Operator and Environment Protection Performance Evaluation of Esco Animal Research Workstation

By: Fajar Mustika and Alexander Atmadi

Esco manufactures 3 models of animal handling workstations, VIVA Dual Access Animal workstation (VDA), VIVA Universal Animal workstation (VA2), and VIVA Bedding Disposal workstation (VBD). These VIVA products were designed to help promote the health and safety issues for the scientists, veterinarians and animal care professionals.

To meet this design objective, the allergen containment performance of these cabinets was studied using actual mice allergen as challenge aerosol. Actual cage changing and bedding disposal operations were performed to simulate how the workstations are used, and allergen concentration at designated areas was sampled. The allergen concentration was quantified using sandwich ELISA method, and operator and environment protection of each type of workstation was evaluated.

1. Introduction

Allergens are complex molecules that can stimulate antibody response in certain or susceptible individuals. Allergens are usually proteins and exposure commonly results in an Immunoglobulin E (IgE) response (immune defense against foreign objects, i.e. bacteria and viruses).

Animal workers experiencing repeated low-level exposure might lead to sensitization, and subsequent to sensitization, a response may occur with further exposure. Continued sensitization may lead to breathing difficulties, chest tightness, fever, and cough. These symptoms usually become a disease called hypersensitivity pneumonitis and innate risk factors are unknown.

The major route of exposure to animal allergens is inhalation of allergen particles less than 10 µm in size, which tend to deposit in lung airways or the thoracic region. Particles less than 4µm are likely to penetrate deeply into the lung and deposit in the pulmonary, or alveolar, region of the lung where gas exchange takes place.

Controlling occupational exposure to animal allergens is the true challenge for ESCO, by providing protective workstations for animal-care workers. Our objectives of exposure control are to prevent and minimize principal route of exposure to animal allergens, particularly from aeroallergens.

2. Material

Three animal handling workstation products were used:
1. VIVA Animal Containment Workstation (VA2),
2. VIVA Bedding Disposal Workstation (VBD),
3. VIVA Dual Access Animal Containment Workstation (VDA).

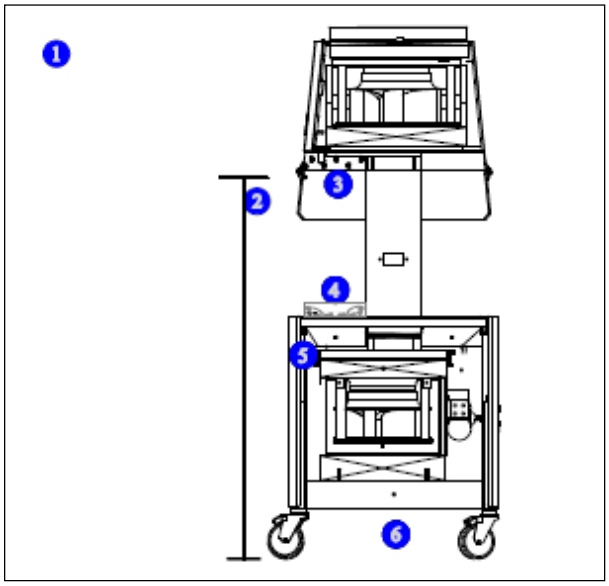
Apparatus used:
1. The dirty cages that are two weeks old. Each of cages contain 5 mice of 2 months old
2. Filter cassettes containing filter membrane with diameter of 25 mm and pore size of 1.0µm, connected by tube to valves, as shown here on the left
3. Valves, to control the flow rate into each sampling cassette
4. BIOS Definer flow meter, to help adjust valves to get 2.0-2.3 liter/min suction for each cassette
5. Vacuum pump, to provide suction to the filter cassettes
6. ELISA kit for Mus m 1 detection system, with buffer and ABTS substrate colorization solutions
7. Rotisserie-type shaker, to extract the allergen from filter membrane into solution in vials
8. Beckman Coulter refrigerated centrifuge, to separate the allergen supernatant in vials
9. Microplate and single & 8-channel micropipettes, to process the allergen solution
10. Microplate reader, to analyze the allergen concentration on microplate

