Primary Containment for Biohazards:

Selection, Installation and Use of Biological Safety Cabinets

3rd Edition

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non-ventilated “boxes” built of wood and later of stainless steel, within which simple operations such as weighing materials could be accomplished.[2]

SECTION III

Biological Safety Cabinets

The similarities and differences in protection offered by WKH YDULRXV FODVVHV RI %6&V DUH UHÀHFW also refer to Table 2 and Section IV for further considerations pertinent to BSC selection and risk assessment.

The Class I BSC

The Class I BSC provides personnel and environmental protection, but no product protection. It is similar in air movement WR D FKHPLFDO IXPH KRRG EXW KDV D +(3$ V\VWHP WR SURWHFW WKH HQYLURQPHQW)L %6& XQ¿OWHUHG URRP DLU LV GUDZQ DFUR 3HUVRQQHO SURWHFWLRQ LV SURYLGHG E\ W ORQJ DV D PLQLPXP YHORFLW\ RI OLQHDU is maintained[6] through the front opening. Because product protection is provided by the Class II BSCs, general usage of the Class I BSC has declined. However, in many cases, &ODVV , %6&V DUH XVHG VSHFL¿FDOO\ WR HQ H J FHQWULIXJHV KDUYHVWLQJ HTXLSPHQ RU SURFHGXUHV ZLWK SRWHQWLDO WR JHQH GXPSLQJ FXOWXUH DHUDWLRQ RU WLVVXH K

7KH FODVVLFDO &ODVV , %6& LV KDUG GXF FRQQHFWLRQ WR WKH EXLOGLQJ H[KDXVW V exhaust fan provides the negative pressure necessary to draw room air into the cabinet. Cabinet air is drawn through a HEPA ¿OWHU DV LW HQWHUV WKH FDELQHW H[KDX ¿OWHU PD\ EH LQVWDOOHG LQ WKH WHUPLQD prior to the exhaust fan.

6RPH &ODVV , %6&V DUH HTXLSSHG ZLWK D fan. The cabinet exhaust fan must be interlocked with the

7 K H & O D V V , , 7 \ S H V $ ⁸ B$SCs pMovid2 Q G %

between the front and rear grilles and two to six inches above the work surface.

The air is discharged through the front and rear grilles under negative air pressure into a blower and pushed into the space EHWZHHQ WKH VXSSO\ DQG H[KDXVW ¿OWHUV RI WKHVH WZR ¿OWHUV DSSUR[LPDWHO\ F WKH H[KDXVW +(3$ ¿OWHU DQG UHFLUFXOD +(3$ ¿OWHU EDFN LQWR WKH ZRUN]RQH 0RV $ FDELQHWV KDYH GDPSHUV WR PRGXODWH

A Class II Type A1 BSC is not to be used for work involving volatile toxic chemicals. The buildup of chemical vapors in the FDELQHW E\ UHFLUFXODWHG DLU DQG LQ W DLU FRXOG FUHDWH KHDOWK DQG VDIHW\ KD

It is possible to exhaust the air from a Type A1 or A2 cabinet outside of the building. However, it must be done in a manner that does not alter the balance of the cabinet exhaust system, and WKHUHE\ GLVWXUELQJ WKH LQWHUQDO FDEL of connecting a Type A1 or A2 cabinet to the building exhaust system is through use of a canopy hood,[8,10] which provides a VPDOO RSHQLQJ RU DLU JDS XVXDOO\ LQFK H[KDXVW ¿OWHU KRXVLQJ)LJXUH 7KH DLU H[KDXVW PXVW EH VXI¿FLHQW WR PDLQWDLQ JDS EHWZHHQ WKH FDQRS\ XQ[a]LW DQG WKH ¿O must be removable or be designed to allow for operational testing RI WKH FDELQHW 6HH 6HFWLRQ 9, &ODVV ,, should never be hard-ducted to the building exhaust system.[8] Fluctuations in air volume and pressure that are common to all EXLOGLQJ H[KDXVW V\VWHPV PDNH LW GLI¿FX UHTXLUHPHQWV RI WKH FDELQHW ([LVWLQJ & ducted exhaust connections can be altered and the room air EDODQFHG WR DOORZ IRU WKH UHFRPPHQGHG ÀXFWXDWLRQV LQ DLUÀRZ 7KLV PD\ UHTXLUH system capacity.

