THE BAKER COMPANY

161 Gatehouse Rd., Sanford, Maine 04073 USA

"Creating Immaculate Atmospheres"

Microbiological Testing Report

BioProtect IV with Becton Dickenson FACS Aria III April 26, 2011

Customer

Becton Dickenson Biosciences

Microbiological Testing

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Data Reference

Document number: BAK172

Baker Company Laboratory Book number # 80

BioPROTECT IV with installed BD FACSAria cell sorter plus Fluidics Cart

Purpose

To report the results of microbiological and physical testing of the BioProtect IV while the BD FACS Aria cell sorter is operating. Ensuring the proper safety performance of the cabinet under A2 style biosafety conditions, while addressing customer needs concerning equipment applications.

Procedure:

All microbiological tests are performed to the NSF/ANSI biosafety standard 49, 2009. Test challenge time had been extended from 5 minutes to 15 minutes, increasing the stringency of each test. Determine the optimal operating range of the cabinet, which in this application through smoke and initial biotesting was identified to be 65 fpm down flow and 105 fpm intake.Develope a performance envelope to reflect passing areas to support the NSF criteria of no test failures plus or minus 10 fpm from nominal set point.

Other testing requested was to microbiologically challenge the plate sorting chamber to ensure when chamber door was in the open position that product cleanliness was not compromised.

Modification

Due to turbulent non laminar down flow air in front of the FACS Aria cell sorter it was necessary to smooth out the air by adding air deflectors to the top front , supporting cabinet safety performance. The deflectors are designed to give customer easy access to make adjustments to the cell sorter during operation.

Air Deflectors on top of FACSAria III

Personnel Protection tests:

These tests are performed to determine the degree to which aerosols will be contained within the cabinet and that outside contaminants will not enter the work area of the cabinet under given test conditions. The cabinet is operated at the manufacturers recommended air flow operating set point. This has been determined to be 65 fpm down flow air and 105 fpm intake velocity. The cabinet is turned on at least 30 minutes before the start of any test and operated continuously throughout all test procedures.

All personnel tests challenged with a bacterial aerosol spore concentration of 5.0 x 10^8 spores per ml.

Personnel Protection Microbiological test

Personnel Protection Biotesting

Test apparatus location, times and passing criteria:

Cabinet set point 65fpm down flow and 105 fpm Intake velocity

All Glass Impingers Filtered: Qty. 6

Nebulizer Location	Test time	Total CFU's allowed passing	Set Point Results
inside, center of view screen	30 min total 15 min nebulizer	<= 10	Pass

2 Slit Samplers	Test time	Total CFU's allowed passing	Set Point Results
6 in from side wall	30 minutes total	<= 5	Pass

- Personnel Protection tests are also shown on the Performance Results graph. (Ref. page – 6).

Product Protection Tests:

These tests are performed to determine the degree to which aerosols will not enter the safety cabinet and contaminants will not enter the work area under given test conditions. Challenged with a bacterial aerosol spore concentration of 5.0 X 10^6 spores per ml.

Product Protection Microbiological Test

Test apparatus location, times and passing criteria:

Nebulizer Location	Test time	Total CFU's allowed passing	Set Point Results
Outside, center view screen	30 min total 15 min nebulizer	<= 5	Pass

- Product Protection tests with results are shown on the Performance Results graph. (Ref. page - 6)

Other Microbiological Testing (ACDU SWING DOOR CHAMBER)

At nominal set point the FACSAria plate sorting chamber was challenged for personnel and product protection directly at the chamber as shown.

Personnel Protection Biotest

Product Protection Biotest

Results: Both Personnel and Product protection testing for the (Automatic cell deposition unit) swing down door **passed** the microbiological challenge

