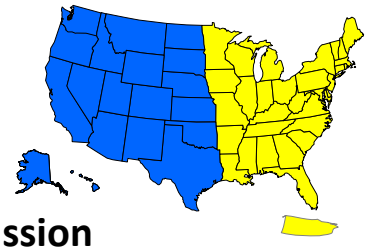
7/21/2009

National Wildlife Health Center



Guidelines for Post-Emergence Bat Submission

Summer 2009 (June-October)

Purpose: The primary mission of the National Wildlife Health Center (NWHC) is to determine the cause of death in free-ranging, migratory wildlife involved in unusual die-off events. Since the emergence of White-nose Syndrome (WNS) among hibernating cave bats in the northeastern US, our lab has targeted sample collection to develop diagnostic tests to more rapidly and accurately identify affected sites. Because characteristic clinical signs observed in the winter are not readily apparent in summer bats, surveillance/early detection of WNS in new states is challenging. It requires large number of samples for which there are few labs able to perform the current diagnostics for WNS and it is not yet known if these assays can detect evidence of WNS in bats collected during the summer. Therefore, NWHC is focusing efforts to: 1) continue investigation of unusual mortality events in bats throughout the US, and 2) evaluate a predefined number of summer bats from select WNS-confirmed areas. Limited evaluation of suspect bats from other areas will be considered based on the guidelines below. As more observational data becomes available, summer and fall sampling criteria may be revised.

Be sure to comply with all Federal and State permits (or authorizations) when capturing and handling bats. These guidelines do not supersede permit requirements.

Unusual mortality of bats observed in any State or US Territory

1. Dead bats found in greater numbers than normally expected for the species, age class, location, or time of year are being accepted at the NWHC for diagnostic evaluation with prior approval. We are currently investigating multiple adult and pup mortalities at maternity colonies throughout the US.

2. Collect 3-5 of the freshest carcasses (intact body, no evidence of scavenging, fur does not pull out easily, wings remain pliable, skin of face not dry or dessicated) which are representative of the affected species at a given site. Follow carcass collection and shipping instructions described at: http://www.nwhc.usgs.gov/mortality_events/reporting.jsp. Keep individual carcasses chilled in separate bags with ID tags containing:
    - date died & date collected (if differs)
    - location name(nearest town, county, state)
    - collector name & phone number
    - species
    - unique animal ID number (standard format: state, MMDDYY, collector, ###; ex: WI061609AEB001)

- found dead or method of euthanasia

Group all individually bagged carcasses destined for laboratory shipment into a 2nd clean bag upon exiting the location but prior to traveling to additional sites.

3. If unable to ship chilled specimens within 48 hours of death for delivery to the lab no later than Thursday, freeze the carcasses and ship early the following week. *NOTE: The general public should be discouraged from handing any live bats due to the risk of rabies exposure and should be instructed to not directly contact any dead bats that they may wish to have examined. If willing, instruct public to double-bag bat carcasses in resealable plastic bags and pack in ice inside a cooler (not a food freezer) as soon as possible until you can arrive. Styrofoam coolers cannot be thoroughly disinfected and should be discarded after specimen retrieval while plastic coolers may be cleaned and decontaminated by following guidelines located at:*
http://www.fws.gov/northeast/whitenose/FINALDisinfectionProtocolforBatFieldResearchJune2009.pdf

4. Contact USGS-NWHC to arrange shipment and further instruction. For eastern states, contact Anne Ballmann (608-270-2445; aballmann@usgs.gov). Western states should contact Krysten Schuler (608-270-2447; kschuler@usgs.gov).

Live bats with visible evidence of fungal growth on muzzle, ears, or wing membranes captured between June – October in any state

It is not anticipated to observe fungus on bats during the summer, however, we do not rule out the possibility. Please ensure any suspicious substance has a fuzzy, fungal-like appearance and is not dust, mud, cobwebs, flaking of roost-site substrate, ectoparasites, etc. If in doubt, e-mail close-up photos to your NWHC contact.

