OMB Control No. 1018-####
Expires ##/##/20##

1STUDY PROTOCOL FOR A COMPASSIONATE AQUACULTURE INVESTIGATIONAL NEW ANIMAL DRUG (INAD) EXEMPTION FOR ERYTHROMYCIN (Erymicin 200 Injection) (INAD 12-781)

Sponsor:

U.S. Fish and Wildlife Service, Fish and Aquatic Conservation

_____ _____

Sponsor Signature Date Approved

Manufacturer/Source of Supply:

Syndel USA
1441 W Smith Rd
Ferndale, WA 98248 USA

Office for Coordination of Erythromycin (Erymicin 200 Injection) INAD:

Aquatic Animal Drug Approval Partnership
4050 Bridger Canyon Road
Bozeman, Mt 59715

Proposed Starting Date	July 1, 2016
Proposed Ending Date	December 31, 2026
Study Director	Ms. Bonnie Johnson

Clinical Field Trial Location:

Facility: _____

Investigator: _____

TABLE OF CONTENTS

STUDY PROTOCOL FOR A COMPASSIONATE AQUACULTURE INVESTIGATIONAL NEW ANIMAL DRUG (INAD) EXEMPTION FOR ERYTHROMYCIN (Erymicin 200 Injection) UNDER INAD 12-781

I. STUDY ID AND TITLE

Clinical field trials to determine the efficacy of erythromycin (Erymicin 200 Injection) treatment to 1) control mortality caused by bacterial kidney disease (BKD; causative agent: *Renibacterium salmoninarum*) in a variety of salmonid species; and 2) to control (prevent) the vertical transmission *of R. salmoninarum* from BKD positive female salmonid broodstock to eggs/progeny. INAD 12-781.

II. SPONSOR

Dr. Marilyn Blair, U.S. Fish and Wildlife Service, Branch Chief, Aquatic Animal Drug Approval Partnership (AADAP) Program, 4050 Bridger Canyon Road, Bozeman, MT 59715; Phone: 406-994-9904; Email: marilyn_j_blair@fws.gov

Manufacturer/Source of Supply:

Syndel USA
1441 W Smith Rd,
Ferndale, WA 98248 USA

Note: Erymicin 200 Injection is a product of **Jurox Pty Limited**, 85 Gardiner Street, Rutherford NSW 2320, Australia

Study Director: Ms. Bonnie Johnson, U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership (AADAP) Program, 4050 Bridger Canyon Road, Bozeman, MT 59715; Phone: 406-994-9905; Email: bonnie_johnson@fws.gov

Principal Clinical Field Trial Coordinator: Ms. Paige Maskill, USFWS – AADAP Program
4050 Bridger Canyon Road, Bozeman, MT 59715;
Phone: 406-994-9911; Email: paige_maskill@fws.gov

INAD Study Monitors: See Appendix II for names and contact information.

III. INVESTIGATORS/FACILITIES

See Appendix IIIa for names and contact information.

IV. PROPOSED STARTING AND COMPLETION DATES:

Proposed Starting Date: July 1, 2016

Proposed Completion Date: December 31, 2026

V. BACKGROUND/PURPOSE

Bacterial Kidney Disease (BKD) is a systemic infection found to occur in both wild and cultured salmonids. Although BKD more typically results in chronic mortality over an extended period of time, it can also cause acute, high-level mortality. The disease is oftentimes slow to become evident, but very difficult to control and virtually impossible to eradicate completely. BKD was first reported in the United States in 1935 (Belding and Merrill, 1935). Although it was initially thought to be limited to cultured fish populations, it was discovered in a wild population of brook trout in 1970 (Post, 1987). To date, BKD has been found in cultured and free-ranging salmonid populations throughout the United States. BKD is particularly prevalent among Pacific salmonids in the western U.S., where it has been a serious fish health problem for many years.

The causative agent of BKD is *Renibacterium salmoninarum*, a small, gram-positive (diplobacillus) bacterium. The bacterium is non-acid-fast, non-motile, and is usually found in pairs. *Renibacterium salmoninarum* is fastidious and slow growing, but can be cultured using specialized media containing L-cysteine and extended incubation. Optimal incubation temperature is 15^{o}C. As traditional culture methodology is not practical for the routine diagnosis of BKD, other techniques are more commonly used for identification of *Renibacterium salmoninarum* including: 1) observation of clinical signs and presence of gram-positive bacilli in tissues; 2) fluorescent antibody techniques (FAT); 3) enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR - confirmatory method). Currently, FAT and ELISA are the most commonly used diagnostic techniques for BKD.

BKD can be transmitted via horizontal transmission (i.e., fish to fish in the same water supply), as well as by vertical transmission through infected gametes (Evelyn et al, 1986; Brown et al, 1990; and Lee and Evelyn, 1994). Routine surface disinfection of eggs with iodophor is an ineffective treatment as *Renibacterium salmoninarum* resides within the egg. Although water-hardening newly fertilized eggs with iodophor treatment may result in partial elimination of the pathogen and is commonly practiced, the overall utility of this methodology is considered variable.

External signs of BKD include hemorrhagic lesions on the body surface, subcutaneous abscesses which may result in open lesions, pimple-like blisters or nodules on the body surface, and exophthalmia. External signs of BKD may commonly be limited to only a few fish in an infected population. Internal signs of BKD include swollen kidneys that often have a corrugated or “rope-like” appearance, white to gray-white cysts or abscesses in the kidney as well as the liver, spleen, and heart, and an accumulation of yellow-brown fluid in the body cavity. Fish with advanced BKD will have little or no visceral fat.

BKD has been found to be one of the more difficult bacterial diseases of fish to treat (i.e., control) with drugs or therapeutic agents. Not only is the disease chronic in nature and the pathogen widespread and egg transmissible, but the bacterium may be intracellular. Additionally, the pathogen has not been found to be highly susceptible to antibiotics commonly, and generally more effectively, used in fisheries management (Post, 1987). Although currently there are no FDA-approved treatments for the eradication of BKD, the disease can be controlled through treatment with antimicrobial compounds (Beitlich et al, 1995). Wolf and Dunbar, 1959 tested 34 therapeutic agents for the control of BKD, and found that only erythromycin resulted in significant control of mortality. Austin, 1985 evaluated 70 antimicrobial compounds (both *in vitro* and *in vivo*) for control of BKD in rainbow trout, and found that erythromycin was one of only 5 compounds potentially effective for use in early clinical cases.

Erythromycin is a macrolide antibiotic with bacteriostatic or bactericidal actions isolated from *Streptomyces erythreus* that is FDA-approved for use in both human and veterinary medicine. Although it is an effective antimicrobial for use against infections caused by a broad range gram-positive bacteria, it has little usefulness against staphylococcal and gram-negative bacteria (Moffit,1991). As most bacterial pathogens affecting aquatic species are gram-negative, the application of erythromycin therapy in aquaculture has been limited primarily to the treatment of BKD caused by *Renibacterium salmoninarum*. Although erythromycin is not approved for use in any aquatic species, experimental use (including under Investigational New Animal Drug exemption) for the treatment of BKD in Pacific salmonids has been both relatively widespread and effective. It has been used both as an injectable treatment, and as an oral (i.e., medicated feed) treatment. Injectable erythromycin has been used primarily to treat pre-spawning broodstock in order to minimize egg transmission of *Renibacterium salmoninarum*. Oral administration of erythromycin is believed to be the most effective (and practical) method of treating juvenile salmonids.

