BAKER
Environments For Science™
175 Gatehouse Road / Sanford, Maine 04073 / USA
800-992-2537 / 207-324-8773 / Fax: 207-324-3869 / bakerco@bakerco.com

MICROBIOLOGICAL TEST REPORT

Becton Dickinson BD400®
Class II type A2 Biosafety Cabinet
for the
BD FACSAria Fusion Cell Sorter
BD FACSAria Fusion Special Order System
BD FACSymphony S6 Special Order System

Figure 1 BD FACSAria Fusion Cell Sorter inside the BD400 BioSafety Cabinet

Table of Contents

I – INTRODUCTION

The Baker Company Class II, type A2 BioSafety Cabinet was designed and built specifically for integration with the BD FACSAria Fusion cell sorter, FACSAria Fusion Special Order System and FACSymphony S6 Special Order Systems. The modified SterilGARD cabinet was designed for the specific purpose of providing product, personnel and environmental protection against potential biohazards.

Figure 2 BD FACSAria Fusion inside safety cabinet

The biosafety cabinet applies airflow technology to provide the following protection:

- Personnel protection or containment by an intake air velocity of no less than 100 feet per minute (fpm) through the front access opening of the cabinet.
- Product protection by high efficiency particulate air (HEPA) filtered downward air inside the cabinet work area.
- Environmental protection by exhausting only HEPA filtered air back into the atmosphere.

The BD400 biosafety cabinet with the FACSAria Fusion cell sorter **(FAF)** installed and operational according to factory designated conditions was microbiologically tested in compliance with the following National and International Biological Safety Cabinet Standards for product and personnel protection against aerosols which may contain potential biohazards.

- NSF/ANSI International Standard 49- 2014
- European Standard (EN 12469:2000)
- British Standard (BS EN 12469:2000)
- South Africa National Standard (SANS 12469:2000)
- French Standard (NF-095:2006)
- China Standard (SFDA YY- 0569:2005)
- Japanese Industrial Standard (JIS K 3800:2009)
- Australian Standard (AS 1807.1:2009)

The following testing was performed with the BD FAF Cell Sorter using the aerosol management system **(AMS)** operating at the low setting unless noted otherwise in this report. ***Typically an AMS aerosol management system is dedicated at the cytometer sorting chamber. NIH (National Institutes of Health) recommends that the AMS system operates continuously at the low setting during all sorting activities in the event of an aerosol or sample stream misalignment. The AMS for this biosafety cabinet application uses the internal vacuum system option integrated within the biosafety cabinet. All testing was performed with the BD FAF flow cytometer in an operational condition.***

Preliminary Testing Referenced:

Testing conducted on a system mock up on Oct.25, 2012 provided an estimate for the airflow set point velocities required in establishing the biosafety cabinet's performance. This testing was done using a model or mock-up that estimated the size, shape and volume of the BD FAF within a prototype Baker Company type A2 style biosafety cabinet. This original model did not take into account the current "open back" BD FAF design and the addition of the work area divider that is currently being implemented for this system. During this test a secondary method was established for measuring the downflow velocities inside the cabinet work area. This secondary method enables the system to be checked for air flow without the removal of the FAF. (*Reference Baker document BAKT192rev.3).*

II– NSF/ANSI Standard 49:2014 International Biosafety Cabinetry Standard

Purpose: The purpose of the following tests (standardized, modified and created specifically for the FAF) is to determine the following:

- whether aerosols from within the biosafety cabinet will be contained.
- contaminants from outside the biosafety cabinet will be excluded from the cabinet work area providing a particulate free environment.
- HEPA filtered exhaust air will be exclusively returned into the environment.

Testing Introduction:

With the BD FAF installed the cabinet was airflow balanced to a set point velocity of 50 feet per minute downflow and 105 [335cfm] feet per minute inflow. Preliminary smoke visualization tests were conducted to identify potential areas of turbulence which may affect cabinet performance. Observation of smoke visualization tests showed the absence of turbulent air around the flow cytometer and very minimal refluxing (turbulence) in the bottom front of the cabinet. It is very important to ensure that smoke does not escape or enter the cabinet as this indicates refluxing internally or externally respectively. This turbulence can be mitigated by adjusting flows and the position of the BD FAF for this segment of the test.

NSF/ANSI 49:2014 Standard Microbiological Testing:

The NSF/ANSI-49 biosafety standard states that personnel and product microbiological testing shall be conducted at an operating range of plus or minus 10 fpm [0.05 m/s] from the biosafety cabinet's nominal airflow set point. This assures a safety range in the event the biosafety cabinet's air balance is hampered such as when HEPA filters load or other unforeseen air disruptions. This safety range is plotted on the Baker Company performance graph on page 10 of this report. Baker uses a more rigorous test criterion exceeding that of NSF/ANSI-49 by going 5 fpm [0.025 m/s] beyond the required test range for the purpose of proving a greater range of safety performance, also plotted on the Baker performance graph.

To determine the optimal air flow setpoint for this biosafety cabinet application a series of tests will be performed at varying airflow settings with the cell sorter operational and the AMS operating at the low setting. To begin the following tests the cabinet airflow balance was set to 50 fpm [0.25 m/s] downflow and 105fpm [0.53m/s] inflow. This is the typical Baker cabinet air balance set point with a front access opening of 8 inches. In section VI the worst case condition is defined as: the AMS operating in the high flow feature with the sort chamber door open.

NSF Personnel Protection Test

The following NSF Personnel Protection microbiological testing was performed with the FAF operational and the AMS at the low setting. All BD FAF doors were in the closed position during this test.

