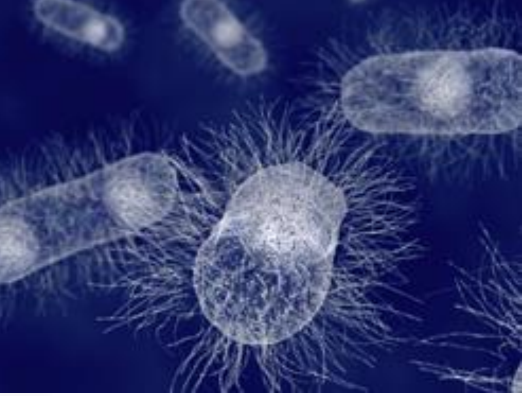

7th external quality assurance (EQA-7) scheme for typing of VTEC in 2015-2016

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EQA-7: 2015/2016

- LOT 1 SALMONELLA
  - PFGE
  - MLVA

- LOT 2 VTEC
  - PFGE
  - O:H serotyping
  - Virulence genes
  - Phenotypic testing

- LOT 3 LISTERIA
  - PFGE
  - Serotyping
    - Conventional
    - Molecular

EQA-7 includes the following methods

- **molecular typing: Pulsed Field Gel Electrophoresis (PFGE)**
- **serotyping O:H**
- **virulence determination:**
  - **genotyping of VTEC virulence genes: *vtx1*, *vtx2*, *eae*, *aaiC and aggR***
  - **subtyping of *vtx1* (*vtx1a*, *vtx1c* and *vtx1d*) and *vtx2* (*vtx2a* to *vtx2g*)**
- **phenotypic testing**
  - **production of Verocytotoxin/Shiga toxin**
  - **production of Extended Spectrum Beta Lactamases (ESBL)**
  - **production of β-glucuronidase**
  - **production of enterohaemolysin**
  - **fermentation of sorbitol**

Test panel

	Serotyping		**Genotyping**								**Phenotyping**						
Strain ID	**O group**	**H type**	***aaiC* gene**	***aggR* gene**	***eae* gene**	***ehxA* gene**	***vtx1* gene**	***vtx2* gene**	***vtx1* subtypes**	***vtx2* subtypes**	**β-glucuronidase production**	**ESBL production**	**Haemolysin production**	**Sorbitol fermentation**	**Verocytotoxin production**	**Additional virulence genes**	**Pathotype**
AA11	**O80**	**H2**	**-**	**-**	**+**	**+**	**-**	**+**	**-**	***vtx2a***	**+**	**-**	**+**	**+**	**+**		**VTEC**
BB12	**O26**	**H11**	**-**	**-**	**+**	**+**	**-**	**+**	**-**	***vtx2a***	**+**	**-**	**+**	**+**	**+**		**VTEC**
CC13	**O78**	**H2**	**+**	**+**	**-**	**-**	**-**	**-**	**-**	**-**	**+**	**-**	**-**	**+**	**-**	***aatA***	**EAEC**
DD14	**O145**	**H34**	**-**	**-**	**+**	**-**	**-**	**+**	**-**	***vtx2f***	**+**	**-**	**-**	**-**	**+**		**VTEC**
EE15	**O166**	**H15**	**-**	**-**	**-**	**-**	**-**	**+**	**-**	***vtx2d***	**+**	**+**	**-**	**+**	**+**	***eltA***	**VTEC-ETEC**
FF16	**O156**	**H4**	**-**	**-**	**-**	**-**	**-**	**+**	**-**	***vtx2d***	**+**	**-**	**-**	**+**	**+**		**VTEC**
GG17	**O146**	**H21**	**-**	**-**	**-**	**+**	**+**	**+**	***vtx1c***	***vtx2b***	**+**	**-**	**+**	**+**	**+**		**VTEC**
HH18	**O157**	**[H7]**	**-**	**-**	**+**	**+**	**+**	**+**	***vtx1a***	***vtx2c***	**-**	**-**	**+**	**-**	**+**		**VTEC**
II19	**O91**	**H14**	**-**	**-**	**-**	**+**	**+**	**+**	***vtx1a***	***vtx2b***	**+**	**-**	**+**	**+**	**+**	***saa***	**VTEC**
JJ20	**O103**	**H2**	**-**	**-**	**+**	**+**	**+**	**-**	***vtx1a***	**-**	**+**	**-**	**+**	**+**	**+**		**VTEC**

30 Participants enrolled

Austria
Belgium
Bulgaria
Cyprus
Czech Republic
Denmark
Estonia
Finland
France
Germany
Greece
Hungary
Iceland
Ireland
Italy
Latvia
Lithuania
Luxembourg
Norway
Poland
Portugal
Republic of Macedonia
Romania
Serbia
Slovenia
Spain
Sweden
The Netherlands
Turkey
United Kingdom

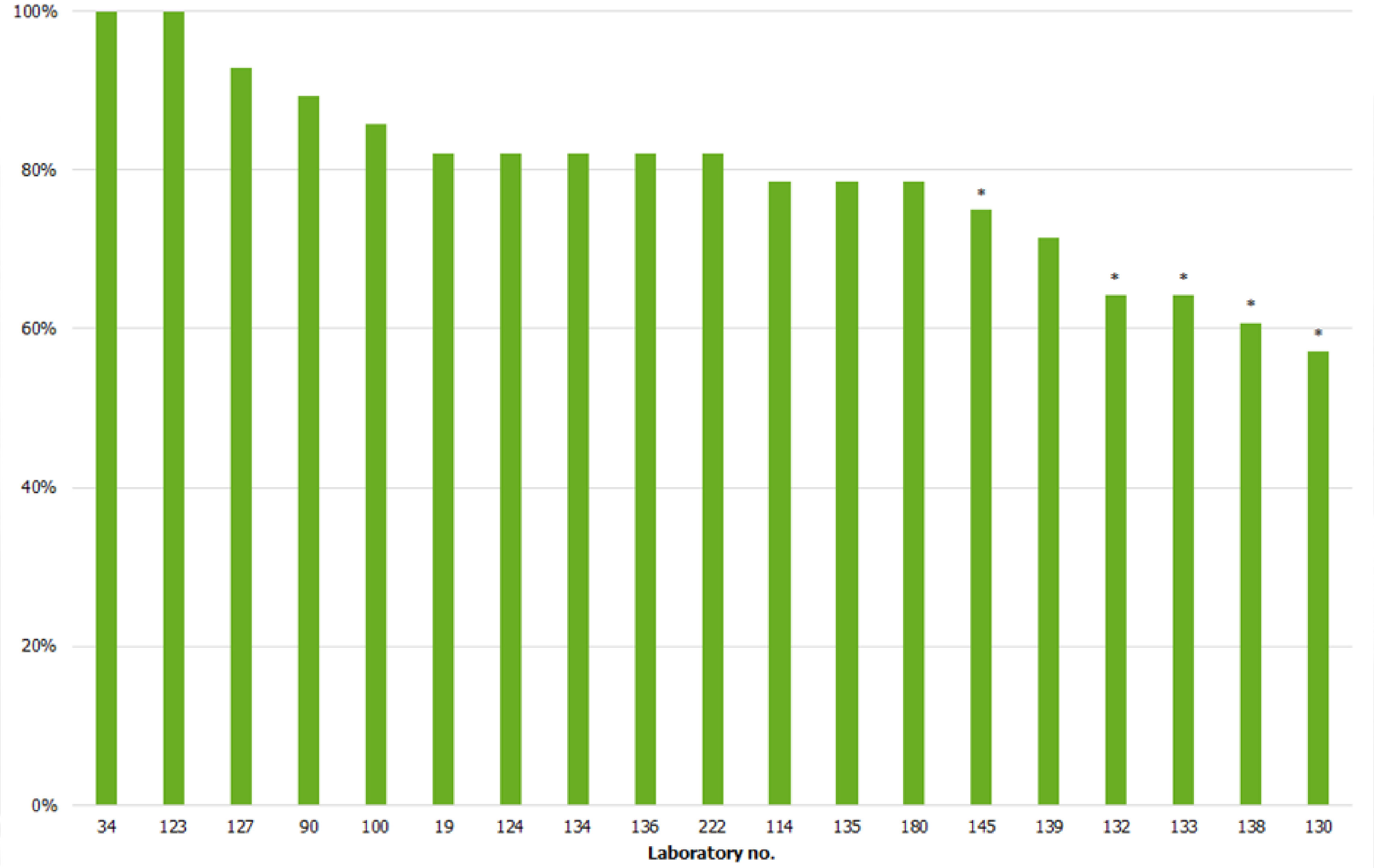
Number and percentage of laboratories submitting results for each part

