

A novel Iodine implant surface treatment demonstrates excellent antimicrobial efficacy *in vitro* that is not affected by serum protein concentration

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This novel Iodine implant surface treatment is effective against bacteria and fungi, and serum protein concentration does not affect test results

Background

Periprosthetic Joint Infection (PJI) remains one of the most common causes of revision, and a challenging complication of Total Joint Arthroplasty (TJA). The high volume of TJA procedures performed annually results in a substantial number of affected patients, contributing to a rising demand for revision surgeries¹. The risk of re-infection following a revision due to infection is about 18%². Notably, the mortality rate associated with PJI exceeds that of breast, melanoma, and prostate cancers³. Iodine is a well-known antimicrobial that is widely used in clinical practice for the management of PJI. Iodine-treated joint replacement devices are designed to reduce microbial adhesion to the device surface and may therefore contribute to infection risk management.

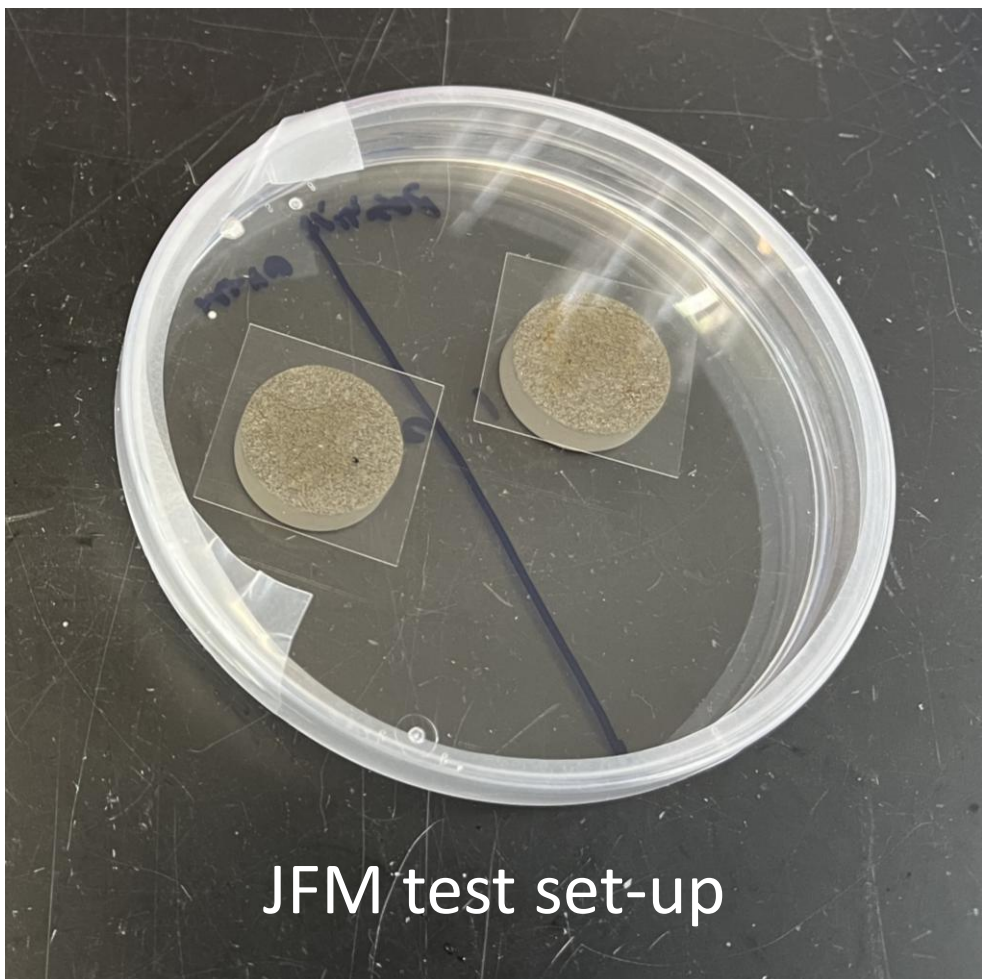
Objectives

This study aimed at assessing the *in vitro* antimicrobial efficacy of a novel Iodine implant surface treatment against bacteria and fungi. The effect of serum protein concentration within the growth medium was also assessed.

