Royal Tropical Institute

Chagas' disease: Challenges in Diagnostics

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Aims of KIT Biomedical Research

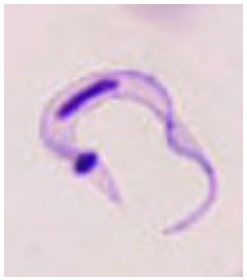
To contribute to the health of people living in low and middle income countries:

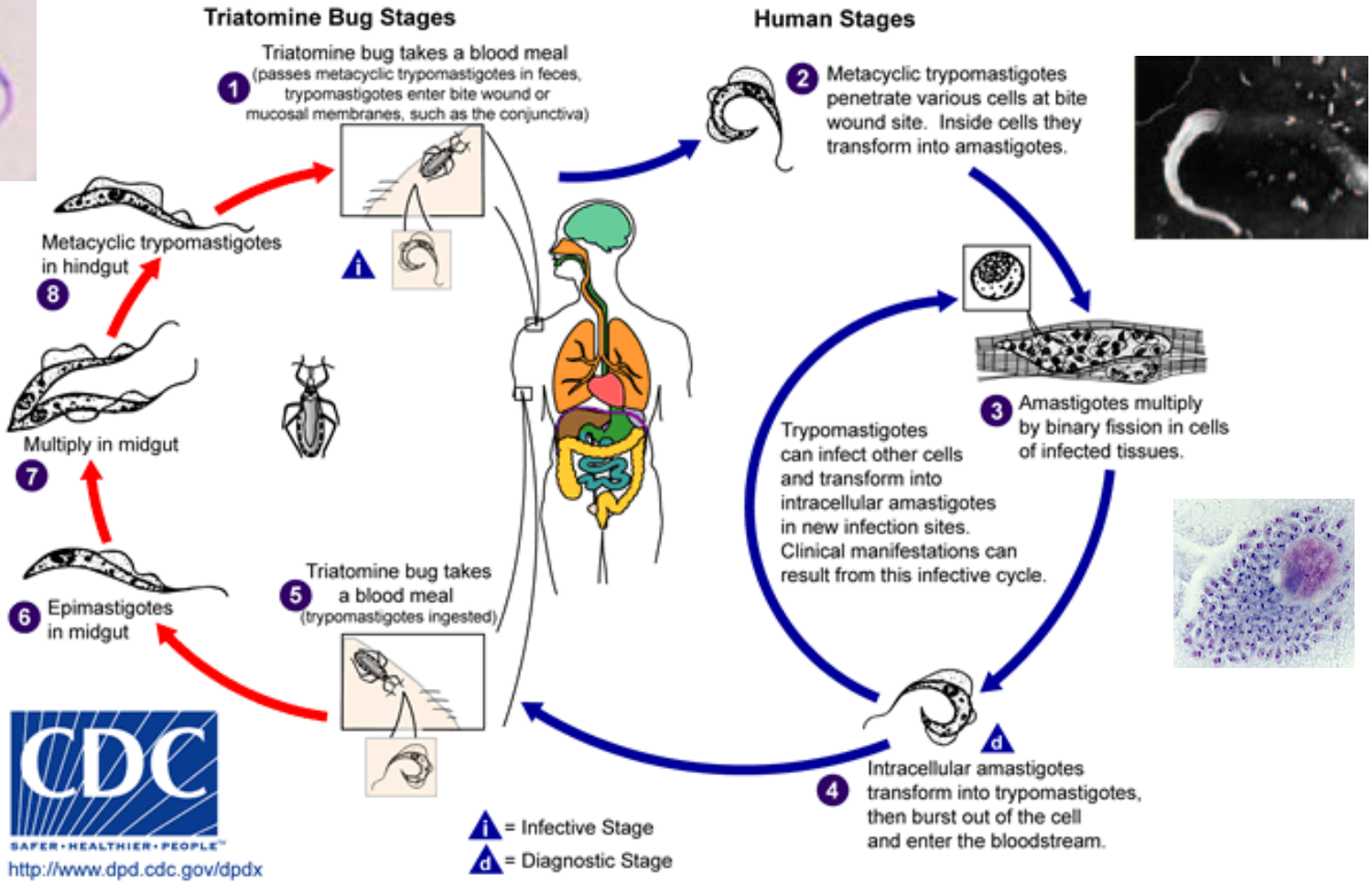
To contribute to infection control by developing and making available simple and robust diagnostic tests and methods

