Running Rings Around Ringworm With PCR

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IDEXX

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Gold standard: Fungal culture

2-3 weeks to final result

75% of RW suspects are negative (Moriello JFMS 16: 419-431 2014)

PCR

1-3 days to final result

Performance?

Interpretation?

Why does this study matter?

- Ringworm is a cosmetic disease but uses a lot of time, space and resources and leads to increased length of stay or even euthanasia
- **Most** ringworm suspects are **negative** for ringworm
- Cutting isolation time for negative cats increases life-saving capacity and reduces euthanasia

IDEXX® PCR panel

- *Microsporum* sp., *M. canis* and *Trichophyton* sp.
- At time of study, *Microsporum* and *Trichophyton* sp. only
- Positive or negative

Original Article

Journal of Feline Medicine and Surgery
1–5

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SAGE

Comparison of real-time PCR with fungal culture for the diagnosis of *Microsporum canis* dermatophytosis in shelter cats: a field study

Linda S Jacobson, Lauren McIntyre and Jenny Mykusz

Original Article

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SAGE

Assessment of real-time PCR cycle threshold values in *Microsporum canis* culture-positive and culture-negative cats in an animal shelter: a field study

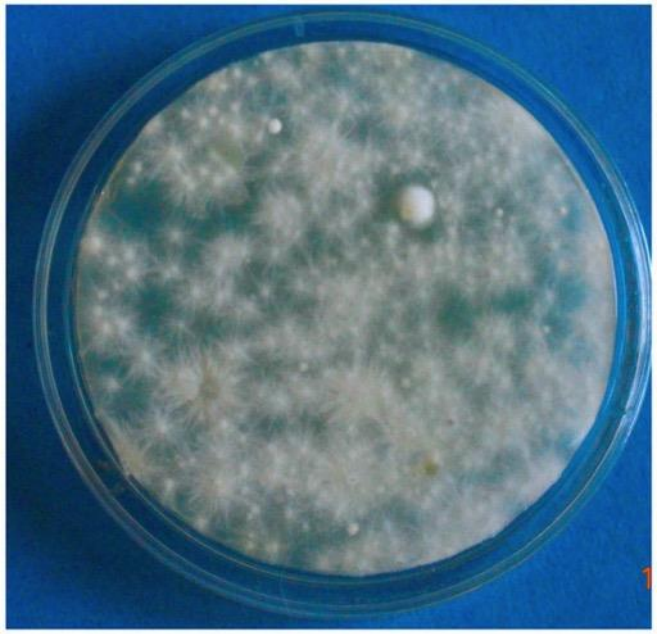
Cats

- Included cats with skin lesions or suspected exposure
  - High-risk: Suspicious skin lesions
  - Exposed: Non-lesional, history of exposure
  - Low risk: Skin lesions not typical for dermatophytosis

Treatment and diagnostics

- Treatment
  - Low-risk – single dip lime sulfur 1:16
  - Exposed and high-risk – lime sulfur twice weekly, itraconazole 5mg/kg PO q24h for 21 days (14 days if first culture was negative)
- Culture and PCR: Weekly until cleared (first culture negative or two negative cultures after initial positive culture)

Tests



- Hair samples were split into two parts
- Cultures were performed at the THS. Positive initial cultures were confirmed by IDEXX®
- PCR was performed by IDEXX®

Case Definitions

- Positive case: *M. canis* was grown on the first fungal culture, regardless or presence or absence of skin lesions
- Mycological cure: Two negative cultures 1 week apart

Culture results for 132 cats (% of subgroup)

	Culture +	Culture -
High risk (61)	**39**	**61**
Exposed (30)	**7**	**93**
Low risk (41)	**5**	**95**

PCR pre-treatment (n=132)

	Culture +	Culture -	Total
PCR +	**28**	**12**	**40**
PCR -	**0**	**92**	**92**
Total	**28**	**104**	**132**
Sensitivity: 100% (87.7-100) **Specificity: 88.5 (80.7-93.9)**			

“False” positives (n=12)

