Brief Study on CPAL Settings
5mm Penetrate and 2mm Retract
Variable Flow Rates
Variable Plunger

Starting Points
for
Method Development Work
for the Researcher

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Outline

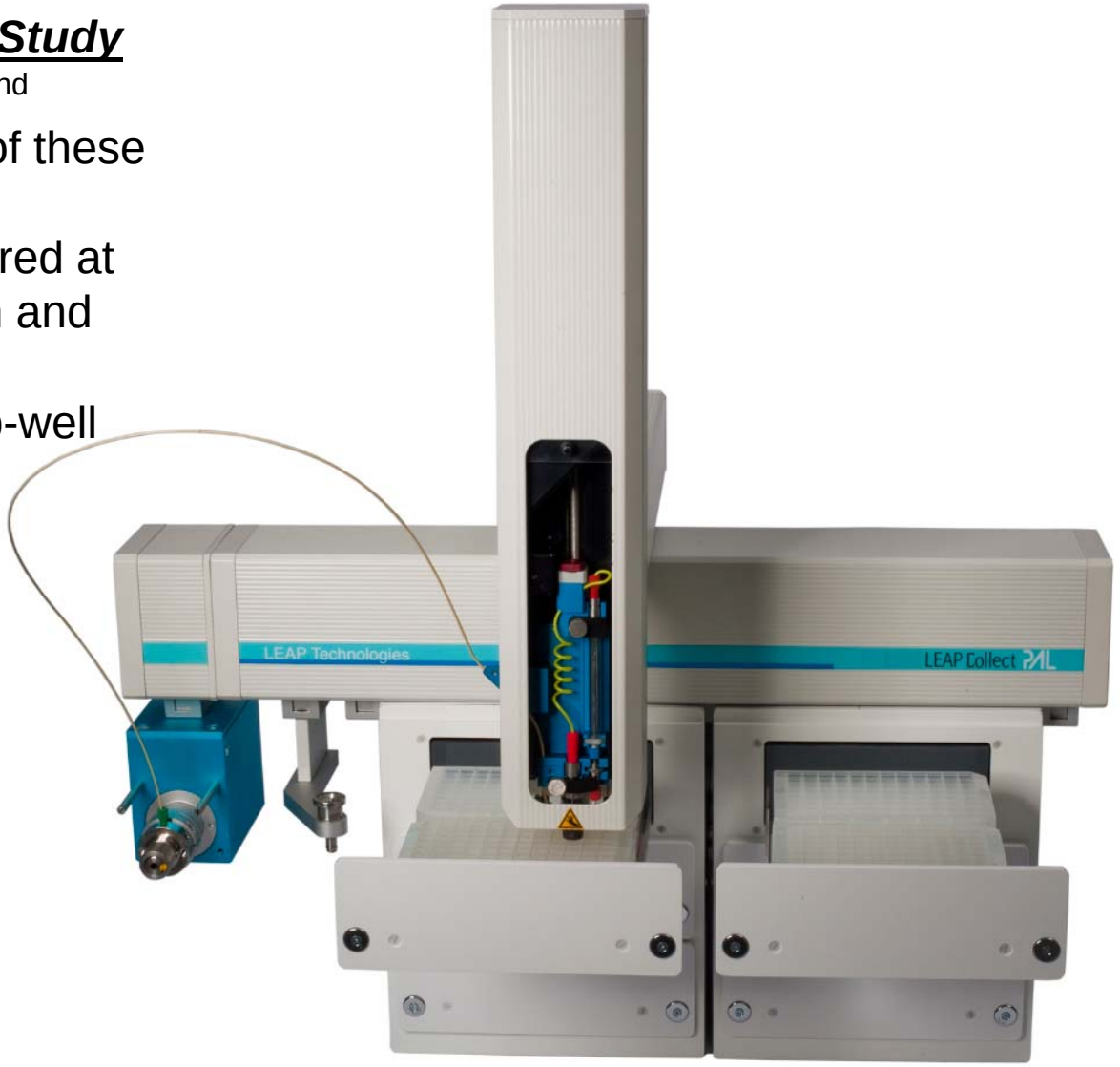
- This study was conducted over a period of three days with various settings to optimize the Collect PAL in house.
- Liquid (50:50 H2O:MeOH) was collected.
- Parameters were selected in order to test for no dripping between well-to-well or waste-to-well movements.
- Version 2.1 Collect PAL Application was used.
- Only 500 uL/min, 1.0 mL/min, and 1.5 mL/min flow rates were measured.
- 70Asp and 70 Dsp settings were tested with 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 sec Collection values.
- 5mm PENETRATION and 2mm RETRACTION values were used for the study.
- A Gilson 305 Pump with manometric module and 5.0 mL head were used. These had been PM'd and QA/QC performed just prior to use by Gilson.
- For these studies the furthest plate location was chosen as a worst case scenario for waste-to-well time movement after a short delay.

Study Overview

- A straight line in the graph means that every well was accessed for the same amount of time per well. It can be deduced from this that the Fraction Time per Well setting is accurate over a wide range of Fraction Sites.
- Both Numerical by Column and Serpentine by Column collection patterns were examined.
- Move from Waste incorporates a new feature in version 2.1 that adds 10mm only for the 1st aspiration to prevent dripping from Waste to Well #1.
- Multiple collection trials were performed at each fraction time to show the penetration and retraction effects on the time per well parameters at these flow rates.
- These findings used in conjunction with the other studies will afford the researcher good starting points for their own method development.

Set Up as Shown for the Study

1) The 3rd Drawer of the 2nd Stack was used for all of these measurements.
2) Flow rates were measured at 500 uL/min, 1.0 mL/min and 1.5 mL/min.
3) Collection into 96, deep-well plates.



