

# *Importance of validating RT-PCR methods for *Dientamoeba fragilis**

Rory Gough, Joel Barratt, John Ellis, Damien  
Stark,

# The history of *D. fragilis*

- 1918 – initial description by Jepps and Dobell – ‘ a harmless commensal’ – although several patients had GIT symptoms with no other cause found
- 1920’s - implicated as a cause of GIT disease
- sporadic ‘for’ and ‘against’ publications over the next 90 years
- pathogenicity still debated

Clin Microbiol Rev. 2016 Jul;29(3):553-80. doi: 10.1128/CMR.00076-15.

**Dientamoeba fragilis, the Neglected Trichomonad of the Human Bowel.**

Stark D<sup>1</sup>, Barratt J<sup>2</sup>, Chan D<sup>2</sup>, Ellis JT<sup>2</sup>.



# “Aber” and *Dientamoeba fragilis*

[Br J Biomed Sci](#). 1998 Sep;55(3):172-5.

## **Incidence of *Dientamoeba fragilis* in faecal samples submitted for routine microbiological analysis.**

[Windsor JJ<sup>1</sup>](#), [Rafay AM](#), [Shenov AK](#), [Johnson EH](#).

### ⊖ Author information

1 Department of Microbiology and Immunology, College of Medicine, Sultan Qaboos University, Muscat, Sultanate of Oman.

[BMJ](#). 1999 Mar 13;318(7185):735.

## **More laboratories should test for *Dientamoeba fragilis* infection.**

[Windsor JJ](#), [Johnson EH](#).

[Br J Biomed Sci](#). 2003;60(2):79-83.

## **Detection of *Dientamoeba fragilis* by culture.**

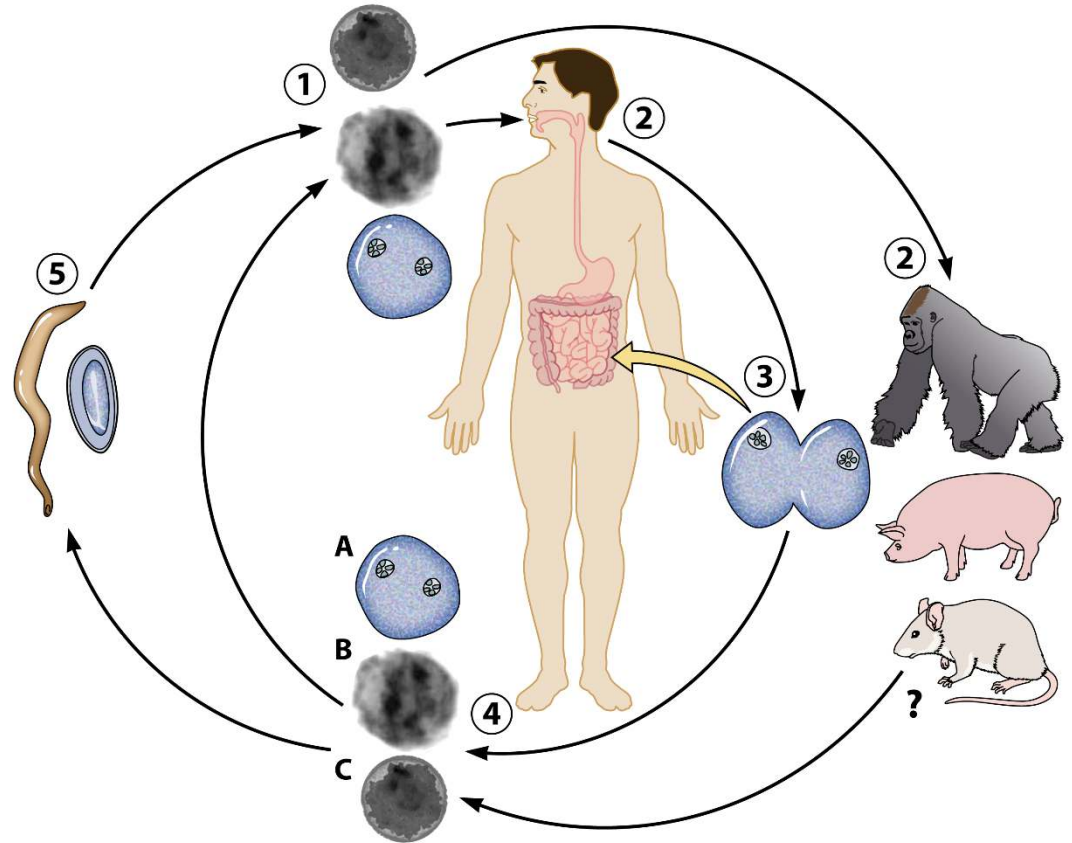
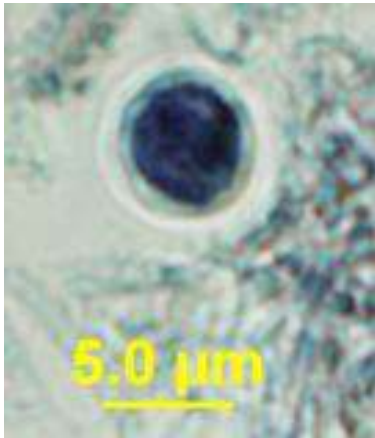
[Windsor JJ<sup>1</sup>](#), [Macfarlane L](#), [Hughes-Thapa G](#), [Jones SK](#), [Whiteside TM](#).

