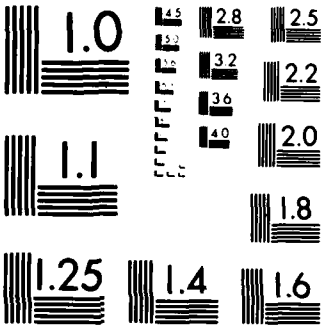
AD-A148 647 RAPID DIAGNOSIS OF ARBOVIRUS AND ARENAVIRUS INFECTIONS BY IMMUNOFLUORESCENCE(U) YALE UNIV NEW HAVEN CONN SCHOOL OF MEDICINE G H TIGNOR 01 FEB 82 1/1

UNCLASSIFIED DAMD17-81-C-1156 F/G 6/5 NL



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RAPID DIAGNOSIS OF ARBOVIRUS AND ARENAVIRUS INFECTIONS BY IMMUNOFLUORESCENCE

ANNUAL PROGRESS REPORT

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February 1, 1982

Supported by

U.S. Army Research and Development Command, Fort Detrick,
Frederick, Maryland 21701-5012

Contract DAMD 17-81-C-1156

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New Haven, Connecticut 06510

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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO. AD A148 647	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Rapid Diagnosis of Arbovirus and Arenavirus Infections by Immunofluorescence		5. TYPE OF REPORT & PERIOD COVERED Annual Progress Report 9/1/81 - 1/31/82
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Gregory H. Tignor, Sc.D.		8. CONTRACT OR GRANT NUMBER(s) DAMD17-81-C-1156
9. PERFORMING ORGANIZATION NAME AND ADDRESS Yale Arbovirus Research Unit, Yale University School of Medicine, 60 College Street, P.O.Box 3333, New Haven, CT. 06510		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62770A.3M162770A870.AQ.006
11. CONTROLLING OFFICE NAME AND ADDRESS U. S. Army Medical Research and Development Command, Ft. Detrick, Frederick, MD 21701 -5012		12. REPORT DATE 1 February 1982
		13. NUMBER OF PAGES 15
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Lassa Fever virus	Crimean Hemorrhagic Fever-Congo viruses
Ebola virus	Immunofluorescence
Marburg virus	ELISA
Rift Valley Fever virus	Equatorial Africa

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

A serologic survey of equatorial Africa for antibodies in man to Crimean-hemorrhagic fever-Congo, Rift valley fever, Ebola, Lassa fever, and Marburg viruses was begun. Positive reactions to Ebola virus have been observed with sera from military recruits from southern Sudan (41.3%) but rarely with sera from military recruits from northern Sudan (1.5%). Ebola positive reactions have also been found in sera from life-long residents of Senegal's lower Fleuve region in the Senegal River Valley. Antibody to Rift Valley Fever and

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

Cont. 20. ABSTRACT

Lassa viruses was also found in Senegal. Human sera from Ethiopia and Senegal have been screened on polyvalent spot-slides. The rate of positive reaction varies with geographic region.

One hundred and fifty-eight (158) bunyavirus supergroup viruses have been screened for reaction on KHF virus-infected spot-slides. Positive reactions have been found with several group Tete viruses and with one antibody preparation to Manawa virus.

Spot-slides for IF tests have been prepared, tested for adequacy and specificity and shipped to USAMRIID.

SUMMARY

A serologic survey of equatorial Africa for antibodies in man to Crimean-hemorrhagic fever-Congo, Rift Valley fever, Ebola, Lassa fever, and Marburg viruses was begun. Positive reactions to Ebola virus have been observed with sera from military recruits from southern Sudan (41.3%) but rarely with sera from military recruits from northern Sudan (1.8%). Ebola positive reactions have also been found in sera from life-long residents of Senegal's lower Fleuve region in the Senegal River Valley. Antibody to Rift Valley Fever and Lassa viruses was also found in Senegal. Human sera from Ethiopia and Senegal have been screened on polyvalent spot-slides. Human sera from Ethiopia and Senegal have been screened on polyvalent spot-slides. The rate of positive reaction varies with geographic region.

One hundred and fifty-eight (158) bunyvirus supergroup viruses have been screened for reaction on KHF virus-infected spot slides. Positive reactions have been found with several group Tete viruses and with one antibody preparation to Manawa virus.