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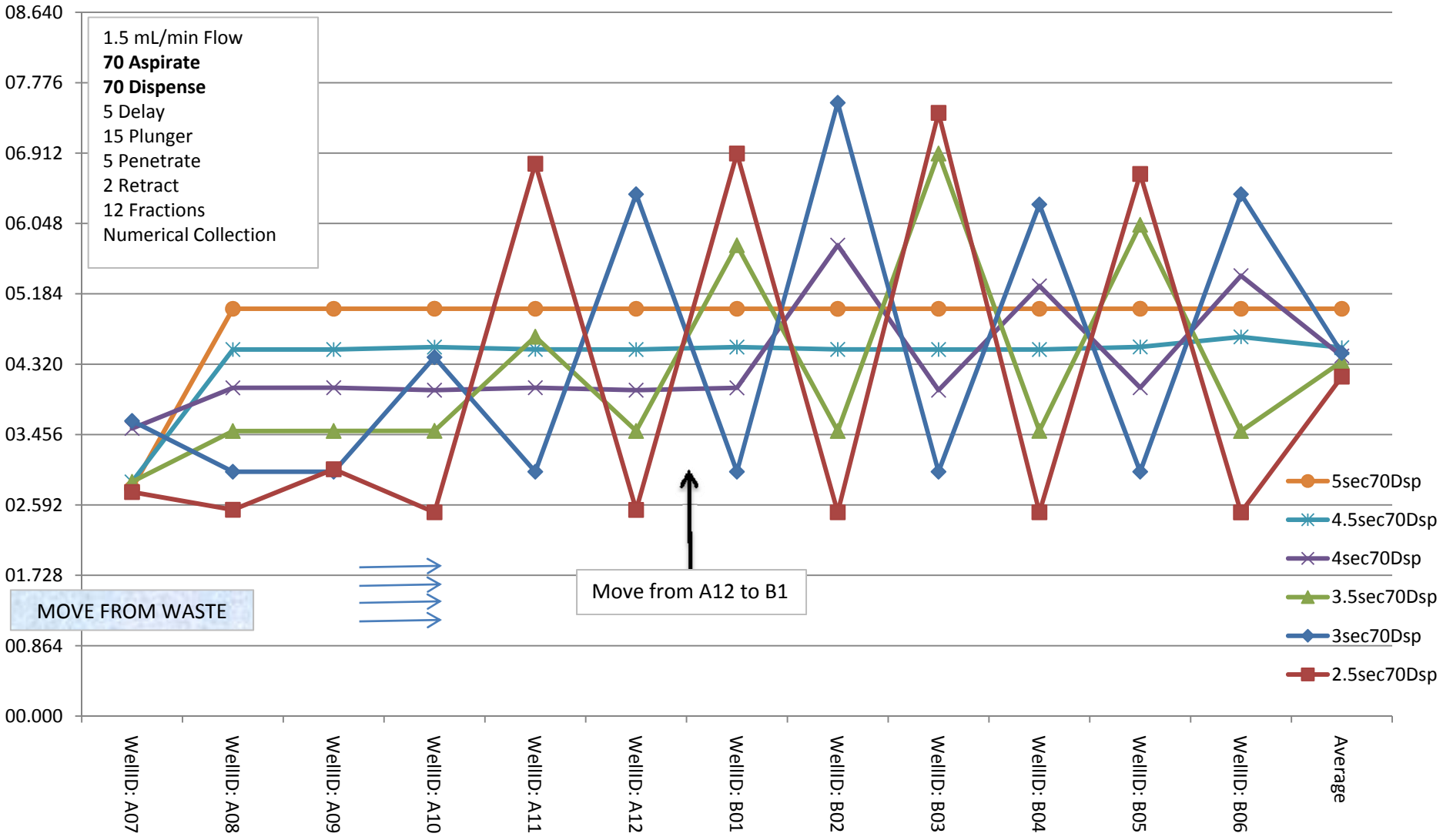
5mm Penetration 2mm Retraction 5sec Delay

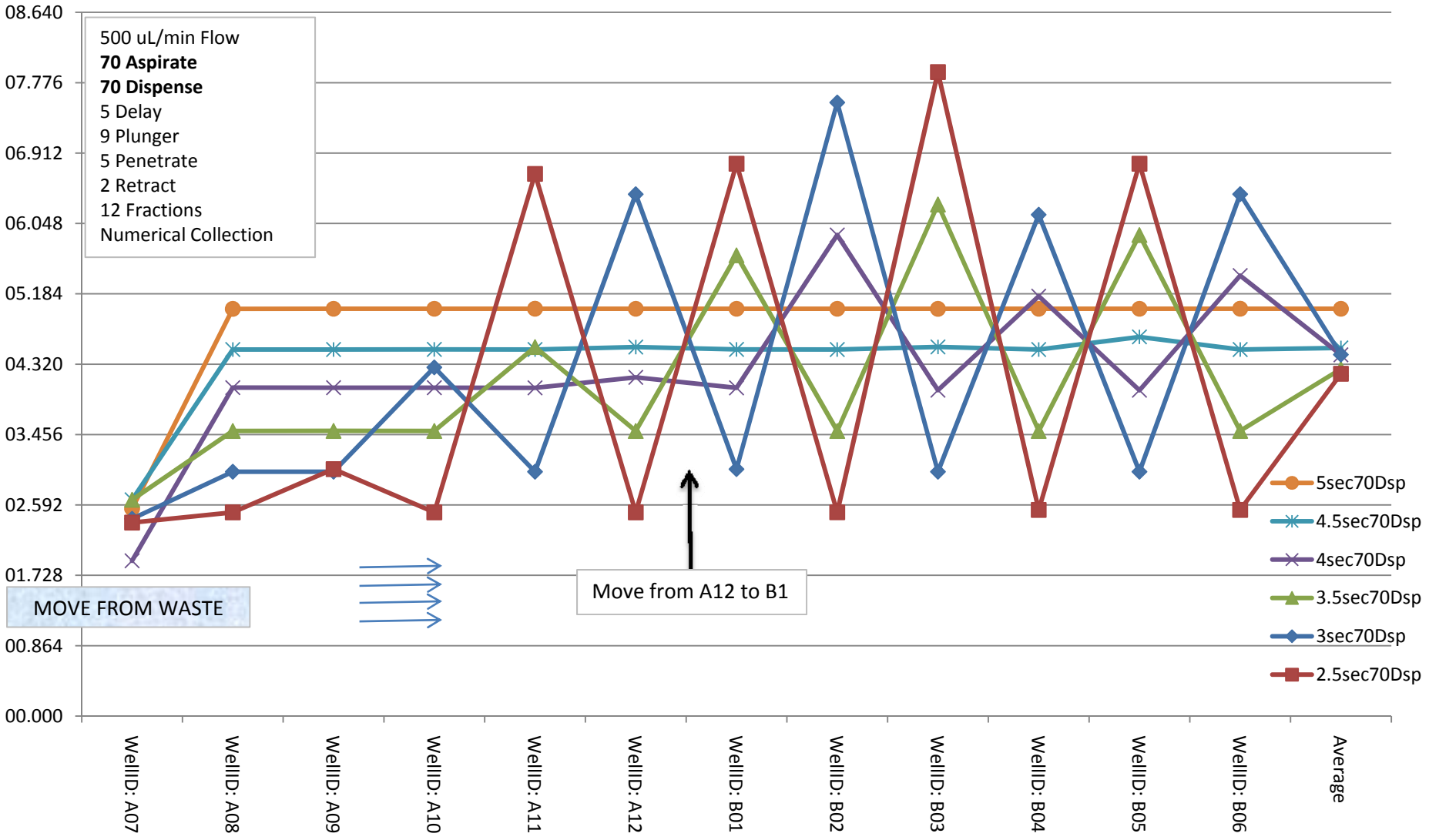
Results

- With the delay you will always have different first well TOTAL FRACTION TIME that does not match to the Fraction Time per Well setting. This time has to take into account the movement of the Head from the Waste position to Well #1. A worst case (furthest distance scenario was used). A longer collection time does not adjust for this first well movement.
- This time is a measure from time=0 (when the valve switches at the waste station) to the aspirate movement of the syringe prior to moving to well #2. The graph does take into account the 5 sec delay. So REAL time for well #1 is the recorded time – delay time.
- For both collection patterns 4.5 sec/well is the fastest setting that passed consistently. It appears that the penetration and retraction is the rate determining step in this process regardless of the other parameters. Faster ASP and DSP setting may help slightly, but these would need to be tested.
- This indicates that more rigorous method development is needed for any methods requiring penetration and retraction into the plate or through capmats or plate seals.

