The use of rapid diagnostic testing for Chagas disease screening in Florida and a case of Chagas in a U.S. born traveler

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Disclosures

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Chembio Diagnostics Incorporated donated test kits for research purposes

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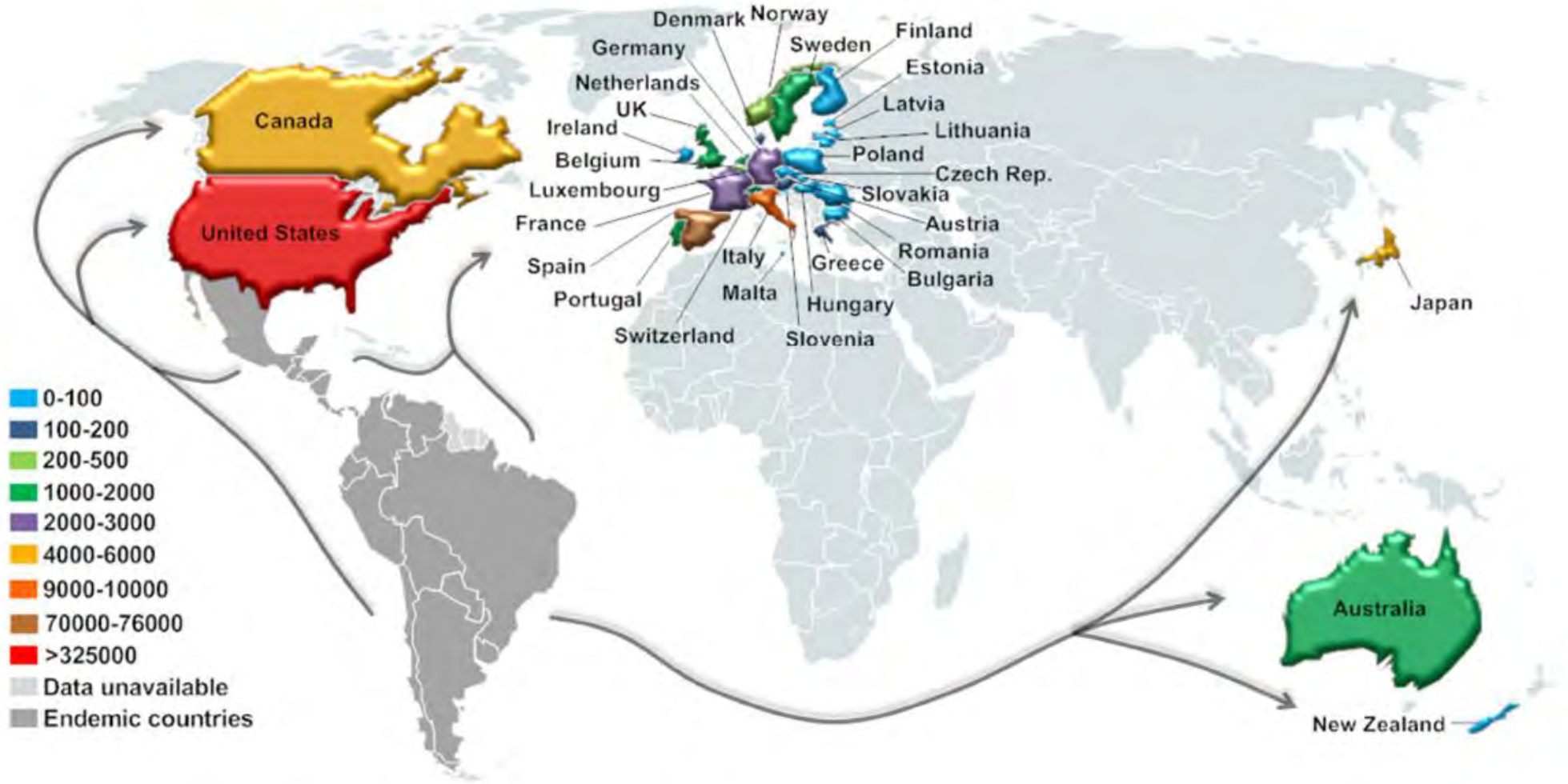
Chagas disease in the United States

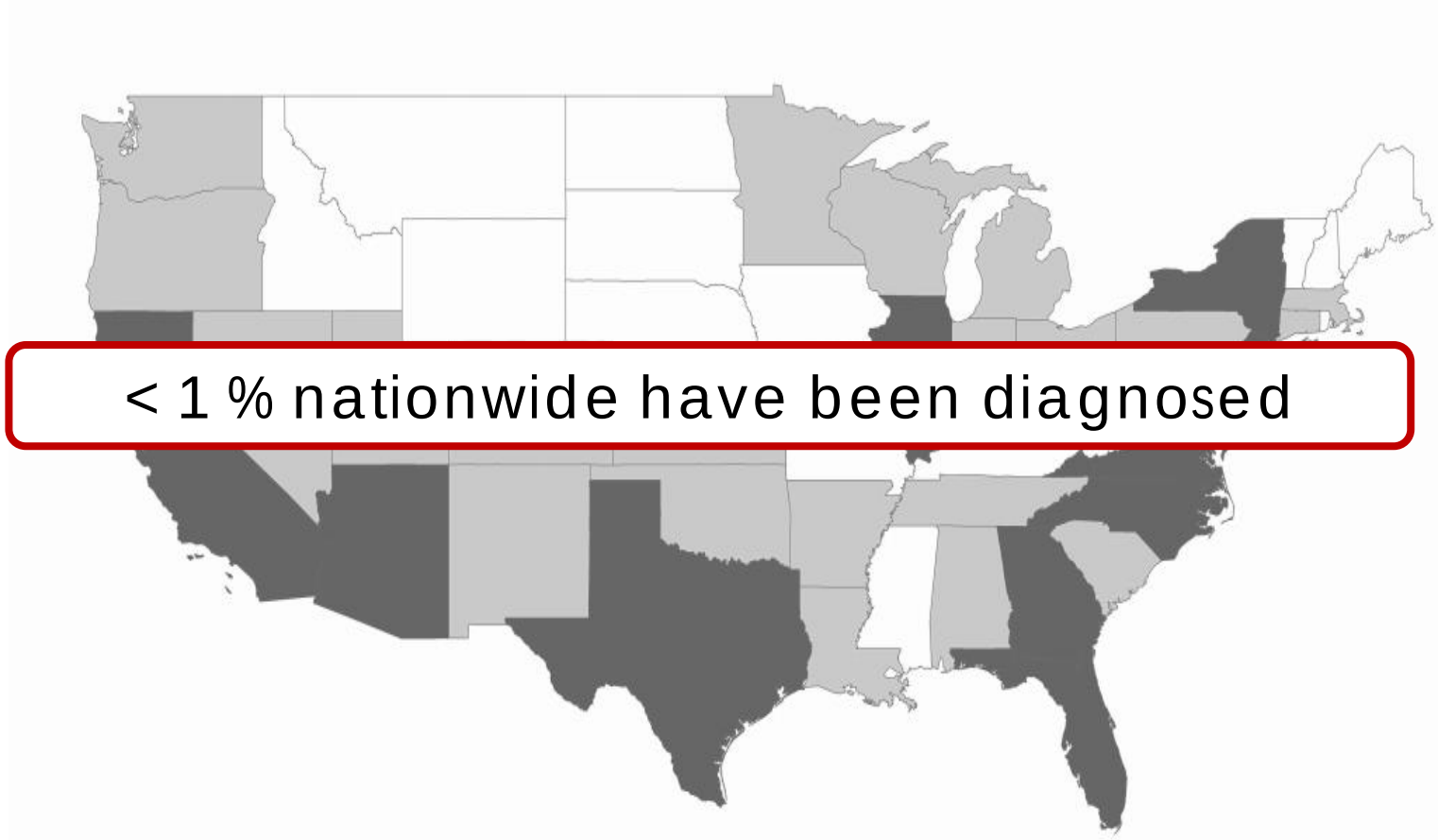


Lidani et al. Chagas disease a Worldwide Health Problem. *Front Public Health* 2019

Chagas disease in the United States

Top regions
estimated by state:

California – 70,000
Texas – 37,000
Florida – 18,000
New York – 17,000
Illinois – 9,000
New Jersey – 8,600
Virginia – 7,300
Arizona – 6,400
Maryland – 6,000



Manne-Goehler J *et al*. PLoS Negl Trop Dis. 2016.

Total estimated burden – 347,000

Chagas testing in the United States*

Wiener Chagatest ELISA Recombinante v.3.0

- Now available in the U.S.
- Quest Diagnostics

Hemagen Chagas' Kit (ELISA)

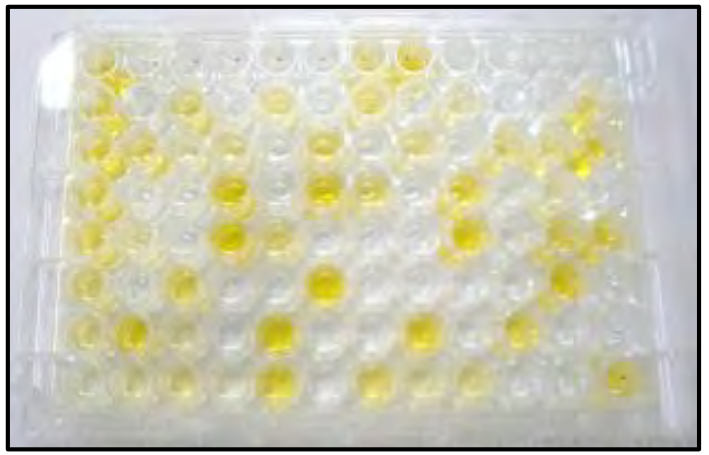
- Widely available throughout the U.S.
- Most common test used by commercial labs

Ortho *T. cruzi* ELISA test system

- Available as stand-alone test or infectious diseases panel with Eurofins-Viracor
- Primary screening test for stem cell and solid organ donation

InBios Chagas Detect Plus (rapid lateral flow assay)

- Available but not widely used



96-well ELISA with reactive results in yellow

*FDA-approved or -cleared testing

Screening for Chagas in the United States

Some key principles to consider:

- Awareness of the disease among healthcare providers and community at-risk
- Access to adequate and reliable testing
- Ease of collecting blood sample for testing
- Ease of ordering test and receiving results
- Cost of testing and coverage by medical insurance
- Health literacy for those who will be screened and screen positive
- Access to care and further testing when screening test is positive

Fingerstick blood being collected for rapid testing in mobile clinic

Forsyth C, et al. Proposed multidimensional framework for understanding Chagas disease healthcare barriers in the United States. *PLoS Negl Trop Dis*. 2019