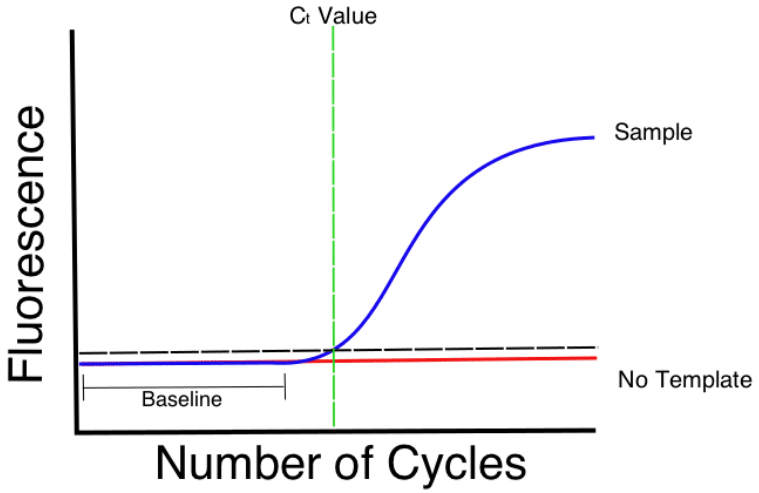
9 had repeat cultures:

- 2/9 - subsequent positive culture
- 5/9 - history of exposure
- 2/9 - could not explain positive PCR; very low amount of fungal DNA present

PCR for confirmation of mycological cure (n=17)

	First negative culture	Second negative culture
PCR +	**82%**	**65%**
PCR -	**18%**	**35%**

Cycle threshold (Ct) values



- Ct value is inversely and exponentially proportional to amount of DNA in the sample
- **Ct 20.26 – 12,565,433 DNA copies; Ct 39.51 – 21 DNA copies**
- Lab reports > 39.99 as negative

Assessment of Ct values: Goals

- In cases with a negative culture and a positive PCR, can a Ct cut-off value be found to help interpret the PCR result?
- The cut-off would differentiate true PCR positives from clinically non-significant PCR positives

