J. clin. Path., 1976, **29**, 305-308

A new serotyping method for *Klebsiella* species: evaluation of the technique

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SYNOPSIS A new indirect fluorescent typing method for *Klebsiella* species is compared with an established method, capsular swelling. The fluorescent antibody (FA) technique was tested with standards and unknowns, and the results were checked by capsular swelling. Several unknowns were sent away for confirmation of typing, by capsular swelling. The FA method was also tried by a technician in the routine department for blind identification of standards.

Fluorescence typing gives close correlation with the established capsular swelling technique but has greater sensitivity; allows more economical use of expensive antisera; possesses greater objectivity as it requires less operator skill in the reading of results; resolves most of the cross reactions observed with capsular swelling; and has a higher per cent success rate in identification.

A new method for typing *Klebsiella* capsular antigens by the use of the indirect fluorescent antibody (FA) technique has been developed and published in another paper (Riser *et al*, 1976). The present paper deals with an evaluation of the new method as compared with an accepted technique, capsular swelling, which essentially involves the same serological reaction.

It was necessary to establish that the new method, which is a quantitative measure of the reaction of the capsular antigens, was accurate and reliable. It was important to show that the results obtained by fluorescence were identical with those of an established method measuring the same reaction. This was done by testing unknowns by both fluorescence and capsular swelling and having some of the capsular swelling results confirmed by Dr Casewell's laboratory, a recognized centre for *Klebsiella* typing.

Weak reactions with capsular swelling or reactions with very small capsules are difficult to interpret. A great deal of experience is required to identify the 9% of isolates that are indistinct in their reaction, and another 10% cannot be typed. Capsular swelling has been shown to require high concentrations of antisera in order for a capsule to be observed, and there are numerous cross reactions which are difficult to separate (Casewell, 1975; Edwards and Ewing, 1968).

The FA method was therefore investigated here not only for its ability to obtain the same results as capsular swelling but also for its practicality, convenience, and ease of interpretation as compared with the other technique.

A trained medical laboratory technician without previous experience of fluorescent work also tested the new method to verify that it could be used in routine clinical laboratories and did not require unique skills.

Received for publication 18 September 1975

Material and Methods

CULTURES

Reference strains of 72 recognized *Klebsiella* capsular types were obtained from Dr I. Ørskov of the Statens Seruminstitut in Copenhagen and were kept at room temperature on nutrient agar slopes. For the experiments, isolates of *Klebsiella* were subcultured onto Worfel-Ferguson Medium (Difco) for 18-24 hours at 37°C to promote capsule production. Strains of *Klebsiella* species isolated in the clinical laboratory from patients and the environment of a urological unit were treated in the same manner.

FLUORESCENT ANTIBODY TECHNIQUE

The materials and methods used are as described by Riser *et al* (1976).

CAPSULAR SWELLING

The method of Ørskov, as described by Casewell

(1972), was used to identify the *Klebsiella* antigenic types by capsular swelling, with some modification.

The cultures were not homogenized, as agitating the bacteria in formol phosphate buffered saline with a Vortex mixer provided a good suspension. Also, a loopful of bacteria was taken from a streak of the culture grown on Worfel-Ferguson plates. Single isolated colonies were not used as it had been found that there was some variation among the colonies from a particular type in their response to individual antisera, and a mixed suspension provided a good average for the tests.

TYPING BY FLUORESCENCE AND CAPSULAR SWELLING

Capsular swelling and fluorescence techniques were used by the same worker for a blind comparison of these two methods. Standard types were first identified and then routine isolates were typed by both techniques. These included strains isolated during an epidemiological study. The antisera were diluted where necessary to separate cross reacting from specific types. Several of these strains were also sent to Dr Casewell's laboratory for comparison of the results of two different workers using capsular swelling and to provide an accepted basis of comparison for the new technique.

FLUORESCENCE WITH CROSS REACTING ORGANISMS UNDIFFERENTIATED BY CAPSULAR SWELLING

The antisera that Casewell (1975) found to cross react with several types were diluted and run against these types using the FA technique to see if this method could resolve the established cross reactions.

The antisera were diluted 1/32, 1/64, 1/128, and 1/256 because the end point of specific fluorescence had been found to occur between 1/160 and 1/320. Each dilution was run in duplicate and some were repeated in quadruplicate and others were repeated as necessary. Where possible a dilution was chosen which gave a very bright fluorescence with the specific capsular type (about + + to + + + +) and a very weak fluorescence with the cross reacting types (about + to + +).

METHOD TESTED BY SECOND WORKER NEW TO THE TECHNIQUE

The new method was tested by a technician from the routine laboratory for a blind typing of 29 coded standards. The established method was followed.

