

INTRODUCTION

Abstract

- The Petri plate was invented by Julius R. Petri while working at Robert Koch's lab more than 120 years ago.
- It is one of the most simple and recognizable devices still widely used in microbiology labs.
- Over the years, a single-use, sterile, polystyrene Petri plate replaced the early glass versions.
- The Petri plate was recently discussed as one of the most successful and inspiring microbiological instrumentation designs that transcends 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".

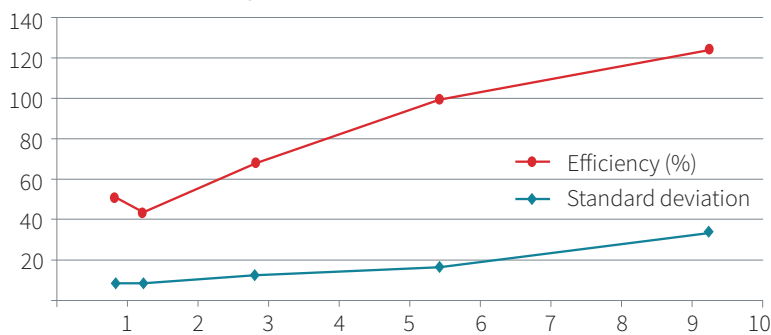
- The work presented here summarizes the validation of a novel, single use, sterile, transparent plastic impactor holding an integrated agar culture media plate.
- This design can be used as an alternative to the stainless steel impactors. The single use device eliminates the need for traditional agar 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

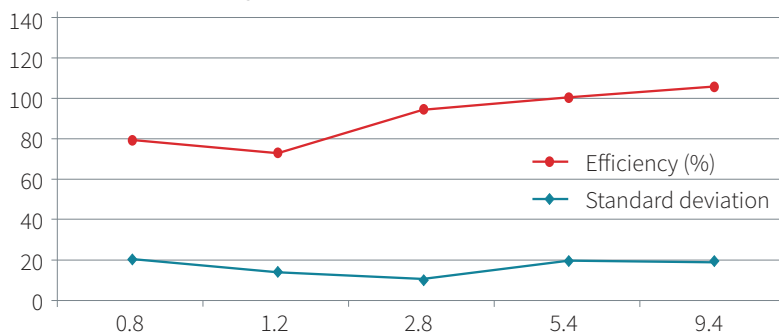
- Aseptic Processing is aimed to maintain the sterility of a product via the assembly from sterile components. It is implemented to prevent microbial contamination.
- This poster presents data supporting the validation of a single use microbial sampler impactor including a preloaded agar plate.

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])

Collection efficiency (%) @ 25 L/min



Collection efficiency (%) @ 50 L/min

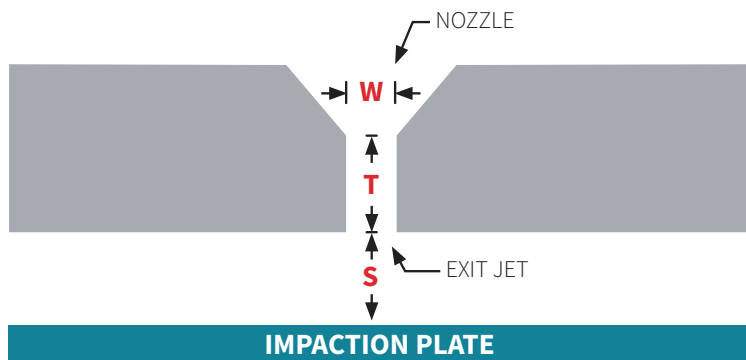


Physical Efficiencies (%) of BioCapt Stainless Steel related to a Filter Sampler (0.8 µm) as a function of the size of particles.

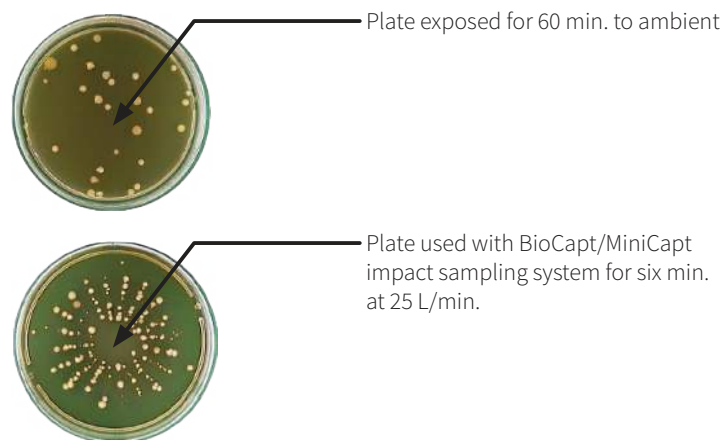
The BioCapt Impactor Atrium Stainless Steel assures a physical efficiency of d50 less than 2 µm @ 25 L/min and less than 1 µm @ 50 L/min.

