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Estimation of Serum Immunoglobulin M as a Screening Technique for Trypanosomiasis

A Field Trial in the Democratic Republic of the Congo

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Field trials in West Africa have shown the value of the estimation of serum immunoglobulin M (IgM) levels as a screening test in endemic trypanosomiasis areas. A further field trial carried out in the Democratic Republic of the Congo is described. The technique used, based on double diffusion on an agar plate, gave consistent results in skilled hands, but the standard required is probably too high for the wide application of the method under normal field conditions. The diameter of the precipitation zones for 200 sera from new patients from a survey population of nearly 10 000 was generally between 8.3 mm and 9.7 mm, while the mean diameter for 210 sera from healthy persons was 6.2 mm. The results for 115 sera from previously treated patients were generally intermediate between these two levels.

Duplicate IgM estimations made in the laboratory on dried blood samples collected on filter-paper in the field gave agreement with the serum IgM test in 96% of the cases. The greater simplicity of the filter-paper method might make it suitable for wide-scale IgM estimations.

In West Africa Mattern and his colleagues (Mattern et al., 1961; Mattern, 1962, 1964, 1968) have demonstrated the importance of the estimation of serum immunoglobulin M (IgM) levels as a screening test for the diagnosis of *Trypanosoma gambiense* infection. Although a raising of the IgM level is not pathognomonic of trypanosomiasis, in their experience it was such a constant finding with this disease that its absence could be used virtually to exclude the diagnosis. Lumsden (1966) showed that a similar increase occurred with infections due to *T. rhodesiense*.

Reports on the use of the IgM estimation in mass surveys have come from Senegal (Mattern & Peretti, 1966), the Ivory Coast (Macario & Bentz, 1963; Rives et al., 1966), and Upper Volta (Bideau, 1966). The results of the test in clinical and survey groups from Uganda and Kenya have also been given by Cunningham et al. (1967) and de Raadt (1966).

The purpose of the present trial was to test under field conditions the technique of IgM estimation adapted by the late Dr T. Webb of the WHO International Reference Centre for Immunoglobulins, Lausanne, Switzerland, from the method of double diffusion on an agar plate worked out by Fahey & McKelvey (1965), and to obtain results from a different geographical area in order to ascertain whether serum IgM levels followed a distribution similar to that recorded for patients from West and East Africa. For this reason an invitation to carry out field trials in a focus of renewed activity in the Democratic Republic of the Congo was gratefully accepted.

The area involved was the subdistrict of Gombe Sud which lies along the border with Angola about 180 km south of Kinshasa (Fig. 1). A long-established endemic focus has existed in this and the neighbouring district of Kimpese around the upper River Kwilu and its tributaries. Although during the time of the Belgian administration trypanosomiasis in this area had been brought under control through regular surveys and mass prophylaxis, the disease had not been eradicated. The last survey figure for the area before independence recorded 75 new cases in 1958. No organized surveys took place during the next three years. In 1962–63 new cases started

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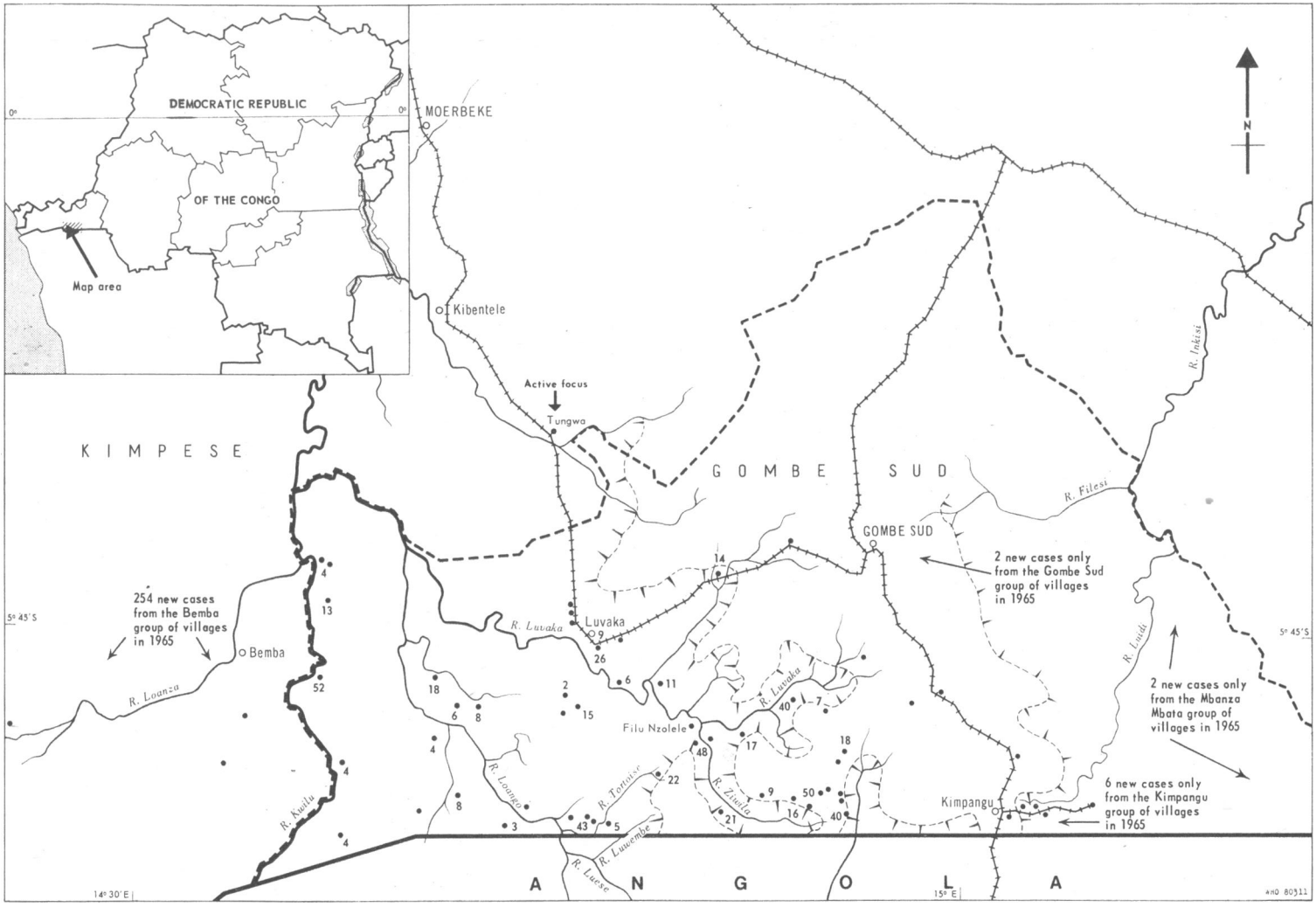
FIG. 1
THE SURVEY AREA



Form-line approximately indicating land above 500 m.
District boundary
Railway
Village
15 Figures marked thus indicate cases of trypanosomiasis diagnosed between January and September 1966.

appearing again. Surveys were then recommenced on a small scale with the limited staff available. With the material, drugs and transport from Belgian sources supplied through the Fonds de Médecine tropicale belge, the medical teams in this and other areas are again becoming mobile and capable of carrying out repeated surveys following a planned schedule. The team in the Gombe Sud area was able to cover the population at greatest risk by two surveys in 1966. The authors accompanied this team for part of the second survey in August and September 1966, helping with the routine work and carrying out additional parasitological and immunological tests where possible.

For the purpose of our investigation on the IgM technique, sera were first collected from apparently normal people to test and standardize the method of estimation. Sera were then collected from as many parasitologically proven cases as possible in order to obtain a wide range of observations in such patients. Further specimens were taken from apparently healthy groups in the endemic area as well as from patients with histories of treatment for trypanosomiasis. Finally a test village was chosen and the IgM estimation used as a screening test in addition to the traditional survey methods employed by the team. Samples of sera from a selection of these patients were also dispatched to the WHO International Reference Centre for Immunoglobulins, Lausanne, Switzerland, for reference purposes and for further testing.

MATERIALS AND METHODS

Plates

These were standard plain-glass photographic plates measuring 8.5 cm × 10 cm.

Buffer

The following materials were weighed out

$Na_2HPO_4 \cdot 2H_2O$	82.0 g
$NaH_2PO_4 \cdot H_2O$	5.5 g
NaCl	85.0 g

and sealed into plastic packets kindly supplied by the CIBA representative, Kinshasa. When required, the contents of 1 packet were made up to 1000 ml with freshly filtered distilled water to give a concentrated stock solution. For use this was further diluted 1 : 10 with filtered distilled water to give a buffer solution of pH 7.6 (0.05 M phosphate; 0.15 M NaCl).

Agar

A 3% solution in the above-mentioned buffer of agarose FF4330 from the Institut Français, Gennevilliers (Seine), was used throughout. Agarose was chosen rather than agar for reasons of purity and transparency. In practice, 100 ml of the buffer solution were heated to approximately 70°C with constant stirring to dissolve the agarose (3 g). The solution was then poured in equal amounts (just over 8 ml) into 12 test-tubes, which were sealed with Parafilm and kept at room temperature (24°C–26°C). It was found by trial that in this form the tubes would remain free of contamination for periods in excess of 3 weeks if kept sealed (and even for over 15 days if left open) in spite of adverse field conditions and lack of refrigeration. Since only about a week's supply of agar was made up at a time the risk of contamination was negligible. For each batch of agar, one control plate was prepared and tested with serial dilutions of the normal serum (see below) to check against the standard curve.

Anti-IgM serum

This specific anti-human-IgM serum, M 690, prepared from sheep, was provided by the late Dr Webb from the WHO Reference Centre in Lausanne in a lyophilized form in ampoules. For the preparation of each plate, the contents of one ampoule were dissolved in 1 ml of distilled water and then transferred to a 25-ml test-tube. The ampoule was rinsed with 7 ml of buffer solution which was then also added to the test-tube. The 8 ml of the final anti-IgM serum solution (1 : 8) were then placed in a flask of water the temperature of which was carefully kept at 56°C by constant checking with a thermometer. All heating was done over a Primus stove.

Preparation of the IgM plate

A tube of the 3% agarose was heated in boiling water until the contents were liquid, then cooled to 56°C in the same water-bath as the anti-IgM serum solution. Using a previously warmed graduated 20-ml pipette, 8 ml of serum solution were added to the 8 ml of agarose and mixed, care being taken to avoid the formation of bubbles; 15 ml of this mixture were poured on to a previously cleaned and prepared glass plate (8.5 cm × 10 cm) placed on a specially made small level table. The plate was allowed to stand for about 10 minutes before being placed in a level humid container and kept at room temperature (24°C–26°C). Under field conditions,

colonies of micro-organisms tended to develop on control plates which had been kept for more than 48 hours. It was therefore considered essential to use only freshly prepared plates so that there would be no risk of any contamination interfering with the final reading at 48 hours.

