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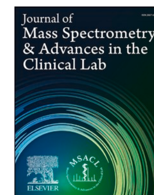

Case Report

Surreptitious pipetting errors on a vendor-programmed liquid handler

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ABSTRACT

Liquid handlers (LHs) have become common in both clinical and academic laboratories for the preparation and manipulation of samples. In theory, these systems offer the potential for reduced error due to the elimination of mis-pipetting errors. In reality, these systems still have potential for mis-pipetting and require careful validation by the end user. In this case report, we describe two instances where inappropriate pipetting by a vendor-programmed LH were observed. In each case, the worklist that was obtained from the LH failed to reflect what had actually been pipetted and as such these instances represented significant near misses with substantial potential for patient harm. Neither of these instances were caught during the laboratory's validation studies of the LH. Laboratories should be aware of the potential for mis-pipetting by LHs. LH vendors should work to ensure the worklists reflect what was pipetted (instead of what was intended to be pipetted) and end users must ensure robust validation studies that take into account as many "real world" scenarios as possible.

Case description

Our clinical laboratory performs confirmatory urine drug testing using a multi-analyte, dilute-and-shoot, liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based laboratory developed test (LDT). Samples are transferred from vacutainer tubes to a 96-well injection plate by a vendor-programmed liquid handler (LH) (configuration shown in Fig. 1). Since we began implementing this LDT analysis, we have encountered two cases with perplexing results that, upon further investigation, showed a potential for patient harm due to inappropriate pipetting by the vendor-programmed LH.

In the first instance, the clinical laboratory scientist (CLS) noticed that three of the calibrators at the start of the run were well outside acceptability criteria (±20 % at the lowest level, ±15 % for other levels, based on reference [1]. Our method has six non-zero calibrators that bracket the run and three levels of quality control (QC) that are included after each set of calibrators. Additional QC samples are also interspersed throughout the batch. The last set of calibrators was within acceptability criteria. Using this set of calibrators, and analyzing the unacceptable calibrators as unknowns, it was noted that the concentration values were within the expected range for the three QC levels used in this method. Because of this close match, this error was initially attributed to a manual sample mix-up, i.e., the CLS added QC instead of calibrator in those positions.

In the second instance, the CLS noted that the calibration curve from the last set of calibrators was problematic, with two of the six calibrators having undetectable levels of all analytes. However, both of the problematic calibrators showed acceptable internal standard recovery, which suggested the issue was not with the LC-MS/MS, or the autosampler not injecting It is important to note that our method includes blank urine samples interspersed throughout the worklist; therefore, the error could have alternately been caused by a sample mix-up where the CLS accidentally added a blank sample instead of a calibrator.

Case discussion

In both instances, the root cause of the problem was thought to be a manual sample mix-up by the CLS. In our method, samples are prepared using a vendor-programmed LH. Briefly, barcode-labelled samples in vacutainer tubes are loaded onto the deck of the LH (racks 1 – 6, Fig. 1). For small volume patient samples, samples are manually added to nesting cups placed on top of the barcode-labeled tubes (allowing for reduced dead volume). The CLS indicates which tubes utilize nesting cups when starting the program. The LH then performs a vendor-

Abbreviations: CLS, Clinical Laboratory Scientist; LC-MS/MS, Liquid Chromatography Tandem Mass Spectrometry; LDT, Laboratory Developed Test; LH, Liquid Handler; QC, Quality Control.

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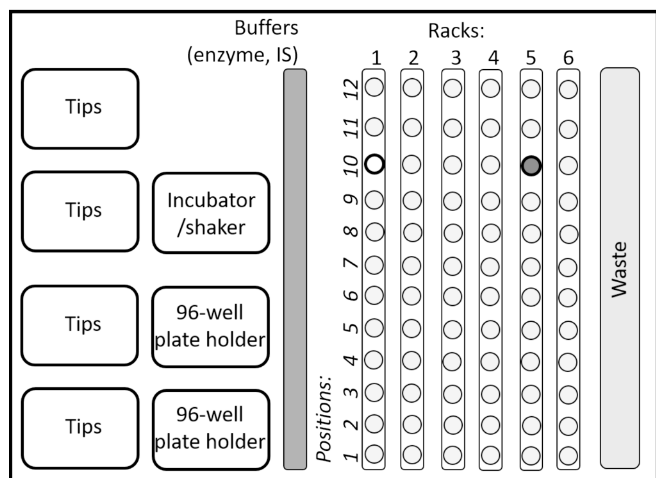