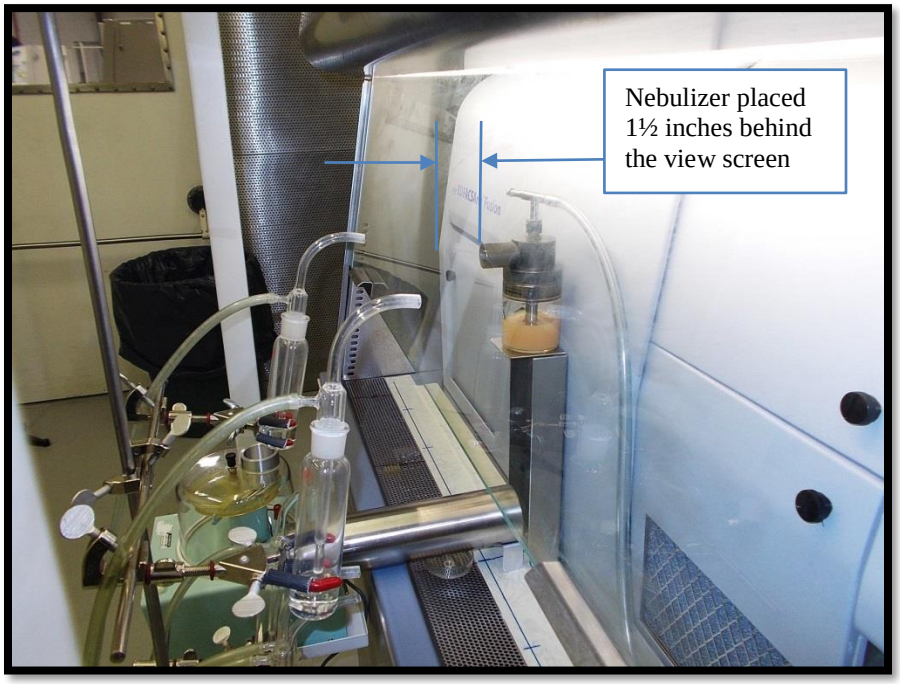


Figure 3 NSF Personnel Protection Microbiological Test Set-Up

Nebulizer placement is closer to the view screen than the NSF standard requires

Testing Method for Personnel Protection:
The system was challenged with *Bacillus Subtilis or also known as Bacillus Atrophaeus* bacterial spores aerosolized at 7.2 x 10^8 spores/ml for each test run. The challenge was delivered via a collison nebulizer which is required to have a discharge velocity of 100 fpm+/-10fpm. The nebulizer was located 1½ inches [38 mm] behind the viewscreen with the horizontal spray axis placed 14 inches [356 mm] above the work surface and centered between the two sides of the cabinet. ***(The NSF standard requires the nebulizer be placed 4 inches behind the view screen 14 inches off of the work surface. The deviation in this test from the standard 4 inches to 1 ½ inches was necessary due to the size of the BD FAF and its location within the cabinet. The new nebulizer location creates a more challenging test. By placing the aerosol spray nozzle closer to the view screen the aerosol is at a higher horizontal velocity as it enters the "safety cabinet velocity air curtain" than it would be if the nozzle were further away. This higher velocity creates more of a challenge by requiring the downflow air and velocity air curtain to capture bacterium that in a normal test would be traveling much slower horizontally)***

The NSF standard states each test is to provide aerosol for 6.5 minutes during the 30 min test. However, this biosafety cabinet was tested with an aerosol challenge of 16.5 minutes which is a standard Baker test and offers a significant increased challenge for the unit to pass. To offer an even higher challenge an airflow disrupter (a challenge cylinder) is introduced into the cabinet 2 ¾ inches [70 mm] above the top of the work surface. NSF/ANSI-49 standard requires this device to be a cylinder of 2.5 inches [63mm] outside diameter, made of stainless steel with closed ends. The challenge cylinder shall be used to disrupt airflow with one end protruding at least 6 inches [150 mm] out of the cabinet's front access opening. (Figures 3, 4) Eight sampling devices shall be placed outside the BCC to capture any bacterial spores which may escape the system.

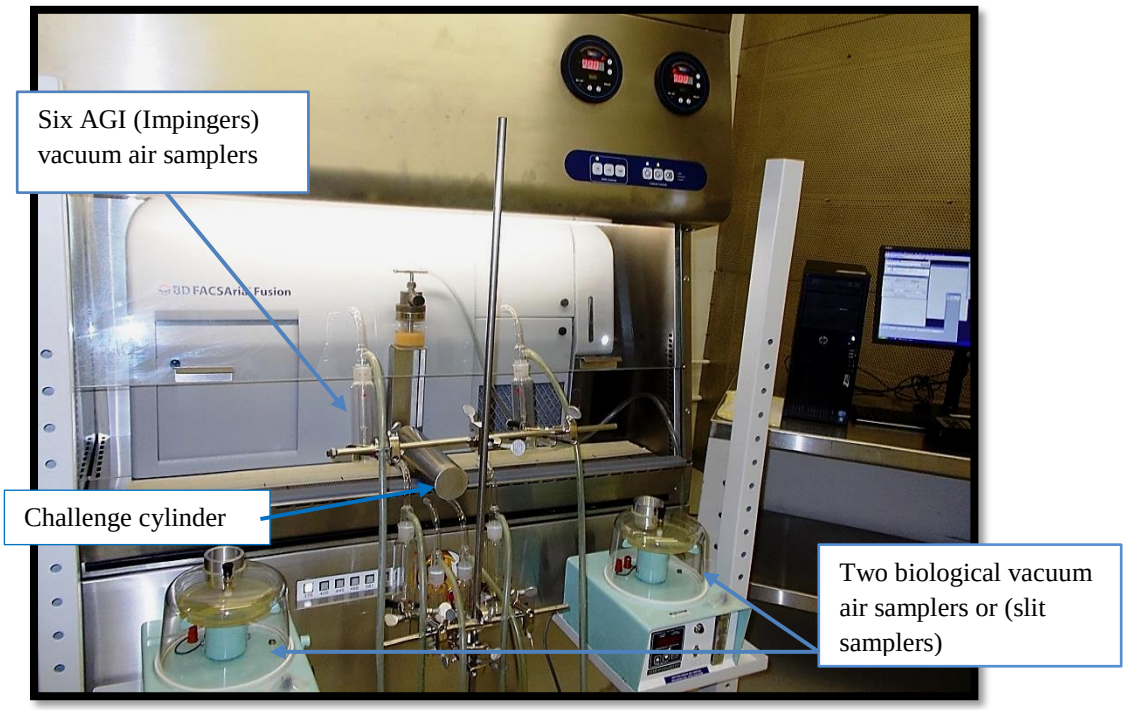


Figure 4 NSF Personnel Protection Biotest

Personnel Protection Acceptance:
The number of *Bacillus Subtilis* **colony forming units (CFUs)** recovered from the six AGI (Impingers) vacuum air samplers (Figure 4) **shall not exceed (10 CFU's)** for each test. Total slit-type vacuum air sampler's (Figure 4) **shall not exceed (5 CFU's)** on the soy agar 150mm media settling plates for a 30 min testing period. Settling plates are incubated for 48 hours before recording the final CFU counts.

