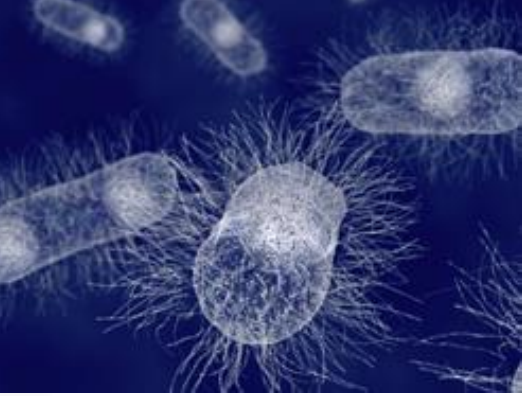


# 8th external quality assurance (EQA-8) scheme for typing of VTEC in 2017-2018

**The EQA-8 includes four parts**

- **O and H Serotyping**
- **Virulence gene determination (*vtx*1, *vtx*2, *eae*, *aai*C, *agg*R)**
- ***vtx* subtyping**
- **Cluster-EQA: Molecular typing-based cluster analyses**

- **DATES TO REMEMBER**

| | |
|---|---|
| **23th of October 2017** | **Deadline to respond to the invitation online** |
| **20th November - 4th of December 2017** | **Shipment of test strains** |
| **23th of February 2018** | **Deadline for submission of results** |

# Test panel

| Strain no. | O group | H type | *eae* geneH | *stx1* gene | *stx2* gene | *stx1* subtypes | *stx2* subtypes | *aggR* gene | *aaiC* gene | Additional virulence genes | Pathotype |
|---|---|---|---|---|---|---|---|---|---|---|---|
| REF1 | O126 | H27/H$^-$ | - | - | - | - | - | + | + | AAFII *aap aar fyuA lpfA pet pic* | EAEC |
| REF2 | O121 | H19 | + | - | + | - | *stx2a* | - | - | *ehxA efa1 lpfA hlyD toxB* | STEC |
| REF3 | O187 | H28 | - | - | + | - | *stx2g* | - | - | *estAp lfpA katP, astA* | STEC-ETEC |
| REF4 | O157 | H7 | + | - | + | - | *stx2a*<br>*stx2c* | - | - | *ehxA iha iss katP toxB* | STEC |
| REF5 | O63 | H6/H$^-$ | + | - | + | - | *stx2f* | - | - | *cif* | STEC |
| REF6 | O146 | H28 | - | + | + | *stx1a* | *stx2b* | - | - | *ireA iha lpfA subA* | STEC |
| REF7 | O8 | H9 | - | - | + | - | *stx2e* | - | - | *iss lpfA fyuA iha iss astA* | STEC |
| REF8 | O104 | H4 | - | - | - | - | - | + | + | AAFI *aap aar fyuA lpfA sigA pic* | EAEC |
| REF9 | O91 | H14 | - | + | + | *stx1a* | *stx2b* | - | - | *ehxA ire iha lpfA saa hlyD* | STEC |
| REF10 | O154 | H31 | - | + | - | *stx1d* | - | - | - | air eilA | STEC |
| REF11 | O111 | H8/H$^-$ | + | + | - | *stx1a* | - | - | - | *ehxA cif efa1 hlyD iha lpfA* | STEC |
| REF12 | O157 | H7 | + | + | + | *stx1a* | *stx2a* | - | - | *ehxA iha iss katP toxB* | STEC |

# 25 Participants

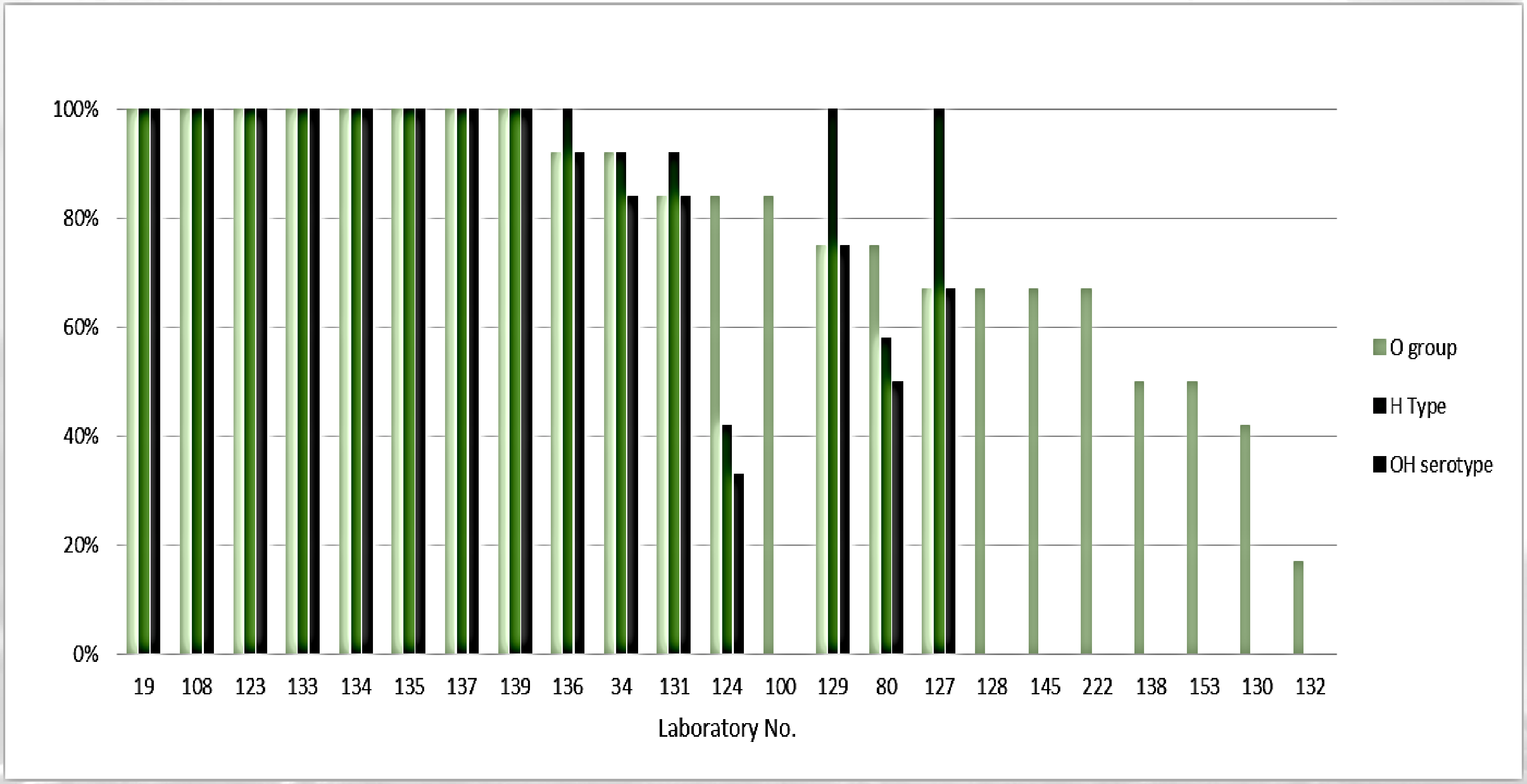
Austria
Belgium
Czech Republic
Denmark
Estonia
Finland
France
Germany
Greece
Hungary
Iceland
Ireland
Italy
Latvia
Lithuania
Luxembourg
Norway
Poland
Portugal
Romania
Slovenia
Spain
Sweden
The Netherlands
United Kingdom

# Number and percentage of laboratories submitting results for each part in EQA-8

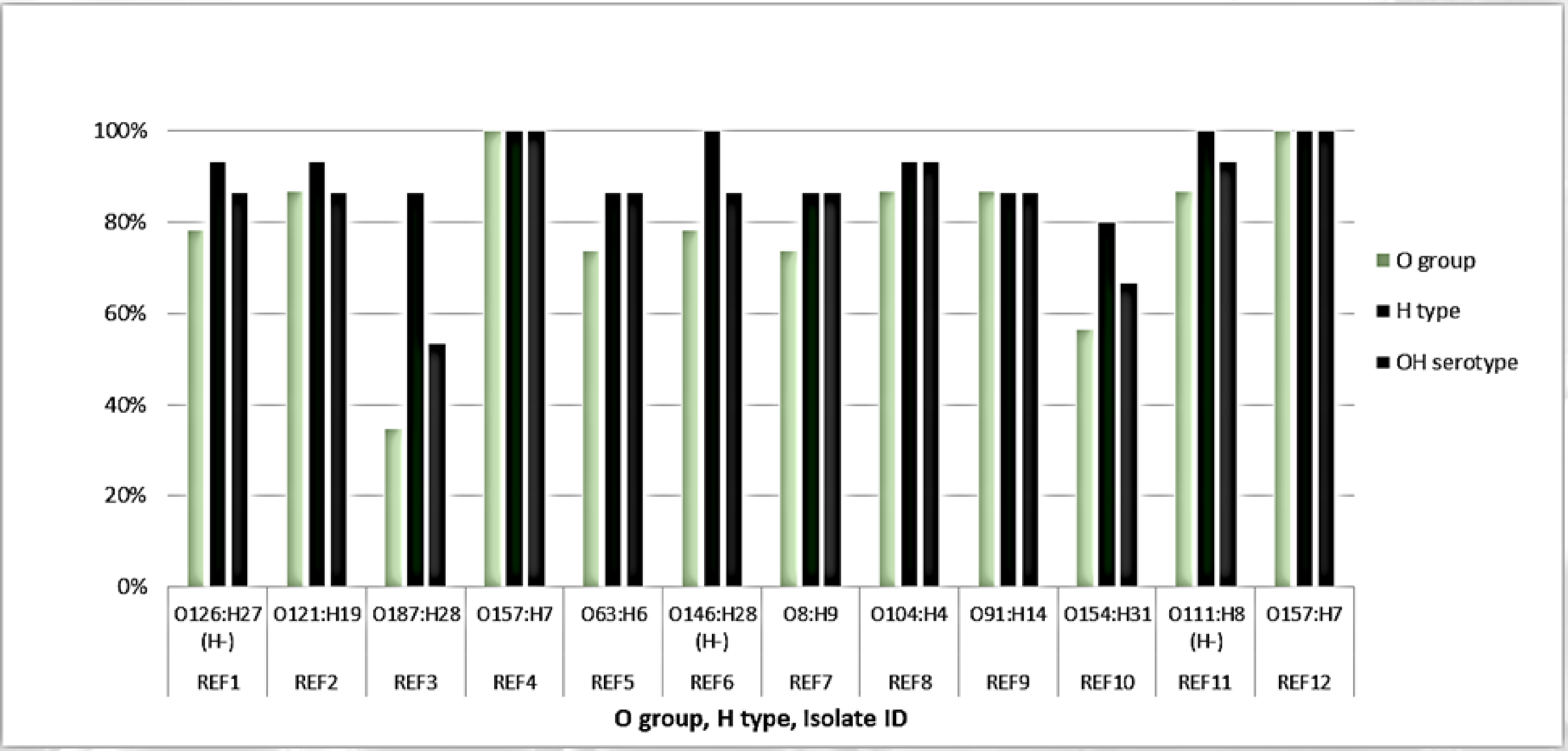
| | Serotyping<br>N=23 | | Virulence profile<br>N=25 | | | | | Cluster analysis<br>N=18 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | O group | H type | *aaiC* | *aggR* | *eae* | *stx1* and *stx2* | *stx* subtyping | PFGE | WGS | Both |
| **Number of participants** | **23[#]** | **15** | **20** | **20** | **25** | **25** | **20** | **9** | **11** | **2** |
| **Percentage of participants** | **100%** | **65%** | **80%** | **80%** | **100%** | **100%** | **80%** | **50%** | **61%** | **11%** |
| **Percentage of participants** | **92%** | **60%** | **80%** | **80%** | **100%** | **100%** | **80%** | **36%** | **44%** | **8%** |