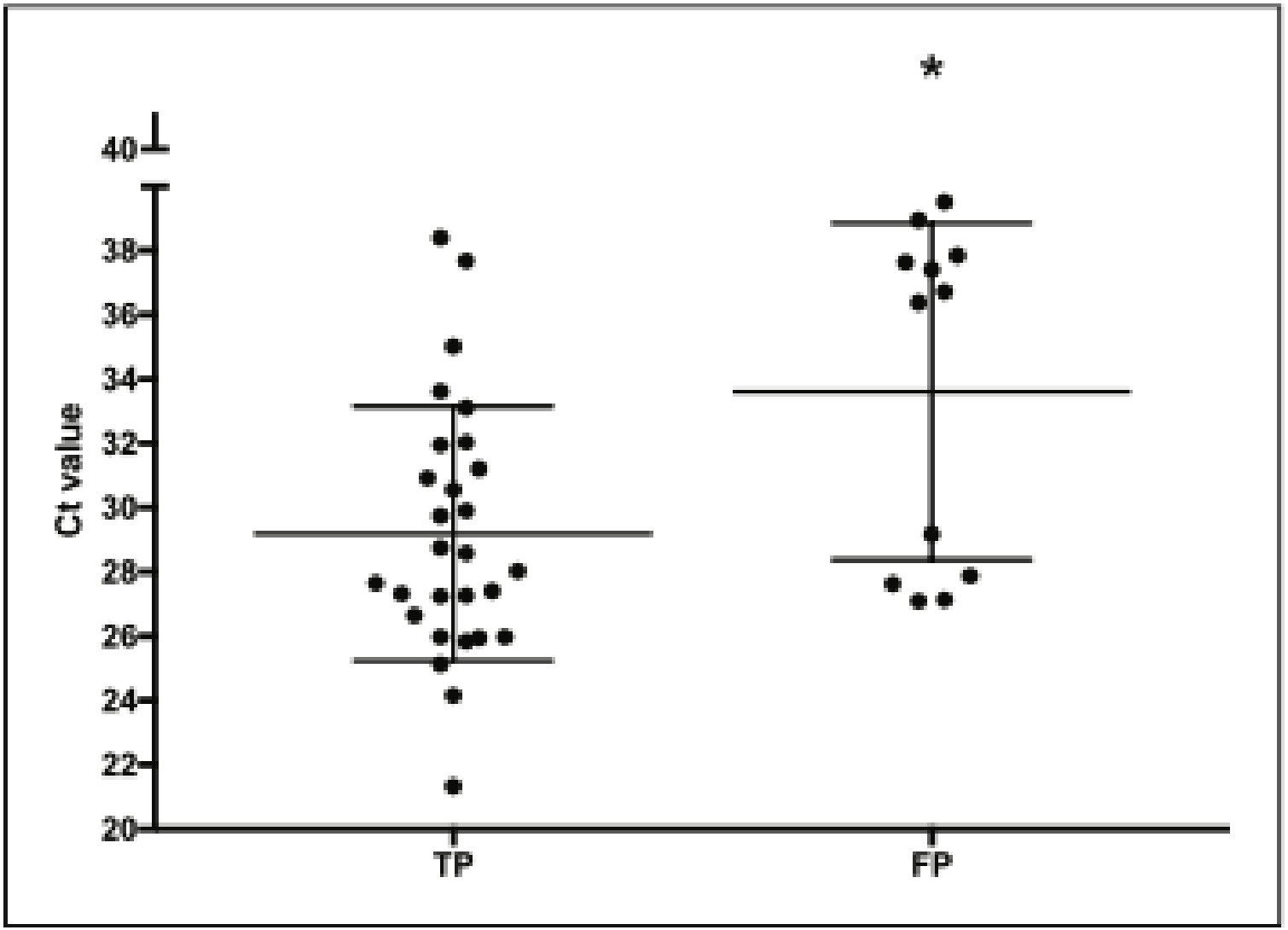
Design

- Pre-treatment (n = 132)
- Treated: Cats that had complete weekly data until the second negative fungal culture (if *M. canis* positive) or until the 14-day culture result (if negative)
  - n = 39 cats; 84 pooled time points for all

