Diagnostic Testing for Parasitic Diseases at CDC

Brian Raphael, PhD, PHLD(ABB)
Lead, Diagnostics and Biology Team
Laboratory Science and Diagnostics Branch (proposed)
Division of Parasitic Diseases and Malaria

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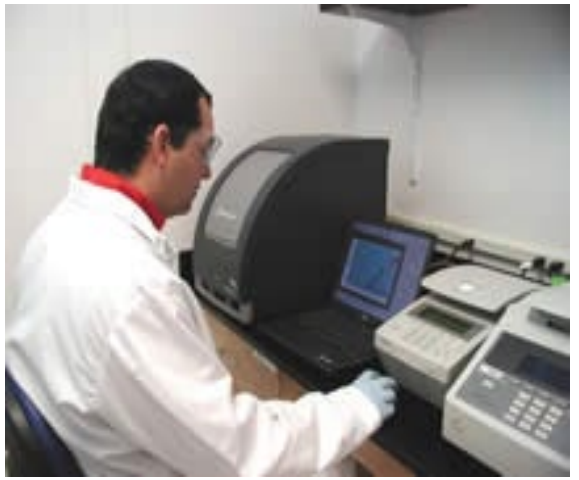
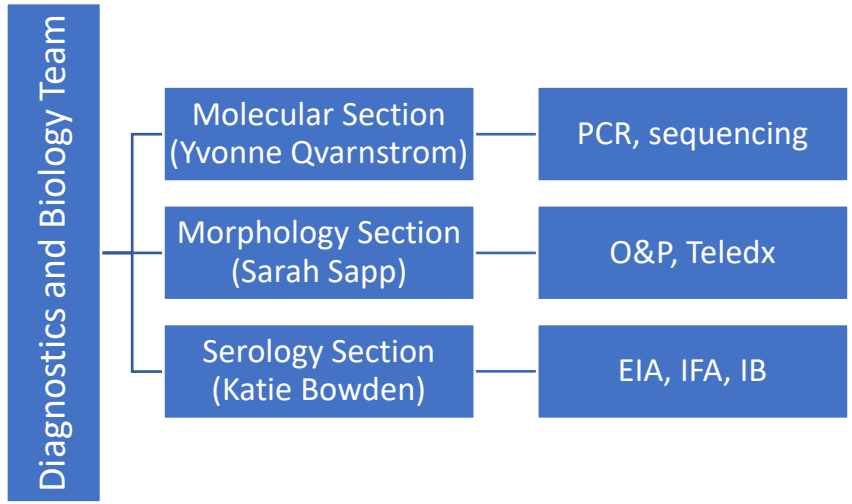


https://www.cdc.gov/parasites/lab_science.html

Diagnostic Parasitology Laboratory

- **Who can submit specimens?**
  - State health departments
    - Original submitters (e.g. hospitals, health care providers) MUST contact local/state health department)
  - Other federal agencies
- **DPDM website**
  - https://www.cdc.gov/parasites/index.html
  - See Information for Specific Groups > Laboratory Scientists
- **Test directory**
  - https://www.cdc.gov/laboratory/specimen-submission/list.html



Current status of test systems – March 2023

Molecular

Chagas disease Molecular Detection
Leishmania Molecular Identification
Malaria Molecular Identification
Babesia Molecular Detection

Angiostrongylus cantonensis Molecular Detection

Serology

Chagas disease (EIA, IB, IFA)
Paragonimiasis (IB)
Fascioliasis (IB)
Baylisascariasis (IB)
Cysticercosis (IB)
Strongyloidiasis (EIA)
Schistosomiasis (IB)
Echinococcosis (IB)
Babesiosis (IFA)
Filariasis (EIA)

Toxocariasis (EIA)

Morphology

Ova and Parasite
Physical specimens
Telediagnosis

Trichomonas Susceptibility

Online
Pending resumption

Submitting specimens to CDC

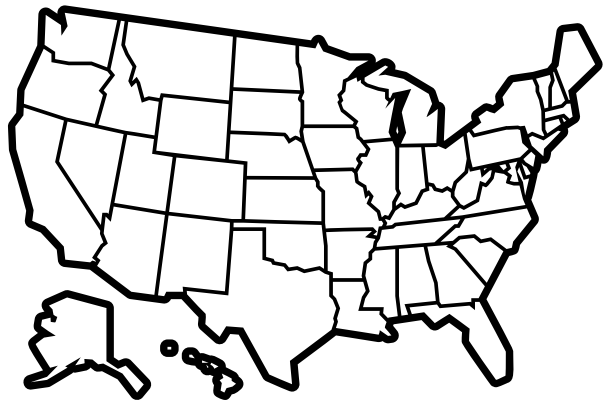
- **See Test Directory for acceptable conditions**
  - Time from collection
  - Storage and shipping temperature
- **Sample submission form; Form 50.34**
- **Send email to CDC POCs or parasiteslab@cdc.gov before sending specimens to obtain pre-approval**
  - Ensures up-to-date submission/shipping information
- **Do not ship prior to federal holidays or weekends**

