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Comparison between the potassium iodide (KI) discus test and the di-octyl phthalate (DOP) containment test for the testing of class II biological safety cabinets (BSC-II)

K Lecamwasam, H Tran (Institute of Medical and Veterinary Science)

Abstract

The two main methods of testing class II biological safety cabinets in Australia are compared in this study. The DOP test equipment, as described in Australian Standard 2252.2 [9], has been used in Australia for over thirty years to verify that a cabinet provides sufficient operator, product and environmental protection. The KI Discus test, described in BSEN12469 [8] and Australian Standard 1807.26-2004 [10], is a European standard test method which is able to determine whether or not the cabinet provides adequate personnel protection. Through decreasing the exhaust velocity of the cabinet and hence weakening the air barrier, this study aimed to determine the more sensitive test. The results showed that both tests were able to reveal weak points in the barrier. In addition, the DOP containment test was more sensitive in detecting a weak barrier. Since the DOP containment test was found to be the more sensitive test, this test is equivalent, if not superior, to the KI Discus test in detecting weak barriers and hence determining adequate personnel protection.

Introduction

Class II biological safety cabinets are designed to protect the user from material within the cabinet, whilst protecting the work area from contamination. They have two types of air flow circulating within the cabinet – down flow air and inflow air.

Down flow air passes through a High Efficiency Particulate Air (HEPA) filter and provides the working area with a continuous flow of sterile filtered air. Inflow air is drawn from the outside environment by the negative pressure at the front grill opening of the cabinet thus preventing particles escaping from within the cabinet. Both inflow- and down- flow air is drawn into a compartment below the work zone and then moved up to the top plenum(s).

Approximately 20 – 40% of air delivered into the top plenum is exhausted into the environment, the remainder passes through the main HEPA filter and is re-circulated as down flow air. In order for the cabinet to function correctly, the same volume of air that is extracted from the cabinet is drawn back into the cabinet as inflow air. As such adjustments made to the exhaust velocity affect the inward velocity and hence the air barrier, with decreased exhaust velocities resulting in a weaker air barrier.

The average down flow air velocity must be between $0.40ms^{-1}$ and $0.45ms^{-1}$ and the air velocity at each point must be $\pm 20\%$ of the average [9]. Since product- and personnel-protection are achieved through the airflows within the cabinet, any airflow disturbances may compromise the level of protection. Disruptions to the normal airflows in a cabinet are generally caused by air conditioning, personnel moving around a cabinet, the user's arm while working inside the cabinet and any drafts through open doors and windows [5].

The cold poly-dispersed di-octyl phthalate (DOP) test determines the containment abilities of a cabinet by releasing DOP at approximately 100mm intervals across the front opening of the cabinet and measuring its penetration across the air barrier. The test equipment contains an aerosol generator and delivery tube which releases $0.7\mu m$ particles 25mm from the opening of the cabinet at 100mm intervals across the front opening of the cabinet [1, 6]. An aerosol photometer is held for 10s at each of these test positions. If the photometer detects a penetration of greater than 0.01% at any of the test positions, this process must be repeated for a 30 second period where there is to be no penetration [6]. If there is any penetration detected, the point tested is classified as a fail. If there is a fail at any individual point, the cabinet will fail the test [6, 9].

The Potassium Iodide (KI) Discus test predicts the containment of the cabinet through the calculation of an A_{pf}. The KI Discus test equipment contains three main components – a spinning disc, air samplers and a peristaltic pump. The pump is used to deliver 20mL of KI solution onto the spinning disc. Once the 38mm diameter disc has reached 28000 revolutions per minute (± 500r/min), it disperses a known number of uniformly sized particles of approximately 7 μm in diameter, from within the cabinet [8]. Air samplers drawing air at a volume flow rate of 100dcm^3/m are positioned at four locations outside the cabinet to collect any particles that may escape through the opening of the cabinet [8]. A cylindrical artificial arm of between 60 - 65mm in diameter is used to mimic a user's arm in disturbing the airflow [2, 8].

After the 20mL of potassium iodide solution has been delivered, filters are removed from the air samplers and soaked in palladium chloride for 30 – 45 seconds. The resultant palladium iodide is visible as grey/brown dots on the filter paper which are counted to calculate the A_{pf} [8].

$$Apf = \frac{62x10^5}{n}$$, where n is the number of particles recovered in the filter

The A_{pf} is defined as the ratio of exposure to airborne contamination generated on the open bench to the exposure resulting from the same dispersal within the containment facility under test [8]. The A_{pf} must be at least $1x10^5$, hence there can not be more than 62 dots picked up on the filter paper. When more than one air sampler is used, the A_{pf} for each individual sampler must be greater than $1x10^5$. If all samplers have an A_{pf} above this value, the results from each of the samplers may be averaged to give the final A_{pf}.

