Challenges of PrEP Regimen Adherence and Monitoring of Kidney Function on Self-collect DBS

Kavinda DeSilva [1*], Shalena Utterback [1], Tami West [1], Diana Do [1], Canan Schumann Ph.D. [1], Brad Thorson[2], Charles Sailey M.D. [1], Mariko Nakano Ph.D.[1] Siobhan McKenny[1]

[1]Department of Research and Development, [2]Business Development, Molecular Testing Labs® (Vancouver, WA)

Objective

Design and develop non traditional samples collection technique for prescription monitoring and kidney functionality evaluation for patients on PrEP medication such as Truvada®.

Background

The CDC reports that Pre-exposure Prophylaxis (PrEP) reduces the risk of contracting HIV through sexual intercourse by 99% and through the use of intravenous drugs by 74%. Unfortunately, PrEP is less effective when regimen adherence is low. Furthermore, PrEP therapy is associated with a risk of moderately reduced kidney function. Tenofovir diphosphate (TFV-DP), the phosphorylated active metabolite of TDF (Truvada) has a half-life of 6-7 days and is an excellent serum biomarker. Thus, it is beneficial to monitor the stable metabolite for therapy adherence as well as serum creatinine for kidney function.

Demand for alternative matrices for prescription monitoring has increased in the recent years. Even though blood and saliva have been used as a more common alternate matrix, dried blood spot (DBS) is a more promising matrix. The use of DBS as an alternate matrix has more advantages than disadvantages due it being non-invasive, easily accessible and improves stability. As a pioneer in DBS testing landscape Molecular® has developed DBS testing to monitor both a adherence as well as kidney functionality.

Experiment

TFV-DP DBS Method:

Samples were extracted using a combination of protein precipitate and liquid-liquid extraction using two 3 mm punches. 15 µL of the extracted sample was injected to LC/MS/MS for analysis. Sciex 5500 QQQ coupled with Shimadzu LC-20 MPX system were used with 100mM Ammonium Acetate as aqueous mobile phase and 75:25 5mM Ammonium Bicarbonate: Acetonitrile as the organic mobile phase.

Creatinine DBS Method:

Samples were extracted using protein precipitation where single 6 mm punch was extracted using Internal standard fortified Acetonitrile-Water mixture. 5 µL of the extracted sample was injected to LC/MS/MS for analysis. Sciex 6500 QQQ coupled with Shimadzu LC-30 system were used with 0.1% Formic Acid in Water as aqueous mobile phase and 0.1% Formic Acid in Methanol was used as the organic mobile phase.

Figure 1. ***(Left)*** **DBS Punch used for Creatinine** ***(Right)*** **DBS Punch used for TFV-DP.**

Analytical measurement ranges (AMR) were evaluated N=6 for creatinine over a range of 0.02 mg/dL to 2 mg/dL. The assay was linear over this range with accuracy ranging from 86.8% - 100.7%, and R^2 0.99993 with %CV < 5%. AMR were evaluated n = 5 for TFV-DP over a range of 300 fmol/punch to 25,000 fmol/punch. TFV-DP assay was linear over this range with accuracy ranging from 81.9% to 106.5%, and R^2 0.99346 with %CV <9.5%.

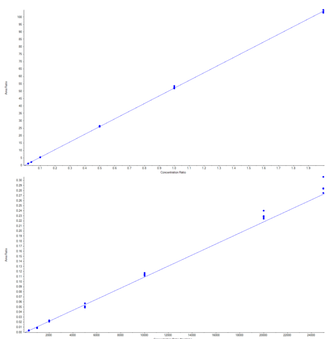

Figure 2. ***(Top)*** **AMR for Creatinine** ***(Bottom)*** **AMR for TFV-DP.**

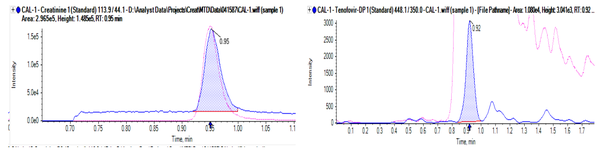

Figure 3. LOQ Standard . ***(Left)*** **Creatinine and** ***(Right)*** **TFV-DP**

