

Herpes

Correspondence

Point Defiance Zoo & Aquarium Research Sample Request

5400 North Pearl St., Tacoma, WA 98407
(253) 404-3680 FAX: (253) 591-5448
kareng@tacomaparks.com

In order to process your sample request please complete the following form. In those instances where the project is extended beyond the completion date, we will continue to collect samples upon the completion of a second form. **Please attach a complete yet concise protocol for sample collection and shipment.** If a protocol is not included, we will collect and process the samples following our standard laboratory techniques. Please return the completed form electronically to the contact at the above email address. The signed signature page can be faxed or mailed but must be returned.

Title of Project: **Seroepidemiological Assessment for Herpesvirus Prevalence in Elephants**

Please provide a brief summary of the project objective including the procedure intended for the requested material:

The National Elephant Endotheliotropic Herpesvirus (EEHV) Lab at the National Zoo provides EEHV PCR and ELISA testing for elephant holding facilities around the world. We are testing serum from many elephants to validate our EEHV ELISA. A positive result in the EEHV ELISA MAY mean that the elephant has been exposed to EEHV in the past, and also that the elephant MAY be protected from future illness caused by EEHV.

Hanako was slightly elevated in the EEHV ELISA in 2004; we would like to test more serum samples from her to determine when her titer went up, and if it is still high. Knowing when her titer went up may allow us to pinpoint what was happening clinically/socially/environmentally at that point in her life, and may help us determine what can trigger EEHV.

Is the project a request of the: SSP TAG Other **International Elephant Foundation** None
Primary Investigator: **Laura Richman**

Contact Person: **Erin Latimer**

Phone Number: **202-633-4252**

Fax Number: **202-673-4660**

Email Address: **latimere@si.edu**

Mailing Address: **National Zoo
Department of Pathology
3001 Connecticut Ave, NW
Washington, DC 20008**

Estimated Project Completion Date: 12/30/06
d d m m y y

Does this request require financial expenditure by us other than basic consumables? Yes No
If yes, please attach a complete budget.

Species/Common name: Elephas maximus
(please list all species)

Sample type: Serum Quantity: 1.0 ml/date
Tissue Source: _____ Quantity: _____
Swab Source _____ Transport media _____
Feces Quantity _____
Urine Quantity _____
Semen Quantity _____
Hair Quantity _____
Egg shell Quantity _____
Rumen contents Quantity _____
Other _____

Whole Blood:
EDTA Quantity: _____
Citrate Quantity: _____
Heparin Quantity: _____
Other _____ Quantity _____

Plasma:
EDTA Quantity: _____
Citrate Quantity: _____
Heparin Quantity: _____
Other _____ Quantity _____

Sample Handling Information: Room Temperature Refrigerator
Freezer Ultra Low freezer
Other _____

Additional Sample Collection and Handling Comments:

Collect one current serum sample using your normal collection procedure. Freeze at -80 asap. We would also like to request banked serum samples from before 2004. Two samples per year from as far back as your bank exists would be very helpful. Please ship on dry ice and email the tracking number when shipping – email to latimere@si.edu.

Specific Labeling Instructions:

name (Hanako) and date collected

TO ENSURE PROPER SAMPLE COLLECTION AND HANDLING PLEASE INCLUDE A COMPLETE AND DETAILED PROTOCOL. see above

Shipping Information:

Please provide your shipping account information:

Courier to be used: FedEx UPS Airborne Other _____

Account Number: 1284 699 40

Will dry ice be required for shipment?

Yes No

Will the project cover dry ice cost?

Yes No

Will you accept Saturday delivery?

Yes No please call ahead if shipping for Saturday delivery

Shipping address (this must be suitable for courier delivery):

National Zoo

Department of Pathology

300 1 Connecticut Ave, NW

Washington, DC 20008

Shipping phone number: 202-633-4252

Erin Lehmer

Signature of Primary Investigator or representative

Julie Lemon.