In recent years, numerous Federal, State, and Tribal hatcheries in the Pacific Northwest have relied heavily on erythromycin treatment of juvenile and adult salmonids to help meet critical management objectives. In the continuing struggle to restore/recover imperiled Pacific salmon stocks, erythromycin treatment has become an extremely important management tool to help mitigate the insidious impacts of BKD (Doug Munson, Idaho Fish and Game, personal communication).

The purpose of this compassionate INAD for erythromycin (Erymicin 200 Injection) is to: 1) provide fish culturists with another mechanism (i.e., in addition to erythromycin medicated feed INAD 6013 held by NRSP-7 and administered by the University of Idaho) to access and use erythromycin to control mortality caused by BKD, 2) provide fish culturists a mechanism to control (prevent) the vertical transmission of BKD from parents (broodstock) to eggs/progeny in a variety of salmonid species, and 3) develop clinical field trial and pivotal data demonstrating the efficacy and safety of erythromycin (Erymicin 200 Injection) treatment.

The USFWS anticipates that it may take several years to complete all technical section data requirements for a NADA for erythromycin (Erymicin 200 Injection). The USFWS is aware

that opportunities for erythromycin (Erymicin 200 Injection) therapy are unpredictable. There is no way of knowing in advance if, when, or where opportunities for pivotal studies will be encountered. The USFWS believes it is likely that data from 3-5 treatment seasons will be required in order to adequately assess the efficacy and safety of erythromycin (Erymicin 200 Injection) treatment, and to generate sufficient data to support a NADA.

VI. SPECIFIC OBJECTIVES

The two major objectives of this study protocol are as follows:

1. Collect clinical field trial data demonstrating the efficacy and safety of erythromycin (Erymicin 200 Injection) treatment. These data will add to, and broaden, the database of publically available information on the efficacy and safety of erythromycin injection therapy.
2. Provide a new mechanism/opportunity for fishery biologists/fish culturists to legally access and use erythromycin (Erymicin 200 Injection) to 1) control mortality caused by bacterial kidney disease in a variety of salmonid species; and 2) to reduce or minimize *R. salmoninarum* or levels in BKD positive female salmonid broodstock in order to control (prevent) the vertical transmission *of R. salmoninarum* to eggs/progeny.

Specifically, erythromycin (Erymicin 200 Injection) will be used in a variety of environmental conditions, at a range of water temperatures, and in a variety of cultured salmonid species to maintain healthy stocks of fish, until such time as a NADA for erythromycin (Erymicin 200 Injection) has been completed.

Within these two relatively broad objectives areas, there are four more specific study protocol objectives:

Objective A: To determine the efficacy and safety Erymicin 200 Injection treatment to control mortality caused by bacterial kidney disease in a variety of salmonid species;

Objective B: To determine the efficacy and safety Erymicin 200 Injection treatment to reduce or minimize *R. salmoninarum* or levels in BKD positive female salmonid broodstock in order to control (prevent) the vertical transmission *of R. salmoninarum* to eggs/progeny;

VII. MATERIALS

A. Test and Control Articles:

1. Drug Identity

a. Active ingredient

Common Name: Erythromycin

Product Name: Erymicin 200 Injection

Chemical Name: Erythromycin ($C_{37}H_{67}NO_{13}$)

CAS Number: 114-04-8

Appearance: Clear, light yellow liquid (not miscible with water)

Odor: None

b. Strength and dosage form: 200mg erythromycin per ml

c. Manufacturer, source of supply

Syndel USA
1441 W Smith Rd,
Ferndale, WA 98248 USA

Note: Erymicin 200 Injection is a product of **Jurox Pty Limited**, 85 Gardiner Street, Rutherford NSW 2320, Australia

2. Verification of Drug Integrity/Strength:

The manufacturer, Jurox Pty Limited, will provide the analytical data necessary to establish the purity of each lot of Erymicin 200 Injection used for treatment under INAD 12-781. The lot number and date of manufacture for each batch of Erymicin 200 Injection will be placed on the label of each container. The form "*Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals*" (Form ERYMICIN-1) will clearly identify the lot number and date of manufacture of Erymicin 200 Injection shipments. If the integrity of the Erymicin 200 Injection is compromised (e.g., spilling, contamination, heat, etc.) the event will be carefully recorded and dated in the Chemical Use Log (Form ERYMICIN-2). The Study Monitor assigned to the Investigator involved will be immediately notified.

3. Storage Conditions

Erymicin 200 Injection will be stored in the original container supplied by the Manufacturer with the appropriate investigational label attached. Erymicin 200 Injection will be stored in a dry, flame-proof storage unit, protected from light, and at temperatures below 30°C. The storage unit should be kept locked at all times. Once opened, unused Erymicin 200 Injection product should be discarded after 28 days. Do not use if the remaining solution is not clear, colorless, and free from particulate

matter. Erymicin 200 Injection should be stored at temperatures and for periods of time not to exceed limits set by the manufacturer.

4. Handling Procedures

Each Study Monitor and Investigator will be required to have a current copy of the Safety Data Sheet (SDS) for Erymicin 200 Injection (see Appendix IV). Each person involved with the study and each person who may be present during the use of Erymicin 200 Injection shall be required to read the SDS. Safety precautions as outlined in the SDS will be followed at all times when working with Erymicin 200 Injection.

5. Investigational Labeling

A copy of the label to be attached to each container of Erymicin 200 Injection is provided in Appendix V. Although investigational labels will be affixed to containers by the manufacturer, it is the responsibility of the Investigator to ensure proper labeling of all containers of Erymicin 200 Injection.

6. Accountability

Syndel USA will be the sole supplier of Erymicin 200 Injection to all Investigators under INAD 12-781.

The Online INAD Database must be used by Investigators for ALL INAD reporting. The online INAD database has a built-in system of checks, balances, and email notifications to ensure that all information/data reporting and accountability follows established INAD Study Protocol guidelines. Unless data is entered directly into the online INAD database (i.e., not captured elsewhere at the time of observation or measurement and transcribed into the online INAD database) investigators must archive hard copies of all raw data.

1. All facilities using Erymicin 200 Injection:

Immediately upon receiving an order/shipment of Erymicin 200 Injection, the Investigator must complete Form ERYMICIN-1 “Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals" (located in the “Manage/View Drug Inventory” section of the investigator account). The Study Director will forward a copy of this form to the FDA. Arrangements should be made between Investigators and Study Monitors to insure completed Form ERYMICIN-1s are received by the Study Director within 10 days of drug receipt.

