Attachment 5: Optional Pharmacokinetic Samples for Testing at Alturas Analytics

During tecovirimat treatment, plasma samples may be collected to monitor tecovirimat levels for adequate drug exposure in patients. Collected pharmacokinetic (PK) s samples s will be assessed at a manufacturer-designated laboratory (Alturas Analytics). The schedule for PK sample timepoints differs for outpatients versus inpatients. Please see below for plasma sample collection timepoints and shipping instructions. Please be informed that the **individual patient results cannot be reported back to the providers** for directly informing individual patient treatment decisions as the PK test is not certified under the Clinical Laboratory Improvement Amendments (CLIA) regulations.

I. TIMING OF PK SAMPLE COLLECTION

Outpatients

For ambulatory patients treated as outpatients (**Figure 1A**), consider collecting at least one PK sample after Day 6 of treatment or sooner for certain patients on a case-by-case (e.g., pediatric, pregnant persons, or those with certain underlying conditions whose drug exposure levels may need to be monitored closely, the timing of PK sample collections may be customized individually as needed). To the extent possible, plasma samples should be drawn just prior (< 30 minutes) to a dose in order to obtain trough values due to the importance of C_{min} for assessing adequate dosing. This could be at the 12-hour time point or the 24-hour time point (i.e., just prior to the second dose in a day or just prior to the first dose the next day). Example plasma sampling day/time is shown in **Figure 1A**. In certain patients (e.g., pediatric, critically ill patients) whose drug exposure levels may need to be monitored, the timing of PK sample collections may be customized individually as needed.

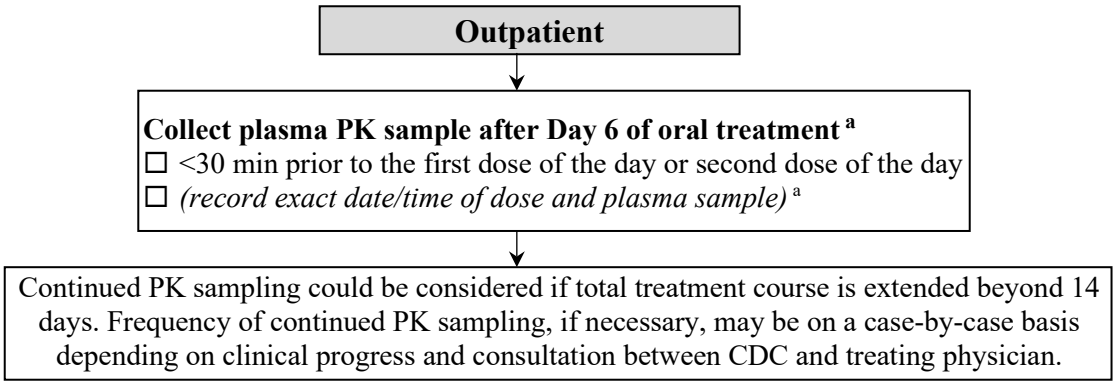
Inpatients

To the extent possible, trough and post-dose plasma samples should be drawn early in the patient's treatment period (Day 2–6) and toward the end of the treatment period (Day 7–14). Patients who receive IV tecovirimat and then are switched to oral tecovirimat therapy should ideally be monitored early at the initiation of IV therapy (day 2–6 of IV therapy) and again early at the initiation of oral therapy (day 2–6 of oral therapy). Those patients should ideally have at least one additional sample drawn toward the end of the treatment period, regardless of the route of administration (Day 7–14). Example plasma sampling days and times are shown in **Figure 1B.**

Trough samples should be collected just prior (<30 minutes) to a dose due to the importance of obtaining C_{min} values. Post-dose samples should be collected 4 hours after a dose. If these time windows are missed, still collect a plasma sample. Plasma samples may be collected up to 48 hours after the last dose, if necessary. Record the exact time the patient took the dose and exact time of all plasma collections.

If feasible, plasma samples should be collected on Day 2, just prior (<30 minutes) to patient's receipt of the third dose and at 4 hours after the third dose. Additional plasma samples should be collected toward the end of the treatment course (e.g., Day 7–14), with one collection occurring prior to a dose and one collection occurring 4 hours after a dose.