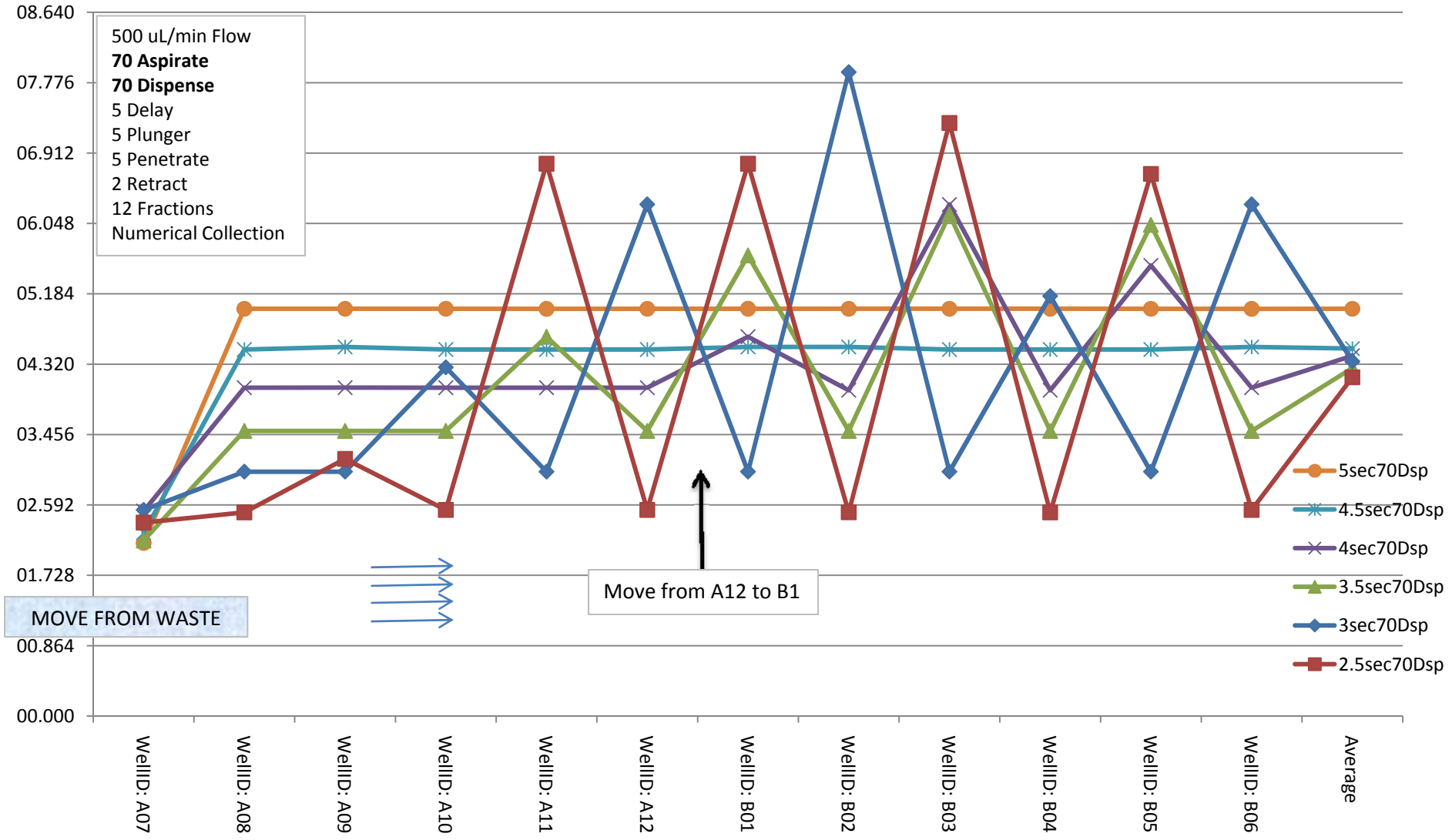
NUMERICAL COLLECTION – 1.5 mL/min Flow Rate



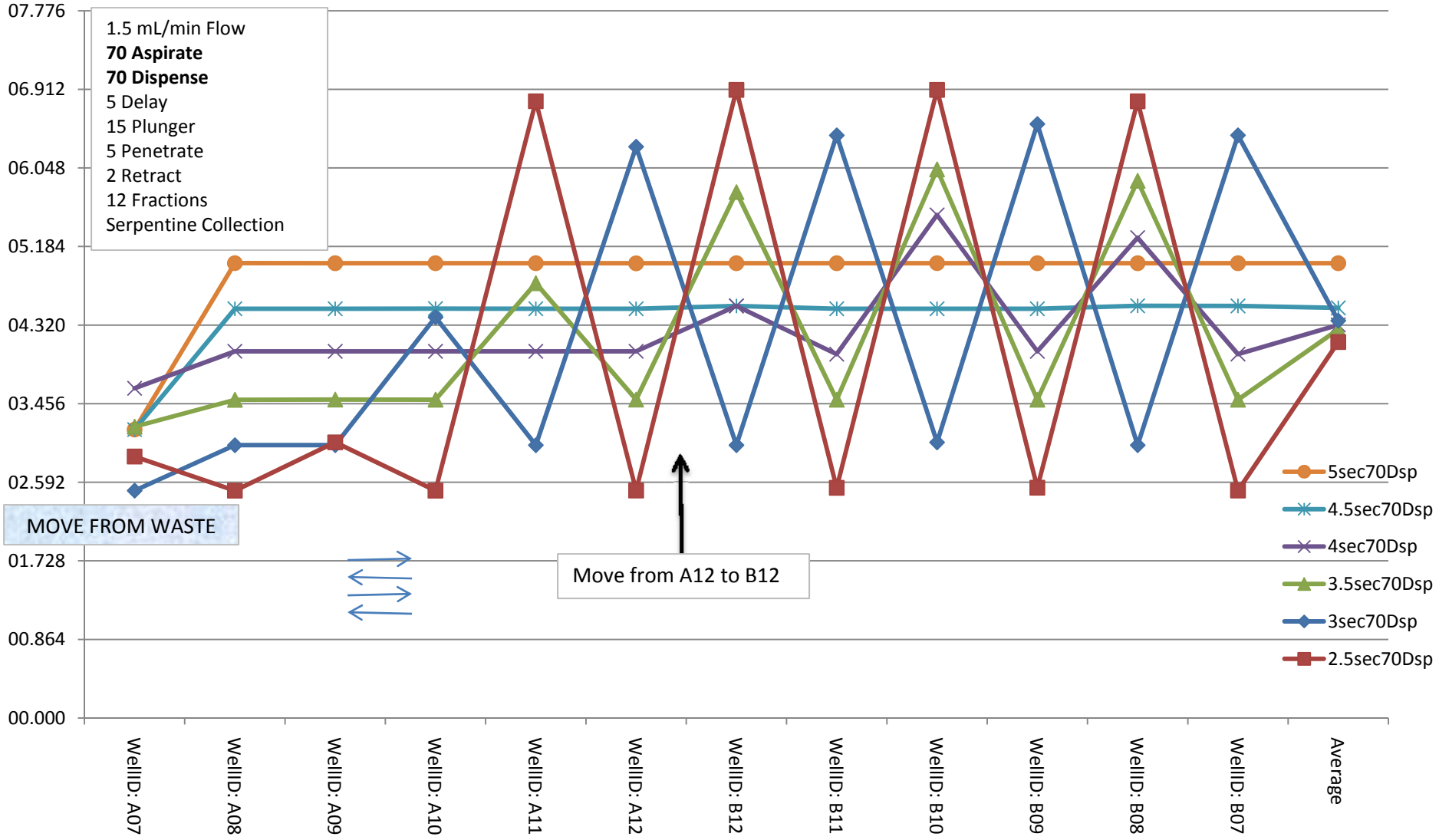
NUMERICAL COLLECTION – 1.0 mL/min Flow Rate



