Evaluation of a multiplex Real-Time PCR panel for the diagnosis of intestinal helminths

N.F.J. Schillaci[1], M. Cuntrò[1], E. Oliva[2,13], L. Clemente[3,13], S. Varani[4,13], N. Menegotto[5,13], V. Besutti [6,13], A. Bruno[7], L. Petrullo[8,13], D. Oggioni[9], A. Carducci [10], C. Ghia [11], P. Mirri[12], C. Farina[1], A. Raglio[1,13], Gdl CoSP-AMCLI[13]

1 ASST-Papa Giovanni XXIII, Bergamo; 2 AUSL -IRCCS Santa Maria Nuova, Reggio Emilia; 3 ASUGI, Monfalcone; 4 Policlinico Sant'Orsola - Malpighi, Bologna; 5 ULSS 2 MarcaTrevigiana, Treviso; 6 Azienda Ospedaliera Universitaria, Padova; 7 IRCCS Policlinico San Matteo, Pavia; 8 AORN Ospedale dei Colli, Napoli; 9 Brianza - Ospedale di Vimercate; 10 ASST Valle-Olona - Presidio ospedaliero di Busto Arsizio; 11 Osp. Fatebenefratelli - Sacra Famiglia di Erba; 12 Ospedale di Legnano; 13 Gdl CoSP-AMCLI.

INRODUCTION

The diagnosis of intestinal parasites requires a high degree of specific skills on the part of the microbiologist. Recent evidence indicates that molecular biology has numerous advantages over microscopy, including greater sensitivity and specificity for protozoa, however, studies using home-made tests reveal some uncertainties for the diagnosis of helminths. The aim of this study was to evaluate a commercial multiplex real-time (RT)-PCR for the detection of intestinal helminths from fecal samples.

Fig. 1 Microscopy of researched helminths

MATERIALS AND METHODS

181 samples were collected from 15 Italian hospitals. They were examined with traditional techniques (macro-microscopic examination after concentration, culture for *Strongyloides stercoralis* (Ss) and serology for Ss and *Schistosoma* spp). From 178 samples (7 were found to be insufficient), DNA was extracted using the Microlab Nimbus IVD (Hamilton, Reno, Nevada) and then examined with RT-PCR (Biorad, CFX96) (Fig. 2) , using the Allplex GI-Helmint Assay kit. (Seegene), which detects *A.lumbricoides* (Al), *Hookworm* (A/N), Ss, *Taenia* spp (TA), *H. nana* (Hn), *T.trichiura* (Tt) and *E.vermicularis* (Ev) (Fig. 1).

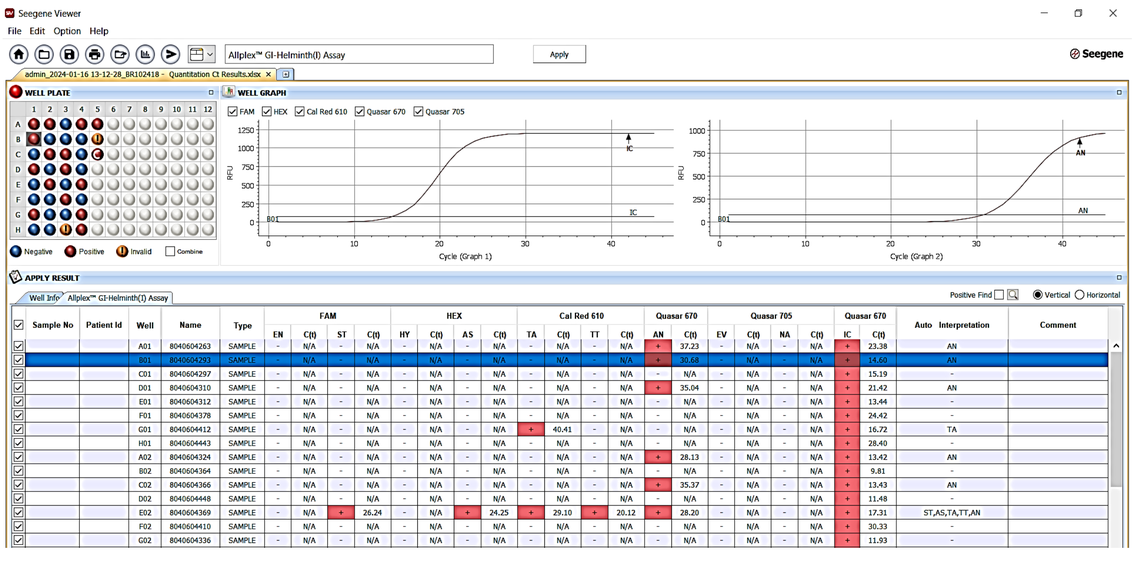
Fig. 2 Microlab Nimbus and Biorad CFX.

