Operator protection test for open – fronted biological safety cabinets: The unique potassium iodide test.

Lin Xiang Qian, Vice-President
Alexander Atmadi, Technical Manager
Amit Kumar Singh, Biomedical Engineer
Esco Micro Pte Ltd, Singapore.

INTRODUCTION

Laboratory-acquired infections (LAIs) are a matter of concern for the microbiological community of the world. There are reports of individuals, particularly laboratory investigators, succumbing to infection transmitted by an aerosol or splash from the material being handled (Kruse *et al.* 1991; Collins 1993). The strategies to eliminate such LAIs include the use of biological safety cabinets. There have been several attempts made by the manufacturers, legislative bodies, and national and international standards bodies to standardize containment testing strategies for open fronted containment systems. Operator protection tests should be an integral part of the routine servicing regime to ensure that the biological safety cabinets meet the required performance levels, and additionally to allow detection and rectification of poor containment, particularly those induced by the environmental factors.

For many parts of Europe and North America, Operator Protection Tests (OPTs) may only be required for initial certification but not following installation, or routinely thereafter, e.g. post servicing. Moreover, the levels of containment required, unfortunately, are not fully comparable between countries, as the recommended testing methodologies and/or protocols vary (Anon. 1992; Richmond and McKinney 1995; Anon 1998). Manufacturers of contamination control equipment have the expense of testing by varying methods in different countries where standards apply. The most common type of open fronted containment system found in laboratories are the biological safety cabinets (BSCs). This paper describes the unique potassium iodide test conducted on biological safety cabinets in a tertiary hospital of Singapore.

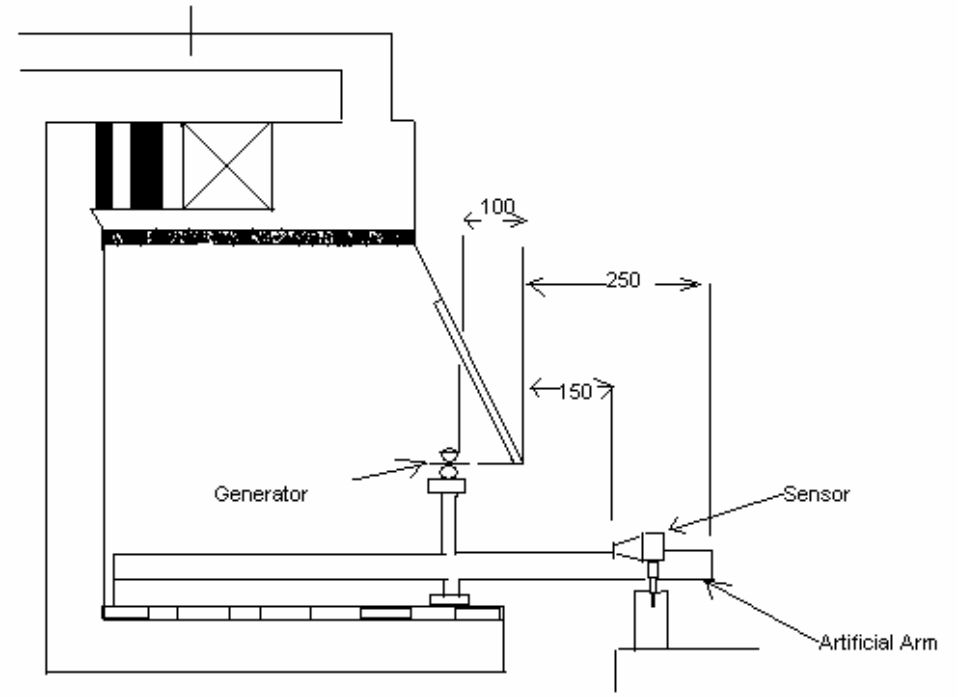
PURPOSE OF STUDY

Users sometime do not realise that the performance of the biosafety cabinets depends greatly on the nature of the installation as well as proper positioning of the cabinet in the laboratory. For example, the inflow air velocity from the laboratory into the cabinet is often of a very low magnitude (around 0.5m/s) and this can easily be disrupted by airflow turbulence in the room, for example, an air-conditioning outlet / diffuser. A safety cabinet may meet all performance criteria in the manufacturer's laboratory after production, as well as in the testing lab of the independent certification agency, but may fail to deliver adequate protection on-site when it is actually installed. A Study was undertaken in one of the tertiary hospitals of Singapore to determine the containment of the biological safety cabinets since it is important to understand that the testing conditions in the manufacturer's laboratory as well as the laboratory of the certification agency are ideal test conditions which sometimes do not correlate with real world conditions. Standard checks carried out on biological safety cabinets includes: Air flow velocity checks (inflow, downflow), airflow smoke pattern tests, noise level tests, vibration level measurement, light level measurement, UV intensity measurements, air cleanliness classification tests using a laser particle counter.