A "control" plate shall be located beneath the challenge cylinder and shall be positive as indicated by containing greater than 300 CFUs of *B.Subtilis*. It can be placed ½" [12.7 mm] above or below the work surface front perforated grill. **Note: The acceptance criteria for all Microbiological Standards throughout this report for Personnel Protection are identical:**

Personnel Protection Test Results

Personnel Protection CFU's acceptance: **(No more than 5 are allowed from the air slit samplers)**
(No more than 10 are allowed from the 6-AGI samplers)

	Cabinet Airflow Settings		Control Plate	Slit-Type Air Samplers	AGI Air Samplers	
Test	**Downflow air**	**Inflow air**	**CFU counts**	**CFU counts**	**CFU counts**	**Results**
1	48 fpm	105 fpm	positive >300	**0**	**0**	**PASS**
2	62 fpm	87 fpm	positive >300	**0**	**0**	**PASS**
3	30 fpm	88 fpm	positive >300	**0**	**2**	**PASS**
4	70 fpm	81 fpm	positive >300	**1**	**0**	**PASS**

Results: All NSF Personnel Protection Microbiological Tests: **PASSED**

The personnel protection testing met or exceeded the safety requirements in accordance with NSF/ANSI Standard 49-2014 for biosafety cabinetry. The microbiological tests passed with the system operating beyond the nominal operating range of plus or minus 10 fpm as required in the standard. (Graph illustrating results on page 10).

NSF Product Protection Test:

The following NSF Product Protection microbiological testing was performed with the BD FAF operational and the AMS at the low setting. All FAF doors were in the closed position during this test.

Figure 5 NSF Product Protection Set UP

Testing Method for Product Protection:
The system was challenged with *Bacillus Subtilis* bacterial spores aerosolized at 7.2 x 10^6 spores/ml for each test run. The challenge was delivered via a collison nebulizer which is required to have a discharge velocity of 100 fpm+/-10fpm. The nebulizer was placed 4 inches [102 mm] in front of the viewscreen with the horizontal spray axis level with the top edge of the work opening and centered between the two sides of the cabinet. The test was operated for a total of 30 min with an increased 15 minute aerosol challenge (the NSF standard states 5 minutes). NSF requires that a challenge cylinder is to be used in this test at the same location noted in the Personnel Protection Test above. The 100mm petri dishes with soy agar media are placed behind the work area perforation grill for all product protection testing. (Figure 5) Settling plates are incubated for 48 hours before recording the final CFU counts.

Product Protection Acceptance:
The number of *Bacillus Subtilis* colony forming units, (CFUs) on the soy agar media 100mm settling (figure 5) plates **shall not exceed (5)CFU's for each test**. The control plate located beneath the challenge cylinder shall be positive, containing greater than 300 CFUs of *B. Subtilis.* It can be placed ½" [12.7 mm] above or below the work surface front perforated grill. **Note: The acceptance criteria for all Microbiological Standards for Product Protection throughout this report are identical.**

Product Protection Test Results

Product Protection CFU's acceptance: **(No more than 5 are allowed on all of the 100mm soy media plates)**

	Cabinet Air flow Settings		Control Plate	100MM Settling Plates	
Test	**Downflow air**	**Inflow air**	**CFU counts**	**CFU counts**	**Results**
1	50 fpm	105 fpm	positive >300	**0**	**PASS**
2	28 fpm	87 fpm	positive >300	**1**	**PASS**
3	33 fpm	120 fpm	positive >300	**1**	**PASS**
4	34 fpm	115 fpm	positive >300	**1**	**PASS**

Results: All NSF Personnel Protection Microbiological Tests: PASSED

The product protection testing met or exceeded the safety requirements in accordance with NSF/ANSI Standard 49-2014 for biosafety cabinetry. The microbiological tests passed beyond the operating range of plus or minus 10 fpm from the nominal set point as required by the standard. (Graph illustrating results on page 10).

The Baker Company established the 'Performance Envelope' as a means of conveying the microbiological performance level of a biosafety cabinet (BSC).The graph is used to illustrate the relationship between a cabinet's airflow and its microbiological safety performance.