### ⊖ Author information

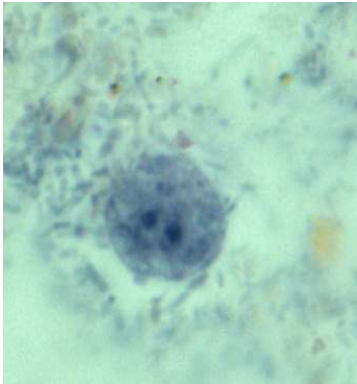
1 NPHS Microbiology Aberystwyth, Bronglais Hospital, Caradoc Road, Aberystwyth, Ceredigion, SY23 1ER, UK.  
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# Transmission

- Involvement of helminth ova ?
- Direct transmission ?



# Laboratory Diagnosis



- Microscopy
- Culture

[Parasitology](#). 2010 Nov;137(13):1867-78. doi: 10.1017/S0031182010000764. Epub 2010 Jul 8.

**Newly defined conditions for the in vitro cultivation and cryopreservation of Dientamoeba fragilis: new techniques set to fast track molecular studies on this organism.**

[Barratt JL<sup>1</sup>](#), [Banik GR](#), [Harkness J](#), [Marriott D](#), [Ellis JT](#), [Stark D](#).

[Parasitology](#). 2012 Jun;139(7):864-9. doi: 10.1017/S0031182012000145. Epub 2012 Feb 16.

**New advances in the in-vitro culture of Dientamoeba fragilis.**

[Munasinghe VS<sup>1</sup>](#), [Stark D](#), [Ellis JT](#).

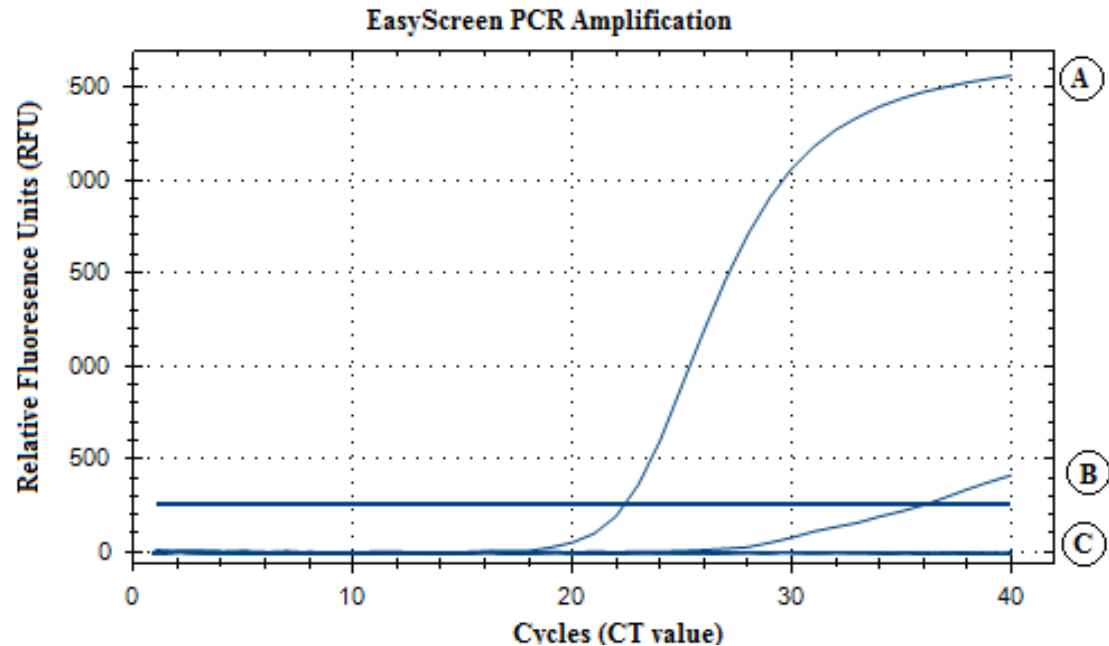


- PCR
  - Conventional
  - Nested
  - RT-PCR



# Genetic Signatures Pty Ltd

- 3 base technology
- Bisulphate conversion
- Primers/probe targeting “new” sequence
- “EasyScreen”

