Fluorescent Antibody Methods
for *Neisseria gonorrhoeae* Identification

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The application of fluorescent antibody procedures to the identification of Neisseria gonorrhoeae—*described in this paper—promises to be of very considerable value in the epidemiology of gonorrhoea. Although there is much still to be finally determined, particularly in relation to the exact nature of the antigenic reaction involved in these procedures, it appears that anti-*N. gonorrhoeae *conjugates may now be prepared and used for the identification of* N. gonorrhoeae *in 16-20 hours, as opposed to as long as 10 days with conventional cultural techniques. The " delayed fluorescent antibody method" is of especial value for the detection of gonorrhoea in females.*

Fluorescent antibody (FA) techniques, as introduced by Coons et al. (1942) and Coons & Kaplan (1950), have been applied to great advantage in many diseases (Coons, 1957; Friou et al., 1958; Goldwasser & Kissling, 1958; Goldwasser et al., 1959; Jackson, 1960). The fluorescent treponemal antibody (FTA) test for syphilis (Deacon, Falcone & Harris, 1957; Deacon, Freeman & Harris, 1958)—the first of two fluorescent antibody applications in the field of venereal disease—has been shown to produce highly sensitive and specific results (US Communicable Disease Center, 1959), and recent publications indicate an increasing awareness of the value of this procedure as a treponemal test for syphilis, both in North America and abroad (Borel & Durel, 1959; Censuales & Garofalo, 1959; Olansky & McCormick, 1960).

An extension of fluorescent antibody methods to *Neisseria gonorrhoeae* identification has recently been announced (Deacon, Peacock et al., 1959, 1960). It is the purpose of the present communication to review this new development and to discuss possible applications.

PRINCIPLES OF FLUORESCENT ANTIBODY
PROCEDURES APPLIED TO *NEISSERIA*

Fluorescent antibody procedures are essentially serological in character and operate in many respects like other conventional serological tests. Since the present discussion has to do with identification of *N. gonorrhoeae* by serological means, it would seem appropriate briefly to consider some of the well-defined principles and concepts presently employed in the differentiation of bacteria by antigenic analysis. The following facts are generally accepted and are well understood. A group of bacteria, classified together on the basis of morphology and cultural similarity, may exhibit sharp differences in serological behaviour. These differences may be of such magnitude that the group can be divided into genera and species. In some instances further subdivisions are possible; in which case, types may be recognized within a species. As examples, the pneumococcus as a species has been shown to consist of over sixty serological types, and the meningococcus has at least three, and possibly four, types. Thus, one must conclude that the subject of bacterial identification by means of antigenic analysis is of more practical importance than is generally realized. In support of this viewpoint, consider the role played by serological typing in expanding epidemiological studies in relation to *Salmonella, Shigella* and *E. coli* infections.

From the foregoing, it is evident that the development of serological identification methods for *N. gonorrhoeae* must be concerned with basic antigenic facts related to the entire *Neisseria* group. It is known that the meningococcus occurs as three or more serological types—A, B, C and possibly D—and that type specificity is determined by the occurrence of capsules or K-like antigens

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(Kauffmann, 1954). It is also known that the meningococci occur as non-encapsulated forms, in which case the type cannot be determined. Non-encapsulated forms, however, are agglutinated by A, B, C and D antisera. Thus, it would appear that the non-encapsulated variant, having lost its K-antigen, has exposed underlying somatic antigens which are common and shared by all of the types. Since the meningococcus and the gonococcus are related species, it seems reasonable to assume that the antigenic behaviour pattern may also be similar.

Wilson (1954, 1956) has shown that meningococci and gonococci share common somatic antigens. Wilson has also reported that freshly isolated *N. gonorrhoeae* cultures are frequently inagglutinable in gonococcal antisera. This observation suggests that K-like antigens (labile surface antigens) occur in freshly isolated cultures and may act as a protective cover over the underlying somatic antigens. Wilson (personal communication, 1957) was unable to demonstrate the antigenic properties of this material, however, and as a result, our original studies were aimed at answering this problem. It was felt that should the gonococcus cell-covering prove to be a K-antigen, it would probably demonstrate a high degree of specificity and prove to be most important as a diagnostic antigen for *N. gonorrhoeae*.