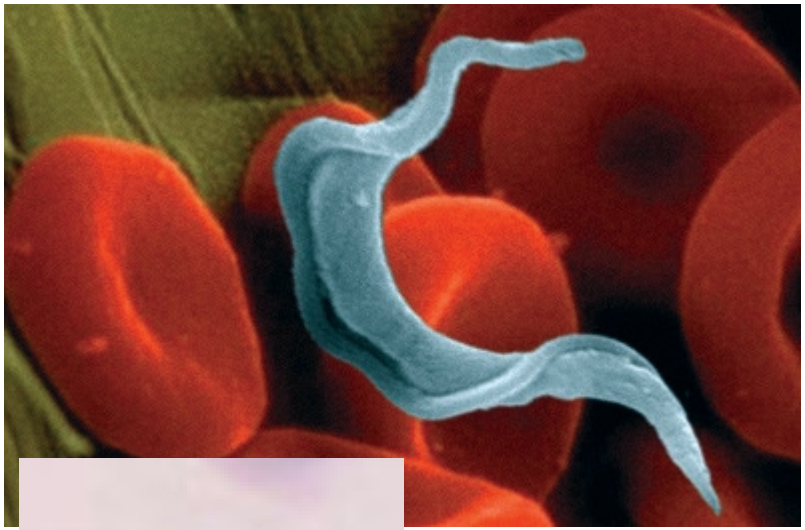
To evaluate and implement new treatment regimes

To contribute to capacity strengthening and policy development

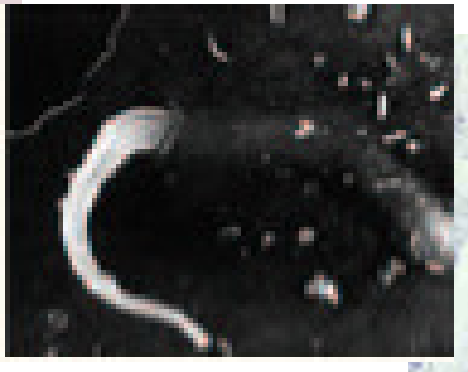
To raise awareness and disseminate information within developed and developing countries