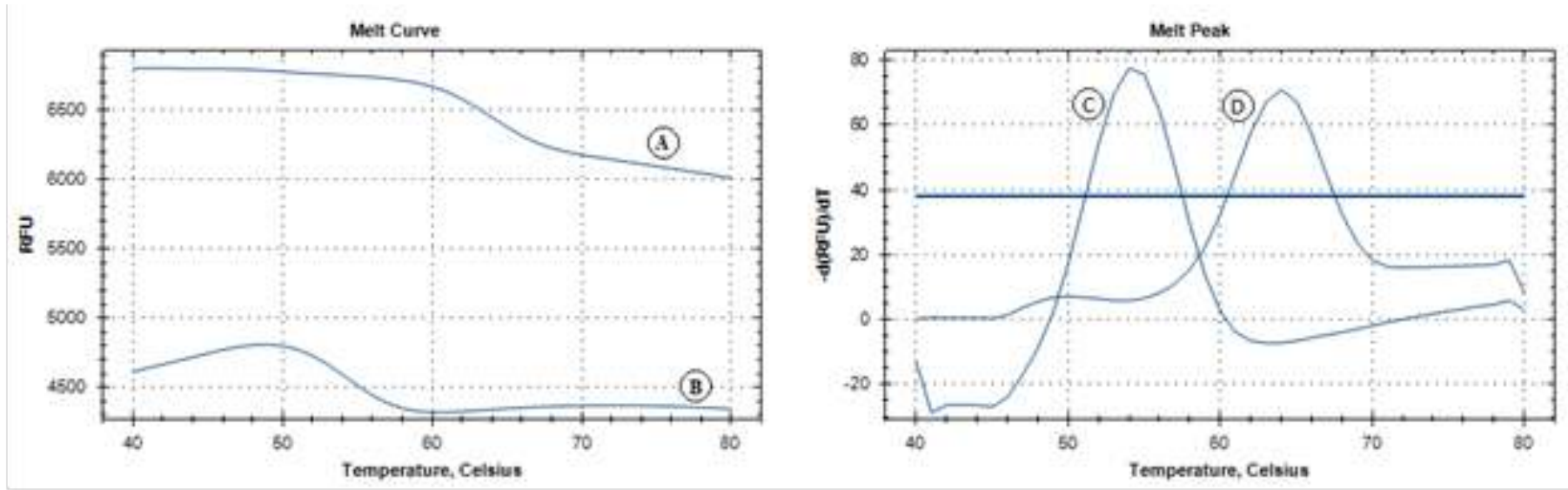


*Diagn Microbiol Infect Dis*, 2014 Feb;78(2):149-52. doi: 10.1016/j.diagmicrobio.2013.10.013. Epub 2013 Oct 23.

**Evaluation of the EasyScreen™ enteric parasite detection kit for the detection of *Blastocystis* spp., *Cryptosporidium* spp., *Dientamoeba fragilis*, *Entamoeba* complex, and *Giardia intestinalis* from clinical stool samples.**



# GS assay specificity



Melt curve analysis of *D. fragilis* (A) compared to *P. hominis* (B),  
*P. hominis* had a melting peak at 54°C (C), compared to *D. fragilis* at 64°C (D)

# Prevalence

- Commonly found in cases of diarrhoea
- More common than *Giardia*!
- Metronidazole

## SVH Sydney (2014-16)

<b>Protozoa</b>	<b>Prevalence (%)</b>
<i>Blastocystis</i> spp.	13.6%
<b><i>Dientamoeba</i></b>	<b>9.2%</b>
<i>Giardia</i>	2.6%
<i>Cryptosporidium</i>	1.3%
<i>E. histolytica</i>	0.3%