In preliminary experiments our agglutination results were similar to those described by Wilson. However, when immunization techniques were altered and greatly prolonged, the K-antigen of the gonococcus was eventually demonstrated. This conclusion is based, in part, on agglutination findings illustrated in Table 1. It will be noted that when living inagglutinable gonococcus cultures (GC-L) and formalin-killed inagglutinable cultures (GC-F) are compared, reactions are identical. That is, both react poorly or not at all in somatic antisera which have been prepared against cultures heated at 100°C or autoclaved at 120°C. On the other hand, both antigens GC-L and GC-F are agglutinated by the antiserum against the formalin-killed antigen. This, it would appear, indicates that the labile K component is preserved by formalin treatment. The hypothesis for the existence of a K-antigen is further supported in Table 1 by the absorption experiment using GC-F antiserum and GC-120°C antigen. Here it is noted that the absorbed antiserum agglutinates GC-L, GC-F and GC-100°C antigens, but does not

TABLE 1

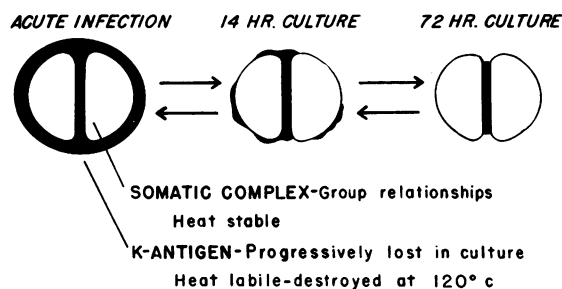### SLIDE AGGLUTINATION RESULTS WITH N. GONORRHOEAE ANTIGENS AND ANTISERA [a]

Undiluted sera	Antigens (5 strains)			
	GC (L)	GC (F)	GC(100°C)	GC(120°C)
GC(F)	+	+	+	+
GC(100°C)	±	±	+	+
GC(120°C)	−	−	+	+
GC(F) absorbed with GC(120°C)	+	+	+	−
GC(100°C) absorbed with GC(120°C)	±	±	+	−
GC(F) absorbed with GC(100°C)	−	−	−	−

[a] Degrees of agglutination are represented by: +, ± and −. L = Living culture. F = Formalin-killed. 100°C = Heat-killed. 120°C = Heat-killed.

agglutinate GC-120°C antigen. The K-antigen, therefore, like Vi-antigen of *Salmonella typhi*, is destroyed at 120°C, but not at 100°C. The *N. gonorrhoeae* K-antigen may be designated as GC-K of the *b* type (Kauffmann, 1954).

The new concept of *N. gonorrhoeae* antigens is depicted in schematic form in Fig. 1. At the left the gonococcus is shown as it occurs in the infectious exudate. The K-antigen is fully developed and appears as an intact covering. The lability of the K-antigen is illustrated in the other drawings at the right. Very young cultures do not contain the fully developed K and, as they become

FIG. 1

SCHEMATIC REPRESENTATION OF NEW CONCEPT OF NEISSERIA GONORRHOEAE ANTIGENS

ACUTE INFECTION   14 HR. CULTURE   72 HR. CULTURE

SOMATIC COMPLEX-Group relationships
Heat stable

K-ANTIGEN-Progressively lost in culture
Heat labile-destroyed at 120° c

older, most of it is lost. As K is lost, somatic antigens are uncovered, serological reactions become non-specific, and cross-reactions usually occur with antisera from other members of the *Neisseria* group. Photomicrographs (Fig. 2, 3 and 4) are used to illustrate K-antigen lability as determined by fluorescent antibody methods. Fig. 2 shows fully developed K-antigen as it appears in acute gonorrhoeal infections. (Note solid staining of the gonococci). Fig. 3 demonstrates considerable K loss in a 12-16-hour culture. The micro-organisms in this photograph have lost much of the surface antigen and therefore have also lost much of the solid staining characteristic. Fig. 4 shows almost complete loss of K-antigen in a 30-hour culture.

Table 2 illustrates the specific staining characteristics of fluorescein-labelled GC-F antisera (the unabsorbed conjugate contains anti-somatic and anti-K antigen). It will be noted that freshly isolated cultures GC-L and gonococci occurring in male urethral smears stain solidly and brilliantly 3+ and 4+ solid). This is illustrated in Fig. 2. It will also be noted that an occasional meningococcus type reacts in a similar manner. However, when the GC-F conjugate is absorbed with serogroup A (heat- and formalin-killed) all but minor staining reactions are removed. From this, it appears that anti-*N. gonorrhoeae* conjugates of specific staining quality may be prepared by the absorption method and used as specific identification reagents for *N. gonorrhoeae* identification.

