High Throughput Assessment of Gut Bacterial Tyrosine Decarboxylase Activity for Predicting Peripheral Levodopa Loss in Parkinson's Disease

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Abstract

Parkinson's disease (PD), a prevalent neurodegenerative disorder, is commonly managed through oral levodopa administration. However, the enzymatic activity of specific gut bacteria, particularly tyrosine decarboxylase (TDC), can metabolize levodopa, potentially leading to diminishing treatment effectiveness over time. Current methods for assessing TDC activity in PD patients focus mainly on genetic markers, lacking a robust and precise assay to measure gut bacterial TDC activity. Building upon prior research demonstrating levodopa conversion to dopamine using bacterial cultures and rat jejunum samples, we present an enhanced protocol to accurately quantify TDC activity in human fecal samples. This high-throughput approach employs fermentation reactions with fecal-derived bacteria, measuring TDC activity by converting supplemented levodopa to dopamine through Ultra-Performance Liquid Chromatography coupled with Electrochemical Detection (UPLC-ED). Serving as a predictive biomarker for levodopa loss, this method allows preparation of 96 fecal sample fermentation cultures within ~7 h. A single individual can conduct the protocol, with completion time varying based on factors such as sample size and time points collected.

Introduction

This procedure allows for the efficient preparation of 96 fecal fermentation cultures in approximately 7 hours. Subsequently, it involves the collection of samples at different time points, their processing, and automated UPLC-ED analysis. It is feasible for a single person to carry out this protocol, although the overall time required for these steps will depend on factors such as the sample size and the individuals familiarity with both UPLC-ED techniques and the protocol itself.

Parkinson's disease (PD) is a highly prevalent, neurodegenerative disorder that commonly occurs in aging people. The global annual incidence of new cases ranges between 5-35 cases per 100,000 individuals. The current gold-standard of treatment involves the pharmacological replacement of dopamine via the oral administration of levodopa (L-3,4-dihydroxyphenylalanine). In previous studies, we and others have demonstrated that gut-associated bacteria, particularly, from the genera Enterococcus, harbor the tyrosine decarboxylase (TDC) enzyme, which can efficiently metabolize levodopa to dopamine in the gut. The peripheral metabolism of levodopa leads to the generation of dopamine, but the inability of dopamine to traverse the blood-brain barrier results in diminished bioavailability of the medication. Consequently, patients undergoing this bacterial metabolization of levodopa experience reduced efficacy of the treatment, compelling them to necessitate higher doses or more frequent administration to attain therapeutic benefits. Hence, the measurement of gut bacterial TDC activity in PD patients could have potential benefits for understanding the potential impact of gut bacteria on the bioavailability of levodopa. By reliably measuring gut bacterial TDC activity, clinicians may gain insights into new strategies for managing PD symptoms and potentially improve treatment outcomes for the patients.

Previous studies have evaluated bacterial TDC abundance using quantitative polymerase chain reaction (qPCR) to investigate potential relationships with levodopa pharmacokinetics, showing varying results.
However, the accuracy of qPCR is influenced by several confounding factors. Moreover, metagenomic analysis of PD can also fail to detect the tdc gene or TDC pathway, likely given the low read counts of the gene, despite significant elevation of *E. faecium* and *E. faecalis* species in PD patients compared to their household controls. Further, sequencing-based methods have the drawback of not necessarily reflecting the actual breakdown of levodopa, as higher tdc gene abundance does not always correlate with increased TDC expression, thus, activity. These limitations underscore the need for alternative methods to achieve a more comprehensive and precise evaluation of gut bacterial TDC activity. In our previous work, we demonstrated that through utilizing fecal fermentation cultures levodopa can be converted to dopamine using bacterial cultures and rat jejunum samples. Here, we have made modifications to present an improved protocol that allows for high throughput determination of TDC activity in human fecal samples. By employing this protocol, portrayed in Figure 1, we have established a reliable and reproducible high-throughput method for assessing TDC activity in human fecal samples which may serve as an objective biomarker that can indicate a peripheral blockade to levodopa, which can open new avenues for designing intervention strategies aimed at circumventing the gut bacterial interference with PD medication.