	PFGE			Serotyping	Genotyping	Pheno typing
	Gel + BN	Gel only	Total			
Number of participants	**13**	**6**	**19**	**27**	**28**	**28**
% of participants	**68**	**32**	**63***	**90***	**93***	**93***

Detailed participation information for the sero-, geno- and phenotyping parts

	Serotyping n=27		Genotyping n=28						Phenotyping n=28				
	O group	H type	*aaiC*	*aggR*	*eae*	*ehxA*	*vtx1* and *vtx2*	*vtx* subtyping	β-glucuronidase	Enterohaemolysin	ESBL	Sorbitol	VT
No. participants	**27**	**17**	**17**	**21**	**28**	**18**	**28**	**25**	**15**	**14**	**18**	**26**	**8**
% of participants	**100**	**63**	**61**	**75**	**100**	**64**	**100**	**89**	**54**	**50**	**64**	**93**	**29**
% of participant	**90**	**57**	**57**	**70**	**93**	**60**	**93**	**83**	**50**	**47**	**60**	**87**	**27**

19 participants PFGE gel quality



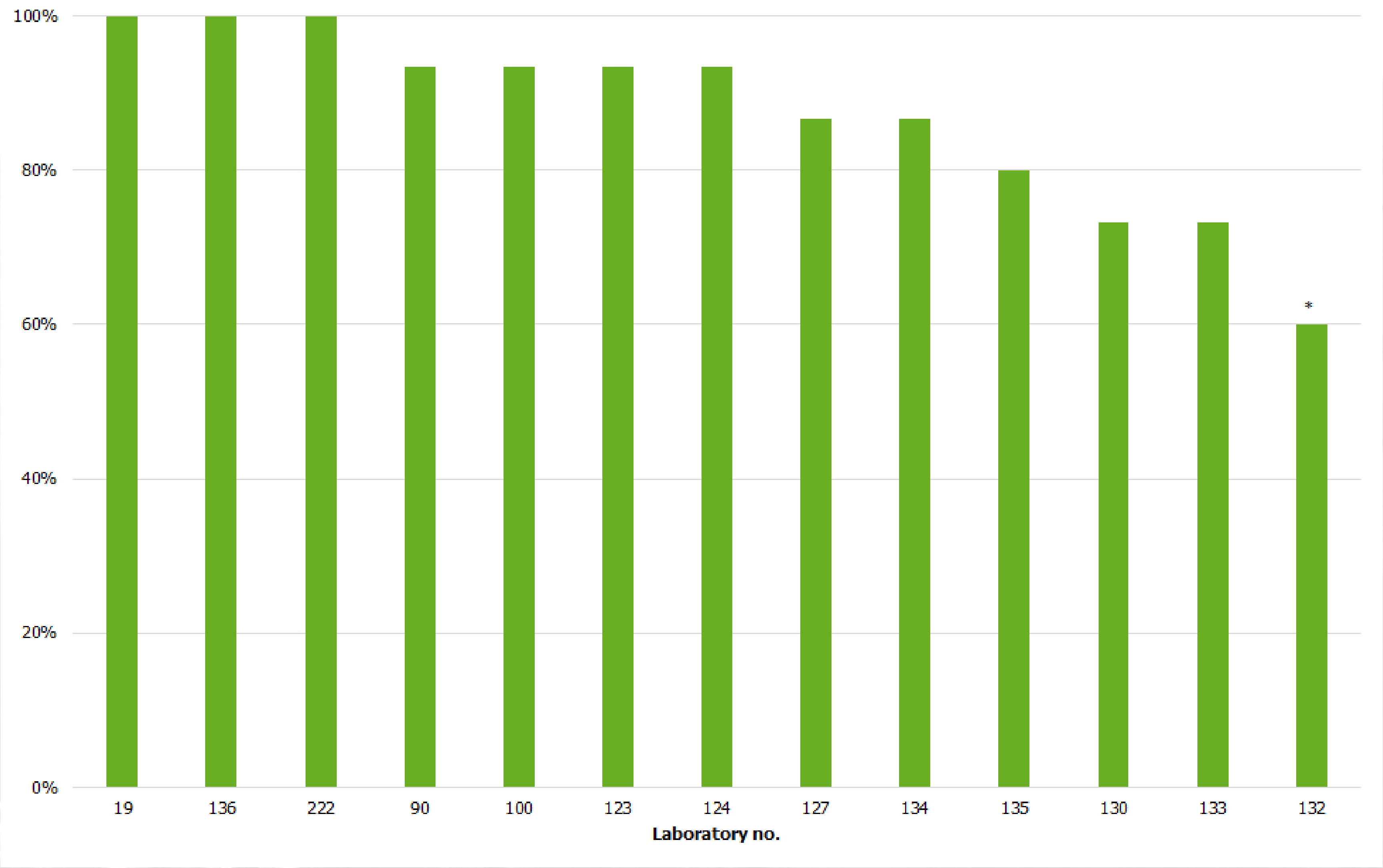
**** Gels unacceptable for inter-laboratory comparison, score of 1 [Poor] in at least one parameter***

