INSTRUCTIONS FOR USE

FLUXERGY TEST KIT SALMONELLA

REF **7068 / 7094**

For Use with the Fluxergy Analyzer CAT #5506-CE

FLUXERGY
30 Fairbanks, Suite 110
Irvine, CA 92618 – USA
+1 (949) 305-4201
customersupport@fluxergy.com
www.fluxergy.com

Instructions For Use (PN: 7036A02)
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1. Intended Use

Fluxergy Test Kit Salmonella PCR is a real-time (RT) polymerase chain reaction (PCR) test intended for the qualitative detection of nucleic acid from Salmonella spp. in environmental samples swabbed from surfaces in veterinary facilities, and equine stalls and equipment in equine practices. Testing should be performed by trained personnel in qualified veterinary facilities and equine practices.

The Fluxergy Test Kit Salmonella PCR is intended to be used in conjunction with the Fluxergy Analyzer and Fluxergy Works software.

2. Explanation of the Test

The Fluxergy Test Kit Salmonella PCR is a qualitative real-time polymerase chain reaction (RT-PCR) test. The primer and probe set are designed to target DNA sequences unique to Salmonella spp. The test performance is monitored by a standardized internal control (IC) to validate the PCR process. Fluxergy Works (software) provides interpretation of results, which are achieved within 1 hour after loading the prepared sample into the Fluxergy Analyzer (instrument).

3. Principles of the Procedure

3.1 Overview

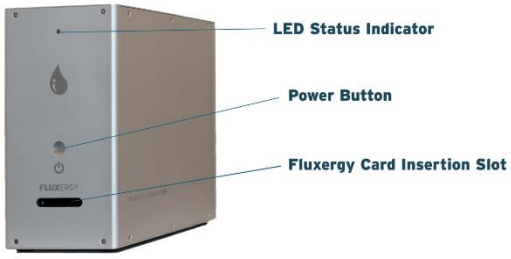
Environmental samples are incubated overnight in enrichment broth. Enriched samples are mixed with the Fluxergy Reaction Mix Salmonella PCR and added to the Fluxergy Card. The Fluxergy Card is then loaded into the Fluxergy Analyzer, and RT-PCR run is started using the Fluxergy Works software. Upon completion of test, Fluxergy Works displays the interpreted result as positive or negative. Result can be printed or downloaded as needed.

3.2 Fluxergy Card

The Fluxergy Card is a disposable card into which the PCR reagents mixed with test samples are manually pipetted in. Each card contains a single sample/reagent input well and microfluidic channels that control the flow of liquid, and reaction wells. The Fluxergy Card is self-contained to prevent cross-contamination between samples.
In the Fluxergy Test Kit Salmonella PCR, fluorescent probes are used together with corresponding forward and reverse primers to amplify Salmonella spp. DNA and exogenous internal control. Two well-conserved regions of the Salmonella spp. genome are targeted to identify Salmonella spp. DNA in the specimen. Internal control is used to detect PCR failure and/or inhibition in addition to monitoring adequate sample processing.

3.3 Fluxergy Analyzer

The Fluxergy Analyzer instrument is a real-time PCR (RT-PCR) thermocycler used for the identification of nucleic acid from biological specimens. The Fluxergy Analyzer performs amplification, detection, and analysis of fluorescent signals generated during PCR.



3.4 Process

In the Fluxergy Test Kit Salmonella PCR test, environmental swab samples collected in neutralizing buffer is incubated and enriched overnight in enrichment broth media. The enriched sample is then mixed with ready-to-use Fluxergy Reaction Mix Salmonella PCR to prepare the complete test master mix. The master mix is then loaded into the Fluxergy Card. After loading the Fluxergy Card into the Fluxergy Analyzer instrument, the test run is initiated. Approximately in 1 hour, Fluxergy Works will complete the thermal cycling and analysis.

3.5 Primer and Probe Sets

Identification of the Salmonella spp. occurs using target specific primers and fluorescent-labeled probes that hybridize to conserved regions of the Salmonella genome.

4. Reagents and Instruments

The Fluxergy Test Kit Salmonella PCR is to be used with the following instrument, reagents, and supplies:

4.1 Materials Provided

The Fluxergy Test Kit Salmonella PCR (single pack) contains sufficient reagents and consumables to test a single specimen or quality control sample. The Fluxergy Test Kit Salmonella PCR contains the following and is available in packs of 10 (CAT #7068) and in packs of 100 (CAT #7094).

- Sample Collection Materials:

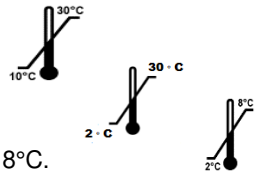
  Sponge-Stick with 10mL Neutralizing Buffer (3M, Cat # SSL10NB)

- Enrichment Media:

  BBL™ Selenite Cystine Broth (Becton, Dickinson and Company, Cat # 297711; Fisher Sci Cat # BB97711) or (Hardy Diagnostics, Cat # K120)

- Fluxergy Reaction Mix Salmonella PCR
- Fluxergy Card

4.1.1 Storage and Handling



- Store the Fluxergy Card at 10 to 30°C.
- Store the Sponge-Stick at 2 to 30°C
- Store the Selenite Cystine Broth at 2 to 8°C.
- Do not open individual Sponge-Stick, Selenite Cystine Broth, and Fluxergy Card packaging until you are ready to test.
- Store the Fluxergy Reaction Mix Salmonella PCR at -30 to -10°C.