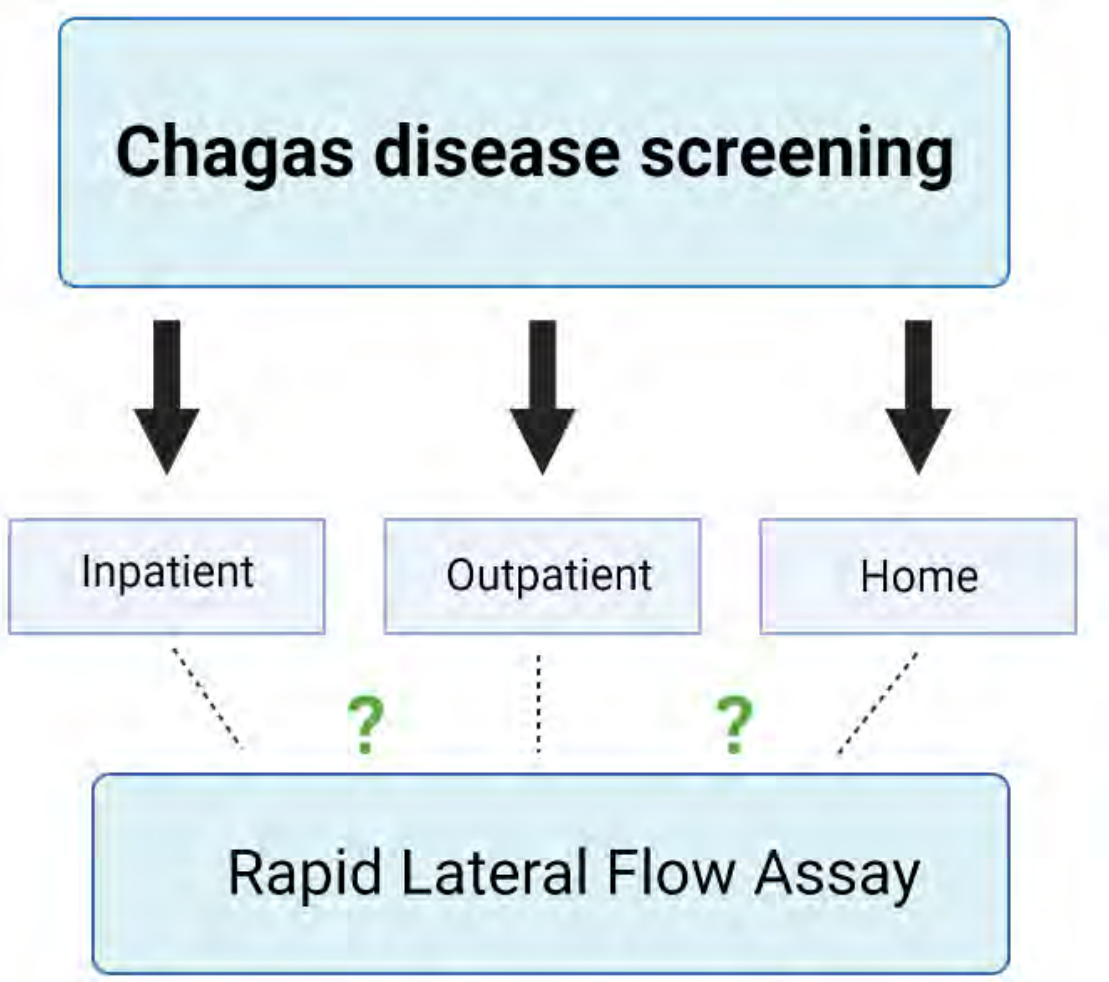
Has anyone used rapid diagnostic testing for screening an infectious disease?

A: Yes

B: No

Rapid HIV testing in community clinic in Brooklyn, New York

How does rapid testing fit into screening model?

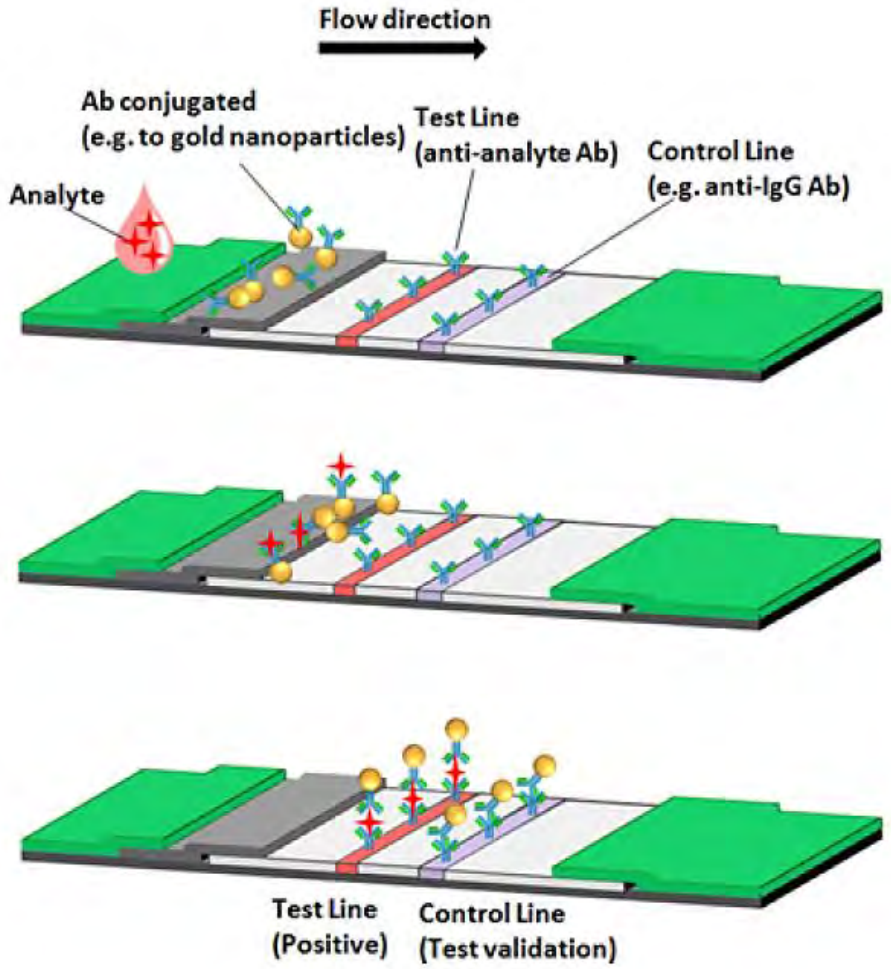


Didactic objectives:

- Benefits
- Limitations
- Current literature
- Our experience at UF

Benefits – Chagas rapid LFA

- Time (minutes to results)
- Simple to administer
- No special equipment needed
- Low cost
- Fingerstick blood stick
- Point of care/need
- Long shelf life
- Kept at room temperature
- Sensitive



Essays in Biochemistry (2016) 60 111-120.

Limitations – Chagas rapid LFA

- Operator error
- Mostly qualitative or semi-quantitative
- High temperature can affect regents/test strips
- Specificity issues (cross reactivity)
- Differences in whole blood and serum sensitivity
- Low signal intensity (weak or faint bands)
- Possible batch to batch variability (any LFA)
- Only one test is FDA-cleared (InBios Chagas Detect Plus)

Rapid Chagas LFA – Studies

How have LFAs performed?

Inbios Chagas Detect Plus (CDP):

- Whitman J *et al*. 2019 (sensitivity 97.4% to 99.3%; specificity: 87.5% to 92.3%) US blood donor network
- Castro-Sesquen Y *et al*. 2020 (sensitivity: 86.8% to 97.0%; specificity: 92.4% to 95%) US Washington DC
- Shah V *et al*. 2014 (sensitivity/specificity: whole blood – 96.2%/98.8%; Serum: 99.3%/96.9%) Bolivia
- Castro-Sesquen Y *et al*. 2021 (sensitivity/specificity: 91.9%/80.3%) US Washington DC

Chembio Stat-Pak:

- Eguez Karina E, et al. 2017 (sensitivity: 100%; specificity: 99.3%) Bolivia
- Suescun-Carrero SH, et al. 2021 (sensitivity: 100%; specificity: 100%) Colombia
- Castro-Sesquen Y *et al*. 2021 (sensitivity/specificity: 89.7%/97.1%) US Washington DC

Geographic variations in test reactivity for the serological diagnosis of *Trypanosoma cruzi* infection

Carine Truyens[*,a], Eric Dumonteil[*#,b], Jackeline Alger[c], Maria Luisa Cafferata[d], Alvaro Ciganda[d], Luz Gibbons[e], Claudia Herrera[b], Sergio Sosa-Estani[f,g], Pierre Buekens[h]

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[b] Department of Tropical Medicine, School of Public Health and Tropical Medicine, and Vector-Borne and Infectious Disease Research Center, Tulane University, New Orleans, LA, USA

[c] Instituto de Enfermedades Infecciosas y Parasitología Antonio Vidal; Departamento de Laboratorio Clínico, Hospital Escuela; Facultad de Ciencias Médicas, UNAH; Tegucigalpa, Honduras

[d] Unidad de Investigación Clínica y Epidemiológica Montevideo (UNICEM), Montevideo, Uruguay

[e] Institute for Clinical Effectiveness and Health Policy, Buenos Aires, Argentina

[f] Drugs for Neglected Diseases initiative-Latin America, Rio de Janeiro, Brazil.

[g] Epidemiology and Public Health Research Center, (CIESP-CONICET), Buenos Aires, Argentina

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JCM

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Geographic differences

- Multiple recent studies have shown differences in performance of RDTs and other serological-based assays based off geographic region
- Some are performing better depending on region
- Some have proposed related to different strains of *T. cruzi* (DTU)
- TcI versus non-TcI
- More research is needed

University of Florida Chagas Project

Design: Reach the people!

- Primary focus was to reach a diverse population of Latin Americans at-risk for Chagas disease.
- Implement Chagas disease screening into a panel of other health screening exams (primary care).

Multidisciplinary approach

- Primary care
- Rural health
- Mobile clinics
- Obstetrician care and Women's health
- Emergency medicine

Screen → Confirm → Linkage to Care

"Boots on the ground"

Team coming together after mobile event at local church

Design: screening tests

Rapid Chagas testing:

- InBios Chagas Detect Plus
- ChemBios DPP (research only)

Comorbidities:

- Diabetes Mellitus: Rapid Hemoglobin A1C
- Hypertension: Blood pressure testing
- Obesity: BMI testing
- Heart disease: 12-lead electrocardiogram
- Depression screening: PHQ-9

Design: inclusion criteria

Inclusion criteria:

>18yo and (one of the following):

(1) Born or have lived in endemic regions (> 6months)

(2) Known family member with CD

(3) Exposure to the vector through a bite or finding the insect in your home

(4) Mother was born in Latin America.

