Travel medicine - Laboratory diagnosis of acute tropical infections

Assoc. Prof. Stuart Blacksell MPH PhD
Mahidol-Oxford Tropical Medicine Tropical Medicine Research Unit (MORU)
Bangkok, Thailand
University of Oxford, Nuffield School of Clinical Medicine, Oxford, UK
WHO dengue diagnosis expert group member

Mahidol-Oxford Tropical Medicine Research Unit (MORU) - AIM

“To develop effective, practical means of diagnosing and treating tropical infections responsible for significant morbidity and mortality in the populous rural areas of Southeast Asia and elsewhere”

MORU’s strategic position....

Half of the world’s population within 2000 miles....
Mahidol-Oxford Tropical Medical Research Unit (MORU)

- **Locations**
  - Thailand (established early 1980s)
  - Bangkok
  - Udon Thani/Ubun Ratchathani (NE)
  - Mae Sai (West)
  - Laos PDR
  - Cambodia, Sri Lanka, Indonesia
- **Diseases studied**
  - Dengue, Rickettsiosis, Malaria, Melioidosis, Leptospirosis
- **Studies**
  - Diagnosis & Treatment
  - Diagnostic development
  - Pathogenesis
  - Epidemiology
  - Molecular characterisation

The diagnostic challenges in the tropical travel medicine

- If we can diagnose we can treat or manage the patients
- Many tropical fevers have similar clinical presentation (Broad differential Dx)
- We need a diagnosis on presentation or soon after
- Most endemic settings have limited laboratory infrastructure with limited staff training
- In Western settings staff may have limited experience in travel medicine
Acute fever diagnostics

Acute tropical fever differential Dx
(Amongst others!)

- Viral
  - Dengue, Chikungunya, Influenza
- Bacterial
  - Leptospirosis, Salmonella Typhi
- Rickettsial
  - Scrub typhus, Murine typhus, Spotted fever
- Parasitic
  - Malaria

Dengue, countries or areas at risk, 2010
Summary of Dengue diagnostics

<table>
<thead>
<tr>
<th>Format</th>
<th>Assay</th>
<th>Quant?</th>
<th>Time</th>
<th>Ease</th>
<th>Setting</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serology</td>
<td>Rapid RDT</td>
<td>No</td>
<td>15 min</td>
<td>++++</td>
<td>Clinic/Hosp</td>
<td>++</td>
</tr>
<tr>
<td>Serology</td>
<td>ELISA</td>
<td>Yes</td>
<td>4 h</td>
<td>+++</td>
<td>Hospital/Ref Lab</td>
<td>++</td>
</tr>
<tr>
<td>Genetic</td>
<td>Real-time PCR</td>
<td>Yes</td>
<td>3 h</td>
<td>+++</td>
<td>Ref Lab/Hosp</td>
<td>++++</td>
</tr>
<tr>
<td>Isolation</td>
<td>Cell culture/Mosq inoculation</td>
<td>No</td>
<td>Days</td>
<td>+</td>
<td>Ref Lab</td>
<td>++++</td>
</tr>
</tbody>
</table>

Summary of Dengue diagnostics – Accuracy and timing

<table>
<thead>
<tr>
<th>Format</th>
<th>Assay</th>
<th>Target</th>
<th>Optimal timing</th>
<th>Primary/Secondary</th>
<th>Sens</th>
<th>Spec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serology</td>
<td>Rapid RDT</td>
<td>IgM</td>
<td>&gt;5 days fever</td>
<td>Yes</td>
<td>++++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NS1</td>
<td>1-5 days fever</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serology</td>
<td>ELISA</td>
<td>IgM</td>
<td>&gt;5 days fever</td>
<td>Yes</td>
<td>++++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NS1</td>
<td>1-5 days fever</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genetic</td>
<td>Real-time PCR</td>
<td>RNA</td>
<td>1-5 days fever</td>
<td>No</td>
<td>+++</td>
<td>++++</td>
</tr>
<tr>
<td>Isolation</td>
<td>Isolation</td>
<td>Virus</td>
<td>1-5 days fever</td>
<td>No</td>
<td>++</td>
<td>++++</td>
</tr>
</tbody>
</table>

Always consider timing of the sample!
Dynamics of Dengue Infection

**Primary dengue infection**
- Longer NS1 window
- Strong IgM response
- Later IgG response
- Viraemia persists for 6 months

**Secondary dengue infection**
- Shorter NS1 window
- Weak IgM response
- Early strong IgG response
- Anti-NS1 Ab
Dengue/acute fever POCT/ELISA

Dengue rapid test evaluations
- SD Dengue Duo rapid test (NS1/IgM/IgG) - Second generation
- Biorad NS1 strip
- Merlin (IgM/IgG)
- Immunoquick (IgM/IgG)
- Panbio rapid test (NS1)
- Panbio rapid test (IgM)

Sri Lanka cohort
- Location: Ragama, Sri Lanka
- 550 adult (>16 yo) fever patients recruited
  - Acute (median days of fever: 5 (IQR:3-8))
  - Conv (median days of fever: 24 (IQR:19-30))
- Disease
  - 24.5% acute dengue
    - DEN3 (67%); DEN2 (28%); DEN4 (3%); DEN1 (1%)
  - 19% Primary, 81% Secondary
  - 17% acute chikungunya, 7% leptospirosis
  - 4% bacteremia, 2% Q fever
Combining antigen (NS1) and antibody (IgM)

Need to combine NS1 and IgM results

Effect of time on rapid test positivity
Differentiating primary/secondary

Interpretation

| IgM-/IgG- | Primary |
| IgM+/IgG+ | Secondary |

Dengue Dx – Conclusion

- Use ELISA or RDT
  - NS1 antigen
  - IgM antibody
  - Combine NS1/IgM results
- Admission Dx
  - Sensitivity 90%
  - Specificity 90%

Rickettsia

Antigenic groups and species

- Scrub Typhus Group
  - Orientia tsutsugamushi (Scrub typhus, Japanese flood fever)
- Typhus Group
  - Rickettsia prowazekii (Epidemic typhus)
  - Rickettsia typhi (Marine typhus)
- Spotted Fever Group (some examples)
  - Rickettsia rickettsii (Rocky Mountain spotted fever)
  - Rickettsia japonica (Oriental spotted fever)
  - Rickettsia akari (Queensland tick typhus)
  - Rickettsia conori (Mediterranean spotted fever)
  - Rickettsia honei (Flinders Island spotted fever)
  - Rickettsia slovaca (Rickettsia poincarei)
  - Rickettsia sibirica (Siberian tick typhus)
  - Rickettsia felis (Cat flea fever)
  - Rickettsia helvetica
O. tsusugamushi and scrub typhus

Genus Orientia: Family Rickettsiales
Obligate intracellular bacterium

<table>
<thead>
<tr>
<th>Scrub typhus</th>
<th>Clinical complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute fever</td>
<td>Pneumonitis</td>
</tr>
<tr>
<td>Eschar 40-60%</td>
<td>Acute respiratory distress syndrome</td>
</tr>
<tr>
<td>Regional lymphadenopathy</td>
<td>Meningo-encephalitis</td>
</tr>
<tr>
<td>Maculo-papular rash</td>
<td>Acute renal failure</td>
</tr>
<tr>
<td></td>
<td>Severe hepatitis</td>
</tr>
</tbody>
</table>

Scrub typhus burden and appropriate diagnostics

- **Global disease burden caused by scrub typhus infection**
  - 771 billion at risk / 1 million cases annually (1%)??
  - Most prevalent yet neglected treatable infectious disease
  - Studies confirm scrub typhus causes 10-25% of non-malaria fevers in rural Asia.

- **Scrub typhus endemic in rural areas**
  - Developing and newly-industrialized countries
  - Tropics, sub-tropics and temperate
  - Dubai/Nepal-Japan

- **Diagnoses that are accurate, simple and cheap**
  - Low resource settings, rural clinics
  - Dx at acute presentation
  - Point-of-care technologies, Multi-analyte
  - Less than $1

Geographic range of Scrub typhus
O. tsutsugamushi type strains - defining variation

- Classical type strains
  - *Karp (New Guinea 1943)
  - *Kato (Japan 1955)
  - *Gilliam (Burma 1943)
  - Kawasaki (Japan 1981)
  - Boryong (Korea 1985)

- Thai type strains - 1963
  - TA763 - Karp related
  - TA686, TA716 - Kato related
  - TH1817
  - Mediated by 56 kDa antigen

* Antigenic pools for serology

Murine typhus - R. typhi

- Rickettsia typhi
- Rats are the natural reservoir (urban disease)
- Transmitted by rat fleas (Xenopsylla cheopis) via faeces
- Symptoms
  - Rash (80%)
  - Nausea & vomiting, abdominal pain
  - Confusion,思路, hallucinations (10%)
  - Acute renal failure, pneumonitis (5-10%)
  - Mortality 1-2%
- Treatment doxycycline

Distribution of R. typhi
Spotted fever group rickettsioses

- Tick-borne - except R. akari (mites) and R. felis (fleas)
- Endothelial cell infection
- Incubation 4 days - 2 weeks
- Non-specific malaise, fever
- Day 3-5 maculopapular rash
- Neurological, pulmonary and renal involvement

Distribution of Spotted Fever Rickettsia

12 tick-borne rickettsioses
12 since 1991 !!
Scrub typhus and other rickettsial diagnostics

<table>
<thead>
<tr>
<th>Format</th>
<th>Assay</th>
<th>Time</th>
<th>Ease</th>
<th>Setting</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolation</td>
<td>In vitro isolation*</td>
<td>7-14</td>
<td>+</td>
<td>Reference lab</td>
<td>+++</td>
</tr>
<tr>
<td>Serology</td>
<td>Indirect Immunofluorescence</td>
<td>5 h</td>
<td>+++</td>
<td>Ref lab/Hosp</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>Weil-Felix (WF)</td>
<td>24 h</td>
<td>+++</td>
<td>Clinic/Hosp</td>
<td>++</td>
</tr>
<tr>
<td>Serology</td>
<td>Rapid test</td>
<td>15 min</td>
<td>+++</td>
<td>Clinic/Hosp</td>
<td>+++</td>
</tr>
<tr>
<td>Serology</td>
<td>ELISA</td>
<td>4 h</td>
<td>+++</td>
<td>Ref lab</td>
<td>++</td>
</tr>
<tr>
<td>Genetic</td>
<td>Nested PCR (34 kDa)</td>
<td>8 h</td>
<td>++</td>
<td>Ref lab/Hosp</td>
<td>+++</td>
</tr>
<tr>
<td>Genetic</td>
<td>Realtime PCR (16S, 56 kDa, 47 kDa, groEL)</td>
<td>3 h</td>
<td>+++</td>
<td>Ref lab/Hosp</td>
<td>+++</td>
</tr>
<tr>
<td>Genetic</td>
<td>Loop amplification (groEL)</td>
<td>1 h</td>
<td>+++</td>
<td>Clinic/Hosp</td>
<td>+++</td>
</tr>
</tbody>
</table>


Well-Felix OX-K serodiagnostic test

- Nonspecific OX-19 and OX-2 antigens from Proteus vulgaris and OX-K (Kingsbury) from Proteus Mirabilis
- Agglutination of the OX-K 'Kingsbury' strain of Proteus vulgaris, rather than X-19
- Very insensitive (<50% on single sera)

Immunofluorescence

Seroology gold standard

- Infected cells (pooled antigens) fixed on a slide
- 4 fold rise in paired specimens is diagnostic
- <50% sensitivity with single acute samples
- False positives due to high background immunity in endemic areas
Scrub typhus rapid tests

- Panbio IgM ICT (Australia)
  - Recombinant 56 kDa Karp antigen
  - Currently out of production
- AccessBio IgM and WA ICT (USA)
  - Recombinant 56 kDa Karp, Kato, Gilliam
- Standard Diagnostics (SD) IgM ICT (Korea)
  - Recombinant 56 kDa Karp and Boryang
- LAMP

---

SCRUB TYPHUS POINT OF CARE TESTS

<table>
<thead>
<tr>
<th>Diagnostic assay</th>
<th>Compared to STIC criteria* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity</td>
</tr>
<tr>
<td>Panbio IgM ICT</td>
<td>46.3 (33-60)</td>
</tr>
<tr>
<td>SD IgM ICT</td>
<td>57.9 (49-79)</td>
</tr>
<tr>
<td>AccessBio IgM ICT</td>
<td>55.5 (46-63)</td>
</tr>
<tr>
<td>AccessBio Total Ab ICT</td>
<td>60.5 (53-68)</td>
</tr>
<tr>
<td>LAMP</td>
<td>51.9 (38-66)</td>
</tr>
</tbody>
</table>

Blacksell et al. 2012 Clinical and Vaccine Immunology 19, 391

Limit of detection: IFA titer vs ICT positivity

Blacksell et al. 2012 Clinical and Vaccine Immunology 19, 391
Useful range

Days of illness: ICT individual positivity

Blacksell et al. 2012 Clinical and Vaccine Immunology 19, 391

Useful range

7-10 days

Diagnostic assay

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panbio IgM ICT</td>
<td>46.3 (33-60)</td>
<td>95.1 (89-98)</td>
</tr>
<tr>
<td>SD IgM ICT</td>
<td>67.9 (50-75)</td>
<td>73.0 (68-78)</td>
</tr>
<tr>
<td>AccessBio IgM ICT</td>
<td>55.8 (46-63)</td>
<td>90.0 (87-94)</td>
</tr>
<tr>
<td>AccessBio Total antibody ICT</td>
<td>65.5 (53-75)</td>
<td>67.0 (62-75)</td>
</tr>
<tr>
<td>LAMP</td>
<td>94.8 (86-98)</td>
<td>69.3 (63-75)</td>
</tr>
<tr>
<td>Panbio IgM ICT &amp; LAMP</td>
<td>66.7 (53-79)</td>
<td>90.6 (83-95)</td>
</tr>
<tr>
<td>SD IgM ICT &amp; LAMP</td>
<td>77.2 (70-84)</td>
<td>68.2 (63-73)</td>
</tr>
<tr>
<td>AccessBio IgM ICT &amp; LAMP</td>
<td>68.5 (61-78)</td>
<td>84.9 (81-89)</td>
</tr>
<tr>
<td>AccessBio Total antibody ICT &amp; LAMP</td>
<td>71.6 (64-78)</td>
<td>63.2 (58-68)</td>
</tr>
</tbody>
</table>

Approx 10-20% sensitivity improvement

Combine LAMP and Antibody based tests

Useful range

6-8 days

Days of illness: LAMP & ICT positivity

Blacksell et al. 2012 Clinical and Vaccine Immunology 19, 391
Real-time PCR for Rickettsial Diagnosis

- Scrub typhus - 47kDa
- Typhus group - gltA
- Spotted fever group - ompB

Comparison of detection limits and relative sensitivities

<table>
<thead>
<tr>
<th>Target</th>
<th>47kDa</th>
<th>gltA</th>
<th>ompB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probe SYBR green</td>
<td>2 copies/μl</td>
<td>3 copies/μl</td>
<td>1 copy/μl</td>
</tr>
<tr>
<td>Probe SYBR green</td>
<td>1 copy/μl</td>
<td>1 copy/μl</td>
<td>1 copy/μl</td>
</tr>
</tbody>
</table>

PCR for rickettsial diagnosis

- Scrub typhus
  - Gene targets
    - 47 kDa, 56 kDa, 16S, groel
- Typhus group – gltA
- Spotted fever group – ompB

Real-time PCR detection of Rickettsia

Melt curve analysis
PCR assays – Generally insensitive

Rickettsial PCR

Eschar samples will give higher sensitivity

Scrub typhus/Rickettsial Dx – Conclusion

- Don’t use Wiel-Felix
- Eschar PCR >100% sens/100% spec
  - Other PCRs less sensitive
- Use ELISA or RDT with caution
  - Admission Dx IgM results
    - Sensitivity 50-75%
    - Specificity 70-90%
- Combine with LAMP or PCR
Malaria microscopy

Plasmodium falciparum  Plasmodium vivax

Malaria: Rapid diagnosis test

Detection of parasite releasing products: HRP-2
(Histidine rich protein-2)

- Commercial Dipsticks are available e.g. ICT, Parasight F test, Paracheck etc.
- Simple, quick, stable for a month
- Sensitivity: 10 parasites/μL
- Species identification as Pf, Pv and others
- False positive after parasite clearance
Malaria: Rapid diagnosis test

Detection of parasite lactate dehydrogenase (pLDH)
- Commercial Dipsticks are available e.g. ICT Optimal tests kit
- Rapid, simple
- Species identification for Pf, Pv
- Sensitivity: 100-200 parasites/μL

Conclusion: Malaria microscopy vs RDT

RDTs at least as accurate as microscopy

<table>
<thead>
<tr>
<th>PCR</th>
<th>Positive</th>
<th>Negative</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopy</td>
<td>18</td>
<td>14</td>
<td>95</td>
<td>50</td>
</tr>
<tr>
<td>Positive</td>
<td>8</td>
<td>296</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RDT</td>
<td>18</td>
<td>0</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>8</td>
<td>310</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* RDT = rapid diagnostic test; PCR = polymerase chain reaction.

Nicastri et al., 2009 Am J Trop Med Hyg 80: 712-717

Epidemiology of leptospirosis
Number of leptospirosis, scrub typhus, and acute undifferentiated febrile illness in Thailand, 1990-2007

![Graph showing the number of cases per year for leptospirosis, scrub typhus, and acute undifferentiated febrile illness (AUFI).](source: Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand, http://epid.moph.go.th)

### Laboratory Diagnosis

- **Microscopic Agglutination Test (MAT)**
  - High sensitivity but not useful for early diagnosis and treatment
  - Only used in reference lab

- **Isolation of Leptospira**
  - Low sensitivity (<10%) using standard EMJH medium and not useful for early diagnosis and treatment
  - Useful source for further studies, microbiology, pathogenesis, epidemiology

- **PCR**
  - useful for early diagnosis and treatment but uncertain sensitivity

- **Leptotex Lateral flow**
  - High sensitivity but doubt specificity

**Microscopic agglutination test (MAT)**

- **MAT using ≥ 20 strains representing serogroup**
  - Insensitive
  - It is a complex test to control, perform and interpret
  - High degree of cross reaction
  - The antibody may persist for years.
Isolation of Leptospira

- Gold standard for diagnosis but rarely done
- Heparin blood, CSF: incubated at room temperature
- Not difficult to culture but prolonged process requiring up to >8 weeks and has low sensitivity
- Positive in patients with fever of less than 10 days
- Median time to blood culture positivity was 21 days (IQR, 14-28 days, range 7-84 days)


Leptospira diagnosis
Recent achievements

Disc or Etest diffusion methods have never been described for Leptospira, only broth dilution techniques were reported.

Optimization of culture

Standard culture medium containing 0.1% bacteriological agar in EMJH medium supplemented with 3% rabbit serum.

The advantages of LVW agar

LVW agar could revolutionize culture of Leptospira in the diagnostic microbiology laboratory:

- Simple to prepare with commercial recipes
- Morphological characteristics
- Standardized quantification of colonies
- Maintenance of isolates
Susceptibility test for *Leptospira* spp.

**LVW agar**

Disc susceptibility  E-test

Salmonella enterica Typhi

- Bacteria causing typhoid fever are mixed with patient serum
- A single Widal test is of little clinical relevance due to the number of cross reacting infections, including malaria
- If no other tests are available look for a fourfold increase in the titer (e.g., from 1:40 to 1:160) in the course of the infection.

