

***Escherichia coli O antigen Grouping***

**Method:**

1. Biochemical identified pure culture of *E. coli* strain streaked out on any non-selective media e.g.
  - sheep blood agar
  - beef agar
  - nutrient agar
  - horse blood agarand incubated overnight at 37°C.
2. A) One smooth colony of the culture will be inoculated in enrichment broth (e. g. BHI, beef broth) and incubated overnight at 37°C.  
or  
B) Plating on non-selective (see 1. above) agar overnight at 37°C. Scrape off the *E. coli* colonies and suspend the bacteria in 2 ml of distilled or de-ionised water.
3. After incubation, the broth (A) or suspension (B) will be heated at  $\geq 90^{\circ}\text{C}$  for 1 hour.
4. Preparation of ready-to-use O antigen suspension for O grouping in microtiterplates (MTP):  
A) The broth (=ready-to-use O antigen suspension) after cooling to room temperature (Note: LEP dilutes this broth 1/2 with formol saline to give the ready-to-use O antigen suspension).  
or  
B) The boiled suspension from 3. B) is added drop-wise (about 0.2 ml) to a tube containing 5 ml of normal saline until a concentration corresponding to an overnight broth culture is reached, which is estimated by the eye. The remaining boiled suspension is stored refrigerated and used to

## **Appendix 1**

### **O Grouping: Standard Operation Procedure (O SOP)**

prepare a ready-to-use O antigen suspension in distilled or de-ionised water if the strain is O rough in normal saline.

#### **Screening:**

5. A minimum of 25 µl of the ready-to-use O antigen suspension and an equal volume of

A) Pooled O antisera (O pools according to WHO Reference Laboratory)

or

B) single O antisera (full range)

is added to the MTP

6. The MTP is incubated in a humid atmosphere at 50-52 °C overnight.

7. Reading of results.

Ranging from	- or +	not significant
	to	
	++ or +++	significant

#### **Presumptive typing:**

8. A minimum of 25 µl of the ready-to-use O antigen suspension and an equal volume of

## **Appendix 1**

### **O Grouping: Standard Operation Procedure (O SOP)**

single O antisera of respective pool is added in a new MTP and incubated again at 50-52 °C overnight.

9. Reading of results of single O antisera from respective O pool (see 7.)

## **Appendix 1**

### **O Grouping: Standard Operation Procedure (O SOP)**

#### **Definitive typing:**

10. A minimum of 25 µl of the ready-to-use O antigen suspension and an equal volume of the reacting O group mono-specific or non-absorbed O antiserum is titrated using doubling dilutions, including negative control in normal saline.

#### **Negative strains:**

If strains are negative in either O pools or O single antisera during screening (5-7.), a fresh broth (2.A) or suspension (2. B)) is made and autoclaved at 122°C for 90 minutes and used as the ready-to-use O antigen suspension.

#### **O rough strains:**

Use the other (non-saline) half of the ready-to-use O antigen suspension from 4. B) or produce fresh suspension from non-selective medium in distilled water as in 2.B) - 4. B).

11. Report.

## ***Escherichia coli H antigen Determination***

**Method:**

**Production of motile *E. coli***

- The same colony as is used for *Escherichia coli* O antigen Grouping, see 2.) is inoculated from non-selective agar to
  - one side of U-tubes with semi-solid agar
  - swarming agar plates
  - tubes with semi-solid agar
  - Craigie tubes with semi-solid agar
- The tubes or plates are incubated at 30°C or 37°C
  - motile strains which show migration through the tubes, should get another passage in a second tube to obtain the optimal formation of H antigen
  - strains which show no swarming or migration through tubes for 2 weeks are defined as non-motile

## Appendix 2

### **H Determination: Standard Operation Procedure (H SOP)**

#### **Ready-to-use preparation of H antigens**

- A) A small amount of tube culture from the migration zone is transferred to any of the following:
  - overnight broth culture as stand-culture (30°C)
  - roller incubation of broth culture for 6-8 hours (37°C)
  - broth culture for 6 hours (30°C)

or

- B) From swarming agar plates:
  - Suspend growth from an actively swarming semi-solid agar plate in broth corresponding to an overnight incubation
  
- Formalin is added to the broth culture to a final concentration of 0.5% resulting in the ready-to-use preparation of H antigen