DETECTION OF GONOCOCCUS IN FEMALES

Detection of the gonococcus in females by fluorescent antibody methods presents certain difficulties not usually associated with acute gonorrhoea in the male. It is well known, for instance, that positive findings by cultural methods are frequently dependent upon techniques requiring great skill and the selection of the proper times for specimen collection. This means that gonococci may occur in very small numbers and may be extremely difficult to demonstrate in fluorescent antibody smear preparations. In order to obviate this foreseen difficulty, a technique of specimen enrichment was developed, whereby a collection swab is placed on a slanted medium capable of supporting good growth of the gonococcus and after a short incubation period (16-20 hours) heavy smears are prepared for fluorescent anti-

TABLE 2

NEISSERIA REACTIONS WITH *N. GONORRHOEAE* (GC-F) FLUORESCEIN-LABELLED ANTISERUM [a]

Antigens	Unabsorbed	Single absorption with serogroup A (100°C)	Single absorption with serogroup A (F)
Gonococcal:			
GC(L) (3 strains)	3+ s, oc 4+ s	3+ s, oc 4+ s	3+ s, oc 4+ s
GC(100°C)	3+, oc 4+	3+, oc 4+	3+, oc 4+
Urethral smears (25 individuals)	4+ s	4+ s	4+ s
Meningococcal:			
Serogroup A(L)	1+, oc 4+ s	—, oc 3+	—
(100°C)	1+, oc 4+ s	1+	—
Serogroup B(L)	1+, oc 4+ s	1+	—
(100°C)	1+, oc 3+ s	1+	—
Serogroup C(L)	1+	1+	1+
(100°C)	2+	1+	—
N. catarrhalis (L)	1+	1+	1+
(100°C)	1+	1+	1+
N. perflava (L)	—	—	—
(100°C)	—	—	—
N. sicca (L)	—	—	—
(100°C)	—	—	—

[a] Degrees of fluorescence are represented by: —, 1+ (barely visible), 2+, 3+, 4+.
s = Solid stain.   oc = Occasional.

body staining. This procedure is designated as the delayed fluorescent antibody method, abbreviated in Tables 3 and 4 to " Delayed FA ".

In order to test the efficiency of the fluorescent antibody detection system in women a comparative study was designed. Females in this study were named contacts of men with gonorrhoea. Specimens were obtained from three sites—the urethra, the vagina, and the cervix. From each site slides were prepared for direct staining (Direct FA), specimens on swabs were placed on slants (Delayed FA) (see Fig. 5, 6 and 7), and Petri plates were inoculated (conventional culture technique). Table 3 details the results obtained in the first study, consisting of 50 female contacts. It will be noted that the culture and the delayed fluo-

TABLE 3

COMPARISON OF CULTURE AND DIRECT AND DELAYED FLUORESCENT ANTIBODY METHODS FOR IDENTIFYING GONOCOCCUS (50 FEMALE CONTACTS)

Method	Positive vagina		Positive cervix		Positive urethra		Total sites positive		Total patients positive	
	No.	%	No.	%	No.	%	No.	%	No.	%
Direct FA	13	26	4	8	5	10	22	14.6	13	26
Delayed FA	24	48	25	50	22	44	71	47.3	29	58
Culture	24	48	23	46	20	40	67	44.6	29	58

TABLE 4

COMPARISON OF DIRECT AND DELAYED FLUORESCENT ANTIBODY METHODS FOR IDENTIFYING GONOCOCCUS (100 FEMALE CONTACTS)

Method	Positive vagina		Positive cervix		Positive urethra		Total sites positive		Total patients positive	
	No.	%	No.	%	No.	%	No.	%	No.	%
Direct FA	17	17	10	10	12	12	39	13.0	24	24
Delayed FA	44	44	46	46	38	38	128	42.6	58	58

rescent antibody techniques each gave 58% positive findings. The delayed fluorescent antibody procedure detected 71 positive sites, however, compared with 67 for culture. It will also be noted that if culture alone had been used for cervical examinations, two women would have gone undetected. One of the unexpected findings disclosed in this study was the positive results obtained in vaginal examinations, 48% by both the culture and delayed fluorescent antibody methods. The value of the fluorescent antibody procedure was particularly impressive in this series of examinations because findings were obtained in from 16 to 20 hours by the delayed fluorescent antibody technique, while cultures frequently required 10 days for completion.

