Original papers

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Identity of *Treponema pallidum* subsp. *pallidum* polypeptides: Correlation of sodium dodecyl sulfate-polyacrylamide gel electrophoresis results from different laboratories

As the first step in a cooperative effort to standardize the identification of the polypeptides of *Treponema pallidum* subsp. *pallidum*, the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) results obtained in 16 laboratories were compared. Although it was possible to correlate the positions of 16 of the major polypeptide bands, the cross-identification of many of the polypeptides was ambiguous, particularly in the low molecular weight range. Two-dimensional electrophoresis provided an improved means of separating and characterizing *T. pallidum* polypeptides as isolated molecular species. An approach to the unambiguous identification of treponemal polypeptides was outlined which will utilize two-dimensional electrophoresis in combination with specific properties attributable to individual proteins, including reactivity with monoclonal antibodies or monospecific antisera, biochemical and structural properties, and sequence information. To demonstrate the feasibility of this approach, two-dimensional electrophoresis in conjunction with immunoperoxidase staining was used to specifically identify three cloned *T. pallidum* proteins.

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1 Introduction

Treponema pallidum subsp. *pallidum*, a bacterium of the family *Spirochaetaceae*, is the causative agent of venereal syphilis and is closely related to the organisms causing endemic syphilis (*T. pallidum* subsp. *endemicum*), yaws (*T. pallidum* subsp. *pertenue*), and pinta (*T. carateum*). Although penicillin and other antimicrobial agents are effective in the treatment of these diseases, treponemal infections continue to be a world-wide problem. Syphilis and the other treponematoses have an extremely complex pattern of pathogenesis, exhibiting multiple disease stages, latency, and a complex immunologic response [1, 2]. A tissue culture system capable of supporting limited multiplication of *T. pallidum* has recently been developed [3, 4]; however, for most research and diagnostic purposes it is necessary to propagate the organism by intratesticular infection of rabbits. The relatively small quantities of *T. pallidum* which can be obtained by this means, together with the problem of removing host tissue components, have limited definitive studies on the structure, physiology, pathogenesis, and immunology of *T. pallidum*.

In recent years, many investigators have taken a molecular approach toward determination of the factors involved in the pathogenesis and immunologic reactivity of *T. pallidum*. Characterization of antigenic structure of *T. pallidum* began with studies in the 1950's and 1960's which demonstrated the presence of both protein and nonprotein antigens in the organism [2]. Identification of individual molecular species did not occur until modern electrophoretic techniques, including sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), crossed immunoelectrophoresis (CIE), and two-dimensional electrophoresis (2DE), were applied to tre-

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Abbreviations: **CIE**, crossed immunoelectrophoresis; **2DE**, two-dimensional gel electrophoresis; **IRS**, infected rabbit serum; **2-ME**, 2-mercaptoethanol; **PBS**, phosphate-buffered saline; **RIP**, radioimmunoprecipitation: **SDS-PAGE**, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; **T**, Tween 20

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ponemal research. Since the first reported SDS-PAGE analysis of *T. pallidum* in 1977 [5], this procedure has been utilized widely in the characterization of *T. pallidum* polypeptides, usually in combination with either radioimmununoprecipitation (RIP) or Western blotting techniques to identify antigenically reactive species. The antibody responses against individual polypeptides during the course of human and experimental syphilis have been studied extensively in this manner. However, the relative importance of these proteins in the induction of immunity, as well as their actual structural locations and functions, is just beginning to be elucidated.

Although the SDS-PAGE patterns of *T. pallidum* reported by different laboratories are generally quite similar (as recently reviewed by Penn *et al.* [6]), considerable uncertainty exists with regard to the identity and molecular weights of polypeptides described by different researchers. Furthermore, no standardized system for the identification of these proteins has been established and accepted by all investigators. As a result, it has not been possible in most cases to conclusively determine whether the proteins described by one laboratory are identical to or different from those studied by another, thereby precluding a direct and meaningful comparison of the information obtained.

The purposes of this collaborative study, which represented the joint effort of sixteen laboratories, were to: 1) cross-identify the major polypeptides of *T. pallidum* subsp. *pallidum* by correlating the SDS-PAGE patterns submitted by the participating investigators; 2) utilize this tentative identification scheme to combine the data accumulated by the different laboratories into a more unified body of information; 3) evaluate 2DE as a means of resolving and identifying *T. pallidum* polypeptides; 4) identify problems and ambiguities existing in the electrophoretic characterization of treponemal polypeptides; and 5) develop an approach for the unambiguous identification of *T. pallidum* polypeptides.

2 Materials and methods

2.1 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

The virulent Nichols strain of *T. pallidum* subsp. *pallidum*, propagated by intratesticular infection of rabbits, was utilized by all investigators in this study. The *T. pallidum* purification methods (for removal of contaminating host proteins) and gel electrophoresis parameters employed by the authors contributing gel patterns are given in Table 1, along with a list of pertinent references. The order in which the investigators are listed is arbitrary and was based on the arrangement which yielded the best correlation of polypeptide patterns (see Fig. 13)*. The Laemmli discontinuous buffer gel electrophoresis system [7] in a slab gel format was used in all of the SDS-PAGE gels presented in this study. The polyacrylamide concentration varied from laboratory to laboratory, and both single concentration and linear gradient gels were employed. Molecular weight standards marketed by Bio-Rad (Richmond, CA), Pharmacia (Uppsala, Sweden), Sigma (St. Louis, MO), New England Nuclear (Boston, MA), or Amersham Arlington, IL) were used to estimate the relative molecular weights (M_r's) of *T. pallidum* polypeptides by the method of Weber and Osborn [8].

2.2 Two-dimensional gel electrophoresis

2DE was performed as described by O'Farrell [9] with minor modifications. Percoll-purified [10] *T. pallidum* (4×10^8) were solubilized by the sonic disruption technique [9], utilizing lysis buffer (Buffer A) containing double the indicated concentrations of Nonidet P-40, 2-mercaptoethanol, and carrier ampholytes. Isoelectric focusing was carried out in 0.25 × 11.5 cm tube gels containing 3.2 % pH 5–7 and 0.8 % pH 3.5–10 Ampholines (LKB, Bromma, Sweden) for 7200 volt-hours. The second dimension consisted of SDS-PAGE utilizing 1 mm thick, 8–20 % linear gradient polyacrylamide gels. *T. pallidum* (2×10^8) and Bio-Rad low and high molecular weight standards were placed in wells at either end of the IEF tube gel, to permit correlation of the resulting single dimension SDS-PAGE profiles with the 2DE pattern. The SDS-PAGE dimension was generally run at 7.5 mA per gel overnight and then changed to 180 V constant voltage until 1 h after the Bromophenol Blue dye front had eluted from the gel. The silver staining technique employed was that described by Guevera *et al.* [11].

2.3 Immunoperoxidase staining

The 2DE gel patterns were transferred to nitrocellulose by the electrophoretic method of Towbin *et al.* [12] at 6 V/cm for 3 h. The electroblots could be stored at −20 °C for several weeks with no apparent loss in antibody binding activity. Following a 30 min preincubation in phosphate-buffered saline (PBS) with 0.05 % Tween 20 (PBS-T), the electroblots were exposed to the monospecific rabbit antisera or murine monoclonal antibodies (as culture supernatant) diluted 1:200 to 1:400 in PBS-T for 2 to 4 h at 25 °C. After washing five times with PBS-T for a total of 30 min, the electroblots were incubated for 1 h with horseradish peroxidase conjugated goat anti-rabbit IgG (heavy and light chain specific, Miles Scientific, Elkhart, IN) or goat anti-mouse IgG + IgA + IgM (Cappel Laboratories, Malvern, PA) diluted 1:1000 in PBS-T. After washing, the electroblots were exposed to 0.06 % 4-chloronaphthol (Sigma) in PBS with 20 % methanol and 0.02 % hydrogen peroxide for 1 to 5 min. The reaction was stopped by rinsing the electroblots thoroughly in deionized water. Secondary staining of the same electroblots with pooled sera from *T. pallidum*-infected rabbits (diluted 1:200) was carried out in the same manner, except that 0.06 % diaminobenzidine (Sigma) in PBS with 0.02 % hydrogen peroxide was used as the peroxidase color reaction substrate.

3 Results

3.1 Comparison of SDS-PAGE patterns

In this study, 12 of the participating laboratories submitted SDS-PAGE profiles of *T. pallidum* in which the polypeptides were visualized by Coomassie Brilliant Blue R-250 or silver staining, radiolabeling, or immunologic techniques. The conditions employed by each group and pertinent references are presented in Table 1. Because many of the photographs submitted contained additional information which could not be adequately presented in a composite, all of the gel patterns

* For figures see Addendum on pp. 86–92

Table 1. Gel electrophoresis parameters utilized by the groups submitting SDS-PAGE gel patterns

Group	Principal investigator(s)	*T. pallidum* Purification method	Gel	M_r Standards	Staining[a)] method	Reference
1	Norris	Percoll purification	8–20 %	Bio-Rad, Pharmacia	C, S	[10, 13]
2	Hanff, Lovett, Miller	Percoll purification	8–20 %	Bio-Rad	C	[10, 14–22]
3	Larsen	Percoll purification	10 %	Pharmacia	S	[7, 10]
4	Baughn	Percoll purification	10 %	Bio-Rad	C	[23, 24]
5	Lukehart, Baker-Zander	Differential centrifugation	12.5 %	Sigma	C	[25–29]
6	van Embden	Urografin purification	12 %	Bio-Rad	C, S	[30–32]
7	Stamm, Bassford	Differential centrifugation	15 %	Bio-Rad	C, R	[33–35]
8	Müller, Moskophidis	Urografin purification	12.5 %	Pharmacia, Amersham (^{14}C)	C, R	[36–39]
9	Penn, Bailey, Cockayne	Differential centrifugation	12.5 %	Bio-Rad	C	[6, 40–42]
10	Norgard	Percoll purification	10 %	New England Nuclear	RIP	[43–47]
11	Basemann, Alderete	Methocel-Hypaque centrifugation	7.5 %	Bio-Rad	C	[5, 48–56]
12	Wicher	Differential centrifugation, filtration	10 %	Bio-Rad, Pharmacia	I	[57]

a) C, Coomassie Brilliant Blue R-250; S, silver stain; R, autoradiography of [^{35}S]methionine labelled *T. pallidum*; I, immunoperoxidase staining; RIP, radioimmunoprecipitation of surface radioiodinated *T. pallidum*.

have been provided (Figs. 1 through 12). Data pertinent to these gel patterns and the polypeptide characterizations previously reported by these groups are given in the figure legends, so that the correlations presented in this paper could be extended to previous publications. To aid in the cross-identification, molecular weight standards were usually included, and the positions of specific *T. pallidum* polypeptides, including reactivity with monoclonal antibodies and expression by cloned *T. pallidum* DNA segments, are also indicated.