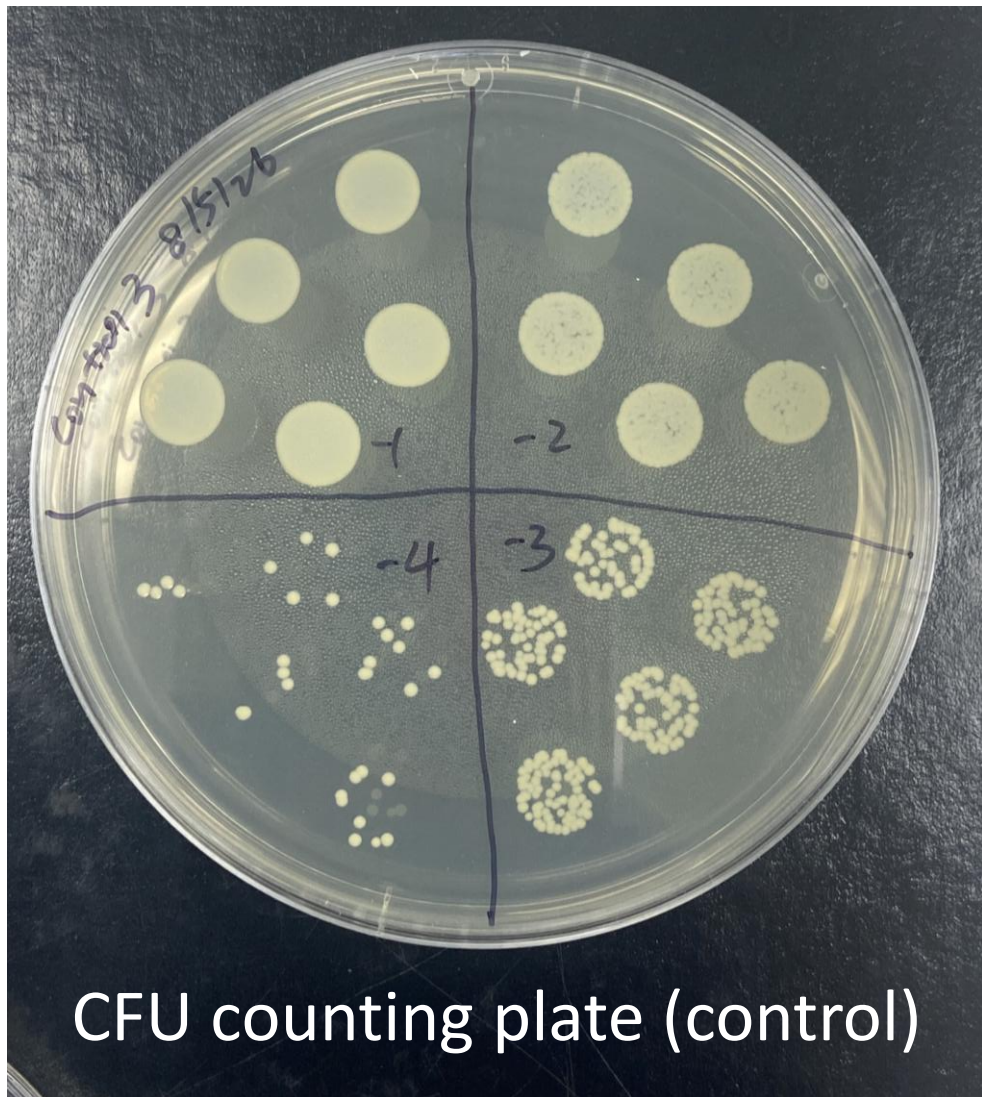
Materials and Methods

Samples: Ti6Al4V discs (Ø20 mm x 6 mm) coated with Porous Plasma Spray (PPS) on one side were used in this study as representative of cementless joint replacement surfaces. All discs received the Iodine surface treatment, which is an electrochemical process developed from the work of Takaya et al⁴. Non-iodine-treated discs were used as controls.

Species	Type	ATCC strain
<i>Staphylococcus aureus</i>	Gram-positive	29213
<i>Staphylococcus epidermidis</i>	Gram-positive	35984
Methicillin Resistant <i>Staphylococcus aureus</i> (MRSA)	Gram-positive	700789
<i>Pseudomonas aeruginosa</i>	Gram-negative	27853
Extended Spectrum β -lactamase (ESBL) <i>Escherichia coli</i>	Gram-negative	BAA-196
Methicillin Resistant <i>Staphylococcus epidermidis</i> (MRSE)	Gram-positive	51625
<i>Enterobacter cloacae</i>	Gram-negative	13047
<i>Enterococcus faecalis</i>	Gram-positive	700802
<i>Candida parapsilosis</i>	Fungus	90018
<i>Candida albicans</i>	Fungus	90027



