

SOP Title: Use and Maintenance of a BioTek Plate Washer in the HPV Serology Laboratory

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1. PURPOSE

- 1.1. The purpose of this procedure is to set instructions in the proper use and handling of the BioTek 405 TS microplate washer.

2. SCOPE

- 2.1. This procedure applies to the Human Papillomavirus (HPV) Serology Laboratory located at the Advanced Technology Research Facility (ATRF), room C2007.

3. REFERENCES

- 3.1. BioTek 405 TS Microplate Washer User Manual
3.2. HSL_EQ_005: Use and Maintenance of a Molecular Device M5 Plate Reader
3.3. HSL_GL_001: Waste Disposal at the Advanced Technology Research Facility
3.4. HSL_GL_006: Reagent Preparation for the HPV Serology Laboratory
3.5. HSL_QS_017: Good Documentation Practices

4. RESPONSIBILITIES

- 4.1. The Research Associate, hereafter referred to as analyst, is responsible for reviewing and following this procedure.
4.2. The Scientific Manager or designee is responsible for training personnel in this procedure and reviewing associated documentation.
4.3. The Quality Assurance Specialist is responsible for quality oversight and approval of this procedure.

5. DEFINITIONS

Term	Definition
ELISA	Enzyme-linked Immunosorbent Assay
OD	Optical Density
SDS	Safety Data Sheets
Type I water	Ultrapure/Reagent Grade/Critical applications
Type II water	Pure/Analytical Grade, used for standard applications

6. REAGENTS, MATERIALS AND EQUIPMENT

- 6.1. Reagents
6.1.1. Tergazyme (VWR, Cat # 21837-118 or equivalent)

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- 6.1.2. Tartrazine Solution (Refer to HSL_GL_006, Section 16)
- 6.1.3. Wash Buffer (Refer to HSL_GL_006)
- 6.1.4. Dulbecco's Phosphate-Buffer Saline (DPBS) (1X) (Gibco, Cat # 14190-136 or equivalent)

6.2. Equipment

- 6.2.1. Balance
- 6.2.2. Microplate Reader, M5 or equivalent
- 6.2.3. Plate Washer (Model 405 TS)
- 6.2.4. Pipettes
- 6.2.5. Small Metal Scooper (VWR, Cat # 13197-386 or equivalent)

6.3. Consumables

- 6.3.1. Measuring Scoop, 10 mL (VWR, Cat # 83008-684 or equivalent)
- 6.3.2. Small Weigh Boats (Thomas, Cat# 9885D37 or equivalent)
- 6.3.3. 96-well MaxiSorp Plate (Nunc, Cat# 439454)
- 6.3.4. Pipette Tips

7. HEALTH AND SAFETY CONSIDERAIONS

- 7.1. Proper safety precautions should be taken while working in a laboratory setting. This includes, but is not limited to, proper protective equipment such as lab coats, safety glasses, closed-toe shoes, and non-latex gloves.
- 7.2. Refer to the respective SDS when working with any chemicals.
- 7.3. Refer to "HSL_GL_001: Waste Disposal at the Advanced Technology Research Facility" regarding waste disposal processes at the ATRF.
- 7.4. While Tartrazine is not toxic, care should be taken to not inhale or ingest it. If inhaled, Tartrazine may produce an allergic or asthmatic reaction. May also irritate skin. If breathing is difficult immediately go to an area with fresh air and alert supervisor. If it comes in contact with skin, wash immediately and watch for any skin irritation. Personnel can wear a mask while working with Tartrazine to help prevent inhalation.

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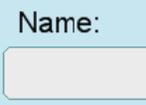
- 7.5. Avoid spilling liquids on the plate washer. Potential shock hazard or instrument damage could result from the internal components being exposed to liquid. If a spill occurs while a program is running, abort the program and turn the instrument off. Immediately wipe up all spills. Do not use the plate washer if the internal components have been exposed to fluid.
- 7.6. Always turn off the plate washer and unplug it before cleaning the outer surface.
- 7.7. Some areas of the plate washer or its components can present pinch hazards while in operation. Keep hands and fingers clear of these areas while operating the instrument.

