How to isolate a rodent: biocontainment caging options

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Interest in research on 'select agents', dangerous federally regulated microorganisms and biological toxins, has intensified in recent years, spurred on by both terrorist threats and natural outbreaks of emerging diseases like SARS, Ebola, and avian influenza. The expanded interest can be easily measured in dollars that are earmarked for research centers, government agencies, and investigators working with select agents. One example: the National Institutes of Health (NIH)-funded Project for the Bioshield Act of 2004, which allotted $5.6 billion dollars for vaccine and drug purchases and research financing over ten years[1]. Moreover, the value of federal research grants for projects involving biosafety levels (BSL)-3 and -4 (the conditions under which *in vitro* select agent work is conducted) increased from $1.2 billion in the two years ending in 2001 to $13.1 billion for the period from 2002 to 2004 (ref. 2).

Such lucrative payouts have increased the demand for the facilities capable of working with these deadly disease agents. Since the National Institute for Allergy and Infectious Diseases (NIAID) began ramping up its biodefense research in 2003, the agency has funded the creation of nine BSL-3 and two BSL-4 facilities[3]. Following that lead, other research institutions across the nation have invested in similar facilities, prompting many companies to increase the availability of biocontainment products. That trend includes the laboratory animal caging industry, which has seen the advent of racks, cages, and other equipment designed for use in the animal research counterpart to BSL, animal biosafety level (ABSL)-3 and -4 facilities. This article provides a review of some of the rack and cage systems available for use in ABSL-3 and -4 facilities or as stand-alone biocontainment equipment.

BIOSAFETY: A DEFINITION

BSL and ABSL guidelines are published in the pamphlet *Biosafety in Microbiological and Biomedical Laboratories* (*BMBL*)[4]. This manual, offered *gratis* on the Centers for Disease Control and Prevention (CDC) website (http://www.cdc.gov/od/ohs/pdffiles/4th%20BMBL.pdf), is jointly produced by the U.S. Department of Health and Human Services, the CDC, and the NIH.

According to the *BMBL* guidelines, ABSL-3 equipment is prescribed for animal research involving certain microorganisms that can be transmitted through the air and pose a serious or possibly lethal threat to human life, such as West Nile virus, *Bacillus anthrasis*, or *Coxiella burnetii*. The measures required for ABSL-3 include autoclaving cages prior to bedding removal, incinerating all waste from the animal room, and ventilating the room such that the direction of airflow is from external 'clean' areas into the research area (creating a negative pressure differential between the external areas and the research area). The *BMBL* allows for a variety of caging options to satisfy its requirement for 'primary containment', including open cages contained in laminar flow cabinets or filtered solid-wall and -bottom cages. Any manipulation of the cages that may disrupt the primary containment system (such as might be called for in the research protocol) should be done in HEPA-filtered Class II or III biological safety cabinets[4].

ABSL-4 precautions are reserved for animal research on certain lethal or exotic aerosolized disease agents that have high or unknown rates of transmission to humans, including Lassa virus and Junín virus. Safety measures for ABSL-4 include those for ABSL-3, plus some additional requirements, such as that all wastes be decontaminated in a double-door autoclave and that research only be done in Class III biological safety cabinets. The *BMBL* also recommends whenever possible using disposable materials that can be autoclaved and incinerated[4].

General specifications

Research involving select agents is often individually tailored to a specific project or laboratory. Because no single system can serve every need or situation, vendors offer many subtly different options to satisfy the sundry research requirements of different facilities (**Table 1**). A number of characteristics are common to the different biocontainment systems in this article:

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TABLE 1 | Selected specifications for ABSL caging from vendors