#### **Screening:**

- A minimum of 25 µl of the ready-to-use preparation of H antigen and an equal volume of
  - A) Pooled H antisera (H pools according to WHO Reference Laboratory )
  - or
  - B) single H antisera (full range)

is added to the MTP

## **Appendix 2**

### **H Determination: Standard Operation Procedure (H SOP)**

- The MTP is incubated in a humid atmosphere at 50-52 °C for one-and-a-half to two (maximum!) hours.
- Reading of results.  
Ranging from        - or +        not significant  
                                 to  
                                 ++ or +++    significant

#### **Presumptive typing:**

- A minimum of 25 µl of single H antisera of respective H pool and an equal volume of the ready-to-use H antigen preparation is added in a new MTP and incubated a humid atmosphere at 50-52 °C for one-and-a-half to two (maximum!) hours.
- Reading of results of single antisera from respective pool (see 7.)

#### **Definitive typing:**

- A minimum of 25 µl of the ready-to-use H antigen preparation and an equal volume of the reacting H group mono-specific or non-absorbed H antiserum is titrated using doubling dilutions, including negative control with normal saline. Incubation in a humid atmosphere at 50-52 °C for one-and-a-half to two (maximum!) hours.
- Report.

## Appendix 2

### H Determination: Standard Operation Procedure (H SOP)

#### D) Special procedures:

- “H rough” cultures are sub-cultured in semi-solid media and incubated again in broth + 1% glucose at room temperature in the dark overnight. Formalin is added to the broth culture to a final concentration of 0.5% resulting in the new ready-to-use H antigen preparation and typed from **Screening 5**.
- Non-typeable strains are passaged several times (up to three months once to thrice a week) in semi-solid medium. Most motile strains will eventually become typeable. If not, refer to reference laboratory or try *fliC* typing.



### Appendix 3

### Test strains

O antigen test strains for reference						
C-number	Original No.	Test	SEROTYPE	Isolated from <sup>a</sup>	Previous designation	Comments
C 559-59	C 559-59	OIF1				Only in use in Copenhagen; Rare
	U5-41	O1,H7	O1:K1:H7	Hu H		K- derivative: D703
	U9-41	O2,K1,H4	O2:K1:H4	Hu U		
	U14-41	O3,K2ab	O3:K2ab:H2	Hu U		
	U4-41	O4,K3,H5	O4:K3:H5	Hu H		
	U1-41	O5,K4	O5:K4:H4	Hu U		
	Bi7458-41	O6	O6:K2a:H1	Hu H		
	Bi7509-41	O7	O7:K1:H-	Hu U		
	G3404-41	O8,K8	O8:K8:H4	Hu B		
	H308b	"O"K84 = O93	O8:K84:H-	Hu F	O93	Former test strain for O93; Temporarily removed (=O8)
	Bi316-42	O9,K9,H12	O9:K9:H12	Hu P		
	Bi8337-41	O10,K5	O10:K5:H4	Hu P		NM by E&E
	Bi623-42	O11,K10,H10	O11:K10:H10	Hu P		NM by E&E
	Bi626-42	O12	O12:K5:H-	Hu P		
	Su4321-41	O13,K11,H11	O13:K11:H11	Hu P		
	Su4411-41	O14	O14:K7:H-	Hu U		O14 is O rough (R4)
	F7902-41	O15,K14	O15:K14:H4	Hu F		
	F11119-41	O16	O16:K1:H-	Hu F		
	K12a	O17,K16,H18	O17:K16:H18	Hu F		
	F10018-41	O18ab,H14	O18ab:K-:H14	Hu F	K76 (B20)	
C 516-59	3219-54 DM (Ewing)	O18ac	O18ac:K5:H7	Hu D	K77	
	F8188-41	O19ab	O19ab:K-:H7	Hu F		
	P7a	O20,K17	O20:K17:H-	Hu F		
	E19a	O21,K20	O21:K20:H-	Hu P		[H4] <sup>b</sup>
	E14a	O22	O22:K13:H1	Hu P		
	E39a	O23,K18ab,H15	O23:K18ab:H15	Hu P		
	E41a	O24	O24:K+:H-	Hu P		
	E47a	O25,K19	O25:K19:H12	Hu P		
	H311b	O26	O26:H11	Hu F	K60 (B6)	H11 by E&E '72
	F9884-41	O27	O27:K-:H-	Hu F		
	K1a	O28ab	O28ab:K-:H-	Hu F		