This approach involves utilizing a fermentation reaction to assess TDC activity. Standardized amounts of fecal samples are suspended in a growth media that selects for small intestinal bacteria, which is where levodopa is taken up *in vivo*, supplemented with kanamycin. The fecal fermentation culture is supplemented with the levodopa substrate and is then incubated for a period of 24 hours at 37 °C in anaerobic conditions, during which the levodopa is metabolized by TDC-producing bacteria. The composition of the media ensures consistent growth conditions that facilitate replicable quantification of discernible differences in levodopa decarboxylation among patients. By measuring the production of dopamine and reduction of levodopa at each time point, the activity of TDC can be inferred.

The underlying rationale of this assay is that individuals with lower TDC activity, reflected by a reduced presence of TDC-producing bacteria, will exhibit lower levels of dopamine production and levodopa reduction in the assay. Analytes in the collected samples are measured by means of ultra-performance liquid chromatography (UPLC) with electrochemical detection (ED) on a C\textsubscript{18} reverse-phase column in a short 9 min data acquisition program. Throughout the assay, 3,4-dihydroxybenzylamine hydrobromide (DHBA) is included as an internal standard, and the calibration standards for UPLC-ED quantification are prepared in a homogenized fecal matrix to account for matrix effects in the detection of levodopa and dopamine.

**Reagents**

- PD patient fecal samples stored at -80 °C upon collection.
- *Enterococcus faecalis* V583, stored as a glycerol stock at -80 °C.
- 3,4-dihydroxybenzylamine hydrobromide (DHBA) (Sigma-Aldrich, cat. no. 858781), stored at RT.
● 2-(3,4-Dihydroxyphenyl)ethylamine hydrochloride or dopamine HCl (dopamine) (Sigma-Aldrich, cat. no. H8502), stored at 4 °C.

● L-3,4-dihydroxyphenylalanine or levodopa (Sigma-Aldrich, cat. no. PHR1271), stored at 4 °C.

● Kanamycin sulfate (Sigma-Adrich, cat. no. 60615).

● Formic acid for analysis, 98-100% (Merck-Millipore, cat. no. 533002), stored at RT. **CAUTION** Formic acid is corrosive and should be handled in a fume hood; use appropriate personal protective equipment.

● Hydrochloric acid (HCl) for analysis, 37% (Merck-Millipore, cat. no. 100317), stored at RT. **CAUTION** Hydrochloric acid is corrosive and should be handled in a fume hood; use appropriate personal protective equipment.

● Perchloric acid (HClO₄), 70-72% (Merck-Millipore, cat. no. 1.00519.1001), stored at RT. **CAUTION** Perchloric acid is corrosive and should be handled in a fume hood; use appropriate personal protective equipment.

● Ethanol **CAUTION** Ethanol is highly flammable and should be handled in a fume hood; use appropriate personal protective equipment.

● Homogenized fecal material (see reagents setup).

● Enriched beef broth with supplemented kanamycin (see reagents setup).

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**Reagent set-up**

**0.7% perchloric acid (HClO₄)**

Dilute 70-72% HClO₄ in MilliQ ultrapure water to reach a 100x dilution such that the final concentration is 0.7%. Store 0.7% HClO₄ at 4 °C.

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**Aqueous UPLC-ED solutions**

All the solutions for UPLC use are prepared using MilliQ ultrapure water. The mobile phase solutions required are:

● MilliQ ultrapure water + 0.1% formic acid (Solution A)

● 100% methanol + 0.1% formic acid (Solution B)
Solutions required for column storage (Solution C) and syringe/needle washes (Solution D) are:

- 50% methanol in MilliQ ultrapure water + 0.1% formic acid (Solution C)
- 20% methanol in MilliQ ultrapure water + 0.1% formic acid (Solution D)

Prepare sufficient solutions to enable analysis of the whole sample set. These aqueous solutions can be stored at RT for up to a year. **CAUTION** All solutions should be prepared in a fume hood.

