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Shelf Life of Fluorescent Treponemal Antibody-Absorption Test Reagents

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Properly prepared, standarized, and stored fluorescent treponemal antibody-absorption (FTA-ABS) reagents have been shown to have stabilities equal to other biological reagents. A liquid antigen over 10 years old has been shown to give a satisfactory reaction. Newer preparations have now been shown to be stable for over 5 years, and the tests on each are being continued. The very new liquid antigens which were originally standardized by the FTA-ABS method have shown no decrease in potency over a 20-month period. Stability studies on antigens dried on slides are now in their eighth month, with no apparent loss in potency. The stability of the conjugate is constant when stored frozen at −20 C or lyophilized. When stored as a liquid at 4 C, the stability is governed by the *p*H and the molarity of the buffer. The standardized and lyophilized sorbent has been shown to be stable for over 1 year.

Information concerning the stability of the fluorescent treponemal antibody-absorption (FTA-ABS) reagents is minimal. Leibovitz et al. (2) have studied the comparative stability of the antigens used in the fluorescent treponemal antibody (FTA) and *Treponema pallidum* immobilization (TPI) tests. They found that an antigen preparation containing 5.0% dimethylsulfoxide and 10% normal rabbit serum and stored in the frozen state was stable for 2 weeks after thawing. Fife et al. (1) found that the FTA test showed a greater reproducibility of results than did the TPI test because the FTA antigen could be kept lyophilized for at least 4 months.

The National Communicable Disease Center (NCDC) (6) has reported that their antigen for the FTA-ABS test may be stored at 6 to 10 C without preservative or it may be lyophilized. Their experimental antigens containing albumin were satisfactory at 5 to 10 C for 4 weeks, although some decrease in the total number of treponemes was noted. Antigens extracted in phosphate-buffered saline (PBS), Hank's balanced salt solution, distilled water, or maintenance media were stable for 2 to 3 months at 5 to 10 C without preservatives. After 39 days at −20 C, these antigens gave essentially the same results as those obtained with fresh preparations, despite a reduction in the total number of treponemes by these methods. In a separate study, Yobs et al. (8) found that antigen fixed to a slide with acetone was satisfactory for at least 2 weeks at −20 C and for 5 days at room temperature.

The fluorescein isothiocyanate (FITC) conjugate of anti-human γ-globulin was found to be stable for at least 50 days when maintained either at 4 C or at −20 C (3). Wickerhauser and Pethel (7) have shown that lyophilization of the conjugate at a 1 to 2% protein concentration did not cause a decrease in titer.

The contribution of each reagent to the background in the FTA-ABS test is the subject of the accompanying paper (5). Of equal importance is the standardization and stability of the reagents. The standardization of each reagent has been described (6). This paper deals with stability studies which have been done with (i) the *Treponema pallidum* antigen, (ii) the sorbent which is an extract of the Reiter treponeme, and (iii) the conjugate which is a FITC-labeled anti-human γ-globulin prepared with rabbits.

MATERIALS AND METHODS

The stability studies were performed on reagents furnished by the NCDC and by J. N. Miller of UCLA or on reagents prepared in this laboratory according to the method described by Miller et al. (4). The commencement (zero) time for aging of the reagents depended to a large extent upon factors associated with the state of the preparation.

Aging of liquid T. pallidum antigens. Dehydrated antigens were reconstituted according to directions and were stored at 4 C; the zero-time was considered to be the day on which the antigen was rehydrated. The zero-time for the antigens prepared in the liquid

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state in this laboratory began 10 days after harvest from rabbit testes.

Aging of T. pallidum antigen fixed on slides. The liquid antigen as prepared in this laboratory (5) or rehydrated as directed was applied to pre-etched methanol-cleaned and flame-dried glass slides. The standard loopful of antigen on the slide was allowed to dry at room temperature and was fixed in acetone, in acetone followed by a distilled water rinse, or in 10% methanol. When acetone was used, the fixation time was 10 min. When the acetone slides were water rinsed, the time was 30 sec followed by rapid drying with warm air. When 10% methanol was used, the fixation was 10 to 20 sec followed by rapid drying with warm air. The fixed antigen was aged at −20 C, 4 C, room temperature, and 37 C. The testing of the preparations on a predetermined schedule continued until the antigen failed to show proper reactivity with a standard 1+ serum in the trypsin digestion (FTA-ABS-T) test (5).

