

To perform the air diffusion integrity test on a membrane cassette system, the cassettes should be flushed with deionized (DI) water before performing the integrity test. If temperature stabilization is required (same temperature between the process fluid and assembly), buffer conditioning should also be performed before the integrity test.

Air diffusion is a quantitative test that measures the rate of air diffusing through the wetted membrane at a given pressure differential. Since the measurements are relative, air diffusion rates can be performed on cassettes wetted with water or buffer solution.

The schematic diagram (Figure 8) shows the setup for a generic integrity test instrument. The instrument regulates the required air flow rate and pressure to perform the integrity test. A highly sensitive mass flow meter measures and displays the rate of air that is diffusing through the membrane.

There are two important parts to the integrity test protocol.

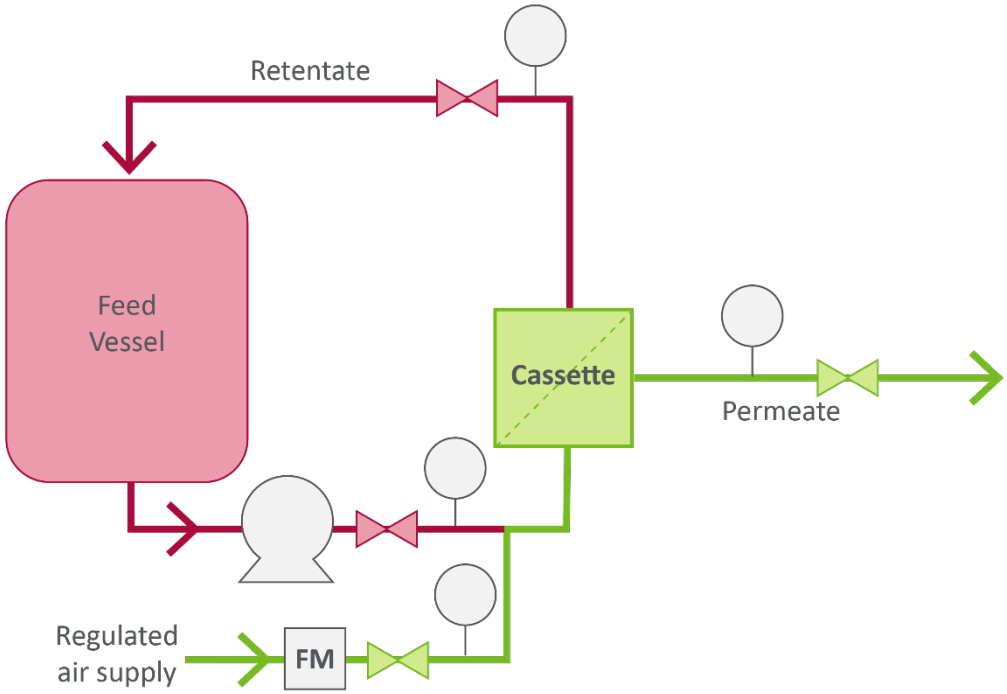
1. System integrity check
2. Membrane integrity check

The system integrity check evaluates the external seals, fittings, plumbing and gasket assemblies for leaks. The membrane integrity check evaluates the membrane and internal seal assemblies.

System integrity checklist

- ☐ Close the feed, retentate and permeate valves on the TFF system (Figure 8).
- ☐ Set air pressure regulator of the integrity test device to the specified test pressure (see Table 4).
- ☐ With the Permeate valve set to drain, open the Permeate valve first then open the valve for the air supply. Purge the liquid from the feed/retentate.
- ☐ When the flow of liquid is followed by air from the Permeate valve, close the valve and let the system charge to the test pressure.
- ☐ As the pressure stabilizes to the test pressure, the air mass flow rate will decrease to zero. (see example graph, Figure 9).
- ☐ If the measured mass flow rate equals zero (approximately), the system integrity passed. Continue to the next step. If the mass flow rate levels above zero, STOP HERE and begin trouble-shooting system integrity failure.
- ☐ Open the Permeate valve to reduce the air pressure very slowly and allow the pressure to reach zero.

Figure 8. Integrity test schematic



Troubleshooting - System integrity failure

Check torque on the nuts on the cassette holder to assure that the recommended setting is maintained.

Manual torque system:

1. Check with torque wrench.

Auto torque system:

1. Check the hydraulic and air pressure to auto-torque pump.
2. Check all sanitary clamp connections and fitting assemblies to assure they are securely tightened.
3. Disassemble cassettes and after rinsing with WFI water, reinstall assembly. If gaskets are more than one month old, replace with new ones.