**Potassium buffered saline (PBS)**

Dissolve 8.01 g of NaCl, 2.01 g of KCl, 17.79 g of Na₂HPO₄·2H₂O, and 2.45 g of KH₂PO₄ anhydrous in 700 mL demiwater, and bring to 1 L. Adjust the pH to 7.4 with either 1 M HCl or NaOH if necessary. Store at 4 °C for up to 6 months.

**DHBA stock**

Prepare 100 mM DHBA using 1 M HCl as solvent. Filter sterilize with a sterile 0.2 µm filter and store at -20 °C for up to 2 months.

**Catecholamine master stocks**

Prepare master stocks of levodopa (200 mM) and dopamine (100 mM) using 1 M HCl as solvent. Filter sterilize with a sterile 0.2 µm filter and store at -20 °C for up to 2 months.

**Levodopa reaction stock for TDC activity assay**

Using the 200 mM levodopa master stock, prepare a reaction stock of levodopa at 10 mM to use in the fermentation reaction, using sterile MilliQ ultrapure water as a solvent. **CRITICAL:** Prepare fresh just before the assay when it is required. Levodopa undergoes spontaneous oxidation at neutral and alkaline pH and will degrade over time unless kept in an acidic environment.

**Standard catecholamine mixture stock for UPLC-ED calibration**

Using 200 mM levodopa and 100 mM dopamine stocks, prepare a standard mixture stock containing both dopamine and levodopa at 10 mM using MilliQ ultrapure water as a solvent. Make a serial dilution
of the standard mixture stock 2-fold in MilliQ ultrapure water to obtain standard mixture stocks containing the following five concentrations: 10 mM, 5 mM, 2.5 mM, 1.25 mM, and 0.625 mM. **CRITICAL:** Prepare fresh just before the assay when it is required. These catecholamines undergo spontaneous oxidation at neutral and alkaline pH and will degrade over time unless kept in an acidic environment\textsuperscript{12,13}.

**Enriched Beef Broth With Supplemented Kanamycin (EBB/K)**

1. Prepare the 1000x vitamin solution following the following steps:

   a. Prepare $10^4$x vitamin solution (pre-stock) by dissolving the indicated weights of ingredients below in 100 mL H$_2$O (Supplementary Table 1).

   b. Adjust the pH of the $10^4$ vitamin pre-stock solution to 7 with 1M NaOH to dissolve folic acid and filter sterilize with a 0.2 µm filter. Store at 4 °C for up to 6 months.

   c. Prepare $10^6$x vitamin K1 solution (pre-stock) by dissolving 5 µL vitamin K1 (0.984 g/mL) in 5 mL 100% ethanol. Store at 4 °C for up to 6 months.

   d. Mix 1,000 µL $10^4$x vitamin pre-stock, 10 µL $10^6$x vitamin K1 pre-stock, and 8,990 µL sterile H$_2$O to obtain the final stock. Store at 4 °C for up to 6 months.

2. Prepare the following stock solutions as described in Supplementary Table 2.

3. Prepare the Enriched Beef Broth (EBB) using the stocks prepared in steps 1-2, supplemented with kanamycin as described in \textsuperscript{6}, Supplementary Table 3 (for 1 L). Store EBB/K at 4 °C for up to 1 month.