Results

TYPING BY FLUORESCENCE AND CAPSULAR SWELLING

Table I shows close agreement in the results obtained

Isolate	*Fluorescence*	*Capsular Swelling*	
		Ours	*Casewell's*
A	21 (11)	11, 21	21, 11
B	44 (18)	44 (18)	44
C	9	?	9
F	44 (18)	44	44
H	9	9	9
K	21 (11)	11, 21, 33	21 (11, 33)
L	10	10, 61	10 (61)
N	1	?	1
O	?	?	?
P	27	?	27
Q	21 (11)	11, 21	21, 11
R	9	9	9
W	15		?
AG	27	?	27
AH	51	51	?
AI	21, 46	?	?
AJ	21, 46, 66, 69	?	?
AK	37 (22)	22, 37	37 (22)
AO	2*	2	2
AP	21 (25, 11)	11, 21	25
AS	9	?	?
AT	16*	16	?
AV	18 (13)	13, 18	64 (66)
AW	?	?	?
AX	?	?	?
AZ	8, 59	8, 59	8
BG	9	9	
BH	21 (11)	11, 21	
BI	21 (11)	11, 21	
BJ	21 (11)	11, 21	
BK	?	25	
R 7	7 (10 65)	7	
R11	68	64, 68	
R48	68 (3)	3, 68	68 (3)
R60	65 (70, 72)*	65, 70, 72	65 (64)
R63	5 (7, 46)	5	5
R67	64 (14, 66)	14, 64, 66	
R69	42 (12, 29, 40)	12, 29, 42	29, 42
R70	27	27	27
R73	60 (18, 31)	60	60 (18, 31)
1	2	2	2
4	?	?	?
5	?	?	?
6	55	55	21, 11
11	31	31	31
14	17	?	17
15	63	63	63
16	19	?	19
24	2	?	2
29	17, 63	?	?
33	18	?	?
38	18	?	?
233	9	?	
362	3 (41, 46)*	3	
365	13*	13	
469	7	7, 10, 61	
489	64 (66)	64, 66	
490	64 (66)	64, 66	
531	61	?	
536	?	66	
561	17	17	
608	8*	8	
639	24	24	
719	27	27	
794	7	7	
896	10 (61)	10, 61	
982	24	24	
1202	51	51	

Table I *Comparison of typing unknowns by fluorescence and capsular swelling*

*Typed on repeat

by capsular swelling and fluorescence. There were a few minor differences. Casewell found results with two unknowns (AV and 6) that differed from both our capsular swelling and fluorescence which were in agreement. In one instance (AP), the type determined by Casewell's capsular swelling was indicated to be a cross reacting type by fluorescence which gave a stronger fluorescence with other type antisera.

In seven cases fluorescence could distinguish between cross reacting strains undifferentiated by capsular swelling. With fluorescence it was possible to type 10 out of 15 isolates that could not be typed by capsular swelling, and conversely, using capsular swelling, it was possible to type one out of six that were untypable by fluorescence.

FLUORESCENCE WITH CROSS REACTING ORGANISMS UNDIFFERENTIATED BY CAPSULAR SWELLING

Use of the FA technique made it possible to separate most specific types from other types which cross reacted with the same antiserum. Table II lists the sera with the cross reacting types as observed with capsular swelling, the dilution to distinguish them using fluorescence, and the remaining types which still react strongly and cannot be separated. Weaker reactions are in parentheses.

Specific Serum	*Type Strains giving Positive Reaction with Capsular Swelling*	*Dilution to resolve Cross Reactions with Fluorescence*	*Remaining Type Strains giving Positive Reaction with Fluorescence*
3	3, 68	1/32	3
10	10, 7	1/128	10
11	11, 21	1/64	11
12	12, 29, 41	1/128	12, 41
14	14, 64	1/64	14
18	18, 44	1/128	18
22	22, 23, 26, 37, 41	1/32	22, 26
23	23, 41	1/128	23
30	30, 13	1/128	30
29	29, 12, 42	1/128	29
33	33, 21	1/64	33
35	35, 34	1/32	35
38	38, 41	1/256	38
41	41, 12	1/32	41, 12
42	42, 12, 29	1/256	42
46	46, 27, 28	1/128	46
49	49, 48	1/64	49
53	53, 15, 17, 47	1/32	53, 17 (47)
61	61, 7, 10	1/128	61
64	64, 14	1/64	64
66	66, 14, 64	1/32	66
68	68, 3	1/128	68
69	69, 2, 30	1/256	69

Table II *Sera with cross reacting types as observed with capsular swelling, the dilution to distinguish them using fluorescence, and remaining types which still react strongly and cannot be separated: weaker reactions in parentheses*

METHOD TESTED BY SECOND WORKER NEW TO THE TECHNIQUE

The laboratory technician correctly identified 95% of the coded standards that were given to her for blind typing. One of the two not identified was accurately typed as far as the pool. None was incorrectly typed.

