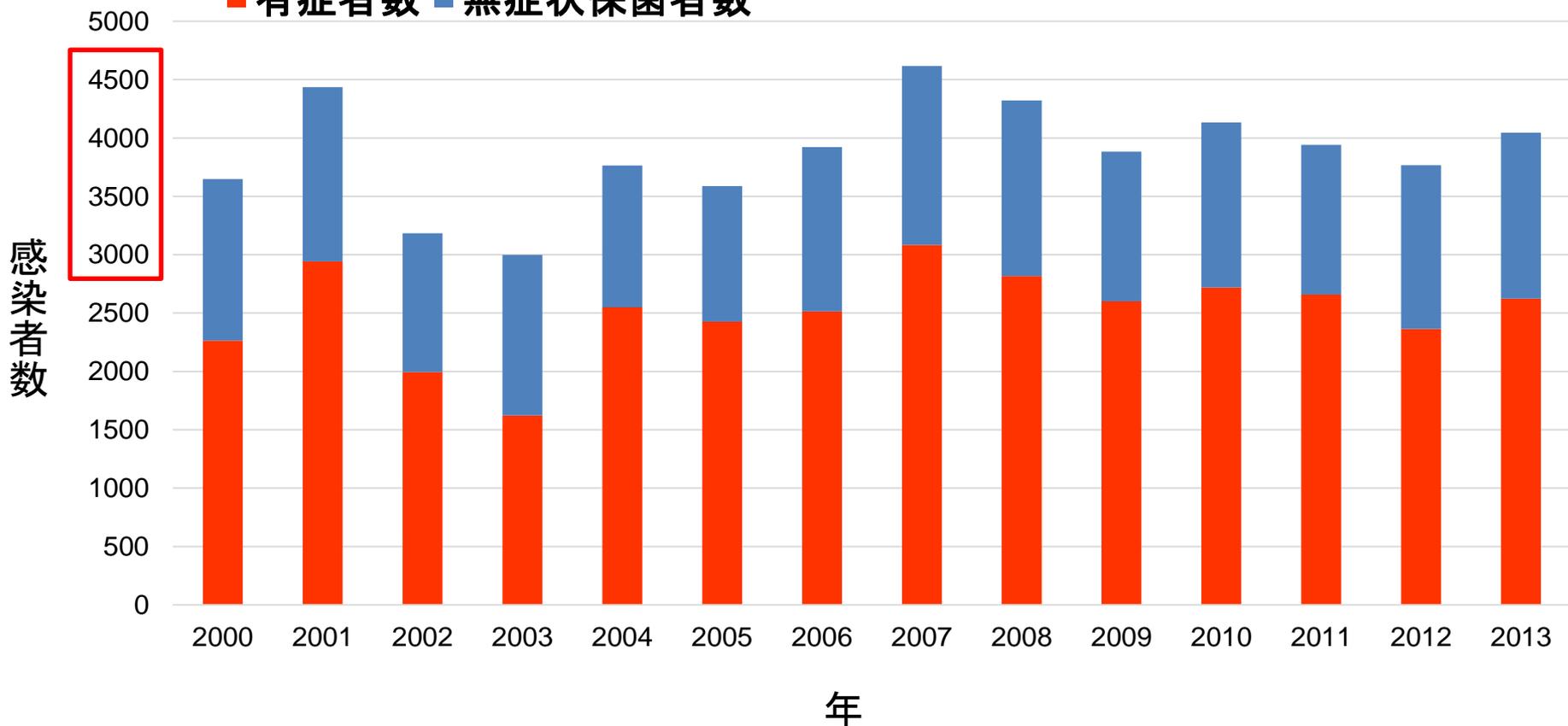


# 「大腸菌」

伊豫田 淳  
感染研・細菌 I 部

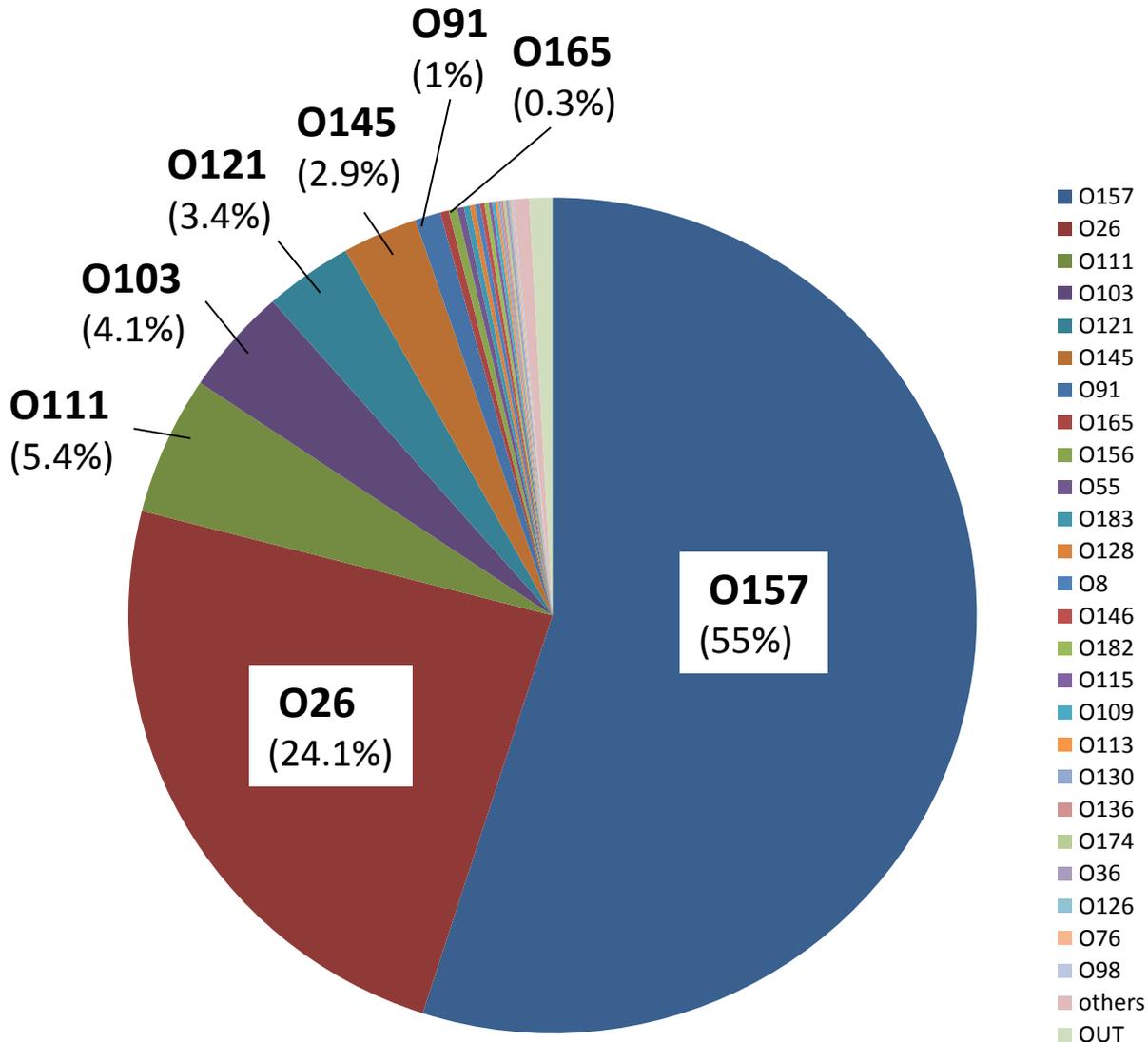
# 腸管出血性大腸菌感染症の年別発生状況

■ 有症者数 ■ 無症状保菌者数



(病原体検出情報の集計)

# 腸管出血性大腸菌のO血清群 (2013年; n=3,069)



(細菌第一部の集計)