Migration of IgM

When the agar was firm the plate was introduced into a special template (Fahey & McKelvey, 1965) and small wells (2.25 mm in diameter and 1.75 mm deep) bored through the guide holes in the template, the contents being aspirated with a Pasteur pipette. The sera to be examined were then introduced according to a predetermined plan into the wells, a separate 5-μl Drummond micropipette being used for each specimen. Dr Webb felt that for reasons of comparison, it was preferable to use standard amounts.

After the sera had been introduced into the wells, the plate was placed in the level humid container at 100% relative humidity and left for 48 hours at room temperature (24°C–26°C). In the laboratory this stage would have been carried out in a refrigerator at 4°C. In the field, provided the plates were freshly prepared, no contamination was observed within 48 hours at room temperature. It was, however, necessary to change the moist filter-paper in the humid chamber daily to avoid growth of any fungal spores or micro-organisms on it.

During the 48 hours, a ring of antibody-antigen precipitate was formed around the well, the diameter reflecting the concentration of IgM in the serum. Since the antiserum was monospecific, only IgM–anti-IgM precipitates were formed.

The first reading was made at 48 hours on the unstained plate, using oblique illumination from a lamp or torch; the diameter of the precipitation zone for each serum tested was measured using an 8× magnifying micrometer.[1]

In setting up the plate a control serum was also included. This control serum was available as a lyophilized powder, the contents of the ampoule being reconstituted with 0.9 ml of distilled water. The serum, kindly provided by Dr Wuilleret of the Swiss Red Cross in Lausanne, had been pooled from 4 normal blood donors, delipidated by ultra-centrifugation, reconstituted to 66% of the original volume and lyophilized (1 ml in each ampoule). The diameter of the precipitation zone given by this control serum was found to be reproducible under field conditions; after incubation for 48 hours, washing and staining, a value of 6.2 mm was obtained. This reproducibility provided evidence that possible day-to-day and plate-to-plate variations were small compared with the range of diameters given by sera from the survey population. In this paper the IgM data are analysed only on the basis of the diameters of the precipitin rings: no conversion from ring diameter to concentration has been made.

Staining

After the reading had been made on the unstained plate, the plate was immersed for 24 hours in 300 ml of buffer solution, the solution being changed three times during this period. After removal, the plate was covered carefully with filter-paper, cut so as just to overlap the plate and soaked in distilled water, any air bubbles being avoided. The plate was then dried at room temperature (24°C–26°C) for about 24 hours. When the paper was *absolutely* dry it was very gently peeled off, stray fibres removed by washing in distilled water, and the plate stained by immersion for 20 minutes in a 0.1% solution of Amido Black in 5% acetic acid. This solution was filtered before use through double-thickness filter-paper to avoid the possible deposition of grains of dye on the plate. After staining, the plate was decolorized with 2% acetic acid until the background became pale enough to contrast clearly with the precipitin rings. Usually, two or three separate washings with 100 ml of 2% acetic acid for 5 minutes each were sufficient. Finally, the plates were dried and the diameter of the stained precipitation zone was then easily measured.

PRACTICAL CONSIDERATIONS

Collection of sera

Since additional serum specimens were required for dispatch to the WHO International Reference Centre at Lausanne for reference purposes, venous blood samples had to be collected; this naturally reduced the number of specimens which could be handled in one day.

Blood samples were collected into 10-ml or 20-ml Vacutainers. These were found to be very practical for the purpose; the disposable sterile needles made for simplicity and the sealed containers reduced the handling and possible contamination of the blood samples.

Blood samples, collected at the day's working centre, were allowed to stand for 3–4 hours for clotting to occur, and then transferred to base each

[1] Flubacher, Horgen, Zürich, Switzerland.

evening for testing, still unopened; the risk of contamination was therefore very small. At base, after removal of the bungs, the Vacutainers themselves were used as centrifuge tubes, thus further reducing handling. The Honda generator and centrifuge used for this stage in the separation of the sera proved both compact and practical.

Serum specimens required for Lausanne were transferred to special ampoules and immersed in liquid nitrogen in a refrigerator-transporter (Guthe, 1965) for dispatch.

Plates

It was found by trial that the agar-coated plates did not stand up to transport at the prevailing atmospheric temperatures (24°C–26°C), since the agar layer was not very firm and distortion was liable to occur after a bumpy journey. They were therefore prepared fresh at base for testing each collection of sera at the end of the day.

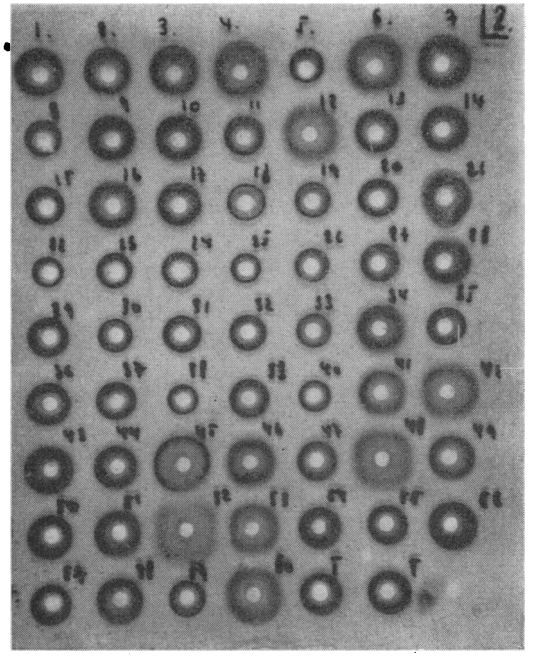
For the first plates prepared, 63 wells were bored in 9 rows of 7, all wells equidistantly placed (exactly 1 cm apart). With 2 wells at each corner unused and 3 occupied by the control serum and its dilutions, 52 sera could be tested on each plate. However, it was found that when large numbers of undiluted sera with high IgM titres were tested together, the neighbouring precipitation zones interfered with each other and some distortion occurred when diameters exceeded 8 mm (Fig. 2; see below under "Results: New cases of trypanosomiasis"). The plates were then modified to take either 19 or 25 wells (Fig. 3). This arrangement gave excellent results but naturally reduced the number of sera which could be tested on each plate, thus increasing the cost of the test per patient. If it was decided to use 52 wells, the test sera would have to be diluted 1: 2.

Timing

The choice of a delay of 48 hours before reading was largely governed by convenience, but it was also felt that 48 hours should not be exceeded because of the rapid increase in the risk of secondary growth on the plates at room temperature after this time.

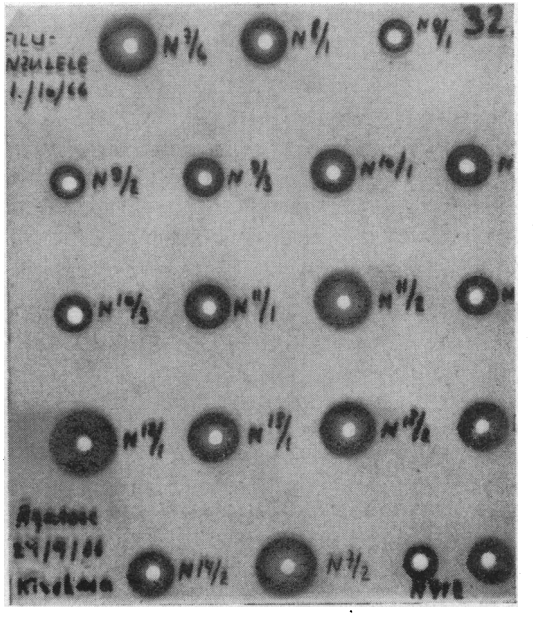
It could usually be decided by inspection of the unstained plate whether the test was indicative of trypanosomiasis or not. However, a comparison between unstained and final stained readings (Fig. 4) showed that around the critical diameter of 8.3 mm (see "Results: New cases of trypanosomiasis") the unstained readings gave approximately 10% of false positives and 10% of false negatives as judged by the

FIG. 2. AGAR PLATE WITH 63 WELLS [a]



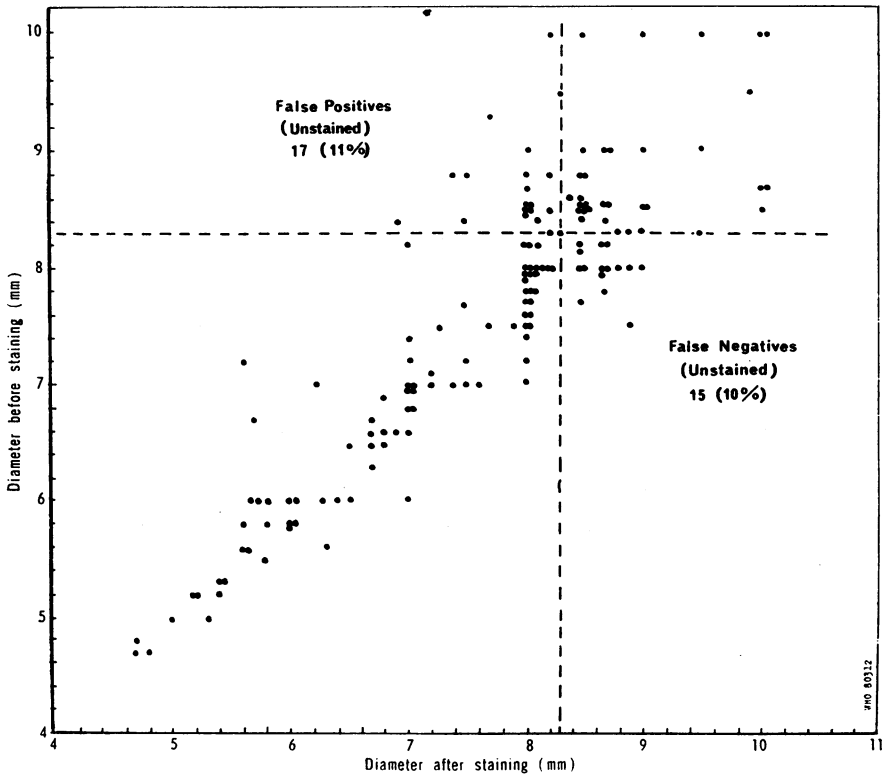
[a] Some distortion of the precipitation zones was produced when neighbouring wells contained sera with high IgM levels (see in particular wells 42, 48 and 52).

