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Morphologic and Molecular Characterization of New *Cyclospora* Species from Ethiopian Monkeys: *C. cercopitheci* sp.n., *C. colobi* sp.n., and *C. papionis* sp.n.

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Appendix I

Stool Processing Procedures

Stool samples collected in 1997 were processed by a conventional formalin-ethyl acetate sedimentation concentration procedure. A portion of the sediment was examined by UV fluorescent microscopy (18). Some positive samples were also stained by the acid-fast or hot safranin techniques (19). For stools collected in 1998, an aliquot of each sample was washed because potassium dichromate suppresses the autofluorescence of the oocysts. Any oocysts observed in the samples examined from the collection of 1998 were graded as either sporulated or unsporulated. Part of the remaining specimen in potassium dichromate was processed over sucrose gradient to harvest oocysts. Purified oocysts were returned to clean 2.5% potassium dichromate solution for storage, and portions of the purified oocysts were used for morphologic studies.

To excyst sporocysts and sporozoites, one of two procedures was used. If the intent was to obtain free sporocysts, but not sporozoites, a small drop of solution containing oocysts was placed on a glass slide and covered. To induce rupture of the oocyst wall, the coverslip was tapped with a blunt glass rod and then rotated on the slide. To obtain free sporozoites, one of two

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excysting fluids were used: either a solution made up in DMEM containing 0.25% trypsin plus 0.75% sodium taurocholate or a solution made up in PBS containing 0.25% trypsin, 0.75% sodium taurocholate, and 20 mM cystine HCl. Both solutions worked equally well. The oocysts were incubated in the excysting fluid for 2 hours in a heat block at 37°C.

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