LLOQ (Figure 3) for Creatinine was 0.02 mg/dL with s/n = 212 and for TFV-DP at 300 fmol/punch with s/n = 104. SCIEX 6500 QQQ was used for Creatinine instead of 5500 QQQ to attain a better s/n ratio, as the baseline on the 5500 instrumentation was higher in comparison to the 6500. Overall, a high s/n for TFV-DP gives us more opportunity to lower the LLOQ for TFV-DP further.

Analyte Name	# of Replicates	Conc.	% CV
Creatinine	20	0.02 mg/dL	1.5%
Creatinine	20	2 mg/dL	0.7%
TFV-DP	15	300 fmol/punch	14.8%
TFV-DP	15	25,000 fmol/punch	2.6%

Table 1. Intra-Day Precision for Creatinine and TFV-DP

Analyte Name	# of Days	Sample	Between Day Precision	Total Precision
Creatinine	6	QC-1	5.4%	6.1%
Creatinine	6	QC-2	1.7%	5.0%
Creatinine	6	QC-3	3.4%	3.0%
TFV-DP	10	QC-1	8.8%	10.2%
TFV-DP	10	QC-2	12.4%	15.4%

Table 2. Repeatability for Creatinine and TFV-DP
Inter-Day repeatability was done over 6 days for Creatinine and over 10 days for TFV-DP. Triplicate matrix QC samples utilized for this evaluation and were assayed twice a day with at least 2hrs between the runs.

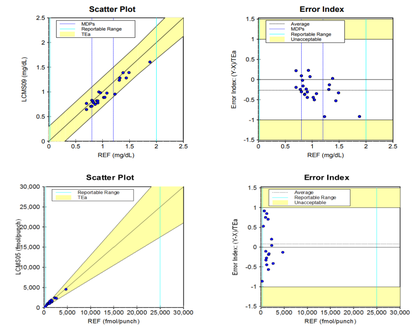

Figure 4. Correlation ***(top)*** **Creatinine** ***(bottom)*** **TFV-DP**
N = 24 samples for used for creatinine; R = 0.9576 and slope = 0.880

N = 20 samples used for TFV-DP; R = 0.9816 and slope = 0.945

Analyte Name	RTP Stability
Creatinine DBS	7 Weeks
TFV- DP DBS	5 Days

Table 3. Stability of Creatinine and TFV-DP on DBS
Creatinine on DBS was pretty stable when compared to TFV-DP. Stability of TFV-DP on DBS drops significantly at RTP conditions.

Conclusion & Future Developments

- Creatinine test on DBS had an excellent correlation with a traditional sample type (serum), which can be utilized for monitoring kidney functions under TFV treatment.
- TFV-DP in DBS also proves to be a standardized methodology to measure TFV-DP and adherence monitoring.
- As DBS successfully utilized as a self-collected specimen, at-home collection will help increase patient care and expedite therapeutic intervention.

Future Development

- Optimize the assay to lower the TFV-DP LOQ monitoring adherence on Descovy® prescriptions.
- Combine Creatinine and TFV-DP to a single extraction to offer more cost effective test to the market.

Acknowledgement:
Craig Sykes, Mackenzie L. Cottrell, Angela DM Kashuba @ UNC

*Contact: Kavinda De Silva, kavindad@moleculartestinglabs.com

Reference:
1. *J Pharm Biomed Anal. 2018 February 05; 149: 40–45; 2. Journal of Antimicrobial Chemotherapy, 66(2), 240–250, 3J Kidney Di s. 201 0; 5 5(4) :622-627..*

MOLECULAR® TESTING LABS