Evaluation of collagenase gold plus BP protease in isolating islets from human pancreata

Bashar Khiatah, Amber Tucker, Kuan-Tsen Chen, Rachel Perez, Shiel Bilbao, Luis Valiente, Leonard Medrano, Jeffrey Rawson, Elena Forouhar, Keiko Omori, Fouad Kandeel, Meirigeng Qi, and Ismail H. Al-Abdullah

Department of Translational Research and Cellular Therapeutics, Diabetes and Metabolism Research Institute, Beckman Research Institute of the City of Hope, Duarte, CA, USA

ABSTRACT
Selection of enzymes for optimal pancreas digestion is essential for successful human islet isolations. The aim of this study was to evaluate the efficacy and outcome of using Collagenase Gold plus BP protease (VitaCyte) (n = 8) by comparing it to two commercially available enzymes, Liberase MTF C/T (Roche) (n = 48) and Collagenase NB1/NP (Serva) (n = 15). The isolation outcomes were assessed by islet counting, viability, glucose-stimulated oxygen consumption rate (OCR), and successful graft-rate following transplantation in diabetic NOD scid mice. The pancreas donor characteristics were not significantly different between the tested enzyme groups regarding their BMI, pancreas weight, cold ischemia time (CIT) and HbA1c. The results show that digested tissue volume was not statistically significant between the VitaCyte enzyme (34.25 ± 5.4 mL) and the Roche enzyme (55.25 ± 3.42 mL, p = 0.073), however, this was significant with Serva enzyme (64.07 ± 7.95 mL, p = 0.020). Interestingly, the islet yields were not statistically different between all enzyme groups. Moreover, when islets were transplanted into NOD scid mice, the reversal rate of diabetes for the VitaCyte enzyme group was similar to all enzyme groups. In conclusion, the effectiveness of Collagenase Gold plus BP protease is comparable to the MTF C/T and the Collagenase NB1/NP enzymes; the low cost could facilitate the use of more pancreata for islet isolations.

KEYWORDS
collagenase gold; collagenase NB1; islet isolation; Liberase MTF C/T; neutral protease

Introduction
Clinical islet transplantation has been shown to be an effective treatment for patients with brittle type 1 diabetes.1 The islet yield and quality from pancreas digestion are critically important for achieving this goal.2 Successful human islet isolation requires many critical decisions to be made by the isolation team depending on donor characteristics.3-8 One of the main decisions is choosing an appropriate enzyme combination of collagenase and neutral proteases.9-15 Roche MTF C/T and Serva collagenase NB1/NP are among a number of enzymes available for pancreas digestion during human islet isolations for research and clinical applications. The standardization of the islet isolation process is critically important for promoting biological licensed applications from the US Food and Drug Administration. For this context, it is highly recommended to use enzymes that are GMP grade, animal tissue-free, or recombinant products.16,17 However, highly standardized GMP enzymes/materials are often costly, especially for research pancreata. Based on the UNOS report, a high number of pancreata were not used due to donor characteristics and/or economic concern for isolation cost.18 Furthermore, large numbers of the pancreata procured were suboptimal and therefore were not used for either whole organ or islet transplantation. Thus, these pancreata were used for research application in the US including our center. Therefore, both efficient and cost-effective enzymes are advantageous to fulfill the increasing demand of islets for research applications. Recently, VitaCyte has released a new product designated Collagenase Gold. This is a non-GMP grade enzyme that has not been fully characterized. The VitaCyte collagenase is prepared from Clostridium...
that was grown in media containing porcine gelatin. This enzyme contains class I collagenase (73%), class II collagenase (27%), and low protease activity.\(^{15}\) Since proteases are required to digest the pancreas, the combination of the Collagenase Gold and BP protease is indicated to free the islets.\(^{20,21}\) This new VitaCyte Collagenase Gold contains the highest percentage of class I collagenase when compared to the Roche (60%) and Serva (50%) enzymes.\(^{15}\) Since proteases are required to digest the pancreas, the combination of the Collagenase Gold and BP protease is indicated to free the islets. Moreover, with a significantly lower price, this enzyme combination may potentiate the utilization of more pancreata for islet isolations, especially for research applications. Therefore, in this study, we hypothesize that the Collagenase Gold plus BP protease is as effective as other standard enzymes in human islet isolation. A retrospective analysis was conducted to compare the VitaCyte Collagenase Gold plus BP protease to Roche MTF C/T and Serva Collagenase NB1/NP enzymes.

