**Characterization, Assessment, and Autoproteolysis in Commercial Blends of Clostridial Collagenases Used for Human Islet Isolation**

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The most commonly used reagent for pancreas digestion and human islet isolation is Liberase HI from Roche Diagnostics, a blend of purified and mixed bacterial $Zn^{2+}$-containing type I and II collagenases – both from *Clostridium histolyticum* -, and the metalloproteinase thermolysin from *Bacilius thermoproteolyticus rokko*. The composition of the mixture is strictly formulated and should provide reliability and consistency in islet isolation procedures. Nevertheless, the enzymes are not stable even in lyophilized form hermetically sealed under nitrogen and stored at -70°C. Their activity is progressively lost, and sometimes new batches must be utilized even in a short, two-month period. Heterogeneity of collagenase type I, which has been shown recently by several investigators (T. Canavagh, 2002; J. Lakey, 2005; F. Bertuzzi, 2006) is especially a concern. Our attempt to address this issue yielded the development of a new HPLC method of separation of distinct components within the collagenase I subgroup. The approach is based on separation of target proteins at a pH close enough to their pIs, where enzymes are still absorbed by the column resin while impurities are eluted in void volume. Subsequent gradient elution results in separation of sub-components (collagenase type Ia and Ib). It was found that collagenase type Ia is rapidly autocatalytically degraded to its Ib form. The relative proportion of these two forms can be a valuable parameter for lot assessment both in blends and individual components of clostridial collagenases. At the same time, we showed that collagenase type II is a

homogeneous and stable component in the blends.  Its chromatographic profile was not changed even after incubation in 1X HBSS for a month at 0°C.