8. PROCEDURE PRINCIPLES

- 8.1. Reagent bottles are dedicated and labeled for Type II water and Tergazyme solution.
- 8.2. Refer to the process-specific procedure for the Wash Protocol needed (step 9.2.3) for the assay being run.

9. OPERATION AND MAINTENANCE

- 9.1. Icons

	Numbers: Touch any field that requires a numeric value to open a field-sensitive number pad. Use the number pad to specify volume, times, and other values. To enter negative numbers use the down (decrement) arrow.
	Pick Lists: touch a field with a down arrow to open a pick list to select an option.
	Text: Touch any text field or comments box to open a keyboard. Use the keyboard to name protocols, input runtime prompts, enter comments, etc.

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Scrolling: Press the down or up arrows to scroll a list with more items than fit on the screen.



Quick
Prime, Wash, ...
Quick access to prime the washer, wash a plate, and when equipped: AutoClean to sonicate the manifold and Verify™ Technology to check for clogged aspirate and dispense tubes.



Define
Protocols ...
To create and modify protocols.



Maintenance
Rinse, QC, ...
To run maintenance and quality control protocols.



Instrument
Config, Options, ...
To change instrument settings, obtain protocols from a memory stick, etc.



Home: Press the home button to return to the Home screen at any time.



Previous: Press the previous button to go to the previous screen.



Help: To learn more about a screen, press its Help button.



Green buttons make the 405 TS perform.

9.2. Daily Use

9.2.1. Startup Maintenance

9.2.1.1. Turn the instrument ON.

9.2.1.2. Make sure the plate washer is connected to a bottle filled with Type II water. Press **Maintenance**.

9.2.1.3. Press **W-DAY_RINSE** then press **START**.

9.2.1.4. Once the program is done running, go back to the main menu by tapping the “Home” button on the upper left corner of the screen.

9.2.1.5. Document maintenance on “HSL_EQ_004.01: Plate Washer Daily Maintenance Form.”

9.2.2. Buffer Prime

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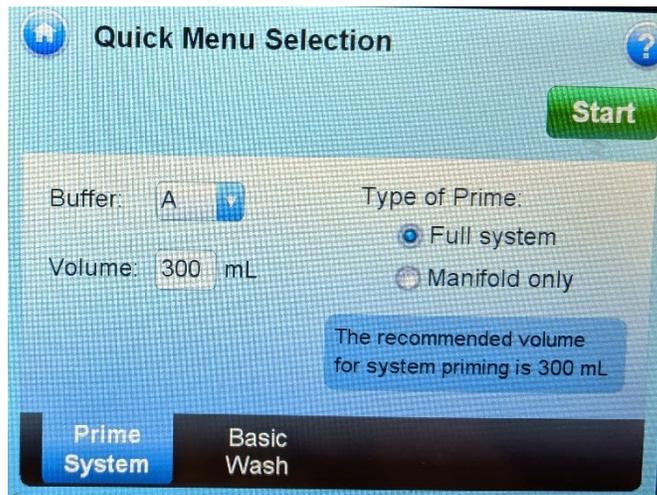
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Note: Prime the washer manifold with buffer only once, prior to start of experiment.

- 9.2.2.1. Connect the plate washer to a reagent bottle filled with washing buffer.
- 9.2.2.2. At the Home screen press **Quick**, select **Prime System**.
- 9.2.2.3. Verify volume is 300 mL and Buffer is connected to the correct Tube (Example: Tube A). Select "Full system" under Type of Prime, then press **START**. See Figure below.



- 9.2.2.4. Once the program is done running, go back to the main menu by tapping the "Home" button on the upper left corner of the screen.
- 9.2.3. Run Assay Wash Protocol
 - 9.2.3.1. Pre-programmed Protocol
 - 9.2.3.1.1. Using the touch screen, choose a predefined protocol from the home screen. See Figure below.