The effect of several electrophoresis parameters, including use of different molecular weight standards, heating of the solubilized *T. pallidum* prior to application to the gel, and visualization by either Coomassie Blue or silver staining, were examined in Fig. 1. The *T. pallidum* profiles were quite similar regardless of treatment prior to electrophoresis or method of staining. Although the same set of prominent polypeptides was evident with both staining techniques, silver staining revealed the presence of a large number of minor proteins which are not detected with Coomassie Blue. However, Coomassie Blue staining appeared to provide a more quantitative estimate of polypeptide concentration, based on the comparison of staining intensity and the relative size of the polypeptide bands. The heated and unheated samples yielded nearly identical patterns, indicating that *T. pallidum* does not contain proteins analogous to the heat-modifiable major outer membrane proteins found in many gram-negative bacteria. A notable exception is the 4D antigen described by Lovett and co-workers [17–20], which is visible on silver-stained gels as a band with an M_r of 180 000 to 190 000 (Fig. 1). This band is not detectable in heated samples, but is replaced by a band at 19 000. These properties are consistent with those previously described by Fehniger *et al.* [18, 19] for the 4D antigen.

As an aid to cross-identification, 16 prominent bands which were consistently detectable in the gels submitted by the co-authors were assigned a letter designation (Fig. 1). These designations are for reference purposes only and do not constitute a proposed identification scheme. In analyzing the SDS-PAGE patterns, it is important to remember that these 'bands' do not necessarily correspond to isolated protein species. Thus properties attributed to a given 'band' may represent the activities of two or more comigrating polypeptides.

A composite of the *T. pallidum* gel patterns obtained by the participating investigators is shown in Fig. 13. An attempt was made to correlate the band positions on the different gels, as indicated by the dashed lines. The cross-identifications were based on the positions of the bands relative to other *T. pallidum* polypeptides and to the molecular weight standards, as well as the M_r values provided by the coauthors. It was possible to correlate most of the prominent higher molecular weight bands (b–j), although in some cases what appeared to be single bands in some gel patterns were resolved as doublets or triplets in some of the higher resolution gels. Thus it would appear that there is a significant degree of overlap of polypeptide species in these areas. In the lower molecular weight range (k–p), it was much more difficult to achieve any clear correlations between the gel patterns, due to both the large number and close proximity of many of the bands and inconsistencies in the migration patterns and staining intensities in the different gels. For this reason, the correlation of the SDS-PAGE patterns in this region should be considered to be tentative.

The molecular weight estimates obtained by each laboratory for the protein bands designated a–p in Fig. 13 are given in Table 2. The values are surprisingly close in most cases. A 'consensus' relative molecular weight (M_r) was determined for each polypeptide by averaging all of the available M_r values. Widely disparate estimates were reported for polypeptide c (due either to incorrect cross-identification or inaccurate M_r determinations), indicating that further analysis of the M_r of this protein is needed. The consensus M_r values may provide a useful means of standardizing *T. pallidum* polypeptide identification, as discussed later.

The effect of using different molecular weight standards is illustrated by comparing the first two columns in Table 2, where the values were determined by utilizing either Bio-Rad or Pharmacia M_r standards on the same gel (Fig. 1). These values were calculated by direct, 'point to point' interpolation of M_r values from the nearest M_r standards, as opposed to using a best-fit straight line based on all of the M_r standard R_f and M_r values. Although many of the values are close, some (most

Table 2. Relative molecular weight ($M_r \times 10^3$) estimates obtained by the contributing authors for the *T. pallidum* polypeptide bands a–p identified in Fig. 13

	Group[a)]														
	1[b)] (BioRad)	1[b)] (Pharm.)	2	3	4	5	6	7	8	9	10	11	12	Mean M_r ± SD	Consensus M_r
a	182														190[c)]
b	96	94.5	94	94	94	90.5			94					93.8 ± 1.8	94
c	81.5	81.5	83	84	87	80			79	80	84	89.5		83.1 ± 3.5	83
d	75	75	70.5	70	72	70.5			69					71.2 ± 2.1	71
e	64	63	60	58	62	64.5	58	61	63	60				61.2 ± 2.4	61
f	48	44.5	46	48	47	46.5	47	48	46	47	47	45		46.9 ± 1.0	47
g	45	43	43	45.8	45	44.0	44.5		43.5					44.5 ± 0.9	44.5
h	42.5	40.5	40	40.8	42	41.5		42	40	42	40			41.2 ± 1.0	41
i	41	39	38.5	39.2		40.0			38.5					39.3 ± 1.2	39
j	38	37	36	38.5	37	37.0	38.5	40	37	37	36		36	37.4 ± 1.2	37
k	36	36	34.2	37.6	35	35.0	34.5		35	34	34		34	34.6 ± 0.6	34.5
l	35	35	33	34.5	33	33.5	32.5		33		32	32		33.1 ± 0.9	33
m	32	31.5	31	31		30.5	29.5		30.5	31.5	29		27	30.2 ± 1.4	30
n	24.5	23	24.5	26.3	25	22.5			22.5		24			24.1 ± 1.2	24
o	17.5	17.7	17.5	19.9	16.5	16		16	16.5					17.1 ± 1.4	17
p	15.5	16.4	16.2	15.2	15.5	14			15.5					15.3 ± 0.7	15.5

a) Group numbers correspond to those given in Table 1 and to Fig. 1–12.
b) M_r determinations based on either Bio-Rad (BR) or Pharmacia (Ph) M_r standards.
c) Based on the results of Fehniger *et al.* [18, 19].

notably f–i) differ by as much as 2000–3000, due largely to the differences in M_r values provided for the standards by the two companies.

Table 3 provides a summary of the previous designations and properties attributed to the polypeptide bands a–p. Although a great deal of uncertainty still exists regarding these identifications, a consensus is gradually developing as to the structural locations and immunologic significance of some of these proteins. For example, evidence is accumulating that the M_r 47 000 polypeptide (band f) is a major outer membrane protein [42, 43] which stimulates a vigorous antibody response [13–16, 25, 29, 36, 42, 43, 47–49, 54, 55]. Similarly, the 4D antigen (band a) appears to be a surface localized protein [20] with a ring-like structure [19] analogous to that of porin molecules in gram-negative bacteria. Polypeptides j, k, l and m (with M_r's of 37 000, 34 500, 33 000 and 30 500) appear to be associated with the periplasmic flagella, based upon the composition of flagella purified by removing the outer membrane and shearing off the flagella [22, 41] or by fractionating sonically disrupted organisms on sucrose density gradients (S. J. Norris, N. W. Charon, and R. J. Limberger, manuscript in preparation). The identity of j as a major periplasmic flagellar protein has been verified by the reactivity of monoclonal antibody IB8 both with intact *T. pallidum* flagella and with polypeptide j in two-dimensional Western blots (M. J. Bailey, A. Cockayne, and C. W. Penn, manuscript in preparation). In addition, antisera directed against *T. phagedenis* biotype Reiter periplasmic flagella reacts specifically with polypeptides k, l, and m (J. D. A. van Embden, unpublished data). Three of the polypeptides which were labelled by incorporation of [^{14}C]glucosamine in studies by Moskophidis and Müller [38] (and thereby reported by these authors to be glycosylated) appear to correspond to these same three putative flagellar proteins.

Most, if not all, of the polypeptides of *T. pallidum* detectable by Coomassie Blue or silver staining are also antigenically reactive with sera from syphilis patients or from *T. pallidum*-infected rabbits, as described in several publications [13–16, 25, 29, 35, 36, 43, 48, 57, 60]. Antisera against *T. pallidum* proteins react with epitopes on many pathogenic and nonpathogenic spirochetes, although certain "pathogen-specific" epitopes which do not generate cross-reactive antibodies have been described [28, 57].

Discrepancies exist with regard to the proposed location and function of the protein corresponding to band c, which is described as a surface-localized cytadhesin with fibronectin-binding characteristics by Baseman and co-workers [48, 51], but has been reported to be the major protein component of the cytoplasmic filaments by A. Cockayne, M. J. Bailey, and C. W. Penn (Proceeding of the 102nd Meeting of the Society of General Microbiology, Birmingham UK, January 1985; manuscript in preparation). A large number of *T. pallidum* proteins have been reported to be surface localized based on the surface radioiodination of intact organisms [13, 38, 43, 44, 46, 49]. Unfortunately, the reliability and reproducibility of this approach is subject to question. The radiolabelling patterns reported by different laboratories vary greatly, and internal proteins have been shown to be iodinated under at least one of the protocols employed. The 39 000 polypeptide reported by Norris and Sell [13] to be preferentially labelled by Iodogen-catalyzed surface radioiodination has since been identified as a putative flagellar protein (j) and is thus internal to the outer membrane (S. J. Norris, Abstract, Annual Meeting American Society of Microbiology, Washington, DC, March, 1986).

Many monoclonal antibodies have been produced against *T. pallidum* antigens [28, 32, 39, 43, 44, 47, 53], some of which appear to be specific for polypeptides c, d, f, g, j, and m (Table 3). In addition, several monoclonal antibodies have been described whose reactivity can not be clearly correlated with those proteins listed in the table, including those reacting with polypeptides with reported M_r values of 102 000, 53 000, 52 000–54 000, 34 000 and 29 000 [44, 46], 12 500 [28], and 24 000 and 21 000 (P. Hindersson and N. H. Axelsen, un-

Table 3. Tentative correlation of properties associated with *T. pallidum* bands a–p as identified in Fig. 13

Letter designation	Consensus $M_r \times 10^3$	Properties [Reference]
a	190	Cloned antigen 4D [17–19]
b	94	
c	83	Designations: P1 [48], 83(6.7) [13], P1 [6] Monoclonal Ab's: 6E10, 5A5–7 [44], PD9 [a] Fibronectin-binding protein [51, 24] Cytadhesin [48] Associated with cytoplasmic filaments [b]
d	71	Designation: 75(5.8) [13]
e	61	Designations: Tp4 [59, 60], P5 [51], 61(5.8) [13], P2 [6], TpA [30] Monoclonal Ab's: HATR-16, HATR124 [c] Cloned antigens TpA [30] and Tp4 are different polypeptides [c, d] Surface radioiodinated [49, 37, 38] Preferentially labeled with [^{14}C]glucosamine [38]
f	47	Designations: P6 [51], 47K [43, 47], 48K [35], 46(5.9) [13], P3 [6], TpS [c] Monoclonal Ab's: C3E5 [30], 8G2 [43, 44, 47], A8, C2, D10, 2G10, and H91 [28], 13F3 [53], 4a [39], AD5 [a] Cloned antigens: 47K [47], P6 [54, 55], TpS [c] Major 'immunodominant' antigen [13, 14, 16, 25, 29, 36, 42, 43, 47–49, 54, 55] Putative outer membrane protein [42, 43] Surface radioiodinated [49, 43, 37, 38]
g	44.5	Resolved as two distinct spots by 2DE (Figure 14) Monoclonal Ab's: 1–14M1 [30, 31], 3a [39], 2B11 and 2D7 [a] Cloned antigens: TmpA [30, 31] (membrane protein, gene sequence determined) and an unrelated 44K protein [47] Surface radioiodinated [37, 38]
h	41	Designations: 41(5.7) [13], P4 [6] Surface radioiodinated [37, 38]
i	39	Designation: 40(6.6) [13], 42K [35] Resolved as three spots by 2DE (Figure 14)
j	37	Designations: 39(6.0) [13], P5 [6], 40K [35] Major flagellar protein [41, 22, e] Monoclonal Ab's: H92 [28], IB8 [a] Surface radioiodinated [13, 43] Contains pathogen-specific epitopes [57] Cloned antigens TmpC [30], P2* [56], and 37–38K [21, 58] appear to possess slightly lower M_r's than this protein.
k	34.5	Designations: P3 [48], 33(6.1) [13], 34K [35], P6 [6], AF-1 [d] Resolved as two spots by 2DE (Figure 14) Flagellar protein [34, 22, d, e] Surface radioiodinated and preferentially labeled with [^{14}C]glucosamine [37, 38] Contains pathogen-specific antigens [57] May comigrate with a cloned, nonflagellar 34K protein which reacts with monoclonal Ab's 3B5, 9B12, and 10G2 [46]
l	33	Designations: 33 (6.3) [13], AF-2 [d] Resolved as two spots by 2DE (Figure 14) Monoclonal Ab's: 2b [39], 2G4 [44] Flagellar protein [41, 22, d, e] Surface radioiodinated [37, 38]
m	30	Designations: P4 [49, 54], P7 [6], AF-3 [d] Monoclonal Ab's: 5H10 [44], JD11 [a] Flagellar protein [22, d, e] Contains pathogen-specific antigens [57]
n	24	
o	17	16K [35]
p	15.5	Monoclonal Ab: 1b [39]

Unpublished results: [a] M. J. Bailey, A. Cockayne and C. W. Penn; [b] A. Cockayne, M. J. Bailey and C. W. Penn, submitted; [c] P. Hindersson and N. H. Axelsen; [d] J. D. A. van Embden; [e] S. J. Norris.

published data). This battery of polypeptide-specific reagents may provide an important means of cross-identifying proteins, as described below.