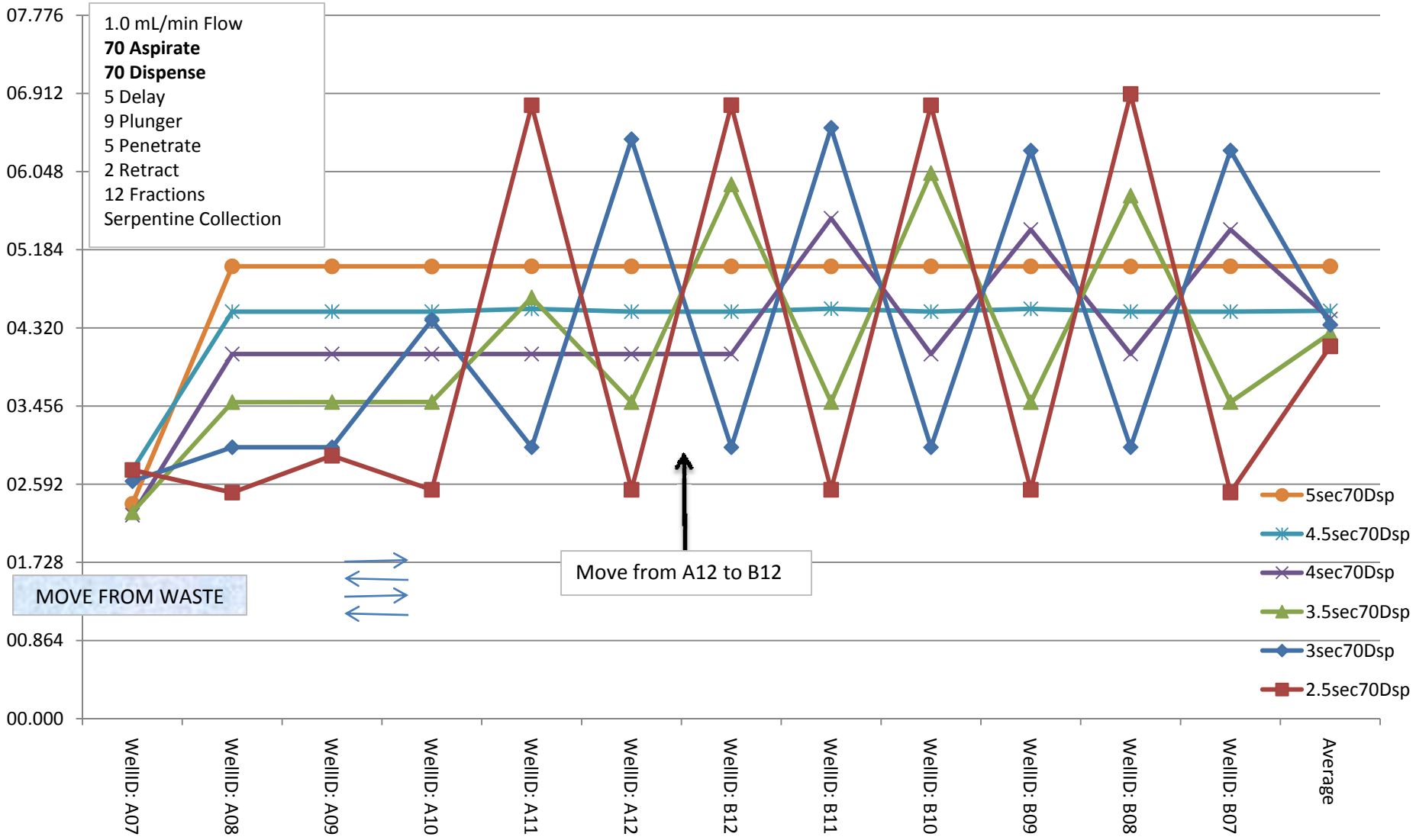
NUMERICAL COLLECTION – 500 uL/min Flow Rate