Results of PFGE gel quality grading
19 participants

22 participants

	Grade [score in points]					
Parameter	**Poor [1]**	**Fair [2]**	**Good [3]**	**Excellent [4]**	**Average**	**EQA-6**
Image Acquisition and Running Conditions	16%	11%	26%	47%	3.1	3.3
Cell Suspension	0%	11%	26%	63%	3.5	3.6
Bands	16%	11%	53%	21%	2.8	2.6
Lanes	0%	11%	42%	47%	3.4	3.7
Restriction	5%	11%	21%	63%	3.4	3.3
Gel Background	0%	16%	53%	32%	3.2	3.6
DNA Degradation	5%	37%	21%	37%	2.9	3.5

13 participants PFGE gel BN analysis



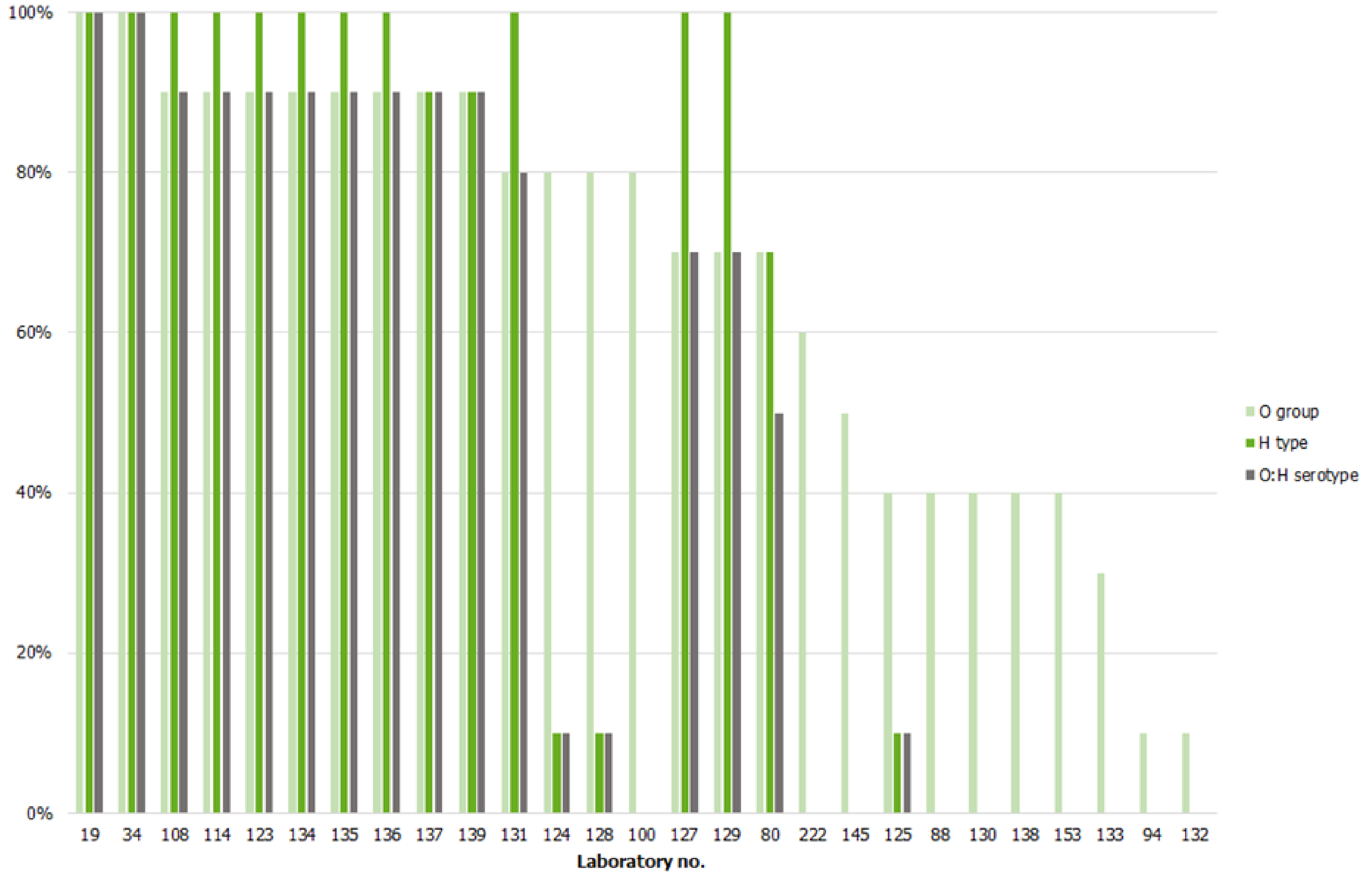
BN analysis not performed according to guidelines