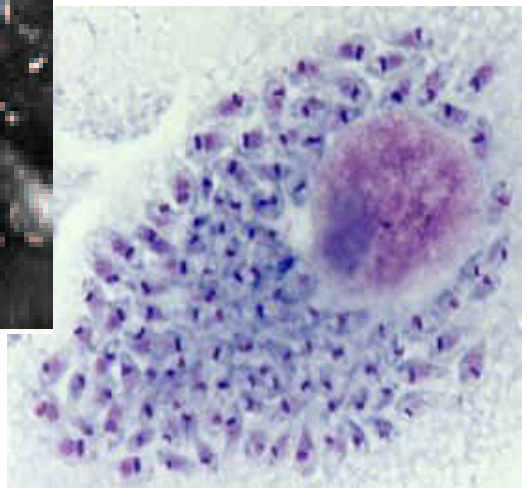


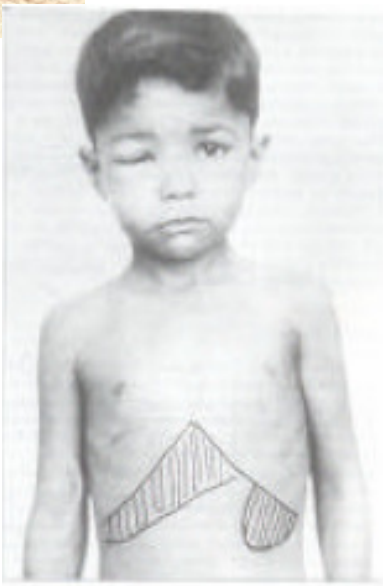

Triatomine Bug Stages
Human Stages
Triatomine bug takes a blood meal
(passes metacyclic trypomastigotes in feces, trypomastigotes enter bite wound or mucosal membranes, such as the conjunctiva)
1
2
Metacyclic trypomastigotes penetrate various cells at bite wound site. Inside cells they transform into amastigotes.
Metacyclic trypomastigotes in hindgut
8
Multiply in midgut
7
3
Amastigotes multiply by binary fission in cells of infected tissues.
Trypomastigotes can infect other cells and transform into intracellular amastigotes in new infection sites. Clinical manifestations can result from this infective cycle.
Triatomine bug takes a blood meal
(trypomastigotes ingested)
5
6
Epimastigotes in midgut
4
Intracellular amastigotes transform into trypomastigotes, then burst out of the cell and enter the bloodstream.
i = Infective Stage
d = Diagnostic Stage
CDC
SAFER·HEALTHIER·PEOPLE™
http://www.dpd.cdc.gov/dpdx



Triatoma infestans







www.DNDi.org

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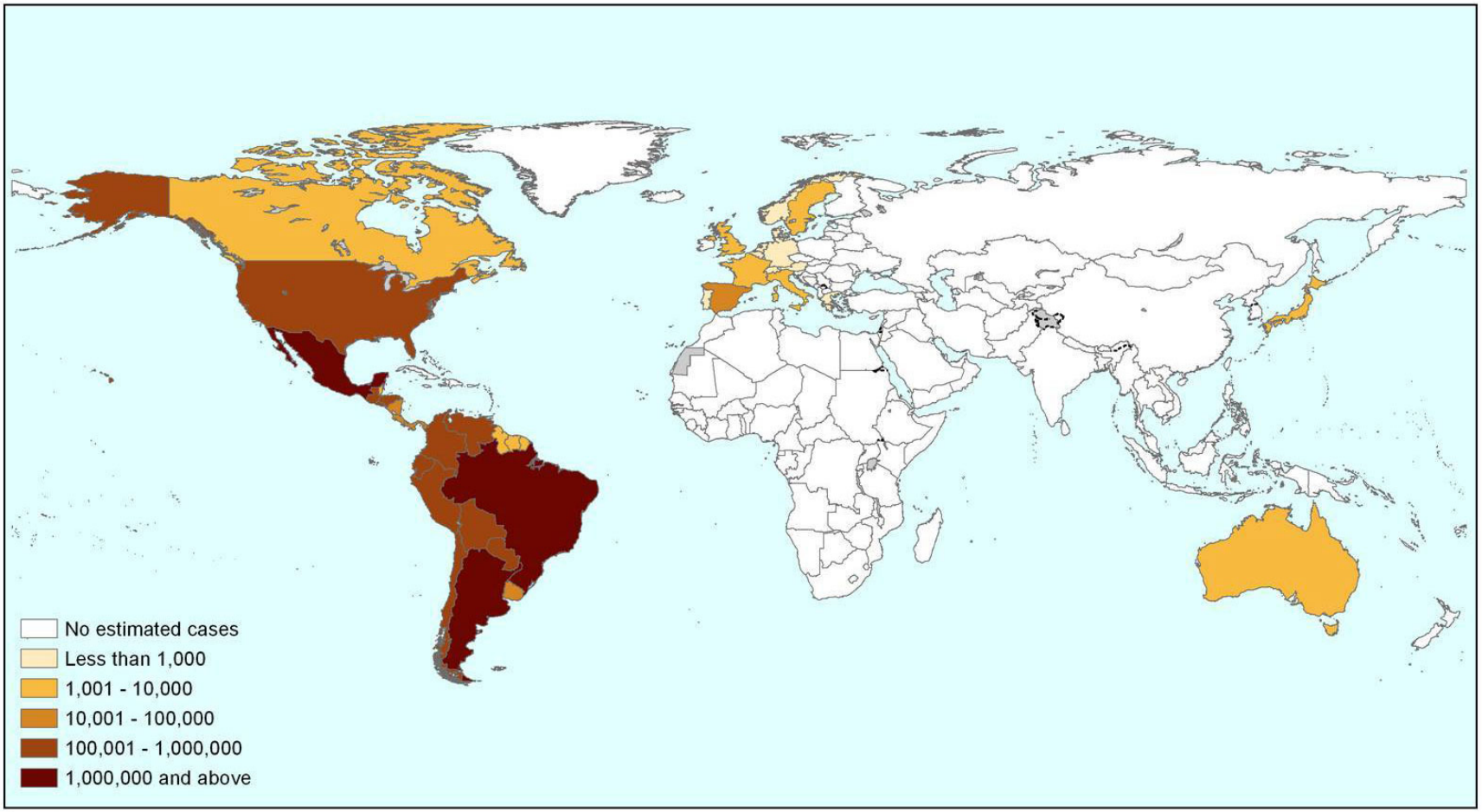
We will update this map regurlarly (version: June 2009)