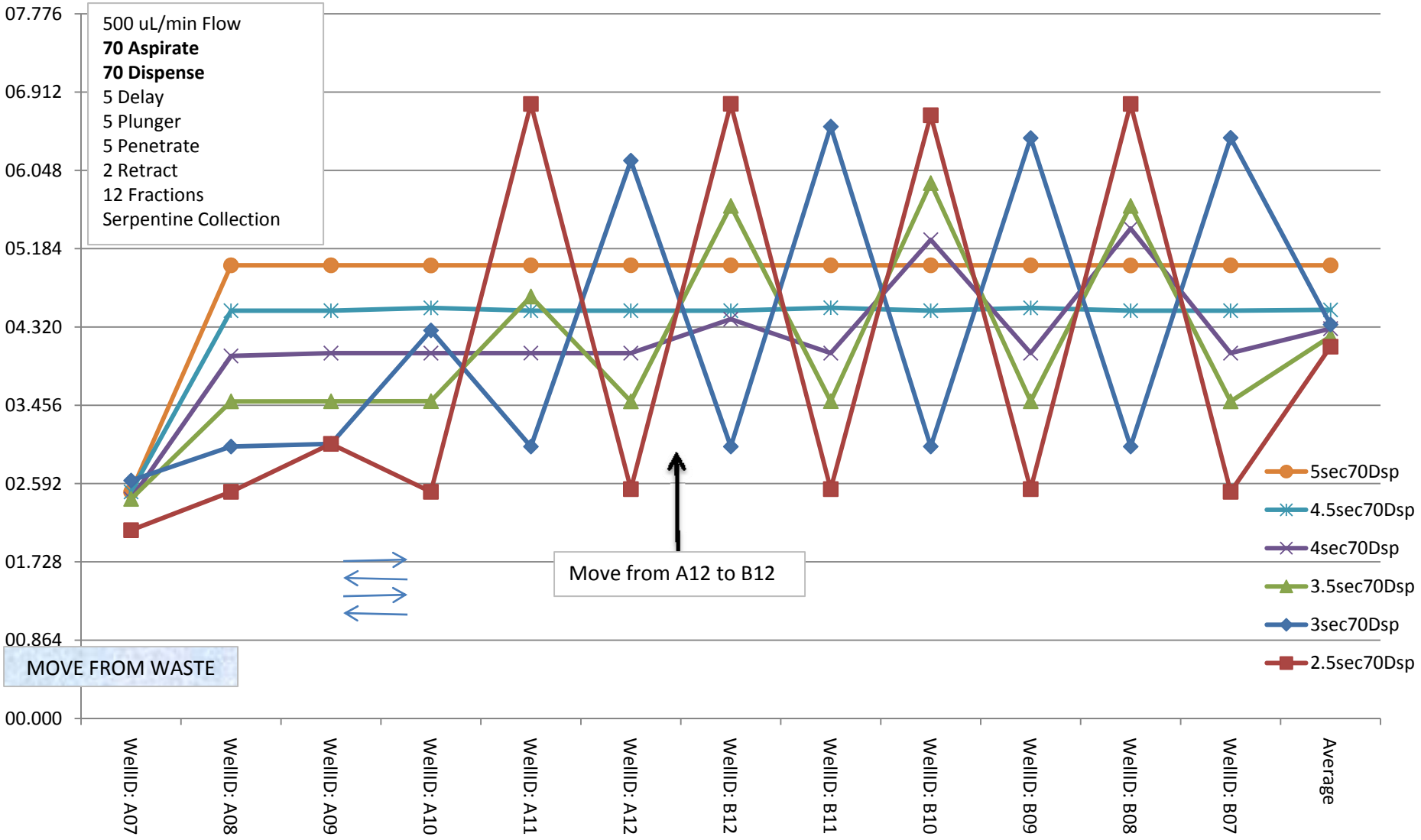
SERPENTINE COLLECTION – 1.5 mL/min Flow Rate