FIG. 3. AGAR PLATE WITH 19 WELLS [a]



[a] Precipitation zones are clear and easy to measure.

FIG. 4
DIAMETERS OF PRECIPITATION ZONES BEFORE AND AFTER STAINING



final stained result. For these high IgM levels the unstained precipitation zone was sometimes diffuse and its diameter difficult to judge. A higher concentration of the anti-IgM serum in the agar might have remedied this, but in view of the limited time at our disposal, further modifications were avoided. All the results in this report therefore refer to the final stained readings.

General techniques

Since the primary object of the mission was to collect suitable sera for testing the IgM technique and for other estimations, the greater part of the time was spent working with the field team in their general trypanosomiasis survey, in examining the population, in selecting patients and in checking the findings of those whose sera were finally collected. Thus the majority of the new patients shown in Table 1 were selected by means other than serum IgM estimation during a survey covering a population of nearly 10 000. The serum IgM estimation was used as a full screening test only at the end of the survey for the examination of the Filu Nzolele area, which comprised three hamlets with a total population of around 250.

All the IgM estimations were carried out by one of us (G. B.), so that there was no observer variation in the final readings, and the various stages of the test could be carefully standardized. The need for scrupulous attention to detail cannot be too strongly emphasized; it is essential if consistent results are to be obtained under field conditions.

All thick blood films were prepared and examined personally by one of us (G. T.), anything up to an hour being spent on individual slides. Slides were stained with Giemsa stain—Gurr's R66 or Hopkin and Williams' Revector brand.

Filter-paper tests

From a proportion of the patients, a blood drop was collected on Whatman No. 1 filter-paper and dispatched to Mr M. P. Cunningham and

Dr N. M. Bailey at the East African Trypanosomiasis Research Organization (EATRO), Tororo, Uganda, who very kindly undertook the estimation of the IgM levels by the filter-paper technique (Cunningham et al., 1967) and carried out the indirect fluorescent antibody test (Bailey et al., 1967) on the same dried blood specimens.

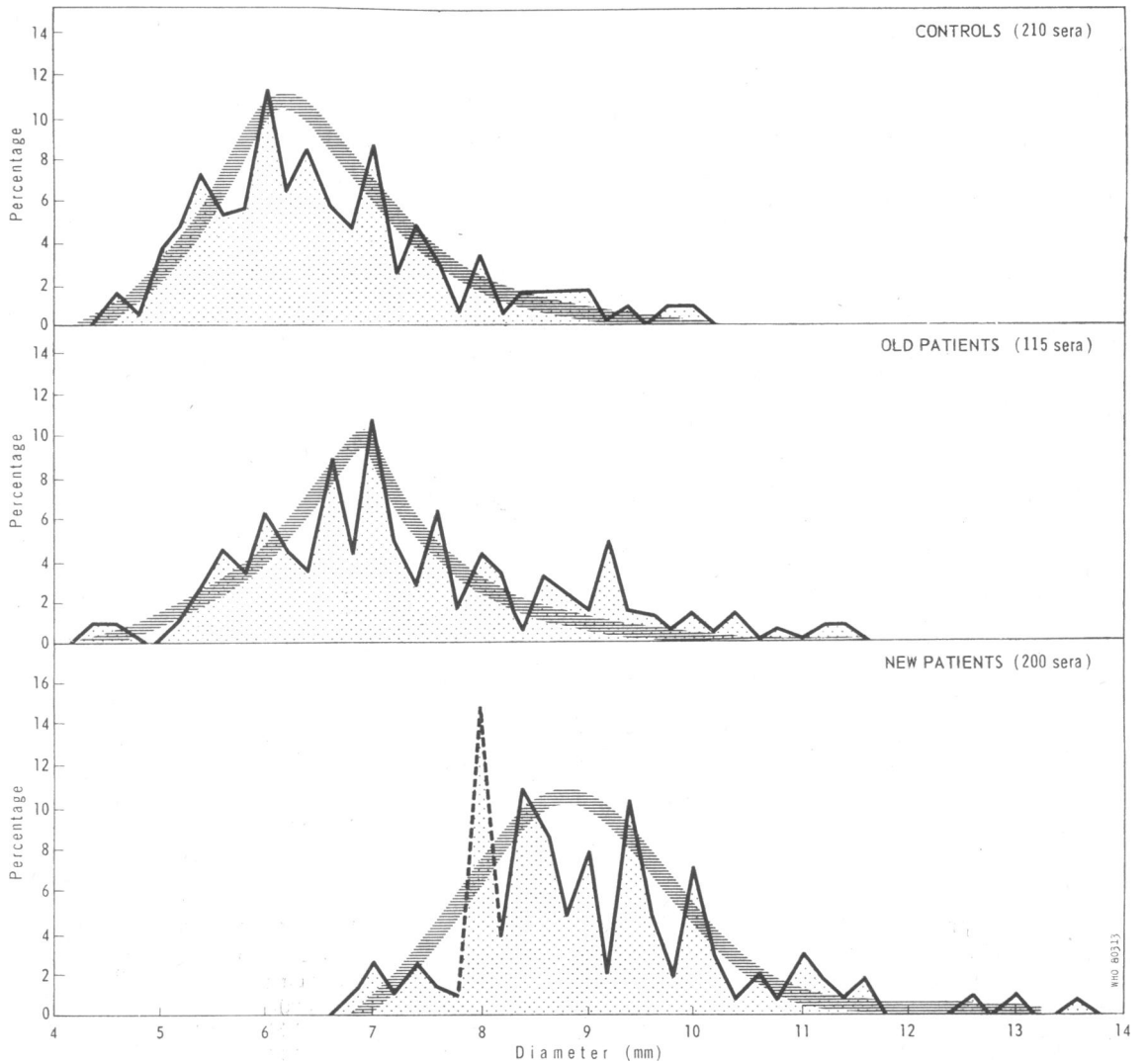
RESULTS OF IgM ESTIMATIONS ON SERUM SAMPLES

New cases of trypanosomiasis

Fig. 5 and Appendix Table 1 show the distribution of IgM precipitin-ring diameters in 200 patients with no history of previous trypanosomiasis treatment and in whom trypanosomes were now found for the

FIG. 5

DISTRIBUTION OF IgM LEVELS FOR CONTROLS, OLD PATIENTS AND NEW PATIENTS [a]



[a] "Controls" were apparently normal individuals, parasitologically negative, who had not been treated for trypanosomiasis previously; "old patients" were individuals who had previously been treated; "new patients" were individuals who had not been treated previously, and who were found during the survey to be parasitically positive.

first time. Trypanosomes had been detected in 39 cases in gland juice only: in 14 in blood film only: and in the remaining 147 in both gland juice and blood.

In the following results, no conversion from ring diameter to concentration has been made: the IgM quantitative data have been analysed on the basis of ring diameter only.

The majority of readings fall between 8.3 mm and 9.7 mm. However, a peak also occurs at 8 mm (shown dotted in Fig. 5). Most of these sera were among the first to be tested, when up to 52 wells were being used on each plate. The wells proved to be too close to each other when a large number of undiluted sera with high titres were being tested together and neighbouring precipitation zones reached a diameter of 8 mm: interference then took place. Some of these early estimations with readings of 8 mm would in fact have shown larger precipitation zones but for this effect. It was as a result of this experience that the placing of the wells was altered; the subsequent distribution of IgM levels in new cases of trypanosomiasis gave the peak between 8.3 mm and 9.7 mm. The alteration was made after serum sample No. 158 had been examined.

Although the majority of new cases showed increases in their serum IgM above the critical level of 8.3 mm, there were 19 with readings below 8 mm. (The critical level of 8.3 mm was chosen because previous laboratory tests had indicated that this represented an approximately fourfold increase in IgM levels over the reading of 6.2 mm obtained with the control serum.) In view of the above-mentioned problem of the interference between large precipitation zones, it is not possible to come to any definite conclusion for the majority of the sera below No. 159, since some of these would probably have shown a higher level if retested with the later modification in the plate. This has been confirmed for sera 148, 149, 150, 151, 153, and 154, all of which gave positive results with the subsequent duplicate estimation of the IgM level made on the dried blood sample (see Appendix Table 4).

It is clear, however, that some patients do exist in whom the presence of a trypanosome infection does not necessarily give rise to a critically high level of serum IgM. Details are given of 5 such cases, together with a sixth in which, although the serum IgM was not estimated, negative results were subsequently obtained with the dried blood sample both for the IgM estimation and the indirect fluorescent antibody test (IFAT).

Case No. 152 (6-year-old female). No complaints but rather puffy features. Small palpable cervical lymph nodes; also enlarged nodes in tonsillar and mandibular regions. Spleen palpable 1½ in (about 4 cm) below costal margin.

GP (gland puncture) +
BF (blood film) + (also *Plasmodium falciparum* rings +)
CSF (cerebrospinal fluid) 2 cells/mm³; total protein 20 mg/100 ml

IgM	(on serum)	7.4 mm	
	(on dried blood)	4.8 mm	(−)
IFAT	(on dried blood)	2	(−)

Case No. 190 (6-year-old male). Large soft cervical lymph nodes on both sides. Liver just palpable, and spleen palpable 2 in (about 5 cm) below costal margin. Stomatitis and glossitis.

GP	+	
BF	+	
CSF	3 cells/mm³; total protein 25 mg/100 ml	
IgM	(on serum)	7.8 mm

Case No. 337 (18-year-old male). Complains of headache and daytime weakness. Looks well but multiple enlarged cervical lymph nodes palpable. Liver and spleen not palpable.

GP	+		
BF	+		
CSF	20 cells/mm³; total protein 30 mg/100 ml		
IgM	(on serum)	7.7 mm	
	(on dried blood)	4.0 mm	(−)
IFAT	(on dried blood)	1	(−)

Case No. 368 (9-year-old female). Complains of fever only. Numerous enlarged cervical lymph nodes on left. Spleen palpable 1½ in (about 4 cm) below costal margin.

GP	+		
BF	+ (1 per 6 fields under 1/12-in (2-mm) objective) (also *P. falciparum* rings ++++)		
CSF	0 cell/mm³; total protein 15 mg/100 ml		
IgM	(on serum)	8.0 mm	
	(on dried blood)	4.0 mm	(−)
IFAT	(on dried blood)	1	(−)

Case No. 439 (8-year-old male). No complaints. Fair condition. Multiple soft enlarged cervical lymph nodes. Spleen palpable.