Estimated global population infected by *Trypanosoma cruzi*, 2009



Sources:
1. OPS/HDM/CD/425-06 Estimación cuantitativa de la enfermedad de Chagas en las Américas.
2. Guerri-Guttenberg RA, Grana D.R., Giuseppe Ambrosio, Milei.J. Chagasic cardiomyopathy: Europe is not spared! European Heart Journal (2008); 29: 2587-2591.
3. Schmunis. G. A. Epidemiology of Chagas Disease in non-endemic countries: the role of international migration. Mem Inst Oswaldo Cruz, Rio de Janeiro, Vol. 102(Suppl. I): 75-85, 2007.
4. De Ayala A.P, Pérez-Molina J.A, Norman F., and López-Vélez R.Chagasic cardiomyopathy in inmigrants from Latin America to Spain. Emerging Infectious Disease Volume 15, Number 4–April 2009.
5. According to the numbers of inmigrants registered for 2007in the website of the Japanese Ministry of Justice and estimated seroprevalence for non endemic countries according to Paricio-Talayero J.M. Vigilancia epidemiológica de la transmisión vertical de la enfermedad de Chagas en tres maternidades de la Comunidad Valenciana. Enferm Infecc Microbiol Clin 2008;26(10):609-13.

Challenges in Diagnosis

• Currently estimates of Chagas patients are between 8-14 million people. Important to have a better estimate of the number of people infected with Chagas' disease.

• Crucial for the blood banks and screening programs to have access to accurate diagnostics that can be used in the field, remote situations – simple, quick tools

• Easy read out systems for remote areas

• Diagnostics than can detect infection in chronic asymptomatic stage of the disease and identify all sub-lineages of *T. cruzi* at equal sensitivity– very low parasitaemia

• Diagnostics that can detect active infection – used for treatment outcome and "test of cure"

• Standardisation and quality control for diagnostics

Serological testing:

Rapid diagnostic test: STATPAK: serological test. Detection of antibodies to *T. cruzi*

Used as screening tool by MSF and control programs in the field

<2$ per test, ~93 - 98% sensitive & 97% specific Chippaux et al.(2009)
– Test time 10 minutes

• Conclusive diagnosis may need multiple serological tests (WHO)
• Call for more RDTs with higher sensitivity and specificity (Roddy *et al.* 2008 MSF)

www.chembiodiagnostics.com/products.html

Serological testing:

ELISA (test used in the film)
Immunosorbent and recombinant
HAI: indirect hemaglutination
IFA: indirect immunofluorescence
Test time 2-3 hours, requires skilled technicians and specialised equipment, not available at the point-of-care.

Triatoma infestans

However, serology can cross react with other disease, *T. rangeli* and Leishmania especially – 2 tests needed (WHO)

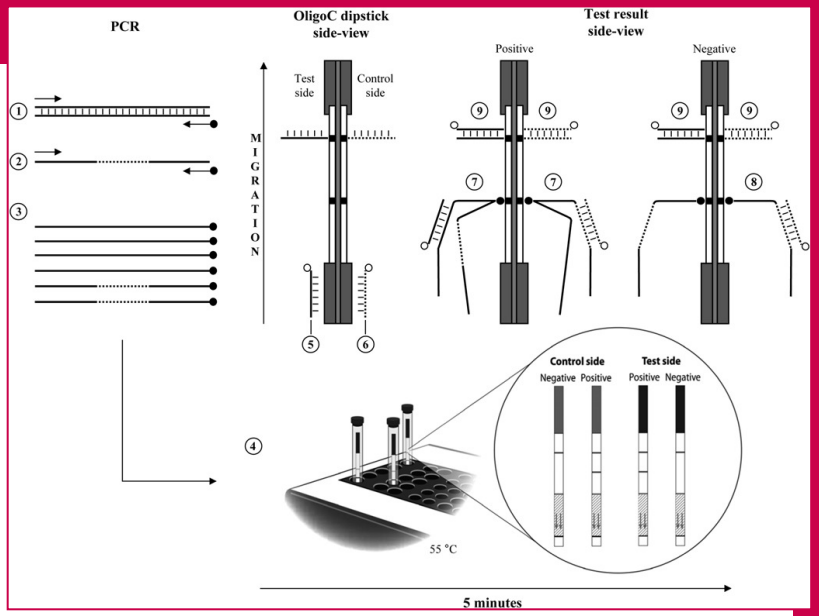
Xeno-diagnostics

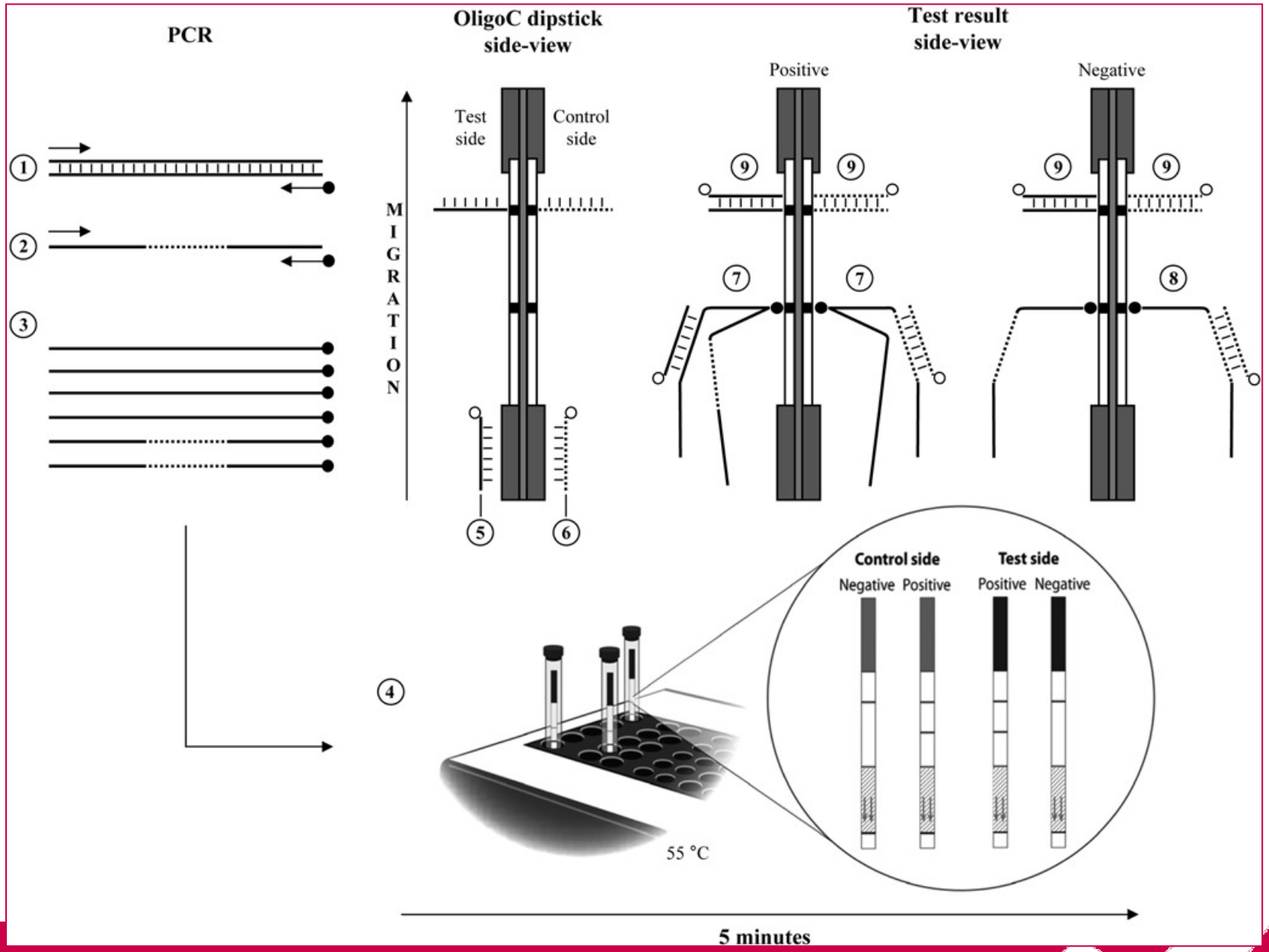
Cages of nymphs (approx 10 per cage) feed on patient
Intestines dissected out, light microscopy used to detect parasites
Test time up to 90 days

