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A collaborative investigation of phages for typing bovine staphylococci

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Seventeen collaborators tested a total of 6 999 cultures. The results were used to assess the usefulness of each phage for typing bovine staphylococci and to classify the phages into lytic groups and the cultures into phage groups. Some marked differences in the distribution of phage patterns from country to country were found. Evidence concerning the stability of the phage patterns is presented. On the basis of the results, a set of phages that would be generally useful for typing bovine staphylococci was selected. This set was established as the international basic set of phages for typing bovine staphylococci by the International Subcommittee on the Phage Typing of Staphylococci.

The international phage set that was selected for typing human coagulase-positive staphylococci [2] has proved inadequate for typing staphylococci from other animals. Workers interested in typing bovine staphylococci have, consequently, selected different phages, including phages isolated from or adapted to bovine cultures (Bertoni & Rosaschino, 1963; Bonin & Blobel, 1967; Coles & Eisenstark, 1959; Davidson, 1961; Frost, 1967; Fujikura & Shibata, 1965; Gedek, 1966; Meyer, 1967; Mondini & Dovadola, 1959; Nakagawa, 1960; Seto & Wilson, 1958; Smith, 1948; Verge et al., 1960).

In order to facilitate the comparison of results and to make the most useful phages generally available, the author submitted proposals to the International Subcommittee on Phage Typing of Staphylococci of the International Association of Microbiological Societies for the development of an international phage set for bovine staphylococci (*Int. Bull. bact. Nomencl.*, 1963, **13**, 119; *Int. J. system. Bact.*, 1967, **17**, 113). These proposals were accepted and all workers known to be active in the field were invited to take part in an investigation of a preliminary selection of phages that showed promise of being generally useful.

Collaborators were asked to use, as a minimum, the following phages:

international phages: 29, 52A, 3A, 6, 53, 75, 77, 84, 42D.

bovine and other phages: 78, 102, 107, 1363/14, S1, S6, 883, AC1.

If possible, all the phages of the international basic set for human staphylococci were to be used. In addition, collaborators were asked to test the stability of the phage patterns of representative cultures. Each collaborator obtained seed material for the propagation of the bovine phages from Weybridge and samples of the international phages from his national representative on the International Subcommittee.

MATERIALS AND METHODS

Data submitted

The 17 collaborators listed in the Annex submitted details of the phage reactions they obtained with a total of 6 999 cultures of coagulase-positive staphylococci. Fourteen of the collaborators used most or all of the international phages and 3 used the more

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[2] Described in the reports of the International Subcommittee on Phage Typing of Staphylococci (*Int. Bull. bact. Nomencl.*, 1959, **9**, 115; *Int. Bull. bact. Nomencl.*, 1963, **13**, 119; *Int. J. system. Bact.*, 1967, **17**, 113).

Table 1. Data used in computer analysis

Collaborating laboratory	No. of cultures	Sources of cultures	Phages used [a]
1. Australia	873	more than 288 herds in 7 districts: normal and mastitis milk	set A except 85 and 187. Additiona phages: 3B, 7,42DA, 101, 367, 425 600, 10, 186, 373
2. Bulgaria	113	milk of individual cows	set A
3. Denmark	812	494 cultures from churn milk samples from 223 herds: 318 cultures from milk of individual cows from 9 herds	set A except 85. Additional phages: 3B, 7, 42F
4. Finland	1 344	650 herds: normal and mastitis milk	set A except 52, 79, 85, and 187
5. Federal Republic of Germany	1 034	more than 40 herds from the north and south of the country: milk of individual cows	set A except 80, 83A, and 85. Additional phages: 7, 825
6. Federal Republic of Germany	200	200 herds: milk of individual cows	set B except 78
7. German Democratic Republic	249	91 cultures from Czechoslovakia from mastitis milk, remaining cultures from 3 districts of the GDR: 124 from cows' milk and 34 from dairy products	set A. Additional phages: 42F, 108, 111, 812, 825
8. Ireland	128	91 cultures from bulk milk of different herds: 37 cultures from milk of individual cows of one herd	set B
9. Israel	97	31 herds: normal and mastitis milk	set B
10. Japan	300	northern and central Japan: milk from individual cows	set A except 85. Additional phages: 3B and 7
11. New Zealand	190	several districts: 10 cultures from cheese, 2 from calves, remainder from normal and mastitis milk	set A except 85. Additional phages: 3B, 7, 101, 105, 108, 110, 111, A13
12. Sweden	226	61 herds: mastitis milk	set A
13. United Kingdom	454	more than 300 herds from all parts of the UK: normal and mastitis milk	set A. Additional phages: 3B, 7 129/16, P42D/E193, 88A, 111, H98, 365, T90, 257
14. USSR A	120	3 herds: 10 cultures from milkers' hands, 22 from milking apparatus, the remainder from normal and mastitis milk	set A, except 80, 3C, 47, 54, 84, and 85. Additional phages: 3B and 7
15. USSR B	180	98 cultures from mastitis milk, 82 from dairy products	set A, except 85. Additional phages: 3B and 7
16. USA A	442	1 herd sampled over 3 years: milk from individual cows	set A except 84 and 85. Additional phage: 3B
17. USA B	237	more than 50 herds in 12 States, 9 cultures from Canada: normal and mastitis milk	set A except 84, 85 and 78. Additional phage: S2

[a] See Table 2.

Table 2. Phages used in computer analysis

Set A
Current international set and bovine phages from provisional bovine set: 29, 52, 52A, 79, 80, 3A, 3C, 55, 71, 6, 42E, 47, 53, 54, 75, 77, 83A, 84, 85, 42D, 81, 187, 78, 102, 107, 1363/14, S1, S6, 883, AC1
Set B
Provisional bovine set: 29, 52A, 3A, 6, 53, 75, 77, 84, 42D, 78, 102, 107, 1363/14, S1, S6, 883, AC1
Additional phages
3B, 7, 42F — phages of international series not in basic international set.
129/16, P42D/E193, 88A — Smith, H. W. (1948)
S2 — Seto, J. T. & Wilson, J. B. (1958)
A13 — Coles, E. H. & Eisenstark, A. (1959)
H98, 365, T90, 257 — Nakagawa, M. (1960)
101, 105, 108, 110, 111 — Davidson, I. (1961)
812, 825 — Meyer, W. (1966)
367, 425, 600, 10, 186, 373 — Frost, A. J. (1967)
42DA — a variant of phage 42D obtained in Australia

limited selection listed above. Details are given in Tables 1 and 2.

