Protocols for Laboratory Verification of Performance of the BioFire® Global Fever Special Pathogens Panel

Laboratory Protocols for Use with ZeptoMetrix NATtrol™ Control Materials

TECHNICAL NOTE

Purpose

The Clinical Laboratory Improvement Amendments (CLIA), passed in 1988, established quality standards for all laboratory testing to ensure the accuracy and reliability of patient test results, regardless of where the test is performed. The CLIA regulations include a requirement for verifying the performance specifications of unmodified, moderate and high complexity tests cleared or approved by the FDA.

This document provides example protocols for the verification of BioFire Global Fever Special Pathogens (GF SP) Panel performance on BioFire® FilmArray® 2.0 and BioFire® FilmArray® Torch Systems. The example verification schemes provide positive and negative tests for each organism detected by the BioFire GF SP Panel and may be easily modified or expanded to meet specific criteria. Day-to-day variation is evaluated by testing each sample on two separate days. To evaluate user-to-user variation, multiple laboratory technicians may test the same sample. In addition, testing patient samples for verification or to evaluate matrix effects on the performance of the panel should be done under the guidance of the Laboratory Director but is not described here.

As per the CLIA regulation, the Laboratory Director is ultimately responsible for ensuring that verification procedures meet the appropriate standards for CLIA and applicable laboratory accrediting agencies.

Intended Use

The BioFire® Global Fever Special Pathogens Panel is a qualitative, multiplexed, nucleic acid-based test intended for use with BioFire FilmArray 2.0 and BioFire FilmArray Torch Systems. The BioFire Global Fever Special Pathogens Panel is for the simultaneous qualitative detection and identification of multiple bacterial, viral, and protozoan nucleic acids directly from EDTA whole blood collected from individuals with signs and/or symptoms of acute febrile illness or recent acute febrile illness and known or suspected exposure to the target pathogens described below.

Technical Note

BioFire Defense, LLC
www.BioFireDefense.com

DFA2-PRT-0221-01

Pathogens identified:

- Chikungunya virus
- Dengue virus (serotypes 1, 2, 3 and 4)
- *Leishmania* spp. that cause visceral leishmaniasis (e.g., *L. donovani* and *L. infantum*)
- *Leptospira* spp.
- *Plasmodium* spp. (including species differentiation of *Plasmodium falciparum* and *Plasmodium vivax/ovale*)
- West Nile virus

Pathogens presumptively identified:

- *Bacillus anthracis*
- Crimean-Congo hemorrhagic fever virus
- *Ebolavirus* spp.
- *Francisella tularensis*
- Lassa virus
- *Marburgvirus*
- Yellow fever virus
- *Yersinia pestis*

The complete intended use statement and additional information about the use of the BioFire Systems can be found in the *BioFire® Global Fever Special Pathogens Panel Instructions for Use*.

Performance Verification Overview

Two different examples of performance verification procedures are described: (1) a Simple Protocol for the verification of the BioFire GF SP Panel and (2) a Blood Protocol that evaluates BioFire GF SP Panel performance in the intended panel matrix. These procedures are provided to assist your laboratory in developing a protocol for the verification of BioFire GF SP performance on BioFire Systems. A BioFire System is defined as all BioFire FilmArray Instruments or Modules that are connected to and controlled by a single computer system.

The verification procedures described here may be used to evaluate the performance of each assay on the BioFire GF SP Panel. The performance verification protocols have been designed to take advantage of the multiplex nature of the test. Verification testing efficiency is maximized by evaluating multiple target organisms in a single test run. The procedures described below will generate multiple positive and negative detections for each of the assays on the test.

The procedures were developed using the NATtrol™ Global Fever Panel available from ZeptoMetrix LLC, Buffalo, NY (Part No. NATGFP-BIO).

If the laboratory director chooses not to perform the entire verification protocol on each individual instrument, it is advised that test replicates are evenly distributed among the instruments or modules. An example of a performance verification workflow using 2, 4, or 6 modules is provided in Figure 2.

Clinical/patient samples may be used in place of, or in addition to the verification schemes described here in order to assess clinical sensitivity/specificity and sample matrix effects as part of the performance verification of the BioFire GF SP Panel.