GP	+		
BF	+ (1 per 30 fields under 1/12-in (2-mm) objective) (also *P. falciparum* rings +)		
CSF	3 cells/mm³; total protein 10 mg/100 ml		
IgM	(on serum)	7.2 mm	
	(on dried blood)	3.6 mm	(−)
IFAT	(on dried blood)	3	(+)

Case No. 579 (9-month-old female). Mother had noticed attacks of fever only. A well-covered child, but

appearance suggestive of early kwashiorkor. No palpable cervical lymph mode. Spleen palpable.

BF	+	(also *P. falciparum* rings+)
IgM		(on dried blood) 4.0 mm (−)
IFAT		(on dried blood) 1 (−)

The first five subjects would not have been missed by the team, since all had enlarged cervical lymph nodes and trypanosomes were present in the gland juice. In the last, however, there was little to suggest trypanosomiasis. Her blood was examined as part of the survey of the Filu Nzolele area when blood films were taken from every person. With the present shortage of trained field staff, it is a physical impossibility for the small local teams to undertake the examination of blood films from every single person. One of the objects of the Filu Nzolele survey was to assess the value of the IgM estimation as a screening test to select possible cases for detailed blood and other examinations (see " IgM estimation as a screening test " below). Case No. 579 would not have been picked out by this means.

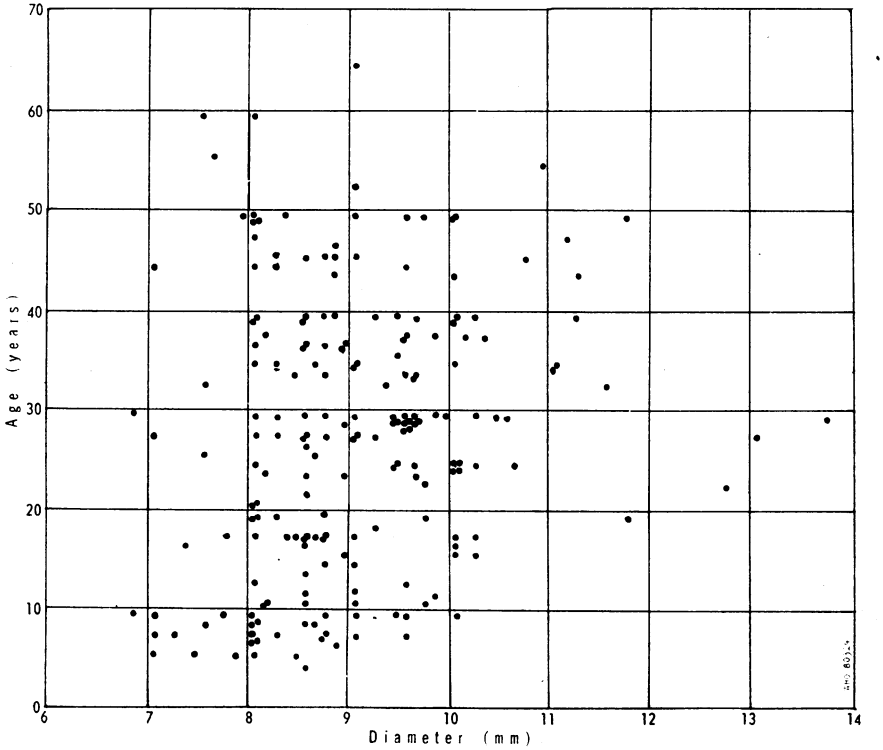
It would appear, therefore, that in this area of the Congo and with this particular strain of trypanosome, at least 3%, and possibly more, of patients with trypanosomiasis do not show IgM levels which are critically high. In the cases quoted above, with the possible exception of the last, there was nothing to indicate that the infection was very recent; in each it had probably been present for at least 2–3 months. It may not be entirely a coincidence that 5 out of the 6 patients were children.

A scatter diagram for the 200 confirmed positive new cases (Fig. 6) shows their estimated ages against the diameter readings. If the patients are divided into children and adults (up to and above 15 years) there is a difference in result:

	Mean diameter ± standard deviation (mm)
All new cases (200)	8.9
44 children (0–15 years)	8.3 ± 0.79
156 adults (over 15 years)	9.1 + 1.12

It is unfortunate that so few results were available for small children. As already mentioned, we did

FIG. 6
DISTRIBUTION OF IgM LEVELS WITH AGE



not make any attempt to take venous samples from the majority of such children as it was an unpopular measure. However, the figures above show a trend towards higher levels with increasing age, and emphasize the need for following up this point in greater detail in any subsequent field trial.

Patients previously treated for trypanosomiasis

Sera were collected from 115 patients with histories of treatment for trypanosomiasis. The distribution of their precipitin ring diameters is shown in Fig. 5 and Appendix Table 2. It will be seen that there is a considerable spread, with a peak above the control level of 6.2 mm but below the critical level of 8.3 mm. The distribution is what one would expect with such a random sample of previously treated patients which includes both relapses and cured cases, and many whose treatment was comparatively recent. However, when it comes to individual readings the interpretation is less simple. A high IgM level should certainly be regarded as indicating the need for further investigation: a single examination showed clinical or parasitological relapse in over 45% of those with readings of over 8.3 mm. The remaining patients merited more lengthy and detailed observation than was possible on the present occasion, even though the results of lumbar puncture were normal; a number came from heavily infected villages and might eventually have proved to be suffering from new infections.

At the other extreme, among those whose IgM precipitin ring diameters were above 6.2 mm but below 8.3 mm, there were 18 patients in whom clinical or parasitological relapse had been confirmed by other means. Details of two cases follow.

Case No. 160 (10-year-old male). First diagnosed in 1964. Received full course of suramin sodium and tryparsamide.

Jan. 1965	GP−; BF−; CSF 16 cells/mm³; total protein 40 mg/100 ml. A further course of suramin sodium.		
May 1965	2 courses of 3 injections of melarsonyl potassium.		
Dec. 1965	CSF 8 cells/mm³; 40 mg% protein. 2 courses of 4 injections of melarsoprol.		
Sept. 1966	GP−; BF + (1 per 10 fields); CSF 178 cells/mm³; 80 mg% protein. Clinically advanced.		
but	IgM (on serum)	7.4 mm	
	(on dried blood)	4.0 mm	(−)
	IFAT (on dried blood)	1	(−)

Case No. 238 (44-year-old male). First diagnosed April 1964. (G.P.+; BF +; CSF 6 cells/mm³; total protein 60 mg/ 100 ml). Received 2 courses of 3 injections of melarsonyl potassium.

Sept. 1966	Clinical deterioration. GP −; BF −; CSF 320 cells/mm³; total protein 60 mg/ 100 ml (trypanosomes present) (IgM on CSF +, 3.7 mm)		
but	IgM (on serum)	7.1 mm	
	(on dried blood)	4.2 mm	(−)
	IFAT (on dried blood)	1	(−)

In the following case, which was very similar as regards the clinical stage of the disease, all the IgM levels were in contrast significantly raised.

Case No. 239 (25-year-old male). First diagnosed in 1964. Received a full course of suramin sodium and tryparsamide.

1965	CSF 0 cell/mm³; total protein 22 mg/ 100 ml.		
Oct. 1966	Clinical relapse. GP −; BF −; CSF 160 cells/mm³; total protein 50 mg/ 100 ml (IgM in CSF + (3.2 mm))		
	IgM (on serum)	10.5 mm	
	(on dried blood)	7.0 mm	(+)
	IFAT (on dried blood)	4	(+)

In the following two patients, a husband and wife, the IgM readings were interesting in that they gave the only correlation with the clinical picture, the CSF findings being equivocal in view of the short interval since treatment.

Case No. 166 (28-year-old male). First diagnosed in August 1964 (GP +; BF +; CSF 3 cells/mm³; total protein 44 mg/100 ml). Received a full course of suramin sodium and tryparsamide.

May 1965	CSF 1 cell/mm³; total protein 10 mg/ 100 ml.		
Jan. 1966	CSF 12 cells/mm³; total protein 56 mg/ 100 ml. Received 2 courses of 4 injections of melarsoprol.		
Sept. 1966	CSF 3 cells/mm³; total protein 46 mg/ 100 ml; BF −. Looks well. No clinical signs or symptoms.		
	IgM (on serum)	6.2 mm	
	(on dried blood)	3.9 mm	(−)
	IFAT (on dried blood)	1	(−)

Case No. 165 (25-year-old female). First diagnosed in August 1964 (GP+; BF+; CSF 7 cells/mm³; total protein 66 mg/100 ml). Received a full course of suramin sodium and tryparsamide.

May 1965	CSF 1 cell/mm³; total protein 22 mg/ 100 ml; GP −; BF −.
Jan. 1966	CSF 10 cells/mm³; total protein 50 mg/ 100 ml. Received 2 courses of injections of melarsoprol.

Sept. 1966 CSF 2 cells/mm³; total protein 40 mg/100 ml; BF −.
Clinical case with shuffling gait, choreiform movements, excessive sleeping.

IgM	(on serum)	9.8 mm	
	(on dried blood)	6.6 mm	(+)
IFAT	(on dried blood)	2	(−)

However, the general inference from this series of observations would appear to be that serum IgM estimation in patients previously treated for trypanosomiasis is not a reliable guide to their present condition. A significantly raised level should indeed continue to be viewed with suspicion and call for a more thorough re-examination, or at least more frequent observation, of the patient. Unfortunately, a negative IgM estimation does not necessarily exclude a relapse. In fact, out of the patients examined in this group, one-fifth of those with serum IgM readings below 8.3 mm had already been shown to have had clinical or parasitological relapses.

Persons without signs of trypanosomiasis

Fig. 5 and Appendix Table 3 give the distribution of serum IgM precipitin ring diameters in 210 persons who were parasitologically negative and showed no clinical evidence of trypanosomiasis. There is a sharp peak around 6 mm–6.4 mm, showing that the control serum, a mixture of European sera, did not differ appreciably in this respect from sera from Africans in this area.

However, there were 23 persons (11%) with a serum IgM level above 8.3 mm, the critical level. In Senegal (Mattern & Peretti, 1966) it has been estimated that up to 5% of the population have significantly raised IgM levels due to reasons other than trypanosomiasis. On this assumption at least 10–12 of these 23 persons should prove to be positive if examined further, and experience in this area suggested that this figure might very well be exceeded. Unfortunately, although this area was excellent for finding new cases easily, it was not good for the purpose of more detailed follow-up studies of individual patients, particularly since around half of the local population was made up of unregistered immigrants who had arrived from Angola during the previous 5 years.