Company	Rack	Rack capacity (cages)	Rack size (mm)	Rack weight (kg) (without cages)	Rack material	Cage material
Allentown	BCU-1000 and -2000 (mouse)	42 or 98	(1676 × 587 × 2038) to (1844 × 813 × 2038)	270–360	Fully seam-welded stainless steel	Polysulfone
Allentown	BCU-3000 and 4000 (rat)	30 or 60	(2038 × 673 × 2273) to (2038 × 965 × 2273)	305–445	Fully seam-welded stainless steel	Polysulfone
Animal Care Systems	OptiMICE	100	1980 × 840 × 990	270	Stainless steel	Polysulfone or smoked polysulfone
BioZone	SX220909RC (mouse)	81	1810 × 620 × 2025	310	Stainless Steel	Polysulphone
BioZone	SX340707RC (rat)	49	1775 × 675 × 2075	290	Stainless Steel	Polysulphone
Innovive	InnoRack (mouse)	32, 56, 112, or 168	(813 × 813 × 1905) to (1753 × 813 × 2032)	114–295	Stainless steel	Polyethylene terephthalate
Innovive	InnoRack (rat)	20, 40, 60, or 80	(1753 × 915 × 635) to (1753 × 915 × 1880)	143–273	Stainless steel	Polyethylene terephthalate
Lab Products	bCON	24 (1161 cm^2) or 48 (453 cm^2)	1918 × 546 × 1481	340	Fully seam-welded stainless steel	Zyfone
Lenderking	Micro-Safe	36–140	(1690 × 1481 × 480) to (1926 × 2434 × 780)	68–250	Stainless steel	Polyethersulphone or polyetherimide
Tecniplast	IsoCage Rack	30–72	(1710 × 550 × 1958) to (1710 × 880 × 1958)	216–363	Fully seam-welded stainless steel	Polyethersulphone

- Fully autoclavable racks and cages;
- Airtight, individually ventilated cages (IVCs) (except BioZone and Lab Products);
- Cage-level pre-filters and other filters;
- Rack-level HEPA filters;
- Cages that remain sealed after removal from the rack (except BioZone and Lab Products).

Each of the biocontainment cages and racks described in this article (with the noted exceptions for BioZone and Lab Products, whose systems use alternative technology to achieve the same level of safety) exhibits the five characteristics listed above. In addition, each of the cages and racks also has unique aspects that to a certain degree set it apart from the other products offered in the field. When choosing a biocontainment system for a given facility or purpose, it is often these differences that prove important in determining which system best suits the research goals. The biocontainment products described below are separated into three categories based on type of ventilation system: negative-pressure blower systems, negative-pressure blower systems that are positive-pressure capable, and non-blower systems.

BLOWER SYSTEMS

Most biocontainment systems are 'blower-driven', which means that they rely on fans to circulate air through the unit's ventilation system. The standard *modus operandi* for these closed systems is to blow HEPA-filtered air into the rack ventilation infrastructure, which then flows through the IVCs and out their exhaust plenums into an exhaust duct. Then the exhaust is filtered and vented outside the building, often through the house ventilation system. All systems that rely on blowers have redundant fans, only one of which is necessary to keep the system running. The blower-driven biocontainment systems described below are variations on this theme.

NEGATIVE-PRESSURE BLOWER SYSTEMS

Allentown, Inc.

Allentown's (Allentown, NJ) Bio-Containment Unit (BCU) comes in both rat and mouse varieties. The mouse version comes in two lines, the BCU-1000 (42 cages, single-sided) and the BCU-2000 (98 cages, double-sided). Likewise, there are two types of rat racks, the BCU-3000 (30 cages, single-sided) and the BCU-4000 (60-cages, double-sided).

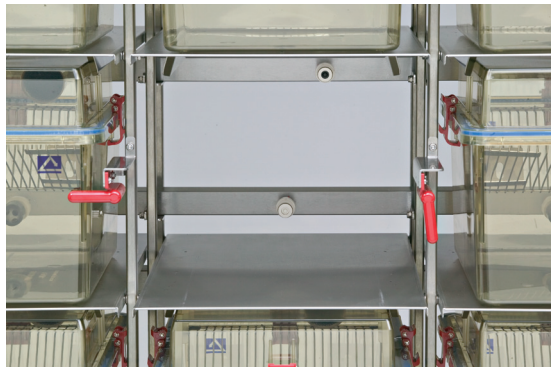

FIGURE 1 | The four-point sealing for Allentown's BioContainment Unit (BCU). One cage on the rack is missing, allowing the two rack sealing points to be visible. Two additonal sealed air fittings on the BCU cage complete the four-point seal, which seals off the cage and rack when the cages are removed.

