IL-6 ELISA Analysis

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Tradename: Probacillus Revive

Code: 16618

CAS #: 9015-54-7

Test Request Form #: 208

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed:
Interleukin (IL)-6 Enzyme-Linked Immunosorbent Assay (ELISA)

Introduction

Interleukin-6 is a proinflammatory cytokine known to play an active role in inflammation, immunology, bone metabolism, reproduction, arthritis, neoplasia, and aging. IL-6 signals through the nuclear factor-kappa B (NF-κB) pathway that results in the transcription of inflammatory mediators, including matrix metalloproteinase-1 (MMP-1). MMP's are responsible for breaking down the extracellular matrix and collagen in the skin leading to wrinkles, fine lines, and loss of skin elasticity. Reducing the level of IL-6 and other inflammatory mediators is believed to slow down degradation of the skin matrix and, possibly, stimulate its replenishment.

Interleukin-6 ELISA was conducted to assess the changes in IL-6 levels in **Probacillus Revive**-treated *in vitro* cultured human dermal fibroblasts.

Assay Principle

This ELISA utilizes a colorimetric reaction employing antibodies with antigen specificity to human IL-6. Monoclonal antibodies specific for IL-6 epitopes are coated on a microtiter plate. In positive samples, IL-6 will bind to these antibodies and are tagged a second time with another IL-6-specific antibody labeled with horseradish peroxidase (HRP). The addition of the chromagen solution, containing 3,3',5,5'-tetramethylbenzidine, provides the colorimetric reaction with HRP that is quantitated through optical density (OD) readings on a microplate spectrometer. The standard curve provides a reference from the OD readings for the amount of collagen in each sample.

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Materials

A. Kit:	IL-6 ELISA Kit (Biosource; KAC1261)
B. Incubation Conditions:	37°C at 5% CO_2 and 95% relative humidity (RH)
C. Equipment:	Forma humidified incubator; ESCO biosafety laminar flow hood; Microplate Reader; Pipettes
D. Cell Line:	Normal Human Dermal Fibroblasts (NHDF) (Lonza; CC-2511)
E. Media/Buffers:	Dulbecco's Modified Eagle Medium (DMEM); Penicillin-Streptomycin (50U-50mg/mL); Fetal Bovine Serum (FBS); Phosphate Buffered Saline (PBS); Amphotericin (45pg/mL)
F. Culture Plate:	Falcon flat bottom 12-well tissue culture treated plates
G. Reagents:	Lipopolysaccharide (LPS) (1µg/mL)
H. Other:	Sterile disposable pipette tips; wash bottles

Methods

Human dermal fibroblasts were seeded into 12-well tissue culture plates and allowed to grow to confluency in complete DMEM. 1%, 0.1%, 0.01% concentrations of **Probacillus Revive** were added to complete DMEM containing 1µg/mL LPS and incubated with fibroblasts for 24 hours. Complete media containing 1µg/mL LPS was used as the positive controls and complete DMEM was used a negative control.

Standards were prepared in concentrations ranging from 2476pg/mL to 0pg/mL. 50µL of Solution B was added to wells for standards and assay controls and 50µL of Solution A was added to experiment wells. 100µL of standards, controls, and samples were added to appropriate wells. After a one hour incubation at room temperature and washing, 50µL Solution A and 100µL anti-IL-6 conjugate was added to all wells. Following a one hour incubation and washing, 100 µL chromagen solution was added for the colorimetric reaction. One-hundred µL stop solution was added to stop the reaction after 15 minutes. The optical density was read at 450nm on the Synergy HT Microplate Reader.

A standard curve was created by reducing the data and generating a linear curve fit. The IL-6 concentration of **Probacillus Revive** treated-fibroblasts was determined by extrapolation from the standard curve and expressed in pg/mL.

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Results

The data obtained from this study met criteria for a valid assay and the positive and negative controls performed as anticipated.

Probacillus Revive was not able to decrease IL-6 production compared to our positive control at higher concentrations but at low concentrations has some anti-inflammatory activity.