**Homogenized Human Fecal Material**

Transfer the experimental batch of fecal samples from the storage freezer to a biosafety cabinet, keeping the material on ice. Weigh equal parts of all experimental fecal samples or a representative fraction, depending on the sample size, and collect them in a sterile 2.0 mL microcentrifuge tube. Use a larger storage vessel if necessary due to the number of samples and volume collected. Avoid gathering large undigested residues of food present in the fecal debris, as this can lead to inflated weight measurements and inaccurate representation of the fecal matrix. While maintaining the fecal material on ice, homogenize it through cycles of thorough manual mixing and brief centrifugation (4 °C, ~5s). Store this homogenized fecal material along with the original samples at -80 °C.
Equipment

- MilliQ water-ultrapure (>18 Ω cm\(^{-1}\)), filtered and deionized water, supplied by a MilliQ system (Merck; Millipore, Bedford, MA, USA). It is important that MilliQ ultrapure water is used throughout the protocol, especially in the UPLC-ED system.

- Disposable pipette tips (Gilson; 2-200 µL, cat. no. F171300; 20-300 µL, cat. no. F161731; 2-30 µL, cat. no. F171303; 100-1000 µL, cat. no. F171703. Sarstedt; 5 mL, cat. no. 70.1183.001).

- Single-channel adjustable volume pipettes (Gilson; P20, P200, P1000, cat. no. F167360).

- Microman electronic pipette controller (Gilson; cat. no. F110120).

- Coy Laboratory anaerobic chamber (neo-Lab Migge GmbH, Heidelberg, Germany).


- Dionex ED40 electrochemical detector (Dionex, Sunnyvale, USA, with a glassy carbon working electrode).

- Reverse-phase C18 column (Kinetex 5 µM, C\(_{18}\) 100 Å, 250 x 4.6 mm, Phenomenex, Utrecht, The Netherlands).

- Sterile 1.5 mL microcentrifuge tubes (FisherScientific; cat. no. 509GRDPFB).

- Sterile 2 mL microcentrifuge tubes (FisherScientific; cat. no. 508GRDPFB).

- Henke-ject 1 mL plastic syringes (HenkeSassWolf, cat. no. 8300034660).

- 15 mL centrifuge tubes (Sigma Aldrich, cat. no. Z707732).

- Cell culture Class II biological safety cabinet (CleanAir, Woerden, NL).

- pH meter (VWR international, cat. no. LM 18772).

- High-performance liquid chromatography 1.5 mL screw neck vials (BGB, cat. no. 080400-W).

- High-performance liquid chromatography silicone screw caps (BGB, cat. no. 070301).

- Microcentrifuge (Eppendorf, cat. no. 5404000014).

- Large centrifuge (Eppendorf, cat. no. 5805000010).
● Incubator (VWR INCU-Line).
● Analytical balance (Sartorius Laboratory).
● 0.2 µm filters (LLG- Syringe filters SPHEROS PES 0.22µm; & Phenomenex PHENEX AF0-3203-12).
● Autoclave.
● 1 mL 96-well 0.2 µm filter plate (AcroprepAdv, cat. no. 8682).
● P300 multi-channel pipette (Gilson, FA10015).
● Zone-Free pierceable plate seal (Excel Scientific, ZAF-PE-50).
● PlatePrep 96-well vacuum manifold (Sigma, 575650U).
● Vacuum pump.
● Chromeleon software (version 6.8 SR13).

**Equipment set-up**

**Incubator and coy laboratory anaerobic chamber**

The incubator should be placed inside the anaerobic chamber and set to 37 °C. The anaerobic chamber should be set up according to manufacturer's instructions, such that the anaerobic conditions are 1.5% H₂, 5% CO₂, balanced with N₂.

**UPLC-ED Set-Up**

Set up the UPLC-ED according to manufacturers instructions. For detailed set-up of the UPLC-ED data acquisition program used in this protocol see Table 1. Before each run, pre-equilibrate the C₁₈ column using a gradient of flow-rates and water/methanol with 0.1% formic acid (solutions A & B):

● 0-10 mins, 50% Solution A, 50% Solution B; flow = 0.4 mL/min
● 10-15 mins, 95% Solution A, 5% Solution B; flow = 0.7 mL/min
● 15-20 mins, 99% Solution A, 1% Solution B; flow = 1 mL/min
Between experiments maintain the C\textsubscript{18} column in a 1:1 ratio of water and methanol with 0.1% formic acid at a flow rate of 0.04 mL/min to maintain column integrity.

