Comparison of 12 Techniques for Detection of *Cryptosporidium Oocysts*

Kh. Arjomandzadeh and A. Dalimi

Parasitology Dept., Medical Faculty, Tarbiat Modarres University, PO Box 14155-4838, Tehran, I.R. of Iran

Summary

To select the most reliable method for detection of Cryptosporidium *oocysts in faecal samples, 12 methods were evaluated. Of the 30 confirmed positive samples tested by Sheather sucrose flotation, auramine-rhodamine, modified Ziehl Neelson, auramine O, acid fast DMSO and safranin methylene blue staining techniques 30 (100%), 30 (100%), 29 (96.6%), 29 (96.6%), 26 (86.6%) and 25 (83.3%) were indentified positive, respectively. Sucrose flotation technique was found to be more sensitive than the others and, due to availability and simplicity of the test, it is recommended as the method of choice.*

Introduction

Cryptosporidium is an intracellular-extracytoplasmic coccidian protosoan. It has been recognized in past decade as a significant entropathogen and causative agent of diarrhoea in human and animals. Different techniques for detection of *Cryptosporidium oocyst* in faecal specimens have been evaluated by different investigators. Giemsa staining technique and negative periodic acid-shciff staining were used by Horen(1), Giemsa staining, Ziehl Neelsen and safranin methylene blue techniques by Baxby et al.(2), safranin staining with heat by Baxby(3), acid fast dimethyl sulfoxide (DMSO) staining by Bronsdon(4) sucrose flotation and formalinethyl acetate sedimentation techniques by McNabb(5), indirect fluorescent antibody, auramine O and modified acid fast DMSO by Stibbs et al.(6), sucrose flotation methylene blue and fluorescent monoclonal antibody techniques by Baxby(7), acid fast, auramine rhodamine, indirect fluorescence method, direct and indirect fluorescent monoclonal antibody,

indirect diaminobenzydine and aminoethylcarbaxole by Arrowood and Sterling (8), ELISA technique by Anusz(9) modified Ziehl Neelsen staining, safranin methylene blue, auramine phenol, fluorescence and Sheather sucrose flotation technique by Moodley(10) . In present study 12 techniques, for detection of *Cryptosporidium oocysts* in faecal samples, were evaluated .

Materials and methods

Oocysts were obtained from faecal specimens from infected calves and lambs. For preparing samples for tests, oocysts were washed and concentrated by Sheather sucrose flotation technique. In each test, 10^4 oocysts per ml were suspended in formalin saline . Thereafter, detection were made by 12 techniques as follows: Formalin ether sedimentation and zinc sulphate flotation described by Adam et al.(11), modified Ziehl Neelsen staining by using the method of Henriksen and Pohlenz(12), safranin methylene blue staining as described by Baxby et al.(3), Sheather sucrose flotation as a method of Sheather(13), auramine-rhodamine by using the method of Arrowood and Sterling(8), acid fast and acridine orange as used by Garcia et al.(14, 15) and auramine O as described by Chermette et al. (16). Each test was repeated 30 times. Quality of oocysts with regard to internal structure, size and colour, were evaluated in each test by randomly choosing and studying at least 50 oocyst under light and fluorescence microscopes .

Results

The efficacy of 12 techniques in demonstrating oocysts of *Cryptosporidium* in 30 postive samples are shown in Table 1.

Sucrose flotation, auramine rhodamine, modified Ziehl Neelsen , auramine O, acid fast DMSO, safranin methylene blue showed the highest sensitivity for detection of *Cryptosporidium* oocysts in decreasing order. Assessment of the quality of the oocysts, with regard to their internal structure, dimensions and colours are presented in Table 2 for each test . The quality of the oocysts were best kept when sucrose flotation, auramine rhodamine , acid fast DMSO, modified Ziehl Neelsen and auramine O were used. Comparison of total time needed for completion of test

procedures showed that sucrose flotation with 11 minutes and modified Ziehl Neelsen staining with 75 minutes take the shortest and the longest time, respectively (Table 3).

With a view to simplicity, not requiring sofisticated laboratory facilities, and the ease with which the tests could be run the following techniques are recommonded: sucrose flotation, formalin ether, modified acid fast, modified Kinyoun carbolfuchsin, modified Ziehl Neelsen , safranin mehtylene blue and Giemsa staining methods (Table 3).