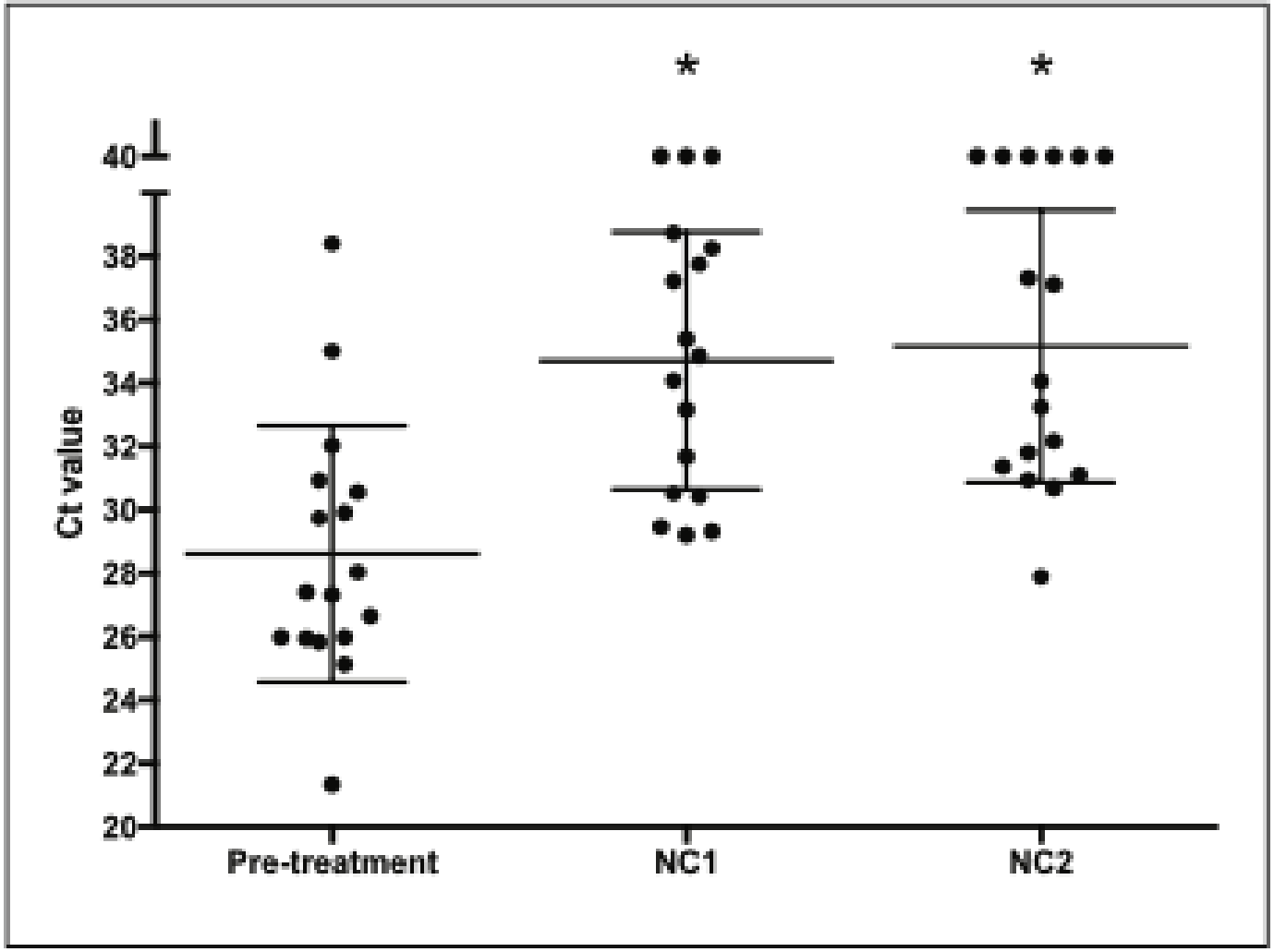
Results

- ROC curve cut-off (for sens and spec both > 90%)
- **Pre-treatment – cutoff was Ct < 35.7 (DNA count approx. 300)**
  - **Sens 92.3, spec 95.2**
- During treatment – no acceptable cut-off value

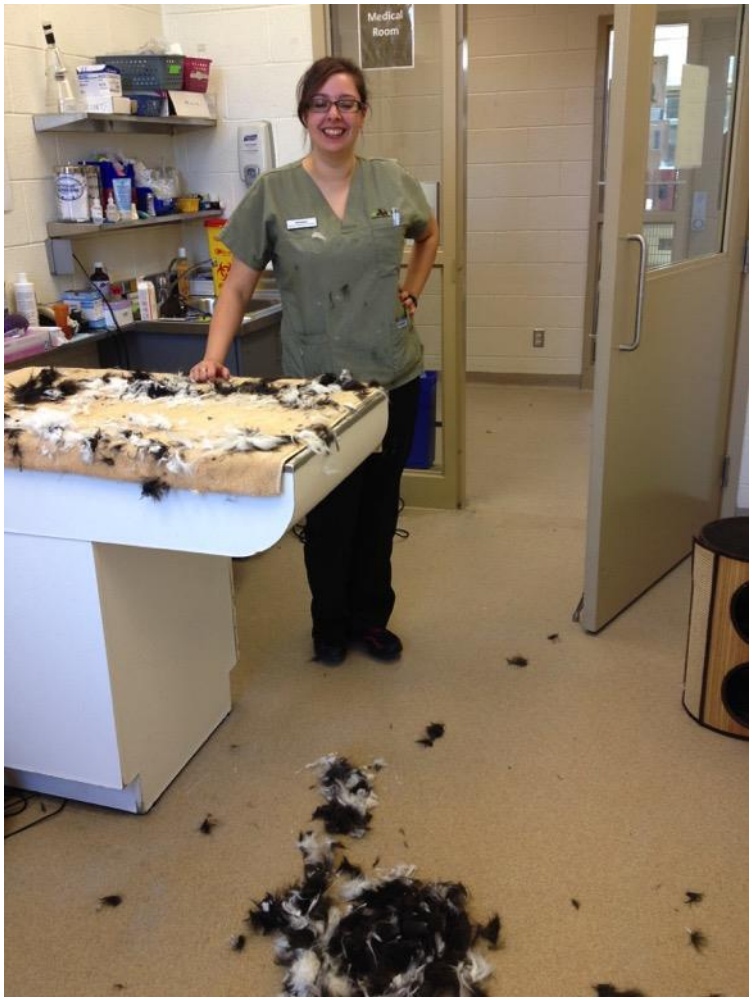
Pre-treatment Ct values - true-positive and false-positive cats



Ct values over time for positive cases



Discussion/Conclusions



Excellent agreement between PCR and culture **before treatment** - consistent with human and veterinary studies

PCR **not** recommended for confirming mycological cure

Many factors could cause false positives – dead organisms, cross-contamination of samples, fomite contamination