3. Cabinet Airflow Description (Please also refer to the schematics on Section 4):

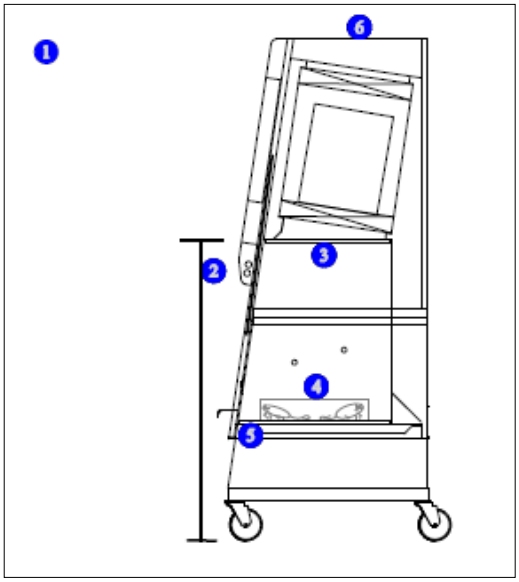
VDA	1. Room air is drawn from top of cabinet, passes through downflow ULPA filter for product protection 2. Downflow is joined by inflow. Both enter through work zone perimeter air grille, creating air curtain 3. The combined airflow passes through carbon filter and ULPA filter before it's exhausted to the room
VA2	1. Room air (Inflow), joins downflow, entering the front grille, creating air curtain 2. The combined air passes underneath tray, back wall, pulled by the blower and blown to the plenum 3. 1/3 of plenum air is exhausted through ULPA filter, and 2/3 becomes downflow, passing ULPA filter
VBD	1. Room air (inflow) enters the work zone, then is exhausted up by the blower through carbon filter 2. The blower pushes the exhaust through ULPA filter on top of the cabinet

4. Allergen Measurement Location

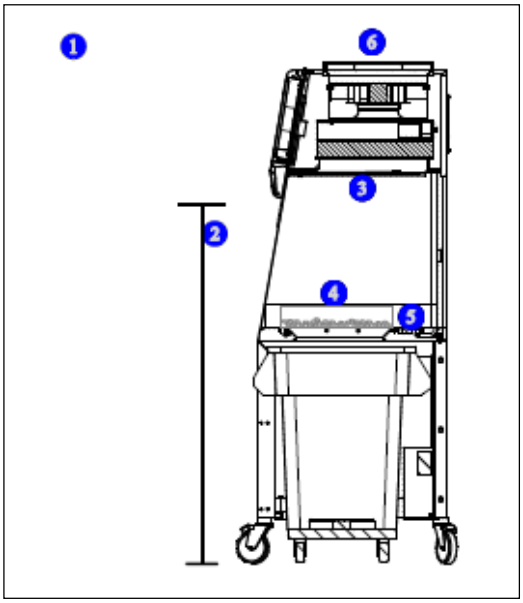
VIVA Dual Access (VDA) Sampling Sites



VIVA Universal (VA2) Sampling Sites



VIVA Bedding Disposal (VBD) Sampling Sites

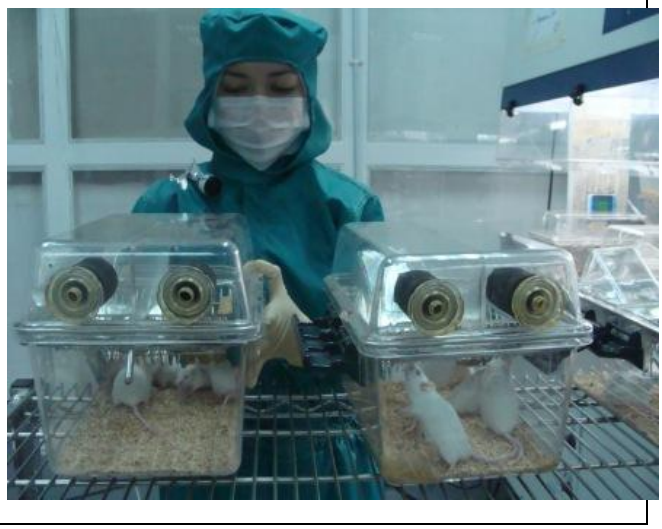


Allergen Sampling Location

No	Location
1	Room, at 1 m (3ft) from front opening, to measure **environment protection**
2	Operator breathing zone, at gown collar to measure **operator protection**
3	Underneath downflow diffuser. Concentration should be close to room on VDA and VA2, and high on VBD
4	On top of the dirty cage, representing the concentration of **released allergen**
5	Underneath the work tray, when air from work zone flows. This is for positive control. Concentration should be high like on the dirty cage.
6	Downstream of exhaust filter. Indicating allergen concentration exhausted to room. Concentration should be close to room air.