- Do not thaw or open Fluxergy Reaction Mix Salmonella PCR reagent until you are ready to test.
- Freeze thaws of Fluxergy Reaction Mix Salmonella PCR will interfere with test results.

4.2 Materials Required but Not Provided

- Fluxergy Analyzer (CAT #5506-CE), sold separately.
- Barcode scanner
  - Light Source: 650 – 670nm
  - Scanner Type: Bi-directional
  - Host System Interfaces: USB
  - Indicators: LED & Buzzer
  - Supported Barcode Formats (minimum requirement): 2D Data Matrix, 2D GS1 Data Matrix
  - Stand with hands-free operation capability.
- Laptop to install Fluxergy Works software.
  - Laptop Computer recommended requirements
    - → Operating System, 64-bit
    - → Windows 10 (build 1151 or later) with Intel Core i5 2.5GHz processor or equivalent
    - → RAM: 8GB DDR4
    - → HDD: 250GB
    - → Screen: 1080p
    - → USB: 2x2.0 port (for scanner and mouse)
    - → Networking: Ethernet port
- Minivortexer, FisherBrand Part Number 14-955-151 or equivalent
- Microcentrifuge, FisherBrand. Part Number 12-006-902 or equivalent
- Micropipettes (20 µL, 200 µL, and 1000 µL) and associated pipette tips
- -20° C manual defrost freezer
- Instructions and Documents
  - Instructions for Use, SDS, and additional resource documents can be found at www.fluxergy.com/downloads.

5. Warnings and Precautions

5.1 General

- Positive results are indicative of the presence of Salmonella spp.
- Ensure that you save your sample in case follow up testing is needed.
- Authorized for use only with the equipment, materials, and supplies indicated in Section 4. Use with equipment, materials, and supplies other than those indicated above in Section 4 may cause errors and erroneous results.
- All biological specimens, including used Fluxergy Cards, used sample collection materials, used sample transfer materials, and used reagents, should be handled as if infectious, using good laboratory procedures as outlined by your local or national authorities, for example: Biosafety in Microbiological and Biomedical Laboratories – 6th Edition (cdc.gov) [1] or Laboratory Biosafety Manual – 4th Edition (WHO) [2].
- Follow safety procedures set by your institution for working with chemicals and handling biological specimens.
- Bleach introduced into a sample may damage DNA in that sample, which may lead to an erroneous result.
- Wear appropriate Personal Protective Equipment (PPE), including (but not limited to) disposable clean powder-free gloves. Protect skin, eyes, and mucus membranes. Change gloves often when handling equipment, reagents, or samples. [3]
- This product may contain components or chemicals that may cause cancer if ingested.
- Dispose of materials used in this assay, including reagents, samples, and used buffer tubes, according to local regulations.

5.2 Test/Reagent

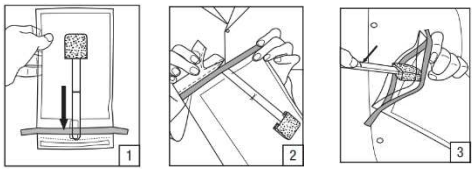
- The Fluxergy Test Kit Salmonella PCR including Fluxergy Card, and Fluxergy Reaction Mix Salmonella PCR are only compatible and for use only with the Fluxergy Analyzer.
- Do not handle samples or Fluxergy Card in a biosafety cabinet which is used for Salmonella spp. culture or immunofluorescence testing.
- Do not use a test kit or components that are damaged.
- Each single-use Fluxergy Card and single-use Fluxergy Reaction Mix are used to process one sample. Do not reuse processed Fluxergy Card and Fluxergy Reaction Mix.
- Each pipette tip is used to transfer one sample.
- Do not use reuse pipette tips for separate pipetting steps.
- Prior to processing samples, thoroughly clean both the work area with a suitable cleaner such as freshly prepared 10% bleach or a similar disinfectant.
- Fluxergy Reaction Mix, Fluxergy Card, and samples should be handled and tested one-at-a-time.
- Always change gloves and clean the work area between using each Fluxergy Card and Fluxergy Reaction Mix.
- Use clean gloves to remove materials from bulk packaging and reseal bulk-packaging when not in use (e.g., Fluxergy Card bulk packaging).
- **Always check the expiration date on the Fluxergy Cards and Fluxergy Reaction Mixes. Do not use kit components after the expiration date.**

6. Sample Requirements

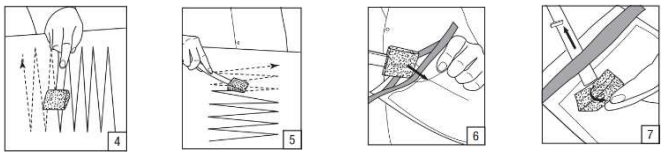
6.1 Sample Collection

Improperly collected, transported, or handled samples risk the potential for false positive, false negative or erroneous results. The detection of bacterial nucleic acid is dependent upon proper sample collection, handling, transportation, storage, and preparation. Follow instructions below to swab and collect environmental sample [4].

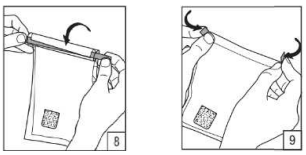
1. Shake stick to end of bag.