### Results

Initially, results from 100 consecutive islet isolations were reviewed and 71 isolation outcomes were ultimately analyzed, as the others were excluded because the donor had an HbA1c >6.5%, was DCD or had cardiac arrest >30 minutes. The choice of enzymes for pancreas digestion using Roche Liberase MTF C/T (Roche) (n = 48) or VitaCyte Collagenase Gold plus BP protease (VitaCyte) (n = 8) were alternately selected, while younger donors were digested using Serva collagenase NB1/NP (Serva) (n = 15). Overall, with the exception of age, the donor characteristics for the three different enzyme groups were not significantly different between the tested enzymes (Table 1).

However, the donors in the Serva enzyme group (25.6 ± 1.8 yrs) were significantly younger than that in the VitaCyte (50.1 ± 3.3 yrs) and Roche (48.5 ± 1.6 yrs) enzyme groups (p < 0.0001, Table 1).

The optimum pancreas digestion time (switch time) was not significantly different between the VitaCyte enzyme (12.63 ± 0.67 min) and the Roche enzyme (12.31 ± 0.30 min, p = 0.896), as well as between the VitaCyte enzyme and the Serva enzyme (12.92 ± 0.27 min, p = 0.936) (Fig. 1A). However the VitaCyte enzyme digested significantly less tissue (34.25 ± 5.40 mL) when compared to the Serva enzyme (64.07 ± 7.95 mL, p = 0.020) but not the Roche enzyme (55.25 ± 3.42 mL, p = 0.073) (Fig. 1B). Digestion percentage showed significant difference between the VitaCyte and Roche enzymes (67.94 ± 4.50% vs 79.12 ± 1.58%, p = 0.026), as well as between the VitaCyte and Serva enzymes (67.94 ± 4.50% vs 84.17 ± 2.67%, p = 0.004) (Fig. 1C).

For the VitaCyte enzyme, the islet yields in total IEQ at pre-purification, post-purification, and post-culture were 277312 ± 51932, 204010 ± 56891, and 163016 ± 47656 IEQ, respectively. The islet yields in IEQ/g at pre-purification, post-purification, and post-culture were 4741 ± 745, 3503 ± 729, and 2752 ± 574 IEQ, respectively. There were no significant differences with regard to islet yields both in total IEQ and IEQ/g when compared the VitaCyte enzyme to either the Roche or Serva enzyme (Fig. 2). Additionally, both islet purity and packed cell volume post-purification of the three enzymes used were not significantly different (Fig. 3). The results of islet assessments showed that there was a significant difference in the viability of the islets obtained with the VitaCyte enzyme (98.13 ± 0.40%) compared to those obtained with the Roche enzyme (94.43 ± 0.41%, p = 0.005) and the Serva enzyme (94.93 ± 0.73%, p = 0.020) (Fig. 4A), though this difference did not

### Table 1. Donor characteristics and enzymes used for islet isolations.

|                | VitaCyte | Roche | Serva | p value        |
|----------------|----------|-------|-------|----------------|
| Number of donors| 8        | 48    | 15    | N/A            |
| Donor age (yrs) | 50.1 ± 3.3 | 48.5 ± 1.6 | 25.6 ± 1.8 | VitaCyte vs Serva: <0.0001 |
| BMI (kg/m²)    | 26.8 ± 1.5 | 30.7 ± 0.7 | 31.4 ± 1.9 | Roche vs Serva: <0.0001 |
| Donor HbA1c (%)| 5.6 ± 0.2  | 5.4 ± 0.1  | 5.3 ± 0.1  | 0.13           |
| CIT (hrs)      | 7.0 ± 0.1  | 7.0 ± 0.0  | 6.9 ± 0.1  | 0.32           |
| Pancreas weight (g)| 84.1 ± 4.8 | 100.3 ± 3.1 | 99.7 ± 8.3 | 0.99           |
| Cost for each isolation ($) | 1130 | 2075 | 4952 | N/A            |

BMI, body mass index; HbA1c, hemoglobin A1c; CIT, cold ischemia time; N/A, not applicable.

