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Importance of validating RT-PCR methods for Dientamoeba fragilis

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

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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[1], Barratt J[2], Chan D[2], Ellis JT[2].

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"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[1], Rafay AM, Shenoy 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[1], Macfarlane L, Hughes-Thapa G, Jones SK, Whiteside TM.

Author information

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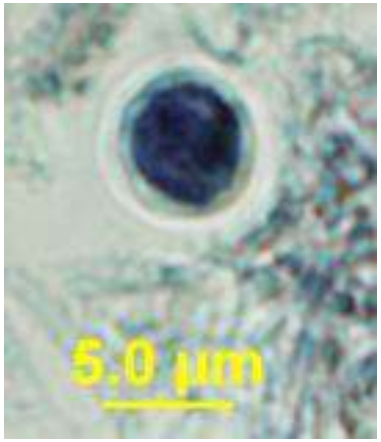
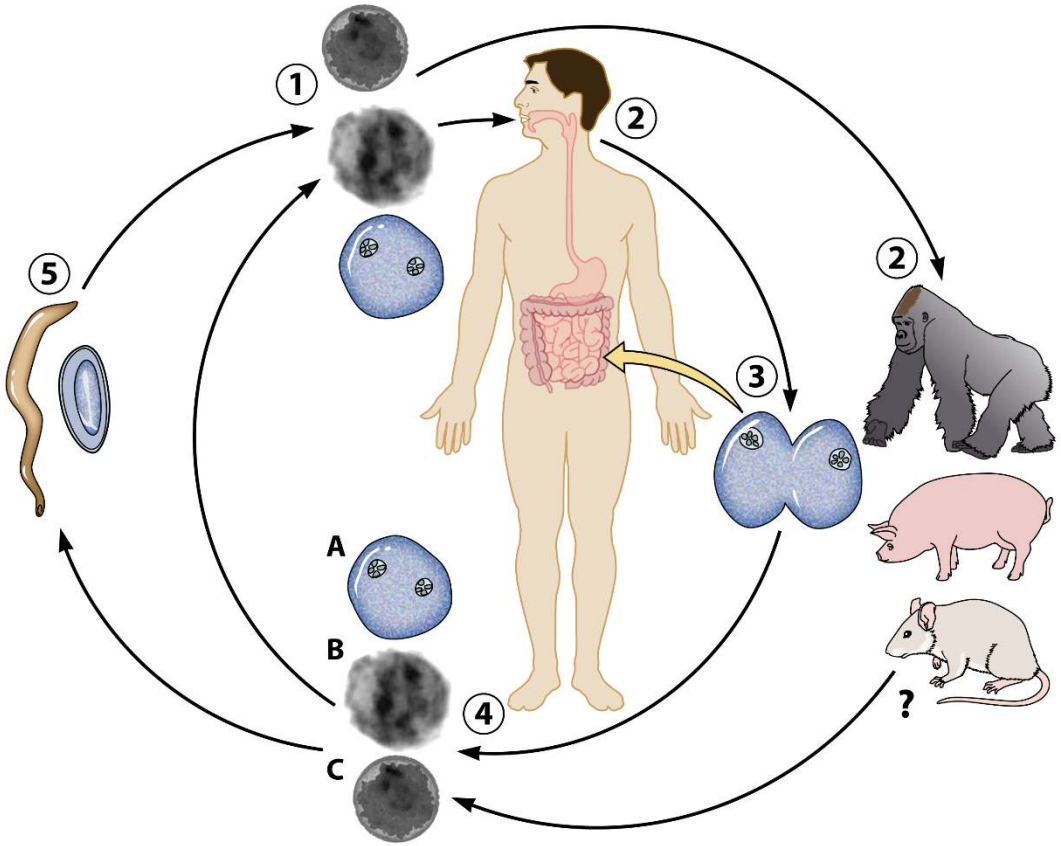
Transmission

- Involvement of helminth ova ?
- Direct transmission ?





Int J Parasitol. 2013 Oct;43(11):879-83. doi: 10.1016/j.ijpara.2013.06.003. Epub 2013 Jul 19.

Cyst formation and faecal-oral transmission of Dientamoeba fragilis--the missing link in the life cycle of an emerging pathogen.

Munasinghe VS[1], Vella NG, Ellis JT, Windsor PA, Stark D.

