The haematocrit centrifuge for the detection of trypanosomes in blood

PATRICK T. K. WOO

Department of Zoology, University of Guelph, Guelph, Ontario

Received May 3, 1969


A capillary tube holder, devised to minimize shadow effects during microscopic examination of haematocrit capillary tubes for trypanosomes, is described. A blood dispenser, designed to remove trypanosomes from centrifuged tubes, resulted in thinner smears of trypanosomes covering a smaller area. The anterior end of centrifuged trypanosomes was abnormally thickened and the kinetoplast displaced posteriorly.

Introduction

The first simple microtechnique for the concentration of trypanosomes by centrifugation was described in 1955 (Devignat and Dresse 1955). Later the haematocrit centrifuge technique was used routinely in the diagnosis of blood protozoa (Bennett 1962).

The main difficulty in the use of the haematocrit for detecting trypanosomes is the diffraction of light which causes shadows at the two sides of the capillary tube when it is examined under a microscope. This shadow effect may not be important in the detection of heavy infections or of rather large trypanosomes (approximately 40-70 \( \mu \) in length, and 10-25 \( \mu \) in width), but it hinders detection of smaller trypanosomes (approximately 15-25 \( \mu \) in length, and 2-3 \( \mu \) in width) especially if the infection is low and the trypanosomes are at, or near, the sides of the capillary tube.

Since the haematocrit centrifuge technique is especially useful for the detection of trypanosomes in low infections, a good method is needed for the recovery of specimens from capillary tubes for staining and eventual identification. Smears made with large amounts of serum often contain cracks after fixation and staining. Also, trypanosomes stain poorly and are widely distributed on the smear.

Bennett (1962) assumed that centrifugation does not alter the morphology of trypanosomes. This assumption should be examined because the haematocrit method is especially useful for the concentration and isolation of trypanosomes for eventual identification.

In the present report techniques are described to minimize shadow effects produced during microscopic examination of capillary tubes and to recover trypanosomes from these tubes. In addition, the effect of centrifugation on the morphology of trypanosomes was investigated.

Materials and Methods

A capillary tube holder (Fig. 1) was devised to minimize shadow effects. Two rectangular pieces of glass (1.2 mm thick) were fixed 1.5 mm apart on a standard glass microscope slide. The capillary tube is placed in the slot and a drop of immersion oil (refractive index 1.524 at 20 \(^{\circ}\)C) is placed on top of the capillary tube. Oil fills the space between the capillary tube and the two pieces of glass. This arrangement reduces the effects of light diffraction caused by the curvature of the capillary tube and facilitates observation of trypanosomes.

A blood dispenser (Fig. 2) was made of a rubber bulb (A) connected to a second rubber bulb (D) by a piece of glass tubing (B) which serves as a handle. A disposable needle (C) (26 G) is inserted into the rubber bulb (D).

After centrifugation, the trypanosomes are usually found in, or just above, the buffy layer. The tube is cut approximately 2 mm below the buffy layer in the packed red cells and the shorter portion discarded. The uncut end of the tube is inserted into a hole (1 mm diameter) in bulb D. Needle C releases the air pressure created in the system when the haematocrit tube E is inserted into the bulb D; this pressure would otherwise force out the contents of the tube prematurely. Needle C also partially releases pressure when bulb A is squeezed to dispense contents of the tube. This partial release of pressure facilitates control of the volume of fluid to be dispensed onto a glass slide from which a smear is made.

By applying gentle pressure to bulb A, an operator can dispense onto slides a small amount of red cells (R), the buffy layer (T) containing the trypanosomes, and a small volume of serum.

Trypanosoma pipientis, a monomorphic trypanosome of the leopard frog (Rana pipiens), was used to study the effects of centrifugation. A frog, naturally infected with T. pipientis, was pithed and 12 blood smears were made.
from blood recovered from the heart. The 12 smears were arbitrarily divided into series A and B (six smears in each). Five haematocrit tubes containing heart blood were centrifuged for 6 minutes at 11 500 r.p.m. (International model MB microcapillary centrifuge). After centrifugation, the tubes were cut and the trypanosomes dispensed onto slides from which smears were made (series H).

The smears, air-dried and fixed in 10% buffered formalin (Lehmann 1964), were later stained in Giemsa's stain.

The image of the trypanosome was projected onto drawing paper with a camera lucida. A line was drawn down the middle of the trypanosome from one extremity to the other (including the free flagellum). The positions of the kinetoplast, the nucleus (at the center), and the anterior end and maximum width of the body were marked on this line. Measurements were made with a pair of fine dividers set so that the points were separated by a distance equal to 1 micron on the projected image.

Results

After they were stained, 43 specimens were measured from series A, 44 from series B, and 44 from series H (Table I). The Student t test was used to compare the means of the three series (Table II).