From: Kathleen Larson [klarson@pdza.org]
Sent: Tuesday, November 28, 2006 1:49 PM
To: Julie Lemon
Subject: FW: Hanako samples

copy to Hanako

Hey Jules,
Just cleaning my e mail inbox. We sent the one additional historic sample that we had,
right?
Kathy

-----Original Message-----
From: Latimer, Erin [mailto:LatimerE@si.edu]
Sent: Thursday, November 09, 2006 9:59 AM
To: klarson@pdza.org
Subject: RE: Hanako samples

Hello,
The five samples from Hanako were all elevated in the EEHV ELISA. Do you have any samples
that are older than '04? I would like to find out when she sero-converted.
Thank you,
Erin

-----Original Message-----
From: Kathleen Larson [mailto:klarson@pdza.org]
Sent: Monday, October 16, 2006 3:32 PM
To: Latimer, Erin
Subject: RE: Hanako samples

Thanks Erin,
I'll ask about Hanako's exposure, but I am unaware of any. Thanks for letting us send the
abbreviated form. We'll be sending this out Wednesday.
Kathy

-----Original Message-----
From: Latimer, Erin [mailto:LatimerE@si.edu]
Sent: Saturday, October 14, 2006 3:54 PM
To: klarson@pdza.org
Subject: RE: Hanako samples

Just send the signed form and the blood. Hanako is the only elephant that has an elevated
OD in the ELISA, that doesn't seem to have had exposure to an EEHV case. I may have
missed it though. Do you know of any contact between Hanako and an EEHV case, or an
elephant that has lived with an elephant that had EEHV?
Thank you,
Erin

-----Original Message-----
From: Kathleen Larson [mailto:klarson@pdza.org]
Sent: Sat 10/14/2006 6:27 PM
To: Latimer, Erin
Subject: RE: Hanako samples

Erin,
Do we need to resubmit the survey with the samples from Hanako or can we just send the
signed agreement and the serum? The forms are signed and the blood is pulled, but the
urvey might take awhile.
anks,
athy

Julie Lemon

From: Latimer, Erin [LatimerE@si.edu]
Sent: Friday, September 28, 2007 7:12 AM
To: Julie Lemon
Subject: RE: fed ex tracking number

*Copy to Hanako ✓
Suki ✓*

I tested the Hanako and Suki serum samples that were sent on 7/20/07. They were both low in the EEHV ELISA. I will send a paper copy of the results shortly.
Erin

From: Julie Lemon [mailto:JulieL@tacomaparks.com]
Sent: Thursday, July 19, 2007 11:54 AM
To: Latimer, Erin
Subject: RE: fed ex tracking number

Erin,

OK let's try this again. The fed ex tracking# is: 8596 0451 8620.
I packed it in ice packs.

Thanks again!

Julie Lemon, RVT
Point Defiance Zoo & Aquarium
5400 N. Pearl Street
Tacoma, WA 98407
253-404-3653

From: Latimer, Erin [mailto:LatimerE@si.edu]
Sent: Thursday, July 19, 2007 4:17 AM
To: Julie Lemon
Subject: RE: fed ex tracking number

FedEx doesn't have info on this package as of 7:15 am our time. Did it get picked up? I'll check it again in a couple hours.
Erin

From: Julie Lemon [mailto:JulieL@tacomaparks.com]
Sent: Wednesday, July 18, 2007 1:49 PM
To: Latimer, Erin
Subject: fed ex tracking number

Hi Erin,

Thanks for accepting our annual Elephant serum for EEHV titer testing. The Fed Ex tracking number is: 8599 1983 3022.

I will be sending more samples for Int'l Elephant Foundation at another time.

Thanks Again!

Julie Lemon, RVT

11/4/2007

Point Defiance Zoo & Aquarium
5400 N. Pearl Street
Tacoma, WA 98407
253-404-3653

11/4/2007

BWW
AC

**National Zoological Park
Department of Pathology
3001 Connecticut Ave., NW
Washington, DC 20008**

**National Elephant Endotheliotropic Herpesvirus (EEHV) Laboratory
202-633-4252**

EEHV Testing Results

*Copies were put in
Mod Records -*

Date 9/28/07
Submitting Institution Point Defiance Zoo
Animal Name/ISIS # Suki 96M004
Samples submitted Serum 7/6/07

PCR positive

negative

primers used terminase 2429/2430 (Asian elephants)

polymerase 2433/2434 (African elephants)

terminase 3024/3025 (African elephants)

ELISA
titer low

The EEHV ELISA is in the developmental stage. We are still working on many aspects of the test, including storage conditions for samples, controls to be used, detection systems, and significance of positive/negative titers. We will be using the results over the next six to twelve months to help us determine the optimal ELISA for EEHV. Please keep in mind that your results are part of this developmental process, and may not reflect the elephants' actual EEHV status. Thank you for sending samples for us to use in this endeavor.