Aging of the sorbent. The sorbent, as prepared in this laboratory (5), was lyophilized and aged at −60, −20, 4, and 48 C. At specified intervals, a sample was removed, rehydrated, and tested by the standard NCDC test to determine the efficacy of the product (6).

Aging of the conjugate. The conjugates used in the stability studies were prepared in this laboratory (5). Each showed a strong γ-globulin line and a very faint albumin line. The fluorescein-protein ratios varied between 13.5 and 15.5, with a protein concentration ranging between 0.9 to 1.1%. One of the conjugates was aged in both the lyophilized and frozen state at −60, −20, and at 4 C. Various buffers, molarities, and pH values were used with these test conditions. At designated intervals, samples were removed and tested as described by the NCDC (6).

RESULTS

Shelf life studies on liquid T. pallidum antigen. The antigen for the FTA-ABS procedure was extracted from the testes of rabbits previously inoculated with the Nichols strain of *T. pallidum*. The method of preparation of the testicle for extraction and composition of the extracting fluid has gone through an evolutionary process over the years. Early in our investigations on the possible automation of the FTA-ABS process, it was found that the best preparations must contain minimal amounts of either mammalian cellular debris or soluble protein. Since the standard NCDC antigen contained more soluble proteins than our preparations, it was necessary to determine the shelf life of each. At zero-time, the antigens were tested against standard 4+ and 1+ sera. These antigens were tested by the trypsin digestion modification of the standard procedure (5), except in a few instances with the early antigen lots. The results of tests on liquid antigens of various ages are shown in Table 1. Although two of the antigens were much older than 20 months, they were not tested by the FTA-ABS method until 20 months ago. Those antigens which showed correct titer with 4+ and 1+ sera in the last three columns of Table 1 are still being tested. They will continue to be tested at intervals until there is a decrease in potency as shown by this test. Two of the antigens shown in Table 1 were quite unstable, as shown by their rapid loss of stainability.

TABLE 1. *Shelf life studies on liquid antigens*[a]

Antigen	Zero-time		Time interval (months)	Titer	
	4+ serum	1+ serum		4+ serum	1+ serum
Lot 1	4+	1+	20	4+	1+
Lot 3	4+	1+	12	4+	1+
Lot 5	4+	1+	10	4+	1+
17059[b]			68	4+	1+
912662[b]	4+	1+	20	4+	1+
TPIA[b]			127	4+	1+
NCDC	3+	1+	2	+	−
271167W[b]	3+	1+	10	4+	1+
Lot 5-22	3+	1+	1	−	−

[a] The trypsin digestion (5) was used in all tests except the early antigen lots. The antibodies were NCDC no. 661 (4+) and (1+) samples. The conjugate was produced at Aerojet-General. Lot 1 antigen was the first production lot produced at Aerojet-General.

[b] Supplied by J. N. Miller of UCLA. Lot 271167W was in Nelson's medium and TPIA contained 1.25% bovine albumin.

Shelf life studies of T. pallidum antigen fixed on slides: NCDC antigens. The NCDC antigens were the standard to which modifications in procedures were compared. The antigen used on these slides was obtained from the NCDC in a dehydrated state. It was rehydrated as directed and was fixed on the slides on the day of rehydration. The unused portion was kept at 4 C for shelf life studies of the fluid product. The antigen on the slide was fixed in acetone and stored at 4 and −20 C (Table 2).

NCDC antigen prepared at UCLA. This antigen was prepared by the NCDC method but not by NCDC personnel. The antigen was fixed to the slides by either acetone or 10% methanol and was stored at room temperature and at −20 C (Table 2).