2. Tear bag open.
3. Squeeze bag to open. Aseptically grasp the stick above the thump-stop line to remove the sponge.
4. Aseptically swab across the entire sampling surface.
5. Turn sponge over. Change direction 90°. Aseptically swab the same sampled surface.



6. Aseptically place sponge into bag up to the thumb-stop.
7. Hold sponge in place inside bag. Bend stick to break. Allow sponge to drop in bag. Discard stick.
8. Fold bag to close.



9. Fold ends of blue wires inward.

6.2 Transport and Storage

Samples should be enriched within 48 hours of collection to maintain the viability. Post-enrichment, it is recommended to test with the Fluxergy Test Kit Salmonella PCR within the next 48 hours.
* Note: Performance is not guaranteed if samples are not tested immediately. Extended heat and fluctuation of temperature will degrade sample and affect detection of nucleic acid.

7. Test Procedure

7.1 Setting up a Fluxergy Analyzer

Refer to the Fluxergy Analyzer IFU [5] for how to:

- Setup a Fluxergy Analyzer
- Managing devices on Fluxergy Works software
- If using multiple Fluxergy Analyzers, ensure that each device is labeled and uniquely named.
  - The Fluxergy Analyzer will not uniquely flash or prompt to identify itself.
- Adding users on Fluxergy Works software

Prior to running a Fluxergy Test Kit Salmonella PCR, make sure the Fluxergy Analyzer is on and connected to the Fluxergy Works software.

7.2 Test Features

Fluxergy Test Kit Salmonella PCR Features	
Sample Type	Enriched (Selenite Cystine Broth) environmental samples in Sponge-Stick w/10mL Neutralizing Buffer (3M, Cat # SSL10NB)
Minimum amount of sample required	1 mL of Neutralization buffer and 100 µL of the cultured sample
Sample Processing Volume	14 µL of cultured sample
Duration of Test	Approximately 1 hour

7.3 Sample Collection

1. Refer to section 6.1 for environmental sample collection.
2. **Do not discard sample after use in case follow-up testing is needed.**

7.4 Sample Culture

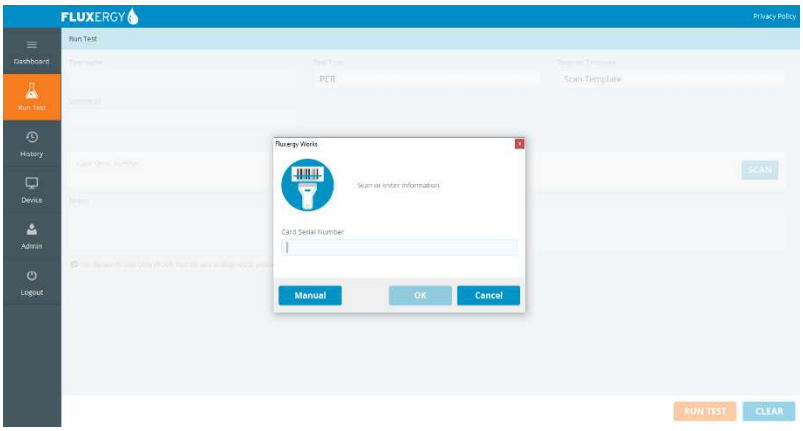
1. Remove the Selenite Cystine Broth Media from the 4°C refrigerator.
2. Remove the cap.
3. Add 1 mL of collected Neutralization Buffer from environmental swab to the culture vial.
4. Screw the cap back onto culture vial and vortex.
5. Slightly loosen the cap to ensure proper aeration of the media. Set the shaking incubator to 35 + 2°C for 20 +/- 4 hours. Coliforms could overgrow the Salmonella if incubated longer than 24 hours.

IMPORTANT:

- **Thaw Fluxergy Reaction Mix Salmonella PCR at room temperature before use (5 min). Ensure that the Reaction Mix is completely thawed and use immediately. (Do not vortex the Reaction Mixes).**
- **Start the test within 4 minutes of adding the sample to the Fluxergy Card.**

7.5 Set Up and Run Your Test on Fluxergy Works Software

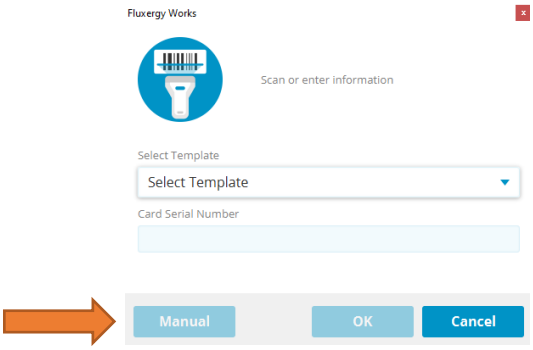
1. Open Fluxergy Works on the laptop and log in using your user ID and password.



