# Liquid Handling and the ELISA Protocol: Making the case for a 96-channel semi-automated pipette in your lab

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**Abstract:** Enzyme-linked immunosorbent assays (ELISA) are commonly used analytical biochemistry assays. The specific techniques vary significantly in the details and sequence of the biological interactions that occur, but they all require a similar range of liquid handling operations. The purpose of this paper is to illuminate the potential benefits of using the Avidien microPro300 semi-automated benchtop pipettor when performing these assays in either a 96 or 384 well microplate format.

## 1 Introduction

Enzyme-linked immunosorbent assays (ELISA) are commonly used analytical biochemistry assays, and for that reason we have chosen a generic ELISA protocol to demonstrate a few of the benefits and time savings that can be realized by using a 96-channel semi-automated benchtop pipette. Frequently used ELISA methods include direct, indirect, sandwich and competition assays. These techniques vary significantly in the details and sequence of the biological interactions that occur, but they all require a similar range of liquid handling operations. The purpose of this paper is to illuminate the potential benefits of using the Avidien microPro300 semi-automated benchtop pipette when performing these assays in either 96 or 384 well microplate formats.

Liquid handling steps required in successfully executing a typical ELISA:

1. **Coat:** apply antigen or antibody to the well surfaces followed by an incubation
2. **Block:** used to block subsequent non-specific binding to uncoated well surfaces
3. **Wash**: removes unbound sample. Multiple washes in an individual step (up to eight or more) and multiple wash steps in an assay are typical.
4. **Sample**: addition of samples and reference standard dilutions
5. **Detection antibody**: addition of conjugated antibody for detection and quantification
6. **Substrate solution**: added for color development
7. **Stop reagent**: added to stop the reaction, with plate reading immediately following

Most of these liquid handling steps occur just once with the exception of washing the unbound compounds from each well, which is usually required at several points in the assay. Readers are

almost certainly keenly aware of how tedious and time consuming each of these steps can be, especially using a handheld multichannel pipette. For the purpose of this analysis, we will focus on using an eight channel handheld pipette versus the Avidien microPro300 pipetting across all 96 channels simultaneously.

**2 Method and Results**

In order to understand the approach we take in this analysis, we will look at an individual liquid handling step required for successful completion of the assay. Washing is a critical and time consuming step that occurs repeatedly in the protocol, so we'll examine the details here.

Materials:

1. SBS footprint reservoir with wash buffer.
2. Empty SBS footprint reservoir (for discarding used wash buffer).
3. Clear 96-well microtiter plate, SBS footprint.
4. Eight channel handheld 300uL electronic pipette and standard pipette tips.
5. Avidien microPro300 96-channel semi-automated pipette, 5 to 300uL.
6. Avidien microPro 300uL pipette tips, standard.

For this comparison, an eight channel electronic handheld pipette was used to execute an ELISA protocol's individual wash step sequence. The wash step required four wash cycles per well in order to ensure complete washing and a successful protocol outcome. Each column of the microplate was washed separately, proceeding column by column. As per the protocol, tips were changed between columns to avoid cross contamination, but not during wash steps within a column; a single tip per well was used. Using the eight channel pipette and breaking this sequence into individual steps, and assigning average elapsed times for each, a basic wash has the following operations:

1. Aspirate wash buffer from the reservoir. (5 seconds)
2. Dispense the wash buffer to a column of the microplate. (5 seconds)
3. Wait the designated soak time. (5 seconds)
4. Aspirate the used wash buffer from the microplate. (5 seconds)
5. Dispense used wash buffer to waste. (5 seconds)

The sum of these liquid handling steps for a single wash cycle is 25 seconds, which has to be multiplied for the four washes (100 seconds) and added to a tip load (3 seconds) and a tip eject (2 seconds) making the total for a single column of the microplate about 105 seconds. Apply that across 12 columns and the total time to execute this one wash step is (105 x 12) 1260 seconds, or 21 minutes. Also note that this one wash sequence has 264 individual steps and the 21 minutes is necessary to execute just one wash step of the multi-step protocol.

Executing the same wash sequence using the microPro300 reveals an almost 90% reduction in the time it takes to execute the sequence. Below is the sequence of steps when using a 96-channel semi-automated pipette:

1. Aspirate wash buffer from the reservoir in the upper nest. (5 seconds)
2. Switch to the microplate in the upper nest and dispense the wash buffer. (11 seconds)
3. Wait the designated soak time. (5 seconds)
4. Aspirate the used wash buffer from the microplate*. (5 seconds)
5. Remove the microplate from the upper nest and dispense used wash buffer to the waste reservoir in the lower nest. (8 seconds)
6. Replace the wash buffer reservoir in the upper nest. (5 seconds)

*The microPro's PDR feature will also maximize wash buffer removal and increase each wash step's effectiveness.

This equivalent wash step sequence for a single wash takes approximately 39 seconds using the microPro300. Multiply that by four washes (156 seconds) and add a tip load (15 seconds) and an automatic tip eject (5 seconds) and the total is 176 seconds, or about 3 minutes. The time saved for this one wash step is almost 18 minutes.