Selected Specimen Conditions

- **Serum for serology**
  - Send frozen (≤ -20°C) on dry ice
  - Up to 8 weeks from collection
- **Whole blood for molecular assays**
  - Send refrigerated (2-8°C) on cold packs
  - Time from collection varies
- **Tissue for molecular assays**
  - Fresh tissue should (preferably) be sent frozen (≤ -20°C) on dry ice
  - Up to 30 days from collection

Coordinating with original submitters

- **Jurisdiction specific requirements**
  - Do specimens need to be sent to SPHL?
  - Assistance with SPHL information on 50.34
- **Reports**
  - Released to SPHL electronically
  - CDC can release to original submitter for urgent patient care purposes and will communicate with SPHL concurrently



Created by Setyo Ari Wibowo from the Noun Project

Highlighted test systems

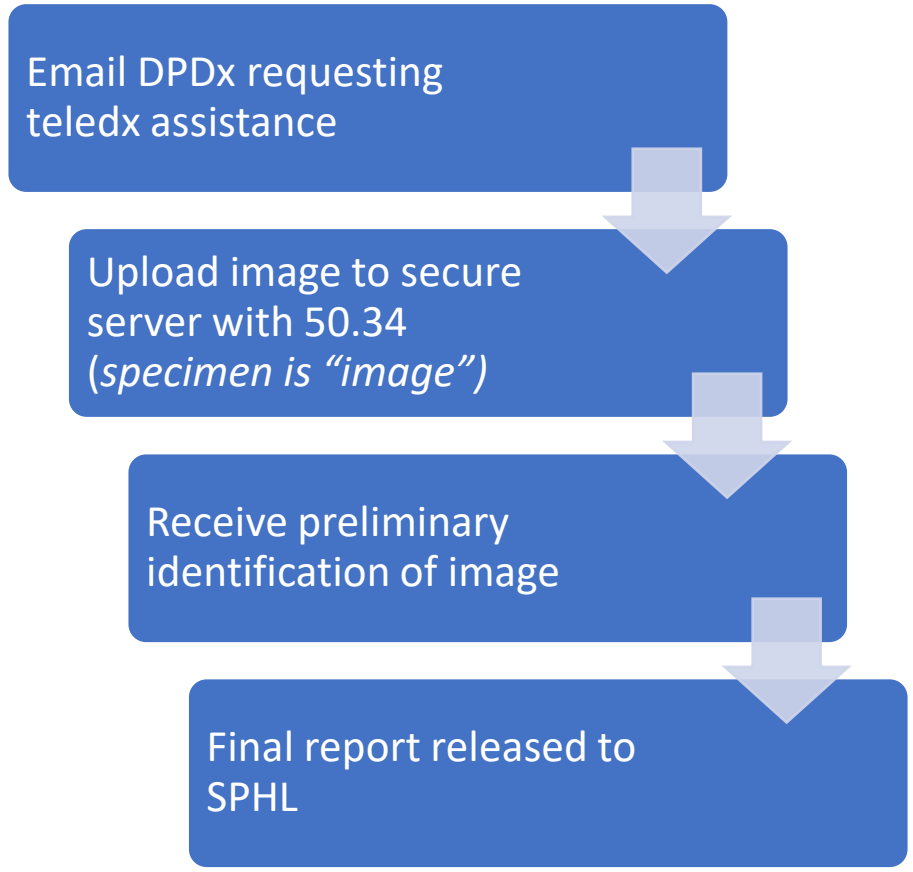
Morphological Identification

- **Test codes**
  - Parasites: Morphological identification (CDC-10234)
  - Malaria: Morphological identification (CDC-10520)
- **Physical specimens – consult with lab for specific conditions (preapproval)**
  - Whole blood
  - Stool*
  - Other fluids*
  - Whole organisms*
  - Slides