Ventilation and filters. The lid-mounted exhaust prefilter prevents debris from building up in the plenums. The BCU's HEPA filters are independently certified and separate from the blower to avoid contamination of blower components. The system functions by means of an 'intelligent' microprocessor that continuously adjusts the blower speed to maintain the preset pressure. The BCU has 'four-point sealing' in the sense that all cages employ quick-connect air fittings that seal them to the supply and exhaust plenums (**Fig. 1**). The exhaust and supply ports on the rack itself are also sealed.

Monitoring and security components. The BioSense cage, which the microprocessor uses to monitor the system's conditions, is habitable, allowing more accurate measurement of the animal environment. In addition, the BCU microprocessor can simultaneously activate the alarms and adjust the blower speed to compensate for a malfunction in one of the blowers. The microprocessor also alerts operators when components need service or replacement. The system comes equipped with a 10-hour battery (20-hour battery upgrade optional) to guard against power failure or related emergency. The BCU has two cage locks and is Bluetooth capable for remote wireless real-time monitoring.

Other features and accessories. The BCU has an optional thimble hookup for the removal of heat and odor, and comes equipped with built-in ports for vaporized hydrogen peroxide (VHP) decontamination. There is also a QDX test fixture for on-site cage challenges.

Lab Products, Inc.

The bCON Biocontainment System has been designed by Lab Products (Seaford, DE) for housing mice in ABSL-3 facilities or below. Prior to the development of IVCs, ABSL-3 facilities used simple non-ventilated static cage systems for biocontainment. Lab Products has built upon this traditional system to create bCON, which consists of negative cages (never positive) surrounded by a HEPA-controlled environment within a cabinet in an animal holding room. The cages come in two interchangeable sizes, Super Mouse 750 (483 cm^2) (**Fig. 2**) or Super Mouse 1800 (1161 cm^2, which takes up the space of two 483 cm^2 cages).

Ventilation and filters. Air is HEPA-filtered and flows into the cabinet air supply system. Once circulating inside the cabinet, the HEPA-filtered air is drawn through the cage filter-top and then evacuated through the airlock cage exhaust connection, then through a HEPA-filtered exhaust unit. The cage exhaust connection has a reusable filter that can be cleaned. Removing cages does not affect the cage airflow: the cabinet supply air replaces the same amount of air that is evacuated from the negative cage. The cages are not sealed but are maintained continuously under negative pressure while in the bCON cabinet and revert to a static cage condition after removal from the rack.

FIGURE 2 | Lab Products's Super Mouse 750 (483 cm^2). After the cage is inserted into the fully docked position, a hinged plate comes down in front of the cage for visual confirmation of proper docking. Two Super Mouse 750s can be replaced by one Super Mouse 1800. The different size cages can be mixed and matched on a single rack.

Monitoring and security components. The bCON unit's parameters are constantly monitored by the E^2 environmental control system, which records the cage air changes, cabinet airflow, air pressure, temperature, humidity, fan performance, and HEPA filter hours. Cages revert to a safe static cage mode if the airflow is disrupted.

Other features and accessories. Uninterruptible power supply (UPS) battery power is available to further protect the bCON against power outages. The E^2 system also provides remote monitoring, a 60-day accessible backlog of data in the on-board memory, and alarm alerts. The bCON system can be decontaminated by either chlorine dioxide gas or VHP.

Tecniplast USA

Tecniplast USA's (Exton, PA) IsoCage System includes a rack, air handling unit, Class II biosafety cabinet (the IsoCage Biosafety Station), and specialized IVCs (the IsoCages) (**Fig. 3**). The rack comes in one of four sizes: 30- or 36-cage racks (both single-sided) or 60-cage or 72-cage racks (both double-sided).