**96-well plate vacuum filtration manifold**

Attach the 96-well filtration manifold to the vacuum pump according to manufacturers instructions. Place a 96-well plate lid on top of the apparatus, turn on the vacuum pump, and slowly close the vacuum gauge. Ensure there are no leaks and a vacuum is pulled in the manifold.

## Procedure

### Preparation of the Fecal Fermentation Culture

1. Transfer the experimental fecal samples and the homogenized fecal material from the storage freezer to a biosafety cabinet and keep them on ice.

2. Weigh 50 mg of each experimental fecal sample and collect them in separate sterile 2.0 mL microcentrifuge tubes. Keep the tubes on ice until they are stored at 4 °C. Wait to proceed further until all samples within the batch have been weighed. **CRITICAL:** Avoid including large undigested residues of food present in the fecal debris as this could lead to inflated weight measurements, inaccurately representing the fecal matrix.

3. Weigh 5 aliquots of 50 mg of homogenized fecal material and collect them separately in 2.0 mL microcentrifuge tubes. Process these tubes together with the rest of the experimental samples. Keep the tubes on ice until they are stored at 4 °C. Wait to proceed further until all samples have been weighed.

4. Briefly centrifuge the 50 mg fecal samples at 4 °C for approximately 5 seconds to pellet them.

5. Add 1 mL of ice-cold PBS and gently pipette up and down to wash the fecal material.

6. Centrifuge the suspended fecal material for 8 minutes at 4,000 x g and 4 °C, then discard the supernatant.

7. Prepare ice-cold EBB/K media supplemented with 50 μM DHBA, which will serve as an internal standard throughout the assay.

8. Add 990 μL of the ice-cold DHBA-supplemented EBB/K to each fecal sample pellet and mix gently by pipetting up and down.
Experimental fermentation cultures and suspended homogenized fecal material can be kept at 4 °C and should be used within the same day.

Tyrosine Decarboxylase Activity Assay

Fermentation assay:

9. Transfer the experimental fermentation culture to the anaerobic chamber while keeping the samples on ice.

10. Add 10 µL of freshly prepared sterile 10 mM levodopa reaction stock to achieve a final levodopa concentration of 100 µM in the fermentation culture.

11. Gently mix the fermentation cultures by pipetting up and down. Collect 150 µL of each culture and immediately stop the reaction by adding it to 750 µL of ice-cold 0.7% HClO4. Collect time-point samples as follows: A. For a small number of samples, use 1.5 mL microcentrifuge tubes. Label each tube with a sample ID and time point indicator (e.g., t0). B. For a large number of samples, use a 96 deep-well plate. When processing more than 12 fermentation cultures (fecal samples), deep-well plates are recommended. Record the sample ID and time point indicator (e.g., t0) for each well.

12. Store the t0 samples at 4 °C.

13. Incubate the remaining levodopa-supplemented fermentation cultures anaerobically (1.5% H2 and 5% CO2, balanced with N2) at 37°C for 24 hours.

14. Collect time-point samples at 12 and 24 hours (t12 and t24, respectively) as described in step 11. Store these time points at 4 °C.

UPLC-ED calibration standards in homogenized fecal matrix

15. For each of the 5 suspended homogenized fecal material mixtures, add 10 µL from one of the five standard catecholamine mixture stocks (Supplementary Table 4). These final standards will be measured by UPLC-ED within the range of 6.25 - 100 µM. They are prepared in the homogenized fecal matrix to account for matrix effects in the electrochemical detection of dopamine and levodopa in UPLC-ED. **CRITICAL:** Prepare these standards fresh for each experiment.