Table 1 also gives the sources of the cultures used. Most of the cultures were obtained from cow's milk but some came from dairy products, milking apparatus, calves, and human contacts. Some collaborators were able to obtain cultures from a large number of different herds in different districts while others tested a more limited selection.

Nine collaborators used the phages at RTD (routine test dilution) and at 1 000 RTD (or in one case, undiluted). Eight collaborators used the phages at RTD only. Nine collaborators conducted studies of the stability of phage patterns. Twelve collaborators submitted the results of their lytic spectra determinations and/or of their determinations of the phage patterns of the propagating strains. Two laboratories submitted, in addition, the results of testing cultures with other selections of phages.

Analysis of phage reactions

To assist in analysing the phage reactions of the 6 999 cultures, a computer programme was used to analyse the results of each collaborator individually and to analyse the combined results. Since not all collaborators used all the phages, the combined results were analysed in three stages. First, the results of those collaborators using all or almost all the phages of set A (see Table 2) were added together. The collaborators concerned (group 1) are no. 1, 2, 3, 7, 10, 11, 12, 13, 15, and 16. They tested 3 839 cultures. Since six of them did not use phage 85, this phage was disregarded in making the analysis. One collaborator in group 1 did not use phage 84 and one did not use phage 187.

Next, the results of those collaborators using a rather smaller number of the phages in set A (collaborators no. 4, 5, and 17) were added to those of group 1 to form group 2 and a total of 6 455 cultures. Phage 85 was again disregarded. Phages 52, 79, 80, 83A, and 78 were not used by one collaborator each in this group and phages 84 and 187 were not used by two collaborators.

Finally, the results of all collaborators were taken together to form group 3. In performing the analysis, due allowance was made for the fact that not all collaborators used all the phages.

The analysis of individual results was carried out at two levels; (*a*) including only strong reactions (i.e., more than 50 plaques) at RTD and (*b*) including all reactions at RTD. The combined results were analysed by the computer at level (*a*) only, since the information needed from the level (*b*) analysis could be obtained fairly easily by adding together the individual analyses.

Collaborator no. 5 did not record weak reactions with the international phages. In this case, confluent lysis at 1 000 RTD was used as level (*b*) but it should be noted that cultures that gave strong reactions at RTD were not tested at 1 000 RTD. Collaborator

no. 17 recorded only two levels of reaction at RTD, i.e., confluent lysis and 10 plaques to semi-confluent lysis, inclusive. The first level was used as level (*a*) and the two together as level (*b*).

The successive stages of the analysis are described below. Stages 1–5 inclusive, 7, and 8 were carried out by the computer and the remainder by hand.

(1) The computer counted the number of cultures reacting with each phage.

(2) For each of the counts at (1), it counted the number of those cultures also reacting with each other phage.

(3) The computer printed the counts at (1) and (2) in the form of two triangular matrices of numbers, one matrix for level (*a*) and one for level (*b*).

Example :

	Phage no.		
	29	52	52A
29	173	142	105
52		156	105
52A			112

Thus 173 cultures reacted with phage 29. Of the 173, 142 also reacted with phage 52, etc.

(4) The computer calculated the counts at (1) as percentages of the total number of cultures (454 in the example), and the counts at (2) as percentages of the counts at (1).

Example :

	Phage no.		
	29	52	52A
29	38 [a]	82 [b]	61 [b]
52	91 [c]	34 [a]	67 [c]
52A	94 [d]	94 [d]	25 [a]

[a] = % of 454
[b] = % of 173
[c] = % of 156
[d] = % of 112
} see example at (3)

(5) The computer examined the results at (4) and printed lists of pairs of phages that showed more than 75%, more than 65%, and more than 50% similarity both ways (i.e., more than 75% of cultures reacting with phage A also reacted with phage B and more than 75% of cultures reacting with phage B also reacted with phage A, etc.).

(6) These results were examined by hand and a set of phages was chosen according to the criteria listed below.

(7) The computer then calculated the percentage of cultures typed (i.e., giving strong reactions) at RTD by the phages chosen at stage (6).

(8) Finally the computer identified cultures not typed by the chosen phages and reexamined their strong reactions at RTD with the excluded phages in the same way as stages (1)–(3) above. Any useful phages that had been wrongly eliminated could then be identified.

(9) The data from stage (4) of the group analyses were used to determine the lytic groups of the phages in the manner of Williams & Rippon (1952). The cultures were then classified, collaborator by collaborator, according to the lytic groups of the phages that lysed them.

(10) A further search for useful phages was made using the data from stage (9).

The aim at stages (6), (8), and (10) was to select a set of phages that would type a high proportion of the cultures at RTD and that would make as many reproducible distinctions between cultures as possible. To this end, the following criteria were applied.

(1) Phages that raised the percentage of typable cultures significantly were chosen. In the case of a collection of 100 cultures, any phage that typed 4% or more of the cultures not typed by the other chosen phages was selected provided that it satisfied the other criteria. For 200 cultures the figure of 2% was used, for 1 000 cultures, 1%, and for 5 000 or more cultures, 0.5%.

Preference was given to those phages that raised the percentage of typable cultures by a large amount relative to the total number of cultures they lysed.

(2) In addition, phages were chosen that subdivided the cultures lysed by those of the other chosen phages that lysed large numbers of cultures. Such "subdividing" phages were required to lyse between 20% and 50% of cultures sensitive to one of the other chosen phages. Generally not more than one "subdividing" phage was chosen for each other phage unless the latter typed a very large number of cultures.

(3) Not more than one of any group of similar phages was chosen. No two phages that showed more than 50% similarity both ways (see stage (5) of computer programme) at level (*a*) or more than 65% similarity at level (*b*) were chosen unless it was necessary to include such phages in order to raise the percentage of typable cultures to an acceptable level.

(4) As far as possible, phages that gave large numbers of weak reactions were excluded. Generally, a phage was excluded if the number of reactions it gave at level (*b*) was more than twice the number at level (*a*).

(5) In choosing between similar phages, preference was given to those from the international set so as to avoid the needless introduction of new phages.