Caution

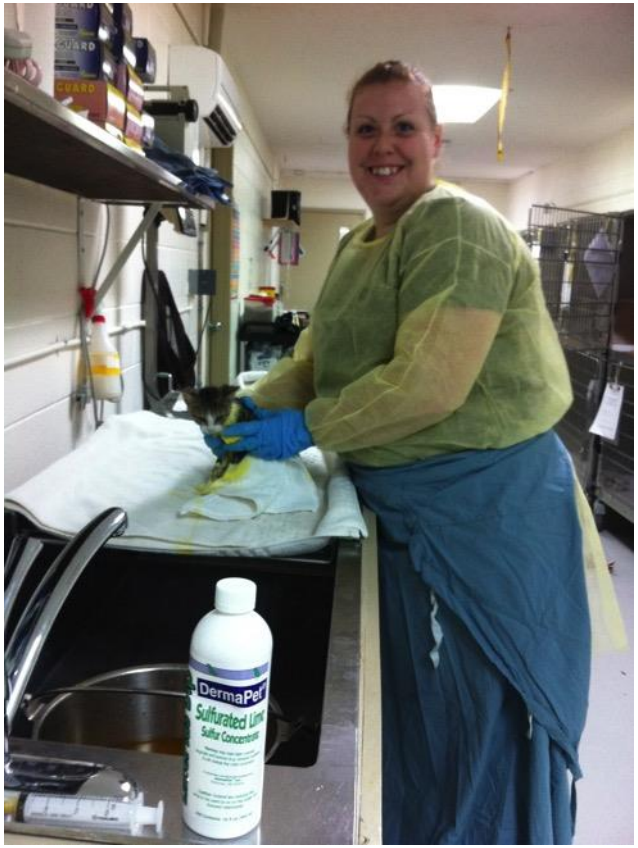
- “NSQ”
- **Interpret all findings** – history, clinical findings, Wood’s lamp; don’t just rely on the PCR
  - We have subsequently seen initial false negatives in a litter of very young kittens in an exposed group
- Extrapolation between labs is risky
- Shelters’ experiences may differ especially based on prevalence and fungal loads

PCR cost analysis

- 92/103 culture-negative cats were PCR-negative
- Iso time = 92 x 14 = **1,288 cat care days**
  - $20/day – $25,760
- Iso time if PCR had been trusted: 92 x 3 = **276 cat care days**
  - At $20/day – cost of $5,520 i.e. savings of $20,240
- Cost of PCR tests – 92 x 56 = $5,152
- Savings = $20,240 - $5,152 = **$15,088 and 1,012 cat care days**

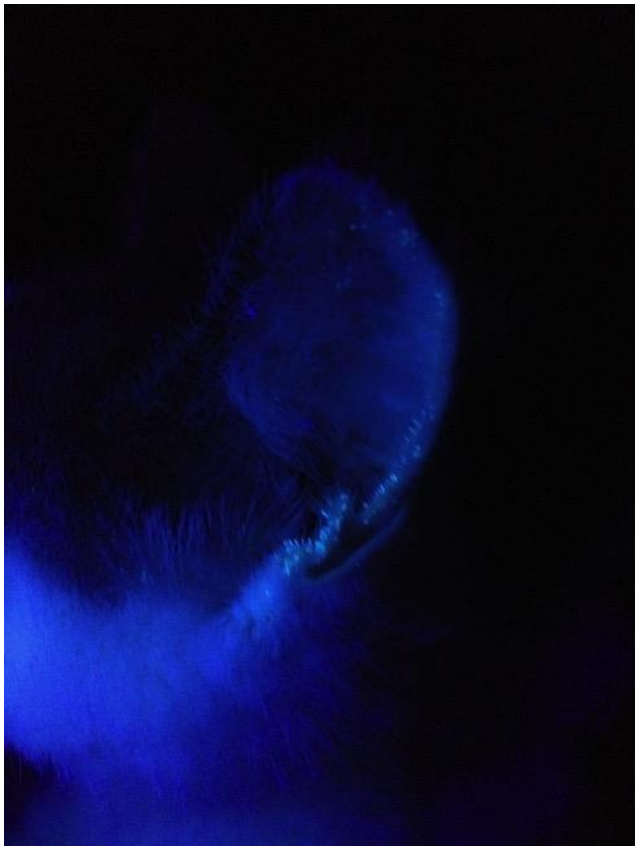
What we used to do

- Exam and Wood's lamp exam at intake
- Isolated and treated all "high-risk" suspects and exposed cats while waiting for culture results
- Cultured, lime dipped (usually once) and monitored "low-risk" suspects



What we do now

- WL for all, stronger focus on lesion checks
- Positive lesion check, positive Wood's lamp – consider positive, isolate
- Positive lesion check, negative Wood's lamp – PCR and lime dip
  - Medical observation until PCR result for most
  - Isolate/quarantine if very suspicious



How is this working for us?

- Very well! E.g. groups of cats from an institutional hoarder with known dermatophytosis
  - Only a few cats per transfer of 20-40 cats have required isolation and treatment; the rest are moved ahead quickly
- The number of cats being isolated for dermatophytosis in our shelter has dropped dramatically

Summary: IDEXX® PCR

- Excellent method to rapidly rule out dermatophytosis and for initial diagnosis
- False positives outweighed by rapid results for true negatives
- Culture remains the method of choice to determine mycological cure
- Ct values can help in decision-making but there is no reliable cut-off during treatment
  - Ct value ≥ 35.7 at intake – in individual cases, **may** suggest a false-positive PCR