Poster Presentation

The American Society of Tropical Medicine and Hygiene

Advancing global health since 1903

#TropMed14

63rd Annual Meeting November 2–6, 2014 New Orleans, LA USA

Viravarn Luvira

OASIS, The Online Abstract...

www.abstractsonline.com/submit/login.asp?mkey={52AE2426-7F12-4D2B-9404-C0D0B5A8EB5A}

Search

The American Society of Tropical Medicine and Hygiene

Advancing global health since 1903

#TropMed14

63rd Annual Meeting November 2–6, 2014 New Orleans, LA USA

Welcome to the
American Society of Tropical Medicine and Hygiene 63rd Annual Meeting
Submission Site.

Click here to create a new user account.

If you are submitting for the first time, please select "Create New Account".

Please note the login information for this site is not the same as your ASTMH login.

Recommended Browsers:
For Windows users, we recommend Internet Explorer 8.0. - 9.0
For Macintosh users, we recommend Safari 3.2. - 5.0

Attention IE 8 or 9 Users:

If you are using Internet Explorer 8.0 or 9.0, you **MUST** display the website using the Compatibility View before you begin your submission.

To run Internet Explorer 8.0 in Compatibility Mode, from the Command Bar, select Tools, and then select the "Compatibility View" option. Add the checkmark to the very last box (View all sites in Compatibility View).

If you are a returning user, please enter your login and password and then click on the Continue button to continue.

Login:

Password:

Continue »

Click here if you have forgotten your password.

Poster presentation instruction

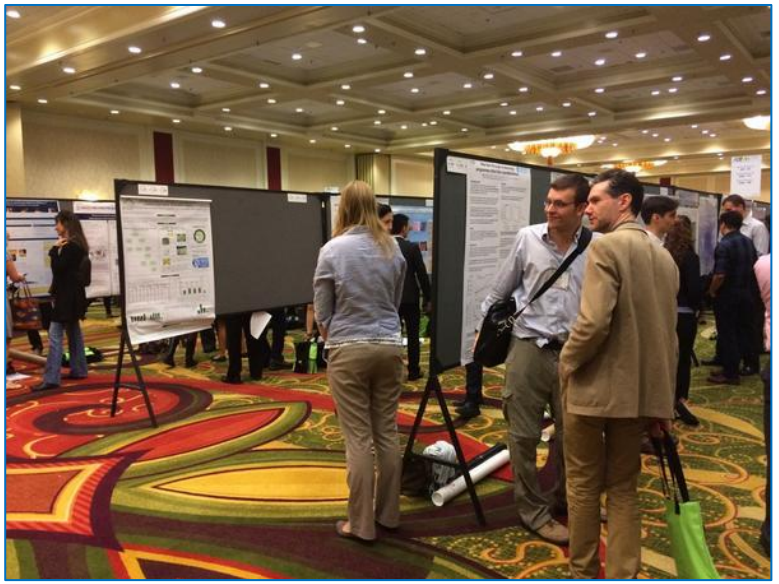
Poster Hall Location

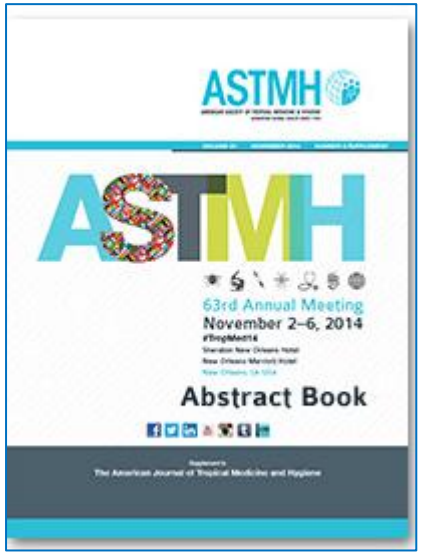
Poster presentations will take place at the New Orleans Marriott in the Grand Ballroom on the third floor.