RESULTS

Phage reactions at RTD

Table 3 shows, for each collaborator, the percentage of cultures lysed strongly by each phage at RTD. There was considerable variation from one collaborator to another. Some of this is no doubt

Table 3. Percentage (to the nearest whole number) of each collaborator's cultures giving a strong reaction with each phage at RTD *

Phage	Collaborator																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
29	5	2	2	9	17	10	0	61	1	6	21	1	38	0	1	2	13
52	7	4	2		13		0			1	23	4	34	1	2	1	11
52A	0	3	2	4	11	2	1	43	4	2	10	3	25	1	1	0	8
79	0	3	0		8		0			1	2	2	2	0	1	0	17
80	1	3	3	6			0			6	8	2	33		1	0	11
3A	1	1	43	0	0	1	1	2	0	0	2	15	2	0	1	0	4
3C	0	1	1	1	1		0			1	3	0	0		0	0	2
55	1	0	0	0	1		2			0	0	0	0	1	0	0	2
71	1	1	0	0	2		0			0	0	0	0	1	0	0	2
6	7	1	1	2	11	4	6	37	6	1	25	8	31	3	2	1	2
42E	30	2	0	6	14		34			1	34	0	37	52	9	0	3
47	5	1	1	1	10		6			3	21	7	30		2	7	11
53	3	3	1	1	4	4	4	21	6	4	14	7	7	0	1	1	4
54	13	2	1	2	11		15			1	20	8	30		2	1	3
75	11	2	1	1	9	3	4	43	2	2	22	4	22	6	1	0	3
77	3	1	0	6	0	2	7	1	2	3	7	4	3	11	1	1	5
83A	7	0	1	1			6			3	2	0	28	3	1	1	5
84	2	3	1	3	4	1	2	9	5	1	10	4	13		0		
85		3					1					3	5				
42D	23	13	4	20	5	40	56	12	2	20	22	0	20	68	34	1	7
81	2	0	2	1	12		3			5	19	3	30	0	7	1	5
187		0	0		0		0			0	0	0	0	0	0	0	3
78	1	0	2	3	0		0	0	0	0	15	1	9	0	0	0	
102	9	25	5	26	30	67	65	27	26	25	29	0	18	32	61	13	24
107	22	19	3	33	25	73	70	9	9	24	18	5	14	24	50	15	22
1363/14	28	18	5	60	30	59	63	32	32	25	41	11	49	67	62	11	16
S1	1	4	0	3	3	2	3	9	2	6	3	0	6	2	2	12	9
S6	4	4	15	9	2	2	0	5	1	0	0	1	9	23	2	52	53
883	2	2	22	0	0	1	60	3	0	11	0	23	2	5	0	0	2
AC1	0	9	1	3	0	1	1	1	1	3	1	0	9	4	1	2	5

* A blank space indicates that the phage was not used by that collaborator.

random variation in the samples of cultures tested, especially when the number of cultures was small. However, there is a tendency for sets of results from the same country and from neighbouring countries to resemble each other more closely. For example, the results from Denmark and Sweden have a high proportion of reactions with phages 3A and 883; both laboratories in the USA found a high incidence of reactions with the phage S6 and the highest incidence of reactions with lytic group I phages was found by the collaborators in Ireland and in England.

Percentage of cultures typed

The percentage of cultures typed with set A or set B at RTD ranged from 40 to 97 (see Table 4). The use of these phages at 1 000 RTD resulted in a considerable rise in the percentage typed when this was low at RTD. The additional phages gave a useful increase in the percentage typed in only two cases. The percentage typed by the international phages alone was always lower and often considerably lower.

Lytic groups of phages

The results of the three groups of collaborators (see above) were used to produce correlation charts showing the frequency with which pairs of phages occurred in the phage patterns. In interpreting the charts it has to be borne in mind that certain phages were not used by all the collaborators. In the charts for groups 2 and 3, this resulted in these phages showing lower correlations with the phages used by all collaborators than they did in the chart for group 1. Since the three charts were otherwise almost identical, only one is reproduced, that for group 3, i.e., the overall results (see Fig. 1).

As has been noted before with bovine staphylococci, the difference between lytic group I and lytic group III was not as great as it is with human staphylococci. Phage 42E, in particular, appeared to be intermediate between the two groups. On the other hand, phage 81, which is placed amongst the miscellaneous phages of the international set, behaved as a group-III phage.

The group-IV phages 42D, 102, 107, and 1363/14 formed a well-defined group.

Phage 883 was highly correlated with phage 3A. Its somewhat lower correlations with the group-IV phages were produced entirely by the results of one collaborator. In the reproducibility tests performed by this collaborator, these correlations with the

Table 4. Percentage of cultures typed with various sets of phages

Characteristic[a]	Collaborator																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
A	74	40	75	91	68	92	83	97	52	58	81	52	89	73	78	78	85
B		85	98[b]		85	98	98				88	81		100		85	
C	85	54	84	91	NR[c]	93	86	97	67	82	82	63	91	73	82	81	89
D	7		0		0		2			1	6		2	0	0	0	0
E	56	17	54	39	27	55	70	74	18	36	60	31	65	69	46	11	53
F		63			71		94				78	71		98		73	

[a] A. Percentage of cultures typed (i.e., giving one or more strong reactions) at RTD by phages of set A or set B.
B. Percentage of cultures typed at 1 000 RTD by phages of set A or set B.
C. Percentage of cultures giving any degree of lysis at RTD with one or more phages of set A or set B.
D. Of the cultures not typed at A, percentage typed at RTD by additional phages.
E. Percentage of cultures typed at RTD by current international phage set.
F. Percentage of cultures typed at 1 000 RTD by current international phage set.
See Table 1 for the phages used by each collaborator.

[b] Undiluted phages.

[c] NR=not recorded.