Agricultural worker health fair in Alachua County, Florida

Community event at local church in Alachua, Florida

Study Setting

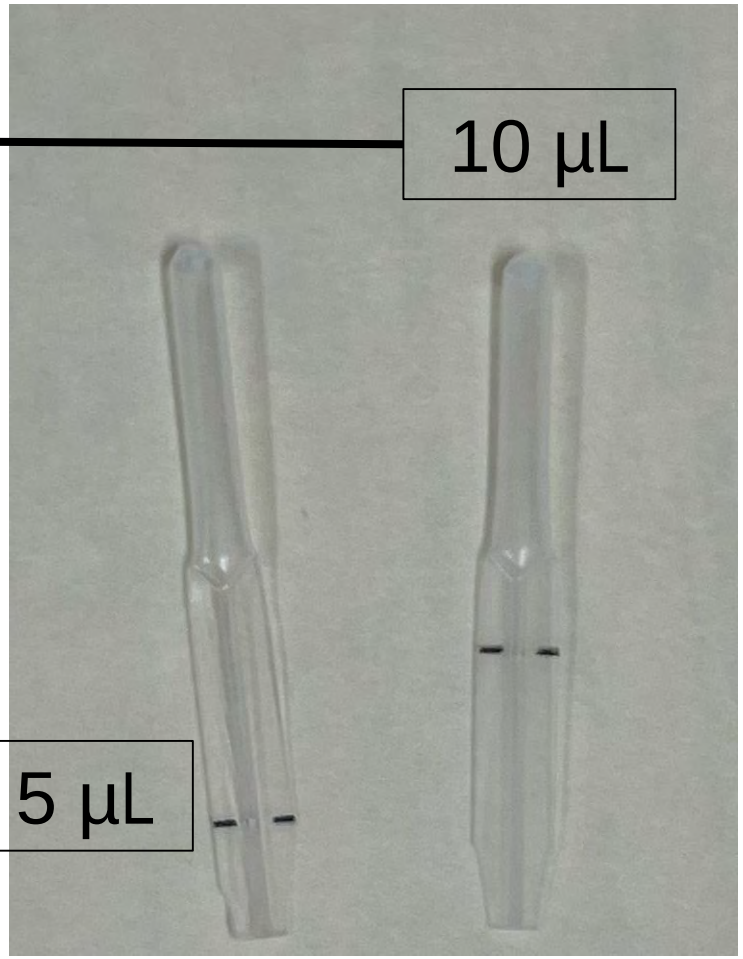
- Internal medicine primary care
- Mobile outreach clinic (bus or local church)
- Traveling Equal Access Clinic (local churches or health fairs)
- Infectious Diseases outpatient clinic
- Stand-alone emergency department
- Women's health primary care clinic

Getting started – logistics

- Space for testing (one or two 6’ tables)
- Review protocol for testing from manufacturer
- Organize materials at table (reagents, kits, results sheets…
- Wrist bands or name tags to keep track of participants
- Easily accessible sharps container
- Single-use retractable lancets
- Protection from sun
- Label maker
- Timers!

Table set-up at local church in Gainesville, Florida

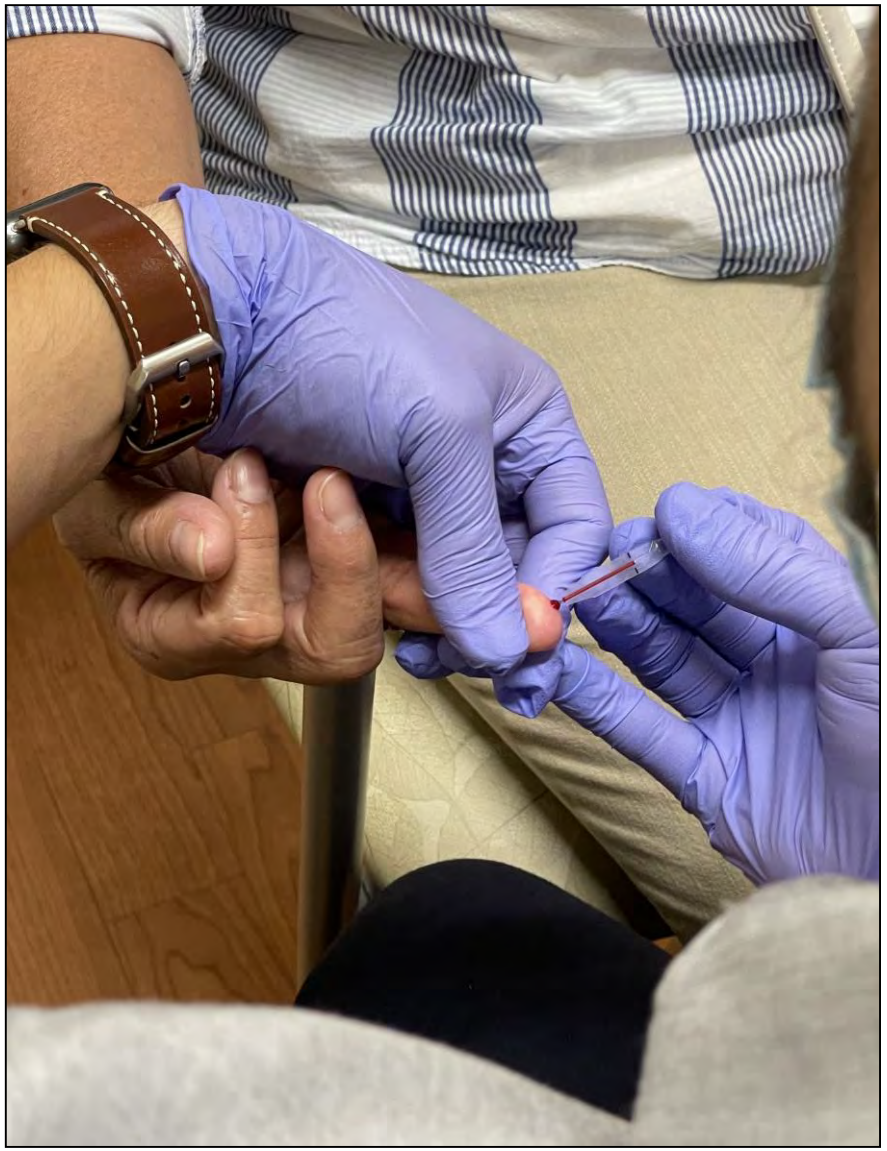
MicroSafe® capillary tubes



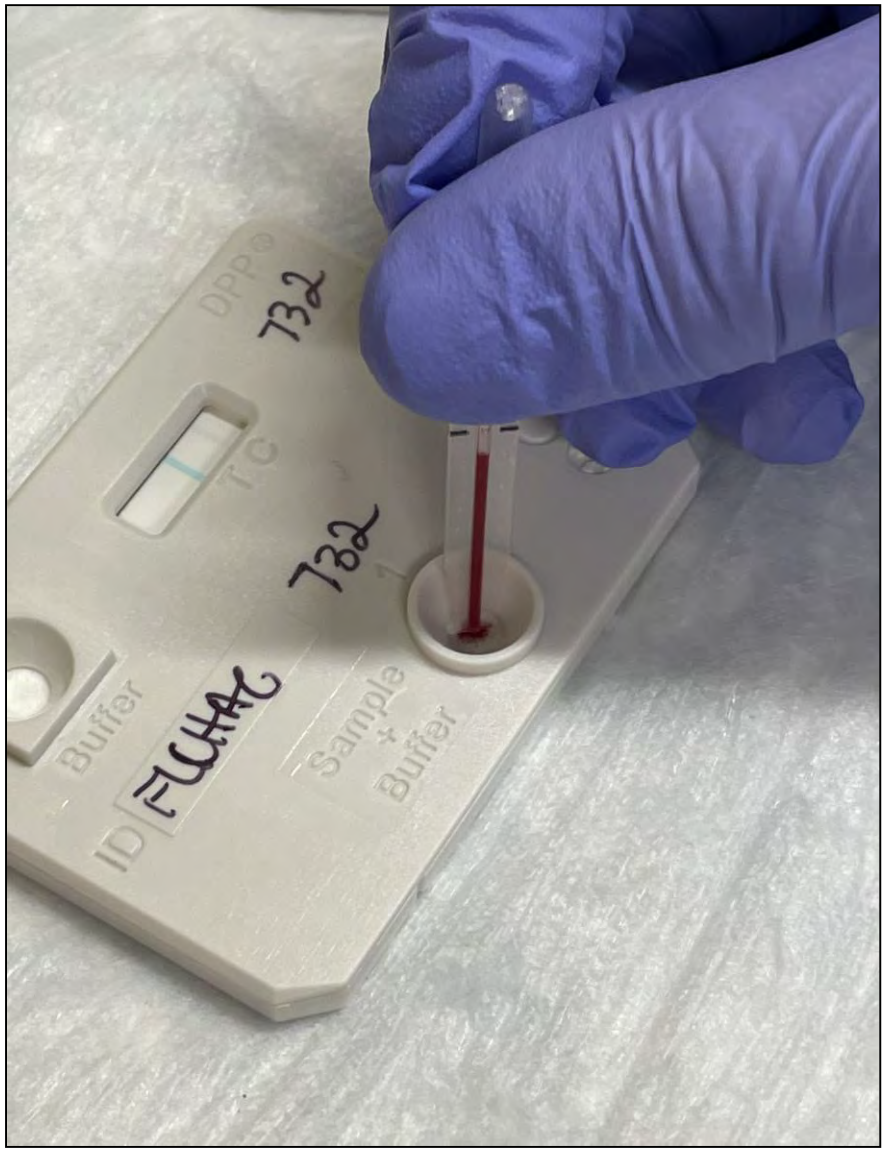
- Prevent capillary tubes from being damaged
- Do not attempt blood collection until enough blood on finger present
- Only use what manufacturer asks for
- Hold capillary tube vertical to well

Hold micropipette horizontal and gently touch blood drop; automatically fills

- Example of micropipette not filling due to blood clotting
- More common in 10µL



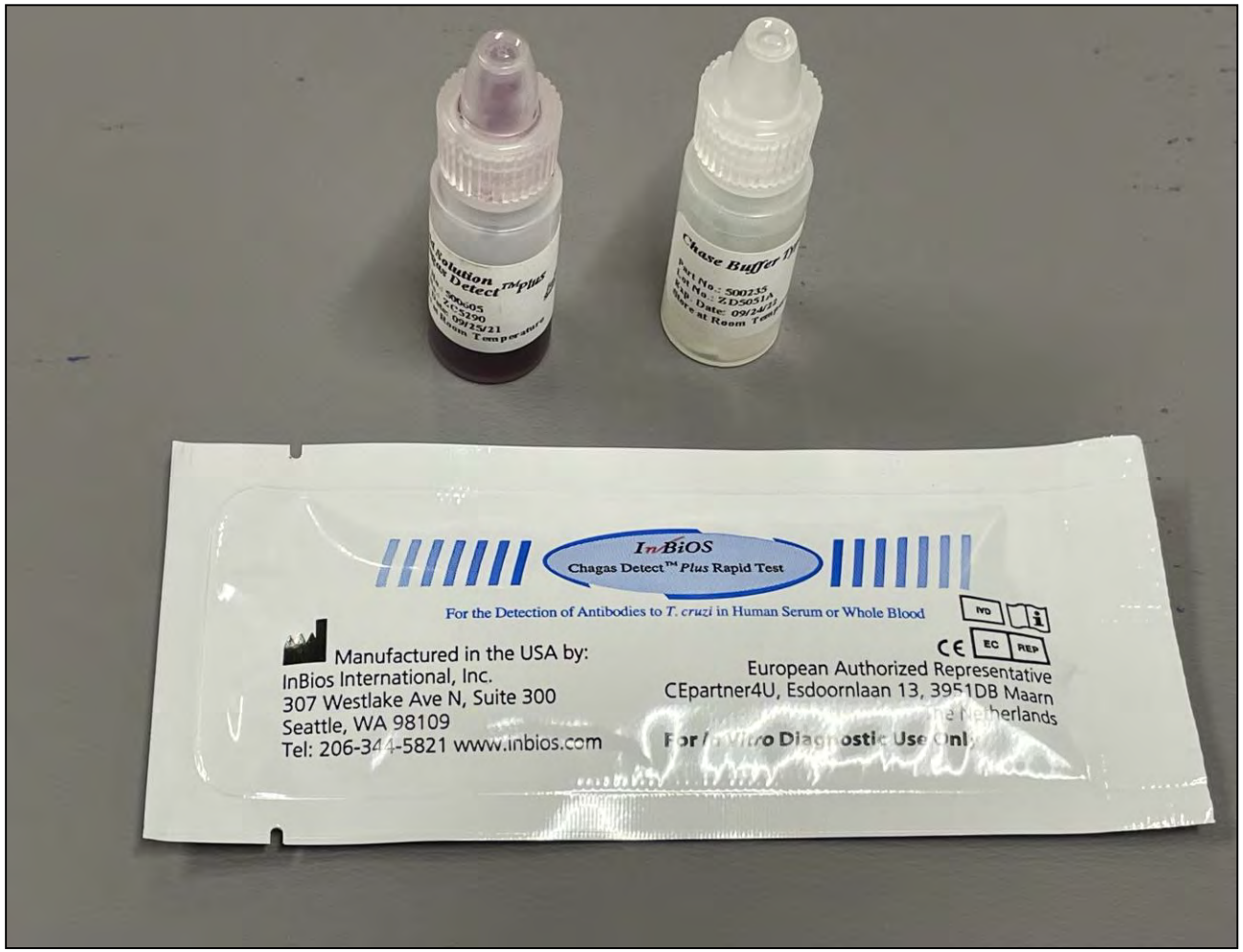
Transfer directly to kit and do not wait; blood may coagulate