JFM test set-up

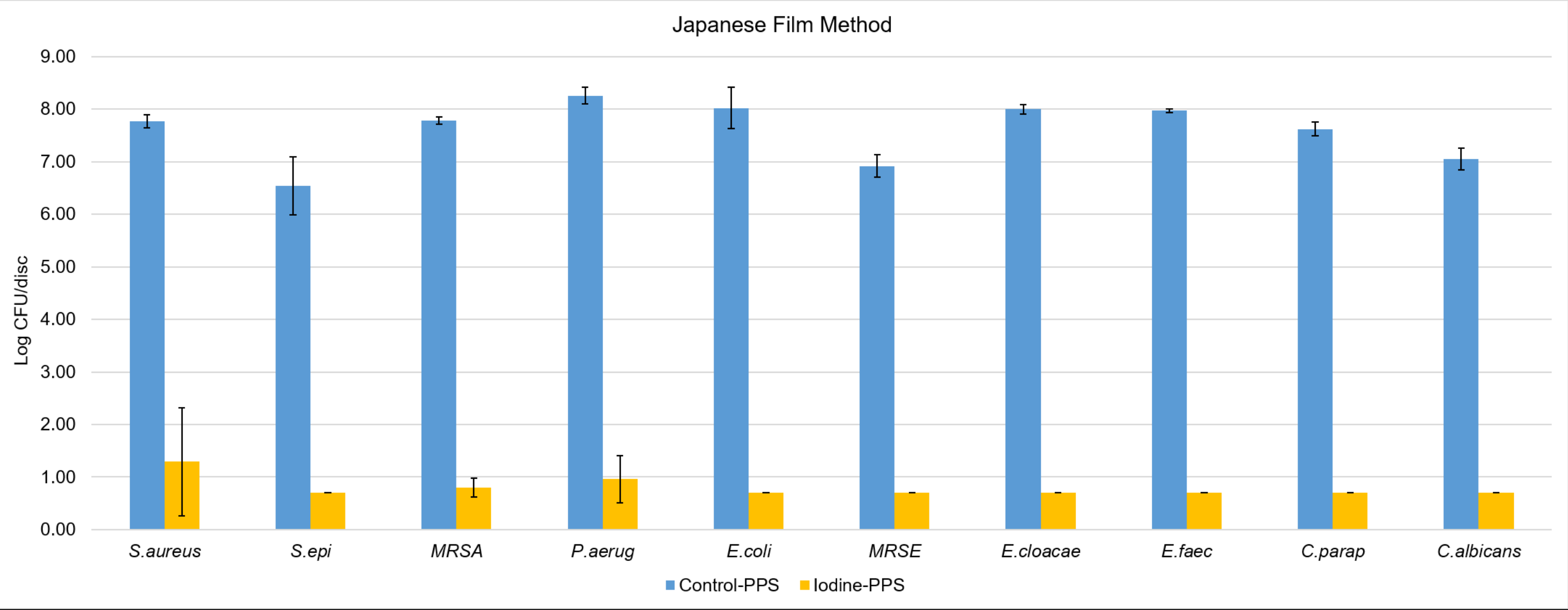


CFU counting plate (control)