Widal Test

<table>
<thead>
<tr>
<th>Title</th>
<th>test</th>
<th>100</th>
<th>200</th>
<th>400</th>
<th>800</th>
<th>1600</th>
<th>3200</th>
<th>control</th>
</tr>
</thead>
</table>

| 1:100 | 1:200 | 1:400 | 1:800 | 1:1600 | 1:3200 | control |

15/10/2012
Sensitivity of PCR for S. Typhi

- 100 Patients with BC positive typhoid (Typhi (54); Paratyphi A (46)
  - PCR - Positive - 41%
- 50 Patients with ‘clinical’ enteric fever but negative BC
  - PCR - All negative
- 28 Bone Marrow samples positive for S. Typhi
  - PCR - Positive - 100%

Conclusions: Lepto and S. Typhl

- Leptospirosis
  - PCRs – low sens (hours)
  - Serology slow (paired sample) and inaccurate
  - Culture (weeks)
- S. Typhi
  - Don’t use Widal serology (inaccurate)
  - Low sensitivity
    - Blood cultures (days)
    - PCR (hours)
    - RDT (minutes)
  - Combine BC and RDT (days)

500 children admitted with fever to AHC, Siem Reap, Cambodia

- 55 children diagnosed as typhoid:
  - 24 children blood culture positive
  - 8 children PCR positive but blood culture negative
  - 12 children blood culture and PCR negative but “probable typhoid”
  - 11 children blood culture and PCR negative but “possible typhoid”

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Culture</td>
<td>44%</td>
<td>100%</td>
</tr>
<tr>
<td>PCR</td>
<td>33%</td>
<td>100%</td>
</tr>
<tr>
<td>Typhoid RDT (≥2+)</td>
<td>44%</td>
<td>98%</td>
</tr>
</tbody>
</table>
Develop antigen-based diagnostics NOW!

There is Need for Antigen-Based Rapid Diagnostic Tests to Identify Common Acute Tropical Illnesses

Henry Wills, MD, FACP* and Cassandra Sandefurty, MD, PhD*
*Queen Sirikit Memorial Institute and Division of Infectious Diseases, King Chulalongkorn University Hospital, The Red Cross Society, British Red Cross, Bangkok, Thailand, Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand

In the traveller returning from overseas consider the following;

- Where has the patient been in the past month?
  - Disease epidemiology
- How long has the patient been ill?
  - First week of illness (PCR/Antigen)
  - >7 days (antibody)
- What is the best sample?
  - Rickettsia eschar, Buffy coat (rickettsial PCR)/plasma (dengue PCR)
- What is the best test?
  - PCR, POCT, ELISA
- Do we need to repeat serology?
  - Rising titer

COLLABORATORS

Bangkok
Dr Daniel Paris
Prof Nick Day
Dr Wiriyong Chierakul
Dr Premchirat Srisuphansakul
Dr Prasert Somsaktham
Prof Narongsak Sophawong

AFRIMS
Dr Rick Jarman
Dr Robert Gibbons

Liverpool School of Tropical Medicine
Dr Janaka de Silva
Dr Ranjan Premaratna

Australian Rickettsial Reference Laboratory
Dr Stephen Graves
Dr John Stenos

Lao PDR
Dr Paul Newton
Dr Rattanaphone Phetsuvanh

US Navy
Dr Allen Richards

University of Kelaniya
Dr Nihal Jayasuriya

Mae Sot
Dr Francois Nosten
Dr Rose McGready
Dr Paul Turner

Udorn Thani and Chiang Rai Hospitals
Dr Apichart Apiwattanaporn
Dr Pacharee Kantipong

Udorn Thani
Dr Paul Turner
Wanda Wannanakorn

Vanaporn Wuthiekanun
Dr Peingchan Sonthayanon
Ampai Tanganuchitcharnchai
Suthatip Jintaworn

Yale School of Public Health
Dr Paul Turner

Thank you