All Investigators are also responsible for maintaining an accurate inventory of Erymicin 200 Injection on-hand. A Chemical Use Log (Form ERYMICIN-2) must be completed and maintained by each Investigator. Each time Erymicin 200 Injection is used, it must be recorded by the Investigator in the Results Report form in the “Amount Of Drug Used”.

At the conclusion of field trials, all remaining Erymicin 200 Injection will be destroyed by following the SDS (note: unless Erymicin 200 Injection is planned for use in another approved field trial, and planned usage is within the storage guidelines established by the manufacturer). Disposition of all Erymicin 200 Injection must be properly recorded and accounted for on the Chemical Use Log (Form ERYMICIN-2). The Study Monitor will be responsible for verifying the quantity of Erymicin 200 Injection remaining on hand versus the amount indicated on Form ERYMICIN-2.

7. Preparation Procedures

Erymicin 200 Injection will be supplied to Investigators in sterile 100 ml glass vials. Erymicin 200 Injection should not be adulterated in any manner prior to use. Erymicin 200 Injection should be administered by sterile syringe.

B. Items Needed for Treatment, Data Collection, Etc.:

Sampling techniques and diagnostic equipment will most likely be provided by trained fish health biologists serving as Study Monitors or their designee(s). Equipment and supplies needed would include items to sample fish and tissues and to 1) culture bacteria and identify culture growths microscopically using fluorescent antibody techniques, and 2) conduct enzyme-linked immunosorbent assays and Polymerase Chain Reaction (PCR) procedures. Syringes or other semi-automated injection systems (e.g., Rapidovacs) will be used for Erymicin 200 Injection administration.

When the Study Protocol has been approved and treatments are scheduled, the Investigator at each facility covered by the Erymicin 200 Injection INAD will need to complete several forms located in the online INAD database. These forms are described in Section XIII. Copies of these forms are attached to this Study Protocol and will be used as a guide only for collecting the data that will be entered into the online INAD database.

VIII. EXPERIMENTAL UNIT

The experimental unit in these clinical field trials will consist of contained or isolated groups of fish. This will generally be groups of fish contained in tanks, raceways, or ponds. However, the experimental unit in clinical field trials may also be **individual animals**. If individual animals are considered to be the experimental unit, treatment response parameters for each animal must be evaluated separately.

IX. ENTRANCE CRITERIA

A. Facilities/Investigators

The proposed facility and the Investigator must be listed in Appendix IIIa of the Study Protocol for the current calendar year before Erymicin 200 Injection can be

ordered and dispensed under this INAD. Last minute deviations can be requested by the Sponsor, Study Director, or by an Investigator in case emergency use-pattern needs should arise (See Section XX). However, poor planning and/or a lack of preparation will not be considered an emergency situation.

B. The characteristics of the study animals (species, number, etc.) are presented in Appendix VIb.

C. Environmental conditions

Environmental conditions will be variable and include a broad spectrum of water temperatures and water quality parameters. Environmental conditions will be reported on Form ERYMICIN-3. Drug discharge must be in compliance with local **NPDES** permitting requirements.

D. Ability of Investigator to fulfill all the requirements of the Study Protocol

See Appendix IIIb for example of knowledge required of hatchery managers (i.e., Investigators).

E. Pathogen/disease considerations (Objective A: Control of Mortality)

1. *Renibacterium salmoninarum* should be presumptively identified by procedures described in Section 1, Chapter 1 of the "Blue Book" (Procedures for the Detection and Identification of Certain Fish Pathogens, Third Edition, Fish Health Section/American Fisheries Society, 1985). Other methods described elsewhere in peer-reviewed references, or as mutually determined by the local fish health biologist, in consultation with the Study Monitor, also may be used. (**Note**: **Diagnostic methods other than those in the Third Edition of the "Blue Book" should be described on a separate sheet attached to Form ERYMICIN-3 "Diagnosis and Treatment Record"**).

2. There should be an increased mortality rate among fish in at least one rearing unit for three or more consecutive days. (**Note**: Station history and the experience of the investigator, monitor, or the fish health biologist may over-ride this criterion to halt potentially explosive disease outbreaks. In such cases, however, careful diagnostic surveillance should be carried out in all rearing units proposed for treatment and controlled tests should be carried out if at all possible).

3. Typical clinical disease signs must be detectable in at least a few fish and the causative agent (i.e., *Renibacterium salmoninarum*) should be identified.

4. Since the efficacy of Erymicin 200 Injection therapy for the control of

mortality caused by BKD is being tested, Investigators must be prepared to make no changes in the fish cultural procedures or environmental conditions and apply no other treatments once a decision has been made to conduct Erymicin 200 Injection therapy. Complicating bacterial or other parasitic diseases should be carefully documented. If necessary, these infections can be treated once Erymicin 200 Injection response (efficacy) data has been collected. However, it may take as long as 10 days after the completion of Erymicin 200 Injection therapy to determine differences between test and control groups and to complete post-treatment evaluations.

F. Pathogen/disease considerations (Objective B: Control of Vertical Transmission)

1. *Renibacterium salmoninarum* should be presumptively identified by procedures described in Section 1, Chapter 1 of the "Blue Book" (Procedures for the Detection and Identification of Certain Fish Pathogens, Third Edition, Fish Health Section/American Fisheries Society, 1985). Other methods described elsewhere in peer-reviewed references, or as mutually determined by the local fish health biologist, in consultation with the Study Monitor, also may be used. (**Note**: **Diagnostic methods other than those in the Third Edition of the "Blue Book" should be described on a separate sheet attached to Form ERYMICIN-3 "Diagnosis and Treatment Record"**).

2. It is anticipated that the majority of Erymicin 200 Injection treatments under this INAD will be used to control (prevent) vertical transmission *of R. salmoninarum* from returning wildstock adult female salmonids to their progeny (i.e., not captive broodstocks). Therefore, historical Enzyme Linked Immunosorbent Assay (ELISA) or Real-time Polymerase Chain Reaction (RT-PCR) data that have quantitatively documented historical levels of *R. salmoninarum* in wildstock adult female broodstocks will be relied upon heavily to determine when treatment is warranted. In many cases, treatment will likely be initiated soon after adult females return to the hatchery or collection site.

3. Since the efficacy of Erymicin 200 Injection therapy to control (prevent) the vertical transmission of *R. salmoninarum* is being tested, Investigators must be prepared to make no changes in the fish cultural procedures or environmental conditions and apply no other treatments once a decision has been made to conduct Erymicin 200 Injection therapy. Complicating bacterial or other parasitic diseases should be carefully documented. However, it should be noted that it may take as long as 60-90 days after the completion of Erymicin 200 Injection therapy to determine differences between test and control groups and to complete post-treatment evaluations.