Ventilation and filters. Each IsoCage in the system has a consistent negative pressure that is maintained even after it is disconnected from the ventilating rack. A soft silicon seal, located between the top and base of the cage, maintains the negative pressure. Blowers are

located off the rack to ensure that the noise and vibration are not transmitted.

Although the IsoCage system should be linked up to a central UPS, the air handling unit has its own UPS that allows the system to run autonomously for at least three hours. The unit can be removed to wash or autoclave the rack.

Monitoring and security components. The IsoCage system is controlled by the combination of the air handling unit and a control cage. The control cage monitors the airflow conditions and relays that data in real time to the air handling unit, which in turn modulates the twin fans to attain the programmed negative pressure. An alarm is activated when the airflow drops below a set point.

Other features and accessories. There is an optional thimble connection for exhausting through the building exhaust system. Moreover, the IsoCage system is capable of *in situ* hydrogen peroxide decontamination.

The IsoCage Biosafety Station (IBS), a Class II laminar flow cabinet, is designed for use with the IsoCages. The IBS is used to manipulate the IsoCages. The outside of the cages (which are both air- and water-tight) can be disinfected in the attached dunk tank or transferred directly into an autoclave via a port that connects to an autoclave transfer chamber. The IBS can also accommodate an anesthetic gas delivery system.

The IsoCage Mini is a small trolley that can hold Isoline racks for 7 or 11 IsoCages (plus a control cage). The IsoCage Mini has supply and exhaust pre-filters and HEPA filters and is ideal for use as a quarantine unit, as a transport trolley, or in laboratories with small autoclaves.

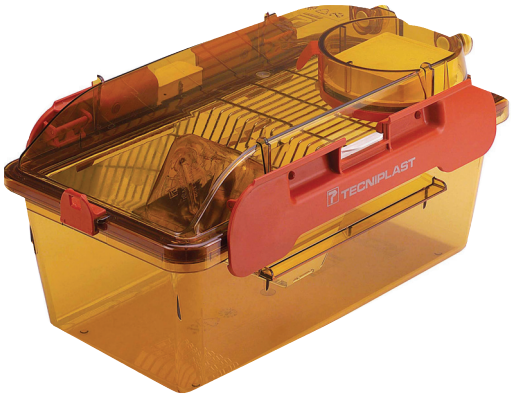

FIGURE 3 | Tecniplast's hermetic IVC, the IsoCage. A silicon o-ring keeps the cage's supply and exhaust values airtight even when the cage is closed or opened. The cage maintains its seal when removed from the rack. Twin clamps on the sides of the cage have safety buttons to prevent incorrect closure or accidental opening.

FIGURE 4 | Lenderking Caging Products's Micro-Safe biocontainment unit, which consists of a controller unit and up to three racks for a maximum of 280 total cages. The unit is depicted attached to one single-sided rack.

DUAL POSITIVE-/NEGATIVE-PRESSURE BLOWER SYSTEMS

These biocontainment systems have the capacity to function in either a positive- or negative-pressure mode. Under ABSL-3 and -4 conditions, these systems would be run almost exclusively in negative-pressure mode, but the dual functionality affords versatility to these products. For instance, such a rack system could initially be used for conventional research or caging needs, then later used with ABSL-3 and -4 cages for select-agent research. Alternatively, the rack might also be used for ABSL-3 or -4 work, then sterilized and autoclaved for conventional research applications.

Lenderking Caging Products

Micro-Safe is Lenderking Caging Products's (Millersville, MD) biocontainment unit, which consists of a controller unit and up to three racks for a maximum of 280 total cages (**Fig. 4**). Micro-Safe is available for both mice and rats.

Ventilation and filters. The Micro-Safe system has the standard ventilation system described under the General specifications section above. At cage-level, the airflow design allows highly efficient ventilation.

Monitoring and security components. The Micro-Safe controller has a touchscreen that monitors the temperature, humidity, and carbon dioxide levels of the room

and the exhaust flow. The system has a standard one-hour backup power supply which can be upgraded to a longer time. For the polyglot, the control unit will display in English, French, German, or Chinese (traditional and simplified characters). The controller is also capable of auto-paging and networking.