An even more extensive list of *T. pallidum* protein products expressed by recombinant DNA vectors in *E. coli* has been described (Table 4). Some of these can be tentatively identified as major polypeptide bands in the *T. pallidum* SDS-PAGE pattern. Others appear to be minor proteins which in many cases co-migrate with more abundant protein species, making their identification less straightforward. Monospecific antisera and monoclonal antibodies have been raised against many of these antigens, which will aid in their specific identification.

Table 4. Proteins of *T. pallidum* expressed by recombinant DNA vectors in *E. coli*

Reported $M_r \times 10^3$	Protein designation	Representative clone	Reference	Properties	Monoclonal reactivities
200	TpF1	pRIT7100	[30]		
190	4D	pAW329	[17–20]	Corresponds to a (Fig. 1) Dissociation into 19 K subunits at 95 C in 0.1 % SDS Ring-like structure	
60	Tp4	pHI1001	[59] [a]	Crossreacts with "Common Antigen" of *Enterobacteriaceae*	HATR-16, HATR124 [a]
58	TpA	pRIT9320	[30]	Antigenically unrelated to Tp4 [a, b]	
47	47K TpS (Tp47) P6	pMN23, 24 pHI1030 R12	[43, 47] [a] [54, 55]	Correspond to f (See Fig. 13, 14)	11E3, 8G2 [43, 47], HATR 14 [a], C3E5 [32], 4a [39], AD5 [c] 13F3 [53]
46		Tp3A	[33]	Also expresses 43 K, 38 K, and other antigens	
43–66		pLVS6	[33]	Several minor proteins expressed	
44		RICB2–1 (pMN7)	[45]		
42	TmpA	pRIT4694 pMJB10	[30, 31] [c]	Corresponds to g Membrane-associated DNA sequence determined [31]	1–14M1 [30, 32], 2B11, 2D7 [b, c] 2B11, 2D7 [c]
42	TpF2	pRIT7100	[30]		
41		pMJB20	[c]	Corresponds to h?	33a [c]
39		pLVS3	[33]	Also expresses 35 an 25 K components Restriction map differs from pAW305	
37–38		pAW305	[21, 58]	Associated with outer membrane in *E. coli*	
37	P2* (P2 star)	B5	[56]		
35		pLVS1	[33]	Identical to pMN20 by restriction mapping, reactivity with monoclonal antibodies	
35	TmpC	pRIT9000	[30]	Membrane-associated	C322 [b]
34	TmpB	pRIT4694 pMJB10	[30, 31] [c]	Membrane-associated	B712 [b], HATR129 [a], 2a [39]
34		pRIT3200 pMJB30	[30] [c]		10c [c]
32		pLVS5	[33]		
29–35	TpD	pRIT3200 pMN20 pHI1002 pMJB30	[30] [46] [a] [c]	Surface-associated Surface-radioiodinated [46] Accessable to Ab in intact organisms [46] Diffuse spot on 2DE (Fig. 17)	HATR1–3 [a] 3B5, 9B12, 10G2 [46] 10c [c]
29		pMJB40	[c]		
25		pMJB50	[c]		
24–28	TpE	pRIT7100	[30]		HATR1–25 [a]
21	TpT	pHI1005	[a]		HATR117 [a]
15		pMJB60	[c]		2b [c]

Unpublished results: [a] P. Hindersson and N. H. Axelsen; [b] J. D. A. van Embden; [c] M. J. Bailey, A. Cockayne and C. W. Penn.

3.2 Two-dimensional electrophoresis

2DE separates on the basis of both charge and molecular weight, greatly enhancing the resolution of molecular species and reducing the likelihood of co-migration [9]. As shown in Fig. 14, 2DE provides a 'fingerprint' of *T. pallidum* proteins in which the spots corresponding to different polypeptides can be easily identified by their relative location. In this silver stained gel, whole *T. pallidum* and Pharmacia low molecular weight standards were run in single dimension SDS-PAGE

lanes on either side of the two-dimensional pattern, allowing the direct comparison of the 2DE spots with the bands present in the SDS-PAGE pattern. In this way, it was possible to correlate the 2DE spots with the letter designation used in Fig. 13. In several cases, what appeared to be single bands by SDS-PAGE could be resolved into two or more distinct spots by 2DE.

By combining 2DE with immunoperoxidase staining using monospecific antibodies, it was possible to clearly identify the reactive polypeptides. To exemplify this approach, the reactivities of monoclonal antibodies and monospecific antisera directed against three cloned *T. pallidum* gene products were studied. The antisera were reacted with 2DE electroblots, and the distribution of bound antibody was determined using the appropriate anti-mouse or anti-rabbit peroxidase conjugate and 4-chloronaphthol as substrate. The electroblots were photographed and then counterstained with serum from infected rabbits, utilizing diaminobenzidine as the color substrate. This infected rabbit serum 'counterstain' detected essentially all of the major polypeptides of *T. pallidum* [13] and was approximately equal in sensitivity to silver staining. In this manner, a 2DE profile of the major polypeptides was revealed, so that the relative location of the antigen reactive with the specific antiserum could be easily identified.

Monoclonal antibodies and monospecific antisera specific for cloned *T. pallidum* polypeptides Tp47, TmpC, and TpD (as indicated in Fig. 6) were provided by van Embden and coworkers [30, 32]. The staining patterns obtained with these reagents are shown in Fig. 15–17. Monoclonal antibody C3E5, which is specific for Tp47 [32], reacted with a large spot on the 2DE pattern corresponding to polypeptide f, a putative major outer membrane protein of *T. pallidum* (Fig. 15). A second smaller spot above the major species was also detected, and may correspond to a precursor molecule or an otherwise modified form of Tp47. TmpC appeared to co-migrate with the major M_r 37 000 flagellar protein (j) by SDS-PAGE (Fig. 6), but was clearly identified as a more acidic polypeptide by 2DE (Fig. 16). Interestingly, this antigen was not usually visible on Coomassie Blue or silver stained gels, indicating that it is either present in very small quantities or is refractory to staining by these techniques. Monospecific rabbit antiserum directed against TpD reacts with a large, diffuse spot at the acid end of the gel, overlapping the position of TmpC (Fig. 17). Despite the close proximity of TpD and TmpC in the 2DE pattern, they could be distinguished easily.

Similar 2DE analyses of the reactivities of monoclonal antibodies have been performed independently by M. J. Bailey, A. Cockayne, and C. W. Penn (submitted for publication), M. J. Fohn, S. A. Baker-Zander and S. A. Lukehart (submitted for publication), and M. V. Norgard (data not shown). The 2DE profiles of *T. pallidum* polypeptides obtained in these laboratories closely resemble those shown in Fig. 14. The monoclonal antibodies PD9, AD5, 2B11, IB8, and JD11 developed by M. J. Bailey reacted specifically with polypeptides c, f, g, j, and m respectively, as confirmed by independent studies performed by S. J. Norris (unpublished data). All of the monoclonal antibodies described previously by Lukehart *et al.* [28] which are specific for a M_r 47 000–48 000 molecule react with three spots in the region of f in Fig. 14. Norgard has observed a similar pattern using monoclonal antibodies 11E3 and 8G2 [43, 44, 47]. It is likely that the broad band which is usually observed for f in single and two-dimensional gel patterns is actually comprised of a cluster of 2–3 closely related molecular species. The monoclonal antibody F5 [28] also defines a low molecular weight species which appears to correspond to the large antigenic spot visible at the bottom of the infected rabbit serum (IRS)-stained electroblots in Fig. 15–17 (M. J. Fohn, S. A. Baker-Zander and S. A. Lukehart, submitted for publication).

4 Discussion

4.1 Analysis of SDS-PAGE results

It is generally accepted that SDS-PAGE alone can not be used to conclusively identify a protein in a mixture, because of the possibility of co-migration of polypeptide species. This conclusion becomes even more evident in the analysis of a complex organism such as *T. pallidum*, which in all probability contains thousands of proteins. Thus most of the correlations of SDS-PAGE patterns and properties of polypeptides given in Fig. 13 and Tables 2 and 3 should be considered to be tentative. Certain polypeptides (most notably c, d, f, and j) are consistently identifiable in the SDS-PAGE profile of *T. pallidum* due to their abundance and to the apparent lack of major comigrating species. Even in these cases, caution should be used in identifying these polypeptides solely by their electrophoretic mobility in SDS-PAGE. For example, the cloned antigen product TmpC co-migrates with j (the major M_r 37 000 flagellar protein) by SDS-PAGE (Fig. 6), but was identified as a totally different protein by 2DE and immunoperoxidase staining (Fig. 16).

All of the gel patterns shown in this study represented the polypeptides of the Nichols strain of *T. pallidum* subsp. *pallidum*, originally isolated in 1910 from a patient with neurosyphilis. SDS-PAGE or 2DE analyses of a more recent isolate of *T. pallidum* subsp. *pallidum*, Street Strain 14 ([35] and S. A. Larsen and W. O. Schalla, unpublished data), and the Gauthier strain of *T. pallidum* subsp. *pertenue* [26, 35, 52] indicate that their polypeptide profiles are nearly identical to those of the Nichols strain, although some differences were noted. Thus the correlations made in this publication may be generally applicable to other strains and subspecies of *T. pallidum*. This does not appear to be the case with regard to other species of *Treponema* such as *T. phagedenis*, whose polypeptide migration pattern seems to differ substantially from that of *T. pallidum* [35, 38, 41] despite the existence of significant antigenic cross-reactivity between the two organisms [15, 25, 38, 60].