1. If the affected bat is a nonendangered or nonthreatened species, consider euthanasia for diagnostic evaluation at NWHC. Take close-up photos of affected individuals prior to handling. Collect up to 5 affected bats per location and contact NWHC prior to submission. Recommend guidelines for humane bat euthanasia are available at:
www.michigan.gov/documents/emergingdiseases/**Humane_Euthanasia**_of_**Bats**-Final_244979_7.pdf

2. If the affected bat is an endangered or threatened species, photograph the affected individual prior to nonlethal sample collection and record information on data sheet (APPENDIX B). Next, collect a nonlethal sample for diagnostic evaluation. Options include a fungal tape lift from the most prolific fungal growth on the bat (APPENDIX D) or a biopsy punch from an affected portion of the wing membrane only (APPENDIX C). Collect samples from up to3 individuals per site, place a wing band ID, and release. Contact NWHC prior to submission.

Live bats with evidence of moderate to severe wing damage (Reichard Scale ≥ 2)

Reichard Wing-Damage Index (http://www.fws.gov/northeast/wnsresearchmonitoring.html)

When observed in States with no prior history of WNS,

1. Photos of the wings should be taken with corresponding geographic, demographic and physical data (see datasheet-APPENDIX B or submit copy of PAGC Summer Maternity Roost Monitoring MEASUREMENTS-SAMPLES-BANDING form available at http://www.pgc.state.pa.us/pgc/cwp/view.asp?a=458&Q=176739&PM=1). If no band identification is present, bats can be released without further sampling. Report this information to your State Wildlife Resources Agency bat biologist **and** the FWS Bat WNS Coordinator (WhiteNoseBats@fws.gov) or contacts at the USGS-NWHC.

2. If the bat has a band ID linking it to a known WNS affected site and evidence of moderate to severe wing membrane damage, consider humane euthanasia of nonendangered bats for full diagnostic evaluation at NWHC. Contact Eric Britzke (601-634-3641; Ebritzke@comcast.net) or Susan Loeb (864-656-4865; sloeb@clemson.edu) for bat banding data in the eastern US. Recommend guidelines for humane bat euthanasia are available at: www.michigan.gov/documents/emergingdiseases/**Humane Euthanasia** of **Bats**-Final_244979_7.pdf

3. If affected animals (Reichard Scale ≥2; with or without ID band) are endangered or threatened species such as Indiana bats, gray bats, or Virginia big-eared bats, consider collecting wing punch biopsies of up to 2 representative lesions per individual bat. See APPENDIX C for detailed description of wing biopsy protocols. Also photograph the wings prior to biopsy and record associated geographic, demographic, and physical data (APPENDIX B). Bats undergoing biopsies should be banded for future identification and can be released following the procedure. Contact NWHC to discuss diagnostic evaluation and arrange shipment. *NOTE: wing punch biopsies are an insensitive diagnostic & surveillance method for detecting Geomyces destructans, the fungus associated with WNS, and negative results do not rule out the possibility of an animal being infected. Use wing punch biopsies only when the index of suspicion for WNS is high.*

4. If injuries or poor condition of a captured bat warrants euthanasia, keep the carcass chilled and contact NWHC within 24 hrs to discuss possible submission on a case-by-case basis. Otherwise, freeze the carcass until contact with NWHC is made. Bats with evidence of moderate to severe wing membranes lesions are of primary diagnostic interest.

