

# Immunity analysis kit for canine: a novel tool for the analysis of NK cell activity using whole blood



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## Abstract

Cancer in adult dogs is the leading cause of death and share many features with human cancers including similar tumor biology. In human and mouse, NK cells play an important role in the innate immune response against tumor cells and infections. NK cells recognize and destroy tumor cells by releasing IFN- $\gamma$ , TNF- $\alpha$  and exocytosis of granzymes (Perforin) containing lytic granules.

In this study, we developed a simple assay to determine canine immunity using a small amount of whole blood. Our assay system was designed on the premise that more potent NK cells secrete higher levels of IFN- $\gamma$  when activated. Using a proprietary stabilized immunomodulatory cytokine, Promoca, NK cells were stimulated in whole blood. After their activation a quantitative sandwich ELISA was used to determine the levels of IFN- $\gamma$ . As expected, Promoca induced IFN- $\gamma$  production and its levels were significantly higher in healthy canine ( $2,377 \pm 224$  pg/mL, n=80), than in unhealthy ones ( $781 \pm 146$  pg/mL, n=80) with various diseases.

These results suggest canine NK cell activity may be a potential tumor biomarker for dogs. Also this novel assay is suitable for high-throughput monitoring of immunity and health condition of dogs.

**Keyword :** Canine NK cell, Immune potency, IFN- $\gamma$ , Assay kit

## Introduction

Natural killer (NK) cells are a type of cytotoxic lymphocyte critical to the innate immune responses against tumor or pathogen-infected cells. NK cells can interact with dendritic cells (DCs), also play a role in adaptive immune response [1]. The role of NK cells in both the innate and adaptive immune response is becoming increasingly important in research involving NK cell activity and regarding potential cancer therapies [2,3].

Cytotoxic ability of NK cells are well-characterized. IL-12, IL-15, IL-18, IL-2, and CCL5 play a crucial role in NK cell activation. Activated NK cells work to control the immune response by secreting IFN- $\gamma$  and TNF- $\alpha$ . IFN- $\gamma$  activates macrophages for phagocytosis and lysis, and TNF- $\alpha$  promotes the destruction of tumor cells. Activated NK cells secrete perforin and granzymes. Perforin can form pores in the membrane of target cell. Granzymes and associated molecules can enter through the pores, inducing either apoptosis or osmotic cell lysis. So, NK cell activity can be a good indicator of the immune state [4,5].