Results of PFGE gel BN analysis
13 participants

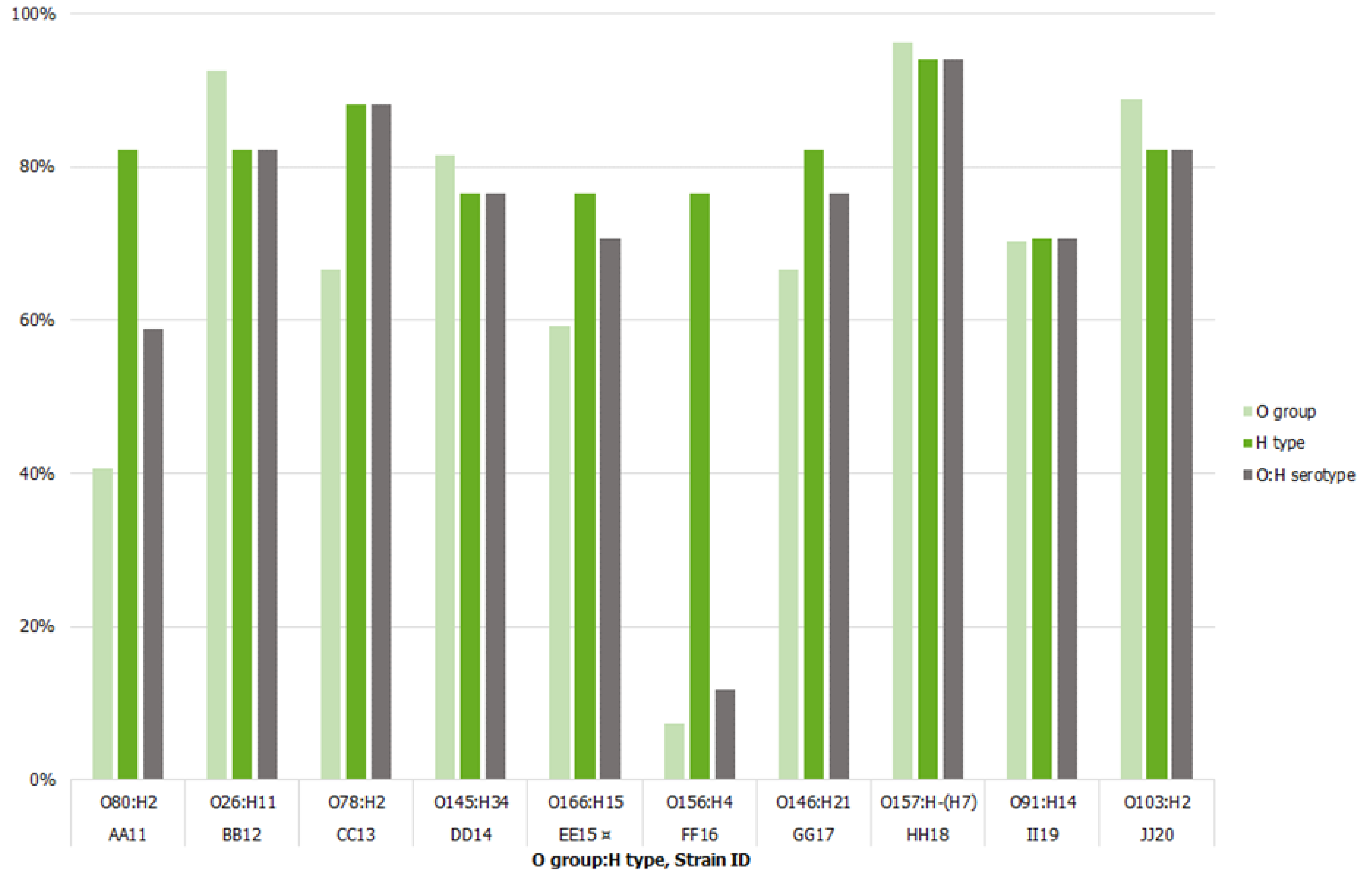
15
participants

	Grade [score in points]				
Parameter	**Poor [1]**	**Fair [2]**	**Excellent [3]**	**Average**	**EQA-6**
Position of Gel Frame	0%	31%	69%	2.7	2.5
Strips	0%	38%	62%	2.6	2.6
Curves	0%	38%	62%	2.6	2.6
Normalization	8%	23%	69%	2.6	2.6
Band Assignment	0%	46%	54%	2.5	2.3

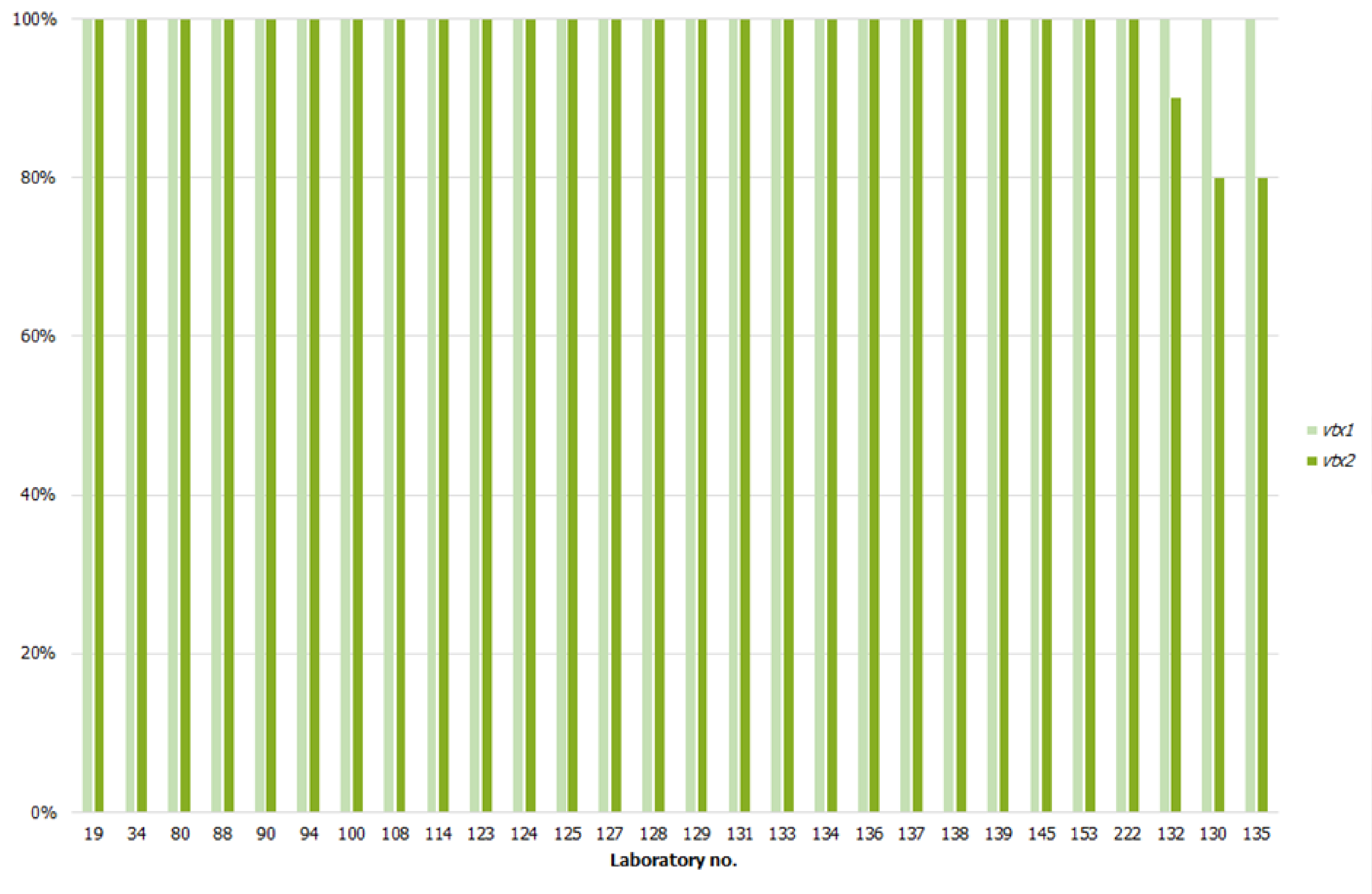
O grouping (27) and H typing (17)