The correlation of SDS-PAGE patterns and M_r values in this article was only possible because the polypeptide bands were clearly labeled in each of the photographs submitted. Unfortunately, this is not the case in many publications. As a result, it is often difficult to identify the specific bands referred to in the text and tables even within the same manuscript, and comparisons with other publications can be difficult or impossible. It is therefore advisable to provide a Coomassie Blue or silver-stained SDS-PAGE profile in which all polypeptides described in the text or tables have been marked clearly in each publication, or to refer to a previous publication where this has been done.

Table 5. Properties potentially useful in the identification of *T. pallidum* polypeptides

I. Electrophoretic mobility
  A) SDS-PAGE
  B) Isoelectric focusing
  C) 2DE

II. Immunologic properties
  A) Western blotting and immunologic staining of electrophoretic patterns with monospecific antisera or monoclonal antibodies
  B) Radioimmunoprecipitation with monospecific antisera or monoclonal antibodies and electrophoresis
  C) Crossed immunoelectrophoresis

III. Biophysical properties
  A) Specific lability patterns (heat modifiability, susceptibility or resistance to proteolytic agents, structural modification by reducing agents)
  B) Enzymatic activities
  C) Association with purified structural components (*e.g.* flagellar proteins)

IV. Sequence information
  A) Amino acid sequence
  B) DNA sequence of gene
  C) Restriction endonuclease cleavage pattern of gene

Assuming that the correct cross-identifications were made, the M_r values obtained by the different laboratories were generally within 1000 to 2000 of the mean value, although certain polypeptides and M_r ranges exhibited greater variability. Undoubtedly part of this deviation was due to the use of M_r weight standards from different manufacturers, which provided different M_r values for the same proteins. It would seem that sufficient amino acid sequence data are currently available for these well characterized proteins to allow a standardization of the M_r values provided by all manufacturers, thus alleviating this problem. Another source of variability was the calculation of M_r values by two different methods, namely: 1) use of a best-fit straight line of all M_r standards to determine the unknown M_r's; or 2) 'point to point' interpolation of M_r values from the nearest M_r standards. The disadvantage of the first method is that it assumes linearity of the log M_r *vs.* R_f plot, which is not generally the case in a typical SDS-PAGE gel, particularly in the high or low M_r ranges. On the other hand, the interpolation method tends to amplify errors in the reported M_r values and/or aberrant mobilities of the protein standards and thus to increase the variability when different M_r standards are used.

4.2 Approaches to standardization

In order to develop a standardized nomenclature for *T. pallidum* polydeptides, it is necessary to identify individual polypeptides unequivocally. In some cases, it may be possible to do this utilizing either SDS-PAGE or 2DE without any confirming evidence. However, such an approach is potentially misleading. By using migration characteristics as the sole criterion for identification, it would be easy to assume that two publications refer to the same molecule, when they actually refer to two different proteins. Thus it is preferable to use a combination of two independent criteria: 1) a technique capable of separating and identifying *T. pallidum* polypeptides as homogeneous molecular species; and 2) a second property which distinguishes the polypeptide in question from other proteins. Examples of properties which can be used in such an identification process are listed in Table 5.

2DE appears to be the best method available for fulfilling the first criterion of effective separation. Most of the major *T. pallidum* polypeptides are resolved well by 2DE, even in cases where M_r's overlap (as exemplified by TpD and TmpC in Fig. 17). In most cases, it is possible to make a clear association between the spots in the two-dimensional pattern and the bands in the SDS-PAGE pattern (Fig. 14), which will aid in the application of the 2DE data to the more widely used SDS-PAGE system. However, further verification of these correlations is warranted and could be accomplished by adding a small quantity of solubilized *T. pallidum* to the agarose sealing the IEF gel onto the second dimensional gel, thus providing a protein 'line of identity' between the 2DE and SDS-PAGE patterns.

The principal requirement for a second property verifying the identity of a polypeptide is that it be specific for the protein in question. Where monospecific reagents are available, use of immunologic reactivity in conjunction with 2DE as exemplified in this study would appear be the most universally applicable method. However, a variety of other properties, including biophysical characteristics and actual sequence data, could also be employed. For example, the heat lability of the 4D antigen in the presence of SDS [19] provides a specific means of identifying this protein. With the continued development and verification of methods for the separation of structural components, association of polypeptides with certain structures (such as the flagella, outer membrane, cytoplasmic membrane, and cytoplasmic fibrils) could be used in their identification. Finally, actual sequence data could provide the most conclusive evidence for protein identity, and gene sequence and restriction mapping data have already been used to determine the identity or nonidentity of cloned *T. pallidum* DNA segments [46].

A second publication is planned in which the combination of 2DE and immunoperoxidase staining with monospecific reagents will be employed to specifically identify a large number of *T. pallidum* polypeptides. Further refinement of the consensus M_r values may also be possible utilizing both a more standardized method of M_r determination and actual sequence data. The gene sequence of the cloned *T. pallidum* protein TmpA was determined recently [31], and it is likely that sequence data and thus more accurate M_r values will be available for several other cloned *T. pallidum* genes in the near future. An attempt will also be made to identify a second characteristic, such as the isoelectric point, which could be used to distinguish between two polypeptides with the same M_r. Using a combination of M_r values and (when necessary) this second criterion, a standardized nomenclature for *T.*

pallidum polypeptides may be established. A similar approach may also be useful in the identification of proteins of other organisms.

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6 Addendum

Figures 1–12. SDS-PAGE polypeptide patterns *Treponema pallidum* subsp. *pallidum* (Nichols strain) submitted by the investigators listed in Table 1. M_r values are $\times 10^3$ as indicated. Tp = *T. pallidum*; M = M_r standards. Figures were provided by the investigators listed in parentheses.

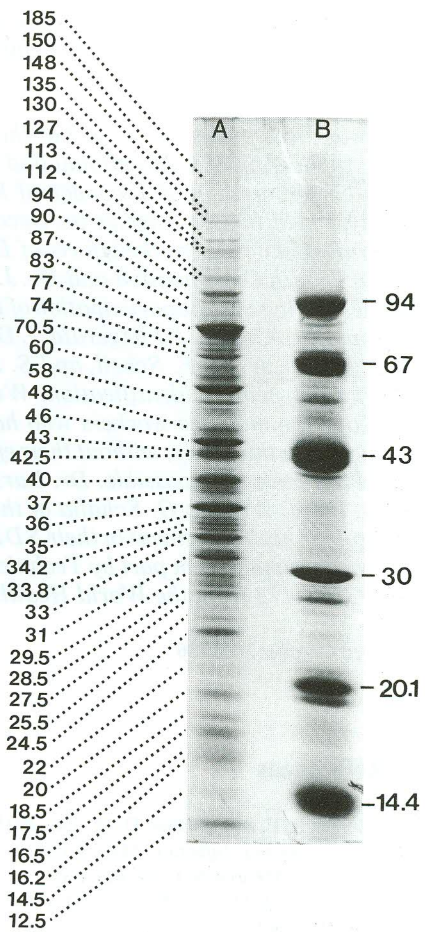


Figure 2. (Hanff, Lovett, Miller) Estimated M_r values of *T. pallidum* polypeptides as determined by comparison with Pharmacia low M_r standards. Coomassie Brilliant Blue R-250 stained gel, from [14].

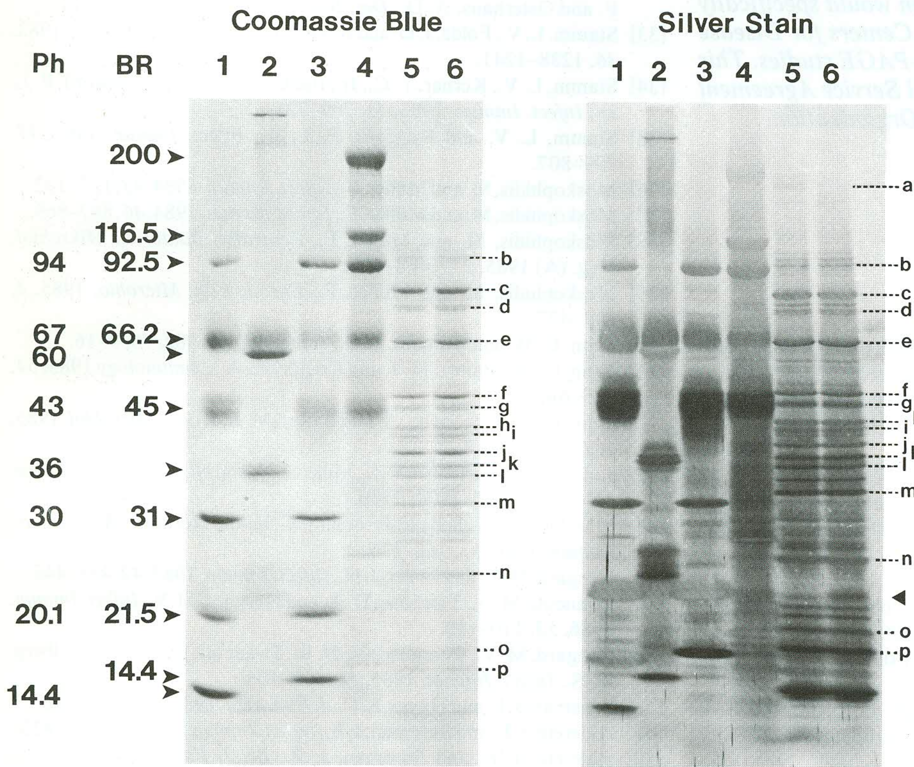


Figure 1. (Norris) A single SDS-PAGE gel with two identical sets of samples (1–6) was divided into two sections and stained with either Coomassie Brilliant Blue R-250 or by the silver technique of Guevera *et al.* [11]. Lanes (1)–(4) correspond to Pharmacia (Ph) low and high M_r standards and Bio-Rad (BR) low and high M_r standards, with the corresponding M_r values supplied by the manufacturers as indicated. Lanes (5) and (6) both contain 2×10^8 Percoll-purified *T. pallidum* solubilized in 0.0625 M Tris buffer, pH 6.8, with 2 % SDS and 5 % 2-mercaptoethanol (2ME). The lane (5) samples were not heated prior to application to the gel, whereas those for lane (6) were heated at 100 °C for 2 min. The letter designations a–p correspond to those shown in Fig. 13. The solid triangle indicates the position of a band with an M_r 19 000 which may represent the dissociated subunits of the heat modifiable 4D antigen (lane 5, band a) described by Lovett and co-workers [17–20].

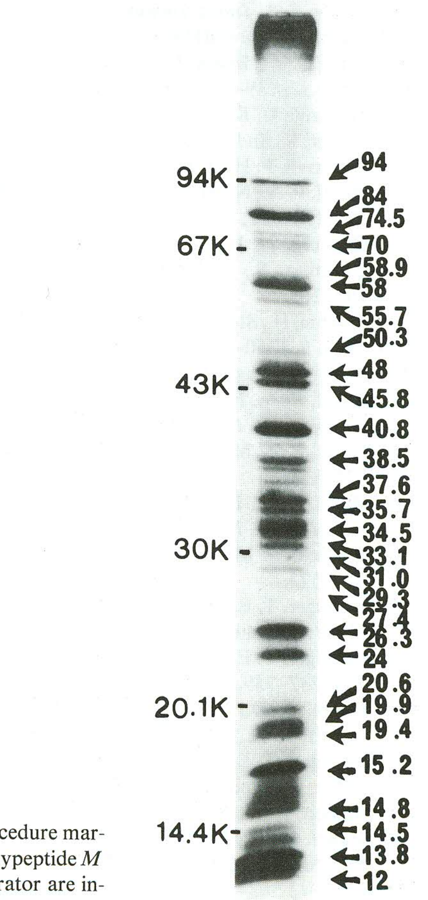


Figure 3. (Larsen) SDS-PAGE pattern of *T. pallidum* polypeptides as revealed by the silver staining procedure marketed by Bio-Rad. The relative positions of Pharmacia low M_r standards (left side) and the *T. pallidum* polypeptide *M* values as determined with a Bio-Rad Model 620 scanning video densitometer and Model 3392A integrator are indicated.

