QIMR Berghofer Medical Research Institute

HOT NORTH
Improving Health Outcomes in the Tropical North

menzies school of health research

Developing a Diagnostic PCR for Scabies

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and James McCarthy

JAMES COOK UNIVERSITY AUSTRALIA

TELETHON KIDS INSTITUTE
Discover. Prevent. Cure.

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Walter+Eliza Hall Institute of Medical Research
DISCOVERIES FOR HUMANITY

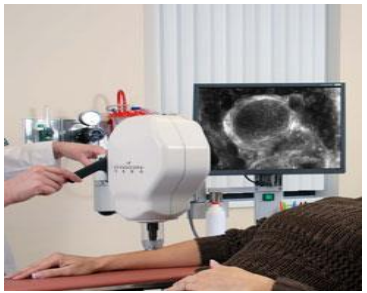
Diagnosis of scabies is challenging

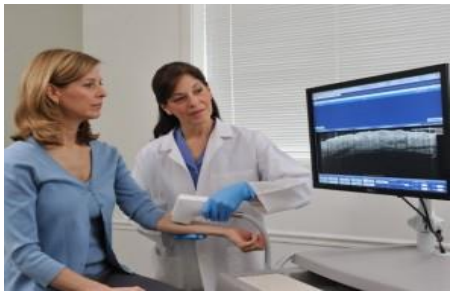
- Disease mimics other skin conditions such as eczema, psoriasis, dermatitis, etc
- Presumptive clinical diagnosis based on nocturnal pruritus, location of papules and history of exposure with infected individuals
- Microscopic confirmation of standard skin scrapings often fails as mites are elusive and very low in numbers

Why are we concerned?

- Misdiagnosis leads to improper treatment, serious disease sequelae and increased chance of epidemics
- Increase in misdiagnosis results in inaccurate assessment of the effects of intervention and leads to failure of control programs

What alternative tests are available for scabies diagnosis ?

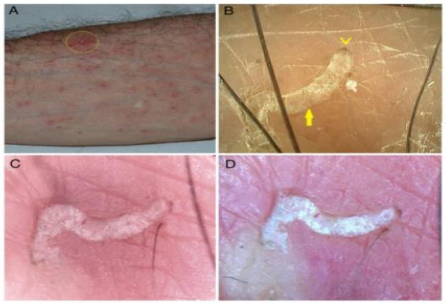




- Dermatoscopy (10x)
- Video dermatoscopy(1000x)
- Reflectance Confocal Microscopy (RCM)
- Optical Coherence Tomography (OCT)

(Thanks to Bart for photos)

What alternative tests are available for scabies diagnosis ?



- Burrow Ink Test (BIT)
- Serology (IgE, Abs to mite Ags)
- PCR (ribosomal DNA, RNA, mitochondrial DNA)

(Thanks to Bart for photos)