Table 1. Sensitivity of 12 techniques in detection of 30 positive samples of Cryptosporidium *oocyst.*

		Oocyst detectibility	
	Techniques	*No.*	*sensitivity (%)*
1	*Sucrose flotation*	*30*	*100*
2	*Auramine rhodamine*	*30*	*100*
3	*Modified Zieh1 Neelsen*	*29*	*96.6*
4	*Auramine O*	*29*	*96.6*
5	*Acid fast DMSO*	*26*	*86.6*
6	*Safranin methylene blue*	*25*	*83.3*
7	*Zinc flotation*	*22*	*73.3*
8	*Acridine orange*	*18*	*60*
9	*Modified Kinyoun carbolfuchsin*	*18*	*60.*
10	*Giemsa staining*	*16*	*53.3*
11	*Modified acid fast staining*	*14*	*46.6*
12	*Formalin ether*	*12*	*40*

Table 2. Quality comparison of Cryptosporidium *oocyst in 12 techniques*

	Techniques	Oocysts with clear internal structure		Oocyst Size	Oocyst colour	Background colour
		No.	%	(μm)		
1	Sucrose flotation	48	100	5.2	brigh	bright
2	Auramine rhodamine	48	96	5	greenish yellow	green
3	Modified Ziehl Neelsen	48	96	5	red	green
4	Auramine O	48	96	5	greenish yellow	green
5	Acid fast DMSO	50	100	4.9	red	green
6	Safranin methylene blue	28	56	4.9	light orange	green
7	Zinc flotation	35	70	5.2	bright	bright
8	Acridine orange	20	40	4.5	yellow orange	
9	Modified Kinyoun carbolfuchsin	40	80	4.8	red	green
10	Giemsa staining	25	50	4.5	blue	blue
11	Modified AF staining	38	76	4.5	red	green
12	Formalin ether	32	64	5.1	bright	light brown

Table 3. Comparison of rapidity , availability and simplicity of 12 techniques for detection of Cryptosporidium *oocyst*

	Techniques	*Time for Test procedure (min)*	*Time for Oocyst detection (min)*	*Total (min)*	*Availability & simplicity*
1	*Sucrose flotation*	*10*	*1*	*11*	*+3*
2	*Auramine rhodamine*	*30*	*1*	*31*	*+1*
3	*Modified Ziehl Neelson*	*75*	*1*	*31*	*+1*
4	*Auramine O*	*30*	*2*	*32*	*+1*
5	*Acid fast DMSO*	*14*	*2*	*32*	*+1*
6	*Safranin M. blue*	*20*	*2*	*22*	*+3*
7	*Zinc flotation*	*15*	*2*	*17*	*+2*
8	*Acridine orange*	*20*	*4*	*24*	*+2*
9	*Modified Kinyoun carbolfuchsin*	*15*	*3*	*18*	*+3*
10	*Giemsa staining*	*15*	*3*	*18*	*+3*
11	*Modified AF staining*	*20*	*4*	*24*	*+3*
12	*Formalin ether*	*10*	*5*	*15*	*+3*

Discussion

For detection of *Cryptosporidium* oocysts depending on the laboratory facilities and the number of specimens and individual experiences, various methods may be used. However, due to increase in number of patients with *Cryptosporidium* infections, selection of a technique to give the result in recovery and identification of the oocysts in faecal samples is very important for clinical laboratories. The results of present study confirmed that Sheather sucrose flotation, auramine rhodamine, modified Ziehl Neelsen , auramin O and acid fast DMSO are the most recommendable techniques for detection of oocysts of *Cryptosporidium*. The Sheather sucrose flotation technique due to the high oocyst detectability,

presentation of clear internal structure of oocysts, availability , speed and easiness, was found to be the best. This is in agreement with Moodley(10). In mild infections and in carrier patient, the Sheather sucrose flotation is recommended.