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O antigen test strains for reference						
C-number	Original No.	Test	SEROTYPE	Isolated from <sup>a</sup>	Previous designation	Comments
	Kattwijk	O28ac	O28ac:H-	HuD	K73 (B18)	Sereny-, EIEC+
	Su4338-41	O29	O29:K-:H10	Hu U		
	P2a	O30	O30:K-:H-	Hu F		
	P6a	O32	O32:K-:H19	Hu F		
	E40	O33	O33:K-:H-	Hu P		
	H304	O34	O34:K-:H10	Hu F		
	E77a	O35	O35:K-:H10	Hu P		
	H502a	O36	O36:K-:H9	Hu F		
	H510c	O37	O37:K-:H10	Hu F		
	F11621-41	O38	O38:K-:H26	Hu F		
	H7	O39	O39:K-:H-	Hu F		
	H316	O40	O40:K-:H4	Hu F		
	H710c	O41	O41:K-:H40	Hu F		
	P11a	O42,H37	O42:K-:H37	Hu F		
	Bi7455-41	O43,H2	O43:K-:H2	Hu U		
	H702c	O44	O44:H18	Hu F	K74	
	H61	O45	O45:K1:H10	Hu F		
	P1c	O46	O46:K-:H16	Hu F		
	U8-41	O48	O48:K-:H-	Hu U		
	U12-41	O49	O49:K+:H12	Hu U		
	U18-41	O50	O50:K-:H4	Hu U		[H4] <sup>b</sup>
	U19-41	O51	O51:K-:H24	Hu U		
	U20-41	O52	O52:K-:H10	Hu U		
	Bi7327-41	O53,H3	O53:K-:H3	Hu U		K4 by E&E; Phase variation to H16
	Su3972-41	O54	O54:K-:H2	Hu U		
	Su3912-41	O55	O55:H-	Hu Pus	K59 (B5)	
	Su3684-41	O56	O56:K+:H-	Hu Mes		
	F8198-41	O57	O57:K-:H-	Hu F		
	F8962-41	O58	O58:K-:H27	Hu F		[H27] <sup>b</sup>
	F9095-41	O59	O59:K-:H19	Hu F		
	F10167a-41	O60	O60:K-:H33	Hu F		[H33] <sup>b</sup>

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O antigen test strains for reference						
C-number	Original No.	Test	SEROTYPE	Isolated from <sup>a</sup>	Previous designation	Comments
	F10167b-41	O61	O61:K-:H19	Hu F		
	F10524-41	O62	O62:K-:H30	Hu F		
	F10598-41	O63	O63:K-:H-	Hu F		
	K6b	O64	O64:K-:H-	Hu F		
	K11a	O65	O65:K-:H-	Hu F		
	P1a	O66	O66:K-:H25	Hu F		Original No. P6a by E&E (=No. For test O32)
	P7d	O68	O68:K-:H4	Hu F		
	P9b	O69,H38	O69:K-:H38	Hu F		
	P9c	O70,H42	O70:K-:H42	Hu F		
	P10a	O71	O71:K-:H12	Hu F		
	P12a	O73	O73:K-:H31	Hu F		
	E3a	O74,H39	O74:K-:H39	Hu P		
	E3b	O75,K95	O75:K95:H5	Hu P		
	E5d	O76	O76:K-:H8	Hu P		
	E10	O77,K96	O77:K96:H-	Hu P		
	E38	O78	O78:H-	Hu P	K80	
	E49	O79,H40	O79:K-:H40	Hu P		
	E71	O80	O80:K-:H26	Hu P		
	H5	O81,K97	O81:K97:H-	Hu F		
	H14	O82	O82:K-:H-	Hu F		
	H17a	O83	O83:K-:H31	Hu F		
	H19 (Knipschildt)	O84	O84:K-:H21	Hu F		
	H23	O85	O85:K-:H1	Hu F		
	H35	O86	O86:K-:H-	Hu F		H25 by E&E '72
	H40	O87	O87:K-:H12	Hu F		
	H53	O88	O88:K-:H25	Hu F		
	H68	O89	O89:K-:H16	Hu F		
	H77	O90	O90:K-:H-	Hu F		
	H307b	O91	O91:K-:H-	Hu F		
	H308a	O92	O92:K-:H33	Hu F		