RESULTS

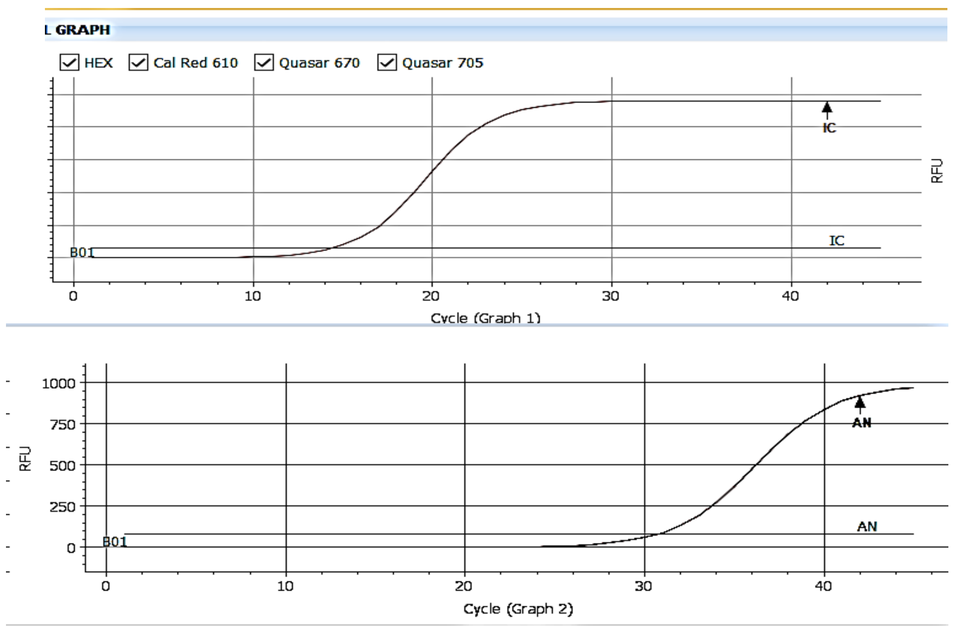
Of the 178 samples enrolled, traditional investigations identified: 4 negative samples, 16 helminths not included in the panel (10 *Schistosoma mansoni*, 5 *Bothriocephalus latus* and 1 *Dicrocelium dentriticum*); 2 only in culture for Ss and 5 only with positive serology for Ss, 15 Al; 11 *Ancylostomids*; 61 CE; 39S; 24 Taenia spp; 5 Hn and 1 Tt. Compared to traditional techniques, the specificity of the RT-PCR kit was 100% while the sensitivity was as follows: 66.6% for Al, 90.9% for Hookworms, 47.9% for Ev, 69.2% for Ss, 80% Hn and 100% for Taenia spp respectively (Fig2). In particular, PCR for Ss was positive in two cases with culture alone and in 2/5 with serology alone.

Results according to traditonal methods	N°	P	N	% positivity
Taenia spp	24	24	0	100%
A. lumbricoides	15	10	5	66,6%
Hookworms	11	10	1	90,9%
E. vermicularis	61	29	32	47,5%
S. stercoralis	38	27	12	69,2%
H. nana	5	4	1	80%
T. trichiura	1	1	0	NV

Fig. 3 Results based on agreement between standard methods and RT-PCR.



Sample No	Patient Id	Well	Name	Type	FAM EN	FAM C(t)	FAM ST	FAM C(t)	HEX HY	HEX C(t)	HEX AS	HEX C(t)	Cal Red 610 TA	Cal Red 610 C(t)	Cal Red 610 TT	Cal Red 610 C(t)	Quasar 670 AN	Quasar 670 C(t)	Quasar 705 EV	Quasar 705 C(t)	Quasar 705 NA	Quasar 705 C(t)	Quasar 670 IC	Quasar 670 C(t)	Auto Interpretation	Comment
		A01	8040604263	SAMPLE	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	+	37.23	-	N/A	-	N/A	+	23.38	AN	
		B01	8040604293	SAMPLE	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	+	30.68	-	N/A	-	N/A	+	14.60	AN	
		C01	8040604297	SAMPLE	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	+	15.19	-	
		D01	8040604310	SAMPLE	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	+	35.04	-	N/A	-	N/A	+	21.42	AN	
		E01	8040604312	SAMPLE	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	+	13.44	-	
		F01	8040604378	SAMPLE	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	+	24.42	-	
		G01	8040604412	SAMPLE	-	N/A	-	N/A	-	N/A	-	N/A	+	40.41	-	N/A	-	N/A	-	N/A	-	N/A	+	16.72	TA	
		H01	8040604443	SAMPLE	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	+	28.40	-	
		A02	8040604324	SAMPLE	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	+	28.13	-	N/A	-	N/A	+	13.42	AN	
		B02	8040604364	SAMPLE	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	+	9.81	-	
		C02	8040604366	SAMPLE	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	+	35.37	-	N/A	-	N/A	+	13.43	AN	
		D02	8040604448	SAMPLE	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	+	11.48	-	
		E02	8040604369	SAMPLE	-	N/A	+	26.24	-	N/A	+	24.25	+	29.10	+	20.12	+	28.20	-	N/A	-	N/A	+	17.31	ST,AS,TA,TT,AN	
		F02	8040604410	SAMPLE	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	+	30.33	-	
		G02	8040604336	SAMPLE	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	+	11.93	-	



CONCLUSIONS

As reported in the literature, the commercial kit also demonstrates a low sensitivity for Al and Ev, an average for Ss and a high value for Hookworms and *Taenia* spp and Hn. In contrast, RT-PCR correctly classified all samples as negative or positive for other species. In conclusion, this study suggests that RT-PCR technology can help in the diagnosis of intestinal helminth infections with the possibility of reducing the coproparasitological investigation to a single sample instead of the traditional three. However, the low sensitivity for some helminths underlines that the experience of the parasitologist is always fundamental for the confirmation of the results with the appropriate traditional techniques (microscopic, culture and serological) and the correct clinical and epidemiological analysis

BIBLIOGRAPHY

Evaluation of the Allplex GI-Helminth(I) Assay, the first marketed multiplex PCR for helminth diagnosis.
Brice Autier, Jean-Pierre Gangneux, and Florence Robert- Gangneux.
Parasite 28,33 (2021)

Sistema Socio Sanitario
Ospedale di Bergamo
Regione Lombardia
ASST Papa Giovanni XXIII

www.asst-pg23.it