# Interesting observations from NeDa

STUDY	COUNTRY	YEAR	FINDINGS
de Wit et al	Netherlands	2001	<i>D. fragilis</i> found more frequently in healthy controls (14.6%) than those GI complaints (10.3%)
Roser et al	Denmark	2013	<i>D. fragilis</i> incidence 43% (n=22,484)
Engsbro et al	Denmark	2014	Prevalence 35-41%
Bruijestein et al	Denmark	2015	Symptomatic patients 37.3% Asymptomatic control group 25.7%
de Jong et al	Netherlands	2015	Healthy controls 50.6% Paediatric patients presenting with abdominal pain 43.2%

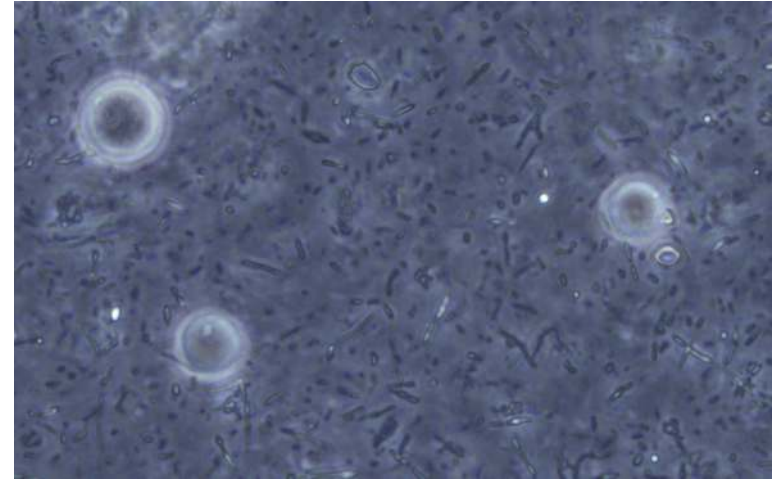
[Mol Cell Probes. 2007 Oct-Dec;21\(5-6\):400-4. Epub 2007 May 29.](#)

**Real-time PCR for the detection of *Dientamoeba fragilis* in fecal samples.**

[Verweij JJ<sup>1</sup>](#), [Mulder B](#), [Poell B](#), [van Middelkoop D](#), [Brienen EA](#), [van Lieshout L](#).

# Sensitivity

- *Cultured* trophozoite cell counts
- Serial dilutions
- Spiked into faecal sample
- DNA extracted
- Limit of Detection Assays



Live *Dientamoeba fragilis* trophozoites from a Loeffler slope with overlay supplemented with rice starch.

# Sensitivity

<i>D. fragilis</i>	Roche	AusDx	GS	Stark	NeDa
<b>Trophozoites</b>					
500	27.22	176,389	30.33	24.67	23.73
50	29.98	8,045	33.02	28.44	27.52
5	33.92	1,804	34.48	30.79	30.64
0.5	Negative	Negative	Negative	Negative	Negative
0.05	Negative	Negative	Negative	Negative	Negative



# Specificity

**Table 2**  
Specificity of each assay when tested against other flagellates.

Flagellate species	Source of DNA	Nested PCR – Cacciò et al. (2012)	Real Time PCR – Verweij et al. (2007)	EasyScreen – Genetic Signatures
<i>Dientamoeba fragilis</i> (Isolate P)	SydPath, St Vincent's Hospital	Positive	Positive	Positive
<i>Tritrichomonas foetus</i>	University of Sydney	Positive	Positive	Negative
<i>Pentatrichomonas hominis</i> (ATCC® PRA-151)	American Type Culture Collection	Positive	Negative	Negative
<i>Trichomonas vaginalis</i> (PNG-21)	University of Technology, Sydney	Negative	Negative	Negative
<i>Tritrichomonas muris</i>	University of Sydney	Negative	Negative	Negative
<i>Hypotrichomonas acosta</i>	University of Sydney	Positive	Negative	Negative
<i>Tritrichomonas mobilensis</i>	University of Sydney	Positive	Negative	Negative
<i>Histomonas meleagridis</i>	University of Technology, Sydney	Positive	Negative	Negative
<i>Chilomastix mesnili</i>	SydPath, St Vincent's Hospital	Negative	Negative	Negative

*Vet Parasitol.* 2016 Aug 30;227:42-7. doi: 10.1016/j.vetpar.2016.07.025. Epub 2016 Jul 20.

## Detection of *Dientamoeba fragilis* in animal faeces using species specific real time PCR assay.

Chan D<sup>1</sup>, Barratt J<sup>2</sup>, Roberts T<sup>3</sup>, Phillips O<sup>3</sup>, Šlapeta J<sup>4</sup>, Ryan U<sup>5</sup>, Marriott D<sup>3</sup>, Harkness J<sup>3</sup>, Ellis J<sup>6</sup>, Stark D<sup>7</sup>.