4. The following should be used as standard levels for Quantitative PCR and ELISA. If a facility uses a different standard then the standard level used needs to be reported in Form ERYMICIN-3.

   a. Quantitative PCR

Negative Cq:	≥ 38
Low Cq:	30.0 – 37.9
Medium Cq:	20.0 – 29.9
High Cq:	<20.0

   b. ELISA

Negative OD:	< 0.095
Low OD:	0.095 – 0.199
Medium OD:	0.20 – 0.99
High OD:	> 1.0

Prior to initiating each treatment event, the Investigator must first complete Form ERYMICIN-W. "Worksheet for Designing Individual Field Trials" (located under the "New Study Request" tab in the investigator account) that pertains to each specific treatment event. The worksheet should be filled out and forwarded to the Study Monitor through the online INAD database. The Study Monitor will review the planned treatment (worksheet) and forward it to the Study Director at the AADAP Office. The Study Director will then review the worksheet, assign the approved treatment a Study Number, and then the online INAD database will notify both the Investigator and the Study Monitor of the assigned number and approval to proceed. In most cases, this entire process should be able to be accomplished within a single working day. After initiation of the field trial, the Investigator should also record the assigned study number on any paper forms that are being used as a guide to collect the data to enter in the online database (i.e., Form ERYMICIN -2 and ERYMICIN -3), as well as on any additional correspondence regarding that specific treatment event. If for some reason the Investigator is unable to reach the Study Monitor with regards to Worksheet approval and the need for treatment is immediate, the Investigator should contact the AADAP Office for permission to proceed.

Note: The online INAD database, which must be used by Investigators for all INAD reporting, has a built-in system of checks, balances, and email notifications to ensure that all information/data reporting follows established INAD Study Protocol guidelines.

X. TREATMENT GROUPS

A. A treatment group or experimental unit may be an entire tank, pond, raceway, or group of fish, or it may be individual animals.

B. Separately confined, untreated control fish will not be required in supplementary

field studies conducted to determine the effectiveness of Erymicin 200 Injection treatment. Fish from a group or lot will first be examined to determine if treatment with Erymicin 200 Injection is warranted. When treatment is underway or has been completed, fish from the same group will be examined to determine the effect of treatment on the parameters used to initially sanction the treatment. Evaluation will in all cases consist of determining fish mortality, although in most cases degree or severity of *Renibacterium salmoninarum* infection will also be quantified.

C. Although untreated control groups are not a required element of treatment under this INAD exemption and are at the discretion of the Investigator, they are strongly encouraged whenever circumstances permit. **Control groups are extremely important to not only document response to treatment, but also to validate potential adverse reactions in treated animals.** Assignment to control and treatment groups should be random and designed to avoid bias. It is important that all test fish are treated/handled in a similar fashion. If fish are physically moved into separate test groups or different rearing units, caution should be used so that handling and rearing conditions are as similar as possible. Control fish should be kept under conditions as similar as possible to treated fish for valid comparison. Use of control groups will help to ensure that results of efficacy studies provide useful information that will support a NADA.

D. Although as stated above untreated control groups are not a required element of treatment under this INAD exemption, **it is important for all investigators to note that field trials conducted under a more stringent study protocol (i.e including requirements for non-treated controls groups, replication, blinding, dose verification, etc.) will ultimately be required in order to support a NADA for Erymicin 200 Injection. It is also important to note that the INAD sponsor fully expects that a limited number of facilities/investigators listed under this INAD exemption will agree to participate in such "pivotal" efficacy studies.** These studies will be initiated only after direct consultation between facilities/investigators and the sponsor. These studies will be conducted under a separate FDA-approved study protocol (i.e. not the INAD study protocol), and will also be conducted with assistance from, and under the direct supervision of, the sponsor. **If for any reason it becomes apparent to the sponsor that facilities/investigators listed under this INAD are not willing to participate in such "pivotal" studies, the sponsor will request that FDA terminate the INAD.**

XI. TREATMENT SCHEDULES

A. Route of administration

Erymicin 200 Injection will be administered by injection treatment. Fish may receive either intramuscular or intraperitoneal injection.

B. Dose to be administered

Erymicin 200 Injection will be administered at a dosage of 10-25 mg

erythromycin per kg of fish biomass per injection.

C. Dosing interval and repetition

Objective A (control mortality): Erymicin 200 Injection will be administered as a single treatment regimen (injection), with no repetition of treatment.

Objective B (control/prevent vertical transmission): Erymicin 200 Injection will be administered as a single or multiple treatment regimen (injection), with a maximum of 3 treatments (injections). **Note**: each injection will be administered at a dosage of 10-25 mg erythromycin per kg fish biomass, and total dosage administered over the entire treatment period will not exceed 75 mg erythromycin per kg fish biomass. The interval between treatments (injections) must be a minimum of **21 days**.

D. Duration of treatment

Not Applicable.

E. Drug preparation and administration procedures

Erymicin 200 Injection will be supplied from the manufacturer as a “ready to inject” product, and will require no further preparation by the Investigator. Erymicin 200 Injection contains 200 mg erythromycin per ml.

Standard personal protective equipment such as gloves, lab coats or aprons, eye protection, etc. should be worn at all times when preparing and administering Erymicin 200 Injection (see Appendix IV: SDS for Erymicin 200 Injection)

F. Feeding regimen

As an injection treatment, Erymicin 200 Injection treatment should cause no significant disruption to normal feeding regimen. However, and at the discretion of the Investigator, fish may be taken off-feed the day prior to handling and treatment.

G. Permissible concomitant therapy

Since efficacy data are being collected during the INAD process, there should be no concomitant therapy. Preferably, there should be no other therapy during a period extending from 2 weeks prior to treatment to 2 weeks after treatment. Investigators must be prepared to make no changes in fish cultural procedures or environmental conditions, and apply no other drug therapy once a decision has been made to conduct Erymicin 200 Injection treatment.

However, if concomitant therapy is required in order to protect valuable fish stocks (i.e., threatened and endangered species not for human consumption) it

should be fully documented and the efficacy data from the Erymicin 200 Injection treatment involved should be appropriately labeled. **Contact the AADAP Office for the information that will need to be provided in the Form ERYMICIN-3 if concomitant therapy is conducted.** This additional information is required by the FDA in order to be able to conduct other drug treatments (either approved or INAD) during the Erymicin 200 Injection study.

XII. TREATMENT RESPONSE PARAMETERS

The collection and reporting of source data begins with the decision to treat valuable fish based on hatchery records or other pertinent species information indicating treatment is warranted. Daily morbidity and mortality records, case history records, as well as any extenuating or mitigating circumstances that may affect treatment response need to be documented. All pertinent treatment response parameters should be reported on Form ERYMICIN-3. Treatment response parameters that should be addressed include the following:

Objective A (control of mortality):

1. Primary Parameters

Morbidity and mortality data, coupled with case history and bacteriological analyses usually indicate when Erymicin 200 Injection treatment is needed. **This source data must be collected for at least 5 days before treatment, during treatment, and for at least 10 days after the treatment period has ended**. Collection of this data is critically important in all cases. Samples of kidney or other tissue will be removed from groups of representative fish and tested by bacteriological, serological, or other methods to determine the presence and bacterial load of *Renibacterium salmoninarum*.

