

Part 1: A general comparison of positive and negative pressure caging systems

Part 2: A comparison of operating parameters of four commercial systems designed specifically for mice.

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Part 1

Purpose: To de-mystify the technical & marketing information in the market place regarding the attributes of caging systems

Terminology

IVC	Individually Ventilated cage (for the housing of small rodents)
ACH	Air Changes per Hour
PSI	Pounds per Sq Inch
CFM	Cubic feet per minute
Lt/sec	Litres per second
1 Pascal	1 Newton/sq Mt (1/100,000 Bar)
HEPA	High Efficiency Particle Arrestor
OHSA	Relating to Occupational Health and Safety
CV/RV	Cage Volume/Room volume.
HVAC	Heat Ventilation Air Conditioning.

The design of all rack systems has been driven by the users need for:

- 1. High isolation integrity of each cage**
- 2. Optimum environmental comfort and hygiene for the mice**
- 3. Ease of handling by technicians**
- 4. High quality air for researchers and technical staff**
- 5. Low operating cost**
- 6. Low capital cost**

High isolation integrity of each cage is in the mind of a researcher and the operating technicians at all times.

The other 5 items above are intrinsic to system selection and facility design and the following is a guide to optimising a system for use in a facility.

There are two types of IVC's in common use. This paper will use the nomenclature type A and type B

Type A: These systems work generally with positive pressure. They have been in use for several years and have a powered air handler to circulate pressurised air through the cages so that it is not likely that pathogens can enter the cage.

Typically **Type A** systems operate with a cage pressure of 5 to 10 Pascals and up to 15 Pascals. This is achieved by having a discrete air handler for each cage rack of up to 120 or so cages. In these systems the air is essentially recirculated through the rack and room with

ambient air added from, and exhaust air extracted to the room, effectively diluting cage gasses to a low value (ammonia carbon dioxide and odour). The degree of dilution depends on the room air conditioning and its ability to sweep away these gasses. With high density racking this dilution can be variable depending on the circulation and mixing within the room

The air handler may have some or all of the following features/equipment

- Air circulating pump
- HEPA Filters
- Temperature control
- Humidity control
- Ambient air inlet from the room
- Air exhaust to the room
- Computer controller
- Gas sensors

The greater the number of options the greater will be the energy demand

Generally, setting of the operating parameters is through a keypad and display.

Inherent in this configuration are the following characteristics.

- The racks can work in a low integrity environment (where ambient air is not pre filtered like an office or normal lab) however the handling of animals for cage change can be high risk to the animals (and the operators) without strict cage handling protocols and air controlled change stations.
- Unless the rack exhaust is directly connected to the room exhaust then there will be an accumulation of cage gasses in the room and in the cages.
- If, as is generally the case, the racks are in an air-conditioned environment, the air conditioning must accommodate the heat load of the rack air handling equipment, the heat load of the mice and operators, the discharge air from each rack together with the gas load emitted from the racks.
- Recirculating a significant part of the air (or any at all) through an air handler back to the cages will lead to a labile level of carbon dioxide, ammonia and odour in the cage air stream. The HEPA filters are to ensure that no **particles** are passed back to cages. If gas filtration is added to the air handler then the heat load will further increase.
- As the cages wear and age, or if cage closing clips are faulty, there is an increasing possibility that there will be direct leakage of air from the cage to the room. This air may constitute a risk to mice and operators and is generally undetectable because the odour will be the same as the HEA filtered air coming from the air handler.
- Part of the positive cage system may be an overpressure escape in each cage. A specific valve may be used or simply provision for leaking from the lid seal or a HEPA filter insert. Some of these air paths in an air handler fault condition can allow unfiltered cage air to enter the room. Note that these cages work typically at 10 to 15 Pascals pressure
- The use of small diameter (< 8mm) air inlet and or outlet devices can cause ultra sonic noise and further, if in one wall of the cage, can lead to poor cage circulation. Usually the air flowing out of an orifice will cause turbulence up to 20 to 30 orifice diameters at about 40 Pascals, but the extraction orifice will cause turbulence only 2 diameters from

the orifice. This means orifice location can significantly affect the air quality in cage extremities and if placed behind food hoppers circulation can be even further impeded.