[#] ***Phenotypic (N=13)/PCR based (N=4)/WGS based (N=6)***

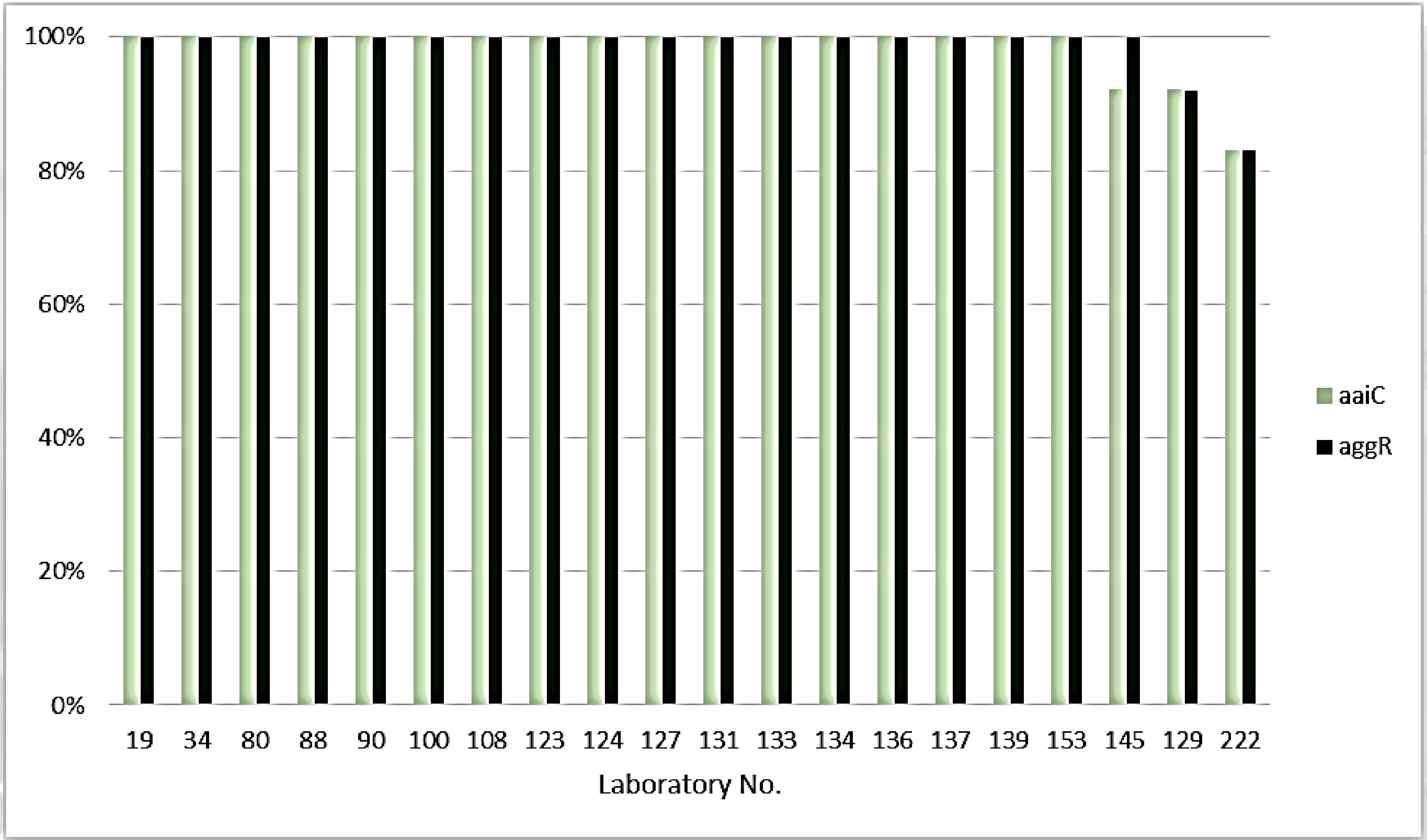
# Participant percentage scores for O grouping and H typing



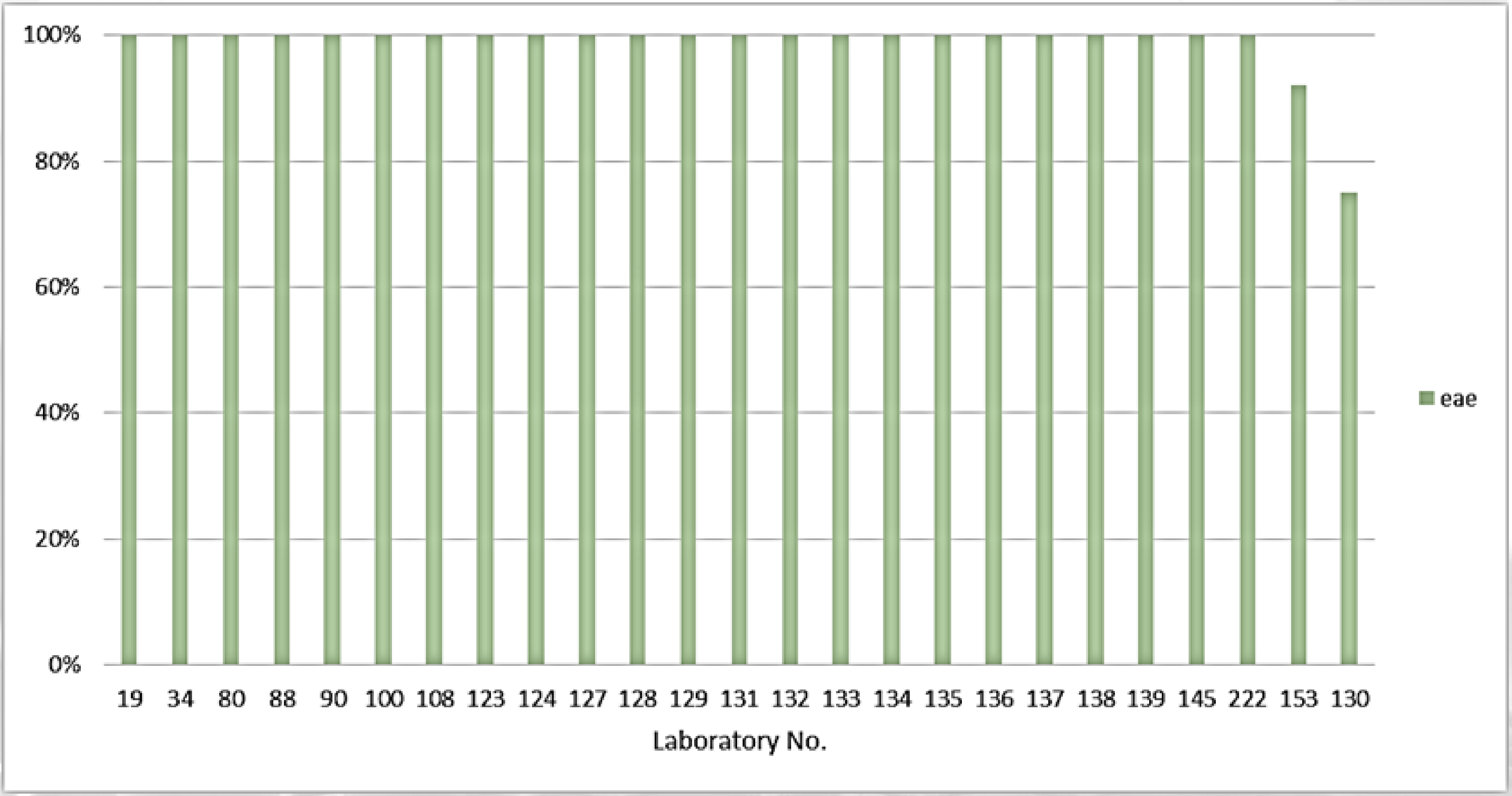
# Average percentage test isolate score for serotyping of O and H



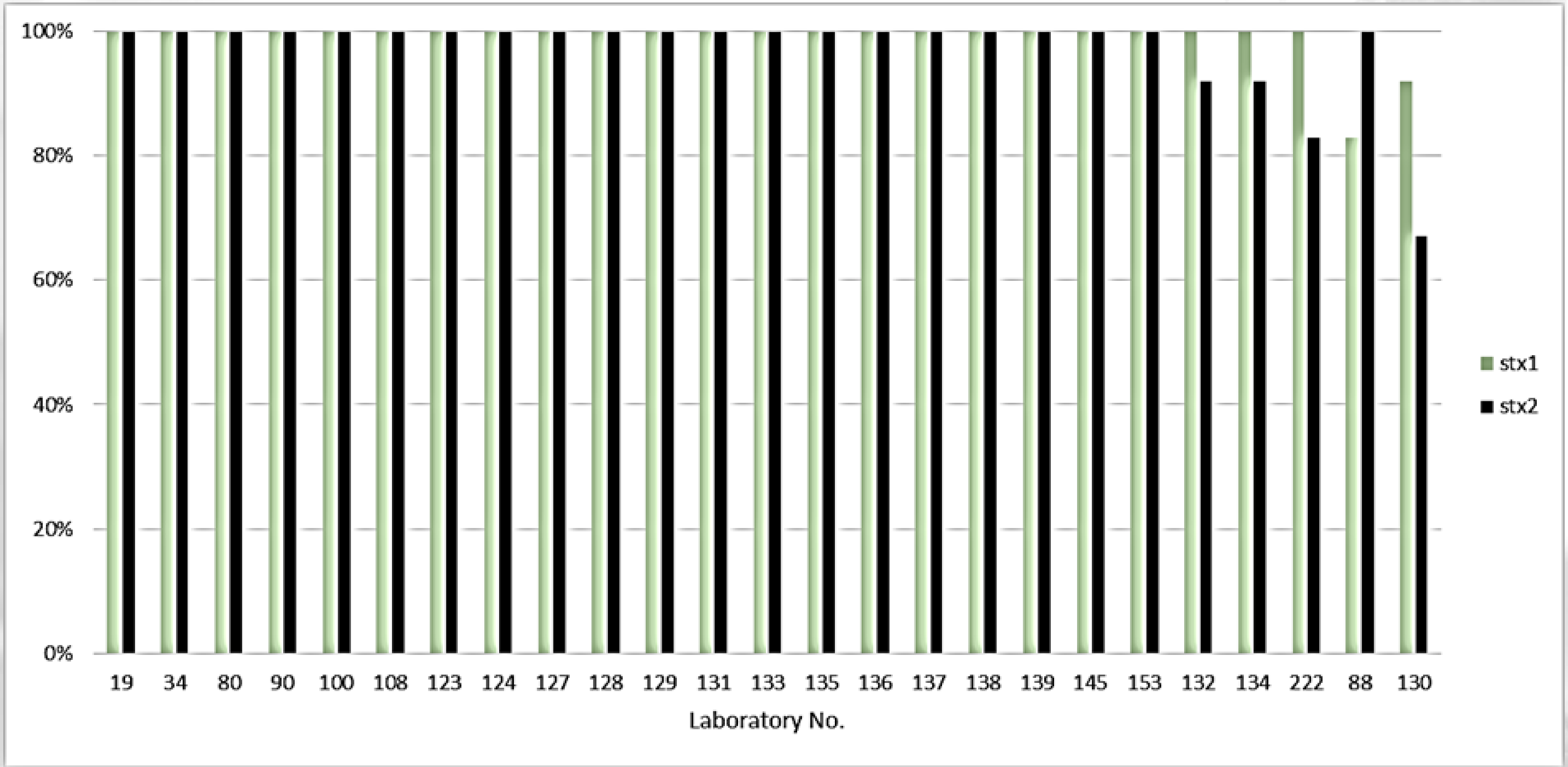
# Participant percentage scores for genotyping of *aaiC* and *aggR*



