



# Elevated levels of interferon gamma in human saliva following ingestion of *Streptococcus salivarius* K12

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## Introduction

Users of the oral probiotic, *Streptococcus salivarius* K12 (BLIS K12 Throat Guard), reported that taking the bacterial lozenges at the onset of flu-like symptoms appeared to help prevent further development of the symptoms. Interferon gamma (IFN- $\gamma$ ) is known to contribute to non-specific immunity against many intracellular bacteria and viruses. In previous studies it has been reported that IFN- $\gamma$  can be detected in saliva samples during viral infections at concentrations up to 25 pg/ml (1). Research in our lab has shown that human and murine leukocyte preparations produced IFN- $\gamma$  when exposed to *S. salivarius* K12 cells.

## Methods and Results

### Materials and Methods

#### *Dosage regime*

Four adult subjects took twelve BLIS K12 Throat Guard lozenges (four lozenges every two hours)

#### *Saliva collection and preparation*

One ml of unstimulated saliva was collected from the subjects before taking the lozenges and 6, 8, 10, 14 and 24 hours later. Saliva samples were frozen until processed. Ten  $\mu$ l of complete protease inhibitor (Roche) was added per ml of saliva. The saliva samples were treated by adding 20  $\mu$ l 2.5M NaCl and 20  $\mu$ l 1.5 M sodium acetate to 200  $\mu$ l of saliva. The samples were incubated on ice for 30 minutes. The saliva was then centrifuged for 5 min at 10,000 rpm, and the supernatant collected for testing.

#### *Interferon gamma ELISA assay*

Interferon gamma was detected in the saliva samples using an ELISA kit (BD Biosciences). One hundred  $\mu$ l of saliva sample supernatant was added to each well. The ELISA assay was conducted according to the manufacturer's protocol.

### Results

Interferon was not detected in the saliva samples until after 8 h. Between 22- and 139 pg of interferon gamma was detected in the 24 h samples (Fig. 1).

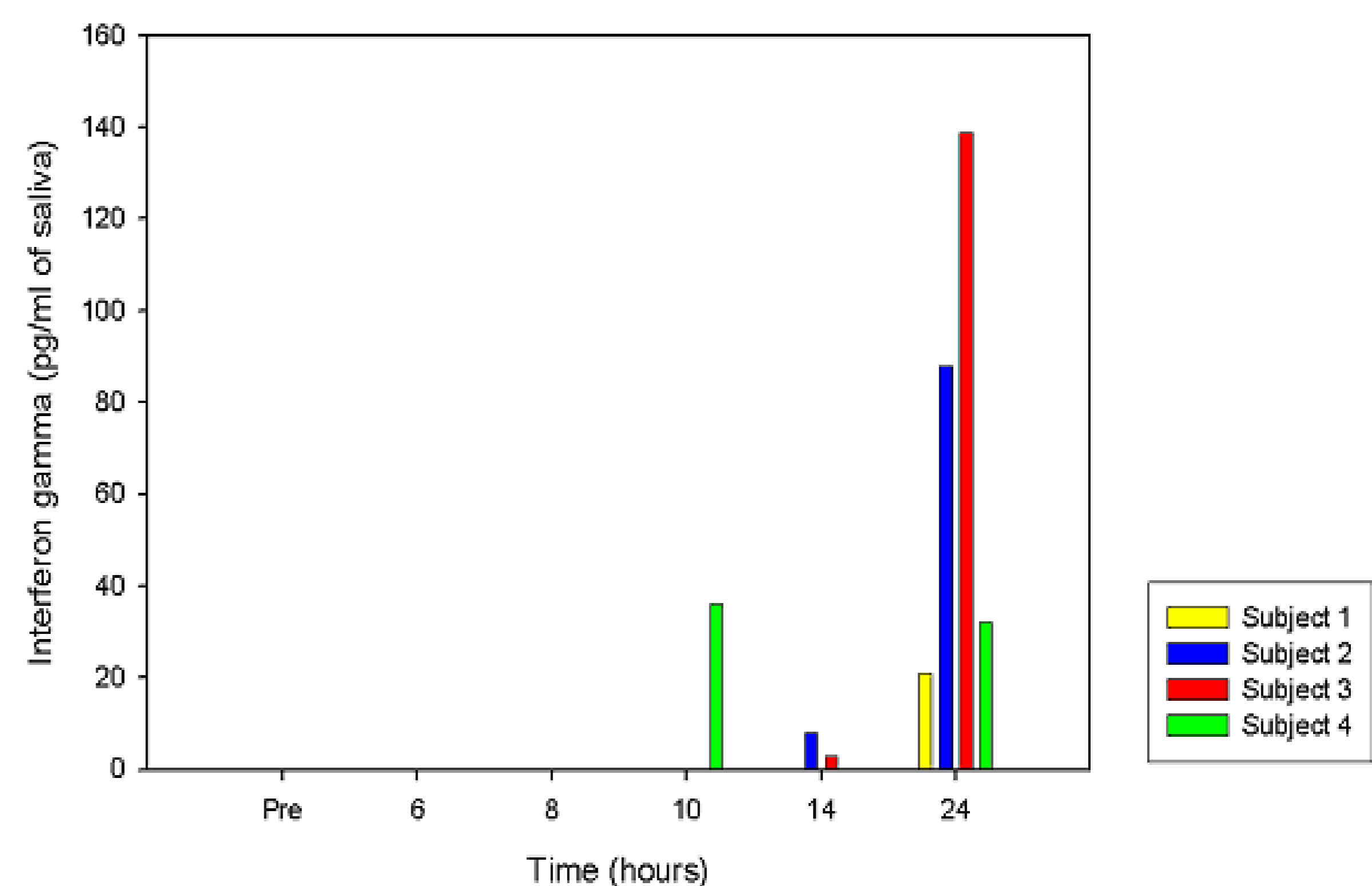


Figure 1. Detection of interferon gamma in saliva following ingestion of *Streptococcus salivarius* K12

During a viral infection the virus titre has been shown to increase during the first two days. An increase in the production of interferon during this time then leads to a decrease in the viral titre.

The induction of interferon gamma by ingestion of *S. salivarius* K12 may help in preventing further development of the symptoms, by reducing the viral titre.

## References

(1) Spear GT, Alves MR, Cohen MH, Bremer J, Landay AL. (2005). Relationship of HIV RNA and cytokines in saliva from HIV-infected individuals. *FEMS Immunol. Med. Microbiol.* 45(2):129-136.



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