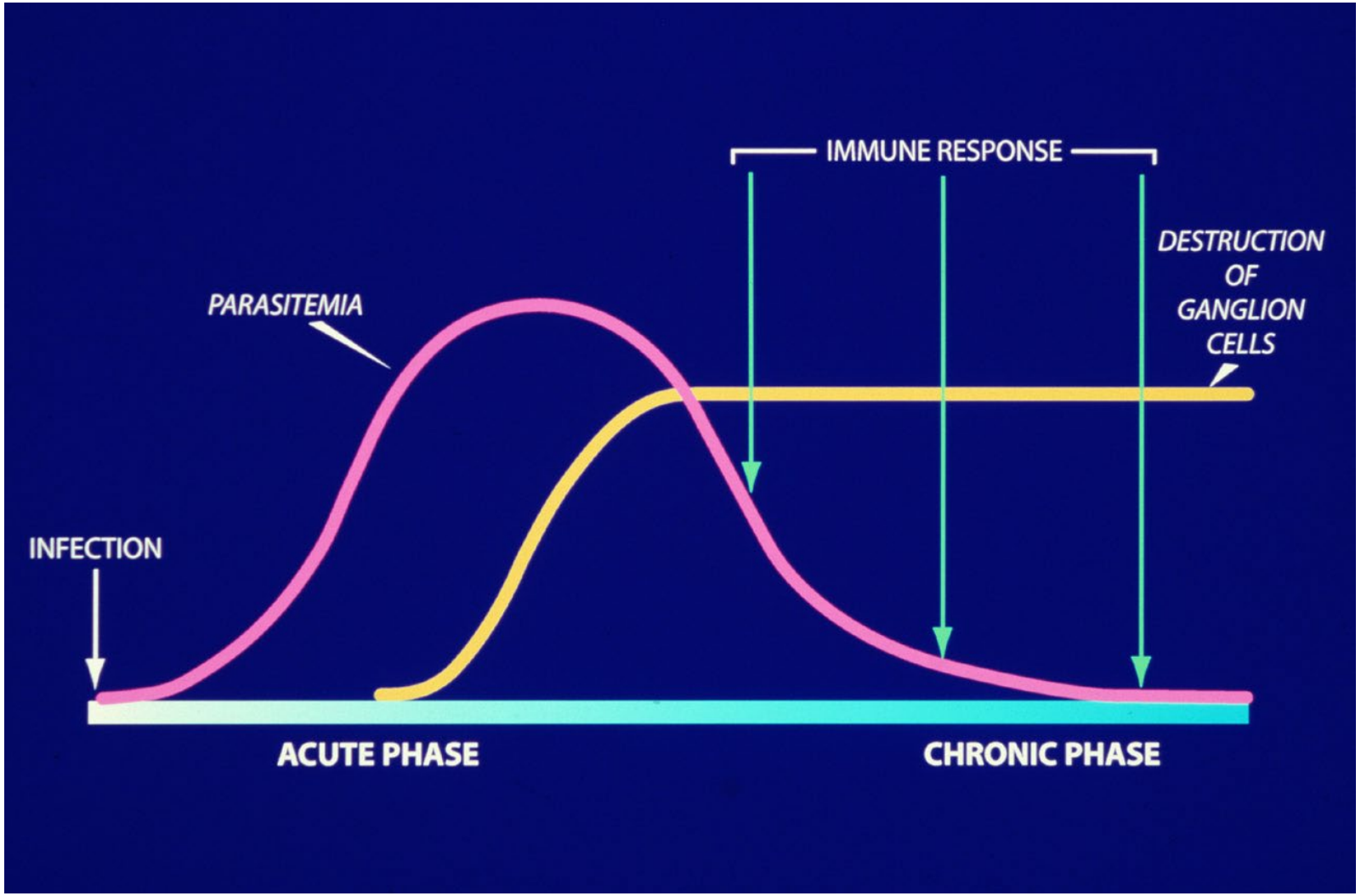
*(in preservative/fixative)

DPDx@cdc.gov

- **DPDx – Telediagnosis service**



Chagas disease



Thanks to Dr. Herb Tanowitz

Chagas disease testing

- **Molecular detection**
  - Test code CDC-10475 (Chagas disease molecular detection)
  - Request preapproval to determine if test request is appropriate for case (acute phase)
  - Specimens: Whole blood (EDTA), CSF, unpreserved heart tissue
  - Methodology
    - Real-time PCR to two *T. cruzi* targets

- **Serology**
  - Test code CDC-10458 (Chagas disease serology)
  - Request preapproval to determine if test request is appropriate for case (chronic phase)
  - Confirmation requires two or more serologic tests using different antigens
  - Specimen: serum
  - Methodology
    - EIA, Immunoblot
    - May request 2nd specimen if divergent
    - Repeat EIA, immunoblot
    - If still divergent; IFA