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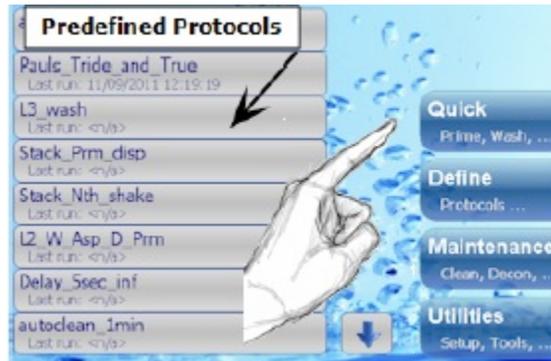
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9.2.3.1.2. Press **START** to run the program.

9.2.3.2. Create or Edit a Protocol

9.2.3.2.1. Select **Define** at the Home/Maintenance screen.

9.2.3.2.2. Press **Create** or highlight the protocol and press **Edit**.

9.2.3.2.3. Naming

9.2.3.2.3.1. New protocols: Touch the name field to open a keyboard. Enter a unique name for the protocol.

9.2.3.2.3.2. Editing protocols: Press the Info button and touch the name field to change the protocol name. This will create a new copy of the protocol being edited. Delete the original protocol if no longer needed.

9.2.3.2.4. Plate Type

9.2.3.2.4.1. New protocols: If needed, touch the plate type field to select a different plate type.

9.2.3.2.4.2. Editing protocols: Press the Info button and touch the Plate Type field to change the plate type.

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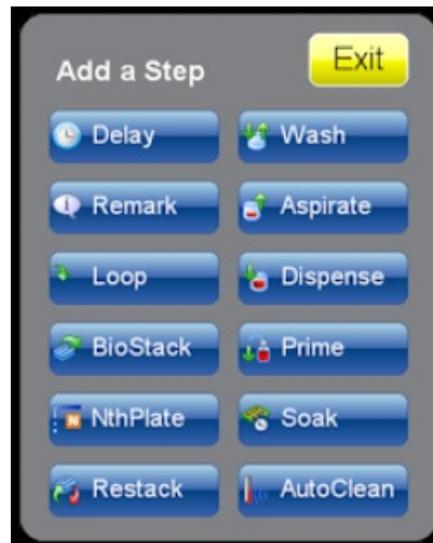
9.2.3.2.5. Press **Save** to save the protocol. All saved protocols are put on the Home screen for easy retrieval.

9.2.3.2.6. Add or Edit Steps

9.2.3.2.6.1. New protocols: To add a step to the protocol, touch the **Add** button and select a step and define its parameters.

9.2.3.2.6.2. Editing protocols: Highlight a step and press **Edit** to modify its parameters.

9.2.3.2.6.3. Adding steps: Highlight the <end of steps> or a step to be preceded by the new step, and press **Add** and the action button to insert a step. See Figure below.



9.2.3.2.6.4. Continue adding or editing steps, as needed.

9.2.3.2.6.5. Press **Save** to save the protocol. All saved protocols

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are put on the Home screen for easy retrieval.

Note: Optionally, do a test run to verify the protocol performs as expected. **Test Run** executes the protocol. Fill reagent bottles with Type II water or disconnect reagent bottles to preserve reagent.

9.2.4. Shutdown Maintenance

Note: Monthly Maintenance can be performed in place of Shutdown Maintenance.

9.2.4.1. Connect the plate washer to a bottle with Type II water. Press **Maintenance**.

9.2.4.2. Press **W-DAY_RINSE** then press **START**.

9.2.4.3. Repeat step 9.2.4.2.

9.2.4.4. Return to the Maintenance Menu and Press **W-RINSE_AND_SOAK** and press **START**. Once the manifold is in the water chamber, turn the machine OFF.

9.2.4.5. Document on HSL_EQ_004.01.

9.2.5. Long Shutdown

9.2.5.1. Long shutdown can be performed when the plate washer will not be used for more than 3 weeks.

9.2.5.2. Perform Monthly Maintenance as described in section 9.3 instead of daily Shutdown Maintenance.

9.2.5.3. Perform Startup Maintenance per section 9.2.1 and Quarterly Maintenance per section 9.4 prior to equipment use after long shutdown.

9.3. Monthly Maintenance

9.3.1. Tergazyme preparation: Dilute 1:100 for typical use; 10 mL of Tergazyme for every 1 L of Type II water. A 10 mL scoop may be used to measure Tergazyme.