Much of the above relates to the mice but the technicians and other people may well be in the mouse room environment for 6 to 10 hours per day and OSHA regulations apart from good management, demands a safe work place.

A number of products from mice are known to produce respiratory and skin reactions in some people and for others normal mouse odour can cause nausea.

Type B: These systems are relatively new and directly extract air from the cages with negative pressure so that gasses and particles can not escape from the cage to the room air. The air is not recirculated in the cage or the room; it operates as a one-pass air system.

Typically the cage pressure runs much lower at 2 to 5 Pascals negative in relation to the room in which the rack is installed. This is achieved by simply extracting most of the room air through the cages.

The obvious difference with such a system is that room air must flow into the cages.

To deal with this:

- The cage must have an inlet filter
- The room air should reasonably free from contaminants.
- The cage lid perimeter seal should be capable of preventing leakage from the room directly to the cage.

For optimum isolation all of these criteria must be met.

The common approach is to provide the room with air at a positive pressure and discharge all or most of the room air through the cages with room extraction equipment. There is also the option to use semi portable containment systems to provide this environment for example a "Bio Bubble"

- Each cage is generally fitted with an inlet filter located in the front plane of the cage typically 10 to 50-micron pore size and typically 200 Sq mm in area.
- For added hygiene the air extracted from the cage at the rear or from the top of the cage and is generally filtered as well to avoid detritus from food and bedding entering the extraction system.
- The airflow is from low front to high back (or top) of the cages and there is no possibility of airflow noise being generated due to the large cross section of the inlet and outlet apertures.
- Dead spots within the cages generally don't exist in rectangular cages, and air mixing is generally excellent. In addition, in the event of air extraction failure some airflow will be maintained by thermal pumping caused by the heat generated by the mice.

Such a system places the emphasis optimising the environment for the benefit of people and mice.

- The people benefit because there is no odour, no ammonia and no other irritants in the room air.
- The mice benefit because they are not exposed to other than fresh filtered air.

- Change stations with extraction to outside are still required to ensure that cage air is swept away when cages are opened and changed and the extraction from the change stations is used to make the room and cage airflow equations balance. (See spread sheet)
- To achieve this degree of room air quality is not difficult and further benefit can be achieved if the air flowing into the room is HEPA filtered with a filter attached externally to the room system such that it can be routinely maintained by building services without non-essential personnel entering the mouse rooms.

Appropriate airlocks to the room are also standard in most mouse facilities.

To make this whole system work efficiently there has to be a satisfactory amount of air flowing to the room.

Before this is addressed the issue of air flow into and out of the cages needs to be clarified.

What is an ACH?

For ease of comparison with systems from non-metric countries, the two terms for airflow, namely Litres per second (**L/sec**) and Cubic feet per minute (**cfm**) will be expressed as follows:

Lt/sec will be first with Cubic feet per min in brackets following. Values will be integers and rounded as follows **28 l/s (60cfm)**

For pressure, because the pressure is so low, it is convenient to work in **Pascals**.

To express an air **quantity in ACH** both the flow and the volume of the room or cage must be known.

For all engineering and air-conditioning purposes 1 ACH is an amount of air moved into and out of a space equal to the volume of that space.

This does not define the **intrinsic quality** of the room/cage air because this is a function of mixing and air flow patterns

As an example if a room is 10X3X4 = 120 units of volume, then 1 ACH is 120 units of volume flowing into and out of the room in one hour. Clearly if the inlet and outlet for the air are close then mixing and refreshing of the air may be less than perfect.

If a mouse cage has a volume of 6 Lt, then, if in one hour, 6 Lt of airflow into and out of the cage then the rate is 1 ACH. Again this may not define the mixing or refreshing in the cage volume

The amount of air that needs to flow through a cage is dependant on

- The relative humidity
- The rate of generation of carbon dioxide (number and size of mice)
- The rate of generation of ammonia. (Number and size of mice)
- The mixing and dispersion of the air in the cage

It is not practical to monitor each cage and modulate airflow to suit each cage so a standard load of six 25-gram mice is used as a base line.

From empirical work done in many places the range of air required for each cage is between 80 and 130 ACH.