2. <u>The Class II, Type B1 BSC</u>. Some biomedical research

as carcinogens. The powdered form of these carcinogens should be weighed or manipulated in a chemical fume hood or a static-
DLU JORYH ER[HTXLSSHG ZLWK D GRXEOH GF
XVHG LQ FHOO FXOWXUH RU PLFURELDO V\VW
and chemical containment.[11]

The Class II, Type B cabinet originated with the National
&DQFHU ,QVWLWXWH [12] &, OD\RXW RI WKH 7\SH
%6&)LJXUH $ DQG ZDV GHVLJQHG IRU PDQ
TXDQWLWLHV RI WKHVH KD]DUGRXV FKHPLFDO
systems. The NSF International NSF/ANSI Standard 49 - 2002
GH¿QLWLRQ RI 7\SH % [8] Includes the Class II, Type B2 cabinet. This design
7\SH % DV ZHOO DV FDELQHWV ZLWKRXW VXS
LPPHGLDWHO\ EHORZ WKH ZRUN VXUIDFH)LJ
ZLWK H[KDXVW UHFLUFXODWLRQ GRZQÀRZ VS

7KH FDELQHW VXSSO\ EORZHUV GUDZ URRP
WKH FDELQHW¶V UHFLUFXODWHG DLU WKURX
WKH VXSSO\ +(3$ ¿OWHUV ORFDWHG LPPHGLD
VXUIDFH 7KLV SDUWLFXODWH IUHH DLU ÀRZV
at each side of the cabinet and then downward to the work area through a back-pressure plate. In some cabinets there is an
DGGLWLRQDO VXSSO\ +(3$ ¿OWHU WR UHPRYH
generated by the blower-motor system.

5RRP DLU LV GUDZQ WKURXJK WKH IDFH RS
DW D PLQLPXP PHDVXUHG LQÀRZ YHORFLW\ R
7\SH $ DQG $ FDELQHWV WKHUH LV D VSOL
air stream just above the work surface. In the Type B1 cabinet,
DSSUR[LPDWHO\ SHUFHQW RI WKH GRZQÀRZ
WKH UHDU JULOOH SDVVHV WKURXJK WKH H[
discharged from the building. The remaining 30 percent of the
GRZQÀRZ DLU LV GUDZQ WKURXJK WKH IURQW
ÀRZV WR WKH UHDU JULOOH LV GLVFKDUJHG
activities that may generate hazardous chemical vapors or particulates should be conducted towards the rear of the cabinet work area.[13]

Type B1 cabinets must be hard-ducted, preferably to a dedicated, independent exhaust system, or to a properly-

designed laboratory building exhaust. As indicated earlier, fans for laboratory exhaust systems should be located at the terminal

GRXEOH GRRU SDVV WKURXJK ER[H J DQ D
GHFRQWDPLQDWHG EHWZHHQ XVHV 5HYHUVL
materials to be removed from the Class III BSC safely. Both
VXSSO\ DQG H[KDXVW DLU DUH +(3$ ¿OWHUHG
([KDXVW DLU PXVW SDVV WKURXJK WZR +(3$

being manipulated on the clean bench potentially resulting in hypersensitivity, toxicity or infection depending on the materials E H L Q J K D Q G O H G + R U L] R Q W D O D L U À R Z ³ F O H D used as a substitute for a biological safety cabinet. Users must

SECTION IV

2WKHU /DERUDWRU\ +D]DUGV DQG 5LV

Primary containment is an important strategy in minimizing exposure to the many chemical, radiological and biological hazards encountered in the laboratory. An overview is provided, in Table 2, of the various classes of BSCs, the level of containment afforded by each and the appropriate risk assessment considerations. Microbiological risk assessment is addressed in depth in BMBL.[1]