2. Secondary Parameters

Secondary parameters may also include general observations on fish behavior and response to routine culture/handling activities. This would include such responses as feeding activity, feed consumption, apparent level of stress, negative fish behavior, etc.

3. Adverse Reactions

Any adverse reaction to treatment should be reported **immediately** to the Study Monitor, who will in turn notify the Study Director. Such responses might include extremely negative responses/behavior by the fish or hazards to the applicator. Although erythromycin treatment has been used fairly extensively with beneficial effect in salmonid culture, it is possible adverse reactions may occur under certain environmental conditions or with respect to specific species/strains of fish. Carefully observe all treated fish for any signs of any adverse reaction to

treatment. The Investigator should carefully document all observations of adverse reactions on Form ERMICIN-3. **If any signs of drug toxicity are detected, they should also be documented and immediately reported to the Study Monitor, who will in turn notify the Study Director.**

Note: Investigators are strongly encouraged to record observations/comments with respect to all phases of treatment. This may include a description of events before, during, and post-treatment. All extenuating or mitigating treatment circumstances need to be described in detail. Such information is imperative so that accurate study/data analysis can be performed.

Objective B (control/prevention of vertical transmission):

1. Primary Parameters

The primary response variable will be the presence or absence of *R. salmoninarum* in adult females at the time of spawning. If *R. salmoninarum* is found, the "bacterial load" will also be quantified. Detection and bacterial load of R. salmoninarum will be will be determined by testing kidney samples using either Enzyme Linked Immunosorbent Assay (ELISA) or Real-time Polymerase Chain Reaction (RT-PCR). ELISA data should be reported as Optical Density (OD), and RT-PCR data should be reported as Cycle Threshold (CT). If using ELISA, a sample will be considered “positive” if it’s OD is two standard deviations above the negative control. If using RT-PCR, a sample will be considered “positive” if it has a positive amplification of <40 CT. All *R. salmoninarum* data should be reported based on samples collected from individual fish.

2. Secondary Parameters

Morbidity and mortality data, coupled with case history and bacteriological analyses usually indicate when Erymicin 200 Injection treatment is needed. **This source data must be collected for at least 5 days before treatment, during treatment, and for at least 10 days after the treatment period has ended**. Collection of this data is critically important in all cases.

Secondary parameters may also include general observations on fish behavior and response to routine culture/handling activities. This would include such responses as feeding activity, feed consumption, apparent level of stress, negative fish behavior, etc.

3. Adverse Reactions

Any adverse reaction to treatment should be reported **immediately** to the Study Monitor, who will in turn notify the Study Director. Such responses might include extremely negative responses/behavior by the fish or hazards to the applicator. Although erythromycin treatment has been used fairly extensively with beneficial effect in salmonid culture, it is possible adverse reactions may occur under certain

environmental conditions or with respect to specific species/strains of fish. Carefully observe all treated fish for any signs of any adverse reaction to treatment. The Investigator should carefully document all observations of adverse reactions on Form ERMICIN-3. **If any signs of drug toxicity are detected, they should also be documented and immediately reported to the Study Monitor, who will in turn notify the Study Director.**

Note: Investigators are strongly encouraged to record observations/comments with respect to all phases of treatment. This may include a description of events before, during, and post-treatment. All extenuating or mitigating treatment circumstances need to be described in detail. Such information is imperative so that accurate study/data analysis can be performed.

XIII. FORMS FOR DATA COLLECTION

When the Study Protocol has been approved and treatments are scheduled, the Investigator at each facility covered by the Erymicin 200 Injection INAD will need to complete the following forms:

Form ERYMICIN-W.	Worksheet for Designing Individual Field Trials under INAD 12-781
Form ERYMICIN-1.	Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals
Form ERYMICIN-2.	Chemical Use Log for Clinical Field Trials Using Erymicin 200 Injection under INAD 12-781
Form ERYMICIN-3.	Results Report Form for use of Erymicin 200 Injection under INAD 12-781

Copies of these forms are attached to this Study Protocol. Actual reporting is accomplished on forms located in the online INAD database.

XIV. RECORD KEEPING PROCEDURES

As stated immediately above, all data reporting are accomplished via forms located in the online INAD database. All current and completed studies conducted under the investigator account will be stored and available in the online INAD database to the current study monitor, study investigator, and AADAP.

XV. DISPOSITION OF INVESTIGATIONAL ANIMALS

Objective A (control of mortality) and **Objective B** (control/prevention of vertical transmission):

Animals that die during treatment should be disposed of by burial or incineration. All fish treated with Erymicin 200 Injection must be maintained in culture facilities for a **minimum of 60 days** following completion of therapy and before stocking/release or harvest. The progeny of any treated fish can be released into open waters zero days after the last injection of broodstock. Fish treated before the 60 days withdrawal period has been met may not be used for stream nutrient enhancement programs.

The Investigator must verify compliance with requirements regarding the disposition of all treated fish on Form ERYMICIN-3.

XVI. DISPOSITION OF INVESTIGATIONAL DRUG

Erymicin 200 Injection will be used only in the manner described, and by the individuals specified in the Study Protocol. If any unused or expired Erymicin 200 Injection remains at the end of the study period, Investigators should contact Study Monitors for instructions regarding drug disposal. Drug disposal information is available in the Safety Data Sheet (SDS) located in Appendix IV of this protocol. Disposition of all Erymicin 200 Injection must be properly recorded and accounted for on the Chemical Use Log (Form Erymicin 200 Injection -2). The Study Monitor will be responsible for verifying the quantity of Erymicin 200 Injection remaining on hand versus the amount indicated on Form Erymicin 200 Injection -2. The investigational drug may not be redistributed to others not specified by the protocol and should not be retained by the Investigator after completion of the study (note: unless Erymicin 200 Injection is planned for use in another approved field trial, and planned usage is within the storage guidelines established by the manufacturer).

XVII. DATA HANDLING, QUALITY CONTROL, MONITORING, ADMINISTRATIVE RESPONSIBILITIES

A. Drug distribution

See Section VII.A.6. Accountability for information and details.

B. Study Monitors

Study Monitors are generally fish health professionals with experience in diagnosing and treating fish diseases, and the ability to monitor overall fish health with respect to ongoing fish culture practices. A study monitor will be selected by each facility that is authorized to treat fish with Erymicin 200 Injection. A list of Study Monitors, along with addresses and phone numbers, can be found in Appendix II. Study Monitors are responsible for supervision of the trials, adherence of the Investigator to the Study Protocol, and inspection of the site.

C. Special equipment and materials

Most of the equipment and materials required for this study (with the exception of the Erymicin 200 Injection itself) are already available at each participating fish hatchery. The use of various drugs, chemicals, and therapeutants to meet management and/or production goals is a common occurrence at most fish hatcheries. Fish hatchery managers (i.e., Investigators) are well trained and well equipped to handle these situations (see Appendix IIIb). If any additional equipment or materials are required, they will be provided by the Study Monitors (See Section VII.B. Items needed for sample collection, observations, etc.).