Direct smear results (Direct FA) indicate that the method may be useful as an adjunct procedure but should not be used alone. Only 26% positive findings are reported for this procedure as compared with 58% for the delayed procedure. Table 4 shows the results of a further series of examinations with the direct and delayed techniques on 100 female contacts. In this group the findings are similar to those previously described.

CONCLUSION

In summary, fluorescent antibody procedures for *N. gonorrhoeae* identification appear to have much to offer to venereal disease programmes. Their application should prove particularly valuable in respect to the epidemiology of gonorrhoea. The K-antigen which is now being used for species identification is under investigation. Slight differences in staining reactions have been observed which indicate that strain antigens may exist. If in the future it is found possible to differentiate strains, the epidemiological application of fluorescent antibody methods should be of further value.

RÉSUMÉ

L'application de la méthode des anticorps fluorescents à l'identification de *Neisseria gonorrhoeae* ouvre des perspectives intéressantes à l'étude épidémiologique de la blennorragie et aux campagnes de lutte antivénérienne. L'auteur décrit les recherches qui l'ont conduit à appliquer au dépistage des infections à gonocoques une technique qui a déjà rendu de grands services dans le diagnostic de diverses maladies.

Rappelant les principes du typage sérologique des bactéries, telles que les *Salmonella*, *Shigella* et *Escherichia*, et de son rôle pratique en épidémiologie, l'auteur décrit ses recherches sur le gonocoque, fondées sur les découvertes faites dans le groupe des méningocoques, étroitement apparenté par les caractères sérologiques. On connaît trois, peut-être quatre, types sérologiques de méningocoques. La spécificité de type du méningocoque repose sur l'antigène capsulaire (antigène K). La perte de l'antigène K chez les variants non capsulés met à découvert les antigènes somatiques sous-jacents, communs à tous les types, d'où perte de la spécificité de type et réaction croisée avec d'autres membres du groupe *Neisseria*. La parenté entre gonocoques et méningocoques permettait de supposer que le gonocoque avait une constitution antigénique analogue.
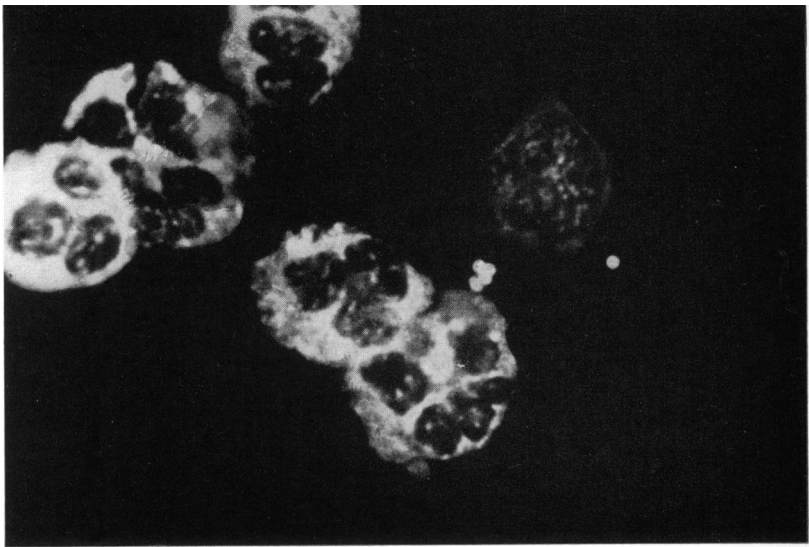
FIG. 2