Acid fast DMSO and auramine rhodamine techniques have been respectively shown by Bronsdon(4) and Arrowood(8) to be very sensitive tests. The present study also showed auramine-rhodamine to be very sensitive and preferable over acid fast DMSO. In auramine rhodamine and auramine O techniques total time for test procedure and detection of oocysts is, relatively, long. Besides, the requirements for laboratory facilities to perform the test may decrease their practicability. In case of acute diarrhoea the acid fast DMSO method, due to clear presentation of internal structure of oocysts and rapid procedure, can be recommended as the test of choice. Our results in this respect is in accordance with Brondson(4). Modified Ziehl Neelsen technique with its characteristics is recognised as one of the most reliable technique for detection of *Cryptosporidium* oocysts. Our results, with this technique, are the same as those of Henriksen and Pohlenz(12). This technique is slow and time consuming, being the slowest of all 12 techniques tested. According to Baxby et al.(3) safranin methylene blue is rapid, simple with slightest chance of error and more sensitive than currently recommended Ziehl Neelsen methods. But our results showed that modified Ziehl Neelsen was more sensitive than safranin methylene blue. For detection of *Cryptosporidium* oocysts formalin ether sedimentation technique, due to to its poor sensitivity, is inconvenient. Other techniques such as zinc flotation, acridine orange, modified kinyoun carbolfuchsin, Giemsa staining and modified acid fast staining are not desirable.

Acknowledgement

The authors wish to express their sincere thanks to Dr. Gharavi for his valuable scientific cooperation in the course of this work.

References

1. *Horen, W.P. (1983). Detection of* Cryptosporidium *in human fecal specimens. Journal of Parasitology. 69: 622-4*

2. *Baxby, D., Blundell, N. (1983). Sensitive, Rapid, simple methods for detecting* Cryptosporidium *in faeces. Lancet. 11: 1149*
3. *Baxby, D., Blundell, N., Hart, Ca. (1984). The development and performance of a simple sensitive method for the detection of* Cryptosporidium *oocyst in faeces. Journal of Hygiene (Cambridge). 93: 317-23*
4. *Bronsdon, M. A. (1984). Rapid dimethyl sulfoxide-modified acid fast strain of* Cryptosporidium *oocysts in stool specimens. Journal of Clinical Microbiology. 19: 952-53*
5. *McNabb, S. J. N., Hensel, D.M., Wehlch, D.F., Heijbe, H., Mckee, G.L. and Istre, G.R. (1985). Comparison of sedimentation and flotation techniques for identification of* Cryptosporidium *spp. oocysts in a large outbreak of human diarrhoea. Journal of Clinical Microbiology. 22: 587-9*
6. *Stibbs, H. H. and Ongerth, J. E. (1986). Immunofluorescence detection of* Cryptosporidium *oocyst in faecal smears. Journal of Clinical Microbiology . 24: 517-521*
7. *Baxby, D., Blundell, N. (1988). Recognition and laboratory characteristics of a typical oocyst of* Cryptosporidium. *Journal of Infectious Diseases. 158: 1938-45*
8. *Arrowood, M. J. and Sterling, C. R. (1989). Comparison of conventional staining methods and monoclonal antibody based methods for* Cryptosporidium *oocysts detection. Journal of Clinical Microbiology. 27: 1490-95*
9. *Anusz, K. Z., Mason, P. H. (1990). Detection of* Cryptosporidium *parvum oocysts in bovine faeces by monoclonal antibody capture enzyme linked immunosorbent assay . Journal of Clinical Microbiology. 28: 2770-4*
10. *Moodley, D., Jackson, T. F., Gathiram, V., Van den Ende, J. (1991). A comparative assessement of commonly employed staining procedures for the diagnosis of cryptosporidiosis. South Africa Medical Jouranl (1991). 79: 314-317*
11. *Adam, K. M. c., Paul, J. and Zaman, V. (1971). Medical and veterinary parasitology. 1st ed. PP.167-168 . Edinburgh and London : Churchill Livingstone*
12. *Henriksen, S. A. and Pohlenz, J. F. L. (1981). Staining of* Cryptosporidium *by a modified Ziehl Neelsen technique. Acta Veterinaria Scandinavica. 22: 594-596*
13. *Sheather, A. L. 1923. The detection of intestinal protozoan and mange*

parasites by a flotation technique. Journal of Comparative Pathology. 36: 266-275

14. *Garcia, L. S., Brucker, D. A., Brever,T. C. and Shimizu,R.y. (1983). Techniques for the recovery and idetification of* Cryptosporidium *oocysts from stool specimens. Journal of Clinical Microbiology . 18: 185-190*

15. *Garcia, L. S. (1986). Laboratory methods for diagnosis of parasitic infections. P.775-858 . In S. M. Finegold and E. J. Baron, Bailey and Scott's diagnostic microbiology, 7th ed. C.V. Mosby Co., St. Louis.*

16. *Chermette, R. and Boufassa-Ouzrout, S. (1988). Cryptosporidiosis. A cosmopolitan disease in animals and in man. (Technical report ser.No.5) 2nd. Office international des Epizooties. Paris.*