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RETENTION OF FLUORESCENT ANTIGENICITY OF VIRUS-INFECTED CELLS ON SPOTSLIDES UNDER VARIOUS CONDITIONS OF STORAGE

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Ten well, epoxy-coated spotslides containing arbo- and arenavirus-infected cells were tested as determined by fluorescent methods for the retention of antigenicity after storage in a nitrogen atmosphere at various temperatures and times. In most cases antigen stability was maintained at -70 or -20°C for periods exceeding 1-3 years. Antigen deterioration was greatest at ambient temperature, and less so at 4°C.

fluorescent antigen stability arboviruses arenaviruses infected cells

INTRODUCTION

During the past few years both indirect (IFA) and direct (FA) fluorescent antibody tests have become very practical and the tests of choice for serologic diagnosis of high hazard arena- and arboviruses. For certain arbovirus antibodies the IFA test is more sensitive than either the hemagglutination-inhibition (HAI) or complement fixation (CF) test but less so than the suckling mouse neutralization test (Rosato, Bagley and Luscri, in prep.). Sensitive, reliable fluorescent tests have been developed for Machupo (MAC, Peters et al., 1973), Junin (JUN, Grela et al., 1975), Lassa (LAS, Wulff and Lange, 1975), Congo-Crimean hemorrhagic (C-CHF, Zgurskaya et al., 1975), Marburg (MAR, Wulff et al., 1978), Ebola (EBO, Bowen et al., 1978), and Korean hemorrhagic fever (KHF, French et al., 1980) viruses.

One of the primary problems (other than the subjectivity of interpretation) associated with the widespread acceptance of fluorescent antigen and antibody techniques as a replacement for CF and HAI tests is the necessity for certain reagents to be stored at -70°C in order to retain activity or antigenicity over long periods of time. Such a requirement for antigen and at times conjugate storage often precludes the widespread use of fluorescent techniques in the field where conditions for low temperature storage do not

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always exist or are sporadic at best. Upon using virus antigen-containing, tissue culture cells fixed onto epoxy-coated spotslides for serodiagnosis, it became apparent that antigen stability at temperatures other than -70°C needed to be determined if such slides were to be stockpiled and/or used in the field. Since some biologicals are better preserved in a nitrogen atmosphere, a procedure was designed to examine IFA or FA antigen stability in such an atmosphere at -70 , -20 , and 25°C (ambient) temperatures.

MATERIALS AND METHODS

Canning procedure

Spotslides were placed in plastic, slide-mailing boxes modified by 'drilling' a hole in each end using a 16-gauge needle and held at -70°C until canned. To can, slideboxes were placed in a tall no. 1 can (Freund Can Co., Chicago, Illinois) and approximately 10–15 ml of liquid nitrogen were added, and the top of the can was placed into position for sealing. Once nitrogen evaporation had ended, the can was sealed and placed at an experimental temperature. After all cans were sealed and held at their respective temperature for 2 h, one was removed from both -20 and 4°C , opened, and stained to obtain base-line data.

General

Virus strains used to infect both cell lines and animals and to prepare spotslides and conjugated antisera are listed in Table 1. Procedures for the preparation and fractionation of sera for conjugation, and conjugation of sera with fluorescein isothiocyanate (FITC) were as described previously (R.R. Rosato, L.R. Bagley and B.J. Luscri, in prep.). Spotslides were prepared as described by Casals for arenaviruses (Casals, 1979a). Slides were read on a 1+ to 4+ scale of intensity in which a 1+ is a definite positive but lacking in intensity.