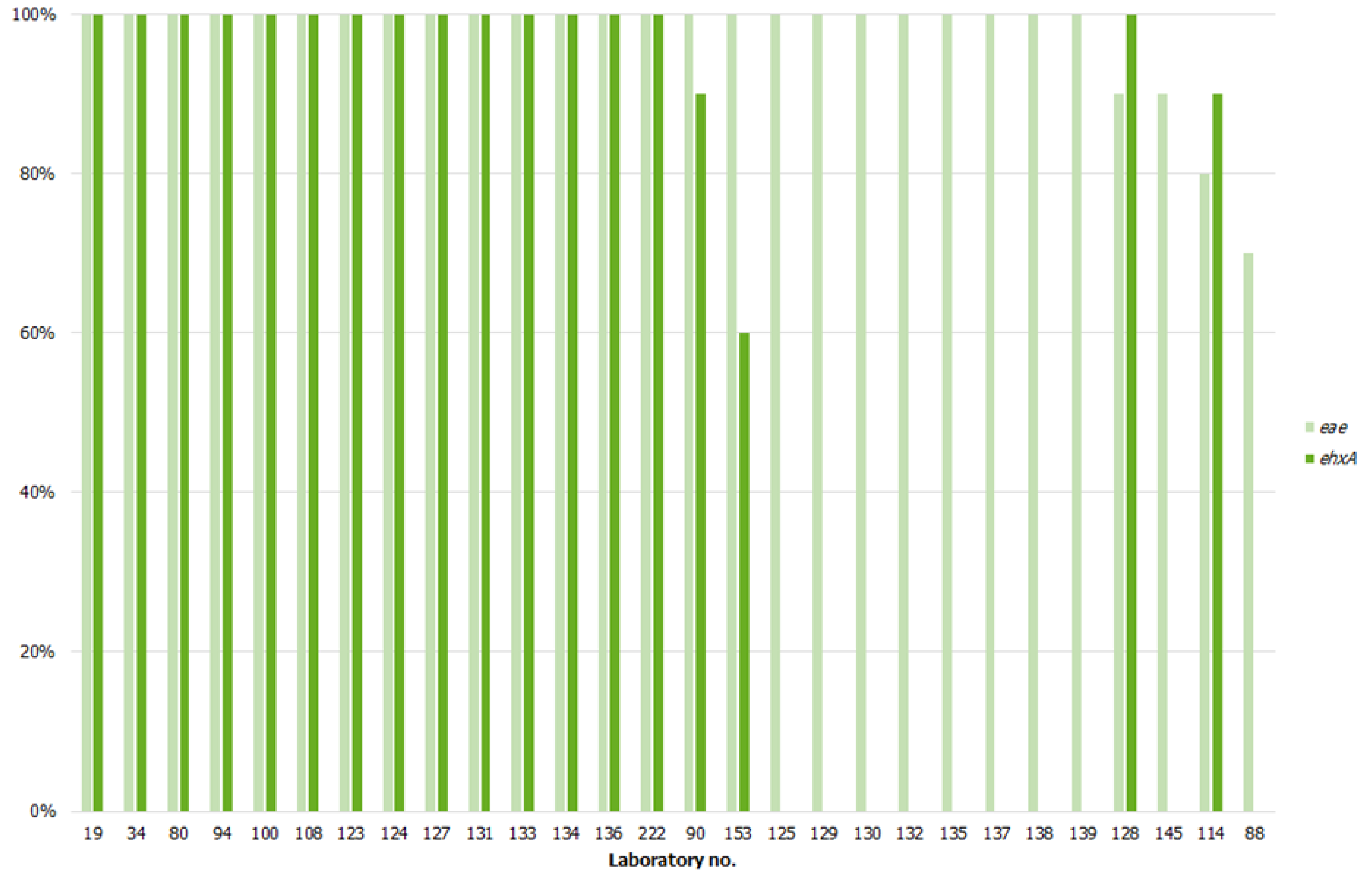
Test strain score for O and H



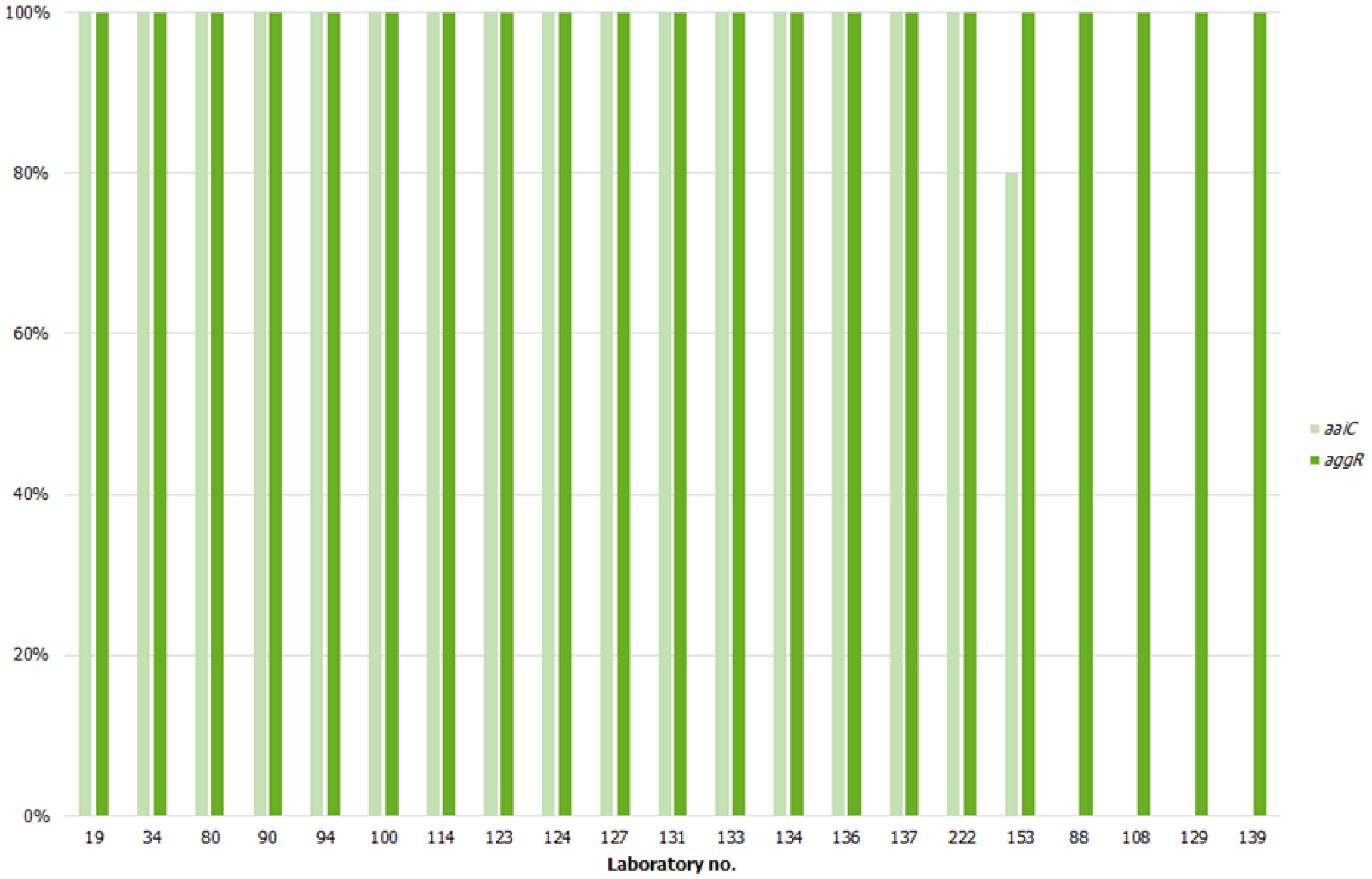
28 participants *vtx1* and *vtx2*