Table 1. Overview of Verification Protocols

Verification Protocol	Organisms per Pool[a]	Number of Sample Pools	Replicates per Sample Pool	Pouches Required	Expected Positive Results[a]	Expected Negative Results	Approximate Days of Testing[b]
Example 1: Simple protocol	5 or 6	4	4	16	≥4 per organism	≤12 per organism	4
Example 2: Blood protocol	5 or 6	4	4	16	≥4 per organism	≤12 per organism	4

[a] The expected number of positives and negatives per organism is dependent upon the number strains of a particular organism used to complete the verification.
[b] The approximate number of days for testing assumes a BioFire System configured with one instrument/module.

Performance Verification Materials

The following materials may be used to perform the verification procedure:

Table 2. Recommended materials for the verification protocols for the GF SP Panel

Material	Part Number
Panel kit	DFA2-ASY-0018
Panel IFU	DFA2-PRT-0136
Panel quick guide	DFA2-PRT-0137
ZeptoMetrix control[1]	NATGFP-BIO
Blood	Any of various biorepository sources
Sample tubes	Various manufacturers
Transfer pipettes	VWR, 414004-024 (or equivalent)

[1] Any appropriate source of organism may be used for verification of any or all of the assays on the BioFire GF SP Panel. However, when alternate organism sources are used (i.e., not the ZeptoMetrix control material), the sample volumes or pooling schemes suggested in the examples below may need to be adjusted.

Simple Protocol

The Simple Protocol evaluates the BioFire GF SP Panel performance when verification materials (NATGFP-BIO) are pooled in the absence of clinical matrix. The proposed organism pooling scheme (Table 3) should be followed to obtain the expected number of positive and negative results for each assay in a time and resource-efficient manner.

NOTE: *Dilution of ZeptoMetrix organisms beyond levels proposed in these guidelines may lead to inconsistent results and is not recommended.*

Figures 1 and 2 illustrate workflow schemes for testing 4 replicates per pool for 4 different pools over multiple days. This produces a total of 16 verification sample test runs and provides at least 4 positive results and as many as 12 negative results per assay. Some organisms, such as dengue virus, are represented multiple times. This is done to ensure all dengue virus assays are represented in the verification protocol.

The number of samples tested per day should be determined by the individual laboratory. This testing scheme can be modified to run more (or fewer) samples per day based on the number of modules in the BioFire System. The pooling scheme in Table 3 provides sufficient volume for retesting if necessary or for testing more replicates if desired. Figure 2 provides an example of user-to-user, day-to-day, and module-to-module testing for labs with multiple BioFire modules.

Pooled samples can be stored overnight (or up to 3 days) at refrigeration temperature (2–8°C) for subsequent testing to evaluate day-to-day variation.

Table 3. Proposed Organism Pooling Scheme for the Simple Protocol

Organism	Approximate Organism Volume	Approximate Pool Volume
Pool 1		
E. coli w/ Marburg Virus plasmid	0.3 mL	1.8 mL
E. coli w/ Ebolavirus Taï Forest/Zaire plasmid	0.3 mL	
E. coli w/ Lassa Virus plasmid	0.3 mL	
E. coli w/ *P. spp/ovale* plasmid	0.3 mL	
E. coli w/ *Y. pestis* plasmid	0.3 mL	
Yellow Fever Virus (17D)	0.3 mL	
Pool 2		
E. coli w/ *B. anthracis* plasmid	0.3 mL	1.8 mL
Chikungunya Virus (R80422)	0.3 mL	

E. coli w/ Dengue Virus Type 2 (Dak Ar A1247) plasmid	0.3 mL	
E. coli w/ Ebolavirus Bundibugyo/Sudan plasmid	0.3 mL	
E. coli w/ *Leishmania* plasmid	0.3 mL	
Dengue virus type 2 (New Guinea C)	0.3 mL	
Pool 3		
E. coli w/ CCHF Virus plasmid	0.3 mL	1.5 mL
Dengue type 3 (H87)	0.3 mL	
E. coli w/ Ebolavirus Reston plasmid	0.3 mL	
E. coli w/ *F. tularensis* plasmid	0.3 mL	
West Nile Virus (NY 2001-6263)	0.3 mL	
Pool 4		
Dengue Virus type 1 (Hawaii)	0.3 mL	1.5 mL
Dengue Virus type 4 (H241)	0.3 mL	
E. coli w/ *Leptospira* plasmid	0.3 mL	
E. coli with *P. falciparum/vivax* plasmid	0.3 mL	
West Nile Virus (B-956 Uganda)	0.3 mL	

Simple Protocol Example

The estimated total time for completion for this Simple Protocol verification example is 4 days for a BioFire System configured with 1 module. A proposed organism pooling scheme is presented above in Table 3.