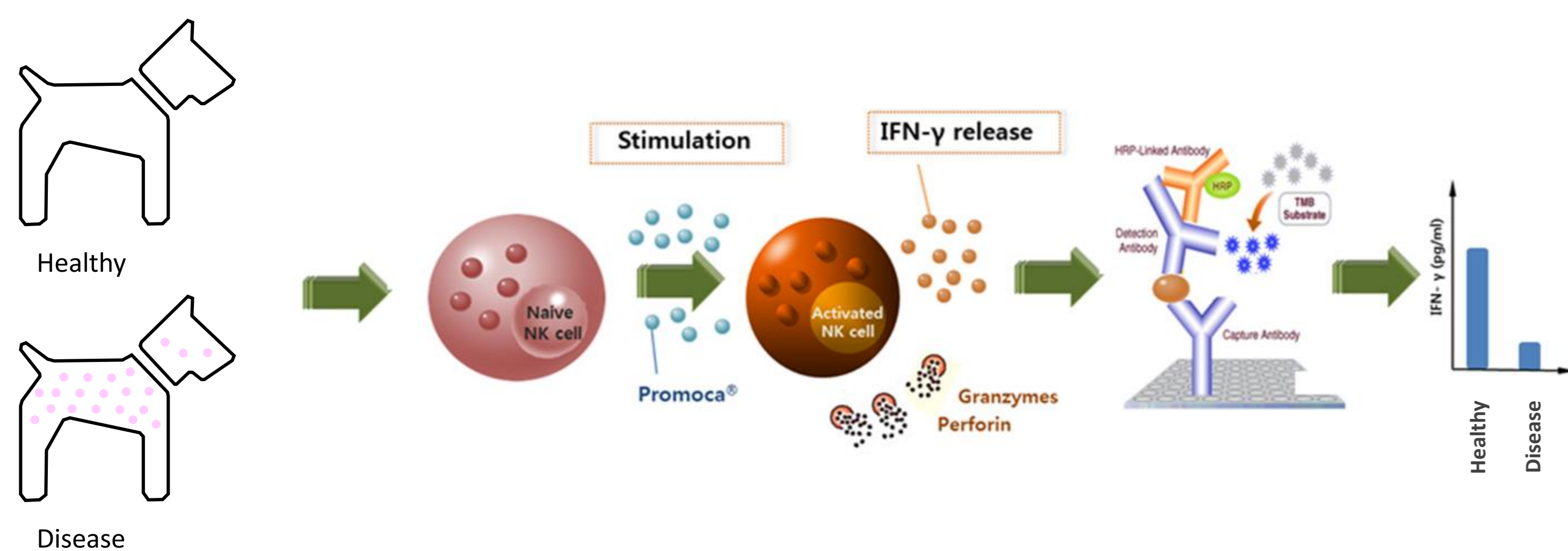


Fig.1. Schematics of NK cell activity measurement using our kit in whole blood. The freshly collected whole blood from dog is activated by engineered-activator (Promoca). During incubation, NK cells secrete IFN- $\gamma$  to their full potency. After incubation, the concentration of secreted IFN- $\gamma$  is measured by ELISA in plasma. This kit can quantify the immune potency of healthy dogs and dogs with immune-mediated diseases by measuring IFN- $\gamma$  that was secreted by Promoca-activated NK cells in whole blood.

## Results

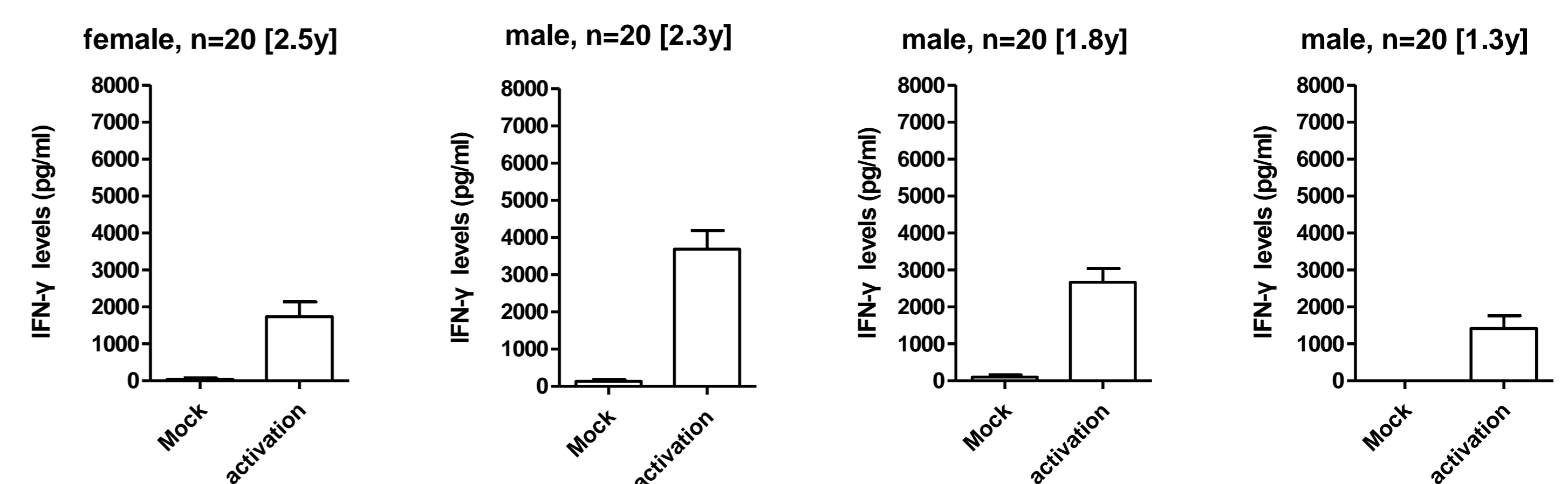


Fig.2. Increased secretion of IFN- $\gamma$  in Promoca-stimulated dog plasma.

Whole blood was incubated with Promoca for 24 h at 37 °C. Plasma was obtained by centrifuging (1000xg, 15 min), and the concentration of IFN- $\gamma$  was determined by enzyme-linked immunosorbent assay (ELISA). Induced level of IFN- $\gamma$  was found in all dogs and considerably higher ( $2377 \pm 2002$  pg/ml), however non-stimulated sample did not show significant levels of IFN- $\gamma$  ( $64 \pm 193$  pg/ml). It appears that Promoca could be utilized as a supportive activator to measure NK cell activity.

