



HAMILTON

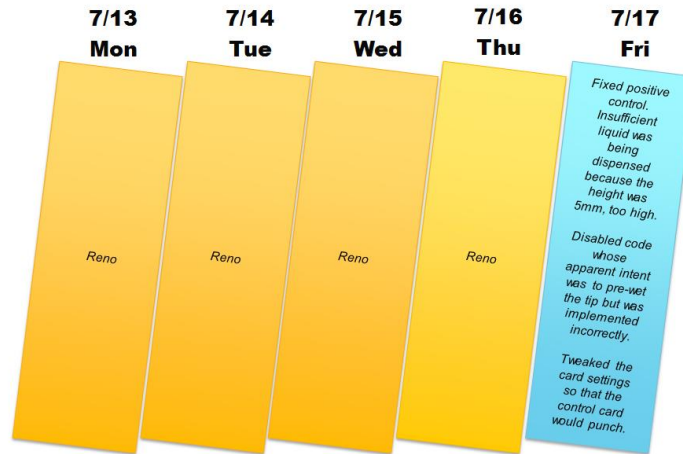
LAS VEGAS UPDATE – 7/17/15



1

GOALS / SUMMARY

Overview of *this* Week



Went to Vegas to resolve three issues: a control card imaging problem, insufficient volumes of the positive control, and a strange double-aspiration. Vegas is really hot this time of year, the sun was scotching, but at least we had the chance to have some fun (do a 10-minute Helicopter ride and I met the local Switch people. Those folks are really excited about the 1 billion USD datacenter in Reno).

Vegas by Helicopter



Right Now:

98°

So Hot!

Today:

Jul 17



Partly cloudy in the afternoon.

102°
79°

Saturday:

Jul 18



Drizzle starting in the afternoon, continuing until evening.

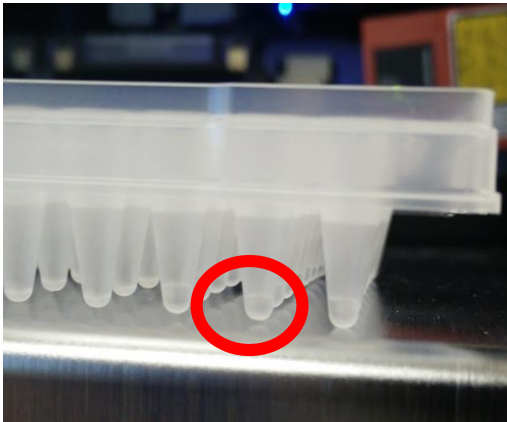
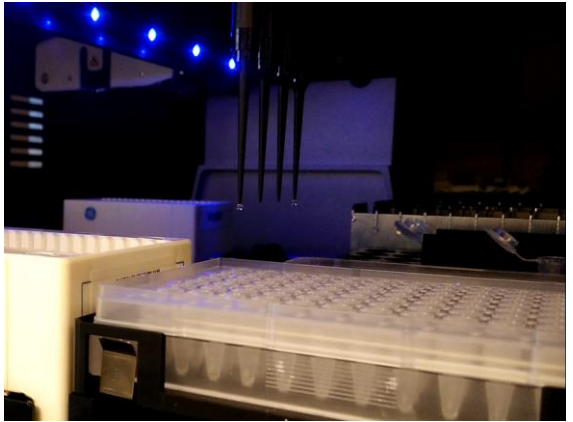



94°
77°



Nevada
Innovation
Center

This document is divided up as follows:

Item	Contents
1	Summary
2	Progress Update
3	Action Items

Item	Description
<p>Positive Control – Incorrect Volumes</p>	<p>[Resolved– 7.17.15]</p> <p>Problem The final volume of the positive control in the plate is incorrect, the wrong volume is being delivered.</p> <div style="display: flex; justify-content: space-around;">   </div> <p>FIGURE. (LEFT) POSITIVE CONTROL VOLUME IN A2 IS LOWER THAN IT SHOULD BE. (RIGHT) LIQUID DROPLETS ON THE TIP AFTER DISPENSE.</p> <p>Otherwise perfectly good cards were being placed into the recovery rack because the imaging software could not read the card.</p> <p>Discussion We ran a plate with 8 samples, 8 positive controls, and 8 negative controls with no allelic ladders (so each group would occupy a column and it would be easy to compare them).</p> <p>Log Files, COM Trace, and Service iData</p> <div style="display: flex; justify-content: space-around; align-items: flex-end;"> <div style="text-align: center;">  HID_4958b7a3d8fa4a02a3100064caa640ad_Trace.trc </div> <div style="text-align: center;">  HxUsbComm20150717.trc </div> <div style="text-align: center;">  ML_STAR_6452_idat a_2015-07-17.pdf </div> </div> <p>Solution We found the positive control was being dispensed at a height of 5mm above the bottom of the PCR plate. Because this is the first dispense into those wells (the PCR mix contains the water), I changed it to dispense at 0.5mm height.</p>

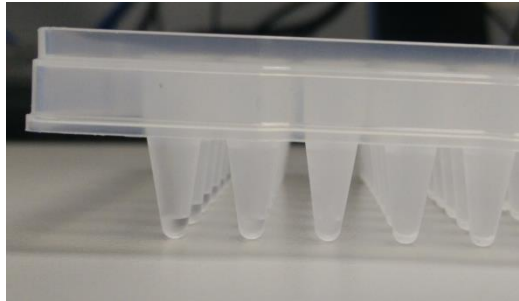
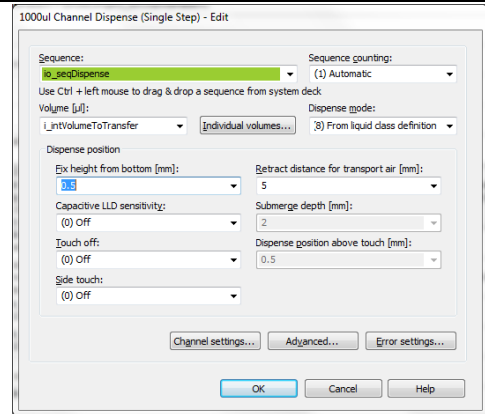
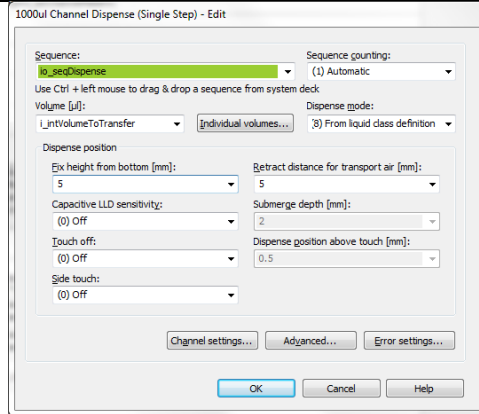


FIGURE. (LEFT) INCORRECT DISPENSE HEIGHT (RIGHT) IMPROVED DISPENSE HEIGHT
(BOTTOM) PLATE WITH CONSISTENT VOLUMES.

[Resolved – 7.17.15]

Problem

During addition of the water to the PCR plate, in the first aspiration, the first tip enters the water reservoir, aspirates, and then dispenses back into the reservoir. This only happens with the first tip, and it only happens on the first aspiration, all return aspirations behave normally.

Discussion

It appears in the method that someone attempted to add a pre-wetting step to the water addition, but the channel pattern variable is not properly set:

Water Addition – Prewetting

bstract	PunchCards	TransferPCRMix	TransferPositiveControls	TransferWater	_AdjustSequencePositionsKeepPlatePattern	_AdjustSequencePositionsMove
TransferWater						
155		<p>Comment</p> <pre><===== FILE NAME: TransferWater AUTHOR: Jeff James PURPOSE: Transfers water onto the PCR plate re-using the PCR function from the logic-layer The procedure is as follows: ** WARNING: the variables in this function were copied from the TransferPCRMix() function ** ** Even though the variable names are PCRMixVolume, PCRMixLiquidClass, etc, they correspond to the water volumes, liquid class, etc ** REVISIONS: 1/29/15 - Jeff James. - Multidispenes water 1/29/15 - Jeff James. - Improved performance with single dispense 1/30/15 - Alex R. - Modified tip reset to tip edit 7/17/15 - Alex R. - Noticed that there is a funky pre-aspirate / dispense build into the water tube, disabled it. Attempt at pre-wetting? ===== ></pre>				
156		Grouping trace				
157		<p>X=0 Assignment 'strFunctionName' = ""</p>				
158		<p>HSL code. strFunctionName = GetFunctionName();</p>				
159		<p>Release_Trace4 of TraceLevel TRACELEVEL:Release_Trace4("====", strFunctionName, "====", "begin")</p>				
160		<p>X=0 Assignment 'strLibraryName' = ""</p>				
161		<p>HSL code. strLibraryName = GetFileName();</p>				
162		Grouping				
163		<p>Sequence: Set Current Position current position of sequence 'io_seqPCRMixOnTargetPlates' = '1'</p>				
164		<p>Sequence: Set Current Position current position of sequence 'l_seqPCRAmplificationMix' = '1'</p>				
165		Grouping tip pickup				
229		<p>Comment</p> <pre><===== ***** COME BACK TO THIS ***** ***** WHO ADDED THIS HERE ***** ===== ></pre>				
230		<p>1000ul Channel Aspirate (Single Step) on ML_STAR Channel (1..4): strChannelPattern, Optimized channel use: All sequence positions, Sequence: l_seqPCRAmplificationMix, Sequence counting: (0) Manually, Liquid class: l_strPCRMixLiquidClassName, Volume [uL]: l_ftVolumePCRMixPerWell, Mix volume [uL]: *, Cycles: 0, Position relative to liquid surface: * mm, LLD settings: On, Capacitive:5, Liquid following: On 3 return value(s) .</p>				
231		<p>1000ul Channel Dispense (Single Step) on ML_STAR Channel (1..4): strChannelPattern, Optimized channel use: All sequence positions, Sequence: l_seqPCRAmplificationMix, Sequence counting: (0) Manually, Liquid class: As in first aspiration of cycle, Volume [uL]: l_ftVolumePCRMixPerWell, Mix volume [uL]: *, Cycles: 0, Position relative to liquid surface: * mm, LLD settings: On, Capacitive:5, Liquid following: On</p>				

Solution

The code was disabled. If after further testing it is determined pre-wetting step is truly required, we can change the code so that it is done correctly.

[Resolved – 7.17.15]

Imaging Optimization

Problem

The imaging settings appear to not detect the sample for control card 5. Admittedly some of these cards are challenging.