If we apply this comparison method over an entire protocol, we conclude that the reduction in time required for all of the full-plate liquid handling steps approaches 90%.

**3 Additional Benefits**

**Pipetting depth recall ensures complete wash buffer removal and reduced background signal:** Correct execution of an ELISA protocol relies on thorough removal of unbound assay compounds. The microPro pipette has a unique feature that optimizes wash efficiency, and in so doing improves your signal to noise ratio and the outcome of the ELISA. Simply stated, the effectiveness of your wash sequence relies on thorough removal of used wash buffer and therefore unbound components from each well. When you use a handheld multichannel pipette, inaccurate and variable placement of the pipette tips in the wells of the microplate results in incomplete and variable removal of used wash liquids. Poor washing commonly results in high background signal due to incomplete, inconstant washing from well to well. The microPro300 is equipped with an automated Pipetting Depth Recall feature (PDR) that ensures correct positioning of the pipette tips in the well. In addition to repeatable assay performance, the PDR feature can be used to reliably set the tips as close as 0.2mm from the bottom of the wells, ensuring thorough removal of unbound components in the wash buffer, without risk of crashing the tips into the microplate. Using the PDR feature, in some cases, may allow a reduction in the number of wash replicates since each wash is more effective in removing unbound components.

**Custom Program reduces pipetting errors:** The microPro controller app includes the ability to build the entire ELISA sequence in a custom program that can exactly match your written protocol, including instruction pop-up panels that describe steps that may have nothing to do with the pipetting. In this way, the microPro becomes the central resource for instructing an

operator through the steps of the ELISA protocol, and not just part of the supporting cast of laboratory instruments. For example, consider an incubation step that follows the addition of your samples. An instruction step built in to your microPro protocol can be used not only to describe the parameters for the incubation (time, temperature, etc.), but the built in count-down timer can be enabled to ensure that the correct time has elapsed before continuing on to the next step.

**Eliminate missed or duplicated columns or rows:** A common problem encountered while executing the pipetting steps of any protocol using a handheld multichannel pipette is losing track of which column is the current one, or simply picking the wrong column accidentally. In many cases, once the error is made, the assay has to be scrapped and run again. Even without pipetting, accidental immersion of the pipette tips into the wrong column can increase your background signal. It is an easy mistake to make given the repetitious nature of the activity. Using a 96 channel pipette eliminates this problem. In addition, the microPro's software is designed to reduce other errors as well. For example, each microplate of the protocol can be named in the software to indicate exactly which to use while executing the protocol (e.g. "wash buffer", "stop reagent", etc.). The user interface displays the labware name clearly, for instant confirmation that the transferred liquid is correct for that step.

**Repetitive Strain Injuries:** As illustrated above, a single wash sequence with four replicates has 264 individual steps when an eight channel pipette is used. Within that sequence are a dozen tip changes. Running ELISA protocols repeatedly means almost continuous and repetitious use of a handheld pipette, which puts employees at heightened risk of developing a repetitive strain injury such as carpal tunnel syndrome. This type of injury can lead to extended down time, lost productivity, unhappiness and significant medical expense. The microPro300 has been designed to reduce this risk through its ergonomically friendly counterbalanced pipetting head design, and simply the reduction in repetitive steps that its 96-channel configuration provides. And contrary to the high physical effort required to successfully load eight pipette tips simultaneously on a handheld pipette, 96 tips can be loaded into the microPro with only a light action. Finally, the microPro has a tip ejection system that is completely automated, requiring only the push of a button to execute.

**Secure Pipette Tip Attachment:** The inventor of the traditional pipette tip surely did not envision their use in a multi-channel pipetting system. As a result, the likelihood of leak-free attachment across all of the tips on a multichannel pipette is difficult to achieve during repetitious pipetting activities. An incorrectly seated pipette tip can fall off in the middle of a transfer, or worse, leak undetectably and deliver incorrect liquid volumes to a well. The tip cartridge system used by the microPro300 has been designed to provide leak-free, secure tip attachment to the instrument in a format that is extremely easy to load and eject.

**Simultaneous Reactions:** Some ELISA protocols (and other types of assays) are sensitive to variation in the time between the last liquid handling step (e.g. stop reagent addition) and reading the plate. In these assays, an unintended variation can be introduced using a handheld

pipette simply by the variation in reaction time from the first column dispense to the last column dispense. A 96-channel pipette dispenses to all wells simultaneously and thus avoids this problem.

## 4 Conclusions

The completion of an ELISA protocol requires a significant number of carefully executed liquid handling steps. Completing these transfers using a multichannel handheld pipette is time consuming, repetitious, error prone and uncomfortable. A 96-channel pipette can be a valuable addition to your lab by significantly reducing the time needed to execute the protocol. In addition to time savings, a 96 channel pipette, in particular the microPro300, can improve your outcomes by taking advantage of its unique and well thought out features. A careful analysis of the microPro's capability will lead you to the conclusion that the instrument represents significant and quantifiable value both when compared to a multichannel handheld pipette, and to other 96 channel benchtop systems that are available. If you are intrigued and have further questions, please do not hesitate to contact Avidien Technologies today.

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