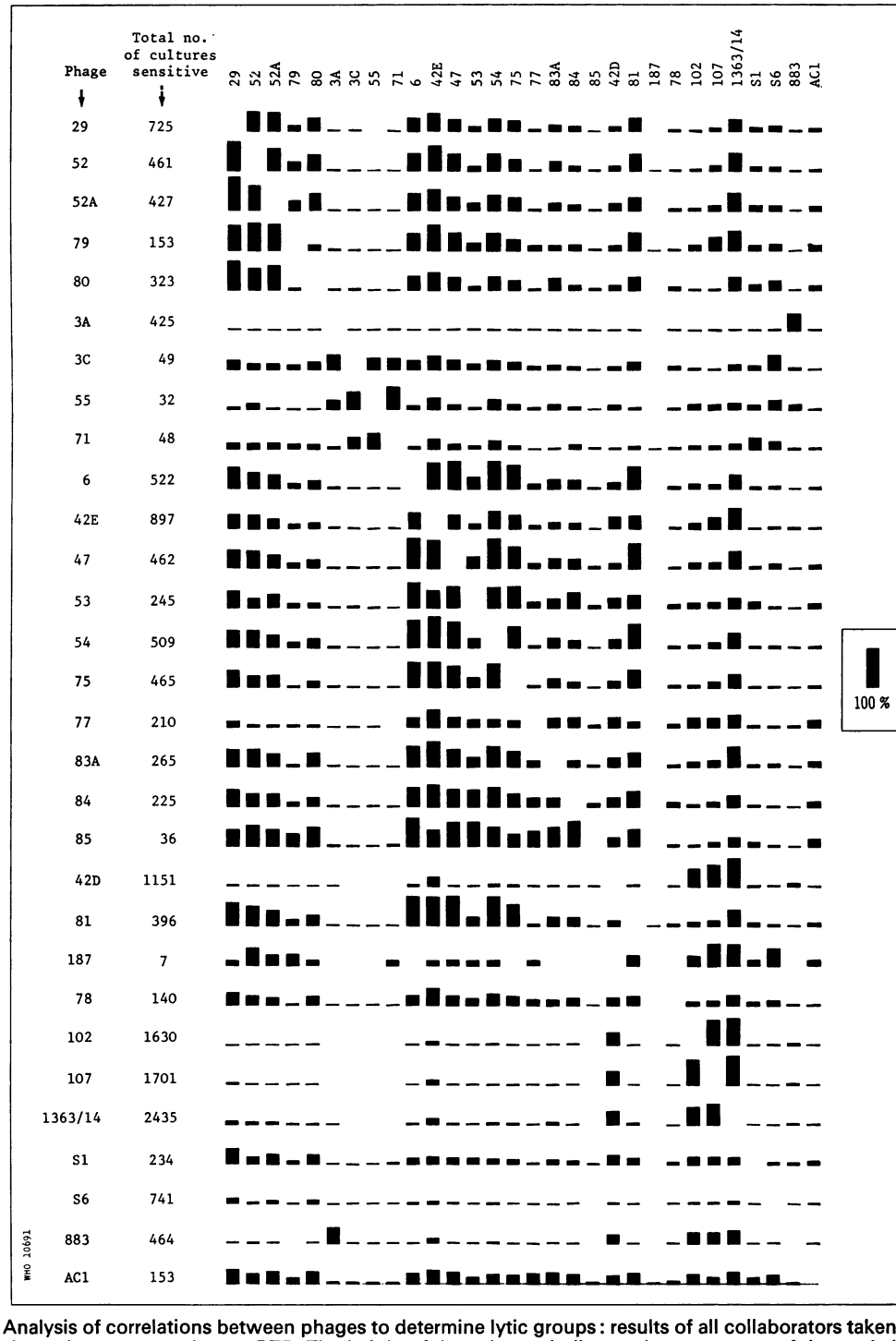

Fig. 1. Analysis of correlations between phages to determine lytic groups: results of all collaborators taken together considering only strong reactions at RTD. The height of the columns indicates the percentage of the total also sensitive to each other phage.

group-IV phages were no longer evident. Phage 883 can thus be allocated, at least provisionally, to lytic group II. The other group-II phages lysed too few cultures for their positions to be assessed.

Phages 78, S1, and AC1 showed low levels of correlation with many phages and thus have to be considered miscellaneous: 29% of the cultures lysed by phage 78 and 76% of those lysed by phage S1 were not sensitive to any other phage.

Phage S6 was not correlated with any other phage.

Phage groups of cultures

For this purpose the phages were regarded as being grouped as follows:

group I: 29, 52, 52A, 79, 80

group II: 3A, 3C, 55, 71, 883

group III: 6, 42E, 47, 53, 54, 75, 77, 83A, 84, 85, 81

group IV: 42D, 102, 107, 1363/14

miscellaneous: 187, 78, S1, S6, AC1

Table 5. Number of cultures in each phage group *

Phage group	Collaborator																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
I	18	1	4	22	29	12	0	12	2	10	4	5	9	0	1	2	8
I/M	13	1	16	75	14	5	0	1	0	4	1	0	10	0	1	6	29
II	21	0	375	6	11	2	5	0	0	36	3	53	2	0	1	0	3
II/M	1	0	1	1	6	0	0	0	0	0	0	0	0	0	0	2	1
III	120	1	20	50	36	10	6	7	9	18	15	26	25	0	3	2	7
III/M	8	0	1	24	0	0	1	0	1	3	6	0	8	0	0	0	14
IV	254	27	53	856	428	149	22	26	34	78	43	24	135	11	104	36	23
IV/M	1	4	0	3	2	0	0	0	0	0	6	0	6	8	3	7	9
I/III	24	0	8	10	59	0	0	29	1	3	10	4	33	0	1	0	2
I/III/M	0	0	0	14	1	0	0	1	0	3	1	0	28	0	0	0	4
I/IV	1	0	0	4	3	1	0	17	0	0	0	0	1	1	0	0	5
I/IV/M	0	0	1	10	0	0	0	1	0	0	0	0	0	0	0	0	10
II/IV	0	0	0	0	1	0	90	1	0	1	0	0	0	5	0	0	0
III/IV	133	1	0	57	21	1	18	3	0	1	22	0	13	42	23	20	2
III/IV/M	5	0	0	7	1	0	6	1	1	1	4	1	3	18	1	9	9
I/II/IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
I/III/IV	18	1	0	2	69	0	0	9	0	0	21	0	59	0	2	0	2
I/III/IV/M	2	0	2	4	4	0	0	8	0	3	9	0	45	0	0	4	6
II/III/IV	0	1	0	0	0	0	56	0	0	0	0	0	0	1	0	0	1
78	2	0	18	5	3	0	0	0	0	0	5	1	7	0	0	0	0
S1	0	0	0	6	11	2	0	0	2	10	0	0	0	0	0	29	2
S6	7	0	102	47	3	0	0	2	0	0	0	2	5	0	1	222	48
others	18	8	5	14	3	2	3	6	0	3	4	2	13	1	0	5	15
NT	227	68	206	127	329	16	42	4	47	126	36	108	52	33	39	98	36
total	873	113	812	1 344	1 034	200	249	128	97	300	190	226	454	120	180	442	237

* The table is based on strong reactions, i.e., more than 50 plaques, at RTD. See text for groups of phages. M = miscellaneous phages; NT = not typable.

