



Single Use Systems in Microbiological Environmental Monitoring: A Sterile, Transparent, Plastic Impactor

Claudio Denoya, Ph.D, and Gilberto Dalmaso, Ph.D.

INTRODUCTION

Abstract

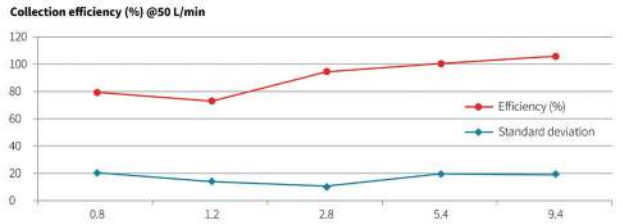
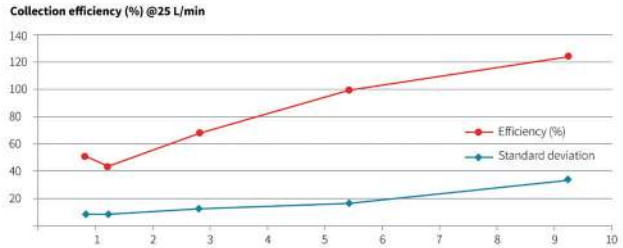
- The Petri plate was recently discussed as one of the most successful and inspiring microbiological instrumentation design that transcend the test of time (Denoya, C., Jan/Feb 2014, American Pharmaceutical Review).
- Petri plates are used in microbial air monitoring where a certain volume of air is forced towards the plate's agar surface (active air sampling). Microbial contaminants present in the air will impact the agar surface and, after incubation, colonies can be enumerated. In this method, the operator manually removes the lid of an agar plate when loading it into a stainless steel sampling device. At this step there is a chance the operator may contaminate the plate resulting in a “false positive”.
- The work presented here summarizes the validation of a novel single use, sterile, transparent plastic impactor holding an integrated agar culture media plate
- This design can be used as an alternative to the stainless steel impactors. The single use device eliminates the use of the traditional agar plates and minimizes operator intervention for plate substitution, exposure and removal.
- Validation performance data and the benefits of this single use device upon implementation in clean rooms for aseptic manufacturing are discussed below.

Goal of this work

- Aseptic Processing is aimed to maintain the sterility of a product, assembled from sterile components. It is implemented to prevent microbial contamination
- This poster presents data supporting the validation of a single use microbial sampler impactor including a preloaded agar plate.

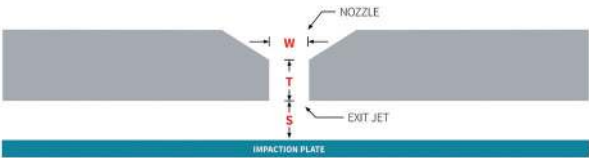
Physical efficiency verification of the BioCapt Impactor Active Microbial Air Sampling Atrium by the Centre for Applied Microbiology & Research (CAMR), U.K. (currently Health Protection Agency [HPA])

Physical Efficiencies (%) of BioCapt SS related to a Filter Sampler (0.8 µm) as a function of the size of particles. The BioCapt Impactor Atrium SS assures a physical efficiency of d50 less than 2 µm @ 25 L/min and less than 1 µm @ 50 L/min.

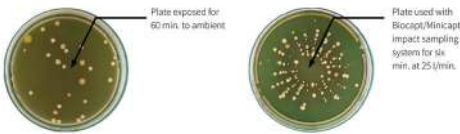


BioCapt Acceleration Channels with Rectangular Geometry

The BioCapt has been designed with the goal of obtaining a very high probability of uptake for all particles of interest in the field of bacterial contamination. By using channels of acceleration in rectangular geometry, the BioCapt operates in laminar regime with very low speed impact; this allows avoidance of any stress condition for the particles and improves the efficiency of recovery.



Slit Arrangement in BioCapt Identification of False Positives



VALIDATION TESTS AND RESULTS

Microbiological media

Results

- All results verified that product complies with specifications for release and regulatory compliance.