When observed in States with prior history of WNS,

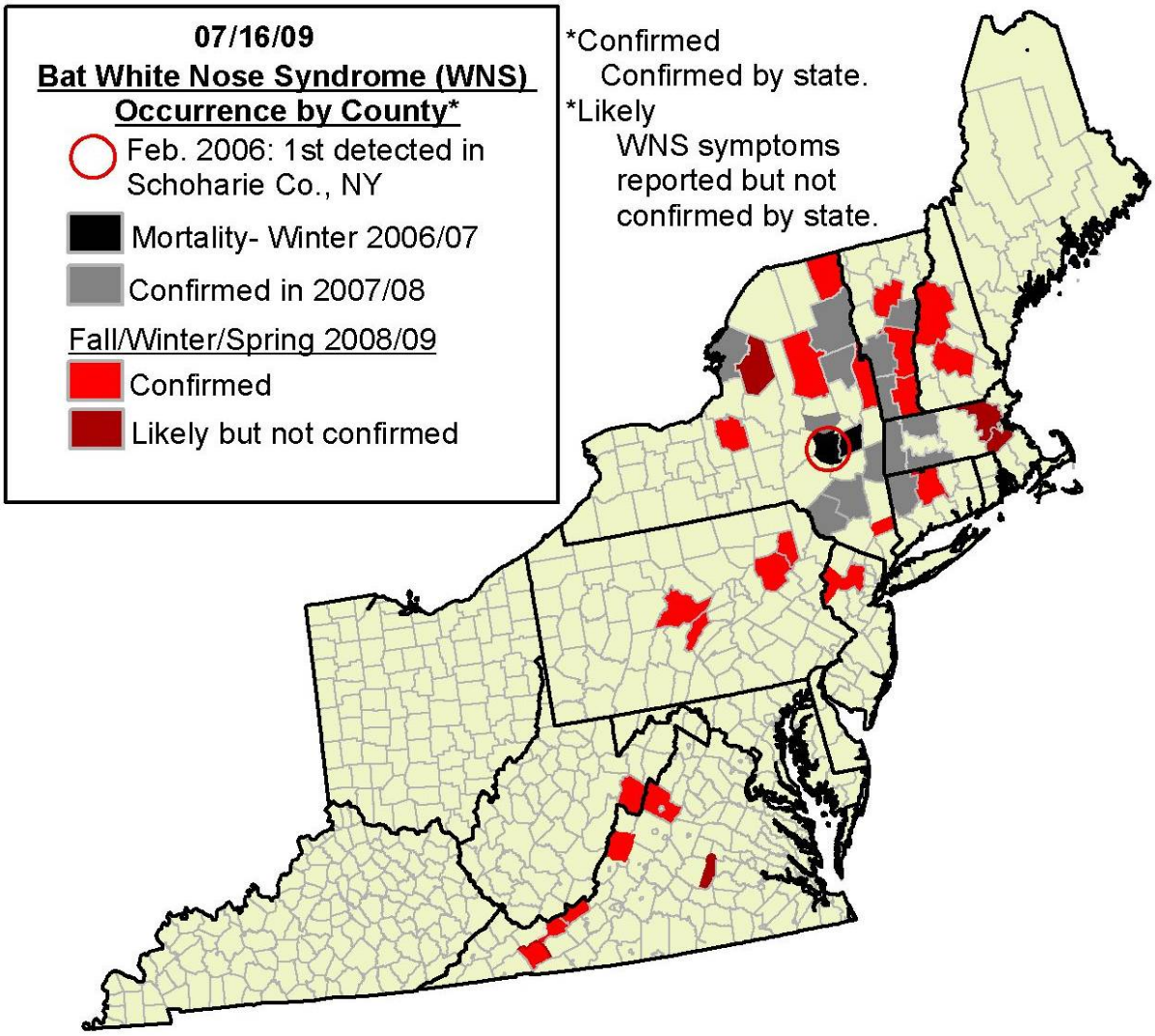
1. Photos of the wings should be taken with corresponding geographic, demographic and physical data (APPENDIX B). If the county in which the affected animal(s) is caught has no prior history of WNS **and** the bat is neither a threatened nor endangered species, no further sampling is requested by NWHC at this time. If a threatened or endangered bat species, consider collecting wing biopsies of 1-2 affected areas (APPENDIX C). Report this information to the State Wildlife Resources Agency bat biologist **and** the FWS Bat WNS Coordinator (WhiteNoseBats@fws.gov) or Anne Ballmann (aballmann@usgs.gov) at the NWHC.