NOTE: *It is important to prepare only the number of sample pools that will be tested within 3 days of preparation. The suggestion to prepare 3 sample pools is based on testing up to 4 pouches per day. The number of samples prepared may be increased or decreased based on the laboratory's work schedule and number of modules connected within a BioFire System.*

Day 1

1. Organize needed materials (Table 2).
2. Prepare two sample pools (i.e., Pools #1 and 2), one at a time, from ZeptoMetrix NATtrol™ Global Fever Panel control materials. Organism vials should be mixed vigorously for 5 seconds prior to preparing each pool. Refer to Table 3 for example organism pooling schemes and specific volumes for each pool. Start with the first pool.
    a. Transfer 0.3 mL of material from the ZeptoMetrix organism vial into a 2 mL tube. Alternatively, a 5mL tube may be used.
    b. Repeat with the second (and subsequent) organisms to combine the appropriate organisms for each pool into a

single tube. The total volume for each pool will be approximately 1.5 or 1.8 mL.

c. Ensure the pooled sample is well mixed prior to removing a sample for testing.

3. Repeat Step 2 for the remaining sample pool (i.e., Pool #2) to be prepared on Day 1.
4. Test 2 replicates from a single sample pool (i.e., Figure 1: Pool # 1 replicates A and B). The replicate samples should be tested in a single day by different users.

   NOTE: *For each sample, follow instructions in the BioFire® Global Fever Special Pathogens Panel Instructions for Use and the BioFire® Global Fever Special Pathogens Panel Quick Guide for pouch preparation, pouch hydration, sample loading, and sample testing. When setting up a test on the BioFire System users are prompted to select a run protocol, verification controls must be tested using the* ***GF Blood*** *protocol.*

5. Repeat Step 4 for the remaining sample pool replicates to be tested that day (i.e., Pool # 2 replicates A and B)
6. Refrigerate samples (2–8°C) for up to 3 days for the evaluation of day-to-day variation.

   NOTE: *The proposed organism pooling scheme (Table 3) provides sufficient material for running samples as described in Figure. 1. The volume is sufficient for testing more samples if desired.*

Day 2

To evaluate day-to-day variation, test replicates from the same sample pools prepared on Day 1 by repeating Step 4 and 5 above (i.e., Pools 1 and 2, replicates C and D for each).

Day 3

Prepare 2 new sample pools (i.e., Pools #3 and 4) as described in Steps 2 and 3. Test replicates as described in Steps 4 and 5 above.

Day 4

To evaluate day-to-day variation, test replicates from the same sample pools prepared on Day 3 by repeating Step 4 and 5 above (i.e., Pool # 3 replicates C and D).

NOTE: *A BioFire GF SP Verification Record is provided and may serve as a template for recording your results.*