URETHRAL (MALE) SMEAR STAINED WITH GC(F), FLUORESCEIN-LABELLED ANTISERUM

Solid staining of gonococci indicated fully developed gonococcal K(B) antigen. Oil immersion. Magnification: approx. 1000 ×.
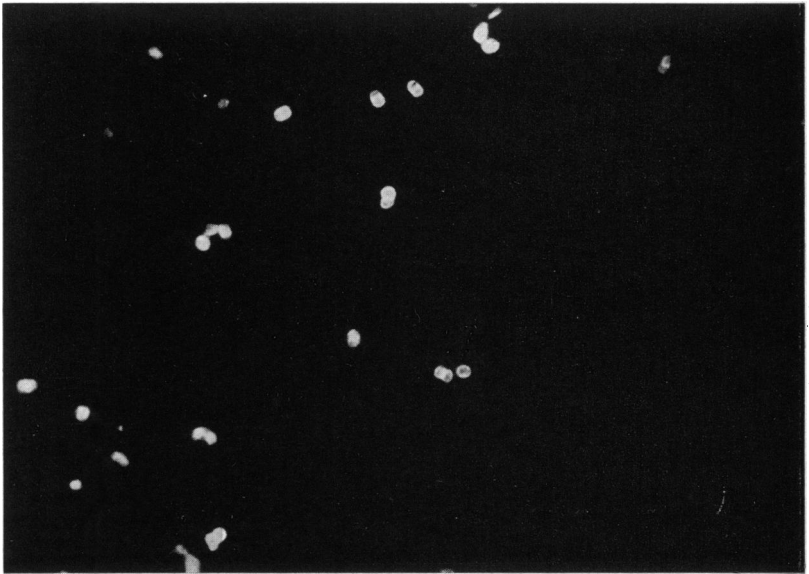

FIG. 3

12-16-HOUR GONOCOCCAL CULTURE STAINED WITH GC(F), FLUORESCEIN-LABELLED ANTISERUM

Gonococcal K(B) antigen is well developed but not as complete as in Fig. 2. Oil immersion. Magnification: approx. 1000 ×.
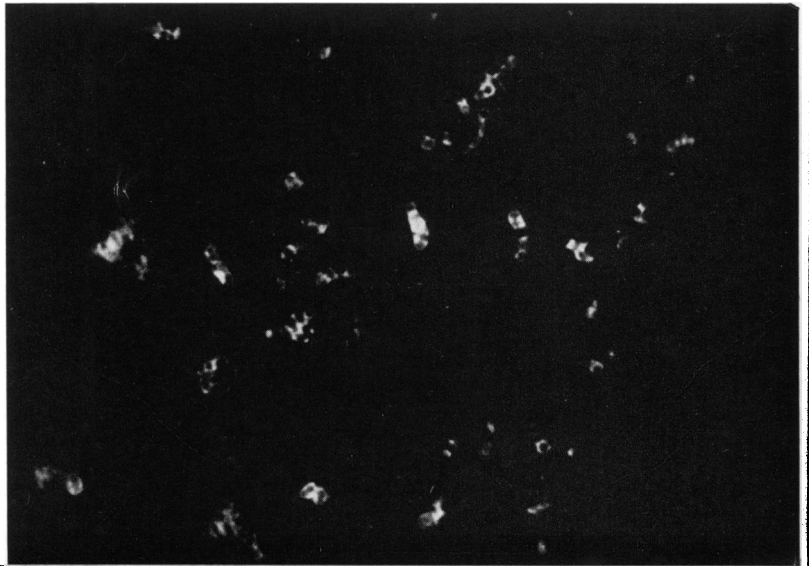

FIG. 4

30-HOUR GONOCOCCAL CULTURE, ILLUSTRATING EXTREME LABILITY OF GONOCOCCAL K(B) ANTIGEN

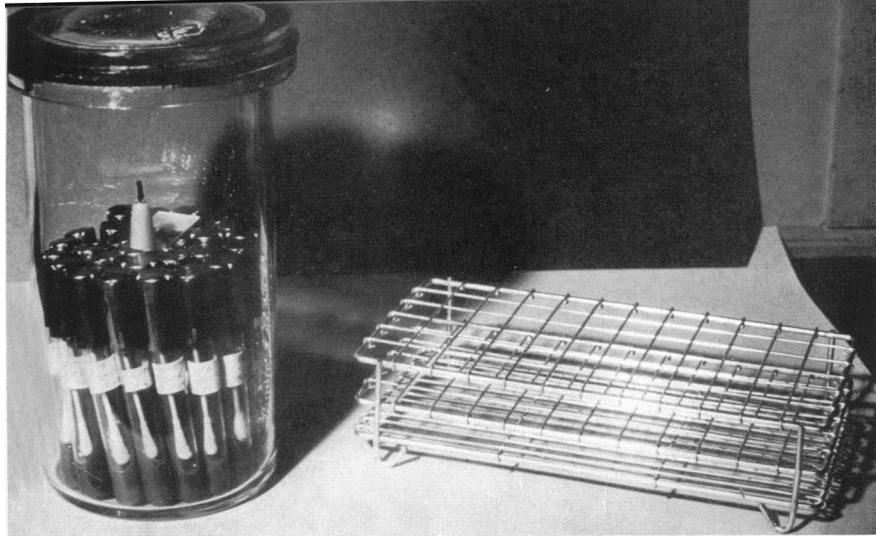Some cells show only a trace of antigen remaining. Oil immersion. Magnification: approx. 1000 ×.