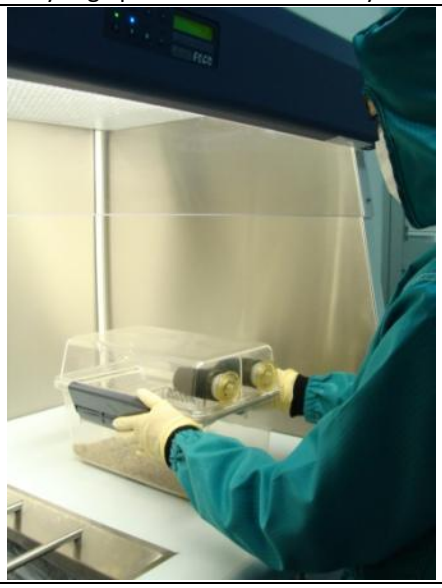
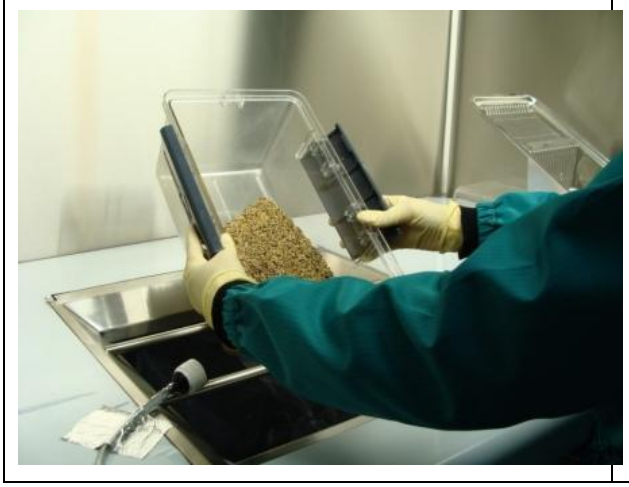
5. Allergen sampling steps on VDA and VA2:

1. Operator wore protective gown.
2. Dirty cages were placed on the left side, and clean cages on the right side of the cabinet.
3. Filter cassettes were placed at designated locations, connected with vacuum pump.
4. Workstation was turned on, and then the work surface was disinfected with 70% IPA.
5. Sterilization solution was poured into a rectangular bowl, and then put in the center of the work zone.
6. Vacuum pump was turned on. The valves have been pre-adjusted to give 2.0 - 2.3 l/min suction.
7. A clean cage and soiled cage were put side by side of the sterilization solution in the work zone.
8. Both cages were opened. The lids were placed next to the cages.
9. The mice were taken by the tail and set into a clean cage, with new bedding that was previously prepared.
10. Mice food and water bottle were placed on top of the clean cage.
11. The cages were closed, and taken out of the work zone.
12. The operator's hands were regularly disinfected with sterilization solution between each cage changing.
13. The mice transfer procedure was performed on 15 pairs of dirty and clean cages for 30 minutes.
14. This procedure was repeated for 4 times by different operators.
15. At the end of sampling, the vacuum pump was turned off, and the sampling cassettes were disconnected from the tubes for subsequent analysis.
16. After the testing finished, the work zone area was cleaned with 70% IPA, and wiped clean.

1.Dirty and clean cages outside of workstation	2. Lids on dirty cages prevent allergen release to room
	
3. Mice were transferred from dirty to clean cage	4.New cages with lids arranged outside of workstation
	

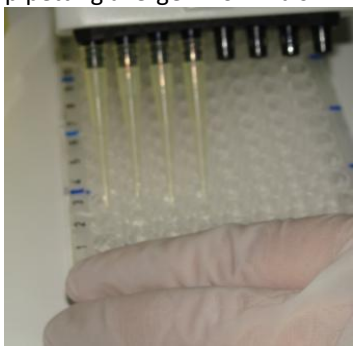
6. Allergen sampling on VBD:

1. Operator wore protective gown.
2. The dirty mice cages were put in a cart, placed outside of the workstation.
3. The filter cassettes were placed at designated sampling locations and connected to vacuum pump.
4. The workstation was turned on.
5. The refuse bag was installed on the disposal bin.
6. The work zone was surface decontaminated with 70% IPA.
7. The vacuum pump was turned on. The valves have been pre-adjusted to give flow rate of 2.0 – 2.3 l/min.
8. The dirty cage was transferred into work zone area. The lid was opened, and placed right next to the cage.
9. The dirty bedding was gently poured out of the cage into refuse bag.
10. The lid was put back on the top of the cage.
11. The cage was taken out of workstation into the cart that was set for empty cages.
12. This bedding disposal procedure was performed on 15 cages for 30 minutes,
13. This procedure was repeated for 4 times by different operators.
14. The vacuum pump was turned off at the end of sampling, and the sampling cassettes removed from tubes.
15. The refuse bag was tied and removed.
16. Finally the work zone was surface decontaminated with 70% IPA.