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O antigen test strains for reference						
C-number	Original No.	Test	SEROTYPE	Isolated from <sup>a</sup>	Previous designation	Comments
	H311a	O95	O95:K+:H33	Hu F		[H33] <sup>b</sup>
	H319	O96	O96:K-:H19	Hu F		
	H320a	O97	O97:K-:H-	Hu F		
	H501d	O98	O98:K-:H8	Hu F		
	H504c	O99	O99:K-:H33	Hu F		
	H509a	O100	O100:K-:H2	Hu F		
	H510a	O101	O101:K-:H33	Hu F		
	H511	O102	O102:K-:H40	Hu F		NOT H8!
	H515b	O103	O103:K+:H8	Hu F		
	H519	O104	O104:K-:H12	Hu F		
	H520b	O105	O105:K-:H8	Hu F		
	H521a	O106	O106:K-:H33	Hu F		
	H705	O107,K98	O107:K98:H27	Hu F		Is not H7 as listed by G&R
	H708b	O108	O108:K-:H10	Hu F		
	H709c	O109	O109:K-:H19	Hu F		
	H711c	O110	O110:K-:H39	Hu F		
	Stoke W	O111	O111:H-	Hu D	K58 (B4)	
C 707-65	6396-62	O111ac	O111ac:H-		K58 (B4)	=C 708-65
	1411-50	O112ab	O112ab:H18	Hu D	K68 (B13)	
	Guanabara (M194) 1685	O112ac	O112ac:H-	Hu D	K66 (B11)	Sereny-, EIEC-
	6182-50 (=32w)	O113	O113:H21	Hu F	K75 (B19)	
	K10 (=26w=H34w)	O114,H32	O114:H32	Ca S	K90ab	Original No. W34 by E&E '72
	27w	O115	O115:K-:H18	Ca S		Original No. W27 by E&E '72
	28w	O116	O116:K+:H10	Ca S		Original No. W28 by E&E '72
	30w	O117	O117:K98:H4	Ca S		Original No. W30 by E&E '72
	31w	O118	O118:K-:H-	Ca S		Original No. W31 by E&E '72
	34w	O119	O119:H27	Ca S	K69 (B14)	Original No. W34 by E&E '72
	35w	O120	O120:K18a:H6	Ca S		Original No. W35 by E&E '72
	39w	O121	O121:K-:H10	Ca S		Original No. W39 by E&E '72
	43w	O123	O123:K-:H16	Ca S		Original No. W43 by E&E '72
	227 (Ewing)	O124	O124:H30	Hu D	K72 (B17)	Sereny-, EIEC-; H32 by E&E

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O antigen test strains for reference						
C-number	Original No.	Test	SEROTYPE	Isolated from <sup>a</sup>	Previous designation	Comments
	2745-53 (Canioni)	O125ab	O125ab:H19	Hu D	K70 (B15)	
C 4-55	2129-54 (Ewing)	O125ac	O125ac:H6	Hu F	K70 (B15)	=C 712-65
	E611 (Taylor)	O126	O126:H2	Hu D	K71 (B16)	6021-50 (Ewing)
	4932-53 (Holcomb)	O127a	O127a:H-	Hu D	K63 (B8)	
C 966-64	56-54 (Cigleris)	O128ab	O128ab:H2	Hu D	K67 (B12)	K68 (B12) by E&E
C 715-65	5564-64	O128ac	O128ac:K-:H12		K67 (B12)	No answer re. source
	178-54 (Seeliger)	O129	O129:K-:H11	Hu D		Original No. 1986-54 by E&E
	4866-53 (Ewing)	O130	O130:K-:H9	Hu?		
	S239 (=H27w)	O131,H26	O131:K-:H26	Ca S		ID to H36w
	N87 (=H30w)	O132,H28	O132:K+:H28	Ca F		
	N282 (=H31w)	O133,H29	O133:K-:H29	Ca F		
	4370-53 (Ewing)	O134,H35	O134:K-:H35	Hu F		
	Coli Pecs	O135	O135:K-:H-	Hu D		
C 408-55	1111-55 (Sakazaki)	O136	O136:H-	Hu D	K78 (B22)	Sereny-, EIEC+
	RVC1787 (Rees)	O137,H41	O137:H41	Ca D	K79	H41 after 14 days (RKI)
C 62-57	CDC62-57	O138	O138:H14	Sw D	K81	
C 63-57	CDC63-57	O139	O139:K12:H1	Sw D	K82	
C 24-58	149-51	O140,H43	O140:K-:H43	Hu D		
C 235-59	E68	O141ab, F4ab	O141:K-:H4; F4ab	Sw D	K85ab (K88ab)	FR. 520 + 875-876, OK85 antiserum is used for typing of O141ab
C 21-58	RVC2907	O141ac	O141ac:H4	Sw D	K85ac	
C 28-58	C771	O142	O142:H6	Hu D	K86	
C 316-58	4608-58 (Ewing)	O143	O143:K-:H-	Hu D		Sereny+, EIEC+; Hatsumoto
C 318-58	1624-56 (Ewing)	O144	O144:K-:H-	Hu D		Sereny+/-, EIEC+
C 52-55	E1385(3) (Taylor)	O145	O145:K-:H-	Hu D		
C 9-55	CDC2950-54	O146	O146:K-:H21	Hu D	OX4	OX4
C 540-60	G1253	O147, F4ac	O147:H19; F4ac	Sw D	K88ac; K89	tox+
C 831-67	E519-66	O148	O148:K-:H28	Hu D		
C 867-64	A1 (Sweeny, Abbotstown)	O149	O149:H10	Sw	K91	=C 929-63 ID m. D615 (F4+); D616 is F4ac- and derived from CS1483 (Wittig)