For a 6 Lt cage this is airflow of **0.13 l/s (0.28 cfm) to 0.22 l/s (0.46cfm)**

Data in the following spreadsheet provides a guide for using negative IVC's with the room and cage parameters and dimensions being variables. The cage volume and the total number of cages in a room together with over supply of air can be inserted to give an indication of the system requirements.

The calculation of room supply and return duct flow and pressure is not covered here because this is the realm of the ventilation engineers who, depending on climate, may include heat exchangers to transfer energy between exhaust air to inlet air to further reduce energy demand. The data below does however form a basis for calculations.

Important parameters

The room must have at least 7 ACH and up to 15 ACH

Most of the air into the room should be extracted through the cages

The surplus or bypass air can be extracted through the change stations or through vents.

	LONG	WIDE	HIGH	room volume	
room size Mt	10	5	3	150,000	Lt
cage volume	6	Lt			
total cages					
	10	racks			
each of	180	cages			
room total of	1,800	cages			
cage ACH	100	Generally between 80 and 130			
total volume of cages	10,800	Lt			
air required for cages	1,080,000	Lt per hour	300	Lt/sec	
room air input required	1,296,000	Lt per hour	120%	over supply	
in airflow units =	771	CFM or	360	lt/sec	
room ACH	9				

For optimum and simple operation a Constant Airflow Regulator (CAR Valves) can be fitted to **each rack** preset to the rack required airflow. (Some systems require special set-up tools) These CAR Valves respond to variations in flow and pressure and maintain **constant volume** flow over a wide pressure range ensuring that regardless of rack location in the room the airflow will be correct

There is a lot of confusion regarding these different systems but the basic physics can be summarised as follows.

- Flow of gas and air-borne particles will always be from high pressure to low pressure (at constant temperature).
- Depending on mixing and distribution of airflow in a room/cage, for practical purposes, it requires from 11 to 30 room volumes or cage volumes to replace all of

the room or cage air. The replacement is in fact asymptotic so is forever incomplete. This is the dilution factor and is not directly linked to ACH

- Cages with small tubes for air in and out at one end of the cage need up to 30 CV's for complete air replacement.
- Cages with large end-to-end air apertures trend towards 11 CV's for complete air replacement due to better in-cage mixing and the flow of air following the natural thermal gradient in the cage from bottom to top.
- From the above it can be seen that if 120 ACH's are set with a caging system the air in the cage will be changed completely **4 times (120/30=4) with a type A cage and 11 times with a type B cage (120/11=11) in an hour.**
- At 120 ACH it takes 30 seconds for a "particle" of air to flow the length of a cage.

Positive cage pressure creates the pressure gradient for all waste cage gas to flow back into the room from the cages as part of normal operation, and, in addition, air borne particles in fault conditions. It takes many hours for such gasses and particles to be swept from the room by the room air conditioning.

Type A cages have the following characteristics

- A high level of complexity that is generally represented by manufacturers as system sophistication.
- Air handlers that consume energy and generate heat
- Air handlers that by design recirculate cage air many times through the cages.
- The room effectively becomes part of the waste air path for the cages
- Ammonia in the cages is generally > 25PPM
- Ammonia and odour in the room is noticeable.
- Air conditioning load is high.
- From a OSHA perspective these systems are only marginally better than Micro isolators
- The high working pressure makes lid sealing very critical

Type B cages have the following characteristics.

- Negative cage pressure creates the gradient for air to enter the cage and for air/gas to exit the cage on a one pass end to end only basis.
- Ammonia in the cages is generally <<5PPM
- There is no ammonia or odour discharged into the room
- Simplicity of design and operation.
- Low working pressure and a large inlet orifices reduces risk of lid leaks providing the lid fits well
- The room air for negative cages is intrinsically clean if it is HEPA filtered and the room properly constructed.
- Air conditioning load is low. (HEPA Filtering of room air will increase air conditioning load)
- Personnel have an environment comparable to a modern office

Additional data

- The use of positive pressure demands that the lid be secured by clips latches or other mechanical devices because the total thrust on the lid at 15 Pascals can be 100 Gms or more.
- Positive systems can run at >10 Pascals
- Negative pressure in the cage prevents cage leakage to the room and internal pressures of -2.5 to -5 Pascals reduces risk of lid leakage.