RESULTS

We had previously determined (O.M.B.) that KHF monolayer spotslides held in sealed cans from which the air had been excluded by the addition of liquid nitrogen were stable as to IFA antigen intensity at various temperatures (-70 , -20 , 4 , and 25°C) for various periods of time (Table 2). After 14 mth at -70 , -20 , or 4°C staining intensities were still 3+ to 4+; at 25°C , intensities became negative at some time between 3 and 6 mth. Results from several groups of spotslides prepared for serodiagnosis showed that KHF slides held at -20°C in slide boxes inside of small self-sealing plastic bags without nitrogen atmosphere lost 50–100% of their fluorescent antigenicity within 3–6 mth (unpubl. data). Casals (1979b) reported that spotslides of eastern equine encephalitis virus (EEE) held at -60°C retained total antigen staining intensity (4+) after 22 or 78 days; those held at 4°C were 4+ at 22 days, but of questionable use after 78 days at which time staining intensity had decreased to 2+. If held at 22°C , slides were usable

TABLE 1

Virus strains, cell types, and animal species used to produce reagents

Virus ^a	Spotslide		Conjugate	
	Strain	Cell	Strain	Species
TCR	TRVL 11573	Vero	TRVL 11573	Guinea Pig
PIC	An 3739	Vero	An 3739	Monkey
EEE	Alabama	Vero	New Jersey	Guinea Pig
DEN-2	NG B	LLC-MK ₂	^c	Human
WN	Egypt 101	Vero	Egypt 101	Guinea Pig
RVF	ZZ 501	BHK-21	^d	Sheep
LAC	^b	PK-15	B 956	Guinea Pig
ORO	TRVL 9760	BHK-21	TRVL 9760	Guinea Pig
KHF	76-118	A-549	human IgG	Goat

^a Tacaribe (TCR), Pichinde (PIC), eastern equine encephalomyelitis (EEE), dengue (DEN), West Nile (WN), Rift Valley fever (RVF), LaCrosse (LAC), Oropouche (ORO), Korean hemorrhagic fever (KHF).

^b ATCC VR 744, no strain designation given.

^c Natural infection.

^d Unknown.

with qualification (2+ intensity) after 22 days but of no use at all after 78 days. Slides of questionable value or usable with qualification were generally of 2+ intensity and the fluorescence in the cytoplasm had migrated toward the periphery and accumulated under the cell membrane resulting in membrane fluorescence or margination of fluorescence. It seems improbable that fluorescence could have migrated in acetone-fixed preparations. Rather, what was observed may have been the differential inactivation or destruction of reactive fluorescent cytoplasmic antigens and the partial retention of membrane antigenicity appearing as margination of fluorescence. Slides from the same lot are still of

TABLE 2

Evaluation of KHF fluorescent antigen^a stability during nitrogen storage at various temperatures

Time	Intensity (1-4+) at various temperatures			
	25°C	4°C	-20°C	-70°C
0 days	4	4	4	4
31 days	2	2	3	3
61 days	1	4	3	4
96 days	3	4	4	4
6 mth	0	4	4	4
14 mth	0	3	3	3

^a IFA using goat antihuman IgG conjugate.

excellent quality after almost 3 yr of storage at -70°C when tested by either FA or IFA tests.

In view of the encouraging results obtained from the KHF study, a similar study was started to test the FA storage stability of Tacaribe (TCR), Pichinde (PIC), eastern equine encephalomyelitis (EEE), dengue-2 (DEN-2), West Nile (WN), Oropouche (ORO), La-Crosse (LAC), and Rift Valley fever (RVF) viruses at 20 and 4°C . The viruses selected represent various serological classifications. The other temperatures were not evaluated for the following reasons: at -70°C , EEE and other viruses are known to be stable for more than 3 yr (unpubl. data); -60 to -80°C is the generally accepted temperature for storage of fluorescent reagents (Wulff and Lange, 1975; Kawamura, 1977); and 25°C was shown not to be a viable option (KHF study).

The data in Table 3 indicate that RVF and PIC viruses are stable at both 4 and -20°C for up to 365 days. TCR virus is stable for 123 days at both 4 and -20°C , and then stability decreases equally between 123 and 240 days at both temperatures. WN reacts similarly to TCR up to 123 days, but shows a more pronounced decrease at 4°C than at -20°C . DEN-2 virus is stable to 123 days at 4°C , decreases at 240 days and is negative at 365 days, whereas at -20°C stability is maintained to 240 days and then decreases slightly (3+ to 2+) at -20°C .