Kam
AC

**National Zoological Park
Department of Pathology
3001 Connecticut Ave., NW
Washington, DC 20008**

**National Elephant Endotheliotropic Herpesvirus (EEHV) Laboratory
202-633-4252**

EEHV Testing Results

*Serum were put
in Metal Refrigerator*

Date 9/28/07

Submitting Institution Point Defiance Zoo

Animal Name/ISIS # Hanako 97M 024

Samples submitted Serum 7/6/07

PCR positive

negative

primers used terminase 2429/2430 (Asian elephants)

polymerase 2433/2434 (African elephants)

terminase 3024/3025 (African elephants)

ELISA

titer low

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Kathy Larson

From: Latimer, Erin [LatimerE@si.edu]
Sent: Wednesday, September 06, 2006 5:31 AM
To: klarson@pdza.org
Subject: RE: Hanako samples

Attachments: Generic sample request formPtDefiance.doc



Generic sample
request formPtD...

Dr. Larson,
I have attached the IACUC sample request form.
Thank you for your help in this,
Erin

-----Original Message-----

From: Kathleen Larson [mailto:klarson@pdza.org]
Sent: Tuesday, August 29, 2006 6:31 PM
To: Latimer, Erin
Subject: RE: Hanako samples

Please let me know if you have difficulties with this format. I can send a hard copy if needed.
Kathy

-----Original Message-----

From: Latimer, Erin [mailto:LatimerE@si.edu]
Sent: Tuesday, August 29, 2006 3:20 PM
To: klarson@pdza.org
Subject: RE: Hanako samples

Dr. Larson,
Thank you for your help. I didn't get an attachment to your last email--it may be because I am checking my emails remotely, but I have always been able to get the attachments remotely before. Anyway, would you mind sending the attachment again?
Thank you,
Erin

-----Original Message-----

From: Kathleen Larson [mailto:klarson@pdza.org]
Sent: Mon 8/28/2006 6:32 PM
To: Latimer, Erin
Subject: RE: Hanako samples

Erin,

Thank you for such a timely reply. In order for us to send you the additional samples and survey information, I have to run your protocol through our IACUC approval process. Please fill out the attachment and return. The more information you provide, the less questions will be asked, and the sooner we can approve the protocol. Our next meeting is the second Wednesday of the month. Upon approval, we should be able to send you the samples shortly.

Dr. Kathy

-----Original Message-----

Sent: Thursday, August 24, 2006 4:06 AM
To: klarson@pdza.org
Subject: Hanako samples

Dr. Larson,

Thank you for finding the Hanako samples for me. I apologize for not checking my messages sooner. I work part-time and usually check them more often. You can send the samples to the address below. Please send overnight, with dry ice, and email me the tracking number (we have a problem with not getting our packages in a timely manner, so if I can track it, I can make sure I get it right away.)

I have attached some forms to use for the shipping of the samples. On the "Request Form for EEHV Testing" form, under "reason for request", you can leave it blank, or put "requested by EEHV Lab". You can use my FedEx number to ship (1284 699 40).

Thank you for your help in our research.

Erin

Erin Latimer

Research Assistant

Department of Pathology

Smithsonian National Zoo

3001 Connecticut Ave, NW

Washington, DC 20008

202-633-4252 (phone)

202-673-4660 (fax)

latimere@si.edu

Kathy Larson

From: Latimer, Erin [LatimerE@si.edu]
Sent: Tuesday, August 29, 2006 3:20 PM
To: klarson@pdza.org
Subject: RE: Hanako samples

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To: Latimer, Erin
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Thank you for your help in our research.

Erin

Erin Latimer

Research Assistant

Department of Pathology

Smithsonian National Zoo

1001 Connecticut Ave, NW

Washington, DC 20008

202-633-4252 (phone)

202-673-4660 (fax)

latimere@si.edu

Kathy Larson

From: Latimer, Erin [LatimerE@si.edu]
Sent: Thursday, August 24, 2006 4:06 AM
To: klarson@pdza.org
Subject: Hanako samples
Attachments: Consent Form for EEHV Testing.doc; Elephant History form.doc; Request Form for EEHV testing.doc

Dr. Larson,

Thank you for finding the Hanako samples for me. I apologize for not checking my messages sooner. I work part-time and usually check them more often. You can send the samples to the address below. Please send overnight, with dry ice, and email me the tracking number (we have a problem with not getting our packages in a timely manner, so if I can track it, I can make sure I get it right away.)

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Thank you for your help in our research.

Erin

Erin Latimer
Research Assistant
Department of Pathology
Smithsonian National Zoo
3001 Connecticut Ave, NW
Washington, DC 20008
202-633-4252 (phone)
202-673-4660 (fax)
latimere@si.edu

Consent Form for EEHV Testing

Department of Pathology
Smithsonian National Zoo
3001 Connecticut Ave. NW
Washington, DC 20008
(202) 633-4252

I give consent for the results of Elephant Endothelial Herpes Virus (EEHV) testing to be used by the Species Survival Plan (SSP) and the National EEHV Laboratory to make recommendations on any elephant related issues (including, but not limited to, recommendations on relocations and breeding).