Other issues that affect the choice of system

Pressure (type A) systems have airflow that is not particularly good at sweeping the whole cage volume uniformly.

Usually air enters and leaves the cage in pressure systems in one plane of the box either the lid or the rear wall. The orifices are generally small and the separation of the inlet and outlet is also small (a few cm). The location and proximity of the in and out tubes does not create good circulation and further, can bypass some of the “fresh” air from inlet to outlet with no benefit to the mice.

If the air enters near the top of the cage then for the air to circulate to the bottom of the cage it must be cooler than the cage air.

A differential of only a few degrees may not be enough for the corners of the cage to be swept clear of stale air and constant inflow of cold air can cause mouse distress.

Pressure (type A) systems recirculate air through HEPA filters and the room air determines the long-term quality of the air. The room is the “sink” for all of the cage gasses and a compromise must be made between mouse and operator environment. Some **Type A** systems are connected such that they discharge all cage air through the air handler directly to outside the room. Such an arrangement can cause uncertainty in the relative cage pressure.

It is not unusual for ammonia levels in positive systems to stabilise at 20 to 50 PPM whereas negative systems as described are generally much less than 5 PPM

If mouse needs are paramount then the room air conditioning must be increased to meet the personnel needs. In addition up to 300 watts of heat generated by the air handlers attached to the cage must also be handled by the room air conditioner. This is expensive because for every KW generated by equipment in the room another KW will be consumed by the air conditioner to off set this heat. 2 KW Hrs costs about 20 cents or \$4.80 per day.

With negative (type B) systems the emphasis shifts out of the room to building services to ensure that filtered humidified air at 22-24 degrees C is fed to the room in sufficient quantities as shown in the chart. There are no heat generators in the room apart from people and mice. People about 400 watts and mice about 0.4 watts each and no gasses or any other matter from the mice enter the workplace.

Finally the level of isolation and bio-containment is a function of the whole system, namely:

- The room
- The room air conditioning
- The filtration
- The rack
- The handling protocols

Part 2

Examination and comparison of the operating parameters of 4 systems namely.

- **Airlaw**
- **Allentown**
- **Optimice**
- **Tecniplast**

There is engineering, and there is the perception of engineering and this is what this paper has attempted to address. There is no suggestion that this is an exhaustive coverage of issues but it is a reasonably comprehensive review of critical issues. Any or all of the claims and conclusions can be verified from published data, data in the public domain, or simple experimentation with readily available test apparatus

Whatever system is used it is the performance of the system after some months of regular use that will be the critical issue. All polymers age, distort, change size and become brittle with use. The environmental stress that cages are exposed with repeated washing and autoclaving is extreme compared with most polymer applications and, because of this, it is vital that these issues are dealt with by engineering design and practice in such a way that they have no impact on the performance of the system.

Unfortunately this is not clearly in evidence with some products and systems where the use of seals and latches can contribute to distortion.

Similarly in products and systems where no clear provision is made to accommodate the normal ageing twisting and distortion occurring in components constantly exposed to thermal and chemical cycling.

An IVC rack should be a complete engineering entity not simply a cage placed on a rack with air pipes added.

The following document is a list of features and attributes of four IVC Rack systems. There are other systems available.

The main focus is operational data that affects how and where a rack may be installed and the consequential issues that affect the mice the workplace and the people who may be working with the racks 8 hours per day.

There is no attempt to examine or quantify the suitability or otherwise of any system in a particular environment. Nor is the list of attributes claimed to be complete