Materials and Methods

All cabinets were set up with average downward air velocities to comply with AS2252.2 [9]. The KI Discus test was performed in accordance with EN12469:2000 [8] and the KI

Discus operating manual. Before each test, the equipment was thoroughly cleaned and background contamination measured using a KI Discus test where no KI is delivered to confirm that there were no KI particles present. The DOP test was performed in accordance with AS1807.22. Poly-alpha olefin (PAO) was used in the place of cold DOP, an acceptable replacement [3]. Figure 1 shows location of test positions for DOP and KI Discuss method.

Test One: Effect of air barrier velocity on the A_{pf}

Testing was conducted through reducing the exhaust air velocity of the BSC-II to determine how a weakened air barrier affected the A_{pf} produced by the KI Discus test. The cabinet had aperture dimension of 1.18m x 0.18m. It was set up in a quiet lab but was in close proximity to an air conditioning duct which had the potential to affect the air flows. The KI Discus test was performed on a cabinet where the average exhaust velocity of the cabinet was set at: 0.85ms^{-1}, 0.75 ms^{-1}, 0.69ms^{-1}, 0.64 ms^{-1}and 0.59 ms^{-1}.

Test Two: Comparing the results from the DOP and KI Discus methods for decreasing exhaust air velocities to determine the more sensitive test.

Three class II cabinets were tested to verify the more sensitive test. Cabinet 1 was the same cabinet as used in test 1 Cabinet 2 has aperture dimensions of 1.18m x 0.20m and was set up in a lab where there were no air conditioning ducts close to the cabinet, but there were some personnel operating in the lab at the time. Cabinet 3 had aperture dimensions of 1.18m x 0.165m and was set up in a lab that had an air conditioning duct close to the right hand corner of the cabinet.

The DOP and KI discus methods were performed over a range of decreasing exhaust velocities in an attempt to determine the exhaust velocity that resulted in a "fail" of the cabinet.

Test Three: Interpreting the results from the KI Discus Test

The developed filters from the KI Discus test for one velocity were analysed by 10 qualified engineers and technicians to compare consistency of A_{pf} calculations. The KI Discus filter used in this evaluation was from a test where the cabinet conditions led to one of the samplers having more than 62 dots and the other three samplers having less than 62 dots. The participants of this test were given the instruction manual from the KI Discus equipment and a x10 magnifying glass and were asked to count the number of dots on each of the four samplers.

Results

Test One.

Figure 2 demonstrates the relationships between average exhaust velocity and the operator protection factor using the KI Discus test method. As expected, increasing the exhaust air velocity results in a general increase in A_{pf}. Failure of the barrier was detected at an exhaust velocity of $0.59ms^{-1}$.

Test two.

Table 1 represents the results from the DOP and KI discus methods in Cabinet 1. Test C and D demonstrated that the DOP test failed the cabinet at point 4. Since the KI Discus test has an air sampler situated at point 4, and still did not fail the cabinet at this point (in tests H and I), it can be deduced that the KI Discus test is less sensitive than the DOP test. Test E failed the barrier at points 2, 3, 4, 5, 6, 7, 8 and 11. Since the air samplers of the KI Discus test are aligned with points 4 and 8, fails at points 2, 3, 5, 6, 7, and 11 were not detected by the KI Discus method.

The results show that the KI Discus test passed the cabinet for average exhaust velocities of $0.64ms^{-1}$ or above. The DOP test, however, required velocities of $0.74ms^{-1}$or above

Results for both Cabinet 2 and 3 (Table 2) demonstrate that the DOP aerosol test was again more sensitive in detecting the weakening containment barrier. For cabinet 2, there were 5 points across the barrier where the containment was found to fail by the DOP test.

At point four, both methods test the barrier and again, this point was failed by the DOP method and passed by the KI Discus method (only 22 dots found on the filter). The same trend was observed in Cabinet 3.

Test Three.

Ten experienced engineering technicians were given the same filter set score for a KI Discus Method test (Test J). Table 3 demonstrates the large variation in results (with standard deviations of the same order as the average value), which demonstrates technicians recording a pass, while others recorded a fail.