D. Administrator of the drug

Erymicin 200 Injection will be administered directly by the fish health personnel, the assigned Investigator (typically a fish hatchery manager), or under the Investigator's direct supervision (see Appendix IIIa for names). Erymicin 200 Injection will be maintained in a secure location, and only the Investigator or persons under his/her direct supervision will have access.

E. Drug accountability records

See protocol Section VII.A.6. Accountability for details and the following forms will be used as guides for data collection: Forms ERYMICIN-W, ERYMICIN-1, ERYMICIN-2, and ERYMICIN-3.

F. Recording observations

The Investigator or a person under his/her direct supervision will be responsible for implementing the Study Protocol, making observations, collecting samples, and recording data during the clinical field trials. After the data have been collected and recorded on the forms, the Investigator will send the data to the Study Monitors who will review the information and ensure that all required data is provided. The Study Monitors will in turn send the data to the Study Director. The Study Director will analyze and summarize the data and prepare a report that will be submitted to the FDA. **Note: If the Study Monitor does not think all required information has been provided, or forms have not been satisfactorily completed, he/she should contact the Investigator and rectify the situation before forwarding the package to the Study Director.**

G. Data storage

The Investigator is responsible for complete and accurate data collection, and must complete all required data forms (see Section XIII). The Investigator should forward all completed forms to the Study Monitor for review. Study Monitors should carefully check each set of data for accuracy and completeness. If a form is incomplete or inaccurate, it should be returned to the Investigator. If a form is complete and accurate, it should be forwarded to the Study Director at the AADAP Office. **Note:**

data that is entered through the online INAD database will be archived in the database. These archived forms will be available as long as the study participant accounts remain open.

XVIII. PLANS FOR DATA ANALYSIS

Data analysis will be completed by the Study Director located at the AADAP Office. Data from the treatment year will be summarized through tabulation and appropriate statistical analysis. INAD reports will be prepared and submitted to the FDA as required. This submission may include a request for an extension of the INAD based on the data collected during that year. When sufficient data are collected, the entire INAD data set will be summarized in a final report for submission to support a full NADA.

XIX. PROTOCOL AND PROTOCOL AMENDMENTS

A signed copy of the Study Protocol must be retained by each Investigator. At any time before the study begins, desired changes in the Study Protocol should be brought to the attention of the Study Director. The desired changes will be fully described in the form of an amendment along with the reason for the change. The amendment will be signed by the Sponsor (or its representative) and forwarder to the FDA for review. Copies of the signed amendment will be attached to each copy of the Study Protocol. **Investigators will be liable for non-compliance violation if drugs are used without a Study Protocol or in a manner different than specified in the Study Protocol, if forms are not filed on time, or if the study data are not properly collected, maintained, and reported.** The Study Monitor is responsible for ensuring that all INAD procedures are being followed as defined by the Study Protocol.

XX. PROTOCOL DEVIATIONS

Deviations from the established Study Protocol occasionally cannot be avoided. If deviations occur, the Study Monitor should be notified immediately. **Protocol deviations should be fully documented and should be accompanied by a written explanation of what happened, why, and what steps were taken to mitigate the deviation**. Deviations should be documented on Form ERYMICN-3 in the *Description of Results* section and in the *Study Deviation* field.

XXI: E.O. 13891

The contents of this document do not have the force and effect of law and are not meant to bind the public in any way. This document is intended only to provide clarity to the public regarding existing requirements under the law or agency policies.

LITERATURE CITED

Beitlich, J. B. Jennings, B. Lasee, R. Nelson, T. Ott, J. Whitney, and S. Woolley. 1995. Introduction to Fish Health Management. U.S. Fish and Wildlife Service Publication. 139pp.

Belding, D.L. and B. Merrill. 1935. A preliminary report upon a hatchery disease of Salmonidae. rans. Am. Fish. Soc. 65:76-84.

Brown, L.L. L.J. Albright, and T.P.T. Evelyn. 1990. Control of vertical transmission of *Renibacterium salmoniarum* by injection of antibiotics into maturing female coho salmon *Oncorhynchus kisutch*. Diseases of Aquatic Organisms. Vol. 9: 127-131.

Evelyn, T.P.T. J.E. Ketcheson, and L. Porsperi-Porta. 1986. Use of erythromycin as a means of preventing vertical transmission of *Renibacterium, salmoninarum.* Diseases of Aquatic Organisms. Vol. 2: 7-11.

Lee, E. G-H. and T.P.T. Evelyn. 1994. Prevention of vertical transmission of the bacterial kidney disease agent Renibacterium salmoninarum by broodstock injection with erythromycin. Diseases of Aquatic Organisms. Vol. 18: 1-4.

Moffit, C. 1991. Oral and injectable applications of erythromycin in salmonid fish culture. Veterinary and Human Toxicology. Vol. 33, Supplement 1. pp. 49-53.

Nelson, R., D. Desens, D. Lloyd, and J. Beitlich. 1989. Introduction to Fish Health. U.S. Fish and Wildlife Service Publication. 123pp.

Peters, K. and C. Moffitt. 1996. Optimal dosage of erythromycin thiocyanate in a new feed additive to control bacterial kidney disease. J. Aquatic Animal Health. 8:229-240.

Post, G. 1987. Textbook of Fish Health. T.F.H. Publications, Inc. Neptune City, NJ. 288pp.

Warren, J. 1981. Disease of Hatchery Fish. U.S. Fish and Wildlife Service Publication. 91pp.

1

Appendix I. Sponsor Contact Information for Erymicin 200 Injection INAD #12-781

Sponsor: Dr. Marilyn Blair, U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership (AADAP) Program
Phone: (406) 994-9904
Fax: (406) 582-0242
Email: marilyn_j_blair@fws.gov

Sponsor Address: 4050 Bridger Canyon Road, Bozeman, MT 59715

Study Director: Ms. Bonnie Johnson
Aquatic Animal Drug Approval Partnership (AADAP) Program
Phone: (406) 994-9905
Fax: (406) 582-0242
Email: bonnie_johnson@fws.gov

Principal Clinical Field Trial Coordinator: Ms. Paige Maskill
Aquatic Animal Drug Approval Partnership (AADAP) Program
Phone: (406) 994-9911
Fax: (406) 582-0242
Email: paige_maskill@fws.gov

1

Appendix II. Study Monitors for Erymicin 200 Injection INAD #12-781

Note: This information will be provided directly to CVM

1**Appendix IIIa. Facilities and Names of Investigators Participating under Erymicin 200 Injection INAD #12-781**

Note: This information will be provided directly to CVM and Syndel

Appendix IIIb. Sample of Knowledge Required for Position of Hatchery Manager (i.e. Investigators)

Professional knowledge of all facets of fishery biology as well as the ability to apply new scientific findings, developments, and advances toward the resolution of critical propagation problems involving the rearing a variety of fish species under a variety of water quality conditions, water temperatures, water chemistry, etc.

Knowledge of general bacteriology, parasitology, and water chemistry sufficient to treat fish for various diseases.

Skill in interpreting biological observations and ability to draw sound conclusions from available data.