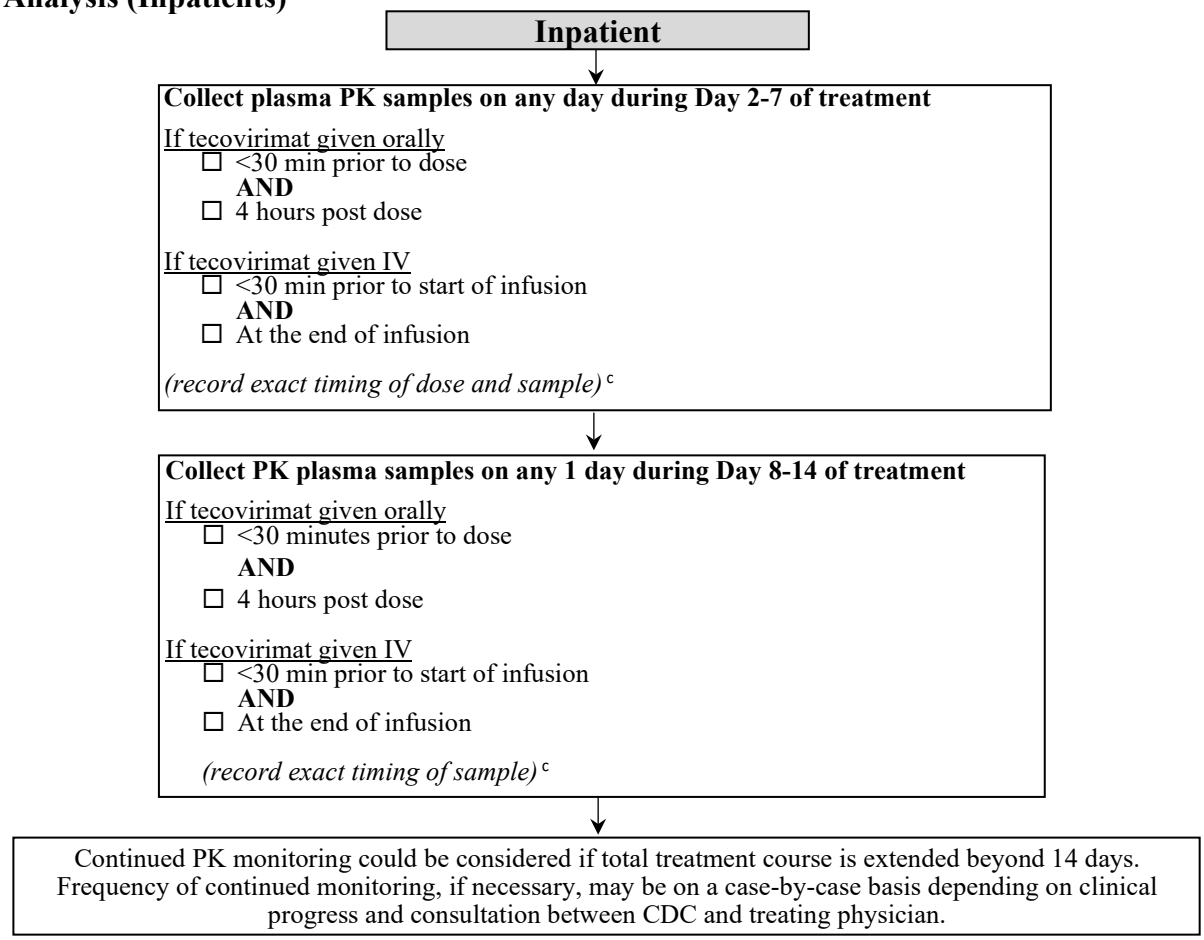
Figure 1A. Example Flow Chart of Plasma Sampling for Pharmacokinetic Testing During Oral Therapy (Outpatients)[a]



[a] In certain patients (e.g., pediatric, pregnant persons, or, those with certain underlying conditions) whose drug exposure levels may need to be monitored, the timing of PK sample collections may be customized individually as needed

[b] Where feasible, schedule patient's in-person follow-up that can allow trough PK plasma collection (blood draw <30 min before the next tecovirimat dose); if not feasible, then collect at 4 hours post-dose. If these time windows are not feasible, then record the time the patient took the last dose and exact time of plasma collection.

Figure 1B. Example Flow Chart of Tecovirimat Plasma Sampling Schedule for Pharmacokinetic Analysis (Inpatients)[a,b]



a Adjustment to frequency of plasma collection may be considered based on patient's clinical progress in consultation between treating physician and CDC.

b If a patient is switched from IV to oral therapy, a PK sample may be obtained just prior (<30 minutes) to patient's receipt of the third **oral dose** and at 4 hours after the third **oral dose**.

c Ideally, timing of plasma sample collection should be approximately at the same time post-dose to allow for relevant comparison.