1.Dirty cages placed outside of workstation	2.One dirty cage placed on the work tray
	
3.Dirty bedding was poured into refuse bag	4.Refuse bag was tied and removed
	

7. ELISA Analysis on Allergen Sampling

Following allergen sampling procedure:
1. Filter membrane from cassettes were removed, put into microcentrifuge vials filled with PBS buffer solution.
2. Vials were shaken overnight, then centrifuged at 2500 rpm, 4°C, 20 min to extract allergen from filter.
3. The microplate was primed for ELISA, then the allergen solution was pipetted from vials to microplate.
4. Two-polyclonal sandwich ELISA method specific for Mus m 1 allergen complex was performed.
5. ABTS solution was pipetted to the microplate. Allergen in wells will turn from clear to green.
6. The green color intensity (allergen concentration) was analyzed using microplate reader.

Shaker and centrifuge were used to extract allergen from filter membrane	Microplate was primed before pipetting allergen from vials	Microplate reader was used to analyze allergen concentration
		

Operator protection efficiency was calculated by comparing the amount of allergen captured by the cassettes worn by the operator against the allergen sampled on top of dirty cages.

Environment protection efficiency was calculated by comparing the amount of allergen captured by the cassettes placed inside the test lab against the allergen sampled on top of dirty cages.

8. Test Results for VIVA Dual Access Animal Containment Workstation (VDA):

Allergen at	Sampling sites	Allergen concentration (ng/m3) at Test Replicate			
		1	2	3	4
1. Protected area	Operator breathing zone	0.491	0.719	0.625	0.563
	Room	0.536	0.444	0.535	1.136
	Downstream exhaust filter	0.438	0.433	0.624	0.658
2. Source	Dirty cage	86.868	88.965	47.727	47.014
3. Inside work zone	Below diffuser	6.796	6.621	8.317	8.514
	Below inflow air grille	53.215	53.379	77.419	77.089
Protection efficiency (%)	Operator	99.43 %	99.19 %	98.69 %	98.80 %
	Environment (Room)	99.38 %	99.50 %	98.88 %	97.58 %

From four testing replicates, Esco Viva Dual Access (VDA) animal workstation provides an average of **99.03 % operator protection** of mice allergen, and **98.80% environment protection**. This high degree of protection would substantially help to prevent the allergen-related sensitization for the users.

Unlike on VA2 and VBD, worst case animal handling scenario with bad technique was not yet performed on VDA. This would be done for future study.

9. Test Results for VIVA Universal Animal Containment Workstation (VA2):

Allergen at	Sampling sites	Allergen concentration (ng/m3) at Test Replicate			
		1	2	3*	4*
1. Protected area	Operator breathing zone	0.832	0.821	6.849	6.741
	Room	5.599	5.260	3.561	4.744
	Downstream exhaust filter	0.816	0.811	1.813	0.937
2. Source	Dirty cage	109.106	105.030	80.833	82.417
3. Inside work zone	Below diffuser	1.851	1.203	3.511	3.398
	Below inflow air grille	89.093	82.667	262.516	266.170
Protection efficiency (%)	Operator	99.24 %	99.22 %	91.53 %	91.82 %
	Environment (Room)	94.87 %	94.99 %	95.59 %	94.24 %

On this cabinet, two different animal handling techniques were tried. Test #1 and #2 were performed using **good technique**, with careful handling and placing the cages on the work tray. The average **operator protection using this good technique was 99.23 %**, and the average **environmental protection was 94.93 %.**

Meanwhile, Test #3 and #4 were performed using bad technique to investigate **worst case scenario** of cabinet usage, with harsh movements that disrupts the airflow curtain, and placing the cages partially outside the work area, covering part of the inflow grille that reduces operator protection. The average **operator protection using bad technique was 91.68 %,** and the **average environmental protection was 94.92 %.**

From the testing, it can be observed that VA2 cabinets provide a high level of containment when combined with good working practice, and yet still manage to provide good protection even with improper handling technique.