All p values were calculated by ANOVA tests; the p values for donor ages were calculated by Tukey’s multiple comparisons test following ANOVA; data expressed as mean ± SEM (standard error of mean).
correlate to a significant difference in OCR consumption as the VitaCyte enzyme was 1.48 ± 0.06 and the Roche enzyme was 1.45 ± 0.03 (p = 0.943), while the Serva enzyme was 1.45 ± 0.06 (p = 0.955) (Fig. 4B). After islet transplantation into diabetic NOD scid mice, there were no significant differences found between the VitaCyte enzyme group and any of the two other enzyme groups in terms of the reversal rate of diabetes (Fig. 5); however, there was a significant difference between the Roche and Serva groups (p = 0.008). The cost per each enzyme preparation for isolation was lower when VitaCyte enzymes are used ($1130) compared to Roche ($2075) and Serva ($4952) enzymes (Table 1).

Discussion

In recent years, the request of islets for research has been rising. Therefore, reducing the cost of processing research pancreata would have a great impact on fulfilling the increasing demand.7,22 The cost associated with non-GMP products is significantly lower than the cost of GMP products. Accordingly, the expense of each isolation using VitaCyte Collagenase plus BP protease was 54% and 23% of the cost compared to Roche MTF C/T and Serva collagenase NB1/NP, respectively. Research pancreata accepted for isolation have been strictly selected due to the cost burden of using expensive GMP products, like the Roche and...
Serva enzymes. In this context, using cost-effective enzymes, like VitaCyte, may facilitate utilizing more organs for islet isolations specifically for research applications. Therefore, this pilot study was initiated to explore the possibility of using less expensive, but indeed effective, enzymes for research applications.

Determining the optimal concentration of collagenase and thermolysin/NP is critical to optimize pancreas digestion and free the islets. While there are many manufacturing variabilities, and even lot-to-lot variability, there is a lack of common assays to detect enzyme activity, which limits standardizing the digestion process across different islet transplant centers. In this case, one-on-one systematic comparisons of the currently available enzymes manufactured by Roche, Serva and VitaCyte are highly important in terms of selecting the ideal enzyme(s). In this study, we evaluated the performance of the VitaCyte Collagenase Gold plus BP protease in human islet isolations. Using VitaCyte Collagenase Gold plus BP protease, we were able to achieve comparable results in terms of islet yield and quality when compared to the Roche Liberase MTF C/T and the Serva collagenase NB1/NP, but with a lower cost (Table 1).

Collagenase and NP are produced from Clostridium histolyticum, while Thermolysin is purified from Bacillus thermoproteolyticus rokko. Collagenases (class I and II isoforms) supplemented with either Thermolysin or NP are currently used for pancreata digestion. VitaCyte Collagenase Gold contains low protease activity and a high percentage of class I collagenase (73%). Additionally, VitaCyte Collagenase Gold retains the flexibility of being combined with different types of proteases to digest pancreata for islet isolations. In our hands, we used VitaCyte Collagenase Gold supplemented with BP protease in human islet isolations. The digested tissue volume and digestion percentage were significantly lower in pancreata

Figure 3. Purity post-purification (A) and packed cell volume post-purification (B) of the three enzymes used. VitaCyte (n = 8), Roche (n = 48), and Serva (n = 15). There are no significant differences in both purity post-purification and packed cell volume post-purification between any two groups of enzymes.