2. Click “Run Test” tab on the side-bar on the left side of your screen.

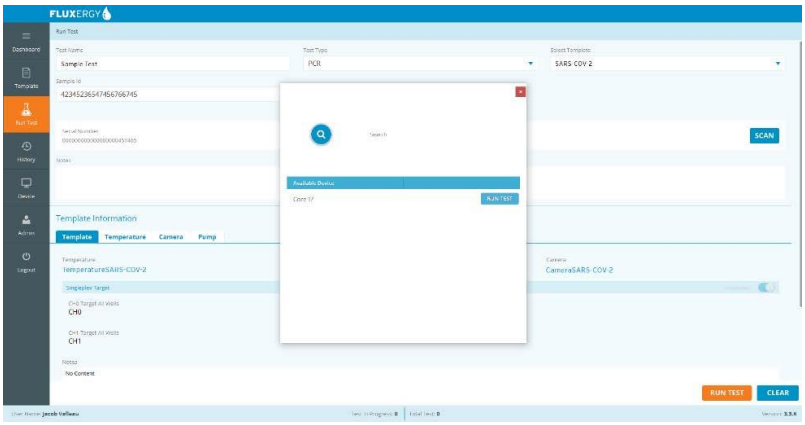
3. Scan the barcode on the Individual Fluxergy Card Package.

   Note: For best results, ensure that the scanning surface is flat, and entire barcode can be captured.



4. If the barcode cannot be scanned a prompt will appear, Click “Manual” and select the correct assay from the dropdown list.

5. Enter the serial number in the “Card Serial Number” section. Click “Ok”.



6. Type in Test Name and Sample ID. Sample specific information can also be included in the Notes section.

7. If a Test is a Retest, append the Test name with “_Retest”

8. Once the Reaction Mix is fully thawed, briefly spin down the Reaction Mix tube using a mini centrifuge.

9. Pipette 14 µL of incubated sample culture into the Reaction Mix tube.

10. Mix the sample and Reaction Mix by gently flicking the bottom of the tube 5 times.

    Caution: DO NOT Vortex Sample Mix and Reaction Mix!

11. Briefly spin down using a mini centrifuge to get residual fluid off cap and walls of the tube.

    Caution: DO NOT use pipette to mix sample and Reaction Mix!

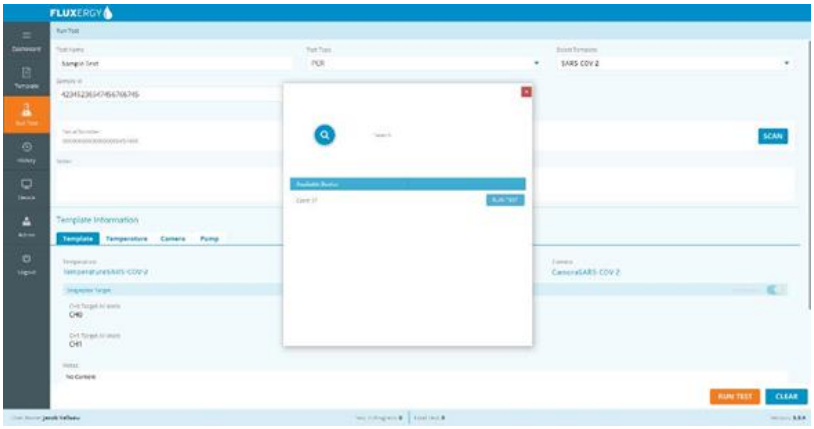
12. Place the Fluxergy Card on a flat surface.

13. Carefully dispense 130 µL into the loading port of the Fluxergy Card.

    Caution: Pipette the sample mix directly onto the loading port. Avoid pipetting onto the loading port's wall.

14. Press the plastic cap onto the loading port of the Fluxergy Card.

15. Once the Fluxergy Card is loaded and cap placed onto the loading port do not invert, shake, or drop the Fluxergy Card.



16. Click “RUN TEST” at the bottom right. Select a device from the list of available devices.

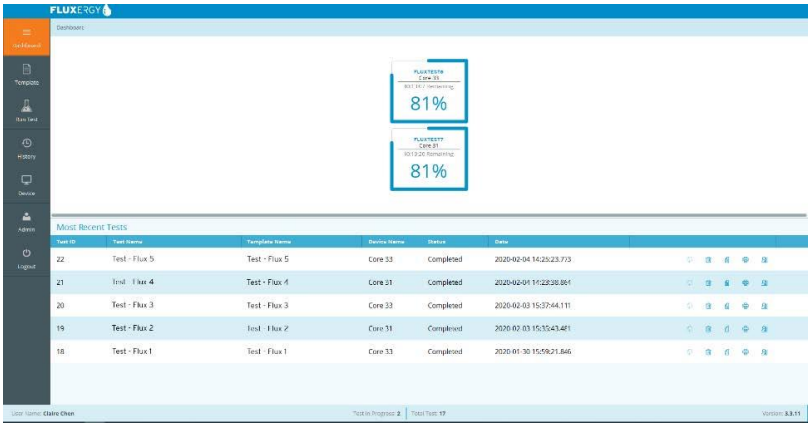
    Note: Fluxergy Works automatically filters unavailable devices.

17. Fluxergy Works will prompt you to insert a loaded Fluxergy Card.

    Note: Immediately proceed to running a test, the user has 4 minutes to insert a Fluxergy Card and run test.

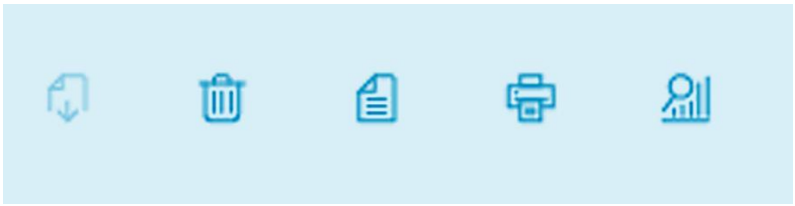
18. Insert the Fluxergy Card with sample and Reaction Mix into Fluxergy Analyzer.