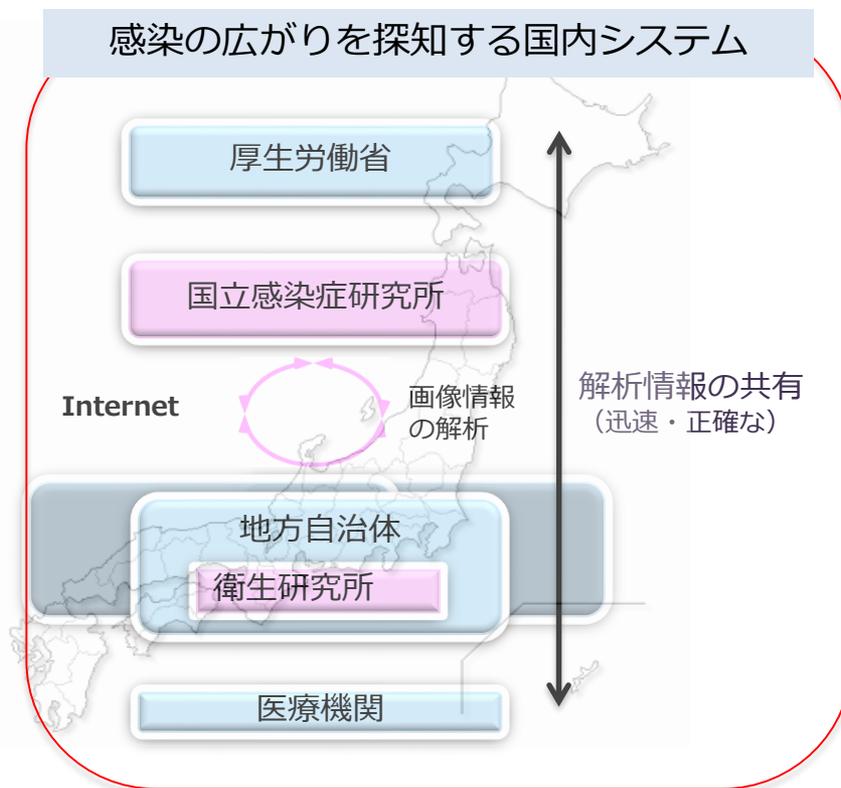
# 腸管出血性大腸菌の型別法



**MLVA:** multilocus variable-number tandem repeat analysis

**PFGE:** pulsed-field gel electrophoresis

# 