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# **Compatibility of BD™ P800 Evacuated Blood Collection Tubes with Insulin Testing for Research Purposes**

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## Purpose

The BD™ P800\* blood collection system is used for the preservation of the following plasma metabolic biomarkers; GLP-1, GIP, glucagon, and ghrelin. The purpose of this study was to demonstrate that the protease inhibitor-containing plasma obtained from P800 tubes is compatible with various assays for another commonly studied metabolic biomarker, insulin. The assays tested utilized the following detection methods: a manual ELISA sandwich assay which uses a horseradish peroxidase conjugated antibody to develop a colorimetric substrate, an automated one-step sandwich assay using an alkaline phosphatase conjugated antibody and a chemiluminescent substrate, and another automated sandwich assay, with a detection antibody conjugated to a ruthenium complex for electrochemiluminescent signal generation.

## Methods

Venous blood was drawn into BD serum, K<sub>2</sub>EDTA (EDTA), and P800 evacuated blood collection tubes from four healthy subjects. Subjects 1-3 were non-fasting and Subject 4 was fasting (only EDTA and P800 tubes drawn). Blood was allowed to clot for 30 minutes prior to centrifugation to obtain serum; plasma tubes (EDTA and P800) were centrifuged immediately at 1600 x g for 20 minutes. Serum and plasma were pooled according to tube type, then aliquotted for the time course study. Samples were maintained under ambient conditions and aliquots were removed and stored frozen at less than -80 °C at t = 0, 7, 24, and 48 hours for automated assays (subjects 1-3 [n=3]) and at t = 0, 2, 5, 7, 24, and 48 hours for the manual ELISA assay (subject 1 and subject 4 [n=2]).

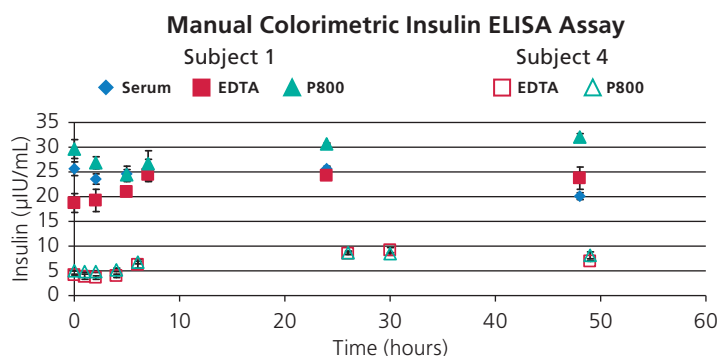
Samples were thawed and endogenous insulin levels analyzed according to manufacturer's instructions for use.

## Results and Discussion

Data from the insulin ELISA are displayed in **Figure 1**. The ELISA showed the most variation between the different tube types with P800 showing nearly 50% more insulin than EDTA and a 16% greater value than serum at the initial time point for Subject 1. It is important to note that the serum sample differed from the EDTA sample by over 25% as well. However, at 7 hours, P800 insulin values were less than 10% greater than the other tube types for this subject and within the error of the assay. In Subject 4, the initial P800 value was about 22% greater than the initial EDTA insulin value.

**Figure 2** shows the data from individual subjects using the automated immunoassays. On average there was less than 10% difference between the insulin values obtained at the initial time point with P800 plasma, serum and EDTA plasma on both analyzers for the three subjects tested. Those differences are similar to the difference in insulin values between serum and EDTA plasma. Also evident in **Figure 2**, particularly with subject 3, is the increased degradation of insulin over time in serum, compared to slower rates of degradation with EDTA and P800 plasma.

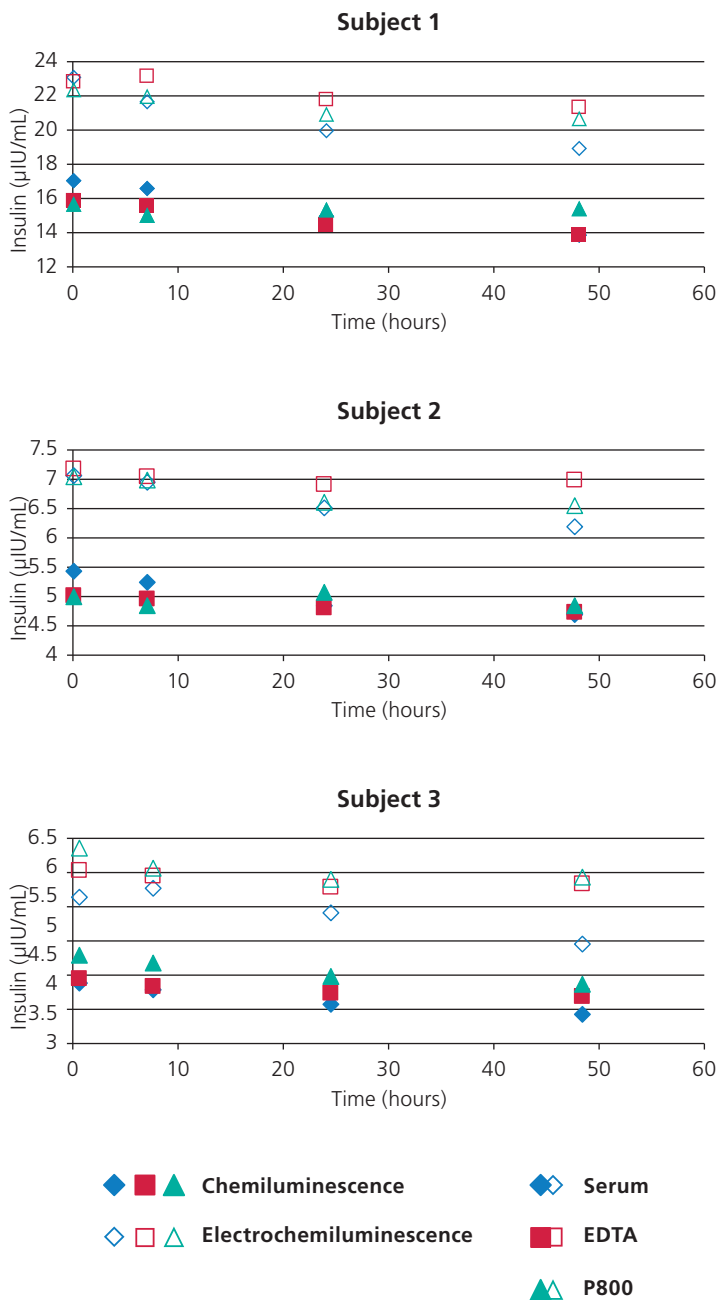
Variation of insulin levels between assay detection methods was expected. This is not a function of the tube type as all tubes showed similar shifts in insulin levels depending on the analyzer used.



**Figure 1** – Insulin manual ELISA data, error bars represent standard deviation for replicate analyses (n = 2). Filled shapes represent Subject 1 (non-fasting), open shapes represent Subject 4 (fasting), no serum tube drawn for Subject 2.

## Conclusion

The data suggests that the components of P800 do not interfere with the insulin assays tested, which included three different detection methods, colorimetric, chemi-luminescence, and electrochemiluminescence detection. P800 plasma showed similar insulin values compared to EDTA plasma and serum, two of the commonly accepted sample types for insulin testing. P800 plasma, which uses EDTA for anticoagulation, also showed similar insulin stability compared to EDTA plasma.



**Figure 2** – Automated insulin assay data for individual subjects (Subjects 1-3, non-fasting). Open shapes represent data collected with electrochemiluminescence detection, filled shapes represent data collected with chemiluminescence detection.

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