For detailed specifications refer to the manufacturers information

Parameter	Airlaw system	ACS Optimice system	Tecniplast system	Allentown system
CAGE AND RACK PHYSICAL PARAMETERS				
Available cage volume	6.0 litres	5.8 litres Internal bottle occupies 0.5 Litres	Approx 7.5 Litres	Approx 7 Litres
Cage floor area	427 sq cm	484 sq cm	530 sq cm	500 sq cm
System	Negative total extraction	Negative total extraction	Positive/negative recirculation.	Positive/negative recirculation
Complete cage weight	1.35 Kg	NA		NA
With food water and 5 mice	2.1Kg		2.4 Kg	
Ease of handling cage	Can be easily held in one hand. Well balanced	Asymmetric weight due to offset food and water. Wedge shape not easy to hold in one hand.	Rack and cage latching requires two handed removal, easy to hold in one hand. Well balanced	Rack and cage latching requires two handed removal, easy to hold in one hand. Well balanced
Bottle size	270 ml external	410 ml internal (lid must be removed for access to water)	250 mL external. other sizes available	250 to 500 mL external
Food hopper contents	380 Gms	470 Gms	> 500 Gms	>500 Gms
Lid fit	Force fit by rack location with edge labyrinth	Gravity only: If the lid twists or distorts with repeated autoclaving/stacking the lid may not seal well on the base.	Nylon Latches and soft seal. There is no rack function that guarantees the closure/sealing of the lid	Nylon Latches. There is no rack function that guarantees the closure/sealing of the lid
Cage shape	Long and narrow	Wedge shaped	Rectangular	Rectangular
Power requirements	None	None	240V 200-300W	240V 200-300W
Installed density or cages per unit of floor area	Very high with mechanical rack drive system providing mobility	High but rack must be pushed by operator for floor cleaning	Low due to poor rack density and requirement for air handling module. Heavy and awkward to move for cleaning, top row difficult to reach if 10 rows installed	Medium density because air handler is mounted on the top of the rack and requires no additional floor space.
Cage Materials available	APEC HT polycarbonate or Polyether sulphone	Polycarbonate Polysulphone	Polysulphone Polyether imide	Polysulphone

Parameter

Airlaw system

ACS Optimice system

Tecniplast system

Allentown system

CAGE AIR FLOW PARAMETERS

Air requirements	800 Lt per cage per hour	Published at 60cfm for 100 cages this equals 1,020 Lt per cage per hour (170 ACH)	Drawn from the room and discharged to the room or directly to outside the room. Parameters programmable	Drawn from the room and discharged to the room or directly to outside the room. Parameters programmable
ACH, Cage air changes per hour	120 ACH preset with regulating device. Pressure at building connection to be -100 Pascals Rack connected to HVAC	Actual rack demand 170 ACH but published at 15 to 20 ACH. No explanation found for this anomaly. Rack connected to HVAC No pressure data available	Generally set between 80 and 90 ACH but can be programmed to a much wider range. System not dependant on HVAC but odour and gas is	Generally set between 60 and 90 ACH but can be programmed to a much wider range. System not dependant on HVAC but odour and gas is.
Cage airflow	Bottom front to top rear with one pass. Large cross section apertures each filtered	Bottom front to high at the rear with one pass with two equal size large apertures each filtered	Top back in and top back out small tubular orifices high velocity and no mechanism in design that forces flow to front bottom corners of the cage.	Low rear inflow with top rear extraction airflow is consistent with thermal gradient in the cage.
Power failure consequences. Building or air handler.	Ammonia and CO2 will increase but air will flow in through front filter. No distress of mice for >72 hours	Ammonia and CO2 will increase but air will flow in through front filter.	Ammonia and CO2 levels will rise. Air can circulate through a filter in the top of the cage. This is then comparable to a micro-isolator.	Ammonia and CO2 levels will rise. Air can circulate through a filter in the top of the cage. This is then comparable to a micro-isolator.
Airflow pattern and mixing in cage	Excellent distribution and mixing with no air noise in cages. large low velocity airways	Good distribution apart from twin front dead spots. No air noise in cages large low velocity airways	Reasonable mixing but small orifice on inlet causes local higher velocity (chilling) airflow. Bedding and paper fragments can block exhaust.	Reasonable mixing but small orifice on inlet causes local higher velocity (chilling) airflow

Parameter	Airlaw system	ACS Optimice system	Tecniplast system	Allentown system
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RACK AND RACK AIR FLOW PARAMETERS