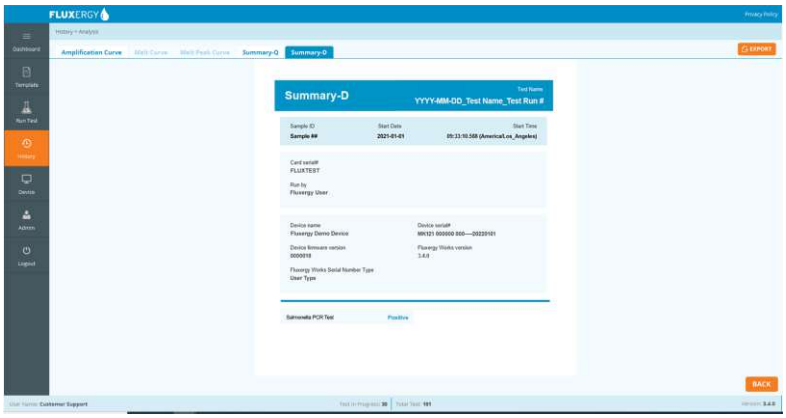
19. Click “OK” on Fluxergy Works to begin the test.



20. The test will be complete in about 1 hour. Tests in progress can be viewed on the Dashboard.

7.6 Accessing your Results





1. Once you select “OK” to run the test, you will be redirected to the dashboard. Here, you can find your tests in progress, as well as a list of your most recent tests.

2. To see all tests, select the “History” tab. On the right-hand side of the window, you can choose to delete, export, print, or view results for each test.

3. Selecting the trash icon will delete test data permanently. Selecting the file icon will allow user to view template information for the test. Selecting the printer icon will enable you to print test summaries. Select the far-right option, the magnification glass and graph icon, to view and export your results.

4. Summary D in each test will contain the qualitative test result.

* For detailed instructions on how to view and print the results, see the *Fluxergy Analyzer IFU*.

8. Quality Control

8.1 Internal Control

An internal control, included as part of the Reaction Mix, is used in the background of every test to validate a true negative. Amplification must occur in the internal control channel for a negative to be qualified as a negative.

8.2 External Control

Fluxergy recommends including one positive and one negative control daily. Fluxergy does not supply External Controls with Fluxergy Test Kit Salmonella PCR. External Controls can be requested from Fluxergy separately.

8.2.1 Negative Control

- A “no template”, negative control is used when a new system is first set up, as well as for training or proficiency testing. The routine QC testing frequency will be based on the labs IQCP requirements.
- The negative control is added to Fluxergy Reaction Mix Salmonella PCR in the same way an environmental sample would be (refer to section 7 of IFU for Test Procedure).
- Based on its internal validation, Fluxergy recommends using Selenite Cystine broth as the negative control.

8.2.2 Positive Control

- A positive template control is used when a new system is first set up, as well as for training or proficiency testing. The routine QC testing frequency will be based on the labs IQCP requirements.
- The positive control is added to Fluxergy Reaction Mix Salmonella PCR in the same way an environmental sample would be (refer to section 7 of IFU for Test Procedure).
- Fluxergy Salmonella PCR External Control (+) available in pack of 1 (CAT #7128) or pack of 10 (CAT #7124), sold separately.

9. Interpretation of Results

9.1 Test Outputs

Test Output	Interpretation
Positive	Salmonella spp. DNA present
Negative	Salmonella spp. DNA not present

9.2 Error Codes

Error Codes	User Action on First Error	User Action on Second Error
600-625, 627-636	1. Restart Fluxergy Analyzer. 2. Retest sample with new card and mix.	Contact Customer Service
626	1. Dilute sample. 2. Retest diluted sample with new card and mix.	Contact Customer Service
Network Error*	Click to retrieve Test Result.	Follow instructions above for any other errors.

** This error can happen when analyzer loses connectivity with PC.*

9.3 Retests

If an ERROR is shown as the result from the test, there is a strong likelihood that you need to retest the original sample. In cases where sample quality may have played a role, you may need to recollect the sample.

The procedure to retest is as follows:

1. If there is no sample recollection required, use the leftover sample from the original swab. If sample recollection is required, collect according to 7.3 and standard procedure.
2. Use a clean pair of gloves as if starting a new test. Vortex the sample for 90 seconds and follow steps 7.4 – 7.5.
3. Make sure to give a different name to test in Fluxergy Works (e.g. Original Test Name_RETEST).
4. If an Error comes back for a second time, contact customer service, and seek confirmatory testing.

9.4 Restarting the Fluxergy Analyzer Device

For instructions on how to Restart, Refer to the Fluxergy Analyzer IFU.