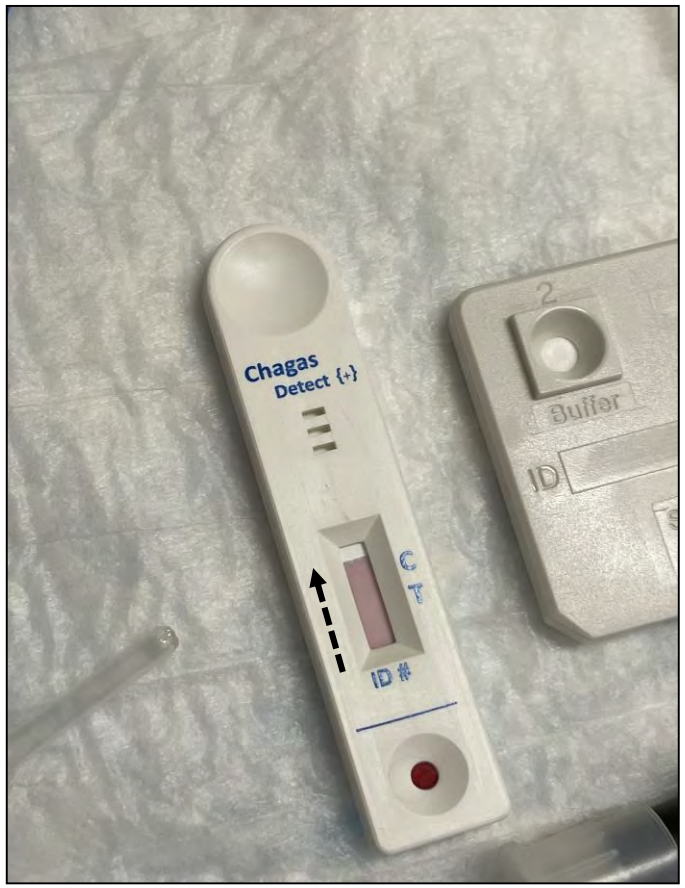


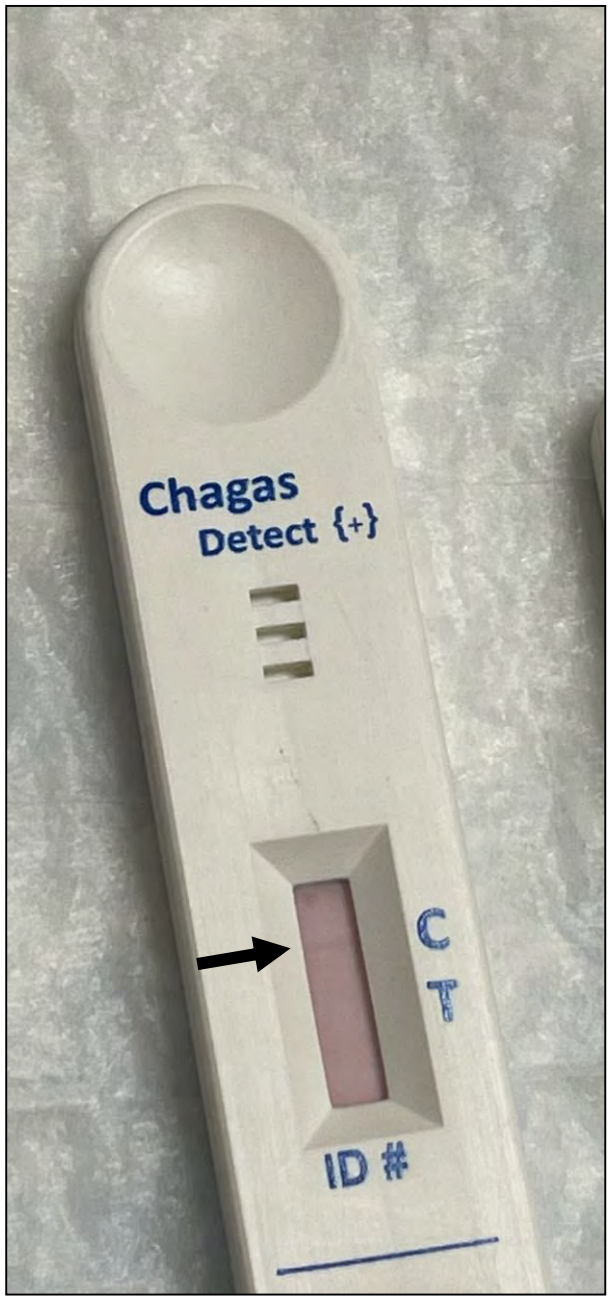
Hold vertical to well; gently push on bulb

Reagents

- Each kit has unique reagents
- Both utilize two steps for adding these reagents
- Simple process but always good to have provided instruction sheets out
- Need to have reagents kept out of the sun and heat
- Need to have a back-up if one of the bottles stops working



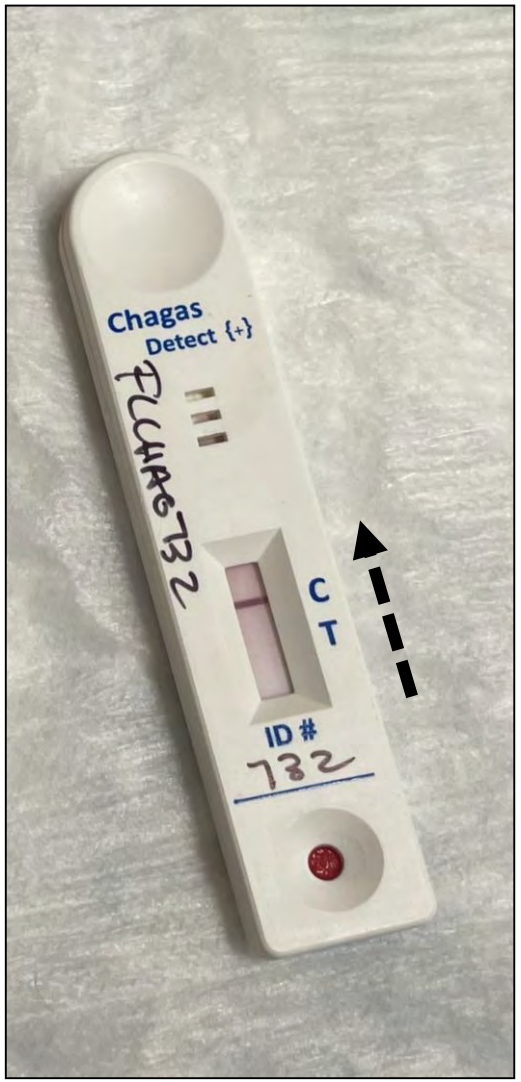


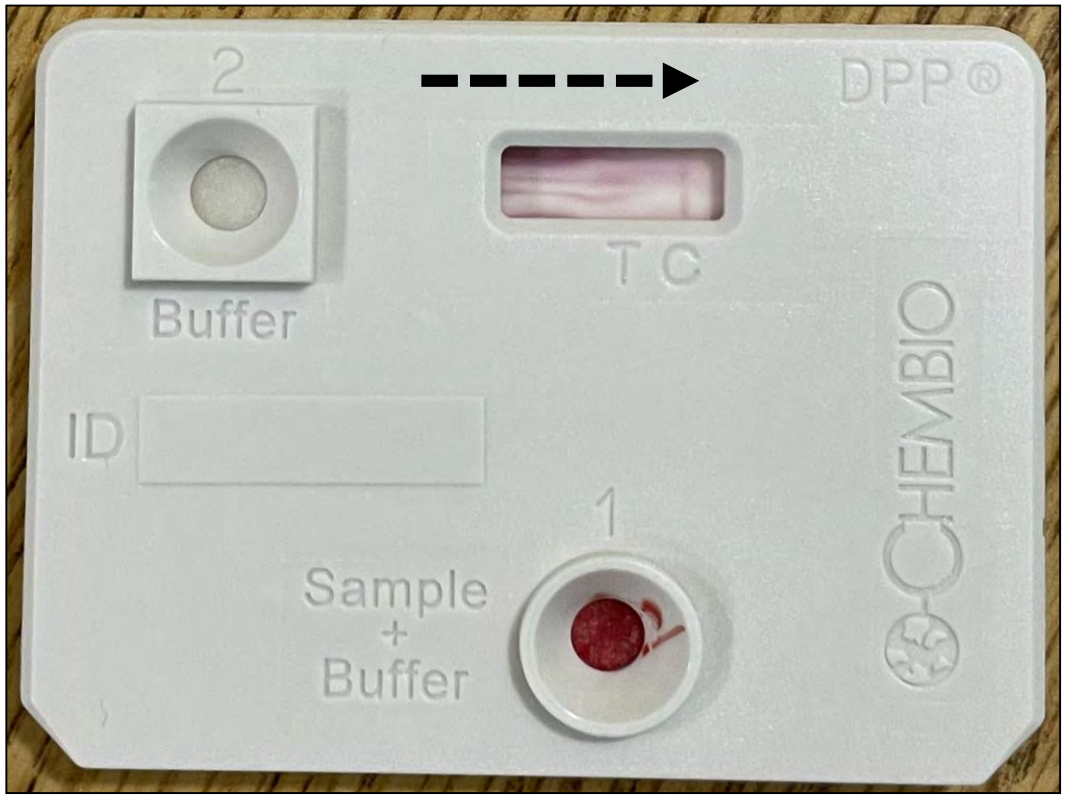


- Very important to transfer blood immediately to well
- Control line should begin to pop up within first 5 minutes if not sooner

Timers are always helpful!

Let tests run!