Spot-slides for IF tests have been prepared, tested for adequacy and specificity and shipped to USAMRIID.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

a) **Serological survey in the Sudan.** (in collaboration with J. Meegan and T. Bucci, NAMRU-3). It is likely that many viruses which circulate in northern and southern Africa are disease problems in Sudan. Few studies have been undertaken in this geopolitically important country. During 1979, 1980, and 1981, over 3400 sera were collected from military recruits in Sudan. These represent collections from all areas of Sudan (age, birthplace, and district of residence are available for all). This survey is one phase of a long-term study to determine the impact of a number of virus infections on humans and animals in Sudan. In addition, since Sudan may act as a tunnel for the movement of viral disease from sub-Saharan Africa to Egypt and beyond, survey for other viruses may give clues as to what diseases to be alert for.

We have used the IF test to screen representative sera from this collection. This year, in collaboration with Drs. K. Johnson and J. McCormick (CDC) who provided inactivated, infected-cell antigen slides, we have screened over 400 sera from 16-21 year-old male military recruits in Sudan for IF antibodies to RVF, Lassa, CCHF, Marburg, and Ebola viruses. We used a polyvalent antigen slide (designated CRE_2LM) containing a mixture of cells which had been individually infected with each of the viruses (including both Sudan and Zaire strains of Ebola virus). Since recruits were bled at various recruit camps throughout the country, the sample contains sera from recruits whose home village was adjacent to the camp, as well as sera from recruits who were transferred into the area. Results of our initial screening on polyvalent slides indicate that sera from residents of many Governorates in southern Sudan have a high prevalence of antibodies detected in this test (69 positives/167 total sera = 41.3%), while recruits from northern Governorates had a low prevalence of antibodies (1 positive/55 total sera =1.8%). Antibody was not found in a small sample of sera collected from Egypt (24 sera). Additionally, soldiers from northern and central Sudan (low endemic areas) who were transferred south for deployment have a significantly higher prevalence of antibody when compared to a control group sent to northern Governorates (9 positives/56 sera vs. 2 positives/56 sera). Currently, we are testing the positive sera on monovalent slides. All positive sera are being retested on monovalent slides. Sixteen of the first 28 positive sera have been positive on monovalent Ebola (Zaire) slides, but negative on all other monovalent slides. The 12 sera not reacting on monovalent slides could represent false positives or sera positive to Ebola (Sudan). These preliminary results suggest that the majority of the sera positive on CRE_2LM slides represent Ebola antibodies.

Table 1. Sudan sera seropositive in immunofluorescence tests using polyvalent antigen slides containing Ebola, Marburg, Lassa, Crimean-Congo, and Rift Valley fever viruses.

Source of Sera (Home of Governorates	Governorate where currently stationed	Number Positive/ Number tested	Percent
Northern Governorates (Nile, Northern, Kassala, Khartoum, El Gezeria, White Nile)	Khartoum	1/55	1.8
Southern Governorates			
Eastern Equatoria	Eastern Equatoria	7/34	26.6
Western Equatoria	Western Equatoria	18/47	38.3
Bahr El Ghazal	Bahr El Ghazal	33/59	56.0
Central Governorates			
Northern and Southern Kordofan	Khartoum	2/56	3.6
Northern and Southern Kordofan	Eastern and Western Equatoria	9/56	16.1

Our testing has been divided between ELM slides and CRE2LM slides. While the CRE2LM slides seemingly offer the advantage of greater breadth in our screening, it was our concern that this advantage might be counterbalanced by the increased danger of decreased reproducibility using the CRE2LM slides. Since the CRE2LM slides contain more viruses, it could be that the chances of error could be significantly increased. In addition, it was our observation that the quality and quantity of infected cells varied somewhat from lot-to-lot. Therefore, a series of coded single-blind experiments were undertaken to test the reproducibility of our test system. Different lots of CRE2LM slides were mixed and used in these experiments. The results are as follows.

Table 2 Reproducibility of IF test on two types of polyvalent slides.