16. Gently mix the calibration standards in biological matrices by pipetting up and down, and collect 150 µL from each mixture. Place the collected mixture into a 1.5 mL microcentrifuge tube containing 750 µL
of ice-cold 0.7% HClO₄. These five samples constitute the final calibration standards for UPLC-ED. Store them at 4 °C.

**PAUSE POINT** Fermentation time points and calibration standards in 0.7% HClO₄ can be stored at -20°C for a maximum of two months before proceeding with UPLC-ED sample preparation.

### UPLC-ED Sample Preparation

17. Centrifuge the reaction time points and calibration standards suspended in 0.7% HClO₄ to pellet the debris. Use 20 minutes of centrifugation at 4,000 x g and 4 °C.

#### Calibration standards

Calibration standards can be processed and filtered simultaneously with the reaction time points. In this protocol, it's assumed that the filter plates have no available wells for additional samples.

18. Using a 1 mL syringe and a 0.2 µm filter, filter 800 µL of the supernatant from each calibration standard. Transfer the filtered liquid into labeled 1.5 mL UPLC vials and securely seal the lids.

19. Store the filtered supernatants in the labeled UPLC vials at 4 °C.

#### Fermentation time points

20. Place a plastic plate seal on the bottom of a 96-well 0.2 µm filter plate and chill the plate on ice.

21. Carefully transfer 800 µL of the supernatants to the filter plate, making sure not to transfer any pellet. Seal any unused wells on the filter plate's top, taking care to prevent cross-contamination. Unused wells can be saved for another experiment. **CRITICAL:** Keep track of which well corresponds to each reaction time point.

22. Set up a 2.0 mL 96 deep-well plate in the lower compartment of the 96-well vacuum manifold apparatus according to the manufacturer's instructions. This plate will collect the filtered supernatants.

23. Remove the bottom seal from the filter plate and place it gently on top of the collection plate within the vacuum manifold apparatus. **CRITICAL:** Ensure both the collection plate and the filter plate are oriented correctly, aligning each well in the filter plate directly above its corresponding well on the
collection plate. This prevents sample bleed into neighboring wells. Always follow the manufacturer's instructions when using a 96-well vacuum manifold.

24. Open the remote vacuum gauge's bleed valve to prevent vacuum suction and activate the vacuum pump.

25. Ensure the filter plate is positioned to cover the rubber edges of the manifold. Gradually close the vacuum gauge while monitoring flow rates through the filter wells. Do not exceed -25 mHg (= -85 kPa or -0.85 bar). **CRITICAL:** Maintain a stable vacuum. Avoid touching the exposed filter plate as it may disrupt the vacuum. Sudden disruptions in the vacuum can cause air flow increases and result in sample bleed into neighboring wells.

26. Maintain a consistent vacuum for about 15 minutes, then slowly release the vacuum by carefully opening the vacuum gauge.

27. Turn off the vacuum pump and return the filter and collection plates to an ice bath to maintain their cold temperatures.

28. Inspect wells that didn't filter completely. If the filtrate collected is insufficient (less than 250 μL), filter the remaining supernatants with a syringe as described in step 18. Add the obtained filtrate to their corresponding wells on the collection plate.

29. Transfer 250 μL of the filtered samples to a 300 μL flat-bottom 96-well plate. Seal the plate with a Zone-Free pierceable sealing film.

**PAUSE POINT** Filtrates can be stored at 4°C for a maximum of two weeks before starting the UPLC-ED analysis.

**UPLC-ED Data Acquisition**

30. Prepare the UPLC-ED for the assay by pre-equilibrating the column following the conditions mentioned earlier. Open Chromeleon and configure the data acquisition program using the parameters outlined in Supplementary Table 5.

31. Position the flat-bottom 96-well plate, containing the samples, on the provided sampling tray of the autosampler, or as indicated in the manufacturer's instructions.

