

# Multiplexed Molecular Diagnostics

Dermatophytes, fungi, and other skin infections

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# AusDiagnostics MT-PCR

- MT-PCR Step 1

Reverse transcription and  
pre-amplification



- Highly multiplexed amplification of up to 24 targets
- Single sample required
- Low cycle number (15 cycles)
- No competition
- Preserve quantification

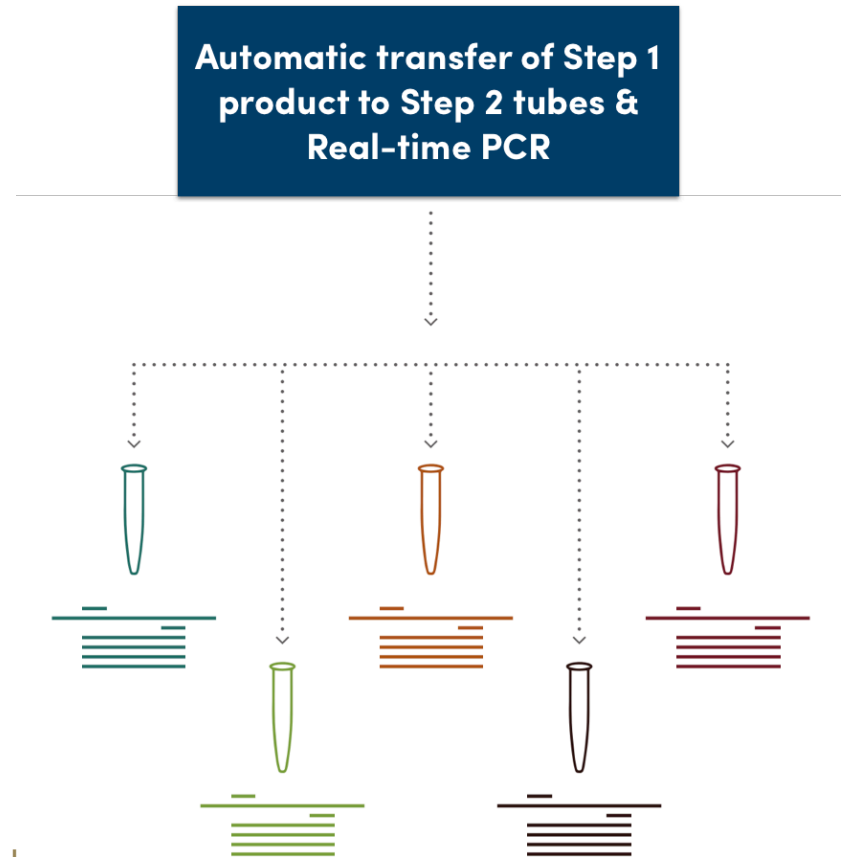
Performed Automatically on Board of Easy-Plex™ Processor

# AusDiagnostics MT-PCR

- MT-PCR Step 2

- Performed in separate wells for each target
- No competition
- Amplification with nested primers
- High specificity
- Real-time data
- Target quantification
- 384-well plate
- High throughput
- Low reaction volume without loss of sensitivity

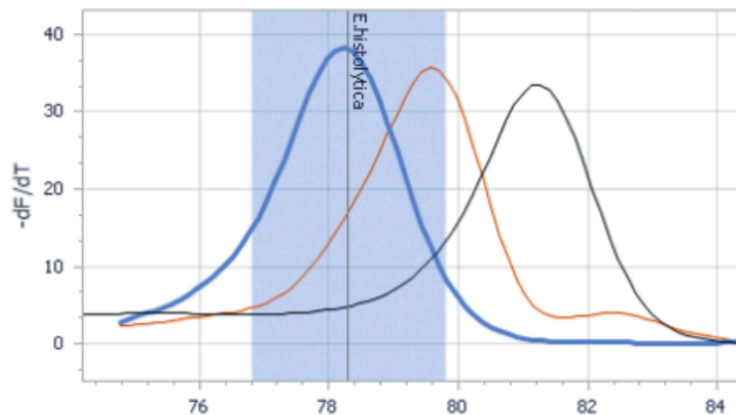
Real-time PCR in *Easy-Plex™* Analyser



# AusDiagnostics MT-PCR

- Amplicon analysis

Immediate step after  
Real-time PCR



- Automated results calling in each well according to temperature
- Detection of several targets in one well
- Easy Genotyping