SERPENTINE COLLECTION – 1.0 mL/min Flow Rate



SERPENTINE COLLECTION – 500 uL/min Flow Rate

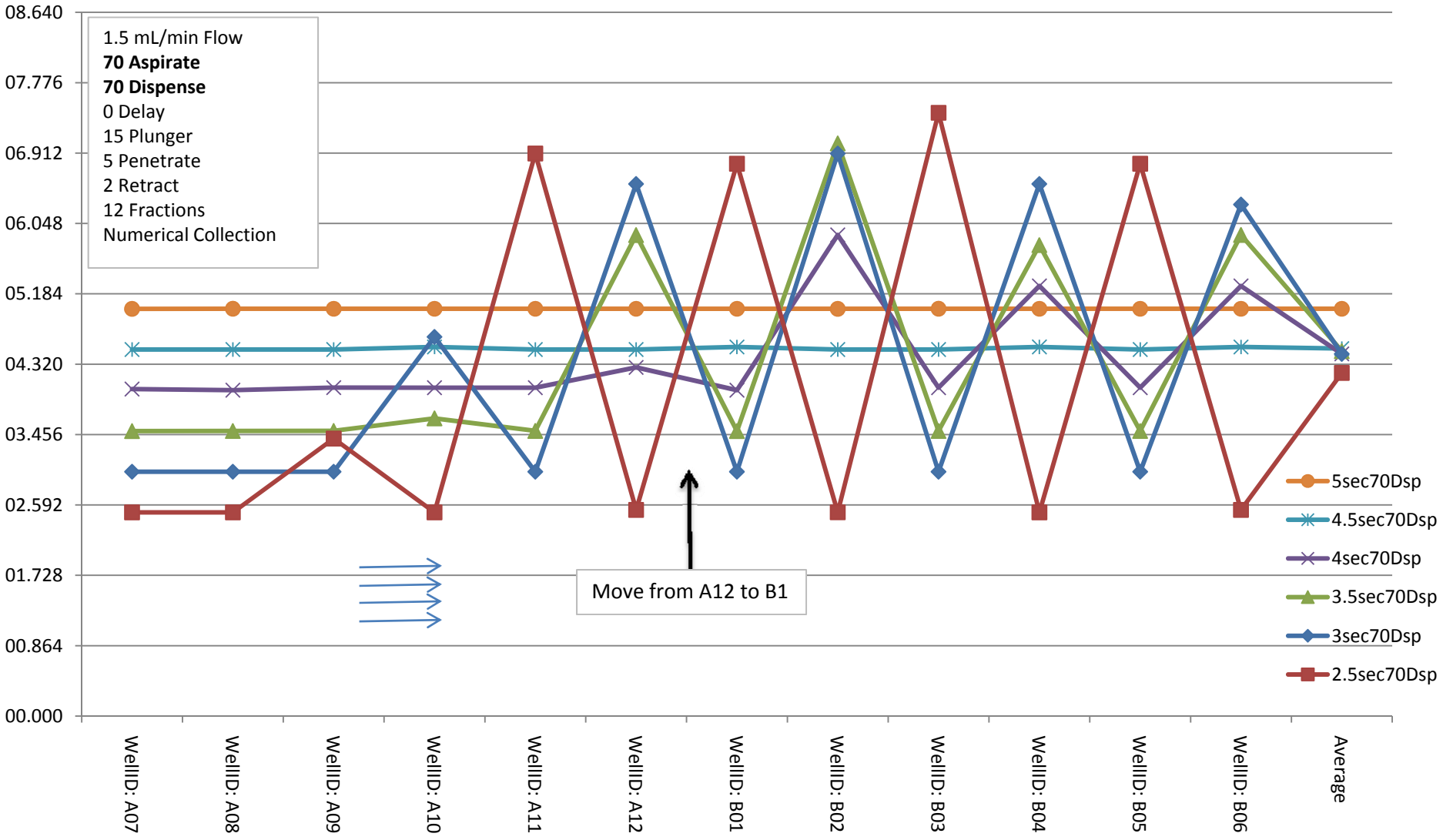


5mm Penetration 2mm Retraction 0sec Delay

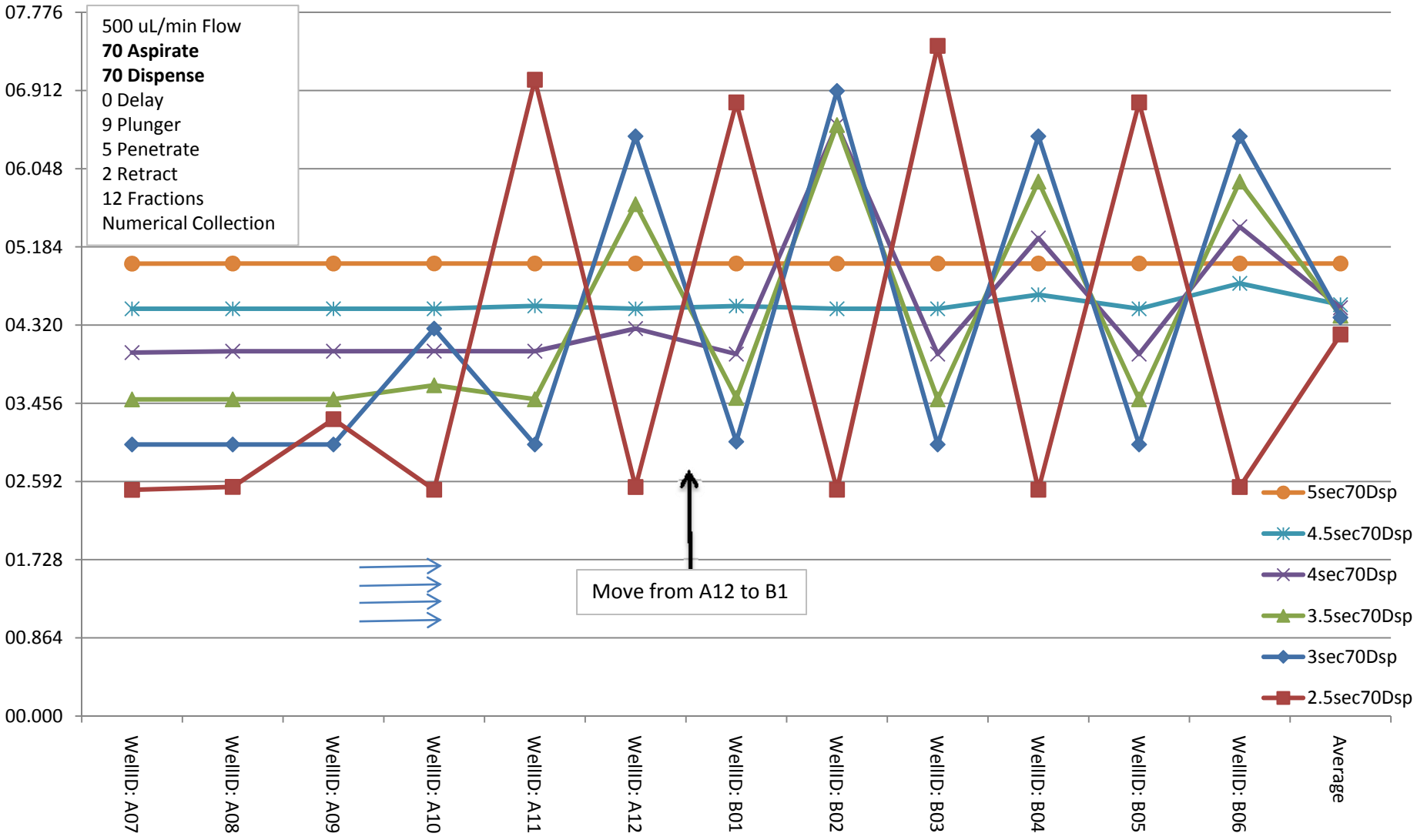
Results

- With the 0 sec delay you should always have the first well TOTAL FRACTION TIME matching to the Fraction Time per Well setting.
- This time is a measure from time=0 (when the valve switches at Well #1) to the aspirate movement of the syringe prior to moving to well #2.
- For both collection patterns 4.5 sec/well is the fastest setting that passed consistently. It appears that the penetration and retraction is the rate determining step in this process regardless of the other parameters. Faster ASP and DSP setting may help slightly, but these would need to be tested.
- This indicates that more rigorous method development is needed for any methods requiring penetration and retraction into the plate or through capmats or plate seals.

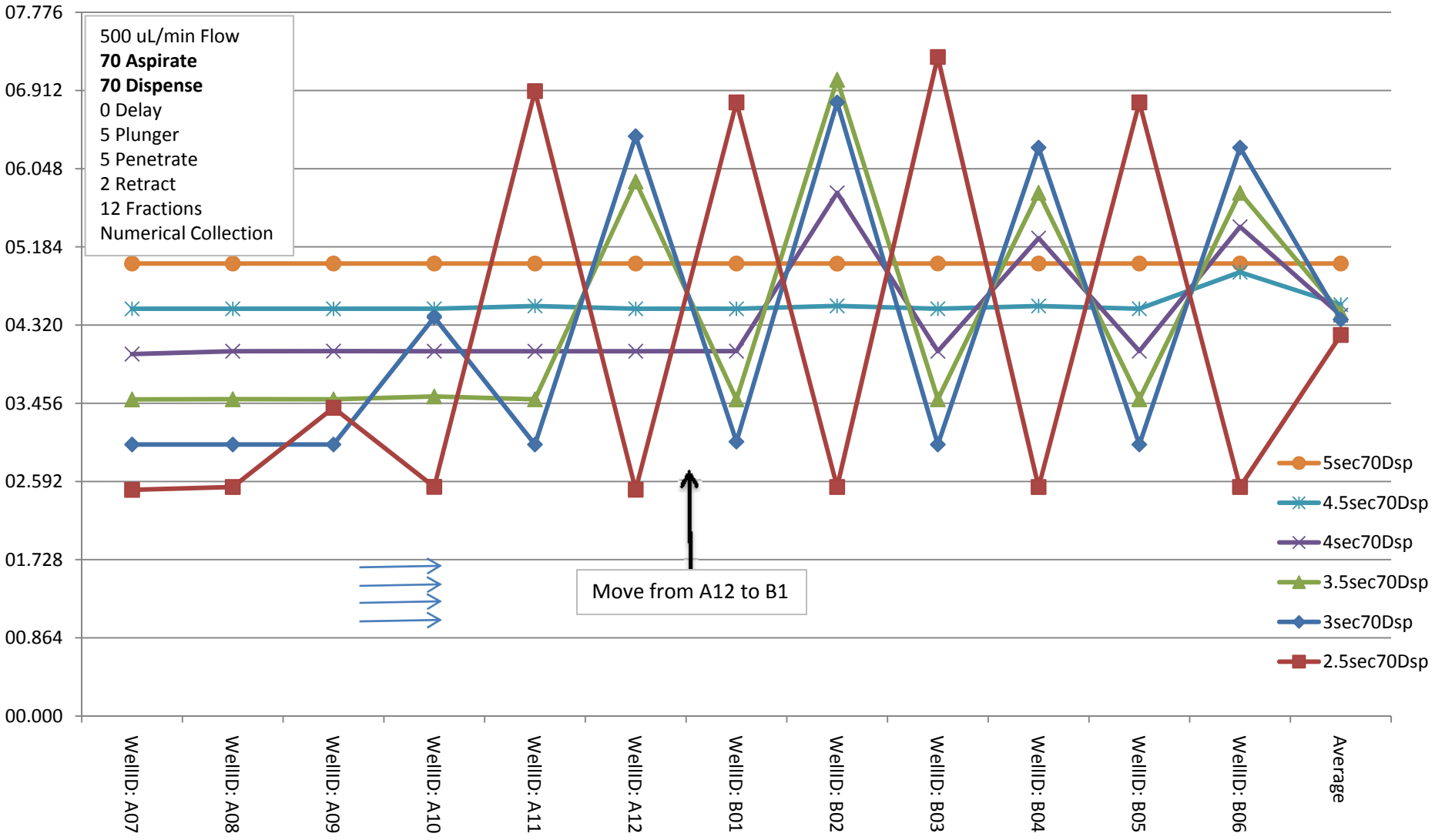
NUMERICAL COLLECTION – 1.5 mL/min Flow Rate