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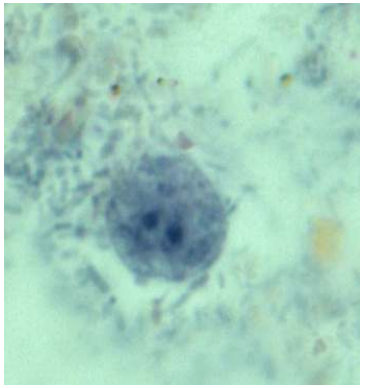
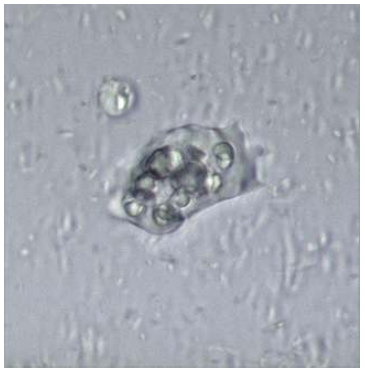
Laboratory Diagnosis





- 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[1], 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[1], Stark D, Ellis JT.

- PCR
  - Conventional
  - Nested
  - RT-PCR

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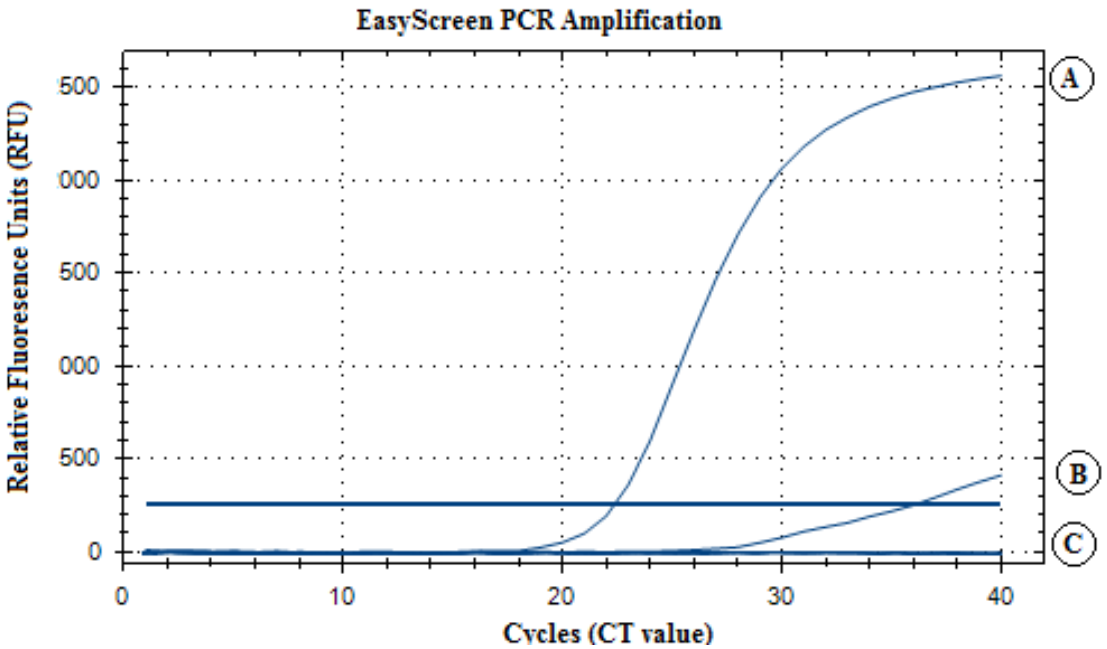
Genetic Signatures Pty Ltd

- 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.