Skill in developing and coordinating available resources to ensure effective management and utilization of manpower, equipment, and funds relative to established priorities and needs.

Skill in coordination of sometimes divergent resource issues to obtain common objectives, including interaction with other Federal, State, Tribal, and private agencies/facilities.

Knowledge of and skill in the use of effective management and supervisory techniques to provide support, guidance, and motivation to hatchery staff.

Appendix IV. Safety Data Sheet (SDS) for Erymicin 200 Injection INAD #12-781

The SDS for Erymicin 200 Injection can be found at the drug sponsor's website:

https://syndel.com/wp-content/uploads/2019/01/Erythromycin-200-Injection-SDS-04-2016.pdf

1

Appendix V. Investigational Label for Erymicin 200 Injection INAD #12-781

1. Investigational label for tests in vitro and in laboratory research animals [511.1(a)]:

   "Caution. Contains a new animal drug for investigational use only in laboratory animals or for tests in vitro. Not for use in humans."

2. Investigational label for use in clinical field trials [511.1(b)]:

   "Caution. Contains a new animal drug for use only in investigational animals in clinical field trials. Not for use in humans. Edible products of investigational animals are not to be used for food unless authorization has been granted by the U.S. Food and Drug Administration or by the U.S. Department of Agriculture."

1

Appendix VIa. Fish Species Treated under Erymicin 200 Injection INAD #12-781

Adult and Juvenile Salmonids

1

Appendix VIb. Table of Facilities and Fish Stocks Treated under Erymicin 200 Injection INAD #12-781

Note: This information will be provided directly to CVM

All data must be entered through the online INAD database:

The following forms are to be used as a guide for collecting data that will be entered into the online INAD database. Any paper forms that are submitted to AADAP will be sent back to the study participants.

Form ERYMICIN-W: Worksheet for Designing Individual Field Trials Under Erymicin 200 Injection INAD 12-781

INSTRUCTIONS

1. Investigator must fill out Form ERYMICIN-W for each proposed treatment under this INAD **before** actual use of Erymicin 200 Injection.
2. Investigator should forward a copy of ERYMICIN Form-W to the Study Monitor for review.
3. After review, the Study Monitor should forward a copy to the AADAP Office for review and assignment of a Study Number.

SITE INFORMATION

Facility			
Address			
Investigator			
Reporting Individual (if not Investigator)			
Phone		Email	

FISH CULTURE AND DRUG TREATMENT INFORMATION

Fish species to be treated			
Disease/pathogen to be treated		**BKD / *Renibacterium salmoninarum***	
Treatment Objective A or C (control of mortality)			
Treatment Objective B or D (control/prevent vertical transmission via eggs)			
Average fish weight (gm)		Average fish length (in)	
Number of fish per rearing unit		Number of rearing units to be treated	
Total number of fish to be treated		Approximate water temperature	
Intended erythromycin dosage (10-25 mg/kg bw) per injection			
Number of injections		Injection interval (days)	
Anticipated date treatment will be initiated			
Anticipated treatment evaluation date			

STUDY DESIGN: Provide a brief description of your planned study. The description should

include the reason you feel fish should be treated, the treatment dates, the number of fish that will be treated, and if the fish are a threatened or endangered species.

Study designed by _____

DISPOSITION OF TREATED FISH (Human Food Safety Considerations):

☐ Investigator should initial here to indicate awareness that fish disposition must be in compliance with FDA-mandated withdrawal times as described in the Study Protocol.

USE AND DISPOSITION OF ERYMICIN 200 Injection (Environmental Safety Considerations):

☐ Investigator should initial here to indicate awareness that Erymicin 200 Injection usage and disposition must be in compliance with requirements described in the Study Protocol.

WORKER SAFETY CONSIDERATIONS:

☐ Investigator should initial here to indicate that all personnel handling Erymicin 200 Injection have read the Safety Data Sheet for Erymicin 200 Injection and have been provided personal protective equipment, in good working condition, as described in the Study Protocol.

Date Prepared: _____ **Investigator:** _____

Date Reviewed: _____ **Study Monitor:** _____

1 Form ERYMICIN-1: Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals

INSTRUCTIONS

1. Investigator must fill out Form ERYMICIN-1 **immediately** upon receipt of Erymicin 200 Injection.
2. Investigator should forward a copy of Form ERYMICIN-1 to the Study Director at the AADAP Office

The sponsor, U.S. Fish and Wildlife Service, submits a notice of claimed investigational exemption for the shipment or delivery of a new animal drug under the provisions of Section 512 of the Federal Food, Drug, and Cosmetics Act. The following information is submitted to FDA:

Name of Drug	**Erymicin 200 Injection**	INAD Number	12-781
Proposed Use of Drug	Objective A: To control mortality caused by bacterial kidney disease in a variety of salmonid species Objective B: To control (prevent) the vertical transmission of Renibacterium salmoninarum via salmonid eggs		
Date of CVM Authorization Letter	08/11/2016		
Date of Drug Receipt		**Amount of Drug Received**	
Drug Lot Number			
Name of Investigator			
Address of Investigator			
Location of Trial			
Approximate Number of Treated Animals			
Study Protocol Number	12-781		
Approximate dates of trial (start/end)			
Species, Size, and Type of Animals			
Maximum daily dose and duration	25mg/Kg body weight		
Methods of Administration	Injection (up to 3 injections)		
Withdrawal Period	60 days following completion of treatment		

[1] **To be filled out by the AADAP Office**

Date Prepared: _____ **Investigator:** _____

Date Reviewed: _____ **Study Monitor:** _____

Date Reviewed: _____ **Sponsor:** _____

1

Form ERYMICIN-2. Chemical Use Log for Clinical Field Trials Using Erymicin 200 Injection Under INAD 12-781

Instructions: 1. Initiate Form ERYMICIN-2 immediately upon receipt of Erymicin 200 Injection.

2. Each lot number of Erymicin 200 Injection may be used for multiple treatment regimens.

Quantity on Hand From Previous Page (ml): _____ **Facility:** _____ **Reporting Individual:** _____

Erymicin 200 Injection Lot Number	Date Received	Amount Received (ml)	Date Used	Study Number	Erymicin 200 Injection Used for Teatment (ml)	Erymicin 200 Injection Transferred[1] (ml)	Erymicin 200 Injection Disposal[2] (ml)	Erymicin 200 Injection On-hand (ml)	Inventoried by (initials)

[1] **Unused Erymicin 200 Injection that is shipped to another facility participating in Erymicin 200 Injection INAD 12-781 (Note: Erymicin 200 Injection can only be transferred to another facility with prior authorization by the AADAP Office).**

[2] **Unused Erymicin 200 Injection that is past expiry date or has been compromised should be disposed of following the SDS.**

Investigator: _____ **Study Monitor:** _____

Signature and Date **Signature and Date**

1**STUDY NUMBER** _____

Form ERYMICIN-3: Results Report Form for Clinical Field Trials Using Erymicin 200 Injection Under INAD 12-781

INSTRUCTIONS

1. Investigator must fill out Form ERYMICIN-3 no later than **10 days** after completion of treatment. Attach lab reports and other pertinent study information.
2. If Erymicin 200 Injection was not used under the assigned Study Number, contact the Study Director at the AADAP Office to close-out the study.
3. Investigator should forward a copy of Form ERYMICIN-3 to the Study Monitor. Within **10 days** of receipt, the Study Monitor should forward a copy to the Study Director at the AADAP Office.