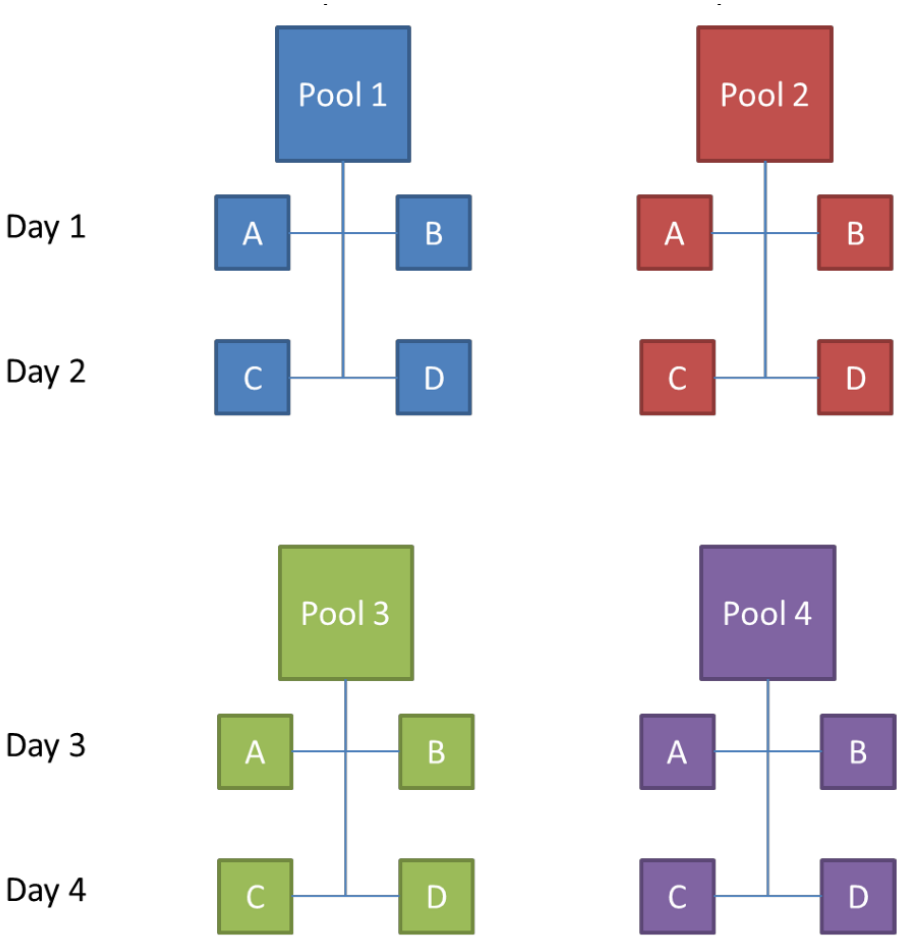

Figure 1. Workflow for the Simple Protocol and the Blood Protocol

Verification with 2 modules	Module 1		Module 2	
Day 1	Pool 1/ User 1	Pool 2/ User 2	Pool 1/ User 2	Pool 2/ User 1
Day 2	Pool 1/ User 2	Pool 2/ User 1	Pool 1/ User 1	Pool 2/ User 2
Day 3	Pool 3 / User 1	Pool 4 / User 1	Pool 3/ User 2	Pool 4 / User 2
Day 4	Pool 3/ User 2	Pool 4 / User 2	Pool 3 / User1	Pool 4 / User 1

Verification with 4 modules	Module 1	Module 2	Module 3	Module 4
Day 1	Pool 1/ User 1	Pool 1/ User 2	Pool 2/ User 1	Pool 2/ User 2
Day 2	Pool 2/ User 2	Pool 2/ User 1	Pool 1/ User 2	Pool 1/ User 1
Day 3	Pool 3 / User 1	Pool 3/ User 2	Pool 4 / User 1	Pool 4 / User 2
Day 4	Pool 4 / User 2	Pool 4 / User 1	Pool 3/ User 2	Pool 3 / User1

Verification with 6 modules	Module 1	Module 2	Module 3	Module 4	Module 5	Module 6
Day 1	Pool 1/ User 1	Pool 1/ User 2	Pool 2/ User 1	Pool 2/ User 2		
Day 2			Pool 1/ User 1	Pool 1/ User 2	Pool 2/ User 1	Pool 2/ User 2
Day 3	Pool 3 / User 1	Pool 3/ User 2			Pool 4 / User 1	Pool 4 / User 2
Day 4	Pool 4 / User 2	Pool 4 / User 1	Pool 3 / User1	Pool 3/ User 2		

Figure 2. Example of a verification workflow for use with multiple BioFire Modules

Blood Protocol

The Blood Protocol evaluates the BioFire GF SP Panel performance when verification materials (ZeptoMetrix NATtrol™ Global Fever Panel) are tested in the presence of the relevant clinical matrix. The proposed organism pooling scheme (Table 4) should be followed to obtain the expected number of positive and negative results for each assay in a time and resource-efficient manner.

NOTE: *Dilution of ZeptoMetrix organisms beyond levels proposed in these guidelines may lead to inconsistent results and is not recommended.*

The protocol and workflow schemes (Figures 1 and 2) illustrate testing 4 replicates per pool for 4 different pools over multiple days. This produces a total of 16 verification sample test runs and provides at least 4 positive results and as many as 12 negative results per assay. Some organisms, such as dengue virus, are represented multiple times. This is done to ensure all dengue virus assays are represented in the verification protocol.

