

**Physician Instructions for Collecting and Handling Clinical Specimens and Data
from Patients with Suspected Tick Bite-Associated Rash Lesions
of Unknown Etiology in the Southern United States**

Background: Lyme disease is due to infection with the tick-transmitted spirochete *Borrelia burgdorferi*. In the United States, the regions with the highest Lyme disease incidences are the Northeast, Upper Midwest, and Pacific Coast. The characteristic annular, macular, erythematous skin lesion of early Lyme disease, erythema migrans (EM), occurs at the site of the infected tick bite, has an incubation period of 3-31 days, and typically expands over time, sometimes to a diameter of ≥ 30 cm.

Tick bite-associated EM-like lesions also occur in the southern United States, but the etiology of such lesions is unknown. Some appear to be associated with bites of the Lone Star tick, *Amblyomma americanum*, which is the most common human-biting tick in the region. Studies to date have consistently failed to etiologically implicate *B. burgdorferi* in these cases [ref. Campbell et al. *J Infect Dis* 1995;172:470-80, Kirkland et al. *Arch Intern Med* 1997;157:2635-41]. Possible etiologies include a novel tick-transmitted spirochete [ref. Barbour et al. *J Infect Dis* 1996;173:403-9] or other infectious agent.

To determine the etiology and epidemiology of tick-associated annular skin lesions in the South, scientists at the Centers for Disease Control and Prevention (CDC) are cooperating with clinicians and scientists in the South to collect appropriate clinical material for research purposes. Specimens will not be tested immediately but will be stored in an appropriate fashion to allow for future testing of various etiologic hypotheses, once test methods are available.

Your cooperation is important to insure that informed patient consent is obtained, appropriate clinical specimens are obtained and appropriately handled, and that standardized and complete clinical data are collected. Please read carefully the following guidelines and any enclosures or attachments. If you have technical questions or wish to inquire about the availability of supplies for specimen collection, please contact one of the CDC scientists listed below.

Patient Eligibility

The following criteria should be used to determine if a patient is eligible for enrollment:

1. A person with acute onset (within 14 days of visit to physician office) of an annular, erythematous, expanding EM-like rash that attains a size of at least 5 cm in diameter, when no alternate explanation for the rash can be found, and
2. a history of tick bite at the rash site or potential exposure to ticks within 14 days prior to rash onset.

Informed Consent for adults and Assent Form for minors:

A CDC investigator will send an informed consent and/or assent form for minors, as appropriate, to your office by facsimile or mail. PRIOR to collecting specimens, please have the patient read and sign this form. For a patient less than 18 years of age, please have a parent or guardian complete and sign an *Informed Consent* form and have an *Assent Form for Minors* completed and signed by the minor patient. **It is extremely important that your office return the signed and witnessed form with the clinical specimens. We cannot accept specimens and enroll patients in the study without a signed informed consent and/or assent form for minors.**

Clinical Data Collection:

Please carefully complete the enclosed or attached *Clinical Data Collection Form: Suspected Tick Bite-Associated Rash Lesions, Southern United States*, including the space for a simple diagram of the skin lesion. The Data Collection Form will have a preassigned patient identification number listed; no personal patient identifiers are included on the form. [Note: Clinical information that is legible and as complete as possible must accompany all specimens.] If possible, a color photograph of the skin lesion should be obtained, properly labeled, and attached to the clinical data collection form.

Clinical Specimen Collection and Handling:

Ideally, the following samples should be collected. A specimen collection kit can be sent to your office prior to the beginning to tick season, or by overnight delivery service at the time a patient presents for care.

- 2 skin biopsies
- clotted blood for serum (acute-phase specimen now, convalescent-phase specimen 3-6 weeks later)
- anticoagulated whole blood
- urine

If a patient does not consent to a skin biopsy, it is still important to collect and submit the blood and urine specimens (and the clinical data).