2. For live bats (little browns, eastern pipistrelles, northern long-eared) with evidence of moderate to severe wing lesions (excluding large wing tears) captured in counties where WNS has been previously confirmed, NWHC would like to receive 3 intact bat carcasses per site (limit of 1 site per state, total of 3 states) to evaluate in June/July and repeat sampling of bats captured at the same locations in late summer prior to Fall swarm. Please contact Anne Ballmann (NWHC) prior to collection with proposed summer sampling locations to coordinate targeted sample areas. Bats meeting these criteria should be humanely euthanized according to AVMA standards and approved protocols. Recommended guidelines for humane bat euthanasia are available at: www.michigan.gov/documents/emergingdiseases/**Humane_Euthanasia**_of_**Bats**-Final_244979_7.pdf

3. For all other bat species with evidence of wing damage captured in WNS confirmed counties, score wings using the Reichard index, obtain photographs of wings, record observations in the datasheet (APPENDIX B), and report observations to NWHC. At this time, NWHC is not soliciting samples from bats meeting these criteria; however, this policy may be revised in the future.

4. Bats with wing bands from WNS affected sites with moderate to severe wing lesions captured outside the targeted areas should be considered for euthanasia and diagnostic submission. Contact Eric Britzke (601-634-3641; Ebritzke@comcast.net) or Susan Loeb (864-656-4865; sloeb@clemson.edu) for bat banding data in the eastern US.

APPENDIX A



Most current map updates are posted at http://www.fws.gov/northeast/white_nose.html

APPENDIX B – USGS NWHC Summer/Fall 2009 Bat Submission Datasheet

Date (MMDDYY):___________________
Location ID:_______________________
(*circle one*: maternity colony; bachelor colony; night roost; day roost; hibernaculum; other______________________)
County:________________
State:_______
Decimal degrees (NAD83): N_______________ E_______________
Collector:_____________________ e-mail: ___________________

Estimated number of live bats at site___________; Estimated number of dead bats__________
Bats present (G. species) & estimated % of total popn: ________________ (____%);________________ (____%); ________________ (___%); ________________ (____%); __________________ (____%)
Percent of total population affected by clinical signs: ______________
Percentage of each species affected: ________________ (____%);________________ (____%); ________________ (____%);________________ (____%);________________ (____%)
Distribution pattern of affected bats at site*:* solitary *vs.* clustered;
(circle one from each row) outer periphery *vs.* inner region *vs.* throughout site

ID or Band# (state, MMDDYY, collector, ###)	**Species**	**Sex** *(circle one)*	**Status** (Live, Dead, Euth)	**Age Class** (Juv, Adult, Unknown)	**Weight (g)**	**Forearm length (mm)**	**Reichard Wing Score** *(circle one)*	**Photo file ID**	**Disposition** (Released, Fungal Tape, Wing Biopsy, Archived, Submitted)	**Comments/Notes**
		M F	L D E	J A U			0 1 2 3		R T B A S	
		M F	L D E	J A U			0 1 2 3		R T B A S	
		M F	L D E	J A U			0 1 2 3		R T B A S	
		M F	L D E	J A U			0 1 2 3		R T B A S	
		M F	L D E	J A U			0 1 2 3		R T B A S	
		M F	L D E	J A U			0 1 2 3		R T B A S	
		M F	L D E	J A U			0 1 2 3		R T B A S	
		M F	L D E	J A U			0 1 2 3		R T B A S	
		M F	L D E	J A U			0 1 2 3		R T B A S	
		M F	L D E	J A U			0 1 2 3		R T B A S	

Additional Notes/Diagrams:

APPENDIX C

Instructions for Taking a Tissue Biopsy

Updated by Pat Ormsbee and Jan Zinck 5/14/09
Modified by Anne Ballmann 6/17/09

1. When taking biopsies it is important to reduce the potential for cross-contamination among other bats, insects, or people. In order to do this, use a clean nonporous biopsy board, a new tissue punch for each sample, sterilized forceps, and disposable gloves. Soak the board before use at a site, and during the site visit if necessary, using a 10% bleach and water solution for 10 min. Thoroughly rinse the biopsy board twice with water to remove the bleach and contaminants. Air-dry the board or use clean gauze or paper towel to wipe it dry. Make sure the board is dry before it is used. When using the board, take punches on different areas of the board to avoid mixing DNA from multiple bats if not cleaned and disinfected between each use.