# AusDiagnostics MT-PCR

## Processor & Analyzer



(Step 1)



(Step 2)

- 384-well Real-Time PCR cycler
- Cost effective for 5+ sample per run
- Maximum load - 24 or 48 samples depending on processor version

# Dermatophytes & other Fungi – via molecular detection

DERMATOPHYTES AND OTHER FUNGI (12-WELL) REF 24115 VER 01



Assay	Target
Trichophyton spp.	<i>Trichophyton</i> spp. (including all isolates)
T.rubrum complex	<i>Trichophyton rubrum</i> complex (all members)
Metagrophytes comp	<i>Trichophyton interdigitale</i> complex (all members)
Microsporum spp.	<i>Microsporum</i> spp. ( <i>M.canis</i> , <i>M.audouinii</i> , and <i>M.ferrugineum</i> )
Microsporum canis	<i>Microsporum canis</i> (including all isolates)
E.floccosum	<i>Epidermophyton floccosum</i> (including all isolates)
Nannizzia gypsea	<i>Nannizzia gypsea</i> (including all isolates)
Scopulariopsis spp.	<i>Scopulariopsis</i> spp. (including all isolates)
Aspergillus spp.	<i>Aspergillus</i> spp. ( <i>A.fumigatus</i> , <i>A.clavatus</i> , <i>A.giganteus</i> , <i>A.niger</i> , and <i>A.flavus</i> )
Candida	<i>Candida albicans</i> (including all isolates), <i>Candida guilliermondii</i> (includes all isolates)
Candida2	<i>Candida parapsilosis</i> (all members, including <i>C.orthopsilosis</i> and <i>C.metapsilosis</i> ), <i>Candida glabrata</i> (including all isolates)
SPIKE	Artificial sequence for assay control

\*Aspergillus will be replaced by *Malassezia furfur*

Nail clippings, hair, skin



~30min Lysis/digestion with heat, quick centrifugation



Use supernatant for DNA extraction ~ 1h



Test with MT-PCR ~3.5h

**Sample to Result in 5 hours**

# Dermatophytes & other Fungi – via molecular detection

## Automatic result calling in MT-PCR analysis:

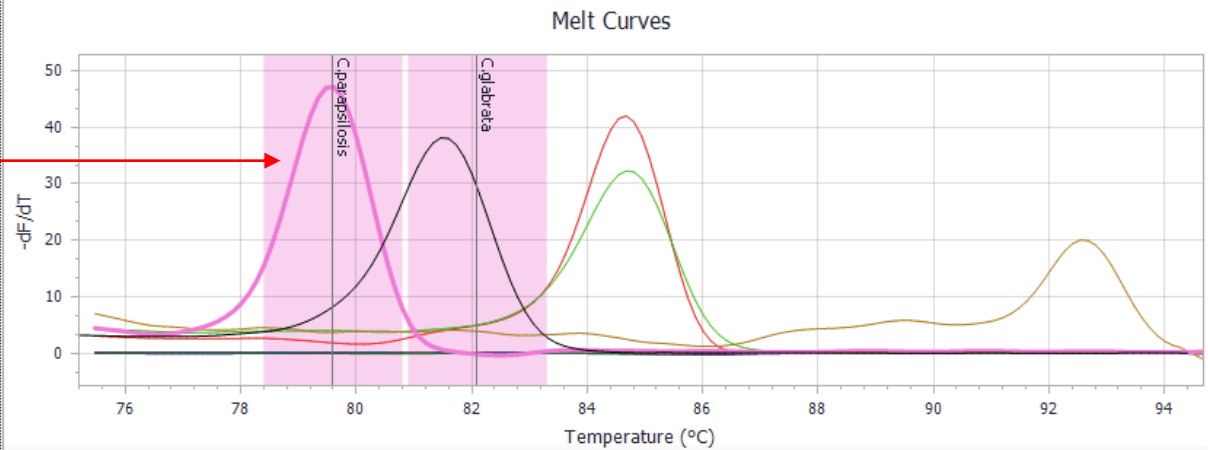
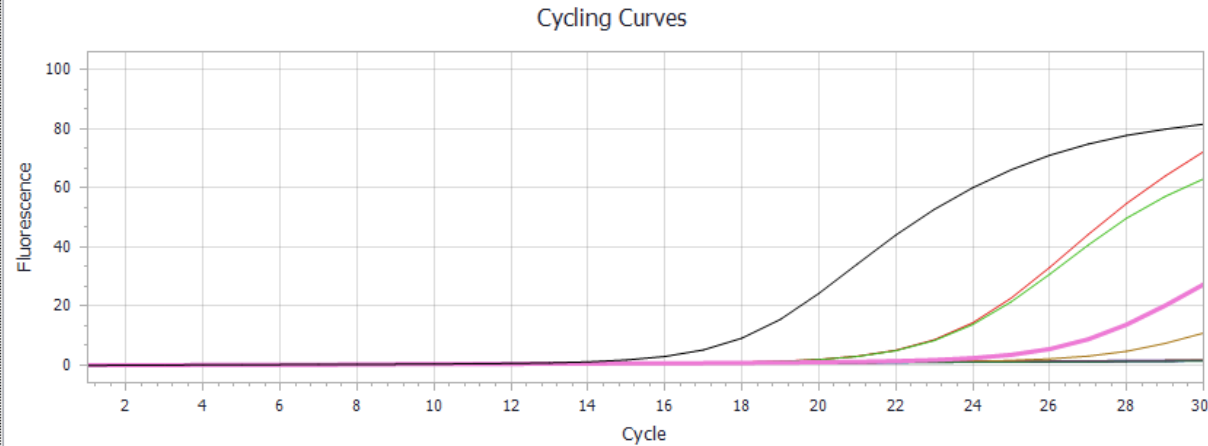
Result



Results Template ID: 7A96

No.	Sample	Gene	Call	Corrected Melt	Take-Off
A16	3487...	Trichophyton spp	Present	84.66	19.87
B16	3487...	T.rubrum complex	Present	84.72	19.63
C16	3487...	Mentagrophytes			
D16	3487...	Microsporum spp			
E16	3487...	Microsporum canis			
F16	3487...	E.floccosum			
G16	3487...	Nannizzia gypsea			
H16	3487...	Scopulariopsis spp			
I16	3487...	Aspergillus spp	Present	92.6	24.67
J16	3487...	Candida			
K16	3487...	Candida2	Present (C.parapsilosis)	79.56	23.19
L16	3487...	SPIKE	Present	81.8	14.67

Species call via melt curve



# Dermatophytes & other Fungi – via molecular detection

Report for clinician / GP :

Sample ID	Gene Target	Call	Intensity
	Trichophyton spp	Present	★★
	T.rubrum complex	Present	★★
	Mentagrophytes		
	Microsporum spp		
	Microsporum canis		
	E.floccosum		
	Nannizzia gypsea		
	Scopulariopsis spp		
	Aspergillus spp	Present	★
	Candida		
	Candida2	Present (C.parapsilosis)	★
	SPIKE	Present	★★★★



## Future multiplex panel (in development)

- A new 24-well panel is being developed and will include a scabies assay.
- Contact us for details

# Future multiplex panel (in development)

## Rationale for scabies assay

- Improve diagnosis – improve treatment



- Scabies mite/eggs/feces ↔ sampling technique/ skin scraping
- Secondary infections: GAS? (S.pyogenes), S.aureus
  - impetigo, cellulitis
  - GAS impetigo and acute rheumatic fever?
  - MRSA
- Drug resistance in scabies?
- Further differential diagnosis – which targets? E.g. viral exanthems, mycetoma, tinea, folliculitis,...



# Thank You for Your Attention!

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