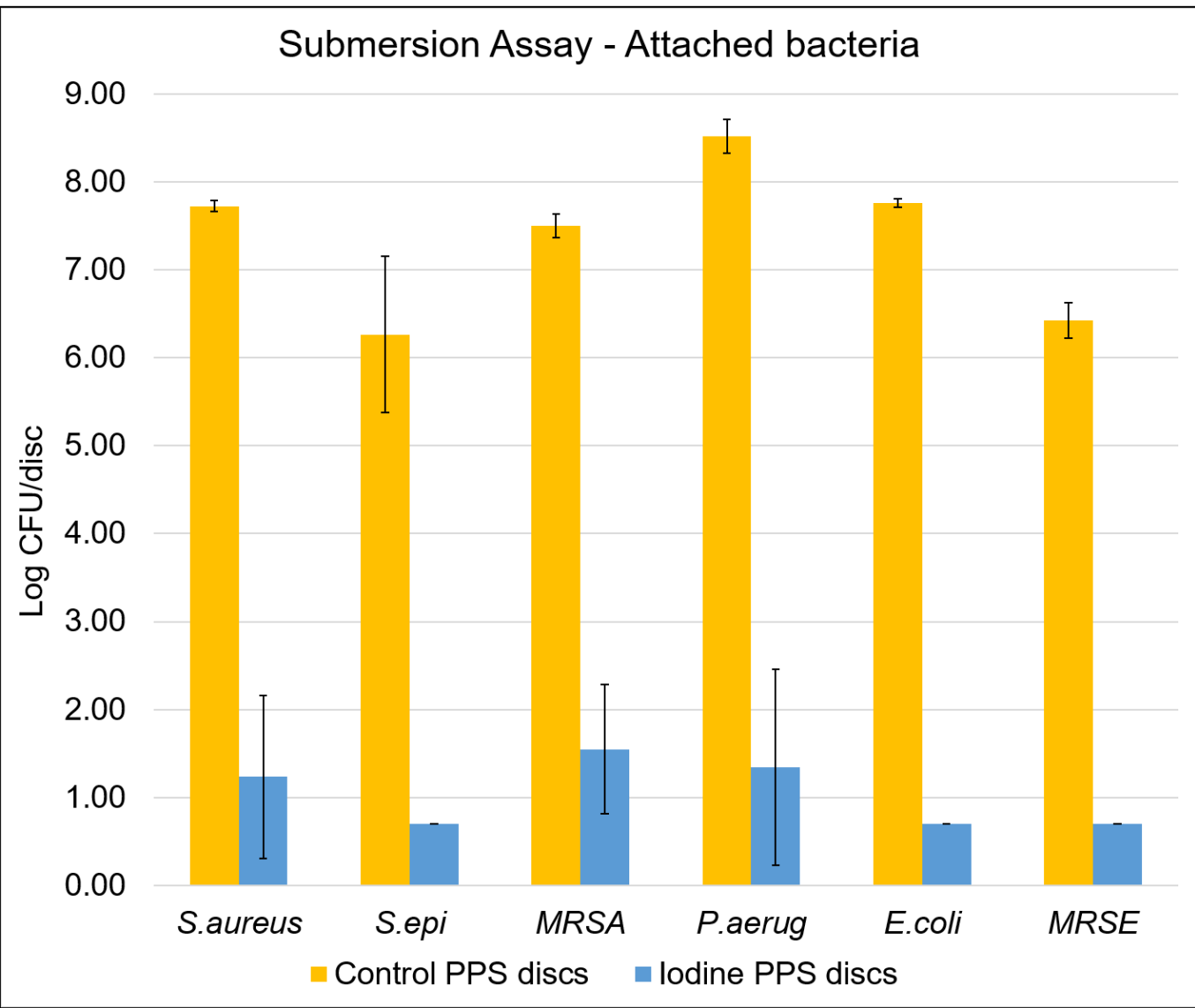
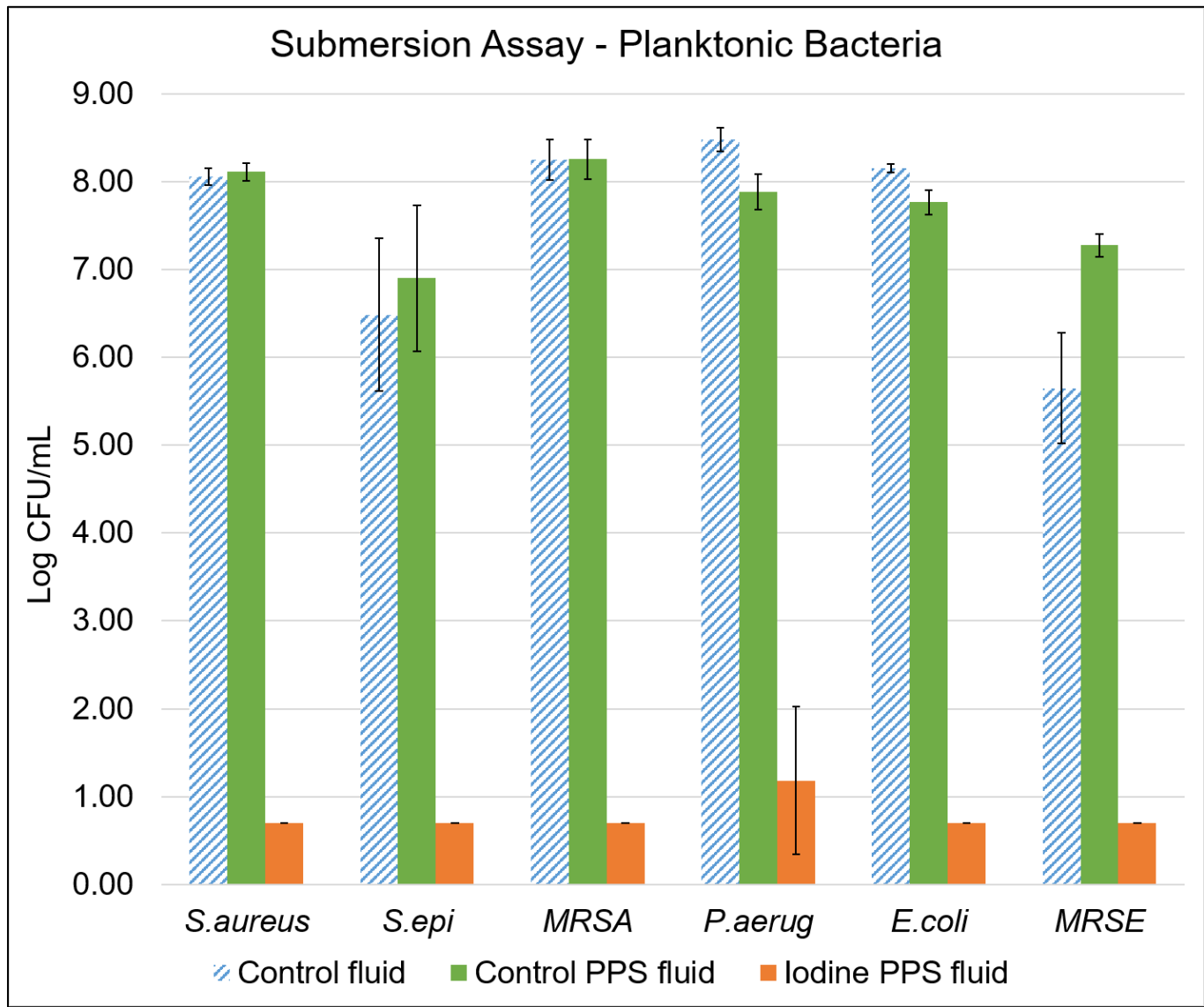
Japanese Film Method (JFM): The antimicrobial performance of the Iodine-treated discs was assessed using a method adapted from the JFM test per JIS Z 2801⁵ and ISO 22196⁶. In the assay, eight bacterial species and two fungal species (see table) were used, which were selected to represent the most common pathogens detected in confirmed PJI cases⁷. Each disc was loaded with 100 μ L of inoculum, covered with a glass coverslip and incubated at 37°C for 24h. Log reductions were calculated based on Colony Forming Unit (CFU)/disc, following plating and counting. Six of the bacterial species were also used in a **Submersion assay** to assess the effect of iodine eluted into a larger 2 mL volume of fluid.