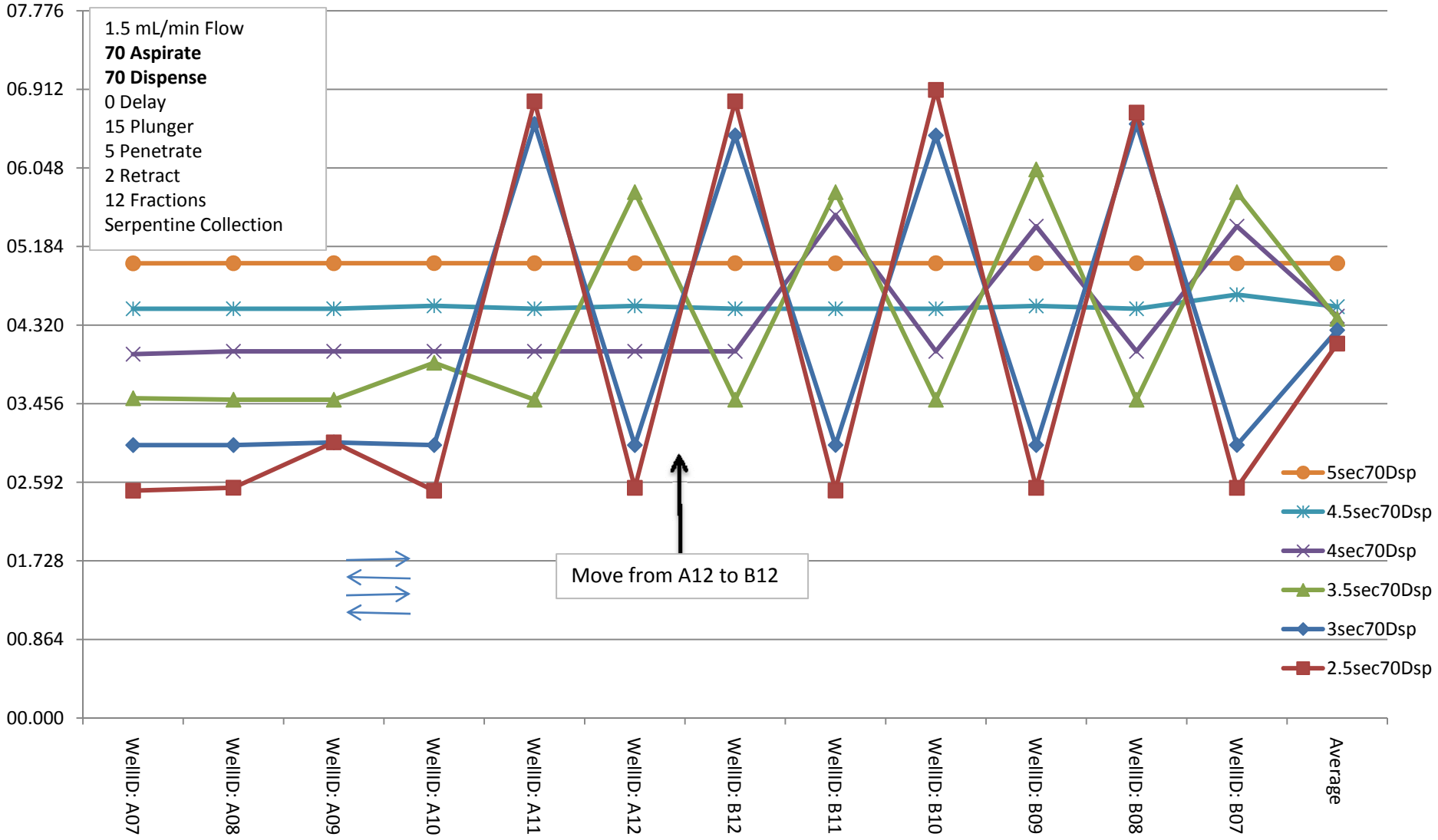
NUMERICAL COLLECTION – 1.0 mL/min Flow Rate