### Appendix 3

### Test strains

O antigen test strains for reference						
C-number	Original No.	Test	SEROTYPE	Isolated from <sup>a</sup>	Previous designation	Comments
C 853-66	1935	O150,K93	O150:K93:H6	Ch S		
C 426-69	880-67	O151	O151:K-:H10	Hu D		
C 432-69	1184-68	O152	O152:K-:H-	Hu D		Sereny+, EIEC+
C2213-69	14097	O153	O153:K-:H7	Hu D		
C 624-70	E1541-68	O154,K94	O154:K94:H4	Hu F		
C 626-70	E1529-68	O155	O155:K-:H9	Hu F		
C 627-70	E1585-68	O156	O156:K-:H47	Hu F		
C1477-68	A2 (Sweeny, Abbotstown)	O157	O157:H19;F4 <sub>ac</sub>	Sw D		
C 161-73	E1020-72 (Rowe)	O158	O158:K-:H23	Hu D		Is not H20 as listed by G&R
C 162-73	E2476-72 (Rowe)	O159	O159:K-:H20	Hu D		Is not H23 as listed by G&R
C 592-73	E110-69 (Rowe)	O160	O160:K-:H34	Hu D		
C 593-73	E223-69 (Rowe)	O161,H54	O161:K-:H54	Hu D		New flagellin gene named <i>flmA54</i>
C 481-73	10B1-1 (Bettelheim)	O162	O162:K-:H10	Hu F		
C 130-74	SN3B-1	O163	O163:K-:H19	Hu F		
C1172-73	DRL145-46	O164	O164:K-:H-	Hu D		(=Sc647); Sereny-, EIEC
C1088-79	E78634 (Maruyama)	O165	O165:K-:H-	Hu D		Outbreak of enteritis, Tokyo, Japan, 1978
C 13-55	3866-54 (Ewing)	O166	O166:K-:H4	Hu D	OX8	(OX8)
C 614-80	E10702 (Rowe)	O167	O167:K-:H5			ST+
C 615-80B	E10710 (Rowe)	O168	O168:K-:H16	Hu D	OX5	(OX5) ST+, LT+
C 7-55	1792-54 (Ewing)	O169	O169:K-:H8	Hu D	OX2	(OX2)
C 6-55	745-54 (Ewing)	O170	O170:K-:H1	Hu D	OX1	(OX1)
C1754-84	198 (Wijuwanta)	O171	O171:K-:H2	Ca	~OX6	LT+; Sri Lanka; (~OX6) First test strain (244-55) replaced because inappropriate due to failing ability to ferment various sugars.
C 70-86	3288-85 (Wachsmuth)	O172	O172:K-:H-	Hu HC		
C 862-87	L119B-10 (Taylor)	O173	O173:K-:H-	Hu BD		Sereny-, EIEC+
C 8-55	2531-54 (Ewing)	O174	O174:K-:H27	Hu D	OX3	(OX3)
C 12-55	2533-54 (Ewing)	O175	O175:K-:H28	Hu D	OX7	(OX7)
C 410-93A	E29518-83 (Rowe)	O176	OX176:H-	Ca F		
C 411-93	E40874-85 (Rowe)	O177	OX177:H25	Ca		

**Appendix 3****Test strains**

<b>O antigen test strains for reference</b>						
<b>C-number</b>	<b>Original No.</b>	<b>Test</b>	<b>SEROTYPE</b>	<b>Isolated from<sup>a</sup></b>	<b>Previous designation</b>	<b>Comments</b>
C 414-93A	E54071-88 (Rowe)	O178	OX178:H7	Raw meat		
C 412-93	E43478 (Rowe)	O179	OX179:H8	Hu 54 Yrs F BD		
C 508-98A	86-381 (Woodward)	O180	OX180H-	Sw		
C 515-98A	92-1250 (Woodward)	O181	OX181:H49	Ground beef		

### Appendix 3

### Test strains

H antigen test strains for reference *							
C-number	Original No.	Test	Flagellar encoding gene(s) Functional (coexist with)	SEROTYPE	Isolated from <sup>a</sup>	Previous designation	Comments
	Su1242	H1	<i>fliC</i>	O2:K2ab:H1	Hu B		
	Bi7455-41	O43,H2	<i>fliC (flkA)</i>	O43:K-:H2	Hu U		
	Bi7327-41	O53,H3	<i>flkA (fliC16)</i>	O53:K-:H3	Hu U		K4 by E&E; Phase var. til H16
	U9-41	O2,K1,H4	<i>fliC (flnA17)</i>	O2:K1:H4	Hu U		
	U4-41	O4,K3,H5	<i>fliC (flIA)</i>	O4:K3:H5	Hu H		
	A 20a	H6	<i>fliC</i>	O2:K1:H6	Hu A		
	U5-41	O1,H7	<i>fliC</i>	O1:K1:H7	Hu H		K- derivative: D703
	App.320c	H8	<i>fliC (flkA)</i>	O2:K-:H8	Hu A		
	Bi7575-41	K25,H9	<i>fliC</i>	O8:K25:H9	Hu U	B2	
	Bi623-42	O11,K10,H10	<i>fliC</i>	O11:K10:H10	Hu P		NM by E&E
	Su4321-41	O13,K11,H11	<i>fliC (flkA)</i>	O13:K11:H11	Hu P		
	Bi316-42	O9,K9,H12	<i>fliC</i>	O9:K9:H12	Hu P		
	F10018-41	O18ab,H14	<i>fliC</i>	O18ab:K-:H14	Hu F	K76 (B20)	
	E39a	O23,K18ab,H15	<i>fliC</i>	O23:K18ab:H15	Hu P		
	F8316-41	K15,H16	<i>fliC (flkA)</i>	O6:K15:H16	Hu F		
	P12b	H17	<i>flnA (fliC4)</i>	O15:K97:H17	Hu F		Phase variation to H4
	K12a	O17,K16,H18	<i>fliC</i>	O17:K16:H18	Hu F		
	A18d	H19	<i>fliC</i>	O9:K36:H19	Hu A		~H29w
	H330b	H20	<i>fliC</i>	O8:K49:H20	Hu F		
	U11a-44	H21	<i>fliC (flkA)</i>	O8:K49:H21	Hu U		
	K42 (=H23w)	H23	<i>fliC</i>	O45:Kne:H23	Ca S		
	K72 (H25w)	H24	<i>fliC (flIA)</i>	O51:K12:H24	Ca S		
	N234 (=H26w)	H25	<i>fliC (flIA)</i>	O15:K16:H25	Ca F		
	S239 (=H27w)	O131,H26	<i>fliC</i>	O131:K-:H26	Ca S		ID to H36w
	K50 (=H28w)	H27	<i>fliC (flkA)</i>	O15:Kne:H27	Ca S		~ H27w
	N87 (=H30w)	O132,H28	<i>fliC</i>	O132:K+:H28	Ca F		
	N282 (=H31w)	O133,H29	<i>fliC (flIA)</i>	O133:K-:H29	Ca F		
	N157 (=H32w)	H30	<i>fliC</i>	O38:Kne:H30	Ca F		
	K15 (=H33w)	H31	<i>fliC</i>	O3:K-:H31	Ca S		