The results that could be reviewed and used by the SSP and the National EEHV Lab include Polymerase Chain Reaction (PCR), Enzyme-Linked Immunosorbent Assay (ELISA), and DNA sequencing of the EEHV virus.

I understand that all results and recommendations will be kept confidential.

_____ Yes, I agree to allow the SSP and the National EEHV Lab to use our testing results.

_____ No, I do not consent to the use of our testing results.

Signature, title

Date

Printed name

Phone number

Institution

Email address

Address

Elephant History for Elephant Endotheliotropic Herpesvirus (EEHV) Testing

Department of Pathology
Smithsonian National Zoo
3001 Connecticut Ave, NW
Washington, DC 20008
202-633-4252
FAX: 202 673-4660

Please fill out this form when submitting samples to the lab for EEHV testing for the first time. There is no charge for the testing, but we do ask for some information for our studies. Please send this history with the samples and the "Request for EEHV Testing" form. Use additional pages, if necessary.

1. Please list all elephants currently at your facility. Please include name, ISIS number, sex, age, breeding history, and move history.

2. Have there been any EEHV cases at your facility? When? Which elephants had direct or indirect contact with the EEHV case? Is there anything that you feel may have contributed to the case (for example, but not limited to: recent move, new source of stress on an elephant, elephant arrival that you think is the source of the EEHV, etc)?

3. Did any of your elephants have direct/indirect contact with a documented EEHV case prior to coming to your facility?

4. Have there been any unexplained illnesses in any herd member in the past? What were the clinical findings?

5. What is the purpose for EEHV testing today (circle all that apply)?

- a. concern about active EEHV illness (viremia)-whole blood PCR requested
- b. upcoming move-ELISA requested
- c. suspicious skin, oral or genital lesion-PCR requested
- d. curious about EEHV titer-either current titer or longitudinal study requested

6. Name/title/email address/telephone number of person filling out history?

7. Institution Name/Address?

Instructions for Elephant Endotheliotropic Herpesvirus (EEHV) Sample Submission

Please submit all samples on plenty of dry ice in a sealed styrofoam container.

- For a sick elephant with suspected active EEHV infection, please send at least 1-2 ml of whole blood (EDTA, lavender top tube). It is best to transfer the blood to a freezer-safe tube (not glass) after thoroughly mixing with EDTA. We have received broken glass tubes in the past.
- We can test any tissue for EEHV from deceased elephants, but it must be frozen (preferably in liquid nitrogen or dry ice, or store in a -70 C freezer). Heart, liver and spleen are the best organs to test for EEHV.
- We would like to test placenta from all newborn/stillborn/aborted elephants. Please freeze a 1 inch³ piece (preferably in liquid nitrogen or dry ice, or store in a -70 C freezer).
- For EEHV titers, please send at least 2 mls of serum (preferably more). Freeze immediately and send on dry ice.
- Please include all pertinent elephant information (including history) on the attached form.
- The best day to ship (FedEx) would be Wednesday for a Thursday arrival. Please call Erin or Laura before shipping samples:

Erin Latimer: 202-633-4252 (W)
703-471-2168 (H)
latimere@si.edu

OR

Laura Richman 301-398-4741 (W)
301-253-8723 (H)
RichmanL@MedImmune.com or richmanl@comcast.net

FedEx to:

**Erin Latimer/Laura Richman
Smithsonian, National Zoological Park
Department of Pathology
3001 Connecticut Ave. NW
Washington, DC 20008
(202) 633-4252**

Consent Form for EEHV Testing

Department of Pathology
Smithsonian National Zoo
3001 Connecticut Ave. NW
Washington, DC 20008
(202) 633-4252

I give consent for the results of Elephant Endothelial Herpes Virus (EEHV) testing to be used by the Species Survival Plan (SSP) and the National EEHV Laboratory to make recommendations on any elephant related issues (including, but not limited to, recommendations on relocations and breeding).

The results that could be reviewed and used by the SSP and the National EEHV Lab include Polymerase Chain Reaction (PCR), Enzyme-Linked Immunosorbent Assay (ELISA), and DNA sequencing of the EEHV virus.

I understand that all results and recommendations will be kept confidential.