腸管出血性大腸菌の分子型別解析結果還元法



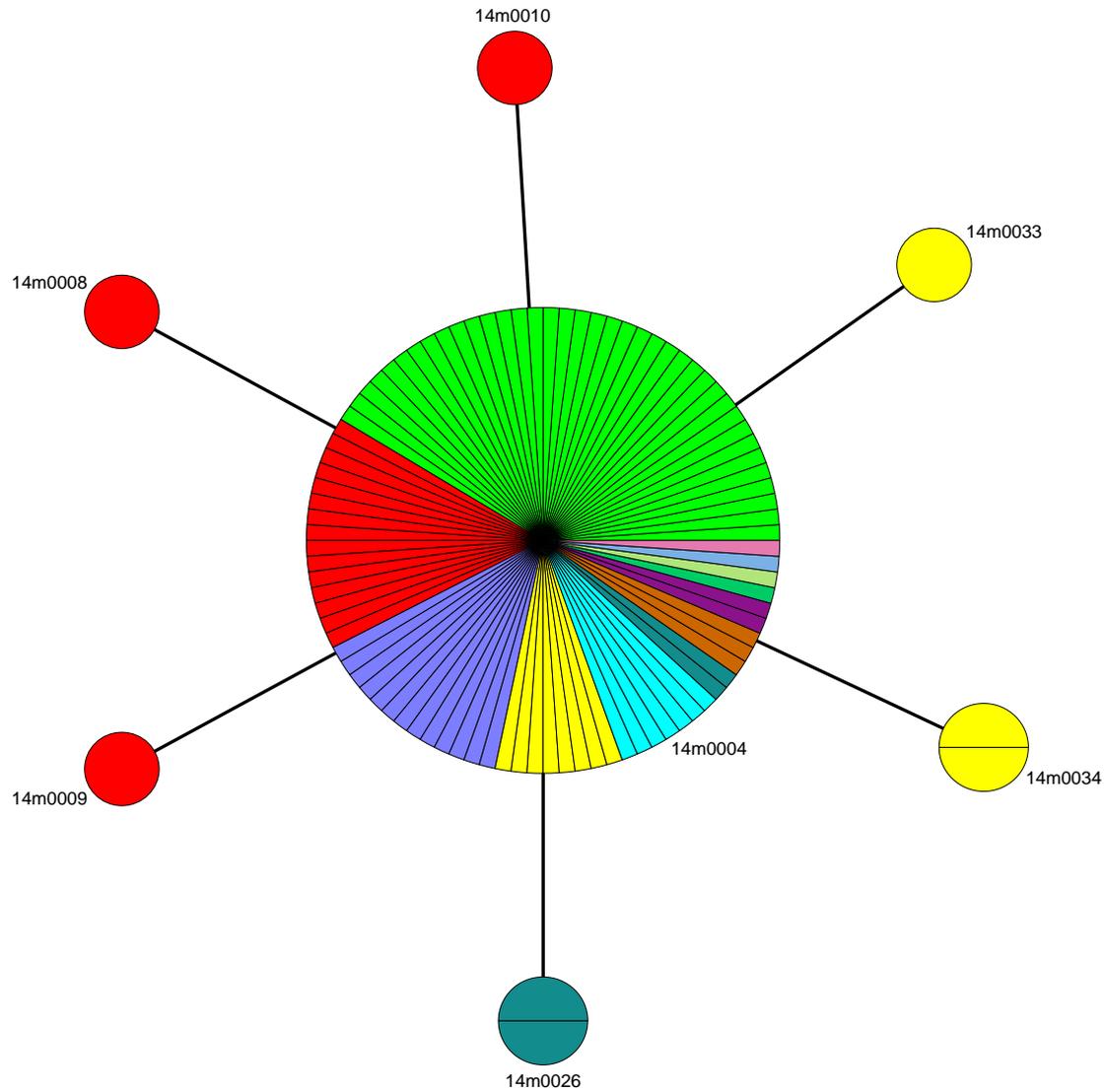
- **O157, O26, O111**は MLVA を利用して迅速に解析結果を報告.
- 広域事例が疑われる場合、**O157, O26, O111**代表株の PFGE データを提示.
- **他のO群**は PFGE 解析結果を報告.