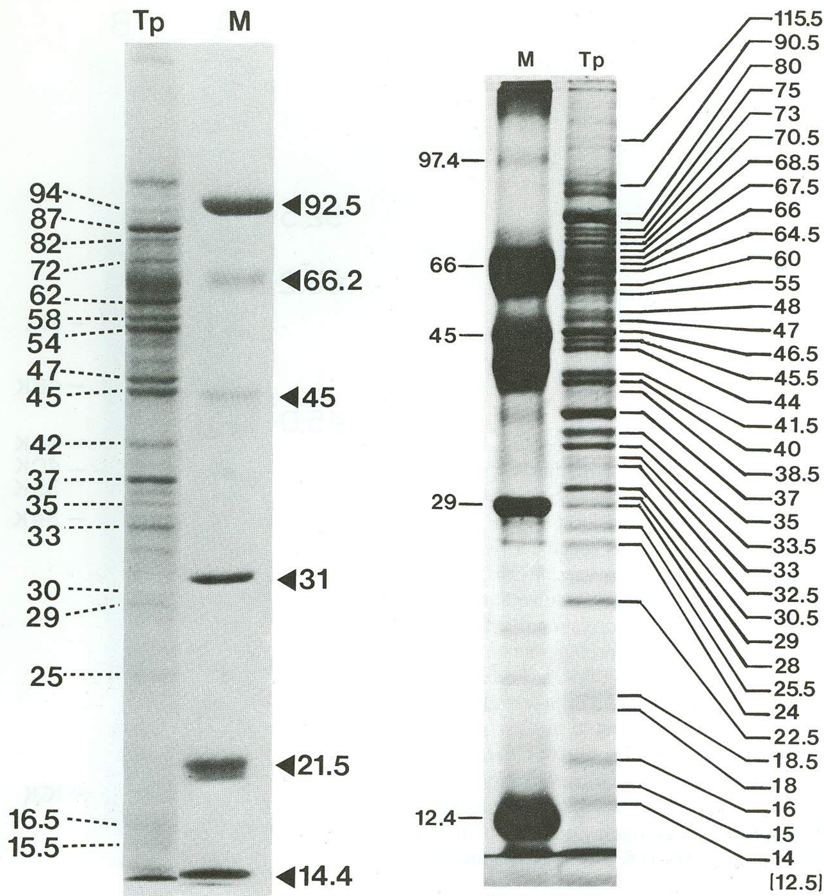


Figure 4. (Baughn) Coomassie Brilliant Blue R-250 stained gel pattern of *T. pallidum* and Bio-Rad high M_r standards, from [24]. *T. pallidum* polypeptide M_r values are indicated, along with the M_r standard values.

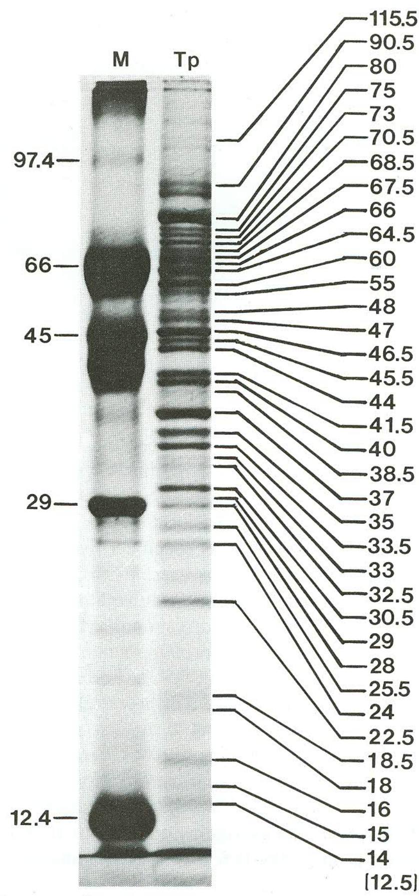


Figure 5. (Lukehart and Baker-Zander) *T. pallidum* polypeptides with M_r values determined by comparison with Sigma M_r standards (from top to bottom, phosphorylase b, bovine serum albumin, ovalbumin, carbonic anhydrase, and cytochrome C). The gel was stained with Coomassie Brilliant Blue R-250 and analyzed with a scanning densitometer (ISCO, Lincoln, NE). Monoclonal antibodies have been obtained which react specifically with the polypeptides with M_r's of 46.5 (monoclonals A8, C2, D10, 2G10, and H91) and 37.0 (H92); monoclonal F5 reacts both with the 46.5 and the 12.5 polypeptides [28]. By Western blotting and radiolabelling with [^{125}I]-*Staphylococcus aureus* protein A [25, 27, 29], it was determined that pooled human serum from secondary and latent stage syphilis patients and pooled serum from *T. pallidum*-infected rabbits reacted with the following polypeptides: high intensity of radiolabelling – 80.0, 64.5, 46.5, 45.5, 40.0, 37.0, 35.0 33.5, 30.5, 14.0, and 12.5; moderate intensity – 75.0, 73.0, 55.0, 50.0, 48.0, 47.0, 41.5, 32.5, 28.0, 25.5, 24.0, 22.5, 18.5, 18.0, 16.0, and 15.0. Based on monoclonal and polyclonal antibody reactivities, the 46.5 and 37.0 polypeptides contain both epitopes specific for *T. pallidum* and determinants common to other pathogenic and nonpathogenic Spirochetes, whereas the 12.5 molecule recognized by F5 appears to lack epitopes cross-reactive with other Spirochetes [27, 28].

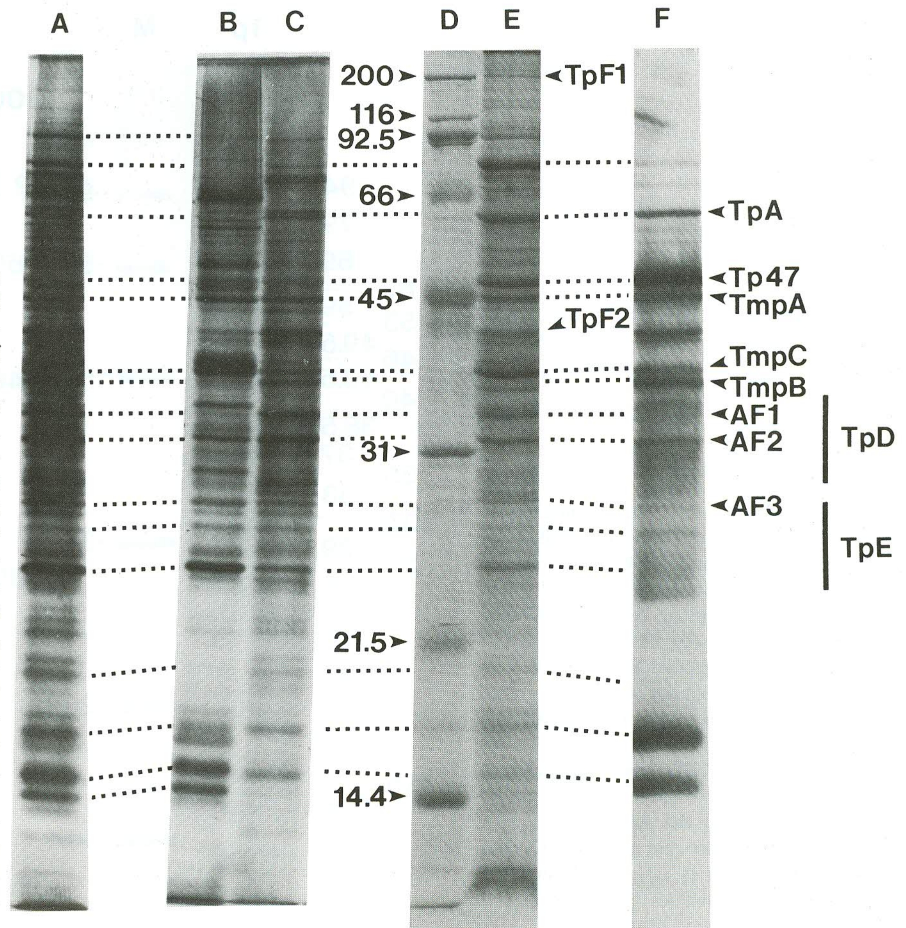


Figure 6. (van Embden) (A) and (E) represent the total *T. pallidum* polypeptide pattern, as revealed by silver staining and Coomassie Blue R-250 staining, respectively. (B) contains the soluble fraction of sonically disrupted *T. pallidum* whereas (C) contains the sedimented (*i. e.* particulate) fraction; both are silver stained. (F) is a Western blot of total *T. pallidum* proteins which has been reacted with rabbit anti-*T. pallidum* antiserum to reveal the pattern of antigenic reactivity. (D) contains Bio-Rad high and low M_r standards with the indicated molecular weights. Products of recombinant DNA clones expressed in *E. coli* [30, 31] (and their respective M_r values $\times 10^{-3}$) include TpFl (200), TpA (58), TpS (Hindersson and Axelsen, unpublished; $M_r = 47$), TmpA (44.5), TpF2 (45), TmpC (38.5), TmpB (37.5), TpD (29–35) and TpE (24–28). Both TpD and TpE migrate as diffuse bands over a broad M_r range, as indicated by the vertical lines. AF1, AF2 and AF3 are three *T. pallidum* polypeptides which react with specific antiserum directed against *T. phagedenis* biotype Reiter periplasmic flagella, indicating that they may represent flagellar proteins of *T. pallidum*. Monoclonal antibodies have been produced against TpS (monoclonal C3E5) and TmpA (monoclonal 1-14M15) [32].

