The Alzheimer's Association flow chart for lumbar puncture and CSF processing

	Procedure	**Comment**
Lumbar puncture	• CT or MRI is performed before the LP	Identify cases with high intracranial pressure or mass lesions in the brain
	• Check medication	LP should not be performed in patients treated with anti-coagulants such as warfarine, but treatment with ASA or NSAIDs is not a contra-indication for LP
	• Perform LP at a standardized time, 8-12 AM	Recommendation to avoid potential diurnal variation for CSF biomarkers
	• The patient can be placed either sitting or lying down • The LP is done in the L3/L4 or L4/L5 interspace	Patient position or the incision point of the needle does not affect the results
	• Disinfect the skin using standard procedures. • Sterile gloves and a mask are used.	Reduce the risk for local infection
	• Local anaesthesia in the skin/subcutaneous tissue may be given	LP can be performed with or without local anaesthetics. Local anaesthetics introduces a risk for side effects of the anaesthetics, but may reduce local pain during LP.
	• Use a needle with a small diameter (0.7 mm / 22 gauge). • Use atraumatic technique, insert the needle with the bevel in parallel to the dura fibres.	A small gauge needle makes a smaller hole in the dura, which will heal more easily if the dura fibres are not cut-off, thus reducing the risk for post-LP headache.
	• Discard the first ½-1 mL of CSF. In the case of a puncture bleeding, a larger volume of CSF may be discarded, until the bleeding has diminished. • The CSF sample is collected in a new tube.	Blood contamination in the CSF sample may interfere with some assays, especially IgM analysis, and possibly also tau and Aβ.
	• Take the CSF sample in a non-adsorbing (polypropylene) tube.	Avoid adsorption of hydrophobic proteins such as β-amyloid to the test tube wall, which will happen if tubes of polystyrene or glass are used.
	• A standardized volume (10-12 mL) is tapped into a single tube. • The tube is then capped, and mixed gently by turning it around a few times.	• A standardized CSF volume and gentle mixing is recommended to avoid possible influence of concentration gradients, 10 mL is the recommended volume in the multiple sclerosis biomarker consensus, but smaller (or larger) volumes may be taken depending on the local routine. • The volume of CSF tapped will not affect the risk of post-LP headache.

	• Leave the patient to rest for ½-1 hour after LP.	Prolonged bed-rest or other procedures (e.g. excess drinking, caffeine) will not influence the risk for post-LP headache.
	• The CSF sample is sent to the local laboratory without delay.	Cells in CSF will start to lysate if the sample is at room temperature, which will affect the CSF cell count.
Laboratory procedures	• Basic CSF analyses - Cell count - CSF/serum albumin ratio (recommended) or total protein - IgG, IgM index, oligoclonal bands - other, e.g. glucose, lactate	• An aliquot is pipetted off for the CSF cell count, which must be performed directly (<1-2 hours). • The CSF sample is centrifuged in the original tube, and the supernatant taken off and aliquoted in tubes made of polypropylene. • Many local laboratories perform basic CSF biomarker analyses. If not, an aliquot of CSF and serum is sent to a specialized laboratory.
	• Sample information	• For samples to be included in future research studies, it is recommended that basic information on the sample is filed, including: - date and timepoint of sampling - conditions (RT or frozen) and time of transportation - date and timepoint of freezing - storage conditions - Red cell count or information on possible blood contamination
	• Core CSF biomarker analyses - Total tau protein - Phosphorylated tau - B-amyloid isoforms (Aβ42, others e.g. Aβ40)	• At the laboratory performing the specific biomarker assays, all CSF samples should be frozen once before assays (-20°C or lower). A) CSF samples sent at room temperature should be frozen (-20°C) upon arrival to the Lab performing the analyses. B) CSF samples sent frozen should be frozen (-80°C) before shipment, sent on dry ice, and kept frozen (-20°C) before assay.
	• Transportation of CSF	• Core CSF biomarkers analyses are performed at a specialized laboratory. • An aliquot of 1 mL is suitable for core CSF biomarkers, to allow re-analysis if needed. • The CSF can be sent by ordinary mail, at room temperature if the shipping time is less than two days. If longer, the sample is frozen and sent on dry ice.
	• Core CSF biomarker assays	• Analyses are performed by experienced Lab technicians • A standard Lab QC program is followed (temperature control, pipett calibration etc.) • A standardized protocol (kit insert) is followed in detail • Analyses are performed in duplicate to increase the precision.
	• Internal QC controls	• Two or more internal control CSF samples (aliquots of pooled CSF) are analyzed each run, to minimize between-assay variability. The mean ± 2SD for these internal control samples is calculated based on a series (6-10)

		runs. For approval of a plate, the internal control samples should fall within this range. • The batch / lot # for both the reagent kit and the calibrators/standards are registered for each run, to identify batch-to-batch variation for the assay.

Abbreviations: AD= Alzheimer's disease; CSF= cerebrospinal fluid; LP= lumbar puncture; NSAID= non-steroidal anti-inflammatory drugs.