2. Label 2 sterile vials: one containing 70% ethanol (5 ml) and one containing nothing. Use a black ultra-fine Sharpie permanent marker and a sticky paper label. Be careful that once the label is adhered to the tube the entire identifier is visible. Use the following naming convention to uniquely identify the bat:

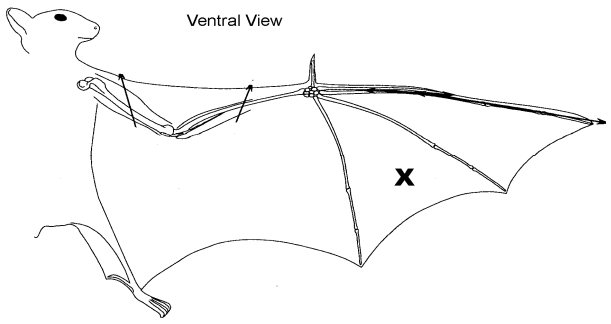
   State, Date (MMDDYY), Collector initials, bat number (ex: WI061609AEB001)

3. Have a clean board, a labeled tube, a new tissue punch, and a sterilized forceps ready. Do not touch (contaminate) the end of the punch, the forceps, or the inside of the tube lid with fingers or environmental debris.

4. Place the bat on the board on its back and extend one wing membrane. Identify 1-2 representative lesions to biopsy on the affected wing of the bat. Avoid sampling from bats with large wing tears. For inexperienced people, it works best when one person holds the bat and another person does the biopsy.

5. Tissue biopsies are taken from the wing membrane, avoiding bones and major blood vessels. (Figure 1). If possible, locate an area with lesions within the lower half of the membrane. Press the punch firmly through the membrane and twist the punch slightly to guarantee a complete punch. Apply direct pressure to biopsy site for several minutes if bleeding occurs.

Figure 1: "X" marks a sample location for a tissue biopsy of the wing membrane.



Modified by P. Ormsbee 6/10/04 from original by Shonene Scott, Portland State University, shonenes@pdx.edu 5/03

6. Lift up the bat carefully and look for the tissue. It will be on the board or inside the tip of the punch. If necessary, you can use a new 25 ga needle or sterile forceps to pick the tissue up and transfer to the tube. The sample placed in the tube with 70% ethanol will be used for histopathology and the sample placed in the empty tube should be frozen for subsequent fungal PCR. Be careful on windy days since the wind can blow the tissue off of the board. Release the bat only after the tissue has been placed in the tube and the tube has been closed and any bleeding has stopped. The same bat can be biopsied more than once using the same equipment without intervening disinfection, although the number of biopsies should be limited to prevent compromising flight.

7. Dispose of the used biospy punch after each animal. DO NOT reuse the same biopsy punch on multiple bats. The punches are very sharp. Be careful to not cut yourself. Change into new gloves before handling each bat.

8. Before reusing a forceps while in the field, follow the flaming sterilization protocols described in "Disinfection Protocol for Bat Field Research/Monitoring, June 2009" (http://www.fws.gov/northeast/wnsresearchmonitoring.html). Upon returning to the office, perform a more thorough cleaning and disinfection of nondisposable biopsy equipment with detergent washing followed by soaking in a 10% bleach solution for 10 min and a clean water rinse. Once dry, the forceps can be placed in a clean hard surface container (not plastic bags), free of contaminates, marked for cleaned forceps, and with handles all pointing in the same direction.