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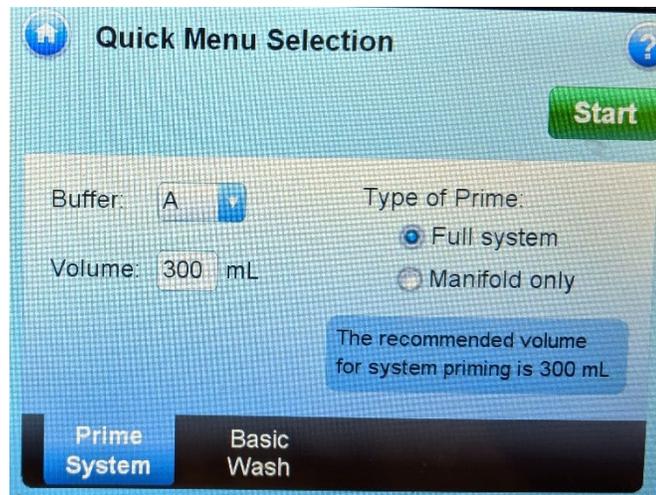
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- 9.3.2. Make sure a reagent bottle is filled to the line with Type II water.
- 9.3.3. Connect the plate washer to a reagent bottle with Tergazyme solution.
- 9.3.4. At the Home screen press **Quick**, select **Prime System**.
- 9.3.5. Verify volume is 300 mL and reagent bottle is connected to the correct Tube (Example: Tube A). Select "Full system" under Type of Prime, then press **START**. See Figure below.



- 9.3.6. Once the program is done running, go back to the main menu by tapping the "Home" button on the upper left corner of the screen.
- 9.3.7. Press **Maintenance**.
- 9.3.8. Press **W-DAY_RINSE**, then press **START**.
- 9.3.9. Once the program is done running, go back to the main menu by tapping the "Home" button on the upper left corner of the screen.
- 9.3.10. Press **W-RINSE_AND_SOAK** and press **START**.
- 9.3.11. Once the program is done running, go back to the main menu by tapping the "Home" button on the upper left corner of the screen.
- 9.3.12. Transfer the tube from the Tergazyme reagent bottle to the Type II water reagent bottle.
- 9.3.13. Press **Maintenance**.
- 9.3.14. Press **W-DAY_RINSE**, then press **START**.

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- 9.3.15. Repeat 9.3.14 twice (In total the W-DAY_RINSE procedure will be performed three times with the Type II water).
- 9.3.16. Finally, clean all bottles with Tergazyme solution and rinse with Type I water and air dry overnight.
- 9.3.17. Document maintenance on “HSL_EQ_004.03: Plate Washer Maintenance Form.”
- 9.4. Quarterly Maintenance (Microplate Washer Carryover Check)
- 9.4.1. The Microplate Washer Carryover Check is performed once per quarter. Record information on “HSL_EQ_004.02: Plate Washer Quarterly Maintenance Form.”
- 9.4.2. Connect plate washer to bottle with any wash buffer used for the HPV assays (1X Wash Buffer or PBS_0.05T).
- 9.4.3. Using a multichannel pipette, add 200 µL of Tartrazine solution to each well of a 96-well MaxiSorp plate.
- 9.4.4. Open “Plate Washer carryover.spr” from the plate reader programs.
- 9.4.5. Save the file as a data file (*.sda). Use the following naming scheme – “Plate Washer carryover EquipmentID# DDMMYY.sda”. Save the file [REDACTED].
- 9.4.6. Verify the wavelength is set to 450 nm using 650 nm as a blank.
- 9.4.7. Insert plate into reader oriented normally with well A1 at the topmost left corner.
- 9.4.8. Read plate as “Pre 1” and save result.
- 9.4.9. Remove plate and turn it 180°. Read the plate again as “Pre 2”. Save results.
- 9.4.10. Remove plate from reader and wash using “HPV ELISA WASH with PRIME” protocol on the washer being tested. See Attachment 1 for protocol parameters. Tap plate on paper towel to remove residual washing buffer.
- 9.4.11. Add 200 µL of 1X DPBS to each well.
- 9.4.12. Insert plate into reader again with A1 oriented to the top left corner.
- 9.4.13. Read as “Post 1” and save results.