II. SAMPLE COLLECTION AND SHIPMENT

Plasma Sample Collection and Preparation Instructions:

1. Please collect target volume of whole blood per time point:
    a. Patients ≤ 10 kg: 1.2 mL of whole blood
    b. Patients 11–20 kg: 2.5 mL of whole blood
    c. Patients > 20 kg: 5 mL of whole blood
    d. **NOTE: preferred collection time is immediately prior to dosing (C_{min})**
        1. This could be at the 12-hour time point or the 24-hour time point (i.e., just prior to the second dose in a day or just prior to the first dose the next day)
    e. NOTE: preferred collection day is after Day 6 of treatment. However, collect plasma sample when feasible, recording tecovirimat treatment day, exact date/time patient took the dose and date/time of plasma collection
2. Anticoagulant: K_3EDTA or K_2EDTA tubes should be chilled (on wet ice) immediately following blood collection (do not use a cryoblock as this could lyse RBCs).
3. Whole blood samples should be centrifuged within 40 minutes of collection.
    a. Samples should be processed to plasma by centrifugation at a speed of 2000–2200g for 10–15 minutes.
    b. NOTE: Split the sample and RETAIN ONE sample as a frozen back up.
4. **For samples collected from a patient with confirmed or suspected mpox, conduct the following methanol inactivation procedure.**
    a. Plasma may be methanol inactivated immediately or must be stored at minus 80°C (+/- 10°C) if methanol is to be added at a later time.
        1. Inactivate virus by addition of 3 parts methanol (HPLC grade) to 1 part plasma. A typical inactivation would be 100 microliters plasma + 300 microliters HPLC grade methanol.
    b. Vortex vigorously long enough to ensure adequate mixing of methanol and plasma (3 to 4 seconds).
        1. Alternately, the sample may be mixed by hand by vigorous shaking for approximately 30 seconds.
    c. The methanol-inactivated plasma must be stored at minus 80°C (+/- 10°C).
        1. Anytime the plasma is thawed (before or after addition of methanol), thawing should occur on the bench at room temperature or in a tepid water bath.
5. Be sure to label samples with the following minimum information:

• Whether or not methanol inactivated • Patient ID • Tecovirimat dose (mg) and frequency	• Tecovirimat treatment day number (dose number) • Date & time of last tecovirimat dose (exact date & military time) • Date & time of plasma collection (exact date & military time)

6. The plasma samples should be shipped **frozen on dry ice.**
7. **NOTE: Ensure sufficient quantity of dry ice is included so that it does not thaw completely during transit. Samples must arrive on dry ice in order for them to be analyzed.**
8. Ship to: Alturas Analytics Inc.
   Attention: Lawrie Malsack and/or Tara O'Brien
   1917 S Main Street
   Moscow, ID 83843

   Please ensure notifying CDC by email (regaffairs@cdc.gov) if PK samples are sent to Alturas. This is necessary to match the patient treatment information submitted to CDC under the IND with the patient PK samples sent to Alturas.

   Send email notification to the following prior to shipment:
   Tara O'Brien (tobrien@alturasanalytics.com); Phone: (208) 883-3400
   Lawrie Malsack (lmalsack@alturasanalytics.com); Phone: (208) 883-3400
   Emmanuel Onua (eonua@siga.com); Phone; (541) 250-6305
   Kady Honeychurch (khoneychurch@siga.com); (541) 908-1868
   CDC Regulatory Affairs (regaffairs@cdc.gov)

   **Do not include personally identifiable information in this email notification unless the email is securely encrypted

III. Include a Shipping Log of Patient Plasma Sample Collected for Tecovirimat PK Testing as Shown below.

Complete and include this shipping log for each patient PK sample(s)

Hospital-Issued Patient ID: _____ Treating Physician Name: _____

Hospital Name/Address: _____

Anticoagulant used *(K_3EDTA is preferred)*: _____

Tecovirimat Dose (mg) and frequency	Indicate Oral, NGT, or IV	Tecovirimat Treatment Day Number	Date & Time of Tecovirimat Dose (exact date & military time)	Date & Time of Plasma Collection (exact date & military time)
			_____/_____/_____ ____:____	_____/_____/_____ ____:____
			_____/_____/_____ ____:____	_____/_____/_____ ____:____
			_____/_____/_____ ____:____	_____/_____/_____ ____:____
			_____/_____/_____ ____:____	_____/_____/_____ ____:____

Name of Person Preparing Shipment (please print):_____

Shipment Preparer's Contact Information:_____