Air management in rack	The best arrangement is for the majority of the air entering the room to be extracted through the cages. The Rack regulator valve then maintains optimum flow to cages	Installation advice is to use the ACS anemometer to set flow velocity at the hose. This must be fitted to each rack and the rack flow adjusted and the tester then removed after the damper is set.	Air is managed by an air handling module but heat load of the modules must be accommodated by the room air conditioning system	Air is managed by an air handling module but heat load of the modules must be accommodated by the room air conditioning system
Rack air control	Rack airflow factory preset with built in automatic airflow regulator. The rack airflow is virtually independent of variations in the building systems. Small cage to cage variation	Mechanical damper provided but no built in device for indicating or controlling actual flow. Must buy additional equipment to adjust and set. No data on cage to cage uniformity	Computer programmable but no data on cage-to-cage uniformity. The assumption is that all air entering the cage exits through the exhaust or through the top filter but air can escape around the lid perimeter if latches or seals are compromised	Computer programmable but no data on cage to cage uniformity. The assumption is that all air entering the cage exits through the exhaust or through the filter but air can escape around the lid perimeter if latches or seals are compromised
Cage to cage uniformity of airflow in the rack	Cage pressure variation across the rack less than 20% this can be easily measured	No data	No data	No data
OPERATOR ENVIRONMENT				
Odour and gasses	No odour or gasses from cages enter the room	No odour or gasses from cages enter the room	Total return of gas and odour to the room unless system is exhausted to outside the room	Total return of gas and odour to the room unless system is exhausted to outside the room
Particulate matter	None returned to room	None returned to room	None returned to room unless cage seals are faulty or clamps not secure	None returned to room unless cage seals are faulty or clamps not secure
Room air requirements	Filtered air to room is best for racks and operators. HEPA not essential. The room can operate down to 8 ACH.	No information available.	Conventional air conditioning adequate but with provision for heat load of air handlers. Room should have 15 to 20ACH	Conventional air conditioning adequate but with provision for heat load of air handlers. Room should have 15 to 20ACH

Parameter	Airlaw system	ACS Optimice system	Tecniplast system	Allentown system
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OTHER CONSIDERATIONS

Maintenance and service within the facility	The rack requires no maintenance other than that routinely done by animal technicians	The rack requires no maintenance other than that routinely done by animal technicians	Non animal personnel required to service air handler filtration and electronic equipment. Contracts offered for airway cleaning	Non animal personnel required to service air handler filtration and electronic equipment
Lighting	The rack is made from dark materials limiting light in the cages	The rack is made from dark materials limiting light in the cages	Open racks made from stainless steel. Light levels in cages generally near room ambient level.	Open racks made from stainless steel. Light levels in cages generally near room ambient level.
Temperature	Controlled by room conditions	Controlled by room conditions	Can be controlled by the air handler but at additional heat load	Can be controlled by the air handler but at additional heat load
Cost per installed cage	As low as \$150 per cage installed	Greater than \$200 per cage plus installation Subject to exchange variations and shipping cost	\$350 to \$450 per cage plus installation. Subject to exchange variations and shipping cost	\$350 to \$450 per cage plus installation. Subject to exchange variations and shipping cost
Service and support external	Australian made with 24 hour availability of all components	Fully imported: lead times for spares variable	Fully imported: lead times for spares variable	Fully imported: lead times for spares variable

In summary

There is no perfect cage system.

The effectiveness of any system in the hands of the user

These parameters should be addressed in system selection

Ergonomics

- The cage must be easy to handle by people probably with small hands and small stature.
- The rack must have ease of removal and insertion of the cage without operator strain or complex functions.
- The rack must be easy to move for floor cleaning and general housekeeping

Operational

- The cage when in the rack must consistently seal well and should do so as a matter of design and not operator diligence
- Placement in the rack should guarantee correct cage operation
- The cage even when cycled many times through the processes of cage changing and cleaning etc. must continue to seal well.
- The cages must be easy to handle, economic to store and convenient to transport when off the rack.
- The materials chosen must meet autoclave requirements and be FDA approved
- The rack should be lightweight and easy to move for operating and cleaning
- The choice of rack should be made in conjunction with the parameters of the available or intended air conditioning

Environment

- The environment for the mice must conform to various formal and informal protocols and harmful gas accumulation in the cages must be near zero.
- The work environment for staff and operators must meet modern OSHA standards
- The energy demand should be low and should provide a satisfactory environment for the mice in the event of a building energy supply failure
- The workplace should be free from odour and noxious gasses.
- The rack should be compatible with conventional air conditioning systems.

Cost and availability

- The cost of a system in the hands of the end user should represent value for money for the whole of life of the product and should be well and promptly supported with the capability from time to time to, by simple testing procedures, verify that the cages are performing to physical requirements.

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