32. Create a new experiment and sequentially input the list of all the samples in duplicate. Include their respective locations on the sampling tray. Ensure that each run begins with a filtered 0.7% HClO4 sample,
followed by the five calibration standard samples. Conclude each run with another set of calibration standards for duplicate analysis. Arrange time points from the same sample together. Running samples in duplicate accounts for UPLC-ED analysis variability.

33. Confirm that the ED cell is powered on, and start the automated UPLC-ED data acquisition.

34. Following the run, store the column in solution C (50% methanol in MilliQ water with 0.1% formic acid) at a flow rate of 0.04 mL/min.

Data Processing

35. After processing the sample, review all chromatograms from the run and verify the following:

i. Ensure accurate labeling of each peak. If necessary, manually correct peak annotations.

ii. Check the accuracy of the area under the curve (AUC) for each peak. If needed, manually adjust peak area annotations.

36. Export all AUC values obtained during the run for DHBA, dopamine, and levodopa, from the summary tab.

37. Optional: export the chromatograms corresponding to all samples in the run for future data visualization.

Determining tyrosine decarboxylase activity:

38. Infer the concentration of each analyte from the obtained chromatogram AUCs (μA · min⁻¹). Normalize the AUC of levodopa and dopamine to the AUC of their respective internal standard (DHBA) using this formula for each analyte in a sample: \[ \text{AUC}_{\text{normalized}} = \frac{\text{AUC}_{\text{analyte}}}{\text{AUC}_{\text{DHBA}}} \]. This normalization accounts for UPLC-ED analysis variations.

39. Construct a calibration curve using the normalized AUCs of the calibration standards, which were run in duplicate, along with their supplemented analyte concentrations.

40. Utilize the formula derived from the linear model of the calibration curve (concentration analyte = a x \(\text{nAUC}_{\text{analyte}} + b\)) to calculate levodopa and dopamine concentrations in the samples, using their respective normalized AUC. Here, ‘a’ and ‘b’ are parameters determined from the linear regression model.

41. Since each sample is injected twice in UPLC-ED, calculate the average concentration of levodopa and dopamine from both measurements. **CRITICAL:** The obtained concentrations should be closely similar,
and significant variations between replicates might indicate equipment or solvent issues.

42. Determine TDC activity for intervals 0-12h and 0-24h using formula 1.

43. Determine the percentage of levodopa depletion in intervals 0-12h and 0-24h using formula 2.

**Formula 1:**

\[
\%\text{levodopa decarboxylated} = \frac{([\text{dopamine 12 h or 24 h}] - [\text{dopamine 0 h}])}{[\text{levodopa 0 h}]} \times 100
\]

**Formula 2:**

\[
\%\text{levodopa depleted} = \frac{([\text{levodopa 0 h}] - [\text{levodopa 12 h or 24 h}])}{[\text{levodopa 12 h or 24 h}]} \times 100
\]

**Troubleshooting**

**Time Taken**

**Fermentation mix preparation (total 6.5 h)**

Weighing samples (4 h), steps 1 - 3

Washing samples (1.5 h), steps 4 - 6

Suspending in media (1 h), steps 7 + 8

**Fermentation assay (total 4 h)**

Substrate supplementation (30 min), steps 9 + 10

Incubation & sampling (45 min, x3), steps 11 - 14

Calibration standard preparation (30 min), steps 15 + 16

**UPLC-ED sample preparation (total 1 h)**
Steps 17 - 29

**UPLC-ED data acquisition (total 90 h)**

UPLC-ED setup (1 h), steps 30 - 32

Automated data acquisition (~89 h), steps 33 + 34

**Anticipated Results**

**References**


**Figures**

Figure 1. **Diagrammatic summary of the entire fecal tyrosine decarboxylase activity assay procedure.** The protocol includes sample preparation, the fermentation of fecal material, UPLC-ED sample preparation, and product and substrate analysis with UPLC-ED.
Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTablesTDCAvivityProtocol.pdf