Nanotechnology and mHealth to reach the smallest and the furthest

By

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Disclosure

• Disclose no financial benefits/gain

• Some of the discussed data are applied for Gates’ foundation grant

• Disclose no off label drug to be discussed
Outlines

• Rapid Bloodless P. Vivax Colorimetric Detection using Gold Nanoparticles

• Toxoplasma Diagnosis by Detecting Antigens in Urine Using Magnetic Based Antibody Nanoparticles Conjugation

• mHealth and SMS Tablet Based Software to Fight Childhood Malnutrition
The evolution!
Rapid Bloodless P. Vivax Colorimetric Detection using Gold Nanoparticles

In Collaboration with
Taryn Clark, Medical Student
Alejandro Gurbillon, Medical Student
RAFAELLA NAVARRO, Medical Student
John Pino, Biotechnology Student
Background

• Malaria is the main infectious disease in the tropics and subtropics regions.

• It is caused by protozoan parasites of the genus Plasmodium that is transmitted by the infected Anopheles mosquitoes.

• Among the human plasmodium, P. Falciparum is the most fatal and P. Vivax is the most prevalent worldwide.

López del Prado et al. –Malaria in developing countries, J Infect Dev Ctries 2014; 8(1):001-004.
• An estimated 2.48 billion people lived at some risk of P. vivax infection in 2010

• Despite being the most prevalent, limited research has been done as the attention was drawn toward P. Falciparum

Geographic Subdivision of the Range of the Malaria Parasite Plasmodium vivax, Li J et al., Vol. 7, No. 1, January–February 2001
P. Vivax Worldwide

Malaria Atlas Project, MAP:
http://www.map.ox.ac.uk/browse-resources/transmission-limits/Pv_limits/world/2009/
• To control malaria, there is a need for rapid, accurate and easy diagnostic tools.

• While most rapid diagnostic tests (RDTs) can detect as low as 100 parasites per microliter, using nanoparticles can decrease this threshold to five parasites per microliter.

• One of the challenges in P. Vivax control is to make a diagnosis.

• It stays dormant in the liver

• Its genetic and geographic diversities add more challenges.

Hulden and Hulden, Malaria Journal 2011, 10:90, Activation of the hypnozoite: a part of Plasmodium vivax life cycle and survival
• Merozite surface protein 10 (MSP10) is used in invading erythrocytes

• It is highly specific to P. Vivax

• It has limited genetic and geographic diversity

• The colorimetric system using AuNPs have been described in the literature to diagnose various infectious and non infectious diseases

• Using blood samples was limited due to the red color of AuNPs

• Urine can offer a good alternative to detect malaria DNA

• Using urine eliminates the need for venipuncture

Urine positive or negative for *P. Vivax*

**MSP10 primer**

Denature urine at 95°C for 5 minutes to break down DS DNA then cool down the urine at RT for 10 minutes.

After adding primer and NaCl solution to the tube, denature and anneal.

**0.1 M NaCl solution**

**AuNPs 15 nm**

If DNA detected, DS DNA induce aggregation and color change.

If no DNA detected, DS will not be formed and gold nanoparticles will stay red.
Positive urine for *P. Vivax*

Negative urine
Spectrophotometer Analysis at different time points for the 3 used primers

P. Vivax Different Primers AuNPs Detection at Different Time Points

- **Primr 3 F**: C-Terminal forward primer
- **Primer 2 F**: Middle segment forward primer
- **Primer 3 R**: C-Terminal reverse primer
Conclusion

- C-Terminal forward primer (containing EGF-like domain) showed higher and earlier color changes observed by naked eye and spectrophotometer.

- Reverse primer showed a slower reaction and inconsistent results (one well changed its color while the other didn’t until 24 hours and never reached blue color).

- 610 nm wavelength showed the highest difference between aggregated vs non-aggregated AuNPs.
Future Plans

• Use C-Terminal primer that contains the most consistent part of MSP10 DNA; EGF-like domain

• Fasten the reaction to reach full color change within 20 minutes

• Investigate cross reactivity with P. Falciparum
Toxoplasma Diagnosis by Detecting Antigens in Urine Using Magnetic Based Antibody Nanoparticles Conjugation

In Collaboration with
- Francesca Schiaffino
- Alejandro Florentini
- NOELIA ANGULO
- Luz agueda perez toma
- Maritza Calderon, PhD
- Manuela Verástegui, PhD
Background

- Toxoplasmosis is caused by parasite infection: Toxoplasma gondii
- More than one third of the world population is infected with it without illness
- It causes serious diseases in newborns and immunocompromised individuals
- The detection of Toxoplasma-specific antibodies is the primary diagnostic method

• Diagnosing Toxoplasma can be challenging especially in immunocompromised and newborns due to insensitivity and poor specificity of antibodies*

• Detecting antigen in urine has been reported by foroghi et al. as early as 4 days post infection**

* Y. Sukthana et al., A promising diagnostic tool for toxoplastic encephalitis: tachyzoite/bradyzoite stage-specific RT-PCR, International Journal of Infectious Diseases 16 (2012) e279–e284

Hypothesis

• Using magnetic advantage of metallic nano/microparticles to absorb antibody on their surface to capture antigen by ELISA, we hypothesize that detecting antigen in urine might serve an earlier and more sensitive tool to diagnose Toxoplasma
Create a magnetic field

Capture antigens

Block unnecessary binding

NPs will absorb Abx

** NPs: nanoparticles, Abx: antibodies
Goals

• Provide a more sensitive diagnostic test

• Overcome the specificity issues of antibodies based diagnosis

• Diagnose Toxoplasma earlier than the reported four days post infection
Detecting Toxoplasma Antigens in Mice Urine Using Antibody-nanoparticles Sandwich ELISA
AuNPs, Rat polyclonal antibodies blocked by Gelatin

AuNPs, Rat polyclonal antibodies blocked by Goat serum

AuNPs, P30 monoclonal antibodies blocked by Gelatin

AuNPs, P30 monoclonal antibodies block by Goat serum

IOMPs, Rat polyclonal antibodies blocked by Milk 5%

OMP, P30 monoclonal Antibodies blocked by Milk 5%
Conclusions

- IOMPs showed antigen capture using both antibodies
- None of the used blocking buffers worked with AuNPs
- P30 antibodies led to AuNPs aggregation and color changes
Future Plans!

• As we showed IOMPs can capture toxoplasma antigens, we will use Iron oxide nanoparticles to increase the sensitivity (70-100 nm in size)

• Test mice urine from day 1 post infection to show an earlier detection than what is known in the literature (day 4).
mHealth and SMS Tablet Based Software to Fight Childhood Malnutrition

In Collaboration with:
Jackie Mountcastle, MPH Candidate of 2015
Malnutrition & Child Growth

• Malnutrition threatens proper growth and cognitive development for children under 5 years of age

• Globally:
  - 24 developing countries still have wasting rates of over 10%
  - 24.5% of children under 5 years old were considered stunted, indicating an urgent global problem.

ENDES 2014

Desnutrición Crónica (OMS) en menores de 5 años

mHealth

• Implementation of mobile devices as a tool for public health initiatives

• Previous programs have been limited to transferring data directly from the field through SMS text messages

• In 2012, 98.8% of Peru’s population owned mobile phones with SMS text capabilities (UNICEF)

Goals and Objectives

- We aim to integrate a table-based software with SMS messaging to...
  - Collect anthropometric data
  - Track developmental milestones
  - Send updates to mothers on child’s development
  - Serve as an educational/training-support tool for CHWs
Collaborations!!

PRISMA

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“I am a clown and that could be a Public Health role: people smile”
Patch Adam