Leishmania species identification

- **Test code: CDC-10238**
- **Preapproval required**
- **Specimens**
  - EDTA-treated blood, bone marrow
  - Unpreserved skin tissue (in sterile buffered medium)
- **Methodology**
  - Real-time PCR targeting ITS2 to detect *Leishmania* spp.
  - Sequencing to identify species
- **Formalin-fixed tissue specimens**
  - May be submitted to Infectious Disease Pathology Branch with Test Order CDC-10365 (Pathologic Evaluation of Tissues for Possible Infectious Etiologies)
  - Extracted DNA may be used in Leishmania species identification test
  - May be difficult to resolve species with this specimen type

Trichomonas Susceptibility

- Test code: CDC-10239
- Request preapproval; CDC can provide InPouchTV
- Specimen is an inoculated InPouch TV
- Must arrive at CDC within 48 hours of collection at ambient conditions (18-25^{o}C)
- Isolate is recovered in culture medium
- Microplate dilution of antimicrobials (metronidazole, tinidazole) to generate Minimum Lethal Concentration

Modernizing the diagnostic laboratory

Need for improved tests

- **Why do we need improved tests?**
  - Improved performance, accuracy
  - Sustainability of reagents
  - Adaptability to changing needs, updated equipment, new targets
- **Actions**
  - Designing new laboratory developed tests
  - Engage partners with new methods, test systems
  - Develop specimen panels to conduct validations

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UPDx

- NGS-based method
- Enrich parasite DNA extracted from specimens
- Conduct deep sequencing of parasite target (e.g. 18S rRNA)
- Dedicated bioinformatics pipeline using reference sequence database

Flaherty *et al. Microbiome* (2021) 9:1
https://doi.org/10.1186/s40168-020-00939-1

Microbiome

RESEARCH Open Access

Sensitive universal detection of blood parasites by selective pathogen-DNA enrichment and deep amplicon sequencing

Briana R. Flaherty[1,2†], Joel Barratt[1,2*†], Meredith Lane[1,3], Eldin Talundzic[4] and Richard S. Bradbury[1,5*]

Abstract

Background: Targeted amplicon deep sequencing (TADS) has enabled characterization of diverse bacterial communities, yet the application of TADS to communities of parasites has been relatively slow to advance. The greatest obstacle to this has been the genetic diversity of parasitic agents, which include helminths, protozoa, arthropods, and some acanthocephalans. Meanwhile, universal amplification of conserved loci from all parasites without amplifying host DNA has proven challenging. Pan-eukaryotic PCRs preferentially amplify the more abundant host DNA, obscuring parasite-derived reads following TADS. Flaherty et al. (2018) described a pan-parasitic TADS method involving amplification of eukaryotic 18S rDNA regions possessing restriction sites only in vertebrates. Using this method, host DNA in total DNA extracts could be selectively digested prior to PCR using restriction enzymes, thereby increasing the number of parasite-derived reads obtained following NGS. This approach showed promise though was only as sensitive as conventional PCR.

Results: Here, we expand on this work by designing a second set of pan-eukaryotic primers flanking the priming sites already described, enabling nested PCR amplification of the established 18S rDNA target. This nested approach facilitated introduction of a second restriction digestion between the first and second PCR, reducing the proportional mass of amplifiable host-derived DNA while increasing the number of PCR amplification cycles. We applied this method to blood specimens containing *Babesia*, *Plasmodium*, various kinetoplastids, and filarial nematodes and confirmed its limit of detection (LOD) to be approximately 10-fold lower than previously described, falling within the range of most qPCR methods.

Conclusions: The assay detects and differentiates the major malaria parasites of humans, along with several other clinically important blood parasites. This represents an important step towards a TADS-based universal parasite diagnostic (UPDx) test with a sufficient LOD for routine applications.

Keywords: Molecular parasitology, Amplicon sequencing, Blood microbiota, Parasite biodiversity, Parasite detection, Molecular diagnosis

Flaherty et al, 2021. Microbiome 9:1

Multiplex Bead Array

- Utilize recombinant antigens to design assays using Luminex instruments
- Allows multiplexing of antigens (e.g. multiple species-specific antigens)
- Increased throughput
- Standardize methodologies and reagents used in serological analysis

How to contact us

SHARED MAILBOXES

Laboratory:
parasiteslab@cdc.gov
dpdx@cdc.gov

Clinical inquiries:
parasites@cdc.gov

TECHNICAL SUPERVISORS

Molecular: Yvonne Qvarnstrom (bvp2@cdc.gov)

Serology: Katie Bowden (wzi1@cdc.gov)

Morphology: Sarah Sapp (xyz6@cdc.gov)

Trichomonas Susceptibility: Evan Secor (was4@cdc.gov)

General inquires, etc:
Brian Raphael
Email: ELX9@cdc.gov
Phone: 404-639-4292