18. Effects of processing and storage conditions on CSF Amyloid-ß(1-42) and Tau concentrations: implications for use in clinical practice

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Introduction: Reported concentrations of amyloid-ß(1-42) (Aß42) and tau in cerebrospinal fluid (CSF) differ among reports. We investigated the effects of storage temperature, repeated freeze/thaw cycles, and centrifugation on the concentrations of Aß42 and tau in CSF.
Methods: Stability of samples stored at -80°C was determined by use of an accelerated stability testing protocol according to the Arrhenius equation. Aß42 and tau concentrations were measured in CSF samples stored at 4, 18, 37, and -80°C. Relative CSF concentrations (%) of the biomarkers after one freeze/thaw cycle were compared with two, three, four, five, and six freeze/thaw cycles. In addition, relative Aß42 and tau concentrations in samples not centrifuged were compared with samples centrifuged after 1, 4, 48 and 72 h.
Results: Aß42 and tau concentrations were stable in CSF when stored for a long period at -80°C. CSF Aß42 decreased with 20% during the first two days at 4, 18, and 37°C compared with -80°C. CSF tau decreased after storage for 12 days at 37°C. After three freeze/thaw cycles CSF Aß42 decreased 20%. CSF tau was stable during six freeze/thaw cycles. Centrifugation did not influence the biomarker concentrations.
Conclusion: Repeated freeze/thaw cycles and storage at 4, 18, and 37°C influence the quantitative result of the Aß42 test. Preferably, samples should be stored at -80°C immediately after collection.

19. A novel time resolved fluormetric assay of anoikis using Europium-labelled annexin V in cultured adherent cells

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Introduction: Adherent cells are dependent for survival from continuous engagement of cellular integrins to the extra cellular matrix. Detachment of adherent cells from the matrix induces almost immediately apoptosis, a phenomenon designated as 'anoikis' or homelessness (1). Anoikis is of pertinent relevance to tissue homeostasis. We developed a new very sensitive method to analyse anoikis in adherent cell cultures using the principles of the DELFIA assays (2).
Methods: A new and sensitive method to analyse apoptosis and anoikis of adherent cell types using a time resolved fluorometric DELFIA assay with Europium-labelled Annexin V was developed. Anoikis was induced with tumor necrosis factor-alpha/cycloheximide and three cell fractions of the cell cultures were prepared and analysed. Fraction 1 consisted of adherent cells, analysed while growing on their support (without detachment by trypsinisation). Fraction 2 contained detached cells due to anoikis (floating cells) and fraction 3 contained apoptotic bodies. Both fractions 2 and 3 were present in the culture medium and were isolated by differential centrifugation.
Results: TNF-alpha treatment of three different types of adherent cell cultures induced a significant increase of the amount of floating cells (anoikis) and apoptotic bodies compared to control cell cultures. Also in the adherent cell fractions a small amount of apoptosis was observed.
Conclusion: The novel time resolved assay provides the ability to analyse the cell death cascade in adherent cell cultures of the same sample at the same time in a sensitive and reproducible way. According to our knowledge this is the first direct quantitative technique to measure anoikis in adherent cell cultures.

Literature: 1. Frisch et al. J Cell Biol 1994, 124: 619. 2. Hemmilä et al. Anal Biochem 1984, 137: 335.

20. External Quality Control of Cerebrospinal Fluid Markers for Alzheimer's Disease

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Introduction: Amyloidß 1-42 (Aß), Tau (tTau) and phosporylated Tau-181 (pTau) are gradually being accepted as biomarkers for Alzheimer's disease (AD). Measurement of these proteins is typically performed in cerebrospinal fluid (CSF), in the absence of reliable methods for their detection in more accessible body fluids. Different marker levels are reported by different groups, pre-analytical factors have been investigated (1), but QC programmes are currently lacking. This investigation was performed to compare results of different laboratories and to establish the feasability of an international QC scheme for AD markers.
Methods: CSF specimens with three different levels of the analytes were distributed to 15 laboratories worldwide involved in AD biomarker measurements, which agreed to participate. Laboratories were asked to report their assay results, information on the assay used and the condition in which the specimens arrived.
Results: Three months after dispatch of the specimens 11 laboratories reported their results. Five measured all three markers, six measured one or two analytes. Sometimes a lack of sample was reported. Coefficients of variation for Aß were 20.3, 52.5 and 27.9% at mean levels of 511, 393 and 755 pg/ml respectively (n=9). For Ttau the CV's were 7.9, 28.6 and 15.4% at 878, 389 and 219 pg/ml respectively (n=7). For pTau at 107, 49 and 35 pg/ml the CV's were 12.1, 9.6 and 16.0% for 7 laboratories. One laboratory reported extremely differing results which were excluded from the present report.
Conclusion: Although CSF marker results differed considerably between laboratories, the preliminary results of this study are encouraging. It is concluded that establishment of an international quality assessment schedule for AD markers in CSF is feasible.

Literature: Schoonenboom et al: Clin Chem 2005 In Press