Clinical Chemistry

# Throughput Considerations for a Sample-Multiplexed LC-MS/MS Assay: Is the Ability to Double the Injection Throughput Always a Time Saver?

**Article:**
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**Guest:** Dr. David Wells is a pharmaceutical scientist who provides consulting services through his company, Wells Medical Research Services.

Bob Barrett: This is a podcast from *Clinical Chemistry,* sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I am Bob Barrett.

An important goal of most clinical laboratories is to optimize throughput, especially for high volume assays using liquid chromatography tandem mass spectrometry. Reducing the turnaround time for patient testing is key, but it must be done in a manner that maintains the quality of the assay. In the September 2020 issue of *Clinical Chemistry*, Julia Colletti and her colleagues at Quest Diagnostics published a paper on increasing throughput for quantification of total testosterone in serum by LC tandem mass spec by sample multiplexing. The same issue contains an accompanying Editorial asking if the ability to double the injection throughput always saves time. Dr. David Wells is a pharmaceutical scientist who provides consulting services through his company, Wells Medical Research Services. He wrote that Editorial and he's our guest in this podcast. So Dr. Wells, why is high throughput important for clinical bioanalysis and what are some of the ways in which high throughput can be achieved?

David Wells: High throughput is important because there's an ever increasing need to analyze more samples in a shorter amount of time. There's more data, more samples. Companies want to shorten their timelines for drug studies, so, having the data available quicker helps all steps of the process. Some ways in which high throughput can be achieved, if you look at the sample preparation as the start of it, just the act of parallel processing on multiple samples at one time is the first step to achieving higher throughput to processing more samples per unit time. And that's commonly done nowadays with the 96-well plate format. So, no longer are people using test tubes or individual columns. So samples can be prepared in parallel using 96 microplate and on top of that, automation processes to send the liquids through the 96-wells at a time and to collect eluates is also important. So, that reduces analysts' hands-on time. Anything to free up analyst time and to process more samples faster is a big plus, and then beyond sample preparation going toward the injection, the LC columns can

 

provide higher throughput. There's shorter columns, there's faster flow rates, there's unique particle chemistries. Those are the three main modules for which high throughput can be achieved.

Bob Barrett: Doctor, what exactly is sample multiplexing? And how was it used in this paper that applied it to the assay of total testosterone?

David Wells: Sample multiplexing, the word "multiplexing" is used in different ways, but in this specific paper, which is the correct way, it's being able to inject, for example, two samples at the same time. So normally, there are Patient A and Patient B, Patient C, each of those will be a single ejection. With sample multiplexing, you can derivatize the samples in different ways so that the mass spectrometer can then differentiate them during the detection process. So, Patient A and Patient B can actually be put into the same injection well and in,ject it together, but because each of them has been derivatized separately, the mass spectrometry will be able to identify each one. So, you reduce the number of samples injected. Instead of a hundred injections, you would do 50 injections. So, that improves your throughput in that way.

Bob Barrett: Would you consider such procedures to be ones that everyone can and maybe should adopt?

David Wells: I would consider it more of an advanced procedure. Number one, it depends on the analyte if it can be derivatized and if there are closely related chemical derivatives to be able to be analyzed together in the same assay. It takes some time and it takes some knowledge of chemistry. So, I would consider it more of an advanced technique because it's not universal for every analyte and it does require some know-how with the instrumentation and the procedure itself. So, I would reserve it for certain situations when you want to improve throughput by another factor, but you've exhausted the more traditional ways of doing so.

Bob Barrett: What are the considerations of using multiple sample preparation procedures prior to analysis? Tell us the pluses and the minuses.

David Wells: Okay. So, typically, I guess an ideal sense, people want one sample preparation procedure that's fast, efficient, produces a clean eluate for injection, and at a reduced cost. But that's not always possible, so there's a sacrifice. So, on the simple end of the scale is protein precipitation, so it could be a quick and straightforward procedure. It doesn't rely on chemistry too much, but you have a high potential for the matrix effect that can give you some reduced signal. Liquid

extraction as an intermediate technique to solid-phase extraction as a more advanced technique, but may require more time and requires more cost for the materials and the device. So, these are all considerations. And then if you let the liquid chromatography do more of the separation, then you can get away with a less efficient sample preparation procedure. So sometimes, people would actually stack the procedures to one and then the other. So, in this particular paper under discussion, there was protein precipitation first and then the derivatization procedure and then solid-phase extraction. So, you can stack procedures, but it adds time. So, it's all a consideration of throughput time and cleanliness of the sample.

Bob Barrett: Doctor, what types of sample preparation techniques usually provide for the highest throughput?

David Wells: Well, I believe protein precipitation, solid phase extraction provide very high throughput because you can minimize the analyst hands-on time. You can also use online solid-phase extraction techniques, either individual disposable cartridges or reusable and something like turbo flow chromatography. So, those would give you the highest throughput. Lowest throughput would be one that requires more hands-on time or manipulation. For example, liquid-liquid extraction can give you very clean eluate, but it's a very labor intensive process analyst hands-on time. You got to evaporate, allot time for that. There's reconstitution and with every step, there's a potential for analyte loss. You want to contain the analyte as much as possible within the sample well.

Bob Barrett: How did the introduction of sample multiplexing affect the throughput of this particular testosterone assay in this report in *Clinical Chemistry*?

David Wells: In this particular assay, the throughput was doubled because they were able to separate the samples out into derivatizing Agent A and B and then combine those for the injection. So, it was doubled. So, it took half the amount of time as earlier.

Bob Barrett: And finally, what suggestions would you have for our listeners if they choose to adopt this multiplexing approach for one of their assays?

David Wells: I would suggest that users adopt a method development approach. Number one, the chemistry needs to be proven if the derivatization will work and provide the signal-to-noise desired and second, the cleanliness of the eluate needs to be identified. So, the derivatization procedure is more of a dirtier procedure in which you are adding different chemicals and you need to extract those out before injection, otherwise, those chemicals will be injected as well. So, it

requires more of a method development, how clean is the eluate, how much time is this saving us? Does it chemically make sense and does the mass spectrometer detect it efficiently? And also, it most likely requires a cleaner sample preparation step up front and maybe even two procedures. So, I would look at the time required, for example, if protein precipitation was done first and then derivatization and then solid-phase extraction. I would justify each of the steps in great detail because once you adopt the method and multiple laboratories use it, then the air or the time is magnified for each individual laboratory. Ideally, I would see if a solid-phase extraction step could be the only sample prep step you would need after derivatization because protein precipitation just adds more time. So, I would justify each of the individual steps. What do we need to add to get the cleanest eluate and can we justify this in terms of time, cost, and overall analyst hands-on time in the assay.

Bob Barrett: That was Dr. David Wells of Wells Medical Research Services. He's the author of an Editorial on throughput considerations for a sample multiplex LC tandem mass spec assay that appears in the September 2020 issue of *Clinical Chemistry,* along with an original scientific paper applying sample multiplexing to an assay for testosterone. I'm Bob Barrett. Thanks for listening.