Working with Chemicals in BSCs

:RUN ZLWK LQIHFWLRXV PLFURRUJDQLVPV of various chemical agents, and many commonly used chemicals vaporize easily. Therefore, evaluation of the inherent hazards of the chemicals must be part of the risk assessment when selecting a BSC. Flammable chemicals should not be used in Class II, Type A1 or A2 cabinets since vapor buildup inside the FDELQHW SUHVHQWV D ¿UH KD]DUG ,Q RUGH chemical concentration which might be entrained in the air stream following an accident or spill, it is necessary to evaluate WKH TXDQWLWLHV WR EH XVHG 0DWKHPDWLF assist in these determinations.[13] For more information regarding the risks associated with exposure to chemicals, the reader VKRXOG FRQVXOW WKH 7KUHVKROG /LPLW 9D chemical substances established by the American Conference of Governmental Industrial Hygienists.[15]

The electrical systems of Class II BSCs are not spark-proof. Therefore, a chemical concentration approaching the lower explosive limits of the compound must be prohibited. Furthermore, since non-exhausted Class II, Type A1 and A2 cabinets return chemical vapors to the cabinet work space and the room, they may expose the operator and other room occupants to toxic chemical vapors.

A chemical fume hood should be used for procedures using volatile chemicals instead of a BSC. Chemical fume hoods

As indicated above, volatile radionuclides such as I^{125} should not be used within Class II BSCs. When using nonvolatile radionuclides inside a BSC, the same hazards exist as if working with radioactive materials on the bench top. Work that has the potential for splatter or creation of aerosols can be done within W K H %ð0 $ P

to provide worker protection. A sloping shield can disrupt the air curtain and increase the possibility of contaminated air being released from the cabinet. A radiation safety professional should

5 L P $ P

The potential for untoward events must be evaluated to eliminate or reduce to the greatest extent possible worker exposure to infectious organisms and to prevent release to the environment. Agent summary statements detailed in BMBL[1] provide data for microorganisms known to have caused laboratory-associated infections that may be used in protocol-driven risk assessment. Through the process of risk assessment, the laboratory environment and the work to be conducted are

ameliorate risks.

$ S U R S H U O \ F H U W L ¿ H G D Q p 0 0 € P @ p À D O %ð

administrative controls to further reduce the risk of exposure to potentially infectious microorganisms. Suggested work practices and procedures for minimizing risks when working in a BSC are detailed in the next section.

SECTION V

BSC Use by the Investigator: Work Practices and Procedures

Preparing for Work Within a Class II BSC

Preparing a written checklist of materials necessary for a particular activity and placing necessary materials in the BSC before beginning work serves to minimize the number and extent of air curtain disruptions compromising the fragile air barrier RI WKH FDELQHW 7KH UDSLG PRYHPHQW RI D sweeping motion into and out of the cabinet will disrupt the air curtain and compromise the partial containment barrier provided by the BSC. Moving arms in and out slowly, perpendicular to the face opening of the cabinet will reduce this risk. Other personnel DFWLYLWLHV LQ WKH URRP H J UDSLG PRY WKH FDELQHW ZDONLQJ WUDI¿F URRP IDQV HWF PD\ DOVR GLVUXSW WKH FDELQHW DLU

Laboratory coats should be worn buttoned over street clothing; latex, vinyl, nitrile or other suitable gloves are worn to provide hand protection. Increasing levels of PPE can be included as determined by an individual risk assessment. For example, a solid front, back-closing laboratory gown provides better protection of personal clothing than a traditional laboratory coat and is a recommended practice at BSL-3.

Before beginning work, the investigator should adjust the stool height so that his/her face is above the front opening. Manipulation of materials should be delayed for approximately one minute after placing the hands/arms inside the cabinet. This allows the cabinet to stabilize, to “air sweep” the hands and arms, and to allow time for turbulence reduction. When the XVHU¶V DUPV UHVW ÀDWO\ DFURVV WKH IURQ RSHQLQJ URRP DLU ODGHQ ZLWK SDUWLFOH the work area, rather than being drawn down through the front JULOOH 5DLVLQJ WKH DUPV VOLJKWO\ ZLOO front grille must not be blocked with toweling, research notes, discarded plastic wrappers, pipetting devices, etc. All operations

should be performed on the work surface at least four inches in from the front grille. If there is a drain valve under the work surface, it should be closed prior to beginning work in the BSC.