10. Limitations

- For use for Salmonella spp. PCR testing only.
- False negatives may occur if the number of bacterial genome copies in the specimen are below the test limit of detection (LoD).
- As with other tests, false positives may occur. Some settings may indicate the need for repeat testing or testing using a different system.
- The test cannot rule out disease or infection caused by other bacterial or viral pathogens. The tests only detect Salmonella spp. DNA.
- As with any molecular test, mutations within the target regions of Salmonella spp. could affect primer binding, resulting in failure to detect the presence of bacteria.
- The test was only validated against the following sample type: environmental swab using Sponge-Stick w/10mL Neutralizing Buffer and cultured in Selenite Cystine Broth.
- Improperly collected, transported, or handled samples risk the potential for false positive, false negative or erroneous results. The detection of bacterial nucleic acid is dependent upon proper sample collection, handling, transportation, storage, and preparation.
- The internal control will not indicate whether nucleic acid has been lost due to inadequate collection, transport, or storage of samples.
- Because the test is a direct PCR, correct mixing of the sample is important for test function.

11. Conditions of Use for the Laboratory

Use of the Fluxergy Test Kit Salmonella PCR must follow procedures outlined in the manufacturer's Instructions for Use and under the conditions set by the health authorities in your country.

- Laboratories using the Fluxergy Test Kit Salmonella PCR will use the materials and equipment identified in the Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use the Fluxergy Test Kit Salmonella PCR are not permitted.
- All laboratory personnel using the Fluxergy Test Kit Salmonella PCR must be appropriately trained in in performing and interpreting the results of the Fluxergy Test Kit Salmonella PCR, use appropriate personal protective equipment when handling this kit, and use the Fluxergy Test Kit Salmonella PCR in accordance with the authorized labeling.
- Fluxergy, authorized distributors, and laboratories using the Fluxergy Test Kit Salmonella PCR will ensure that records are maintained. Such records will be made available to their national authorities for inspection upon request.

12. Performance Evaluation

12.1 Assay Performance Evaluation [6]

The performance evaluation of the Fluxergy Test Kit Salmonella PCR was conducted with serially diluted Salmonella spp. in negative pooled environmental NB swab matrix down to 1, 3, and 5x LoD. One mL each of the samples were spiked into separate BD BBL™ Mycoflask™ Selenite Cystine Broth (Contrived samples). The samples were enriched overnight an incubator (G-Biosciences Incubator Shaker Equipment Asset #300138) and tested as per the IFU. Another aliquot of the sample was enriched and tested per the IFU of Thermo Scientific SureTect Salmonella species PCR Assay (PT0100A, the comparator assay). A total of 117 enriched environmental swab samples (60 Salmonella positive and 57 Salmonella negative) were collected and tested by both the assays.

The samples were randomized and blinded before testing with the Fluxergy Test Kit Salmonella PCR using the Fluxergy Analyzer to generate the Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) estimates. PPA and NPA were determined by comparing results of the Fluxergy Test Kit Salmonella PCR against the expected results. Results of these 117 archived clinical enriched environmental swabs samples are shown in table below.

Performance Comparison of Fluxergy Test Kit Salmonella PCR			
Fluxergy Test Kit Salmonella*	**Expected Results**		
	Positive	**Negative**	**TOTAL**
Positive	60	0	***60***
Negative	0	57	***57***
TOTAL	***60***	***57***	***117***
PPA	*100.00% (60/60); 92.50% to 100.00%*		
NPA	*100.00% (57/57); 92.13% to 100.00%*		

** During the study, any samples with errors were retested as instructed in IFU.*

12.2 Analytical Performance Evaluation

12.2.1 Limit of Detection (LoD) [7] – Analytical Sensitivity

The LoD of the Fluxergy Test Kit Salmonella PCR test was conducted with serially diluted Salmonella stock in negative pooled environmental NB swab matrix. One mL of each dilution was spiked into separate BD BBL™ Mycoflask™ Selenite Cystine Broth Prepared Medias (BD 297711). The samples were cultured an incubator (G-Biosciences Incubator Shaker Equipment Asset #300138) at 37C for 16-24 hours.

After the preliminary LoD was determined by testing serially diluted and enriched samples in triplicate, a confirmation testing was done with 20 replicates. The limit of detection for Salmonella was defined as the lowest concentration at which each target is detected at least 95% of the time. The claimed LoD of the Fluxergy Test Kit Salmonella PCR is 1 CFU/mL.

Confirmatory LoD of the Fluxergy Test Kit Salmonella PCR in pooled environmental swab matrix.

LoD Determination using Salmonella Strain	
Concentration Tested	**# Tested / # Detected (%)**
1CFU/mL	19/20 (95%)

12.2.2 Inclusivity – Analytical Reactivity

The analytical reactivity of the Fluxergy Test Kit Salmonella PCR was evaluated using *Salmonella serotypes* that have been extracted and diluted to a concentration of 7.14 copies/μL (100 copies/card).

Testing was performed with the Fluxergy Test Kit Salmonella PCR for the presence of clinically relevant *Salmonella serotypes*, within the environmental swab matrix. Using the Fluxergy's Test card and on the Fluxergy Analyzer.

The below results show the evidence that the Fluxergy Test Kit Salmonella PCR detected all available serovars, the analytical reactivity has been successfully met.

All 20x *Salmonella serotypes* were detected at a concentration of 100 copies per test (per card).