The number of samples tested per day should be determined by the individual laboratory. This testing scheme can be modified to run more (or fewer) samples per day based on the number of modules in the BioFire System. The pooling scheme provides sufficient volume for testing more replicates if desired.

Pooled samples can be stored overnight (or up to 3 days) at refrigeration temperature (2–8°C) for subsequent testing to evaluate day-to-day variation.

Table 4. Proposed Organism Pooling Scheme for the Blood Protocol

Organism	Approximate Organism Volume	Volume Blood	Approximate Pool Volume
Pool 1			
E. coli w/ Marburg Virus plasmid	0.3 mL	1.8 mL	3.6 mL
E. coli w/ Ebolavirus Taï Forest/Zaire plasmid	0.3 mL		
E. coli w/ Lassa Virus plasmid	0.3 mL		
E. coli w/ *P. spp/ovale* plasmid	0.3 mL		
E. coli w/ *Y. pestis* plasmid	0.3 mL		
Yellow Fever Virus (17D)	0.3 mL		
Pool 2			
E. coli w/ *B. anthracis* plasmid	0.3 mL	1.8 mL	3.6 mL
Chikungunya Virus (R80422)	0.3 mL		
E. coli w/ Dengue Virus Type 2 (Dak Ar A1247) plasmid	0.3 mL		

E. coli w/ Ebolavirus Bundibugyo/Sudan plasmid	0.3 mL		
E. coli w/ *Leishmania* plasmid	0.3 mL		
Dengue virus type 2 (New Guinea C)	0.3 mL		
Pool 3			
E. coli w/ CCHF Virus plasmid	0.3 mL	1.5 mL	3.0 mL
Dengue type 3 (H87)	0.3 mL		
E. coli w/ Ebolavirus Reston plasmid	0.3 mL		
E. coli w/ *F. tularensis* plasmid	0.3 mL		
West Nile Virus (NY 2001-6263)	0.3 mL		
Pool 4			
Dengue Virus type 1 (Hawaii)	0.3 mL	1.5 mL	3.0 mL
Dengue Virus type 4 (H241)	0.3 mL		
E. coli w/ *Leptospira* plasmid	0.3 mL		
E. coli with *P. falciparum/vivax* plasmid	0.3 mL		
West Nile Virus (B-956 Uganda)	0.3 mL		

Blood Protocol Example

The estimated total time for completion of this Blood Protocol verification example is 4 days for a BioFire System configured with 1 module. A proposed organism pooling scheme is presented above in Table 4.

NOTE: *It is important to prepare only the number of sample pools that will be tested within 3 days of preparation. The suggestion to prepare 2 sample pools is based on testing up to 4 pouches per day. The number of samples prepared may be increased or decreased based on the laboratory's work schedule and number of modules connected within a BioFire System.*

Day 1

1. Organize needed materials (Table 2).
2. Prepare two sample pools (i.e., Pools #1 and 2), one at a time, from ZeptoMetrix NATtrol™ Global Fever Panel control material. Organism vials should be mixed vigorously for 5 seconds prior to preparing each pool. Refer to Table 4 for example organism pooling schemes and specific volumes for each pool. Start with the first pool.
    a. Transfer 0.3 mL of material from the ZeptoMetrix organism vial into a 5mL tube.
    b. Repeat with the second (and subsequent) organisms to combine the appropriate organisms for each pool into a single tube. The total volume for each pool will be approximately 1.5 to 1.8 mL.

c. Add 1.5 or 1.8 mL of blood (as described in Table 4) to the tube containing the organism pool (step b). The total volume will be approximately 3.0 to 3.6 mL.

3. Repeat Step 2 for the remaining sample pool (i.e., Pool #2) to be prepared on Day 1.
4. Test 2 replicates from a single sample pool (i.e., Figure 1: Pool # 1 replicates A and B). The replicate samples should be tested in a single day by different users.

   NOTE: *For each sample, follow instructions in the BioFire® Global Fever Special Pathogens Panel Instructions for Use and the BioFire® Global Fever Special Pathogens Panel Quick Guide for pouch preparation, pouch hydration, sample loading, and sample testing. When setting up a test on the BioFire System users are prompted to select a run protocol, verification controls must be tested using the* ***GF Blood*** *protocol.*

5. Repeat Step 4 for the remaining sample pool replicates to be tested that day (i.e., Pool # 2 replicates A and B).
6. Refrigerate samples (2–8°C) for up to 3 days for the evaluation of day-to-day variation.