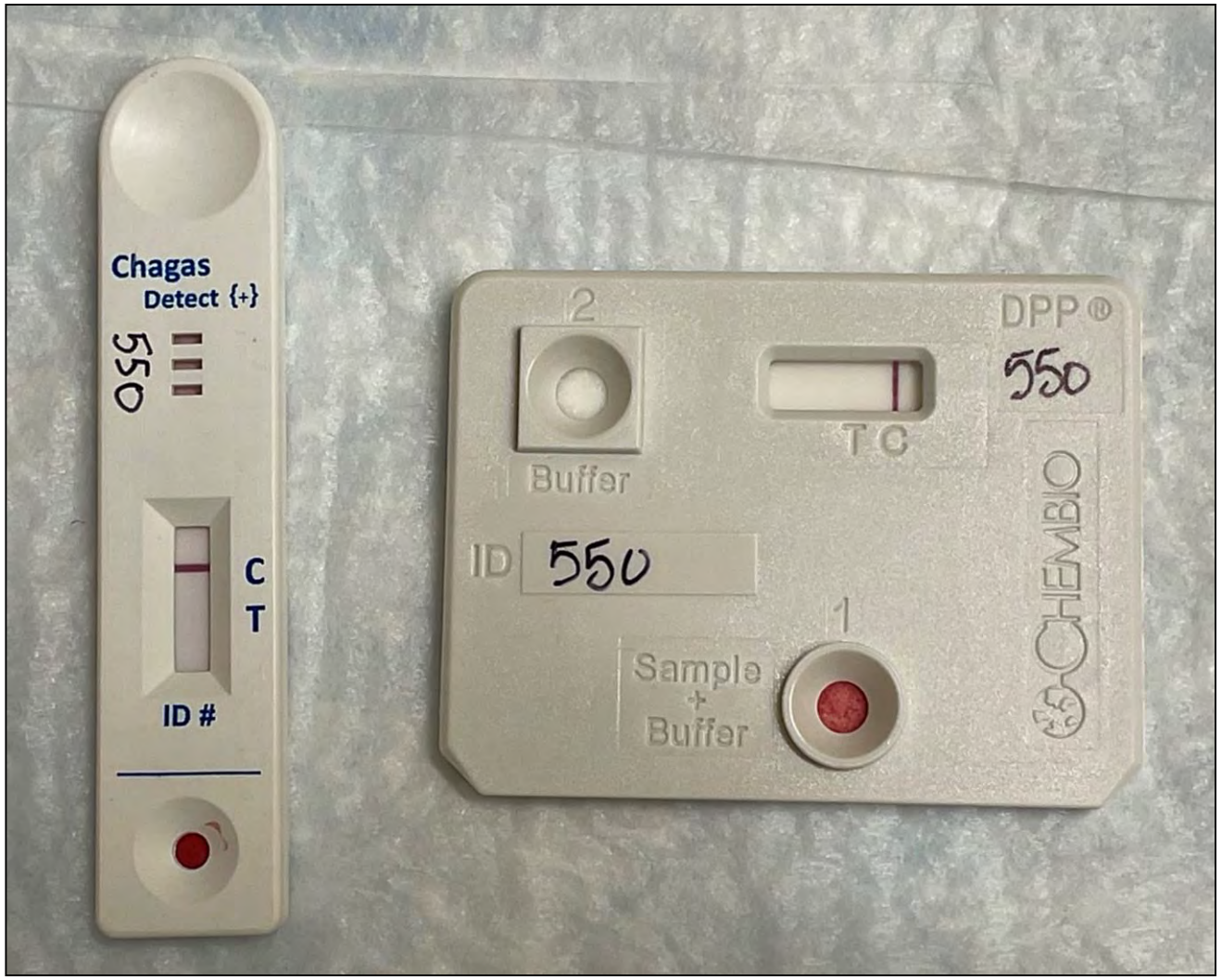




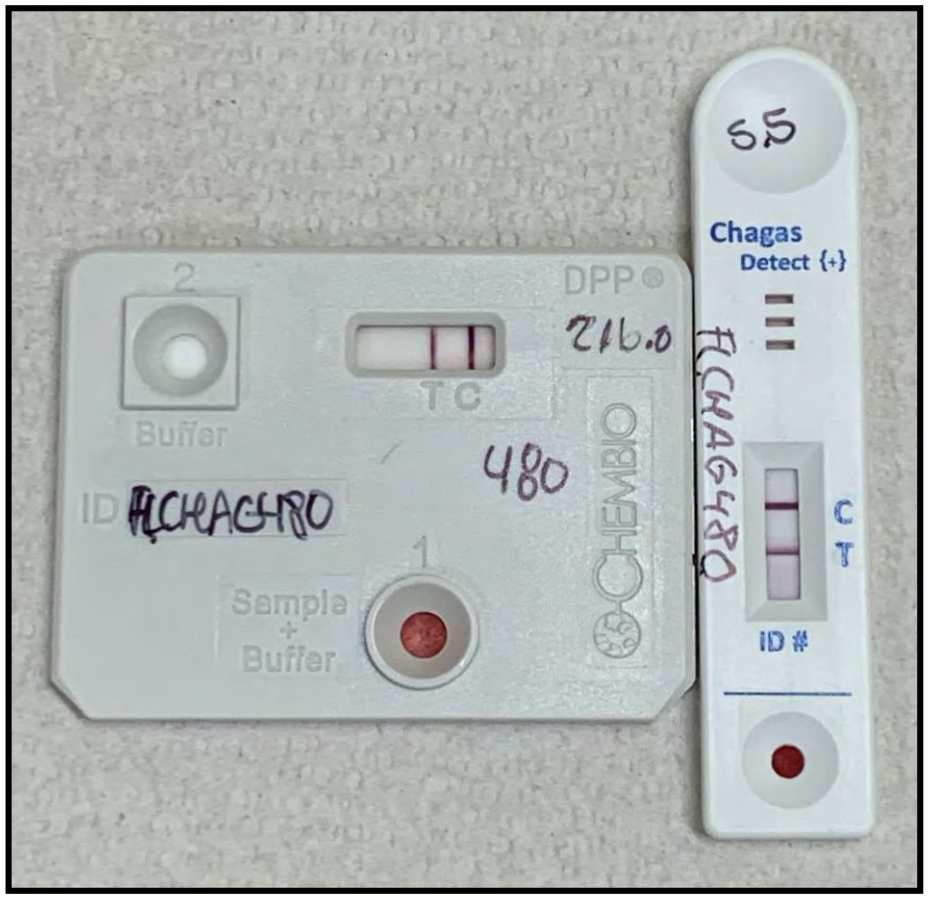
Do not interpret results until manufacturer designated time

- Some samples run slower than others
- Timers are helpful!
- Make sure kit is lying completely flat
- No exposure to sun or wind
- Inbios: 20 minutes
- Chembio: 15 minutes

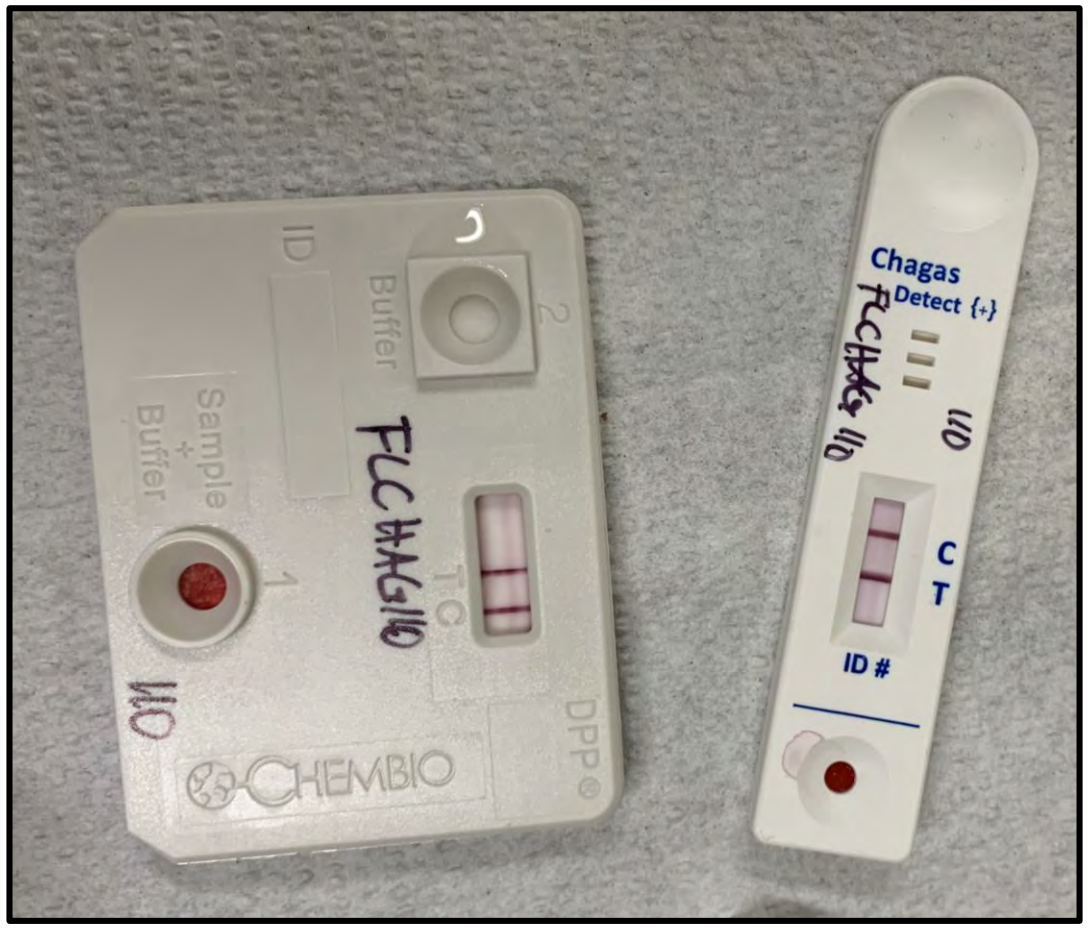
Negative results



Positive results

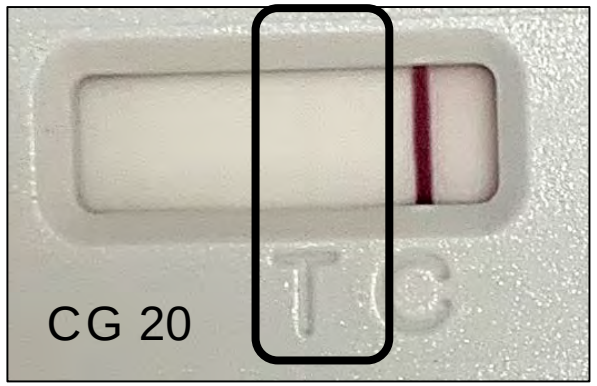


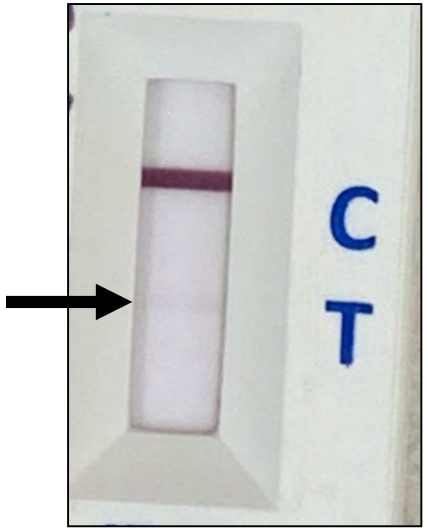
47yo Male: Born in Paraguay and frequent travel to Gran Chaco