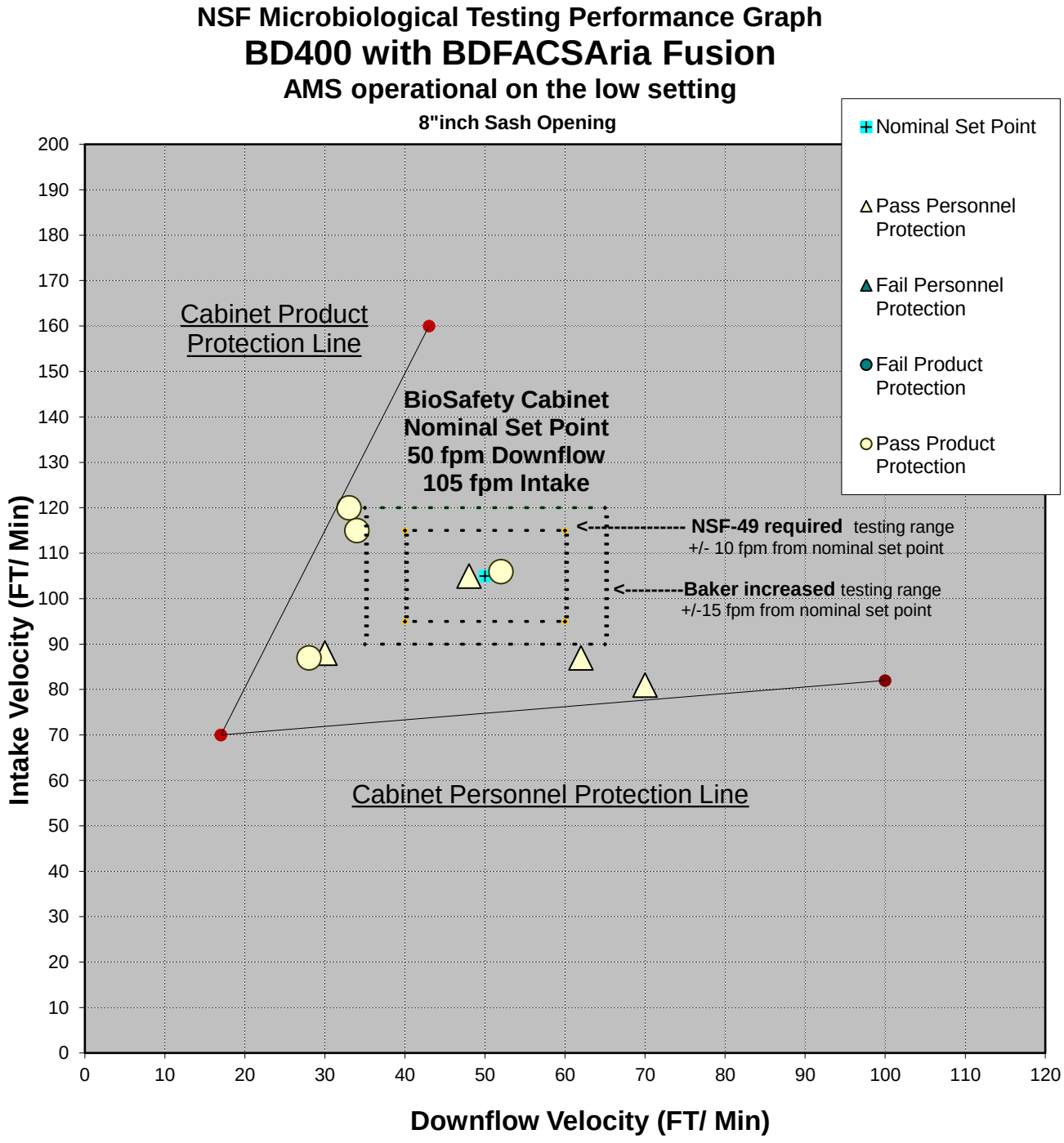
Microbiological Performance Envelope

As previously stated the NSF/ANSI Standard 49 microbiological tests determine whether aerosols will be contained within a BSC (*Personnel Protection*) , outside contaminates will not enter the BSC (*Product Protection*) and aerosol contamination of other equipment or samples inside the BSC will be minimized (*Cross Contamination).* The requirement states Class II Type A2, BSCs must maintain a minimum intake velocity of 100 feet per minute (FPM) or 0.51 meters per second (M/S). Currently there is no minimum NSF requirement for the average downflow velocity; therefore this value is selected based on the final results of the personnel and product microbiological tests. Once this data is inserted into the Performance Envelope graph the Baker Engineering Test Department selects the cabinets' optimal airflow setpoint.

The NSF/ANSI Standard 49 also requires passing microbiological test results at an 'NSF safety range' of 10 feet per minute (0.05 meters per second) outside of the nominal setpoint velocity of a biosafety cabinet. The microbiological tests are identified within the Performance Envelope with a circle for product protection, a triangle of personnel protection and a square for cross contamination. All passing results are indicated by an un-shaded symbol, all failed results are indicated by a shaded symbol. The Baker Company makes every effort to exceed the 'NSF Safety Range' by testing at a level of 15 FPM (0.08 M/S) outside the nominal setpoint velocity.

We call this the 'Baker Safety Range'. The results of both the NSF and BAKER safety ranges are shown in every performance envelope and indicated by the boxed outlines.

It is the Baker Company's policy to identify any unsafe condition of an application in relationship to biological safety and test beyond its intended means. When demands for large equipment installations or robotic style applications with moving parts are placed within the BSC work area. Microbiological cross contamination testing will not be performed due to the physical constraints or air disruptions of the application.



III – *European Microbiological Safety Cabinet Standard (EN12469:2000)

The following microbiological safety standards are equivalent to and reference EN12469:2000 as an acceptable testing method with some modifications to the technical content: **British Standard** (BS EN 12469:2000), **South Africa National Standard** (SANS 12469:2000), **French Standard** (NF-095:2006), **Australian Standard** (AS 1807.1:2009) **Japanese Industrial Standard** (JIS K 3800:2009)

**Reference: The EN European Standard was approved by CEN (European committee of standardization) on January 3, 2000. CEN members are the national standards bodies of Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and United Kingdom. It exists in three official versions (English, French, and German)*

Testing Introduction:

The International Standard Biological tests in this section are to be performed with the nebulizer placed at the location where the operator would be located; this is for cabinets up to 1.5m wide. The following European Standard tests were conducted at the cabinet nominal airflow balance of 50fpm (.25m/sec.) downflow and 105fpm (.533m/sec.) inflow. **The increased nebulizer duration that Baker uses with NSF/ANSI-49 standard testing was not applied to be in compliance with the EN 12469:2000 standard. Tests using the increased challenge will be identified in the method section.**

The significant variations between the NSF/ANSI 49:2014 Standard and the EN Standard 12469:2000 for cabinet biosafety are:

- The nebulizer height for personnel protection is located at sash or viewscreen opening level versus the NSF Standard location of 14 inches [356 mm] above the work area platform.
- To determine the number of Bacillus Subtilis spores delivered, EN12469:2000 requires weighing the nebulizer before and after each test with a known spore concentration to determine the amount of challenge or (CFU's) spores sprayed for each test. NSF calibrates the nebulizer itself.

- Five replicate tests are required for personnel protection at nominal cabinet set point an increase from the NSF required three replicate tests.

- Unlike NSF/ASNI Standard 49, the EN Standard does not require a performance safety range of plus or minus 10 fpm [0.05m/s] from the nominal airflow setpoint. According to EN12469 the microbiological testing is performed at the nominal setpoint only. TUV Nord the Nationally Recognized Testing Laboratory which conducts the microbiological testing to the EN12469:2000 Standard requires that an additional location shall be tested which is at the low air alarm limit, based on a 20% reduction in cabinet downflow. Baker performed the recommended additional testing and the results are displayed on the performance graph on Page 17. All other EN microbiological test requirements remain unchanged in relationship to NSF/ANSI Standard 49.

- All additional international testing will be plotted altogether on one graph located on page 17 of this report.

EN 12469:2000 Retention of Front Aperture or Personnel Protection Test

The following Personnel Protection microbiological testing was performed with The BD FAF operational and the AMS system at the low setting during the challenge testing. All cytometer doors were closed.

Figure 6 EN Standard Personnel Protection Microbiological Test

Testing Method for Retention of Front Aperture

The cabinet was challenged with *Bacillus Subtilis* bacterial spores of 7.2 x 10^8 spores/ml for each test run. The challenge was delivered via a collison nebulizer. The nebulizer was placed 2inches behind the view screen with the horizontal spray axis level with the upper edge of the front aperture. ***(The 10 degree slant of the view screen and lower height relative to the work***

area, places the nebulizer further behind the view screen than the NSF modified location by 1½ inches from previous testing. The EN standard requires the nebulizer be placed 3.937in (100mm) behind the view screen. The deviation from the EN requirement in this test was unavoidable due to the size and location of the BD FAF within the cabinet. However the nebulizer deviation creates a more challenging test, by placing the aerosol spray nozzle closer to the view screen the aerosol is at a higher horizontal velocity as it enters the "velocity air curtain" than it would be if the nozzle were further away. This higher velocity creates more of a challenge by requiring the down flow air and velocity air curtain to capture bacterium that in a normal test would be traveling much slower horizontally).

The standard requires an aerosol challenge of 5 minutes during the 30 min test. To offer a higher challenge an airflow disrupter is introduced into the cabinet 2 ¾ inches above the work surface. EN (as NSF) requires that this device be a cylinder 2.5 inches (63mm) outside diameter stainless steel cylinder with closed ends and shall be used to disrupt airflow. During testing one end of the challenge cylinder must protrude at least 6.0 inches (15mm) out of the cabinet and into the room through the work access opening.

