Comparison of Use of Enzyme-Linked Immunosorbent Assay-Based Kits and PCR Amplification of rRNA Genes for Simultaneous Detection of Entamoeba histolytica and E. dispar

D. MIRELMAN,* Y. NUCHAMOWITZ, AND T. STOLARSKY
Department of Membrane Research and Biophysics, Weizmann Institute of Science, Rehovot 76100, Israel

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A comparison of the use of three commercially available enzyme-linked immunosorbent assay-based kits and PCR amplification of rRNA genes to detect and differentiate Entamoeba histolytica from E. dispar was carried out. Only the Techlab kit did not cross-react with E. dispar antigens, but it was 100 times less sensitive than PCR in detection of and differentiation between the two types of Entamoeba.

A number of epidemiological studies and recent reports have shown that (i) all cases of invasive amebiasis are caused by Entamoeba histolytica and that the nonpathogenic type, E. dispar, is never detected in extraintestinal lesions (2, 11, 14, 21, 22, 26); (ii) persons infected with E. histolytica are occasionally asymptomatic (11); (iii) a significant percentage of individuals, especially in areas of endemicity, can be simultaneously infected with both E. dispar and E. histolytica (1, 19, 24); and (iv) persons found to be infected with E. dispar sometimes have intestinal symptoms and/or high titers of antiamebic antibodies (12, 13). Among the main reasons for the unclear picture of the epidemiology and frequently quoted world rates of amebiasis (12, 13) are the uncertainties in the detection and differentiation of E. histolytica and E. dispar. The main purpose of the present study was to evaluate three commercially available diagnostic enzyme-linked immunosorbent assay (ELISA)-based kits for E. histolytica for their levels of sensitivity to detect and ability to differentiate between small amounts of E. histolytica and E. dispar and to compare them to an established PCR procedure which selectively amplifies the different rRNA genes of the two types of amebae (5, 19, 24).

ELISAs. Axenic cultures of E. histolytica HM-1:IMSS and xenic cultures of E. dispar SAW 1734R c1AR were grown in TYI-S-33 medium as previously described (6). Trophozoites were counted in a hemocytometer and lysed by freeze-thawing in phosphate-buffered saline containing a mixture of various protease inhibitors (leupeptin, 100 μg/ml; phenylmethylsulfonyl fluoride, 2 mM; iodoacetic acid, 5 mM; and phenanthrolene, 1 mM [all from Sigma Aldrich]).

Samples containing different amounts of trophozoite lysates were tested with three commercially available kits for detection of E. histolytica (a kit from Alexon Co., Sunnyvale, Calif.; a kit from Techlab, Blacksburg, Va.; and the Optimum S kit from Merlin Diagnostica, Bornheim, Germany) according to the manufacturers' instructions and with the reagents supplied in each kit by the manufacturer. Optical density was determined in an ELISA reader (Biotek Instruments). As an additional comparison, ELISAs were also performed with two monoclonal antibodies (MAbs) prepared in our laboratory. MAB 318-28 was prepared as previously described (4). MAB 116 was prepared by immunizing BALB/c mice according to the method of Galfre and Milstein (7) with a lipophosphoglycan (LPG) preparation, extracted and purified by hydrophobic and anion exchange from membranes of strain HM-1:IMSS trophozoites as described previously (18).

PCR. PCR was performed for 30 cycles and used as a template a DNA fraction that was solubilized after boiling a suspension of trophozoites (2 × 10⁶/ml in phosphate-buffered saline) in a water bath (10 min) followed by centrifugation (10,000 × g for 10 min). Each of the two sets of selective oligonucleotide primers, for the E. histolytica and E. dispar small subunit rRNA genes, generated a product of 870 bp, as previously described (5, 15).

The results obtained with the different commercially available ELISA-based kits (Table 1) show that the most sensitive one is the Merlin Optimum S kit. It can easily detect antigen from 100 trophozoites of E. histolytica/well. On the other hand, this kit appears not to be sufficiently selective, as the antibodies against the serine-rich antigen, which are reportedly specific for E. histolytica (23), were found to cross-react, at higher concentrations (100 to 1,000 trophozoites/well), with antigens of E. dispar. This lack of selectivity can be a serious drawback, as it could lead to some false-positive E. histolytica results, especially since the majority of infected persons harbor E. dispar (2, 25, 26).

The Techlab kit, which uses MAbs against the Gal-specific lectin of E. histolytica strains (20, 21), was very selective and reacted, as reported, only with E. histolytica. However, its level of detection of amebic antigen, as also indicated by the manufacturer, was quite low and required approximately 1,000 trophozoites per well (8, 9). The high levels of E. histolytica antigen needed for detection suggest that this kit may not be able to detect low-level E. histolytica infections, especially in asymptomatic individuals in areas of endemicity who may be simultaneously infected with both types of parasites (1, 19, 24). The Alexon kit, which consists of anti-E. histolytica polyclonal antibodies which do not differentiate between E. histolytica and E. dispar, has fair detection sensitivity, and amebic antigens of either type can be detected at 100 trophozoites/well.

