# Purpose

This document describes how to make a worklist to run an experiment on the Hamilton STAR liquid handling robot. The experimental design described here is specifically for running and/or optimizing lateral flow assays (LFAs).

# Scope

This document applies to the Hamilton STAR liquid handling robot and its operators.

# Definitions

|  |  |
| --- | --- |
| **TERM** | **DEFINITION** |
| PPE | Personal Protective Equipment |

# Related Documents

1. Hamilton STAR Operator Manual
2. Microlab STAR Software Programmer Manual
3. DROP SOP-001: Hamilton STAR operation
4. DROP Protocol-002: LFA Image Analysis

# Roles and Responsibilities

|  |  |
| --- | --- |
| **Role** | **Responsibility** |
| Principal User | * Establish and implement this procedure * Ensure users are adequately trained in use of this instrument * Complete Training Form and provide to Quality Manager for recordkeeping * Review procedure periodically for necessary updates |
| Instrument User | * Complete training on use of the instrument * Perform tasks as specified in procedure |
| Lab Manager | * Review procedure periodically for necessary updates |

# Worklist Background

## The worklist is a .csv file that is input into the Hamilton Run Control to the robot what actions to follow and in what order.

## The file containing the worklist must end with ‘worklist.csv’ otherwise the Hamilton software will not recognize it. The file containing the worklist must end with worklist.csv; otherwise, the Hamilton software will not recognize it.

## The file must be a plain .csv file (not a UTF-8 or any other csv version).

## A worklist can be made manually or by coding. For LFA optimization work, we have python-based code called the “Robot Worklist Generator”.

## The worklist is composed of 21 columns (two of which are optional) and infinite rows. The identity and definition of each of these columns is described in Section 8. The order of the columns does not matter.

# Worklist Column Definitions

## **step** – a text string to describe what the step is (e.g. conjugate addition, sample addition, buffer addition, imaging)

## **volume\_µL** – volume to be pipetted. Must be smaller than the volume associated with the liquid class (50, 300, or 1000 depending). If volume is set to 0, the robot will take a picture instead of pipetting.

## **liquid\_class** – liquid class associated with the liquid to be pipetted. These classes have been designed specifically for the liquids that we pipette and the volumes we plan to use. There is a list of possible liquid classes found in the CO-RE liquid editor. The specific liquid classes designed for LFAs can be found in the LFA documentation.

## **tip\_type** – this value is associated with the liquid class selected and the volume to be pipetted. This value will be either 50, 300, or 1000. This value needs to be larger than the volume to be pipetted.

## **dispense\_type** – two dispense types, “Surface\_Empty” and “Jet\_Empty” are used. Surface\_Empty goes to the tip of the liquid, detected using a capacitive measurement, and dispenses 2mm below that height. This feature struggles when there are bubbles at the top of a liquid. Jet\_Empty goes inside the well and dispenses at a set height above the bottom of the well plate. Jet\_Empty tends to be accurate at faster speeds than Surface Empty.

## **asp\_mixing** – programs the number of mixing cycles performed after a dispense step. This value needs to be the same value for an entire group. This feature only works for Surface\_Empty liquid classes.

## **source** – the name of the liquid that is being pipetted. By using specific descriptors, source can be useful for the user when designing the worklist.

## **group\_number** – the “group” classification is an important one. Each group needs to be from the same tip\_type (50, 300, 1000). The group number is used by the robot to control the order and timing of pipetting steps. It is recommended to keep groups of 8 rows if you want to control the order in which the robot completes each step.

## *Note: if one item in a group has mixing step, for example, then all items in that group will be mixed. However, using too many groups will make the method too slow. Therefore, group numbers need to be assigned wisely.*

## **timer\_delta** – If the assay has time dependent steps, a value to program a time delay between the step and the next (associated) step, defined in timer\_group\_check. The value here is in seconds.

## **timer\_group\_check** – this value will tell the program which step from which the time should refer to, in seconds. For example, if you wanted group 3 to occur 10 minutes after group 1 is complete, then your group\_number=3, timer\_group\_check=1, and timer\_delta (for group 1) = 600.

## **touchoff\_dis** –used to change the distance vertically that the pipette tip moves up after touching a surface before dispensing. Set value to -1 when not in use.