Molecular diagnostics

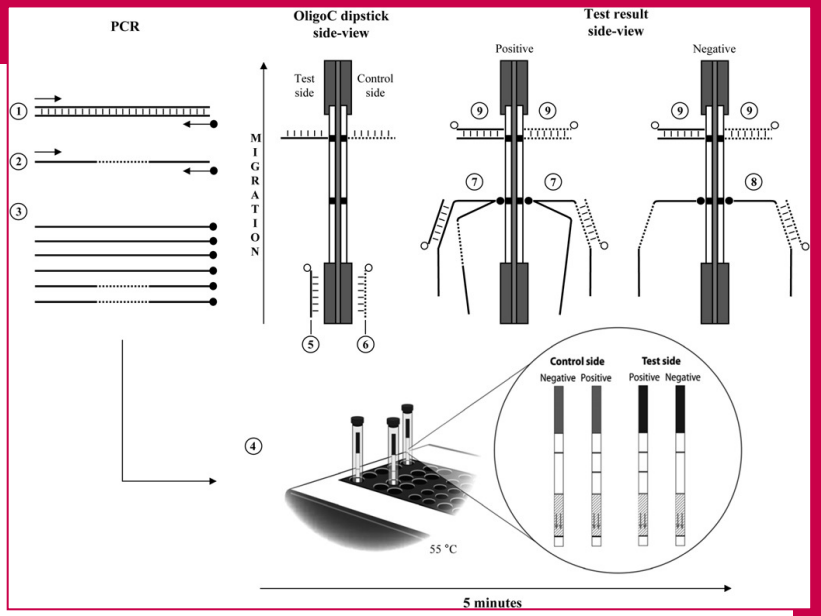
- PCR

PCR – exponential amplification of DNA from single strand of DNA
Sensitive and specific
Many PCR strategies, “in-house primer sets”
629 articles
Deborggraeve *et al.* 2009 developed easy read out PCR oligo-chromatography dipstick in standardised assay.




PCR
OligoC dipstick side-view
Test result side-view
Positive
Negative
Test side
Control side
MIGRATION
1
2
3
4
5
6
7
8
9
Control side
Test side
Negative Positive
Positive Negative
55 °C
5 minutes

Molecular diagnostics



- PCR

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- Quantitative PCR

Several rt-PCRs developed, quantify parasitaemia
Test for treatment outcome
Duffy *et al.* 2009 recently recommended an RT-PCR strategy for monitoring clinical reactivation and treatment outcome for Chagas' patients: closed tube, standardised commercial kit. Follow up of patients is possible

All of these methods are expensive, specialised equipment, staff & DNA extraction.