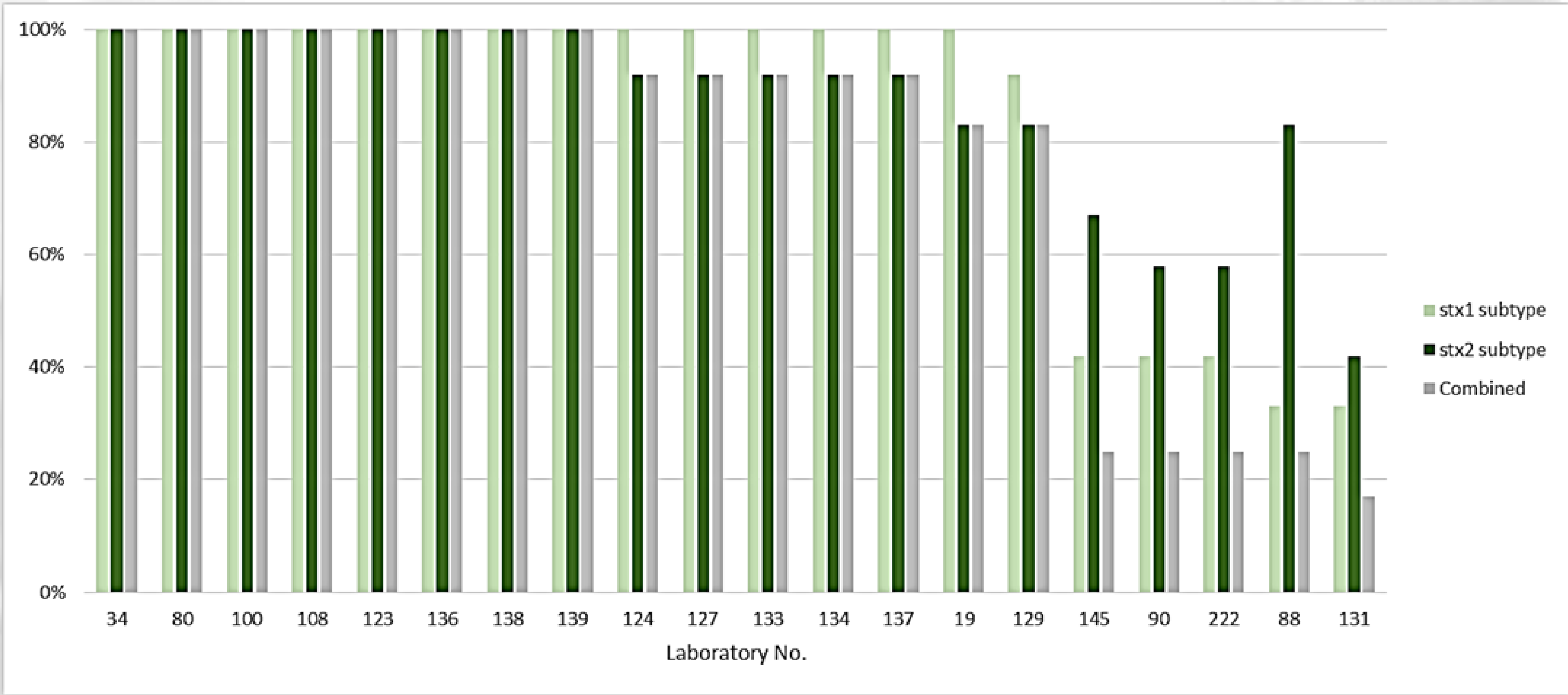
# Participant percentage scores for genotyping of *eae*



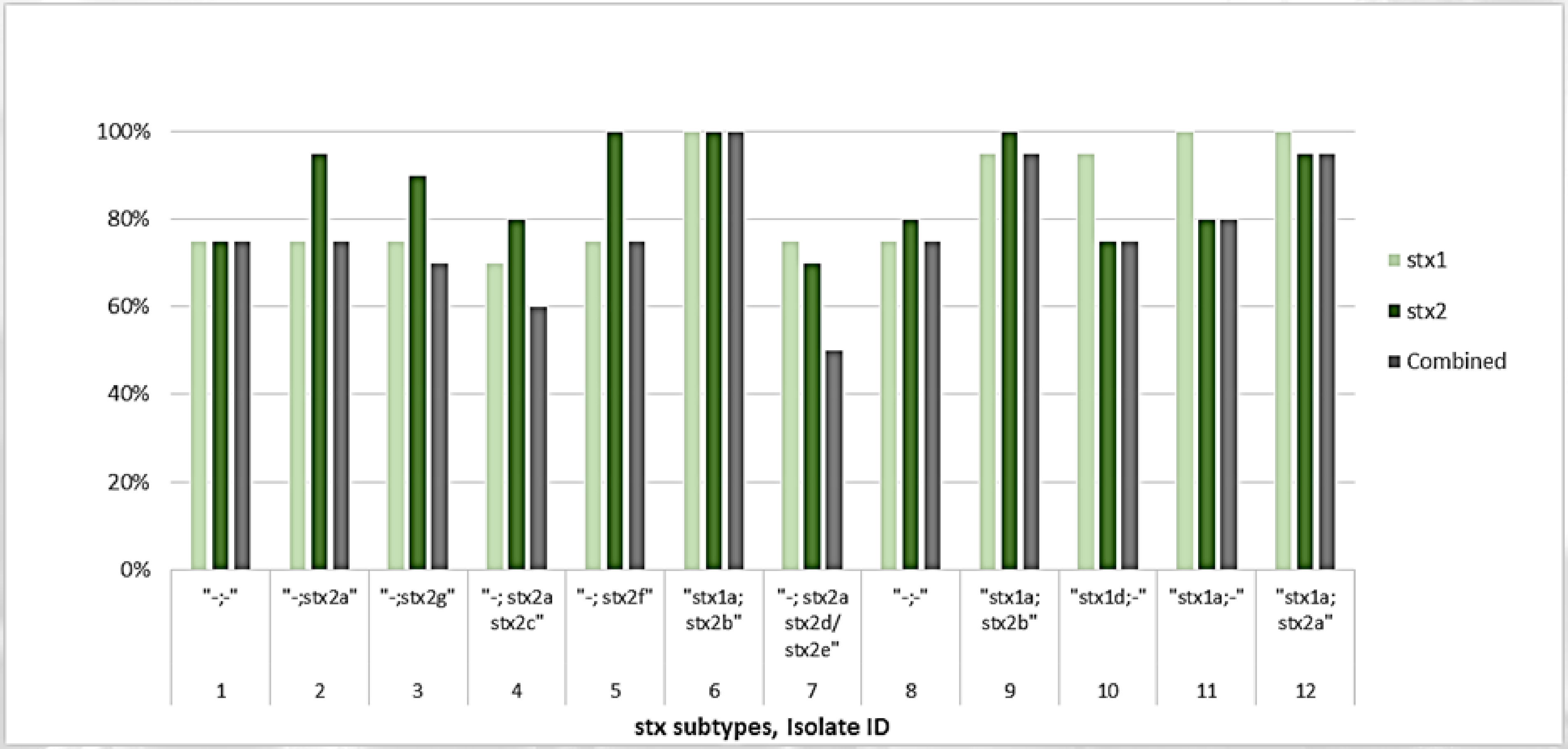
# Participant percentage scores for detection of *stx1* and *stx2*



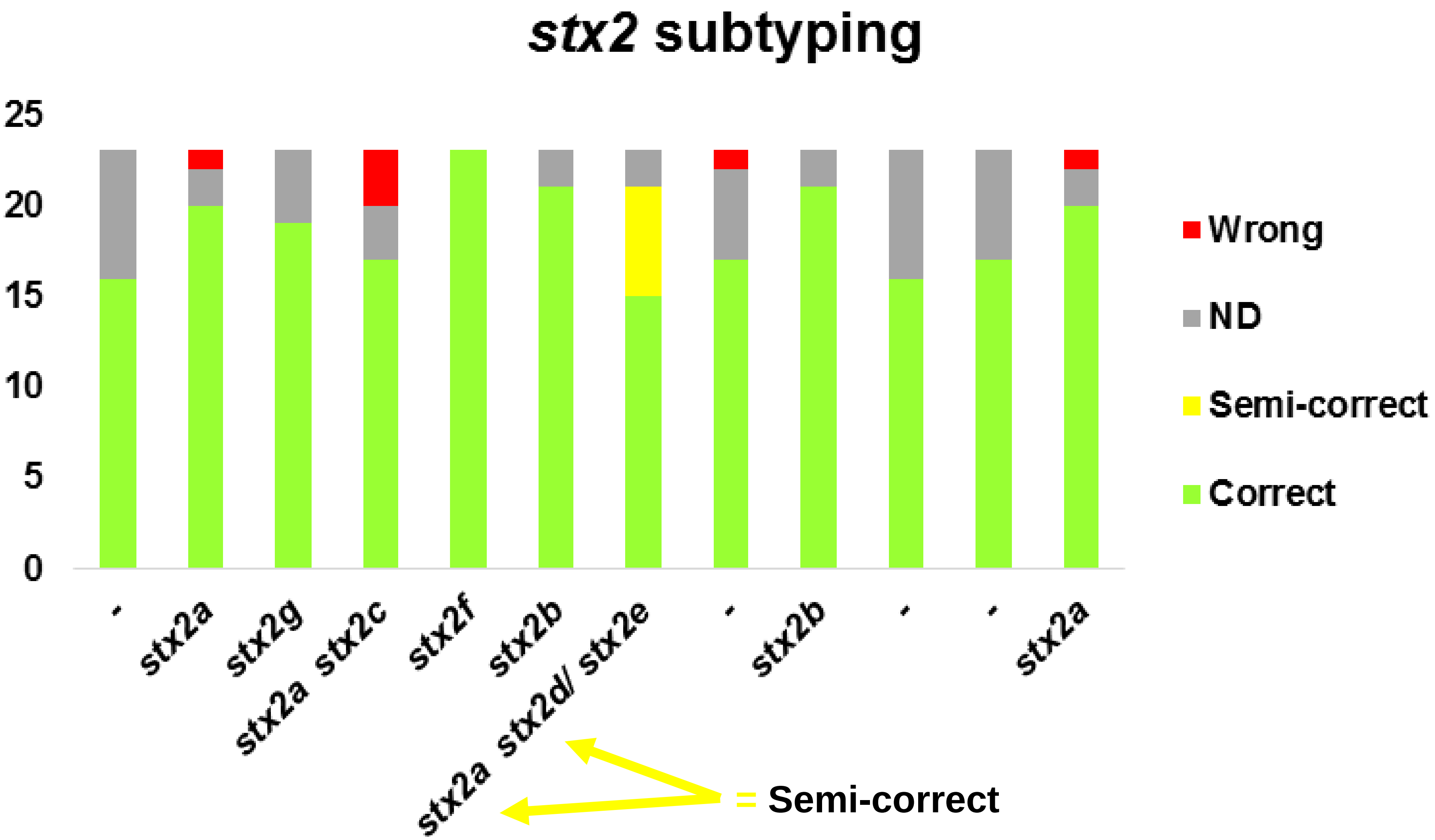
# Participant percentage scores for subtyping of *stx1* and *stx2*