Discussion

The FA method for typing *Klebsiella* capsular antigens was evaluated in this paper by comparison with capsular swelling, both of which measure a serological reaction with capsular antigens.

Both methods gave very close results when typing unknown strains; however, with fluorescence it was possible to distinguish more easily between cross reacting types, and more of the unknowns could be typed.

Whereas capsular swelling is either strongly positive, weakly positive, or negative, fluorescence has a wider range of positive reaction so that the antisera can be diluted until there is a quantitative difference in fluorescence. The more sensitive method of fluorescence is probably detecting an actual difference between the antisera. Therefore, when there is a difference in the results of the two methods, it is possible that fluorescence is more accurate.

Basically, our capsular swelling results supported those observed with fluorescence. Casewell's capsular swelling results are in close agreement with the fluorescence results and provide admissible evidence that the FA method is reliably measuring the same phenomenon as capsular swelling.

Fluorescence has a success rate of 91·1% to date which included typing which was done before the method was completely developed. When the strains that could not be typed were repeated, this was increased to 94·5%. This compares favourably with our 81·1% success of typing by capsular swelling. Casewell (1975) claims about 90% success with capsular swelling. The difference between the success rates of the two laboratories with capsular swelling could be attributed to experience. When the fluorescence method was tested by a routine technician who had no previous experience with fluorescence, 95% of the unknowns were correctly identified. This was without any previous practice with the method. The experience was gained during the typing.

Of the cross reactions observed with capsular swelling 85·7% could be resolved by the use of fluorescence, thereby making it possible to make a more confident identification of an unknown.

Capsular swelling was found to be a difficult method to interpret when the results were not clear and definite. Experience is necessary to recognize a

positive reaction when the capsule is not readily discernible. Also, a negative result does not indicate whether or not the organism was, in fact, a *Klebsiella*.

A fluorescing organism can easily be seen and the intensity of the fluorescence determines a positive reaction. After some practice in quantitating fluorescence interpretation becomes simple and rapid. The FA technique usually demonstrates a low residual fluorescence with *Klebsiello* species and any *Klebsiella* antiserum, which may be a nonspecific reaction of O antigens; but it gives a completely black picture with other organisms. Although there may be considerable fluorescence of some cross reacting types, this is usually easy to distinguish from the fluorescence of specific types.

The FA method involves testing 18 serum pools against an unknown and choosing the pool or pools which give the strongest reaction. The three to five component antisera of these pools are tested with the unknown and the specific antiserum(a) is chosen which gives the strongest fluorescence. Most cross reactions can then be eliminated by titring out the antisera.

Pools of sera are used at a dilution of 1/40 and specific antisera at 1/32 or 1/64-1/256 if necessary. This makes fluorescence typing significantly less expensive than the capsular swelling method which uses pools of undiluted sera and specific antisera either undiluted or diluted up to 1:16. The fluorescein conjugate is used at a dilution of 1/400. The approximate cost to type one organism is under 2½p by fluorescence, whereas it is 10p by capsular swelling.

Large numbers of slides may be prepared at one time with fluorescence and stored at −20°C until they can be conveniently read. This takes advantage of the large amount of material made available by the greater dilutions. The remaining diluted antisera may be kept at 4°C for a long period of time and still retain their activity.

A single run of 44 slides can be performed in this laboratory in 3-3½ hours while an alternating double run of 88 slides takes 4½-5 hours. There are 10 wells per slide. Eighteen wells are used per organism to test the pools and usually 4-8 wells for specific antisera of positive pools in the next run.

Fluorescence can require about 4 hours for one person to type a single organism but when larger numbers are run at once, the average time estimated for identification is two organisms per hour. An experienced team of two can type 10 organisms per hour by capsular swelling (Casewell, 1975), but in this case two people working together are more than twice as fast as two individuals. This also involves testing each pool only until a positive pool is found, and then the component antisera and the established cross reacting antisera are checked. This may be faster, but it can miss positive reactions of mutants or wild types not yet investigated.

In this department a single person with some experience can type two organisms per hour by capsular swelling. Capsular swelling has the advantage of giving a result within half an hour and is, therefore, suitable for any single rapid identification. Fluorescence is more convenient for testing larger numbers, which is often necessary in epidemiological studies.

Fluorescence and capsular swelling were compared with respect to the factors most important in a typing method. They both require the same average time for a single worker to identify an unknown and they both give the same results. The fact that fluorescence is less expensive to run, possesses greater objectivity, resolves most of the cross reactions observed with capsular swelling, and has a higher percent success rate in identification indicates that fluorescence would be a more practical, convenient, and effective method for typing *Klebsiella*.

This method has been successfully employed in an epidemiological study in a urological unit which is reported elsewhere.

We wish to express our gratitude to Dr M. W. Casewell of the Department of Microbiology, St Thomas' Hospital, for his interest in the new method and his co-operation in typing many of the unknown strains.

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