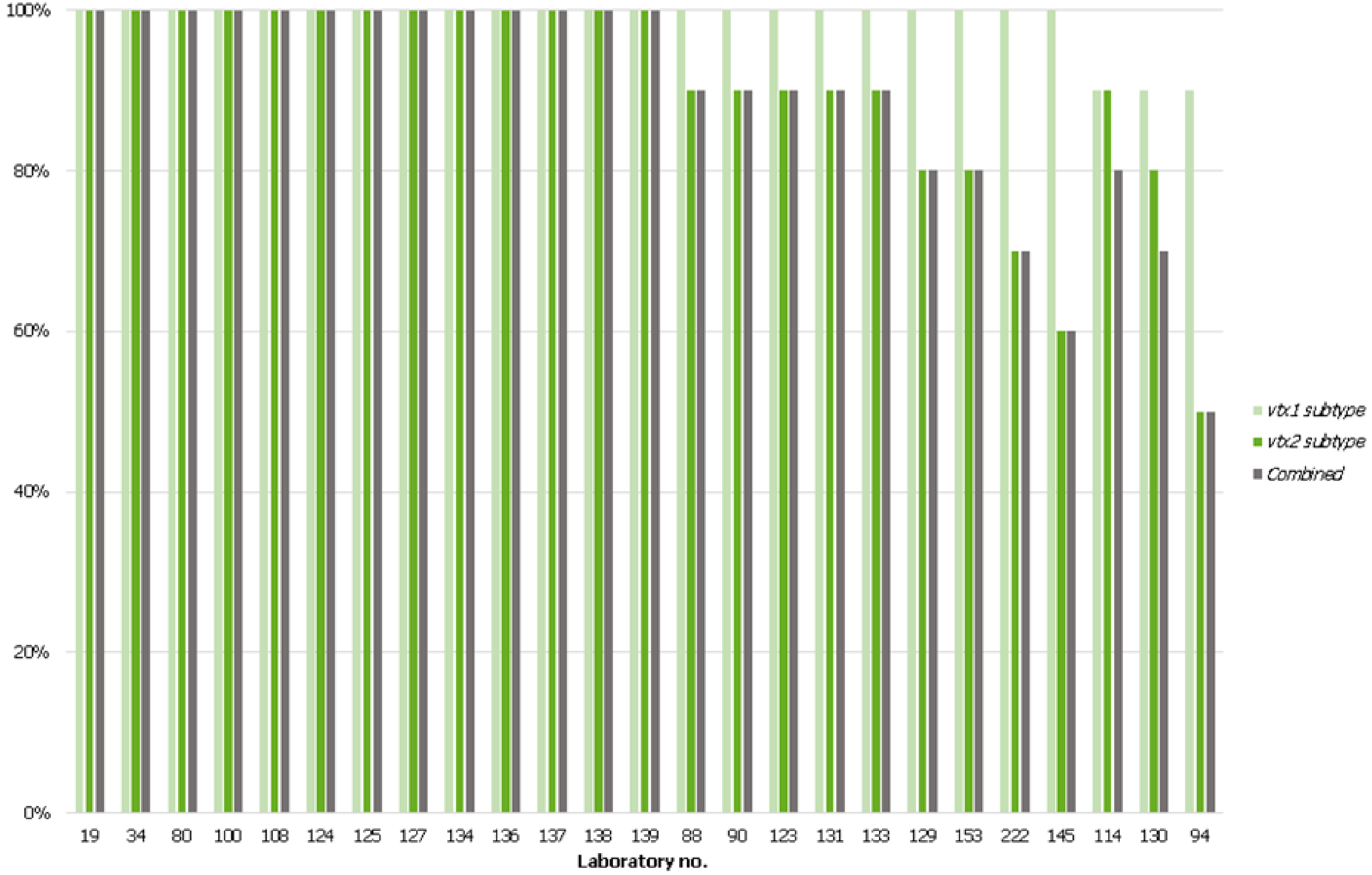
eae (28) and *ehxA* (18)



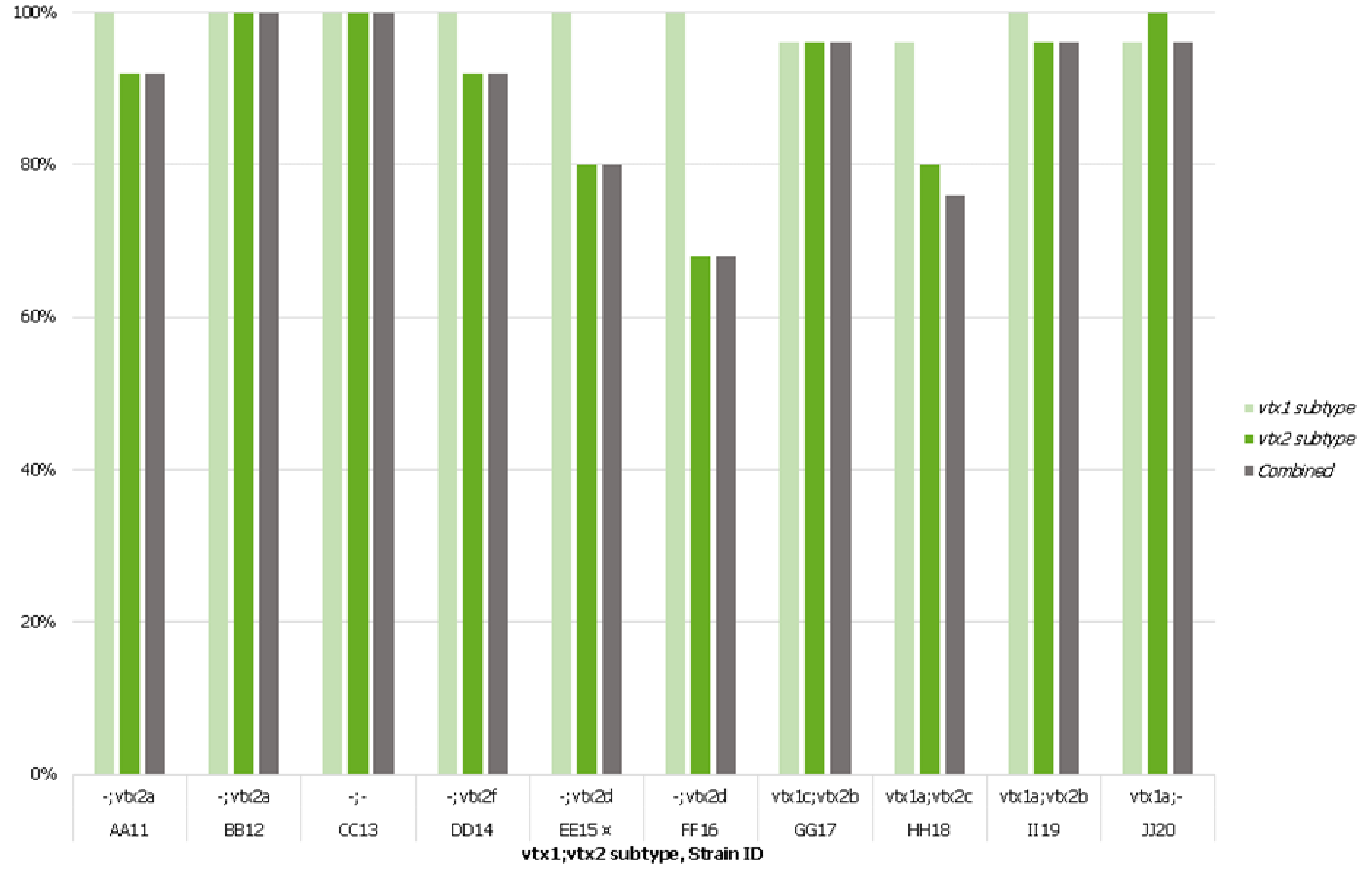
aaiC (17) and *aggR* (21)