# Average percentage test isolate score for subtyping of *stx1* and *stx2*



# Test isolate score for subtyping of *stx2* *with a twist*

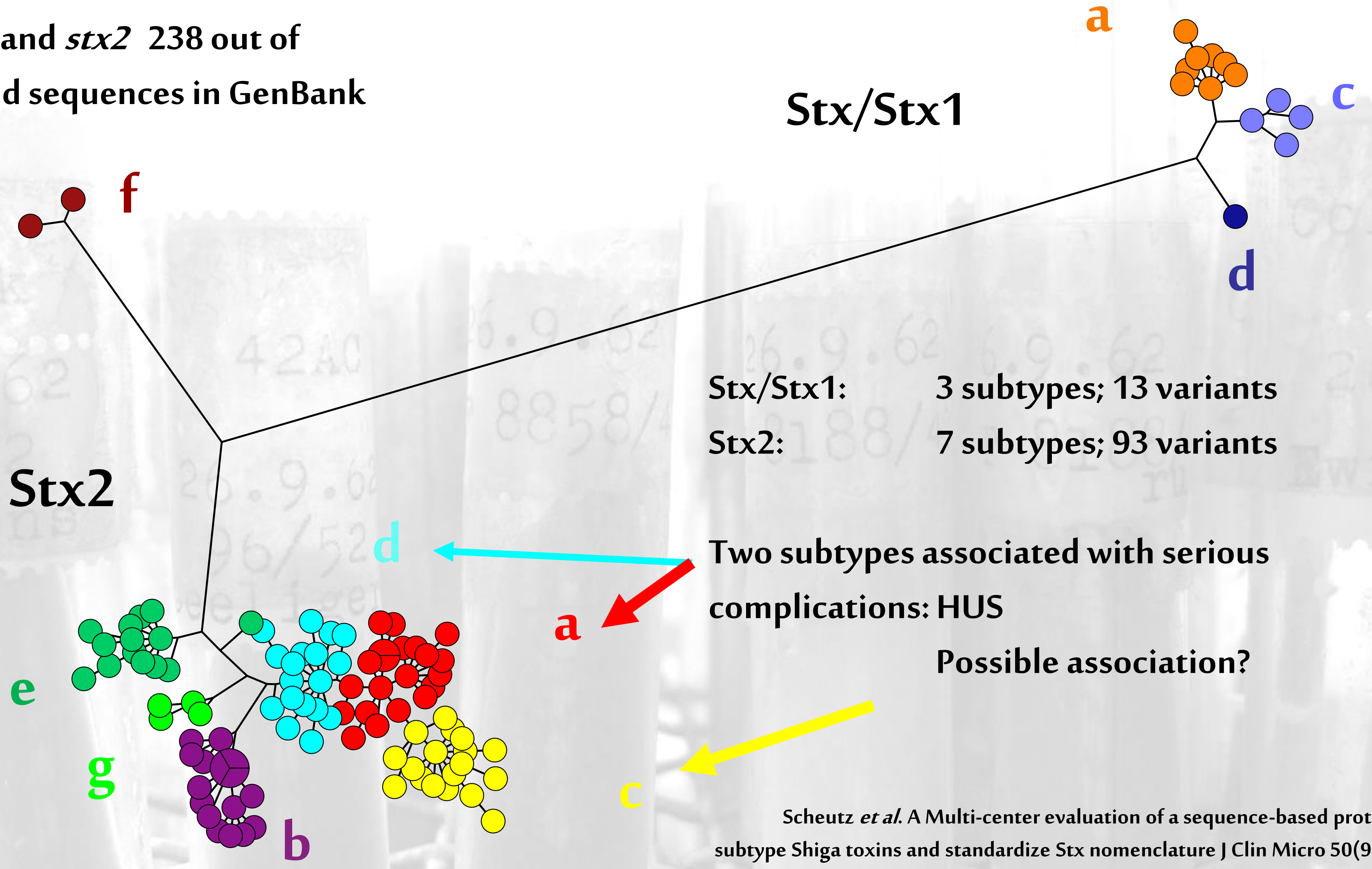


= **Semi-correct**

# Stx subtypes and variants

47 *stx1* and *stx2* 238 out of

285 valid sequences in GenBank



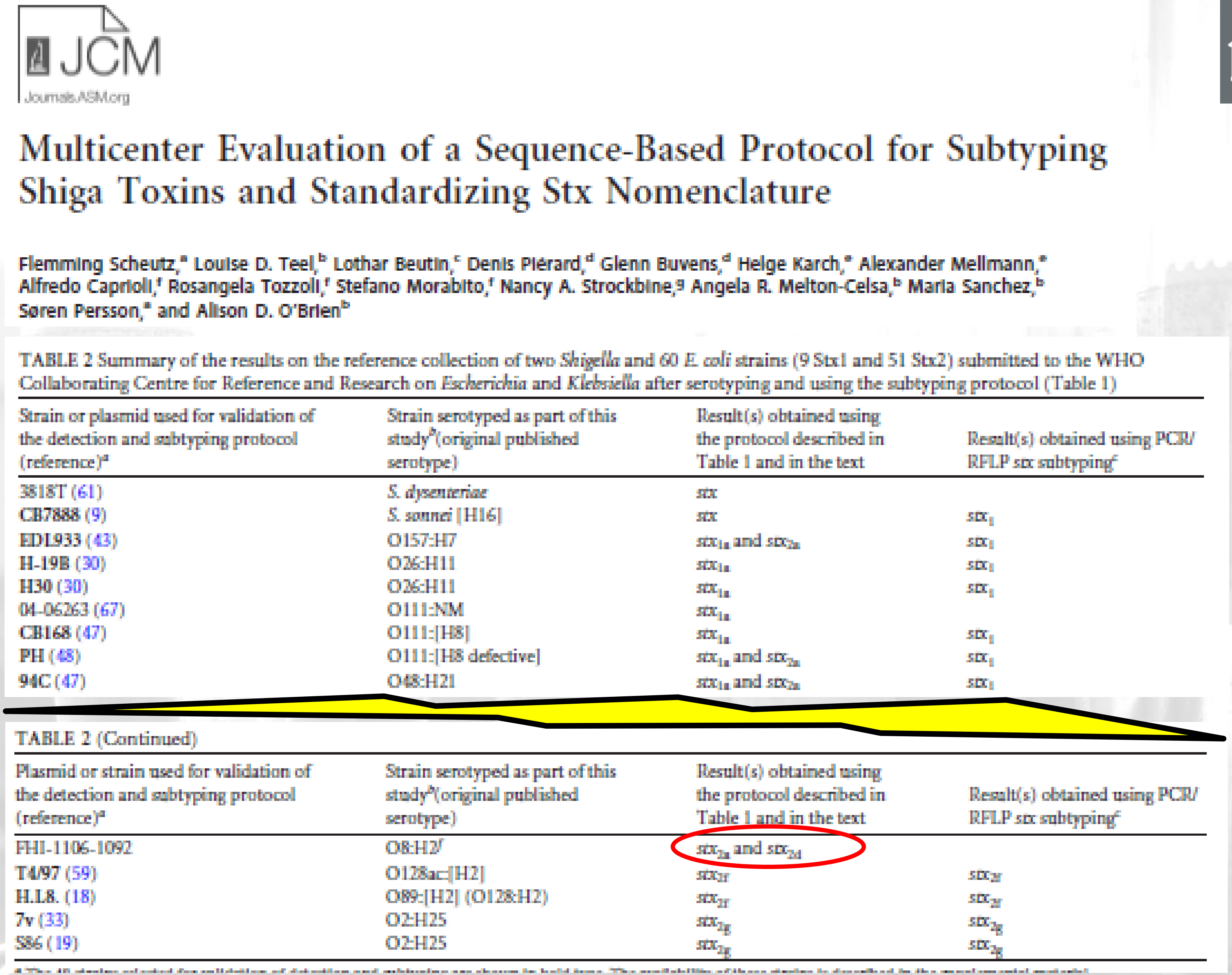
Stx/Stx1: 3 subtypes; 13 variants

Stx2: 7 subtypes; 93 variants

Two subtypes associated with serious complications: HUS

Possible association?

Scheutz *et al.* A Multi-center evaluation of a sequence-based protocol to subtype Shiga toxins and standardize Stx nomenclature J Clin Micro 50(9). 2012



JCM
Journals.ASM.org

# Multicenter Evaluation of a Sequence-Based Protocol for Subtyping Shiga Toxins and Standardizing Stx Nomenclature

Flemming Scheutz,[a] Louise D. Teel,[b] Lothar Beutin,[c] Denis Piérard,[d] Glenn Buvens,[d] Helge Karch,[e] Alexander Mellmann,[e] Alfredo Caprioli,[f] Rosangela Tozzoli,[f] Stefano Morabito,[f] Nancy A. Strockbine,[g] Angela R. Melton-Celsa,[b] Maria Sanchez,[b] Søren Persson,[a] and Alison D. O'Brien[b]

TABLE 2 Summary of the results on the reference collection of two *Shigella* and 60 *E. coli* strains (9 Stx1 and 51 Stx2) submitted to the WHO Collaborating Centre for Reference and Research on *Escherichia* and *Klebsiella* after serotyping and using the subtyping protocol (Table 1)

| Strain or plasmid used for validation of the detection and subtyping protocol (reference)[a] | Strain serotyped as part of this study[b](original published serotype) | Result(s) obtained using the protocol described in Table 1 and in the text | Result(s) obtained using PCR/RFLP *stx* subtyping[c] |
|---|---|---|---|
| **3818T** (61) | *S. dysenteriae* | *stx* | |
| **CB7888** (9) | *S. sonnei* [H16] | *stx* | $stx_1$ |
| **EDL933** (43) | O157:H7 | $stx_{1a}$ and $stx_{2a}$ | $stx_1$ |
| **H-19B** (30) | O26:H11 | $stx_{1a}$ | $stx_1$ |
| **H30** (30) | O26:H11 | $stx_{1a}$ | $stx_1$ |
| **04-06263** (67) | O111:NM | $stx_{1a}$ | |
| **CB168** (47) | O111:[H8] | $stx_{1a}$ | $stx_1$ |
| **PH** (48) | O111:[H8 defective] | $stx_{1a}$ and $stx_{2a}$ | $stx_1$ |
| **94C** (47) | O48:H21 | $stx_{1a}$ and $stx_{2a}$ | $stx_1$ |

TABLE 2 (Continued)

| Plasmid or strain used for validation of the detection and subtyping protocol (reference)[a] | Strain serotyped as part of this study[b](original published serotype) | Result(s) obtained using the protocol described in Table 1 and in the text | Result(s) obtained using PCR/RFLP *stx* subtyping[c] |
|---|---|---|---|
| FHI-1106-1092 | O8:H2[f] | $stx_{2a}$ and $stx_{2d}$ | |
| **T4/97** (59) | O128ac:[H2] | $stx_{2f}$ | $stx_{2f}$ |
| **H.I.8.** (18) | O89:[H2] (O128:H2) | $stx_{2f}$ | $stx_{2f}$ |
| **7v** (33) | O2:H25 | $stx_{2g}$ | $stx_{2g}$ |
| **S86** (19) | O2:H25 | $stx_{2g}$ | $stx_{2g}$ |

JCM
Journals.ASM.org

# Multicenter Evaluation of a Sequence-Based Protocol for Subtyping Shiga Toxins and Standardizing Stx Nomenclature

Flemming Scheutz,[a] Louise D. Teel,[b] Lothar Beutin,[c] Denis Piérard,[d] Glenn Buvens,[d] Helge Karch,[e] Alexander Mellmann,[e] Alfredo Caprioli,[f] Rosangela Tozzoli,[f] Stefano Morabito,[f] Nancy A. Strockbine,[g] Angela R. Melton-Celsa,[b] Maria Sanchez,[b] Søren Persson,[a] and Alison D. O'Brien[b]