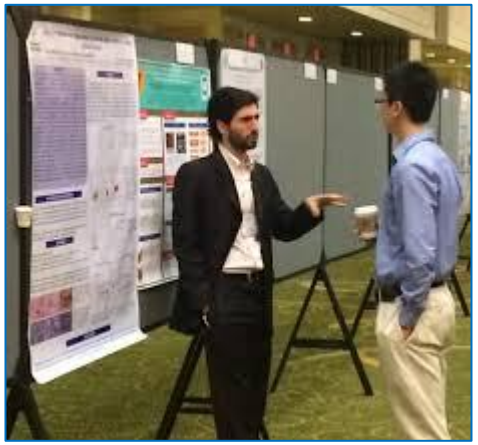
Set-Up, Viewing, Presentation and Dismantle Schedule

	Poster Session A Monday, November 3 (Presentation #69 – 574 and Late Breakers)	**Poster Session B Tuesday, November 4 (Presentation #719 – 1171 and Late Breakers)**	**Poster Session C Wednesday, November 5 (Presentation #1291 – 1795 and Late Breakers)**
Set-Up	9:45 a.m. – 10:15 a.m.	9:45 a.m. – 10:15 a.m.	9:45 a.m. – 10:15 a.m.
Morning Viewing	10:15 a.m. – Noon	10:15 a.m. – Noon	10:15 a.m. – Noon
Presentations (Presenters in attendance)	Noon – 1:45 p.m.	Noon – 1:45 p.m.	Noon – 1:45 p.m.
Afternoon Viewing	1:45 p.m. – 7 p.m.	1:45 p.m. – 7 p.m.	1:45 p.m. – 7 p.m.
Dismantle	7 p.m. – 8 p.m.	7 p.m. – 8 p.m.	7 p.m. – 8 p.m.

> Poster sessions were unique; great diversity of research with a very high standard of scientific content.







ASTMH

Malaria Monday A - 268 | Malaria Tuesday B - 916 | Malaria Wednesday C - 1486

268 - Persistence of *P. falciparum* diagnostic antigens after treatment with artemisinins: association with parasite stage and mechanism of clearance

Elizabeth A Ashley[1,2,3], Kasia Stepniewska[2,3], Carole Fogg[4], Marion Barends[1], Roger Twesigye[4], Lily Keereecharoen[1], James Kiguli[4], Carit Ier Moo[1], Carolyn Nabasumba[4], Anchalee Jaidee[1], Vincent Batwala[4], Khin Maung Lwin[1], Patrice Piola[4], Rose McGready[1,2,3], Philippe J Guerin[4], Nattwut Ekapirat[2], Kesinee Chotivanich[2], Hugh Kingston[2,5], Arjen Dondorp[2,3], Nicholas J White[2,3], François Nosten[1,2,3], Charles J Woodrow[2,3].

Background

- Antigen-based rapid diagnostic tests (RDTs) play an increasing role in achieving parasite-based diagnosis for all suspected cases of malaria
- The detection of *P. falciparum* by most brands of RDT is based on detection of PfHRP2, a histidine-rich protein expressed by parasites in high quantities
- PfHRP2 persists in the circulation for several weeks after successful treatment, confounding the diagnosis of febrile illness
- **Why does PfHRP2 persist after treatment ?**
- Previous suggestions: latent, viable parasites, gametocytes, plasma...

Hypothesis

- Early ring-stage parasites are cleared by extraction from the surrounding red cell in a process mediated by the spleen termed "pitting" (Figure 1a)
- Pitting is enhanced in patients receiving artemisinins
- We hypothesised that after pitting PfHRP2 persists in once-infected cells (Fig. 1b)
- Circulation of once-infected red cells might explain the persistence of PfHRP2
- Our central prediction was that parasite stage at the time of artemisinin treatment would be pivotal in determining the rate of pitting and persistence of PfHRP2

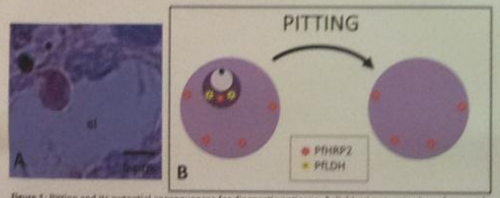


Figure 1: Pitting and its potential consequences for diagnostic antigens; **A**: light microscopic view of removal of *P. falciparum* pitting in the spleen taken from Buffet et al. 2006 (co = cord, sl = sinus lumen); **B**: illustration of theory that removal of the parasite by pitting removes purely intraparasitic proteins such as PfLDH but not exported antigens such as PfHRP2

Methods

- We followed antigen levels in 84 artemisinin-treated patients with *P. falciparum* in Uganda and Thailand in 2005 and 2006 (before artemisinin resistance emerged)
- Whole blood antigens quantified by ELISA
- Baseline staging of parasites
- Antigen clearance quantified by calculation of area-under-curve (AUC) after normalization to baseline levels
- RDT positivity and haematocrit monitored over 9-weeks of follow-up
- Ethical approval from relevant local ethics committees and OXTREC

PITTING

- Described in malaria more than 40 years ago
- The spleen removes the parasite from the red cell without its destruction
- Enhanced with artemisinins

Results

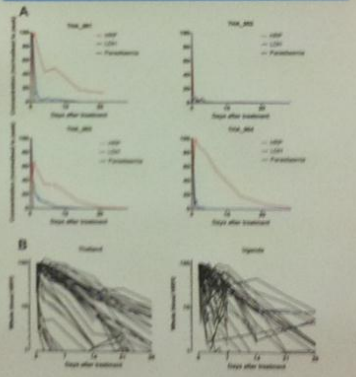


Figure 2 **A**: Example clearance profiles for parasitaemia and whole blood PfHRP2 and PfLDH from the first four Thai patients recruited; **B**: PfHRP2 clearance for all patients in each site

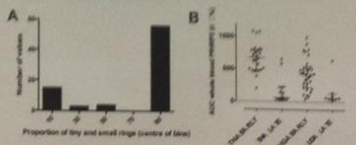


Figure 3 **A**: Histogram showing bimodal distribution of parasite stage across the pool of patients (Thailand and Uganda combined). **B**: AUCs for PfHRP2 in the two sites, further stratified by admission stage

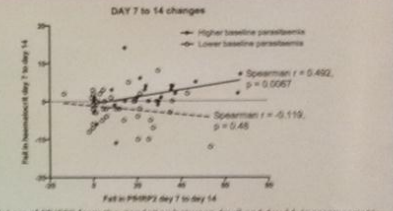


Figure 4: Loss of PfHRP2 from the circulation between day 7 and day 14 is accompanied by a fall in haematocrit even in the absence of frank haemolysis. Cases were stratified according to ranking of baseline parasitaemia

Conclusions

- In both study sites, infections with a majority of large rings or pigmented stages at baseline had lower AUCs for PfHRP2 by approximately one log-order of magnitude
- Higher falls in PfHRP2 between day 7 and day 14 were associated with reduced haematocrit over the same time period
- These findings support the hypothesis that once-infected erythrocytes are the location of persisting PfHRP2
- PfHRP2 persistence and late haemolysis are manifestations of the same process – artemisinin-induced pitting (Figure 5)

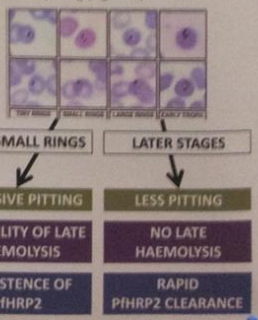


Figure 5: Effect of baseline stage on events after administration of artemisinins

Presented at ASTMH Annual Meeting November 2-6, 2014, New Orleans, USA

Monday A - 511 Do Not Remove | Tuesday B - 1126 Do Not Remove | Wednesday C - 1728 Do Not Remove

BURROUGHS WELLCOME FUND
ASTMH
Fellowship Recipient

Utility of String Test and Stool Sample for Diagnosis of Pulmonary Tuberculosis Using Gene Xpert® MTB/RIF

Andrew DiNardo, Andrew Hahn, Jacinta Leyden, Charles Stager, Edward Graviss, AnnaMandalakas*, Elizabeth Guy*

*Co--Last authors equally contributing

Baylor College of Medicine | HARRIS HEALTH SYSTEM | Texas Children's Hospital | Methodist

Abstract

- Diagnosis of pulmonary Tuberculosis (pTB) has improved with the advent of Gene Xpert® MTB/RIF assay, however challenges remain:
  - Sensitivity of smear in non-HIV patients is 50-80%
  - Sensitivity of smear in HIV patients is 40%
  - Xpert® MTB/RIF has an overall sensitivity of 88%
  - While Xpert® MTB/RIF improves diagnostic accuracy, it still fails to yield a diagnosis in 33% of adult smear-negative and 45% of pediatric culture positive cases
- We sought to evaluate the utility and feasibility of Xpert® MTB/RIF using 2 minimally-invasive clinical specimens, stool and string test
- From August 2013- March 2014, 13 participants with presumed pulmonary TB were enrolled
  - 8 of 13 participants were found to have microbiologically confirmed pulmonary TB by liquid culture
  - String test was positive in 8 of 8 (100%) of participants with microbiologically confirmed pTB
  - At least 1 of the 2 stool collection methods, when tested by Xpert® MTB/RIF detected TB in 6 of 6 culture positive participants

Objectives

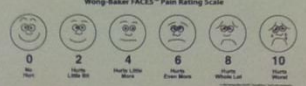
- Assess the feasibility of using string test and 2 stool methods for diagnosing pulmonary TB
- Assess the tolerability of the string test
- Compare the yield of Xpert® MTB/RIF using string and stool samples to the yield of sputum smear and culture

Materials & Methods

- Xpert® MTB/RIF is a fully automated nucleic acid amplification technology (NAAT) that detects *M. tuberculosis* and rifampicin resistance in under two hours with <5 minutes of processing time for sputum and string specimens
- August 2013- March 2014, patients from Ben Taub General Hospital (Houston, TX, USA) with presumed pulmonary TB had induced or expectorated sputum collected as routine standard of care. An additional sputum was collected for Xpert® MTB/RIF
- String test was performed first thing in the morning after nil per mouth the evening before
  - A gelatin capsule containing 140 cm of nylon string was swallowed with the trailing end taped to the cheek until it was removed 4 hours later with gentle traction
  - The string was vortexed in 2 mL of dPBS and 1 mL was used for Xpert testing and 1mL was cultured (BACTEC MGIT 960, BD Diagnostic Systems, Sparks, MD)
- Stool was collected and processed by two low-technology methods: 1) Sugar flotation and 2) MicroSense Beads ® (Microsense Medtech Ltd, London, UK)
  - Sugar flotation method: 5g of stool was emulsified in 10 mL of 50% Sheathens solution, vigorously shaken manually , filtered through funnel paper, and then allowed to settle by gravity for 40 minutes. The top 0.5 mL was then added to Xpert® MTB/RIF decontamination fluid and run on the GeneXpert® system
  - MicroSense Beads ® methods: 5g of stool was mixed with MicroSense decontamination solution, then filtered using coarse material, mixed with MicroSense Beads and washed twice with MicroSense wash solution using a magnet to retain the TB bacilli.

Sputum Smear Status	Sputum Culture	Sputum Xpert®	String Xpert®	String Culture	MicroSens eBead Xpert®	Sugar Xpert®	Stool Culture	Wong Baker Face
***MTB* CULTURE POSITIVE, SMEAR POSITIVE**								
2-4+	*Mtb*	Positive	Positive	Positive	Positive	Positive	Negative	1
4+	*Mtb*	Positive	Positive	Positive	Positive	Invalid	Positive	6
1+	*Mtb*	Positive	Positive	Negative	Negative	Positive	Negative	2
3+	*Mtb*	Positive	Positive	Positive	Positive	Invalid	Positive	2
3+	*Mtb*	Positive	Positive	Positive	Positive	Positive	Positive	2
1+	*Mtb*	Positive	Positive	Positive	Positive	Positive	Positive	2
***MTB* CULTURE POSITIVE, SMEAR NEGATIVE**								
Negative	*Mtb*	Positive	Positive	Positive	Unable to produce			4
Negative	*Mtb*	Positive	Positive	Positive	Positive	Invalid	Negative	0
***MTB* CULTURE NEGATIVE**								
1+	*M. avium*	Negative	Negative	Negative	Negative	Invalid	Positive	1
3+	*M. kansasii*	Negative	Negative	Positive	Negative	Negative	Positive	2
Negative	Negative	Unable to produce	Negative	Negative	Unable to produce			2
Negative	Negative	Negative	Negative	Negative	Unable to produce			0
Negative	Negative	Negative	Negative	Negative	Invalid	Invalid	Negative	2