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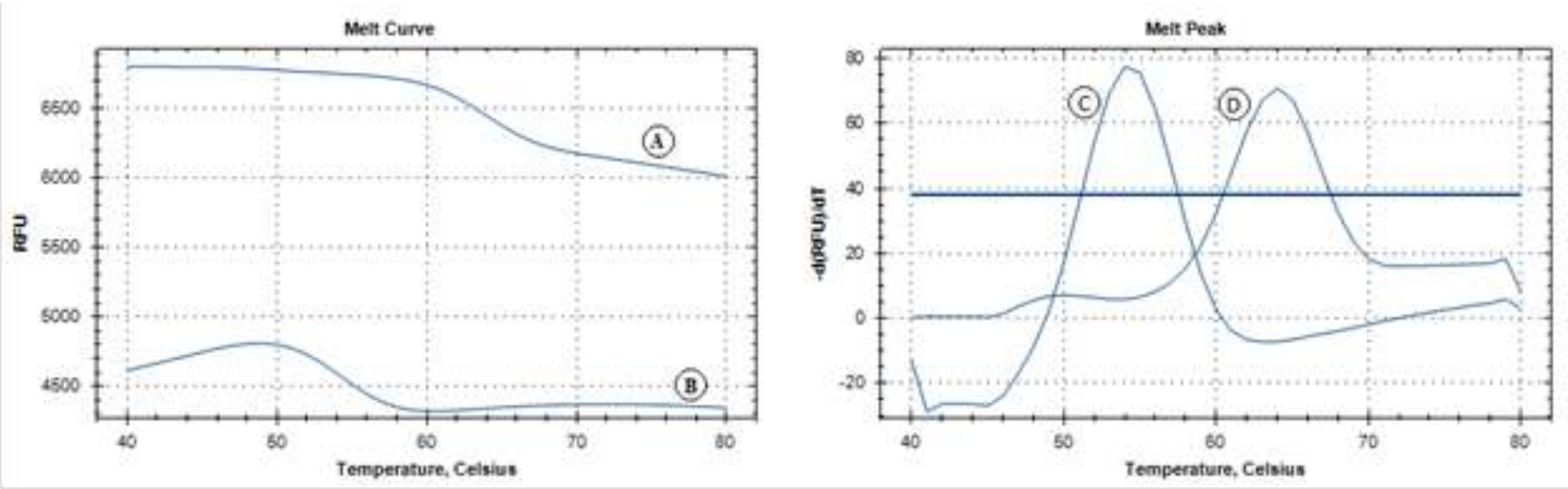
GS assay specificity



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

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Prevalence

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

SVH Sydney (2014-16)

Protozoa	Prevalence (%)
Blastocystis spp.	13.6%
Dientamoeba	**9.2%**
Giardia	2.6%
Cryptosporidium	1.3%
E. histolytica	0.3%

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Interesting observations from NeDa

STUDY	COUNTRY	YEAR	FINDINGS
de Wit et al	Netherlands	2001	*D. fragilis* found more frequently in healthy controls (14.6%) then those GI complaints (10.3%)
Roser et al	Denmark	2013	*D. fragilis* 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%

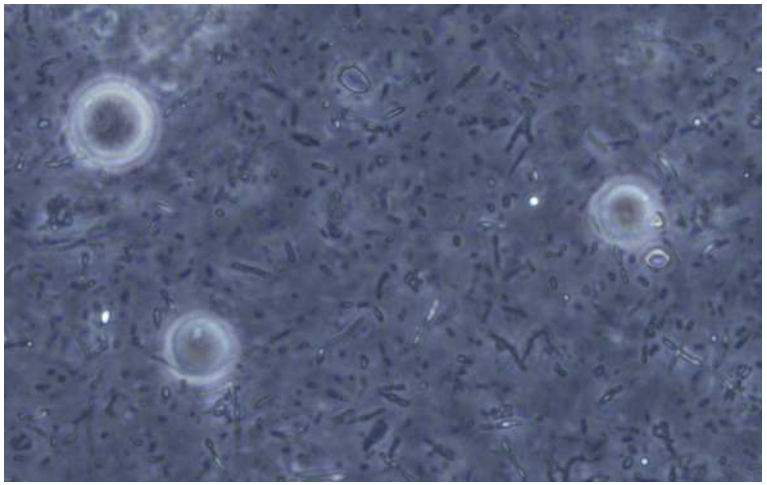
Mol Cell Probes. 2007 Oct-Dec;21(5-6):400-4. Epub 2007 May 29.

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

Verweij JJ[1], 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.

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Sensitivity

D. fragilis Trophozoites	Roche	AusDx	GS	Stark	NeDa
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

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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
Dientamoeba fragilis (Isolate P)	SydPath, St Vincent's Hospital	Positive	Positive	Positive
Tritrichomonas foetus	University of Sydney	Positive	Positive	Negative
Pentatrichomonas hominis (ATCC® PRA-151)	American Type Culture Collection	Positive	Negative	Negative
Trichomonas vaginalis (PNG-21)	University of Technology, Sydney	Negative	Negative	Negative
Tritrichomonas muris	University of Sydney	Negative	Negative	Negative
Hypotrichomonas acosta	University of Sydney	Positive	Negative	Negative
Tritrichomonas mobilensis	University of Sydney	Positive	Negative	Negative
Histomonas meleagridis	University of Technology, Sydney	Positive	Negative	Negative
Chilomastix mesnili	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[1], Barratt J[2], Roberts T[3], Phillips O[3], Šlapeta J[4], Ryan U[5], Marriott D[3], Harkness J[3], Ellis J[6], Stark D[7].