Salmonella Analytical Reactivity Testing Results		
Organisms Tested (Serotype/ Serovar)	Results	Results
Salmonella enterica subsp. enterica, ATCC® 6994™ (Serovar Typhimurium)	Pos (3/3)	Positive
Salmonella enterica subsp. enterica, A36 (Serovar Typhimurium)	Pos (3/3)	
Salmonella enterica subsp. enterica, 15/5 (Serovar Abortusovis)	Pos (3/3)	
Salmonella enterica subsp. enterica, G4639 (Serovar Montevideo)	Pos (3/3)	
Salmonella enterica subsp. enterica, Strain IN01 (Serovar Tennessee)	Pos (3/3)	
Salmonella enterica subsp. enterica, 9640 (Serovar Dublin)	Pos (3/3)	
Salmonella enterica subsp. enterica, SARA23 (CDC B1722) (Serovar Saint Paul)	Pos (3/3)	
Salmonella enterica subsp. enterica, SL473 (CVM19633) (Serovar Schwarzengrund)	Pos (3/3)	
Salmonella enterica subsp. enterica, SL475 (CVM29188) (Serovar Kentucky)	Pos (3/3)	
Salmonella enterica subsp. enterica, SL485 (CVM35947) (Serovar Hadar)	Pos (3/3)	
Salmonella enterica subsp. enterica, SL491 (CVM36357) (Serovar Virchow)	Pos (3/3)	
Salmonella enterica subsp. enterica, 2004 Pennsylvania Tomato Outbreak, Serovar Anatum, Isolate 3	Pos (3/3)	
Salmonella enterica subsp. enterica, 2004 Pennsylvania Tomato Outbreak, Serovar Javiana, Isolate 8	Pos (3/3)	
Salmonella enterica subsp. enterica, 2004 Pennsylvania Tomato Outbreak, Serovar Muenchen, Isolate 3	Pos (3/3)	
Salmonella enterica subsp. enterica, 2004 Pennsylvania Tomato Outbreak, Serovar Thompson, Isolate 1	Pos (3/3)	
Salmonella enterica subsp. enterica, Hopkins 26 (Serovar Typhi)	Pos (3/3)	
Salmonella enterica subsp. enterica, Ty2 (Serovar Typhi)	Pos (3/3)	
Salmonella enterica subsp. enterica, ATCC® 9150™ (Serovar Paratyphi-A)	Pos (3/3)	
Salmonella enterica subsp. diarizonae, CDC 01-0005	Pos (3/3)	
Salmonella enterica subsp. enterica, BL6802 (Serovar Typhi)	Pos (3/3)	

12.2.3 Assay Reproducibility

The reproducibility testing was conducted to assess the Inter-assay precision (reproducibility) of Fluxergy Test Kit Salmonella PCR to detect the presence of *Salmonella spp.* within environmental swab matrix.

Salmonella spp. was serially diluted within negative pooled environmental NB swab matrix to 1, 2, and 3x LoD. One mL of the contrived sample was tested per instructions in the IFU. The study found the assay to be reproducible (100% concordant calls) and low variability (<10% CV for observed FAM Ct values (Table 1.0).

Table 1.0: Assay Reproducibility Testing per card run (test)

Sample	Replicates	Mean (FAM Ct)	Std Dev (FAM Ct)	% CV
1x LoD	32	20.30	1.60	7.88%
2x LoD	32	20.21	1.83	9.07%
3x LoD	32	19.90	0.89	4.46%

12.2.4 Cross-Reactivity - Analytical Specificity

The cross-reactivity testing was conducted to validate the specificity of Fluxergy Test Kit Salmonella PCR designs i.e. it specifically detects *Salmonella spp.*, but does not detect other environmentally relevant organisms.

Cross-reactivity of Fluxergy Test Kit Salmonella PCR was evaluated by wet-lab testing of organisms listed below. All cross-reactive micro-organisms were extracted and diluted to a concentration of (1E+5 copies/mL).

None of the organisms tested in the specificity study listed below were detected by Fluxergy Test Kit Salmonella PCR, thereby demonstrating the assay specificity.

Cross-Reactivity of Fluxergy Test Kit Salmonella PCR Testing Results			
Organism	**Strain**	**Cat # (BEI Resources)**	**Results**
Enterococcus Faecalis	B3119	NR-31884	No detection
Escherichia Coli	KTE181	NR-32771	No detection
Klebsiella Oxytoca	MIT 10-5244	HM-625	No detection
Acinetobacter Radioresistens	WC-A-157	NR-17788	No detection
Micrococcus Luteus	SK58	HM-114	No detection
Burkholderia Pyrrocinia	2327	NR-708	No detection
Enterococcus Faecium	TX1330	HM-204	No detection
Pseudomonas Aeruginosa	MRSN 315	NR-51515	No detection
Shigella sp	D9	HM-87	No detection
Citrobacter Portucalensis	4_7_47CFAA	HM-299	No detection
Escherichia Coli	B6914-MS1 (Serotype O157:H7)	NR-6	No detection
Listeria Monocytogenes	FSL J1-194	NR-13229	No detection
Streptococcus Agalactiae	MNZ933	NR-43896	No detection
Listeria Ivanovii	WSLC3009	NR-51326	No detection
Citrobacter Freundii	GED7749C	HM-1280	No detection
Staphylococcus Epidermidis	VCU013	NR-46376	No detection
Acinetobacter Baumannii	H72721	NR-9667	No detection
Shigella Dysenteriae		NR-520	No detection
Bacillus Subtilis	168	NR-607	No detection