# 200 human faecal specimens that were negative by GS assay (NB: no other pathogens)

Assay	Platform	No. positive	No. negative	% positive	Ct range	No. Ct >35
NeDa	Cepheid SmartCycle r II	17	183	9.2%	12.79 – 45.16	12
	Roche LightCycler 480	4	196	2.0%	39.59 – 45.00	4
	Bio-Rad 7500	14	186	7.5%	37.53 – 47.07	14

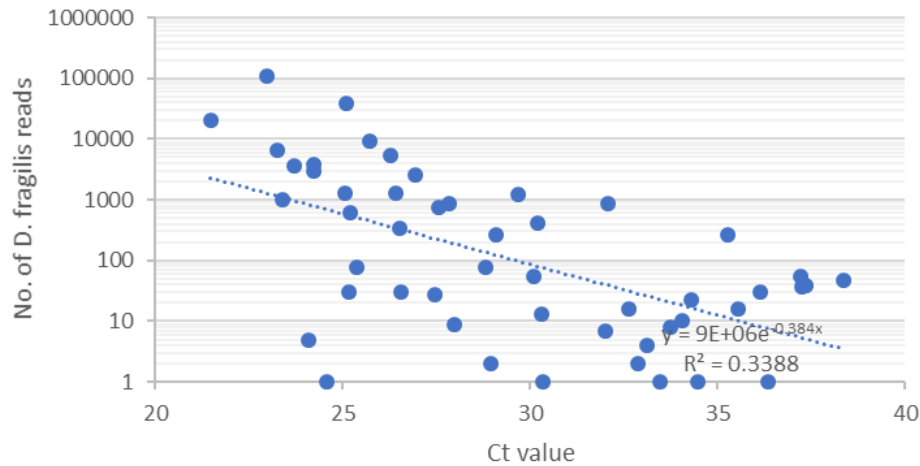
# 50 faecal specimens positive by GS

Assay	Platform	No. positive	No. negative	% positive	Ct range	No. Ct>35
<b>Genetic Signatures</b>	GS XXXX	50 (42) <sup>1</sup>	0 (8)	100%	21.47 – 38.37	8
<b>Verweiji</b>	Cepheid SmartCycler II	50 (47) <sup>1</sup>	0 (3)	100%	17.36 – 36.83	3

Ct cut-offs: 40 (35);

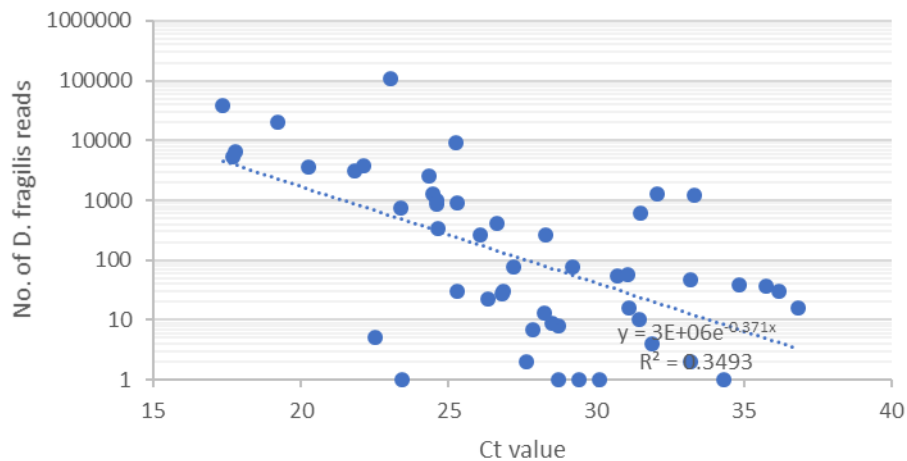
# 18S rDNA diversity profiling

GS Ct value vs No. D. fragilis seq reads



Empowering Australian Genomics

Verweiji Ct value vs No. D. fragilis seq reads



# Conclusion

- More study is required on *D. fragilis* for recognition as a pathogen
- High prevalence rates reported from NeDa
  - RT-PCR assessment
  - Re-evaluate cut off values
  - Recommend 40 not 50
- Comparative data from GS and NeDA; move to standardize assays
- NeDa cannot be used with animal samples – *T. foetus*





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- Rory Gough