In the first " control " sample of 59 young adults, both male and female, without palpable glands and in apparent good health, 6 (10%) had a significantly high serum IgM level. Only 2 could be retraced, but in each case clinical re-examination was negative and repeated blood films failed to show any trypanosomes. In a second group of 18 apparently healthy young males, 4 (22%) had raised serum IgM levels, 2 being particularly high. Again there was lack of success in tracing these men. Only in the test village was it possible to follow up some of the subjects and out of 24 who originally were found with high serum IgM levels but parasitologically negative, 9 were eventually shown on re-examination to be positive in the limited time available; undoubtedly more would have come to light if more prolonged study had been possible. Of the remaining 15 subjects, 13 are included in the group of 23 shown in Appendix Table 3 as having readings above 8.3 mm. The other 2 are not shown there, since the high readings were obtained on their dried blood sample only.

Facilities were lacking for more detailed investigation and the people were in any case suspicious at first of any repeated examinations, even those as simple as the collection of repeat blood films. In fact it took considerable persuasion before the test village would agree to the collection of venous blood samples —and even then small children had to be excluded, dried blood samples on filter-paper alone being taken from them. With familiarity this type of suspicion would very soon have been dissipated (as it was indeed towards the end of our study), but for this short-term project it made the checking of persons who were apparently normal but had raised serum IgM levels both unsatisfactory and incomplete. Blood samples from 18 such persons were injected into white mice, but none of these showed up positive up to the time of our departure. The period was too short, however, to dismiss these results as negative, especially as trypanosomes had in the interval been found by other means in 5 of these subjects.

Malaria, predominantly due to *P. falciparum*, was endemic in the area, but there was nothing to suggest that its presence had any bearing on the general level of serum M immunoglobulins. Similarly microfilariae, chiefly *Acanthocheilonema perstans* but also *Loa loa*, were common but again there was no correlation between their presence and the serum IgM level.

Thus, in this endemic trypanosomiasis area the general level of serum IgM in healthy controls would appear to fall within the limits found elsewhere. There was evidence from the final village examined (see also the next section) to suggest that undetected trypanosomal infection accounted for at least half of those apparently healthy persons whose serum IgM

was raised above the critical level. No other common condition appeared to play a prominent part.

IgM estimation as a screening test

The village of Filu Nzolele is made up of three hamlets. Trypanosomiasis had been endemic here for at least the previous 3 years, the annual incidence of new cases being around 8%. The last survey was carried out at the beginning of 1966, when 21 new cases were diagnosed. On the present occasion 244 persons were examined. Blood samples for sera were collected from 206; venous blood specimens were not taken from the 38 children in the group, but the results of the IgM estimation are available from dried blood samples on filter-paper which were examined at EATRO by courtesy of Mr Cunningham and Dr Bailey.

The serum IgM estimation was considered positive if it gave a diameter of the precipitation zone at

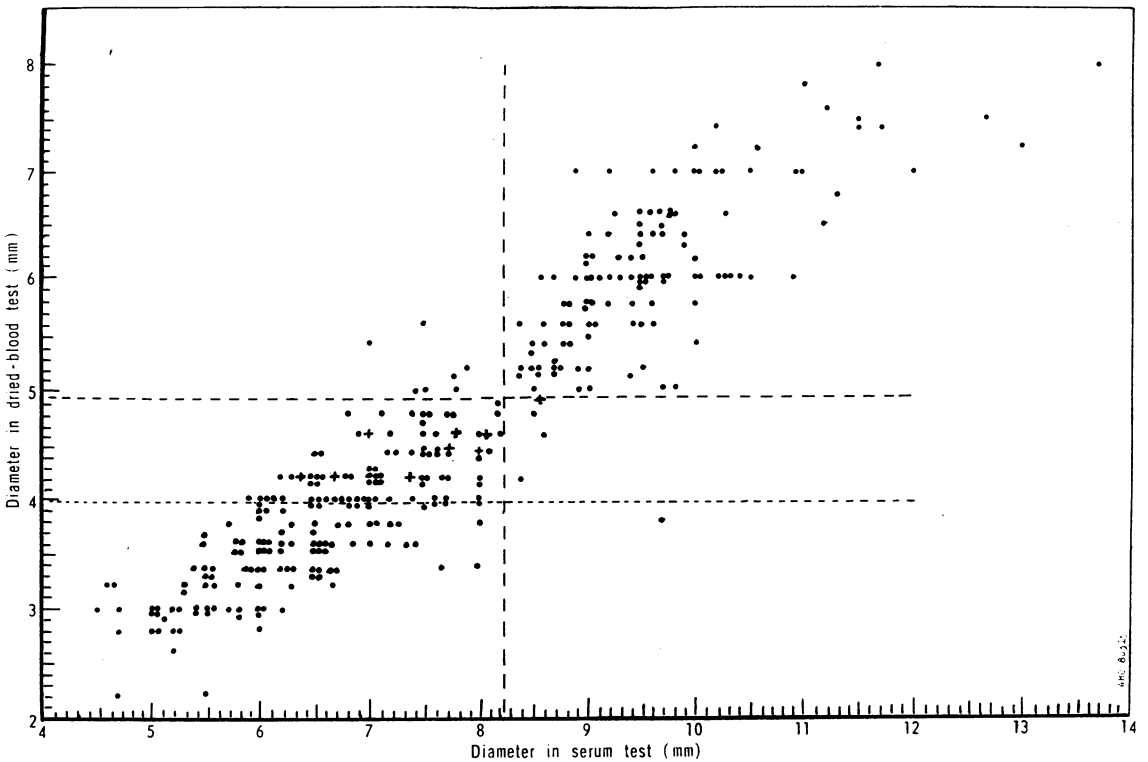
TABLE 1

RESULTS OF IgM TEST IN SURVEY OF FILU NZOLELE AREA

Description	No. of cases		
	IgM −	IgM +	Total
Patients previously treated for trypanosomiasis			
Now apparently well	28	14	42
Relapses	3	5	8
New cases diagnosed by field team	2	14	16
Apparently normal	154	24	178
Total	187	57	244

FIG. 7

CORRELATION BETWEEN RESULTS OF THE SERUM IgM TEST AND THE DRIED-BLOOD IgM TEST [a]



[a] The vertical broken line at 8.3 mm divides positive serum IgM levels from negative ones; the horizontal broken lines at 4.0 mm and 5.0 mm divide positive dried-blood IgM levels (top) from doubtful ones (middle) and negative ones (bottom).

48 hours of 8.3 mm or over. A positive result on the dried blood sample was considered to be a diameter reading of 5.0 mm and over. In addition, a doubtful positive reading between 4.0 mm and 5.0 mm was taken as positive if the indirect fluorescent antibody test was also positive (Cunningham, personal communication.) The survey results are summarized in Table 1.

The chief interest lay in the apparently normal subjects. Out of the 154 without a significant rise in their IgM levels, 1 positive case was found with trypanosomes in the thick blood film (case No. 579, already referred to above). Among the 24 persons with a rise in IgM level, all in apparent good health and negative in the team survey, 9 were subsequently shown to have trypanosomes in their blood. With the limited time available, examination of the remaining 15 had to be curtailed. Mice inoculated with blood from 11 of the 15 were not positive up to the time of our departure. The names of these people were noted and it was hoped that during the following treatment session the local team would be able to re-examine some of them. It was expected that more of this group would eventually prove to be positive.

It is clear from these results that the present teams are missing a considerable proportion of infected persons, particularly those recently infected who are asymptomatic, and infective to tsetse flies. This would account for the lack of success of the repeated surveys as a control measure. The teams at present are too small to be able to carry out universal blood-film examination, or were so at the time of the survey (Burke, 1966). The use of the IgM estimation in the Filu Nzolele area would have cut the number of

TABLE 2

RESULTS OF IgM ESTIMATIONS ON SERUM AND DRIED BLOOD SAMPLES FROM 328 TRYPANOSOMIASIS PATIENTS

Result of IgM estimation		Patients [a]	
Serum	Dried blood	No.	%
	Agreement		
+	+	119 (120)	36.3 (36.6)
−	−	196 (186)	59.8 (56.7)
Total		315 (306)	96.0 (93.3)
	Disagreement		
+	−	5 (4)	1.5 (1.2)
−	+	8 (18)	2.4 (5.5)
Total		13 (22)	4.0 (6.7)

[a] The serum IgM level was considered positive at 8.3 mm or over, and the dried-blood IgM level at 5 mm or over. For the figures in parentheses, doubtful results in the dried-blood test (IgM level between 4.0 mm and 5.0 mm) were also considered positive if the result of the IFAT was positive.

TABLE 3

ANALYSIS OF CASES IN WHICH THE IgM ESTIMATIONS ON SERUM AND DRIED BLOOD SAMPLES DISAGREED

Serum No.	Description	Result of IgM test (mm)		Result of IFAT
		Serum	Dried blood	
	IgM (serum) +; IgM (dried blood) −			
312	Relapse	9.7	3.8	3 (+)
356	Previously treated	8.5	4.8	1 (−)
458	New case	8.4	4.2	1 (−)
468	Previously treated	8.6	4.6	1 (−)
557	New case	8.6	4.9 [a]	3 (+)
	IgM (serum) −; IgM (dried blood) +			
273	Relapse	7.9	5.2	1 (−)
274	Previously treated, now with trypanosomes in blood	7.5	5.0	2 (−)
300	Relapse	7.5	5.6	1 (−)
303	Previously treated	7.0	5.4	3 (+)
313	Previously treated	6.0	7.0	1 (−)
561	Previously treated	7.4	5.0	1 (−)
595	Previously treated	7.8	5.1	1 (−)
597	Previously treated	7.8	5.0	2 (−)
	IgM (serum) −; IgM (dried blood) doubtful			
268	Previously treated	8.2	4.8	3 (+)
271	Previously treated	7.0	4.2	3 (+)
293	Previously treated	6.7	4.2	4 (+)
294	Previously treated	7.7	4.4	4 (+)
357	Apparently normal	7.4	4.2	3 (+)
455	Apparently normal	7.8	4.6	4 (+)
457	Previously treated	8.0	4.4	4 (+)
501	Apparently normal	6.5	4.1	3 (+)
507	Previously treated	7.0	4.6	3 (+)
536	Previously treated	8.0	4.6	4 (+)

[a] This would be considered positive in view of the IFAT result.

persons requiring special examination from 178 to 24 (13%), a proportion which could reasonably have been examined with care. Without still resulting in the diagnosis of every new case, IgM estimation would have greatly reduced the proportion at present being missed. Of the 194 persons with no previous history of trypanosomiasis, 16 (8.2%) were diagnosed by the survey team; if IgM estimation had been used to select those of the remaining 178 who came into consideration for special examination, 9 (4.6%) more, at least, would have been diagnosed, and 1 would have been missed.