Fig. 1. Liquid handler (LH) configuration used in the method. For this configuration, samples are taken from number racks and number positions using a four channel pipette. The pipette tip boxes are in an 8 x 12 layout.

programmed, laboratory-validated set of steps whereby enzyme solution and samples are transferred to a 96-well plate and incubated. Following the incubation, an internal standard solution is added, and the plate is mixed before being manually removed from the LH for centrifugation. After centrifugation, the plate is put back on the LH, which transfers a defined aliquot of supernatant to a fresh 96-well injection plate. The fresh plate is then manually loaded into the autosampler, and a worklist is copied from the LH and pasted into the acquisition software. In this workflow, opportunities for manual error included: (1) incorrect pipetting and worklist generation by the LH, (2) pipetting incorrect samples into nesting cups that sit in the barcode-labeled tubes, (3) improper loading of the 96-well plate (i.e., well A1 in the correct corner), and (4) incorrect autosampler plate location selection. The proper orientation of the plate and selection of autosampler location are confirmed if QC samples that are randomly interspersed with patient samples are all acceptable. Ultimately, the source of these errors was incorrect pipetting of samples by the LH.

For the first incident, the observed agreement between the questionable calibrators and QC suggested that the CLS had incorrectly pipetted QC instead of the calibrator. The CLS strongly denied this, but did admit to using QC in nesting cups for the last set of QC because the remaining volume was insufficient to place the whole tube onto the LH. To further understand the error, the trace from the LH was opened (the programming tells the LH what it should do, and the trace records what it actually did). The trace showed that the LH had pipetted from the wrong rack of samples. Instead of pipetting the three calibrators in rack 1, positions 2 – 4, it pipetted samples from rack 6. It is worth noting that the samples it pipetted from rack 6 were the three QC samples in nesting cups. The laboratory was able to replicate this error by placing nesting cups in any position in racks 2 – 6 and observing that the LH would always take from these later racks instead of rack 1 (Fig. 2). So, if there was a nesting cup in rack 2, position 3, when it came time for the LH to add the sample from rack 1, position 3, it would instead take the sample from rack 2, position 3 (never even pipetting the actual sample in rack 1, position 3). This error did not depend on the position of the sample but on the rack number. So, as shown in Fig. 1, if a nesting cup was in rack 5, position 10, then the LH would incorrectly pipette that sample, instead of the correct sample in rack 1, position 10. Most disconcertingly, the LH would output an incorrect worklist that did not correlate to what was actually pipetted. Fortunately, an exhaustive review of records showed no instances of nesting cups being used outside of rack 1 in our health system, making this error a near miss.

In the second instance, it was discovered that the LH had never actually added samples to the well, and that this error was also not reflected in the worklist. Fortunately, the CLS was watching the LH when this event occurred. At this time, we were experiencing a high frequency of pipette tips not being picked up from the tip box, which caused pipette tip mismatch errors. Typically, this only affected one of the four channels, but in this case, it impacted two. In response to this error, the CLS selected the "exclude channel" option for the two channels that did not have pipette tips. Unfortunately, this led to the LH omitting those channels and not pipetting samples.

Case resolution

Investigation revealed that these two cases were caused by pipetting errors on the vendor-programmed LH that were not reflected in the worklist and were not detected during validation. While no patient results were impacted by these errors, they represented significant near misses. These issues were brought to the vendor's attention, and they agreed that there were programming errors that they could fix. Unfortunately, they never fully explained what the programming errors were.

While validation cannot cover every possible scenario, these errors highlight the need to validate various situations that the method may

A

```
p - start;
p - progress; Pickup tips, Channel Pattern: 1111, Sequence Positions: Ham_FTR_300_0001(53); Ham_FTR_300_0001(54); Ham_FTR_300_0001(55); Ham_FTR_300_0001(56);
p - complete;
Aspirate - start; Individual Volumes: (15.00uL, 15.00
Aspirate - progress; Aspirate, Channel Pattern: 1111 Sequence Positions: Samples_01(1); SmallTubes_02(2); SmallTubes_02(3); Samples_01(4); Liquid Classes: StandardVolu
Aspirate - complete;
Dispense - start; Individual Volumes: (15.00uL, 15.00uL, 15.00uL, 15.00uL);
Dispense - progress; Dispense, Channel Pattern: 1111, Sequence Positions: Plate_Intermediate(A1); Plate_Intermediate(B1); Plate_Intermediate(C1); Plate_Intermediate(D1);
Dispense - complete;
Tip Eject - start; Default waste
Tip Eject - progress; Eject Tips, Channel Pattern: 1111, Sequence Positions: DefaultLongWaste_0001(1); DefaultLongWaste_0001(2); DefaultLongWaste_0001(3); DefaultLongWas
Tip Eject - complete;
```