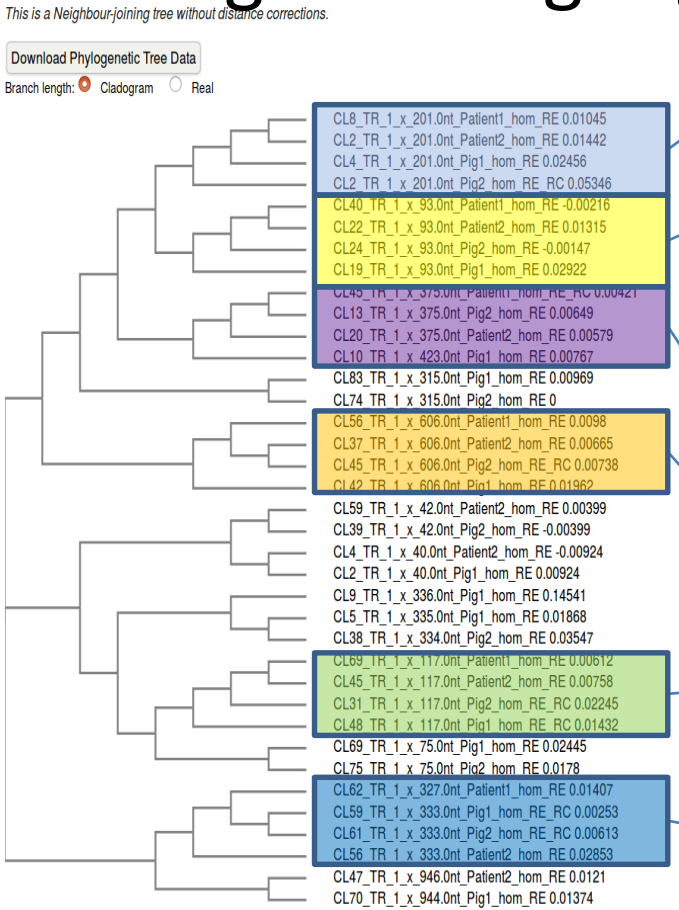
This Project: A DNA test for Scabies

- AIMS
  - Develop a highly sensitive and specific diagnostic probe-based qPCR assay for the detection of scabies targeting more abundant, high copy number DNA sequences
  - Determine optimal gDNA extraction method for mite samples to ensure maximum yield for molecular assay
  - Test efficacy of swabs as alternative, non-invasive methods for sample collection to ease diagnostic workflow

Methods

- Mining of scabies mite genomes for highly abundant DNA sequences
- Identify two candidate DNA sequences as qPCR targets
- Design primers and probes for qPCR assay

High Scoring Highly Abundant Groups



-Top scoring putative satellite of 201bp with ~98-99% confidence
-Found in all four samples

-2nd top scoring putative satellite of 93bp with ~96-98% confidence
-Found in all four samples

-5th scoring putative satellite of 375-423bp with ~76-96% confidence
-Found in all four samples

-Top scoring putative LTR of 606bp with ~90-99% confidence
-Found in all four samples

-3rd top scoring putative satellite of 117bp with ~95-99% confidence
-Found in all four samples

-4th top scoring putative satellite of 327-333bp with ~95-99% confidence
-Found in all four samples

Highly Abundant Region Target

- Promising group families underwent further analysis using the NCBI Nucleotide BLAST tool (blast.ncbi.nlm.nih.gov/Blast.cgi,
- Primers and probes designed and confirmed by unpublished Pac Bio Seqs
- Select conserved region between *Sarcoptes scabiei var hominis* (human) and *Sarcoptes scabiei var suis* (pig) mite sequences

Methods

- Compare various mite disruption methods

  - 0.5mm Zirconium/Silica beads

  - Tissue Homogeniser

  - Motorised pestle

- And various DNA extraction protocols

QiaAMP DNA Kit

HotShot plus Thermal Shock

Methods

- Optimise qPCR using mites from scabies pig model from QASP-UQ at Gatton, QLD

- Compare two types of swabs for sample collection

Catch- All Swab

FLOQ Swab

Methods

- Check performance of optimised probe-based qPCR assay on clinical samples collected at RDH and Darwin Dermatology

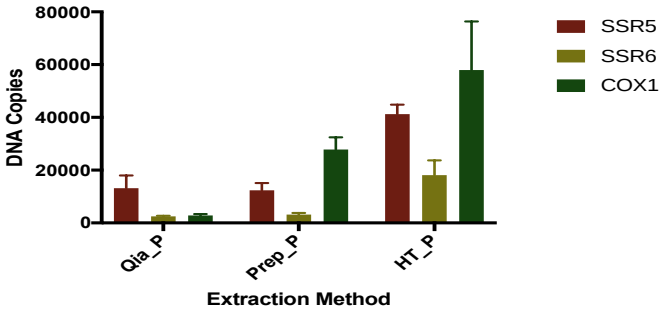
(Bart Currie, Josh Francis, Angela Wilson, Anja Hohls, Sudharsan Venkatesan)

Darwin
Dermatology

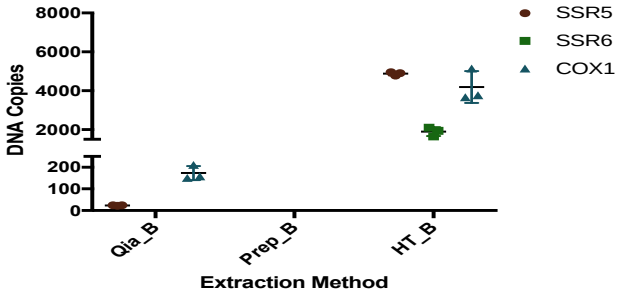
(Dev Tilakaratne)