0DWHULDOV RU HTXLSPHQW SODFHG LQVLG GLVUXSWLRQ RI WKH DLUÀRZ UHVXOWLQJ LQ contamination and/or breach of containment. Extra supplies H J DGGLWLRQDO JORYHV FXOWXUH SODW should be stored outside the cabinet. Only the materials and HTXLSPHQW UHTXLUHG IRU WKH LPPHGLDWH the BSC.

the work surface. When deemed absolutely necessary, touch-
SODWH PLFUREXUQHUV HTXLSSHG ZLWK D SL
on demand may be used. Internal cabinet air disturbance and heat buildup will be minimized. The burner must be turned off when work is completed. Small electric “furnaces” are available for decontaminating bacteriological loops and needles and
DUH SUHIHUDEOH WR DQ RSHQ ÀDPH LQVLGH
recyclable sterile loops should be used whenever possible.

$VSLUDWRU ERWWOHV RU VXFWLRQ ÀDVNV
DQ RYHUÀRZ FROOHFWLRQ ÀDVN FRQWDLQLQ
DQG WR DQ LQ OLQH +(3$ RU HTXLYDOHQW ¿C
combination will provide protection to the central building vacuum system or vacuum pump, as well as to the personnel who
VHUYLFH WKLV HTXLSPHQW ,QDFWLYDWLRQ
EH DFFRPSOLVKHG E\ SODFLQJ VXI¿FLHQW FK
VROXWLRQ LQWR WKH ÀDVN WR LQDFWLYDWH
DUH FROOHFWHG 2QFH LQDFWLYDWLRQ RFFX
disposed of as noninfectious waste.

Investigators must determine the appropriate method of decontaminating materials that will be removed from the BSC at the conclusion of the work. When chemical means are
DSSURSULDWH VXLWDEOH OLTXLG GLVLQIHF
discard pan before work begins. Items should be introduced into the pan with minimum splatter and allowed appropriate contact
WLPH DV SHU PDQXIDFWXUHU¶V LQVWUXFWLR
can be autoclaved prior to disposal. Contaminated items should be placed into a biohazard bag, discard tray, or other suitable container prior to removal from the BSC.

When a steam autoclave is to be used, contaminated materials should be placed into a biohazard bag or discard pan containing enough water to ensure steam generation during the autoclave cycle. The bag should be taped shut or the discard pan should be covered in the BSC prior to transfer to the autoclave. The bag should be transported and autoclaved in a leak proof tray or pan. It is a prudent practice to decontaminate the exterior surface of bags and pans just prior to removal from the cabinet.

KRVH EDUE DQG ÀH[LEOH WXEH VKRXOG EH D
YDOYH DQG EH RI VXI¿FLHQW OHQJWK WR DO

SECTION VI

be discharged away from supply air intakes, to prevent re-entrainment of laboratory exhaust air into the building air supply V\VWHP 5HIHU WR UHFRJQL]HG GHVLJQ JXLG

to allow easy access for maintenance and to ensure that the

when hazardous chemicals or radionuclides have been used in the BSC, and provides protection against exposure for the maintenance personnel and the environment. Note, however, W K D W W K L V U H T X L U H P H Q W P X V W E H L G H Q W L and installation; a bag-in/bag-out assembly cannot be added to a cabinet after-the-fact w ao4h70nt entesivhe egbineringd

SECTION VII

&HUWL¿FDWLRQ RI %6&V

Development of Containment Standards

7KH HYROXWLRQ RI FRQWDLQPHQW HTXLSP
and diagnostic applications created the need for consistency in construction and performance. Federal Standard 209[32,33], was developed to establish classes of air cleanliness and methods for monitoring clean work stations and clean rooms where HEPA ¿OWHUV DUH XVHG WR FRQWURO DLUERUQH S

7KH ¿UVW ³VWDQGDUG´ WR EH GH[12]YHORSHG VHUYHG DV D)HGHUDO SURFXUHPHQW VSHFL &ODVV ,, 7\SH QRZ FDOOHG 7\SH $ %6& Z hinged front window or a vertical sliding sash, vertical downward ODPLQDU DLUÀRZ DQG +(3$ ¿OWHUHG VXSSO\ JXLGHOLQH VSHFL¿HG GHVLJQ FULWHULD DQ IRU PLFURELRORJLFDO DHURVRO FKDOOHQJH WHVWLQJ RI WKH +(3$ ¿OWHUV $ VLPLODU SU was generated[31] ZKHQ WKH &ODVV ,, 7\SH QRZ FD BSC was developed.