IGM ESTIMATIONS ON DRIED BLOOD SAMPLE COMPARED WITH THOSE ON SERA

Although the present survey demonstrated that the serum IgM estimation could be carried out under field conditions with consistent results and without the use of a refrigerator or a great deal of bulky equipment, it was also equally clear that a high technical standard was required if such results were to be reliable. From experience in the field it was evident that the serum estimation would present too difficult a problem for the local technicians at present available. The provision of a special highly trained team to assist the normal survey team could be one solution. It would, of course, be essential to collect only capillary samples of serum if the special team was to keep pace with the survey team. The alternative would be to collect the material in the field and to carry out the estimations in the laboratory. Long distances, bad communications and heat make the problem of transporting large numbers of sera an insuperable one except for a very limited survey; it would not be practicable for large-scale use. It is for this reason that the possibility of estimations carried out on a dried blood sample, easily collected on filter-paper by relatively unskilled assistants and easily transported, becomes of great interest.

On the present occasion duplicate estimations were made for 352 patients. The IgM estimations on the sera were carried out in the field. The dried blood samples, collected on Whatman No. 1 filter-paper, were despatched to EATRO for testing as already described. The results are given in Appendix Table 4. In Fig. 7 the duplicate results for serum and dried-blood-sample estimations have been plotted for patients commencing at No. 160; the results for the first 24 (before No. 160) have not been included, since, for the reasons given above, these early readings on serum specimens were considered to be low in a number of cases. For the remaining 328 patients the results are summarized in Table 2. Details of the cases in which the two methods gave discordant results may be found in Table 3.

Of the 328 patients considered, it is interesting that there was disagreement on only 2 new parasitologically confirmed cases (No. 458 and No. 557), and on only 1 if the result of the IFAT is also taken into account (see Table 3). This represents only 1.25% of the 80 confirmed new cases for whom duplicate results are available, and compares with disagreement on 3 out of 133 apparently normal individuals (2.3%), and on 17 out of 115 patients with a history of previous treatment for trypanosomiasis (14.8%). Thus, with the exception of the results on previously treated cases which in any case may be equivocal, there is close correlation between the conclusions reached by these two methods of IgM estimation.

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We wish to pay special tribute to the late Dr T. Webb who did so much to make the survey possible. The conception had been largely his and we are deeply indebted to him for all the careful preparation and thought which had gone into equipping the team to carry out the essential estimations in the field. His critical interest in the work, sustained up to the very end, was a constant inspiration to us.

We are grateful to the very many in the Democratic Republic of the Congo who assisted us in so many ways. In particular we would like to make warm acknowledgements to the following persons:

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RÉSUMÉ

On connaît l'importance de la détermination de la teneur du sérum en immunoglobuline M (IgM) en tant que moyen de dépistage de l'infection à *Trypanosoma gambiense*. Au cours de l'essai pratique décrit dans le présent article — essai mené d'août à octobre 1966 dans le sous-district de Gombe sud, en République démocratique du Congo — on a mis à l'épreuve une méthode de recherche. La technique employée a été celle de la double diffusion en gélose décrite par Fahey & McKelvey en 1965 et modifiée au Centre international OMS de référence pour les immunoglobulines, Lausanne, Suisse. Elle met en présence le sérum à examiner et un sérum anti-IgM humaine préparé sur le mouton. La présence d'IgM dans le sérum se marque par l'apparition d'un anneau de précipitation antigène-anticorps dont le diamètre varie en fonction de la concentration d'immunoglobuline. Certaines difficultés pratiques, dues en particulier aux conditions climatiques, ont pu être surmontées.

Au cours d'une enquête portant sur près de 10 000 personnes, 200 nouveaux cas de trypanosomiase ont été découverts et confirmés par l'examen parasitologique du sang ou du suc ganglionnaire. La plupart des sérums de ces malades ont donné des anneaux de précipitation dont le diamètre variait de 8,3 à 9,7 mm. Il est apparu cependant que chez 3% au moins des trypanosés infectés pour la première fois par la souche locale de trypanosomes, les taux sériques d'IgM n'étaient pas augmentés dans une mesure significative. On a également prélevé du sérum chez 115 patients déjà traités. L'analyse des taux d'IgM relevés parmi ces malades a montré que le titrage de l'immunoglobuline sérique ne fournit aucune indication valable sur le stade de l'infection. Un taux élevé d'IgM doit certes être considéré comme suspect et doit conduire à des investigations plus poussées ou à une mise sous surveillance plus stricte du sujet; en revanche, la constatation d'un taux faible ne permet pas d'exclure une éventuelle rechute.

Les taux d'IgM ont été déterminés chez 210 personnes apparemment saines: les valeurs observées le plus couramment (6,0 à 6,4 mm) ne différaient guère de celle relevée sur un sérum témoin obtenu par mélange d'un certain nombre de sérums prélevés chez des Européens.

Lors d'opérations de dépistage portant sur les 244 habitants d'un village, 16 nouveaux cas de trypanosomiase ont été découverts par les méthodes classiques. L'emploi de la méthode immunologique a montré chez 24 sujets apparemment sains une élévation significative du taux d'IgM et neuf d'entre eux ont, par la suite, été reconnus atteints de trypanosomiase à l'examen parasitologique. Il est probable que si l'étude de ce groupe avait pu être poursuivie, d'autres cas d'infection auraient été décelés.

On a noté une corrélation étroite (96% de résultats concordants) entre les résultats des titrages effectués sur le terrain et ceux des déterminations pratiquées à l'East African Trypanosomiasis Research Organization (Tororo, Ouganda) sur des échantillons de sang séché expédiés sur papier-filtre. Selon les auteurs, cette dernière technique, d'un emploi facile, pourrait être utilisée à l'occasion d'enquêtes étendues.

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APPENDIX TABLE 1
RESULTS OF IgM TEST FOR NEW PATIENTS

Diameter of IgM precipitation zone (mm)	Serum No.[a]
6.8	*122*, 149
7.0	103, 113, 124, 148, 150
7.2	123, 439
7.4	109, 121, 152, 153, 154
7.6	132, *151*, *337*
7.8	*112*, 190
8.0	61, *63*, 65, 69, 70, 71, *74*, *75*, 80, 82, *84*, 87, 89, 90, *94*, 95, 96, *97*, 100, *107*, 116, 129, 131, 135, 136, 141, 146, *156*, 157, 368
8.2	62, 64, 85, *88*, *98*, *147*, 155, 158
8.4	67, *73*, *78*, 79, *83*, 86, 91, *92*, 99, 114, 115, 120, *126*, *127*, 139, *193*, 200, *205*, 216, 328, 458, 527
8.6	*68*, *93*, 101, *106*, 110, 117, 119, *125*, 130, 137, 138, 140, 191, *195*, 230, 287, 524, 557
8.8	77, *134*, *142*, *192*, *211*, 228, *323*, 358, *556*, 560
9.0	*60*, 102, 105, *118*, *143*, 188, 189, *203*, 206, 210, 229, *326*, *327*, *331*, 483, *518*
9.2	227, 281, 428, 520
9.4	76, *104*, *194*, *198*, 202, 208, 214, 224, 225, 243, 244, 279, 319, 330, 339, 360, 460, 472, 481, 502, 529
9.6	133, 234, *280*, *282*, 286, *335*, 336, *340*, 361, 370
9.8	162, 212, 215, *253*
10.0	81, 111, *128*, 144, 145, 186, *187*, *199*, 204, 217, 251, 256, 259, *285*, 324
10.2	*196*, 213, *320*, 321, *333*, 486
10.4	201, *249*
10.6	322, 573
10.8	*332*
11.0	*197*, *222*, 318
11.2	284, *334*
11.4	283
11.6	*257*, 329
11.8–12.4	
12.6	260
12.8	
13.0	*250*
13.2	
13.4	
13.6	252

[a] The italicized numbers indicate patients who, in addition to being parasitologically positive, also showed changes in the cerebrospinal fluid with raised cell count or an increase in total protein as judged by the Sicard-Cantaloube method.

APPENDIX TABLE 2
RESULTS OF IgM TEST FOR PATIENTS PREVIOUSLY TREATED FOR TRYPANOSOMIASIS

Diameter of IgM precipitation zone (mm)	Serum No. [a]
4.4	248
4.6	395
4.8–5.0	
5.2	220
5.4	241, 577, 587
5.6	
5.8	*232*, 297, 309
6.0	276, 288, 291, 313, 379
6.2	166, 231, 265, *467*
6.4	219, 221, 233, 301, 375, 591, 592
6.6	235, 293, *315*, *503*, 586
6.8	295, 509, 541, 596
7.0	*238*, 271, 292, 302, 303, 304, *371*, 463, *499*, 507, 519
7.2	247, 380, 407, 521, 544
7.4	*160* (+), 240, *267*, *272*, 274 (+), *300*, 305, 308, 317, 425, 508, 561
7.6	290, 294, 296, *366*, 385, 562
7.8	273, *595*, 597
8.0	*270*, 299, *390*, 457, 490, 536, 598 (+)
8.2	268, 306
8.4	237 (+), 356, 406, *444*, *549*
8.6	167, 402, *419*, 468
8.8	246
9.0	262, 314, 411, 513
9.2	*264*, 266, *420* (+)
9.4	*218* (+), 307
9.6	242, *261*, *275*, *312*, 496, 568 (+)
9.8	*165*
10.0	311, *404*
10.2	*161* (+)
10.4	*239*, 316
10.6	
10.8	263
11.0	
11.2	269
11.4	163
11.6–11.8	
12.0	*278*

[a] The italicized numbers indicate patients who showed changes in the cerebrospinal fluid since treatment, with raised cell count or an increase in total protein as judged by the Sicard-Cantaloube method; (+) = trypanosomes detected in blood or gland juice.