To determine the number of Bacillus Subtilis spores delivered, the EN12469:2000 Standard requires weighing the nebulizer before and after each test with a known spore concentration. 5 replicate tests shall be carried out at the center of the front aperture only.

EN Standard Personnel Protection Test Results

Personnel Protection CFU's acceptance: **(No more than 5 are allowed from the air slit samplers)**
(No more than 10 are allowed from the 6-AGI samplers)

	Cabinet Air flow Settings		Control Plate	Slit-Type Air Samplers	AGI Air Samplers	Spores Sprayed	
Test	Downflow air	Inflow air	CFU counts	CFU counts	CFU counts	CFU	Results
1	50 fpm	105 fpm	positive >300	**0**	**0**	**$2.2x10^9$**	**PASS**
2	50 fpm	105 fpm	positive >300	**0**	**0**	**$2.2x10^9$**	**PASS**
3	50 fpm	105 fpm	positive >300	**0**	**0**	**$2.2x10^9$**	**PASS**
4	50 fpm	105 fpm	positive >300	**0**	**0**	**$1.9x10^9$**	**PASS**
5	50 fpm	105 fpm	positive >300	**0**	**0**	**$1.9x10^9$**	**PASS**
6	40 fpm	99 fpm	positive >300	**0**	**0**	**$2.2x10^9$**	**PASS**
7	40 fpm	99 fpm	positive >300	**0**	**0**	**$1.9x10^9$**	**PASS**
8	40 fpm	99 fpm	positive >300	**0**	**1**	**$2.2x10^9$**	**PASS**
9	40 fpm	99 fpm	positive >300	**0**	**0**	**$1.9x10^9$**	**PASS**
10	40 fpm	99 fpm	positive >300	**0**	**0**	**$2.2x10^9$**	**PASS**

Results: All EN Personnel Protection Microbiological Tests: **PASSED**

EN Product Protection Test

The following Product Protection microbiological testing was performed with The BD FAF and the AMS system at the low setting during the challenge testing. All cytometer doors were closed.

Test Method for Product Protection:
All tests are performed to the European Standard EN12469:2000. The cabinet was challenged with Bacillus Subtilis spores at 7.2 x 10^6 spores/ml for each test run. One 2.5 in (63mm) outside diameter stainless steel cylinder with closed ends shall be used to disrupt airflow. The end protrudes at least 6.0 in (15mm) into the room through the work access opening of the cabinet.

To determine the number of Bacillus Subtilis spores sprayed, EN12469:2000 Standard requires weighing the nebulizer before and after each test with a known spore concentration.

For cabinets up to 1.5m wide, 3 replicate tests shall be carried out at the center of the front aperture only.

EN Standard Product Protection Test Results

Product Protection CFU's acceptance: (No more than 5 are allowed on all of the 100mm soy media plates)

	Cabinet Air flow Settings		Control Plate	100MM Settling Plates	Spores Sprayed	
Test	**Downflow air**	**Inflow air**	**CFU counts**	**CFU counts**	**CFU**	**Results**
1	50 fpm	105 fpm	positive >300	**0**	**$1.5x10^7$**	**PASS**
2	50 fpm	105 fpm	positive >300	**0**	**$1.5x10^7$**	**PASS**
3	50 fpm	105 fpm	positive >300	**0**	**$1.5x10^7$**	**PASS**
4	40 fpm	99 fpm	positive >300	**0**	**$1.8x10^7$**	**PASS**
5	40 fpm	99 fpm	positive >300	**0**	**$1.5x10^7$**	**PASS**
6	40 fpm	99 fpm	positive >300	**0**	**$1.5x10^7$**	**PASS**

Results: All EN Product Protection Microbiological Tests: PASSED

Additional Biosafety Testing to the European Microbiological Safety Cabinet Standard (EN12469:2000).

BD requested additional biosafety testing at the BDFACSAria Fusion sorting chamber location to the EN Biosafety Cabinet Standard. The sort chamber door was in the opened position with the required biotesting stainless steel cylinder placed inside the chamber. (Figures 8, 9, 10, 11) All tests performed were witnessed by TUV NORD the nationally recognized testing laboratory which conducts microbiological testing and auditing to the EN Standard 12469:2000. TUV NORD required a more stringent criterion then the EN Standard by requiring additional testing at the safety cabinet low alarm limit which is based on a 20% reduction of cabinet downflow air. 16 more tests were performed with PASSING results. A copy of the testing results (Document BAKT202) may be provided by BAKER upon request.

IV- China Microbiological Safety Cabinet Standard (SFDA YY- 0569:2005)

Testing Introduction:

The China Standard (SFDA YY- 0569:2005) testing and set up criteria is very similar to the NSF/ANSI Biosafety Standard 49- 2014 (**reference Section II)** of this report. The only difference between the NSF and SFDA YY standards for microbiological testing are the placement of the impingers for personnel protection. (See Figure 7 below) The placement of the two top impingers are lowered and located in line with the middle impingers, then separated further apart from each other, from 12 inches to 14¼ inches. The nebulizer also has a slight dimensional variation placed 14¼ inches above the work area rather than the NSF-49 Standard of 14 inches. All other NSF-49 biosafety cabinet methods and acceptance criterion apply.