Table 5 shows the phage patterns of the cultures classified according to these lytic groups and Table 6 shows the number of cultures sensitive to one phage only.

Differences between collaborators similar to those already noted in Table 3 are evident. To explore the reason for these differences more fully, each collaborator's results that gave sufficient information were examined to determine whether differences in the sources of the cultures might be responsible. The results of this examination are summarized below.

Table 6. Number of cultures giving a strong reaction at RTD with only one phage of set A or set B *

Phage	Collaborator																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
29	1	0	0	6	5	11	0	6	0	3	0	0	2	0	0	2	0
52	10	0	0		2		0			0	0	0	0	0	0	0	1
52A	1	0	0	1	2	0	0	2	0	0	0	0	0	0	0	0	0
79	0	0	0		0		0			0	0	0	0	0	0	0	2
80	0	0	1	0			0			5	0	1	1		0	0	0
3A	1	0	198	0	0	0	0	0	0	0	0	1	0	0	1	0	0
3C	0	0	0	0	0		0			3	0	0	0		0	0	0
55	0	0	0	0	0		0			1	0	0	0	0	0	0	0
71	2	0	2	0	2		0			0	0	0	0	0	0	0	0
6	0	0	0	3	2	1	0	0	2	0	0	0	1	0	0	0	0
42E	43	0	0	5	0		0			1	1	0	0	0	2	0	0
47	0	0	0	0	1		0			0	0	0	1		0	0	2
53	1	0	2	0	1	2	0	0	1	3	1	0	1	0	0	0	0
54	7	0	0	1	0		1			0	0	6	0		0	0	0
75	10	0	0	1	4	1	0	1	0	1	0	0	0	0	0	0	0
77	0	0	0	18	0	0	0	0	0	0	0	0	0	0	0	1	0
83A	3	0	0	1			0			1	0	0	0	0	0	1	0
84	1	0	3	2	3	1	0	0	2	1	1	0	1		0		
85		0					0					0	0				
42D	59	2	0	19	7	0	2	0	0	1	1	0	11	1	2	0	1
81	0	0	6	0	2		1			4	0	1	3	0	0	0	1
187		0	0		0		0			0	0	1	0	0	0	0	0
78	2	0	18	5	3		0	0	0	0	5	1	7	0	0	0	
102	6	0	0	27	127	2	2	11	3	4	3	0	10	0	5	1	3
107	26	0	5	10	46	2	0	0	0	2	3	0	6	0	1	5	0
1363/14	46	0	3	256	11	2	0	0	10	0	8	13	37	0	2	8	0
S1	0	0	0	6	11	2	0	0	2	10	0	0	0	0	0	29	2
S6	7	0	102	47	3	0	0	2	0	0	0	2	5	0	1	222	48
883	16	0	19	4	0	1	2	0	0	32	0	18	2	0	0	0	2
AC1	3	5	1	2	0	0	0	0	0	3	0	0	0	0	0	2	1

* A blank space indicates that the phage was not used by that collaborator.

Collaborator 1. Phage patterns were predominantly III, IV, and III/IV but there was a high proportion of 883 and untypable cultures as well as group-III and group-IV cultures from Western Australia.

Collaborator 3. Group-II and S6 cultures predominated in both churn samples and milk from individual cows. There was a greater variety of phage patterns amongst the former but this was to be expected since they represented 223 herds while the individual cow samples represented only 9.

Collaborator 5. Cultures from the southern part of the Federal Republic of Germany were mostly group IV. Amongst those from the northern part of the Federal Republic of Germany, however, there were more I/III/IV and III/IV cultures than group IV cultures.

Collaborator 7. No significant differences were found between cultures from the German Democratic Republic and those from Czechoslovakia. A higher proportion of untypable cultures was obtained from dairy products than from fresh milk, possibly because staphylococci from human sources were present in the former.

Collaborator 11. The two cultures from calves and the ten from cheese had phage patterns similar to those of cultures from milk.

Collaborator 13. There was little difference between phage patterns of cultures from different areas or between those of cultures from clinical mastitis and herd samples.

Collaborator 14. There were no significant differences between cultures from different sources.

Collaborator 15. Group IV and III/IV cultures predominated in cultures from both mastitis milk and dairy products.

Collaborator 17. There were no significant differences between areas except that there was a higher proportion of S6 cultures from three of the four herds sampled in New Hampshire.

Thus there is evidence that, in some cases, phage patterns varied from district to district in the same country as well as from country to country. There is no evidence that the way the samples for culture were selected influenced the results.

Stability of phage patterns

These tests took three main forms: (1) testing at different times subcultures of the same original culture, (2) testing simultaneously single-colony subcultures derived from the same culture, and (3) testing repeated isolations from the same site.

Although the tests revealed some instability of phage patterns, most cultures varied only slightly or not at all. No phage was markedly more variable than the others in this respect.

The results summarized in the tabulation below refer to tests at RTD unless otherwise stated. The phages used were those listed in Table 1 unless otherwise stated. The results with the additional phages are not recorded below. The figures at A are the numbers of cultures that gave a strong (i.e., ++) reaction on at least one occasion. The figures at B are the numbers of cultures that gave a ++ reaction on at least one occasion and no reaction at all on at least one occasion.

Collaborator 3

Four cultures were tested 10 times and one culture 9 times over an 18 day period. With one exception, variations were noted only in weak reactions.

	29	52	52A	80	3A	3C	55	71	6	42E	47	54	75	83A	84	81	78	102	1363/14	S1	S6	883
A	3	3	3	5	1	1	1	1	1	2	1	2	1	1	1	2	1	2	2	1	3	2
B	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0

Six single-colony subcultures from each of 6 cultures and 4 from each of 2 cultures were tested. Three of these single-colony subcultures showed some variation and a further series of 3 or 4 single-colony subcultures from each was tested. Six of these 8 cultures varied only in their weak reactions.

		29	52	52A	79	80	3A	3C	55	71	6	42E	47	54
RTD	A					1	2				1		1	1
	B					0	0				0		0	0
RTD+1 000 RTD	A	2	1	3	1	2	4	2	2	2	4	3	4	3
	B	0	0	0	0	0	0	0	0	0	0	0	0	0

		75	77	84	42D	81	78	102	107	1363/14	S1	S6	883	AC1
RTD	A	1					1	1	1				1	
	B	1					0	0	0				0	
RTD+1 000 RTD	A	2	1	1	2	3	3	3	1	1	2	2	3	1
	B	0	0	0	0	0	0	0	0	0	0	0	0	0

Cultures were isolated on more than one occasion from each of 101 different sites, i.e., quarters of cows' udders: 91 sites yielded series of 2 cultures each, 4 sites series of 3 cultures each, 3 sites series of 4 cultures each, and 3 sites series of 5 cultures each. Of these series of cultures, 44 showed no variation at all and 47 varied only in their weak reactions. In two series, completely different phage patterns were found and this was presumed to indicate infection with a different strain. These two series are not recorded below.

	29	52A	3A	S6	883
A	1	2	89	11	30
B	0	0	0	0	8

Collaborator 4

Seventeen cultures were tested in duplicate using, respectively, the original bovine phages from Weybridge plus the international phages and the local propagations of the bovine phages plus the international phages. Seven cultures did not vary at all and 6 varied only in their weak reactions.

	80	3C	6	42E	47	54	84	42D	102	107	1363/14	S6
A	1	1	1	11	1	1	1	8	4	11	9	1
B	0	0	0	2	0	0	0	0	1	3	0	0

Collaborator 5

A total of 1 034 cultures were tested twice with the phages shown below.

	29	52A	3A	6	53	77	84	42D
A	214	123	5	130	51	16	49	65
B	59	16	2	33	10	14	12	29

Collaborator 7

Nine cultures were retested after an 11-month interval, 4 were tested 5 times over a 6-month period, and 2 were tested 4 times over a 6-month period. Three cultures varied only in their weak reactions.

		52	52A	79	80	3A	3C	55	6	42E	47	53	54	75
RTD	A							1	3	6	3	3	6	3
	B							1	1	4	0	0	2	0
RTD+1 000 RTD	A	1	2	3	4	4	1	5	7	11	6	6	9	6
	B	0	0	0	2	2	0	2	2	6	0	1	2	0

		77	83A	84	85	42D	81	78	102	107	1363/14	S1	883	AC1
RTD	A	1	4	1		7	1		10	10	10	4	7	
	B	0	0	1		1	0		1	2	2	4	5	
RTD+1 000 RTD	A	6	7	2	2	10	5	1	10	10	11	6	8	4
	B	0	1	2	2	3	0	0	2	3	4	6	6	2

Collaborator 10

Three direct subcultures and five single-colony subcultures from each of 10 original cultures were tested with the set B phages. Four cultures did not vary at all and the remaining 6 varied only in their weak reactions.

	29	52A	42D	102	107	1364/14	S1	S6	883	AC1
A	3	1	3	3	3	3	1	1	2	1

Collaborator 11

Five single-colony cultures were prepared from each of 23 cultures and tested along with the original cultures. Four cultures did not vary and 6 varied only in their weak reactions.

	29	52A	3A	6	53	75	77	84	42D	78	102	107	1363/14	S1	AC1
A	7	3	1	5	4	5	1	5	3	1	8	5	15	3	1
B	2	1	1	0	3	0	1	2	2	0	1	3	4	2	1

Collaborator 13

Three subcultures made at weekly intervals and 3 single-colony subcultures from each of 20 cultures were tested with the set B phages. Two cultures did not vary at all and 3 varied only in their weak reactions.

	29	52A	6	53	75	77	84	42D	78	102	107	1363/14	S1	S6
A	8	5	5	3	3	1	4	8	1	9	5	16	3	3
B	3	1	2	0	1	1	1	4	0	3	2	7	1	0

Collaborator 16

311 cultures, including 65 untypable cultures, were retested at 1 000 RTD, using a slightly different technique designed to improve the detection of reactions, with certain of the phages with which they had given no reactions previously. In some of the cases where, on retesting, a reaction was found, tests were also made at RTD.

	52A	79	42E	47	53	42D	81	102	1363/14	S1	S6	AC1
No. of cultures retested at RTD	17	4	5	1	3	1	3	16	4	2	6	29
No. ++ at retest	0	0	0	0	0	0	0	0	1	1	6	0

	29	52	52A	79	80	3A	3C	55	71	6	42E	47	53	54
No. of cultures retested at 1 000 RTD	109	66	253	132	70	66	66	65	66	66	280	243	128	221
No. ++ at retest	3	0	28	16	0	0	0	0	0	0	13	29	28	30

	75	77	83A	42D	81	187	78	102	107	1363/14	S1	S6	883	AC1
No. of cultures retested at 1 000 RTD	68	247	153	282	151	65	69	201	273	262	66	66	70	141
No. ++ at retest	0	3	24	4	8	0	0	27	3	4	2	6	0	59

Collaborator 17

In all, 154 cultures were retested with the 16 phages listed below after the cultures had been stored for 1 year. Many cultures had completely different phage patterns on retest. Reactions were both lost and gained although all the differences with phage 79 were losses. There were more losses than gains with the other group I phages also.

	29	52	52A	79	80	42E	47	77	42D	81	102	107	1363/14	S1	S6	AC1
A	27	27	20	33	26	22	28	39	39	10	60	55	36	26	82	14
B	17	13	17	24	10	12	19	32	29	6	16	22	12	13	34	11

Lytic spectra of phages and phage patterns of propagating strains

Tables 7 and 8 show the results obtained at Weybridge. The lytic spectra on the " human " test strains were determined on at least 3 separate occasions on different batches of medium. Those on the other test strains were determined on at least 2 occasions. The phage patterns were determined on at least 6 occasions.