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 a laminar regime with very low impact speed. This allows the avoidance of any stress conditions for the particles and improves the efficiency of recovery.



Slit Arrangement in BioCapt Identification of False Positives



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, removing the lid, replacing the head, lifting the head again after collection, replacing the lid on the 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



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
- Reduced 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 materials, 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.



VALIDATION TESTS AND RESULTS

Microbiological media

Tests

- Stability for 9 months at 2 different storage conditions
- Growth Promotion of TSA and SDA media without inactivators and penicillinase
- Penicillinase effectiveness
- Appearance Check
- Hydration
- pH check (each media)
- Sterility Controls

Results

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

Leakage test

Goals:

- To assure the environment airflow impacting the agar plate in the BioCapt Single-Use (BSU) 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:

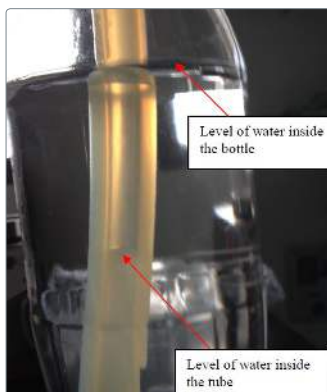
- Seal the lid to the cover with slits using a silicone glue.
- Connect a silicone tube to the vacuum barb fit of the BSU.
- Place the BSU with its silicone tube into a bottle with 1.5 liters of water.
- Repeat the steps after the sterilization cycle with Beta and Gamma rays.

Results

- After waiting several seconds, the level of water inside the tube did not increase, and was under the level of the water in the bottle.
- All six samples had no leakage, and their water level inside the silicone 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 BSU 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 BSU by comparing it to 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 μ L) and *S. epidermidis* suspension of bacteria (50 cfu/100 μ L) to use in aerosol mode.
- Impactors: BioCapt Stainless Steel and BioCapt Single-Use
- Petri dishes: Tryptic Soy Agar
- Incubation: 37 °C for 1 day before counting the colony forming units (CFU)
- Vacuum: Aspiration system @ 25 L/min and 50L/min.
- Test Chamber (Plexiglas) with a volume of 0.26 m³. It is located in a laboratory with the temperature of 22 $\pm$ 2 °C and a relative humidity of 50% $\pm$ 10%.
- Inputs: Connect BSS/filter holder and BSU inside the chamber to the vacuum system (outside).
- Input with absolute filters to insert clean air
- Input to insert aerosol of bacteria/spore
- Fan system to mix air and bacterial/spore aerosol and maintain particles homogeneous distribution

RESULTS

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

Sampling Flow Rate	Volume (Liters)	Run	BSS (CFU)	BSU (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	
25 L/min	200	Run 1	19	15	78.95	
		Run 2	23	18	78.26	
		Run 3	29	19	65.52	
		Run 4	29	23	79.31	
		Run 5	27	34	125.93	

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

Sampling Flow Rate	Volume (Liters)	Run	BSS (CFU)	BSU (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	
50 L/min	200	Run 1	10	12	120.00	
		Run 2	24	17	70.83	
		Run 3	19	17	89.47	
		Run 4	21	41	195.24	
		Run 5	31	41	132.26	

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

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

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

Sampling Flow Rate	Volume (Liters)	Run	BSS (CFU)	BSU (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	
50 L/min	100 L	Run 1	55	52	94.55	
		Run 2	30	34	113.33	
		Run 3	31	24	77.42	
50 L/min	200 L	Run 1	126	120	95.24	
		Run 2	98	83	84.69	
		Run 3	90	93	103.33	
		Run 4	90	99	110.00	
		Run 5	79	73	92.41	

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 to the 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.
 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

2. FDA: “GUIDANCE FOR INDUSTRY, STERILE DRUG PRODUCTS PRODUCED BY ASEPTIC PROCESSING” SEP 2004

3. EU: “ANNEX 1, MANUFACTURE OF STERILE MEDICINAL PRODUCTS”, MAR 2009

4. USP 36 <1116> MICROBIOLOGICAL CONTROL AND MONITORING OF ASEPTIC PROCESSING ENVIRONMENTS, DECEMBER 1ST, 2013

5. ISO 14644-1. CLEAN ROOMS AND ASSOCIATED CONTROLLED ENVIRONMENTS. PART 1: CLASSIFICATION OF AIRBORNE PARTICLES. GENEVA, INTERNATIONAL ORGANIZATION FOR STANDARDIZATION. (CURRENT VERSION)

6. DENOYA, C., IMPLEMENTATION OF RAPID MICROBIOLOGICAL METHODS: SOME TECHNICAL CHALLENGES STILL AHEAD. JAN/FEB 2014, AMERICAN PHARMACEUTICAL REVIEW