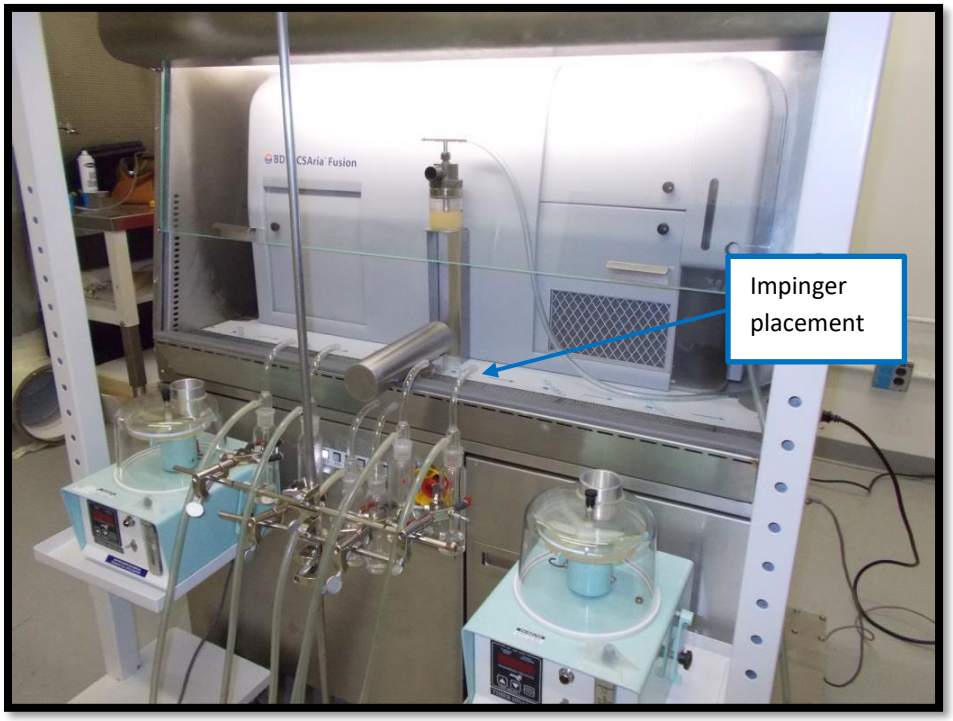


Figure 7 CHINA Standard Personnel Protection Set Up

China Standard Personnel Protection Test Results

Personnel Protection CFU's acceptance: **(No more than 5 are allowed from the air slit samplers)**
(No more than 10 are allowed from the 6-AGI samplers)

	Cabinet Airflow Settings		Control Plate	Slit-Type Air Samplers	AGI Air Samplers	
Test	Downflow air	Inflow air	CFU counts	CFU counts	CFU counts	Results
1	48 fpm	105 fpm	positive >300	**0**	**0**	**PASS**
2	62 fpm	87 fpm	positive >300	**0**	**0**	**PASS**
3	30 fpm	88 fpm	positive >300	**0**	**0**	**PASS**

Results: All 3 China Personnel Protection Microbiological Tests: PASSED

China Product Protection Test Results

The product protection biological testing criteria for the SFDA YY-0569 China Standard are identical to the NSF-49 Standard and have been reported on pages 8, 9 of this report.

V – Japanese Industrial Standard (JIS K 3800:2009)

The Japanese biological safety cabinet standard uses a combination of both NSF/ANSI 49:2007 and the EN12469:2000 Standard set up requirements; (EN) nebulizer placement positioned at sash level for the personnel protection test and requires weighing the nebulizer with spore concentration before after each test. (NSF) requires personnel and product protection testing at a range of plus or minus 10 fpm [0.05m/s] beyond the cabinet nominal airflow set point. **Baker used the combination of the increased nebulizer duration for this testing with the weighing of the nebulizer at the required zones that are to be tested in compliance with JIS K 3800 standard**.

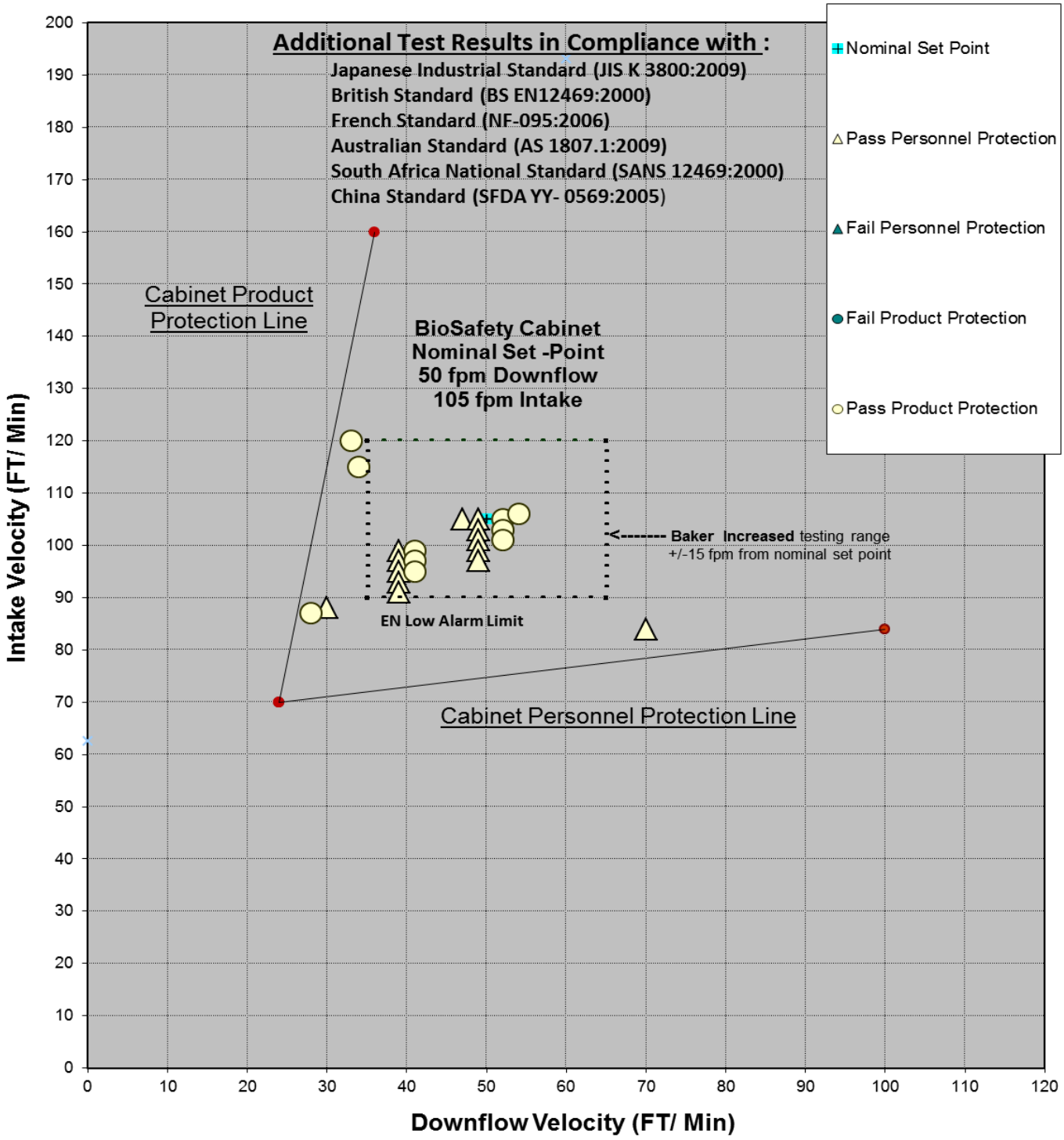
The personnel and product protection testing have met and or exceeded the safety requirements in accordance with JIS K 3800:2009. *Note: The Product testing has also been plotted on the graph on page 10 due to the same set up criterion with the exception of weighing the nebulizer. Testing at nominal safety cabinet set point was not repeated due to quantified passing results from previous testing for the EN BioSafety Standard.*