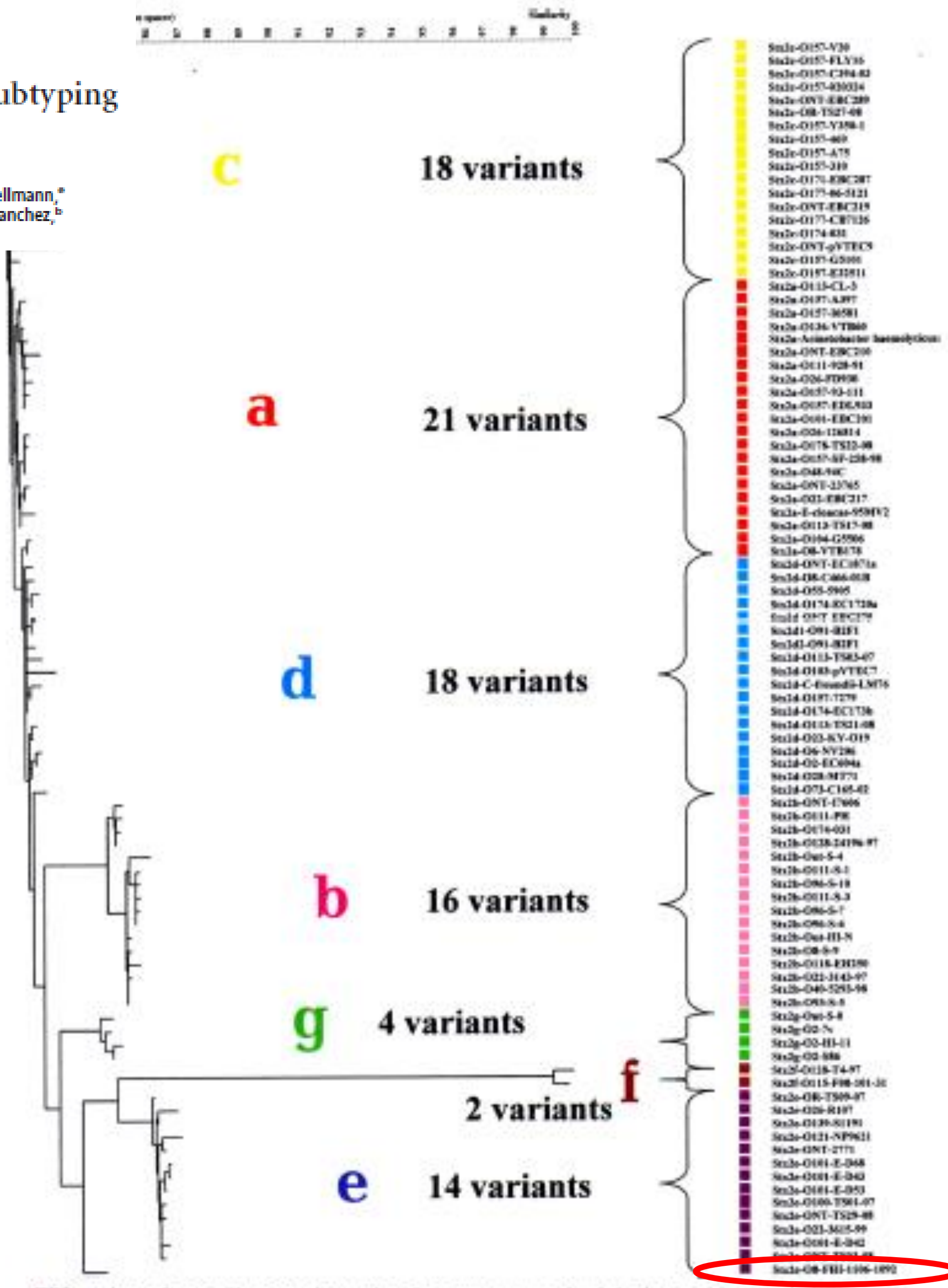
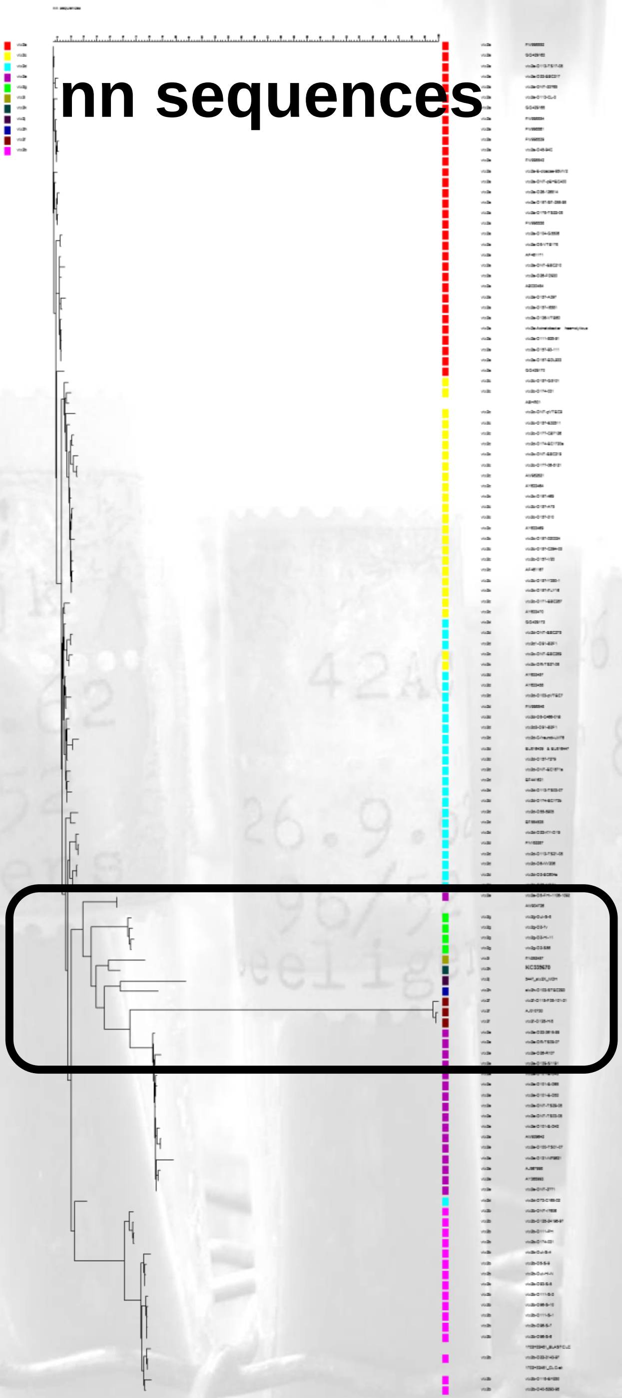
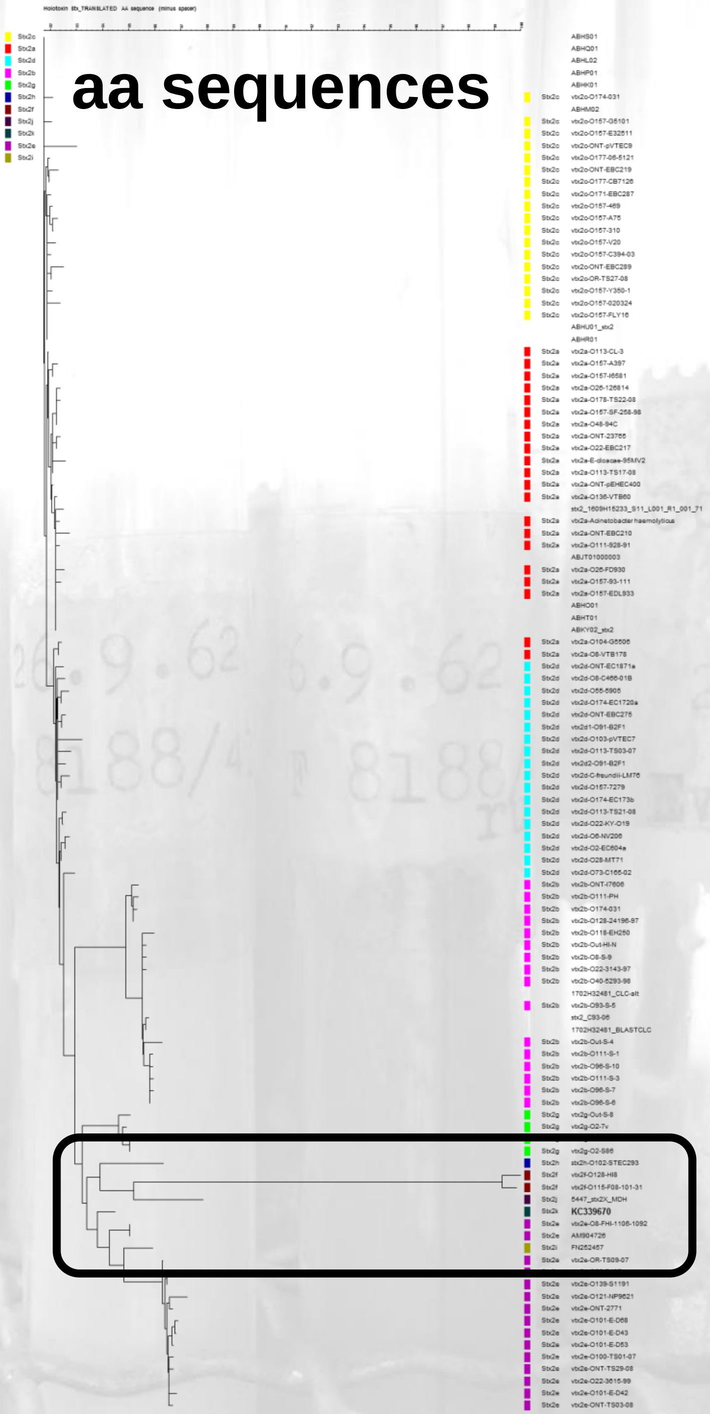


FIG 2 Five clusters of Stx2: neighbor-joining cluster analysis of 95 unique sequences as described in the text and the proposed new designations.

