Test Report

No. B2212101-001

February 9,2023

Attn : TOREMY Co.,LTD.

Subject

The result of Preservatives- Effectiveness Test

Summary of Test Results (See the attached sheet for details)

1. Name of test sample
   Jevie Lotion(WBF - 22B - 2)

2. Testing period
   Dec 26,2022 ～ Feb 9,2023

3. Test Method
   The test was performed according to the Japanese Pharmacopoeia 18th edition.

4. Conclusion
   Preservative efficacy of the test sample conformed to the criteria of the Japanese Pharmacopoeia.

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1. Name of test sample
   Jevie Lotion(WBF - 22B - 2)

2. Test Methods
   The test was performed according to the Japanese Pharmacopoeia 18th edition.

A. Test Microorganisms
   The following strains are used as the test microorganisms.

Table 1 : The test microorganisms

Binominal name	NBRC No.
Escherichia coli	3972
Pseudomonas aeruginosa	13275
Staphylococcus aureus	13276
Candida albicans	1594
Aspergillus brasiliensis	9455

B. Preparation of inoculum
   Inoculate each of the five test strains on the surface of agar slants. For growth of bacteria, use Soybean-Casein Digest Agar Medium, and for yeasts and moulds, use Sabouraud Glucose Agar Medium. Incubate bacterial cultures at 32.5℃ for 20 hours, the culture of *C. albicans* at 22.5℃ for 48 hours and the culture of *A. brasiliensis* at 22.5℃ for 6 to 10 days. Harvest these cultured cells aseptically using a platinum loop. Suspend the collected cells in sterile physiological saline and adjust the viable cell count to about 10^8 CFU/mL. However, in the case of spores of *A. brasiliensis*, suspend in sterile physiological saline containing 0.05 w/v% of polysorbate 80 and adjust the spores count to about 10^7 CFU/mL. Filter the spore suspension though a sterilized gauze to remove hyphae. Use these suspensions as the inoculum.

C. Test Procedure
   1. Put each 20 g of the test sample in five sterile vials and inoculate 0.15mL of each cell suspensions.
      (The concentration of viable cell count would be 10^5 to 10^6 cells per gram of the test sample.)

   2. Incubate these inoculated vials at 22.5℃ and calculate the viable cells of 1 g of the test sample at 7, 14, 21, 28 days subsequent to inoculation.

   3. The viable cell count are determined by the pour plate methods. Soybean-Casein Digest Agar with Lecithin and Polysorbate 80 Medium is used for bacterial cells and Sabouraud Glucose Agar with Lecithin and Polysorbate 80 Medium is used for the yeast and mould cells.

3.Test Results

The test results are shown in Tables 2 and 3.

Table 2 : Viable cell count at the time of receipt of the test sample

Name of Test sample	pH	Cell count (CFU/g)	
		Bacteria	Fungi (yeast and mould)
Jevie Lotion(WBF - 22B - 2)	5.2	$<10^1$	$<10^1$

Table 3 : Change of the cell count in the sample after inoculation

Name of Test sample	Micro-organ-ism	Initial inoculum (CFU/g)	Cell count (CFU/g)			
			7days	14days	21days	28days
Jevie Lotion (WBF-22B-2)	E.c	8.9×10^5	$<10^1$	$<10^1$	$<10^1$	$<10^1$
	P.a	7.5×10^5	$<10^1$	$<10^1$	$<10^1$	$<10^1$
	S.a	6.6×10^5	$<10^1$	$<10^1$	$<10^1$	$<10^1$
	C.a	2.9×10^5	$<10^1$	$<10^1$	$<10^1$	$<10^1$
	A.b	1.6×10^5	1.7×10^3	1.2×10^3	1.1×10^3	1.1×10^3

* E.c: *Escherichia coli*, P.a: *Pseudomonas aeruginosa*, S.a: *Staphylococcus aureus*, C.a: *Candida albicans*, A.b: *Aspergillus brasiliensis*

4.Conclusion

Preservative efficacy of the test sample conformed to the criteria of the Japanese Pharmacopoeia.

End