A kit for in vitro isolation of trypanosomes in the field: first trial with sleeping sickness patients in the Congo Republic

D. Aerts¹, P. Truc², L. Penchenier³, Y. Claes⁴


Short Report

A kit for in vitro isolation of trypanosomes in the field: first trial with sleeping sickness patients in the Congo Republic

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Table. Trypanosome isolation by KIVI and by rat inoculation from ten sleeping sickness patients in the Congo Republic

<table>
<thead>
<tr>
<th>Patient</th>
<th>CATTa</th>
<th>LNb</th>
<th>MHCc</th>
<th>Ratd</th>
<th>Anticoagulant²</th>
<th>KIVI</th>
<th>R1f</th>
<th>R2g</th>
<th>Stock no.</th>
</tr>
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<tr>
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<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Minja</td>
<td>+</td>
<td>NS</td>
<td>+</td>
<td>41</td>
<td>Li</td>
<td>27-36</td>
<td>20-4</td>
<td></td>
<td>ITMAP 2202</td>
</tr>
<tr>
<td>Balpa</td>
<td>+</td>
<td>NS</td>
<td>+</td>
<td>NEG</td>
<td>He+Li</td>
<td>24-36</td>
<td>20-4</td>
<td></td>
<td>ITMAP 2203</td>
</tr>
<tr>
<td>Silou</td>
<td>+</td>
<td>T+</td>
<td>++</td>
<td>38</td>
<td>He+Li</td>
<td>31-36</td>
<td>20-16</td>
<td></td>
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<tr>
<td>Bissi</td>
<td>+</td>
<td>T+</td>
<td>+</td>
<td>NEG</td>
<td>Li</td>
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<td>20-4</td>
<td></td>
<td>ITMAP 2205</td>
</tr>
<tr>
<td>Pave</td>
<td>+</td>
<td>NS</td>
<td>+</td>
<td>NEG</td>
<td>Li</td>
<td>NEG</td>
<td></td>
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<tr>
<td>April 1990</td>
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<tr>
<td>Dicar</td>
<td>+</td>
<td>T+</td>
<td>+</td>
<td>NEG</td>
<td>Li</td>
<td>18-25</td>
<td>15-5</td>
<td></td>
<td>ITMAP 2208</td>
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<tr>
<td>Koa</td>
<td>NEG</td>
<td>T+</td>
<td>++</td>
<td>26</td>
<td>He</td>
<td>NEG</td>
<td></td>
<td></td>
<td>ITMAP 2209</td>
</tr>
<tr>
<td>Bousa</td>
<td>+</td>
<td>T+</td>
<td>++</td>
<td>NEG</td>
<td>Li</td>
<td>15-54</td>
<td>15-3</td>
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<tr>
<td>Houm</td>
<td>+</td>
<td>NEG</td>
<td>+++</td>
<td>ND</td>
<td>Li</td>
<td>NEG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Babi</td>
<td>+</td>
<td>T+</td>
<td>+</td>
<td>NEG</td>
<td>Li</td>
<td>NEG</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

¹Card agglutination test for trypanosomes (MAGNUS et al., 1978).
²Lymph nodes: NS, not swollen; T+, trypanosomes seen in lymph fluid.
⁴Rat inoculation: time (days) to positive by thin blood film examination; NEG: negative by wet smear over 2 months; ND, not done.
⁵Li: Liquide Roche (sodium polyanetholesulphonate) anticomplementary anticoagulant (Le Ray et al., 1970), or a Monovette® (Sarstedt) syringe containing heparin (lithium salt). The blood mixture was then dispensed equally into 2 vials (R1), each containing 10 ml of GLSH-DCA; one also contained a supplement of antibiotics (penicillin, 5000 i.u/ml; gentamycin, 200 µg/ml; 5-fluorocytosine, 50 µg/ml). The vials were mixed by gentle manual agitation and kept at room temperature. In the initial laboratory tests, a minimum concentration of 2.5 × 10⁴ trypanosomes per ml could be transformed and cultured.

Results from 10 patients sampled on 2 separate occasions (1989, 1990) in the Bouenza focus, Republic of the Congo.
Congo, are presented in the Table; at the same time, 1 ml of blood from each patient was inoculated into a rat. The inoculated KIVIs were sent or brought back to Europe, where 2 to 4 weeks after the initial inoculation, they were examined. Subinoculation (R2) was performed into blood-agar (TOBIE, 1949) and Cunningham’s medium (CUNNINGHAM, 1977). R1 and R2 vials were kept under observation for one month.

Of the 10 sleeping sickness patients with low-grade parasitaemias, 7 provided a positive culture in KIVI whereas only 3 were infective to rats. Isoenzyme characterization for 24 loci (TRUC, 1991) showed that all the isolates belonged to classical T. b. gambiense. Our results also confirm the low infectivity of T. b. gambiense in Central Africa to rodents.

In this preliminary study, KIVI was more effective than rat inoculation in isolating human parasites. Liquoïde (Roche) was confirmed to be the best anticoagulant (WEINMAN, 1960). The operational value of KIVI in field work was demonstrated by the long period during which it sustained the growth and viability of procyclic trypanosomes (25-54 d; average 40 d). Work is now in progress to improve the KIVI and test it in other areas of Africa, and to evaluate its diagnostic value for hosts with subpatent infections.

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References


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