16) 6WDQGDUG IRU &ODVV ,, %6&V ZDV ¿ LQ SURYLGLQJ WKH ¿UVW LQGHSHQGHQW manufacture and testing of BSCs. This standard “replaced” the 1,+ VSHFL¿FDWLRQV ZKLFK ZHUH EHLQJ XVHG and organizations purchasing BSCs. NSF/ANSI 49 – 2002, [8] LQFRUSRUDWHV FXUUHQW VSHFL¿FDWLRQV U construction, and testing. This Standard for BSCs establishes performance criteria and provides the minimum testing UHTXLUHPHQWV WKDW DUH DFFHSWHG LQ WK ZKLFK PHHW WKH 6WDQGDUG DQG DUH FHUWL

&HUWL¿FDWLRQ RI %LRVDIHW\ &DELQHWV

NSF/ANSI 49 – 2002 pertains to all models of Class II FDELQHWV 7\SH $ $ % % DQG SURYLGH VSHFL¿FDWLRQV UHJDUGLQJ

- ‡ Design/construction
- ‡ Performance
- ‡ Installation recommendations
- ‡ 5HFRPPHQGHG PLFURELRORJLFDO GHFR procedure
- ‡ 5HIHUHQFHV DQG VSHFL¿FDWLRQV SHUW Biosafety Cabinetry.

$QQH[ ) RI 16) $16, ZKLFK FRYHUV ¿H BSCs, is now a normative part of the Standard. This Standard is reviewed periodically by a committee of experts to ensure that it remains consistent with developing technologies.

The operational integrity of a BSC must be validated before it is placed into service and after it has been repaired or relocated. 5HORFDWLRQ PD\ EUHDN WKH +(3$ ¿OWHU VH WKH ¿OWHUV RU WKH FDELQHW (DFK %6& VK FHUWL¿HG DW OHDVW DQQXDOO\ WR HQVXUH

2Q VLWH WHVWLQJ WKDW IROORZV WKH UHI WHVWLQJ 16) $16, ± $QQH[ ) SOXV $GG EH SHUIRUPHG E\ H[SHULHQFHG TXDOL¿HG S information is included in the Standard to assist in understanding WKH IUHTXHQF\ DQG NLQGV RI WHVWV WR EH EHJDQ D SURJUDP IRU DFFUHGLWDWLRQ RI F and practical examinations. Education and training programs IRU SHUVRQV VHHNLQJ DFFUHGLWDWLRQ DV ¿HOG FHUWL¿FDWLRQ WHVWV DUH RIIHUHG E Selecting competent individuals to perform testing and FHUWL¿FDWLRQ LV LPSRUWDQW DQG LW LV %62 EH FRQVXOWHG LQ LGHQWLI\LQJ FRPSDQ WKH QHFHVVDU\ ¿HOG SHUIRUPDQFH WHVWV

d The standard can be ordered of NSF for a nominal charge at NSF ,QWHUQDWLRQDO 1RUWK 'L[ERUR 5RDG 3 2 %R[

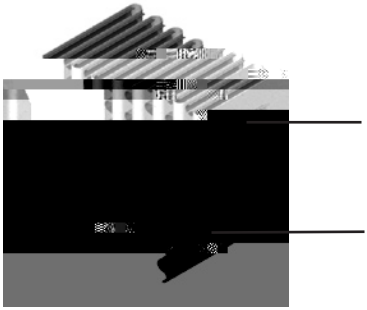
It is strongly recommended that, whenever possible,
DFFUHGLWHG ¿HOG FHUWL¿HUV DUH XVHG WR
LQ KRXVH SHUVRQQHO DUH SHUIRUPLQJ WKH

