An important recent development in the field of HIV/AIDS care and treatment is the discovery that taking an antiretroviral medication 2–24 h before high risk sexual activity followed by daily doses for 2 days significantly lowers risk for HIV transmission (1). This strategy, called preexposure prophylaxis or PrEP, is naturally changing behavior amongst high-risk groups who are HIV negative. The particular approach of taking pills just before and after a high-risk encounter is known as “on demand” or “intermittent” use and has important practical implications. The conventional dictate for antiretroviral therapy has been that maximal viral suppression occurs when patients take at least 90% of their doses. For PrEP, although the recommendation is still that largest protection is upon daily dosing, high-risk individuals may take the medication around only high-risk encounters.

To understand how we can know when PrEP users have adequate protection to guide clinical, or in this case, preclinical care, we spoke with Dr. Peter Anderson, professor of pharmaceutical sciences at the University of Colorado, who has had a long interest in nucleoside analogs and has developed an assay to measure adherence to PrEP.

What Is the Innovation?

To determine if an HIV positive patient is taking a medication, or more importantly having a clinical effect, HIV viral load can be measured. In HIV negative patients (potential PrEP candidates), viral load, however, clearly has no role. Another approach is needed, and that is where the work of Anderson and his colleague Lane Bushman comes in.

The US Food and Drug Administration approved medication for PrEP is a combination of nucleoside analogs tenofovir (TFV) and emtricitabine (FTC), which as a class have been shown to undergo phosphorylation in red blood cells (RBCs) (2). Phosphorylation increases the half-life of the drug forms, allowing them to be measured in the blood as a marker for adherence. The investigators who demonstrated this effect, however, concluded that the drug forms in RBCs were actually contaminants of the main cells in focus at the time, peripheral blood mononuclear cells. Although Anderson and Bushman thought the RBC was owed a second look, they believed purified RBCs were not practical for widespread deployment. So they characterized the pharmacokinetics of intracellular TFV diphosphate (TFV-DP) and FTC triphosphate (FTC-TP) off RBCs and dried blood spots (DBS) (3).

The team found that TFV-DP had a long half-life over 17 days. “The long half-life is perfectly suited for assessing cumulative adherence to TFV-based therapy, and the plentiful red blood cells and ease of obtaining the DBS matrix make it practical for clinical/research settings,” says Anderson.

“To show that we could use DBS, we had to demonstrate reproducible extraction, stability, etc. along with the results being correlative to purified red blood cell concentrations,” explains Bushman. “These validation studies required the use of clinical samples because quality controls cannot adequately mimic TFV-DP within red blood cells from humans.”

“To the iPrEx (Preexposure Prophylaxis Initiative) study’s open label extension, we said - let’s add dried blood spot. At the end we found a strong relationship between DBS level and efficacy of PrEP,” says Anderson.

What Is the Method?

A 3-mm punch is taken from the DBS (Fig. 1). Next it is extracted in 70% methanol and then loaded onto a strong anion exchange, solid phase extraction column.
TFV-DP is isolated with salt gradients after which it is dephosphorylated. The dephosphorylated TFV is put through a final purification via reversed-phase solid phase extraction. Finally LC-MS/MS analysis is performed. The result of these steps gives a TFV-DP concentration. Adherence, reported in average doses per week and PrEP efficacy, are calculated based on standards derived from pharmacokinetics studies (3) and PrEP studies on men who have sex with men (4, 5).

Where Does It Fit in Clinical Practice?

Kenneth Mayer, professor of medicine at Harvard Medical School and leader in the field, appreciates the practical potential of DBS. “DBS has the advantage that the specimen in theory could be self-collected and sent in, and/or stored and run in batch, depending on the monitoring schema. Saving plasma or sera requires more laboratory effort, and will not measure intracellular drug levels,” he says.

Mayer’s colleague, Douglas Krakower from the Division of Infectious Diseases at the Beth Israel Deaconess Medical Center at Harvard, believes that DBS may “hone the accuracy of adherence assessment technologies” through its objectiveness. He sees value in the use of DBS for developers of adherence strategies as patient-reported adherence measures are limited by strong bias.

“There is a need for ways to accurately measure adherence on an individualized basis during routine care so that clinicians and programs can focus their resources to provide the most intensive adherence support to those who are risk of non-adherence. Studies suggest that persons who initiate PrEP and then use it continuously, with high levels of adherence, can greatly reduce their risk for HIV acquisition, so technologies that can support individuals to make optimal decisions about whether or not to utilize or discontinue PrEP will be of great value,” says Krakower.

An obvious closing question is whether DBS is ready for “prime time.” Acknowledging that today’s method takes “significant expertise and technology to obtain the needed level of sensitivity, accuracy and precision,” Anderson mentions that he has collaborated with a laboratory in South Africa to “transfer the technology including a successful laboratory cross-validation.”

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures or Potential Conflicts of Interest: No authors declared any potential conflicts of interest.
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DOI: 10.1373/clinchem.2015.253179