Other features and accessories. Lenderking offers bedding-free IVC cages for mice, which eliminate the need to dispose of contaminated bedding. The cages also have built-in cage-level flood protection and a floor that allows waste to pass through to a snap-on waste tray.

Innovive, Inc.

Innovive (San Diego, CA) offers disposable IVC cages (**Fig. 5**) with the InnoRack, their biocontainment rack. The system is available in mouse and rat versions, both of which are modular. Each mouse rack module (up to three per rack) can hold up to 56 cages (double-sided), for a total of 56, 112, or 168 cages depending on the number of modules. The rat rack (double-sided) can be configured with modules (up to four per rack) to hold 20, 40, 60, or 80 cages. Single modules (both mouse and rat) can be mounted on a desktop stand for smaller studies.

Ventilation and filters. The Innovive cage lid snaps onto the cage bottom to form an airtight seal. Single-use HEPA filters in the lid secure both the exhaust and supply ports. The cage-level exhaust HEPA filters also protect a vent at the rear of the cage to ensure the animals will survive for at least 48 hours in case of power failure. A disposable adhesive gasket seals the interface between the cage and the water bottle.

FIGURE 5 | Innovive's disposable IVC cage. The cages are irradiated and double-bagged to ensure sterility. Used cages are bagged under the hood, wiped down, and then autoclaved or incinerated. The cage lid snaps onto the cage bottom to form an airtight seal. Single-use HEPA filters in the lid secure the exhaust and supply ports.

The InnoRack has less than 8% airflow variability between cages because of constant-pressure airflow connections at the cage-to-rack interface. The system is powered by dual HEPA-filtered blowers for both air supply and exhaust. The blowers are regulated by a single intuitive digital controller.

Monitoring and security components. The system provides digital readouts for set points and real-time performance for air changes per hour and pressure differential. Alarms notify researchers of fan failure, filters that need to be replaced, or pressure failure. The 2007 InnoRack allows upgrades for remote wireless management and alerts.

Other features and accessories. Innovive's IVC cages come irradiated and double-bagged to ensure sterility. The system is unique in that dirty and used cages are bagged under the hood, wiped down, and then autoclaved or incinerated. This eliminates cage washing and, in many instances, autoclaving. The cages are then replaced with fresh, irradiated ones. The cages are compatible with all varieties of safety hoods. Visibility of the animals in the cages is enhanced by pivoting transparent cardholders. An optional heating, ventiliation, and air conditioning (HVAC) exhaust adapter is available for the rack. A 32-cage small-footprint mouse rack is available beginning February 2007.

BioZone, Ltd.

Biozone's (Fort Mill, SC) equipment for ABSL-3 and -4 laboratories relies on differential pressure/directional airflow and not on seals to provide the required biocontainment. To this end, BioZone's IVCs can be run in negative pressure for ABSL-3 and -4 applications. Additionally, BioZone's DuoZone system can be used for biocontainment in conjunction with three other BioZone systems: with a BioZone IVC rack enclosed in a BioZone MiniRoom, with a BioZone IVC rack contained in a cabinet, and with individual rows of a BioZone IVC rack (**Fig. 6**).

Ventilation and filters. DuoZone technology uses the Digiflow microprocessor to produce two independent zones of differential pressure, one zone occupied by animals, the other zone (the 'SurroundZone') surrounding the first . DuoZone is capable of creating any combination of positive- or negative-pressure systems, such as two negative-pressure zones, two positive-pressure zones, or one negative- and one positive-pressure zone. The SurroundZone can be created by using the MiniRoom or other cabinet around any BioZone IVC rack or by using a row enclosure around individual rows of a mouse or rat IVC rack. The latter option separately isolates single rows of cages to create small quarantine or biocontainment units. All supply and exhaust air is pre- and HEPA-filtered by large, long-life filters.