Membrane integrity checklist

- ☐ Set air pressure regulator to the specified test pressure (see Table 4).
- ☐ Open the Permeate valve.
- ☐ The air flow rate displayed on mass flow meter will increase at first then level off to a constant flow rate. The measured mass flow rate is the air diffusion (see Figure 9).
- ☐ If the mass flow rate is below the recommended specification and consistent with previous measurements, the membrane integrity check passes.
- ☐ If the mass flow rate is above the recommended specification or substantially above previous measurements, STOP HERE and begin trouble-shooting membrane integrity failure.

Troubleshooting - Membrane integrity failure

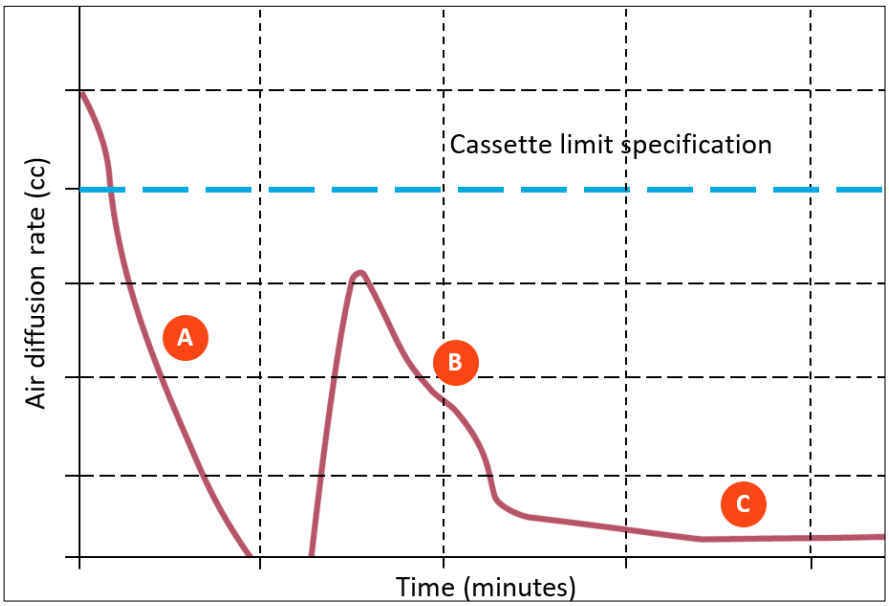
Check torque on the nuts on the membrane cell to assure that the recommended setting is maintained.
Manual torque system:

1. Check with torque wrench.

Auto torque system:

2. Check the hydraulic and air pressure to auto-torque pump.
3. Check all sanitary clamp connections and fitting assemblies to assure they are securely tightened.
4. Disassemble cassettes and after rinsing with WFI water, reinstall assembly. If gaskets are more than one month old, replace with new ones.
5. If air diffusion rate continues to exceed specification limits, disassemble the stack of cassettes and test the integrity of each cassette to determine if integrity is a problem with one of the cassettes. Contact Repligen with the results to receive further advice.

Figure 9. Integrity test air diffusion rate



Example: Typical air (mass) flow rate plotted for an integrity check of a system and membrane cassettes: A = System test B = Membrane test C = Diffusion rate.

System test

As the system fills with air to the targeted test pressure, the air flow rate will decrease to zero (baseline). If it does not reach zero, then the system has an external air leak. Check all fitting and valves.

Membrane test

When the permeate valve is open, the air flow rate will rise due to expansion associated with the membrane elements (cassette) and then the flow rate will decrease to a steady state which is the true air diffusion rate.

Specifications

Table 4. TangenX® Cassette integrity specifications

Cassette channel type	Membrane type (1)	Specification (2)
HP - Fine Screen LP - Medium Screen EP - Screen for higher viscosity feeds S - Suspended Screen J - 0.5 mm Open	Ultrafiltration ≤ 5 kD	≤ 323 ccm/m^2 at 1 bar Or ≤ 30 ccm/ft^2 at 15 psi
	Ultrafiltration 10 kD - 300 kD	≤ 323 ccm/m^2 at 0.5 bar Or ≤ 30 ccm/ft^2 at 7.3 psi
	Microfiltration ≥ 0.1 µm	≤ 323 ccm/m^2 at 0.2 bar Or ≤ 30 ccm/ft^2 at 3 psi

(1) Applies for ProStream and HyStream
(2) 1 ccm = 1 mL/min

Conclusion

The specificity and repeatability of the air integrity test was determined to establish that the method is acceptable for use. The air integrity test procedure is used to verify the cassette air diffusion rate. This information provides the basis for establishing the integrity of the cassette and its fitness for use.