_____ Yes, I agree to allow the SSP and the National EEHV Lab to use our testing results.

_____ No, I do not consent to the use of our testing results.

Signature, title

Date

Printed name

Phone number

Institution

Email address

Address

Kathy Larson

From: Holly Reed [hreed@pdza.org]
Sent: Thursday, June 22, 2006 6:59 PM
To: kl Larson@pdza.org; a case@pdza.org; Julie Lemonshark
Subject: FW: Hanako elephant serum

Hi All,

I am forwarding this to you so that you can act on it. You can get location history from Marla or the elephant barn crew to send along with the samples. It would also be good to let KGB know about this – nothing to worry about but to keep her informed. Talk to Erin Latimer for perspective before you talk to KGB.

Holly

Holly Reed, DVM
Head Veterinarian
Point Defiance Zoo & Aquarium
5400 N. Pearl St.
Tacoma, WA 98407

(253) 404-3639 (o)
(253) 404-3631 (to be contacted by radio)
(253) 591-5448 (FAX)

-----Original Message-----

From: Latimer, Erin [mailto:LatimerE@si.edu]
Sent: Friday, June 02, 2006 5:21 AM
To: Holly Reed
Cc: Julie Lemonshark
Subject: Hanako elephant serum

Hello,

We're starting to put together elephant histories and EEHV serology results to do a retrospective EEHV study. You sent some serum from Hanako in 2004 and she had a titer to EEHV. She is the only positive elephant that I have found so far who has not been in direct contact with an EEHV case. Do you have any banked serum from her that I could test? I would like it from as far back as you have it, about 1-2 ml, if you can spare it. Also, any history that you have on her would be great—where she has been, any illnesses, lesions, etc

Thank you,
Erin

Erin Latimer
Research Assistant
Department of Pathology
Smithsonian National Zoo
3001 Connecticut Ave, NW
Washington, DC 20008
202-633-4252 (phone)
202-673-4660 (fax)

Julie Lemon

From: Latimer, Erin [LatimerE@si.edu]
Sent: Friday, June 02, 2006 5:21 AM
To: Holly Reed
Cc: Julie Lemon
Subject: Hanako elephant serum

*A Copy to Hanako Arch
Serum record*

Hello,

We're starting to put together elephant histories and EEHV serology results to do a retrospective EEHV study. You sent some serum from Hanako in 2004 and she had a titer to EEHV. She is the only positive elephant that I have found so far who has not been in direct contact with an EEHV case. Do you have any banked serum from her that I could test? I would like it from as far back as you have it, about 1-2 ml, if you can spare it. Also, any history that you have on her would be great—where she has been; any illnesses, lesions, etc

Thank you,
Erin.

Erin Latimer
Research Assistant
Department of Pathology
Smithsonian National Zoo
3001 Connecticut Ave, NW
Washington, DC 20008
202-633-4252 (phone)
202-673-4660 (fax)
latimere@si.edu

From: Holly Reed [mailto:hreed@pdza.org]
Sent: Friday, April 28, 2006 12:27 PM
To: Latimer, Erin
Cc: Julie Lemonshark
Subject: RE: elephant serum

Erin,

I can't believe this week. I have passed your info to our tech Julie and she might be contacting you about quantities of dry ice necessary to keep the samples good. In one week we have had 2 (yours is the 3rd) disastrous Fed EX shipments that have ruined important serum samples for animals that are waiting shipments and diagnostic information. We may also start using UPS. We will get other samples to you. Thanks for letting us know.

Holly

Holly Reed, DVM
Head Veterinarian
Point Defiance Zoo & Aquarium
5400 N. Pearl St.
Tacoma, WA 98407

11/4/2007

(253) 404-3639 (o)
(253) 404-3631 (to be contacted by radio)
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-----Original Message-----

From: Latimer, Erin [mailto:LatimerE@si.edu]

Sent: Friday, April 28, 2006 9:07 AM

To: hreed@pdza.org

Subject: elephant serum

I just received serum from Bamboo and Suki. Unfortunately, the dry ice was gone and they were warm. Can you send it again next week, with more dry ice? Adding some ice packs can help too.

Thank you,

Erin

Erin Latimer

Research Assistant

Department of Pathology

Smithsonian National Zoo

3001 Connecticut Ave, NW

Washington, DC 20008

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latimere@si.edu

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Sent: Friday, April 28, 2006 12:27 PM
To: Latimer, Erin
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Subject: RE: elephant serum

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Holly

Holly Reed, DVM
Head Veterinarian
Point Defiance Zoo & Aquarium
5400 N. Pearl St.
Tacoma, WA 98407

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Sent: Friday, April 28, 2006 9:07 AM
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Subject: elephant serum

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Thank you,
Erin

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