Leakage test

Goals:

- To assure the environment air flow impacting the agar plate in BioCapt® Single Use comes exclusively through the slits in the cover
- *Demonstrate that there is no air leakage in the junction between the base, the O-ring and the cover*
- *To assure that the irradiation process does not affect the seal between the base and the cover*

Test description:

- Seal the lid to the cover with slits using a silicon glue
- Connect a silicon tube to the vacuum barb fit of the BioCapt®
- Place the BioCapt® Single Use with silicon tube into a bottle full with 1.5 liters of water
- Repeat the steps after the sterilization cycle with Beta and Gamma rays

Results

- The level of water inside the tube, after few seconds, did not increase and it was under the level of the water in the bottle
- All the samples (6) had no leakage, their water level inside the silicon tube remained stable and under the level in the bottle for over 10 minutes

Conclusions

- The leakage test showed that the sampling air is drawn exclusively through the slits in the sampling head
- The sterilizing irradiation treatment used for The BioCapt® Single Use has no effect on the design.
- No leakage was found either before or after the irradiation steps

Efficiency performance

BioCapt Stainless Steel vs Single Use

Goal: The goal of this study is to verify the efficiency performance of the BioCapt® Single Use (BSU) by comparing it with the certified BioCapt Stainless Steel (BSS) used as a control

Physical and biological efficiency

Bacillus atrophaeus (previously known as *Bacillus subtilis* var. niger) spores were used for the Physical Collection Efficiency test because they are robust and unaffected during sampling by drying or stress
Staphylococcus epidermidis was used for the Biological Efficiency test because this culture is a better example of a typical microbial culture affecting cleanroom environments.

The efficiency performance test was conducted inside a test chamber located inside a laminar flow hood.

Method

- Microorganisms: *B. subtilis* subsp. *Spizizenii* spores (50 cfu/100 µL) and *S. epidermidis* suspension of bacteria (50 cfu/100 µL) to use in aerosol mode.
- Impactors: BioCapt® stainless steel (BSS) and BioCapt® Single-Use (BSU)
- Petri dishes: Tryptic Soy Agar
- Incubation: 37°C for 1 day before counting the colony forming unit (CFU)
- Vacuum: Aspiration system @ 25 L/min and 50L/min.

BioCapt® Stainless Steel Microbial Impactor

The BioCapt® Stainless Steel is composed of:

Head

- 20 slits stage
- Slits stage holder

Base

- Pins to locate petri dish
- Holder
- O-ring

Vacuum connection

- Barbfit or TC connection

Head and base fit each other with a conic connection that, with the O-ring, prevent leakage between them

Certification

- Certified by CAMR (Centre for Applied Microbiology & Research [now Health Protection Agency (HPA), UK])
- Report # 670/00 (December 15, 2000)



- Biological efficiency with optimal impaction velocity at 25L/min and 50L/min
- Slit design with minimal microorganism stress on impaction to agar surface

Stainless steel sampling head

Manipulations and risk of false positives

- Too many steps: “Lifting the head, placing an agar plate, taking up the lid, replacing the head, lifting the head again after collection, replacing the lid on agar plate and removing it for incubation

- Higher risk of microbial contamination during the manipulation by the operator

- Disinfection between uses

- Sterilization of sampling head is time and energy consuming



RESULTS

St. epidermidis - BioCapt Stainless Steel vs Single Use CFU recoveries at 25L/min flow rate

Sampling Flow Rate	Volume (Liters)	Run	BSS (CFU)	BioCapt Single Use (CFU)	Recovery (%)	Average
25 L/min	200	Run 1	7	11	157.14	98.66
		Run 2	8	11	137.50	
		Run 3	15	10	66.67	
		Run 1	19	15	78.95	
		Run 2	23	18	78.26	
25 L/min	200	Run 3	29	19	65.52	98.66
		Run 4	29	23	79.31	
		Run 5	27	34	125.93	

Bacillus subtilis (spore) - BioCapt Stainless Steel vs Single Use CFU recoveries at 25L/min flow rate

Sampling Flow Rate	Volume (Liters)	Run	BSS (CFU)	BioCapt Single Use (CFU)	Recovery (%)	Average
25 L/min	50 L	Run 1	21	13	61.90	99.84
		Run 2	21	21	100.00	
		Run 3	24	21	87.50	
		Run 1	27	21	77.78	
		Run 2	13	15	115.38	
25 L/min	100 L	Run 3	18	16	88.89	99.84
		Run 1	107	104	97.20	
		Run 2	49	41	83.67	
		Run 3	8	15	187.50	
		Run 1	22	23	104.55	
25 L/min	200 L	Run 2	69	72	104.35	99.84
		Run 1	52	48	92.31	
		Run 2	75	73	97.33	
		Run 3	127	130	102.36	
		Run 4	98	92	93.88	
25 L/min	200 L	Run 5	72	74	102.78	99.84