Discussion

While the KI Discus and DOP Methods are both used to detect inadequate personnel protection in BSC-II's, it is difficult to compare the two tests directly as their methodologies are so different. When challenging the air barrier, there are fundamental differences between the tests. These include the size of the particle used to penetrate the barrier, the number of particles challenging the barrier and the force with which the particles challenge the barrier

The KI Discus Method generates a known number of particles within the cabinet and the number of particles that escape at specific locations are captured and counted. Due to the particles being generated on a spinning disc,a significant proportion of the particles produced will be released in the direction of the 3 walls of the cabinet rather than the air barrier. This has implications for clean up of the cabinet work space after completion of the test. In contrast the DOP aerosol test aims all particles directly at the barrier and measures the percentage of the original sample that escapes.

The time taken to carry out each test is an important consideration for technicians who carry out the routine testing of these cabinets. For a 1.2m cabinet, the DOP aerosol test will be carried out at 11 points for 10 seconds at each point. If all of the points pass, this will take less than five minutes. If slight penetration is detected at a point, the test must be repeated for a further 30 seconds at this point [6]. Even allowing for all 11 points to be tested for this further time period, and accounting for the set up time of five minutes, the test will take approximately 15 minutes.

Including the recommended background test, a KI Discus test takes approximately 1.5 hours to complete. If the cabinet does not pass the KI Discus test, adjustments to the velocities of the cabinet are required. However, a minimum of 24 hours between testing is necessary before the next test can be performed to minimise background contamination from KI particles in the environment [8]. Potentially, a BSC-II requiring multiple adjustments may result in the cabinet being out of service for many days, resulting in substantial inconvenience.

Ideally, the test result must be as objective and operator interpretation/variability minimized. The DOP method provides results in real time. Each of the 11 points being tested will either receive a pass or a fail. At the conclusion of the ten minute test, the technician can determine immediately whether the BSC-II has passed or failed. The DOP method, however, does not provide a quantitative result of the protection offered to the user by the cabinet.

Results from the KI Discus method are available soon after the conclusion of the test However, interpretation of what constitutes a 'dot' on the filter exposes the method to operator variability. This is exacerbated when filters have a large amount of dots present, making it difficult to count the dots. Accurate scoring of the number of dots is critical in calculating the A_{pf}. A reported advantage of the KI Discus test is that the test can be carried out by anyone [2]. However, when the results were interpreted by 10 engineers and technicians (table 3) it was found that there were substantial variations in the numbers of dots counted on the same filters. In practice, a cabinet could be passed when it is unfit for use. In summary, the results of the KI Discus method were difficult to interpret consistently and the accuracy of the resultant A_{pf} was uncertain. In contrast the DOP method had a consistent and objective "fail" point which is completely independent of the person undertaking the test.

The DOP test challenges the barrier of a 1.2m cabinet at eleven points across the opening. In contrast, the KI Discus method tests at four points, however since two of the samplers are positioned directly below another sampler, only two points across the barrier are tested. Both points are close to the centre and neither end is tested. Test G demonstrated a circumstance under which both the DOP and KI Discus tests passed the barrier at points 4 and 8 yet not at all positions were passed by the DOP test.

Since BSC-II's provide personnel, product and environmental protection, it is important that all three components of this protection are tested; only the DOP aerosol method can test all three. The DOP aerosol is used to test the exhaust filter to ensure adequate environmental protection. This test can also easily test the work zone integrity of the cabinet due to the flexibility of the equipment, as described in AS1807.5 [7]. Due to the fixed nature of the KI Discus equipment, the work zone integrity test cannot be performed and an alternative test must be used .

Conclusion

Since the DOP aerosol test equipment can be used to test all aspects of the cabinet, and can be used to carry out type, installation and routine testing, it is the more effective and convenient test method to deploy. This study provides an initial comparison between the KI Discus and DOP containment methods of testing the integrity of air barriers in class II biological safety cabinets. Although initial results have shown that the DOP containment test is more sensitive in the detection of weak air barriers, further testing should be carried out on a large range of cabinets of different designs. This study also highlights the inherent variability in results from KI Discus tests as they are dependant upon the individual, while the DOP test is completely independent.

References

~~[1]. Carlton, H. R. and Guelta, M. A. (1995), New performance data for "EMERY 3002" and "EMERY 3004", two army approved safe materials to replace DOP in mask and filter testing, Proceedings of the 23rd DOE/NRC Nuclear air cleaning conference, Buffalo, New York, Vol.1, Page 743 – 756.~~

[2]. Clark, R. P and Goff, M. R. (1981). The Potassium Iodide Method for Determining Protection Factors in Open-Fronted Microbiological Safety Cabinets. Journal of Applied Bacteriology, 51, 439 – 460.

[3]. Moore, D. R., Jeffrey, P. E., Marshall, G and Kennedy, M. A. (1994). Comparative Testing of Challenge Aerosols in HEPA Filters with Controlled Defects. Pharmaceutical Engineering, 14.