IL-6 levels are expressed by the following formula:

$$= Average_{IL-6\ Concentrations} \times Dilution\ Factor$$

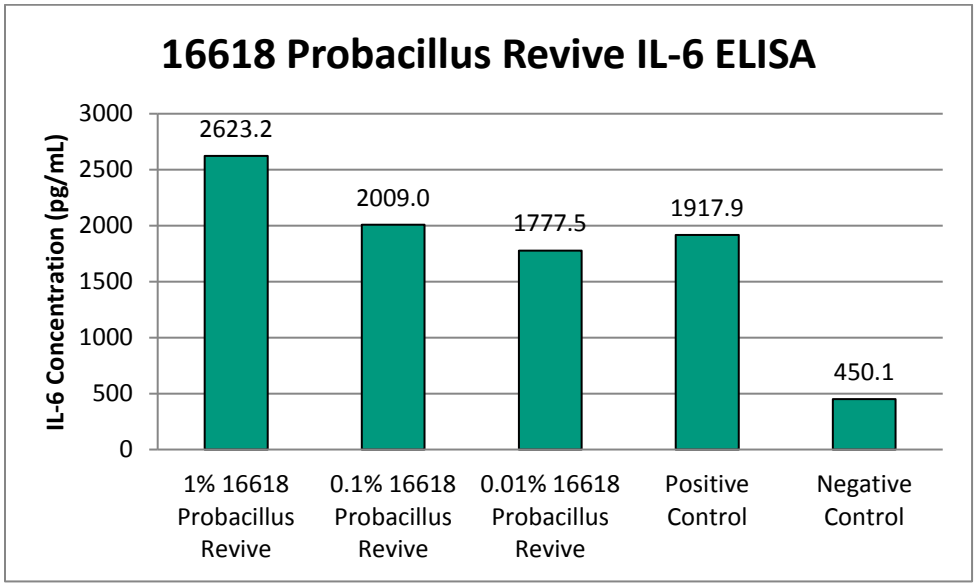

Figure 1: Probacillus Revive -treated fibroblasts IL-6 concentrations

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IL-6 production percent decrease is calculated by the following formula:

$$= \frac{Positive\ Control_{\ Avg.Concentration} - Sample_{\ Avg.Concentration}}{Positive\ Control_{\ Avg.Concentration}} \times 100$$

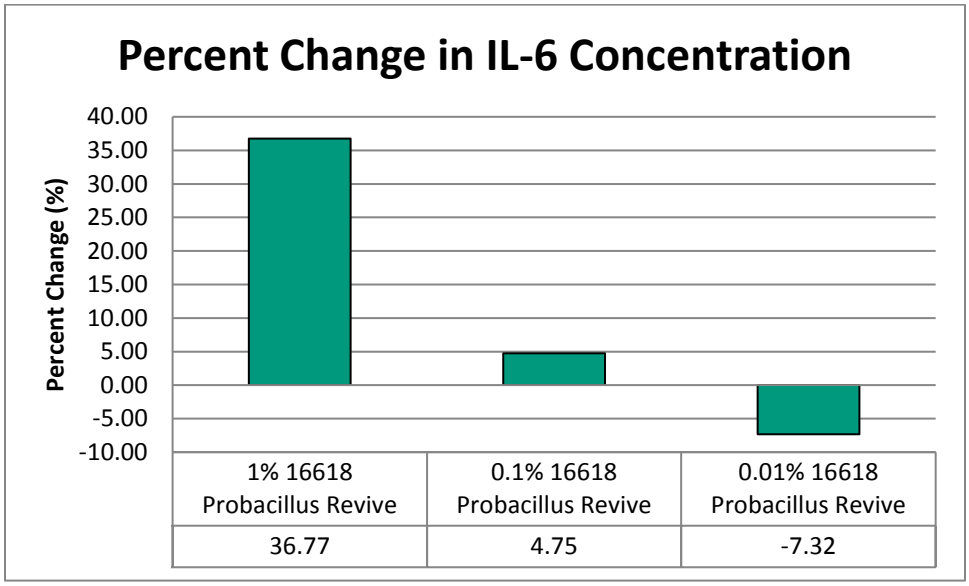

Figure 2: Percent change in IL-6 production compared to positive control

Discussion

As shown in figure 1, **Probacillus Revive** did not exhibited anti-inflammatory effects on LPS-treated fibroblasts at higher concentrations. As expected, the changes in IL-6 production using **Probacillus Revive** appears to be dose dependent and at lower concentrations seems to be moderately anti-inflammatory. Therefore, **Probacillus Revive** is more suitable for cosmetic applications other than anti-inflammation when higher concentrations are used.