Figure 4. Viability (A) and OCR (B) results of the three enzymes used. VitaCyte (n = 8), Roche (n = 48), and Serva (n = 15). There is a significant difference in viability between VitaCyte and Roche (**p = 0.005), as well as between VitaCyte and Serva (p = 0.020) (Fig. 3A). OCR data showed no significant difference between any two groups of enzymes (Fig. 3B).
digested with Collagenase plus BP protease compared to the digested tissue volume produced from pancreata digested with Serva. However, islet yields were not statistically different among the three tested enzymes. It is conceivable that the use of BP protease with Collagenase Gold may have contributed to digesting pancreatic tissues more efficiently which resulted in lower digested tissue volumes. The VitaCyte enzyme group had a similar reversal rate of diabetes in mice compared to the Roche and Serva enzyme groups, albeit islets isolated using the VitaCyte enzyme maintained better viability post culture. However, the Serva enzyme group had better in vivo function than the Roche group; this may be due to the fact that a higher percentage of young donor pancreata were digested using Serva enzymes supporting that islets from young donors have superior in vivo function. It has been reported that islets from young donors are difficult to free from acinar tissue since most of the islet are embedded/mantled. It is suggested that the Roche MTF C/T contains 60% of class I and 40% of class II collagenases (protein content by weight), while the Serva Collagenase NB1 contains 50% of class I and II collagenases (protein content by weight), respectively. The difference in vivo islet functions may also stem from the fact that a neutral protease was used with the Serva enzymes while thermolysin was used with the Roche enzymes. These neutral proteases were produced by different bacterial sources, Clostridium histolyticum and Bacillus thermoproteolyticus rokko, respectively. Hence, the mode of action to digest pancreatic tissue may be different. As mentioned previously, the VitaCyte BP protease is produced by Bacillus polymyxa, which is suggested to be a Dispase equivalent enzyme. It is conceivable that the enzymes used may have contained different Class I and II collagenases and different proteases, which might have an effect on the islet yield, viability and digested tissue volume. The percentages of class I and class II collagenases present in each vial supplied between different companies are still debatable, thus further studies are need to substantiate the optimal enzyme concentrations. Overall, the VitaCyte Collagenase Gold plus BP protease were found to be as effective as two high-quality digestion enzymes when tested in human islet isolations.

In this study, VitaCyte Collagenase Gold plus BP protease were found to be effective for pancreas digestion from donors >38 years of age. It is tempting to speculate that such an enzyme may also be efficient to digest pancreata from younger donors, especially when multiple proteases such as Clostripain were used. Further investigation is required to substantiate this hypothesis. Roche MTF C/T and Serva NB1/NP enzymes are GMP products and are often the most suitable for clinical use. Comparatively, VitaCyte Collagenase Gold is sterile but is a non-GMP product and therefore used as a research grade enzyme. However, in this study, the Collagenase Gold/BP protease was filtered in the GMP facility prior to use according to our SOP and Quality Assurance regulations at City of Hope. Nevertheless, improving islet isolation outcomes need to be continuously enhanced, so that ultimately every pancreas could be utilized for clinical applications; this can only be achieved with GMP enzymes once the initial results of low cost enzyme(s) show positive outcomes. In fact, the VitaCyte Company has made progress in developing a recombinant collagenase class I and II, in addition to Collagenase Gold, which would show great potential for isolating islets.

The data generated from this study is promising because 8 isolations using Collagenase Gold/BP protease resulted in comparable outcomes as those obtained utilizing GMP grade enzymes, indicating the potency of Collagenase Gold/BP protease. However, more data from Collagenase Gold/BP Protease would further substantiate that using these enzyme combination would be beneficial to isolate islets from suboptimal isleds.
pancreata with low cost, especially from type 2 diabetic and younger donors.

In conclusion, we have shown that using Collagenase Gold plus BP protease results in equal islet quality and yield as the counterpart of Liberase MTF C/T and Collagenase NB1/NP enzymes. Furthermore, due to the lower cost, more pancreata can be utilized for isolations for advancing islet research.