   NOTE: *The proposed organism pooling scheme (Table 4) provides sufficient material for running samples as described in Figure. 1. The volume is sufficient for testing more samples if desired.*

Day 2

To evaluate day-to-day variation, test replicates from the same sample pools prepared on Day 1 by repeating Step 4 and 5 above (i.e., Pools 1 and 2, replicates C and D).

Day 3

Prepare 2 new sample pools (i.e., Pools #3 and 4) as described in Steps 2 and 3. Test replicates as described in Steps 4 and 5 above.

Day 4

To evaluate day-to-day variation, test replicates from the same sample pools prepared on Day 3 by repeating Step 4 and 5 above (i.e., Pool # 3 replicates C and D).

NOTE: *A BioFire GF SP Panel Verification Record is provided and may serve as a template for recording your results.*

Software Protocol Compatibility

It is necessary to select the appropriate software protocol prior to running the test. The Positive External Control and the Negative External Control protocols are only for use with the BIOFIRE SHIELD Control Kit for the BioFire GF SP Panel and should not be used to test clinical specimens or other types of controls.

The BioFire System prompts the user to select a software protocol after scanning the pouch barcode. Verification testing using the ZeptoMetrix NATtrol™ Global Fever Panel described in this technical note must be completed using the **GF Blood** software protocol.

Expanding the Protocols

The protocols described above can be expanded by increasing the number of tests from each of the organism pools. Each organism pool contains sufficient volume for retesting or for testing additional replicates.

Verification of Loaner, Repaired, and Permanent Replacement Instruments or Modules

If it becomes necessary to verify the performance of a loaner, repaired, or permanent replacement instrument or module, the following protocol may serve as a guideline but should be verified by the Laboratory Director.

1. Select a few specimens and/or proficiency samples (any combination of positives and negatives) previously tested on the panel. The Laboratory Director should determine the appropriate number of samples to test. Proficiency samples should not be pooled or diluted.

2. Select a set of controls that verify detection of all targets on the BioFire GF SP Panel.

Test the selected samples on the loaner, repaired, or permanent replacement instrument or module and document the results.

Technical Support Contact Information

BioFire Defense provides the best customer support available. If you have any questions or concerns about this process, please contact the BioFire Technical Support Team for assistance.

General Information
Email: support@biofiredefense.com
Phone: 1-801-262-3592
Fax: 1-801-447-6907

BioFire Global Fever Special Pathogens (GF SP) Panel Verification Record

Kit Part # _____ **Module Serial #** _____ **Module Serial #** _____

Lot # _____ **Module Serial #** _____ **Module Serial #** _____

	Organism and Representative Strain	Replicate Testing - Record Organism Detections																Summary					
		1-A	1-B	1-C	1-D	2-A	2-B	2-C	2-D	3-A	3-B	3-C	3-D	4-A	4-B	4-C	4-D	# Positives	# Negatives	# Users	# Days	# Modules	Patient Samples
Pool 1	E. coli w/ Marburg Virus plasmid																						
	E. coli w/ Ebolavirus Taï Forest/Zaire plasmid																						
	E. coli w/ Lassa Virus plasmid																						
	E. coli w/ P. spp/ovale plasmid																						
	E. coli w/ Y. pestis plasmid																						
	Yellow Fever Virus (17D)																						
Pool 2	E. coli w/ B. anthracis plasmid																						
	Chikungunya Virus (R80422)																						
	E. coli w/ Dengue Virus Type 2 (Dak Ar A1247) plasmid																						
	E. coli w/ Ebolavirus Bundibugyo/Sudan plasmid																						
	E. coli w/ Leishmania plasmid																						
	Dengue virus type 2 (New Guinea C)																						
Pool 3	E. coli w/ CCHF Virus plasmid																						
	Dengue type 3 (H87)																						
	E. coli w/ Ebolavirus Reston plasmid																						
	E. coli w/ F. tularensis plasmid																						
	West Nile Virus (NY 2001-6263)																						
Pool 4	Dengue Virus type 1 (Hawaii)																						
	Dengue Virus type 4 (H241)																						
	E. coli w/ *Leptospira* plasmid																						
	E. coli with *P. falciparum/vivax* plasmid																						
	West Nile Virus (B-956 Uganda)																						

Reviewed by: _____

Signature Date