EXPERIMENT 1

Number tested on ELM (JC) retested on ELM (RC)	42
# (+) on orig ELM and (+) on repeat	11
# (+) on orig ELM and (-) on repeat	1
# (-) on orig ELM and (-) on repeat	27
# (-) on orig ELM and (+) on repeat	3

EXPERIMENT 2

Number tested on CRE2LM (RC) retested on CRE2LM (RC) 7 days later	42
# (+) on day 1 and (+) day 7	17
# (+) on day 1 and (-) day 2	2
# (-) on day 1 and (-) day 2	15
# (-) on day 1 and (+) day 2	8

EXPERIMENT 3

Number tested on ELM (RC) retested on CRE2LM 7 days later	186
# (+) on ELM and (+) on CRE2LM	28
# (+) on ELM and (-) on CRE2LM	7
# (-) on ELM and (-) on CRE2LM	110
# (-) on ELM and (+) on CRE2LM	41

Retesting on ELM slides gave 90% reproducibility; however, retesting on CRE2LM gave only 76% reproducibility. These results confirm our suspicions and suggest a good rationale for screening with ELM slides. Nevertheless, we will screen with CRE2LM slides since our results will be confirmed by continued IF tests and by either ELISA or neutralization tests.

Senegal human serosurvey for Rift Valley fever, Ebola, Marburg, Lassa, and Crimean-Congo hemorrhagic fever

A serological survey of human sera collected in Senegal by a YARU team headed by W.G. Downs in 1977 was done using indirect immunofluorescence and spot-slides. The slides were provided by Dr. K.M. Johnson, CDC. Polyvalent slides were used to screen 283 sera for RVF, Ebola, Marburg, Lassa, and CCHF. Not all sera were tested for all viruses. Of 283 sera tested, 37 were positive to one or more antigens.

Six sera were positive with spot-slides containing all 5 viruses; 4 out of 5 of those tested for RVF were mono-specific for RVF. Only 4 of 31 positive to the polyvalent Ebola-Marburg-Lassa spot-slides were tested further.

The cumulative positive results obtained by J. Casals and G. Tignor so far indicated Ebola antibody in 6 persons (2 were children aged 5 and 8 years), Lassa antibody in one person, and RVF antibody in 4 persons. All of these persons claimed to be life-long residents of Senegal's lower Fleuve region in the Senegal River valley. This is the site where multi-national construction teams are about to start building a series of dams.

In view of the surprising finding of RVF antibody and the known cross-reaction by IFA with other phlebotomus fever group viruses, tests were done to determine the specificity of the reaction with 2 of the sera. The results indicated full specificity for RVF . In addition, PRNT performed by Dr. C.J. Peters, USAMRIID, confirmed the RVF positive reactions.

Serosurvey in Ghana

Two hundred forty-seven human sera collected in Ghana in 1975 were screened on ELM slides during the past year. We have found many positives with sera from individuals in the Eastern (42%) and Western (34%) regions and fewer positives from the Ashanti (18%) region. We have not yet screened sera from the Brong-Ahafo or the Northern regions.

Ghana positives on ELM slides by geographic region

Region	Results		
	Positive	Tested	Percent
Eastern	35	83	42
Western	35	102	34
Brong-Ahafo	Not tested		
Ashanti	11	62	18
Northern	Not tested		

Serosurvey in Ethiopia

We have screened 201 sera from the Ethiopian collection and all of these sera were tested on ELM slides.

Ethiopian survey results by region on ELM slides

Region	Positive	Negative	Per Cent
Assab	3	17	15
Blue Nile	26	44	37
Sidamo-Borena	10	50	17
Ogaden	0	4	0
L'Aquache	14	33	30

The breakdown of positives by region shows more positives in the Blue Nile and Valle deL'Aquache regions than in the Assab, Sidamo-Borena regions. (Only 4 sera have been tested from Ogaden). However, within the Blue Nile region, 8 of 26 total positives (31%) come from two villages where each individual tested was positive. There were 25 villages sampled in the region. Similarly, in the Valle deL'Aquache, 8 of 14 total positives (57%) came from 2 villages. There were 12 villages sampled in this region. It appears from these preliminary data that there is a marked localization of virus activity within these broad geographic regions. Again, in the Sidamo-Borena region, 8 of 10 positives came from 3 villages of 25 sampled.

b.) Early diagnosis by IF

1. Spot-slides for IF tests have been prepared, tested for adequacy and specificity and shipped to USAMRIID. Two-hundred (200) spot-slides for immunofluorescence (IF) were prepared with a mixture of six cell cultures individually infected with the following group B mosquito-borne viruses: St. Louis (Parton), Rocio, JBE (Nakayama), Sepik (MK 7168), Yellow Fever (Asibi), and Dengue 2 (NGB). The cells used were Vero clone C-1008 for Sepik, St. Louis, yellow fever, (Asibi), and dengue 2 (NGB). The cells used were Vero clone c-1008 for Sepik, St. Louis, yellow fever, Rocio, JBE; and LLC-MK2, supplied by Dr. S. Buckley at YARU, for dengue 2. CPE for all viruses ranged from one to two plus at the time the cells were processed. This preparation, designated Lot #518, was tested for efficacy and specificity in the IF test and shipped to USAMRID in September 1981.