## **to\_plate** – the ID of the plate pipetting step is going to. For most cases, there are more than one of each type of plate on the deck, indicated by the number at the end of the to\_plate ID. Plates used in LFA applications are the following:

**Table 1.** Plate IDs with descriptions.

|  |  |  |
| --- | --- | --- |
| **Type of plate** | **to\_plate ID** | **Max volume per well (practical 80%)** |
| 96 well DW plate | dw\_96\_0001, dw\_96\_0002, dw\_96\_003 | 1000 µL (800) |
| 96 well flat bottom plate | flat\_96\_0001, flat\_96\_0002, flat\_96\_0003 | 300 µL (240) |
| 384 well flat bottom plate | flat\_384\_0001, flat\_384\_0002, flat\_384\_0003 | 100 µL (80) |
| Multichannel reservoir | flat\_reservoir\_0001, flat\_reservoir\_0002, flat\_reservoir\_0003 | 50-100 mL |

## *Note: Any standard plate will work for each of the categories above.*

**Table 2.** Plate IDs for LFA holders.

|  |  |
| --- | --- |
| **Type of custom LFA- hardware** | **to\_plate ID** |
| LFA strip holder | *16 strip holders: 8 x 2*  IVL\_Plate\_v2a\_16strips\_plastic\_col\_first\_0001 to 012 |
| LFA cassette holder | *96 cassettes: 16 x 6*  IVL\_Plate\_v3\_96cassettes\_ABformat\_0001  *80 cassettes: 16 x 5*  IVL\_Plate\_v1\_80cassettes\_ABformat\_0001 |

## **to\_well** –where pipetting step is going to. The ID ranges from 1-96 for 96 well plates, and 1-384 for 384 well plates. Independent of plate size, the numbering is always top to bottom, left to right. Examples of the layout for each plate type are shown below.

**96 well plate**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **96** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** |
| **A** | 1 | 9 | 17 | 25 | 33 | 41 | 49 | 57 | 65 | 73 | 81 | 89 |
| **B** | 2 | 10 | 18 | 26 | 34 | 42 | 50 | 58 | 66 | 74 | 82 | 90 |
| **C** | 3 | 11 | 19 | 27 | 35 | 43 | 51 | 59 | 67 | 75 | 83 | 91 |
| **D** | 4 | 12 | 20 | 28 | 36 | 44 | 52 | 60 | 68 | 76 | 84 | 92 |
| **E** | 5 | 13 | 21 | 29 | 37 | 45 | 53 | 61 | 69 | 77 | 85 | 93 |
| **F** | 6 | 14 | 22 | 30 | 38 | 46 | 54 | 62 | 70 | 78 | 86 | 94 |
| **G** | 7 | 15 | 23 | 31 | 39 | 47 | 55 | 63 | 71 | 79 | 87 | 95 |
| **H** | 8 | 16 | 24 | 32 | 40 | 48 | 56 | 64 | 72 | 80 | 88 | 96 |

**384 well plate**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **384** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** |
| **A** | 1 | 17 | 33 | 49 | 65 | 81 | 97 | 113 | 129 | 145 | 161 | 177 | 193 | 209 | 225 | 241 | 257 | 273 | 289 | 305 | 321 | 337 | 353 | 369 |
| **B** | 2 | 18 | 34 | 50 | 66 | 82 | 98 | 114 | 130 | 146 | 162 | 178 | 194 | 210 | 226 | 242 | 258 | 274 | 290 | 306 | 322 | 338 | 354 | 370 |
| **C** | 3 | 19 | 35 | 51 | 67 | 83 | 99 | 115 | 131 | 147 | 163 | 179 | 195 | 211 | 227 | 243 | 259 | 275 | 291 | 307 | 323 | 339 | 355 | 371 |
| **D** | 4 | 20 | 36 | 52 | 68 | 84 | 100 | 116 | 132 | 148 | 164 | 180 | 196 | 212 | 228 | 244 | 260 | 276 | 292 | 308 | 324 | 340 | 356 | 372 |
| **E** | 5 | 21 | 37 | 53 | 69 | 85 | 101 | 117 | 133 | 149 | 165 | 181 | 197 | 213 | 229 | 245 | 261 | 277 | 293 | 309 | 325 | 341 | 357 | 373 |
| **F** | 6 | 22 | 38 | 54 | 70 | 86 | 102 | 118 | 134 | 150 | 166 | 182 | 198 | 214 | 230 | 246 | 262 | 278 | 294 | 310 | 326 | 342 | 358 | 374 |
| **G** | 7 | 23 | 39 | 55 | 71 | 87 | 103 | 119 | 135 | 151 | 167 | 183 | 199 | 215 | 231 | 247 | 263 | 279 | 295 | 311 | 327 | 343 | 359 | 375 |
| **H** | 8 | 24 | 40 | 56 | 72 | 88 | 104 | 120 | 136 | 152 | 168 | 184 | 200 | 216 | 232 | 248 | 264 | 280 | 296 | 312 | 328 | 344 | 360 | 376 |
| **I** | 9 | 25 | 41 | 57 | 73 | 89 | 105 | 121 | 137 | 153 | 169 | 185 | 201 | 217 | 233 | 249 | 265 | 281 | 297 | 313 | 329 | 345 | 361 | 377 |
| **J** | 10 | 26 | 42 | 58 | 74 | 90 | 106 | 122 | 138 | 154 | 170 | 186 | 202 | 218 | 234 | 250 | 266 | 282 | 298 | 314 | 330 | 346 | 362 | 378 |
| **K** | 11 | 27 | 43 | 59 | 75 | 91 | 107 | 123 | 139 | 155 | 171 | 187 | 203 | 219 | 235 | 251 | 267 | 283 | 299 | 315 | 331 | 347 | 363 | 379 |
| **L** | 12 | 28 | 44 | 60 | 76 | 92 | 108 | 124 | 140 | 156 | 172 | 188 | 204 | 220 | 236 | 252 | 268 | 284 | 300 | 316 | 332 | 348 | 364 | 380 |
| **M** | 13 | 29 | 45 | 61 | 77 | 93 | 109 | 125 | 141 | 157 | 173 | 189 | 205 | 221 | 237 | 253 | 269 | 285 | 301 | 317 | 333 | 349 | 365 | 381 |
| **N** | 14 | 30 | 46 | 62 | 78 | 94 | 110 | 126 | 142 | 158 | 174 | 190 | 206 | 222 | 238 | 254 | 270 | 286 | 302 | 318 | 334 | 350 | 366 | 382 |
| **O** | 15 | 31 | 47 | 63 | 79 | 95 | 111 | 127 | 143 | 159 | 175 | 191 | 207 | 223 | 239 | 255 | 271 | 287 | 303 | 319 | 335 | 351 | 367 | 383 |
| **P** | 16 | 32 | 48 | 64 | 80 | 96 | 112 | 128 | 144 | 160 | 176 | 192 | 208 | 224 | 240 | 256 | 272 | 288 | 304 | 320 | 336 | 352 | 368 | 384 |