# 集団事例調査における解析結果の判定基準

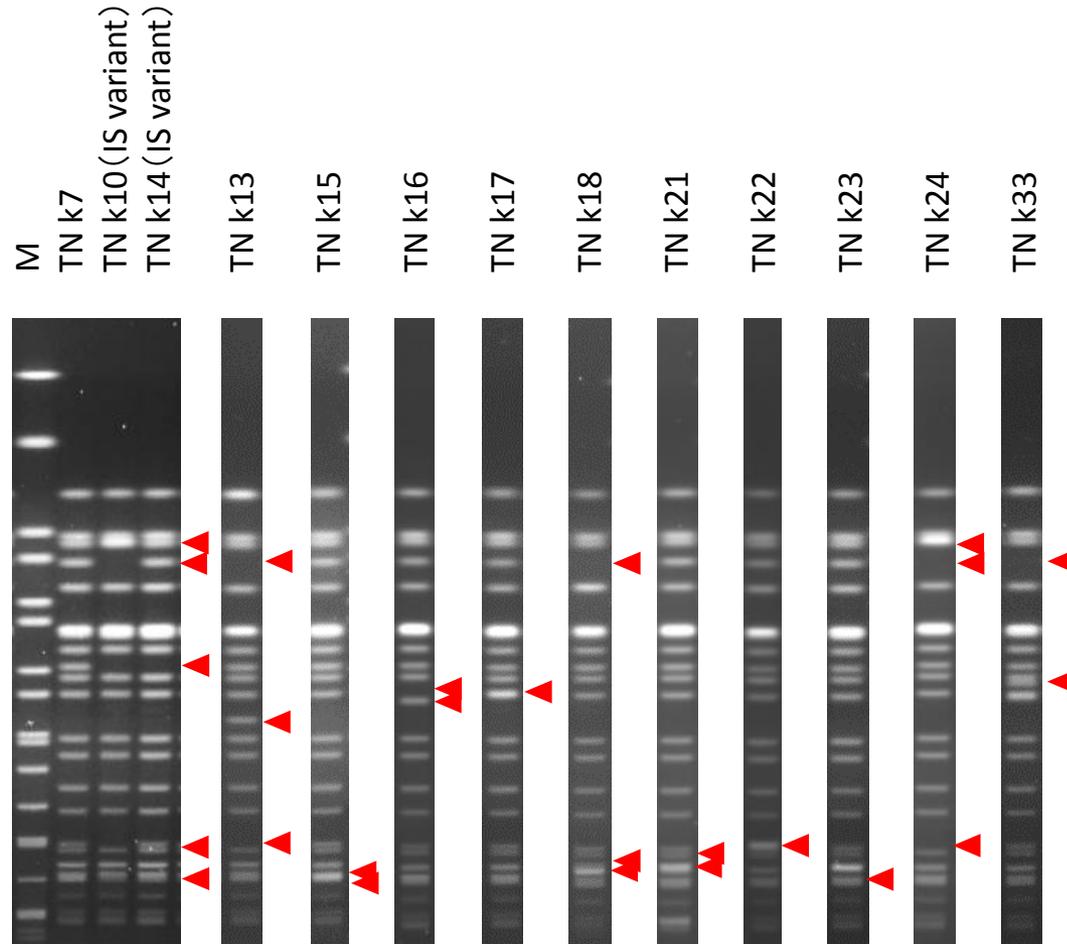
MLVA法では、相違する部位数が0の場合は一致、1部位違いの場合(SLV: single locus variant)は PFGEの2-3バンド違いと同様に集団発生の一部であると解釈します。

| 分類                            | PFGE   | MLVA    | 解釈                   |
|-------------------------------|--------|---------|----------------------|
|                               | 断片の相違数 | 相違部位数   |                      |
| 一致                            | 0      | 0       | 分離菌株は集団発生の一部である      |
| 密接に関係<br>(closely related)    | 2 - 3  | 1 (SLV) | 分離菌株はほぼ確実に集団発生の一部である |
| 関係する可能性<br>(possibly related) | 4 - 6  | 2       | 分離菌株は集団発生の一部かもしれない   |
| 不一致                           | 7以上    | 3以上     | 分離菌株は集団発生の一部ではない     |

# 馬刺し関連O157 MLVA パターン



# 馬刺し関連O157 PFGE パターン



(TN k10およびk14の ◀ 差異バンド位置はまとめて表示)

# 馬刺し関連 O157 型別まとめ

| IS           | MLVA type | PFGE type | 分離株数 | PFGE コメント                   |
|--------------|-----------|-----------|------|-----------------------------|
| Major type   | 14m0004   | K7        | 78   |                             |
| Variant type |           | K10       | 1    | five bands differ. from k7  |
| Variant type |           | k14       | 1    | two bands differ. from k7   |
| Major type   |           | k13       | 1    | three bands differ. from k7 |
| -            |           | k15       | 1    | two bands differ. from k7   |
| -            |           | k16       | 1    | two bands differ. from k7   |
| Major type   |           | k17       | 2    | one band differ. from k7    |
| -            |           | k21       | 1    | two bands differ. from k7   |
| -            |           | k22       | 2    | one band differ. from k7    |
| -            |           | k23       | 1    | one band differ. from k7    |
| -            |           | k24       | 1    | three bands differ. from k7 |
| -            |           | k33       | 1    | two bands differ. from k7   |
| Major type   | 14m0008   | k7        | 1    |                             |
| Major type   | 14m0009   | k7        | 1    |                             |
| Major type   | 14m0010   | k18       | 1    | three bands differ. from k7 |
| -            | 14m0026   | k7        | 2    |                             |
| -            | 14m0033   | k7        | 1    |                             |
| -            | 14m0034   | k7        | 2    |                             |