25: subtyping of *vtx1* and *vtx2*



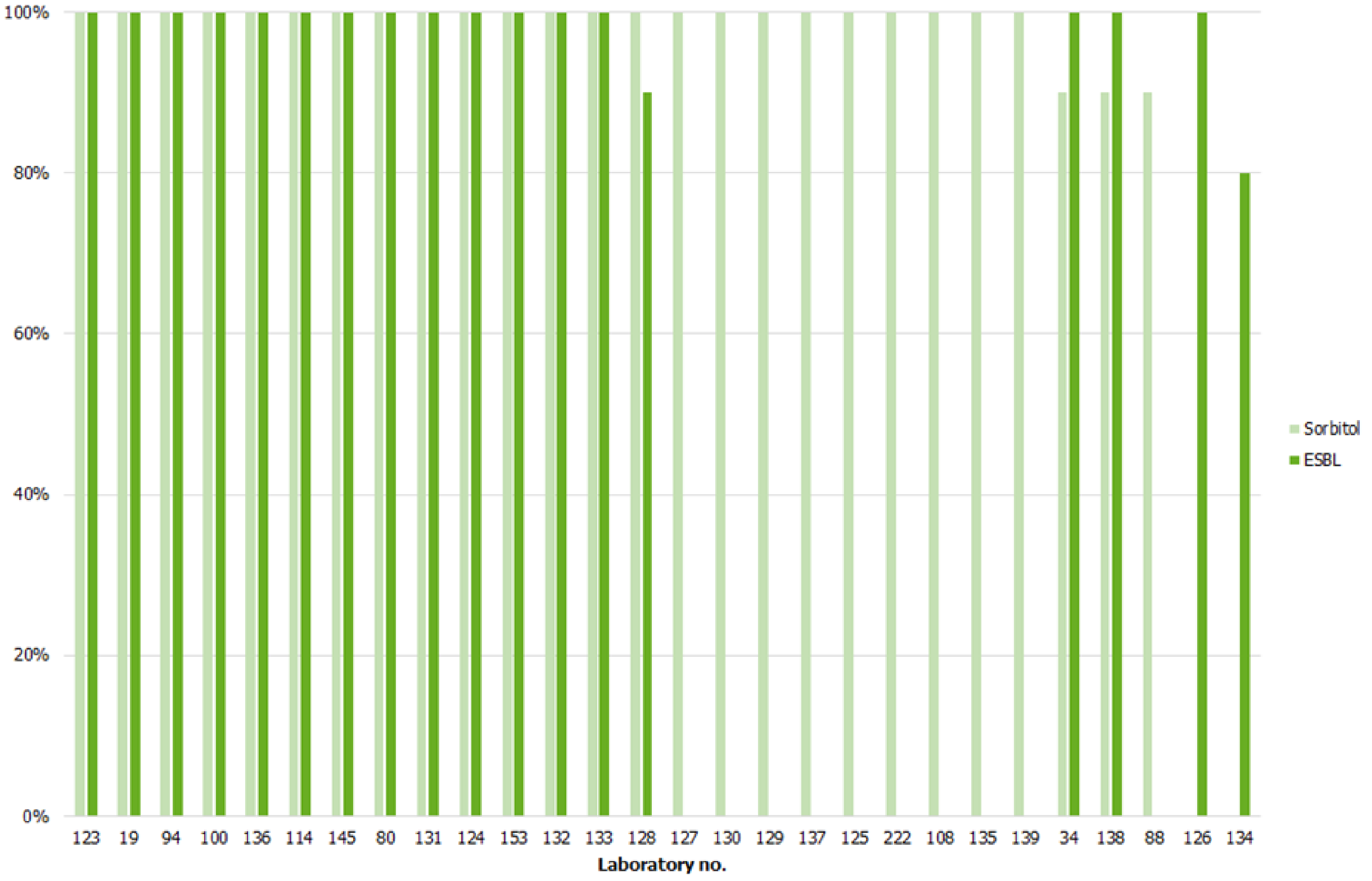
Subtyping of *vtx1* and *vtx2*



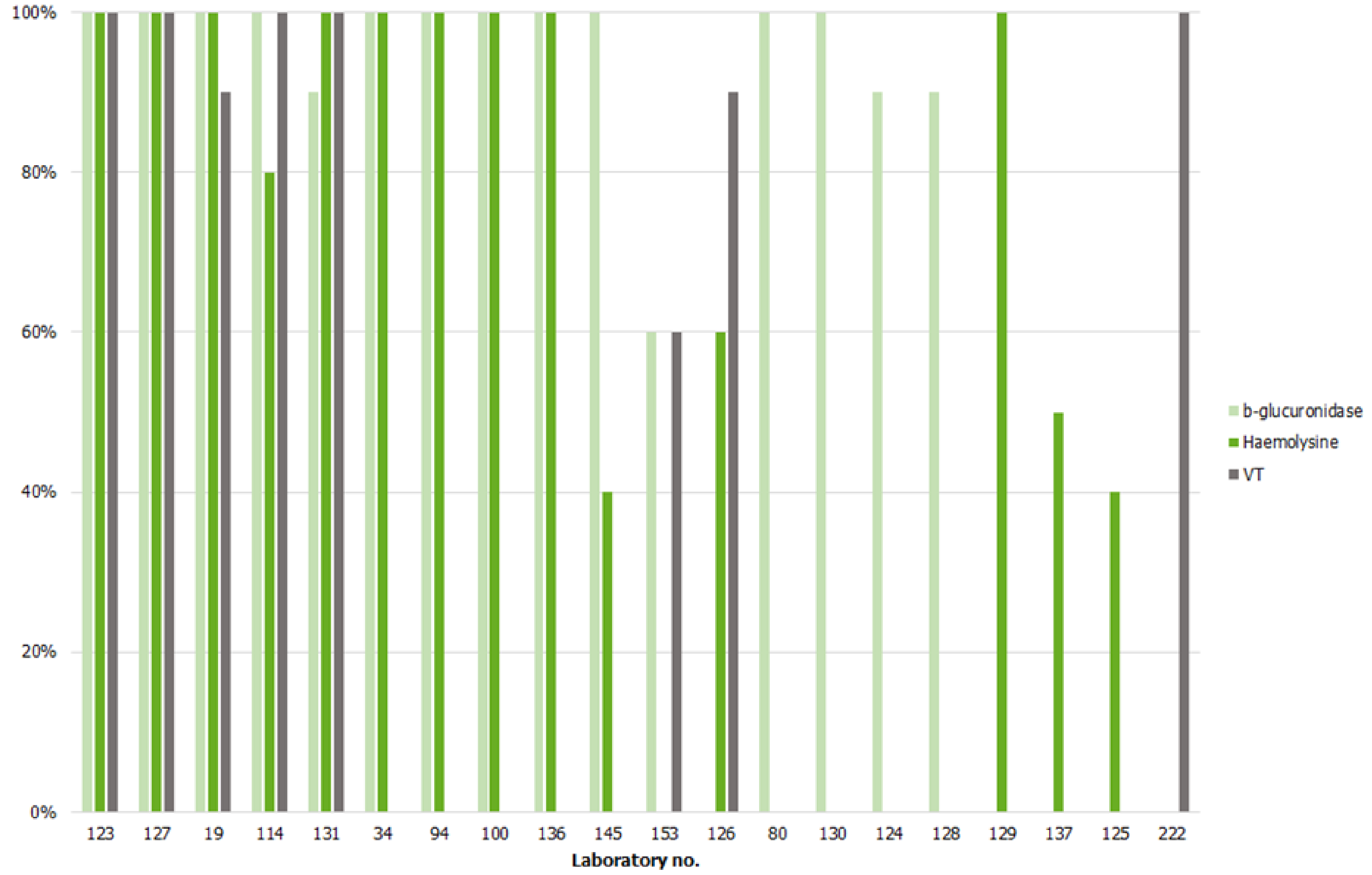
Incorrect *vtx2* subtype results

Strain ID	Original	Incorrect *vtx2* subtype results				
		False negative	One incorrect	Correct + incorrect	Two incorrects	Total no.
AA11	*vtx2a*			*vtx2a* + *vtx2b* (1), *vtx2a* + *vtx2c* (1)		2
BB12	*vtx2a*					0
CC13	-					0
DD14	*vtx2f*		*vtx2e* (1)	*vtx2f* + *vtx2e* (1)		2
EE15	*vtx2d*			*vtx2d* + *vtx2a* (1), *vtx2d* + *vtx2c* (4)		5
FF16	*vtx2d*	1	*vtx2a* (3), *vtx2c* (2)		*vtx2a* + *vtx2c* (2)	8
GG17	*vtx2b*		*vtx2g* (1)			1
HH18	*vtx2c*	1		*vtx2c* + *vtc2d* (2)	*vtx2a* + *vtx2d* (2)	5
II19	*vtx2b*		*vtx2g* (1)			1
JJ20	-					0
Total		2	8	10	4	24

sorbitol (26) and ESBL (18) production



β-glucuronidase (15), haemolysin (14) and VT (8) production



participation

	EQA-7	EQA-5	EQA-4
Full O:H serotyping	63%	66%	64%
β-glucuronidase	50%	52%	68%
haemolysin	47%	48%	54%
Verocytotoxin	27%	28%	39%
Fermentation of sorbitol	↓ 87%	90%	93%
eae	93%	90%	86%
vtx1	↓ 93%	97%	89%
vtx2	93%	97%	89%
vtx subtyping	↑ 83%	76%	71%
ehxA gene	↓ 60%	66%	71%
ESBL	60%	62%	61%
aggR	70%	69%	61%
aaiC	↑ 57%	55%	39%

Conclusions

EQA-7	EQA-5	EQA-4	
71%	69%	93%	O:H serotype correctly
73%	90%	89%	β-glucuronidase
64%	94%	80%	haemolysin
63%	86%	87%	Verocytotoxin
88%	97%	95%	Fermentation of sorbitol
89%	100%	100%	ESBL

Genotypic detection

86%	98%	94%	*eae*
100%	98%	96%	*vtx1;* subtyping: 88% 98% 89%
