

APPLICATION INFORMATION

Centrifugation Biosafety

BIOCONTAINMENT CAPABILITY OF THE AEROSEAL™ COVERS FOR THE JS-5.3 ALLSPIN ROTOR

Health Protection Agency
Porton Down, United Kingdom

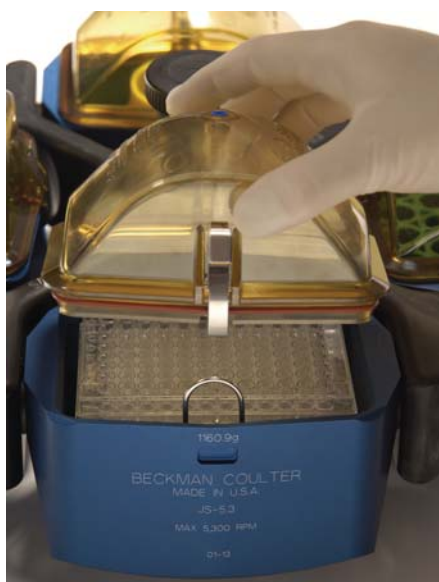


Figure 1. AeroSeal covers for use with the AllSpin JS-5.3 rotor buckets

Summary

The Beckman Coulter AeroSeal covers (PN 368417) for use with the AllSpin JS-5.3 rotor buckets (PN 368706) have been tested in triplicate for biocontainment using the new draft of Annex AA of IEC 1010-2-20 and have been shown to be capable of containing large volumes of concentrated micro-organisms in all three tests.

Introduction

The new AeroSeal covers for use with the JS-5.3 rotor buckets, developed by Beckman Coulter, were provided to HPA Porton Down for biocontainment testing. The tests were based on a recent draft of Annex AA of IEC 1010-2-020. Four of the JS-5.3 buckets were filled with a concentrated spore tracer.

The AeroSeal covers (fitted with new O-rings and air-vent filter) were placed onto the buckets, then rotated and placed on the JS-5.3 rotor and spun in an Avanti® J-Series centrifuge from Beckman Coulter.

Materials and Methods

The centrifuge containing the JS-5.3 fitted with AeroSeal covers was placed in an environment room (dimension 3 m x 3 m x 2 m high) fitted with a filtered extract and supply ventilation system. The four buckets, with covers, were moved into a laboratory where each of the buckets was filled with 500 mL of a suspension greater than 10^{10} spore per mL of *bacillus subtilis* var niger NCTC10073. This suspension was re-assayed before the first test and after the last test. The AeroSeal covers were clipped onto the buckets.



Figure 2. Avanti J-26 XPI

A wetted cotton swab was taken of the outside of each of the covered bucket assembly seals and the air-vent filters and plated out onto a Tryptone Soya Broth agar (TSBA) plate. Each of the covered bucket assemblies was turned to allow the tracer to challenge the seal, and another swab was taken of

Results

	cfu* before centrifugation			cfu after centrifugation		
	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3
Swab Seal 1	0	0	0	0	0	0
Swab Seal 2	0	0	0	0	0	0
Swab Seal 3	0	0	0	0	0	0
Swab Seal 4	0	0	0	0	0	0
Swab top filter 1	0	1	0	0	0	0
Swab top filter 2	0	0	0	0	0	0
Swab top filter 3	0	0	0	0	0	0
Swab top filter 4	0	0	0	0	0	0
Swab Seal after turn 1	0	0	0	-	-	-
Swab Seal after turn 2	0	0	0	-	-	-
Swab Seal after turn 3	0	0	0	-	-	-
Swab Seal after turn 4	0	0	0	-	-	-
Swab top filter after turn 1	0	0	0	-	-	-
Swab top filter after turn 2	0	0	0	-	-	-
Swab top filter after turn 3	0	0	0	-	-	-
Swab top filter after turn 4	0	0	0	-	-	-
Swab of rotor chamber	0	0	0	0	0	0
Swab rotor	0	0	0	0	0	0
Controls						
+ ve 1	-	-	-	TNTC	TNTC	TNTC
+ ve 2	-	-	-	TNTC	TNTC	TNTC
+ ve 3	-	-	-	TNTC	TNTC	TNTC
+ ve 4	-	-	-	TNTC	TNTC	TNTC
Average Concentration of Spore Suspension = 4.00×10^{10} /mL						

* = colony forming units, - = not applicable, TNTC = too numerous to count ($>10^3$)

each of the bucket seals and air-vent filters. The covered bucket assemblies were then returned to the room and placed on the rotor in the centrifuge. A swab was taken of the rotor and the circumference of the rotor chamber at the level in which any release from the covered bucket assemblies would be found, which were also plated out on TSBA plates. Prior to each test the room and equipment were decontaminated with formaldehyde vapor (boiled off from a mixture of 300 mL formalin and 3 liters water) for four hours and vented overnight.

The rotor was then run in the centrifuge at its maximum speed of 5,300 rpm for seven minutes. After the run was complete swabs were taken of the outside of each of the covered bucket assembly seals and the air-vent filters and of the rotor and circumference of the rotor chamber as before, and they were plated out onto TSBA plates.

A swab was taken of the inside of each of the buckets as a positive control. All the TSBA plates had been previously incubated at room temperature for at least 72 hours to ensure sterility. The batch of

TSBA plates had been quality controlled to ensure they were capable of supporting the growth of low concentrations of the challenge microorganism. All plates were incubated aerobically at 37° C ($\pm 2^\circ$) for between 18 and 24 hours before being counted. After each test the covered bucket assemblies were cleaned, refilled and returned to the environmental room, which was fumigated with formaldehyde as previously described.

Discussion

The AeroSeal covers for use with the JS-5.3 rotor buckets have been shown to be capable of containing a large spill using the technique described in Annex AA of IEC 1010-2-020 in all three tests. 500 mL of a 1010 spore/mL suspension challenged each of the covered bucket assembly seals and no leakage was detected across the seal. Therefore, if the AeroSeal covers used with the JS-5.3 rotor buckets are operated and maintained according to the manufacturer's instructions, they can prevent the release of microorganisms in the event of a spill within the bucket.

Reference

1. IEC 1010-2-020. (2003): Safety requirements for electrical equipment for measurement, control and laboratory use. Part 2. Particular requirements. Section 2.20 Specification for laboratory centrifuges. BS 7687: Section 2.20: Annex AA

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