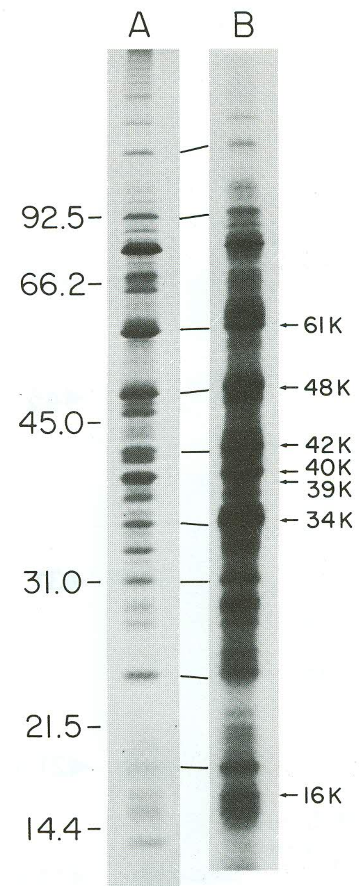


Figure 7. (Stamm and Bassford) SDS-PAGE comparison of the (A) Coomassie Brilliant Blue R-250 stained protein profile of *T. pallidum* with (B) that obtained by fluorography of *T. pallidum* proteins intrinsically radiolabelled with [^{35}S]-methionine, from [35]. The numbers to the left indicate the positions of Bio-Rad M_r standards run on the same gel, whereas those to the right represent the M_r values of specific *T. pallidum* polypeptides. The 61 polypeptide is precipitated by the anti-61 monospecific rabbit antiserum provided by Norris. The 48 protein is strongly recognized in Western blots by mouse anti-*T. pallidum* antiserum and by the monoclonal antibody 13F3 provided by Thornburg *et al.* [53], and most likely corresponds to the "immunodominant" 47 protein identified by Jones *et al.* [43] and the 48 treponemal protein recognized by monoclonal H9-1, as reported by Lukehart *et al.* [28]. The 42, 40, 39, and 34 polypeptides can be extracted from aged treponemes by 0.04 % SDS and are therefore thought to be surface-exposed in these organisms. However, the 34 protein is strongly recognized by three different antisera prepared against purified periplasmic flagella of *T. phagedenis b.* Reiter (provided by R. E. Baughn, D. R. Blanco, and N. Charon) and is also recognized by monospecific rabbit antiserum against a 33 flagellar protein of *T. pallidum* provided by S. J. Norris. The gene encoding the 39 protein has been cloned by Stamm *et al.* [33, 34] and the recombinant product purified by W. Dallas, P. Ray, L. Stamm and P. Bassford (manuscript in preparation). It appears to be a relatively minor, basic (p*I* 9.5) treponemal membrane protein. The 16K protein is prominently surface-exposed in aged treponemes [35].

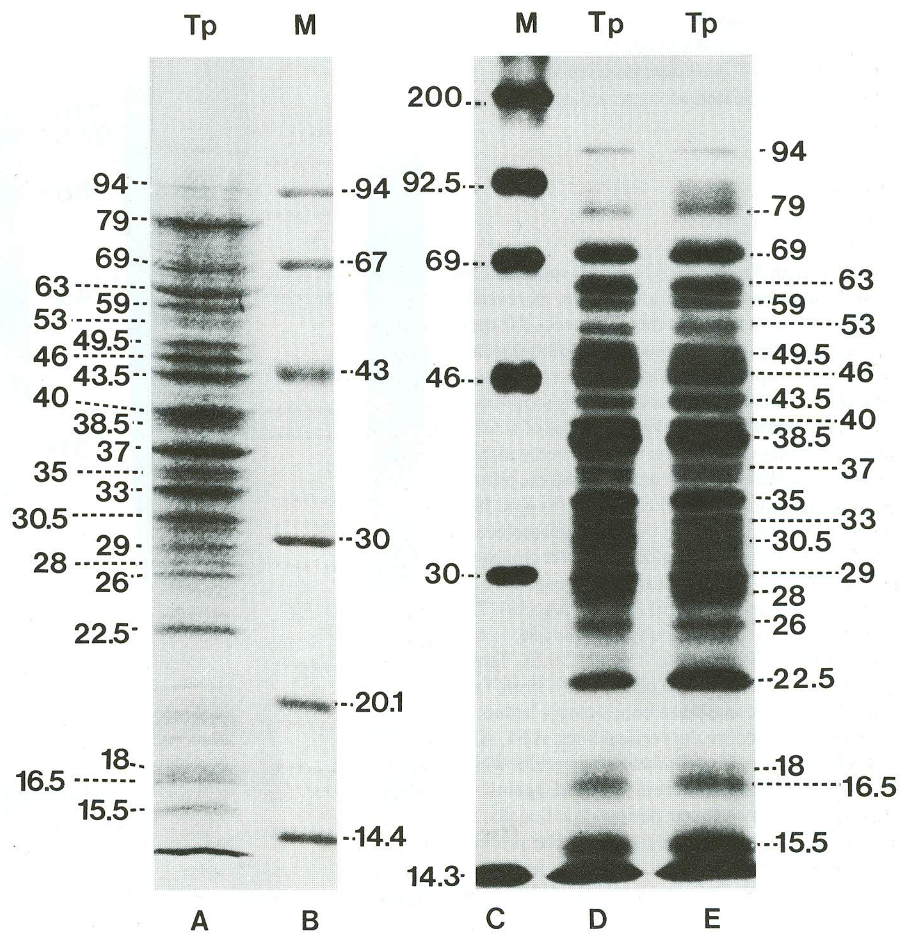


Figure 8. (Müller and Moskophidis) *T. pallidum* polypeptides as demonstrated by (A) Coomassie Brilliant Blue R-250 staining, or autoradiography following intrinsic labelling with (D) [^{35}S]-methionine or (E) ^{14}C-labeled protein hydrolysate. (B) Pharmacia and (C) Amersham ^{14}C-methylated M_r standards were also included. Polypeptides labeled by surface radioiodination [38] are those with indicated M_r values of 69, 59, 49.5, 46, 43.5, 40, 35, 33, 30, 28, 26, and 15.5. [^{14}C]Glucosamine is preferentially incorporated into the 59, 35, 33, and 30 polypeptides, leading the authors to suggest that these proteins are glycosylated [37, 38]. Monoclonal antibodies directed against the polypeptides with M_r values of 46, 43.5, 33, and 15.5 have been isolated [39].

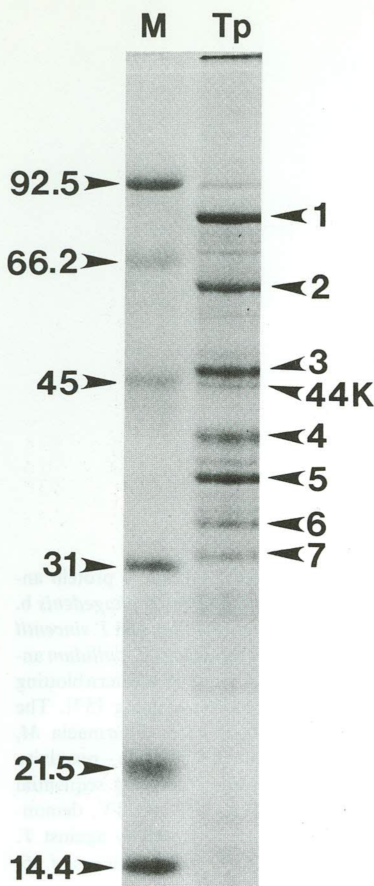


Figure 9. (Penn, Bailey, and Cockayne) Coomassie Brilliant Blue R-250 stained SDS-PAGE pattern of *T. pallidum* and Bio-Rad M_r standards with the M_r values indicated. The numerical designations 1 through 7 correspond to *T. pallidum* polypeptides with M_r values of 80, 60, 47, 42, 37, 34 and 31.5, as previously described by Penn *et al.* [6]. Monoclonal antibodies directed against the 80, 47, 37, and 31.5 polypeptides, and the indicated 44 polypeptide, have been isolated. The anti47 and anti44 monoclonal antibodies are reactive in the *T. pallidum* immobilization test and appear to bind to the surface of methanol-fixed *T. pallidum* (M. J. Bailey, A. Cockayne, and C. W. Penn, manuscript in preparation). The 80 polypeptide is thought to be the major protein component of the cytoplasmic filaments (A. Cockayne, M. J. Bailey, and C. W. Penn, manuscript in preparation); it also has the property of exhibiting a lower M_r following treatment with Triton X-100 [42]. Solubilization of the outer membrane of "aged" *T. pallidum* with 1 % Triton X-100 results in the selective release of the 47 polypeptide, leading the authors to conclude that it is a major outer membrane-associated protein [40, 42]. The 37 polypeptide and a doublet of bands at 34 are major constituents of purified preparations of *T. pallidum* periplasmic flagella [41].

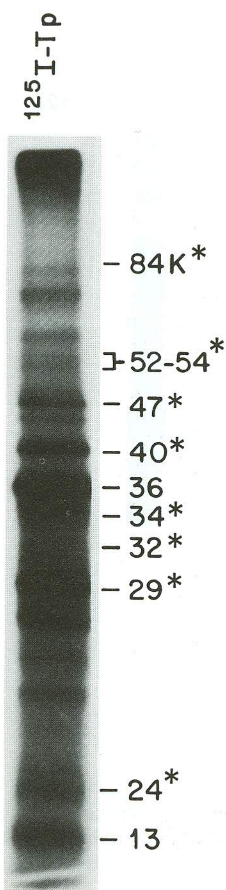
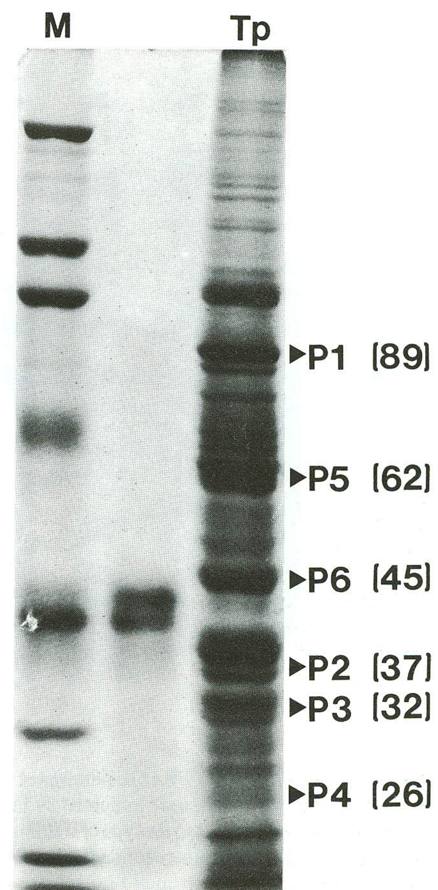


Figure 10. (Norgard) *T. pallidum* protein antigens, as demonstrated by radioimmunoprecipitation [43]. Percoll-purified *T. pallidum* were subjected to lactoperoxidase-catalyzed surface radioiodination and solubilized in detergents. The solubilized antigenic proteins were then precipitated with serum from *T. pallidum*-infected rabbits and *Staphylococcus aureus* (Cowan I strain), and the resulting complex was analyzed by SDS-PAGE and autoradiography. Antigenic polypeptides thus identified include those with M_r values of 84, 52–54, 47, 40, 36, 34, 32, 29, 24, and 13. Monoclonal antibodies have been obtained which react specifically with the polypeptides marked with an asterisk [44]. DNA recombinants expressing *T. pallidum* polypeptides with M_r's of 47, 44, and 34 have been isolated and characterized [45–47]. The 47 polypeptide is thought to be a surface-localized, "immunodominant" antigen, and monoclonal antibodies directed against the 47 antigen are capable of eliciting complement-mediated immobilization and neutralization of the organism and of blocking attachment to mammalian cells [43].