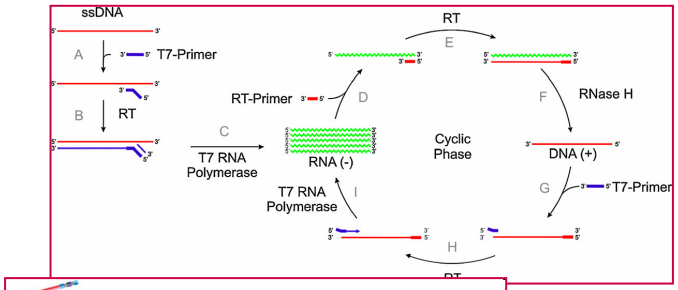
Isothermal reactions

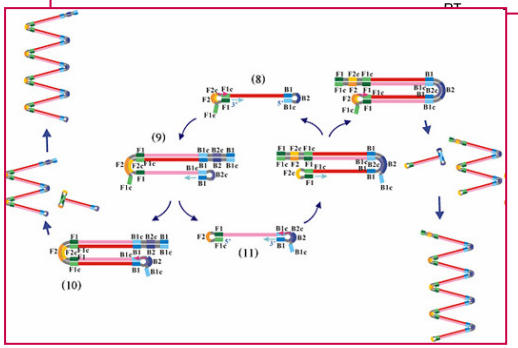
Isothermal reactions operate at one temperature and, therefore, do not require PCR machine – can be used in more remote settings
As sensitive and specific as PCR

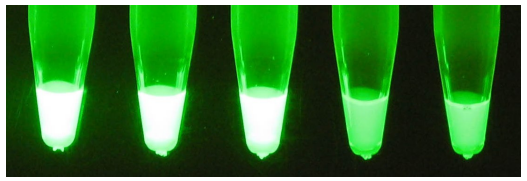
NASBA
Nucleic-Acid sequence based amplification
RNA amplification, - 41°Test of cure

LAMP – Loop-mediated Isothermal amplification
60-65°for 40-60 minutes.
Complex design of primers

RPA
Re-combinase polymerase activity
~40°60 minutes stable, dipsticks developed







Bohmer et al 2009; Adams et al. 2009 in submission; Piepenburg et al. 2006

Does this affect the Netherlands?

Screening programs

Contamination of blood supplies in Latin America, the US and Spain has lead to testing of blood donors.
Many countries in Latin America screen with two serological tests for antigens to Chagas from blood donors.
FDA (US) released guidance in March 2009 using ELISA testing on all new blood donors.
In the film the public health workers had no clue what Chagas was

Few reports in the Netherlands (Marcu *et al.* 2007)
No screening in place, so can not say if there are more people infected.

Some case reports in Suriname (Oostburg *et al.* 2003)

What happens if we/ USA / Spain/ Latin America screen?

What happens when patients are positive? No effective treatments, drugs may not be licensed

Should pregnant women previously living in endemic areas of Latin America be screened to prevent vertical transmission?

Recent study in Switzerland revealed out of 72 pregnant women from Latin America 9.7% infected with Chagas – now screen all pregnant women from Mexico, Central and South America (Jackson *et al.* 2009, PloS NTD)

Similar prevalence found in Spain 4.3% (Arandes *et al.* 2009), however no control program yet initiated.

Drugs are more effective in the acute stage – so early diagnosis best

Should the Netherlands screen pregnant women from endemic areas?

Challenges in Diagnostics

Serological tests available:

Need quality control, comparison tests – very useful at point of care

Molecular tests also available:

Offer sensitive, specific diagnostics, can be standardised

Test of cure can be developed - Specialised staff, expense of equipment

Isothermal reactions:

Not yet available, a good alternative?

Discussion points…

Can we contribute to Chagas' diagnostics with expertise in this room?

Should the Netherlands screen for Chagas'?

Should pregnant women from endemic areas be screened?