Ten collaborators submitted the results of their lytic spectra determinations and ten recorded the phage patterns given by the propagating strains. Only a few differences were noted between the phage patterns determined at Weybridge and those determined elsewhere. The lytic spectra were more variable, however. In particular, phages found to lyse propagating strains 6, S1, and S6 at Weybridge often gave no reactions on these strains at other laboratories. Two collaborators compared the original seed material from Weybridge with their own propagations and found the two to be identical although, in each case, several of the reactions with strains 6, S1, and S6 were missing. Thus these differences appear to have been caused by differences in media and techniques rather than by changes occurring in the phages on propagation.

Selection of phages

Table 9 shows the phages selected for individual collaborators and groups of collaborators by the process described under " Analysis of phage reactions". Where there was little to choose between very similar phages, they are shown as alternatives. Phages that were only marginally excluded by the criteria used are listed as " possibly also useful".

The percentage of cultures typed at RTD with the phages definitely selected ranged from 36 to 91. In each case, these percentages are fairly close to the maximum obtainable with the available phages (see Table 4).

Where a collaborator tested only a small number of cultures or where there was only a limited variety of phage patterns, it was possible to select only a few phages. Such cases, however, provide valuable evidence as to the extent to which each phage is generally useful.

The following appear to be clear choices as generally useful phages:

group I : 29
group II: 883
group III: 6 or 75
group IV: 42D, 102, 1363/14
miscellaneous: S6

Each was chosen by the results of between 5 and 14 collaborators and by the overall results.

The other phages that made a useful contribution are as follows:

52A —subdivided phage group I and I/M cultures.

3A —important for two collaborators: it significantly increased the overall percentage of cultures typed.

6 and 75 —chosen by the same number of individual results, but the overall results suggested them only as alternatives.

42E —important for two collaborators. Subdivided cultures of phage groups I, III, and IV. Significantly increased the overall percentage of cultures typed.

53 and 84—each subdivided phage group III cultures and each significantly increased the overall percentage of cultures typed.

107 —chosen by almost as many individual results as 102 but overall overlapped more than 50% with 102 and 1363/14. Significantly increased the overall percentage of cultures typed.

78 and S1—each was chosen by several individual results. Each increased significantly the overall percentage of cultures typed.

Additional results

Collaborator 5 tested a further 878 bovine cultures with the following phages: 29, 52A, 79, 3A, 6, 42E, 53, 54, 75, 84, 42D, 78, 102, 107, 1363/14, S1, AC1, 129/16, P42D/E193, 88A, 825. The results were ana-

Table 7. Lytic spectra of " bovine " phages as determined at Weybridge *

Phage	Standard " human " test strains																Other strains										
	29	52	52A/79	80	2009	3A	3B	71	8719	42C	42E	47	53	54	75	77	6	84	42D	78	102	105/107	M8	S1	S6	HAC1/2	94
102	—	(0)	(0)	—	(0)	0/1	—	—	—	(1)	0	3	—	—	(1)	1	(0)	(0)	2	—	5	5	4/5	3	3/4	2/3	—
107	—	—	(0)	—	—	(1)	—	—	—	(2)	—	1	(1)	(1)	—	—	(2)	—	1	—	4	5	5	3	3	–/5	(1)
1363/14	—	—	—	—	—	(1)	—	—	—	—	(0)	—	(1)	0/2	(0)	(0)	3	(0)	(2)	—	—	5	5	—	4	—	—
S1	—	(0)	0/2	—	(0)	0/2	—	(0)	—	4	2	—	—	1	—	1/2	3	—	(0/1)	—	1	—	—	5	2/3	(0)	(1)
S6	—	—	—	—	(1)	1	—	—	—	3	1/2	—	(1)	1/2	—	(1)	2	—	(1)	1	—	—	—	2	5	—	—
AC1	(0)	(0)	3	—	(0)	0	—	(0)	—	(0)	0	0/2	(1)	(1)	(0)	1	0	(0)	(0)	(0)	2	1	1	—	3	5	—
883	—	—	—	—	—	5	3	2	—	—	(1)	1	1	1	(1)	—	1	—	—	—	—	—	—	—	—	—	5

* 5 = titre equal to that on propagating strain
4 = titre 10 – 10^2 times less than that on propagating strain
3 = titre 10^2 – 10^4 times less than that on propagating strain
2 = titre 10^4 – 10^6 times less than that on propagating strain
1 = weak reaction with undiluted phage
0 = inhibition reaction
( ) = reaction not always present.

Table 8. Phage patterns of " bovine " propagating strains as determined at Weybridge *

Propagating strain	Strength of phage	Phage																	
		29	52A	3A	6	42C	53	75	77	84	42D	78	102	107	1363/14	S1	S6	AC1	883
102	RTD	—	—	—	—	—	—	—	—	—	(+)	—	SCL	++	—	—	—	—	—
	1 000 RTD	(+)	—	—	—	(±)	—	—	(+)	—	SCL	—	CL	CL	—	—	—	(±)	—
105/107	RTD	—	—	—	—	—	—	—	—	—	(±)	—	SCL	SCL	SCL	—	—	—	—
	1 000 RTD	—	—	—	—	—	—	—	(+)	—	SCL	—	CL	CL	CL	—	—	—	—
M 8	RTD	—	—	—	—	—	—	—	—	—	++	—	SCL	SCL	SCL	—	—	—	—
	1 000 RTD	—	—	—	—	—	—	—	(±)	—	CL	—	CL	CL	CL	—	—	—	—
S 1	RTD	—	—	—	—	(±)	—	—	(±)	(±)	—	—	(±)	—	—	SCL	—	—	—
	1 000 RTD	(0)	—	—	++	CL	(±)	—	++	SCL	—	—	++	++	—	CL	—	—	—
S 6	RTD	—	—	—	—	—	—	—	—	(±)	(±)	—	++	(±)	—	—	SCL	—	—
	1 000 RTD	0/CL	0/++	(0)	(0)	—	+	(0)	+	0/CL	SCL	0/++	CL	CL	CL	—	CL	CL	(0)
HAC 1/2	RTD	SCL	—	—	—	—	±	—	++	(+)	—	—	—	++	—	—	—	SCL	—
	1 000 RTD	CL	0/+	—	—	(±)	CL	+	CL	CL	(0)	(0/+)	+	CL	—	(0)	—	CL	—
94	RTD	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	SCL
	1 000 RTD	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	CL