Figure 11. (Baseman and Alderete) SDS-PAGE profile of *T. pallidum* as compared to Bio-Rad M_r standards, demonstrated by Coomassie Brilliant Blue R-250 staining. P1 through P6 (with M_r values as indicated) represent major antigenic polypeptides of *T. pallidum*, as described previously by this group [48–56]. P1, P2 and P3 have been implicated as surface-localized proteins involved in the attachment of *T. pallidum* to mammalian cells [48, 49, 51] and the interaction with fibronectin [51]. Hybridomas secreting monoclonal antibodies directed against P6 have been characterized [53], and recombinant plasmids containing the genes for P6 [54, 55] and a protein called P2* (star) [56] have been isolated.



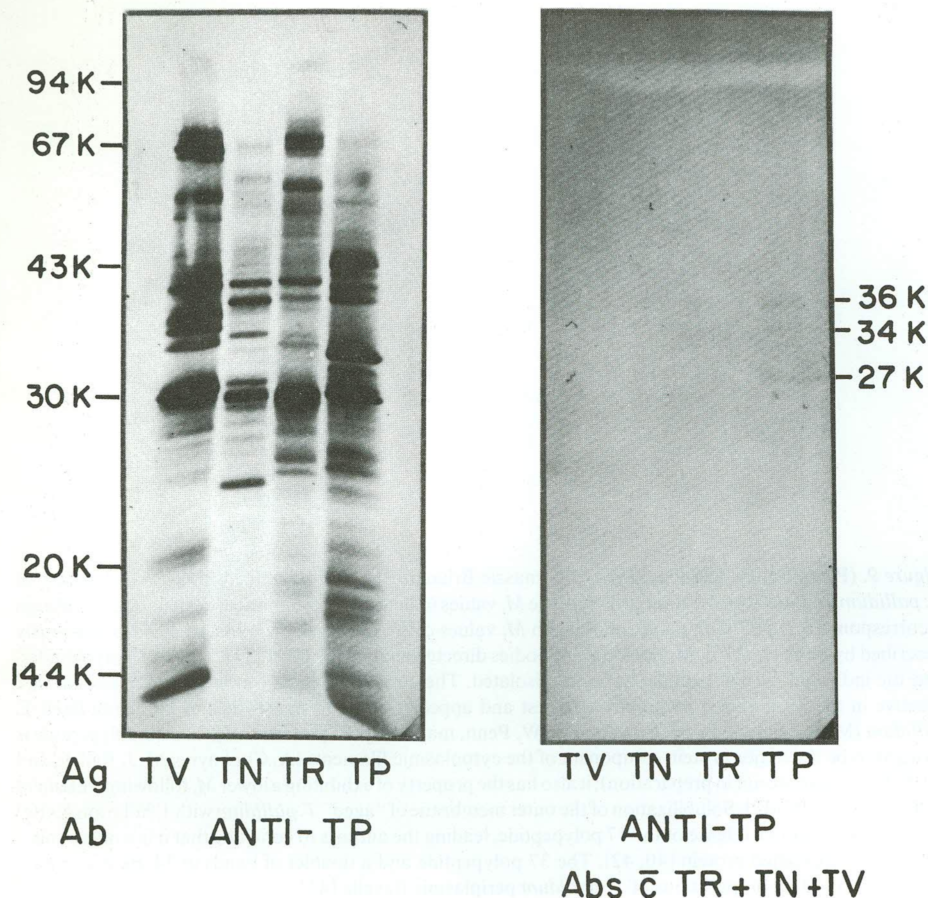


Figure 12. (Wicher) Left side – protein antigens of *T. pallidum* (TP), *T. phagedenis* b. Reiter (TR), *T. noguchi* (TN), and *T. vincentii* (TV) reactive with rabbit anti-*T. pallidum* antiserum, as demonstrated by Western blotting and immunoperoxidase staining [57]. The corresponding positions of Pharmacia M_r markers are indicated. Right side – reactivity of the same antiserum following sequential adsorption with TR, TN and TV, demonstrating the retention of activity against *T. pallidum* polypeptides with M_r values of 36, 34, and 27. From [57].

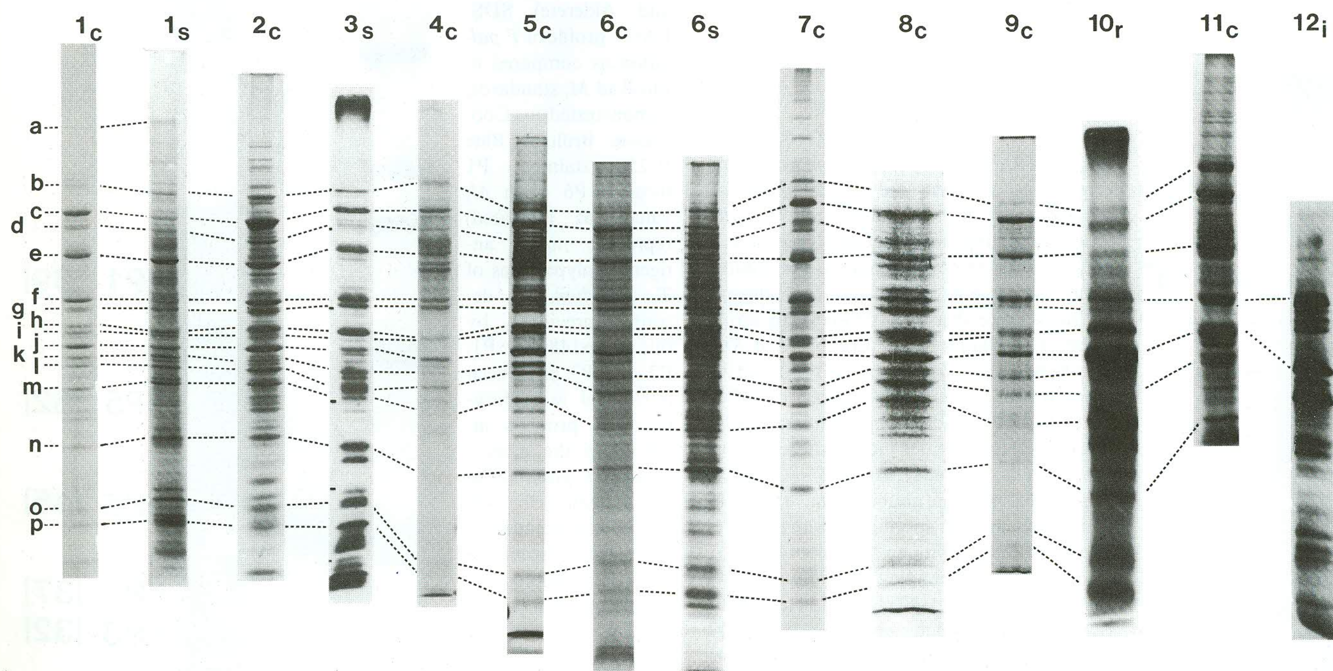
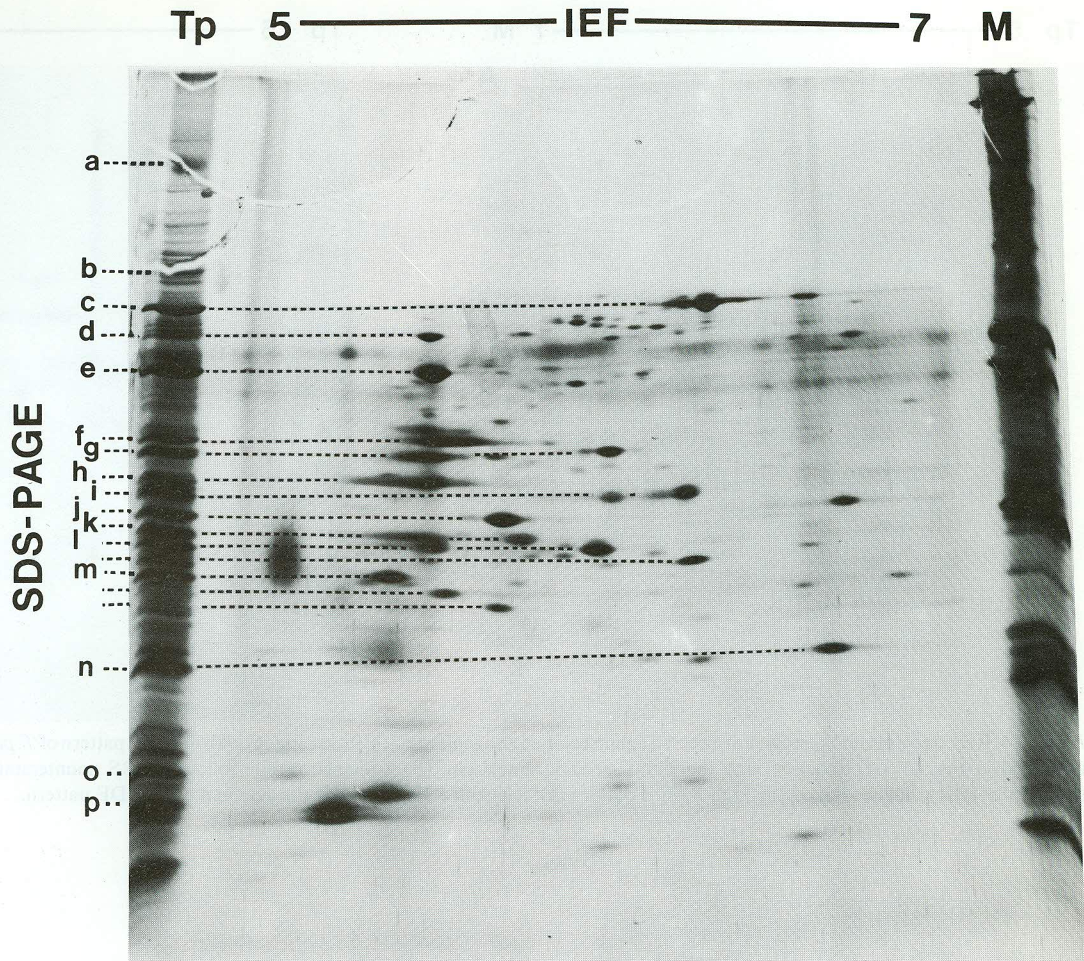
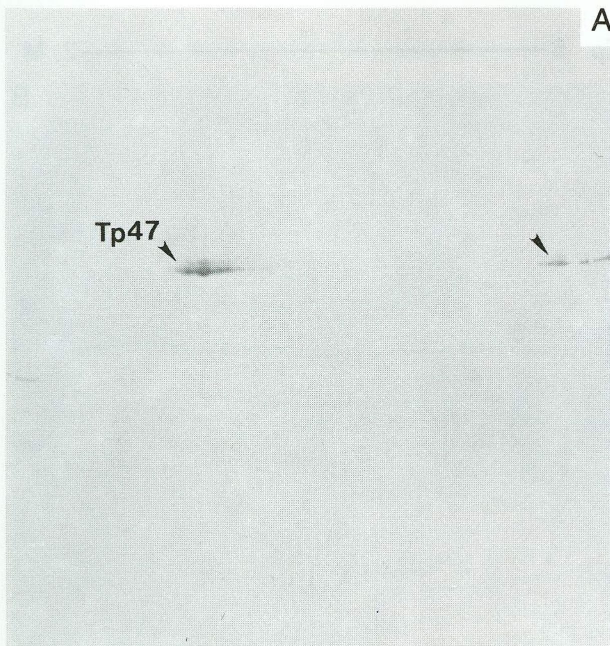


Figure 13. Composite of SDS-PAGE patterns of *Treponema pallidum* indicating a tentative correlation of the bands a through p in the different gel profiles. The contributing laboratories are indicated by the number at the top of each of the lanes, corresponding to Figs. 1 through 12 and the group numbers in Table 1. The subscript refers to the type of staining: c = Coomassie Blue; s = silver stain; or i = immunologic reactivity as demonstrated by autoradiography of a radioimmunoprecipitation reaction (Lane 10) or by immunoperoxidase staining (Lane 12). The letter designations assigned to the different polypeptide bands correspond to those given in Fig. 1.

