Supplementary material to article by A. Rystedt et al. "Effect of Botulinum Toxin Concentration on Reduction in Sweating: A Randomized, Double-blind Study"

## Study limitations

This study has the following limitations: how to stimulate sweating in a standardized manner, how to compensate for different levels of sweating on the back, and how to exclude small anhidrotic/hypohidrotic areas, which may be artefacts. Heat stimulation was carried out in a sauna, as sweating from the trunk is thermo-regulated. When the sweating from the back began, an iodine-starch imprint was performed. It was not important to keep external factors, such as the temperature in the sauna and the duration of the visit to the sauna, constant between subjects, since the rate of sweating was individual and the quality of the imprints would be inadequate if the back was too dry or too wet when performing the iodine-starch test. However, on an individual basis the autonomic sudomotor neurone signalling to the sweat glands on the back was homogenous, which enabled us to compare the 16 measuring points. It is well known that sweating is more prominent in the middle of the back, down the spine, than on the lateral parts of the back. We compensated this heterogeneity by randomizing the subjects to different treatment sequences, which minimizes any potential bias. In addition, position on the back (lateral/medial) was incorporated in the statistical models, hence adjusting the treatment effects for position effects. In our study approximately 20% of imprints were excluded because of uncertain margins and artefacts due to too much or too little sweat on the back. To eliminate possible bias the exclusion was carried out prior to un-blinding.