When Lot #518 was tested for reactivity against group B mosq[illegible]orne viruses using mouse hyperimmune sera, positive reactions were obtaine[illegible] t titer 1:16 or higher with each of the viruses represented in the slides. I[illegible] #518 was also tested against the following: Banzi, Bussuquara, dengue 1, [illegible]ue 3, dengue 4, Ilheus, MVE, Spondweni, West Nile, Tamana, Zika, and [illegible] MML, Spondweni, and Tamana viruses did not react, while the others reacted at titer 1:8 or higher.

A second set of slides was prepared with dengue 4 (H241) using the LLCMK2 cell line supplied by Dr. S. Buckley. This preparation was designated Lot #520. CPE was questionable at the time of processing (Day 8). The IF test using mouse hyperimmune serum gave good fluorescence at titer 1:64. Lot #520 was shipped to USAMRID in September 1981.

2. E.M. studies with KHF virus suggested that KHF virus might be a Bunyavirus-like particle. Subsequently, spot-slides of KHF-infected cells were prepared and delivered by Dr. R. Rosato to Dr. G.Tignor by instruction of Dr. K. Johnson for IF screening using antibody to all known members of the Bunyamwera supergroup. In our tests, most members were tested by FA or complement fixation against their homologous sera or ascitic fluids. Grouping sera or ascitic fluids were not used in these tests by instruction of Dr. K. Johnson. A summary of our results is presented below.

TESTED	POSITIVE	QUESTIONABLE	NOT TESTED
158	4	18	43

All of the questionable reactions have been eliminated as tissue reactions since these sera reacted with uninfected cells. One positive reaction which we report is with Manawa virus (strain JC 791) antibody made with infected mouse brain tissue on KHF (Rosato) slides. Only one of several antibody preparations to Manawa virus reacted positively. It may be that this particular strain of Manawa virus contains more than one agent. The hypothesis is supported by the fact that our other positive reactions occur within one virus group. The other positive reactions which remain to be pursued are to Bahig, Matruh, Tete, all group Tete viruses. Antibody to these three viruses also reacted positively on KHF (Lee) slides. We propose experiments to determine whether or not these reactions are bona fide. For positive controls, we used human (KHF) sera received by Dr. J. Casals from Dr. N.H. Wiebenga on June 6, 1969. Any one of the strongly positive sera was routinely used at dilution 1:4 as a positive control when screening with the new (Rosato) KHF slides.

Human immune sera (from Wiebenga) were tested in parallel on KHF slides from Lee and Rosato. The following reactions were observed.

SERUM #	OLD KHF SLIDES		NEW KHF SLIDES (ROSATO)	
	Acute	Convalesc	Acute	Convalesc
KHF 1*	++	++	+	++
KHF 2*	++	++	NT	+
KHF 3*	++	++	++	NT
KHF 4*	++	++	NT	++
KHF 5	0	0	0	0
KHF 6	++	++	+	++
KHF 7	++	++	NT	++
KHF 8	++	++	++	++
KHF 9	++	++	++	++
KHF 10	++	++	++	++
Loomis	NT	++	NT	++

NT was not tested; * was negative with uninfected cells

The detailed cumulative results of our tests are presented below. A positive reaction is indicated by (+) and a negative by (0). Tests which were inconclusive were repeated and these results are indicated by (-). Most of these reagents also reacted with uninfected cells.

IF REACTIONS WITH KHF SPOT SLIDES

IMMUNE REAGENT	RESULT
Anopheles A	
Anopheles (CoAr 3624)	NT
Lukuni	0
ColAn 57389	-
Tacaiuma (BeAn 73)	0
Bunyamwera Group	
Bunyamwera	0
Germiston	0
Shokwe	NT
Batai (Calovo)	-
Birao	0
Tensaw	0
Cache Valley	0
Maguari	-
Northway	0
Santa Rosa	0
Lokern	-
Wyeomyia	0
Taiassui	NT
Anhembi	0
Sororoca	0
Main Drain	0
Kairi	0
Guaroa	0
76V-25880	0
Macaua	0
ACRE	0
Mojui Dos Campos	0
AG 80-381	0
AG 80-504	0
PARA	0
Virgin River	0
Bwamba Group	
Bwamba	0
Pongola	0
C Group	
Caraparu (BeAn 3994)	-
Caraparu (BeH 5546)	NT
Caraparu (Trinidad)	NT
Ossa	-
Apeu	-

