



Natural Killer Cell Activity (NKA) *In Vitro* Diagnostic Device (IVDD) – a Fast and Simple Method that Brings NKA Testing to the Clinic

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ABSTRACT

Background and Objectives. For several decades, patients with different types of cancers have been shown to have lower NKA compared to controls. To date, NKA has been measured with the Research Use Only 51Cr or immunofluorescence-based cytotoxicity assays, requiring PBMC isolation. Herein, a novel, simple IVDD is described (NK Vue®; ATGen Canada). It uses standard blood collection tubes containing a proprietary formulation engineered to activate NK cells in whole blood. NKA is determined by the amount of interferon-gamma released into the plasma by ELISA after incubation. Assay reproducibility, sample stability, and precision of the quantitative ELISA test were evaluated.

Methods. Blood was drawn from healthy donors, NK cells were activated, and NKA determined using NK Vue® as per the manufacturer's recommendations.

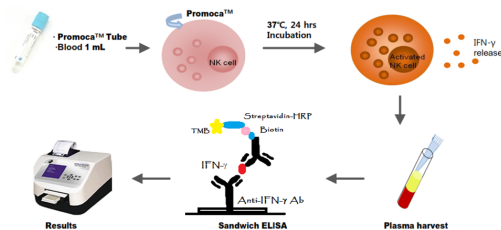
Results. Plasma samples were stable at least 6 months at -80°C ($p = 0.98$) and interferon-gamma determination was reproducible (between-run precision of 6%). NKA and NKA per NK cell values were consistent for same-patient samples taken on three different days. Data from 40 healthy adults provided a potential reference range for NKA.

Conclusions. The NK Vue® tubes are an easy and efficient way to activate NK cells. Samples obtained with this IVDD were stable for a minimum of 6 months frozen, and the provided ELISA assay offers a highly reproducible method. A preliminary NKA reference range in the normal Canadian population was obtained and could prove useful in patient assessment. Additional studies are required to optimize the ELISA part of the test on different automated platforms.

INTRODUCTION

- Patients with different types of cancers have been shown to have lower NK cell activity (NKA), than healthy patients.
- NKA is normally evaluate by measuring the quantity of interferon-gamma (INF- γ) released compared to the number of NK cells.
- Previous methods to assess NKA usually involved Research Use Only Cytotoxicity Assays (51Cr cytotoxic gold standard assay or immunofluorescence assay).
- To facilitate NK cell activation, Promoca® tubes (ATGen Canada) have been developed, allowing normal procedure for blood collection and in-tube cell activation after an easy overnight incubation.

Figure 1: Schematics of the activated NK cells using Promoca® tubes and NK Vue® ELISA kit.



RESULTS

Figure 2: Effect of collection tubes and incubation on IFN- γ release

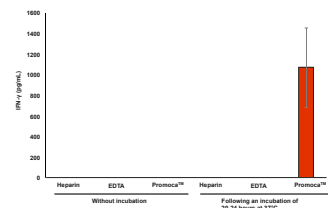


Figure 3: Daily variations in NK cells and IFN- γ release in two young healthy patients

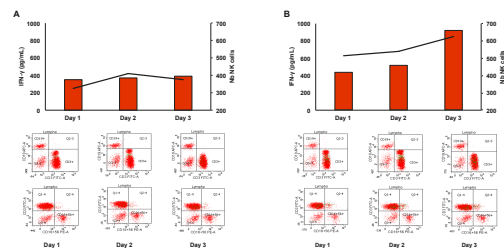


Figure 4: Stability of samples at -80°C over a 6 months period

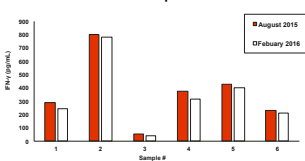
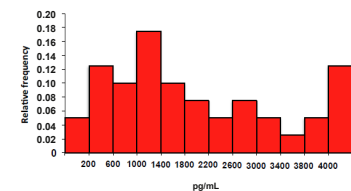


Table 1: Imprecision of the NK Vue™ ELISA kit (36 different plates, 17 days, 2 lots of ELISA kits)

	Control Low	Control High
Expected value (pg/mL)	150	1000
Average measured (pg/mL)	146	1035
Imprecision within runs (%)	2.74	4.35
Imprecision between runs (%)	6.96	5.94

RESULTS

Figure 5: NKA distribution for healthy patients (n=40)



Estimated NKA reference range using bootstrap strategy	Relative distribution of samples			
100% one-sided confidence interval	< 297 pg/mL	297 - 1000 pg/mL	1000 - 4000 pg/mL	> 4000 pg/mL
> 297 pg/mL	5%	22.5%	60%	12.5%

CONCLUSIONS

- The NK Vue tubes are an easy and efficient way to activate NK cells.
- Samples obtained with this IVDD were stable for a minimum of 6 months frozen, and the provided ELISA assay offers a highly reproducible method.
- A preliminary NKA reference range in the normal Canadian population was obtained and could prove useful in patient assessment.
- Additional studies are required to optimize the ELISA part of the test on different automated platforms.

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