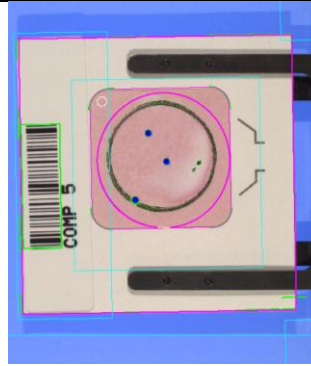
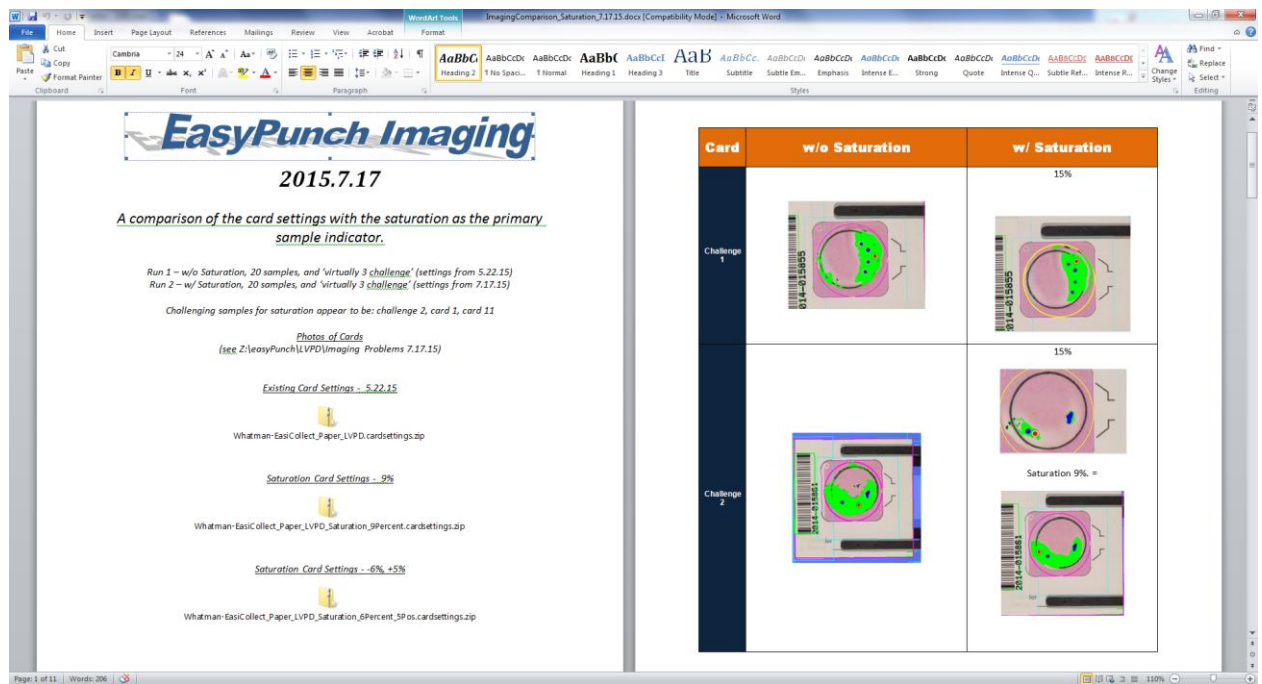


FIGURE. CONTROL 5

Discussion

It appears in the method that someone attempted to add a pre-wetting step to the water addition, but the channel pattern variable is not properly set:



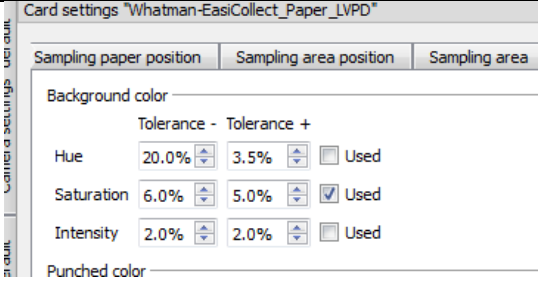
Below is a report file that contains the analysis shown above:



ImagingComparison_Saturation_7.17.15.c

Solution

Changed the card settings to use saturation instead of Hue. The last cardsettings change was 5.22.15

	<p><u>Cardsettings</u></p>  <p>Whatman-EasiCollect_Paper_LVPD.cardsettings.zip</p>
<p>Cosmetic Improvements</p>	<p>[Not Started]</p> <p>Two cosmetic issues pointed out by Jen:</p> <ol style="list-style-type: none"> 1) The loading graphic should show that both cleaning card positions are required even if only one card is going to be punched. 2) The master mix loading graphic positions do not match the positions in the loading instructions.

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ACTION ITEMS

Task	Priority	Description
Image Testing	High	The new image settings need to be tested for robustness, we evaluated them against control card 5, and 20 historical cards in the database, but that's not enough to be sure. Some of these samples have sparse and oddly positioned sample making it very difficult for the robot to know where to place the next punch.
Performance Check	Medium	A performance check needs to be run on the system to confirm the changes to the positive control and water pipetting are correct and do not impact anything else in the method.
Cosmetic Items	Low	The loading graphic should show that both cleaning card positions are required even if only one card is going to be punched. The master mix loading graphic positions do not match the positions in the loading instructions.