89%	94%	97%	*vtx2;* subtyping: 52% 94% 87%
83%	98%	99%	*ehxA*
75%	100%	100%	*aggR* (21/20/17 participants)
61%	80%	65%	*aaiC* (17/20/17 participants)
-	65%	89%	ETEC gene *elt*

Test panel: > two errors

Strain ID	Serotyping		Genotyping								Phenotyping					Additional virulence genes	Pathotype
	O group	H type	*aaiC* gene	*aggR* gene	*eae* gene	*ehxA* gene	*vtx1* gene	*vtx2* gene	*vtx1* subtypes	*vtx2* subtypes	β-glucuronidase production	ESBL production	Haemolysin production	Sorbitol fermentation	Verocytotoxin production		
AA11	**O80**	**H2**	-	-	+	+	-	+	-	*vtx2a*	+	-	+	+	+		**VTEC**
BB12	**O26**	**H11**	-	-	+	+	-	+	-	*vtx2a*	+	-	+	+	+		**VTEC**
CC13	**O78**	**H2**	+	+	-	-	-	-	-	-	+	-	-	+	-	*aatA*	**EAEC**
DD14	**O145**	**H34**	-	-	+	-	-	+	-	*vtx2f*	+	-	-	-	+		**VTEC**
EE15	**O166**	**H15**	-	-	-	-	-	+	-	*vtx2d*	+	+	-	+	+	*eltA*	**VTEC-ETEC**
FF16	**O156**	**H4**	-	-	-	-	-	+	-	*vtx2d*	+	-	-	+	+		**VTEC**
GG17	**O146**	**H21**	-	-	-	+	+	+	*vtx1c*	*vtx2b*	+	-	+	+	+		**VTEC**
HH18	**O157**	**[H7]**	-	-	+	+	+	+	*vtx1a*	*vtx2c*	-	-	+	-	+		**VTEC**
II19	**O91**	**H14**	-	-	-	+	+	+	*vtx1a*	*vtx2b*	+	-	+	+	+	*saa*	**VTEC**
JJ20	**O103**	**H2**	-	-	+	+	+	-	*vtx1a*	-	+	-	+	+	+		**VTEC**

Personel from SSI

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& EQA Reports
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Head of laboratory
Mia Torpdahl

VTEC
Flemming Scheutz

PFGE/MLVA/Molecular serotype
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VTEC testing
Susanne Jespersen