Figure 14. 2DE profile of *Treponema pallidum* subsp. *pallidum*, as detected by silver staining. Isoelectric focusing (IEF, pH 5–7) was performed in tube gels which were then applied to 8–20 % linear gradient SDS-PAGE gels for the second dimension. Single dimension SDS-PAGE separations of *T. pallidum* (Tp) and Pharmacia low M_r standards (M) were performed in the same gel as a means of correlating the SDS-PAGE and 2DE patterns, as indicated by the dashed lines. The letter designations are the same as those used in Fig. 1 and 13. Note that many of the SDS-PAGE bands appear to represent two or more co-migrating species, which are separated on the basis of charge in the 2DE patterns. The cross-correlation of polypeptides not consistently detected in the SDS-PAGE profiles shown in Fig. 13 are shown as unlabeled lines. The positions of a, o and p in the 2DE pattern were uncertain and therefore were not indicated.





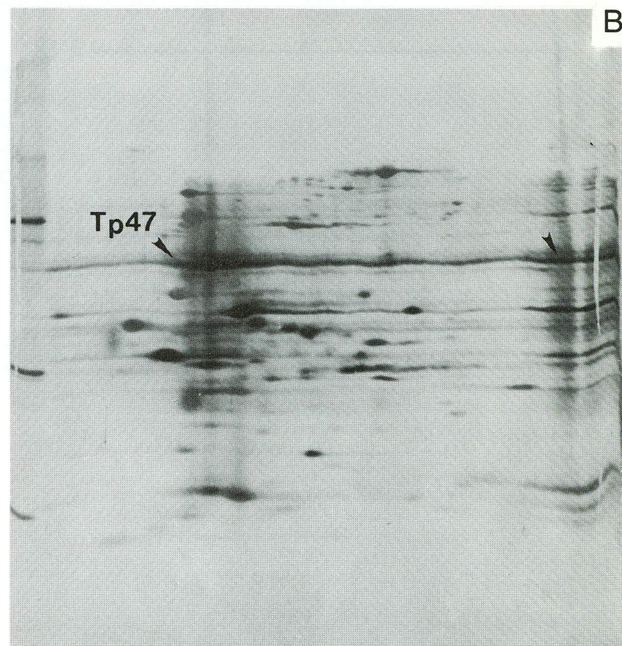


Figure 15. Location of a 47 antigen in the 2DE profile of *Treponema pallidum*, as determined by immunoperoxidase staining with monoclonal antibody C3E5 [30]. (A) Immunoperoxidase staining of a 2DE electroblot of *T. pallidum* with C3E5. An electroblot of the 2DE pattern obtained with *T. pallidum* (with flanking single dimension *T. pallidum* and M_r standard lanes) was prepared as described in Fig. 14. The electroblot was reacted sequentially with a 1:400 dilution of culture supernatant from the C3E5hybridoma, a 1:1000 dilution of peroxidase conjugated goat anti-mouse IgG, IgA, and IgM, and 4-chloronaphthol as the peroxidase substrate. (B) The same electroblot following immunoperoxidase staining with serum from *T. pallidum*-infected rabbits (IRS) to reveal the distribution of most *T. pallidum* polypeptide antigens. The position of the 47 antigen, as determined by direct comparison of the staining patterns before and after counterstaining, is indicated by the arrow. This antigen corresponds to the major polypeptide designated as 'f' in Fig. 1, 13, and 14.

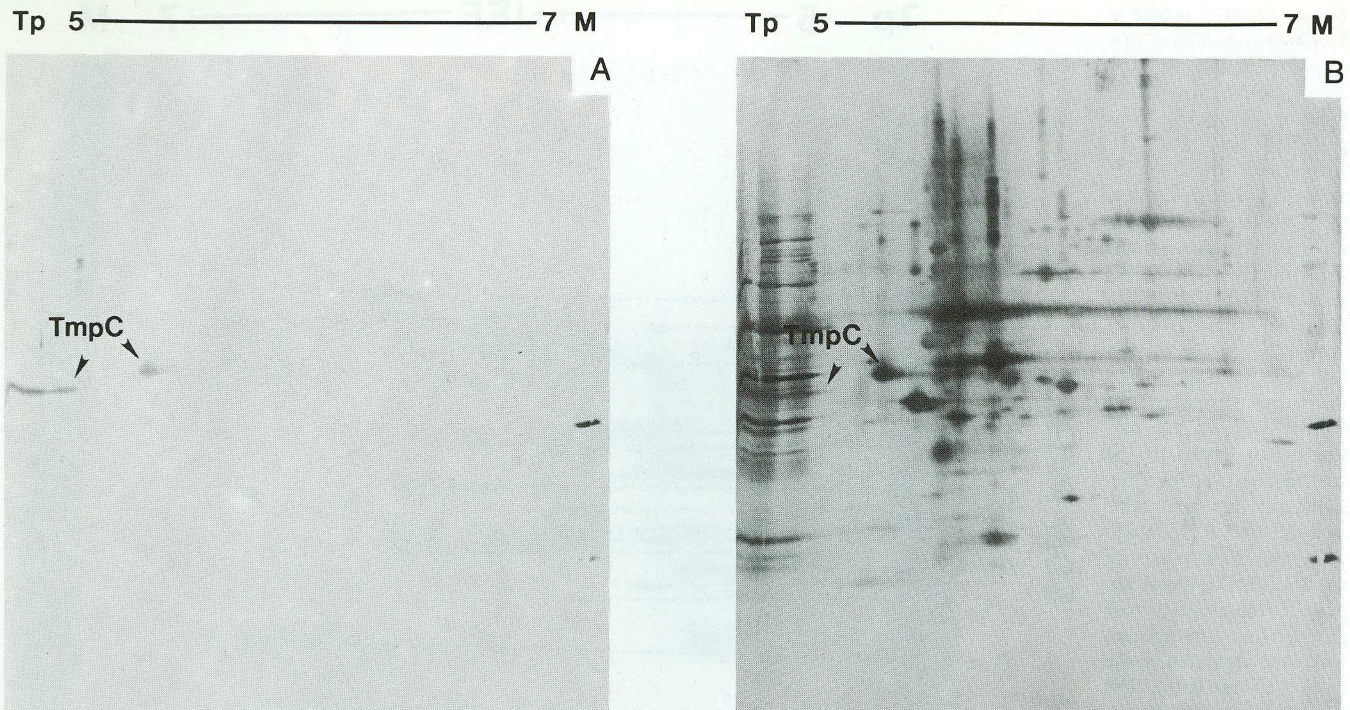


Figure 16. Reactivity of rabbit antiserum directed against cloned antigen TmpC ([30], see Fig. 6) with the 2DE pattern of *T. pallidum*. The procedure was as described in Fig. 15. (A) Reaction with the TmpC-specific antiserum. (B) Antigenic profile following IRS counterstaining. The antiserum reacted specifically with a single band on the SDS-PAGE pattern (far left) and a single spot at the acid end of the 2DE pattern.

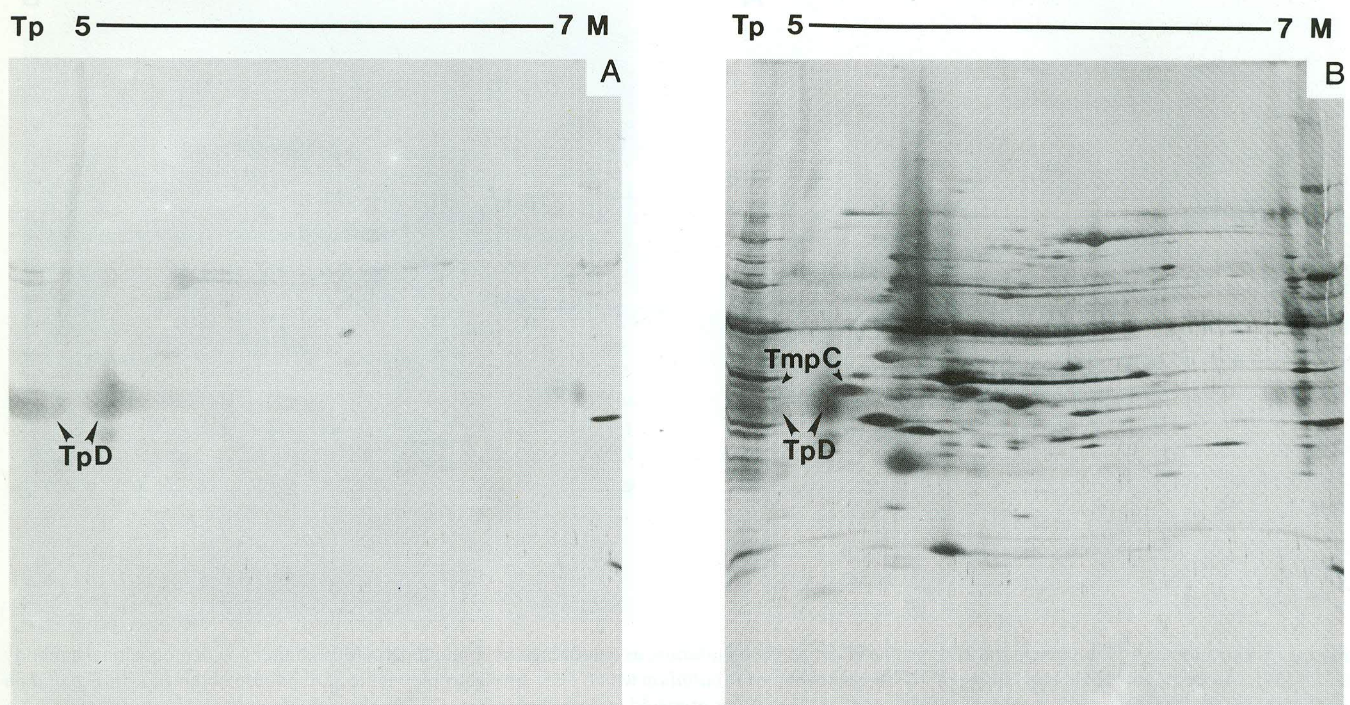


Figure 17. Localization of cloned antigen TpD ([30], see Fig. 6) in the 2DE profile of *T. pallidum*, utilizing a specific rabbit antiserum prepared by affinity chromatography. (A) Reactivity of TpD-specific antiserum. Due to prolonged incubation with the color reagent, background reactivities of the antiserum to TmpC and the major 61 polypeptide were also detectable in this case. (B) Antigenic profile following IRS counterstaining. The positions of TpD and TmpC in the 2D and single dimension SDS-PAGE profiles are indicated by the arrows.