SITE INFORMATION

Facility	
Reporting Individual	

FISH CULTURE AND DRUG TREATMENT INFORMATION

Erymicin 200 Injection Lot Number		Total amount of drug used (ml)	
Treatment **Objective A**		Treatment **Objective B**	
Fish species treated		Disease treated	**BKD**
Average fish weight (gm)		Average fish length (in)	
Number of rearing units treated		Number of fish per treated rearing unit	
ID of all treated rearing units (e.g. Tank 5, Pond 6B)			
Total number of treated fish			
Number of control rearing units		Number of fish per control rearing unit	
ID of all control rearing units (e.g. Tank 5, Pond 6B)			
Total number of control fish			
Treatment dosage (mg/kg)		Treatment date(s)	
Injection method (IM or IP)			
Number of injections		Injection interval (days)	
Evaluation date(s)		Evaluation interval (time from treatment until evaluation; days)	

Daily Mortality Record (use for treatments under both Objectives A and B)

1. Investigator should fill out the Daily Mortality Record as completely as possible.

2. Prior to initiation of the trial, fill out Rearing Unit ID, whether a rearing unit is **T**reated or **C**ontrol, and the number of fish in each rearing unit.
3. Use additional copies of this form if more than 6 rearing units are involved in the trial and/or the post-treatment period exceeds 21 days

Facility										
	Rearing Unit ID									
	Treated or **C**ontrol									
	Number of fish									
	Day	Date	Water Temp (°F)	Mortality (# of fish)	Mortality (# of fish)	Mortality (# of fish)	Mortality (# of fish)	Mortality (# of fish)	Mortality (# of fish)	Observer Initials
Pre-Treatment Period	5									
	4									
	3									
	2									
	1									
Treatment Day(s)[1]										
	0									
Post-Treatment Period	1									
	2									
	3									
	4									
	5									
	6									
	7									
	8									
	9									
	10									
	11									
	12									
	13									
	14									
	15									
	16									
	17									
	18									
	19									
	20									
	21									

[1] If more than 1 treatment (injection) is used under Objective B, please note additional treatment date(s) on form

(use only for treatments under Objective B)

1. Investigator should fill out this form as completely as possible.
2. Prior to initiation of the trial, fill out Rearing Unit ID, whether a rearing unit is **T**reated or **C**ontrol, and the number of fish in each rearing unit.
3. Use additional copies of this form if more than 6 rearing units are involved in the trial and/or in order to include (report) data for all fish sampled.
4. Standard levels to follow. ELISA - Negative OD: <0.095; Low OD: 0.095 – 0.199; Medium OD: 0.2 – 0.99; High OD: >1.0. qPCR – Negative Cq: ≥38; Low Cq: 30.0 – 37.9; Medium Cq: 20.0 – 29.9; High Cq: <20.0

Facility									
	Rearing Unit ID								
	Treated or **C**ontrol								
	Number of fish								
Fish #	Date sampled	**E**LISA or RT-**PCR**	**P**ositive or **N**egative and OD or CT[1]	**P**ositive or **N**egative and OD or CT	**P**ositive or **N**egative and OD or CT	**P**ositive or **N**egative and OD or CT	**P**ositive or **N**egative and OD or CT	**P**ositive or **N**egative and OD or CT	Observer Initials

[1] OD = optical density from ELISA; CT = cycle threshold for RT=PCR

WATER QUALITY PARAMETERS

Mean treatment water temperature (^oF)		Dissolved Oxygen (mg/L)	
Hardness - $CaCO_3$ (mg/L)		pH	

RESULTS: Please describe treatment results in as much detail as possible. Was treatment successful? If treatment did not appear to be successful, explain why not? Describe general fish behavior, including feeding behavior. Were there any mitigating environmental conditions that may have impacted treatment results? Were there any deviations from the Study Protocol?

Pathology Report: Attach pathology report to this form. Report should include: 1) a description of how the pathogen(s) was identified; 2) disease identification records that confirm the presence of the pathogen; and 3) the name and title of the individual performing the diagnosis.

Pathology Report included: ☐ pre-treatment ☐ post-treatment

Toxicity observations: Report **<u>any</u>** apparent drug toxicity that was observed during the study period, including a detailed description of unusual or abnormal fish behavior.

OBSERVED WITHDRAWAL PERIOD:

Observed withdrawal period: ☐ **60 days** Investigator should initial here to indicate compliance with established withdrawal period

Estimated number of days between last treatment and first availability of fish for human consumption (ensure this time period meets the withdrawal period). _____

DISPOSITION OF Erymicn 200 Injection

☐ Use and disposition of all Erymicin 200 Injection followed Study Protocol guidelines and has been clearly identified on Form ERYTHRO-2 (Investigator should initial)

☐ **NEGATIVE REPORT:** Erymicin 200 Injection was not used at this facility under this Study Number during the reporting period. The study will be closed out in the online INAD database.

Date Prepared: _____ **Investigator:** _____

Date Reviewed: _____ **Study Monitor:** _____

NOTICES

Paperwork Reduction Act

In accordance with the Paperwork Reduction Act (44 U.S.C. 3501 *et seq.*), the U.S. Fish and Wildlife Service collects information necessary to permit the use of an investigational new animal drug to generate data to support a new animal drug approval (NADA) as part of the Fish and Aquatic Conservation fish health network. Your response is voluntary, but is required to obtain or retain a benefit. According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor and a person is not required to respond to a collection of information unless it displays a currently valid OMB control number. OMB has approved this collection of information and assigned Control No. 1018-####.

ESTIMATED BURDEN STATEMENT

We estimate public reporting for this collection of information to average 4 hours, including time for reviewing instructions, gathering and maintaining data, and completing and reviewing the form. Direct comments regarding the burden estimate or any other aspect of the form to the Service Information Clearance Officer, Fish and Wildlife Service, U.S. Department of the Interior, 5275 Leesburg Pike, MS: PRB (JAO/3W), Falls Church, VA 22041-3803, or via email at Info_Coll@fws.gov. Please do not send your completed form to this address.

FREEDOM OF INFORMATION ACT STATEMENT

Information provided to the Service is generally subject to release to the public under the Freedom of Information Act (FOIA). Certain information, however, may be subject to withholding if the Service determines that the information is a trade secret and/or commercial or financial information that is privileged or confidential. To the extent you are submitting business information that falls into one of these categories, you must clearly mark this information as "Business Confidential" in order for the Service to assess the applicability of FOIA Exemption 4. Any information provided by you that is not marked as “Business Confidential” will be considered releasable to the public under the FOIA [43 CFR 2.26 – 2.33].