### Appendix 3

### Test strains

H antigen test strains for reference *							
C-number	Original No.	Test	Flagellar encoding gene(s) Functional (coexist with)	SEROTYPE	Isolated from <sup>a</sup>	Previous designation	Comments
	K10 (=26w=H34w)	O114,H32	<i>fliC</i>	O114:H32	Ca S	K90ab	Original No. W34 by E&E '72
	K181 (H35w)	H33	<i>fliC (fliA)</i>	O11:Kne:H33	Ca S		
	BP12665 (=F48/51)	H34	<i>fliC</i>	O86:H34	Hu D	K61 (B7)	
	4370-53 (Ewing)	O134,H35	<i>flkA</i> (IS in <i>fliC11</i> )	O134:K-:H35	Hu F		
C 519-59	5017-53 (Ewing)	H36	<i>flkA</i>	O86:H36	Hu D	K64 (B9)	
	P11a	O42,H37	<i>fliC</i>	O42:K-:H37	Hu F		
	P9b	O69,H38	<i>fliC (fliA)</i>	O69:K-:H38	Hu F		
	E3a	O74,H39	<i>fliC (fliA)</i>	O74:K-:H39	Hu P		
	E49	O79,H40	<i>fliC (flkA8)</i>	O79:K-:H40	Hu P		
	RVC1787 (Rees)	O137,H41	<i>fliC</i>	O137:H41	Ca D	K79	H41 after 14 days (RKI)
	P9c	O70,H42	<i>fliC (fliA)</i>	O70:K-:H42	Hu F		
C 24-58	149-51	O140,H43	<i>fliC</i>	O140:K-:H43	Hu D		
C 25-58	781-55	H44	<i>fliA (fliC4)</i>	O3:K19:H44	Hu D		New flagellin gene named <i>fliA44</i>
C 26-58	4106-54	H45	<i>fliC</i>	O52:Kne:H45	Hu D		
C 27-58	5306-56	H46	<i>fliC</i>	O26:H46	Ch F		K60 (B6)
C 184-59	1755-58	H47	<i>flkA (fliC21)</i>	O86:Kne:H47	Hu D		
C 716-59	P4 (Chapple)	H48	<i>fliC</i>	O16:Kne:H48	Sw D		Original No. P48 by G&R
C 133-61	2147-59	H49	<i>fliC</i>	O6:K13:H49	Hu F		
C218-70	AB1767	H51	<i>fliC</i>	O8:K50:H51	Hu S		H2S+; strain 669-58 listed as test H51
C2187-69	3219	H52	<i>fliC</i>	O11:Kne:H52	Hu U		H2S+
C 56-70	E480-68 (Taylor)	H53	<i>flkA</i>	O148a:Kne:H53	Hu D		
C 593-73	E223-69 (Rowe)	O161,H54	<i>flmA</i> (IS in <i>fliC21</i> )	O161:K-:H54	Hu D		New flagellin gene named <i>flmA54</i>
C1371-75	E2987-73 (Rowe)	H55	<i>fliA (fliC38)</i>	O75:Kne:H55	?		New flagellin gene named <i>fliA55</i>
C 688-74	SN2N-1 (Bettelheim)	H56	<i>fliC (fliA)</i>	O139:K-:H56	Hu F		SN3N-1 by Ø&Ø '84 is incorrect; grow at 30°C

\* H13, H22 and H50 are obsolete.

### Appendix 3

### Test strains

K antigen test strains for reference						
C-number	Original No.	Test	SEROTYPE	Isolated from <sup>a</sup>	Previous	Comments
	U9-41	O2,K1,H4	O2:K1:H4	Hu U		
	U14-41	O3,K2ab	O3:K2ab:H2	Hu U		
	U4-41	O4,K3,H5	O4:K3:H5	Hu H		
	U1-41	O5,K4	O5:K4:H4	Hu U		
	Bi8337-41	O10,K5	O10:K5:H4	Hu P		NM by E&E
	Bi7457-41	K6	O4:K6:H5	Hu U		Hu A
	Pus 3432-41	K7	O7:K7:H4	Hu P		
	G3404-41	O8,K8	O8:K8:H4	Hu B		
	Bi316-42	O9,K9,H12	O9:K9:H12	Hu P		
	Bi623-42	O11,K10,H10	O11:K10:H10	Hu P		NM by E&E
	Su4321-41	O13,K11,H11	O13:K11:H11	Hu P		
	Su65-42	K12	O4:K12:H-	Hu Pus		[H5] <sup>b</sup> ; Hu U
C 515-59	Su4344-41	K13	O6:K13:H1	Hu H		
	F7902-41	O15,K14	O15:K14:H4	Hu F		
	F8316-41	K15,H16	O6:K15:H16	Hu F		
	K12a	O17,K16,H18	O17:K16:H18	Hu F		
	P7a	O20,K17	O20:K17:H-	Hu F		
???	H67	K18a	O23:K18a:H15	Hu F	K22	= former K22
	E39a	O23,K18ab,H15	O23:K18ab:H15	Hu P		
	E47a	O25,K19	O25:K19:H12	Hu P		
	E19a	O21,K20	O21:K20:H-	Hu P		[H4] <sup>b</sup>
C 518-59	H38	K-	O23:(K21):H15	Hu F		K21 lost and therefore deleted
	H54	K23	O25:K23:H1	Hu F		
	H45	K24	O83:K24:H31	Hu F		Formerly assigned to O22
	Bi7575-41	K25,H9	O8:K25:H9	Hu U	B2	
	Bi449-42	K26	O9a:K26:H-	Hu P		
	E56b	K27	O8:K27:H-	Hu P		[H10] <sup>b</sup>
	K14a	K28	O9ab:K28:H-	Hu F		
	Bi161-42	K29	O9:K29:H-	Hu P		
	E69	K30	O9:K30:H12	Hu P		H1 by E&E
	Su3973-41	K31	O9:K31:H-	Hu U		[H4] <sup>b</sup>