Japanese Industrial Standard (JIS K 3800:2009) Testing Results: PASSED

BAKER

Environments For Science™

Microbiological International Testing Performance Graph
BD400 with BDFACSAria Fusion
AMS operational on the low setting
8"inch Sash Opening

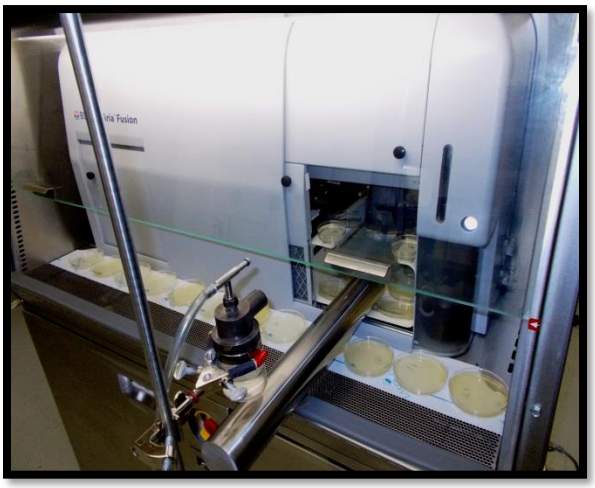


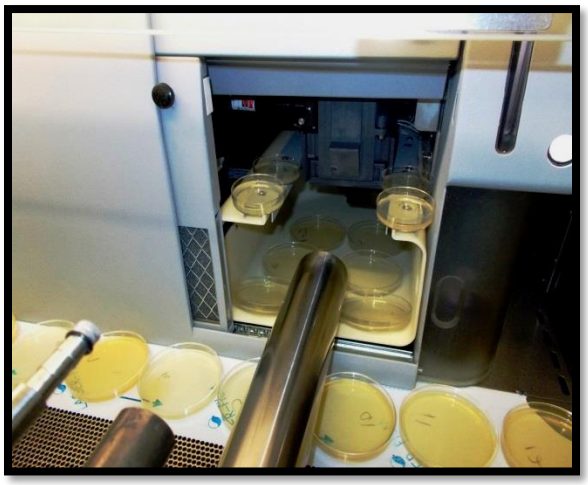
VI - Customer Specified Testing using NSF 49 Standard Testing methods

Additional microbiological tests were performed to look for limits of operation.These tests were performed with the cabinet operating at the nominal airflow set point only.

Product Protection Testing at the Sorting Chamber Location

The collison nebulizer was positioned at the centerline of the BD FAF sort chamber.While using the more challenging 15 minute nebulizer challenge, the agar collection plates were located inside sort chamber area and along the front of the work surface where space was available. The challenge cylinder was applied to create an air disruption.





Figures 8, 9 Product Protection Microbiological Test at the Sort Chamber

The AMS, operating at the high setting feature with sort door open, has been identified as worst case condition for product protection. However during daily sorting operations the sort chamber door would not be opened during the process.

The purpose of this research test is to evaluate the effectiveness of excluding aerosols from the sort chamber under these conditions.

Product Protection Test Results

The AMS operating at the high setting increased the cabinet intake airflow from 268cfm to 290 cfm. Bacterial spores were detected on agar collection plates on each side of the challenge cylinder and on some plates inside the sort chamber. No spores were detected on any other plates.The test provided evidence that with the AMS on high setting and the sort door open there is the potential for a high degree of containmation inside the sort chamber. The tests indicated that the contaminants entering the cabinet under this condition were due to the open door and the air suction created from the high flow of theAMS.

Personnel Protection Testing at the Sorting Chamber Location

The worst case for personnel protection has been identified as the system operating: with the AMS off and the sort door open. However during daily sorting operations the sort chamber door would not be opened during the process.

The collison nebulizer was positioned at the centerline of the BD FAF inside the sort chamber.The more challenging 15 minute nebulizer challenge was performed and the challenge cylinder was applied to create an air disruption.

Figures 10, 11 Personnel Protection Microbiological Test at the Sort Chamber

The purpose of this research test is to evaluate the effectiveness of aerosols being contained within the sort chamber in case of a stream misalignment or other unforseen occurance.

Personnel Protection Test Results

No bacterial spores were detected outside the the biosafety cabinet.

ReadySafe™Mode Testing

The “Ready Safe Mode" is a Baker Company Cabinet energy efficiency feature. As with all the testing in this section; testing is not required by NSF or any other agency.

Static Test: The Biosafety cabinet was balanced to the nominal set point and the view screen placed in the closed position. Placing the view screen in the closed positon to the armrest intiates the “ready safe mode” feature.While in ready safe mode the cabinet intake air was reduced to 75cfm through the armrest and reduced the motor amperage from 3.5 amps to 1.0 amp. Although these Personnel and Product Protection tests are not an NSF required biosafety test. The NSF Standard and the spore concetration used in previous testing will be used as a guideline to evaluate the degree of protection the cabinet will provide. A few deviations will apply such as no challenge cylinder, an impinger location change and the increase in duration of the bacterial aerosol challenge.

Figures 12, 13 "Ready Safe Mode" Product and Personnel Protection Microbiological Specialty Tests

Personnel Protection CFU's acceptance: **(No more than 5 are allowed from the air slit samplers)**
(No more than 10 are allowed from the 6-AGI samplers)

	Cabinet Air flow Settings		Control Plate	Slit-Type Air Samplers	AGI Air Samplers		
Test	Downflow air	Inflow air	CFU counts	CFU counts	CFU counts		Results
1	48 fpm	105 fpm	>300	**0**	**0**		**PASS**

Product Protection CFU's acceptance: **(No more than 5 are allowed on all of the 100mm soy media plates)**

	Safety Cabinet Air flow Settings		Control Plate	100MM Settling Plates		
Test	Downflow air	Inflow air	CFU counts	CFU counts		RESULTS
1	48 fpm	105 fpm	>300	**0**		**PASS**

ReadySafe™ Mode Testing Results: PASSED

Although these Personnel and Product Protection tests are not required by NSF/ANSI 49 Standard or any other international standard, Baker determined that if the front viewscreen was closed to the armrest bypass and the motor amperage reduced, the system would continue to provide product and personnel through all inflow openings while in the Ready™ Safe Mode energy efficiency feature.

Dynamic Personnel and Product Protection Testing

A dynamic personnel protection test was performed beginning with the view screen at the 8 inch operating height, when the nebulizer and impingers were in operation for 1 minute then the view screen was closed, i.e. in contact with the armrest, for the remainder of the test.

A second dynamic personnel protection test was performed with the view screen closed at the start of the test and after 1 minute when the nebulizer and impingers are in operation , the view screen was opened to the operating height of 8 inches.