**Materials and methods**

**Study design**

In this study, outcomes of 100 consecutive research-designated human islet isolations (June 2015 to April 2017) from pancreas donors were retrospectively analyzed. Isolations were categorized into three groups based on the enzymes used for digesting the pancreas: i) Collagenase Gold Plus BP protease (VitaCyte, Indianapolis, USA); ii) Liberase Mammalian Tissue Free Collagenase/Thermolysin (Liberase MTF C/T) (Roche Diagnostics, Roche Applied Science, Indianapolis, IN, USA); iii) Collagenase NB1 with Neutral Protease (SERVA Electrophoresis GmbH, Heidelberg, Germany). For the VitaCyte enzyme, one vial of Collagenase Gold (Specific FALGPA Activity 1138 Units in 1 g amount, Cat # 011–1060) plus two vials of BP protease (each vial contains 1.1 million NP activity, Cat # 003–1000) were used for each isolation according to manufacturer recommendation. For the Roche enzyme, average amount of MTF collagenase and thermolysin used in each isolation were 2862 Wunsch units and 163288 units respectively. For the Serva enzyme, average amount of collagenase NB1 and neutral protease used in each isolation were 2542 Wunsch units and 266 units respectively. Donors with an HbA1c > 6.5%, donation after cardiac death (DCD) or a downtime > 30 minutes were excluded from this study. Research consent was obtained from donor nearest relatives or other person authorized legally to make the consent decision. In addition, the study was approved by the Institutional Review Board of Beckman Research Institute of the City of Hope.

**Human islet isolation**

The human islet isolation procedures were performed in state-of-the-art cGMP facility at the City of Hope using the standard islet processing procedure previously described. Briefly, the pancreas was trimmed, decontaminated, and subsequently cannulated pancreatic lobes were perfused with digestion enzymes using an automatic perfusion apparatus (BioRep Technologies, Miami, FL, USA) with one of the aforementioned enzymes. Afterwards, the pancreas was cut into 7–10 pieces and placed into a semi-automatic Ricordi’s digestion chamber for digestion. Purification of the pooled, digested, tissue containing islets was performed using a COBE 2991 with continuous density gradients. Samples were taken and stained with DTZ for counting and determination of the purity of the islets microscopically; this was performed before and after purification. Islet Equivalents (IEQ) were used to express the total islets count.

Purified islets were cultured at 37°C/5% CO₂ for 24–72 hours as previously described. Post-culture islet samples were taken for quality assessments, including islet count, viability, glucose-stimulated oxygen consumption rate (ΔOCR), and islet transplantation into diabetic NOD scid mice.

**Islet viability and ΔOCR**

Viability of post-cultured islets was assessed through the use of fluorescent microscopy with FDA and PI as previously reported. Islet oxygen consumption rate (OCR) was measured using a Seahorse XFe analyzer (Seahorse Bioscience, North Billerica, MA). Briefly, islets were washed with modified Seahorse XFe assay media containing 3 mM glucose and 1% FBS, and equilibrated in same assay media for 3 hrs at 37°C. Then, 70–100 IEQ islets were handpicked and plated into Seahorse XFe islet capture plates (Seahorse Bioscience). Islet OCR was measured at basal level (3 mM glucose), upon glucose stimulation (20 mM glucose), and on mitochondrial respiration inhibition (Oligomycin 5 nM). OCR fold increase was calculated by OCR upon glucose stimulation/OCR at basal level. Minimum of 4 islet samples were measured simultaneously in each experiment.

**Transplantation of human islets in diabetic NOD scid mice**

Islet transplantation into NOD scid mice was carried out as previously developed method. Briefly, male NOD scid mice (Jackson Laboratory, Bar Harbor, ME, USA) that were 10–12 weeks of age were used as islet recipients. Mice were housed at the Animal Resources Center, Beckman Research Institute of the City of Hope.
Hope. Diabetes was induced in mice by injecting mice intraperitoneally with 50 mg/kg of Streptozotocin (STZ; Sigma-Aldrich, St Louis, MO, USA) for three consecutive days. Mice with glucose levels >350 mg/dL for at least 48 hours were used for islet transplantations. For each isolation, 2–5 mice were transplanted with 1200 IEQ/each under the left kidney capsule. We used this islet number because our empirical study showed that approximately 60% of transplanted mice reversed diabetes (unpublished data). Blood glucose levels were followed for 30 days using a glucometer (LifeScan, Inc., Milpitas, CA, USA). All glucose blood levels readings <200 mg/dL for two weeks were considered a successful transplant. For each enzyme, the success graft-rate was calculated by dividing the amount of successful transplants by the total number of recipients.