Comparison of DNA Extraction Methods

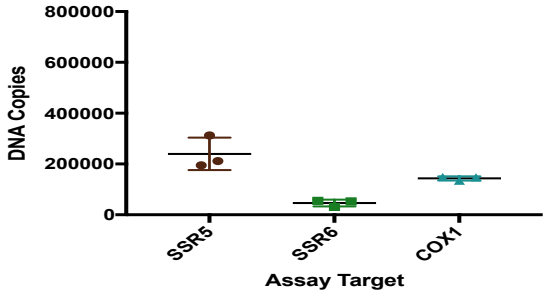
Motorised Pestle +



0.5mm Beads +



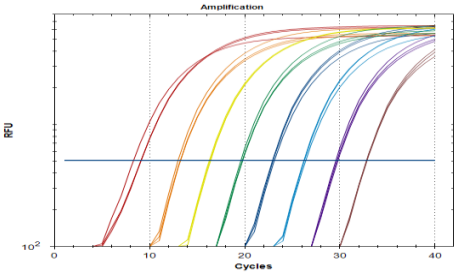
Tissue homogeniser + Qiagen

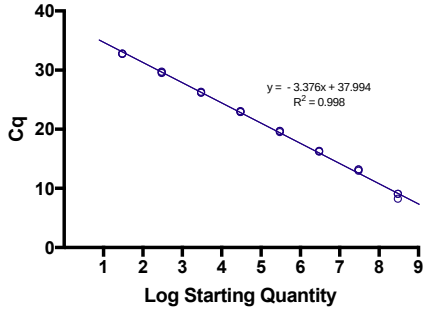


Assay Sensitivity

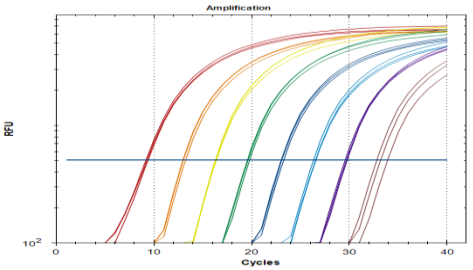
1. Standard Curves and LOD

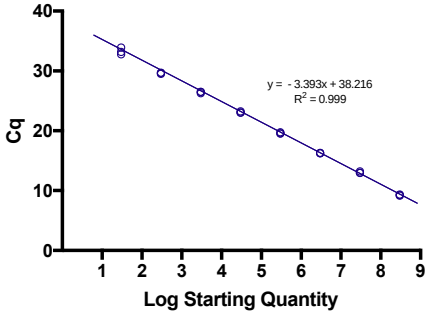
SSR5





SSR6



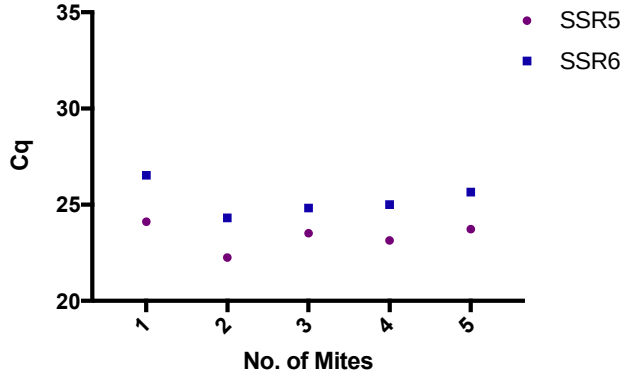


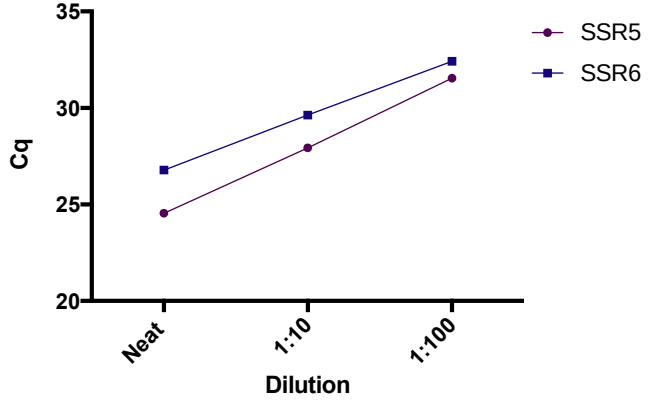
Amplification curves for dilution series from 3 x 10^8 to 30 copies/µl

Standard curves with $\log_{10}$ of each dilution against Cq

LOD for both assays is determined to be 30 copies per 10µl reaction

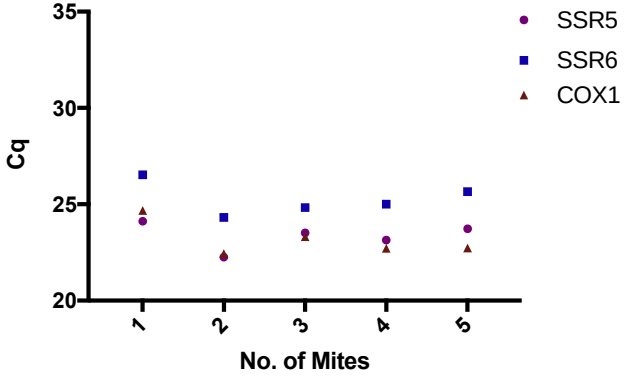
2. gDNA Testing (Mite Pool/Single Mite Serial Dilution)

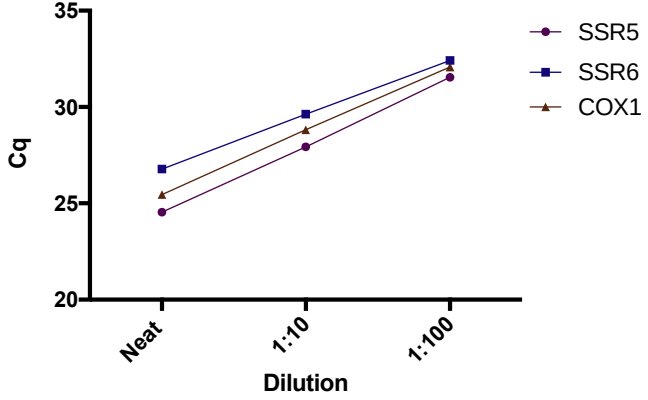




Paired t-tests were done to compare Cq:

- SSR5 vs SSR6 (Mite Pools)
  - p = 0.0004
- SSR5 vs SSR6 (Dilution)
  - p = 0.055





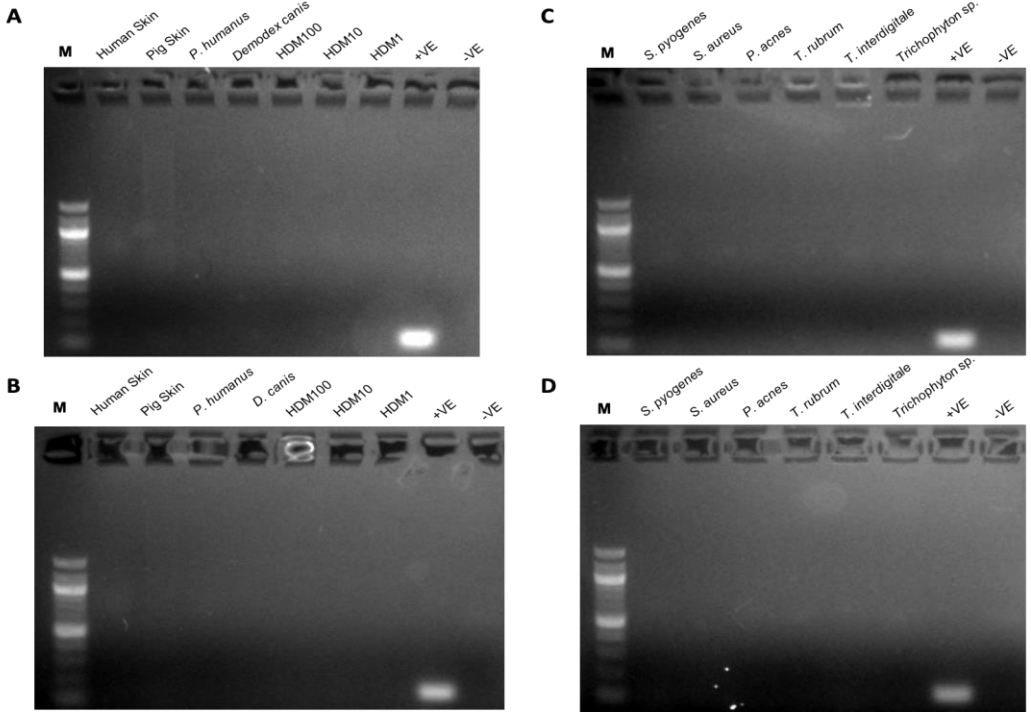
Paired t-tests were done to compare Cq:

- SSR5 vs Cox1 (Dilution)
  - p = 0.0229
- SSR6 vs Cox1 (Dilution)
  - p = 0.1014

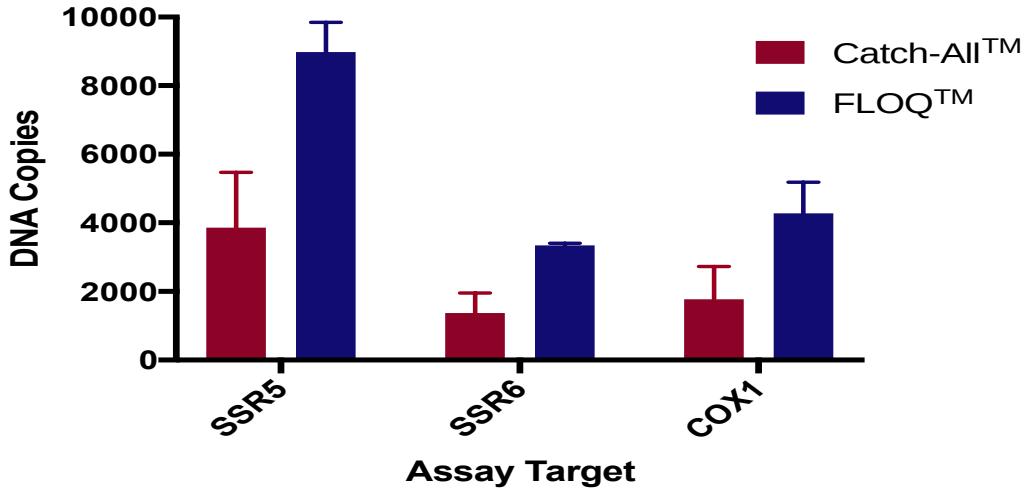
Assay Specificity

- No amplification of SSR5 or SSR6 on DNA extracted from the following:
  - Other mite species : *D. farinae* (house dust mite), canine *Demodex sp* (eye lash mite in humans)
  - *Pediculus humanus capitis* (head louse)
  - Other skin pathogens: *Staph. aures, Strep. pyogenes*, *P. acnes, T. rubrum, T. interdigitale*
  - Normal human skin and pig skin
- No amplification of both targets on DNA extracted from samples collected from patients with other skin conditions: dermatitis, eczema, psoriasis, tinea nigra, pityriasis versicolor, seborrheic keratosis