METHODOLOGY

The KI (potassium iodide) discus test is defined in the European Standard for microbiological safety cabinets, EN12469:2000 as a test method for validating the operator protection capabilities of the cabinet. The KI Discus test has been designed to enable operator protection factors to be measured for class I and Class II open-fronted biological safety cabinets. Unlike test methods employing a micro-biological aerosol challenge this technique enables cabinets to be evaluated without the risk of microbial contamination of either the biological safety cabinet or the laboratory.

A fine mist of potassium iodide droplets, produced by a spinning disc, is used as a challenge aerosol to measure the containment of the biological safety cabinet. Centripetal collectors sample the air and deposit the potassium iodide particles that are entrained in the sampled air on to filter membranes. At the end of the sampling period the filter membranes are placed in a solution of palladium chloride whereupon the potassium iodide "develops" to form clearly visible and easily identified brown dots. Knowledge of the number of droplets in the challenge produced by the mist generator and of the number collected in the air samplers enables the aperture protection factor (Apf) for the cabinet to be evaluated. The aperture protection factor (A_{pf}) is the ratio of how many particles can escape through the cabinet front aperture compared to how many particles are liberated if the experiment is performed on an open bench.



The Unique Potassium Iodide Test
(All dimensions in mm)

On pressing the button marked auto the suction blower in the lower part of the KI discus cabinet is started along with the spinning disc and the digital timer. After 15-16 seconds an indication appears on the control panel showing that the spinning disc has reached the correct running speed of 28,000 rpm. At this stage the peristaltic pump starts. The digital timer determines the quantity of fluid delivered to the spinning disc. Once the peristaltic pump is running, 20 ml of potassium iodide will be delivered from the reservoir bottle to the spinning disc. The digital timer indicates the time elapsed time from the start to completion of the delivery of 20 ml of potassium iodide. The digital timer was

programmed to stop the pump after 20ml of KI is dispensed, which takes about 9 minutes. The blower is turned off 5-6 seconds later.

The filter membrane composed of cellulose nitrate, pore size 3 micron, diameter 25mm is removed from the samplers after 20 seconds so that the suction has completely stopped. The filter membrane is placed in a Petri dish containing the palladium chloride solution, with the surface that has been exposed to the air flow facing upwards. Within 10 seconds the membrane becomes saturated with palladium chloride and any potassium iodide particle if present becomes visible as brown dots. The filter membrane is then removed and immersed in distilled water for 3-4 seconds before being placed on a clean water absorbing paper for examination.

RESULTS AND DISCUSSION

According to EN 12469:2000 A_{pf} of not less than 100,000 for each collectors or there should not be more than 62 brown dots on the KI discus filter membrane after development in palladium chloride. The formula to calculate A_{pf}:

$$A_{pf} = \frac{N \times V}{10^4 \times n}$$ Therefore $$= \frac{6{,}200{,}000}{n}$$

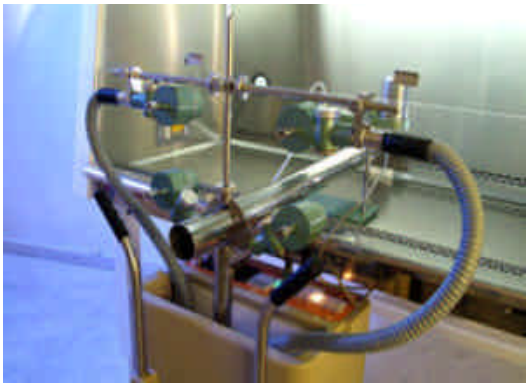
N: number of particles liberated = 3.1 x 10^7 x M
M: volume of KI dispensed = 20 ml
V: sampling flow rate = 100 dm^3/min
n: the number of brown dots captured

Sample of 28 biological safety cabinets were subjected to the KI discus test. Sample of biological safety cabinets were from different manufacturers. It was found that only one BSC failed the test as it surpassed the 62 brown dots (See table 1). The main reason for the failure of the cabinet was attributed to its weak inflow velocity which was measured as 0.36 m/s, the minimum inflow velocity defined by the standard EN 12469:2000 is 0.4 m/s. The study result was promptly conveyed to the concerned hospital authorities who took the necessary action and informed the manufacturer to correct the airflow velocity as the cabinet was under their annual maintenance contract. An understanding of poor cabinet containment is a valuable asset for reinforcing laboratory discipline and help combat the recognized hazards emanating form operator complacency (Rake 1978; Kruse et al. 1991).