FIG. 5

ENRICHMENT MEDIUM AND SWABS
USED IN DELAYED FLUORESCENT
ANTIBODY METHOD

FIG. 6

PREPARATION FOR DELAYED
FLUORESCENT ANTIBODY METHOD
AS SEEN BY TUNGSTEN
ILLUMINATION
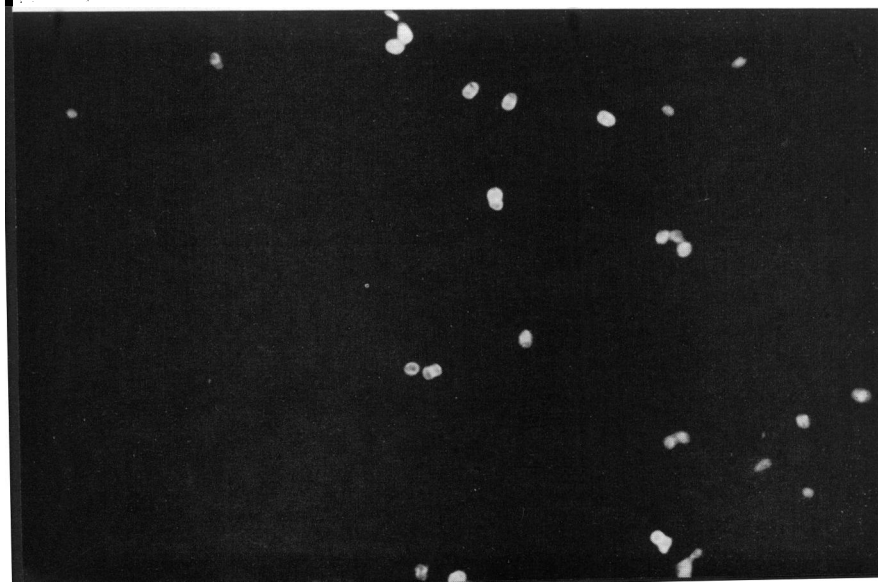Note heavy contamination. Oil immersion. Magnification: approx. 1000 ×.

FIG. 7

SAME FIELD AS IN FIG. 6 AS SEEN
BY ULTRAVIOLET ILLUMINATION
Gonococci are well defined. Oil
immersion. Magnification: approx.

Grâce à la modification des techniques adoptées jusqu'alors, un antigène K a pu effectivement être mis en évidence dans le gonocoque. Comme l'antigène Vi de *S. typhi*, il n'est pas détruit à 100° C, mais à 120° seulement. L'antigène K est labile. Dans les cultures très jeunes, il n'est pas complètement formé, et dans celles de plus de 30 heures il a complètement disparu. Il est en revanche pleinement développé dans les gonocoques des exsudats.

C'est précisément à ce stade que la technique des anticorps fluorescents permet de le mettre nettement en évidence, et de poser un diagnostic rapide. Le gonocoque en possession de son antigène K se colore de façon intense par les conjugués fluorescéinés après absorption par le sérum antiméningococcique A (qui retient les antigènes somatiques).

La recherche du gonocoque chez la femme par la méthode des anticorps fluorescents présente des difficultés, attribuables en particulier au petit nombre de gonocoques présents. Une technique d'enrichissement sur gélose est proposée. Seize à vingt-quatre heures après ensemencement du prélèvement sur gélose inclinée, il est possible d'observer la culture en frottis épais par la méthode de fluorescence. Cette méthode, dite « retardée » raccourcit cependant beaucoup le temps nécessaire au diagnostic, qui, par la méthode classique des cultures, est de 3-10 jours.

D'autre part, de légères différences dans les réactions colorées semblent indiquer la présence d'antigènes spécifiques de souches. Si la technique des anticorps fluorescents permettait de différencier des souches entre elles, elle présenterait un nouvel avantage, sur le plan épidémiologique.

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