IMMUNE REAGENT	RESULT
C Group contd.	
Madrid	0
Marituba	0
Murutucu (BeAn 974)	NT
Restan	0
Nepuyo	0
Oriboca	NT
Itaqui	-
Gumbo Limbo	0
California Group	
California	0
Tahyna	0
Inkoo	0
San Angelo	0
La Crosse	NT
Melao	0
Serra do Navio	NT
Keystone	NT
Jamestown Canyon	0
Trivittatus	0
Capim Group	
Capim	0
Guajara	0
Bush Bush	NT
BeAn 84381	-
Gu 71 u 344	NT
Juan Diaz	0
Acara	0
Moriche	NT
BeAn 153564	NT
Guama Group	
Guama	0
Moju	0
BeAn 109303	NT
Mahogany Hammock	0
Bimiti	0
BeAn 116382	0
Catu	0
Bertioga	0

IMMUNE REAGENT	RESULT
Koongol Group	
Koongol	0
Wongal	NT
Mirim Group	
Mirim	0
Minatitlan	0
Olifantsvlei Group	
Olifantsvlei	0
Bobia	NT
Botambi	0
Patois Group	
Patois	0
Group	
Shark River	NT
Zegla	0
Pahayokee	NT
Simbu Group	
Akabane	0
Yaba-7	0
Facey's Paddock	NT
Shamonda	NT
Sango	0
Sabo	NT
Sathuperi	0
Shuni	0
Aino	0
Simbu	0
Thimiri	0
Nola	0
Peaton	NT
Manzanilla	0
Ingwavuma	0
Mermet	0
Inini	0
Buttonwillow	0
Oropouche	0
Utinga	0

IMMUNE REAGENT	RESULT
Tete Group	
Bahig (EgB 90)	+
Matruh (EgAn 1047-61)	+
Truruse	NT
Batama	NT
Tete (EgAn 4511)	+
Anopheles B	
Anopheles B	-
Boraceia	0
Bakau Group	
Bakau	0
Ketapang	0
Crimean hemorrhagic Fever	
Congo	0
Hazara	0
Kaisodi Group	
Kaisodi	0
Lanjan	0
Silverwater	0
Mapputta Group	
Mapputta	0
Maprik	NT
Gangan	NT
Trubanaman	NT
Nairobi Sheep Disease Group	
Ganjan	0
Dugbe	-
Phlebotomus Fever Group	
Candiru	0
Itaituba	0
Nique	0
Punto Toro	0
Buenaventura	-
Saint Floris	0

IMMUNE REAGENT	RESULT
Phlebotomus Fever Group cont.	
Goril	-
Aqucate	0
Anhanga	0
Arumowot	0
Bujaru	0
Cacao	0
Caimito	0
Chagres	0
Chilibre	0
Frijoles	0
Icoaraci	0
Itaporanga	0
Karimabad	0
Pacui	0
Rio Grande	0
Salehabad	-
Gabek Forest	NT
SF Naples	0
Toscana	0
SF Sicilian	0
Urueuri	0
Alenquer	NT
Tehran	0
Joa	0
Muwguba	0
Oriximа	0
Turuna	-
Rift Valley Fever	0
Belterra	0
Sakhalin Group	
Sakhalin	NT
Tillamook	NT
Taggert	NT
CloMor	0
Avalon	-
Thogoto Group	
Thogoto	0
SiAr 126	NT
Turlock Group	
Turlock	0
Umbre	0
M'Poko	NT
Barmah Forest	NT
Marweh	NT

IMMUNE REAGENT	RESULT
Uukuniemi Group	
Uukuniemi	0
Ocean Side	0
Grand Abaud	0
Manawa	+
Zalin Terpeniya	-
Ponteves	NT
Egan 1825-61	-
Unassigned Viruses	
Belmont	0
Bhanja	0
Khasan	NT
Kowanyama	0
Lone Star	0
Razdan	NT
Rift Valley Fever	0
Sudany Canyon	NT
Tamdy	NT
Tataquine	NT
Witwatersrand	0
Gamboa	0
Guaratuba	0
Kaeng Khai	0

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