**Appendix 3**

**Test strains**

K antigen test strains for reference						
C-number	Original No.	Test	SEROTYPE	Isolated from <sup>a</sup>	Previous	Comments
	H36	K32	O9:K32:H19	Hu F		
	App.289	K33	O9:K33:H-	Hu A		
	E75	K34	O9:K34:H-	Hu P		
	A140a	K35	O9:K35:H-	Hu A		
	A198a	K36	O9:K36:H19	Hu A		
	A84a	K37	O9:K37:H-	Hu A		
	A262a	K38	O9:K38:H-	Hu A		
	A121a	K39	O9:K39:H9	Hu A		
	A51d	K40	O8:K40:H9	Hu A		
	A433a	K41	O8:K41:H11	Hu A		
	A295b	K42	O8:K42:H-	Hu A		[H4] <sup>b</sup>
	A195a	K43	O8:K43:H11	Hu A		
	A168a	K44	O8:K44:H-	Hu A		
	A169a	K45	O8:K45:H9	Hu A		
	A236a	K46	O8:K46:H30	Hu A		
	A282a	K47	O8:K47:H2	Hu A		
	A290a	K48	O8:K48:H9	Hu A		
	A180a	K49	O8:K49:H21	Hu A		
	PA80c	K50	O8:K50:H-	Hu P		[H9] <sup>b</sup>
	A183a	K51	O1:K51:H-	Hu A		[H1] <sup>b</sup>
	A103	K52	O4:K52:H4	Hu A		Ca S
	PA236	K53	O6:K53:H-	Hu P		
	A12b	K54	O6:K54:H10	Hu A		
	N24c	K55	O9:K55:H-	Hu NA		
	H509d	K57	O9:K57:H32	Hu F	B3	
C 64-57	CDC134-51	K83	O20:K83:H26	Hu D		
C 65-57	CDC2292-55	K84	O20:K84:H26	Hu D		2292-55, O20:H26 by E&E '72
	D227(=G:7,F4-)	K87	O8:K87:H19	Sw P		CG9, O141:NM by E&E '72
C1407-67	6181-66	K92	O73:K92:H34	Hu D		
C 853-66	1935	O150,K93	O150:K93:H6	Ch S		
C 624-70	E1541-68	O154,K94	O154:K94:H4	Hu F		
	E3b	O75,K95	O75:K95:H5	Hu P		

### Appendix 3

### Test strains

K antigen test strains for reference						
C-number	Original No.	Test	SEROTYPE	Isolated from <sup>a</sup>	Previous	Comments
	E10	O77,K96	O77:K96:H-	Hu P		
	H5	O81,K97	O81:K97:H-	Hu F		
	H705	O107,K98	O107:K98:H27	Hu F		H7 by G&R
	F147	K100	O75:K100:H5	Hu D		
C1209-75	1413	K101, F6	O20:K101:H-; F6	Sw D	987P	
C1345-75	6CB10-1	K102	O8:K102:H-			
C1342-75	8CE275-6	K103	O101:K103:H-	Hu F		

<sup>a</sup> Abbreviations: Hu, human; Ca, Calf; Sw, Swine (piglet); Ch, chicken; U, urine; A, appendix (appendicitis); NW, normal appendix; S, blood (septicaemia); P, peritoneum (most often appendicitic peritonitis); B, bile; F, faeces from healthy individual; D, diarrhoea; HC, Haemorrhagic colitis; BD, bloody diarrhoea; Mes, mesenterial lymph node

<sup>b</sup> [Hxx] in the comments column refers to H antigens listed by E&E in brackets. Please check if your version is motile and - if yes - which type. *fliC* typing should also be done on these in order to check