Supplies

- 5mm biopsy punches
- Thumb forceps
- 25 gauge needles
- 10% bleach solution (can be made fresh each time, or can be stored in opaque containers for 24 hours, it begins to break down after this)
- Sterile rinse water
- plastic storage container with lid for soaking punches
- 5 ml sterile plastic vials with caps
- 70% ethanol
- Fine point permanent marker
- Vial labels
- Disposable gloves
- Paper towels/gauze
- Cigarette lighter for disinfecting forceps
- Nonporous cutting board

Modified by P. Ormsbee 6/10/04 from original by Shonene Scott, Portland State University, shonenes@pdx.edu 5/03

APPENDIX D - **Fungal tape-lift protocol for bats**

Tape-Strip Sampling of Bat Skin for Identification of WNS-Associated Cutaneous Fungal Infection

Authors: David S. Blehert and Anne Ballmann, USGS – National Wildlife Health Center

Date: 19 November, 2008

Purpose: The following procedure is designed to collect WNS-associated fungi from the skin of bats for later microscopic analyses while minimizing harm to the sampled bat.

Required materials:

1) Glass microscope slides with white label (25 mm (W) X 75 mm (L); 1 mm thick). Fisher Scientific Catalog #12-552. Fisher list price $58.34 pack (144/pack).

2) Fungi-Tape (25 yards X 1 inch; approximately 1 mm thick). Fisher Scientific Catalog #23-769-321 (Scientific Device Laboratory No. 745). Fisher list price $35.59 per box.

3) Plastic 5-slide transport mailers. (Maximum capacity is 10 slides per mailer – see instruction #9 below). Fisher Scientific Catalog #12-569-35 ($31.00 for pack of 25) or #12-587-17B ($185.35 for pack of 200).

Procedure:

1) Wear new disposable gloves when handling each individual bat to reduce the risk of cross-contamination.

2) Label the end of a microscope slide in pencil with an animal ID number, date, and anatomical sample location (i.e. muzzle, ear, wing, or tail).

3) Remove a precut piece of Fungi-Tape from the box being careful not to contaminate the adhesive surface.

4) Bend the tape-strip (without creasing), adhesive-side out, between your thumb and index finger so that the tape forms the shape of a “U” (Fig. 1).

5) Select an area on a bat with obvious fungal growth (whitish, powdery appearance) for sampling.

6) Lightly touch the adhesive surface of the tape-strip, at the bottom of the “U”, to an area of suspect fungal growth on bat surface (Fig. 2). DO NOT use your finger to press the tape down onto the bat’s skin or fur. Attempt to maximize adherence of fungus to the tape adhesive while minimizing adherence of hair (Fig. 3).

7) If only a small area is transferred to the tape, use a different portion of the same tape "U" to touch another affected area on the skin. DO NOT attempt to obtain more than 3 lifts per tape strip. **Collect only 1 tape-strip per live bat.**

8) Align the tape-strip containing the fungal sample, adhesive-side down, over the microscope slide. Ensure that the edges of the tape-strip do not protrude beyond the edges of the microscope slide when laid flat, and do not remove any portion of the tape-strip from the glass slide once it has adhered (Fig. 4).

9) Lightly wipe over the top surface of the tape-strip using a clean paper or cloth towel to consistently adhere the strip to the slide. Hold the slide up to a light source and circle the region of the tape strip that came into contact with the fungus sample with permanent ink.

10) Place each slide into a slide mailer for safe transport. If 2 slides are placed per slot, ensure that the tape surfaces of each slide are facing outwards (only the non-tape sides should be in contact so as not to crush the tape). Seal the slide mailer shut with standard tape or rubber bands prior to shipment.

11) Place slide mailer(s) into a clean Ziploc bag and seal closed to transport from the hibernaculum. Place in a second Ziploc bag

12) The slide mailers can now be held at ambient temperature and shipped to the NWHC for staining and microscopic examination. Ship mailers in a padded envelop with a completed specimen history form. If including slide mailers in a cooler shipment with bat carcasses, ensure that the slide mailers are not in contact with the blue ice. Send an electronic copy of the completed specimen history form to Anne Ballmann (aballmann@usgs.gov). Contact Anne (608-270-2445) if you have any additional questions.