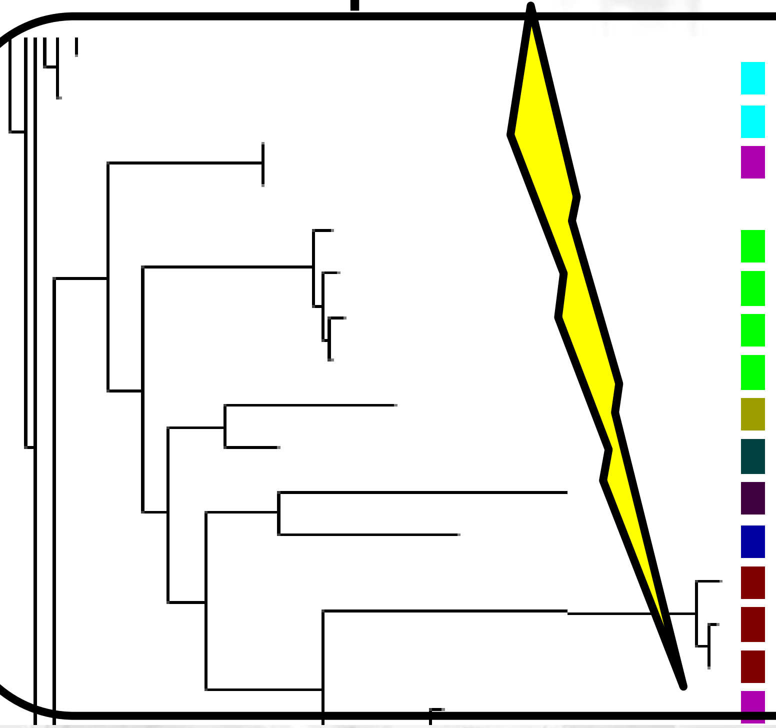
## nn sequences



## aa sequences

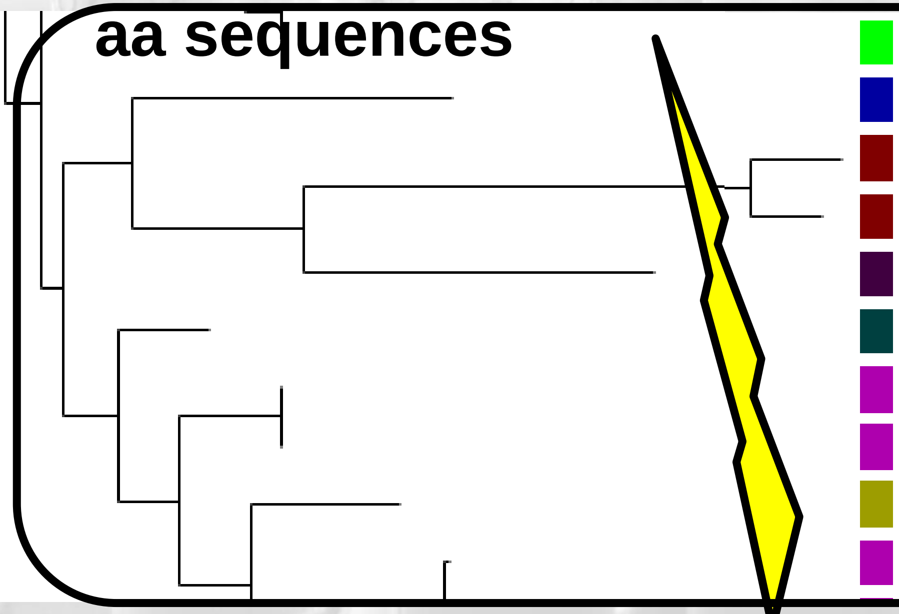


# nn sequences



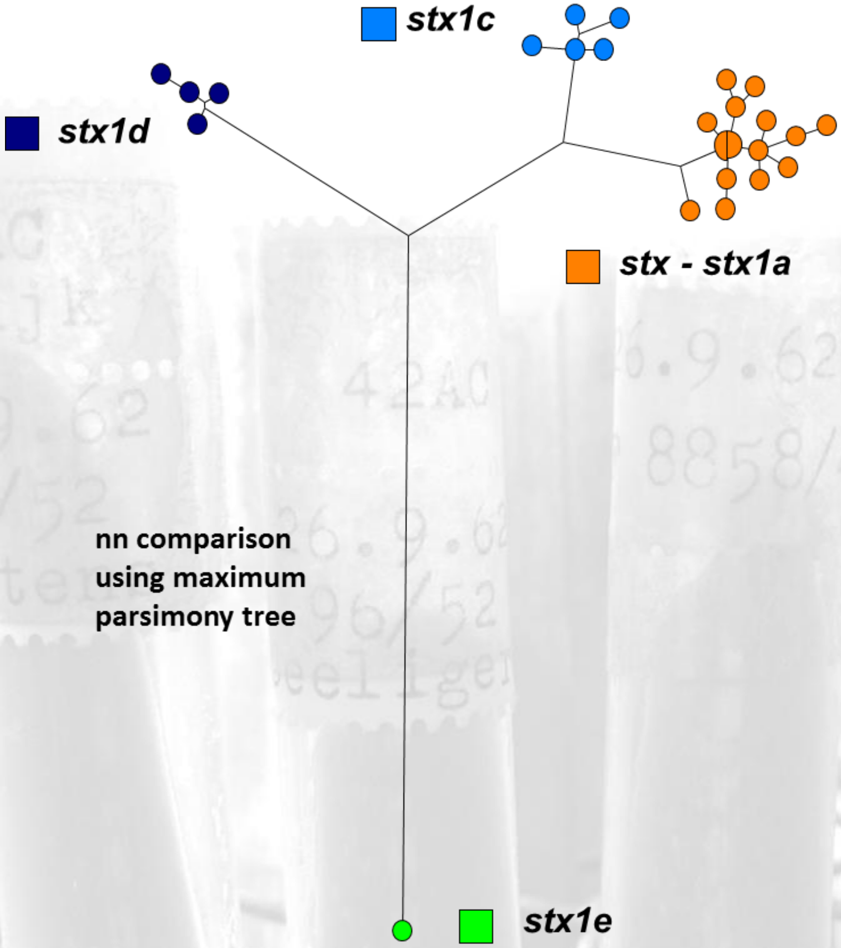
| | | |
|---|---|---|
| 🟦 | stx2d | stx2d-O2-EC804a |
| 🟦 | stx2d | [illegible] |
| 🟪 | stx2e | AM904726 = ***stx2l*-O8-FHI-1106-1092** |
| | | AM904726 |
| 🟩 | stx2g | stx2g-Out-S-8 |
| 🟩 | stx2g | stx2g-O2-Tv |
| 🟩 | stx2g | stx2g-O2-HI-11 |
| 🟩 | stx2g | stx2g-O2-S86 |
| 🟨 | stx2i | FN252457 - **stx2*i*-ONT-CB10366** |
| ⬛ | stx2k | KC339670 = ***stx2k*-O159-12GZSW01** |
| 🟪 | stx2j | unpublished - ***stx2j*-ONT-5447** |
| 🟦 | stx2h | CP022279 = ***stx2h*-O102-STEC299** |
| 🟥 | stx2f | stx2f-O115-F08-101-31 |
| 🟥 | stx2f | AJ010730 |
| 🟥 | stx2f | stx2f-O128-HI8 |
| 🟪 | stx2e | stx2e-O22-2815-99 |

# aa sequences

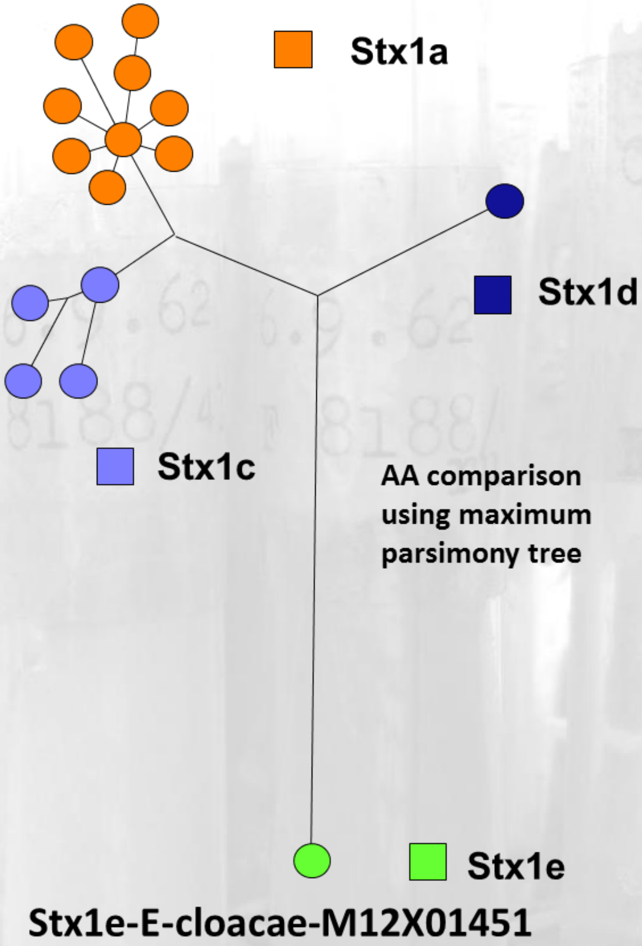


| | | |
|---|---|---|
| 🟩 | Stx2g | stx2g-O2-S86 |
| 🟦 | Stx2h | CP022279 = ***stx2h*-O102-STEC299** |
| 🟥 | Stx2f | stx2f-O128-HI8 |
| 🟥 | Stx2f | stx2f-O115-F08-101-31 |
| 🟪 | Stx2j | unpublished = **Stx2j-ONT-5447** |
| ⬛ | Stx2k | KC339670 = **St2k-O159-12GZSW01** |
| 🟪 | stx2l | AM904726 = **Stx2l-O8-FHI-1106-1092** |
| 🟪 | Stx2e | AM904726 |
| 🟨 | Stx2i | FN252457 = **Stx2i-ONT-CB10366** |
| 🟪 | Stx2e | stx2e-OR-TS09-07 |

# Stx – Stx1 family tree

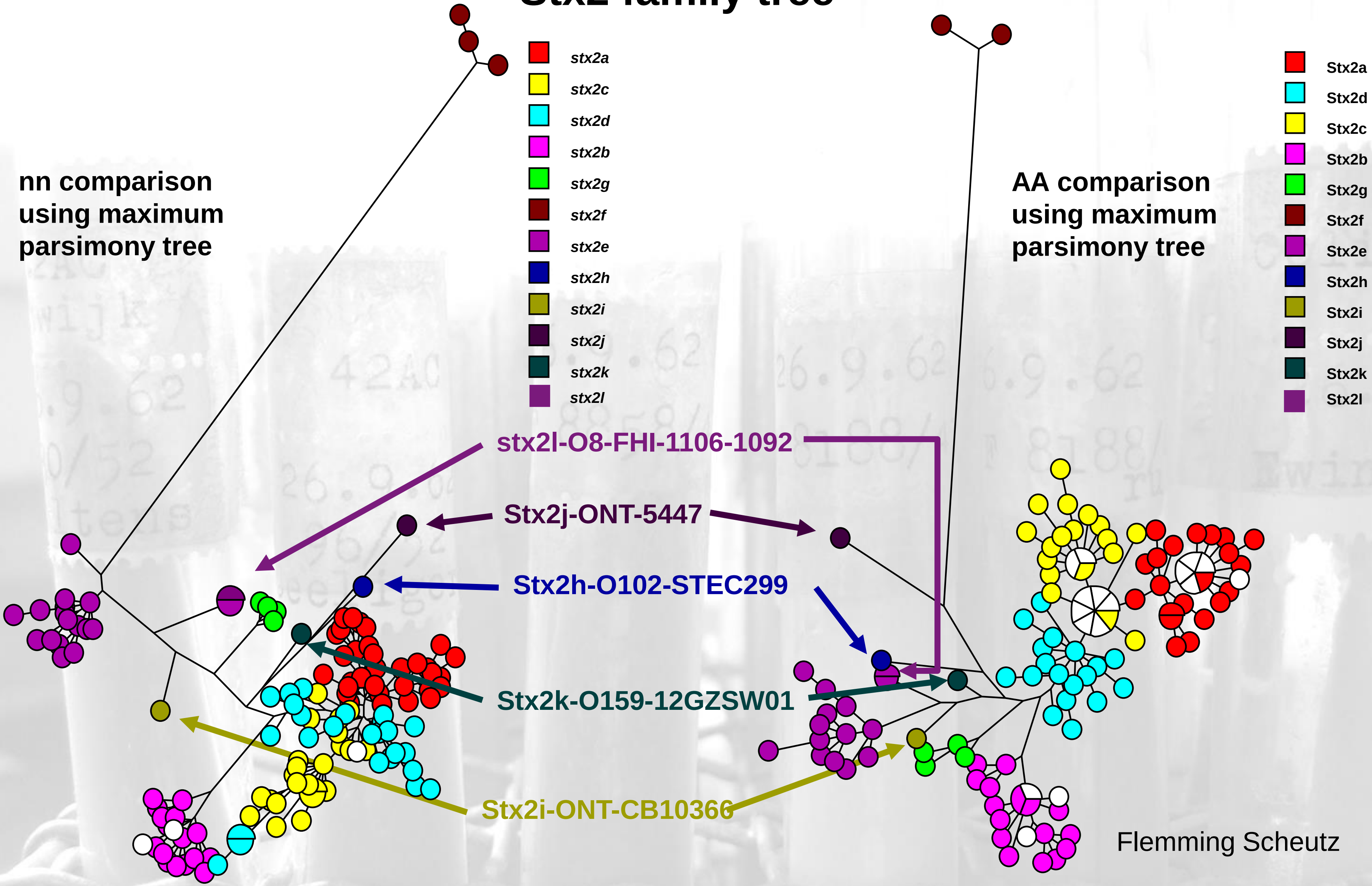


nn comparison
using maximum
parsimony tree



AA comparison
using maximum
parsimony tree

# Stx2 family tree



**nn comparison using maximum parsimony tree**

■ *stx2a*
■ *stx2c*
■ *stx2d*
■ *stx2b*
■ *stx2g*
■ *stx2f*
■ *stx2e*
■ *stx2h*
■ *stx2i*
■ *stx2j*
■ *stx2k*
■ *stx2l*

**AA comparison using maximum parsimony tree**

■ Stx2a
■ Stx2d
■ Stx2c
■ Stx2b
■ Stx2g
■ Stx2f
■ Stx2e
■ Stx2h
■ Stx2i
■ Stx2j
■ Stx2k
■ Stx2l