The levels of detection and selectivity observed with the two laboratory MAbs, MAB 318-28, which is specific for a lysine-rich surface antigen that is expressed on E. dispar strains (4), and MAB 116, which is specific for an LPG molecule present on the surfaces of virulent E. histolytica strains (17), were in the range of the manufactured kits (Table 1).
The levels of detection observed with the various antibody-based systems were found to be >100-fold less sensitive than those that can be attained by PCR amplification of the rRNA genes of amebae. As shown in Fig. 1, PCR amplification can clearly detect the DNA from one single ameba in the sample. Moreover, the selectivity of PCR amplification of rRNA genes appears to be far superior to that of the antibodies, as it can detect one trophozoite of *E. histolytica* even in the presence of a 1,000-fold excess of *E. dispar* and vice versa. This is not surprising, since trophozoites have multiple copies of the rRNA genes (5, 10, 16).

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Our results clearly indicate the advantages of PCR over ELISA-based kits in the ability to both detect and determine the type of amebae. Among the frequently mentioned arguments in favor of the use of ELISA versus PCR techniques are the convenience and lower price of ELISA-based kits, especially for the routine diagnostic laboratory in areas of endemcity. In view of the considerable improvements in the automation and simplification of PCR procedures for clinical sampling directly from stools (1, 3), as well as in the prices of equipment, reagents, and product detection systems that have been recently achieved, its comparison with ELISA has to be

**TABLE 1. Comparison of different antibodies for detection of *E. histolytica* and *E. dispar* by ELISA**

<table>
<thead>
<tr>
<th>Antibody source or antibody</th>
<th>No. of trophozoites/well</th>
<th>Detection of <em>E. histolytica</em></th>
<th>Detection of <em>E. dispar</em></th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E. histolytica</td>
<td>E. dispar</td>
<td>SAW</td>
</tr>
<tr>
<td>Techlab kit</td>
<td>$10^4$</td>
<td>1.058 ± 0.07</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$10^3$</td>
<td>0.102 ± 0.01</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$10^2$</td>
<td>0.01</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Alexon kit</td>
<td>$10^4$</td>
<td>0.874 ± 0.03</td>
<td>0.648 ± 0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$10^3$</td>
<td>0.480 ± 0.05</td>
<td>0.350 ± 0.03</td>
<td></td>
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<tr>
<td></td>
<td>$10^2$</td>
<td>0.164 ± 0.03</td>
<td>0.085 ± 0.01</td>
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</tr>
<tr>
<td>Merlin Optimum S kit</td>
<td>$10^4$</td>
<td>1.764 ± 0.01</td>
<td>0.683 ± 0.02</td>
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<tr>
<td></td>
<td>$10^3$</td>
<td>1.692 ± 0.06</td>
<td>0.149 ± 0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$10^2$</td>
<td>0.419 ± 0.02</td>
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<tr>
<td>Weizmann MAb 116</td>
<td>$10^4$</td>
<td>0.626 ± 0.03</td>
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<td></td>
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<tr>
<td></td>
<td>$10^3$</td>
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<td></td>
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<td>0.02</td>
<td>ND</td>
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<tr>
<td>Weizmann MAb 318-28</td>
<td>$10^3$</td>
<td>0.02</td>
<td>0.776 ± 0.04</td>
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<tr>
<td></td>
<td>$10^2$</td>
<td>0.01</td>
<td>0.410 ± 0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>ND</td>
<td>0.03</td>
<td></td>
</tr>
</tbody>
</table>

* Wells contained lysates.

* Values given in optical density units after color development of triplicate samples. ND, not detected.

FIG. 1. Agarose gel separation of PCR-amplified products of the small subunit rRNA genes of *E. histolytica* (*E. h.*) and *E. dispar* (*E. d.*) (5, 15). DNA was prepared and diluted from a predetermined number of trophozoites (see Materials and Methods). (Left) DNA from one *E. histolytica* trophozoite detected in the presence of $10^5$ *E. histolytica* trophozoites; (right) DNA from one *E. histolytica* trophozoite detected in the presence of $10^5$ *E. dispar* trophozoites.
carefully reevaluated by impartial experts. With respect to the epidemiology of amebiasis, it is important to accumulate data that are more accurate on the prevalence of *E. histolytica* in carriers and patients in various parts of the world. Based on the present comparative study on the available detection systems, this should be achieved preferably by PCR.

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REFERENCES