T. pallidum antigen prepared in the Aerojet-General Corp. The antigen was fixed on glass slides in the usual manner, and on glass cover slips on the slides used in the semiautomated syphilis diagnostic machines. The fixing agents were acetone, acetone followed by a water rinse,

TABLE 2. *Shelf life studies with fixed antigens on slides*[a]

Antigen	Fixant	Storage temp (C)	Weeks of proven antigenicity	Weeks to potency failure
NCDC	Acetone	−20	31	
		4	31	
NCDC method[b]	Acetone	−20	30	
	Acetone	RT[c]		4
	10% methanol	−20	30	
Lot 5 on glass slides	Acetone	−20	30	
	Acetone	4		22
	Acetone	RT		8
	Acetone-water	−20		26
	Acetone-water	4		5
	Acetone-water	RT		2
	10% methanol	−20	30	
	10% methanol	4	30	
	10% methanol	RT		5
Lot 5 on plastic slides	Acetone	−20	21	
	Acetone	4		17
	10% methanol	−20	21	
	10% methanol	4	21	
Lot 4 purified antigen	10% methanol	4		18

[a] These tests were performed with NCDC standard 4+ and 1+ antisera, trypsin (1:30) digestion, and conjugate lot no. 12 at a 1:1,000 dilution. When the antigen did not give a 1+ reaction with the 1+ serum, it was considered as a sign of antigen potency loss.

[b] Prepared by J. N. Miller at UCLA.

[c] Room temperature.

and 10% methanol. The storage temperatures were room temperature, 4 C, and −20 C (Table 2).

Purified T. pallidum antigens. These antigens were prepared from the regular *T. pallidum* antigens by centrifugation procedures in order to reduce the cellular debris. The antigen was fixed to glass slides using acetone, acetone followed by a water rinse, or 10% methanol. The fixed antigen was stored at room temperature and 4 C (Table 2).

The various antigen preparations were satisfactory for 7.5 months when maintained at 4 or −20 C (Table 2). Those antigens remaining in the "weeks of proven antigenicity" column were still being tested for potency at regular intervals. The antigens were not stable at room temperature. Antigens at incubation temperatures of 37 C, not shown in Table 2, decreased in potency in 2 weeks. At the present time, fixation in either 10% methanol or pure acetone appears satisfactory. For the semiautomated machine, the 10% methanol fixation is preferable.

The instability of the antigen at room temperature and at 37 C was thought to be due to residual enzymes remaining in the preparation. At the present time, no method of heating or of chemical inactivation of enzymes, or both, has increased the shelf life of fixed antigens at room temperature or at 37 C.

Shelf life studies on conjugate. The most extensive shelf life studies have been done with conjugate (lot 9A) prepared in this laboratory (5). The initial FTA titer of lot 9A was 1:400. Portions of this lot were put in dialyzing bags and were dialyzed against the solutions shown in Table 3. These portions were then lyophilized and stored at −20 C, frozen and stored at −60 C, or stored at 4 C.

There was little or no deterioration of the samples maintained at −20 and −60 C after 4 to 6.5 months of storage, as a difference in titer between 1:200 and 1:400 is probably not significant (Table 3). The samples which had been lyophilized and rehydrated were stored at 4 C for 5 months. It was found that the borate and carbonate buffers maintained the appearance and the titer of the conjugate. The conjugates in tris-(hydroxymethyl)aminomethane (Tris), phosphate, and PBS buffer showed deterioration, as measured by the various quantities of precipitate contained in each sample together with a reduction in the titer of the supernatant fluid.

The results of other shelf life studies on conjugate showed that when the final protein concentration of the conjugate was 3%, rather than 1%, the rate of precipitate formation in PBS buffer was very slow, but the deterioration of the FTA titer continued at about the usual rate. In addition, lyophilized conjugates maintained at 37 and 45 C lost about half their titers in 10 weeks.

Shelf life studies on sorbent. A lot of sorbent, as prepared in this laboratory (5), was subjected to a series of stability tests. A portion of the lot was dialyzed against PBS until the soluble and dialyzable material was removed. This dialyzed solution was lyophilized, as was the remainder of the original lot. A portion of the total number of bottles of lyophilized sorbent was heated at 48 C for 26 days and was tested in accordance with the NCDC standard procedure.

As shown in Table 4, the lyophilized sorbent was extremely heat stable. The heated control sample was very dark. After heating, the dialyzed sample was lighter in color. Heating also caused a small loss of potency of the dialyzed product, but the dialysis appeared to affect the potency more than the heat. It has been our experience that after a sorbent is standardized and lyophilized, it is a stable biological product.

TABLE 3. *Shelf life study on fluid and lyophilized conjugate*

Storage buffer (molarity and *p*H)	Frozen samples (stored for 6.5 months at −60 C)		Lyophilized samples (stored for 4 months at −20 C)		Previously lyophilized samples (stored for 5 months at 4 C)	
	Appearance	Titer	Appearance	Titer	Appearance	Titer
0.1 M BO_3; *p*H 8.5	Clear	1:400	Clear	1:400	Clear	1:400
0.01 M BO_3; *p*H 8.5	Clear	1:200	Clear	1:400	Clear	1:400
0.1 M $CO_3 \cdot HCO_3$; *p*H 8.5	Clear	1:400	Clear	1:400	Clear	1:400
0.01 M $CO_3 \cdot HCO_2$; *p*H 8.5	Clear	1:400	Clear	1:400	Clear	1:400
0.1 M $CO_3 \cdot HCO_3$; *p*H 7.2	Clear	1:400	Clear	1:400	Colloidal	1:400
0.01 M $CO_3 \cdot HCO_3$; *p*H 7.2	Clear	1:400	Clear	1:400	Clear	1:400
0.1 M Tris; *p*H 8.5	Clear	1:400	Clear	1:200	Precipitate	1:200
0.01 M Tris; *p*H 8.5	Clear	1:400	Clear	1:200	Precipitate	1:200
0.1 M Tris; *p*H 7.2	Clear	1:400	Clear	1:400	Precipitate	1:200
0.01 M Tris; *p*H 7.2	Clear	1:400	Clear	1:400	Precipitate	1:200
0.1 M PO_4; *p*H 8.5	Clear	1:200	Clear	1:400	Precipitate	1:200
0.01 M PBS; *p*H 8.5	Clear	1:400	Clear	1:400	Precipitate	1:200
0.01 M PBS; *p*H 7.2	Clear	1:400	Clear	1:200	Precipitate	1:200

TABLE 4. *Shelf life results on sorbent lot 1 and lot 1A*[a]

Serum[b]	NCDC ref. no. 672	PBS	Lot 1						Lot 1A					
			Control			Heated at 48 C for 26 days			Control			Heated at 48 C for 26 days		
			UD[c]	1:2	1:4	UD	1:2	1:4	UD	1:2	1:4	UD	1:2	1:4
NCDC No. 661	3+	4+	2–3+	2–3+	3+	1+	2+	3+	2–3+	3+	3+	2–3+	3+	3+
Nonspecific No. 651	−	2+	−	−	±	−	−	±	−	±	±	±	±	1+
Standardizing No. 643	Trace	3+	−	1+	1–2+	−	1+	2+	1+	1+	2+	1+	1+	2+
Standardizing No. 671	2–3+	4+	2–3+	2–3+	3+	2–3+	3+	3–4+	3+	3+	3+	2–3+	3+	3+
Nonspecific stain	−	−	−	−	−	−	−	−	−	−	−	−	−	−
2 + Serum		2+												
1 + Serum		1+												

[a] Lot 1 was the undialyzed sorbent; lot 1A was the dialyzed sorbent.
[b] Sera furnished by NCDC for the standard sorbent test.
[c] Undiluted.

DISCUSSION

The shelf life of a biological product is dependent upon a variety of factors. When the product is prepared by standard procedures, as with the FTA-ABS reagents, the aging, which can be regulated, is based on certain controllable factors such as temperature, humidity, gas composition, amount of residual moisture, and pH, molarity, and composition of suspending vehicles.

The shelf life of liquid *T. pallidum* antigen appears to depend primarily on the composition of the vehicle in which the harvested treponemes are suspended. Many complicated formulae have been devised in an effort to increase the stability and usefulness of the treponemes, but it appears that the simplest medium is the best. In semiautomated and automated procedures, where success depends on no background, all unnecessary protein must be eliminated from the medium; the added protein does not aid in the efficacy of the product.

The shelf life of the antigen fixed to a glass slide is not as dependent on the original vehicle as is the liquid antigen. It appears that the shelf life of antigens from various vehicles are equally satisfactory once they are fixed on slides. It was found that either acetone or 10% methanol was equally satisfactory as a fixative, but we prefer the methanol procedure. The antigen on the slide is stable at −20 or 4 C for at least 5 months.

The conjugate appears to possess a relatively long shelf life when it is lyophilized or frozen and stored at −60 C. The stability at 4 C appears to depend on the type of buffer, its molarity and *p*H. In this respect, borate or carbonate buffers are better than Tris and phosphate buffers.

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