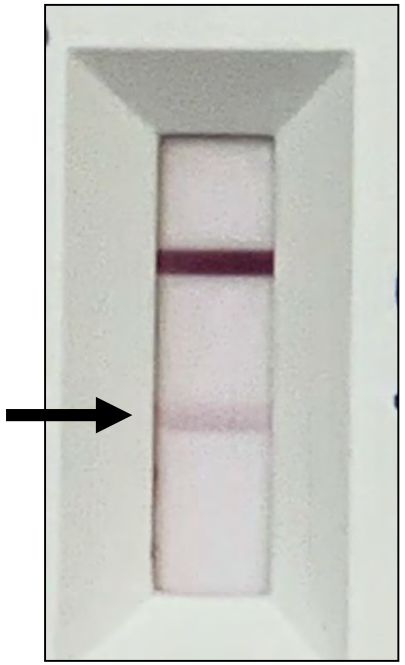


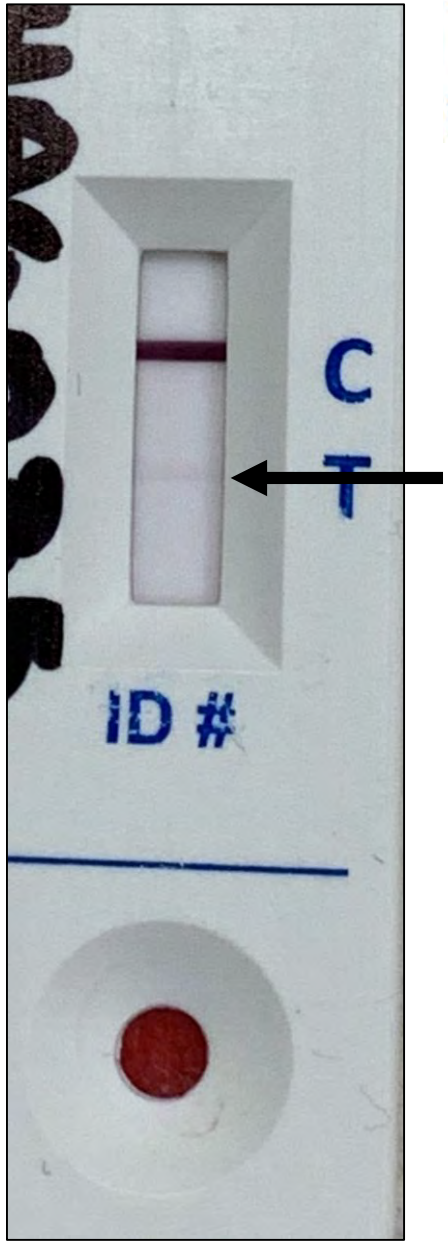
40yo Female: Born in El Salvador and emigrated in 1998

Faint bands









- All visible bands have been worked up with further serological testing
- Time-to-positivity has been associated with higher likelihood of true infection
- In our study so far, minority of faint bands represented true infection but cannot dismiss

Performance of CDP and Stat-Pak

Open Forum Infectious Diseases

BRIEF REPORT

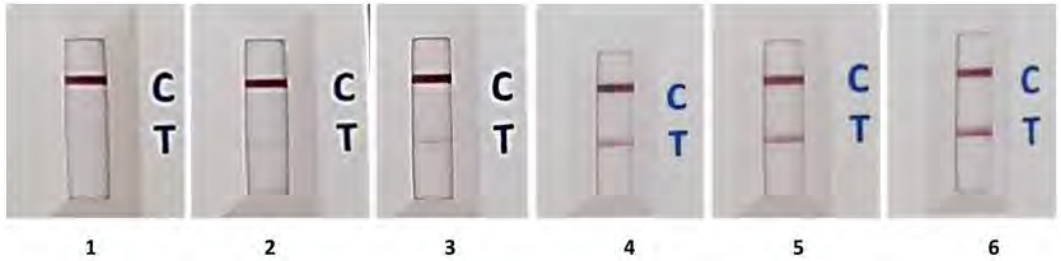
Evaluation of 2 Lateral Flow Rapid Tests in the Diagnosis of Chagas Disease in the Washington Metropolitan Area

Yagahira E. Castro-Sesquen,[1] Antonella Saldaña,[1] Dhayanna Patino Nava,[1] Diana Paulette Evans,[1] Tabitha Bayangos,[1] Kelly DeToy,[1] Alexia Trevino,[1] Rachel Marcus,[2] Caryn Bern,[3] Robert H. Gilman,[1] and Kawsar R. Talaat[1]; the Chagas Working Group in Peru and the United States

[1]Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA, [2]MedStar Washington Hospital Center, Washington, DC, USA, and [3]Department of Epidemiology and Biostatistics, School of Medicine, University of California, San Francisco, California, USA

- False positives had higher time-to-positivity and lower band intensities compared to true positives

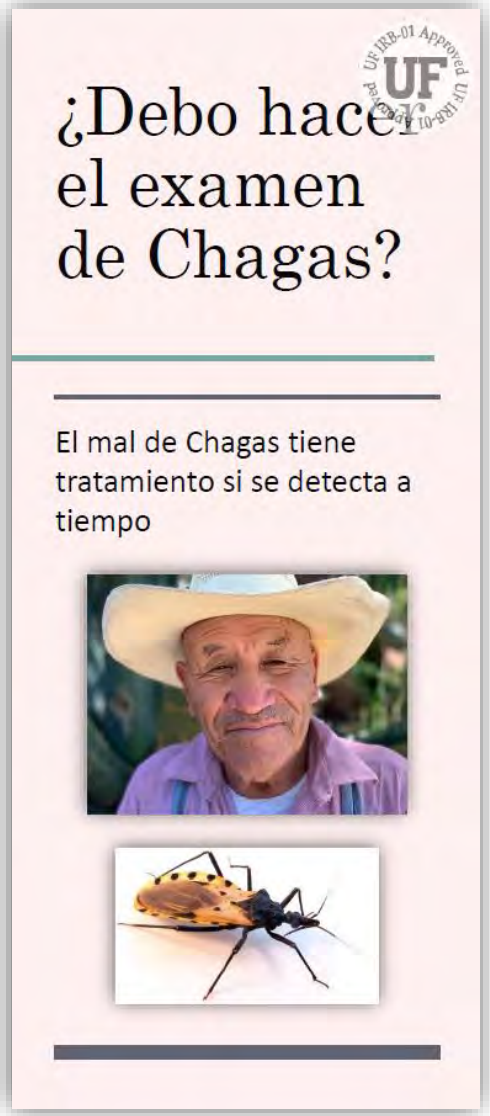
Figure S1. Band intensity card.



Band intensities in the test line were categorized into six different scores, with lower scores detonating lower band intensities. C: Control line, T: Test line, 1 to 6: Score of band intensity.

What is the plan for positive result?

- What resources are available for linking to care if needed? Referral to specialists?
- How will you send for further testing and who will pay for it?
- Who will interpret results of further testing?
- Does the participant have medical insurance and a primary care provider?
- Citizenship – resources available at your institution, county, or state, for those who have emigrated to the United States
- Is Chagas disease reportable to local and state health departments?




Mobile Outreach Clinic

Questions?


WORLD
CHAGAS DAY
APRIL 14TH 2021
Gainesville FL-USA

Case # 1

- 25yo M presents with concerns for Chagas disease; referral sent from primary care provider
- No past medical history; No family history of disease; No allergies
- Lived in Chile for 2 years from 2015-2017 for a church mission
- Traveled throughout Chile; primarily stayed in southern regions; lived rural settings and rudimentary housing, earthen floors, and mud walls
- Born in Utah; No other international travel in Latin America; No h/o blood transfusions or organ transplantation; Mother born in Utah
- No known exposure to triatomines; multiple "flu-like illnesses" while living in Chile that self-resolved
- Works as a medical assistant; planning to attend PA school in the Fall

Letter in the mail

- Donated blood at local blood drive 06/2021
- Received a letter from blood servicer approx. three weeks later informing him the "Chagas tests were positive".
- After discussion with primary care provider, it was decided to "just treat" and patient was started on benznidazole after "looking on uptodate".
- Prescribed 5mg/day (500mg divided into two doses)
- Began to feel ill after one week; week two developed severe maculopapular rash throughout his body that worsened, mild tongue swelling.
- Provider started him on 60mg oral prednisone; approx. two weeks into course
- Urgent referral sent and he is in the infectious diseases clinic

What would be your next step in this case?

A. Send commercial *T. cruzi* serological testing

B. Continue benznidazole and steroids

C. Stop benznidazole and continue steroids

D. Stop benznidazole and steroids

E. A & B

F. A & C

G. A & D

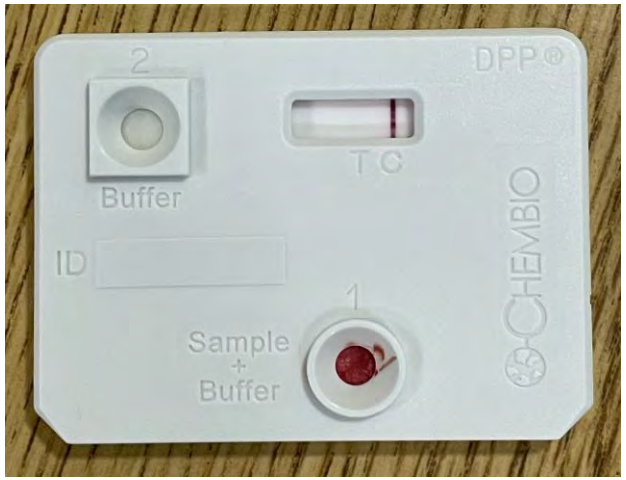
Rash has improved on steroids

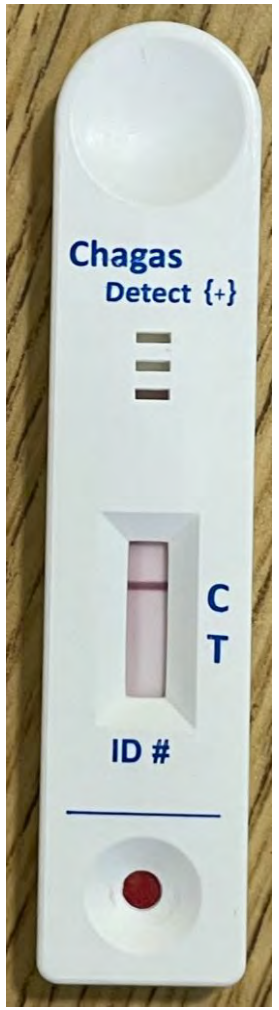
If you had access to rapid Chagas testing would you use to help guide next steps?

A. Yes – if results negative, I would stop benznidazole
B. Yes – if results positive, I would continue benznidazole
C. No – no matter the results, I would stop benznidazole
D. No – no matter the results, I would continue benznidazole
E. A & B

Rapid testing and outcome Case # 1

- CDP and Chembio both negative
- Routine blood work did not reveal eosinophilia, transaminitis, or renal dysfunction
- Strongyloides IgG negative (thank goodness!)
- Commercial *T. cruzi* serology sent to Quest (Weiner v.3.0) and ARUP (Hemagen IgG) were both negative
- Three FDA-cleared assays negative
- Likely not Chagas disease
- Benznidazole was stopped
- Steroids being weaned





Questions or comments about case #1 ?

Case # 2

- 67 y.o. female with history of breast cancer with surgical resection and chronic aromatase inhibitor use, partial blindness in the left eye in 2016 due to extreme coughing spells and retinal tear, who presents to infectious diseases clinic as a new patient for evaluation.
- Patient reports that she went to donate blood in September 2020 for the pandemic at local blood servicer. She received a letter in the mail stating that she was positive for Chagas disease on screening.
- She was referred for further evaluation

National Geographic June 2019;
Illustration by Mark Burckhardt

Review of Systems

- Constitutional: no fevers, chills or rigors
- HEENT: + left eye partial blindness; no new visual disturbances, no new hearing loss
- Respiratory: No cough, sputum, hemoptysis. No new or worsening shortness of breath
- Cardiovascular: No chest pain, denies syncope, denies palpitations; no extremity edema
- Gastrointestinal: + problems swallowing, + intermittent constipation; No nausea, vomiting or diarrhea
- Genitourinary: No dysuria or hematuria
- Musculoskeletal: No new or worsening neck pain or back pain
- Neurologic: No headaches, denies seizures; denies focal deficits
- Hematologic: No easy bruising or bleeding
- Endocrine: No night sweats or acute weight loss
- Integumentary: No new rashes, denies jaundice

Dysphagia

- Reports history of dysphagia for the past 5+ years that's been slowly progressively worsening.
- Describes transient "choking" sensation with solids/liquids.
- Was initially treated with PPI without improvement and then switched to gabapentin with some improvement in symptoms.
- Has seen several providers for this problem but diagnosis is unclear
- Never required an EGD for food bolus removal.