# 配布可能菌株 (1) : 下痢原性大腸菌PCRコントロール用菌株

| 菌株番号 | 保有遺伝子                                     | PCRサイズ (bp)       | プライマーセット    |
|------|---|-------------------|-------------|
| 1290 | <i>elt</i><br><i>estA2</i><br><i>astA</i> | 123<br>178<br>109 | ExEC        |
| 1297 | <i>estA1</i><br><i>astA</i>               | 179<br>109        | ExEC        |
| 1298 | <i>invE</i>                               | 379               | ExEC        |
| 1303 | <i>stx1/2</i><br><i>eae</i>               | 234<br>310        | ExEC        |
| 1733 | <i>stx2f</i><br><i>eae</i><br><i>astA</i> | 296<br>310<br>109 | ExEC        |
| 1782 | <i>afaD</i>                               | 207               | EpALL       |
| 1923 | <i>eae</i>                                | 310               | EpALL       |
| 1924 | neg control                               | ---               | ExEC, EpALL |
| 2279 | <i>aggR</i><br><i>astA</i>                | 254<br>109        | EpALL       |

# 配布可能菌株 (2) : *stx* PCRコントロール用菌株



## Appendix 2

### List of reference strains harbouring the *vtx* gene subtypes

| SSI collection D number | Strain  | Control for toxin subtype | Toxin variant designation | GenBank accession No. | Results obtained using the present method |
|-------------------------|---------|---------------------------|---------------------------|-----------------------|---|
| D2653                   | EDL933  | VT1a                      | VT1a-O157-EDL933          | M19473                | <i>vtx1a + vtx2a</i>                      |
| D3602                   | DG131/3 | VT1c                      | VT1c-O174-DG131-3         | Z36901                | <i>vtx1c + vtx2b</i>                      |
| D3522                   | MHI813  | VT1d                      | Stx1d-O8-MHI813           | AY170851              | <i>vtx1d</i>                              |
| D2435                   | 94C     | VT2a                      | VT2a-O48-94C              | Z37725                | <i>vtx1a + vtx2a</i>                      |
| D3428                   | EH250   | VT2b                      | VT2b-O118-EH250           | AF043627              | <i>vtx2b</i>                              |
| D2587                   | 031     | VT2c                      | VT2c-O174-031             | L11079                | <i>vtx2b + vtx2c</i>                      |
| D3435                   | C165-02 | VT2d                      | VT2d-O73-C165-02          | DQ059012              | <i>vtx2d</i>                              |
| D3648                   | S1191   | VT2e                      | VT2e-O139-S1191           | M21534                | <i>vtx2e</i>                              |
| D3546                   | T4/97   | VT2f                      | VT2f-O128-T4-97           | AJ010730              | <i>vtx2f</i>                              |
| D3509                   | 7v      | VT2g                      | 2g-O2-7v                  | AY286000              | <i>vtx2g</i>                              |

\* May result in both fragments at 179 bp and 280 bp

***stx1: stx1a, stx1c, stx1d,***

***stx2: stx2a, stx2b, stx2c, stx2d, stx2e, stx2f, stx2g***

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# 配布可能菌株 (3) : EHEC, EQA(2013-2014)用菌株