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9.4.14. Remove plate from reader and turn it 180°. Read as “Post 2”. Save results.

9.4.15. Print results and attach to HSL_EQ_004.02 per “HSL_QS_017: Good Documentation Practices.”

9.4.16. Quarterly Maintenance Acceptance Criteria

9.4.16.1. “Pre 1” and “Pre 2” OD value must be between 2.20 and 3.00.

9.4.16.2. Carry over percent (%) must be ≤1.0%. Carry over percent should be calculated using the following equation:

$$\left(\frac{\text{Max OD post}}{\text{Min OD pre}} \right) \times 100$$

9.4.17. If OD or carry over value acceptance criteria is not met, perform carryover test again to verify the test was done correctly.

9.4.18. If washer still does not pass after the second test, perform the following:

9.4.18.1. Plate washer Shutdown Maintenance per section 9.2.4 of this procedure.

9.4.18.2. Plate reader monthly plate calibration per “HSL_EQ_005: Use and Maintenance of a Molecular Device M5 Plate Reader” to verify the plate reader performance. If the plate calibration step fails, troubleshoot according to HSL_EQ_005.

9.4.18.3. Repeat the carryover check using freshly prepared Tartrazine solution once shutdown maintenance and plate calibration is complete.

9.4.19. If the OD or carry over value does not pass for the third time, label the plate washer “out of service” and notify Scientific Manager. Confer with Scientific Manager and QA for next steps.

9.4.20. When plate washer passes criteria, record performance of quarterly maintenance on HSL_EQ_004.03.

10. ATTACHMENTS

10.1. Attachment 1: HPV ELISA WASH with PRIME Protocol

10.2. Attachment 2: HSL_EQ_004.01: Plate Washer Daily Maintenance Form

10.3. Attachment 3: HSL_EQ_004.02: Plate Washer Quarterly Maintenance Form

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10.4. Attachment 4: HSL_EQ_004.03: Plate Washer Maintenance Form

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Attachment 1: HPV ELISA WASH with PRIME Protocol

Page: 1 Printed: 5/23/2019 5:20:16 PM

File Name: HPV ELISA Wash with Prime.LHC
File Location: D:\Protocols
Last Saved: 4/26/2019 11:04:48 AM

LHC Version: 2.19.1

Instrument: EL406
Port: USB EL406 sn:16061715
Settings: 96-tube Dual Washer manifold
Buffer Switching
Cell Washing

Plate Type: 96 Well Plate
Protocol Name: HPV ELISA
Protocol Version: <no data>
Archive Revision: 43
Comments: <no data>

Step Details: W-Wash
Pre-dispense before washing: No
Bottom Wash: Yes
Buffer: A
Volume: 300 µL/well
Flow Rate: 7
Z Offset: 121 steps (15.37 mm above carrier)
X Offset: 0 steps (center of well)
Y Offset: 0 steps (center of well)
Pre-dispense: not available
Number of Wash Cycles: 3
Aspirate per cycle
Travel Rate: 3 7.3 & 1.0 mm/sec
Delay: 0 msec
Z Offset: 13 steps (1.65 mm above carrier)
X Offset: 0 steps (center of well)
Y Offset: 38 steps (2.81 mm front of center)
Secondary Aspirate: No
Dispense per cycle
Buffer: A
Volume: 350 µL/well
Flow Rate: 7
Z Offset: 100 steps (12.70 mm above carrier)
X Offset: 0 steps (center of well)
Y Offset: 0 steps (center of well)
Pre-dispense: not available
Delay start of Vacuum until Volume dispensed: 0 µL/well
Shake/Soak after dispense: No
Pre-dispense between cycles: No
Final Aspirate: No
Shake/Soak
Move carrier home: No
Shake: No
Soak: Yes
Duration: 00 min, 05 sec
W-Aspirate
Travel Rate: 3 7.3 & 1.0 mm/sec
Delay: 0 msec
Z Offset: 10 steps (1.27 mm above carrier)
X Offset: -20 steps (0.91 mm left of center)