Constipation

- Reports chronic constipation issues since childhood and young adult.
- Intermittent in nature - sometimes has normal frequency but will go 5+ days in between bowel movements.
- No melena, hematochezia, or mucus in stool.
- Over-the-county fiber and/or stool softener supplementation has not helped much.
- Reports she has a colonoscopy ~ 2 years ago that was normal.

Further History

- Patient reports when she was 10-11 years old her family went on a road trip to New Mexico and Northen Mexico in the state of Nuevo Leon and visited Monterrey. They slept outdoors and camped on the ground. She remembers being in very rural circumstances and being bitten by insects.
- On their way back to their home in Nashville, TN, the patient developed high fever and acute illness that led to her being admitted to Vanderbilt Hospital for several weeks. She recalls having a swelling of her face and particularly around one eye. She was never given a diagnosis that she recalls and got better.

Monterrey, Mexico

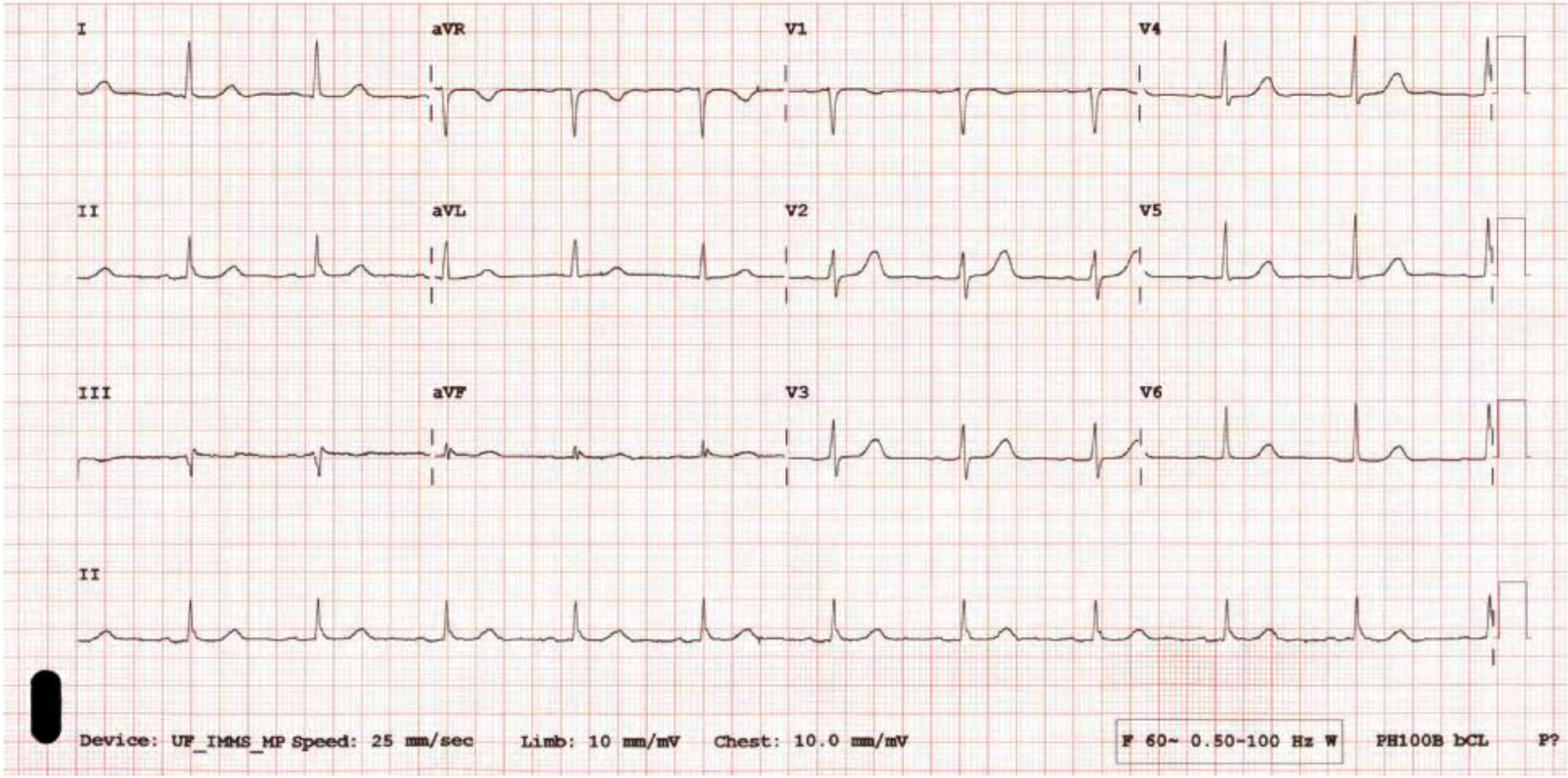
Epidemiological history

- She was born in Nashville, TN; lived in suburban neighborhoods; no exposure to rural lifestyle
- No other travel to Latin America since that childhood trip
- Her mother was born in Tennessee
- Patient denies having ever received blood transfusion and she is not an organ donor recipient.
- Patient does not camp outdoors, even in teenage years or in adulthood; no exotic foods; no organic foods and no raw meat or game meat
- She has never seen a kissing bug in her home; She moved to Florida in her 50s
- She has one Daughter in her 40s who lives in Tennessee and healthy.
- Her only Grandson was born premature and required NICU admission for 6 weeks. He currently has "enlarged GI tract" but she does not know the details.

Physical exam

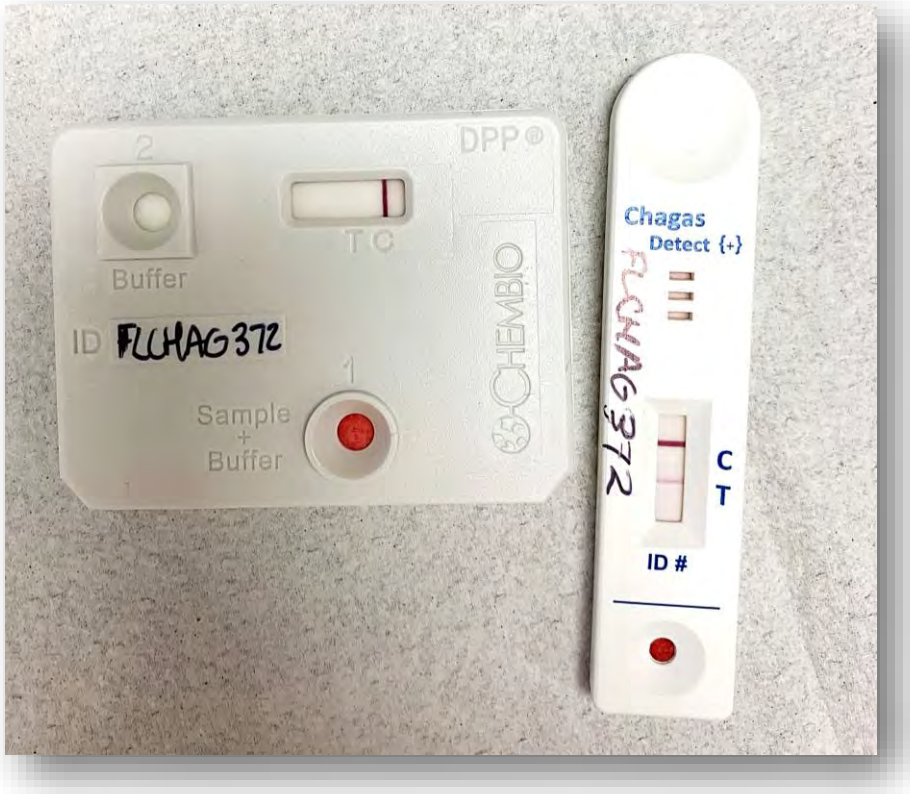
- Vitals: BP 146/75 | Pulse 70 | Temp 36.5 °C (97.7 °F) | RR 16 | Body mass index is 26.26 kg/m².
- Constitutional: no distress; comfortable
- Eyes: extra-ocular muscles intact, anicteric, and lids/conjunctiva normal.
- Ears/Nose/Mouth/Throat: no oral ulcers and no thrush; supple neck
- Cardiovascular: normal s1, s2 , no murmurs and no lower extremity edema
- Respiratory: no rales or crackles at bases, no wheezing and no accessory muscle use
- Gastrointestinal: non-tender throughout, non-distended and soft
- Musculoskeletal: no joint swelling or erythema
- Lymphatic/ Hem: no ecchymoses no petechiae, no lymphangitis
- Psych: mood ok, non-anxious and oriented
- Skin: no rashes or cutaneous lesions, warm and dry in all 4 extremities

ECG done in clinic: No major abnormalities; PR interval 188;
R-wave early transition



Summary of Case # 2

- 67yo F PMH Breast cancer treated with surgery and aromatase inhibitor
- Chronic worsening dysphagia for past 5+ years and mild constipation
- H/O unusual childhood illness after camping in a rural setting in northern Mexico
- No other international travel and no known exposure to triatomines in United States
- Screening tests for Chagas are positive via blood donation



- Rapid Chagas LFA testing is positive in the clinic
- Chembio test was positive (reading was 20) but line is not visible
- Commercial *T. cruzi* IgG positive (Hemagen IgG)
- Confirmatory testing at CDC positive (Wiener v.3.0 and TESA Blot)

Given this patient's presentation and confirmed Chagas what further testing would you get?

A. Transthoracic echocardiogram

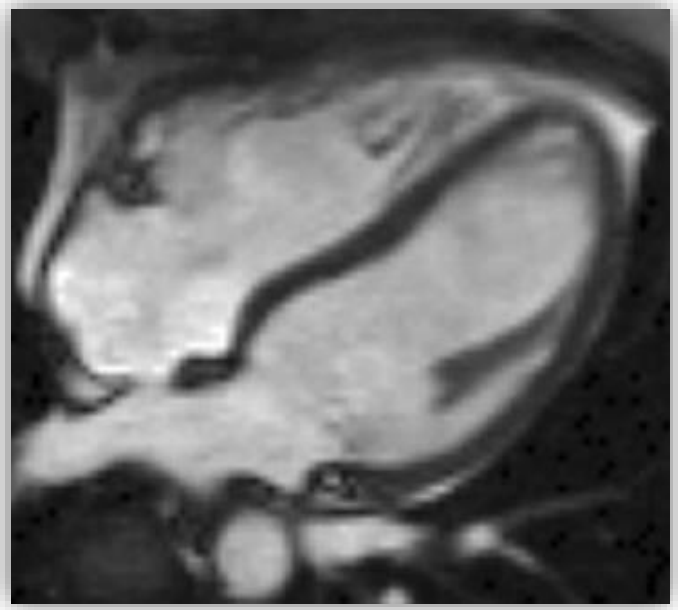
B. Cardiac MRI

C. Esophagogastroduodenoscopy (EGD)

D. Esophageal manometry

E. Colonoscopy

Cardiac studies



Transthoracic echocardiogram

- Normal LV size and systolic function.
- Ejection fraction 60%.
- No regional wall motion abnormalities.
- No left ventricular hypertrophy. No apical aneurysm
- Normal right ventricular size and systolic function.
- No significant valvular disease.
- Insufficient tricuspid regurgitation to estimate PA pressure.