## **from\_plate** – plate ID where pipetting step is coming from. See to\_plate above for more information about plate IDs.

## **from\_well** – well ID where pipetting step is coming from. See to\_well above to see more information about well IDs.

## **step\_index** –this does not matter. Set value to 0.

## **destination** – this value can be used to name the destination location, which can be useful when making the worklist. Not required by Hamilton software and can be set to default value of 0 if desired.

## **guid** – ID number associated with that specific well or LFA. guid is used for tracking when multiple steps are associated with one assay or assay type.

## **from\_path** – string value used to track events. Currently not integrated into workflow. Recommend setting to default value, which is “some path” and use other columns to do so instead.

## **dx** – denotes pipetting location in the x-axis. Origin (0,0) coordinates are hardware dependent, but generally located in the bottom left side of the geometry. Need to be manually verified prior to use.

## **dz** – denotes pipetting location in the z-direction. This value is hardware and instrument dependent. Needs to be manually verified prior to use.

# Making A Worklist

## Manual process for making an LFA worklist.

### Define the number of assay steps, timing delays between steps, reagent types, reagent volumes required for each step. This includes addition of antibodies, addition of sample, addition of running buffer, and LFA imaging. This information is used to fill out the ‘step’, ‘volume\_ul’, ‘liquid\_class’, ‘timer\_delta’, ‘source’, ‘step\_index’, ‘group\_number’, ‘timer\_group\_check’ columns in the worklist.csv file.

### Based on the total volume of each reagent type, identify the appropriate hardware plate to hold each reagent, and layout reagents accordingly. Include an additional 30% volume to account for losses during pipetting. Volumes ≥ 200 uL should be placed in a 96 deep well plate, volumes < 200 uL should be placed in a 96 well plate, volumes < 100 uL should be placed in a 384 well plate. This information is use to fill out the ‘from plate’ and ‘from\_well’ columns in the worklist .csv file.

### Identify the custom LFA-hardware to be used based on the architecture of the RDT (i.e, LFA strips with or without cassette). Measure or note the locations where pipetting needs to take place. This information is used to fill out ‘dx’, ‘dz’, ‘to\_plate’, and ‘to\_well’ columns in the worklist .csv file.

### Prepare the worklist. In a “…worklist.csv” file, translate calculations and plate layout into line-by-line commands in the worklist. Use the definitions described in Section 7 to make sure the worklist has all necessary components.

### Test the worklist in simulation mode. Watch simulation closely to make sure all commands are correct. Iterate as needed.

# Records

This SOP does not generate any records.

# Document Revision History

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **REV.#** | **DATE** | **AFFECTED SECTNS.** | **CHANGE DESCRIPTION** | **AUTHOR/OWNER** |
| 00 | 01 July 2022 | N/A | (Initial Release) | Caitlin Anderson and Luis Alonzo |
| 01 | 22 October 2024 | 7 | Updated plate names | Caitlin Anderson |
|  |  |  |  |  |

Only the four most recent revisions are listed. Archived documents are stored per *SOP-0001 Document Control.*

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