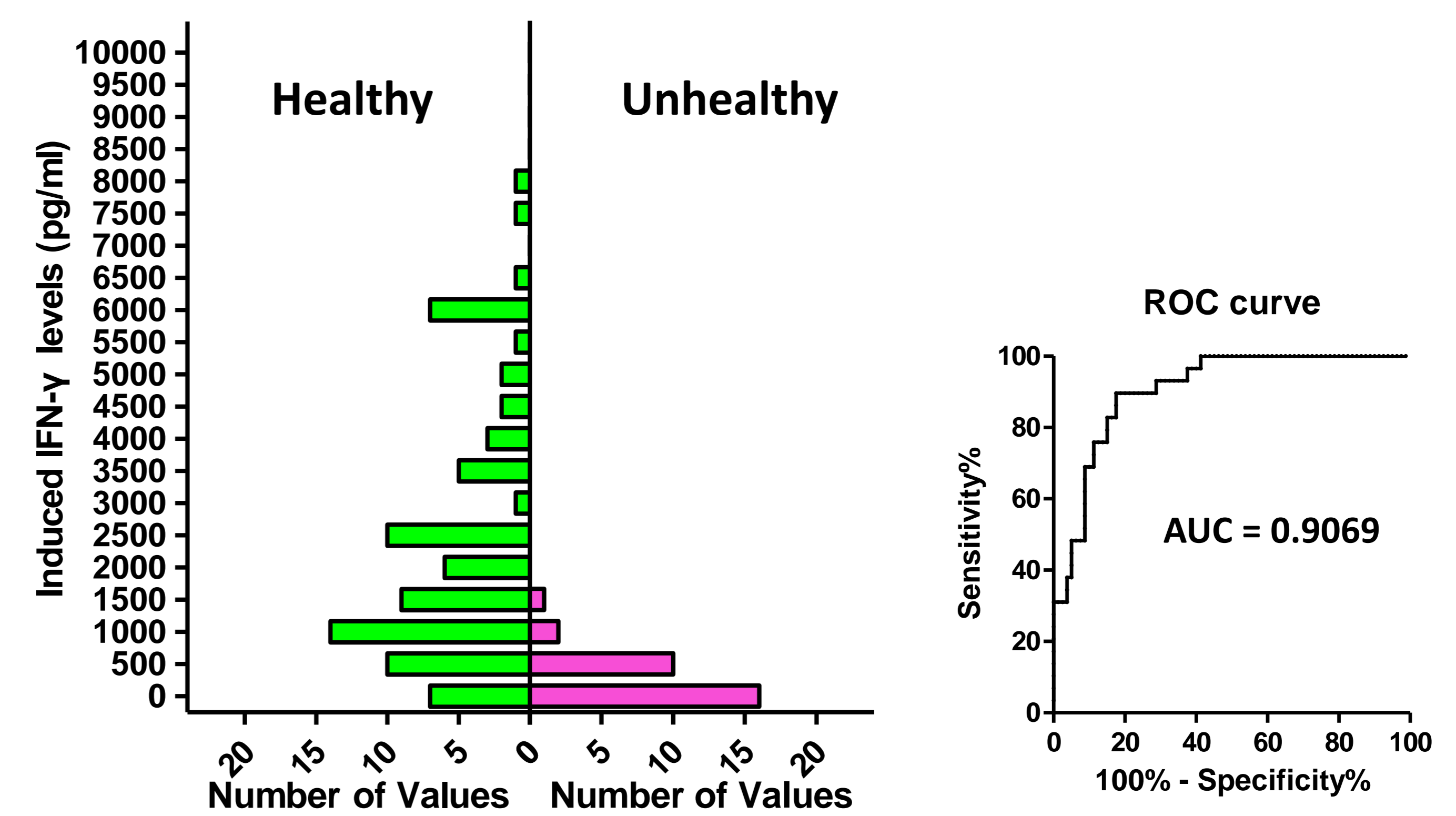


Fig.3. Promoca induced IFN- $\gamma$  in healthy and unhealthy dogs.

Increased level of IFN- $\gamma$  was found in healthy dogs, however, unhealthy dogs with disease such as cancer and severe skin illness, their blood produced very low amount of IFN- $\gamma$  by Promoca. (healthy n=80, unhealthy n=29). ROC analysis was performed to determine the sensitivity and specificity with the value of AUC in the right panel.

## Conclusion

We have developed a high-throughput assay to assess NK cell activity in small amount of whole blood. It is capable of measuring NK cell activity and is simpler than other established methods. This ELISA-based assay could be useful to help in diagnosing and in monitoring diseases associated with functional inhibition of NK cells, such as malignant tumors, viral diseases, and immune-mediated disorders. It will be further validated by clinical studies, especially cancers. This kit should be a good monitoring tool for health condition of pet dogs.

## References

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