IMPROVEMENTS IN PRECISION OF LOW-VOLUME PIPETTING ON AN AUTOMATED ANALYZER

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INTRODUCTION

Precise and accurate delivery of patient sample is a critical step in obtaining accurate test results. Automated analyzers have improved precision over the years, but there is still opportunity to improve further. Additionally, there is a desire to conserve sample collected from patients, leading to a need for even smaller volume sample delivery. This team set out to develop motion profiles that would achieve very high precision (<1% CV) for small volume delivery and maintain fast throughput while eliminating sample carryover by employing disposable pipette tips.

BACKGROUND

Building a high throughput immunoassay analyzer that is very fast and without sample carryover is a challenge. One solution is to employ disposable pipette tips (dispo-tip). Avoiding sample carryover is important for all immunoassays.

Problem Statement

The UniCel Dxl 800 was introduced in 2003 as the highest throughput immunoassay analyzer. Over the years the focus has shifted from speed to continuous improvement of assay performance, especially with the introduction of high-sensitvity assays. To that end, one key element of assay performance is sample delivery precision. The current specification of CV <2.5% for 10 µL delivery is adequate, but the capability of using smaller delivery volumes is desired for future development.

Goals

The team's aim was to focus on four key performance areas with a new pipetting subsystem:

1. Fast Cycle time (throughput > UniCel Dxl 800)
2. Accurate, small sample delivery volumes (</= 2 µL)
3. Sample delivery precision of < 5% CV at 2 uL delivery
4. Absence of sample carryover (dispo-tips)

ACRONYMS & DEFINITIONS

SV – Sample vessel
DV – Dilution vessel
RV – Reaction vessel
Dispo-tip – Disposable tips
WB – UniCel Wash Buffer II
RLU – Relative light units
Pa·s – SI unit for Viscosity

PROTOCOL & MATERIALS

Test process

A colorimetric method for determining precision was employed. An orange-colored dye (OG) solution containing a known volume of 7% bovine serum albumin solution was introduced to the system via a sample cup. The new pipettor was programmed to deliver varying volumes into a reaction vessel (RV). Concentration of the dye was calculated, and measurements of the delivery were performed using a spectrophotometer. Three different motion profiles were created for varying ranges of volume delivery (250 µL, 25-100 µL and 2-24 µL targets). All tests, with 6 different target values at both high and low viscosity, were conducted with 10 replicates per sample, using an 8 second pipetting cycle, on 5 different pipetting subsystems.

Colorimetric method

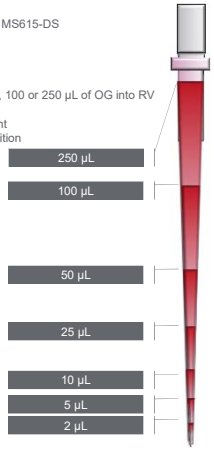
Dye ingredient : Orange-G (C16H10N2NaO7S2)

Instrument

Spectrophotometer : Hitachi U-3900H
Auto dispenser : Hamilton microLAB MS615-DS
Prototype for Sample Pipetting
Dxl with AU pumps for Reagent pipetting

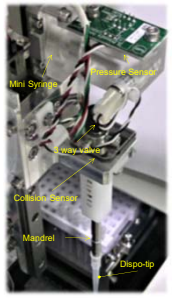
Protocol

Program instrument to dispense 2, 5, 10, 25, 50, 100 or 250 µL of OG into RV (O.D.50 for <25 µL, O.D.25 for ≥ 50 µL)
Fill WB to get total 500 µL for Spec measurement
Run 10 Reps, 3 Runs for each OG volume condition



This product is in development and is not available for sale. Pending achievement of CE compliance; not yet available for in vitro diagnostic use. Pending clearance by the United States Food and Drug Administration; not yet available for in vitro diagnostic use in the US. For Investigational Use Only.