**Statistical analysis**

Data was analyzed with GraphPad Prism (GraphPad Software 7.1, La Jolla, CA, USA). ANOVA one-way analysis of variance was used to compare the three groups of enzymes followed by Tukey multiple comparisons test to compare the mean values between any two groups. All the values were expressed as mean ± standard error of mean (SEM).

**Abbreviations**

- Cgmp: Current Good Manufacturing Practice
- CIT: Cold Ischemia Time
- DCD: Donation after Cardiac Death
- GSIS: Glucose-Stimulated Insulin Secretion
- HbA1c: Hemoglobin A1c
- IEQ: Islet Equivalent
- MTF C/T: Mammalian Tissue Free Collagenase/Thermolysin
- NOD: Non-Obese Diabetic
- NP: Neutral Protease
- OCR: Oxygen Consumption Rate
- SCID: Severe Combined Immunodeficient
- STZ: Streptozotocin

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

**Acknowledgments**

Human pancreatic islets were provided by the NIDDK-funded Integrated Islet Distribution Program (IIDP) at City of Hope. We extend our thanks and appreciation to all islet isolation team members at City of Hope.

**Funding**

This work was supported by a grant from the National Institute of Health (NIH), the Southern California Islet Cell Resources Center (SC-ICR) 5U42RR016607 and the City of Hope (COH).

**ORCID**

Bashar Khiatah [http://orcid.org/0000-0002-6870-8094](http://orcid.org/0000-0002-6870-8094)

Shiela Bilbao [http://orcid.org/0000-0003-0523-5629](http://orcid.org/0000-0003-0523-5629)

**References**

1. Hering BJ, Clarke WR, Bridges ND, Eggerman TL, Alejandro R, Bellin MD, Chaloner K, Czarniecki CW, Goldstein JS, Hunsicker LG, et al. Phase 3 trial of transplantation of human islets in type 1 diabetes complicated by severe hypoglycemia. Diabetes Care. 2016;39:1230-40. doi:10.2337/dc15-1988. PMID:27208344.

2. Ricordi C, Goldstein JS, Balamurugan AN, Szot GL, Kin T, Liu C, Czarniecki CW, Barbaro B, Bridges ND, Cano J, et al. National institutes of health-sponsored clinical Islet transplantation Consortium Phase 3 trial: Manufacture of a complex cellular product at eight processing facilities. Diabetes. 2016; 65:3418-28. doi:10.2337/db16-0234. PMID:27465220.

3. Hering BJ, Kandaswamy R, Ansite JD, Eckman PM, Nakano M, Sawada T, Matsumoto I, Ihm S-H, Zhang H-J, Parkey J, et al. Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes. JAMA. 2005;293:830-5. doi:10.1001/jama.293.7.830. PMID:15713772.

4. Froud T, Ricordi C, Baidal DA, Hafiz MM, Ponte G, Cure P, Pileggi A, Poggioli R, Ichii H, Khan A, et al. Islet transplantation in type 1 diabetes mellitus using cultured islets and steroid-free immunosuppression: Miami experience. Am J Transplant. 2005;5:2037-46. doi:10.1111/j.1600-6143.2005.00957.x. PMID:15996257.

5. Shapiro AMJ, Ricordi C, Hering BJ, Auchincloss H, Lindblad R, Robertson P, Secchi A, Brendel MD, Berry T, Brennan DC, et al. International trial of the Edmonton protocol for islet transplantation. N Engl J Med. 2006;355:1318-30. doi:10.1056/NEJMoa061267. PMID:17005949.

6. Ryan EA, Paty BW, Senior PA, Bigam D, Alfadhlhi E, Kneteman NM, Lakey JRT, Shapiro AMJ. Five-year follow-up after clinical islet transplantation. Diabetes. 2005;54:2060-9. doi:10.2337/diabetes.54.7.2060. PMID:15983207.

7. Qi M, McFadden B, Valiente L, Omori K, Bilbao S, Juan J, Rawson J, Oancea AR, Scott S, Nair I, et al. Human pancreatic islets isolated from donors with elevated HbA1c levels: Islet yield and graft efficacy. Cell ISLETS 57
Transplant. 2015;24:1879-86. doi:10.3727/096368914X683548. PMID:25198342.