Significant differences between the means of the two groups of uncentrifuged trypanosomes (A and B) were not detected (Table II). The kinetoplast was displaced toward the posterior end in centrifuged trypanosomes (Table I). Also, the anterior end of centrifuged trypanosomes was distorted (cf. Figs. 3-4). Because of this distortion, the greatest width of the centrifuged trypanosome was in the anterior region of the body instead of the middle. The greatest width of centrifuged trypanosomes was significantly greater than that of uncentrifuged trypanosomes (Table II). The position of the nucleus, the total length, and the length of the free flagellum were not significantly different in centrifuged and uncentrifuged trypanosomes (Table II).
TABLE I

<table>
<thead>
<tr>
<th>Sample size</th>
<th>PK</th>
<th>KN</th>
<th>PN</th>
<th>PA</th>
<th>FF</th>
<th>BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Series A (not centrifuged) 43</td>
<td>5.99 ± 0.9459</td>
<td>16.64 ± 1.9766</td>
<td>22.63 ± 2.2878</td>
<td>43.03 ± 3.3183</td>
<td>25.54 ± 3.9021</td>
<td>3.03 ± 0.7514</td>
</tr>
<tr>
<td></td>
<td>(5-8)</td>
<td>(13-17.5)</td>
<td>(18.75-24)</td>
<td>(35.8-48)</td>
<td>(18.5-30.5)</td>
<td>(2.0-4.25)</td>
</tr>
<tr>
<td>Series B (not centrifuged) 44</td>
<td>5.81 ± 0.9761</td>
<td>16.93 ± 1.8624</td>
<td>22.55 ± 2.1574</td>
<td>42.95 ± 3.2186</td>
<td>26.02 ± 3.8125</td>
<td>3.11 ± 0.8215</td>
</tr>
<tr>
<td></td>
<td>(4.5-9)</td>
<td>(15-19.5)</td>
<td>(19-25.5)</td>
<td>(39-50.5)</td>
<td>(17.5-29.75)</td>
<td>(2.5-4.0)</td>
</tr>
<tr>
<td>Series H (centrifuged) 44</td>
<td>5.23 ± 1.0832</td>
<td>17.52 ± 2.2964</td>
<td>22.76 ± 3.0212</td>
<td>43.65 ± 6.4661</td>
<td>25.06 ± 3.0632</td>
<td>3.31 ± 0.7670</td>
</tr>
<tr>
<td></td>
<td>(3.0-7.0)</td>
<td>(14.5-22.5)</td>
<td>(19.75-27.5)</td>
<td>(35.8-51)</td>
<td>(19.5-30.75)</td>
<td>(2.5-4.25)</td>
</tr>
</tbody>
</table>

*Mean followed by standard deviation and range (mean and range in microns). Abbreviations in Tables I–II: PK, the distance from posterior end to kmetoplast; KN, the distance of the klnetoplast to the center of the nucleus; PN, the distance from the posterior end to the center of the body excluding the free flagellum; PA, the length of the body excluding the free flagellum; FF, the length of the free flagellum; BW, the maximum width excluding the undulating membrane.

TABLE II

<table>
<thead>
<tr>
<th></th>
<th>PK</th>
<th>KN</th>
<th>PN</th>
<th>PA</th>
<th>FF</th>
<th>BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>B/A</td>
<td>0.87</td>
<td>0.70</td>
<td>0.16</td>
<td>0.11</td>
<td>0.58</td>
<td>0.25</td>
</tr>
<tr>
<td>B/H</td>
<td>2.64*</td>
<td>1.32</td>
<td>0.37</td>
<td>0.64</td>
<td>1.30</td>
<td>3.96*</td>
</tr>
<tr>
<td>A/H</td>
<td>3.48*</td>
<td>1.91</td>
<td>0.22</td>
<td>0.56</td>
<td>0.06</td>
<td>4.91*</td>
</tr>
</tbody>
</table>

*Significantly different at 5% level.

Discussion

Various inefficient techniques to detect and count trypanosomes in low infections have been used. Ross and Thomson (1910) made a thick film of a measured quantity of blood (1 cc), haemolyzed the erythrocytes, and counted, after staining them, all the trypanosomes in the film. Reichenow (1921) made a thick film of an unknown quantity of blood and counted the number of trypanosomes in the thick film for 30 minutes as a measure of parasitaemia. Finally, the number of trypanosomes has been compared to the number of leucocytes in a thick blood smear, or to the number of erythrocytes in a thin blood smear. This last technique is faulty because the number of blood cells may vary according to the parasitaemia.

The present study shows that trypanosomes recovered from haematocrit tubes after centrifugation were distorted and such trypanosomes should not be used for detailed morphological studies unless this is taken into consideration. However, the technique is still the most rapid and reliable for the detection of low parasitaemias and the determination of the number of trypanosomes in a given volume of blood.

Acknowledgments

The critical comments of Dr. R. C. Anderson and the assistance of Miss Lynda-Lou Tibbles, Department of Zoology, University of Guelph, were much appreciated. Financial support in the form of an operating grant to Dr. Anderson was provided for this work by the National Research Council of Canada.