**stx2l-O8-FHI-1106-1092**

**Stx2j-ONT-5447**

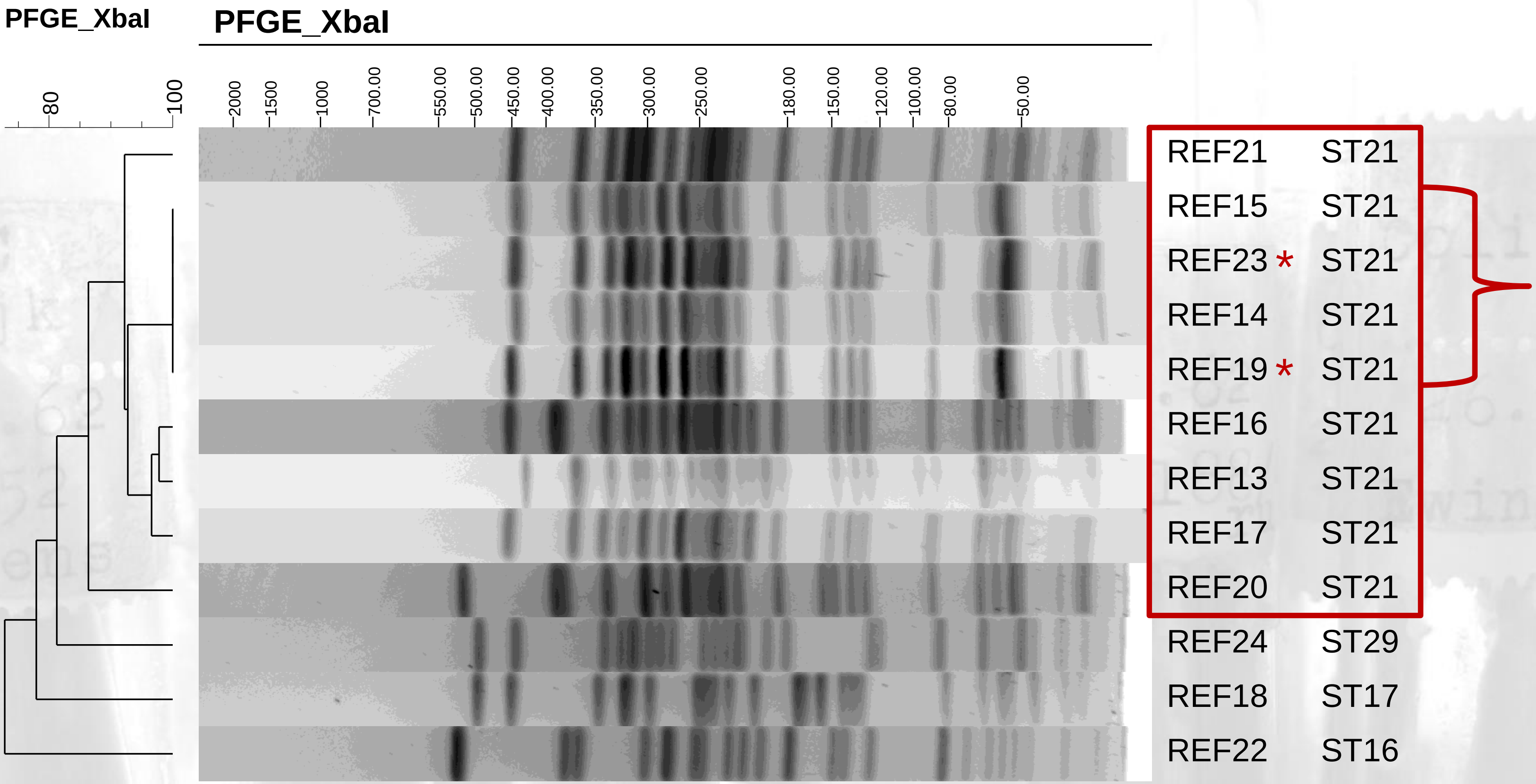
**Stx2h-O102-STEC299**

**Stx2k-O159-12GZSW01**

**Stx2i-ONT-CB10366**

Flemming Scheutz

# Cluster analysis based on PFGE-derived data

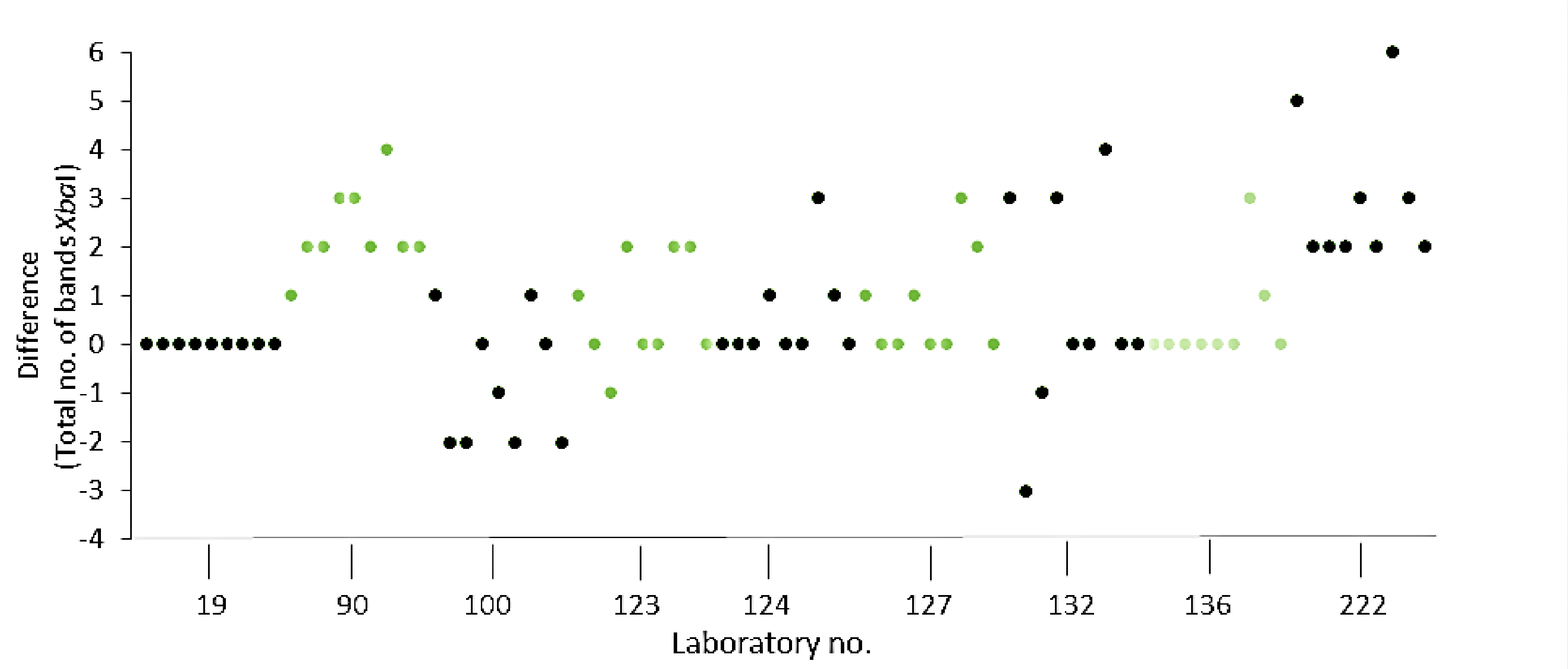


| | |
|---|---|
| REF21 | ST21 |
| REF15 | ST21 |
| REF23 * | ST21 |
| REF14 | ST21 |
| REF19 * | ST21 |
| REF16 | ST21 |
| REF13 | ST21 |
| REF17 | ST21 |
| REF20 | ST21 |
| REF24 | ST29 |
| REF18 | ST17 |
| REF22 | ST16 |

**Cluster of closely related isolates: REF14, REF15, REF19 and REF23**

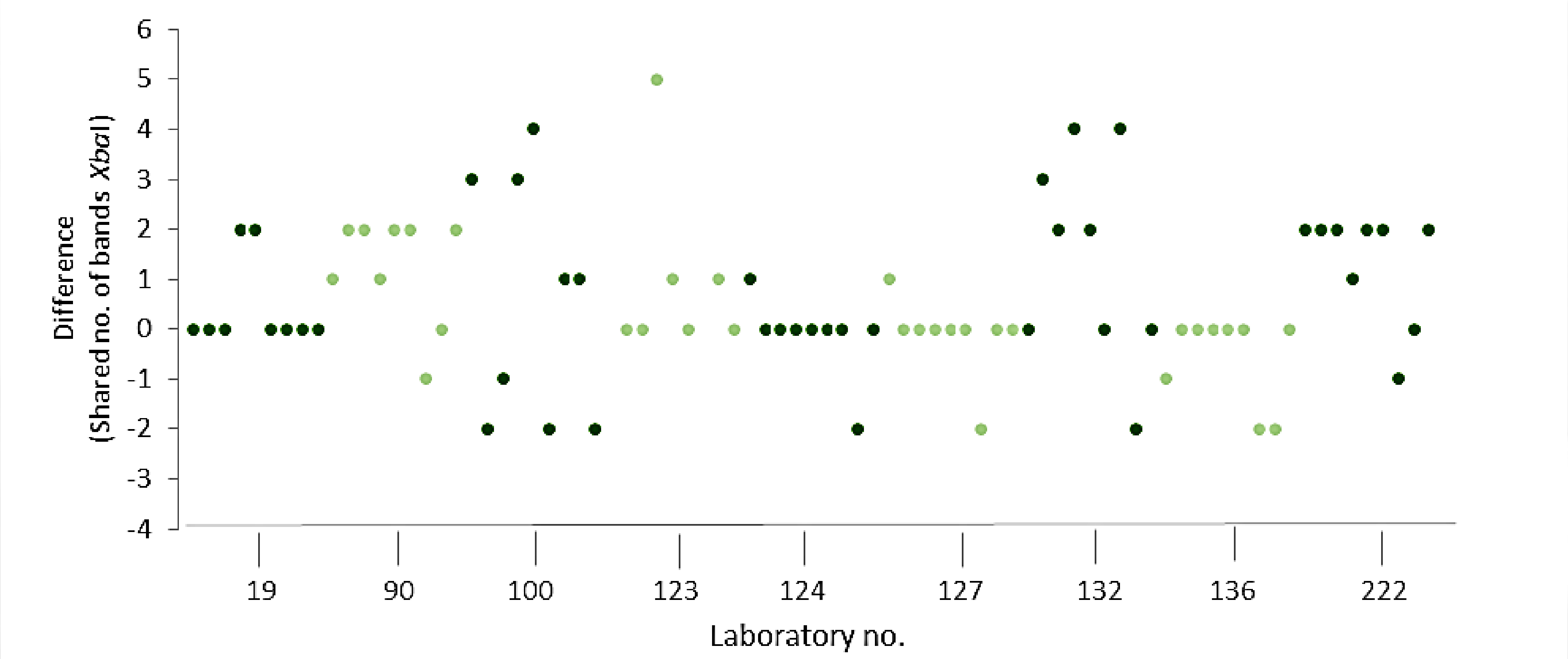
***REF19 and REF23 are technical duplicates.**