There is a need of for continuous monitoring to ensure that the cabinets regularly return the desired level of operator protection (Clark et al. 1990; Osborne and Durkin 1991). The principle factors which contributes to the poor containment of biological safety cabinets are careless technique and in adequate installation/testing to detect and overcome environmental factors that could compromise performance. Nevertheless, it is critical that BSC's repeatedly return high levels of operator protection as the handling of hazardous micro-organisms becomes more widespread; such work is often performed by relatively uninformed, though highly educated people, who may not have a thorough understanding of the mechanisms of the transmission of the infectious agents.

Biological Safety Cabinets – The Unique Potassium Iodide Test

Number	Sensors (Acceptable Limit = 60 brown dots)				Result	Comments
	Xi	X	Yi	Y		
1	>100	>100	>100	>100	Fail	The main reason for the failure of the cabinet was attributed to its weak inflow velocity which was measured as 0.36 m/s, the minimum inflow velocity defined by the standard EN 12469:2000 is 0.4 m/s.
2	27	10	11	6	Pass	
3	3	10	1	46	Pass	
4	4	8	6	15	Pass	
5	0	2	4	0	Pass	
6	2	0	3	6	Pass	
7	2	7	0	5	Pass	
8	0	0	1	2	Pass	
9	0	0	0	2	Pass	
10	3	2	5	2	Pass	
11	1	0	0	0	Pass	
12	0	1	0	0	Pass	
13	0	3	4	0	Pass	
14	1	0	1	1	Pass	
15	1	1	0	1	Pass	
16	5	8	0	6	Pass	
17	3	2	0	0	Pass	
18	9	2	8	2	Pass	
19	0	4	0	3	Pass	
20	0	1	3	0	Pass	
21	3	6	8	0	Pass	
22	3	0	1	0	Pass	
23	1	5	3	0	Pass	
24	2	0	0	1	Pass	
25	11	7	2	5	Pass	
26	1	4	2	0	Pass	
27	3	0	2	0	Pass	
28	0	6	4	1	Pass	



KI Discus Testing

Result Analysis:

Overall the containment results were found to be excellent except a single BSC. The results were promptly conveyed to the concerned hospital authorities who took the necessary action and informed the manufacturer to correct the airflow velocity as the cabinet was under their annual maintenance contract

Table: 1

ACKNOWLEDGEMENT

The authors would like to express their appreciation to Dr. Ling Ai Ee, director, Asia-Pacific Biosafety Association for helpful discussions, support and/or encouragement during the course of this study. Asia-Pacific Biosafety Association (A-PBA) is a newly formed professional association to congregate practitioners of biological safety for the promotion of biological safety and to facilitate the sharing of biosafety information. Finally they would like to thank Mr. Lim Lay Yew, CEO, Esco Micro Pte Ltd, Singapore for sponsoring this study.

REFERENCES

Anon. (1992) Microbiological Safety Cabinets: Part 4. Recommendations for Selection Use and Maintenance.
Clark, R.P., Osborne, R.W., Pressy, D.C., Grover, F., Keddie, J.R. and Thomas, C. (1990) Open fronted safety cabinets in ventilated laboratories. Journal of Applied Bacteriology 69, 338-358.
Collins, C.H. (1993) Laboratory Acquired Infections. History, Causes and Prevention, 3rd edn. London: Butterworth
Kruse, R.H., Puckett, W.H. and Richardson, J.H. (1991) Biological safety cabinetry, Clinical Microbiological Reviews 4, 207-241.
Osborne, R.W. and Durkin, T.A. (1991) Continued successful operation of open-fronted microbiological safety cabinets in a force-ventilated laboratory. Journal of Applied Bacteriology 71, 434-438.
Rake, B.W. (1978) Influence of cross drafts on the performance of a biological safety cabinet. Applied and Environmental Microbiology 36, 278-283.
Richmond, J.Y. and McKinney, R.W. (1995) Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets. Atlanta, Georgia, USA: US Department of Health and Human Services, Public Health Services Centers for Disease Control and Prevention and National Institutes for Health.