DIFFERENTIAL STAINING OF CRYPTOSPORIDIA BY ANILINE-CARBOIL-METHYL VIOLET AND TARTRAZINE IN SMEARS FROM FAECES AND SCRAPINGS OF INTESTINAL MUCOSA

Cryptosporidiosis is one of the new zoonoses of increasing importance in both human and veterinary medicine. In the last years, the protozoa causing this disease have rapidly spread among various animal species and even in man (Taipori R., Microbiol. Rev. 47: 84—96, 1983).

Cryptosporidiosis is usually an intestinal disease. Rapid and simple methods including the examination of faeces smears and scrapings of intestinal mucosa are preferably used in its diagnostics. The samples were previously stained with Giensa method, either in its classical form or modified by Wollbach, but this method was found to be little suitable for this purpose due to some deficiencies. Among others it is the fact that the protozoa can be hardly distinguished from the other components of smears and can be easily mistaken for yeasts (Angus K. W. et al., Vet. Rec. 95: 173, 1981). Moreover, the method by Giensa requires an experience in the diagnostics of cryptosporidiosis. For those reasons other staining methods for the identification of cryptosporidium are still being searched and tested. For the time being, the only methods without the above deficiencies of Giensa method are the modifications by Zoth—Nielson staining after Kinnyoun (Ma P. and Soave R., J. infect. Dis. 147: 824—828, 1983) and by Henriksen and Poblenz (Acta vet. scand. 22: 594—596, 1981). Cryptosporidium selectively stain red on a green background. The smears and scrapings must be thin, otherwise the preparation is stained too deeply and it is little transparent. According to our experience, differential staining of cryptosporidium in smears from faeces and scrapings of intestinal mucosa by aniline-carbol-methyl violet followed by tartrazine staining was proved to be suitable.

Preparation of solutions: Solution of aniline-carbol-methyl violet: 0.6 g methyl violet, 1 ml aniline, 1 g phenol, 30 ml 96 % alcohol, distilled water added after solution up to 100 ml. The solution is filtered on the next day and can be stored for several months.

Additional staining solution: 1 % solution of tartrazine in 1 % acetic acid.

Staining process: 1. Dry smears or scrapings fix in methyl alcohol for 5 min at room temperature.
2. Stain with a solution of anilin-carbol-methyl violet for 30 min and rinse in tap water.
3. Differentiate in 1—2 % sulphuric acid for 30 sec up to 2 min, till the sample is pale blue-violet and then again rinse in tap water.
4. Counterstain with tartrazine for 30 sec—1 min, briefly rinse in tap water and leave to dry.
5. Smears or scrapings used for the examination can be dry or covered with a thin layer of paraffin oil or mounted in Canada balsam.

Result of staining: Cryptosporidium stain blue to blue-violet on a yellow to yellow-green background.

Our staining method for the identification of cryptosporidium is reliable, relatively simple, markedly contrast and does not stain yeasts and other components of smears. The preparations are transparent even if thicker smears or scrapings are used.

Methyl violet (Merek, Darmstadt, C.I.Nr. 42535 or Lachema, Brno) and tartrazine (Lachema, Brno) were used in staining solutions.

Methyl violet can be replaced by the same quantity of gentian violet or crystal violet and orange G can be used instead of tartrazine.

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