



DR | KOZIEJ
IN VIVO TESTING
—

EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF ORAL SPRAY
AGAINST
Streptococcus mutans ATCC 25175
Z-12225/ZBP-21608

Product	Oral spray for adults (CariSpray Adult)
Person Responsible	OralAdvance Labs d.o.o. Zadarska ulica 1, 42000, Varaždin, Hrvatska
Date	25.08.2025

Table of contents

1.	RESEARCH BASIS	3
2.	SUBJECT OF THE STUDY	3
2.1.	INCI	3
2.2.	Product characteristics.....	3
3.	RESEARCH METHODOLOGY	4
4.	PURPOSE.....	4
5.	RESULTS	4
6.	CONCLUSIONS.....	4
7.	STUDY INVESTIGATORS.....	5

1. RESEARCH BASIS

Research time frame	01.08.2025 – 08.08.2025
Order number	Z-12225
Report issue date	11.08.2025

2. SUBJECT OF THE STUDY

2.1. INCI

Product name	Oral spray for adults (CariSpray Adult)
Ingredients	Aqua, Xylitol, Glycerin, Polysorbate 20, Sodium Bicarbonate, Hydroxyapatite (nano), Aroma, Potassium Chloride, Citric Acid, Limonene, Menthol, Potassium Sorbate, Sodium Benzoate

2.2. Product characteristics

Packaging	Replacement
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The Responsible Person is responsible for conformity with the declared qualitative and quantitative composition and microbiological purity of the delivered samples. The Responsible Person confirms that the product is compliant with the law as at the date of the order.

3. RESEARCH METHODOLOGY

A standardized suspension of *Streptococcus mutans* ATCC 25175 was prepared at a concentration of 30×10^6 cfu/ml.

Two samples were tested:

- control sample: 9 ml of sterile 0.85% NaCl + 1 ml of the bacterial suspension
- test sample: 9 ml of the tested preparation + 1 ml of the bacterial suspension

The contact time between the bacteria and both the saline and the tested preparation was 3 minutes. After this period, the number of viable cells (cfu/ml) in both samples was determined using serial dilutions and surface plating on Mueller-Hinton agar supplemented with sheep blood. The plates were incubated at 37°C for 3 days. The effectiveness of the preparation was evaluated as the percentage reduction in viable cells after 3 minutes of contact with the tested product.

Research standard: EXT/P, GLP

GLP - Good Laboratory Practice

A - PCA accreditation (accreditation number)

P - Non-accredited method

EXT / A - Subcontractor Accreditation

EXT / P - Subcontractor's non-accredited method

4. PURPOSE

The aim of the study was to assess the efficacy of the Z-12225 ZBP_21608 preparation in reducing the number of viable *Streptococcus mutans* ATCC 25175 cells after a 3-minute exposure.

5. RESULTS

The results of the study are presented in Table 1.

Table 1. Colony-Forming Units (cfu/ml) and percent reduction of *S. mutans* ATCC 25175 after 3-minute treatment

Sample	cfu/ml after 3 min	Reduction (%)
Control (0.85% NaCl)	28×10^6	6,67%
Tested product	$11,5 \times 10^6$	61,67%

6. CONCLUSIONS

The Z-12225 ZBP_21608 oral spray demonstrated moderate antimicrobial activity against *Streptococcus mutans* ATCC 25175, achieving a 61.67% reduction in viable cells after 3 minutes of contact. The control sample showed minimal natural reduction (6.67%), confirming the effect of the tested preparation.

In microbiology, an antimicrobial product is typically considered effective if it achieves a reduction of ≥ 3 log (99.9%). In this case, the observed reduction was approximately 0.42 log (below 1 log), indicating partial but insufficient bactericidal activity in the context of biocidal standards.

However, for cosmetic product or hygiene products intended for daily use—where the goal is to limit rather than eliminate microorganisms—the observed effect can still be considered beneficial, especially considering the short and realistic contact time (3 minutes).

7. STUDY INVESTIGATORS

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