Serum protein concentration: The potential impact of protein-rich environments on iodine's antimicrobial properties and assay outcomes was evaluated using the same JFM assay, where the culture media contained either 0.5% (as per the standard assay), 10% or 20% of adult bovine serum (ABS), against *S. aureus* and *E. coli*.

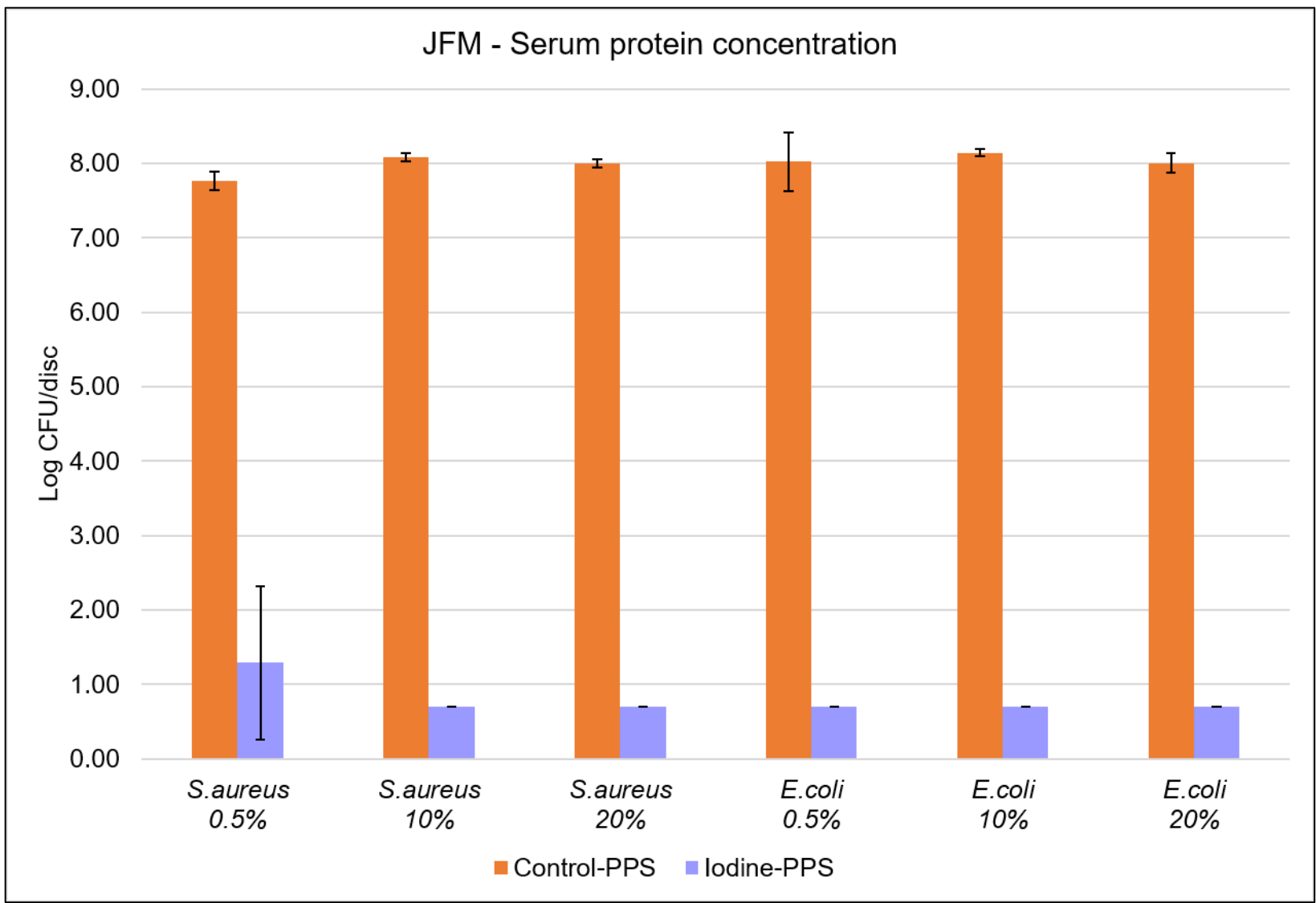
Results



All tested samples showed excellent efficacy in the JFM against all the microbes, which included Gram-positive bacteria, Gram-negative bacteria and fungi. **Log Reductions spanned between 5.84 and 7.32 for bacteria.** The tested PPS discs demonstrated **100% killing** against both *C. parapsilosis* and *C. albicans*, with Log Reductions of 6.92 and 6.35, respectively. A count of zero CFU/disc corresponds to a 0.7 Log count on the graphs.



In the Submersion Assay, where the eluted iodine is diluted into a larger volume of media, all test groups showed high efficacy, with **Log Reductions between 6.20 and 7.55** for planktonic bacteria, and **between 5.56 and 7.17** for attached bacteria. Most samples presented no viable bacterial cells at the end of the test.



The presence of increased protein concentrations in the JFM test media did not appear to significantly affect the outcome of the test, compared to 0.5% ABS used in the standard test.

Conclusions

The PPS discs that received the novel Iodine surface treatment demonstrated excellent antimicrobial properties when tested *in vitro* with a modified-JFM test against both Gram-positive and Gram-negative bacteria, as well as against fungi. Testing in a Submersion assay, using a larger volume of medium, also demonstrated the ability of the Iodine treatment to significantly reduce bacterial growth on the sample surface and within the surrounding fluid. When the concentration of serum proteins was increased in the culture media, the results of the JFM test were not affected for the two bacterial species tested, *S. aureus* and *E. coli*. Testing with *Cutibacterium acnes*, which is relevant for shoulder PJI, is ongoing.

References

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- JIS Z 2801 *Antibacterial Products – Test for Antimicrobial Activity and Efficacy* (Amendment 1, May 2012)
- ISO 22196:2011 *Measurement of Antibacterial Activity on Plastics and Other Nonporous Surfaces*
- Data internal to Zimmer Biomet, from the clinical lab summary of organisms recovered in confirmed PJI cases