8. Qi M, Luis V, Bilbao S, Omori K, Rawson J, McFadden B, Juan J, Nair I, Mullen Y, El-Shahawy M, et al. Sodium levels of human pancreatic donors are a critical factor for determination of islet efficacy and survival. Am J Physiol Endocrinol Metab. 2015;308:E362-9. doi:10.1152/ajpendo.00443.2014. PMID:25537495.

9. Sabek OM, Cowan P, Fraga DW, Gaber AO. The effect of isolation methods and the use of different enzymes on islet yield and in vivo function. Cell Transplant. 2008;17:785-92. doi:10.3727/096368908786516747. PMID:19044205.

10. O’Gorman D, Kin T, Imes S, Pawlick R, Senior P, Shapiro AM. Comparison of human islet isolation outcomes using a new mammalian tissue-free enzyme versus collagenase NB-1. Transplantation. 2010;90:255-9. doi:10.1097/TP.0b013e3181e117ce. PMID:20463640.

11. Kin T, Johnson PRV, Shapiro AMJ, Lakey JRT. Factors Influencing the collagenase digestion phase of human Islet isolation. Transplantation. 2007;83:7-12. doi:10.1097/01.tp.0000243169.09644.e6. PMID:17220782.

12. Breite AG, Dwulet FE, McCarthy RC. Tissue dissociation enzyme neutral protease assessment. Transplant Proc. 2010;42:2052-4. doi:10.1016/j.transproceed.2010.05.118. PMID:20692405.

13. Brandhorst H, Friberg A, Andersson HH, Felldin M, Foss A, Salmela K, Lundgren T, Tibell A, Tufveson G, Korsgren O, et al. The importance of tryptic-like activity in purified enzyme blends for efficient islet isolation. Transplantation. 2009;87:370-5. doi:10.1097/TP.0b013e31819499f0. PMID:19202441.

14. Anazawa T, Balamurugan AN, Bellin M, Zhang HJ, Matsumoto S, Yonekawa Y, Tanaka T, Loganathan G, Papas KK, Beilman GJ, et al. Human Islet isolation for autologous transplantation: Comparison of yield and function using SERVA/Nordmark versus roche enzymes. Am J Transplantation. 2009;9:2383-91. doi:10.1111/j.1600-6143.2009.02765.x.

15. Qi M, Valiente L, McFadden B, Omori K, Bilbao S, Juan J, Rawson J, Scott S, Ferreri K, Mullen Y, et al. The choice of enzyme for human pancreas digestion is a critical factor for increasing the success of Islet Isolation. Transplant Direct. 2015;1:1-9. doi:10.1097/TXD.0000000000000522. PMID:26146662.

16. Balamurugan AN, Naziruddin B, Lockridge A, Tiwari M, Loganathan G, Takita M, Matsumoto S, Papas K, Trieger M, Rainis H, et al. Islet product characteristics and factors related to successful human islet transplantation from the Collaborative Islet Transplant Registry (CITR) 1999–2010. Am J Transplant. 2014;14:2595-606. doi:10.1111/ajt.12872. PMID:25278159.

17. Brandhorst H, Brandhorst D, Hesse F, Ambrosius D, Breidel M, Kawakami Y, Breidel RG. Successful human islet isolation utilizing recombinant collagenase. Diabetes. 2003;52:1143-6. doi:10.2337/diabetes.52.5.1143. PMID:12716744.

18. Bartlett ST, Markmann JF, Johnson P, Korsgren O, Hering BJ, Scharp D, Kay TW, Bromberg J, Odorico JS, Weir GC, et al. Report from IPITA-TTS opinion leaders meeting on the future of beta-cell replacement. Transplantation. 2016;100 Suppl 2:S1-44. doi:10.1097/TP.0000000000001055. PMID:26840096.

19. Collagenase Gold. Low cost collagenase for human islet isolation. http://www.vitacyte.com/.

20. McCarthy RC, Breite AG, Green ML, Dwulet FE. Tissue dissociation enzymes for isolating human islets for transplantation: factors to consider in setting enzyme acceptance criteria. Transplantation. 2011;91:137-45. doi:10.1097/TP.0b013e3181f1f7df. PMID:21116222.