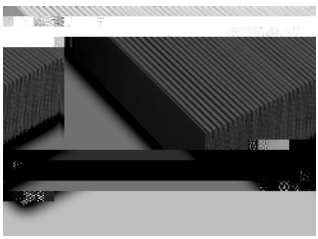
D. HEPA Filter Leak Test

Table 1. Selection of a Safety Cabinet through Risk Assessment				
Biological Risk Assessed	**Protection Provided**			**BSC Class**
	Personnel	**Product**	**Environmental**	
BSL 1-3	Yes	No	Yes	I
BSL 1-3	Yes	Yes	Yes	II $ $ % %
BSL 4	Yes	Yes	Yes	III II - When used in suitroom with suit

FIGURES

Figure 1. +(3$ ¿OWHUV DUH W\SLFDOO\ FRQ
thin sheets of borosilicate medium, pleated to increase surface
DUHD DQG DI¿[HG WR D IUDPH $OXPLQXP VH
added for stability.





&RQWLQXRXV VKHHW RI ÀDW ¿C

Figure 4. &DQRS\ WKLPEOH XQLW IRU GXF
7\SH $ %6& $ EDODQFLQJ GDPSHU % ÀH[LE
H[KDXVW V\VWHP & FDELQHW H[KDXVW +(3$
canopy unit, E. BSC. Note: There is a 1" gap between the
FDQRS\ XQLW ' DQG WKH H[KDXVW ¿OWHU K
room air is exhausted.

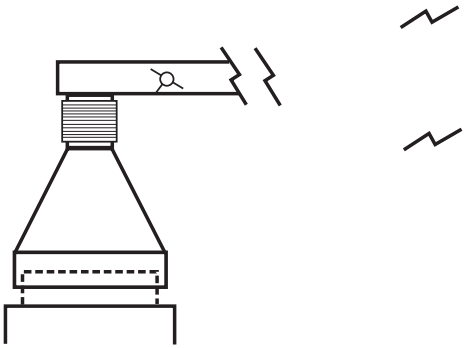


Figure 5A. 7KH &ODVV ,, 7\SH % %6& FODV
IURQW RSHQLQJ % VDVK & H[KDXVW +(3$ ¿
¿OWHU (QHJDWLYH SUHVVXUH GHGLFDWHG
* DGGLWLRQDO +(3$ ¿OWHU Note: The cabinet exhaust
needs to be hard connected to the building exhaust system.

Figure 5B. 7KH &ODVV ,, 7\SH % %6& EHQF

Figure 6. The Class II, Type B2 BSC. A. front opening, B. VDVK & H[KDXVW +(3$ ¿OWHU ' VXSSO\ +(3$ pressure exhaust plenum. Note 7KH FDUERQ ¿OWHU LQ W system is not shown. The cabinet needs to be hard connected to the building exhaust system.

Figure 7. The tabletop model of a Class II, Type A2 BSC. $ IURQW RSHQLQJ % VDVK & H[KDXVW +(3$ +(3$ ¿OWHU (SRVLWLYH SUHVVXUH FRPPRQ pressure plenum. The Class II Type A2 BSC **is not equivalent WRDKDWDVIRUPHUOFDOOHG Class II Type B3 unless it is connected to the laboratory exhaust system.** Note: The A2 BSC should be canopy connected to the exhaust system.

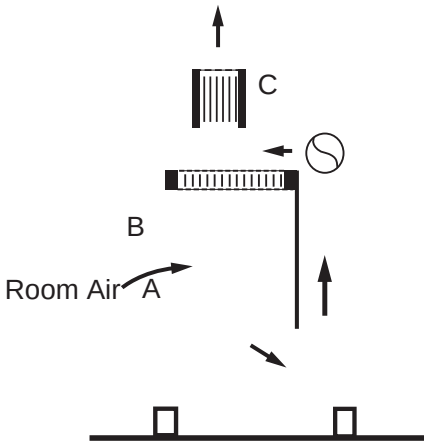


Figure 9A. 7KH KRUL]RQWDO ODPLQDU ÀRZ ³ IURQW RSHQLQJ % VXSSO\ JULOOH & VXSS plenum, E. blower.

