

# RECORD

AEH-09-MAS-02

Isolation Logbook

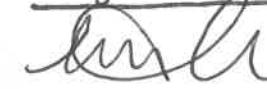
Principal Investigator: Marvin T. Tuttle-Law  
Supervisory Biologist: Mark P. Gajkowski

7530-00-222-3525  
FEDERAL SUPPLY SERVICE  
(GPO)

Printed Name

Maren Tattie-Lam

Signature



Initials

MTL

Date

20JUL11

Abbreviations

SLFH - Spirit Lake Fish Hatchery

UMESC - Upper Midwest Environmental Sciences Center

TSA - Trypticase Soy Agar

MUE - Muskelunge

WAE - Walleye

Equipment Abbreviations

20JUL11 - TSA plates were transported from SLFH to UME and placed at 4°C for storage until subculturing 20JUL11

LATE ENTRY (15-18JUL11) - Plates inoculated on 15JUL11 by Donn Muham (SLFH) were placed into incubation at 30°C on 16JUL11. Plates were returned to UMESC. Then on 17JUL11 plates were placed at 4°C for temporary storage. On 18JUL11 plates were observed for growth. Any plates indicating growth typical of MAS (creamy white to tan, round, raised) were identified and inoculated/subcultured onto a new TSA plate. Only plates had characteristic growth. Plates were subcultured placed into 30°C incubation. On 19JUL11, plates were removed from 30°C and placed at 4°C for storage. LATE ENTRY - 15-18 JUL11 MTL on 20JUL11 MTL

25JUL11 - Plates taken from 16JUL11 to 18JUL11 for WAE study were observed and colonies identified for subculturing. Colonies were circled and new TSA plates labeled and prepared. The colony was aseptically subcultured with a flame sterilized loop onto a new TSA plate. All plates were placed at 30°C incubation. Original plates were retained. 25JUL11 MTL -

27JUL11 - Plates were observed for growth. Plates were transferred to 4°C for storage. 27JUL11 MTL

28JUL11 - Approximately 30 WAE from the Florfenicol tanks and 30 WAE from the OTC tanks were euthanized with N for sampling. WAE were weighed (g) and measured (cm)

②

28JUL11-CONT'D and a kidney sample taken and inoculated onto a TSA plate. Each fish was observed for any obvious external/internal signs. Notes were made on the raw data sheet. Kidney samples were taken on the posterior kidney and inoculated with a sterile loop onto a TSA plate. Plates were placed in a cooler (room temp, no ice) for transport to UMESC for culturing. When returned to UMESC, plates were incubated at 30°C. 28JUL11-MTL —————→  
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AEH-09-MAS-02

WAE 2012  
SLFH

ISOLATION/CULTURE  
RECORD

Logbook IC-2

7530-00-222-3521  
FEDERAL SUPPLY SERVICE  
(GPO)

①

12Jun12 - TSA plates were prepared by dissolving the indicated 40g of TSA (calibrated balance) powder into 1 L of dH<sub>2</sub>O. Media was heated to dissolved <sup>12Jun12</sup> poured into 2-500 mL Wheaton flasks to autoclave for sterilization. Once cool enough to pour ( $\leq 180^{\circ}\text{C}$ ) plates were poured in the laminar flow hood with aseptic technique. Plates were allowed to cool and bagged with date and initials. Four batches of TSA plates were prepared for use in AEH-09-MAS-02.

12Jun12 MTL —————→

14Jun12 - WAE INSPECTION: Approx. 30 WAE were obtained during SLFI grading and sorting procedure. Each fish was weighed, measured and an external/internal observation made. A kidney stab was done on each fish and plated on TSA for culture of MAS. A necropsy <sup>14Jun12</sup> data recording form was used to record raw data. Plates were returned to UMESC and incubated @ 28-30°C for 24-48 hrs upon return. 14Jun12 MTL —————→

(2)

15Jun12 - TSA plates were observed for growth, little to none found. Plates were returned to incubation. 15Jun12 mT

18Jun12 - Plates were observed again for growth. Nothing on the plates indicated MAS present - so no further identification will be done. Plates were discarded. 18Jun12 mT

25JUN12 - 34 WAE were removed from the test system, a fish tank. Fish were weighed + measured and a kidney sample taken aseptically + placed on a TSA plate. WAE were observed externally and internally for any clinical signs of disease. Plates were labeled and brought back to UMEC for incubation at 28°C. Samples taken by MW were also brought back to UMEC for incubation + identification 25Jun12 mT

(3)

26Jun12 - Plates were observed for growth. Some of the 34 plates had small growth at 24 hrs indicative of MAS. Plates from MW also had presumptive MAS. Rimler-Shotts will be prepared to determine if MAS is present. 26Jun12 mT

27Jun12 - Plates from 25Jun12 were observed again for growth. Plates with growth characteristic of MAS were indicated with a  $\oplus$  on the plate label. Plates were placed in storage at 4°C.

RS Plates - Two plates, one from pre-treatment sampling and one from the inspection were positive on RS plates (yellow growth). PTA9-5 and inspection 5 plates were placed in storage at 4°C. 27Jun12 mT

02Jul12 - TSA plates were prepared by dissolving 40g of media into 1L of dH<sub>2</sub>O as described in 12Jun12 entry. Plates were labeled & bagged. 02Jul12 mT

03JUL12 - TSA plates were prepared as  
described in 12JUN12 entry. 03JUL12 MTL