# PFGE total number of bands



**Data from all nine ST21 isolates**

# PFGE number of shared bands

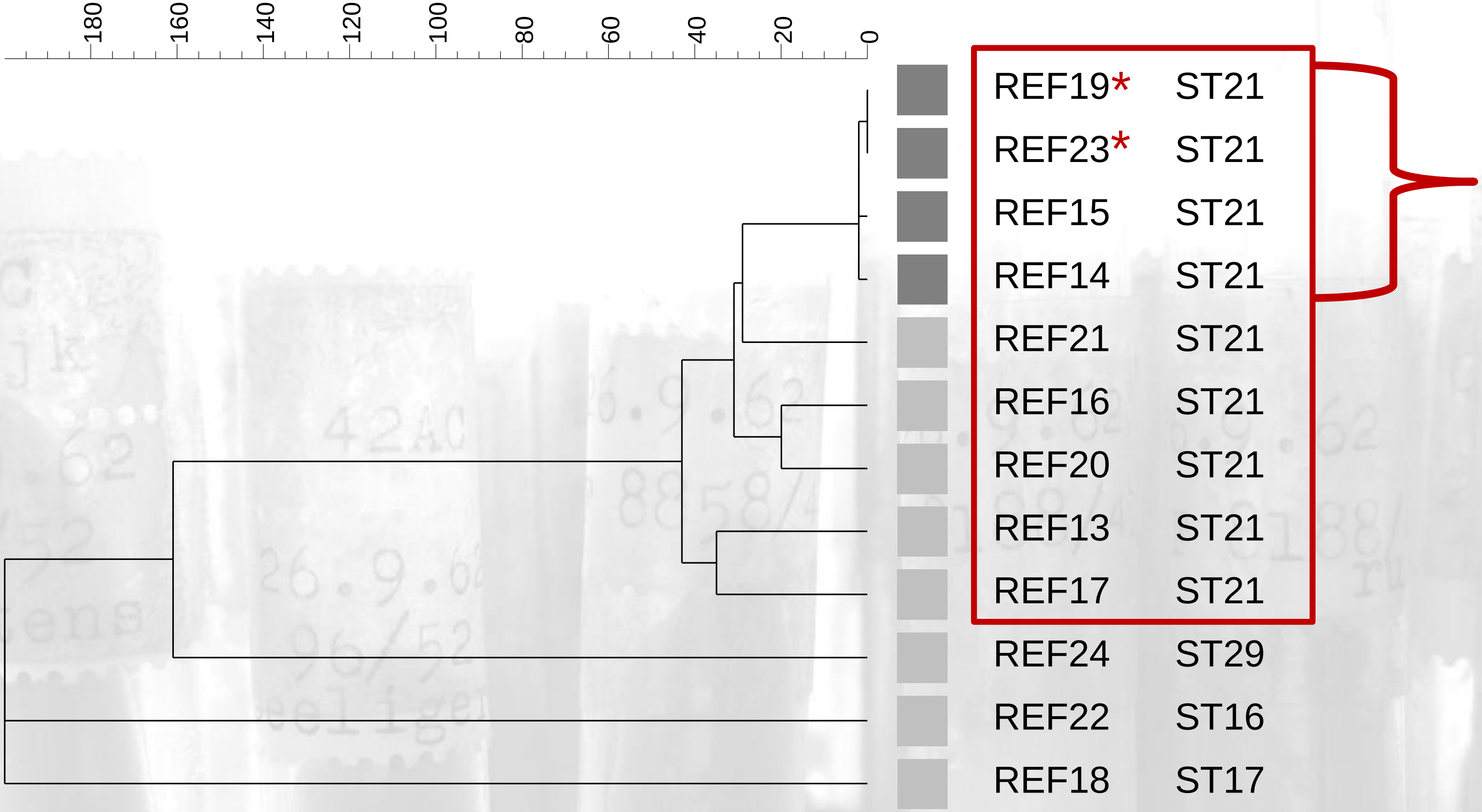


**Data from all nine ST21 isolates**

# Reported cluster of closely related isolates based on PFGE-derived data

| Lab No. | Reported cluster | Corresponding REF isolates | Correct |
|---|---|---|---|
| Provider | | REF14, REF15, REF19, REF23 (19 and 23 technical duplicates) | |
| 19 | 9191, 9211, 9523, 9649 | REF15, REF14, REF19, REF23 | Yes |
| 90 | 9009, 9190, 9647, 9691 | REF15, REF23, REF14, REF19 | Yes |
| 100 | 9136, 9623 | REF19, REF 23 | No |
| 123 | 9479, 9886, 9067, 9159 | REF23, REF14, REF19, REF15 | Yes |
| 124 | 9038, 9052, 9934, 9995, 9929 | REF14, REF19, REF15, REF23, REF21 | No |
| 127 | 9152, 9209, 9704, 9793 | REF15, REF23, REF19, REF14 | Yes |
| 132 | 9095, 9383, 9388, 9637, 9754, 9828, 9881, 9899 | REF14, REF19, REF23, REF15, REF17, REF21, REF16, REF13 | No |
| 136 | 9507, 9797, 9826, 9864 | REF15, REF14, REF19, REF23 | Yes |
| 222 | 9149, 9060, 9330, 9360 | REF14, REF15, REF23, REF19 | Yes |

# Cluster analysis based on WGS-derived data

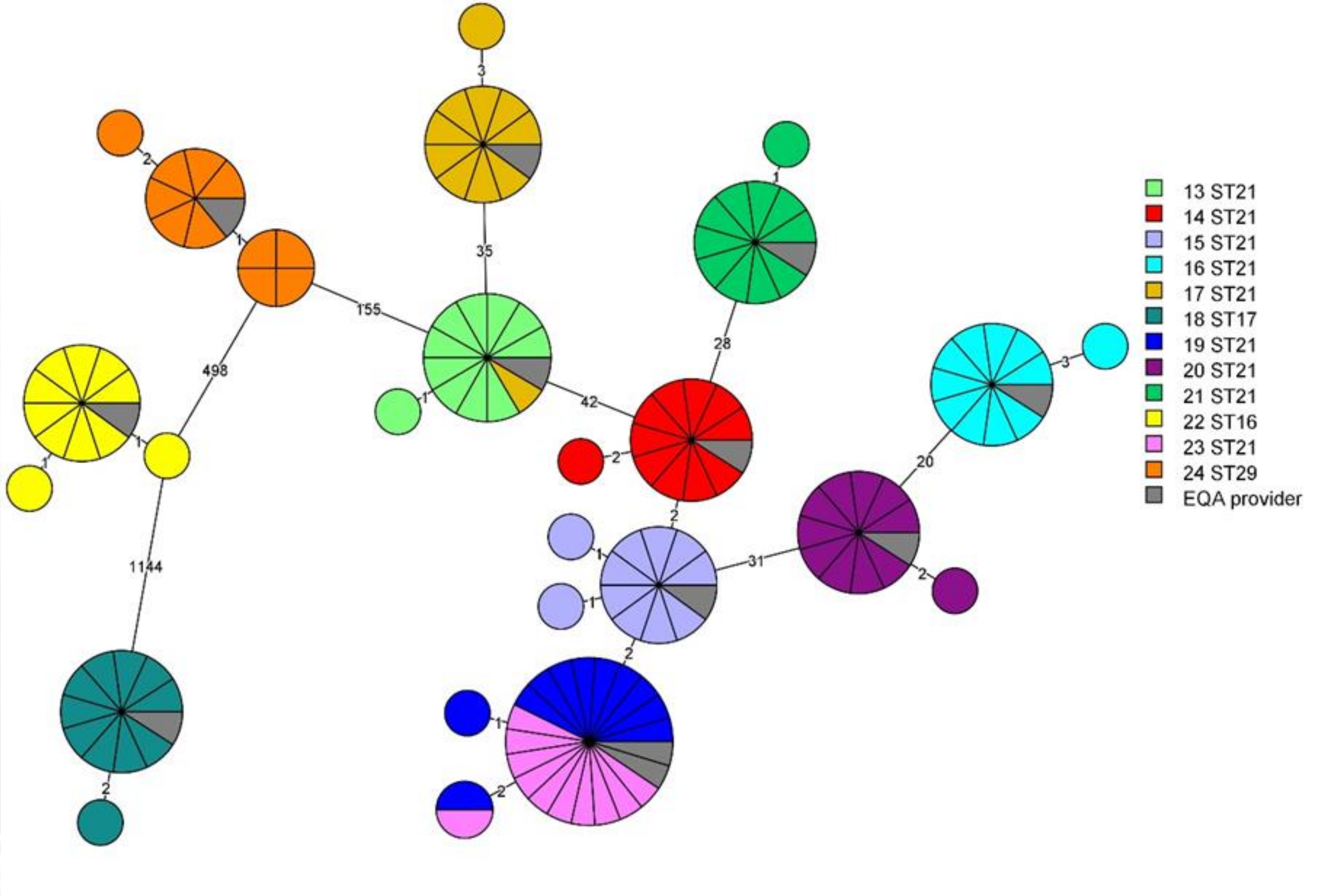

wgMLST (Core (EnteroBase))

**Single linked dendrogram of core genome multi-locus sequence typing (cgMLST) profiles of STEC EQA-8 isolates (cgMLST, EnteroBase, https://enterobase.warwick.ac.uk)**
**Analysed in BioNumerics: Maximum distance of 200 has been exceeded; results were clipped. Cluster isolates dark grey, outside cluster isolates light grey**
* **REF19 and REF23 are technical duplicates.**

# Cluster identification based on WGS-derived data

| | | Laboratory No. | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Isolate ID** | **ST** | **19** | **34** | **80** | **108** | **123** | **129** | **133** | **134** | **135** | **137** | **139** |
| REF13 | 21 | No | No | No | No | No | No | No | No | No | No | Yes |
| REF14‡ | 21 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| REF15‡ | 21 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| REF16 | 21 | No | No | No | No | No | No | No | No | No | No | Yes |
| REF17 | 21 | No | No | No | No | No | No | No | No | No | No | Yes |
| REF18 | 17 | No | No | No | No | No | No | No | No | No | No | No |
| REF19#‡ | 21 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| REF20 | 21 | No | No | No | No | No | No | No | No | No | No | Yes |
| REF21 | 21 | No | No | No | No | No | No | No | No | No | No | Yes |
| REF22 | 16 | No | No | No | No | No | No | No | No | No | No | No |
| REF23#‡ | 21 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| REF24 | 29 | No | No | No | No | No | No | No | No | No | No | Yes |
| Main analysis | | Allele (cgMLST) | Allele (cgMLST) | Allele (cgMLST) | SNP | Allele (cgMLST) | Allele (cgMLST) | Allele (wgMLST) | Allele (cgMLST) | Allele (wgMLST) | SNP | SNP |
| Additional analysis | | SNP | SNP | | | | | | | | | |
| Identified cluster | | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |

# Minimum spanning tree of core genome multi locus sequence typing (cgMLST) participant FASTQ files



Minimum spanning tree (MST) in log scale of core genome multi-locus sequence typing (cgMLST) [12] based on submitted raw reads (FASTQ files)

# Sero- and virulence typing: Summary

| Strain no. | O group | H type | *eae* geneH | *stx1* gene | *stx2* gene | *stx1* subtypes | *stx2* subtypes | *aggR* gene | *aaiC* gene | Additional virulence genes | Pathotype |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **REF1** | **O126** | **H27/H$^-$** | **-** | **-** | **-** | **-** | **-** | **+** | **+** | **AAFII *aap aar fyuA lpfA pet pic*** | **EAEC** |
| **REF2** | **O121** | **H19** | **+** | **-** | **+** | **-** | ***stx2a*** | **-** | **-** | ***ehxA efa1 lpfA hlyD toxB*** | **STEC** |
| **REF3** | **O187** | **H28** | **-** | **-** | **+** | **-** | ***stx2g*** | **-** | **-** | ***estAp lfpA katP, astA*** | **STEC-ETEC** |
| **REF4** | **O157** | **H7** | **+** | **-** | **+** | **-** | ***stx2a*** <br> ***stx2c*** | **-** | **-** | ***ehxA iha iss katP toxB*** | **STEC** |
| **REF5** | **O63** | **H6/H$^-$** | **+** | **-** | **+** | **-** | ***stx2f*** | **-** | **-** | ***cif*** | **STEC** |
| **REF6** | **O146** | **H28** | **-** | **+** | **+** | ***stx1a*** | ***stx2b*** | **-** | **-** | ***ireA iha lpfA subA*** | **STEC** |
| **REF7** | **O8** | **H9** | **-** | **-** | **+** | **-** | ***stx2e*** | **-** | **-** | ***iss lpfA fyuA iha iss astA*** | **STEC** |
| **REF8** | **O104** | **H4** | **-** | **-** | **-** | **-** | **-** | **+** | **+** | **AAFI *aap aar fyuA lpfA sigA pic*** | **EAEC** |
| **REF9** | **O91** | **H14** | **-** | **+** | **+** | ***stx1a*** | ***stx2b*** | **-** | **-** | ***ehxA ire iha lpfA saa hlyD*** | **STEC** |
| **REF10** | **O154** | **H31** | **-** | **+** | **-** | ***stx1d*** | **-** | **-** | **-** | **air eilA** | **STEC** |
| **REF11** | **O111** | **H8/H$^-$** | **+** | **+** | **-** | ***stx1a*** | **-** | **-** | **-** | ***ehxA cif efa1 hlyD iha lpfA*** | **STEC** |
| **REF12** | **O157** | **H7** | **+** | **+** | **+** | ***stx1a*** | ***stx2a*** | **-** | **-** | ***ehxA iha iss katP toxB*** | **STEC** |

# Conclusions EQA-8

**O grouping:**

- **O187 has strong cross-reactions with both O74, O103, and O175**
- **PCR does not react with O187.**

***stx*** **subtyping:**

- **Revision of nomenclature and development of refined PCR protocol for the detection of one new *stx1-* and five new *stx2* subtypes.**

**PFGE-derived data:**

- **Nine (36%) performed cluster analysis using PFGE**
- **Six participants (66%) identified the cluster.**

**WGS cluster analyses:**

- **Eleven (44%) performed cluster analysis using WGS-derived data**
- **ten (91%) correctly identified the cluster**
- **eight (73%) reported an allele-based method (0-3 allele difference cgMLST)**
- **three (27%) reported a SNP analysis (0-5 SNPs).**

# Recommendations EQA-8

- **Meet the deadline**
- **No swapping and mislabelling of isolates**
- **Perform test on ALL isolates regardless of results obtained in the screening and detection or any other test *i.e.* the NDs!!**
- **standard cgMLST scheme (e.g. Enterobase) gives a very high degree of homogeneity in the results**
- **allele-based methods seem to be useful for inter-laboratory comparability and communication about cluster definitions**