10. Test Results for VIVA Bedding Disposal Workstation (VBD):

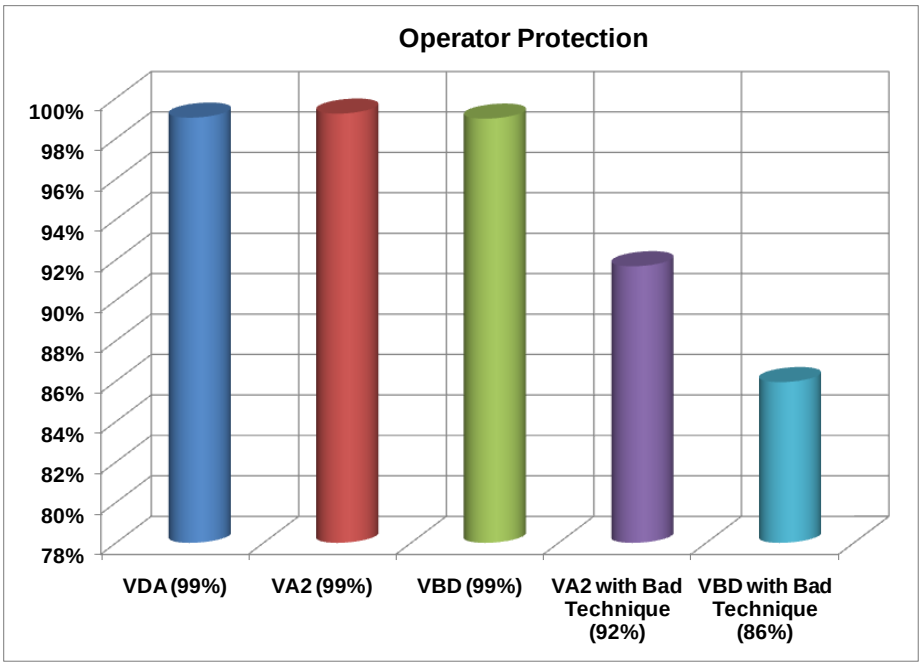
Allergen at	Sampling sites	Allergen concentration (ng/m3) at Test Replicate			
		1	2	3*	4*
1. Protected area	Operator breathing zone	0.785	0.785	6.779	6.930
	Room	0.778	0.778	0.824	0.824
	Downstream exhaust filter	0.771	0.796	0.812	0.812
2. Source	Dirty cage	76.612	77.518	50.484	47.230
3. Inside work zone	Below diffuser	20.727	20.902	18.701	18.484
	Below inflow air grille	72.732	84.520	64.880	64.765
Protection efficiency (%)	Operator	98.97 %	98.99 %	86.57 %	85.33 %
	Environment (Room)	98.98 %	99.00 %	98.37 %	98.26 %

On this cabinet, two different animal handling techniques were tried. Test #1 and #2 were performed using **good technique**, with careful handling and placing the cages in the middle of work tray. The average **operator protection using this good technique was 98.98 %**, and average **environmental protection was 98.99 %.**

Meanwhile, Test #3 and #4 were performed using bad technique to investigate **worst case scenario** of cabinet usage, with harsh movements that disrupts the airflow containment, and placing the cages at the front edge of the work area, close to the operator, that reduces operator protection. The average **operator protection using bad technique was 85.95 %,** and the **average environmental protection was 98.32 %.**

From the testing, it can be observed that VBD cabinets provide a high level of containment when combined with good working practice, and yet still manage to provide good protection even with improper handling technique.