21. Balamurugan AN, Breite AG, Anazawa T, Loganathan G, Wilhelm JJ, Papas KK, Dwulet FE, McCarthy RC, Hering BJ. Successful human islet isolation and transplantation indicating the importance of class 1 collagenase and collagen degradation activity assay. Transplantation. 2010;89:954-61. doi:10.1097/TP.0b013e3181d121e9a. PMID:20300051.

22. Integrated Islet Distribution Program.

23. Wang Y, Paushter D, Wang S, Barbaro B, Harvat T, Danielson K, Kinzer K, Zhang L, Qi M, Oberholzer J. Highly purified versus filtered crude collagenase: Comparable human Islet isolation outcomes. Cell Transplant. 2011;20:1817–25. doi:10.3727/096368911X564994. PMID:21396158.

24. Bertuzzi F, Cainarca S, Marzorati S, Bachi A, Antonioli B, Nano R, Verzaro R, Ricordi C. Collagenase isoforms for pancreas digestion. Cell Transplant. 2009;18:203-6. doi:10.3727/096368909788341270. PMID:19499708.

25. Linetsky E, Bottino R, Lehmann R, Alejandro R, Inverardi L, Ricordi C. Improved human islet isolation using a new enzyme blend, liberase. Diabetes. 1997;46:1120-3. doi:10.2337/dba.x6.1120. PMID:9200645.

26. Al-Abdullah IH, Bagramyan K, Bilbao S, Qi M, Kalkum M. Fluorogenic peptide substrate for quantification of bacterial enzyme activities. Sci Rep. 2017;7:44321. doi:10.1038/srep44321. PMID:28287171.

27. Green ML, Breite AG, Beechler CA, Dwulet FE, McCarthy RC. Effectiveness of different molecular forms of C. histolyticum class I collagenase to recover islets. Islets. 2017;9(6):177-181. doi:10.1080/19382014.2017.1365996. PMID:28933628.

28. Niclauss N, Bosco D, Morel P, Demuylder-Mischler S, Brault C, Milliat-Guitard L, Colin C, Parnaud G, Muller YD, Giovannoni L, et al. Influence of donor age on islet isolation and transplantation outcome. Transplantation. 2011;91:360-6. doi:10.1097/TP.0b013e31820385e6. PMID:21344706.

29. Stahl M, Foss A, Gustafsson B, Lempinen M, Lundgren T, Rafael E, Tufveson G, Korsgren O, Friberg A. Clostripain, the missing link in the enzyme blend for efficient human Islet isolation. Transplant Direct. 2015;1:e19. doi:10.1097/ TXD.0000000000000528. PMID:27500221.

30. Balamurugan AN, Green ML, Breite AG, Loganathan G, Wilhelm JJ, Tweed B, Vargova L, Lockridge A, Kuriti M, Hughes MG, et al. Identifying effective enzyme activity targets for recombinant class I and
Class II collagenase for successful human Islet isolation. Transplant Direct. 2016;2:e54. doi:10.1097/TXD.0000000000000563. PMID:27500247.

31. Loganathan G, Venugopal S, Breite AG, Tucker WW, Narayanan S, Dhanasekaran M, Mokshagundam S, Green ML, Hughes MG, Williams SK, et al. Beneficial effect of recombinant rC1rC2 collagenases on human islet function: Efficacy of low dose enzymes on pancreas digestion and yield. Am J Transplant. 2017. doi:10.1111/ajt.14542.

32. Ricordi C, Hering B, London NJ, Rajotte R, Gray D, Socci C, Alejandro R, Carroll P, Bretzel R, Scharp D. Islet isolation assessment. In: Ricordi C, ed. Pancreatic Islet Cell Transplantation. Austin, TX: R.G. Landes. 1992:133-42.

33. Miyamoto M, Morimoto Y, Nozawa Y, Balamurugan AN, Xu B, Inoue K. Establishment of fluorescein diacetate and ethidium bromide (FDAEB) assay for quality assessment of isolated islets. Cell Transplant. 2000;9:681-6. doi:10.1177/096368970000900514. PMID:11144965.