B

```
 - start;
 - progress; Pickup tips, Channel Pattern: 1111, Sequence Positions: Ham_FTR_300_0001(5); Ham_FTR_300_0001(6); Ham_FTR_300_0001(7); Ham_FTR_300_0001(8);
 - complete;
 start; Individual Volumes: (15.00uL, 15.00uL, 15.00uL, 15.00
Aspirate - progress; Aspirate, Channel Pattern: 1111, Sequence Position Samples_01(1); SmallTubes_06(2); SmallTubes_06(3); SmallTubes_06(4); iquid Classes: StandardVolume_Water_
Aspirate - complete;
Dispense - start; Individual Volumes: (15.00uL, 15.00uL, 15.00uL, 15.00uL);
Dispense - progress; Dispense, Channel Pattern: 1111, Sequence Positions: Plate_Intermediate(A1); Plate_Intermediate(B1); Plate_Intermediate(C1); Plate_Intermediate(D1);  Liquid Cla
Dispense - complete;
Tip Eject - start; Default waste
Tip Eject - progress; Eject Tips, Channel Pattern: 1111, Sequence Positions: DefaultLongWaste_0001(1); DefaultLongWaste_0001(2); DefaultLongWaste_0001(3); DefaultLongWaste_0001(4);
Tip Eject - complete;
```

Fig. 2. Screenshots of trace files showing the mis-pipetting errors the laboratory was able to replicate. In this program, samples from rack 1 position 4 are called Samples_01(4) or SmallTubes_01(4) (for nesting cups). **A.** Trace from samples where nesting cups were placed in rack 2, positions 2 – 3 resulting in pipetting of samples from rack 2, position 2 instead of rack 1, position 2. **B.** Trace from samples where nesting cups were placed in rack 6, positions 2 – 4 causing the liquid handler (LH) to incorrectly pipette from rack 6, positions 2 – 4 instead of rack 1, positions 2 – 4.

encounter, such as different sample types, positions, and racks, as well as performance after error recovery. The publication of guidance on what constitutes adequate validation of LHs would greatly benefit the field. A review of our validation records revealed that testing had only been conducted with nesting cups in rack 1, which explains why the programming error was not detected during validation. Additionally, our validation studies did not encounter pipette tip mismatches, so we could not evaluate the LH's performance in these instances. Furthermore, since validation cannot address all issues, these instances underscore the importance of post-go-live monitoring [2]. Ideally, clinical laboratories should have in-house expertise in LH programming and editing; however, not all labs can provide the necessary staffing and training.

An exhaustive review of the literature found no published examples of pipetting errors by vendor-programmed LHs. Reports of biases between automated LHs and manual pipetting were found, but no reports of actual pipetting errors by LHs [3]. We hope that these cases serve as an important warning to clinical and academic laboratories implementing these systems. The LH will do what it is programmed to do, even if the programming is wrong. For some LHs, this programming is managed almost entirely by the vendor, which leaves the lab at the mercy of the vendor's expertise. While this is understandably how programmed instruments work, it is something that clinical laboratorians need to be aware of. Too often, we expect our robots to be smarter than they actually are. The reality is they are only as smart as their programming, and any laboratory utilizing these instruments should be vigilant in their efforts to understand and uncover coding shortfalls.

Points of interest

1. Clinical laboratorians should be aware that mis-pipetting and non-pipetting are potential errors that can be encountered with automated LHs
2. The worklist may not always reflect what was pipetted and it would be beneficial for LH vendors to make it best practice to only generate worklists reflective of what was pipetted.
3. As much as possible, validation studies must take into account "real world" scenarios including placement of samples and LH performance when dealing with errors.

CRediT authorship contribution statement

Kelly Britt-Rodriquez: Investigation. **Jamie Daniel:** Investigation. **Joshua Hayden:** Writing – review & editing, Writing – original draft, Supervision, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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