~~[4]. Osborne, R., Durkin, T., Shannon, H., Dornan, E and Hughes C. (1999). Performance of open-fronted microbiological safety cabinets: the value of operator protection tests during routine servicing. Journal of Applied Microbiology, 86, 962 – 970.~~

[5]. Rake, B. W. (1978). Influence of Crossdrafts on the Performance of a Biological Safety Cabinet. Journal of Applied and Environmental Microbiology, 36, 278-283.

[6]. Committee ME/60, Cleanrooms, workstations, safety cabinets and pharmaceutical isolators-Methods of test 2000, Method 22: Determination of air barrier containment of laminar flow safety cabinets, (AS1807.22-2000), Standards Australia, Strathfield, NSW.

[7]. Committee ME/60, Cleanrooms, workstations, safety cabinets and pharmaceutical isolators-Methods of test 2000, Method 5: Determination of work zone integrity, (AS1807.5-2000), Standards Australia, Strathfield, NSW.

[8]. Committee CEN/TC 233, Biotechnology – Performance criteria for microbiological safety cabinets, 2000, (BS EN12469:2000), British Standards Institution, London, UK.

[9]. Committee ME/60, Biological safety cabinets, 2004, Part 2: Laminar flow biological safety cabinets (Class II) for personnel, environment and product protection, (AS2252.2), Standards Australia, Strathfield, NSW.

[10]. Committee ME/60, Cleanrooms, workstations, safety cabinets and pharmaceutical isolators-Methods of test 2004, Method 26: Determination of air barrier containment of laminar flow safety cabinets – Potassium iodide discus test, (AS1807.26-2004), Standards Australia, Strathfield, NSW.

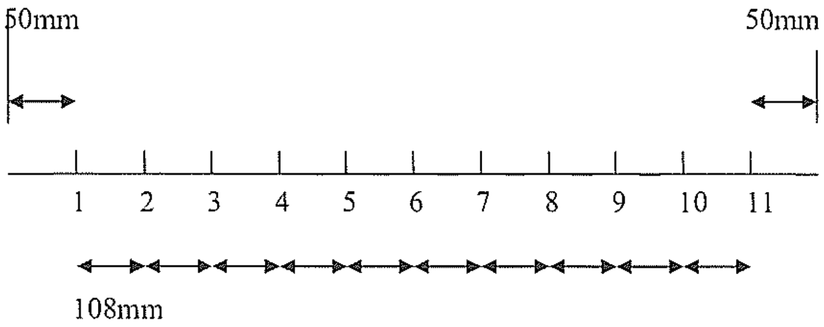

Figure 1: Layout of test points for the DOP containment test. The air samplers of the KI Discus were positioned at approximately points 4 and 8.