Assay Specificity

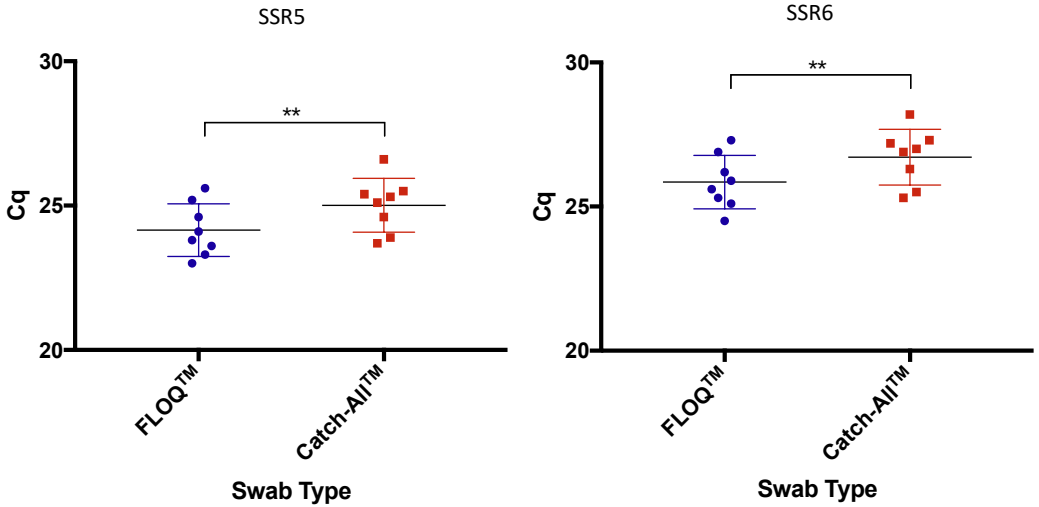


Comparison of performance of swabs from samples collected from mange pigs



Utility/Performance of Swabs

6 FLOQ/Catch-All swabs collected from both ears of 4 porcine scabies models



Paired t-tests performed to compare FLOQ vs Catch-All

- SSR5: p = 0.0058
- SSR6: p = 0.0049

Table 1. Clinical Data and qPCR Results from samples collected from patients with other skin conditions

Patient ID	Clinical Diagnosis	Sample Collection Site	Scabies Tx Given?	Microscopy Diagnosis	qPCR Diagnosis
C1	Dermatitis	Left Palm	NA	NA	Negative
C2	Dermatitis	Left Knee	NA	NA	Negative
C3	Bowen's Disease	Left Calf	NA	NA	Negative
C4	Eczema	Right Palm	NA	NA	Negative
C5	Seborrheic keratosis	Right Arm	NA	NA	Negative
C6	Tinea nigra	Right Palm	NA	NA	Negative
C7	Seborrheic dermatitis	Midline Scalp	NA	NA	Negative
C8	Dermatitis	Anterior Neck	NA	NA	Negative
C9	Pityriasis versicolor	Right Flank	NA	NA	Negative
C10	Psoriasis	Dorsum of hand	NA	NA	Negative
C11	Psoriasis	Left Thigh	NA	NA	Negative
C12	Psoriasis	Left Leg	NA	NA	Negative
C13	Psoriasis	Right Elbow	NA	NA	Negative
C14	Dermatitis	Mid Back	NA	NA	Negative
C15	Plaque psoriasis	Left Forearm	NA	NA	Negative
C16	Psoriasis	Right Knee	NA	NA	Negative
C17	Psoriasis	Right Arm	NA	NA	Negative
C18	Eczema	Left Elbow	NA	NA	Negative
C19	Tinea	Right Forearm	NA	NA	Negative

Table 2. Clinical Data and qPCR results from samples collected from Scabies patients

Patient ID	Clinical Diagnosis	Sample Collection Site	Scabies Tx Given	Microscopy Diagnosis	qPCR Diagnosis
S1	Ordinary Scabies	Right Leg	Yes	Negative	Negative
S2	Ordinary Scabies	Right Foot Left Foot	No	Positive (?) Negative	Negative Negative
S3	Ordinary Scabies	Left Axillary Right Hand	No	Positive Negative	Positive Negative
S4	Ordinary Scabies	Left Foot	Yes	Positive (?)	Negative
S5	Ordinary Scabies	Right Palm	No	Negative	Positive
S6	Ordinary Scabies	Right Thumb	No	Negative	Negative
S7	Ordinary Scabies	Left Palm	No	Negative	Positive
S8	Ordinary Scabies	Left Middle Finger	No	Negative	Positive
S9	Ordinary Scabies	Abdomen	Yes	Negative	Negative
S10	Ordinary Scabies	Left Foot	Yes	Negative	Negative

Summary

- A simple DNA extraction method has been optimised to ensure maximum yield suitable for molecular assay

- The use of swabs as an alternative, non-invasive sample collection tool from patients will ease diagnostic workflow for scabies

Summary

- Highly sensitive – qPCR assay can detect single mite or less (0.1-0.01 mite material)
- Highly specific for human scabies
- New probe-based qPCR assay targeting highly abundant regions of the scabies genome performs better than the published assay

Future Work

- Test performance of new DNA test for scabies on samples collected by swabs in scabies endemic communities

Thanks to all who make this Collaborative Project Happen

- QIMR Berghofer: James McCarthy, **Cielo Pasay**, Lena Ch'ng ; Katja Fischer
- James Cook University, Cairns: Matt Field
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- Menzies School of Health Research: Deb Holt, Vanessa Rigas , Celeste Woerle
- RDH/Darwin Dermatology: Bart Currie, Josh Francis, Dev Tilakaratne, Angela Wilson, Anja Hohls, Sudy Venkatesan
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