DISCUSSION

The retention of fluorescent antigen stability, during prolonged periods of storage at temperatures available in most laboratories and field units, will greatly enhance the FA test applicability and acceptance as the primary serological test for arbo- and arena-

TABLE 3

Stability of FA antigenicity after storage at various temperatures

Virus	Intensity (1-4+) ^a											
	4°C by days						-20°C by days					
	0	30	61	123	240	365 ^c	0	30	61	123	240	365
TCR	4	3	4	3	1	1	3	3	4	3	1	1
PIC	4	4	4	4	4	4	4	4	4	4	4	4
EEE	3	2	4	3	2	1	3	2	4	4	3	4
DEN-2	2	1	4 ^b	4	2	0	2	1	4 ^b	4	3	2
WN	3	4	2	3	1	1	3	3	2	3	2	2
RVF	4	4	4	3	4	3	4	4	4	4	4	4

^a Average of 8 spots.

^b Conjugate changed for both temperatures.

^c Moisture on all slides when opened.

viruses. All FA reagents can be prepared in the primary laboratory, packaged and shipped to the field or other laboratories as required. Constant procurement, preparation and standardization of reagents such as fresh goose erythrocytes or complement for the HAI or CF tests will not be necessary. All antigen fixed spotsides examined are stable at -20°C under the conditions described for a minimum of 123 days and some for more than a year. This is more than adequate for most anticipated field studies. Similar reagents held at -60 to -70°C are stable for 1 to more than 3 yr, perhaps indefinitely.

One potential problem arising from the canning method may be the retention of residual moisture on the slides that is picked up during transfer from -70°C storage to the slide boxes and then into the cans, and the moisture produced by the liquid nitrogen during evaporation. Such residual moisture was found in some cans when opened. To preclude introduction of moisture, it is proposed to warm and dry the slides in a 37°C incubator, a procedure shown not to be detrimental through 3 to 5 cycles, place them in the slide boxes without holes and then into cans, flush with nitrogen gas and complete the canning procedure in a nitrogen atmosphere. Such a modification has been used to ship slides at ambient temperatures to Egypt and Greece (J. Casals, pers. comm.).

The determination of the specific slides to be placed in a given can, or set of cans will be based on the projected use of the slides. Those for use in North America will probably consist of a group A polyvalent slide containing EEE, western (WEE) and Venezuelan (VEE) equine encephalomyelitis, and Chikungunya (CHIK), and monovalent specific slides for EEE, WEE, VEE, LAC, Saint Louis encephalitis (SLE), and yellow fever (YF) viruses; South and Central America, monospecific slides for JUN, MAC, YF, ORO, EEE, SLE, and Rocio (ROC) viruses; Africa, polyvalent slides of LAS, MAR, EBO and monospecific LAS, EBO, MAR, Congo, YF, CHIK and RVF viruses. Polyvalent group B slides containing Japanese encephalitis (JE), ROC, YF, langat (LGT), DEN-1, and DEN-2, and a tick-borne polyvalent group B containing Powassan, LGT, Karshi, Tyuleny and Kadam are also available as are monospecific slides for Mayaro, O'nyong-nyong, DEN-3, DEN-4, sandfly fever Naples, and Sicilian, lymphocytic choriomeningitis and Hazara for use in preparing tailor-made groups of slides for a given geographic area.

It is suggested that the next step in the evolution of geographically tailored spotsides for seroepidemiological studies proceeds along the lines of those for trachoma organisms for which immunofluorescence 'microdot' tests were developed (Wang, 1971). Such a microdot system has been developed for YF virus infected LLC-MK₂ cells and has been shown to be usable (unpubl. data).

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