* CL = confluent lysis
SCL = semiconfluent lysis
++ = more than 50 plaques
+ = 20 – 50 plaques
± = less than 20 plaques
0 = inhibition reaction
( ) = reaction not always present

Table 9. Phages selected for each collaborator and for groups of collaborators

Collaborator	Definitely selected (including alternatives)	Possibly also useful	% typed [a]
1	52, 42E, 75,[b] 42D, 107, 1363/14, S6,[b] 883, 367		73
2	42D, 102 or 107 or 1363/14, AC1		36
3	3A, 42D, 81, 78, 107, S6, 883	1363/14 or 42F	71
4	29, 42E, 77, 42D, 102, 1363/14, S6	80, 6 84, 78	86
5	29, 71, 75, 102, 1363/14, S1	84, 42D, 107	60
6	29, 6, 42D, 102 or 107 or 1363/14, S6		86
7	6, 54, 42D, 883		80
8	29, 6, 102, 1363/14	42D, 107, S6	91
9	52A, 6, 53, 84, 1363/14	107, S1	46
10	29, 47 or 75 or 77 or 83A, 53, 42D or 102 or 107 or 1363/14, S1, 883, 3B	80 [b]	50
11	29 or 52, 6 or 47 or 75, 77, 42D, 78, 102, 107, A13	42E, 1363/14 [b]	78
12	52 or 52A, 3A, 6 or 47 or 53, 54, 75, 107, 1363/14, 883		49
13	29 or 52 or 52A or 80, 75, 84, 42D, 102, 107, 1363/14, 78, S6		84
14	42D or 1363/14, 102, 107	42E,[b] S6 [b]	70
15	42D, 102 or 107 or 1363/14		70
16	102 or 107 or 1363/14, S1, S6	47	74
17	29, 79, 47, 102, 1363/14, S6	52 or 52A or 80, 42D	78
Group 1	29 or 52, 3A, 6 or 47 or 54 or 75 or 81, 42E, 42D, 78, 107, 1363/14, S1, S6, 883	84, AC1	69
2	29, 52A, 3A, 6 or 47 or 54 or 75, 42E, 84, 42D, 78, 102, 1363/14, S1, S6, 883	77, 107, AC1	72
3	29, 52A, 3A, 6 or 47 or 54 or 75, 42E, 53, 84, 42D, 78, 102, 1363/14, S1, S6, 883		73

[a] Percentage of cultures typed at RTD with phages definitely selected. [b] Added after classification of cultures into lytic groups.

lysed as described above and the following phages, which typed 89% of the cultures at RTD, were selected: 29 or 52A, 3A, 6 or 54, 42D, 102, S1.

Collaborator 6 tested a further 413 group IV cultures, each from a different herd, with 10 group IV phages: 42D, 42F, 102, 107, 108, 111, 1363/14, 129/16, P42D/E193, 88A. Of these, 139 cultures were sensitive to all the phages and 42 were untypable at RTD. Every pair of phages except those including 42D showed more than 75% similarity both ways. Phage 42D showed more than 50% similarity with the other phages. The greatest differences were shown by phages 42D, 102, and 1363/14. These three together typed 362 of the 371 typable cultures at RTD.

CONCLUSION

After considering the investigation reported above, the International Subcommittee on Phage Typing of Staphylococci established an international basic set of phages for typing bovine staphylococci (*Int. J. system. Bact.*, 1971). Those phages without international numbers were given the following numbers in the international series.

Original no.	*International no.*
883	116
1363/14	117
S1	118
S6	119

The international basic set for typing bovine staphylococci is thus constituted as follows:

group I: 29, 52A
group II: 3A, 116
group III: 6, 42E, 53, 75, 84
group IV: 42D, 102, 107, 117
miscellaneous: 78, 118, 119

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RÉSUMÉ

RECHERCHE COLLECTIVE DE PHAGES DESTINÉS AU TYPAGE DES STAPHYLOCOQUES BOVINS

La série de phages choisie et utilisée internationalement pour le typage des staphylocoques humains s'est révélée peu appropriée au typage des staphylocoques bovins. Il était devenu nécessaire de définir une batterie de phages mieux adaptée à ces dernières déterminations. Sur l'initiative de l'auteur, des chercheurs travaillant dans ce domaine ont participé à une étude collective visant à sélectionner des phages convenant à une utilisation générale. Ils ont été invités à tester au moins les phages internationaux 29, 52A, 3A, 6, 53, 75, 77, 84 et 42D, les phages bovins ou autres 78, 102, 107, 1363/14, S1, S6, 883 et AC1, et, dans la mesure du possible, tous les phages appartenant à la série de typage des staphylocoques humains.

Au total, 17 collaborateurs ont éprouvé 6 999 cultures de staphylocoques bovins. On a relevé de fortes variations d'un expérimentateur à l'autre, dues sans doute en partie à des différences aléatoires entre les échantillons, surtout lorsque le nombre des cultures était peu élevé. Néanmoins, les séries de résultats obtenus dans un même pays ou dans des pays voisins avaient tendance à concorder davantage. Par exemple, la proportion des réactions relatives aux phages 3A et 883 était élevée au Danemark et en Suède; aux Etats-Unis d'Amérique (2 laboratoires) celle des réactions relatives au phage S6; et la plus forte proportion des réactions avec le groupe I de phages était observée en Irlande et au Royaume-Uni.

L'ensemble des données a été analysé pour évaluer l'utilité de chaque phage pour le typage des staphylocoques bovins et pour sélectionner un jeu de phages capable de classer une forte proportion des cultures et d'assurer le maximum de différenciations reproductibles.

Sur la base de ces résultats, le Sous-Comité international pour la lysotypie des staphylocoques a constitué une série internationale de phages pour le typage des staphylocoques bovins. Les phages ci-dessous, dépourvus à l'origine d'un numéro international, en ont reçu un:

N° d'origine	*N° international*
883	116
1363/14	117
S1	118
S6	119

La série internationale de base des phages à utiliser pour le typage des staphylocoques bovins s'établit dès lors comme suit:

groupe I :	29, 52A
groupe II :	3A, 116
groupe III:	6, 42E, 53, 75, 84
groupe IV:	42D, 102, 107, 117
divers:	78, 118, 119,

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Annex

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