## EQA PART: SEROTYPING, PHENOTYPING, GENOTYPING AND *STX*/*VTX* SUBTYPING

### RESULTS TABLE

|                    | O group | H type | Vero Cell assay | ESBL prod. | Haemolysin prod. | Beta-glucuronidase prod. | Sorbitol ferm. | <i>eae</i> gene | <i>ehxA</i> gene | <i>vtx1</i> gene | <i>vtx2</i> gene | <i>vtx</i> Subtypes | Additional virulence genes | Pathogenic group |
|--------------------|---------|--------|-----------------|------------|------------------|--------------------------|----------------|-----------------|------------------|------------------|------------------|---------------------|----------------------------|------------------|
| AA1                |         |        |                 |            |                  |                          |                |                 |                  |                  |                  |                     |                            |                  |
| BB2                |         |        |                 |            |                  |                          |                |                 |                  |                  |                  |                     |                            |                  |
| CC3                |         |        |                 |            |                  |                          |                |                 |                  |                  |                  |                     |                            |                  |
| DD4                |         |        |                 |            |                  |                          |                |                 |                  |                  |                  |                     |                            |                  |
| EE5                |         |        |                 |            |                  |                          |                |                 |                  |                  |                  |                     |                            |                  |
| FF6                |         |        |                 |            |                  |                          |                |                 |                  |                  |                  |                     |                            |                  |
| GG7                |         |        |                 |            |                  |                          |                |                 |                  |                  |                  |                     |                            |                  |
| HH8                |         |        |                 |            |                  |                          |                |                 |                  |                  |                  |                     |                            |                  |
| II9                |         |        |                 |            |                  |                          |                |                 |                  |                  |                  |                     |                            |                  |
| JJ10               |         |        |                 |            |                  |                          |                |                 |                  |                  |                  |                     |                            |                  |
| KK11               |         |        |                 |            |                  |                          |                |                 |                  |                  |                  |                     |                            |                  |
| LL12               |         |        |                 |            |                  |                          |                |                 |                  |                  |                  |                     |                            |                  |
| MM13               |         |        |                 |            |                  |                          |                |                 |                  |                  |                  |                     |                            |                  |
| NN14               |         |        |                 |            |                  |                          |                |                 |                  |                  |                  |                     |                            |                  |
| OO15 <sup>n)</sup> |         |        |                 |            |                  |                          |                |                 |                  |                  |                  |                     |                            |                  |

Pos.: Positive, Neg. Negative, alfa: pos. for alfahaemolysin, but entero/alfahaemolysin results is accepted for all strains.

Intermediate result noted in the Vero cell assay is accepted as a positive result. H- result noted in the H type is also accepted for all strains.

<sup>qq)</sup> Lactose negative

<sup>nn)</sup> The strain has been observed to lose the ESBL plasmid. Therefore, both results will be accepted.

#### Gene abbreviations

*eae*: CVD434. *E. coli* attaching and effacing gene probe.

*ehxA*: CVD419. Plasmid encoded O157-enterohaemolysin.

*vtx1*: NTP705. Verotoxin1; Almost identical with the Shiga toxin.

*vtx2*: DEP28. Verotoxin2; Variants exist. Approx. 60% homology to *vtx1*.

*aggR*: Gene encoding the master regulator in Enteroaggregative *E. coli*.

*aaiC*: Chromosomal gene marker for Enteroaggregative *E. coli*.

*eht*: G119. Heat labile enterotoxin (LT). Almost identical to cholera toxin.

*aatA*: PCR fragment. The gene encodes the dispersin (*aap*) transporter protein, which is a good plasmid marker for Enteroaggregative *E. coli*.

*estAp*: DAS101. Heat stable enterotoxin (porcine variant) ST<sub>p</sub> (ST1a).

*ipaH*: WR390. Invasion plasmid antigen. These genes are found in several copies chromosomally as well as on plasmids.

# ○157チェック「LPS抗体」

- 血清中の大腸菌O-157LPS抗体の検出
  - ラテックス・スライド凝集法
- 販売元:シオノギ製薬(株)、製造販売元:(株)LSIメディエンス
- LPS抗体検出の唯一の体外診断用医薬品 175点
  
- 採用施設数、出荷数が極少のため、2015年3月末をもって販売終了を検討中(2014年7月最終回製造を検討中)
  - ○抗原凝集抗体価測定が、代替検査になり得るか？
  - 販売終了による影響は？

大腸菌に関するお問い合わせは、一括して

[ehec@niid.go.jp](mailto:ehec@niid.go.jp) へお願いします。

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泉谷 秀昌 [izumiya](#)