SUMMARY AND CONCLUSIONS

Summary of efficiency tests

Recoveries (%) BioCapt® Single Use Versus BioCapt® stainless steel:

S. epidermidis 98.66% (±35.80) @25 L/min
S. epidermidis 115.73% (±37.04) @50 L/min
B. subtilis spores 99.84% (±26.52) @25 L/min
B. subtilis spores 92.11% (±16.61) @50 L/min

Total recoveries

Total average @ 25 L/min: 99,44 % (±29,14)
Total average @ 50 L/min: 102.05% (±28.81)

Conclusion

BioCapt® Single Use has an efficiency performance comparable with BioCapt® Stainless Steel

1. The BioCapt® Single Use sampling device has a radial slit impactor design showing physical and biological efficiency performances comparable with the BioCapt® Stainless Steel sampling head

BioCapt® Single Use Microbial Impactor

The BioCapt® Single Use is composed of:

- The base including a barbed fitting connector and a compartment filled with the agar medium
- The cover in which there are 20 inlet slits for suction
- An O-ring to seal the junction between base and cover
- A lid to protect the slits before and after the sampling

A new EM tool - BioCapt® Single Use

- Unique design, combination of impactor and agar plate
- Reduces exposure to operator intervention Air is drawn through slits in the sampling head using a vacuum pump.
- The organisms are impacted on the agar surface in the pattern of the sampling head.
- Sterilization of sampling head is not required
- Reduced risk of contamination by improper handling (diminish risk of false positives)

BioCapt® Single Use characteristics and advantages

- Unique compact design, combination of impactor and agar plate
- Sterile triple bagged, gamma irradiated including validation documentation
- The agar plate incorporated in the disposable impactor head reduces operator handling
- No touch on the agar surfaces significantly reduces the risk of a “false positive” contamination
- Cost saving (time and labor) due to the elimination of cleaning and sterilization of the stainless steel, re-usable impactor heads
- Collection design with improved physical/microbiological efficacy. Validated (HPA, UK)
- Easy to use with any air sampling systems
- Slit design enables easy identification of false positives and is specifically designed to ensure maximum physical and biological collection efficiencies



St. epidermidis - BioCapt Stainless Steel vs Single Use CFU recoveries at 50L/min flow rate

Sampling Flow Rate	Volume (Liters)	Run	BSS (CFU)	BioCapt Single Use (CFU)	Recovery (%)	Average
50 L/min	200	Run 1	16	17	106.25	115.73
		Run 2	17	18	105.88	
		Run 3	17	18	105.88	
		Run 1	10	12	120.00	
		Run 2	24	17	70.83	
50 L/min	200	Run 3	19	17	89.47	115.73
		Run 4	21	41	195.24	
		Run 5	31	41	132.26	

Bacillus subtilis (spore) - BioCapt Stainless Steel vs Single Use CFU recoveries at 50L/min flow rate

Sampling Flow Rate	Volume (Liters)	Run	BSS (CFU)	BioCapt Single Use (CFU)	Recovery (%)	Average
50 L/min	50 L	Run 1	30	16	53.33	92.11
		Run 2	18	16	88.89	
		Run 3	23	23	100.00	
		Run 1	55	52	94.55	
		Run 2	30	34	113.33	
50 L/min	100 L	Run 3	31	24	77.42	92.11
		Run 1	126	120	95.24	
		Run 2	98	83	84.69	
		Run 3	90	93	103.33	
		Run 4	90	99	110.00	
50 L/min	200 L	Run 5	79	73	92.41	92.11

2. The leakage test demonstrated that 100% of the air sampled comes from the air drawn through the slits of the sampling head by the suction effect of a vacuum pump
3. The BioCapt® Single Use complies with ISO 14698-1 (Clean rooms and associated controller environments – Biocontamination control – Part 1: General principles and methods (ISO14698-1:2003))
4. The quality of the microbiological ready-to-use culture media used to fill the BioCapt Single-Use system complies with the corresponding specific release (Validation study)

REFERENCES

1. Study on Leakage of BioCapt Single Use. Davide Recchia. PMS internal document. Feb 11, 2013.
6. Denoya, C., Implementation of Rapid Microbiological Methods: Some Technical Challenges Still Ahead. Jan/Feb 2014, American Pharmaceutical Review.