Skin biopsies:

NOTE: Please exclude persons with hemophilia or other coagulopathies, including patients taking potent anticoagulants such as warfarin. (Patients taking NSAIDs alone need not be excluded.) Also, please do not biopsy facial lesions for this study.

To collect the two skin biopsy specimens, the use of a standard sterile 2 mm punch instrument and standard sterile technique are recommended. Empirically, these should be taken from 4-6 mm inside the outer margin of the annular EM-like skin lesion and within 2.5 cm of each other and to a depth of 3-4 mm. The biopsy sites are anesthetized (\approx 0.5 ml of a 1% solution of lidocaine and epinephrine 1:100,000 at each site) and then disinfected with a tincture of iodine followed by an alcohol swab. Using a gentle twisting action, the punch instrument is used to cut the skin to a depth of 3-4 mm. The punch instrument is then removed. Repeat for the second biopsy sample. Each skin sample is then grasped with fine-tipped forceps, pulled gently away from the body, and snipped at its base with iris

scissors. If necessary, place the samples on a sterile gauze patch momentarily while attending to hemostasis. Hemostasis is usually achieved by pressure alone; a butterfly bandage or single nonabsorbable suture can be applied if necessary. One biopsy specimen (biopsy specimen "A") is placed directly into the enclosed tube containing BSK-H transport medium and refrigerated. The other biopsy specimen (biopsy specimen "B") is placed directly into the enclosed tube containing Streck tissue fixative, to be stored at room temperature and shipped on a gel-type ice pack (see below).

If only one biopsy specimen is obtained, please place it in BSK-H transport medium.

Phlebotomy:

Phlebotomy site(s) should be disinfected with a tincture of iodine followed by an alcohol swab. To minimize the risk of hematoma, phlebotomy site(s) should be subjected to direct pressure with arm straight and elevated for 3-4 minutes following phlebotomy.

Clotted blood for serum:

A 10-ml acute-phase sample should be collected in the enclosed standard red-topped serum separator tube and centrifuged in the standard fashion, to be stored at approx. 4°C and shipped to CDC on a gel-type ice pack. Following centrifugation, decanting of the serum from the clot is unnecessary. *It is important that a convalescent-phase sample be collected 3-6 weeks later in a similar fashion and shipped on a gel-type ice pack.*

Uncoagulated whole blood:

A 5-ml blood sample should be collected in the enclosed standard EDTA-coated (purple-topped) tube in the standard fashion, to be stored at approx. 4°C and shipped on a gel-type ice pack.

Urine:

A 2.5-ml sample of urine should be collected and place in the enclosed tube containing 2.5 ml 95% ethanol, then stored at approx. 4°C and shipped on a gel-type ice pack.

Shipping Address: use CDC Federal Express account number only!

Bacterial Zoonoses Branch
CDC
Foothills Campus (Rampart Road)
Fort Collins, CO 80521-2087
ATTN: Mr. Steve Sviat
(970) 221-6400

NOTE: Please contact one of the CDC scientists listed below to obtain a Federal Express account number to cover the cost of overnight shipment of specimens.

Mailing Address:

Bacterial Zoonoses Branch
CDC
P. O. Box 2087
Fort Collins, CO 80522-2087

CDC Scientist-contacts:

Dr. Ned Hayes (Epidemiology Section)
Dr. Barbara Johnson (Molecular Bacteriology Section)
Dr. David Dennis (Bacterial Zoonoses Branch)
Bacterial Zoonoses Branch
Division of Vector-Borne Infectious Diseases
CDC
P.O. Box 2087
Fort Collins, CO 80522-2087

Tel (970) 221-6474 (Hayes)
Tel (970) 221-6463 (Johnson)
Tel (970)-221-6418 (Dennis)
FAX (970) 221-6476

Email: ebh2@cdc.gov (Hayes)
bjj1@cdc.gov (Johnson)
dtd1@cdc.gov (Dennis)

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