# **Annals of Clinical Case Reports**

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# **Quality Changes of Deep-Freeze Serum Thymidine Kinase**

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#### Abstract

**Background:** Serum Thymidine Kinase 1 Concentration (STK1p) is benefit for a long-term followup of cancer patients.

Aim: To determine the store time of serum thymidine kinase 1 at -20°C.

Methods: The STK1p were determined by chicken anti-human TK1-IgY-polyclonal antibodies.

**Results:** The data of STK1p were analyzed from serum samples (n=109) in breast, lung and head & neck malignant patients at -20°C. The samples (n=69), randomly collected from the cohort of 109 samples were reanalyzed. No significant changes in STK1p values were found (p>0.2).

**Conclusion:** Store at -20°C did not affect the quality of thymidine kinase 1 serum significant and thus make it possible for a long-term follow-up of the cancer patients or early tumour risk progress.

Keywords: Thymidine kinase 1; Serum Thymidine Kinase 1 concentration (STK1p); Serum samples

## Introduction

Early risk assessments of development human tumors are important. It is necessary to use serum biomarkers for assessment of long-time follow-up [1]. To be able to perform such a study it is often needed to work with stored serum samples for shorter or longer times. In a previous reported in 1990 it was shown that the stability of TK1 in serum probably was due to that TK1 formed a native macromolecule complex (730 KD) [2]. Incubation at 56°C for 30 min remained about 70% of the enzyme activity, showing the rather stability of TK1 in serum [3]. In respect to serum thymidine kinase 1, analysis within 3 hours after preparing the fresh serum is recommended [1]. The collected serum samples can be stored in -80°C, for years before one-time analysis. There is absence of deep freezing at -80°C in some hospitals; serum samples were stored at -20°C for some years. However, no extensive investigation has been done to prove at -20°C works well. Here we performed an investigation on serum TK1 concentration (STK1p) to see how the quality is affected after storage at -20°C for 10 years.

# **Material and Methods**

#### Patients and serum samples

The serum samples from breast and head & neck malignant patients were collected from Karolinska University Hospital, Sweden and the serum samples of the lung malignant patients were collected at Hubei Tumor Hospital, Wuhan, China. Serum samples were collected in the morning on fasting condition, collected without anticoagulant and then centrifuged at 800 × g for 5 to 8 min. STK1p was determined within 3 hours. The results of the STK1p of patients with breast [4], lung, head & neck carcinomas [5] have been reported. The sera were divided into 4 to 6 tubes of 200  $\mu$ l each, at dry-ice and then kept at -20°C and stored for 10 years. At the time for reanalysis after 10 years, 69 serum samples were randomly collected from the cohort of 109 serum samples and re-analyzed for STK1p. The hemolysis, lipolysis, precipitation and repeated thawing of serum samples shall not be used.

#### STK1p assay

Serum samples were probed with chicken anti human TK1 IgY polyclonal antibody raised against a peptide (residue 195 225 of human TK1, amino acid sequence: GQPAG PDNKE NCPVP GKPGE AVAAR KLFAPQ). From the intensities of the TK1-calibrator of known concentrations, the STK1p concentration value was calculated and expressed as pM. Serum samples were probed without the antibodies as controls[1]. Samples were in duplicate analysis.

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**Figure 1:** Changes in thymidine kinas1 concentration in serum after storage at -20°C during the time of 10 years. Of 69 serum samples were randomly collected from the cohort of 109 serum samples and re-analyzed for STK1p. Breast Ca.: Breast carcinomas groups; lung Ca.: Lung carcinomas groups; Head & Neck Ca.: Head & neck carcinomas groups.

#### **Statistics**

Mean values, standard deviation and T-test were determined by SPSS Statistics (V20.0, IBM, USA). P  $\leq$  0.05 was considered to indicate a statistically significant value.

### **Results**

Serum samples (n=109) were collected from breast, lung and head & neck malignant patients, analyzed for Serum Thymidine Kinase 1 Concentration (STK1p) and stored at -20°C. The serum samples (n=69) were randomly collected from the cohort of 109 serum samples and re-analysed for STK1p.. The mean values of STK1p concentration decreased by 3%-15% in the breast, lung and head & neck carcinomas groups however not statistically significant (P> 0.2, Figure 1). The results showed that no signal can be detected when the serum samples were probed without the antibodies as controls (Data not shown).

# **Discussion and Conclusion**

Although fresh biological material is to be preferred when analysis clinical samples, it is not always possible when some hospitals are not available for the deep-freezing at -80°C. An alternative option is to store serum samples at -20°C. We have been working with serological tumor biomarkers during the last 30 years and particular with thymidine kinase 1, a proliferation serum biomarker [1]. Most of time our co-works were able to use fresh material. We mostly stored

the samples at -80°C; sometimes the samples were store at -20°C. However, we did not investigation on the quality effect on TK1 stored at -20°C for years. In this study we therefore reanalyzed the serum samples that have been stored at -20°C for 10 years. No significant changes in the STK1p concentration values were found.

## Conclusion

We conclude that the quality of TK1 in serum is not affected significantly by store at -20°C for at least 10 years. This opens up for the use of deep-freezing clinical serum samples less than -80°C, at least at -20°C, makes it possible to perform long-time follow-up of tumour patients or determine early tumor risk progress for evaluating the prognosis of patients.

# **Ethical Conduct of Research**

All patients gave informed consent to participate in this study, which was conducted in accordance with the Declaration of the 1964 Helsinki declaration and the Harmonized Tripartite Guideline for Good Clinical Practice from the International Conference on Harmonization. The collection of the serum samples was performed by permission of the Committee on Research Ethics at Karolinska University Hospital, Sweden (No. 388/01).

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