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Effects of temperature on presepsin assessment in biological fluids

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Abstract. Thermal stabilization is important for assuring a sample quality and prevent protein denaturation. Presepsin is a new reliable biomarker of sepsis produced in response to bacterial infections. However, before its inclusion into clinical practice several criteria need to be fulfilled. One of these regards the samples thermostability after a long-term refrigeration in different biological fluids (blood, urine, saliva) that can constitute a confounding factor for Presepsin reliability as diagnostic test. On this light, this review offers an update of studies analyzing presepsin after sample thawing.

Keywords: presepsin, temperature, biological fluids, sample storage.

1. INTRODUCTION

A proper sample management (collection, processing and storage) is essential for assuring a consistent quality for the intended analyses and study goal. In particular, consideration must be given to the storage condition to maintain sample quality until analyses are completed (Vaught J.B. et al).

Thermal stabilization is essential to prevent protein denaturation; in human fluids, slow freezing and fast thawing represent the recommended procedure (He X; Cuhadar S. et al).

Presepsin (P-SEP) is a protein produced in response to bacterial infections (Botondi V et al). P-SEP is a reliable biomarker for bacterial infections in adults, infants and newborns because of its measurability in biological fluids (i.e. blood, urine, saliva), its rapid activation and quick result output (Botondi V et al; Koh J.H. et al; Prester L. et al).

Recently, specific criteria for biomarkers inclusion in clinical practice have been stated according to official institutions (Food and Drug Administration, FDA; European Medicine Agency, EMA; the National Institute of Health, NIH) including the thermostability and results reproducibility (U.S Department of Health and Human Services et al; Hunter D.J. et al).

There are several studies in Literature focused on P-SEP detection, in which samples have been frozen (from -20°C to -80°C) until analysis (Kim S.Y.

et al; Lee S. et al; Aliu-Bejta A. et al; Al-Kindi S.G. et al). Nonetheless, according to some Authors and to the package insert of different P-SEP kit (PATHFAST and ELISA) a long-term refrigeration could somewhat represent a bias for P-SEP stability (Lee S. et al; Wagner B. et al, Khan M.).

In the present review we offer an overview of studies using refrigeration as a sample storage method before P-SEP analysis.

2. RESEARCH STRATEGY

We searched in the PubMed database for the period 2010 to 2023 all records matching the terms “Temperature”, “sCD14”, “storage conditions” and “frozen samples”. We found 10 records in whom P-SEP samples were stored from -20 to -80 °C.

3. CONTENT

3.1 P-SEP molecule

P-SEP is a truncated form of a cell surface glycoprotein (CD14) expressed by innate immunity cells such as monocytes and neutrophils. CD14 receptor has a high-affinity for bacterial lipopolysaccharides (LPS) and activates the proinflammatory signaling cascade. At the end of the process, P-SEP is released in the blood stream (Botondi V et al).

3.2 P-SEP sampling and storage

Characteristics of Studies in which P-SEP samples have been frozen for maintenance are shown in Table 1.

In detail, P-SEP was collected in different study-population such as:

- i) adults, in 5 out of the 10 series (Aliu-Bejta A. et al; Kim S.Y et al; Al-Kindi S.G. et al; Khan M. et al),
- ii) children, in 3 out of 10 series (Nishana E. et al; Maya-Barrios A. et al; Bhat S.S. et al),
- iii) newborns 2 out of 10 series (Topcuoglu S. et al; Pons S. et al).

Moreover, P-SEP was measured/stored in different biological fluids such as:

- iv) blood, in 7 out of 10 series (Topcuoglu S. et al; Aliu-Bejta A. et al; Kim S.Y et al; Pons S. et al; Al-Kindi S.G. et al; Lee S. et al; Khan M. et al)
- v) saliva, in 3 out of 10 series (Nishana E. et al; Maya-Barrios A. et al; Bhat S.S. et al),

Notably, CLEIA assay was performed in 3 out of 10 studies (Topcuoglu S. et al; Kim S.Y et al; Lee S. et al) and ELISA assay in 7 out of 10 reports (Aliu-Bejta A. et al; Pons S. et al; Al-Kindi S.G. et al; Al-Kindi S.G. et al; Nishana E. et al; Maya-Barrios A. et al; Bhat S.S. et al).

In all studies, samples were stored at a temperature of $\leq -20^{\circ}\text{C}$ and only 4 out of 10 studies provide information on the duration of freezing (Aliu-Bejta A. et al; Al-Kindi S.G. et al; Lee S. et al; Khan M. et al).

Finally, none of the studies object of evaluation analyzed the samples at different times after thawing.

4. DISCUSSION

Today, the determination of thermostability constitutes an important issue in the evaluation of biomarkers suitable for inclusion in clinical daily practice. The aim is to guarantee sample quality preventing protein denaturation and subsequent reliability of out-put results (He

Table 1. Characteristics of studies.

Population	BF	Assay	Storage T(°C)	Freezing Time	Ref
N	B	C	-80°C	NA	Topcuoglu S. et al
A	B	E	-40°C	7 d	Aliu-Bejta A. et al
A	B	C	-70°C	NA	Kim S.Y et al
N	B	E	-80°C	NA	Pons S. et al
A	B	E	-70/-80 °C	> 2 y	Al-Kindi S.G. et al
C	S	E	-20°C	NA	Nishana E. et al
C	S	E	-80°C	NA	Maya-Barrios A. et al
A	B	C	-80°C	3 m	Lee S. et al
C	S	E	-20°C	NA	Bhat S.S. et al
A	B	E	-20/-80 °C	34 d / 311 d	Khan M. et al

Abbreviations: BF, biological fluid; A, adults; C, children; N, newborn; B, blood; S, saliva; C, CLEIA; E, ELISA; d, days; y, years; m, months NA, not available.

X; Cuhadar S. et al). The issue is noteworthy especially for assessment, storage and measurement of P-SEP, a new promising early sepsis biomarker (Botondi V et al).

Among a series of assays currently used for P-SEP measurement the most available is the PATHFAST P-SEP device. Data on sample storage modalities and potential bias are at this controversial and still matter of debate. For example, according to manufacturer' instructions plasma samples are reported to be stable for 3 days at +2 to +8 °C and 9 months at -20 °C or lower, respectively. Notably, when samples are stored for a period > 9 months the result output reliability can somewhat be affected.

Data is corroborated by those reported by Khan M. et al who stored P-SEP samples at a prolonged temperature ranging from -20 to -80 °C and reported that caution should be exercised while analyzing samples for sCD14 after a so prolonged time-storage length.

Another issue that still require further investigation was within which time-period the samples were frozen from collection or were analyzed for the first time soon after thawing. Literature data showed in Table 1 report that samples were stored at a temperature $\leq -20^{\circ}\text{C}$ in all the studies. It is important to underline that only 4 out of 10 studies (Aliu-Bejta A. et al; Al-Kindi S.G. et al; Lee S. et al) provided information about the duration of sample freezing, moreover no study has analyzed the P-SEP at different time points after thawing.

In conclusion, on the basis of the present data:

- i) there are no studies focusing on the effects of thawing samples after a long-term storage on the P-SEP stability;
- ii) there are no series investigating the P-SEP thermostability at short/long time from refreshment, and
- iii) further research is needed in order to clarify the biomarker's stability at different time points and at different storage conditions.

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