Illustrations – Fungal tape-lift protocol for bats

-Photographs by D. Berndt and D. Johnson, USGS – NWHC

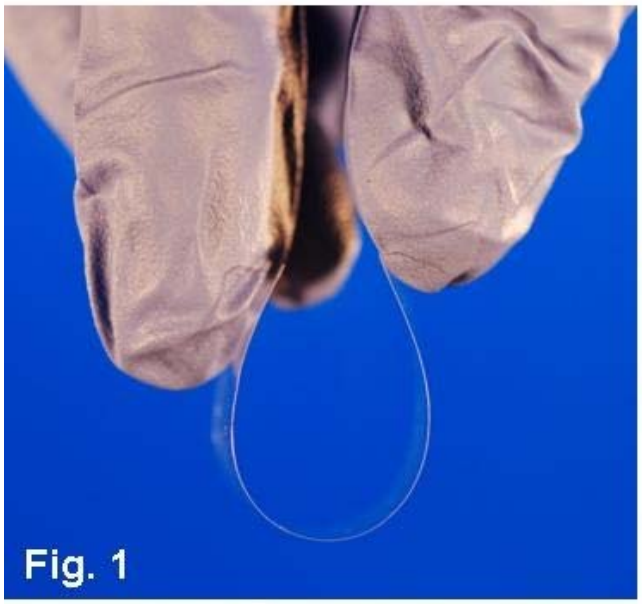


Fig. 1

Fig. 2

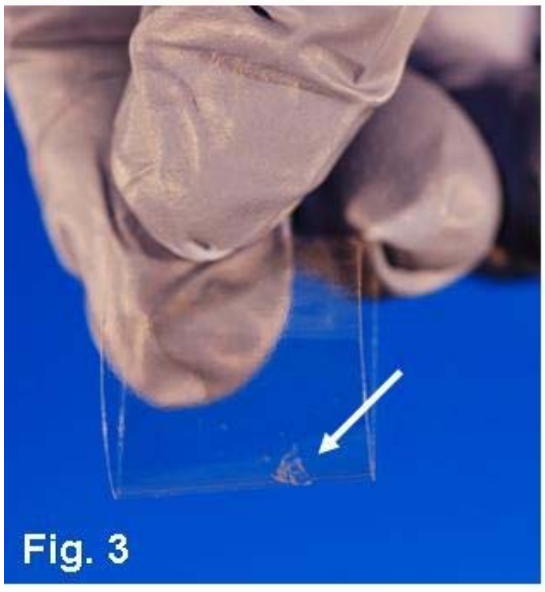


Fig. 3

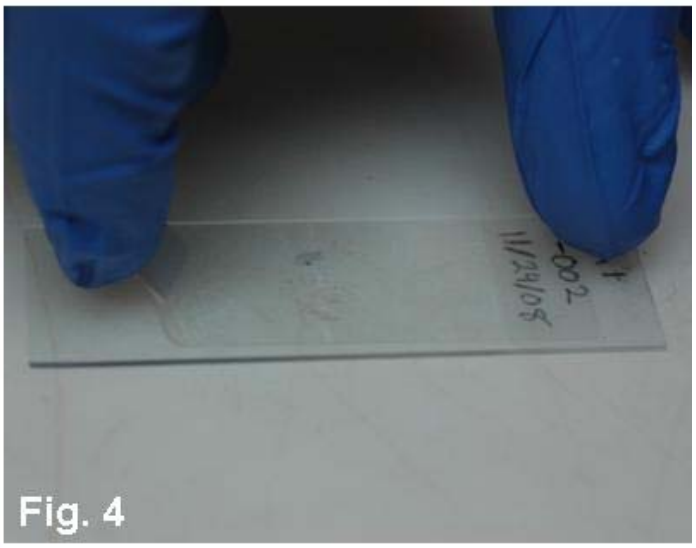


Fig. 4