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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	Cephid SmartCycler 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
Genetic Signatures	GS XXXX	50 (42) [1]	0 (8)	100%	21.47 – 38.37	8
Verweiji	Cephid SmartCycler II	50 (47) [1]	0 (3)	100%	17.36 – 36.83	3

Ct cut-offs: 40 (35);

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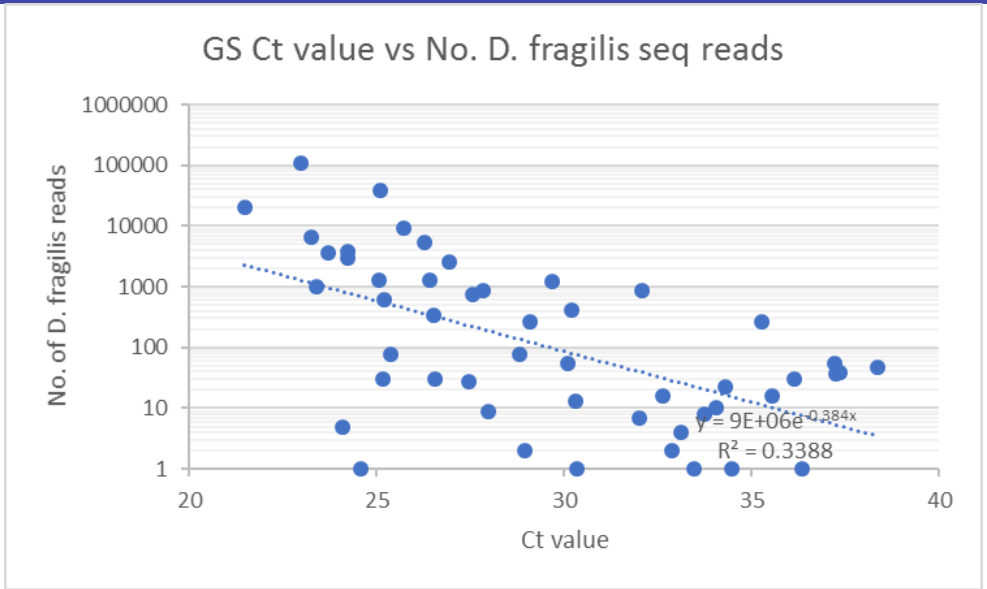
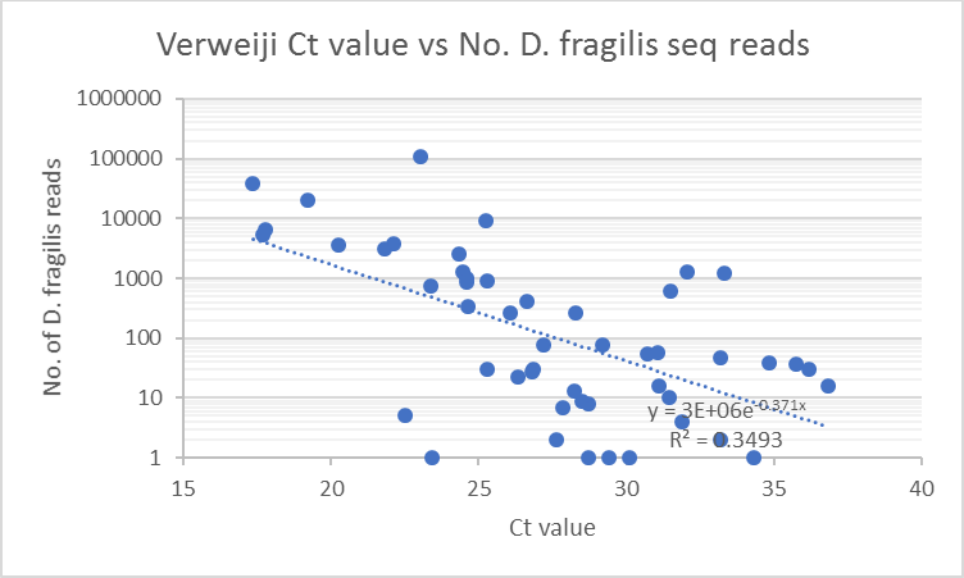
18S rDNA diversity profiling





agrf Empowering Australian Genomics

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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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