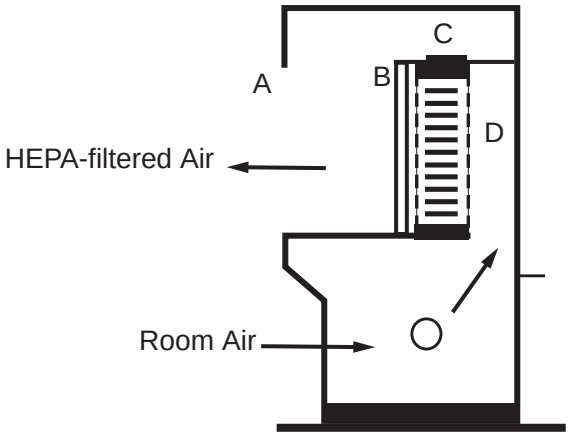


Figure 9B. 7KH YHUWLFDO ODPLQDU ÀRZ ³FO IURQW RSHQLQJ % VDVK & VXSSO\ +(3$ ¿OW 6RPH YHUWLFDO ÀRZ FOHDQ EHQFKHV KDYH U front and/or rear perforated grilles.

Figure 11. A typical layout for working “clean to dirty”

ACKNOWLEDGEMENTS

We gratefully acknowledge the Baker Company, Filtration
*URXS ,QF)ODQGHUV)LOWHUV DQG)RUPD
VRPH GUDZLQJV DQG ¿JXUHV UHSURGXFHG K

5()(5(1&(6

1. Centers for Disease Control and Prevention; The National Institutes of Health. Biosafety in microbiological and biomedical laboratories. 5th ed. Washington, DC. 2007.

2. .UXVH 5+ 3XFNHWW :+ Biological safety cabinetry &OLQ 0LFURELRO 5HY

3. First MW.)LOWHUV KLJK FDSDFLW\ ¿OWHUV DQG ¿OWHUV UHYLHZ. In Place Filter Testing Workshop; 1971; Boston, Massachusetts. 1971.

4. 7KH 1DWLRQDO ,QVWLWXWHV RI +HDOWK Engineering Services, Dow Chemical USA. A Workshop for &HUWL¿FDWLRQ RI %LRORJLFDO Safety Cabinets. The ,QVWLWXWH 2I¿FH RI %LRKD]DUGV DQG (Q 1974.

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7. :KLW¿HOG A new approach to clean room design. Final UHSRUW $OEXTXHUTXH 10 6DQGLD &RUS 1R 6& 55

8. 16) ,QWHUQDWLRQDO 16) $PHULFDQ 1DW ,QVWLWXWH NSF/ANSI 49-2004. Class II ODPLQDU ÀRZ ELRVDIHW\ FDELQHWU\ $QG

9. 2FFXSDWLRQDO 6DIHW\ DQG +HDOWK $GPL of Occupational Medicine. Guidelines for cytotoxic DQWLQHRSODVWLF DJHQWV. Appendix A. Standard 01-23-001. 1986.

29. - R Q H V 5 / - U 6 W X D U W ' * Effects of H V R Q ' H W ceiling height on determining calculated intake air velocities for biological safety cabinets. Appl Occup Environ Hyg. 1991;6:683.

30. 5 D N H %Q À X H Q F H R I F U R V V G U D I W V R Q W K H biological safety cabinet. Appl Env Microbiol. 1978;36:278-83.

31. 6 S H F L ¿ F D W L R Q V I R U J H Q H U D O S X U S R V H F C cabinet % H W K H V G D 7 K H 1 D W L R Q D O , Q V W L W X National Cancer Institute; 1973.

32. Airborne Particulate Cleanliness Classes in Clean rooms and Clean Zones) H G H U D O 6 W D Q G D U G 1 R (

33. & O H D Q 5 R R P D Q G : R U N 6 W D W L R Q 5 H T X L U H I Environment) H G H U D O 6 W D Q G D U G 1 R %