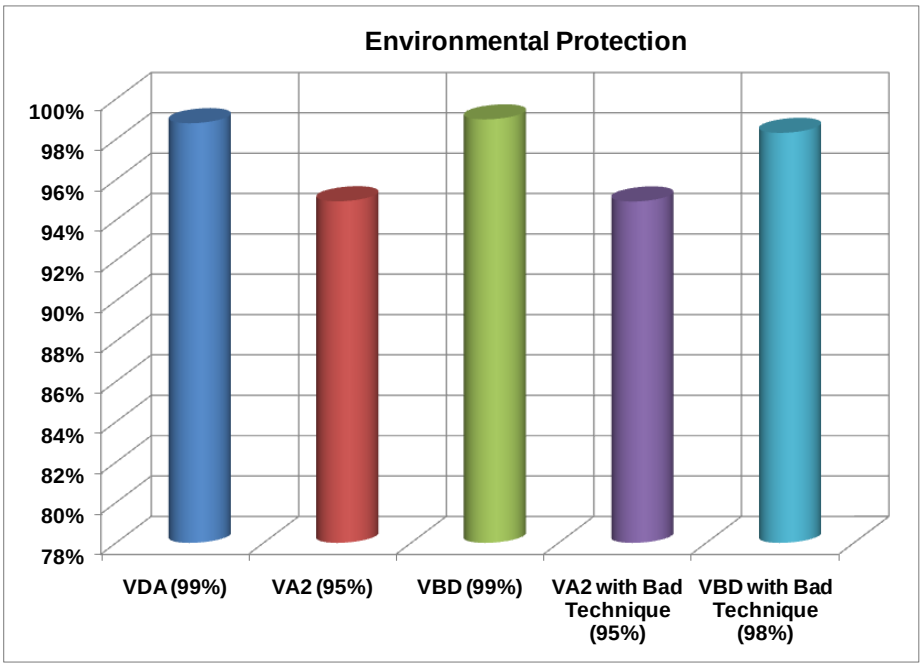
11. Observation of ESCO Animal Handling Workstations Performance



1. All three tested cabinets (VDA, VA2, VBD) have same high operator protection level of 99% when operated using good technique.

2. Even when bad technique was used, the VA2 cabinet still provides good operator protection level of 92%, and the VBD still provides a respectable protection level of 86%.

3. When bad technique is used, VA2 offers higher protection level than VBD, because VA2 has inflow and downflow air curtain like a Class II biosafety cabinet, whereas VBD only has inflow like a Class I biosafety cabinet, and therefore is more susceptible to airflow disruption.



4. The VDA and VBD have high degree environment protection of 99% because unlike VA2, they are equipped with activated carbon filters that help the ULPA filters to further absorb the allergens.

5. Despite having no activated carbon filters, the ULPA filters installed on the VA2 still provide a good environment protection level of 95%.

6. Even when bad technique was employed, the environment protection level of both the VA2 and VBD was unchanged, suggesting that the allergen leak only occurs in minute quantity, and it's immediately diluted in the room air.

12. Conclusion

1. The VIVA Dual Access, Universal, and Bedding Disposal (VDA, VA2, VBD) Animal Workstations offer high levels of operator and environment protection, that can substantially help to minimize the allergen exposure.

2. Despite the Animal Workstations' respectable performance with improper work technique especially in environmental protection; good technique is still important to minimize the operators' exposure to allergens.

3. ULPA filters used in Esco Animal Workstations can effectively absorb the allergens, however this can be further enhanced by activated carbon filters, present on VDA and VBD cabinets.

References

Harrison. DJ,. 2001. *Controlling Exposure to Laboratory Animal Allergens*. ILAR Journal, Vol 42, NO. 1.

Krohn. TC, Hansen. AK,. 2004. *Reduction in the Spread of Rodent Urinary Allergens During Cage Changing by Laminar Airflow Cabins*. Scandinavian Journal Lab Animal Science, Vol 31, No.3

Krohn. TC, Itter. G, Fosse. R, Hansen. AK,. 2006. *Controllling Allergen in Animal Rooms by Using Curtains*. Journal of the American Association for Laboratory Aninal Science, Vol 45, No. 3

Ottesen. JL, Mikkelsen. LF, Bertelsen. T, Krohn.T, Velschow. S, Hau. J, Mollegard. H, Dragsted.N, Hansen. AK,. 2007. *Collaboration between Academia and Industry with Focus on Improvement of the Welfare of both Animals and Humans in Laboratory Animal Facility*. AATEX 14, Special issues.

Standard Operating Procedure AC209. 2004. *Micro-Isolator Techniques*. Division of Laboratory Animal Resources (DLAR), University of Kentucky Medical Center.

Korpi. Anne et.al,. 2003. *Detection of Mouse and Rat Urinary Aeroallergens with an Improved ELISA*. Environmental and occupational disorders - American academy of allergy, Vol 113, No.4

Thulin.H, Bjorkdahl.M, Karlson.AS, Renstrom.A,. 2002. *Reduction of Exposure to Laboratory Animal Allergens in a Research Laboratory*. American occupational hygiene, Vol 46, No 41