FIGURE 6 | BioZone's system uses two independent levels of differential pressure to create ABSL-3 biocontainment. Each row of IVCs, for example, can be enclosed in its own independently ventilated pressure zone, allowing quarantine or laboratory facilities to separately house animals of different provenance in separate rows within the same rack without any risk of cross-contamination.

Monitoring and security components. All of BioZone's IVC or MiniRoom systems monitor and control airflow, differential pressure, and filter conditions. These parameters are displayed on the DigiFlow touchscreen. Alarm contacts are also available to be connected to remote building management systems. Forty-eight hours of battery-powered backup is available for BioZone's systems.

Other features and accessories. BioZone's systems can also monitor and control temperature, humidity, and lighting. Moreover, the system can monitor ammonia levels in the exhaust airflow. Operators can set alarms on either high or low temperatures, relative humidity, or ammonia level. This information is displayed on the DigiFlow touchscreen.

NONBLOWER SYSTEM

Animal Care Systems, Inc.

Animal Care Systems's (Littleton, CO) biocontainment system is compatible with both their M.I.C.E and OptiMICE racks. The M.I.C.E system consists of 14-cage modules configured into either a double- or single-sided arrangement. The rack can accommodate a maximum of 5 modules (70 cages) for the single-sided configuration or 10 modules (170 cages) for the double-sided arrangement. The OptiMICE rack is a circular carousel of 100 cages (**Fig. 7**). The carousel rotates on maintenance-free, low-friction bearings so that operators can access any cage in the unit from a single side.

Ventilation and filters. Animal Care Systems's biocontainment unit does not have blowers, but instead relies on fresh or HEPA-filtered air from the house HVAC system to deliver convection-assisted, low-velocity, one-pass air across its cages. Thus, instead of mixing and diluting air, this system draws HVAC air into the rack through high-efficiency polyester air filters, then delivers that air across each cage and out the exhaust air nozzle at the back of the cage, where it enters a negative-pressure steel plenum that is directly connected to the facility's negative-exhaust air system. Cages removed from the rack do not interrupt the one-pass airflow of those cages left on the rack.

Monitoring and security components. Animal Care Systems's biocontainment unit can be equipped with alarms. Also, the cages themselves are protected by a

FIGURE 7 | Animal Caging Systems's OptiMICE rack. The rack is a circular carousel of 100 cages that rotates on maintenance-free, low-friction bearings so that operators can access any cage in the unit from a single side. The rack does not have blowers, but instead relies on fresh or HEPA-filtered air from the HVAC system to deliver convection-assisted, low-velocity, one-pass air across its cages.

ramped positive-locking system that confirms proper cage insertion and prevents cages from being accidentally removed. In the event of power failure, the heat generated by the animals will keep the system ventilated for 24 hours while still connected to the building exhaust system. If the power outage persists beyond 24 hours, the exhaust drop should be disconnected and allowed to vent freely, which will permit the system to operate for 3–5 days, at which point moisture accumulation necessitates that the cages be changed.

Other features and accessories. Because the rack does not have blowers, the system is free of noise and vibrations. Rack cleaning and draining are facilitated by bottom-drain doors and removable top caps on each cage plenum. Optional features include: cages equipped with stainless-steel floor inserts for bedding-free caging; stainless-steel water-valve grommets on the cage bottoms for automatic watering system access; an inhalation nozzle for delivery of 'test' air directly into the cage; and a trough tunnel situated under the feeder for enrichment.

CODA

The best cage and rack system for a given laboratory or research goal is contingent on variables unique to each facility. In some instances, an institution may need to alter the physical premises of the laboratory to accommodate biocontainment products. In other cases, however, depending on what type of study will be performed and the choice of biocontainment system, the facilities may not have to be altered. A decision about which system to use should be guided by internal discussions among the researchers and facility managers at an institution, as well as consultations with caging vendors, who can offer additional insight and information about their products and how those products might function under specific circumstances. To find out more about any of the products described in this article, we suggest perusing the vendor websites and contacting them directly to answer any questions or concerns (**Box 1**).

COMPETING INTERESTS STATEMENT
The authors declare that they have no competing financial interests.

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