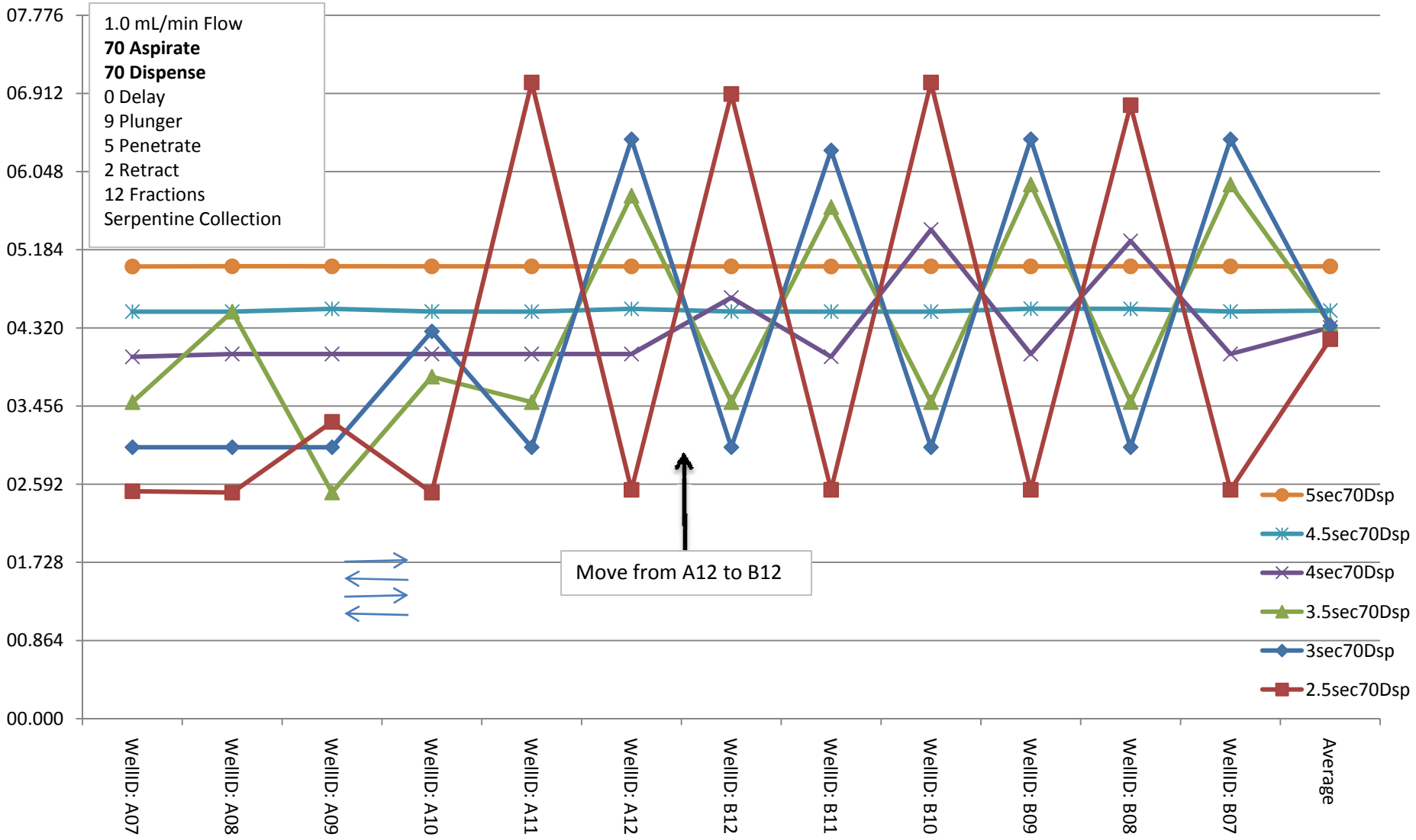
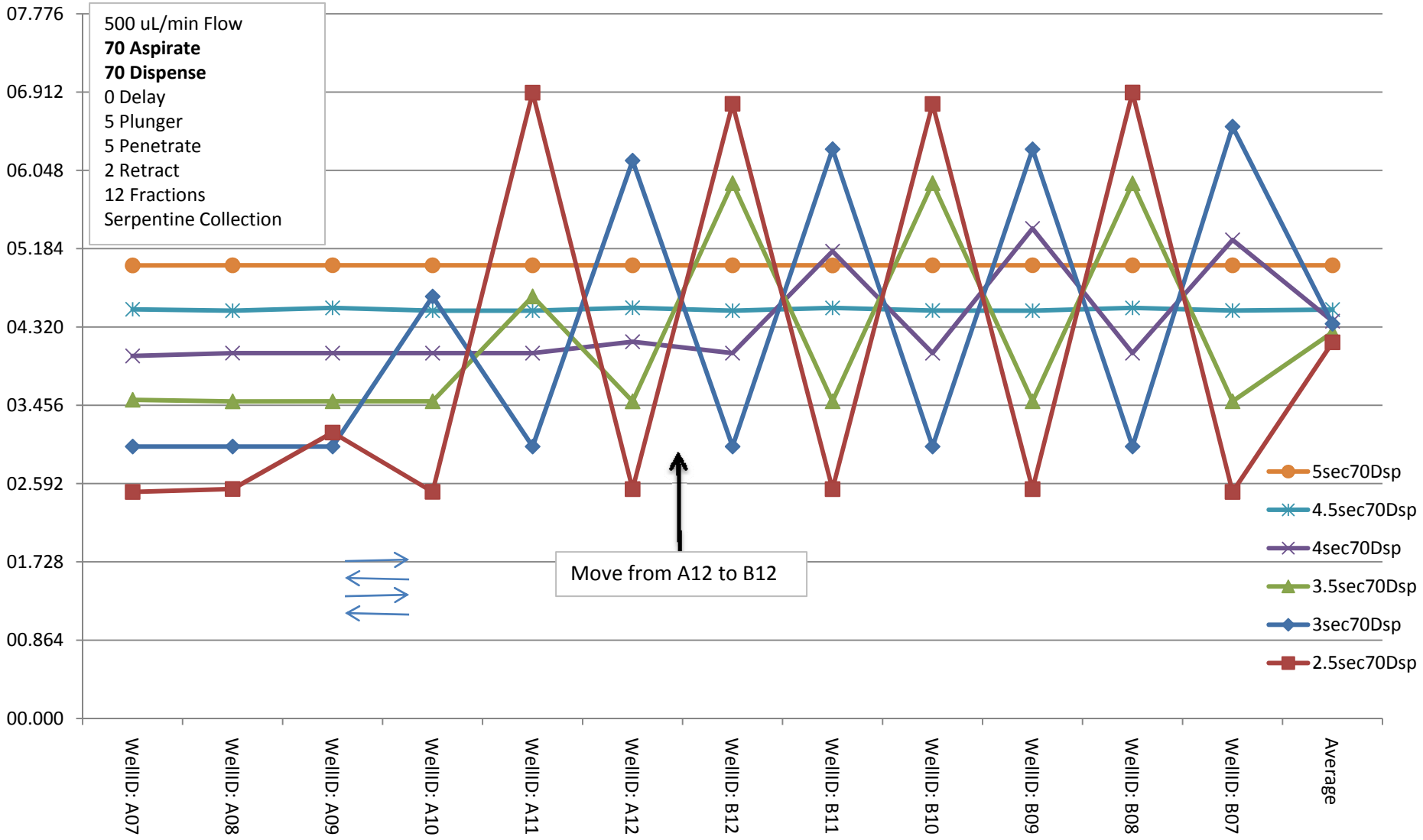
NUMERICAL COLLECTION – 500 uL/min Flow Rate



SERPENTINE COLLECTION – 1.5 mL/min Flow Rate



SERPENTINE COLLECTION – 1.0 mL/min Flow Rate



SERPENTINE COLLECTION – 500 uL/min Flow Rate