APPENDIX TABLE 3

RESULTS OF IgM TEST FOR PARASITOLOGICALLY NEGATIVE SUBJECTS

Diameter of IgM precipitation zone (mm)	Serum No.
4.6	25, 38, 382, 474, 482
4.8	22
5.0	40, 387, 391, 396, 431, 435, 537, 574
5.2	5, 8, 23, 171, 376, 426, 427, 515, 525, 588
5.4	26, 32, 33, 170, 177, 181, 373, 381, 400, 423, 473, 488, 516, 534, 570, 578
5.6	15, 20, 21, 24, 30, 35, 57, 410, 434, 454, 553
5.8	19, 37, 56, 169, 173, 367, 372, 506, 530, 531, 540, 543
6.0	11, 18, 27, 31, 55, 178, 179, 353, 383, 384, 389, 397, 403, 440, 447, 453, 464, 487, 514, 522, 554, 558, 576, 585
6.2	29, 45, 47, 415, 430, 446, 470, 492, 504, 512, 538, 545, 550
6.4	13, 39, 50, 168, 182, 359, 365, 413, 445, 485, 493, 501, 528, 533, 546, 547, 582, 594
6.6	28, 36, 49, 54, 398, 399, 401, 408, 469, 548, 552, 564
6.8	9, 14, 44, 393, 465, 478, 497, 559, 563, 581
7.0	1, 2, 16, 17, 41, 43, 51, 58, 438, 452, 495, 498, 500, 539, 580, 590, 593
7.2	10, 34, 174, 185, 433
7.4	3, 56, 184, 357, 364, 424, 442, 459, 476, 532
7.6	46, 378, 405, 421, 477, 523, 569
7.8	455
8.0	7, 53, 175, 437, 461, 480
8.2	392
8.4	6, 12, 176, 422
8.6	4, 172, 369, 475
8.8	42, 351, 352, 551
9.0	355, 388, 436, 479
9.2	412
9.4	180, 350
9.6	
9.8	48, 510
10.0	52, 183

APPENDIX TABLE 4

RESULTS OF IgM ESTIMATIONS ON SERUM AND DRIED BLOOD SAMPLES, AND OF THE INDIRECT FLUORESCENT ANTIBODY TEST (IFAT) ON DRIED BLOOD SAMPLES, FOR 352 SUBJECTS [a]

Serum No.	Type of case [b]	Result of IgM test (diameter in mm; + or −)		Result of IFAT	Serum No.	Type of case	Result of IgM test (diameter in mm; + or −)		Result of IFAT
		Serum	Dried blood				Serum	Dried blood	
60	N	9.5 +	5.6 +	3+	215	N	9.9 +	6.4 +	2−
136	N	8.0 −	5.5 +	2−	216	N	8.4 +	5.1 +	2−
137	N	8.7 +	4.9 −	1−	217	N	10.0 +	7.0 +	3+
138	N	8.6 +	5.2 +	2−	218	TR	9.5 +	7.0 +	2−
139	N	8.5 +	5.6 +	3+	219	T	6.5 −	4.4 −	1−
140	N	8.7 +	5.0 +	1−	220	T	5.3 −	3.1 −	1−
141	N	8.0 −	5.2 +	1−	221	T	6.5 −	4.4 −	1−
142	N	8.8 +	5.0 +	1−	222	N	11.0 +	7.8 +	3+
143	N	9.0 +	4.8 −	1−	223	S	4.6 −	3.2 −	1−
144	N	10.0 +	6.0 +	1−	224	N	9.5 +	6.4 +	1−
145	N	10.0 +	5.0 +	1−	225	N	9.0 +	6.0 +	1−
146	N	8.0 −	5.0 +	2−	227	N	9.3 +	6.6 +	3+
147	N	8.2 −	5.6 +	3+	228	N	8.9 +	6.0 +	1−
148	N	7.0 −	5.2 +	1−	229	N	9.0 +	6.2 +	3+
149	N	6.9 −	5.0 +	1−	230	N	8.6 +	5.6 +	4+
150	N	7.0 −	5.0 +	1−	231	T	6.2 −	3.7 −	4+
151	N	7.6 −	6.0 +	2−	232	TR	5.8 −	3.6 −	4+
152	N	7.4 −	4.8 −	2−	233	T	6.5 −	3.5 −	4+
153	N	7.5 −	5.5 +	2−	234	N	9.6 +	7.0 +	4+
154	N	7.5 −	6.6 +	2−	235	T	6.7 −	3.8 −	1−
155	N	8.2 −	6.2 +	2−	237	TN	8.4 +	5.6 +	2−
156	N	8.0 −	5.8 +	1−	238	TR	7.1 −	4.2 −	1−
157	N	8.0 −	6.4 +	4+	239	TR	10.5 +	7.0 +	4+
158	N	8.2 −	5.2 +	1−	240	T	7.5 −	4.2 −	2−
160	TR	7.4 −	4.0 −	1−	241	T	5.5 −	3.4 −	1−
161	TR	10.3 +	6.0 +	1−	242	TN	9.7 +	6.0 +	1−
162	N	9.8 +	5.0 +	1−	243	N	9.5 +	6.0 +	4+
163	TR	11.5 +	7.4 +	1−	244	N	9.5 +	6.6 +	1−
165	TR	9.8 +	6.6 +	2−	246	T	8.8 +	5.8 +	1−
166	T	6.2 −	3.9 −	1−	247	T	7.2 −	4.6 −	1−
167	TR	8.6 +	5.4 +	1−	248	T	4.5 −	3.0 −	2−
210	N	9.0 +	5.8 +	3+	249	N	10.4 +	6.0 +	2−
211	N	8.8 +	5.6 +	2−	250	N	13.0 +	7.2 +	4+
212	N	9.8 +	6.6 +	3+	251	N	10.0 +	5.4 +	3+
213	N	10.2 +	7.4 +	3+	252	N	13.7 +	8.0 +	3+
214	N	9.4 +	6.2 +	3+	253	N	9.8 +	5.6 +	4+

[a] The result of an IgM estimation on serum was considered positive if the diameter of the precipitation zone (stained) at 48 hours was 8.3 mm or more. The result of an IgM estimation on a filter-paper sample of dried blood was considered positive if the stained precipitation zone had a diameter of 5.0 mm or more at 48 hours; and in the IFAT, a score of 3 or more was considered positive. Serum No. 350 and the following sera were collected during the Filu Nzolele Survey.

[b] The abbreviations in this column have the following significance:

N = New patient; trypanosomes present.
T = Patient previously treated for trypanosomiasis and now clinically normal with normal CSF and no trypanosomes detected in blood or gland juice.
TR = Patient previously treated for trypanosomiasis, and now relapsed.
TN = Patient previously treated for trypanosomiasis; now parasitologically positive but with normal CSF and asymptomatic; probably a reinfection.
S = Survey " control ", parasitologically negative and with no clinical evidence of trypanosomiasis.

APPENDIX TABLE 4
(*continued*)

Serum No.	Type of case [b]	Result of IgM test (diameter in mm; + or −)		Result of IFAT	Serum No.	Type of case	Result of IgM test (diameter in mm; + or −)		Result of IFAT
		Serum	Dried blood				Serum	Dried blood	
256	N	10.0 +	6.0 +	2 −	307	T	9.4 +	5.6 +	1 −
257	N	11.7 +	7.4 +	4 +	308	T	7.5 −	4.2 −	1 −
259	N	10.0 +	6.0 +	1 −	309	T	5.8 −	3.6 −	1 −
260	N	12.7 +	7.5 +	4 +	311	T	10.0 +	7.2 +	4 +
261	TR	9.7 +	6.5 +	3 +	312	TR	9.7 +	3.8 −	3 +
262	T	9.0 +	6.0 +	3 +	313	T	6.0 −	7.0 +	1 −
263	T	10.9 +	7.0 +	3 +	314	T	9.1 +	6.0 +	2 −
264	TR	9.2 +	6.4 +	3 +	315	T	6.7 −	4.0 −	2 −
265	T	6.2 −	3.6 −	1 −	316	T	10.5 +	6.0 +	1 −
266	T	9.2 +	7.0 +	1 −	317	T	7.5 −	4.8 −	1 −
267	TR	7.5 −	4.6 −	2 −	318	N	11.0 +	7.0 +	4 +
268	T	8.2 −	4.8 −	3 +	319	N	9.5 +	5.6 +	3 +
269	T	11.3 +	6.8 +	1 −	320	N	10.2 +	7.0 +	1 −
270	TR	8.0 −	4.6 −	1 −	321	N	10.2 +	6.0 +	4 +
271	T	7.0 −	4.2 −	3 +	323	N	8.8 +	5.8 +	4 +
272	TR	7.4 −	4.8 −	2 −	324	N	10.0 +	6.2 +	4 +
273	TR	7.9 −	5.2 +	1 −	326	N	9.0 +	6.2 +	2 −
274	TN	7.5 −	5.0 +	2 −	328	N	8.5 +	5.2 +	2 −
275	TR	9.6 +	5.8 +	1 −	329	N	11.7 +	8.0 +	4 +
276	T	6.0 −	3.6 −	1 −	330	N	9.5 +	6.0 +	1 −
278	TR	12.0 +	7.0 +	4 +	331	N	9.0 +	5.8 +	4 +
279	N	9.4 +	6.0 +	3 +	332	N	10.9 +	6.0 +	3 +
280	N	9.6 +	6.4 +	4 +	333	N	10.2 +	6.0 +	2 −
281	N	9.2 +	6.0 +	2 −	334	N	11.2 +	7.6 +	4 +
282	N	9.7 +	6.5 +	3 +	335	N	9.7 +	6.4 +	2 −
283	N	11.5 +	7.5 +	3 +	336	N	9.6 +	6.6 +	3 +
284	N	11.2 +	6.5 +	3 +	337	N	7.7 −	4.0 −	1 −
285	N	10.0 +	7.0 +	4 +	339	N	9.5 +	6.0 +	4 +
286	N	9.7 +	6.0 +	4 +	340	N	9.7 +	6.0 +	4 +
287	N	8.7 +	5.2 +	2 −	341	N	10.2 +	7.0 +	3 +
288	T	6.0 −	3.8 −	1 −	350	S	9.5 +	6.0 +	2 −
290	T	7.7 −	4.4 −	1 −	351	S	8.9 +	5.2 +	1 −
291	T	6.0 −	3.8 −	1 −	352	S	8.8 +	5.4 +	1 −
292	T	7.0 −	4.2 −	2 −	353	S	6.0 −	3.4 −	1 −
293	T	6.7 −	4.2 −	4 +	355	S	9.0 +	5.5 +	1 −
294	T	7.7 −	4.4 −	4 +	356	T	8.5 +	4.8 −	1 −
295	T	6.9 −	4.0 −	1 −	357	S	7.4 −	4.2 −	3 +
296	T	7.6 −	4.0 −	1 −	358	N	8.9 +	5.0 +	3 +
297	T	5.9 −	4.0 −	2 −	359	S	6.5 −	3.6 −	1 −
299	T	8.0 −	4.1 −	1 −	360	N	9.4 +	5.8 +	1 −
300	TR	7.5 −	5.6 +	1 −	361	N	9.6 +	5.6 +	3 +
301	T	6.5 −	4.0 −	1 −	364	S	7.5 −	4.4 −	1 −
302	T	7.0 −	4.2 −	1 −	365	S	6.5 −	3.5 −	2 −
303	T	7.0 −	5.4 +	3 +	366	T	7.7 −	3.4 −	1 −
304	T	7.0 −	4.0 −	2 −	367	S	5.8 −	2.9 −	1 −
305	T	7.5 −	4.2 −	1 −	368	N	8.0 −	4.0 −	1 −
306	T	8.2 −	4.6 −	1 −	369	S	8.6 +	5.0 +	1 −