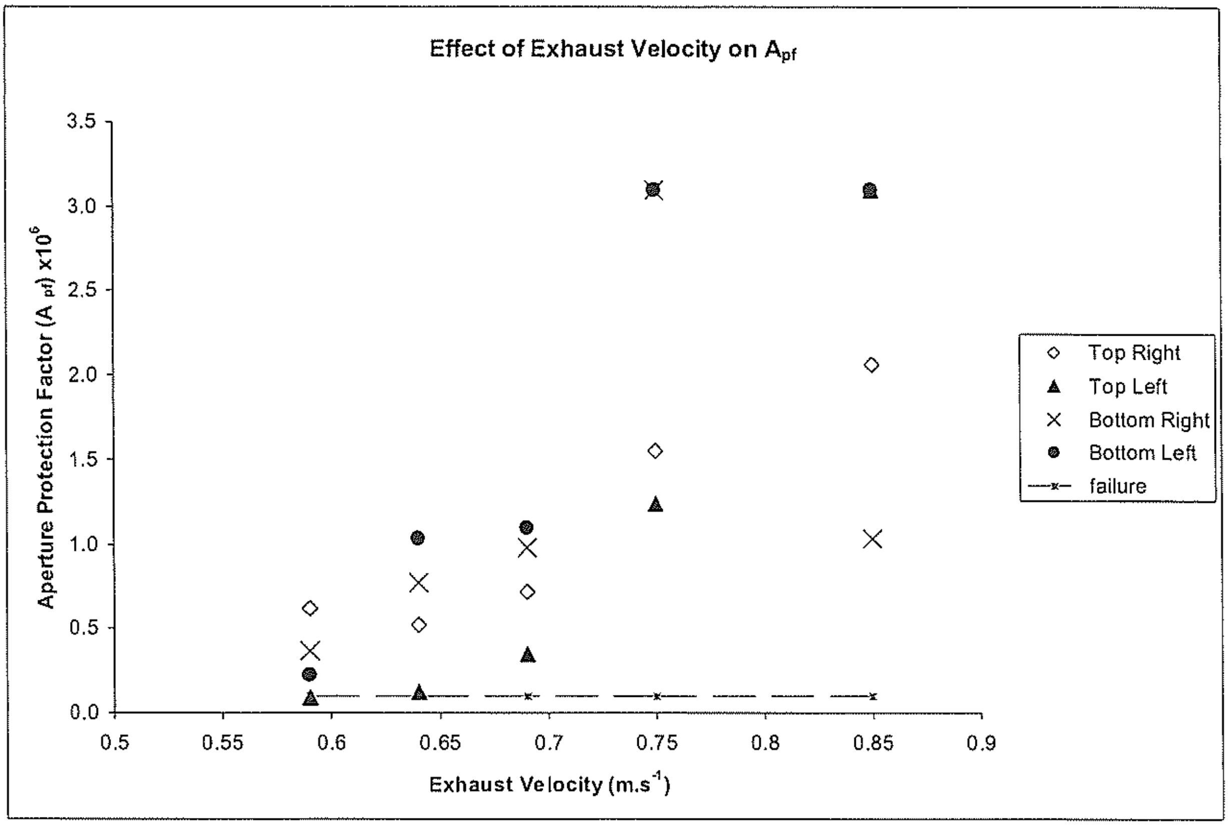

Figure 2: How changes in exhaust velocity effects the APF

Table 1: Summary of DOP and KI discus aerosol test results for different average exhaust velocities in Cabinet 1.

DOP Test results				
Test	Average Exhaust Velocity	Number of Pass/Fail points	Position(s) of Points Failed	Overall Result
A	0.85 ms^{-1}	11/0	-	PASS
B	0.75 ms^{-1}	11/0	-	PASS
C	0.69 ms^{-1}	10/1	4	FAIL
D	0.64 ms^{-1}	9/2	3,4	FAIL
E	0.59 ms^{-1}	3/8	2,3,4,5,6,7,8,11	FAIL

KI Discus Test results							
Test	Average Exhaust Velocity	Number of Dots				A_{pf}	Overall Result
		Left Top	Right Top	Left Bottom	Right Bottom		
F	0.85 ms^{-1}	2	3	2	6	$2.3x10^6$	PASS
G	0.75 ms^{-1}	5	4	2	2	$2.3x10^6$	PASS
H	0.69 ms^{-1}	18	9	6	6	$7.8x10^5$	PASS
I	0.64 ms^{-1}	51	10	6	8	$6.4x10^5$	PASS
J	0.59 ms^{-1}	66	12	28	17	$9.4x10^4$	FAIL

Table 2: Results of both tests for Cabinet 2 and 3 at two different exhaust velocities. The positions of the points failed can be seen on figure 1.

Biological Safety Cabinet 2									
	DOP Results			KI Discus Results					
Average Exhaust Velocity	Number of Pass/Fail Points	Positions of Points Failed	Overall Result	Left Top (dots)	Right Top (dots)	Left Bottom (dots)	Right Bottom (dots)	A_{pf}	Overall Result
$0.83 ms^{-1}$	5/6	2,4,5,9,10	FAIL	22	8	7	4	$8.73x10^5$	PASS
Biological Safety Cabinet 3									
	DOP Results			KI Discus Results					
Average Exhaust Velocity	Number of Pass/Fail Points	Positions of Points Failed	Overall Result	Left Top (dots)	Right Top (dots)	Left Bottom (dots)	Right Bottom (dots)	A_{pf}	Overall Result
$0.93 ms^{-1}$	6/5	5,6,7,9,10	FAIL	8	7	15	3	$1.04x10^6$	PASS

Table 3: Number of dots counted by different people for the average exhaust velocity of $0.59ms^{-1}$.

Person	Top Left	Top Right	Bottom Left	Bottom Right	A_{pf}	Pass/Fail
1	66	10	9	17	0.94×10^5	Fail
2	43	9	10	13	4.82×10^5	Pass
3	53	10	15	24	3.52×10^5	Pass
4	36	7	7	20	7.29×10^5	Pass
5	34	8	12	6	6.27×10^5	Pass
6	31	7	10	16	5.23×10^5	Pass
7	68	9	28	20	9.12×10^4	Fail
8	40	8	22	18	3.89×10^5	Pass
9	39	23	88	41	7.05×10^4	Fail
10	19	4	3	2	1.76×10^6	Pass
Mean	**42.9**	**9.5**	**20.4**	**17.7**		
St Dev	***15.4***	***5.1***	***24.9***	***10.6***		