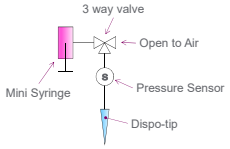
PIPETTOR DESIGN



Sample Pipettor Design Details

There are several locations to aspirate and deliver patient samples. The key to maintaining good precision with high throughput is to make all pipetting elements packaged and move them over multiple locations together.

The left picture is showing the sample pipettor module together with the piping diagram below.

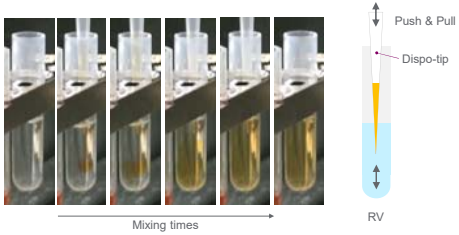


Piping Diagram

PIPETTOR MIXING PROCESS

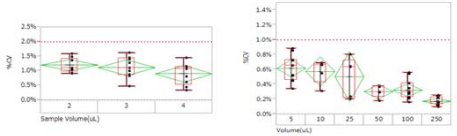
Unique Rinsing Motion

A sample is dispensed into a reaction vessel followed by a rinsing motion where a pipettor syringe pushes and pulls sample + reagent together repeatedly to achieve good precision for low volume delivery. This enables washing out residual inside of dispo-tip to get high accuracy sample delivery.



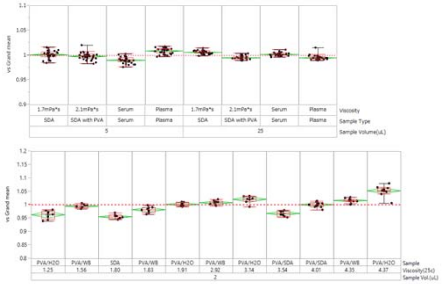
RESULT (NON-DILUTION)

The experimental subsystem with dispo-tip + pipettor mixing successfully improved sample delivery precision across dispensing range.



Precision profile summaries by target value

Several different sample types were studied as well as different levels of viscosity. The results below suggested that there was very little impact from sample type changes.



* Human Serum / Plasma Viscosity: 1.7 ~ 2.0

DILUTION PROCESS

Dilution Process

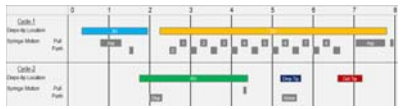
For the purpose of very low sample volume delivery <2µL, an extra process is provided to ensure the 10-200 times dilution is performed before being dispensed into RV.

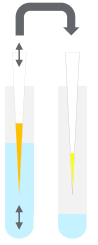
Timing Chart

In order to maintain good precision with high dilution factor, a number of pipettor mixing steps was increased taking 2x 8sec per cycle, shown as below.

Non-dilution (8 sec cycle = 450 tests/hr process speed)

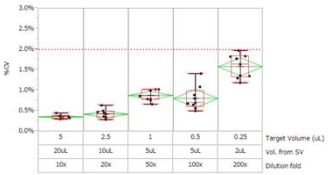
Dilution



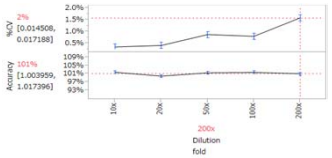


RESULT (DILUTION)

Although the results indicated increasing imprecision with high dilution rates, the highest dilution rate, 200x, still showed CV <2% from the additional pipettor mixing process.



Precision profile summaries by target value on Dilution Process



CONCLUSION

The experimental subsystem has demonstrated performance with dispo-tip as listed below

- CV <1.5% for 2~5 µL
- CV <1.0% for 5~250 µL
- 1.0~4.0 mPa·s
- 10~200x dilution rate

There are several key elements to achieve high performance for the pipetting system using the dispo-tips.

- Miniaturized Components to move together over multiple locations
- Pipettor Mixing to wash out residuals inside dispo-tip
- Number of Pipettor Mixing to mix sample + reagent to be uniform before aliquoting

Having these key functionalities, the experimental subsystem would support improved assay performance when employed on an immunoassay analyzer.