Wong-Baker FACES™ Pain Rating Scale



Results

- The String test was well tolerated with a "Wong Baker Faces" median and mean score of 2 (range 0-6).
- The diagnostic accuracy of the **String test** using Xpert® MTB/RIF was excellent, with a sensitivity of 100% and specificity of 100% (n=13)
- From **stool**, *Mtb* DNA was detected in 7 of 7 participants with pulmonary TB
  - MicroSense Beads diagnosed 6 of 7 participants who had pulmonary TB.
  - The sugar floatation method diagnosed 4 of 7 participants with culture positive pulmonary TB
  - Neither stool method yielded a false positive when tested by Xpert® MTB/RIF, including the two cases of non-tuberculous mycobacteria.
- There were 7 cases of Rifampicin sensitivity and one case of resistance with 100% concordance of Xpert® MTB/RIF with liquid culture phenotypic testing

Acknowledgements

We are grateful for the technical knowledge and Xpert® MTB/RIF cartridges given by Cepheid and TB MicroSense Beads ® donated by Microsens Medtech Ltd , London, UK . We would like to acknowledge the Ben Taub General Hospital Pulmonary Laboratory for use of their isolation suite, the TAMC Houston microbiology department for use of the Gene Xpert machine and laboratory and the Houston Methodist Hospital for culturing all non-clinical specimens.

Discussion

- The study highlights the utility of gastrointestinal (GI) sources to aid in the diagnosis of pulmonary tuberculosis. GI samples allow for minimally invasive specimen collection. Species specific PCR probes decrease the odds of non-tuberculous mycobacteria resulting in false-positives.
- Stool is another non-invasive means to rapidly diagnose pulmonary TB when using highly specific technology. Improved means of separating organisms from stool roughage may improve diagnostic accuracy through decreased rates of invalid tests.
- The string test was well-tolerated, associated with only a brief gagging sensation as the string is removed. The string method performed equivalent to sputum, without requiring a trained respiratory therapist or electricity to induce sputum.
- We achieved our objective of evaluating the feasibility and tolerability of these two alternative means of diagnosing pulmonary tuberculosis using Xpert® MTB/RIF. Further evaluations of these methods should occur in studies powered to statistically compare their accuracy to the current standard of care.

Select References

1. Vargas, et al. "Diagnosis of Smear-Negative Pulmonary Tuberculosis in Lima, Peru." Lancet. 361 (2003): 1205-7.
2. M. P. Wood, et al. "Xpert MTB/RIF Testing of Stool Samples for the Diagnosis of Pulmonary Tuberculosis in Children." Clin Infect Dis. 57 (2013): e18-21.
3. K. P. Cain, et al. "An Algorithm for Tuberculosis Screening and Diagnosis in People with HIV." N Engl J Med. 362 (2010): 707-16.
4. D. Helb, et al. "Rapid Detection of Mycobacterium Tuberculosis and Rifampin Resistance by Use of on-Demand, near-Patient Technology." J Clin Microbiol. 48 (2010): 229-37.
5. Hillemann, et al. "Xpert Molecular Detection of Extrapulmonary Tuberculosis by the Automated Genexpert Microbiology System." J Clin Microbiol. 49 (2011): 1202-5.
6. F. Mesquita, et al. "Point-of-Acid-Fast Smear and Mycobacterial Culture for Tuberculosis Diagnosis in People with Human Immunodeficiency Virus." Am J Respir Crit Care Med. 187 (2013): 703-14.
7. M. Ismail, and M. Pai. "Tuberculosis Diagnosis: Time for a Game Change." N Engl J Med. 363 (2010): 1070-1.
8. K. R. Steingart, et al. "Xpert(R) MTB/RIF Assay for Pulmonary Tuberculosis and Rifampicin Resistance in Adults." Cochrane Database Syst Rev. 1 (2013): CD009593.
9. B. Tiwari, et al. "Can a Simple Floatation Method Lower the Limit of Detection of Mycobacterium Tuberculosis in Extrapulmonary Samples Analyzed by the Genexpert MTB/RIF Assay?." J Clin Microbiol. 50 (2012): 2774-6.


1120
Mahidol University
Factors associated with failure in smear positive pulmonary tuberculosis: using symptoms plus sputum smear and chest radiography
Introduction :
Method :
Results :
1121
Prevalence of Latent Tuberculosis Infection a
Factors in an Urban African Setting

Thank you


WELCOME
TO
OLD ALGIERS
FOUNDED 1719
New Orleans' Best Kept Secret