Cross-Reactivity of Fluxergy Test Kit Salmonella PCR Testing Results			
Organism	**Strain**	**Cat # (BEI Resources)**	**Results**
Bacillus Cereus	G9241	NR-9564	No detection
Serratia sp.	Ag2	NR-50123	No detection
Streptococcus pyogenes	MGAS9882	NR-15272	No detection
Genomic DNA from Yersinia pestis	PB6	NR-2718	No detection

** copies/mL, calculated based on the total nucleic acid concentration of extracted stock material.*

12.2.5 Interference Study [8]

The assay interference from exogenous substances potentially present in the horse stables as well as remaining cleaning agents were tested in this study. No interference was observed with any of the substances tested. The interfering substances were spiked into 3x LoD Salmonella (3CFU/mL) and were added directly to the Selenite Broth and cultured overnight for 16hrs. Testing was performed with the Fluxergy Test Kit Salmonella PCR using the Fluxergy Analyzer and included triplicate testing per substance at the indicated levels. A positive detection of test samples indicated lack of interference.

Potential Interfering Substance	Concentration Tested	Salmonella Detection (#Detected / #Tested)	IC % Detection (#Detected / #Tested)
Hydrogen Peroxide	0.2%	100% (3/ 3)	100% (3/ 3)
Dodecylbenzenesulfonic Acid	0.12%	100% (3/ 3)	100% (3/ 3)
Tannic Acid	90 ng/uL	100% (3/ 3)	100% (3/ 3)
Humic Acid	90 ng/uL	100% (3/ 3)	100% (3/ 3)
Collagen	100 ng/uL	100% (3/ 3)	100% (3/ 3)
Bleach	0.5%	100% (3/ 3)	100% (3/ 3)
Para-tertiary-Amylphenol	0.008%	100% (3/ 3)	100% (3/ 3)
Ortho-Benzyl-para-chlorophenol	0.02%	100% (3/ 3)	100% (3/ 3)
2-Phenylphenol	0.02%	100% (3/ 3)	100% (3/ 3)
Potassium peroxymonosulfate	0.02%	100% (3/ 3)	100% (3/ 3)
Sodium Chloride	0.002%	100% (3/ 3)	100% (3/ 3)
Urea	100mM	100% (3/ 3)	100% (3/ 3)
Didecyl Dimethyl Ammonium Chloride	0.013%	100% (3/ 3)	100% (3/ 3)
Chlorhexidine Gluconate	0.008%	100% (3/ 3)	100% (3/ 3)

13. Symbols and Marking

13.1 Symbols on Packaging

Symbol	Meaning
	The instructions for use are available for download electronically from the website shown.
	This symbol indicates the number of pieces in the package (10).
	Indicates that the transport package shall be kept away from rain and in dry conditions.

Symbol	Meaning
YYYY-MM-DD	Indicates the expiration date, after which the product is not to be used. The date format is YYYY-MM-DD where YYYY represents the four (4) digit year, MM is the two (2) digit month and DD is the two (2) digit day.
YYYY-MM-DD	Indicates the manufacturing date, when the product is manufactured. The date format is YYYY-MM-DD where YYYY represents the four (4) digit year, MM is the two (2) digit month and DD is the two (2) digit day.
UDI	Indicates the unique device identification (UDI) number.
REF	Indicates the Fluxergy catalogue number so that the device can be identified.
SN	Indicates the Fluxergy serial number so that a specific device can be identified.
LOT	Identifies the manufacturer's batch or lot code.
	Indicates the container's net volume in specific unit of measure.
	Indicates that the item is for single use only and must not be used more than once.
	Indicates the medical device manufacturer, as defined in EU Directive 98/79/EC. This symbol is used to identify the name and address of company that manufactured the product.
	Indicates the temperature limits to which the product can be safely exposed and gives the maximum and minimum storage temperatures.
	Indicates that the user should not use the product without inspecting the contents of the package if the package is badly damaged.
	Indicates methods to contact customer support.
	Test Card Image
	Reaction Mix Image
	Selenite Broth Image
	Sponge Swab Image

13.2 Symbols used in this IFU

Symbol	Meaning
	Indicates that there are potential biological risks associated with the device after use. This symbol is used to remind the user that the Fluxergy Test Kit Salmonella PCR is considered hazardous waste after it has been used to perform a test. The used Fluxergy Card should be disposed of as required by national authorities.

14. Contact and Legal Information

14.1 Fluxergy Headquarters Location

FLUXERGY
30 Fairbanks, Suite 110
Irvine, CA 92618, USA

14.2 Customer and Technical Support

14.2.1 Contact us by Mail

Attn: Fluxergy Customer Support
30 Fairbanks, Suite 110
Irvine, CA 92618, USA

14.2.2 Contact us by Email

customersupport@fluxergy.com

14.2.3 Contact us by Phone

+1 (949) 305-4201 US & International

15. References

[1] U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020.

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[3] Clinical And Laboratory Standards Institute. Protection Of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline - Fourth Edition. CLSI M29-A4.

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[5] Fluxergy Inc., *IFU for the Fluxergy Analyzer System*, Current Revision.

[6] CLSI. User Protocol For Evaluation Of Qualitative Test Performance; Approved Guideline-Second Edition. CLSI EP12-A2.

[7] CLSI. Protocols For Determination Of Limits Of Detection And Limits Of Quantitation; Approved Guideline. CLSI EP17-A.

[8] CLSI. Interference Testing In Clinical Chemistry - 3rd Edition. CLSI EP07-Ed3.