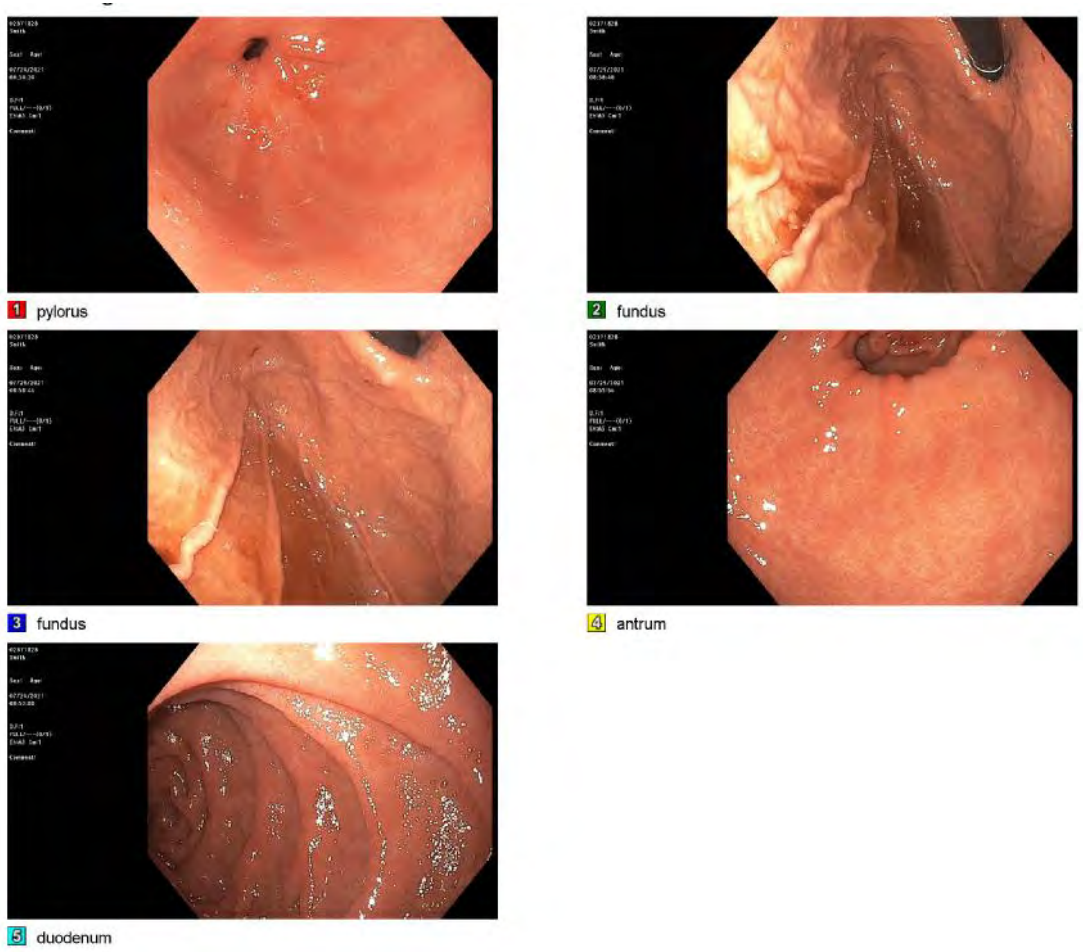
Cardiac MRI

- Normal LV and RV size and systolic function.
- No pathologic enhancement.
- Normal T1 and T2 maps.
- No evidence of infiltrative cardiomyopathy

Gastrointestinal studies

Endoscopy with biopsy

- Larynx and hypopharynx normal.
- Normal mucosa in the entire esophagus.
- Entire stomach and duodenum was normal appearing
- Duodenum, biopsy: Duodenal mucosa with intact villous architecture and no significant histologic abnormality.
- Stomach, biopsy: Antral and oxyntic mucosa with mild chronic inactive gastritis. Immunostain negative *Helicobacter pylori*
- Esophagus, distal, biopsy: Squamous mucosa with no significant histologic abnormality.
- Esophagus, proximal, biopsy: Squamous epithelium with mild reactive features.



Does this patient need esophageal manometry?

A. Yes – possible esophageal Chagas involvement; EGD can be normal

B. No – need ENT follow-up for dysphagia likely oropharyngeal esophageal disorder

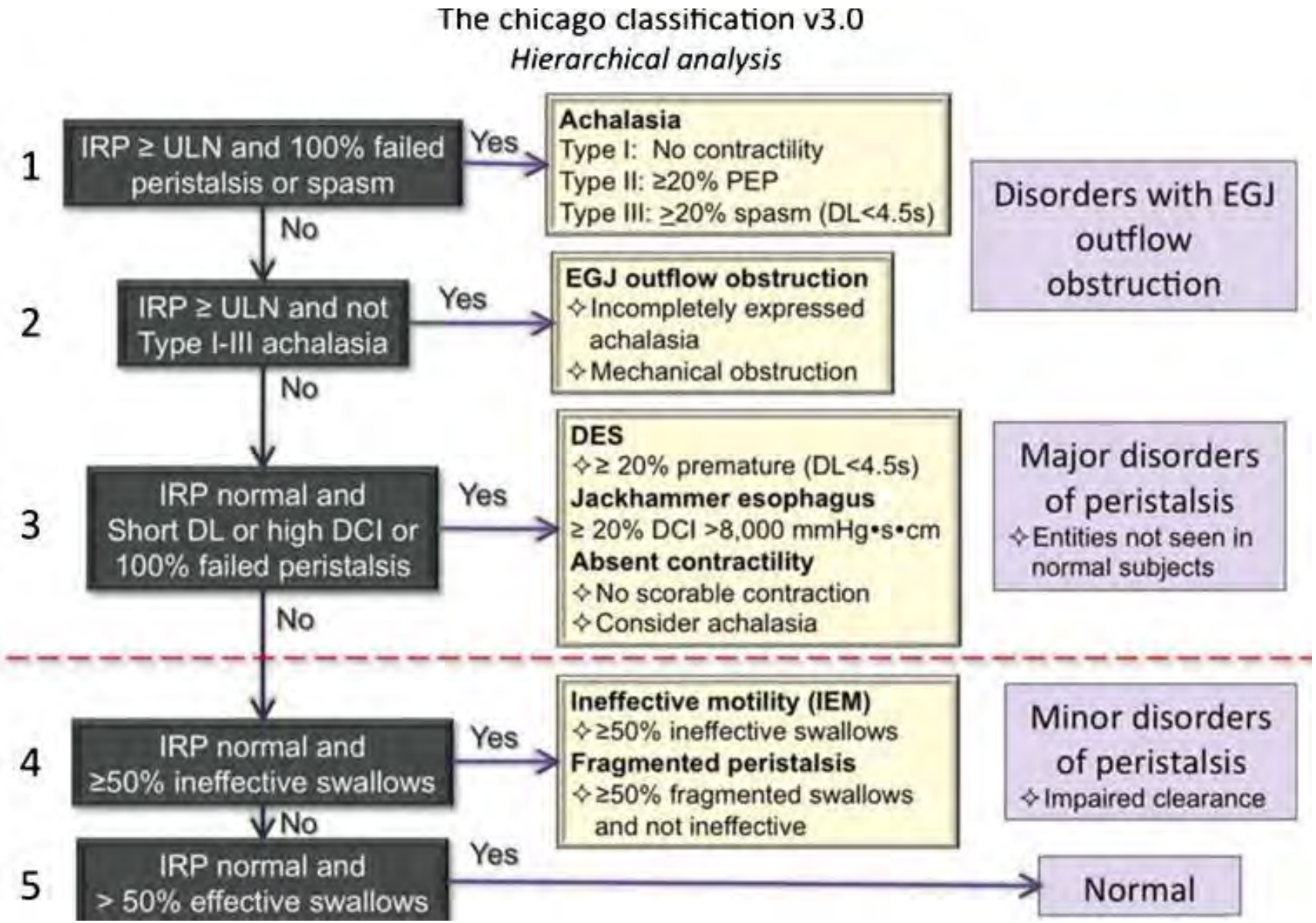
C. Unsure

Esophageal manometry

- EGJ: Normal Relaxation. Median Integrated Relaxation pressure (IRP) was 12.6 mmHg (Normal <15 mmHg)
- Esophageal body: Intact swallows (10%),failed 60%, weak 30%
- Impedance analysis: Incomplete bolus clearance in 60% of the swallows
- UES: Normal tone, relaxation and residual pressures
- Multiple rapid swallows: Good peristaltic reserve
- Rapid drink challenge: Complete EGJ/deglutitive inhibition.

Impression from esophageal dysmotility specialist:

- This is an ineffective esophageal body motility disorder (based on the Chicago classification for high resolution esophageal manometry,).
- 90% of the swallows were ineffective. This is a minor Motility disorder.
- Some swallows showed esophageal pressurization and retrograde peristalsis. Which could represent early autonomic disruption by Chagas disease which could evolve to an achalasia phenotype.



Curr Gastroenterol Rep. 2017; 19(8): 37.

Assessment and Plan for case # 2

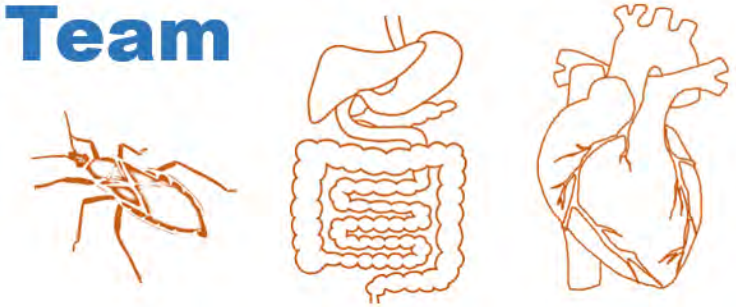
Probable esophageal dysmotility disorder due to chronic Chagas disease

- Scheduled for Timed Barium Emptying Study (TBES)
- Clinical monitoring for now; patient will keep a food diary and avoiding dense foods like steak and other meats (avoid impaction)
- GI considering esophageal electrical stimulation therapy in future
- Will consider antiparasitic treatment; discussing in the clinic on future visit

Summary

- Rapid diagnostic tests for Chagas disease screening can play an important role in certain clinical scenarios – primary care, mobile clinics, and low-resource settings.
- Tests are simple and results are available in minutes; large numbers of people can be screened easily for a low cost compared to other methods.
- Sensitivity is greater than 90% in majority of studies but more research is needed; especially among field studies using fingerstick blood samples and diverse populations at-risk for Chagas from different genetic backgrounds and regions.
- Some challenges exist with subjectivity of interpreting the results and false positive likely due to cross-reactivity with other pathogens.
- When screening for Chagas disease a plan for those who are positive is needed which includes linkage to care provider, further confirmatory Chagas testing and other testing needed for clinical work-up, and access to potential treatment.

Chagas Research Team



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Drugs for Neglected Diseases *initiative*

Thanks to all my collaborators!