The purpose of these tests is to evaluate the effectiveness of aerosols being contained within the cabinet or wheather aerosols may enter the cabinet as the view screen is opened and closed.The AMS was operating in the low flow setting during all dynamic testing.

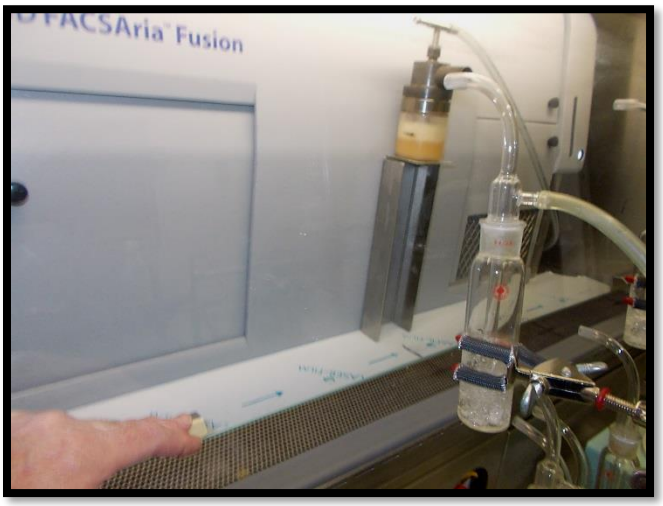


Figure 14 Example of Dynamic Personnel Protection test while closing the cabinet viewscreen

After **1 minute of CFU** challenge the opened view screen was closed to armrest

Personnel Protection CFU's acceptance: **(No more than 5 are allowed from the air slit samplers)**
(No more than 10 are allowed from the 6-AGI samplers)

	Cabinet Air flow Settings		Control Plate	Slit-Type Air Samplers	AGI Air Samplers		
Test	Downflow air	Inflow air	CFU counts	CFU counts	CFU counts		Results
1	48 fpm	105 fpm	>300	**0**	**0**		**PASS**

After **1 minute of CFU** challenge the closed view screen was opened to the 8 inches operating height

	Cabinet Air flow Settings		Control Plate	Slit-Type Air Samplers	AGI Air Samplers		
Test	Downflow air	Inflow air	CFU counts	CFU counts	CFU counts		Results
1	48 fpm	105 fpm	>300	**0**	**0**		**PASS**

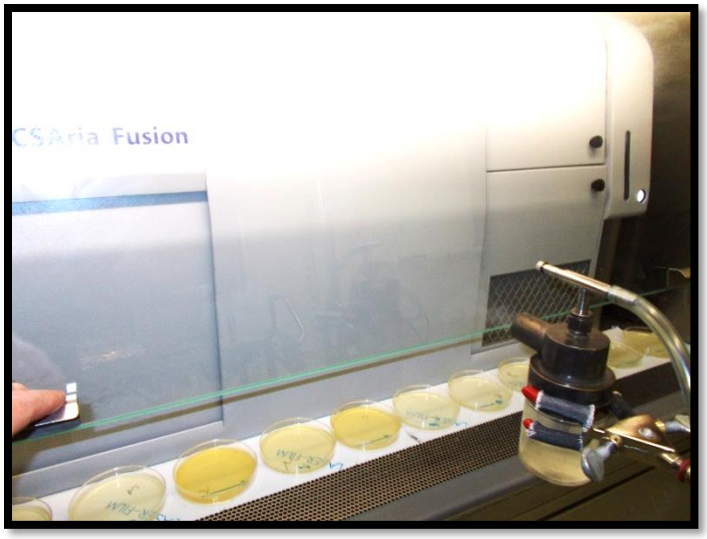


Figure 15 Example of Dynamic Product Protection test while closing the viewscreen

A dynamic product protection test was performed beginning with the view screen at the 8 inch operating height, after the nebulizer was in operation for 1 minute the view screen was closed for the remainder of the test.

A second dynamic product protection test was performed with the view screen closed at the start of the test and after 1 minute of the nebulizer in operation , the view screen was opened to the cabinet operating height of 8 inches.

After 1 minute of CFU challenge the opened view screen was closed to armrest

Product Protection CFU's acceptance: (No more than 5 are allowed on all of the 100mm soy media plates)

	Safety Cabinet Air flow Settings		Control Plate	100MM Settling Plates		
Test	**Downflow air**	**Inflow air**	**CFU counts**	**CFU counts**		**RESULTS**
1	48 fpm	105 fpm	>300	**2**		**PASS**

After 1 minute of challenge the closed view screen was opened to 8 inches operating height

	Safety Cabinet Air flow Settings		Control Plate	100MM Settling Plates		
Test	**Downflow air**	**Inflow air**	**CFU counts**	**CFU counts**		**RESULTS**
1	48 fpm	105 fpm	>300	**3**		**PASS**

DynamicTesting Results

Both Personnel and Product Protection biotesting with the increased 15 minute aerosol challenge showed good results when the cabinet view screen was dynamically opened and closed into the night time set back mode Baker trade name of "ready safe".

Consideration should be observed on how non-diliberate movements and or speed of the view screen may have on the testing. The air flow in the cabinet and the subsequent protection provided by this air flow is easliy effected by movement into and out of the cabinet as well as air flow with in the room and other factors like personnel walking by doors opening and closing, etc. For instance hands making sweeping movements into and out of the cabinet will cause air to flow into and out of the cabinet respectivley. This will cause any biologicals in the air to be transferred into or out of the cabinet, again respectivley.

Final Test Results Overview:

The microbiological testing conducted on the BD400 Biosafety Cabinet exceeded all safety requirements in accordance with the National and International Standards for biosafety cabinetry. Microbiological cross contamination tests were not performed on this biosafety cabinet system due to the overall volume of the BD FACSAria Fusion cell sorter within the cabinet workarea.

Acceptable results were demonstrated while the FAF was installed and operational. All applications under end user conditions are in compliance with the following National and International Biological Safety Cabinet Standards.

- **NSF/ANSI International Standard 49- 2014**
- **European Standard (EN 12469:2000)**
- **British Standard (BS EN 12469:2000)**
- **South Africa National Standard (SANS 12469:2000)**
- **French Standard (NF-095:2006)**
- **China Standard (SFDA YY- 0569:2005)**
- **Japanese Industrial Standard (JIS K 3800:2009)**
- **Australian Standard (AS 1807.1:2009**

Addendum:

In February 2018 a comprehensive biosafety evaluation was performed by the BAKER Company which deemed the **BDFACSAria Fusion Special Order System** and the **BD FACSymphony S6 Special Order Systems** acceptable applications inside the BD400 Biosafety Cabinet.

Microbiological Testing

Robert A.Thibeault