APPENDIX TABLE 4
(*continued*)

Serum No.	Type of case [b]	Result of IgM test (diameter in mm; + or −)		Result of IFAT [a]	Serum No.	Type of case	Result of IgM test (diameter in mm; + or −)		Result of IFAT
		Serum	Dried blood				Serum	Dried blood	
370	N	9.6 +	6.0 +	2 −	426	S	5.2 −	2.8 −	1 −
371	T	7.0 −	4.2 −	2 −	427	S	5.2 −	2.6 −	1 −
372	S	5.8 −	3.0 −	1 −	428	N	9.2 +	5.8 +	2 −
373	S	5.5 −	3.0 −	2 −	430	S	6.2 −	4.2 −	1 −
375	T	6.5 −	3.4 −	1 −	431	S	5.0 −	3.0 −	1 −
376	S	5.2 −	2.8 −	2 −	433	S	7.2 −	3.8 −	1 −
378	S	7.6 −	4.4 −	1 −	434	S	5.6 −	3.2 −	1 −
379	T	6.0 −	3.9 −	1 −	435	S	5.0 −	3.0 −	1 −
380	T	7.2 −	4.0 −	1 −	436	S	9.0 +	5.2 +	1 −
381	S	5.5 −	3.0 −	2 −	437	S	8.0 −	3.4 −	2 −
382	S	4.7 −	3.0 −	1 −	438	S	7.0 −	3.8 −	1 −
383	S	6.0 −	3.6 −	1 −	439	N	7.2 −	3.6 −	3 +
384	S	6.0 −	3.0 −	1 −	440	S	6.1 −	4.0 −	1 −
385	T	7.7 −	4.0 −	2 −	442	S	7.5 −	4.8 −	1 −
387	S	5.0 −	2.8 −	1 −	444	TR	8.5 +	5.2 +	4 +
388	S	9.0 +	5.0 +	1 −	445	S	6.5 −	4.0 −	1 −
389	S	6.0 −	3.2 −	2 −	446	S	6.3 −	4.2 −	1 −
390	T	8.1 −	4.4 −	2 −	447	S	6.0 −	3.4 −	1 −
391	S	5.1 −	2.9 −	1 −	452	S	7.0 −	4.0 −	1 −
392	S	8.2 −	4.9 −	1 −	453	S	6.0 −	4.0 −	1 −
393	S	6.8 −	3.8 −	1 −	454	S	5.7 −	3.8 −	1 −
395	T	4.7 −	2.8 −	1 −	455	S	7.8 −	4.6 −	4 +
396	S	5.0 −	3.0 −	1 −	457	T	8.0 −	4.4 −	4 +
397	S	6.0 −	3.0 −	1 −	458	N	8.4 +	4.2 −	1 −
398	S	6.6 −	3.5 −	2 −	459	S	7.4 −	3.6 −	3 +
399	S	6.7 −	3.4 −	1 −	460	N	9.4 +	5.1 +	1 −
400	S	5.5 −	3.0 −	1 −	461	S	8.0 −	4.0 −	1 −
401	S	6.7 −	3.4 −	1 −	463	T	7.0 −	4.2 −	1 −
402	T	8.7 +	6.0 +	1 −	464	S	6.0 −	2.8 −	2 −
403	S	6.0 −	4.0 −	1 −	465	S	6.8 −	4.0 −	1 −
404	TR	10.0 +	5.8 +	3 +	467	T	6.3 −	3.2 −	1 −
405	S	7.7 −	4.8 −	2 −	468	T	8.6 +	4.6 −	1 −
406	T	8.5 +	5.4 +	2 −	469	S	6.7 −	3.2 −	1 −
407	T	7.2 −	4.4 −	2 −	470	S	6.2 −	3.4 −	1 −
408	S	6.7 −	4.0 −	1 −	472	N	9.5 +	6.0 +	2 −
410	S	5.6 −	3.4 −	1 −	473	S	5.5 −	2.2 −	1 −
411	T	9.1 +	5.6 +	3 +	474	S	4.7 −	2.2 −	1 −
412	S	9.3 +	6.0 +	4 +	475	S	8.7 +	5.2 +	1 −
413	S	6.4 −	4.2 −	3 +	476	S	7.5 −	3.9 −	1 −
415	S	6.2 −	3.0 −	1 −	477	S	7.6 −	4.0 −	1 −
419	TR	8.7 +	5.2 +	3 +	478	S	6.9 −	3.6 −	1 −
420	TR	9.3 +	6.2 +	2 −	480	S	8.0 −	4.4 −	1 −
421	S	7.7 −	4.2 −	1 −	481	N	9.5 +	5.2 +	1 −
422	S	8.5 +	5.2 +	1 −	482	S	4.6 −	3.2 −	1 −
423	S	5.4 −	3.0 −	1 −	483	N	9.0 +	5.6 +	2 −
424	S	7.4 −	3.6 −	1 −	485	S	6.5 −	4.0 −	1 −
425	T	7.4 −	4.4 −	2 −	486	N	10.3 +	6.6 +	3 +

APPENDIX TABLE 4
(concluded)

Serum No.	Type of case [b]	Result of IgM test (diameter in mm; + or −)		Result of IFAT	Serum No.	Type of case	Result of IgM test (diameter in mm; + or −)		Result of IFAT
		Serum	Dried blood				Serum	Dried blood	
487	S	6.0 −	3.6 −	4+	541	T	6.8 −	4.0 −	2−
488	S	5.5 −	3.4 −	1−	543	S	5.8 −	3.2 −	1−
490	T	8.0 −	3.8 −	2−	544	T	7.2 −	3.8 −	1−
492	S	6.2 −	4.0 −	2−	545	S	6.2 −	3.5 −	1−
493	S	6.5 −	4.0 −	1−	546	S	6.5 −	3.4 −	1−
495	S	7.0 −	4.0 −	1−	547	S	6.5 −	3.6 −	2−
496	T	9.7 +	6.6 +	3+	548	S	6.7 −	3.6 −	3+
497	S	6.8 −	4.0 −	1−	549	TR	8.5 +	5.0 +	3+
498	S	7.0 −	3.8 −	1−	550	S	6.3 −	3.6 −	1−
499	T	7.0 −	4.2 −	2−	551	S	8.8 +	5.6 +	1−
500	S	7.0 −	4.2 −	1−	552	S	6.6 −	3.4 −	3+
501	S	6.5 −	4.1 −	3+	553	S	5.7 −	3.0 −	2−
502	N	9.5 +	6.2 +	4+	554	S	6.0 −	3.6 −	1−
503	TR	6.6 −	3.0 −	2−	556	N	8.8 +	5.4 +	4+
504	S	6.2 −	3.4 −	1−	557	N	8.6 +	4.9 −	3+
506	S	5.9 −	3.4 −	1−	558	S	6.0 −	3.2 −	2−
507	T	7.0 −	4.6 −	3+	559	S	6.9 −	4.6 −	1−
508	T	7.5 −	4.7 −	1−	560	N	8.9 +	7.0 +	2−
509	T	6.8 −	4.0 −	1−	561	T	7.4 −	5.0 +	1−
510	S	9.8 +	7.0 +	3+	562	T	7.6 −	4.6 −	1−
512	S	6.3 −	3.8 −	3+	563	S	6.8 −	4.8 −	1−
513	T	9.0 +	6.4 +	1−	564	S	6.6 −	4.2 −	1−
514	S	6.0 −	3.6 −	1−	568	TR	9.7 +	5.0 +	1−
515	S	5.2 −	3.0 −	1−	569	S	7.7 −	4.8 −	1−
516	S	5.4 −	3.0 −	1−	570	S	5.5 −	3.7 −	1−
518	N	9.0 +	5.8 +	3+	573	N	10.6 +	7.2 +	3+
519	T	7.0 −	4.0 −	1−	574	S	5.0 −	3.0 −	1−
520	N	9.2 +	6.2 +	4+	576	S	6.0 −	4.0 −	1−
521	T	7.2 −	4.4 −	1−	577	T	5.5 −	3.6 −	1−
522	S	6.0 −	4.0 −	1−	578	S	5.4 −	3.4 −	1−
523	S	7.6 −	4.4 −	1−	580	S	7.1 −	4.8 −	2−
524	N	8.6 +	6.0 +	4+	581	S	6.8 −	4.2 −	1−
525	S	5.2 −	3.0 −	1−	582	S	6.5 −	4.2 −	1−
527	N	8.5 +	5.4 +	3+	585	S	6.0 −	3.6 −	1−
528	S	6.5 −	3.6 −	1−	586	T	6.6 −	4.0 −	1−
529	N	9.5 +	6.4 +	1−	587	T	5.5 −	3.4 −	1−
530	S	5.8 −	3.5 −	1−	588	S	5.3 −	3.2 −	1−
531	S	5.8 −	3.5 −	1−	590	S	7.0 −	4.0 −	1−
532	S	7.5 −	4.4 −	1−	591	T	6.5 −	3.8 −	1−
533	S	6.5 −	3.6 −	1−	592	T	6.5 −	4.2 −	1−
534	S	5.5 −	3.2 −	1−	593	S	7.0 −	4.4 −	1−
536	T	8.0 −	4.6 −	4+	594	S	6.5 −	4.2 −	1−
537	S	5.0 −	2.8 −	1−	595	T	7.8 −	5.1 +	1−
538	S	6.3 −	3.4 −	1−	596	T	6.9 −	4.0 −	2−
539	S	7.0 −	3.6 −	1−	597	T	7.8 −	5.0 +	2−
540	S	5.9 −	3.4 −	1−	598	TR	8.0 −	4.2 −	1−