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Attachment 2: HSL_EQ_004.01: Plate Washer Daily Maintenance Form

Frederick National Laboratory for Cancer Research <small>sponsored by the National Cancer Institute</small>		HPV Serology Laboratory Standard Operating Procedure Form	
Form Title: BioTek Plate Washer Daily Use Maintenance Form			
Document ID: HSL_EQ_004.01		Version:	3.0
Associated SOP: HSL_EQ_004		Effective Date:	
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Month / Year:			
Equipment ID:	HSL_		
Day	Start Up Initials/date	Shutdown Initials/date	
<input type="checkbox"/> N/A 1			
<input type="checkbox"/> N/A 2			
<input type="checkbox"/> N/A 3			
<input type="checkbox"/> N/A 4			
<input type="checkbox"/> N/A 5			
<input type="checkbox"/> N/A 6			
<input type="checkbox"/> N/A 7			
<input type="checkbox"/> N/A 8			
<input type="checkbox"/> N/A 9			
<input type="checkbox"/> N/A 10			
<input type="checkbox"/> N/A 11			
<input type="checkbox"/> N/A 12			
<input type="checkbox"/> N/A 13			
<input type="checkbox"/> N/A 14			
<input type="checkbox"/> N/A 15			
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<input type="checkbox"/> N/A 26			
<input type="checkbox"/> N/A 27			
<input type="checkbox"/> N/A 28			
<input type="checkbox"/> N/A 29			
<input type="checkbox"/> N/A 30			
<input type="checkbox"/> N/A 31			

Reviewed by/date: _____

QA Reviewed by/date: _____

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Attachment 3: HSL_EQ_004.02: Plate Washer Quarterly Maintenance Form

Frederick National Laboratory for Cancer Research <i>sponsored by the National Cancer Institute</i>		HPV Serology Laboratory Standard Operating Procedure Form	
Form Title: BioTek Plate Washer Quarterly Maintenance Form			
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Equipment

Description	Equipment ID	Calibration Due Date
BioTek Plate Washer	<input type="checkbox"/> HSL_002 <input type="checkbox"/> HSL_003 <input type="checkbox"/> HSL_004 <input type="checkbox"/> Other:	
Plate Reader	<input type="checkbox"/> HSL_018 <input type="checkbox"/> Other:	

Reagents

Description	Lot Number	Expiration Date
Tartrazine Solution		
1X DPBS		
1X Wash Buffer		

Results

File Name:		
Reading	O.D. Values (2.20 - 3.00)	Carryover (%) (≤ 1.0%)
Pre-1	<input type="checkbox"/> Pass <input type="checkbox"/> Fail	<input type="checkbox"/> Pass <input type="checkbox"/> Fail
Pre-2	<input type="checkbox"/> Pass <input type="checkbox"/> Fail	<input type="checkbox"/> Pass <input type="checkbox"/> Fail

Comments:

- N/A
- First fail, retest.
- Second fail, perform Shutdown maintenance on Plate Washer and monthly calibration on Plate Reader. Retest using fresh Tartrazine Solution.
- Third fail, equipment placed Out of Service.

Performed by/date:	
Reviewed by/date:	
QA Reviewed by/date:	

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Attachment 4: HSL_EQ_004.03: Plate Washer Maintenance Form

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Maintenance Year:	
Equipment ID:	HSL_

Monthly Maintenance

Month	January	February	March	April	May	June
Tergazyme lot #:						
Tergazyme Expiration Date:						
Performed by/date:						
Reviewed by/date:						
Month	July	August	September	October	November	December
Tergazyme lot #:						
Tergazyme Expiration Date:						
Performed by/date:						
Reviewed by/date:						

Quarterly Maintenance (HSL_EQ_004.02)

Quarter:	Q1	Q2	Q3	Q4
Recorded by/date:				
Reviewed by/date:				

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Form Title: Plate Washer Maintenance Form

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Unscheduled Maintenance

Date	QE Number	Activity Performed	Recorded by/date	Reviewed by/date

QA Reviewed by/date: _____

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