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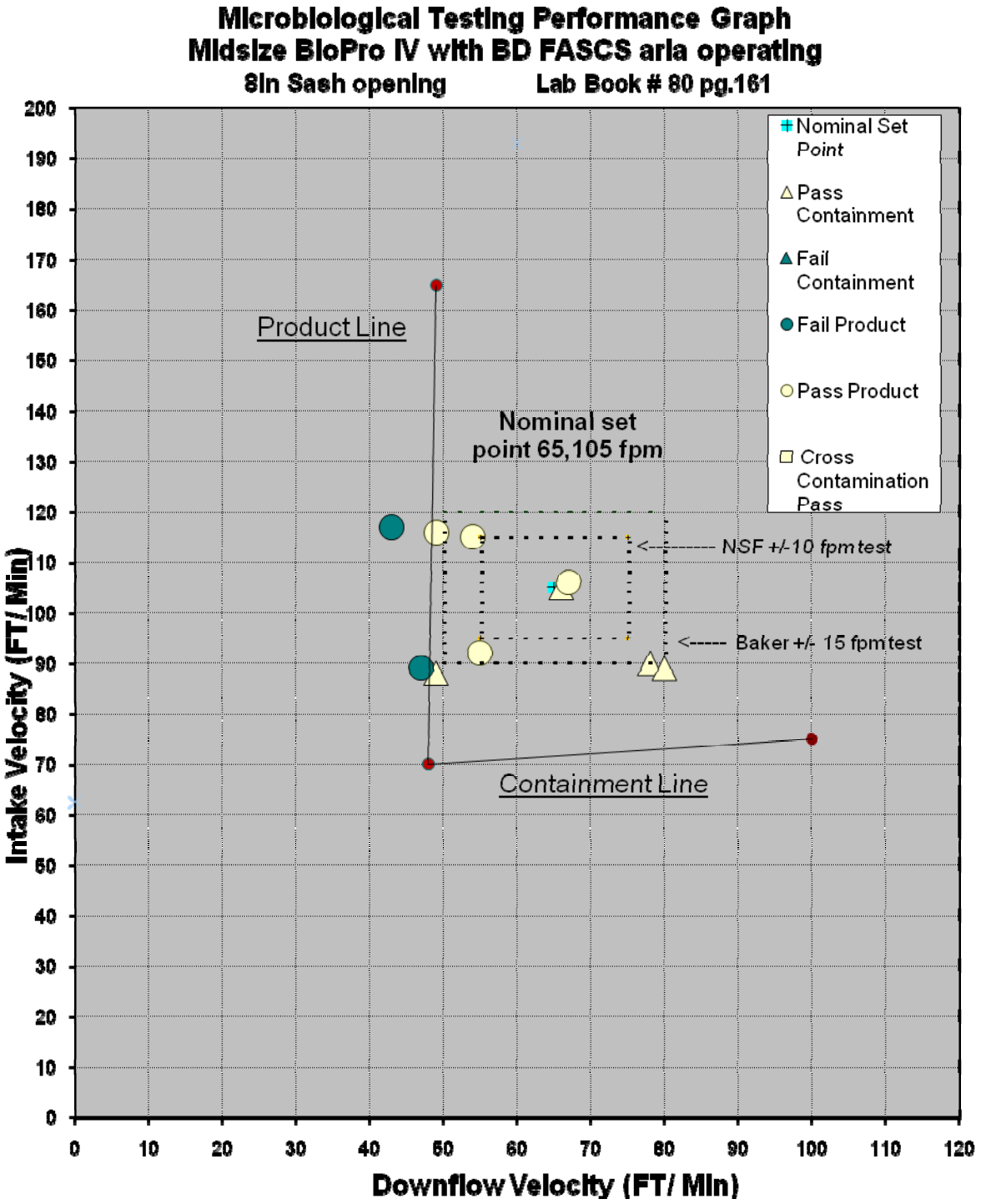
Microbiological Testing Performance Graph
Midsize BioPro IV with BD FASCS aria operating
8in Sash opening
Lab Book # 80 pg.161
Nominal Set Point
Pass Containment
Fail Containment
Fail Product
Pass Product
Cross Contamination Pass
Product Line
Nominal set point 65,105 fpm
<------- NSF +/-10 fpm test
<----- Baker +/- 15 fpm test
Containment Line
Intake Velocity (FT/ Min)
Downflow Velocity (FT/ Min)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200

Conclusion:

Test results reveal a safe nominal operating set point of 65 fpm down flow velocity and 105 intake velocity for the cabinet. The set point air flow range chosen is, 55 – 75 fpm down flow velocity, and 100 to 110 fpm intake velocity.

Testing results demonstrate a strong passing performance envelope – the airflow balance of intake and down flow velocities where the cabinet passes both product and personnel protection microbiological tests.

Additional Information:

Product Protection Test
The cabinet was challenged with Bacillus Subtilis spores at 5.0 X 10^6 spores/ml for each test run. Each test was run for a total of 30 min with a 15 min nebulizer challenge. Each test run shows total count of CFUs from all the plates on the work surface. "CFUs" means number of Bacillus Subtilis colony forming units counted.

Personnel Protection Test
The cabinet was challenged with Bacillus Subtillis bacterial spores at 5.0 X 10^8 spores/ml for each test run. Each test was run for a total of 30 min with a 16.5 min nebulizer challenge, impingers sampling for 15 minutes. The filter count shows the total CFUs obtained after pooling and filtering 6 all glass impingers (AGIs). The Slit sampler count shows the total count of CFUs obtained from the 2 slit samplers.

Down flow values
Due to area constraints for down flow readings a single line grid pattern had to be developed to support certifiers in the field when equipment is in place.

Sound values

At nominal set-point	FACSAria only	66 db
	Cabinet and FACSAria	69 db

Amperage 1.9A

Mag.Gauge - .26 w.c.

Temperature Testing

The purpose of the test is to determine:

- Temperature rise inside cabinet with the cell sorter running at full power.
- The amount of heat generated by the cell sorter.

Baseline test: Cabinet operating only.

71F* – 22C* Temp avg.

1280 BTUH

Main Test: Cabinet and cell sorter both operating.

76F* – 25C* Temp avg.

1875 BTUH